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University of Thi-Qar, Iraq
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Protective Role of Alkaloid Compound Isolated from *Hapalosiphon aureus* against Paracetamol Induced Hepatotoxicity in Male Rats

Khalid G. Al-Fartosi
Department of Biology, College of Science, University of Thi-Qar, Iraq
khalidalfartosi@yahoo.com

Auhood Kadhim Zaid
Department of Chemistry, College of Science, University of Thi-Qar, Iraq

Alyaa Majid
Department of Chemistry, College of Science, University of Thi-Qar, Iraq

Mohammed A. Auda
Department of Chemistry, College of Science, University of Thi-Qar, Iraq

Abstract **– The present study investigated the effect of alkaloid compound isolated from Hapalosiphon aureus against paracetamol induced toxicity in male rats. Twenty four white male rats were used, these animals were divided into four groups each group contain six animals as a following: the first group I is the control group treated with(1 ml/kg) of normal saline for two weeks, the second group (II) treated with (1 ml/kg) of alkaloid compound isolated from Hapalosiphon aureus for two week, the third group III treated (I.P.) with paracetamol (1 ml/ kg) at the first day and fourth day of the first week , the fourth group IV treated (I.P.) with paracetamol (1 ml/ kg) at the first day and the fourth day of the first week , and then treated orally with Hapalosiphon aureus for one week of the fourth group . The result indicate that the paracetamol caused a significant increased ($p < 0.05$) in the level of the serum alanin transferase (ALT), aspartate transferase (AST), cholesterol, and triglyceride levels in the third group comparison with control group. Also, there was a significant decreased in albumin. Protective activity of alkaloid compound isolated from Hapalosiphon aureus against toxicity of paracetamol observed in decreasing of ALT, AST, cholesterol, and triglyceride levels, and increased in the albumin levels in the fourth group comparison with third group.**

Keywords **– Hapalosiphon Aureus, Hepatotoxicity, Paracetamol, Rat.**

I. INTRODUCTION

Acetaminophen (Paracetamol) is used worldwide for its analgesic and antipyretic properties. It is widely available and present in many prescription and non prescription medications. Unfortunately, however, acetaminophen toxicity remains the most common cause of drug-induced hepatic failure. Repeated supratherapeutic misuse, non-intentional misuse, and intentional ingestion may all result in hepatic toxicity.

The mechanism of acetaminophen toxicity has been well studied. Following ingestion a majority (>90%) of acetaminophen undergoes phase II metabolism (via glucuronidation and sulfation) to produce non-toxic metabolites. A small fraction (<5 10%) of acetaminophen is metabolized by CYP450 isoforms (predominately CYP2E1) to N-acetylp- benzoquinoneimine (NAPQI), a toxic metabolite. Under normal conditions NAPQI is detoxified through conjugation with glutathione. With acetaminophen toxicity, cellular glutathione is depleted resulting in the availability of NAPQI to bind to cellular macromoleclues, the consequences of which are hepatocellular injury and cell death. Hepatic toxicity is generally thought to occur when glutathione stores are depleted to less than 30% of normal(1). Children may be less susceptible to acetaminophen toxicity(2)(3). consequent to a developmentally associated increase in sulfation ability (4).

Cyanobacteria are a very old group of organisms and represent relics of the oldest photoautotrophic vegetation in the world that occur in freshwater, marine and terrestrial habitats (5). Cyanobacteria have drawn much attention as prospective and rich sources of biologically active constituents and have been identified as one of the most promising groups of organisms to be able of producing bioactive compounds (6). Cyanobacteria are known to produce metabolites with diverse biological activities such as antibacterial (7), antifungal (8), antiviral (9), anticancer (10), antiplasmodial, (11), algicide (12), antiplatelet aggregation (13) and immunosuppressive (14) activities. Screening of cyanobacteria for antibiotics and other pharmacologically active compounds, has received ever-increasing interest as a potential source for new drugs (6). Cyanobacteria from local habitats seem to be a source of potential new active substances that could contribute to reduction of the number of bacteria, fungi, viruses and other microorganisms (14).

II. MATERIALS AND METHODS

A. Prepare Abstract Alkaloid

Used the method of the Samurai (1983)(15) to prepare the extract, which includes a process of escalation Torgiei Reflux for 24 hours and using ethanol acidified acetic acid as a solvent and then nominated and Concentrated filtrate by Rotary Vacuum Evaporator degree 50 m then acid with sulfuric acid and then adjusted to pH 9 by adding ammonia concentrated and then transferred to a separating funnel Added him and the same volume of chloroform and isolated organic layer, the process repeated 5 times at room temperature

B. Isolating the compound Alkaloid using column chromatography

Method is used Harborne (1984) (16) for this purpose and the use of solvent consisting of (Ethyl acetate, formic acid, water) and by (1:1:8) and respectively and then tested the samples by the technique of chromatography thin layer TLC and collected samples of flow rate relative of Like together in a dish glass and left at room temperature have a person of this compound by (17).

C. Experimental animals

Twenty four white male rats weighing (130-150 g), with (5-6 weeks) of old were used in this study. All animals were obtained from animal house of biology department / college of science / university of Thi-Qar / Iraq. The rats were divided randomly into four groups of six rats.

Group I : the control group , received orally a daily dose of(1 ml /kg) normal saline for two weeks

Group II: was given orally a daily dose of (1ml/kg) alkaloid compound isolated from Hapalosiphon aureus for two weeks

Group III: was given two doses of paracetamol intraperitoneally (1ml/kg), first dose at the 1st day of experimental and the second dose at the 4th day of the first week.

