The Role of Quercetin against *o*-Anisidine Toxicity in Some Physiological, Biochemical and Histopathological Parameters of Laboratory Rats (*Rattus norvegicus*)

A Dissertation Submitted to the Council of the College of Veterinary Medicine, University of Basrah in Partial Fulfillment of the Requirements for the Philosophy Degree of Doctorate of Science in Physiology

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Confirmation of Supervisor

I certify that the preparation of this thesis was prepared under our Supervision at Department of Physiology, Veterinary Medicine College/University of Basrah as a Partial Fulfilment of the Requirements for The Degree of Physiology Doctorate of Sciences in Physiology.

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Certification of Examining Committee

We, the members of examining committee , certify after reading thesis ((**The Role of Quercetin against *o*-anisidine Toxicity in Some Physiological, Biochemical and Histopathological Parameters of Laboratory Rats (*Rattus norvegicus*)**). And after examining the student in it's content ,we found it is adequate for the Doctorate Science degree in Physiology. With excellent degree.

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DEDICATION

To that man….Who burned the night oil for me…My deceased father.

To the woman….Who was the warm lap and sacrificed everything for me…My darling mother.

To that gem…. Who hold me up along this difficult road…my beloved wife.

To the blooming daffodils for them I had every chip on my shoulder…my children.

And to those…who were a real shelter to which I resort…My brothers.

Wissam Al-Uboody

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IN THE NAME OF ALLAH THE MOST GRACIOUS THE MOST MERCIFUL

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Summary

The present study was conducted at Veterinary Medicine College– University of Basrah to investigate the effects of ortho-anisidine hydrochloride and the ameliorating effect of quercetin dihydrates in laboratory rats. The experiment was divided into two parts. The first was dealing with hematological and biochemical parameters and histopathological changes, whereas the second was dealing with the reproductive efficiency.

In the first part, forty eight male rats were used. They were divided into three groups of sixteen male rats to each. The first group was the control group, the animals of which were maintained on a standard ration for fifteen days, and then eight rats of them were terminated for the perquisite hematological, biochemical, and histopathological tests. The first treated group was (T1) group, in which the animals were maintained on a ration, contains 7g/kg/diet ortho-anisidine hydrochloride. The second treated group was (T2) group, in which the animals were maintained on a ration, contains 7g/kg/diet o-anisidine hydrochloride + 50mg/kg/diet quercetin dihydrates. After a period of 15 days, 8 animals of each of the two groups were terminated to perform the required tests and the remaining 8 animals were terminated after additional 15 days. The investigation of blood parameters included Red Blood Cells count (RBC), Hemoglobin concentration (Hb), Packed cells volume (P.C.V.), total and differential White Blood Cells count (W.B.C.), Mean Corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin

concentration (MCHC), Mean corpuscular volume (MCV), and Mean platelets volume (MPV); biochemical parameters included Total Serum Cholesterol (TSCH), Triglycerides (TGs), High density lipoprotein (HDL), Low density lipoprotein (LDL), Very low density lipoprotein (VLDL); liver function enzymes like Alanine amino transferase (ALT), Aspartate amino transferase (AST), alkaline phosphatase (ALP), Malondialdehyde (MDA); antioxidant enzymes like Catalase (CAT), Superoxide dismutase (SOD), and Glutathione peroxidase (GPX) were all performed . Histopathological changes of the liver and kidneys were also done.

In the second part of the experiment, 16 male and 32 female rats were divided into eight similar groups; they were maintained on different rations either containing o-anisidine hydrochloride or quercetin dihydrates or a mixture of them.

Parents were allowed to mate, and then separated after 16 days to let females deliver. Semen aspects were reported for all male rats. Female rats' reproductive abilities were estimated after delivery.

The results revealed that the treatment with o-anisidine hydrochloride for 15 days caused significant decrease in the R.B.C. count, Hb concentration, P.C.V. percentage, neutrophil and lymphocyte counts and it caused significant increase in platelets count, total leukocytes, monocytes, eosinophil and basophil counts, as compared with control group. When quercetin dihydrates was offered as a protective agent, it showed a significant ameliorating effect by increasing the R.B.C. count, Hb concentration, P.C.V. percentage, neutrophil and lymphocyte counts and it caused significant decrease in platelets count, total leukocytes, monocytes, eosinophil and basophil counts, as compared with the 1rst treated group. For the blood indices (RDW, MCH,

MCHC, MCV, and MPV) there were no significant differences among all the experiment groups except for the mean platelet volume (MPV), where o-anisidine hydrochloride caused significant decrease in the MPV of the 1rst treated group as compared with control and 2nd treated group at ($P \leq 0.05$).

The histopathological changes of livers and kidneys of the animals of the 1rst experiment were done and studied well. The histopathological examinations revealed that o-anisidine hydrochloride caused different deleterious effects in tissues of liver and kidney in both treatment periods such as swelling, fatty degeneration, hemorrhage, and infiltration of inflammatory cells. While the renal changes involved degeneration of renal tubules, destruction of renal tubules, hemorrhage, edema, infiltration of inflammatory cells specially the messangial cells, and presence of hyaline droplets in the cortical renal tubules. When quercetin was administered, it really ameliorated the histological texture of liver and kidney toward the normal, where it decreased clearly the damage caused by o-anisidine.

The results of the second part of the experiment revealed that the treatment with o-anisidine hydrochloride for 30 days (T1, T2 and T3 groups) caused significant decrease in the males body weights, sperm count, individual and massive sperm motility, testes weights, and epididymis weights as compared with control and (T4, T5, T6 and T7) groups at ($P \leq 0.05$).When quercetin dihydrate was offered as an ameliorating agent, it showed a significant ameliorating effect by increasing the body weights, sperm count, individual and massive sperm motility, testes weights, and epididymis weights . When quercetin dihydrates was offered alone in the ration of the sixth treated group (T6), it caused clear significant ameliorating effect on all sperm parameters comparing with all treated groups and the sperm count was even significantly higher than that of control group while the other aspects were similar to those of control group at ($P \leq 0.05$). Beside, o-anisidine caused significant decrease in the number of pregnant females, number of delivered litters, weight of

litters and sex ratio, and it prevented the pregnancy from being occurred in the group where both male and females are treated with it (T1). When quercetin was mixed with o-anisidine in the ration of (T7), it significantly ameliorated the pregnancy chances, number of litters and the sex ratio as compared with the other groups but it didn't reach to significant level with control group at ($P \leq 0.05$).

It was concluded from our study that *o*-anisidine has very bad effects on different body systems and it was a major cause of anemia, hyperlipidemia, immune deficiency and infertility. On the other hand, quercetin was found to be a potent antioxidant which prevents or ameliorates all the deleterious effects of *o*-anisidine.

1-Introduction

Ortho-anisidine is one of the aromatic amines EU (2006). It was produced in Armenia, China, France, Germany, India, Japan, the Ukraine and the United Kingdom, CIS (1995). WHO (1999) mentioned that the principal commercial use of o-anisidine is an intermediate in the manufacture of dyes and pharmaceuticals and as an intermediate in the manufacture of synthetic guaiacol and its derivatives. Ortho-Anisidine is also used as a corrosion inhibitor for steel storage, NTP (1991). It has been known that o-anisidine is a potent carcinogen which is classified as group 2B carcinogen, IARC (1982, 1999). Furthermore, it may be released from textiles and leather goods colored with these azo dyes and a large part of the population may be exposed and as a constituent of cigarette smoke, Stabbert *et al.* (2003). O-anisidine was found in human urine sample in the general population, in concentrations of 0.22 µg/l (median), Weiss and Angerer (2003). In addition, hemoglobin adducts of o-anisidine were detected in blood samples of persons living in urban or rural areas of Germany, Richter *et al.* (2001). Stocker (2002) mentioned that young children are exposed by oral suckling clothes which are colored with o-anisidine based dyes. She added that in addition to these specific situations, the risk assessment shows that residual risks cannot be excluded concerning all the populations and uses of the substance with relevant exposure, since o-anisidine is identified as a genotoxic carcinogen for which a threshold cannot be reliably

identified and in particular this applies to the following situations: Workplace exposure by inhalation in the production and processing of o-anisidine; workplace exposure by inhalation and by dermal contact during the formulation of o-anisidine based printing inks; general population in dermal contact with packing materials printed with o-anisidine based pigments; and man exposed indirectly via the environment, Stocker (2002).

Flavonoids are a group of naturally occurring polyphenolic compounds widely distributed as secondary metabolites in plant kingdom, Vidhya and Indira (2009). Quercetin (3, 5, 7, 3, 4-pentahydroxy flavon), is one of the most prominent dietary antioxidants, Paolillo *et al.* (2011). Quercetin occurs in glycosylated form in French beans, broccoli, apples and especially in onions, Pandey and Rizvi (2009). Quercetin is the most abundant antioxidant in the nature and has an antioxidant potential four times that of vitamin E, Sangai and Verma (2012). Quercetin has been reported to increase the genomic stability in rats and enhance the antioxidative defense system by up regulating antioxidant enzymes, Tieppo *et al.* (2007). It has many beneficial effects in human health, including cardiovascular protection, anticancer activity, anti-ulcer effects, anti-allergy activity, cataract prevention, antiviral activity and anti-inflammatory effects, Reutrakul *et al.* (2007). Quercetin prevents oxidant injury and cell death by several mechanisms, such as scavenging oxygen radicals, protecting against lipid peroxidation and chelating metal ions, Inal *et al.* (2002).

The aim of this study was to investigate the bad deleterious effects of *o*-anisidine hydrochloride on different parameters of rats and to detect the ameliorating ability of quercetin dihydrates against these effects in

order to recommend quercetin as an antioxidant for protection and treatment of different disorders.

2-Literature Review

2.1. Ortho-anisidine

O-Anisidine is an aromatic amine that exists at room temperature as a liquid with an amine-like odor and ranging in color from colorless to yellowish, pink, or reddish. It is soluble in water, miscible with ethanol, benzene, diethyl ether, and acetone, and soluble in dilute mineral acids. O-Anisidine hydrochloride is a salt of o-anisidine. It is a gray-black crystalline solid or light gray powder at room temperature and is soluble in water, NTP (2011). The oral LD50 of ortho-anisidine has been reported to be 2000 mg/kg BW in rats, IARC (1999). O-Anisidine is produced from o-nitroanisol (2-methoxy-nitrobenzene) by catalytic reduction with hydrogen under pressure in an inert liquid medium and it is estimated that less than 1,000 tones are produced annually within the European committee, EC (2011). O-Anisidine was produced commercially in the United States from the 1920s until 1957, Lunn (2011). In 2009; six manufacturers of *o*-anisidine were identified worldwide. O-Anisidine was available from 44 suppliers, including twenty U.S. suppliers, and the hydrochloride salt was available from eight suppliers, including five U.S. suppliers, Lunn (2011). U.S. imports of *o*-anisidine and its hydrochloride salt are reported in the category

"*o*-anisidine, *p*-anisidine, and *p*-phenetidine," and U.S. exports are reported in the category "anisidines, dianisidines, phenetidines and their salts." From 1989 to 2008, imports in the category ranged from a high of over 4.6 million kilograms (10.1 million pounds) in 1996 to zero in 2007 and 2008, and exports ranged from zero to 262,000 kg (577,000 lb), Gately (2009). Reports filled under the U.S. Environmental Protection Agency's Toxic Substances Control Act Inventory Update Rule indicated that U.S. production plus imports of *o*-anisidine totaled 500,000 lb to 1 million pounds in 1986, 1990, and 2006; 1 million to 10 million pounds in 1990 and 1998; and 10,000 to 500,000 lb in 2002, EPA (2009). Figures (1) and (2) show the chemical formulas of o-anisidine and o-anisidine hydrochloride.

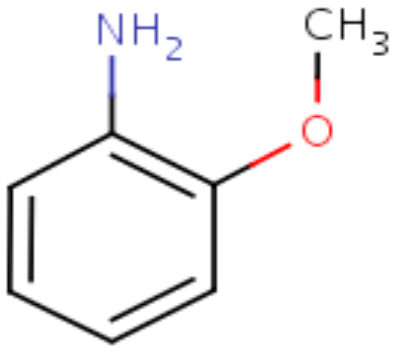


Figure (1). Ortho-anisidine (2-Methoxyaniline)

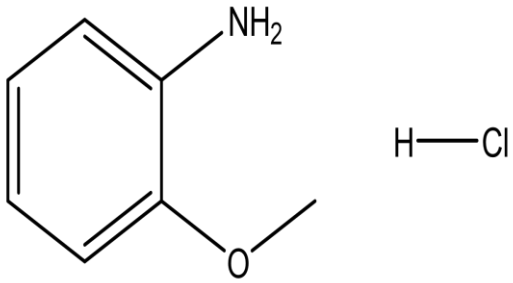


Figure (2). Ortho methoxy aniline

2.1.1. Exposure

Occupational exposure to o-anisidine and its hydrochloride salt may occur during their production and use as a chemical intermediate, corrosion inhibitor, or antioxidant, IARC (1999). The National Occupational Exposure Survey (conducted from 1981 to 1983) estimated

that 705 workers in the Chemicals and Allied Products industry potentially were exposed to o-anisidine and 1,108 workers in the same industry potentially were exposed to o-anisidine hydrochloride, NIOSH (1990).

The primary routes of potential human exposure to o-anisidine hydrochloride are inhalation and dermal contact; exposure may also occur by ingestion, HSDB (2009). Individuals in the population could be exposed to o-anisidine in the environment. O-Anisidine was detected at concentrations ranging from less than 0.05 to 4.2 μg/L (median = 0.22 μg/L) in urine samples from 20 members of the general population in Germany, Weiss and Angerer (2002). O-Anisidine occurs in cigarette smoke where the mean concentrations of o-anisidine in smoke from market, reference, and other cigarettes were reported to range from less than 0.2 to 5.12 ng per cigarette and as an environmental pollutant in wastewater from oil refineries and chemical plants, Stabbert *et al.* (2003). If released to air, o-anisidine is expected to remain in the vapor phase and to be degraded by reaction with hydroxyl radicals, with a half-life of 6 hours. If released to surface water, it is expected to bind to sediment or suspended solids with high organic matter content and to volatilize from water with an estimated half-life of 31 days from streams and 350 days from lakes. O-Anisidine has little potential to bio accumulate in aquatic organisms. If released to soil, it will likely bind to humid materials; at low concentrations, it will be subject to rapid biodegradation under aerobic conditions, HSDB (2009). Arylamines and nitroarenes are important intermediates used in the industrial manufacture of dyes, pesticides and plastics, and are significant environmental pollutants (e.g. from car exhausts and technical spills).

They rank among potent toxic or carcinogenic compounds, presenting a considerable danger for human population (IARC, 1989).

O-anisidine (2-Methoxyaniline) is important pollutant and potent carcinogen for rodents, Rydlovaa *et al.* (2005). The chemical is used chiefly in the manufacture of dyes, one method being the diazotization of o-anisidine and coupling with other aromatic amines or phenols to yield a large number of the azo dyes, Noller (1965). Different derivatives of o-anisidine like Chloro, nitro, alkyl, and aryl exist and are used similarly in the synthesis of other azo dyes. O-Anisidine is listed as a possible ingredient in permanent oxidation hair dyes, Wall (1972). Another use of o-anisidine is as a starting material in the synthesis of guaiacol (o-methoxyphenol), Stecher (1968). O-toluidine, is an aromatic amine analog of o-anisidine, was carcinogenic in long-term feeding studies, Russfield *et al.* (1973).Textile dyes are widely used in the world today and textile industry plays an important role in the nations' economy. Direct dyes are the most popular class of dyes owing to easy application, wide color range, and available at modest cost. Most direct dyes have diazo and triazo structures, Li-Ying *et al.* (2012). Azo dyes are the main constituents of such pollution because of their wide applicability and usages, and therefore, these are present extremely in textile industrial effluents. Mammalian and microbial enzyme systems have been reported to degrade azo dyes. Direct oxidation of azo linkage of azo dyes will activate them to highly reactive electrophilic diazonium salts, Xu *et al.* (2010).

2.1.2. Toxicokinetics

In the mammalian liver, including man, azo compounds are enzymatically cleaved by cytosolic and microsomal enzymes to the corresponding amines, figure (3). Some aromatic amines can be metabolically activated to DNA binding intermediates that are mutagenic and carcinogenic to the human hepatic cell by azoreductase. It is a non-specific enzyme, it is found in various micro-organism (such as in intestinal bacteria) that catalyze a nicotinamide adenine dinucleotide phosphate NADPH dependent reductions, Al-Mashedy (2013). The microbiota also plays roles in the degradation of azo dyes. In many cases the products formed after the degradation of the parent azo dye molecule are more toxic. These products are mainly in aromatic amine form. Azo dyes have been shown to be mutagenic to the human hepatic cell with azo reduction which being the most important reaction related to toxicity and mutagenicity, Jadhav *et al*. (2011). Ring oxidation, N-glucuronidation, N-acetylation, and N-oxidation are the major metabolic pathways of arylamines in mammals, Beland and Kadlubar (1990). N-Oxidation is a crucial step in the metabolism of arylamines and aromatic amides to toxic products. Arylamines are metabolized in the liver by mono-oxygenases to highly reactive N-hydroxyarylamines. Nitroarenes are reduced by microorganisms in the gut or by nitroreductases and aldehyde dehydrogenase in hepatocytes to nitrosoarenes and N-hydroxyarylamines, Rickert (1987). N-Hydroxyarylamines can be further metabolized to N-

sulfonyloxyarylamines, N-acetoxyarylamines or N-hydroxyarylamine N-glucuronide. These highly reactive intermediates are responsible for the genotoxic and cytotoxic effects of this class of compounds, Neumann *et al.* (1994). Xanthine oxidase is the principal enzyme responsible for the reduction metabolism of o-nitroanisole, catalyzing formation of N-(2-methoxyphenyl) hydroxylamine and o-anisidine, Mikšanova *et al.* (2004). Human hepatic microsomal cytochrome P450 (CYP) enzymes as well as these of experimental animals participate in the detoxification metabolism of o-nitroanisole, leading to its demethylation, which enables its excretion from the organism, Rydlovaa *et al.* (2005). O-Anisidine is oxidized by human hepatic microsomes to N-(2-methoxyphenyl) hydroxylamine, which is the same active intermediate as that formed from o-nitroanisole by nitroreduction. The major enzyme participating in this reaction is CYP2E1, followed by CYP1A and 2B6 and such an o-anisidine activation leads to the formation of DNA adducts. Furthermore, similar DNA adduct patterns were obtained also in vivo in urinary bladder, liver, kidney and spleen of rats treated with o-anisidine or o-nitroanisole, Stiborova *et al.* (2005). 2-Nitroanisole metabolite 2-nitrophenol is the major metabolite generated by rabbit and rat microsomal enzymes, but 2, 5-dihydroxynitrobenzene is the predominant product formed in human microsomal cytochromes P450, Svobodova *et al.* (2008). Therefore, hepatic microsomal P450 enzymes participate in detoxication of this environmental carcinogen. O-anisidine is oxidized by human, rat and rabbit hepatic microsomes containing cytochromes P450 not only to N-(2-methoxyphenyl) hydroxylamine, but that this compound is a subject of complex redox cycling reactions, forming also o-aminophenol, 2- nitrosoanisole and one additional

metabolite, the exact structure of which has not been identified as yet, Naiman *et al.* (2008). N-(2-methoxyphenyl) hydroxylamine might also be a subject of complex reactions, and its fate is dependent on the environment, in which it occurs. N-hydroxyarylamine intermediates can be further metabolized by phase II enzymes, such as N,O-acetyltransferases (NATs) or sulfotransferases (SULTs), leading to the formation of reactive esters, e.g. N-acetoxy- or N-sulfoxyarylamines which undergo heterolysis of the N--O or S--O bond to produce electrophilic nitrenium ions, Arlt *et al.* (2003). It can be further metabolized to o-aminophenol, o-nitrosoanisole and parental o-anisidine or when nucleophiles such as DNA or proteins are present in the cell, form the adducts, Stiborova *et al*. (2005). O-Demethylation of 2-nitroanisole to 2-nitrophenol and its hydroxylated products, 2, 5-dihydroxynitrobenzene and 2, 6-dihydroxynitrobenzene, Dračinska *et al.* (2006). Following intraperitoneal administration of radio-labelled O-anisidine, about 70% of the administered dose was eliminated in urine after 72 hours, Sapota *et al*. (2003). The metabolite Nitrobenzene undergoes further metabolic activation where it forms a number of phenolic compounds by oxidation and nitroxides by reduction, Holder (1999). Reduction of nitro group plays a more potent role in NB carcinogenicity and toxicity. Nitroreduction, which is driven by microsomal P-450s and NAD(P)H, can produce reactive nitroxides intermediates aromatic nitroso- and hydroxylamine compounds, e.g. NOB and PH, associated with their reactive free radicals, e.g., the nitro-anion free radical and superoxide free radical, Mason and Holtzman (1975).

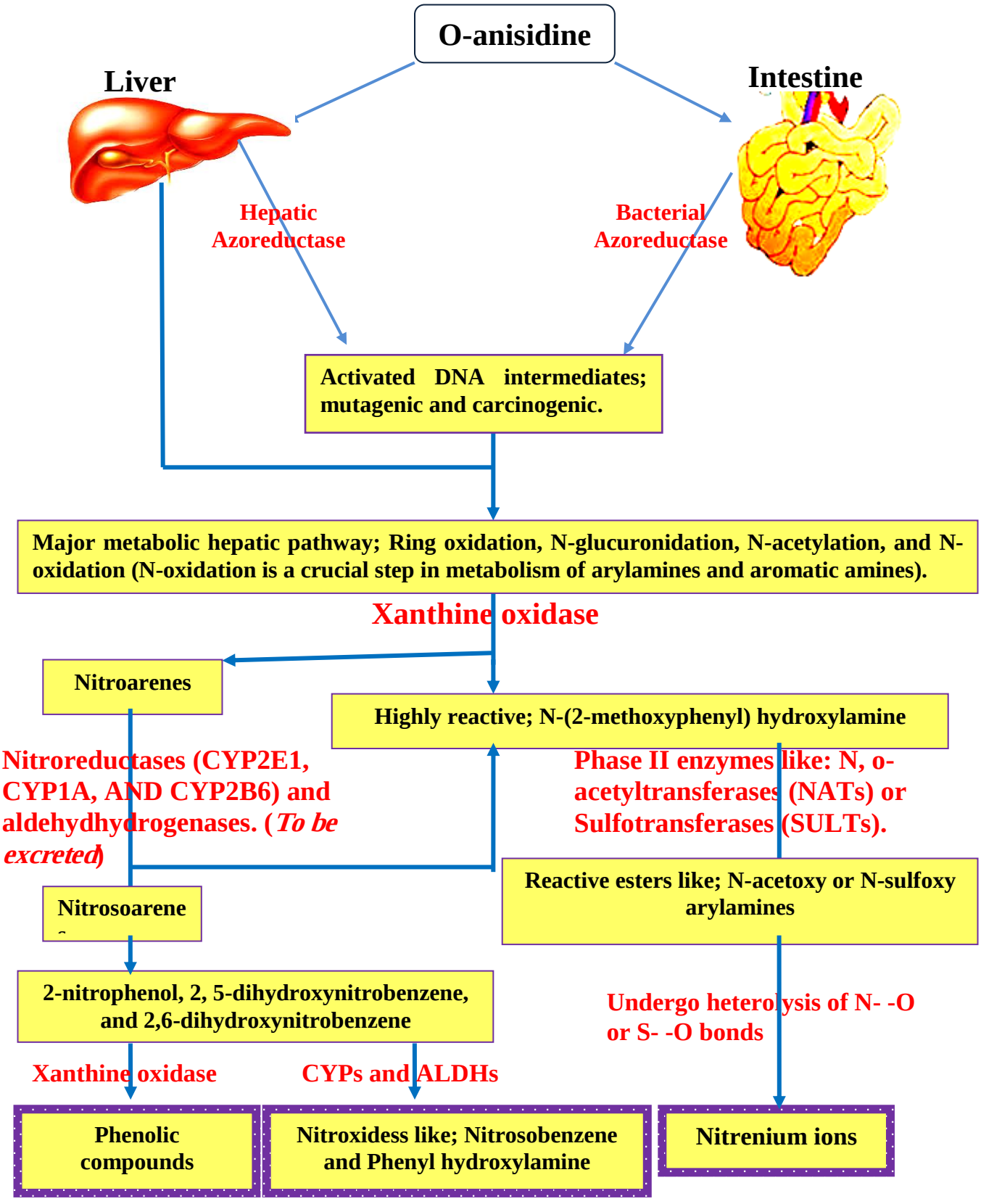

O-anisidine
Liver
Intestine
Hepatic Azoreductase
Bacterial Azoreductase
Activated DNA intermediates; mutagenic and carcinogenic.
Major metabolic hepatic pathway; Ring oxidation, N-glucuronidation, N-acetylation, and N-oxidation (N-oxidation is a crucial step in metabolism of arylamines and aromatic amines).
Xanthine oxidase
Nitroarenes
Highly reactive; N-(2-methoxyphenyl) hydroxylamine
Nitroreductases (CYP2E1, CYP1A, AND CYP2B6) and aldehydhydrogenases. (To be excreted)
Phase II enzymes like: N, o-acetyltransferases (NATs) or Sulfotransferases (SULTs).
Nitrosoarene
Reactive esters like; N-acetoxy or N-sulfoxy arylamines
2-nitrophenol, 2, 5-dihydroxynitrobenzene, and 2,6-dihydroxynitrobenzene
Undergo heterolysis of N- -O or S- -O bonds
Xanthine oxidase
CYPs and ALDHs
Phenolic compounds
Nitroxidess like; Nitrosobenzene and Phenyl hydroxylamine
Nitrenium ions

Figure (3). Metabolism of o-anisidine.

2.1.3. Effects of o-anisidine on different body systems

2.1.3.1. Effects of o-anisidine on circulatory and immune systems

Significantly elevated methaemoglobin levels were found in mice and rats following exposure to o-anisidine by gavage to doses of 690 or 1380 mg/kg, Ashby *et al* (1991). Burns *et al.* (1994) found a decrease in erythrocyte number with a concomitant increase in mean corpuscular Hb and mean corpuscular volume due to exposure of female mice to nitrobenzene. In cats a single intravenous injection of 7.7 mg/kg resulted in significantly elevated methaemoglobin levels in samples taken 1 to 5 hour after administration, McLean *et al*. (1969). Grant (1959) studied the effect of O-aminophenol on pulmonary ventilation in rats. Twelve anaesthetized tracheotomized rats could be studied at a time, allowing the ventilation of each to be measured four times/hr. Wistar albino rats were used, in the age range 3 to 5 months. The mean weights of male were 270 g and mean weight of females were 190 g. Ventilation in the control period averaged 125 ml. /min., with a mean rate of 78 respirations in each 30 second period. In the test period, ventilation increased significantly with all the compounds. In seven instances both rate and depth of respiration were increased, but in two the increased minute volume was produced entirely by tachypnea. In a report made by SCCS (2010), the scientific committee mentioned that exposure to o-aminophenol in Sprague Dawley causes orange discoloration of the urines with orange discoloration of the fur. Signs of regenerative macrocytic anemia were also seen. Increase of GOT activity was registered. Increase of blood urea nitrogen was observed as well as an

increase of urinary proteins. Another study by Sabbioni (1994) on female Wistar rats. In general, lower hydrolysable hemoglobin- adduct levels were found in rats that were given nitroarenes than in rats that were dosed with an equimolar amount of the corresponding arylamines. Clinical signs of toxicity, such as ataxia, head tilt, lethargy, and trembling, were evident. Santi *et al.* (2012) Mentioned in a study on dogs that oxidative stress causes neutropenia and accelerates the apoptosis of neutrophils. Neutrophils produce reactive oxygen species (ROS) when nicotinamide adenine dinucleotide phosphate oxidase is activated, generating the superoxide anion essential to the bactericidal function of neutrophils, Huimin *et al.* (2000). Although the ROS derived from superoxide are required for the defense mechanism of neutrophils, free radicals produced in excess can damage a number of cellular structures, thus inducing lipid peroxidation and accelerating apoptosis, Kato *et al.* (2008). Barbosa and Mori (2010) verified in vitro that, as in humans, the oxidative metabolism and apoptosis of neutrophils is affected in uremic dogs. Oxidative stress due to a decrease in plasma antioxidant capacity and an increase in neutrophil oxidative metabolism has also been observed in cats with chronic kidney disease (CKD), Keegan and Web (2010). It is accepted that the viability and function of human neutrophils are affected by oxidative stress and uremic toxins, Chonchol (2006).

A study by Abousalem and Elgerwi (2012) to explore the hazardous effect of occupational exposure to air pollutants rose from benzene station. A total of 48 albino rats were used. Results indicated a pronounced time-dependent reductions in RBCs, Hb, PCV, total and differential (neutrophil and lymphocyte) leucocyte counts. Total protein,

albumin, globulin and immunoglobulins IgG, IgA and IgM showed marked lower levels in animals exposed to air pollutants in the benzene station. Organ function tests revealed elevations of the levels of AST, ALT, ALP and GGT that indicate impaired liver function compared to those of the corresponding control. Similar results were recorded for creatinine kinase, urea and creatinine indicating toxic effects on the heart and kidneys. Uboh *et al.* (2008) reported that gasoline exposure caused weight loss, growth depression and hemotoxicity in the exposed rats. Uboh *et al.* (2012) reported significant decrements in hematological parameters including RBCs count, HB, PCV, MCV, MCH, MCHC and neutrophils to rats administered nitrocellulose thinner orally as a single daily dose for 30 days. On the other hand, they found significant increments in other parameters including leukocyte count, platelets and lymphocytes. In acute nitrobenzene poisoning, an increase in the number of leukocytes has been reported, with a relative lymphopenia, Parkes and Neill (1953); there were increases in methaemoglobin and reticulocytes in all treated groups, most evident at the high dose (300 mg/kg of body weight per day), with decreases in hemoglobin, hematocrit and red blood cells at 150 and 300 mg/kg of body weight per day; at 75 mg/kg of body weight per day, hemoglobin was decreased. Male mice exhibited leukopenia at 150 mg/kg of body weight per day and leukocytosis at 300 mg/kg of body weight per day. Similarly, lymphopenia was seen in all treated males except at 300 mg/kg of body weight per day, at which dose lymphocytosis was seen. The increase in white blood cells may be a compensatory response to nitrobenzene-induced leukocytopenia or a response to increased infections as a result of the immunotoxicity of the compound, WHO (2003). Repeated administration of benzene (440

mg/kg/day, s.c.) to 6-week-old male Fischer-344 rats resulted in a progressive decline in the number of circulating lymphocytes, Greenlee and Irons (1981). NB could make the mice lymphocyte generate oxidative stress and induce cellular apoptosis, indicating that a certain relationship between oxidative stress and apoptosis might exist, Bai-Chen *et al.* (2008). High level of lipid peroxidation LP may be due to excessive generation of free radicals. Lipid peroxidation, being a free radical reaction, it occurs when the hydroxyl radicals, possibly oxygen, react with the unsaturated lipids of the bio-membranes, resulting in the generation of lipid peroxide radicals (ROO•), lipid hydroperoxide (ROOH) and fragmentation products such as MDA, Pandey *et al.* (2012). With increased LP, the cellular damage is also increased and the viability of lymphocytes will be decreased, Karmakar and Adhikari, (2012). Lymphocytes are important immunological cells, present in blood in large quantity, has played a significant role in acquired immune system. These immune cells use ROS for carrying out their normal functions but an excess amount of ROS can attack cellular components that lead to cell damage. ROS generation and lower antioxidants status in cell leads to cellular oxidative damage, Gautam *et al.* (2010). Glutathione is an important cellular reductant, which offers protections against free radicals, peroxide and toxic compounds. It is reformed from GSSG by donation of hydrogen from NADPH, the reaction being catalyzed by glutathione reductase (GR), John (2003). Adequate concentrations of GSH are required for a variety of immune functions. It has been suggested that oxidative stress and deficiency of thiol compounds may play an important pathogenic role in the development of immune deficiency, Aukrust *et al.* (1995). Depletion of intracellular

GSH may inhibit T-cell Function, Droge *et al.* (1994). Decreased glutathione levels and GPx activity are coupled to increased oxidative damage to DNA, lipids and proteins, Cakatay *et al.* (2001). Siraki *et al.* (2002) found that incubation of hepatocytes with aromatic amines caused a decrease in the mitochondrial membrane potential before cytotoxicity ensured, hepatocyte GSH was also depleted by all aryl amines tested and extensive GSH oxidation occurred with o-anisidine and aminofluorene. Eyong *et al.*, (2004) suggested that ingestion of shellfish exposed to crude oil-polluted water or the polluted perse to rats resulted in hematotoxicity in the form of significant changes in blood parameters. Proctor *et al.*, (1988) mentioned that the exposure to O-anisidine causes different blood discaryasis such as sulfhemoglobin, methaemoglobin and Heinz bodies and it also causes dermal lesions such as dermatitis and sensitization. Elevated levels of ROS such as hydroxyl radicals, superoxide, and peroxides may induce a variety of pathological changes that are highly relevant in nasal and airway mucosa. These include lipid peroxidation, increased airway reactivity, increased nasal mucosal sensitivity and secretions, production of chemo attractant molecules, and increased vascular permeability, Grisham *et al.*, (2000). Inflammatory disorders such as asthma and allergic rhinitis (AR) may be mediated by oxidative stress, Bowler and Crapo (2002). There is increasing evidence that oxidative status and antioxidant capacity play a role in allergic inflammation, but most studies have focused on the relationship with asthma, Cakmak *et al.*, (2009). Basophils are increased in the blood in several states including myeloproliferative neoplasms, hypersensitivity reactions, hypothyroidism, iron deficiency, and renal disease. Particularly large numbers of eosinophil may be seen in foreign

body reactions, parasitic infection, and introduction of air into a body cavity. Basophils and mast cells are not normally found in body fluids, but when present, are most commonly associated with inflammatory conditions, foreign body reactions, and parasitic infestations, George *et al.*, (2012). A linkage between oxidative stress and inflammation is believed to contribute to the pathogenesis of some allergic diseases, Emin *et al.*, (2012). It is known that eosinophil is the most dominant inflammatory cells in allergic disease, Chen *et al.*, (2006). When activated, eosinophil have an even greater ability of free oxygen radical synthesis than higher neutrophils, Emin *et al.*, (2012). Eosinophil develops in the bone marrow and IL-3, IL-5 and GM-CSF are essential for their differentiation. The eosinophilic granulocyte is able to secrete or express a wide range of receptors, cytokines, chemokines, cytotoxic enzymes, lipid mediators and neuromediators, and are normally involved in host defense against parasites, as modulators of innate and adaptive immunity, inflammatory responses and tissue repair, and affect mast cell activation and T-cell function, Blanchard and Rothenberg (2009). The most common cause of eosinophilia in the western world seems to be allergy and in the developing countries invasive parasite infections. The hematopoietic system is (naturally) involved in every case, due to eosinophilia but neutrophilia, basophilia, dysplastic features and immature white blood cells, anemia, thrombocytopenia or thrombocytosis may also be found in blood samples, Gotlib *et al.*, (2004). Eosinophils have normal functions and they may increase in numbers in blood or accumulate in tissues due to relevant stimuli, primarily allergy and infections. This hyper eosinophilic state may thus be a physiological phenomenon and cause reactive or secondary

eosinophilia. However, the number of eosinophil may also increase secondary or as a reaction to a benign or malignant, hematological or non-hematological disorder, primarily due to cytokine-driven eosinophilia. Autonomous clonal proliferations of eosinophil (neoplasms associated with rearrangements of platelet derived growth factor receptors, PDGFR, or fibroblasts growth factor receptors, FGFR1 or chronic eosinophilic leukaemia (CEL) with other clonal markers) are very rare diseases. Finally, the cause of persisting symptomatic hypereosinophilia may remain unclear and then carries the name "true" idiopathic hypereosinophilic syndrome (HES). HES thus remains a diagnosis of exclusion, Bjerrum *et al.*, (2012).

The EC (2011) mentioned that workers exposed to o-anisidine by inhalation to a concentration of 2 mg/m3 for 3.5 hours/day for 6 months developed headaches, vertigo, and anemia. High levels may interfere with the ability of the blood to carry Oxygen causing fatigue, dizziness, and a blue color to the skin and lips (methemoglobinemia). Higher levels may cause trouble breathing, collapse and even death (New Jersey Department of Health and Senior Services, (2004). Phenol compounds induce immunotoxicity by inhibition of lymphocytes proliferation, Iwata *et al*., (2004). Phenols also modulate the activity of ion channels in the nervous system. It was noted that simple phenols may block ion channels in a micro molar concentrations range, Roy *et al.*, (1998). In an experiment damage of epithelium cells of colon was induced by phenols. As the authors suggest, the above process may lead to chronic inflammation of large intestine, Pedersen *et al.*, (2002). The investigations led by, Bukowska *et al*., (2006) have revealed numerous toxic effects caused by phenols on human erythrocytes. Bukowska

(2004) mentioned that phenol compounds affect the antioxidant enzymes and cause decreased superoxide dismutase activity in human. In addition, hemoglobin adducts of o-anisidine were detected in blood samples of persons living in urban or rural areas of Germany, Richter *et al.*, (2001).

Harrison (1977) described the case of a 19-year-old male who consumed a brown liquid while pipetting that apparently contained nitrobenzene. On examination, the patient was unconscious, his lips, tongue, and mucous membranes were navy blue, almost black, and his skin was slate gray. A strong smell similar to that of mothballs or bitter almond was noted. Profound signs of methemoglobinemia were associated with an initial metHb level of 65% and the characteristic chocolate brown coloration of the blood. Analysis of gastric aspirate revealed the presence of aniline and nitrobenzene. Approximately 12 hours after admission, the patient's metHb was 25%. Seven days after admission, hemolytic anemia became apparent.

The characteristic signs of acute nitrobenzene poisoning (coma, cyanosis, and a smell of bitter almonds on the breath) were evident in a 24-year-old female who had ingested an unreported quantity of nitrobenzene, Ajmani *et al.*, (1986). During day 6 of the recovery phase, the subject developed mild jaundice and anemia, yet fully recovered within 2 weeks. Kumar *et al.* (1990) described a 21-year-old male who consumed 30–40 mL of "varnish," a nitrobenzene-containing dye used in screen printing. The patient was in a deeply comatose state with very shallow breathing. Blood samples were obtained that were dark brown in color, and a diagnosis of methemoglobinemia was made, secondary to nitrobenzene consumption, when there was no change in the blood

sample color after it was placed on white filter paper and bubbled with oxygen. Abbinante *et al.* (1997) reported nine cases of nitrobenzene poisoning in Venezuela in people ingesting bitter almond oil containing nitrobenzene. A range of clinical manifestations was observed in affected subjects, including vomiting, dizziness, cyanosis (oral, distal, or general), respiratory depression, convulsions, and generalized weakness. Biochemical findings included anemia, hemolysis, and high levels of metHb. Two articles by Chongtham *et al.* (1999) describe a 24-year-old female whose metHb level was measured as 56.5% as a result of drinking nitrobenzene. The patient was cyanotic and gasping and had a pulse of 120/minute.

Wentworth *et al.* (1999) described the case of a 2-year-old girl who presented with toxic methemoglobinemia, most likely as a result of consuming a nitrobenzene-containing product. The patient was in shock, with marked cyanosis, a heart rate of 170 beats/minute, blood pressure of 80/50 mm Hg, a respiration rate of 28/minute, and a grade II systolic murmur. While the precise source of the toxicosis remained unknown. Gupta *et al.* (2000) reported the case of a 5-year-old boy who died as a result of consuming some screen-printing material that contained nitrobenzene. However, the patient later died of cardiac arrest. Exposure of human research subjects to nitrobenzene vapor resulted in an average absorption of 87% at the blood: gas barrier, Salmowa *et al.*, (1963). The incident described by Ikeda and Kita (1964) most likely also involved dermal contact. The patient presented with a range of typical symptoms of nitrobenzene toxicosis, including headache, nausea, weakness, hyperalgesia, and cyanosis. The woman had been employed for 17 months in a small paint firm where she painted and polished lids of pans

with a red paint containing nitrobenzene as a solvent. The authors determined the nitrobenzene content of the paint solvent to be 97.7% by gas chromatography. Apparently, the workshop was remodeled, and the ventilation became quite poor. The patient started to complain of severe headache, nausea, vertigo, and numbness in the legs approximately 2 months later. After 5 days of bed rest, she returned to work. Nearly 3 months later, the patient experienced the same bout of symptoms, and she was admitted to the hospital the following day. On physical examination, she was emaciated and in a state of distress. Her lips and oral mucosa were cyanotic and the sclerae were slightly jaundiced. The liver and spleen were palpable. During the woman's 2-week stay in the hospital, the nitrobenzene metabolites p-amino- and p-nitrophenol gradually disappeared from her urine. Cody *et al*. (1993) investigated the hematological effect of Benzene caused by job exposure in rubber workers. Significant lower average of white and red blood cell counts had been recorded at each month during the first year of work in workers exposed to the above median benzene exposure when compared with workers exposed below the median. Jacobs *et al*. (1993) recorded that during a 3 year period 229 patients in U.K. with hematological disorders thought to be associated with occupational hazards were notified to the British Society for Hematological/Health and Safety Executive Office. Most were suffering from malignant, premalignant or aplastic anemia. Benzene and ionizing radiation were the most common agent recorded. Ruiz *et al.* (1993) found that macrocytosis and lymphopenia are the earliest hematological signs of benzene toxicity and Ruiz *et al*. (1994) recorded alteration in bone marrow and neutropenia in patients due to chronic exposure to organic solvents (Benzene). Immunologically, many

workers exposed to benzene were studied, the results showed that serum complement levels, IgG and IgA were depressed but IgM levels were slightly higher. These observations taken together with well-known ability of benzene to depress leukocyte counts, may explain why benzene-intoxicated individuals are readily succumb to infection, Abousalem and Elgerwi, (2012). In several human pathologies (e.g. cancer, rheumatoid arthritis, AIDS and leprosy) oxidative stress induces T cell hypo responsiveness, Saso *et al.*, (2003). It was reported that, the levels of GSH was decreased in aging in human lymphocytes, Van Lieshout and Peters, (1998).

Stress can affect all animals. It comes from all non-specific biological phenomena presented by adverse external influences. Free radicals and ROS are unwanted products of aerobic metabolism and are formed endogenously. In addition, they are produced exogenously by chemicals, drugs, and pollutants. Free radicals and ROS lead to biochemical and physiological lesions that may result in cell death from oxidative damages to lipids, proteins, and DNA, Shugaba, (2012). Cellular antioxidant defenses control the levels of endogenous ROS under normal conditions, Sahu, (2002). When there is imbalance between free radicals and antioxidants due to either excessive production of free radicals or decreased antioxidant defense, or both, this might result in a pathological condition called ‘‘oxidative stress’’, Demirkol *et al.*, (2012). Oxidative stress causes oxidation of DNA, membrane lipids, and proteins. Cellular proteins, particularly sulfur-containing proteins, can easily be oxidized. This then results in inactivation of the enzymes, Sahu, (2002). Lipid peroxidation that is caused by oxidative damage may inactivate cellular compounds which may lead to the development of various disorders,

including aging. The antioxidants, SOD and the associated CAT enzyme comprise a first line of defense against oxidative stress. They can protect organisms from oxidative damage by partial relieving of ROS. SOD, the first line of defense against oxygen-derived free radicals, catalyzes superoxide anion radical ($O_2^{\cdot-}$) into less-toxic H_2O_2 and O_2, while CAT reduces H_2O_2 to non-toxic H_2O and O_2, Bakan *et al.*, (2003). Hence, the activities of these enzymes can be served as early indicators of exposure to contaminants that cause oxidative stress, Li-Ying *et al.*, (2012). Oxygen is considered as the major oxidant in metabolic reactions which is designed to gain energy from the oxidation of a miscellaneous of organic molecules. Hence, oxidative stress results from the metabolic reactions that use oxygen, and it has been defined as a disturbance in the equilibrium status of prooxidant/ anti-oxidant systems in intact cells. The implied meaning of this definition of oxidative stress exerts that cells have intact prooxidant/ anti-oxidant systems that continuously generate and detoxify oxidants during normal metabolism. When additional oxidative processes occur, the pro-oxidant systems overcome the anti-oxidant, potentially producing oxidative damage to lipids, proteins, carbohydrates, and nucleic acids, finally leading to cell death in severe oxidative stress. Moderate and chronic oxidative stress may alter the anti-oxidant systems by inducing or repressing proteins that participate in these systems, and by depleting cellular stores of anti-oxidant materials such as glutathione and vitamin E. A disturbance in pro-oxidant/anti-oxidant systems results from a wide range of different oxidative challenges, including radiation, alcohol ingestion, metabolism of environmental pollutants and administered drugs (these are xenobiotic, i.e., foreign materials), and immune system response to

disease or infection, Sies (1997). Whatever the cause the body reacts to stress in a fairly consistent way called the “stress response” or “the general adaptation syndrome”. The response generally involves elevated levels of epinephrine and glucocorticoids especially cortisol: some physiologists define stress as any situation that raises the cortisol level, Saladin, (2003). Almost any type of stress, whether physical or neurogenic, causes an immediate and marked increase in ACTH secretion by the anterior pituitary gland, followed within minutes by greatly increased adrenocortical secretion of cortisol, one possibility is that the glucocorticoids cause rapid mobilization of amino acids and fats from their cellular stores, making them immediately available both for energy and for synthesis of other compounds, including glucose, needed by the different tissues of the body. Indeed, it has been shown in a few instances that damaged tissues that are momentarily depleted of proteins can use the newly available amino acids to form new proteins that are essential to the lives of the cells. Also, the amino acids are perhaps used to synthesize other essential intracellular substances such as purines, pyrimidines, and creatine phosphate, which are necessary for maintenance of cellular life and reproduction of new cells, Guyton and Hall (2006).

Neutrophils produce reactive oxygen species (ROS) when nicotinamide adenine dinucleotide phosphate oxidase is activated, generating the superoxide anion essential to the bactericidal function of neutrophils, Huimin *et al.*, (2000). Although the ROS derived from superoxide are required for the defense mechanism of neutrophils, free radicals produced in excess can damage a number of cellular structures, thus inducing lipid peroxidation and accelerating apoptosis, Kato *et al.*,

(2008). Peripheral blood leukocytes are composed of polymorphonuclear cells, including monocytes as well as lymphocytes. Polymorpho- and mononuclear leukocytes can be activated by advanced glycation end products, oxidative stress, angiotensin II, and cytokines in a state of hyperglycemia. Leukocytes may be activated through the release of cytokines, such as tumor necrosis factor (TNF), transforming growth factor , superoxide, nuclear factor _B (NF-B), monocyte chemo attractant protein 1, interleukin-1, and others, Chung *et al*., (2005), to participate in the pathogenesis of micro and macro vascular complications. Elevated differential cell counts, including counts of eosinophil, neutrophils, and monocytes, also predict the future incidence of coronary artery disease CAD. Neutropenia (decreased measured blood neutrophil concentration) occurs due to many factors including inflammation, margination in vessels, granulocytic hypoplasia, viral infections, toxication, and chronic idiopathic neutropenia, Stockham and Scott (2002). Leukocyte recruitment into inflamed tissue is an essential physiologic process to remove the inflammatory stimulus, such as during wound repair or invasion by infectious microorganisms. However, this beneficial response can lead to a chronic and detrimental inflammatory process if the stimulus is not properly eliminated. Therefore, leukocyte recruitment is also a key factor in the pathogenic process of inflammation. A series of nitrobenzene compounds has been discovered as potent inhibitors of vascular cell adhesion molecule-1 (VCAM-1) expression. Structure–activity relationship (SAR) studies showed that a nitro group and two other electron-withdrawing groups are essential for these compounds to be potent inhibitors of VCAM-1 expression, Meng *et al*., (2001).

Increase in monocyte count seen in chronic myelomonocytic leukaemia with eosinophilia, circulating blasts seen in acute leukaemia, dysplastic changes in neutrophils seen in myelodysplastic syndrome, Bjerrum *et al.,* (2012).

2.1.3.2. Effects of o-anisidine on reproductive and histological aspects

Study done by N.C.I. (1978) in which o-anisidine hydrochloride administered to rats and mice at doses equivalent to 0, 75, 225, 750 or 2,250 mg/kg/day in rats and 0, 150, 450, 1,500 or 4,500 mg/kg/day in mice. Doses of 750 mg/kg/day or greater in rats resulted in dose-dependent reductions in weight and moderate enlargement of the spleens which were black and granular. At 75 mg/kg and 225 mg/kg, spleens of male rats became granular. In mice, doses 450 mg/kg or greater caused dose-dependent reduction in weight and at doses of 1,500 mg/kg/day or greater, the spleens were black and enlarged. O-anisidine was found to be carcinogenic and causes DNA adducts in rat, Naiman *et al.,* (2012).

The effect of 2-nitrophenol in rats was also studied by Koerdel *et al.,* (1981) in a 28-day study (five animals per sex per dose group; daily oral doses of 0, 22, 67, or 200 mg/kg body weight via gavage). In this study, food intake decreased in high-dose males and in mid- and high-dose females, and final body weight decreased non-significantly in all dosed animals. The absolute liver and kidney weights were decreased in mid-dose animals, and the relative testes weight increased in low- and mid-dose males and decreased in high-dose males. In all dosed animals, the relative and absolute weights of the adrenal glands increased. Another study by Hazleton (1984) to evaluate the effect of inhalation of 2-nitrophenol in Sprague-Dawley rats (15 per sex per group), no mortality

was observed after exposure to 0, 5, 30, or 60 mg 2-nitrophenol vapor/m^3 for 6 h/day, 5 days/week, over a period of 4 weeks. Except for squamous metaplasia of the epithelium lining the maxilloturbinates and nasoturbinates in all high-dose animals, the clinical and histopathological examinations gave no consistent exposure-related effects.

In a range-finding study with Charles River rats (five dams per group; application of 0, 50, 125, 250, 500, or 1000 mg/kg body weight via gavage from day 6 to day 15 of gestation; uterine examination on day 20), dose levels of 500 and 1000 mg/kg body weight caused signs of maternal toxicity (transient but dose-related decrease in weight gain early during treatment). Renal cells were seen in the urine in males. Increased relative liver and kidney weights. Kidneys were pale or mottled at macroscopic examination and showed renal tubular lesions at histopathological examination .Increased vacuolization of the urothelium of the bladder. WHO (2000) mentioned that 2-nitrophenol causes testicular atrophy and inhibition of spermatogenesis in rats after oral exposure. Michalowicz and Duda (2006) have studied in details the phenols compounds including 2-aminophenol. These authors mentioned that 2-aminophenol toxicity is expressed by the effect of their action in the formation of phenoxy radicals and intermediate metabolites – semiquinones and quinone methides that interact with biomolecules in the cell. In these reactions reactive oxygen species (ROS) like superoxide radicals or hydrogen peroxide also are formed. Phenols also affect the function of the hormonal system. Phenols are also capable of disturbing sexual hormones function, which finally may lead to sterility of animals and humans, Jung *et al.*, (2004). Histopathological lesions

were observed in brain, liver, lung, kidney, and spleen in rats and mice, though at unstated dose levels. Organ weights appeared to have been dose dependently affected by nitrobenzene exposure, most notably in the case of liver, kidney, and testis (males) and their ratios to body weight were dose dependently increased over control levels and achieved statistical significance compared with controls at all dose levels. At necropsy, rats receiving nitrobenzene had enlarged spleens. Males had enlarged livers, and signs of testicular atrophy. Histopathological examination of the major organs and tissues revealed compound-related effects in the spleen, which appeared to be congested. Splenic corpuscles were small, and the red pulp contained hemosiderin (Sabbioni, 1994).

Administration to rodents of aromatic amine-type chemicals (e.g., aniline) may cause splenic congestion and hemorrhage, which are accompanied by hemosiderin deposition (brown intracellular pigmentation due to insoluble iron), fatty change, and extra medullary hematopoiesis and fibrosis. These changes have been suggested to result from methemoglobinemia or accumulation in erythrocytes of toxic metabolites that are released in the spleen when RBCs are broken down in the red pulp. Sustained congestion causes the spleen to become more firm, enlarged, and fibrotic and renders the organ susceptible to trauma. Spleen enlargement in humans may be caused by a variety of diseases and, in some instances, is associated with increased workload (such as in hemolytic anemia) or hyper function in response to destruction of abnormal RBCs, with symptoms of abdominal pain and early satiety, Greer *et al.*, (2003). The red pulp (also called splenic pulp), which may act as a reservoir for storing blood, is a soft mass of dark reddish-brown color resembling coagulated blood, and it is made of a fine reticulum of

fibers divided into splenic sinuses and splenic cords. The splenic red pulp may undergo changes due to a variety of factors, including immune stimulation, changes in circulation, accumulation of macrophages, and connective tissue or pigment, and in response to increased demand for filtration of abnormal RBCs, Guyton and Hall (2006). The extent to which some observed histopathological effects in the liver were compound related is unclear, because hematopoietic foci and hepatocellular necrosis were evident in both treated and control rats. Hyaline droplets were noted in the cortical tubule cells of the kidney, and some pigmented granules were evident in the cells of a few treated rats. There were obvious compound-related histopathological effects on the seminiferous tubules of the testis of male rats. In some cases, the tubules contained spermatogonia and spermatocytes, while in others there were very few or no spermatids, spermatozoa, and Sertoli cells. Some tubules appeared to contain only a lacy fibrinous material, and others contained multinucleate giant cells. Histopathological changes in the brains of treated rats included hemorrhage, vacuolization, and a wide range of inconsistent degenerative changes. Similarly, a statistically significant increase in ALT was observed only in female rats receiving the high dose. Males also showed a reduction in body weight and an increase in relative liver and kidney weight whereas females showed increased glutamic pyruvic transaminase levels. Both sexes showed increased drinking water consumption, increased bilirubin and urea-nitrogen levels in blood and increased relative spleen weights, EC (2011).

2.2. Quercetin (3, 3', 4', 5, 7-pentahydroxyflavone)

Bioflavonoids were first discovered by Nobel Prize laureate Albert Szent Gyorgyi in the year 1930, Lakhanpal and Rai (2007). Research on flavonoids received an added impulse with the discovery of the French paradox, i.e., the low cardiovascular mortality rate observed in Mediterranean populations in association with red wine consumption and a high saturated fat intake. The flavonoids in red wine are responsible, at least in part, for this effect, Formica and Regelson (1995). Flavonoids are low molecular weight, polyphenolic compounds available in practically all-dietary plants, Cook and Samman (1996). They are primarily recognized as the pigments responsible for autumnal burst of hues and yellow, orange and red shades in flowers and fruits, Harborne and Williams (2000). Over 4000 structurally unique flavonoids have been identified in plants, Ren *et al.*, (2003). The association between flavonoid intake and the long term effects on mortality was studied subsequently, Hertog *et al.*, (1995) and it was suggested that flavonoid intake is inversely correlated with mortality due to coronary heart disease, Knekt *et al.*, (1996).

Flavonoids have existed for over one billion years and survived in vascular plants throughout evolution clearly indicating their importance in nature. The prolonged association between plant flavonoids and various animal species throughout evolution contributes to their wide range of biological activities in mammalian and other biological systems, Ebadi (2002). Flavonoids are major component of citrus fruits and several other medicinal plants and have been used in traditional medicine around the world, Di Carlo *et al.*, (1999). Flavonoids are found to be stored in RBCs, Fiorani *et al.*, (2003). They are effective antioxidants because of their free radical scavenging properties and

because they are chelators of metal ions, Kandaswami and Middleton, (1994); thus, they may protect tissues against free oxygen radicals and lipid peroxidation. Flavonoids exert several biological activities, which are mainly related to their ability to inhibit enzymes and/or to their antioxidant properties, and are able to regulate the immune response. These activities may explain the beneficial effects that flavonoid intake exerts in different human pathologies, including hypertension, inflammatory conditions and even cancer, Comalada *et al.*, (2005). Flavonoids may also be activated by mechanisms that apparently are not directly dependent on their antioxidative properties. A wide range of different biological activities, including antibacterial, antithrombotic, vasodilatory, anti-inflammatory, and ant carcinogenic effects mediated by different mechanisms, are associated with flavonoid compounds, Middleton *et al.*, (2000). Flavonoids are also a kind of natural product and antioxidant substance capable of scavenging free superoxide radicals, reducing the risk of cancer and protecting biological systems against the harmful effects of oxidative processes on macromolecules, such as carbohydrates, proteins, lipids and DNA, Halliwell and Gutteridge, (1990). Antioxidants are substances that may protect cells from the damage caused by unstable molecules known as ROS (reactive oxygen species) and free radicals. Free radicals are responsible for causing a wide number of health problems which include cancer, heart diseases and gastric problems. Flavonoids and phenolics may assist make available security against these diseases by contributing, along with antioxidant vitamins and enzymes, to the total antioxidant defense system of the human body even though Phenolics and flavonoids possess diverse biological activities, for instance, antiviral, Cody *et al.*, (1986),

antiulcer and anti-inflammatory, Lee *et al.*, (1993), antidiabetic, Vessal *et al.*, (2003), antioxidant, Ghasemzadeh and Jaafer, (2011), cytotoxic and antitumor, Paliwal *et al.*, (2005). These compounds have been studied mainly for their properties against oxidative damage leading to various degenerative diseases, such as cardiovascular diseases, inflammation and cancer. Indeed, tumour cells, including leukaemia cells, typically have higher levels of reactive oxygen species (ROS) than normal cells so that they are particularly sensitive to oxidative stress, Battisti *et al.*, (2008). Many papers and reviews describe studies on bioavailability of phenolic acids, emphasizing both the direct intake through food consumption and the indirect bioavailability deriving by gastric, intestinal and hepatic metabolism, Lafay and Izquierdo, (2008). In addition Phenolic acid compounds and functions have been the subject of a great number of agricultural, biological, chemical and medical studies. In recent years, the importance of antioxidant activities of phenolic compounds and their potential usage in processed foods as a natural antioxidant compounds has reached a new level and some evidence suggests that the biological actions of these compounds are related to their antioxidant activity, Saxena *et al.*, (2012).

In vitro studies indicate considerable differences in the antioxidative potential of different flavonoid subgroups, depending on their chemical structures and because of differences in their chemical structure, bioavailability, distribution, and metabolism, Rice and Miller, (1996), different flavonoid compounds may have different effects on human health. There is an inverse association between flavonoid intake and subsequent occurrence of ischemic heart disease, cerebrovascular

disease, lung and prostate cancer, type2 diabetes, and asthma, Paul *et al.*, (2002).

Quercetin belongs to an extensive class of polyphenolic flavonoid compounds almost ubiquitous in plants and plant food sources. Frequently Quercetin occurs as glycosides (sugar derivatives); e.g., rutin in which the hydrogen of the R- 4 hydroxyl group is replaced by a disaccharide. Quercetin is termed the aglycone, or sugarless form of rutin, Baghel *et al.*, (2012). The LD50 of Quercetin in rat is 161 mg/kg BW, James, (1992). It is estimated that flavonoids account for approximately two thirds of the phenolics in our diet and the remaining one third are from phenolic acids. More than 4000 distinct flavonoids have been identified. They commonly have a generic structure consisting of two aromatic rings (A and B rings) linked by 3 carbons that are usually in an oxygenated heterocycle ring, or C ring. Differences in the generic structure of the heterocycle C ring classify them as flavonols, flavones, flavanols (catechins), flavanones, anthocyanidins, and isoflavonoids, Liu (2003). Quercetin belongs to the subclass of flavonols and is an important dietary flavonoid that is present in herbal food like apples, broccoli, onions and many more herbal diets, Pietsch (2011). The chemical formula of quercetin is shown in figure (4).

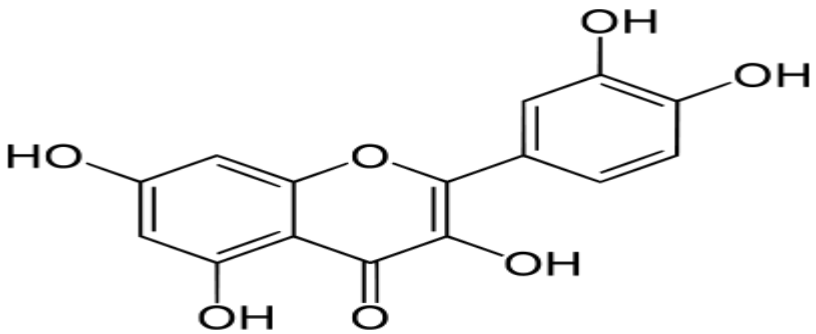


Figure (4). Quercetin (3, 5, 7, 3', 4'-pentahydroxyflavone)

Molecular formula: $C_{15}H_{10}O_7$

2.2.1. Pharmacokinetics

Small intestine of humans absorbs appreciable amount of Quercetin, Hollman *et al*., (1994). It is found in human plasma as conjugates with glucuronic acid, sulfate or methyl groups, with no significant amounts of free Quercetin and it was found to reach 0.1-10 μmol/lit (micromole per liter) in the circulation, Lakhanpal and Rai, (2007). According to Murota and Terao (2003) the concentration of Quercetin was mainly due to the presence of Quercetin metabolites rather than its aglycon. Regarding the pharmacokinetics of Quercetin glucosides conjugates; it seems that the main determinant of absorption of these conjugates is the nature of the sugar moiety. For example Quercetin glucoside is absorbed from small intestine, whereas Quercetin rutinosides is absorbed from the colon after the removal of carbohydrate moiety by bacterial enzymes and the fat content of the diet also influences oral bioavailability of Quercetin. Besides, Quercetin bioavailability from each diet was always higher from the glucoside than from the aglycon but irrespective of the chemical form applied, the bioavailability of Quercetin was also found to be higher in the 17% fat diet compared with the 3% fat diet, Lesser *et al*., (2004). The absorption of Quercetin is enhanced by the enzyme bromelain. Bromelain is also known to have many of the same histamine

and Leukotriene-inhibitory properties as Quercetin. In this way they enhance each other properties.

After getting absorbed in small intestine, Quercetin is transported to the liver via portal circulation, where it undergoes first pass metabolism. Quercetin and its metabolites are distributed to various tissues in the body. Quercetin is strongly bound to the albumin in plasma. Peak plasma level reaches in seven hours following its ingestion. The elimination half-life of Quercetin is approximately 25 hours, Young *et al.*, (1999). The elimination of Quercetin was significantly delayed after its application with fat-enriched diets.

It is important to note that the effects of Quercetin may be mediated substantially by its metabolites, Graf *et al*., (2006). Upon absorption in the gastrointestinal tract, Quercetin is metabolized by Phase II enzymes in gastric and intestinal epithelial cells, and conjugated metabolites are further metabolized in the liver and kidney, De Boer (2005). The B-ring catechol structure is methylated at the 3′ or 4′ hydroxyl site by catechol-O-methyl transferase (COMT), resulting in the formation of isorhamnetin and tamarixetin, respectively, Van der woud, (2004). Quercetin metabolites appear to accumulate in tissues even after short-term ingestion of Quercetin-rich vegetables, Morand *et al*., (1998).

Like many other compounds absorbed Quercetin is probably extensively modified before being excreted by kidneys, Nakamura *et al*., (2000).

Quercetin aglycone and its glucosides are absorbed better than Quercetin administered in non glucosidic forms. The bioavailability of 2 Quercetin glucosides, 3-glucoside and 4'- glucoside, do not differ When Quercetin and its derivatives are provided for consumption along with their natural sources in which these compounds are dispersed in the matrix, Quercetin

aglycone is more bioavailable than its glucosides. This finding suggests that in some cases, bioavailability of isolated food components consumed as food supplements could be less than when they are consumed with the food matrix, Pietsch (2011). Authors Graefe *et al*. (2001) in theirs scientific study observed that the bioavailability of Quercetin from onion, in which a variety of Quercetin glucosides is present, is comparable to the bioavailability of isolated Quercetin 4-glucoside. The lipophilic character of Quercetin suggests that it can cross enterocyte membranes via simple diffusion, Wiczkowski *et al.*, (2008). Urinary excretion of Quercetin seemed to be a small but constant function of Quercetin intake, Young *et al.*, (1999). Nowadays is known that humans absorb appreciable amounts of Quercetin and that absorption is enhanced by conjugation with glucose, Hollman *et al.*, (1995).

2.2.2. Therapeutic properties

Aktoz *et al.*, (2012) has studied the protective effect of Quercetin against renal toxicity induced by cadmium in rats. Twenty four healthy male Wistar albino rats, (weighing 200-250 g and averaging 16 weeks old) were used. Normal structure of the renal cortical tissue was observed in control rats. The animals exposed to Cd showed severe changes in the renal tubules and glomeruli. Hypertrophy and degeneration of renal tubules epithelia with infiltration of mononuclear cells, increased amounts of messangial matrix and dilatation of glomeruli were evident in all animals treated with Cd. With the Quercetin treatment, despite the presence of only a few swollen glomeruli and tubuli, there was a marked protection in renal structure when compared with the Cd-treated rats. Moreover, the renal histology in Cd-treated rats showed an enlargement

of the glomeruli, messangial expansion, thickening of capsular basement membranes (CBMs), glomerular basement membranes (GBMs), and tubular basement membranes (TBMs) as compared with control animals. Treatment of Quercetin reduced the glomerular size, thickening of CBMs, GBMs and TBMs as compared with the Cd-treated group. A study done by Vieira *et al.*, (2011) on 28 male Wistar rats for 30 days in Brazil to clarify the ameliorating effect of Quercetin on cirrhotic rats. The results of the study showed that the cirrhotic group had higher levels of AST, ALT, and ALP compared to the control group. Treatment with Quercetin significantly reduced the higher levels of aminotransferases and ALP induced by biliary obstruction. The histological analysis of liver tissue from the animals in the control group (CO) showed normal architecture of the parenchyma. In animals with cirrhotic liver, there were a loss of normal architecture and a presence of regenerative nodules, cellular necrosis and fibrosis. In contrast, necrosis and fibrosis were minimal in animals from the group treated with Quercetin. For the parameters of lipid Peroxidation, The TBARS concentration was significantly increased in the common bile duct ligated rats, whereas this increase was inhibited by Quercetin treatment. Lipid peroxidation was not modified by Quercetin in normal rats. The SOD enzymatic activity in erythrocytes was measured and verified a reduction in enzymatic activity in the CBDL group compared to the CO and CO + Q and to the cirrhotic group treated with Quercetin (CBDL + Q). In the evaluation of the antioxidant enzymes GPx and CAT, both exhibited a significant reduction in the cirrhotic group CBDL. When animals were treated with Quercetin, significantly increased enzyme activity, restoring values similar to those of the control group.

Adult male Swiss albino mice weighing 25–30 g (10–12 weeks old) were used by Attia, (2010) to study the impact of Quercetin on cisplatin-induced clastogenesis and apoptosis in murine marrow cells. When pretreatment of different doses of Quercetin was given prior to cisplatin treatment, decreased rates of clastogenic changes were observed and the higher dose of Quercetin gave the more effective reduction in the total chromosomal aberrations and abnormal metaphases. In addition, reduction in cisplatin-induced clastogenicity by Quercetin was evident at 24 h and to a much greater extent at 48 h of cell cycle. All types of chromosomal aberrations induced by cisplatin, including breaks, fragments, rings and other damages, were found to be reduced by Quercetin.

The role of Quercetin and Rutin on Serum and hepatic lipid concentrations, fecal steroid excretion and serum antioxidant properties of rats was studied by Nakamura *et al*., (2000). In this study, male rats of the Wistar strain (4 weeks old) were used. Animals weighing 121–144 g were used in Experiment 1 and those weighing 118–137 g were used in Experiments 2 and 3. Each group in Experiments 1–3 contained 5 rats. Significant decrease in serum HDL-cholesterol and triglyceride levels was observed in animals administered rutin at 0.5 g/kg and 1.0 g/kg, respectively, compared to the control rats. A significant increase in the percentage of murodeoxycholic acid (MDCA) was observed in rats administered 0.2 and 0.5 g/kg of Quercetin, and a significant increase of the percentages of deoxycholic acid (DCA), isodeoxycholic acid (IDCA), α-muricholic acid (αMCA). A decrease of 12-keto lithocholic acid (12KLCA) was observed in rats administered 0.5 g/kg of Quercetin, compared to the control rats. The fecal coprostanol/cholesterol ratio

decreased significantly in those administered 0.01, 0.5 and 1.0 g/kg compared to the control rats.

Evaluation of antioxidant and immunity activities of Quercetin in isoproterenol-treated rats was accomplished by Liu *et al.*, (2012). Compared with normal control, electrocardiogram T wave height in ISO-treated rats was significantly decreased, whereas heart index was markedly increased. Compared with ISO-treated rats, three doses of Quercetin treatment significantly enhanced T wave height and decreased heart index .There was a significant increase in plasma AST, CKMB, LDH and TNF-α activities in ISO control rats as compared to normal control group. There was a significant decrease in plasma AST, CKMB, LDH and TNF-α activities in group ISO + Quercetin (50, 100 and 150 mg/kg b.w.) as compared to ISO control. There was a significant decrease in plasma NO, NOS and increase in plasma IL-1, IL-8 and IL-10 levels in ISO control rats as compared to normal control group. There was a significant increase in plasma NO, NOS, IL-10 and decrease in IL-1, IL-8 levels in group ISO + Quercetin (50, 100 and 150 mg/kg b.w.) as compared to ISO control group. There was significant increase in myocardial TBARS level and decrease in myocardial GSH level, SOD, CAT, GSH-Px activities in the ISO control group when compared to the normal control. Significant decrease in the level of myocardial TBARS and increase in the level of myocardial GSH and the activities of SOD, CAT and GSH-Px were observed in groups ISO + Quercetin (50, 100 and 150 mg/kg b.w.) in comparison to the ISO control group. On histopathological examination, large areas of coagulative necrosis were seen in isoproterenol treated rats, with neutrophilic infiltrate, diffused interstitial edema and pale myocytes with fading nuclei and decreased

striations. Pathological features of the infarct area became apparent with widespread necrosis, including the presence of contraction bands, polymorph nuclear leukocytes infiltration, capillaries compressing and a lot of hemorrhage. After three doses of Quercetin treatment, the histological features became typical of normal cardiac structure or mild architectural damage, characterized by interstitial edema and localized necrotic areas.

Sudjarwo (2011) studied the role of Quercetin in endothelial protection in hypercholesteroleamia in New Zealand White rabbits 6 to 8 weeks old weighing between 1.8 and 2.0 kg. The negative control group was fed a standard diet; the positive control group was fed the same diet with 2% cholesterol; the Quercetin group was fed the same diet with 2% cholesterol and Quercetin 50 mg/kg BW/day, 100 mg/kg BW/day or 150 mg/kg BW/day. The lipid peroxidation production was 0.12 nmol/mg protein in negative control; 0.67 nmol/mg in positive control; and 0.71; 0.41, 0.26 nmol/mg protein in Quercetin treatments at dose 50, 100 and 150 mg/kg BW, respectively. In the positive control (hypercholesterolemic) group, the level of TBARS was significantly increased compared to negative control group. Treatment with Quercetin at dose 100 and 150 mg/kg BW but not at dose 50 mg/kg BW markedly reduced aorta TBARS in hypercholesterolemia which was significantly different from the positive control. Endothelium-dependent relaxation evoked by acetylcholine was significantly impaired in aortic ring from the cholesterol-fed (positive control) group as compared to those in the negative control group. The aorta from hypercholesterolemic rabbits treated with Quercetin at dose 100 and 150 mg/kg BW but not at dose 50 mg/kg BW showed marked improvement of the impaired endothelium-

dependent relaxation which was significantly different from positive control group.

The effects of Quercetin were also evaluated in human by Edwards *et al*., (2007) where he studied the role of Quercetin in reducing blood pressure in hypertensive patients. The study was approved by the University of Utah Human Use Review Committee, University of Utah Institutional Review Board, and written informed consent was obtained from each participant. Recruitment efforts in the greater Salt Lake City area targeted males and females with prehypertension (120–139 mm Hg systolic/80–89 mm Hg diastolic) and stage 1 hypertension (140–159 mm Hg systolic/90–99 mm Hg diastolic) as defined by the 7th Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure . The results revealed that Quercetin supplementation reduced systolic, diastolic, and mean arterial pressure in stage 1 hypertensive subjects. The antihypertensive effect of Quercetin was independent of gender, age, and BMI (body mass index) in stage 1 hypertensive subjects.

Another study on human by Kalogerometros *et al.*, (2008) to establish the protecting role of Quercetin against niacin induced flushes in human. Erythema and burning sensation were quite intense, with the highest corresponding scores of 4.75 and 2.25, respectively; the least impressive symptom was edema with a score of 0.50. After 150 mg Quercetin administration, both the erythema and burning scores were reduced from the maximum of 4.75 to 2.5 (50% inhibition. Pruritus was reduced from 2.25 to 1.25. The duration of symptoms was reduced from 3.63 hours before Quercetin to 1.68 hours (55% inhibition). Plasma serotonin was increased from an average of 120 pg/ml to 140 pg/ml, (16% increase;

individual values are shown as a scatter gram). When Quercetin was administered before niacin on Day 6, plasma serotonin was decreased. Given the symptoms of erythema, burning and itching, the above mentioned authors investigated whether methylnicotinate could stimulate human mast cell secretion. Methylnicotinate (1 mM) resulted in significant increase in ProstoglandinD2 release. Pretreatment of mast cells for 15 min with Quercetin reduced PGD2 release by 64%.

Quercetin has a broad range of activities within cells, Lamson and Brignall (2005). As an antioxidant, it prevents oxidation of low-density lipoproteins and the expression of metalloproteinase 1, thus inhibiting the disruption of atherosclerotic plaques and contributing to plaque stabilization and also brings about the regeneration of the pancreatic islets and probably increases insulin release in streptozocin-induced diabetic rats; thus exerting its beneficial antidiabetic effects, Vessal *et al.*, (2003).

It is examined that a single oral daily dose of the bioflavonoid Quercetin reduced blood pressure and heart rate, the cardiac and renal hypertrophy, the endothelial dysfunction and the oxidant status in a rat model of spontaneous hypertension, but had no effect on normotensive rats. This report showed the chronic antihypertensive effect of a Quercetin, Duarte *et al.*, (2001).

Protective effect of Quercetin against various diseases such as osteoporosis, certain forms of cancer, pulmonary and cardiovascular diseases but also against aging was also observed, Boots *et al.*, (2008). Short-term, high intake of black currant and apple juices had a prooxidant effect on plasma proteins and increased glutathione peroxidase activity, whereas lipid oxidation in plasma seemed to

decrease, Young *et al.*, (1999). Quercetin (12, 5-50 mg/kg) reduced the area of gastric ulcer but not the number. It is suggested that α2-adrenergic receptors mediate the effect of Quercetin on intestinal motility and secretion, Di Carlo *et al.*, (1994). Flavonoids may have therapeutic effects on disease conditions caused by oxidative stress, such as coronary atherosclerosis, ischemic damage, diabetes mellitus, aging processes and cancer, Haraguchi *et al.*, (1996). Flavonoids reduce the risk of developing ischemic heart disease and coronary atherosclerosis by inhibiting low-density lipoprotein oxidation, Katan and Hollman, (1998). Other studies have shown that consumption of bioflavonoid antioxidants in animals to be effective in preventing oxidative stresses, Nagata *et al.*, (1999). Quercetin protects the cells against free radicals, Boots *et al.*, (2008). Quercetin due to its high antioxidant activity prevents the formation of ROS, i.e. through the chelation of transition metal ions. It also has the ability to inactivate already produced ROS (superoxide anion, hydroxyl radical, singlet oxygen and lipid radicals), to increase the activity of antioxidant enzymes (superoxide dismutase, catalase and glutathione peroxidase), to inhibit lipid peroxidation and to reduce the effects of irradiation, Adam *et al*., (2012). In a study to evaluate the protective effect of Quercetin against oxidative stress caused by dimethoate in human peripheral blood lymphocytes, Gargouri *et al.*, (2012) has found that SOD and CAT activities were significantly increased by different concentrations of dimethoate. Lymphocytes pre-treated with Quercetin providing significant protection against the higher SOD and CAT activities that were induced by different concentrations of dimethoate. These data show that the induction of antioxidant activities of SOD and CAT, directly mediated by dimethoate, accelerated the

conversion of superoxide radicals to hydrogen peroxide and the decomposition of hydrogen peroxide and probably antioxidant Quercetin may directly scavenge free radicals or modulate the biochemical markers of oxidative stress and antioxidant enzymes. However, Quercetin could effectively ameliorate the dimethoate-induced oxidative stress to a large extent. Consequently, supplementation of Quercetin may act as a protective agent against the toxicity effect of dimethoate in human lymphocytes.

Phagocytes include neutrophils and monocytes (in the blood) and macrophages (in the tissues). Widely distributed, macrophages are strategically situated at the interfaces of tissues with blood or cavity spaces. An initial influx of PMNs should occur within 2 h, and then be replaced by monocytes within 24 h. When exposed to flowing blood, chronic inflammatory processes that are associated with tissue factor expression by monocytes or macrophages may initiate thrombosis, Berkow *et al.*, (1999). Atherosclerotic plaque consists of accumulated intracellular and extracellular lipids, smooth muscle cells, connective tissue, and glycosaminoglycans. The earliest detectable lesion of atherosclerosis is the fatty streak (consisting of lipid-laden foam cells, which are macrophages that have migrated as monocytes from the circulation into the sub endothelial layer of the intima), which later evolves into the fibrous plaque (consisting of intimal smooth muscle cells surrounded by connective tissue and intracellular and extracellular lipids). An atherosclerosis model has been studied in monkeys fed a cholesterol-rich diet. Within 1 to 2 weeks of inducing hypercholesterolemia, monocytes become attached to the surface of the arterial endothelium through the induction of specific receptors, migrate

into the sub endothelium, and accumulate lipid (hence, foam cells). Proliferating smooth muscle cells also accumulate lipid. As the fatty streak and fibrous plaque enlarge and bulge into the lumen, the sub endothelium becomes exposed to the blood at sites of endothelial retraction or tear, and platelet aggregates and mural thrombi form. Release of growth factors from the aggregated platelets may increase smooth muscle proliferation in the intima, Berkow *et al.*, (1999). Prevailing concepts view monocytes as intermediary cells that continuously develop in the bone marrow, circulate in the bloodstream, and migrate unselected into tissue, where they become macrophages, dendritic cells, or other tissue descendants, Swirski *et al.*, (2007). Studies in atherosclerotic mice have shown that bone marrow– derived circulating monocytes populate atherosclerotic lesions, Lessner *et al.*, (2002), and many studies support an active role for monocytes/ macrophages in atherosclerosis. Indeed, both human and mouse monocytes fall into at least 2 phenotypically distinct subsets: Ly-6Chi and Ly-6Clo mouse monocytes correspond to human monocytes, respectively (19, 21–24). Ly-6Chi cells selectively populate sites of experimentally induced inflammation, while their Ly-6Clo counterparts can enter lymphoid and nonlymphoid tissues under homeostatic conditions. Circulating monocytes, the precursors of macrophages, display heterogeneity in mice and humans. The Ly-6Chi monocyte subset increased dramatically in hypercholesterolemia apoE–deficient mice consuming a high-fat diet, with the number of Ly-6Chi cells doubling in the blood every month. Ly-6Chi monocytes adhered to activated endothelium, infiltrated lesions, and became lesional macrophages, Murphy *et al.*, (2011).

A study done by Jamshidzadeh and Mehrabadi, (2010) on patients with G6PD-deficiency to evaluate the role of Quercetin against oxidative stress made by H2O2 exposure, the author mentioned the antioxidant activities of flavonoids, as hydrogen-donors and free-radical scavengers and Quercetin is a very potent free radicals scavenger and upon challenge with H2O2, there was a significant decrease in GSH and an increase in TBARS level in G6PD-deficient erythrocytes. With Quercetin, it managed to preserve concentrations of GSH and TBARS levels of normal and G6PD-deficient erythrocytes against H2O2-induced oxidative damage. In addition to its well-established antioxidant effects, Quercetin was also found to have cytoprotective properties. Quercetin was also more efficient than vitamin C in protecting against lipid peroxidation and GSH depletion in G6PD deficient and normal erythrocytes.

Flavonoids are polyphenolic substances derived from plants that play several pharmacological activities. They possess anti-viral, anti-microbial, anti-inflammatory and anti-allergic potential that can be expressed on different cell types, both in animal and human models. Many of these properties prove inhibitory to huge panoply of molecular targets in the micromolar concentration range, either by down-regulating or suppressing many inflammatory pathways and functions. Flavonoids exert their properties both as purified aglycone molecules and as plant extracts. Depending on little changes in the flavone-backbone and on subtle mechanisms of cell behavior and responsiveness, flavonoids can play a modulating, biphasic and regulatory action on immunity and inflammation; in this context only few flavones and flavonols have been

assayed, mainly because of their chemical similarity with Quercetin, so evidence reported in the literature about the action of flavonoids is limited to a restricted group of molecules. Many of the effects reported about flavonoids regard Quercetin, as probably the most diffused and known nature-derived flavonols. Quercetin has shown a biphasic behavior in basophils at nan molar doses and hence its action on cells involved in allergic inflammation. Like many other molecules sharing a flavone ring, Quercetin affects immunity and inflammation by acting mainly on leukocytes and targeting many intracellular signaling kinases and phosphatases, enzymes and membrane proteins often crucial for a cellular specific function. This overview collects and discusses the role of flavonoids as ant infectious and anti-inflammatory compounds, trying to focus on the complex and modulating interaction of these polyphenolic substances with cell function. However, the wide group of intracellular targets and the elevated number of natural compounds potentially effective as anti-inflammatory therapeutic agents asks for further insights and evidence to comprehend the role of these substances in animal cell biology, Chirumbolo (2010). Quercetin improved dyslipidemia, hypertension, and hyperinsulinemia in obese rats. It also inhibits oxidation of low density lipoprotein (LDL) cholesterol in vitro, probably by inhibiting LDL oxidation itself and actions of Quercetin is also including cardio-protection, cataract prevention, anti-cancer activity, anti-ulcer effects, anti-inflammatory, anti-allergic, antiviral and antibacterial activities, Abd El-Baky (2011).

3-Materials and Methods

3.1. Experimental animals

The experiment was conducted at the animal house of the Veterinary Medicine College–University of Basrah, where 32 females and 64 male rats (*Rattus norvegicus*) of 170–175 grams weights were used. The experiment conditions were unified for all animals, where the room temperature was set between 20–25 C by the use of an air conditioner, and the daily light period was 12 hours by the use of two fluorescent lamps, and the humidity rate was about 50 %.

3.2. Diets of animals

Animals were fed an (AIN-93) standard rat diet, Norlin *et al.*, (1995) on four different sort of diet such as, control diet, quercetin dihydrates supplemented diet, ortho-anisidine hydrochloride diet, and o-anisidine diet supplemented with quercetin. The o-anisidine was added in amount at 7g/kg diet. Quercetin was added in amount at 0.05g/kg (50mg/kg) diet. Quercetin dose was calculated depending on LD50 of quercetin administrated orally in the rats which it was 161mg/kg B.W., James (1992). O-anisidine dose was calculated based on LD50 of o-anisidine administered orally to rats, IARC (1999). Diet components were mixed together as pasta and then cut into small pieces and dried by oven for about 15 minutes in 100 c°. For the quercetin diet, quercetin was first suspended in distilled water and Tween 20 solution (Polysorbate 2%solution) and then mixed with other components of diet. Rats were given free access to the different dietary formulations (Appendix - 1) and water ad labium for four weeks.

3.3. Experimental design

The experiment was divided into two parts.

The first part was divided into three groups; each group consisted of 16 male rats. The hemato–biochemical tests and Histopathological changes studies were done after 15 and 30 days of treatment. The groups were:

- **Control group:** In this group, 16 male rats were fed with standard diet for 15 days then 8 rats of them were terminated for the necessary tests and the remaining were fed with the same diet for additional 15 days.
- **The first treated group (T1) group:** This group consisted of 16 male rats which were fed with a diet contains o-Anisidine hydrochloride (7gm/kg/diet) for 15 days, then 8 rats of them were terminated for the necessary tests and the rest were fed with the same diet for additional 15 days.
- **The second treated group (T2) group:** This group consisted of 16 male rats which were fed with a diet contains (7gm/kg/diet) o-Anisidine hydrochloride and (50mg/kg/diet) quercetin dihydrates for 15 days, then 8 rats of them were terminated for the necessary tests and the remaining rats were fed with the same diet for additionally 15 days.

The second part of the experiment was related with the reproductive parameters and it was done after the first part had been accomplished. It consisted of 16 male and 32 female rats which were separated from each other during the 30 days of treatment then they were gathered to allow them to mate and left together for 16 days then the males were separated to allow the females to deliver freely. All the females of this part of experiment had

delivered during the pre-experiment period. The animals were divided randomly into eight groups of 2 males and 4 females each.

The groups of the second part were as follows:

- **Control group:** Animals were maintained on a standard diet for 30 days.
- **Treatment 1 (T1) group:** In this group, both males and females were fed (7gm/kg/diet) o-anisidine diet for 30 days.
- **Treatment 2 (T2) group:** In this group, the males only were fed (7gm/kg/diet) o-anisidine hydrochloride diet and females on a standard diet for 30 days.
- **Treatment 3 (T3) group:** In this group, males were fed (7gm/kg/diet) o-anisidine hydrochloride diet and females were fed (50mg/kg/diet) quercetin dihydrates diet for 30 days.
- **Treatment 4 (T4) group:** In this group, males were fed (50mg/kg/diet) quercetin dihydrates diet and females were fed (7gm/kg/diet) o-anisidine hydrochloride diet for 30 days.
- **Treatment 5 (T5) group:** In this group, males were fed (50mg/kg/diet) quercetin dihydrates diet and females were fed a standard diet for 30 days.
- **Treatment 6 (T6) group:** In this group, both males and females were fed (50mg/kg/diet) quercetin dihydrates diet for 30 days.
- **Treatment 7 (T7) group:** In this group, both males and females were fed (50mg/kg/diet) quercetin dihydrates + (7gm/kg/diet) o-anisidine hydrochloride diet for 30 days.

3.4. Specimens collection

All experimental rats were sacrificed at the end of experiment period by anaesthetizing them, and placing rat in closed glass container which contain

cotton soaked with chloroform as anesthesia. After that, the abdominal cavity was opened by midline incision and take samples as following:

3.4.1. Blood samples:

Blood samples were collected via cardiac puncture by using 5ml disposable syringe according to the method of Janis, (2009). 2ml of the blood were poured into anti-coagulant containing tubes to be used later for hematological examinations and the remainder (6 - 7ml) were poured into plan tubes to be centrifuged at (3000 rpm for 15 minute) to obtain the serum which then transferred into numerous Ependorf tubes to use in analyses of different parameters and stored at -4c°.

3.4.2. Organs :

Liver, kidney, and testes were removed and weighed with an electronic balance. The weight of the two kidneys and testes were calculated by take the average value of the two. The organs were fixed by using 10% formalin for histological examination. The tail of epididymis was kept in concave watch glass contain 5 ml normal saline to be used for total sperm account and sperm availability.

3.5. Study parameters

3.5.1. Physiological parameters

3.5.1.1. Hematological parameters

All the hematological parameters- (Red corpuscles count (RBC), Total white blood cells count (WBC), Differential leukocyte count, Packed cells volume (PCV), Hemoglobin concentration (HB), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), Mean corpuscular volume (MCV), Mean platelets volume (MPV), and Red

corpuscles distribution width (RDW))- in this study were obtained by the use of a highly developed hematology analyzer (CELL-DYN/Ruby®, Germany made), where 2ml of the non-coagulated blood is aspirated by a special needle equipped within the device and then the analyzed results would appear in a detailed printed report. The principle of the device work is by the use of MAPSS Laser Technology (Multi-Angle Polarized Scatter Sepadiet) and special kits to perform the analysis which were; WBC Lyse, HGB Lyse, and Diluent/Sheath – 09H04-01Kit.

3.5.1.2. Biochemical parameters

The biochemical tests were done in Central Research Unit of Veterinary Medicine College –Basrah University, by using special kits and U.V. Spectrophotometer.

3.5.1.2.1. Total serum cholesterol estimation

Total serum cholesterol (TSCH) is enzymatically measured by using a chemical kit (SPINREACT/CHOD – POD, SPAIN).

Test principle

The principle of this measurement is presented in the following equation (Naito *et al.*, 1984).

$$\text{Cholesterol ester} + H_2O \xrightarrow{\text{Cholesterol Esterase}} \text{Cholesterol} + \text{fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{Cholesterol oxidase}} \text{4-Cholestenona} + H_2O_2$$

$2H_2O_2$ + phenol + 4 aminophenazone $\xrightarrow{\text{Peroxidase}}$ quinineimine + $4H_2O$

♦ The color intensity is directly proportional to the concentration of cholesterol and can be determined photometrically.

Testing procedure

Solution	**Blank**	**Standard**	**Sample**
Standard (µl**)**	—	10	—
Sample (µl**)**	—	—	10
Working reagent (ml)	1.0	1.0	1.0

♦ The tubes were incubated 5 minutes at 37 C. Within 60 minutes, the absorbance of calibrator and sample against reagent blank at 505 nm (500-550) wave length is read.

♦ Total cholesterol concentration was calculated by following equation

$$\frac{\Delta A \text{ Sample}}{\Delta A \text{ Standard}} \times \text{Standard concent.} = \text{TC concent.}$$

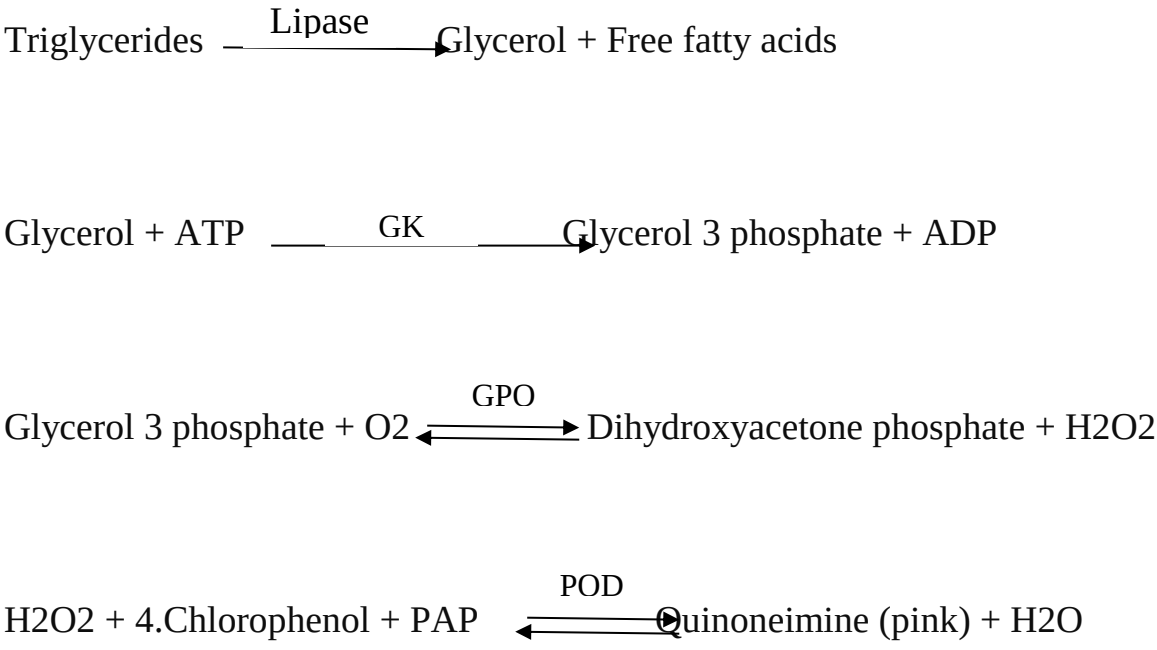
♦ Standard concentration: 200 mg/dl.

3.5.1.2.2. Triglycerides (TGs)

Triglycerides (TGs) are enzymatically measured by using a chemical kit (TRIGLYCERIDES (GPO) / BIOLABO SA, FRANCE).

Test principle

The principle of this measurement is presented in the following equation (Fossati and Prencipe, 1982).



♦The absorbance of the coloured complex (Quinoneimine), proportional to the amount of the Triglycerides in the specimen, is measured at 500 nm.

♦ **Testing Procedure**

Pipette into well identified test tubes	Blank	Standard	Assay
Reagent	1mL	1mL	1mL
Demineralized water	10µL		
Standard		10µL	
Specimen			10µL
Mix. Let stand for 10 minutes at room temperature. Record absorbance at 500 nm (480-520) against reagent blank. Reaction is stable for 1 hour.			

♦ **Calculation**

$$\text{Result} = \frac{\text{Abs (Assay)}}{\text{Abs (Standard)}} \times \text{Standard Concentration}$$

3.5.1.2.3. Serum High density lipoprotein- cholesterol (HDL-C) mg/dl.

Serum high density lipoprotein – cholesterol (HDL-C) is measured by using a chemical kit (HDL-CHOLESTEROL (PTA) / BIOLABO SA, FRANCE) according to the method of (Tietz, 1999).

Test principle

The principle of this measurement is that the HDL-C from the specimen is precipitated by phosphotungstic acid (PTA) and magnesium chloride. The HDL-C obtained in the supernatant after centrifugation is then measured with total cholesterol reagent (i. e.: CHOLESTEROL CHOD-PAP BIOLABO).

♦ **Manual Procedure**

Specimen, Calibrator and Control prepadiet:

Pipette into centrifuge tubes	**Macro-method**	**Micro-method**
Specimen(*)	1mL	0.5mL
Precipitant	100µL	50 µL
Mix vigorously. Let stand for 10 minutes at room temperature. Centrifuge 15 minutes at 3500-4000 RPM.		
Then apply next procedure		

♦ **Assay:**

Let stand supernatant and reagents at room temperature.

Calibrate with standard enclosed in the kit (Cholesterol standard)

Pipette into well identified test tubes	**Blank**	**Standard**	**Assay**
Reagent	1mL	1mL	1mL
Demineralized water	25µL		
Standard 100 mg/dl		25µL	
Supernatant (*)			25µL
Mix. Let stand for 10 minutes at room temperature. Record absorbance at 500 nm (480-520) against reagent blank. Colour is stable for 1 hour.			

♦ **Calculation**

$$\text{Result} = \frac{\text{Abs (Assay)}}{\text{Abs (Standard)}} \times \text{Standard Concentration} \times 1.1$$

∗Standard remaining undiluted, 1.1 factor takes into account dilution of the specimen during the precipitation step.

3.5.1.2.4. Serum low density lipoprotein- cholesterol (LDL-C) mg/dl.

Serum LDL was calculated by Friedewald formula (Friedewald, *et al.*, 1972):

LDL-C = TC – HDL-C – TAG/5

3.5.1.2.5. Serum very low density lipoprotein-cholesterol (VLDL-C) mg/dl.

Serum very low density lipoprotein was calculated by dividing serum TAG by five ((Friedewald, *et al.*, 1972).

VLDL = TAG / 5

3.5.1.2.6. Serum transaminases activity determination

Serum transaminases activity GOT or GPT is determined by using a special kit (BIOMÉRIEUX ® SA Transaminases-Kit, France) (Reitman and Frankel, 1957).

Test principle

Colorimetric determination of GOT or GPT activity is obtained according to the following reactions:

GOT: Aspartate + α keto glutarate $\xrightleftharpoons{\text{GOT}}$ oxaloacetate + glutamate

GPT: Alanine + α keto glutarate $\xrightleftharpoons{\text{GPT}}$ Pyruvate + glutamate

The pyruvate or oxaloacetate formed is measured in its derivative form, 2.4-dinitrophenylhydrazone.

Test procedure

Wave length: ——————————505 nm (490 – 520 nm)

1. Standard curve

Pipette into test tubes (ml):

Tube no.	1	2	3	4	5	6
Distilled water	0.2	0.2	0.2	0.2	0.2	0.2
Reagent 1 or R2	1	0.9	0.8	0.7	0.6	0.5
Reagent 4	—	0.1	0.2	0.3	0.4	0.5
Reagent 3	1	1	1	1	1	1
Mix. Let stand for 20 minutes at room temperature						
NaOH 0.4 N	10	10	10	10	10	10
Mix. Wait 5 minutes. Measure.						
GOT units/ml	0	22	55	95	150	215
GPT units/ml	0	25	50	83	126	—

Plot the standard curve:

— Abscissa: number of units/ml

— Ordinate: OD (millimitre paper)

2. Measurement

The following tubes are set up for each serum:

	GOT	**GPT**
Reagent 1 Reagent 2	1 ml —	— 1 ml
Incubate for 5 minutes at 37 C		
Serum	0.2 ml	0.2 ml
Mix and incubate at 37 C for :	Exactly 1 hour	Exactly 30 minutes
Reagent 3	1 ml	1 ml
Mix. Let stand for 20 minutes at room temperature		
NaOH 0.4 N	10 ml	10 ml
Mix. Wait 5 minutes. Measure under conditions identical to those used for the standard curve.		

The color intensity is stable : ——————— 1 hour

The number of GOT and GPT units/ml of serum are calculated using the standard curve.

3.5.1.2.7. Glutathione Peroxidase

The activity of the glutathione peroxidase enzyme in the serum was calculated based on the procedure described by (Flohe and Gunzler, 1984) and by the use of special kit (SZA kits, Germany).

Procedure:

Step1

Sample (µl)	Phosphate buffer (0.1M, pH7.4) (µl)	Glutathione reduced (2mM) (µl)	Sodium azide (10mM) (µl)	Hydrogen peroxide (1mM)
300	300	200	100	100

Set sample tubes at water bath 37C° for 15 minutes. Add 300 (µl) of 5% TCA. Cool sample tubes and centrifuge at 1500 for 5 minutes.

Step2

Sample (µl)	Phosphate buffer (0.1M, pH7.4) (µl)	DTNB (0.4mg/ml) (µl)
100	300	700

Read the supernatant at a wave length in the range 420 nm. Read the blank (100(µl) distilled water instead of sample) at the same wave length.

Calculations of Glutathione peroxidase

Glutathione peroxidase activity (U/L) = $\left((AT-AB) \times 1000000\right) / (E \times L)$

GPx activity (micromoles/min/L) = $\left((AT-AB) \times 1000000\right) / (622000 \times l)$

Where:

AT= Absorbance for test at 420 nm.

AB= Absorbance for blank at 420 nm.

L= Cuvette length= 1cm

E= Extinction coefficient= 6.22×100000/M.cm

3.5.1.2.8. Catalase

The activity of catalase enzyme in serum was measured based on method described by (Beers and Sizer, 1952; modified by Aebi 1984).

Test Principle:

Catalase enzyme activity can be measured by monitoring the consumption of H_2O_2 substrate at 240 nm.

$$2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2$$

Procedure:

Phosphate buffer (50mM, pH7.0) (µl)	Hydrogen peroxide (30mM) (µl)	Sample (µl)
2700	300	100

Read sample after 60 seconds at a wave length in the range 240 nm.

Calculation of enzyme activity

Activity (micromoles/min/ML) = (AXD)/E

Activity (IU/ML) = (AX300)/40

Activity (IU/ML) = (AX7.5)

Where:

A = Absorbance at 240 nm

D = Dilution factor

E = Extinction coefficient

3.5.1.2.9. Superoxide Dismutase (SOD)

Super oxide dismutase enzyme in the serum was calculated based on the procedure described by (Flohe and Gunzler, 1984) and by the use of special kit (SZA kits, Germany). Where, the method is based on the SOD ability to inhibit the epinephrine oxidation to adrenochrome. Assay reactions are performed at 37 centigrade in air.

Procedure:

- 0.1 mL of sample is diluted in 1.8 mL (50 mM, carbohydrate buffer (pH = 10.2))
- Mix with 0.1 mL epinephrine and 1 mL of 10 mM EDTA (pH = 10.2).

Absorbance is determined at λ = 480 nm immediately after addition of epinephrine and after 5 minutes.

Calculation:

$$\% \text{ inhibition} = \frac{\text{A control} - \text{A sample}}{\text{A control}} \times 100$$

∗ One unit of SOD is defined as the amount of enzyme that inhibits the oxidation 50%. Blank sample is used in order to exclude different spontaneous degrees of oxidation. The absorbance of blank sample is subtracted from the absorbance of the sample to calculate the real absorbance for each sample.

3.5.1.2.10. Malondialdehyde (MDA)

Malondialdehyde serum was measured according to the method of (Beuge and Aust, 1978) which was modified by (Wysocka *et al.*, 1995).

Procedure:

Sample (µl)	**Thiobarbaturic acid (0.375 gm) diluted in hydrochloric acid (0.25M) (µl)**	**Trichloroacetic acid (15%) (µl)**
200	**1000**	**4000**

Set sample tubes at water bath 100 C for 15 min. Cool sample tubes and centrifuge for 5 min. Read the supernatant at a wave length in the range 532 nm. Read the Blank (0.2mL distilled water instead of sample) at the same wave length.

Calculation of MDA

Malondialdehyde (micromoles/L) = $\left((AT-AB) \times 1000000\right) / (E \times L)$

MDA conc. (micromoles/L) = $\left((AT-AB) \times 1000000\right) / (165000 \times 1)$

Where:

AT = Absorbance for Test at 532 nm.

AB = Absorbance for Blank at 532 nm.

L = Cuvette length = 1cm

E = Extinction coefficient = 1.56 × 100000/M.cm

3.5.1.2.11. Alkaline Phosphatase

ALP was measured according to method of (Wenger, *et al*., 1984) by the use of a special kit (ALP, Cypress Diagnostics).

Principle

Alkaline phosphatase (ALP) catalyzes the hydrolysis of p-nitrophenyl phosphate at pH 10.4, liberating p-nitrophenol and phosphate, according to the following reaction:

P-nitrophenylphosphate + H2O $\xrightarrow{\text{ALP}}$ P-nitrophenol + phosphate

Procedure

1.Wavelength 405 nm; Temperature 25, 30, 37°C; Cuvette 1 cm light path.

2. Adjust the instrument to zero with distilled water or air.

3. Pipette into a cuvette:

Working Reagent	**1,2 ml**
Sample	**20 µl**
Mix and wait 1 min. Read initial absorbance (abs) , start the stopwatch and read absorbances every minute for 3 min. Calculate the difference between the absorbances and the average absorbance differences per minute (∆ abs./min).	

Calculation

ALP (U/l) = ∆ Abs/min x 3300

One international unit (IU) is the amount of enzyme that transforms 1 µmol of substrate per minute, in standard conditions. The concentration is expressed in units per litre of sample (U/l).

3.5.2. Reproductive parameters

These parameters were done for the animals of the second part of the experiment. Once the female rats give birth, the males are killed. Testes are cut and the epididymi are removed for the sperms viability measurements, and the rats babies are weighed by a sensitive balance. The fertility and sex ratio are documented and the corpora lutea were counted. The parents of each group were mixed after the treatment period diminished, and they were left together for 16 days (Al–Zobaidy, 2003). Then the parents were separated again and females were left alone to deliver. The necessary tests such as massive and individual sperms movement, total sperms concentration, dead and alive sperms and the sperms malformations were done after that.

3.5.2.1. Massive sperms motility measurement.

This test is done according to Evans and Maxwell (1987) method.

1. Raw epididymis is dropped on a warm clean slide.

2. The slide is examined under the light microscope using 4 X power. 3. The swirling motion is observed from the density and speed of the wave motion then the estimation is converted into the percentage as follow :

Type of movement	*Degree*	*Percentage*
Dense semen, very fast moving waves, no individual movement.	5	90-100
Strong motion but the waves and swirls are not like the previous.	4	75-85
Little motion, slow waves, individual movement is seen.	3	45-65
No waves, but some sperms move.	2	20-40
Very few sperms to be alive, weak movement.	1	10
No movement.	0	0

Evans and Maxwell (1987)

3.5.2.2. Individual sperms motility measurement.

The individual motility of the epididimical sperms is measured depending upon the graduation basis suggested by Chemineau *et al*., (1991) as follows :

1. Drops of the diluted semen are dropped on a warm clean slide at 37 c and covered with a cover slide.

2. The sperms are examined under the light microscope using 40 X power.

3. Depending upon the progressive forward sperms movement and the strength and speed of their motion are converted into a percentage.

Type of movement	*Degree*	*Percentage*
Sperms move rapidly and straightly.	5	90-100
Sperms are rapid, moving fast and some of them move in a circle.	4	75-85
Sperms go on a straight slope, without a shivering motion.	3	45-65
Simple irregular shivering motion, some of them move.	2	20-40
Movement is very slow, sperms shiver with swinging tail.	1	10
No movement.	0	0

3.5.3. Histopathological study

Sorted fragments of different organs (liver, kidneys) were collected from all groups and prepared for histological study according to (Mescher, 2010) method with aid of the light microscope as the following steps:

- **Fixation**

The specimen fixated in the natural buffered formalin 10 % for 24 – 48 hours.

- **Washing and dehydration**

After fixation the specimens washed with water to remove the fixative in order to avoid the interaction between the fixative and staining materials used later.

By dehydration the water had been completely extracted from fragments by bathing them successively in a graded series of ethanol and water (70 %, 80 %, 90 %, and 100 % ethanol).

- **Clearing**

Bathing the dehydrated fragments in solvent (xylene) for 30 – 60 minutes, this step was repeated 3 times .As the tissues clearing, they generally became transparent.

- **Infiltration and embedding**

Once the tissue fragments were impregnated with the solvent, they were placed in melted paraffin in an oven, typically at 52 – 60 C^0. The heat causes the solvent to evaporate, and the space within the tissues becomes filled with paraffin.

- **Sectioning**

After hold from the oven the specimen let at room temperature to be solid and removed from their containers in order to sectioning they were put in the rotary microtome and were sliced by the microtome, s steel blade into sections 5 micrometers thick, then the sections were floated on water bath (50 – 55 C^0 and then transferred into glass slides coated with Mayer's albumin as adhesive substance and left to dry.

- **Staining**

The histological sections of the studying organs were stained with Hematoxylin - Eosin stain.

3.5.4. Statistical Analysis

In this study, ANOVA Analysis and LSD tests are used according to (IBM SPSS, version 20) program at the ($P \leq 0.05$) to find the means for all treatments (IBM SPSS, 2011).

4. Results

4.1. Hematological results

4.1.1. Red blood corpuscles count (R.B.C.)

The results showed that the use of ortho-anisidine 7g/kg of diet for 15 days caused a significant decrease in the R.B.C. count at ($P \leq 0.05$) as compared to the control group as it`s shown in table (1). The table also shows that when Quercetin was offered as treatment for 15 days as 50mg/kg diet it caused the R.B.C. count to elevate significantly as compared to the 15 days of o-anisidine treatment and there was no significant difference as compared to the control group at ($P \leq 0.05$).

Table (1) also shows that when o-anisidine was used for 30 days as 7g/kg of diet it caused further significant decrease in R.B.C. count as compared to the control group and there was no significant difference as compared to the o-anisidine treatment for 15 days. When Quercetin was offered as treatment for 30 days, it caused further significant elevation in R.B.C. count as compared to the o-anisidine treatment of both periods and there was no significant difference as compared with the15 days of quercetin treatment and the control group at ($P \leq 0.05$).

4.1.2. Hemoglobin concentration (Hb)

Considering the hemoglobin concentration, The results showed that the use of o-anisidine for 15 days caused a significant decrease in the Hb concentration at ($P \leq 0.05$) as compared to the control group as it`s shown in table (1). It`s also clear from the table that when Quercetin was offered as treatment for 15 days it caused the Hb concentration to elevate significantly as compared to the 15 days o-anisidine treatment but it was still significantly less than that of the control group at ($P \leq 0.05$).

Table (1) also shows that when o-anisidine was used for 30 days it caused further significant decrease in Hb concentration as compared to the control group and there was also significant decrease as compared to the o-anisidine treatment of 15 days. When Quercetin was offered as treatment for 30 days it caused further significant elevation in Hb concentration as compared to the o-anisidine treatment of the both periods and the 15 days Quercetin treatment and there was no significant difference as compared with the control group at ($P \geq 0.05$).

4.1.3. Packed cells volume (P.C.V.)

The effect of o-anisidine was obvious on the packed cells volume, where it caused a significant decrease in the p.c.v. of the 15 days of o-anisidine treatment as compared to the control group as it`s shown in table (1). The ameliorating effect of quercetin was also obvious as it caused the p.c.v. to elevate significantly as compared to the 15 days of o-anisidine alone treatment but it was still significantly less than that of the control group at ($P \leq 0.05$).

Table (1) also shows that when o-anisidine was used for 30 days it caused further significant decrease in p.c.v. as compared to the control group and there was also significant decrease as compared to the 15 days o-anisidine treatment. When Quercetin was offered as treatment in the 30 days Quercetin+anisidine treatment it caused further significant elevation in p.c.v. as compared to the o-anisidine treatments of the both periods and the 15 days Quercetin+anisidine treatment and there was no significant difference as compared with the control group at ($P \leq 0.05$).

4.1.4. Platelets count (Plat)

Blood platelets inflicted clearly by o-anisidine as the later caused a significant increase in the platelets count of the 15 days of treatment as compared to the control group. Treatment with quercetin exerted its virtues by decreasing the elevated platelets count significantly as compared to the 15 days o-anisidine alone treatment but it remained significantly higher than that of the control group at ($P \leq 0.05$).

Looking at the same table (1) reveals that the 30 days of treatment with o-anisidine caused more significant increase in platelets count as compared to the control group but there was no significant increase as compared to the 15 days o-anisidine treatment. Quercetin was able to cause the platelets count to decline more when it was offered as treatment in the 30 days Quercetin+anisidine as compared to the o-anisidine alone treatment of the both periods and there was no significant difference as compared to the 15 days Quercetin+anisidine treatment, yet; it remained significantly higher than that of the control group at ($P \leq 0.05$).

Table (1). The effect of o-Anisidine and the role of quercetin on blood parameters of male rats (n=16) for 15 and 30 days.

PERIODS	PARAMETERS / GROUPS	RBC ($n\times10^6/\mu l$)	Hb (g/dl)	PCV (%)	Plat ($n\times10^3/\mu l$)
15 DAYS	Control	a 8.6 ±1.1	a 14.6 ±1.2	a 44.5 ±3.4	b 542.6 ±20.6
	T1 (Anisidine)	b 6.6 ±0.9	b 10.7 ±1.2	b 33.4 ±3.8	a 876.7 ±19.62
	T2 (Anisidine+Quercetin)	a 7.7 ±1.0	c 12.6 ±1.7	c 38.8 ±2.3	c 586.5 ±8.6
30 DAYS	Control	a 8.5 ±1.1	a 14.6 ±1.8	a 44.0 ±2.0	b 546.7 ±27.7
	T1 (Anisidine)	b 5.4 ±1.1	d 9.2 ±1.1	d 29.2 ±1.8	a 891.8 ±10.3
	T2 (Anisidine+Quercetin)	a 8.1 ±1.6	a 14.3 ±2.7	a 43.4 ±4.1	b 567.2 ±25.8
	LSD	1.4	1.9	5.7	24.6

The numbers represent the mean ± Standard Deviation. The different letters refer to significant differences among groups ($P\leq0.05$).

4.1.5. Total and Differential leukocytes count

4.1.5.1. Total White blood cells count

For the white blood cells it`s clear from table (2) that o-anisidine treatment for 15 days caused a significant increase in the total white blood cells count of the 15 days o-anisidine alone treatment at ($P \leq 0.05$) as compared to the control group. The table also shows us that treatment with Quercetin for 15 days caused the white blood cells count to decrease significantly as compared to the 15 days o-anisidine alone treatment but the values remained significantly higher than that of the control group at ($P \leq 0.05$).

When o-anisidine was used for 30 days more significant increase in white blood cells count was seen as compared to the control group beside a significant increase as compared to the 15 days o-anisidine treatment. The 30 days of quercetin caused much more significant decrease in white blood cells count as compared to the 30 days o-anisidine treatment, yet; it was still significantly higher than that of the control group and the 15 days quercetin+anisidine treatment at ($P \leq 0.05$).

4.1.5.2. Differential White blood cells count

4.1.5.2.1. Neutrophils

Considering the neutrophils, it`s clear from table (2) that o-anisidine treatment for 15 days caused a significant decrease in the neutrophils count at ($P \leq 0.05$) as compared to the control group. Table (2) also shows that when Quercetin was offered as treatment in the 15 days treatment it caused the neutrophils count to increase significantly as compared to the

15 days o-anisidine treatment but it was still significantly less than that of the control group at ($P \leq 0.05$).

As the treatment with o-anisidine continued for 30 days it caused additional significant decrease in neutrophils count as compared to the control group but there was no significant difference as compared to the 15 days o-anisidine treatment. Treatment with quercetin for 30 days caused a significant increase in neutrophils count as compared to the 30 days treatment with o-anisidine but it was still significantly less than that of the control group and the 15 days quercetin+anisidine treatment at ($P \leq 0.05$).

4.1.5.2.2. Lymphocytes

Lymphocytes also subjected to the bad effect of o-anisidine where O-anisidine treatment for 15 days caused a significant decrease in the lymphocytes count as compared to the control group. The same table (2) also shows that when Quercetin was offered as treatment for 15 days it caused the lymphocytes count to increase significantly as compared to the 15 days o-anisidine treatment and there was no significant difference comparing with the control group at ($P \leq 0.05$).

When o-anisidine was used for 30 days it caused further significant decrease in white lymphocytes count as compared to the control group but there was no significant difference as compared to the 15 days o-anisidine treatment. Quercetin ameliorating effect was obvious, where it caused significant increase in lymphocytes count after 30 days of treatment as compared to the 30 days o-anisidine treatment and there

was no significant difference comparing with the control group at ($P \leq 0.05$).

4.1.5.2.3. Acidophils

Acidophils count was elevated significantly after 15 days treatment with o-anisidine comparing with control group. This increase in acidophils count was retarded significantly after treatment with quercetin for 15 days as compared to the 15 days o-anisidine treatment and there was no significant difference as compared to that of the control group at ($P \leq 0.05$). Sequencing the sight again into table (2) will reveal that o-anisidine treatment for 30 days caused more significant increase in acidophils count as compared to the control group and there was also significant increase as compared to the 15 days o-anisidine treatment. Vice versa, Quercetin treatment for 30 days led to significant declination in acidophils count as compared to the o-anisidine treatments of both periods and there was no significant difference as compared to that of the control group at ($P \leq 0.05$).

4.1.5.2.4. Basophils

The same table (2) inform us that o-anisidine treatment for 15 days caused a significant increase in the basophils count as compared to the control group and that the Quercetin treatment caused the acidophils count to decrease significantly as compared to the o-anisidine treatment and there was no significant difference as compared to that of the control group at ($P \leq 0.05$).

Table (2) also shows that the treatment with o-anisidine for 30 days caused more significant increase in basophils count as compared to the control group and there was also significant increase as compared to the 15 days o-anisidine treatment. Quercetin role was ameliorating as treatment in the 30 days with Quercetin+anisidine caused significant decrease in acidophils count as compared to the o-anisidine treatments of both periods and there was no significant difference as compared to that of the control group at ($P \leq 0.05$).

4.1.5.2.5. Monocytes

Monocytes count was also increased significantly after treatment with o-anisidine treatment for 15 days as compared to the control group. When Quercetin was offered as treatment it caused the monocytes count to decrease significantly as compared to the 15 days o-anisidine treatment and there was no significant difference as compared to that of the control group at ($P \leq 0.05$). Backing again to table (2) will show that when o-anisidine was used for 30 days it caused enormous significant increase in monocytes count as compared to the control group and there was also significant increase as compared to the 15 days o-anisidine treatment. Quercetin caused further significant decrease in acidophils count as compared to the 30 days o-anisidine treatment and there was no significant difference as compared to that of the control group at ($P \leq 0.05$).

Table (2). The effect of o-Anisidine and the role of quercetin on total and differential leukocyte count of male rats (n=16) for 15 and 30 days.

PERIODS	PARAMETERS / GROUPS	WBC ($n\times10^3/\mu l$)	Neutro (%)	Lympho (%)	Acidophil (%)	Basophil (%)	Monocyte (%)
15 DAYS	Control	b 6.2 ±1.2	a 12.1 ±1.3	a 80.1 ±1.3	b 1 ±0.5	b 0.3 ±0.7	b 4 ±0.8
	T1 (Anisidine)	c 9.3 ±1.1	b 8.3 ±1.1	b 70.4 ±3.0	c 4 ±0.6	c 8.5 ±1.4	c 6.2 ±0.9
	T2 (Anisidine+Quercetin)	d 7.2 ±1.3	a 11.1 ±1.5	a 77.9 ±4.1	b 0.9 ±0.6	b 1.1 ±1.1	b 4.6 ±1.3
30 DAYS	Control	b 6.6 ±1.5	a 12.7 ±2.1	a 80.4 ±7.7	b 1 ±0.08	b 0.3 ±0.5	b 4.1 ±0.9
	T1 (Anisidine)	a 13.5 ±1.4	b 7.3 ±1.8	b 68.2 ±6.4	a 6.1 ±0.7	a 9.5 ±0.3	a 7.7 ±0.7
	T2 (Anisidine+Quercetin)	c 9 ±2.7	c,b 9.2 ±1.9	a 76.5 ±5.7	b 1.4 ±0.5	b 1.1 ±0.1	b,d 5 ±0.5
	LSD	1.7	1.9	6.1	2.06	0.9	0.9

The numbers represent the mean ± Standard Deviation. The different letters refer to significant differences among groups ($P\leq0.05$).

4.1.6. Red corpuscles indices

4.1.6.1. Red cells distribution width (RDW)

O-anisidine as it`s seen in table (3) caused no effect on RDW when its used for 15 days and also the same for Quercetin when it`s used with anisidine. When o-anisidine was used for 30 days it caused the RDW to elevate significantly as compared to all groups of the 15 days but there was no significant difference comparing with the 30 days control group. In the Quercetin+anisidine group, it`s seen that there is significant elevation in RDW as compared to all groups of the 15 days and the 15 days control group but there was no significant difference as compared with the 30 days o-anisidine treatment at ($P \leq 0.05$).

4.1.6.2. MCH, MCHC, MCV and MPV

For the mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration and the mean corpuscular volume; there was no significant differences in all treatments of the both periods at ($P \leq 0.05$).

Mean platelets volume (MPV)

Table (3) shows that o-anisidine caused significant decrease in the mean platelets volume in the 15 days o-anisidine treatment as compared to the control group and when Quercetin was offered as treatment in the 15 days quercetin+anisidine treatment it caused significant increase in the MPV as compared to the 15 days anisidine treatment and there was no significant difference comparing with the control group at ($P \leq 0.05$).

At 30 days of treatment, o-anisidine caused further significant decrease in the MPV and the Quercetin increased it significantly but there was no significant difference as compared with control group at ($P \leq 0.05$).

4.2. Biochemical results

4.2.1. Total serum cholesterol (Tch)

O-anisidine showed deleterious effects on the biochemical parameters as it was shown in table (4). From that table, it`s obvious that o-anisidine caused a significant increase in the total serum cholesterol in the 15 days o-anisidine treatment compared to the control group. in the 15 days Quercetin+anisidine treatment, the Quercetin caused the total serum cholesterol to decrease significantly as it`s compared to the 15 days o-anisidine treatment but it was still significantly higher than that of the control group at ($P \leq 0.05$).

On 30 days of treatment with o-anisidine caused further high significant increase in (Tch) as compared to the control group and to the other groups of both periods. Quercetin continued showing its ameliorating effect by decreasing the (Tch) significantly as compared to the 30 days o-anisidine treatment but it was still significantly higher than that of the control group at ($P \leq 0.05$).

4.2.2. Triglycerides (TGs)

Considering the TGs it`s clear from table (4) that o-anisidine treatment for 15 days caused a significant increase in the TGs as compared to the control group. It`s also clear from the table that when Quercetin was offered as treatment it caused the TGs to decrease significantly as compared to the 15 days o-anisidine treatment and there was no significant difference as compared to that of the control group at ($P \leq 0.05$). Table (4) also shows that when o-anisidine was used for 30 days it caused further significant increase in TGs as compared to the control group and all the other groups. When Quercetin was offered as treatment in the 30 days it caused further significant decrease in TGs as compared to the 30 days o-anisidine treatment but it was significantly higher than all other groups at ($P \leq 0.05$).

4.2.3. High density lipoprotein (HDL)

Considering the HDL it`s clear from table (4) that o-anisidine treatment for 15 days caused a significant decrease in the HDL as compared to the control group. It`s also clear from the table that when Quercetin was offered as treatment it caused the HDL to increase significantly as compared to the 15 days o-anisidine treatment and it was still significantly less than that of the control group at ($P \leq 0.05$). Table (4) also shows that when o-anisidine was used for 30 days it caused significant decrease in HDL as compared to the control group and all the other groups. When Quercetin was offered as treatment for 30 days it caused significant increase in HDL as compared to the 30 days o-

anisidine treatment but it was significantly less than the control groups at ($P \leq 0.05$).

4.2.4. Low density lipoprotein (LDL)

Considering the LDL, it`s clear from table (4) that o-anisidine treatment for 15 days caused a significant increase in the LDL as compared to the control group. It`s also clear from the table that when Quercetin was offered as treatment it caused the LDL to decrease significantly as compared to the 15 days o-anisidine treatment but it was still significantly higher than that of the control group at ($P \leq 0.05$). Table (4) also shows that when o-anisidine was used for 30 days it caused further significant increase in LDL as compared to the control group and all the other groups. When Quercetin was offered as treatment for 30 days it caused further significant decrease in LDL as compared to the 30 days o-anisidine treatment but it was significantly higher than the control group at ($P \leq 0.05$).

4.2.5. Very low density low density lipoprotein (VLDL)

For VLDL, it`s clear from table (4) that o-anisidine treatment for 15 days caused a significant increase in the VLDL as compared to the control group. It`s also clear from the table that when Quercetin was offered as treatment it caused the VLDL to decrease significantly as compared to the 15 days o-anisidine treatment and there was no significant difference as compared to the control group at ($P \geq 0.05$). Table (4) also shows that when o-anisidine was used for 30 days it caused further significant increase in VLDL as compared to the control group and all the other groups. When Quercetin was offered as treatment

it caused significant decrease in VLDL as compared to the 30 days o-anisidine treatment but it was significantly higher than the control group at ($P \leq 0.05$).

Table (3). The effect of o-Anisidine and the role of quercetin on Blood indices of male rats (n=16) for 15 and 30 days.

PERIODS	PARAMETERS / GROUPS	RDW (%)	MCH (pg)	MCHC (g/dl)	MPV (fl)	MCV (fl)
15 DAYS	Control	b 10.5 ±1.0	a 16.6 ±1.05	a 32.7 ±1.8	a 4.24 ±0.22	a 50.8 ±1.7
	T1 (Anisidine)	b 10.3 ±1.0	a 16.4 ±1.2	a 32.1 ±2.2	b 3.77 ±0.16	a 51.0 ±1.3
	T2 (Anisidine+Quercetin)	b 10.5 ±1.0	a 16.5 ±1.0	a 32.6 ±1.6	c,a 4.06 ±0.48	a 50.7 ±1.1
30 DAYS	Control	b 10.9 ±1.3	a 17.4 ±2.9	a 33.3 ±4.1	a 4.38 ±0.31	a 53.4 ±4.0
	T1 (Anisidine)	a,c 11.9 ±2.1	a 17.7 ±4.9	a 31.4 ±3.1	d 3.35 ±0.21	a 54.9 ±4.4
	T2 (Anisidine+Quercetin)	a 12.4 ±1.2	a 17.3 ±5.0	a 32.5 ±5.4	c,a 4.06 ±0.09	a 52.9 ±6.1
	LSD	1.43	00	0.0	0.28	00

The numbers represent the mean ± Standard Deviation. The different letters refer to significant differences among groups ($P \leq 0.05$).

Table (4). The effect of o-Anisidine and the role of quercetin on Lipid Profile of male rats (n=16) for 15 and 30 days.

PERIODS	PARAMETERS / GROUPS	TCh (mg/dl)	TGs (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
15 DAYS	Control	b 74.2 ±4.05	b 58.4 ±4.9	b 42.3 ±2.1	b 20.1 ±2.8	b 11.3 ±1.4
	T1 (Anisidine)	c 93.1 ±6.5	c 73.9 ±3.3	c 20.8 ±2.4	c 57.5 ±8.0	c 14.8 ±0.9
	T2 (Anisidine+Quercetin)	d 82.05 ±5.8	b 62.3 ±2.6	d 36.8 ±3.4	d 32.7 ±6.1	b 12.4 ±1.5
30 DAYS	Control	b,d 79.4 ±4.7	b 64.2 ±2.6	a 48.4 ±4.4	b 21.5 ±4.8	b 12.9 ±1.5
	T1 (Anisidine)	a 150.6 ±7.6	a 120.5 ±15.1	e 13.2 ±3.8	a 113.2 ±8.3	a 24.1 ±3.0
	T2 (Anisidine+Quercetin)	e 105.1 ±6.6	d 87.05 ±5.1	c 22.8 ±5.1	c 64.9 ±10.8	d 17.4 ±1.4
	LSD	7.7	9.6	5.5	7.4	1.9

The numbers represent the mean ± Standard Deviation. The different letters refer to significant differences among groups ($P \leq 0.05$).

4.2.6. Aspartate aminotransferase (AST)

It`s clear from table (5) that o-anisidine treatment for 15 days caused a significant increase in the AST as compared to the control group. It`s also clear from the table that when Quercetin was offered as treatment it caused the AST to decrease significantly as compared to the 15 days o-anisidine treatment but it was still significantly higher than that of the control group at ($P \leq 0.05$). Table (5) also shows that when o-anisidine was used for 30 days it caused further significant increase in AST as compared to the control group and all the other groups. When Quercetin was offered as treatment for 30 days it caused further significant decrease in AST as compared to the 30 days o-anisidine treatment but it was significantly higher than the control group at ($P \leq 0.05$).

4.2.7. Alanine aminotransferase (ALT)

For the ALT, it`s clear from table (5) that o-anisidine treatment for 15 days caused a significant increase in the ALT as compared to the control group. It`s also clear from the table that when Quercetin was offered as treatment it caused the ALT to decrease significantly as compared to the 15 days o-anisidine treatment but it was still significantly higher than that of the control group at ($P \leq 0.05$). Table (5) also shows that when o-anisidine was used for 30 days it caused further significant increase in ALT as compared to the control group and all the other groups. When Quercetin was offered as treatment for 30 days it caused further significant decrease in ALT as compared to the 30 days o-anisidine

treatment but it was significantly higher than the control group at (P≤0.05).

4.2.8. Alkaline phosphatase (ALP)

The results also revealed that o-anisidine treatment for 15 days caused a significant increase in the ALP as compared to the control group. It`s also clear from the table that when Quercetin was offered as treatment for15 days it caused the ALP to decrease significantly as compared to the 15 days o-anisidine treatment but it was still significantly higher than that of the control group at (P≤0.05). Table (5) also shows that when o-anisidine was used for 30 days it caused further significant increase in ALP as compared to the control group and all the other groups. When Quercetin was offered as treatment for 30 days Quercetin+anisidine treatment it caused further significant decrease in ALP as compared to the 30 days o-anisidine treatment but it was significantly higher than the control group at (P≤0.05).

Table (5). The effect of o-Anisidine and the role of quercetin on Liver function enzymes of male rats (n=16) for 15 and 30 days.

PERIODS	PARAMETERS / GROUPS	AST (U/L)	ALT (U/L)	ALP (U/L)
15 DAYS	Control	b 55.6 ±1.3	b 29.4 ±1.2	b 113.1 ±3.6
	T1 (Anisidine)	c 84.4 ±1.9	c 51.8 ±5.7	c 137.5 ±3.4
	T2 (Anisidine+Quercetin)	d 65.6 ±2.2	d 46.5 ±3.3	d 120.6 ±3.0
30 DAYS	Control	b 57.2 ±3.4	b 31.5 ±2.8	b 112.6 ±3.2
	T1 (Anisidine)	a 90.1 ±2.6	a 62.3 ±4.4	a 160.2 ±3.2
	T2 (Anisidine+Quercetin)	e 80.3 ±6.1	c 53.4 ±4.1	c 136.1 ±1.6
	LSD	4.1	5.3	7.4

The numbers represent the mean ± Standard Deviation. The different letters refer to significant differences among groups ($P \leq 0.05$).

4.2.9. Malondialdehyde (MDA)

The stress biomarker (MDA) increased significantly due to treatment with o-anisidine as compared to the control group. When Quercetin was offered as treatment it caused the MDA to decrease significantly as compared to the 15 days o-anisidine treatment and there was no significant difference comparing that of the control group at ($P \leq 0.05$). Table (6) also shows that when o-anisidine was used for 30 days it caused further significant increase in MDA as compared to the control group and all the other groups. When Quercetin was offered as treatment it caused significant decrease in MDA as compared to the 30 days o-anisidine treatment but it was significantly higher than the control group at ($P \leq 0.05$).

4.2.10. Glutathione peroxidase (GPx)

We can see in table (6) that o-anisidine treatment for 15 days caused a significant increase in the GPx of the 15 days o-anisidine treatment as compared to the control group. It`s also clear from the table that when Quercetin was offered as treatment in the 15 days it caused the GPx to decrease significantly as compared to the 15 days o-anisidine treatment but it was still significantly higher than that of the control group at ($P \leq 0.05$). Table (6) also shows that when o-anisidine was used for 30 days it caused significant decrease in GPx as compared to the control group and all the other groups. When Quercetin was offered as treatment it caused significant increase in GPx as compared to the 30 days o-

anisidine treatment but there was no significant difference as compared to the control group at (P≤0.05).

4.2.11. Superoxide Dismutase (SOD)

Another antioxidant enzyme the (SOD) was significantly increased after 15 days of o-anisidine treatment at as compared to the control group. It`s also clear from the table that when Quercetin was offered as treatment for 15 days it caused the SOD to decrease significantly as compared to the 15 days o-anisidine treatment but it was still significantly higher than that of the control group at (P≤0.05). Table (6) also shows that when o-anisidine was used for 30 days it caused significant decrease in SOD as compared to the control group and all the other groups. When Quercetin was offered as treatment for 30 days it caused significant increase in SOD as compared to the 30 days o-anisidine treatment and the control group at (P≤0.05).

4.2.12. Catalase (CAT)

o-anisidine caused the (CAT) enzyme to elevate significantly as compared with control group after 15 days of treatment but after 30 days of treatment the value of this enzyme decreased significantly comparing with the 15 days treatment, however it was significantly higher than other treatments of both periods. For quercetin treatment, it caused a significant decrement in the elevated values of the enzyme but in both periods of treatment it was significantly higher than that of control group at (P≤0.05).

Table (6). The effect of o-Anisidine and the role of quercetin on Antioxidant enzymes of male rats (n=16) for 15 and 30 days.

PERIODS	PARAMETERS / GROUPS	MDA (µm / L)	GPx (µm / L)	SOD (µm / L)	CAT (IU/ml)
15 DAYS	Control	d 1.7 ±0.4	c 82.6 ±2.2	d 31.7 ±1.5	c 2.3 ±0.5
	T1 (Anisidine)	c 5.3 ±0.7	a 128.5 ± 2	a 71.3 ±1.5	a 6.7 ±0.7
	T2 (Anisidine+Quercetin)	d 1.8 ±0.3	b 90.7 ±2.7	b 41.1 ±1.1	b 3.5 ±0.5
30 DAYS	Control	d 1.8 ±0.3	c 82.5 ± 2	d 31.8 ±2.2	c 2.6 ±0.5
	T1 (Anisidine)	a 12 ± 1	d 41 ±1.4	e 14.3 ±0.7	b 4 ±0.3
	T2 (Anisidine+Quercetin)	b 7.7 ±0.4	c 84.3 ± 2	c 33.8 ±1.1	c 2.8 ±0.6
	LSD	2.37	6.37	2	0.62

The numbers represent the mean ± Standard Deviation. The different letters refer to significant differences among groups ($P \leq 0.05$).

4.3. Reproductive parameters

4.3.1. Semen aspects

4.3.1.1. Sperm count

O-anisidine was obvious to cause malicious effects on reproductive parameters. Table (7) shows that when o-anisidine was used for 30 days of treatment caused the sperm count to decrease significantly as compared to the control group and other groups at ($P \leq 0.05$). In the Quercetin+anisidine group, the sperm count was increased significantly as compared to the o-anisidine group but it was still significantly less than that of the control group at ($P \leq 0.05$). In the Quercetin only group, the Quercetin elevated significantly the sperm count more than that of the control group and other groups at ($P \leq 0.05$).

4.3.1.2. Massive sperm motility

Table (7) also shows that o-anisidine caused the massive sperm motility to decrease significantly as compared to the control group and other groups at ($P \leq 0.05$). In the o-anisidine+quercetin group, the massive sperm motility was increased significantly as compared to the o-anisidine group but it was still significantly less than that of the control group at ($P \leq 0.05$). In the Quercetin only group, the Quercetin elevated significantly the massive sperm motility more than that of the o-anisidine and the o-anisidine+quercetin groups and there was no significant difference comparing with the control group at ($P \leq 0.05$).

4.3.1.3. Individual sperm motility

It`s clear from table (7) that o-anisidine caused the individual sperm motility to decrease significantly as compared to the control group and other groups at (P≤0.05). In the o-anisidine+quercetin group, the individual sperm motility was increased significantly as compared to the o-anisidine group but it was still significantly less than that of the control group at (P≤0.05). In the Quercetin only group, the Quercetin elevated significantly the individual sperm motility more than that of the o-anisidine and the o-anisidine+quercetin groups and there was no significant difference comparing with the control group at (P≤0.05).

4.3.1.4. Dead sperms

It`s also clear from table (7) that o-anisidine caused the dead sperm ratio to increase significantly as compared to the control group and other groups at (P≤0.05). In the o-anisidine+quercetin group, the dead sperm ratio was decreased significantly as compared to the o-anisidine group but it was still significantly higher than that of the control group at (P≤0.05). In the Quercetin only group, the Quercetin decreased significantly the dead sperm ratio less than that of the o-anisidine and the o-anisidine+quercetin groups and there was no significant difference comparing with the control group at (P≤0.05).

4.3.1.5. Abnormal sperms

It`s also obvious from table (7) that o-anisidine caused the abnormal sperm ratio to increase significantly as compared to the control group and other groups at ($P \leq 0.05$). In the o-anisidine+quercetin group, the abnormal sperm ratio was decreased significantly as compared to the o-anisidine group but it was still significantly higher than that of the control group at ($P \leq 0.05$). In the Quercetin only group, the Quercetin lowered significantly the abnormal sperm ratio less than that of the o-anisidine and the o-anisidine+quercetin groups and there was no significant difference comparing with the control group at ($P \leq 0.05$). The deformities of the sperms which were caused by o-anisidine were very clear under microscopic examination and appeared as: double head sperms, headless sperms, double tails sperms, and curved sperms as it`s seen in picture (24).

Table (7). The effect of o-Anisidine and the role of quercetin on Sperm viability of male rats.

PARAMETERS / GROUPS	Sperm count ($nx10^6/mm^3$)	Massive motility (%)	Individual motility (%)	Dead Sperm (%)	Abnormal Sperm (%)
Control	b 195.2 ±0.3	a 90 ±0.4	a 90 ±0.6	b 11 ±1.4	b 12.5 ±2.1
Anisidine	c 66.5 ±1.0	b 10 ±0.7	b 10 ±0.6	a 96 ±1.4	a 31 ±1.4
Anisidine +Quercetin	d 125.7 ±1.5	c 77.5 ±3.5	c 47.5 ±3.5	c 32.5 ±3.5	c 20 ±1.4
Quercetin	a 198.1 ±0.7	a 90 ±0.3	a 90 ±0.2	b 9 ±1.4	b 9 ±1.4
LSD	2.9	12.5	37.5	21.5	7.5

The numbers represent the mean ± Standard Deviation. The different letters refer to significant differences among groups ($P \leq 0.05$).

4.3.2. Fertility aspects

4.3.2.1. Number of pregnant females

O-anisidine was obviously affecting the number of pregnant female rats as it`s seen in table (8) where it caused no pregnant females in the group of both males and females o-anisidine treated and caused pregnancy to occur only for one female in the other groups whether the females or males only treated with o-anisidine. For the Quercetin it caused the females to be normally pregnant in the Quercetin treatments either where one gender was treated with Quercetin and the other was normal or where both genders were treated with Quercetin.

4.3.2.2. Number of offspring

O-anisidine was also obviously affecting the number of offspring as it`s seen in table (8) where it caused number of offspring in all the groups treated with o-anisidine. For the Quercetin it caused the offspring in the Quercetin treatments either where one gender was treated with Quercetin and the other was normal or where both genders were treated with Quercetin to increase clearly even more than that of control group.

4.3.3.3. Birth weights

Table (8) shows that o-anisidine caused the birth weight of the offspring to decrease clearly and in all groups treated with o-anisidine. For the Quercetin it caused the birth weight of the offspring in the Quercetin treatments either where one gender was treated with Quercetin and the other was normal or where both genders were treated with Quercetin to increase clearly even more than that of control group.

4.3.3.4. Fertility ratio

Considering the fertility ratio, it was obvious from table (8) that o-anisidine decreased the fertility ratio in all groups treated with o-anisidine and the Quercetin was normally affecting the fertility ratio as it`s the same as that of the control group.

Table (8). Effect of o-anisidine and role of quercetin on fertility aspects of rats.

Aspects / Treatments	No. of Pregnant females	No. of litters	Birth weight of litters	No. of corpora lutea	No. of male litters	No. of female litters	Sex ratio %	Fertility percent %
Control	a **4** ± 0.0	b **36** ± 1.8	a **4.82** ± 1.0	a **39** ± 0.8	c **20** ± 1.2	b **16** ± 0.9	a **55.5** ± 0.9	a **100** ± 0.0
A.m.+A.f.	d **0** ± 0.0	d,e **0** ± 0.0	b **0** ± 0.0	d **0** ± 0.0	f,e **0** ± 0.0	e **0** ± 0.0	b **0** ± 0.0	d **0** ± 0.0
A.m+n.f.	c **1** ± 0.5	d **4** ± 2.0	a **4** ± 2.0	b **27** ± 13.5	e **1** ± 0.5	d **3** ± 1.5	b,a **25** ± 12.5	c **25** ± 12.5
A.m+Q.f.	c **1** ± 0.5	d **5** ± 2.5	a **4** ± 2.0	b,a **30** ± 15.0	e **2** ± 1.0	d **3** ± 1.5	a **40** ± 20.0	c **25** ± 12.5
Q.m+A.f	c **1** ± 0.5	d **2** ± 1.0	a **3.5** ± 2.0	c,b **15** ± 7.5	e **1** ± 0.5	e **1** ± 0.5	a **50** ± 25.0	c **25** ± 12.5
Q.m+n.f.	a **4** ± 0.0	b **37** ± 1.7	a **4.75** ± 1.4	a **41** ± 1.2	b **22** ± 0.8	b **15** ± 0.8	a **59.4** ± 0.5	a **100** ± 0.0
Q.m+Q.f	a **4** ± 0.0	a **42** ± 1.3	a **4.9** ± 1.7	a **46** ± 0.9	a **24** ± 1.2	a **18** ± 1.2	a **57.1** ± 0.8	a **100** ± 0.0
(A+Q M)+(A+Q F)	b **3** ± 0.5	c **23** ± 3.9	a **4.26** ± 0.8	b,a **32** ± 1.2	d **10** ± 0.8	c **13** ± 1.2	a **43.4** ± 0.5	b **75** ± 0.0
LSD	**0.75**	**3.2**	**3.0**	**13.7**	**1.5**	**1.7**	**30.7**	**25.0**

Key words: Am=anisidine treated males; A.f.=anisidine treated females; Q.m.=quercetin treated males; Q.f.=quercetin treated females; n.f.= normal females.

4.3.3. Growth aspects

4.3.3.1. Body weights

The effect of o-anisidine was clear on the body weights of the experiment animals. The initial weights were the same for all the experiment animals; the control, o-anisidine, and Quercetin+anisidine treated groups. The weights of the animals of o-anisidine group were significantly less than the other groups from day 7 to day 28 of the experiment as it`s shown in table (9) at ($P \leq 0.05$). In the Quercetin+anisidine group, the weights of the animals were significantly higher than that of the o-anisidine group along the experiment period and it was with no significant difference from the control group on days 7 and 14 of the experiment but on days 21 and 28 they were significantly less than that of the control group at ($P \leq 0.05$).

4.3.3.2. Liver, spleen, and kidney weights

The weights of livers, spleens, and kidneys were clearly increased significantly as a result of treatment with o-anisidine as compared with the control group and other groups and quercetin was able to decrease their weights significantly towards normal but did not reach the control or quercetin alone values as it is seen in table (10).Besides, grossly enlargements were seen in the livers, spleens, and kidneys of o-anisidine treated animals, pictures (21, and 22).

4.3.3.3. Testes weight

For the testes weight, table (10) shows that o-anisidine caused the testes weight to decrease significantly as compared to the control group and other groups at ($P \leq 0.05$). In the Quercetin+anisidine group, the testes

weight was increased significantly as compared to the o-anisidine group but it was still significantly less than that of the control group at ($P \leq 0.05$). In the Quercetin only group, the Quercetin elevated significantly the testes weight more than that of the control group and other groups at ($P \leq 0.05$). In addition, grossly atrophy was seen in the testes of o-anisidine treated animals, picture (23).

4.3.3.4. Epididymis weight

For the epididymis weight, table (10) shows that o-anisidine caused the epididymis weight to decrease significantly as compared to the control group and other groups at ($P \leq 0.05$). In the Quercetin+anisidine group, the epididymis weight was increased significantly as compared to the o-anisidine group but it was still significantly less than that of the control group at ($P \leq 0.05$). In the Quercetin only group, the Quercetin elevated significantly the epididymis weight more than that of the other groups but with no significant difference as compared to the control group at ($P \leq 0.05$).

4.3.3.5. Food intake

Starting from day 7 of treatment, the food intake was decreased significantly in animals who were maintained on o-anisidine alone diet as compared with all other animals while the food intake was significantly increased in animals maintained on o-anisidine+quercetin diet but not reached the control group and for the animals which were maintained on quercetin alone diet they were consuming significantly higher amount of diet than other groups and the control. From day 14 till the day 28 of treatment, food intake was significantly retarding and less than all other groups in animals maintained on o-anisidine only, and it

was significantly higher in animals fed on o-anisidine+quercetin diet but it was less than that of control and quercetin alone feeding animals which were of no significant difference at (P≤0.05) and as it is seen in table (11).

Table (9). The effect of O-Anisidine and the role of Quercetin on Body weights of male rats.

PARAMETERS / GROUPS	Initial weight (gm)	Day 7 weight (gm)	Day 14 weight (gm)	Day 21 weight (gm)	Day 28 weight (gm)
Control	a 177.06 ±1.29	a 183.26 ±1.28	a 188.46 ±1.29	a 195.66 ±1.27	a 201.86 ±1.29
Anisidine	a 176.90 ±1.45	b 179 ±1.46	c 180.08 ±1.58	c 181.08 ±1.56	c 181.18 ±1.58
Anisidine+ Quercetin	a 177.18 ±1.70	a 182.28 ±1.69	b 187.28 ±1.70	b 192.18 ±1.68	b 197.18 ±1.66
Quercetin	a 176.88 ±1.12	a 184.51 ±0.96	a 190.33 ±0.94	a 197.28 ±0.87	a 204.98 ±1.11
LSD	0.4	3.28	2.17	3.47	4.67

The numbers represent the mean ± Standard Deviation. The different letters refer to significant differences among groups (P≤0.05).

Table (10). The effect of O-Anisidine and the role of Quercetin on organs weights of male rats.

The numbers represent the mean ± Standard Deviation. The different

PARAMETERS / GROUPS	LIVER WEIGHT (gm)	KIDNEY WEIGHT (gm)	SPLEEN WEIGHT (gm)	TESTES WEIGHT (gm)	Testes /BW (gm)	Epididymis /BW (gm)
Control	b 4.75 ±0.88	b 1.38 ±0.51	c 0.25 ±0.08	a 1.50 ±0.49	b 0.79 ±0.01	a 0.29 ±0.01
Anisidine	a 7.38 ±1.06	a 3.13 ±0.64	a 2.25 ±0.7	b 0.75 ±0.24	c 0.43 ±0.02	b 0.10 ±0.0
Anisidine +Quercetin	b 5.38 ±0.51	b 1.88 ±0.64	b 1.13 ±0.35	b,a 1.13 ±0.35	d 0.64 ±0.04	c 0.26 ±0.01
Quercetin	b 4.88 ±1.24	C,b 1.25 ±0.46	c 0.38 ±0.11	a 1.88 ±0.34	a 0.86 ±0.05	a 0.29 ±0.0
LSD	2	0.62	0.75	0.75	0.15	0.03

letters refer to significant differences among groups (P≤0.05).

Table (11). Effect of anisidine and role of quercetin on food intake of male rats.

Food Intake (gm) / GROUPS	Day 7	Day 14	Day 21	Day 28
Control	b 28.13 ±1.12	a 29.38 ±0.51	a 31.25 ±1.38	a 34 ±2.20
Anisidine	d 14.25 ±1.48	c 13.63 ±1.06	c 12.75 ±0.88	c 11.50 ±1.77
Anisidine+ Quercetin	c 20.5 ±0.75	b 21.88 ±3.35	b 22.50 ±4.37	b 26 ±3.85
Quercetin	a 29.75 ±0.70	a 30.63 ±0.74	a 32.50 ±1.85	a 33.88 ±3.04
LSD	1.62	7.50	8.75	7.87

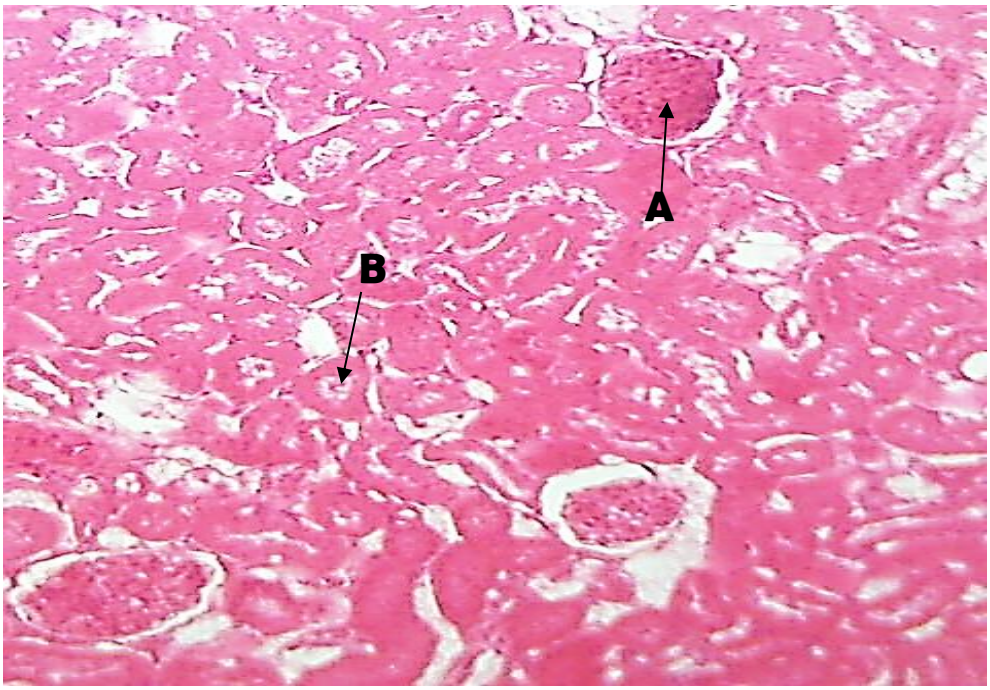
The numbers represent the mean ± Standard Deviation. The different letters refer to significant differences among groups (P≤0.05).

4.4. Histological parameters

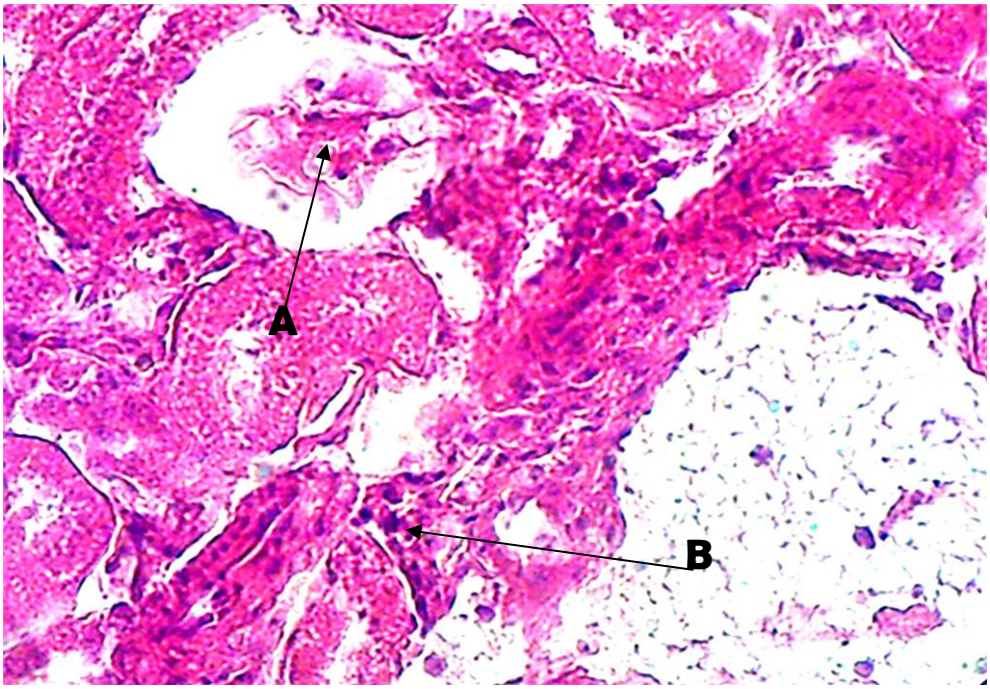
4.4.1. Kidneys

It was clear from the results that o-anisidine caused different degrees of renal damage when it was used for 15 days and the deleterious effect of which was more clear when it was used for 30 days as compared with kidney of the control group in picture (1). Where, it caused atrophy of glomeruli and infiltration of inflammatory (picture, 2), complete disappearance of glomerulus and aggregation of messangial cells (picture, 3), Furthermore, the effect of anisidine after 30 days of treatment was represented by closure of renal tubules, tubular edematous changes, clotting in the glomeruli, and presence of hyaline droplets (picture, 4), degeneration of corticomedullary tubules and edema around tubules (picture, 5).

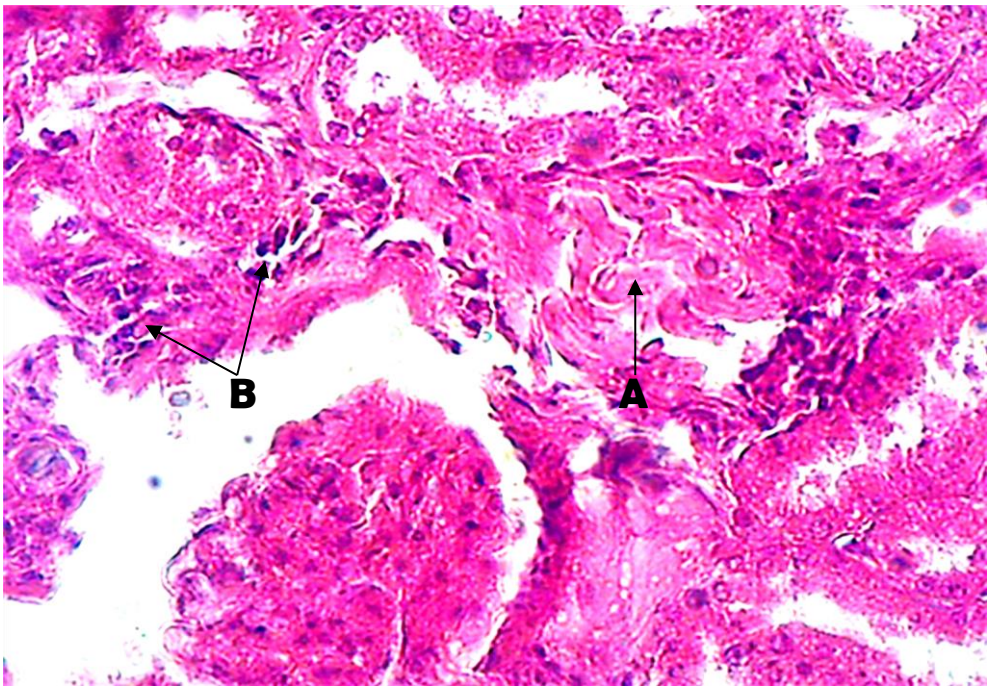
When Quercetin was offered it showed ameliorating effect in both periods of treatment where the damage was lesser than that caused by o-anisidine and parts of the renal tissues were looking like normal tissues (pictures, 6, 7, 8).



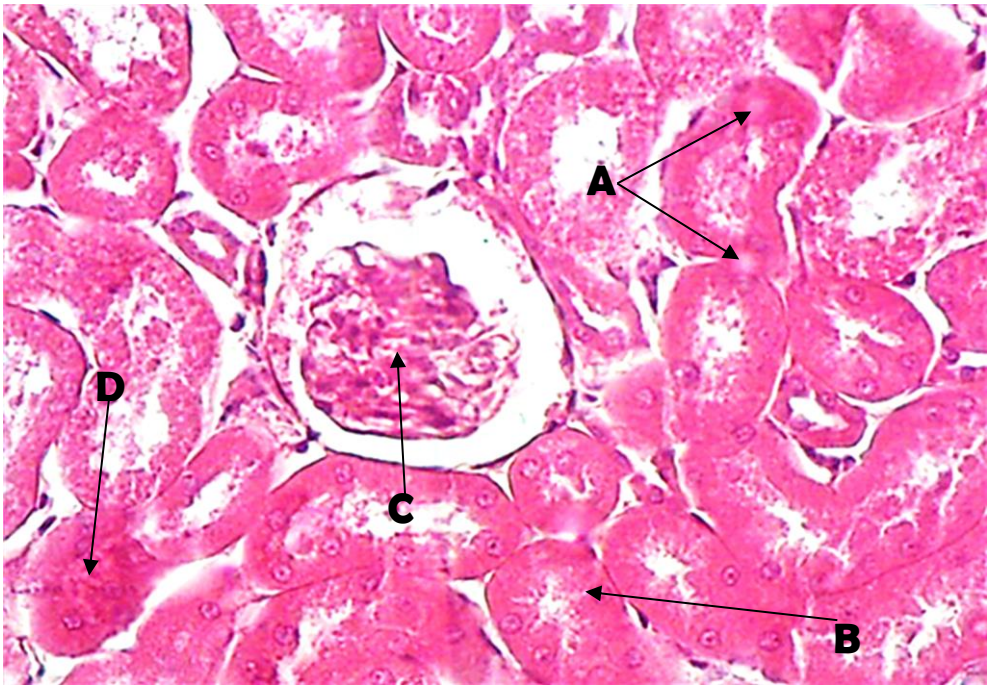
Picture (1). Control Kidney (H&E Stain, 400X).Normal glomerulus (A), and normal renal tubule (B).



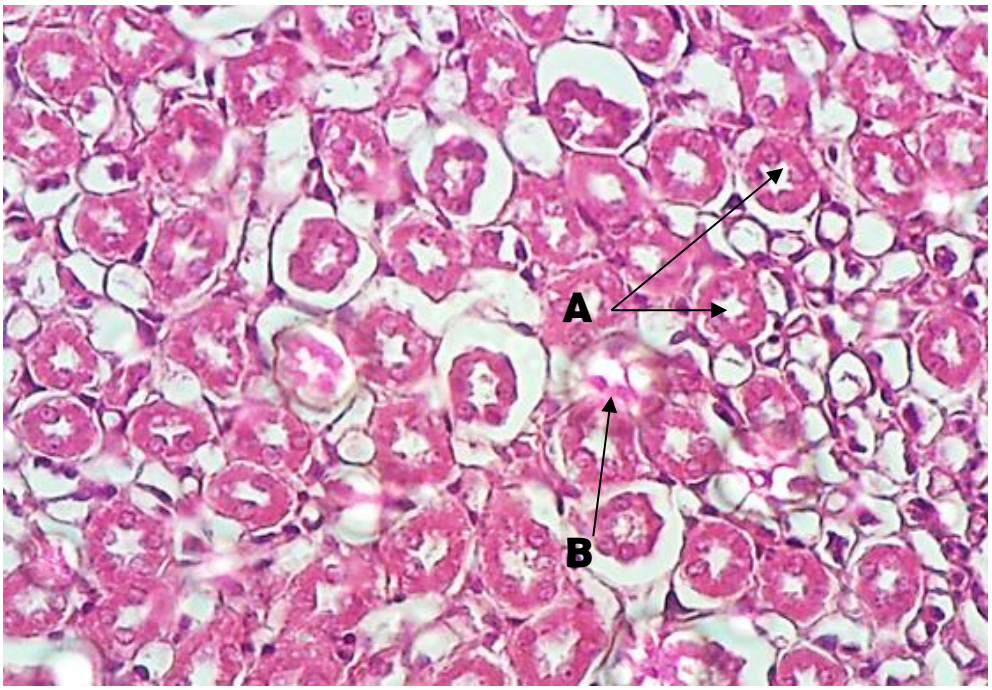
Picture (2). Anisidine 15 days, Kidney (H&E Stain, 400X). Atrophy of glomeruli (A) and infiltration of inflammatory cells (B).



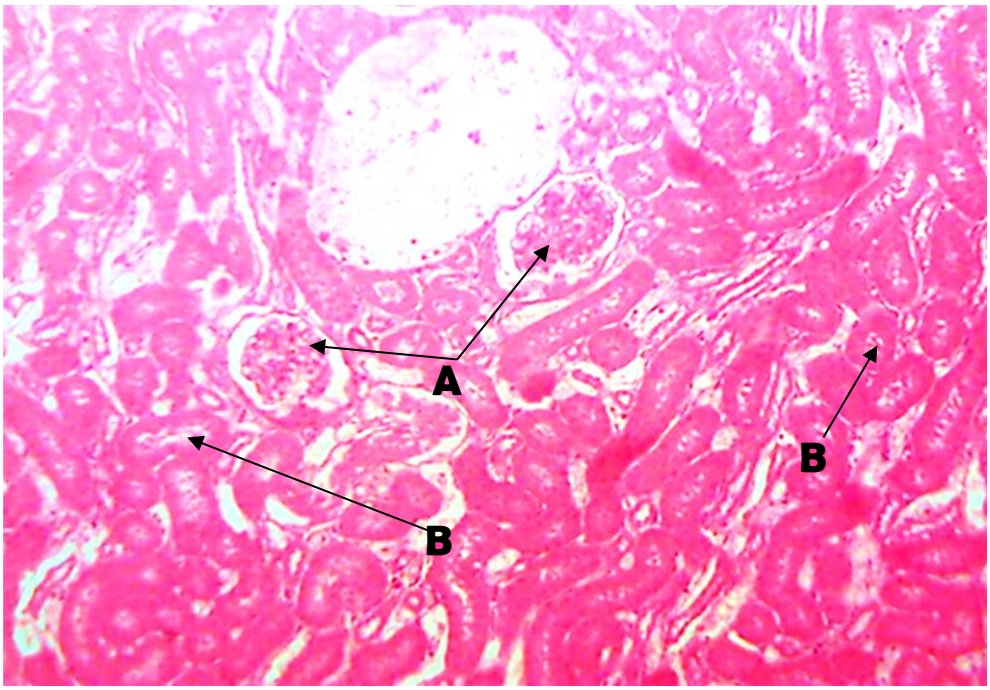
Picture (3). Anisidine 15 days, Kidney (H&E Stain, 400X). Complete disappearance of glomerulus (A) aggregation of messangial cells (B).



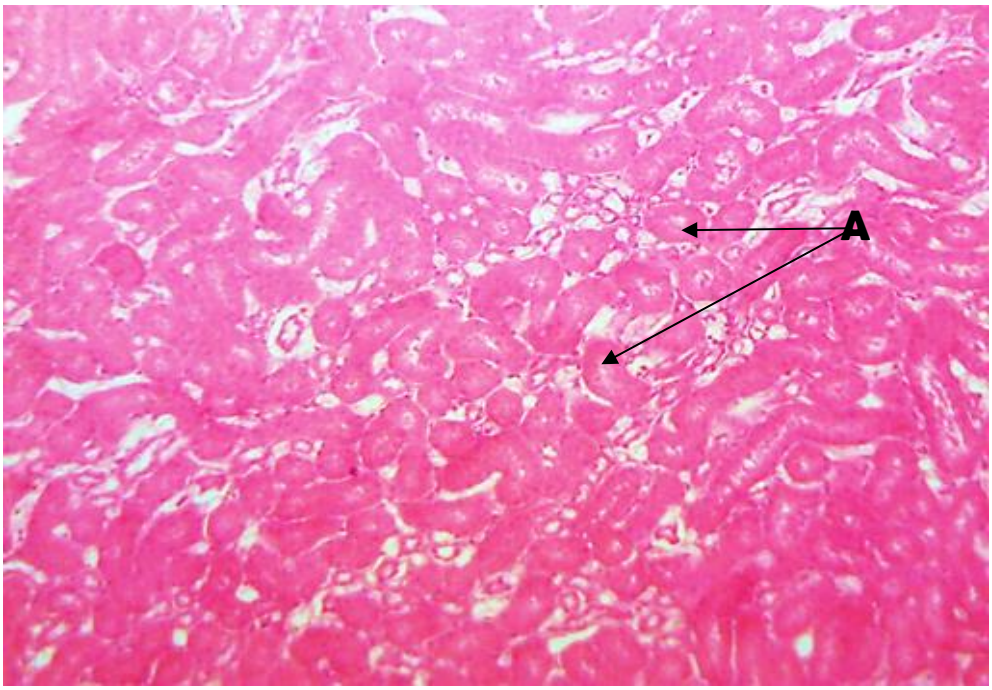
Picture (4). Kidney, Anisidine 30 days, (H&E Stain, 400X). Note the hyaline droplets in the cortico-medullary tubules (A), tubules associated with edematous changes (B) resulting in appearance of structures with clotting in the center of glomeruli (toxic effect)(C) and complete closure of tubules (D).



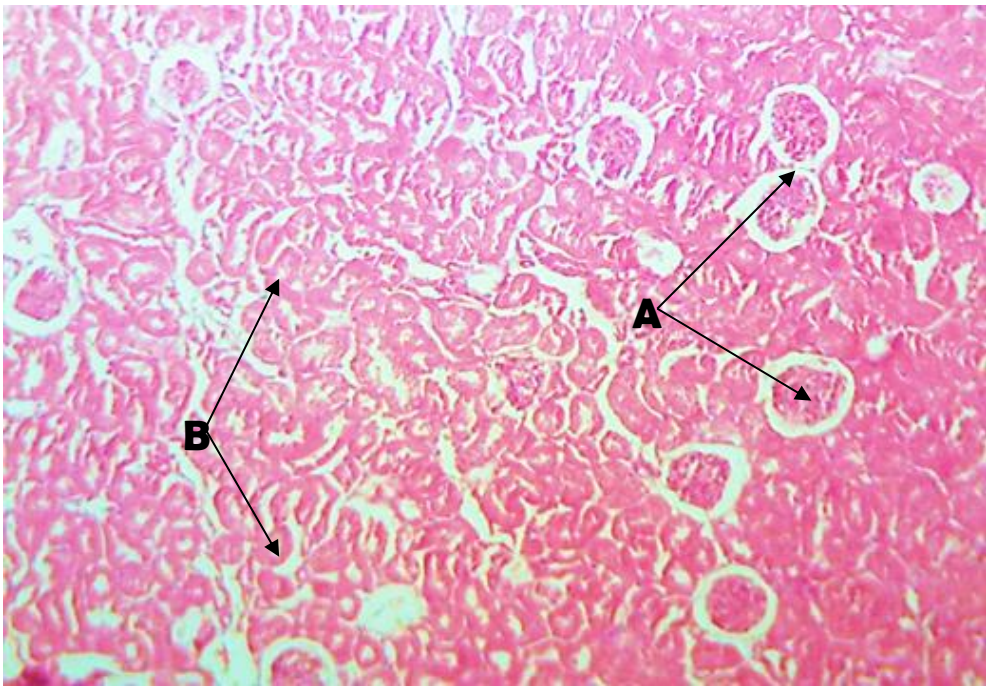
Picture (5). Kidney, Anisidine 30 days (H&E Stain, 100 X). Degeneration of corticomedullary tubules (A) and edema around tubules (B).



Picture (6). Renal cortex, Quercetin 15 days (H&E Stain, 100 X). Restoring some normal glomeruli(A) and renal tubules(B).



Picture (7). Renal medulla, Quercetin 15 days (H&E Stain, 100 X). Appearance of some normal renal tubules (A).



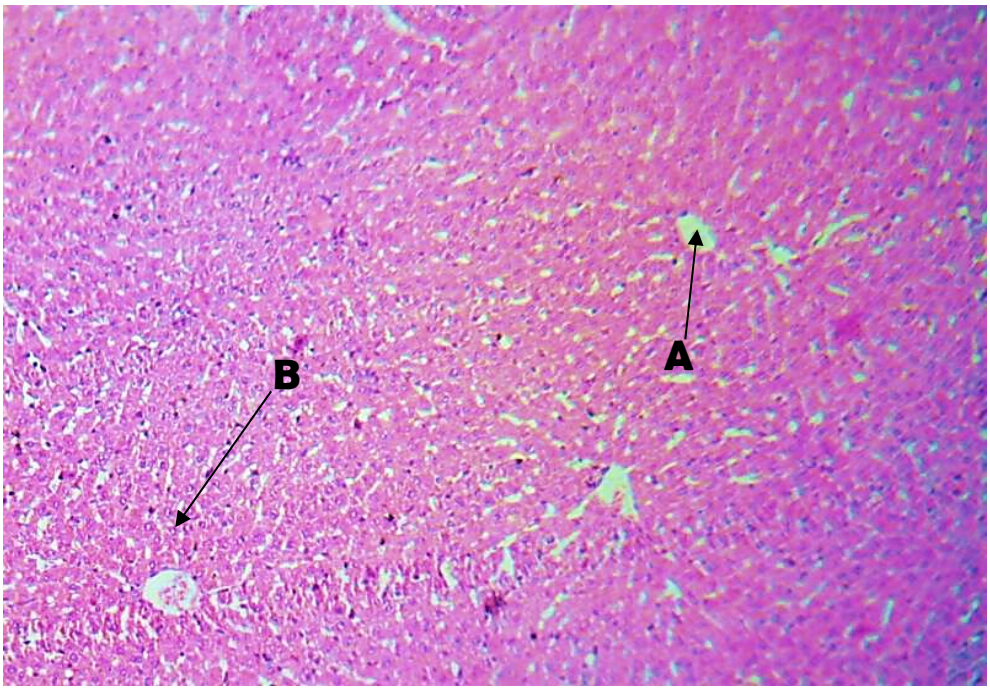
Picture (8). Kidney, Quercetin 30 days (H&E Stain, 100 X). More Quercetin effect represented by appearance of normal glomeruli (A) and renal tubules (B).

4.4.2. Liver

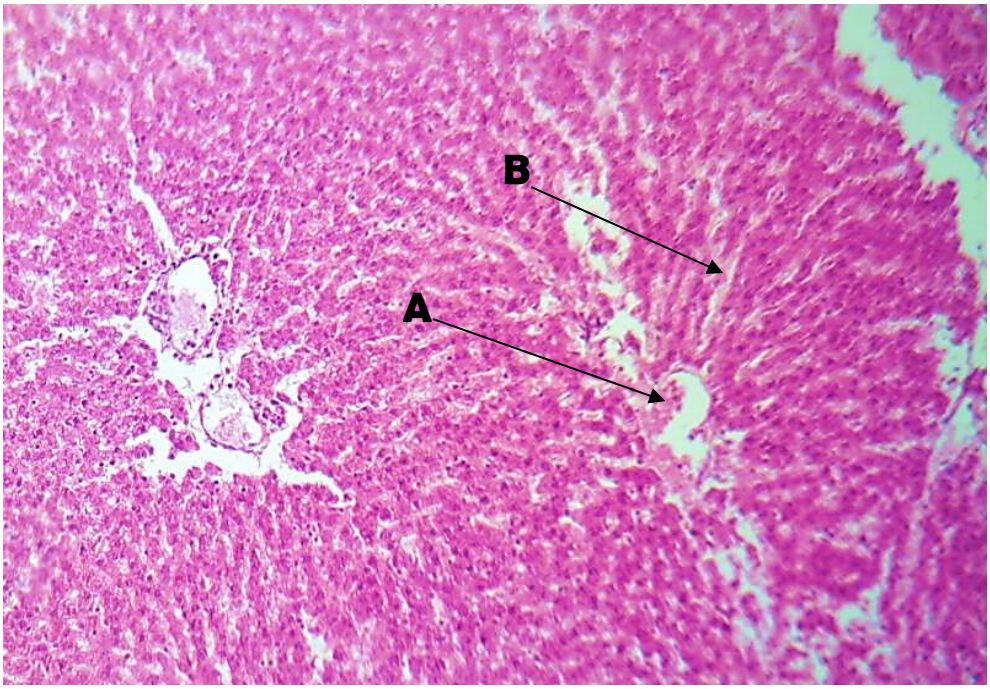
O-Anisidine effect was very humiliating on the liver texture and tissue in both periods of treatment as it compared with the control group (picture, 9). O-Anisidine caused miscellaneous degrees of damage like dilation of central vein and sinusoids (picture, 10), hemorrhage in the sinusoids and cloudy swelling of hepatocytes (picture, 11).

After 30 days of treatment with anisidine, hemorrhage, and clear fatty degeneration (picture, 12), hemorrhage and infiltration of inflammatory cells especially kupfer cells (picture, 13), very clear fatty degeneration, spread hemorrhage and infiltration of kupfer cells (pictures, 14, 15).

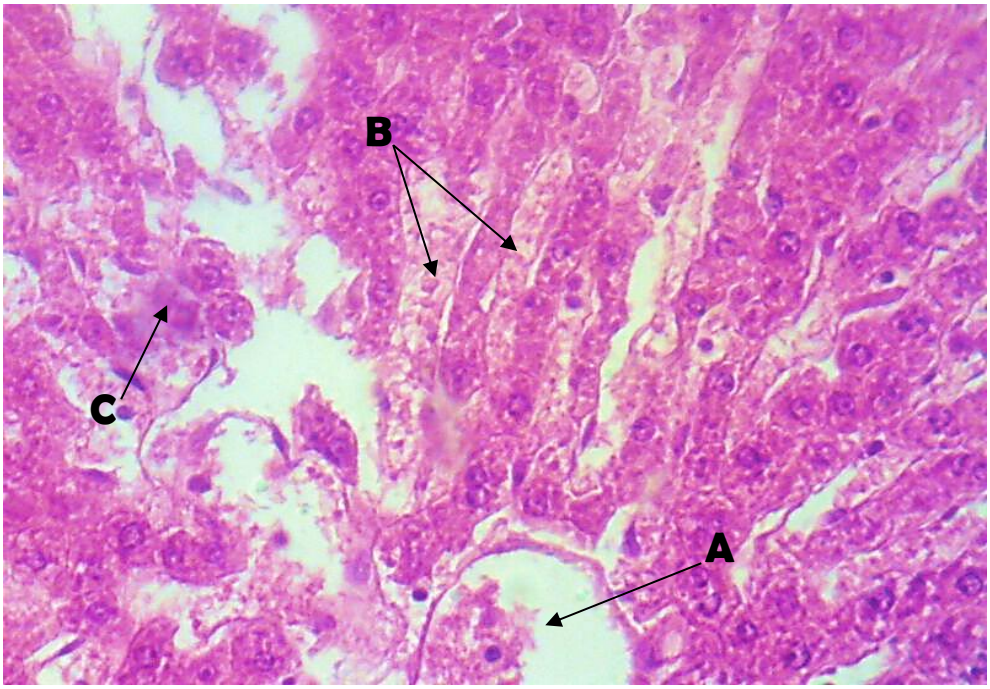
When Quercetin was offered it exerted a very clear ameliorating effects specially after 30 days of treatment were the hepatic tissue was near or like control with clear disappearance of fatty degeneration and the central vein was toward normal (pictures, 16, 17, and 18).



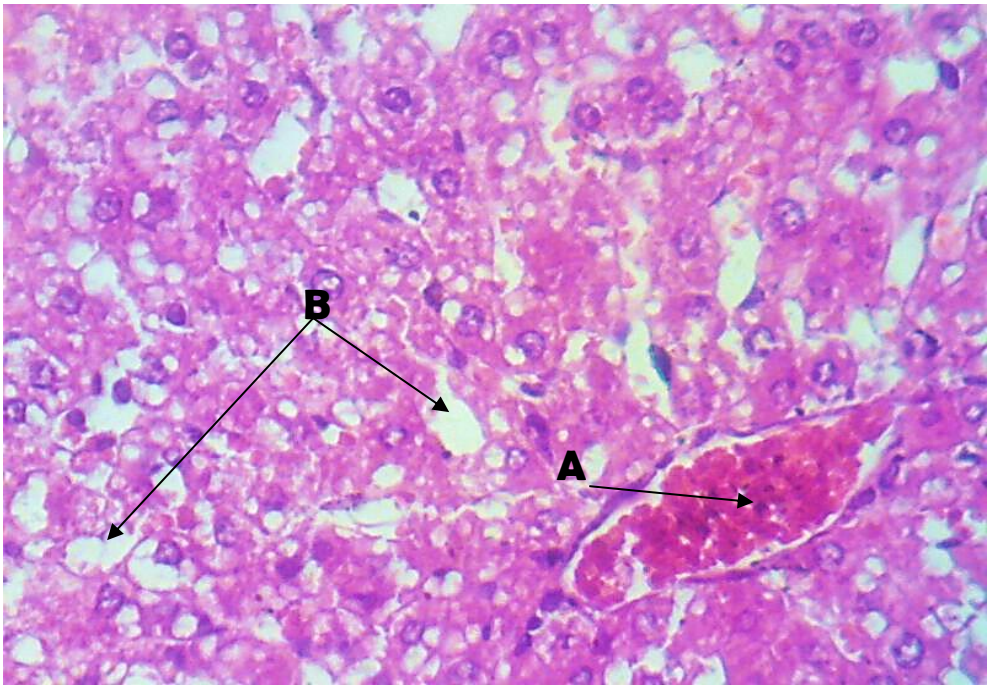
Picture (9).Control liver (H&E Stain, 100X). Normal central vein (A), and normal hepatocytes (B).



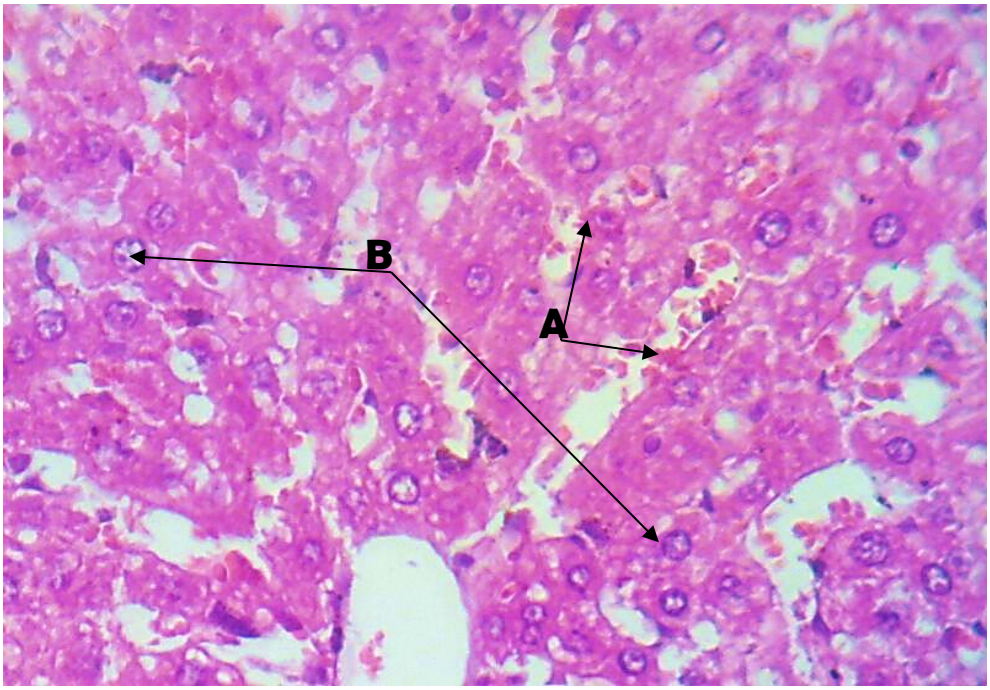
Picture (10). Liver, Anisidine 15 days (H&E Stain, 100X). Dilated central vein (A), and dilated sinusoids (B)



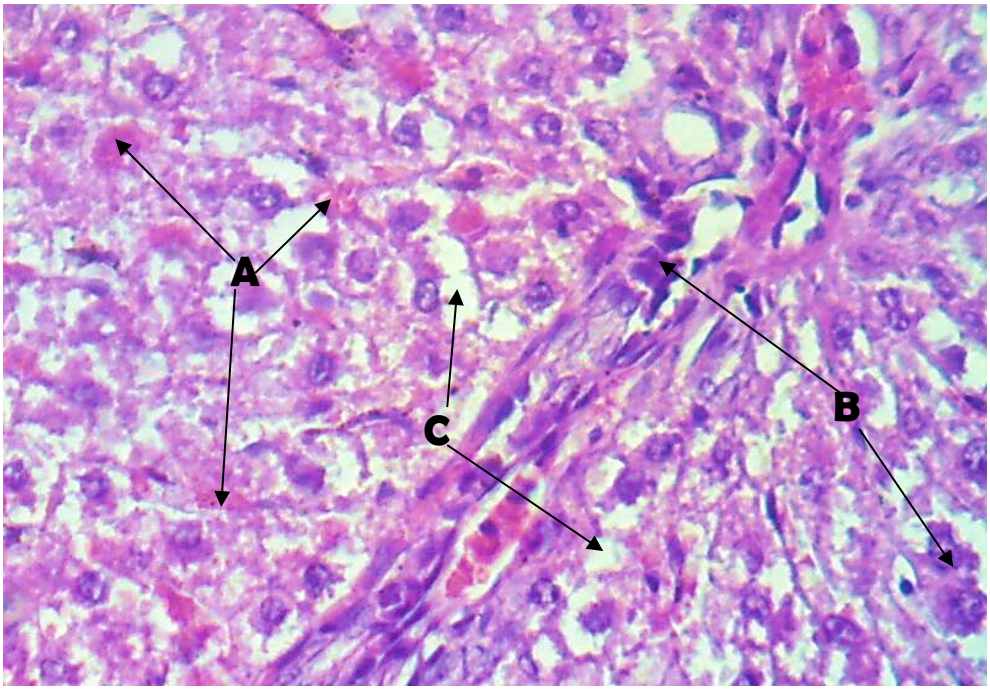
Picture (11). Liver, Anisidine 15 days (H&E Stain, 400X). Dilated central vein (A), dilated sinusoids with hemorrhage (B), and cloudy swelling of hepatic cells (C).



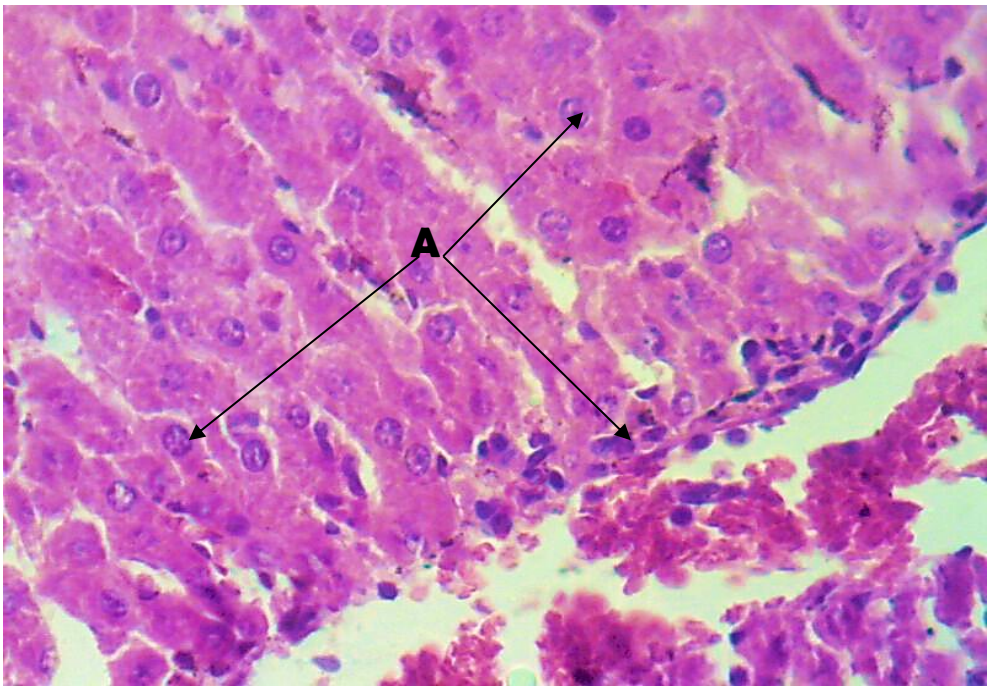
Picture (12). Liver, Anisidine 30 days (400X). Dilated congested central vein (A), and clear fatty degeneration (B).



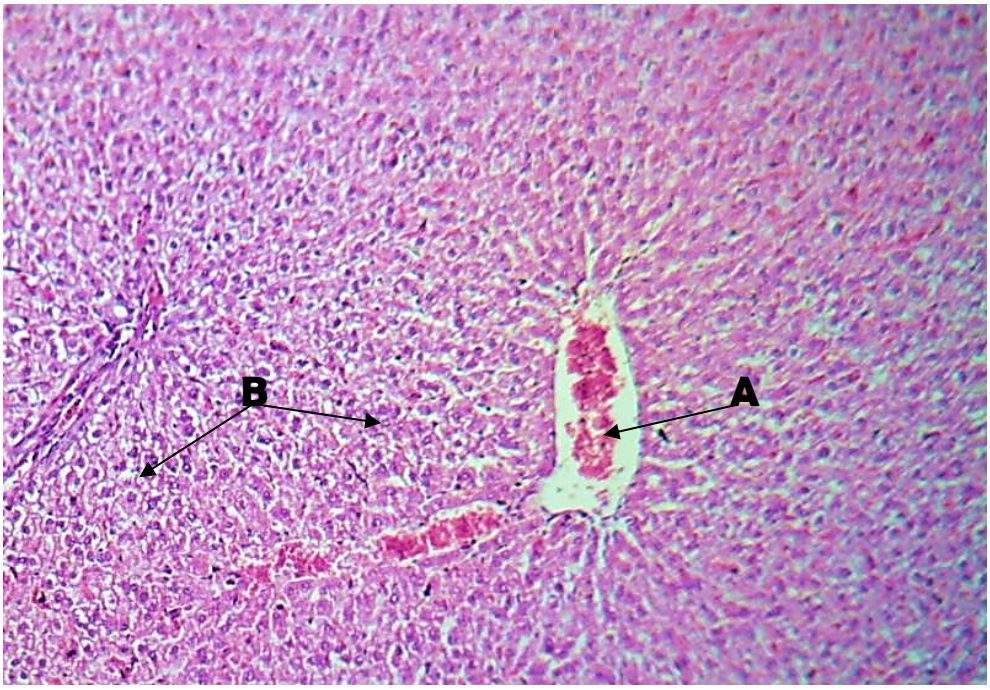
Picture (13). Liver, Anisidine 30 days (H&E Stain, 400X). Hemorrhage (A), infiltration of inflammatory and cells especially macrophage (B).



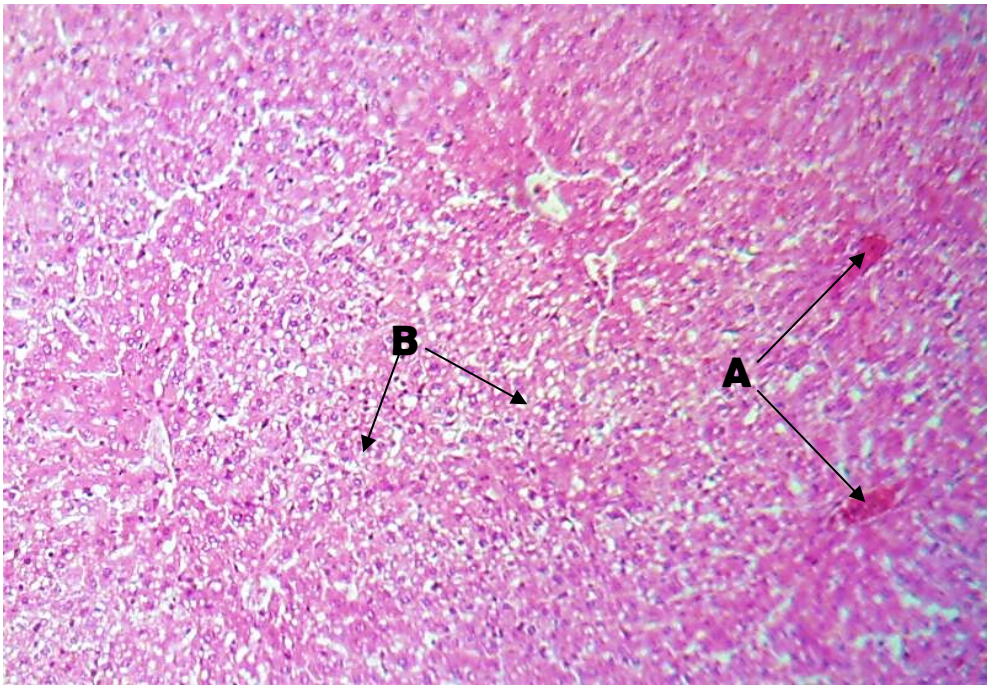
Picture (14). Liver, Anisidine 30 days (H&E Stain, 400X). Wide spread Hemorrhage (A), infiltration of inflammatory cells especially macrophage (B) and very clear fatty degeneration (C).



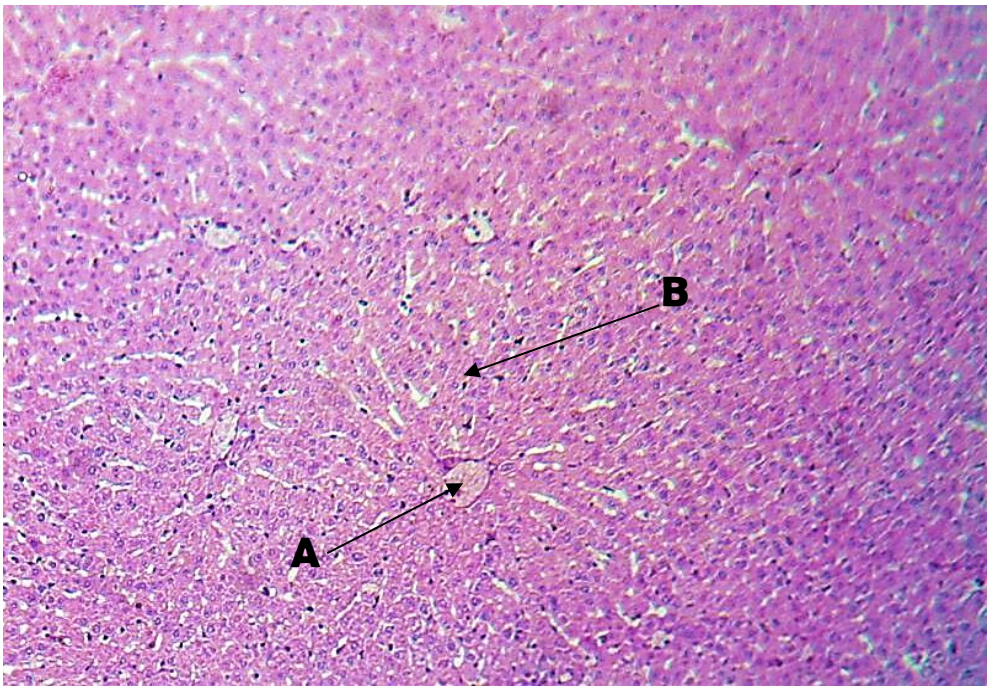
Picture (15). Liver, Anisidine 30 days (H&E Stain,400X). Clear aggregation of inflammatory cells especially kupfer cells (A).



Picture (16). Liver, Quercetin 15 days (H&E Stain, 100X). Appearance of central vein (A), and normal hepatocytes (B).



Picture (17). Liver, Quercetin 15 days (H&E Stain, 100X). Central vein (A), and normal hepatocytes (B).



Picture (18). Liver, Quercetin 30 days (H&E Stain, 100X). Normal central vein (A), and normal hepatocytes (B).

5-Discussion

5.1. Physiological parameters

5.1.1. Hematological parameters

5.1.1.1. R.B.C., Hb, P.C.V. and Platelets

The results showed that the use of Ortho-anisidine for 15 days caused a significant decrease in the R.B.C. count, Hb, and P.C.V. and it caused a significant increase in platelets count of the 15 days o-anisidine treated group as compared to the control group. When Quercetin was offered as treatment in the 15 days quercetin+anisidine treated group it caused the R.B.C. count, Hb, and P.C.V. to elevate significantly and the platelets count to decrease significantly as compared to the 15 days o-anisidine treated group and there was no significant difference as compared to the control group except the platelets count which was still higher than that of control.

When o-anisidine was used for 30 days it caused further significant decrease in R.B.C. count, Hb, and P.C.V. and significant increase in the platelets count as compared to the control group. These results agreed with the results obtained by (EC, 2011). When Quercetin was offered as treatment in the 30 days Quercetin+anisidine treated group it caused further significant elevation in R.B.C. count, Hb, and P.C.V. and a significant decrease in the platelets count as compared to the o-anisidine treated groups of both periods and there was no significant difference as compared with the15 days quercetin+anisidine treated group and the control group except the platelets count which was still higher than that

of control group. The effect of O-anisidine on blood parameters in this study agreed with the results obtained by (Sabbioni, 1995) while the ameliorating role of Quercetin on R.B.C. count, Hb, and P.C.V. agreed with the results obtained by (Petruška *et al.*, 2013).

5.1.1.2. R.B.C. indices (MCH, MCHC, RDW, MPV and MCV)

The hematological results also have shown that O-anisidine did not effect the red cell indices except that it caused the mean platelets volume (MPV) to decrease significantly in both periods of treatment as compared with control group and when Quercetin was used it caused the MPV to elevate significant and reached the control values.

These deleterious effects of O-anisidine on the blood parameters can be explained pending upon either the direct effect of O-anisidine or indirectly by the effects of the metabolites which are resulted from its metabolism in the liver of rat. Where, O-anisidine is oxidized by human, rat and rabbit hepatic microsomes containing cytochromes P450 not only to N-(2-methoxyphenyl)hydroxylamine, but that this compound is a subject of complex redox cycling reactions, forming also o-aminophenol, 2- nitrosoanisole and one additional metabolite, the exact structure of which has not been identified as yet (Naiman *et al.*, 2008). N-(2-methoxyphenyl) hydroxylamine might also be a subject of complex reactions, and its fate is dependent on the environment, in which it occurs. It can be further metabolized to o-aminophenol, o-nitrosoanisole and parental o-anisidine or when nucleophiles such as DNA or proteins are present in the cell, form the adducts (Stiborova *et al*, 2009). O-Demethylation of 2-nitroanisole to 2-nitrophenol and its hydroxylated products, 2, 5-dihydroxynitrobenzene and 2, 6-dihydroxynitrobenzene,

(Dračinska *et al*., 2006). N-Hydroxyarylamines can be further metabolized to N-sulfonyloxyarylamines, N-acetoxyarylamines or N-hydroxyarylamine N-glucuronide. These highly reactive intermediates are responsible for the genotoxic and cytotoxic effects (Neumann *et al.*, 1994) of this class of compounds. One of the most effects of these metabolites is the formation of reactive oxygen species (ROS) especially by the metabolite O-aminophenol like superoxide radicals or hydrogen peroxide (Michalowicz and Duda, 2006). The metabolite Nitrobenzene undergoes further metabolic activation where it forms a number of phenolic compounds by oxidation and nitroxides by reduction (Holder, 1999). Reduction of nitro group plays a more potent role in NB carcinogenicity and toxicity. Nitroreduction, which is driven by microsomal P-450s and NAD(P)H, can produce reactive nitroxides intermediates aromatic nitroso- and hydroxylamine compounds, e.g. NOB and PH, associated with their reactive free radicals, e.g., the nitroanion free radical and superoxide free radical (Mason and Holtzman, 1975). ROS can induce oxidative damage to the cell and can form a very stable structure by extracting electrons from other sources. ROS are also able to generate other forms of ROS. Superoxide can be dismutated into H2O2 and oxygen. H2O2 has the ability to form the more damaging _OH, through a combination of the Fenton and Haber-Weiss reactions (Kohen and Nyska, 2002). The ROS which are not neutralized, can target biological molecules such as DNA, lipids, proteins, and carbohydrates, which can result in cell dysfunction or cell death. Red blood cell (RBC) membranes contain lipids rich in unsaturated fatty acids. RBCs are more frequently exposed to oxygen than other body tissue and, thus, are more susceptible to oxidative

damage. Erythrocytes are highly susceptible to the oxidative damage due to the high cellular concentration of oxygen and hemoglobin—a potentially powerful promoter for the oxidative processes (Adam *et al.*, 2012). Invasion of the RBC membrane by peroxidants may lead to cell hemolysis. Moreover, the hemoglobin in RBCs is a strong catalyst which may initiate lipid peroxidation. In addition to lipid peroxidation, oxidants affect vital –SH groups of proteins which are highly active and may be targeted during oxidative stress (Asgary *et al.*, 2005). During the binding of oxygen to form oxy-hemoglobin (oxy-Hb), one electron is transferred from iron to the bound oxygen forming a ferric-superoxide anion complex. The shared electron is normally returned to the iron when oxygen is released during deoxygenation. However, the electrons can remain and transform oxygen into superoxide anions. In this process, iron is left in the ferric state and Hb is transformed into methemoglobin (met-Hb). The autoxidation of Hb occurs spontaneously and transforms 0.5–3% of Hb into met-Hb per day. In addition to this physiological process, met-Hb can be produced by endogenous oxidants, such as H2O2, nitric oxide (NO), and hydroxyl radicals. Since met-Hb cannot bind oxygen, this is the first step in the formation of harmful hemichromes (Rice-Evans & Baysal, 1987). In normal conditions, spontaneous production of met-Hb from autoxidation and conversion of met-Hb back to Hb are in balance. However, in pathological conditions, increased oxidative stress or impaired antioxidant defense will enhance production of met- Hb and generation of ROS. Hemichrome formation depends on the amount of met-Hb formed and is accelerated by ROS such as superoxide or H2O2. Superoxide produced by one electron reduction of oxygen would reduce ferri-hemichrome to ferro-

hemichrome. In the Fenton reaction, ferro-hemichrome catalyzes decomposition of H2O2 to hydroxyl radical. Hydroxyl radical is an extremely reactive free radical that can react with various biomolecules such as membrane lipids. Peroxidation of membrane lipids, most notably the polyunsaturated fatty acids arachidonic acid and linoleic acid, generates a wide array of molecules, such as lipid hydroperoxides, which are secondary lipid peroxidation products (for example, malondialdehyde and 4-hydroxynonenal, HNE). Lipid peroxidation products can damage membrane structure with the formation of membrane pores, alter water permeability, decrease cell deformability, and enhance IgG binding and complement activation. Finally, disruption of the normal asymmetrical distribution of membrane phospholipids occurs. This may enhance exposure of phosphatidylserine (PS) on the outer cell surface. Erythrocytes that have PS exposed on the outer surface are recognized and engulfed by macrophages with PS-specific receptors, resulting in their degradation (Nur *et al.*, 2011). The increased intra- and extra-erythrocytic oxidative stress induces lipid peroxidation and membrane instability, contributing to accelerated hemolysis. Increased levels of hydroperoxides cause erythrocyte membrane damage and deformity and, ultimately, lead to cell death. Band 3, also termed the anion exchanger, is a major erythrocyte membrane protein, constituting 25% of the total erythrocyte membrane protein. It has two independent domains: the membrane-spanning domain, which catalyzes anion exchange and contains the antigenic determinants recognized by naturally occurring antibodies (NAbs), and the cytoplasmic domain (Pantaleo *et al.*, 2008). A very important feature of hemichrome/free heme/iron damage is its non-random occurrence in space. The highly

damaging feature of hemichromes is their tight association with the cytoplasmic domain of band 3, which, following their binding, leads to band 3 oxidation and clusterization. These band 3 clusters show increased affinity for NAbs, which activate complement and finally trigger phagocytosis-mediated erythrocyte removal. This band 3/hemichrome complex was found not only in pathological conditions in which oxidative stress in erythrocytes is thought to be elevated, but also in senescent erythrocytes (Arese *et al.*, 2005).

The main molecules that are involved in redox signaling are called as reactive oxygen species (ROS), in which we may include hydrogen peroxide (H2O2), nitric oxide (NO), hydroxyl radical, superoxide (O2.−) and peroxynitrite. Current redox signaling investigations indicate that all the vascular constituents, including vascular smooth muscle cells (VSMCs), endothelial and adventitial cells and macrophages, produce ROS (Papaharalambus and Griendling, 2007). ROS are involved in signal transduction which is related to relaxation and contraction of blood vessels, migration, growth and death of vascular cells, and also extracellular matrix (ECM) alterations. Particularly, it was previously demonstrated that nitric oxide (NO) produced by NO synthase (eNOS) in the vascular endothelium modulates blood flow and pressure and presents important antiatherogenic effects on platelets, vascular smooth muscle and endothelial cells (Umans and Levi, 1995).

The endothelium comprises a simple squamous layer of cells that lines the inner surface of all blood vessels from the heart to the smallest capillary. It forms an interface between circulating blood and the vascular wall. The healthy endothelium prevents platelet aggregation

and leucocyte adhesion and controls vascular permeability and it is highly responsive to ROS by releasing endothelial-derived mediators, such as nitric oxide, prostacyclin (PGI2), platelet-activating factor (PAF), C-type atrial natriuretic peptide and ET-1 to maintain vascular tone and structural integrity (Mensah, 2007).Nitric oxide, produced from endothelial nitric oxide synthase (eNOS) has potent vasodilatory, anti-inflammatory and anti-thrombotic characteristics. Endothelial cells also control the coagulation pathway through the production of molecules with anti-coagulant activities such as tissue plasminogen activator, urokinase plasminogen activator, tissue factor pathway inhibitor and thrombo modulin (Libby *et al.*, 2006). Under pathological conditions, the endothelium becomes decompensated such that protective mechanisms are overwhelmed by injurious processes leading to impaired endothelium- mediated vasodilation, increased vascular reactivity, platelet activation, thrombus formation, increased permeability, and monocyte migration into the vascular wall (These events underlie endothelial impairment, which by definition, is a functional and reversible alteration of endothelial cell function. Molecular mechanisms contributing to this include increased expression of adhesion molecules, increased synthesis of pro-inflammatory and pro-atherosclerotic factors, activation of the local reninangiotensin system and increased ET-1 secretion (Endemann and Schiffrin, 2004). Increased ROS bioavailability and dysregulated redox signaling (oxidative stress) together with decreased nitric oxide production because of reduced eNOS activity and increased nitric oxide consumption by ROS contribute to many of the molecular events underlying endothelial injury (Giles TD, 2006).

Platelets, a major player in thrombus formation, are obviously a prime target for oxidants produced or released in the vascular lumen (Del Principe *et al.*, 1991).

So, the elevated platelets count in the results of our study can be explained by the following mechanisms: Production of hydrogen peroxide seems to promote thromboxane synthesis, and hence platelet aggregation, in response to arachidonic acid stimulation. This conclusion is also supported by data obtained by Del Principe *et al.* (1991) and by Pratico` *et al.* (1993), who showed that low concentrations of hydrogen peroxide could potentiate the aggregatory response to arachidonic acid or collagen. In summary, the effects of reactive oxygen metabolites on in vitro platelet function are complex. Low levels of HO may promote thromboxane synthesis and aggregation.

Role of other platelet agonists. Another possible mechanism by which oxidants may influence platelet aggregation is through potentiation of the effects of platelet-activating factor _PAF. PAF is an autacoid released by platelets and other cell types _e.g. endothelium, leukocytes, which acts on platelets at ex- tremely low concentrations (Jiuseppe *et al*., 1997). Superoxide and hydroxyl radicals can rapidly _i.e. within seconds and irreversibly inactivate plasma PAF-acetylhydrolase, the enzyme that catabolizes PAF. Once PAF is formed or released in the blood, inhibition of PAF-acetylhydrolase would enhance concentrations and prolong half-life of this powerful agonist. Thus, it may be speculated that oxygen radicals may indirectly enhance platelet aggregation, through local increases in PAF concentrations secondary to reduced breakdown of PAF. In vivo this hypothesis is indirectly supported by

data derived from a study by (Yao *et al.*, 1993), in which administration of superoxide dismutase was associated with a significant reduction in PAF-mediated aggregation of platelets resuspended in plasma, consistent with preserved activity of plasma acetylhydrolase.

Quercetin has a high antioxidant potential, the effect that is mainly based on inactivation of reactive oxygen species (ROS). Thus, Quercetin protects the cells against free radicals (Boots *et al.*, 2008).Quercetin, inhibit glycosylation by 52%. This is an important effect, as flavonoids are stored in RBCs (Fiorani *et al.*, 2003). Pure Quercetin inhibits hemolysis, Quercetin decreases hemolysis in a dose-dependent manner. This was also shown by (Kitagawa *et al.*, 1992). Bioflavonoid antioxidants in animals are known to be effective in preventing oxidative stresses (Nagata *et al.*, 1999). Earlier studies have demonstrated that induced hemolysis in RBCs is effectively inhibited by natural antioxidants (Zou *et al.*, 2001). Flavonoids are shown to protect biological membranes against free radical-induced oxidative damage (Kitagawa *et al.*, 1992). Additionally, flavonoids have inhibitory effects on the functions of platelets and leukocytes. They also protect endothelial cells, and counterbalance the interactions between the blood stream and vascular wall, which may lead to thrombosis. The latter effect is mediated through the effect of

flavonoids on human monocyte tissue factor, which itself may trigger blood coagulation (Lale *et al.*, 1996). The mechanism of action of Quercetin has been attributed largely to the antioxidant properties, which are known to augment GSH and antioxidant enzyme levels and scavenge lipid peroxides. A concept is now emerging of “adaptogenic drugs” -

drugs that increases non-specific resistance to variety of stresses. Quercetin also shows effects via adaptation which included augmentation of antioxidant enzymes (SOD, CAT& GPx), endogenous antioxidants (GSH) and stress proteins (HSP72) in the heart (Hui *et al.*, 2012). For the effect of O-anisidine on MPV, the decreasing effect which was caused by O-anisidine can be explained by two ways: the first; is that MPV usually decreases when there is increased platelets count (Thrombocytosis) which was evident in our results and was explained in details above, the second; is that the chemical substances and chemotherapies usually effect the bone morrow leading to increased production of platelets with a small volume (MPV). The previous causes of increased MPV was mentioned by (Stockham and Scott, 2008).

5.1.1.3. Total and Differential leukocytes count

It`s clear from the results that O-anisidine treatment caused a significant increase in the total white blood cells, monocytes, acidophils, and basophils counts of the 15 days o-anisidine treated group as compared to the control group. It also caused further significant increase in these cells counts when it was used for 30 days of treatment as compared to the control group. It is also clear from the results that O-anisidine treatment caused a significant decrease in the neutrophil, and lymphocytes counts of the 15 days o-anisidine treated group as compared to the control group. It also caused further significant decrease in these cells counts when it was used for 30 days of treatment as compared to the control group.

The results show that when Quercetin was offered it caused a significant decrease in the total white blood cells, monocytes, acidophils, and basophils counts of the 15 days Quercetin+anisidine treated group as compared to the 15 days O-anisidine treated group. It also caused further significant decrease in these cells counts when it was used for 30 days of treatment as compared to the O-anisidine treated group. It is also clear from the results that Quercetin treatment caused a significant increase in the neutrophil, and lymphocytes counts as compared to the O-anisidine group. It also caused further significant decrease in these cells counts when it was used for 30 days of treatment as compared to the O-anisidine group.

The neutropenia in the results which was caused by O-anisidine treatment came in accordance with the results obtained by (Adriana *et al.*, 2013) who found that oxidative stress caused neutropenia and accelerated neutrophil apoptosis in dogs, and also agreed with the results obtained by (Abousalem *et al.*, 2012) who reported that exposure to benzene derivatives causes lymphopenia and neutropenia in rats.

The neutropenia and lymphocytopenia in our results can be explained by two mechanisms; the first is related with the oxidative stress that is caused by O-anisidine and its metabolites and the second is related to the inhibitory effect of the nitrobenzene metabolite of O-anisidine on the neutrophils.

For the first mechanism, Adriana *et al.*, (2013) mentioned that oxidative stress causes neutrophils to produce reactive oxygen species (ROS) when nicotinamide adenine dinucleotide phosphate oxidase is activated, generating the superoxide anion essential to the bactericidal function of

neutrophils (Huimin *et al.*, 2000). Although the ROS derived from superoxide are required for the defense mechanism of neutrophils, free radicals produced in excess can damage a number of cellular structures, thus inducing lipid peroxidation and accelerating apoptosis (Kato *et al.*, 2008). This approach to the cause of neutropenia is also agreed with what mentioned by (Chonchol, 2006) who reported that the viability and function of neutrophils are affected by oxidative stress and uremic toxins.

For the second mechanism by which O-anisidine treatment caused neutropenia is raised from O-anisidine metabolite; the nitrobenzene. As O-anisidine and its metabolites effect directly the vascular endothelium (Gabriele, 1994), nitrobenzene has been discovered as potent inhibitors of vascular cell adhesion molecule-1 (VCAM-1) expression. Structure–activity relationship (SAR) studies showed that a nitro group and two other electron-withdrawing groups are essential for the nitrobenzene to be potent inhibitor of VCAM-1 expression and the nitro group is also found to be suppressor to the bone marrow, and by this mechanism the nitrobenzene will cause neutropenia (Charles *et al.*, 2001).

Lymphocytes, an important immunological cell, have played a significant role in acquired immune system. The immune cell functions are specially linked to ROS generation and are strongly influenced by the redox potential. Therefore the oxidant/antioxidant balance is an important determinant of immune cell activity. The antioxidant levels in immune cells play a pivotal role in protecting them against oxidative stress and therefore preserving their adequate function (Nandeslu *et al.*, 2012). Lipid peroxidation is one of the important phenomenon and has

been implicated in a number of deleterious effects, such as, increased osmotic fragility, decreased membrane rigidity and cellular deformation (Thambi *et al.*, 1991). Studies on cellular injury implicate peroxidation of polyunsaturated fatty acids (PUFA), leading to the degradation of phospholipids and increased levels of Malondialdehyde (MDA) (Nandeslu *et al.*, 2012). High level of lipid peroxidation (LP) may be due to excessive generation of free radicals. With increased LP, the cellular damage is also increased and the viability of lymphocytes will be decreased (Karmakar and Adhikari, 2012). Glutathione is an important cellular reductant, which offers protections against free radicals, peroxide and toxic compounds. Adequate concentrations of GSH are required for a variety of immune functions. It has been suggested that oxidative stress and deficiency of thiol compounds may play an important pathogenic role in the development of immune deficiency (Aukrust *et al.*, 1995). Depletion of intracellular GSH may inhibit T-cell function (Droge *et al.*, 1994). Decreased glutathione levels and GPx activity are coupled to increased oxidative damage to DNA, lipids and proteins (Cakatay *et al.*, 2001).

The direct effect of the metabolite nitrobenzene which was argued early and the oxidative stress that is caused by O-anisidine and its metabolites as it is proved by the elevated MDA levels and the deteriorated levels of antioxidant enzymes SOD, and GPx in our results might be the preponderant explanation for the neutropenia and lymphocytopenia in our results.

The increased monocyte count (monocytosis) in our study due to treatment with O-anisidine can be related to the increased total

cholesterol, LDL, TGs and VLDL and the monocytosis is also appear to be a predisposing factor for the thrombocytosis (Eitzman *et al.*, 2000) which was discussed previously.

The lipid hypothesis postulates that an elevation in plasma LDL levels results in penetration of LDL into the arterial wall, leading to lipid accumulation in smooth muscle cells and in macrophages (foam cells). LDL also augments smooth muscle cell hyperplasia and migration into the sub intimal and intimal region in response to growth factors. LDL is modified or oxidized in this environment and is rendered more atherogenic. Small dense LDL cholesterol particles are also more susceptible to modification and oxidation. The modified or oxidized LDL is chemotactic to monocytes, promoting their migration into the intima, their early appearance in the fatty streak, and their transformation and retention in the sub intimal compartment as macrophages. Scavenger receptors on the surface of macrophages facilitate the entry of oxidized LDL into these cells, transferring them into lipid-laden macrophages and foam cells (Berkow *et al.*, 1999). Oxidized LDL is also cytotoxic to endothelial cells and may be responsible for their dysfunction or loss from the more advanced lesion. Alternatively, organization and incorporation of the thrombus into the atherosclerotic plaque may contribute to its growth. The chronic endothelial injury hypothesis postulates that endothelial injury by various mechanisms produces loss of endothelium, adhesion of platelets to sub endothelium, aggregation of platelets, chemotaxis of monocytes, and release of platelet-derived and monocyte-derived growth factors that induce migration of smooth muscle cells from the media into the intima, where they replicate, synthesize connective tissue and proteoglycans, and form a fibrous

plaque. Other cells (e.g., macrophages, endothelial cells, arterial smooth muscle cells) also produce growth factors that can contribute to smooth muscle hyperplasia and extracellular matrix production. These two hypotheses are closely linked and not mutually exclusive. Modified LDL is cytotoxic to cultured endothelial cells and may induce endothelial injury, attract monocytes and macrophages, and stimulate smooth muscle growth. Modified LDL also inhibits macrophage mobility, so that once macrophages transform into foam cells in the sub endothelial space they may become trapped. In addition, regenerating endothelial cells (after injury) are functionally impaired and increase the uptake of LDL from plasma (Berkow *et al.*, 1999).

Macrophage accumulation participates decisively in the development and exacerbation of atherosclerosis. Hypercholesterolemia-associated monocytosis (HAM) developed from increased survival, continued cell proliferation, and impaired Ly-6C^{hi} to Ly-6C^{lo} conversion and subsided upon statin-induced cholesterol reduction. It`s believed that Ly-6C^{hi} monocytes represent a newly recognized component of the inflammatory response in experimental atherosclerosis (Murphy *et al.*, 2011).

As it is clear from the results, when we used Quercetin as a protective agent against O-anisidine it rendered the total and differential leukocytes count toward normal; where it decreased the monocytosis, basophilia and eosinophilia and caused increased in the lowered neutrophil and lymphocyte counts.

Quercetin can exert its effects by different mechanisms. One of these mechanisms it can act directly as a potent antioxidant agent and free radical scavenger with a potency more than that of vitamin C

(Jamshidzadeh and Mehrabadi, 2010) and by four times more than that of vitamin E (Neha and Ramtej, 2012). Another mechanism of Quercetin action is that it is capable of preventing low-density lipoprotein (LDL) oxidation and platelet aggregation; crucial factors that prevent the formation of atherosclerotic plaques which predispose individuals to myocardial infarction (Haraguchi *et al.,* 1996). The latter mechanism supports the hypothesis of the role of hyperlipidemia in causing monocytosis and thrombocytosis which was explained before. Furthermore, Quercetin also acts directly by inhibiting xanthine oxidase, lipid peroxidation, and chelating metal ions (Abd El-Baky; 2011). And by its direct inhibitory effect on xanthine oxidase - xanthine oxidase is the major hepatic enzyme responsible for metabolism of O-anisidine into its toxic metabolites, Mikšanova *et al.*, (2004) – Quercetin can abolish or at least strongly weaken the effects of O-anisidine on different blood cells.

5.2. Biochemical parameters

5.2.1. (MDA, TCH, TGs, LDL, HDL, and VLDL)

It is clear from the results that O-anisidine caused a significant increase in the serum malondialdehyde (MDA) concentration, total serum cholesterol (TCH), triglycerides (TGs), low density lipoprotein (LDL) and very low density lipoprotein (VLDL) values. While, it caused a significant decrease in the high density lipoprotein (HDL) value. These effects of O-anisidine and / or its metabolites on lipid profile in our study agreed with the results obtained by (Himri *et al.*, 2011) who studied the oral toxic effects of a synthetic food dye in a 90 days study on Wistar rats, (Kate and Lucky, 2012) in their study on the effects of

an azo dye on lipid profile of rats, and also agreed with the findings mentioned by (Al-Mashedy, 2013) who studied the effect of textile dyes on lipid profile of textile workers.

Stress has effect on almost all animals. It is the sum of all non-specific biological phenomena elicited by adverse external influences (Martin, 2000). If the balance between free radicals and antioxidants is disrupted due to either over production of free radicals or decreased antioxidant defense, or both, this can result in a pathological condition called ‘‘oxidative stress’’ (Demircol *et al.*, 2012).Oxidative stress causes oxidation of DNA, membrane lipids, and proteins. Cellular proteins, particularly sulfur-containing proteins, can easily be oxidized. Cellular antioxidant defenses control the levels of endogenous ROS under normal conditions (Sahu, 2002). Lipid peroxidation that is caused by oxidative damage may inactivate cellular compounds which may lead to the development of various disorders (Demircol *et al.*, 2012). The lipid peroxidation product, malondialdehyde (MDA) is commonly used as a measure of the oxidative stress in cells. Lipid peroxidation, being a free radical reaction, it occurs when the hydroxyl radicals, possibly oxygen, react with the unsaturated lipids of the bio-membranes, resulting in the generation of lipid peroxide radicals (ROO•), lipid hydroperoxide (ROOH) and fragmentation products such as MDA (Pandey *et al.*, 2012), therefore; the increased levels of MDA in our study is a good indicator for the oxidative stress and lipid peroxidation which is caused by O-anisidine.

The increased levels of TCH, TGs, LDL and VLDL might be due to the sever declination in the levels of HDL-C in our study. AL-Yahiya *et al.*,

(2013) mentioned in his study on the oxidative stress in rats that the oxidative stress impedes the formation of HDL-C (High Density Lipoprotein Cholesterol) throughout increased lipid peroxidation, hereon; as the major role of HDL is the transportation of TCH to the liver for catabolism and as HDL promotes cholesterol efflux and has the ability to inhibit the oxidation of LDL via its anti-inflammatory role (Padmini and Rani, 2011) , so the diminished HDL-C will lead to aggregative increased levels of TCH, TGs, LDL and VLDL.

After the Quercetin was used for 15 and 30 days of treatment, it ameliorated all the lipid profile aspects, where it decreased significantly the TCH, TGs, LDL, and VLDL and it increased the HDL significantly as compared with the values of O-anisidine and for both periods but the ameliorated values were still higher than that of control.

The ameliorating effect of Quercetin on lipid profile in our study goes in the same direction of the results obtained by many searchers who mentioned the ameliorating effect of Quercetin in lipid profile like: Coşkun *et al.*, (2004) in their study on Quercetin in rats with gastric ulcer, Jeong et al., (2012) who studied the ameliorating effect of Quercetin in dyslipedimic mice, and with (Seiva et al., 2012) who studied the role of Quercetin in ameliorating lipid profile, and metabolic disturbances in rats.

Flavonoids have been described as modulators of lipid homeostasis in the adipose tissue and liver, through the inhibition of phosphodiesterases (Peluso, 2006). These protective effects can be mainly attributed to the antioxidant properties of quercetin (Arai *et al.*, 2000), indirectly acting on the cholesterol levels. Therefore, LDL-chol and VLDL-chol would be

protected against deleterious effects arising from oxidative process and its recognition by target cells would be determined (Carrero *et al.*, 1998). Other hypothesis includes the role of quercetin on decreasing HMG-CoA reductase activity, an enzyme required for cholesterol synthesis, the potential of reducing atherogenic index and lipoperoxidation (Lapointe *et al*., 2006). Furthermore, there is growing evidence that quercetin and its glycoside acts by altering hepatic cholesterol absorption and triglyceride assembly and secretion as well as through inhibition of phosphodiesterases in the adipose tissue and liver (Rivera *et al.*, 2008). These events could, at least partially, explain the great improvement in lipid profile.

5.2.2. (CAT, GPx, SOD, AST, ALT, and ALP)

From the results, it is clear that O-anisidine caused a significant increase in the antioxidant enzymes GPx, SOD, and CAT when it was used for 15 days, then after 30 days of treatment the levels of these enzymes declined significantly comparing with control group except for CAT which also declined but was still higher than the control. On other hand, when Quercetin was used as a mixture with the O-anisidine diet for 15 days it caused the antioxidant enzymes to decrease but they were significantly higher than the control group. Furthermore, when Quercetin was used for 30 days it caused the antioxidant enzymes to elevate significantly as compared with the 30 days Anisidine treated group and with the control group but they were significantly less than those of 15 days Anisidine and 15 days Quercetin treated groups except for the CAT which was not significantly less than 30 days Anisidine and significantly higher than 15 days Quercetin treated group.

For the liver function enzymes, it was clear from results that O-anisidine caused the levels of AST, ALT, and ALP to elevate significantly in the 15 days of treatment and to elevate much more in the 30 days of treatment as compared to the control group. By other side, the Quercetin caused these enzymes to decline significantly on the 15 days treatment but was significantly higher than that control. The use of Quercetin for 30 days also decreased the levels of these enzymes significantly comparing with the 30 days O-anisidine treated group but they were significantly higher than the 15 days Quercetin and control treated groups.

The elevated antioxidant enzymes in our results after 15 days of treatment agreed with the results obtained by (Menon *et al.*, 2010) who studied the effect of 10 days treatment with nitrobenzene on rat liver, and the increased in SOD activity in the results mentioned by (Liu *et al.*, 2009) in their study on the effect of an aromatic amine on gold fish., and with the results obtained by (Al-Mashedy, 2013) who studied the effect of azo dyes on Iraqi textile workers. The declination in antioxidant enzymes after 30 days of treatment in our study agreed with the findings mentioned by (Ansari *et al.*, 2011) who studied the effect of oxidative stress on Wistar rats in a 28 days study. Besides, the retrogression in CAT enzyme after 30 days of treatment in our study is also agreed with the declined CAT values obtained by (Kate and Lucky, 2012) in their study on the effects of an azo dye on lipid profile of rats.

The enzymatic antioxidants such as SOD, CAT or GPx, can scavenge reactive oxygen species and free radicals or stop their formation (Husain *et al.*, 2005). The superoxide anion (O-2) is a key peroxidative molecule.

The chief scavenger of superoxide anions is the cellular antioxidant enzyme, SOD which catalyzes the dismutation of superoxide to hydrogen peroxide that in turn is removed by another antioxidant enzyme, GPx (Juul *et al.*, 2004). GSH represents the first line of defense against free radicals and is also responsible for the maintenance of protein thiols and acts as a substrate for GPx and GST (Prakash *et al.*, 2001). GPx activity is considered to symbolize the initial protective response required for adjusting the H2O2 concentration under physiological condition as well as after oxidative insult (Izawa *et al.*, 1996).

The three major antioxidant defense enzymes are inducible enzymes. They can be induced by even slight oxidative stress due to compensatory responses. However, a severe oxidative stress suppresses the activities of these enzymes due to oxidative damage and a loss in compensatory mechanisms (Zhang *et al.*, 2004). The possibility that change in antioxidant enzymes could be due to the presence of xenobiotics in organisms has been considered. The increased activities of SOD, CAT, and GPx are known to serve as protective response to eliminate reactive free radicals. Reduction of superoxide radicals by SOD and of H2O2 and ROOH by CAT and GPx, respectively, prevent the formation of radical intermediates by oxygen reduction mechanisms. GSH is involved in scavenging free radicals, and, thereby, blocking the propagation of lipid peroxidation. At first, SOD converts superoxide anion to hydrogen peroxide in a cellular antioxidant reaction. CAT is mainly located in the peroxisomes and responsible for the reduction of hydrogen peroxide produced from the metabolism of long-chain fatty acids; while GPx catalyzes the reduction of both hydrogen peroxide and lipid peroxides,

and is considered to be an efficient protective enzyme against lipid peroxidation (Liu *et al*., 2009). The mentioned above role of these enzymes and the high oxidative stress in our study which was caused by O-anisidine either directly or indirectly by its metabolites and it was clear from the extensive elevated MDA levels in our results could explain why these antioxidant enzymes were increased significantly after 15 days of treatment with O-anisidine.

The antioxidants, SOD and the associated CAT can protect organisms from oxidative damage by partial remediation of ROS. SOD, the first line of defense against oxygen-derived free radicals, catalyzes superoxide anion radical ($O2^{-\cdot}$) into less-toxic H2O2 and O2, while CAT reduces H2O2 to non-toxic H2O and O2 (Bakan *et al*., 2003). O-anisidine causes extensive oxidation to the GSH leading to its depletion (Siraki *et al.*, 2002) and the decreased glutathione levels and GPx activity are coupled to increased oxidative damage to DNA, lipids and proteins (Cakatay *et al.*, 2001). Here, this could explain why the GPx levels in our study were elevated on 15 days of treatment with O-anisidine and then declined extensively after 30 days of treatment.

On the other hand, the increasing of CAT and SOD activities are more indicative for oxidative stress and elevated of ROS especially $O2^{\cdot-}$ which scavenging with SOD to produce H2O2 , the substrate for catalase enzyme (Frank *et al.*, 2000) which decomposed to water and oxygen. The estimation of CAT activity could be considered a biomarker for oxidant-antioxidant status (Al-Mashedy, 2013). Hence, this elucidate the elevated levels of CAT even after 30 days of treatment where CAT levels were significantly higher than that of control group and the

treatment with Quercetin decreased but not significantly the CAT levels after 30 days of treatment as compared to the O-anisidine treated group which reflects the high oxidative stress caused by O-anisidine. Furthermore, another cause should be taken in consideration for the cause of declined antioxidant enzymes after 30 days of O-anisidine treatment which is the increased lipid peroxidation status which was obvious by the elevated levels of MDA in our study, where the increased lipid peroxidation status lead to depression in antioxidant enzymes activity (Ansari *et al.*, 2011).

When Quercetin was used as a mixture with O-anisidine it showed a clear ameliorating effect on the levels of the antioxidant enzymes where it caused these enzymes to decline significantly after 15 days of treatment as compared with elevated levels caused by O-anisidine alone, and then after 30 days of treatment it caused the levels of the antioxidant enzymes to elevate markedly and significantly as compared with the declined values caused by O-anisidine alone after 30 days of treatment even the levels of GPx where near the control level while the levels of SOD and CAT were significantly higher than that of control.

The ameliorating effect of Quercetin on antioxidant enzyme and liver function biomarkers in our study came in accordance with the results mentioned by many searchers like: Abd El-Baky (2011) in his 8 weeks study on the oxidative stress in rats, Hui *et al.*, (2012) in their study on the antioxidant properties of Quercetin in rats, Regulska and Rafal, (2008) in their study on the role of Quercetin in selective antioxidant enzymes in rats, and Reddy et al., (2012) in their study on Quercetin in Benza-anthracin induced cancer in rats.

The main mechanism of action of flavonoids, as a rule, is antioxidant activity, and a number of quercetin effects appear to be due to its antioxidant activity.

Quercetin scavenges oxygen radicals (Saija *et al*., 1995), and inhibits xanthine oxidase and lipid peroxidation in vitro (Chang et al., 1993). Quercetin efficacy has been attributed largely to the antioxidant properties, which are known to augment GSH and antioxidant enzyme levels and scavenge lipid peroxides (Akhtar *et al*., 2010).

A concept is now emerging of "adaptogenic drugs"-drugs that increases non-specific resistance to variety of stresses (Oyedemi *et al.*, 2010). Chronic administration of quercetin resulted in an increase GSH level, SOD, GSH-Px & CAT activity (Bhattacharya *et al.*, 2000). This adaptogenic property may be contributing to its protective effect and strengthen the defense mechanisms of the organism. Quercetin demonstrates protective activity, may also be due to its direct free radical scavenging activity.

5.3. Reproductive Parameters

5.3.1. Growth aspects, Organs weights, Fertility aspects, and Semen aspects.

It is obvious from the results that the effect of o-anisidine on body weights was clear from the day 7 of treatment. Where the initial weights of all treated groups were approximately equal and without any significant differences; yet, in the o-anisidine treated group the weights began to regress significantly from day 7 till day 28 of the experiment as compared with all the other treated groups. When quercetin was used as

a mixture with o-anisidine it caused these declined body weights to elevate significantly but did not reach the values of control group exactly from day 14 and forth. On other hand, the use of quercetin alone ameliorated the body weights significantly as compared with other groups and it was also higher but not significantly than that of control group along the experiment period.

The organs weights were not in a safe shelter from the effect of o-anisidine, where it caused a significant increase in the weights of liver, kidney and spleen and caused a significant decrease in the testes absolute weights, testes relative weights, and epididymi relative weights as compared to all other treated groups. Quercetin ameliorating effect was brilliant whether it was used as a mixture with o-anisidine or alone, where it increased significantly the testes and epididymi weights and decreased the elevated liver, kidney, and spleen weights significantly and reached to the control levels.

Furthermore, o-anisidine treatment caused a significant decrease in the food intake comparing with other groups. When quercetin was used as admixture with o-anisidine, it ameliorated the food intake ability significantly but not to the control values, while quercetin alone increased significantly the food intake and also increased it evens more than the control group but not in a significant consideration.

The effects of o-anisidine and/or its metabolites on growth aspects, food intake, and organs weights in our study was in a high accordance with the results obtained by many searchers such as: Koerdel *et al.*, (1981) in their 28 days study on the effect of o-anisidine metabolite (o-nitrophenol) in rats, (Loprieno, 1983) in his 30 days study on the effect

of o-anisidine metabolite (o-aminophenol) in rats, (Stocker, 2002) in her elaborated study on o-anisidine and its effects in mice and rats, Uboh *et al.*, (2008) in their study on the effects of exposure to gasoline in rats, and the European Committee (EC, 2011) in its report on o-anisidine effects in rats.

The reduction in body weights of rats in our study can be correlated with the decreased food intake caused by o-anisidine treatment which is in turn might be caused by decreased palatability of the diet containing o-anisidine and this preponderant cause was also suggested by (Kate and Lucky, 2012) in their study on the effect of an azo dye in rats and by (Himri *et al.*, 2011) in their study on the effect of the azo dye (tartrazine) in Wistar rats. The increased liver, and kidney weights due to O-anisidine treatment can be explain in the frame which was mentioned by (Yamazaki *et al.*, 2005) in their two weeks toxicity study on the effect of dichloronitrobenzene in rats, where the increased liver, spleen, and kidney weights are correlated and might be due to the hyperlipidemia caused by nitrobenzene compounds which in turn impairs the suitable hepatic metabolism and catabolism of cholesterol and related lipoproteins leading to accumulation of lipids in liver and hence increase the liver weight, and also by the histological changes caused which included swelling, edema, hydrobic degeneration, aggregation of inflammatory cells, hyaline droplets accumulation in the proximal renal tubules and others which will be discussed in details later in the explanations of histopathological findings might also participate in elevation of the weights of these organs. Besides, the declined blood parameters which were discussed earlier in this chapter like hemolytic anemia might be the cause of increased spleen weights due to

haemeosiderosis and the increased splenic processes towards the disorders inflicted the blood cells (Gabriele, 1994). Spleen enlargement may be caused by a variety of diseases and, in some instances, is associated with increased workload (such as in hemolytic anemia) or hyper function in response to destruction of abnormal RBCs (Greer, 2003). The red pulp (also called splenic pulp), which may act as a reservoir for storing blood, is a soft mass of dark reddish-brown color resembling coagulated blood, and it is made of a fine reticulum of fibers divided into splenic sinuses and splenic cords. The splenic red pulp may undergo changes due to a variety of factors, including immune stimulation, changes in circulation, accumulation of macrophages, and connective tissue or pigment, and in response to increased demand for filtration of abnormal RBCs (Guyton and Hall, 2006). Furthermore, the decreased testicular weight is due to testicular atrophy caused by o-anisidine and/or its metabolites (Gabriele, 1994).

The bad effect of o-anisidine on sperm aspects and testes weight was very clear in our study and it was in accordance with the results obtained by Yamazaki *et al.*, (2005) in their study on the effect of nitrobenzene in rats and mice, Sayed *et al.*, (2012) in their study on effects of azo dyes on mice and rats, and Camargo and Morales (2013) in their review study about azo dyes. These effects of o-anisidine might be due to the direct effect of its metabolite nitrobenzene on the testicular tissues, where it induces testicular germ cells necrosis and atrophy, Yamazaki *et al.*, (2005); or due to its direct effect as azo dye, where the azo dyes can induce germ cells (spermatocytes) chromosomal alterations rapidly after administration which lead to decrease sperm count and abnormal sperms, Sayed *et al.*, (2012); or also as azo dye it causes cytotoxic and

genotoxic effects in the chromosome aberrations test, micronucleus test and mitotic index in bone marrow cells and spermatozoids of rats, Camargo and Morales (2013).

The ameliorating effect of quercetin in the aspects of sperms in our study came in accordance with the results obtained by Moretti *et al.*, (2012) in their study on quercetin and other flavonoids on lipid peroxidation of sperms in human. The ability of quercetin as antioxidant is well established by many searchers and it was found to prevent the lipid peroxidation greatly and this might be dependent as explanation for the ameliorating role of quercetin against o-anisidine on sperms, where the sperm membranes are rich with poly unsaturated fatty acids which render them to be an easy goal for the oxidative damage caused by o-anisidine and the antioxidant role of quercetin can prevent this damage. Besides, another mechanism is involved in the role of quercetin in increasing the sperm motility which is that the sperm has receptors for estrogen in the midpiece and the tail of sperm (ERs) and it is found that quercetin binds with affinity to these receptors and might act as agonist for estrogen which then will increase the motility of sperms, Moretti *et al*., (2012).

5.4. Histological parameters

5.4.1. Liver and kidney

The effects of o-anisidine were very obvious and deleterious on the livers and kidneys of the treated animals whether after 15 or 30 days of treatment. After 15 days of treatment with o-anisidine the hepatic changes were miscellaneous including infiltration of inflammatory cells, degeneration, hemorrhage vaculation of hepatic cells and edema. When

the treatment with o-anisidine continued for 30 days more hepatic changes were seen like very clear fatty degeneration and intra sinusoidal hemorrhage. The same was seen in renal tissues such as edema in renal tubules, hemorrhage, aggregation of inflammatory cells, closure of renal tubules, atrophy of glomeruli, vaculation and aggregation of messangial cells and cloudy degeneration. Beside, after 30 days of o-anisidine treatment presence of hyaline droplets were obvious in the corticomedullary tubules. These observations were in accordance with those of (Gabriele, 1994), (WHO, 2003), (EC, 2011) and (Abousalem *et al.*, 2012).

After quercetin had been used either for 15 days or for 30 days it clearly ameliorated the texture of kidney and liver which were annihilated by o-anisidine. For the kidney, presence of normal glomeruli and renal tubules began to be seen and the ameliorating effect was very astonishing in the liver where the hepatic texture was approximately as the same as that of the control group.

The previously noticed hepatic and renal changes which were caused by o-anisidine treatment can be explained pending upon the major route of metabolism of o-anisidine which is the liver and kidney and due to the metabolites which are the byproducts and end products of o-anisidine metabolism like nitrobenzene, o-aminophenol and N-(acetyl hydroxylamine), where the metabolism of these compounds results in formation of highly reactive nitroxide intermediates aromatic nitroso- and hydroxylamine compounds like NOB and PH which are associated with their very reactive free radicals like nitroanion and superoxide free radicals (Menon *et al.*, 2010). The previously proposed mechanism is

supported in our study by other parameters like the elevated MDA values of both periods of treatment with o-anisidine and the elevated values of antioxidant enzymes after 15 days of treatment then the declination of these enzymes after 30 days of treatment with o-anisidine. The presence of hyaline droplets in the corticomedullary tubules might suppose another mechanism to explain the renal and hepatic damages caused by o-anisidine treatment, where these hyaline droplets have a tendency to bind (α2υ-globulin) which is induced to be produced by many hydrocarbon compounds including the metabolites of anisidine and hence; the accumulation of these droplets which are impregnated with α2υ-globulin are responsible for nephropathy (Yamazaki *et al.*, 2005).

Quercetin as it was mentioned before belongs to a widely spread family of naturally occurring compounds known as flavonoids. Quercetin has an anti-oxidant efficacy equals to four times more than that of vitamin E (Sangai and Verma, 2012). Furthermore, quercetin is well known to ameliorate and treat many disorders and functional disturbances like; protection against gastric ulcer (Coşkun *et al*., 2004), rehabilitation of antioxidant enzymes (Regulska−Ilow and Ilow, 2008), improving the hyperglycemia, dyslipidemia and antioxidant status (Jeong *et al*., 2012), ameliorating inflammation and fibrosis (Marcolin *et al.*, 2012), as a chemotherapeutic agent in treatment of breast cancer (Reddy, *et al.*, 2012) and ameliorating lipid and glucose metabolism (Seiva *et al*., 2012).

These ameliorating effects of quercetin and its role in rehabilitating the renal and hepatic tissues might be due to its ability to scavenge the

radicals of arylamines compounds like carbonyl radicals because of its content of phenolic groups (Cheng *et al.*, 2007), or due to its direct effect on rejuvenating the antioxidant enzymes via its effect on NF-kB activation which is related with enzymes expression and its role against the genotoxic agents (Edremitlioğlu *et al*., 2012). Other mechanism by which quercetin plays a major role in regulating the apoptosis of cells when quercetin acts on subcellular level, in other words; quercetin induce Bcl-2 gene expression leading to over production of Bcl-2 related proteins which lead to regulation of cellular apoptosis and hence rehabilitating the damaged tissues. Another mechanism by which quercetin can improve the histological damage which is that the increased levels of oxidative stress will increase the monocytes and macrophages counts and then the levels of nitric oxide (NO) produced by macrophages -(monocytosis and increased tissue macrophages were noticed in our study)- as macrophages contain nitric oxide synthase which is induced by oxidative stress and then NO will react with free radicals to yield the more damaging peroxynitrite which is highly destructive to cell membranes and can directly oxidize the LDL. Quercetin might directly interfere and inhibits the nitric oxide synthase and here it will prevent the formation of the tissue damaging peroxynitrite (Nijveldt *et al*., 2001). Besides, quercetin participates in prevention of subcellular structural alterations including distribution of mitochondrial fine structures (Arash, 2010).

Conclusions

Some conclusions can be summarized from our current study as follows:

1. O-anisidine has a bad effect on blood parameters like the red cells, white blood cells, platelets and hemoglobin.
2. O-anisidine is a causative agent of hyperlipidemia.
3. O-anisidine has a retrogressive effect on reproductive aspects of males and females rats.
4. O-anisidine causes different degrees of tissue necrosis and damage.
5. Quercetin has very potent antioxidant ability and was able to ameliorate all the deleterious effects of o-anisidine on different parameters.

Recommendations

Based on the results of the current study, we suggest some recommendations which may be important to be taken into consideration in future studies:

1. Study the effect of o-anisidine in the populations who dwelt by the rivers sides close to the packaging branches of clothes, dyes and medicaments factories.
2. The effect of quercetin on other o-anisidine- destructed tissues of animals.
3. Study the effects of the other isomers of anisidine; m-anisidine and p-anisidine in human and animals.
4. Study the effects of o-anisidine in term of different doses.
5. We can recommend quercetin to be used as antioxidant, fertility booster and anti-hyperlipidemia drug or food supplement.

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♦ *Appendix No. 1*

Components of experimental diets (gm / kg diet)

Ingredients / **groups**	**Control**	**T1 0.7 % Anisidine**	**T2 0.005 % Que. + 0.7 % Anisidine**	**T6 (2nd experiment) 0.005 % Que.**
Casein	200	200	200	200
Corn starch	650	643	642.4	649.4
Vitamins and minerals mix.	50	50	50	50
Corn oil	50	50	50	50
Cellulose	50	50	50	50
O-Anisidine	0	7	7	0
Quercetin	0	0	50mg	50mg

♦ *Appendix No. 2*

Chemicals

Chemical	Producing company	Country
Ortho-Anisidine hydrochloride	BDH	England
Quercetin dihydrate	Himedia Labs	India
Methanol alcohol	BDH	England
Glutathione Peroxidase kit	Fluka	Germany
Superoxide Dismutase kit	Fluka	Germany
Malondialdehyde kit	Qualikemes	India
Alkaline phosphatase kit	Langdorp-Cypress Diagnostics	Belgium
Catalase kit	GCC	England
Tween 20 (Polysorbate)	SCRC	China
WBC Lyse	Abbott Diagnostics	Wiesbaden-Germany
HGB Lyse	Abbott Diagnostics	Wiesbaden-Germany
Diluent	Abbott Diagnostics	Wiesbaden-Germany
Mercuric oxide	BDH	England
Chloroform	BDH	England
$Alk(So_4)_2.12H_2O$	LRG	England
Ethanol alcohol	Merck	Germany
Glacial acetic acid	Merck	Germany
Eosin stain	Merck	Germany
Paraffin wax	Merck	Germany
Xylole	Scharlau	Spain
Negrosin	Merk	Germany

♦ *Appendix No. 3*

Instruments

Instrument	Trade mark	Manufacture
Incubator	Galen Kamp	England
Oven	Galen Kamp	England
Rotary Microtome	Anglia	England
Sensitive balance AE 2000	Sartouris	Germany
Centrifuge	Hettich — EBA20	Germany
Water bath	STUART	UK
Light microscope	Olympus	Japan
Goblin Jar	Olympus	Iraq
Hot plate	Lassco — India	India
Spectrophotometer	APEL – PD 303 UV	Japan
Hematology analyzer	CELL-DYN-Ruby	Wiesbaden-Germany

ألخـلاصــة

أجريت هذه ألدراسة في كلية ألطب ألبيطري – جامعة ألبصرة لمعرفة تأثير الكويرستين في إزالة التأثير السمي الناتج من التعرض للأورثوأنيسيدين في بعض المعايير الفسيولوجية، التغيرات النسيجية، و الكفاءة التناسلية للجرذان المختبرية.

قسمت التجربة إلى جزئين. الجزء الأول يتعلق بالمعايير الفسلجية والتغيرات النسيجية. في حين إن الجزء الثاني يتعلق بالكفاءة التناسلية.

في الجزء الاول تم استخدام 48 ذكرا من الجرذان. تم تقسيم تلك الجرذان الى ثلاث مجاميع بواقع 16 جرذ لكل مجموعة. المجموعة الاولى مثلت مجموعة السيطرة وتم تغذية الحيوانات فيها على عليقة قياسية لمدة 15 يوما ومن ثم تم قتل 8 جرذان منها لاجراء الفحوصات اللازمة وال8 جرذان المتبقية تم الاستمرار بتغذيتها على نفس العليقة لمدة 15 يوما اضافية ومن ثم تم قتلها لاجراء الفحوصات اللازمة.

مجموعة المعاملة الاولى (T1) تم تغذية الحيوانات فيها على عليقة تحتوي اورثو انيسيدين هيدروكلورايد بتركيز 7 غم\كغم . مجموعة المعاملة الثانية(T2) تم تغذية الحيوانات فيها على عليقة تحتوي على اورثو انيسيدين هيدروكلورايد 7 غم\كغم + كويرستين داي هيدريت 50ملغم\كغم. وبعد فترة 15 يوما تم قتل 8 جرذان من كل مجموعة لاجراء الفحوصات المختبرية المطلوبة والمتبقي ال8 جرذان من كل مجموعة تم قتلها بعد 15 يوما اضافية.

الجزء الثاني من التجربة تضمن 16 ذكرا و32 انثى من الجرذان وقسمت عشوائيا الى 8 مجاميع بواقع ذكرين واربع اناث لكل مجموعة وتم تغذيتها على علائق مختلف محتوية على الانيسيدين او على الكويرستين او على مزيج منهما.

تركت الذكور والاناث لتتزاوج وبعد 16 يوما فصلت الذكور عن الاناث لتركها تلد. تم فحص معايير النطف للذكور ومعايير الخصوبة للاناث.

شملت الفحوصات الدمية كل من عدد كريات الدم الحمراء والهيموغلوبين، حجم الخلايا المرصوص، عدد الكريات البيضاء الكلي، عدد الكريات البيضاء التفريقي وألأسس الحسابية للدم مثل تركيز الهيموغلوبين في الكريات والتوزيع العشوائي للكريات وحجم الصفيحات الدموية. وشملت الفحوصات الكيمياوية الكولستيرول الكلي في المصل، الكولستيرول عالي الكثافة، الكولستيرول منخفض الكثافة والكليسيريدات الثلاثية بالاضافة الى انزيمات المضادة للاكسدة وانزيمات وظائف الكبد. وتم كذلك اجراء الفحوصات النسيجية المرضية للكبد والكلية.

أظهرت النتائج انخفاضا معنويا ($P\leq0.05$) في عدد كريات الدم الحمر ، تركيز الهيموغلوبين ، قيمة حجم الخلايا المرصوص ، عدد الخلايا العدلة وانخفاضا في الحركة الجماعية والفردية لنطف الذكور المعاملة بالاورثو انيسيدين، كما انخفض معدل الخصوبة للإناث المعاملة ووزن صغار الجرذان بالاضافة الى انخفاض في الكولستيرول عالي الكثافة. بينما كان هنالك زيادة معنوية ($P\leq0.05$) في عدد كريات الدم البيض ،الخلايا وحيدة النواة ،الخلايا الحمضة، الكولسترول الكلي في المصل، الكليسيريدات الثلاثية، الكولستيرول منخفض الكثافة، انزيمات وظائف الكبد ALTو AST ، الانزيمات مضادة الاكسدة CAT, SOD, GPX, MDA, ALP والنطف المشوهة والميتة للذكور المعاملة.

بالإضافة إلى ذلك، عندما استخدم الكويرستين مع عليقة الانيسيدين سواء لمدة 15 يوما او لمدة 30 يوما، لوحظ زيادة في عدد كريات الدم الحمر وتركيز الهيموغلوبين وقيمة حجم الخلايا المرصوص، ولكنها بقت منخفضة معنويا مقارنة مع مجموعة السيطرة عند مستوى احتمال ($P\leq0.05$). وفيما يخص المعايير الكيمياوية فقد لوحظ ان الكويرستين ادى الى انخفاض معنوي في الكولستيرول الكلي، الكلسيريدات الثلاثية، الكولستيرول منخفض الكثافة، وانخفاض مستوى انزيمات وظائف الكبد AST و ALT في حين سبب انخفاض مستوى الانزيمات مضادة الاكسدة في الفترة الاولى من المعاملة ومن ثم ارتفاع في مستوى تلك الانزيمات.

لوحظ زيادة في الحركة الجماعية والفردية للنطف وفعالية النطف للذكور المعاملة ولكن لايزال اقل بصورة معنوية من مجموعة السيطرة عند مستوى احتمال ($P\leq0.05$). وعندما

استخدم الكويرستين على حده في العليقة حسن وبصورة معنوية جميع المعايير التكاثرية وبصورة اكثر حتى من مجموعة السيطرة.

لوحظت زيادة معنوية في عدد كريات الدم البيض الكلي والخلايا اللمفية والخلايا العدلة وانخفاض معنوي في خلايا الحمضة والقعدة. لوحظ انخفاض النطف المشوهة والميتة للذكور المعاملة ولكن لاتزال أعلى بصورة معنوية من مجموعة السيطرة عند مستوى احتمال ($P \leq 0.05$) .

الفحص النسيجي اظهر بان الانيسيدين سبب درجات مختلفة من النخر، الانتفاخ، التفجي، تجمع الخلايا الالتهابية ونزف وتلف دهني في جميع أكباد وكلى الجرذان المعاملة، وان المعاملة بالكويرستين أظهرت علامات مختلفة من الإصلاح وتسبب في تقليل تأثير الأنيسيدين في الأنسجة بصورة واضحة جدا.

نستنتج من هذه الدراسة أن ألأورثو أنيسيدين ذو تأثير سلبي جدا على أجهزة الجسم كافة وكونه سببا رئيسيا في حدوث فقر الدم، أرتفاع مستوى الدهون في الدم، تلف الانسجة والعقم. من جانب اخر أظهر الكويرستين قدرة عالية بكونه مانع أكسدة وأسهم بشكل واضح في منع او تقليل تأثيرات ألأورثوأنيسيدين كافة.

دور الكويرستين في أزالة التسمم الناتج من التعرض للأنيسيدين في بعض المعايير الفسلجية، الكيموحيوية والنسيجية المرضية للجرذان المختبرية (*Rattus norvegicus*).

أطروحة مقدمة إلى مجلس كلية الطب البيطري – جامعة البصرة وهي جزء من متطلبات نيل درجة دكتوراه فلسفة في علم الفسلجة

من قبل

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بكالوريوس طب وجراحة بيطرية عامة، ماجستير في علم الفسلجة

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