Group IV: was given two doses of paracetamol intraperitoneally (1 ml /kg), first dose at the 1st day of experimental and the second dose at the 4th day of the first week (1 ml/kg) of alkaloid compound isolated from Hapalosiphon aureus daily oral intake of the second week

D. Biochemical parameters

At the end of the experiment, the overnight fasted animals (the control and experimental animals) were sacrificed under light ether anesthesia. Blood samples were collected by cardiac puncture, 5 ml of blood samples were collected from heart and put tubes without EDTA and centrifugation at3000x for 15 minutes for obtained serum. The biochemical parameters included serum alanin transferase (ALT), serum aspartate transferase (AST), cholesterol, triglyceride and albumin.

III. Results

Table (1) showed the results of the effect of alkaloid compound isolated from Hapalosiphon aureus against some biochemical parameters of male rats treated with paracetamol. The results indicated a significant increase in concentration of serum ALT and AST in group(III) in comparison with control group group(I) ($p<0.05$).In the same table there is no significant differences in concentration of serum ALT and AST in group (II) in comparison with group(I)($p<0.05$).Whereas, there is a significant differences can be observed in group(IV) as compared to control group(I), also there is a significant decrease in concentration of serum ALT and AST in group (IV) in comparison with group(III) and there is a significant differences in concentration of serum ALT and AST in group(IV) in comparison with group (II)($p<0.05$).

The results showed a significant increase in concentration of serum cholesterol and triglyceride in group(III) in comparison with group(I) ($p<0.05$). While no significant differences can be observed between each one of groups (I,II and IV) after having been treated with (1mL/Kg B.W) of Hapalosiphon aureus for two week .The levels of serum cholesterol and triglyceride decreased significantly in (II and IV) groups after they were treated with (1mL/Kg B.W) of alkaloid compound isolated from Hapalosiphon aureus for two week compared to group(III).

Albumin was decreased significantly ($p<0.05$) in group(III) in comparison with group(I) On the other hand , a significant increase in concentration of serum albumin in group (IV) in comparison with group(III) .Also there is no a significant differences in concentration of serum albumin in group (II) in comparison with group (I).

IV. Discussion

Over dosage of paracetamol (PCM) led to the saturation of conjugation pathway leading to glutathione depletion and increase in the formation of toxic reactive metabolites. High level of reactive metabolites increase the level of hepatotoxicity with increase level of protein adducts formation, mitochondrial dysfunction and oxidative stress. Assessment of liver toxicity is done by measuring the marker enzymes such as ALT and AST which are originally present in high concentration in the cytoplasm. When there is hepatic injury these enzymes leak into blood stream inconformity with extend of hepatotoxicity. Elevated levels of serum enzymes are indicative of cellular leakage and loss of functional integrity of cell membrane in liver (18), but the elevated levels of enzymes are decreased to near normal levels after seven days treatment of alkaloid compound isolated from Hapalosiphon aureus indicates that it offered protection by preserving the structural integrity of the hepatocellular membrane against paracetamol. Elevation of triglycerides level during paracetamol intoxication could be due to increased availability of free fatty acids, decreased hepatic release of lipoprotein and increased desertification of free fatty acids. Administration of Hapalosiphon aureus significantly decreased serum lipid profile in paracetamol toxicity induced rats because of its hypolipidemic effects. Hapalosiphon aureus supplementation enhanced desertification effect through hepatoprotective property by inhibiting the free radicals effect on liver cells. Albumin is decreased in chronic liver disease and is generally accompanied by an increase in the β and γ globulins as a result of production of IgG and IgM (6). Hypoproteinemia was observed after paracetamol ingestion but the trend turns towards normal after Hapalosiphon aureus treatment. The protective effect of Hapalosiphon aureus could be attributed to its antioxidant activity and it may possibly have chelating effects on aluminum. It has been reported that Hapalosiphon aureus contains high levels of vitamins A, B2, C and E and is very rich in magnesium (Mg) and other trace elements (19). These vitamins are antioxidants that have been found to be useful in preventing tissue injury caused by toxic agents. Mg protects cells from heavy metals such as aluminum, mercury, lead, cadmium, beryllium and nickel, which explains why re-mineralization is so essential for heavy metal detoxification and chelating. In fact, Mg deficiency has been associated with production of ROS(20). Additionally, Mg protects cells against oxyradical damage and assists in the absorption and metabolism of vitamins B, C and E (21). Which are antioxidants important in cellular protection. Recent evidence suggests that vitamin E enhances glutathione levels and may play a protective

role in Mg deficiency induced cardiac lesions (21). Magnesium protects the cell against oxy radical damage and assists in the absorption and metabolism of B vitamins, vitamin C and E (21), which are antioxidants important in cell protection. Recent evidence suggests that vitamin E enhances glutathione levels and may play a protective role in magnesium deficiency-induced cardiac lesions (21). Also, it has been reported that magnesium is very essential for biosynthesis of glutathione, because the enzyme Glutathione synthetase requires γ-glutamyl cysteine, glycine, ATP and magnesium ions to form glutathione(22). Vitamin C is a strong antioxidant (12). The detoxification effect of vitamin C is manifested by the removal or minimization of free radicals produced by mercury (23). Also, vitamin C protects DNA from oxidative damage (24), reduces DNA damage exerted by irradiation (25) and also reduces micronucleus (MN) frequencies in polychromatic erythrocytes of bone marrow in rodents exposed to heavy metals and radiation (26, 27). Furthermore, Hapalosiphon aureus exhibits a range of biological activities. These biological activities are mainly due to peptides and protein in Hapalosiphon aureus . Bioactive peptides are produced during the digestion of Hapalosiphon aureus in the gastrointestinal tract (28). The beneficial health effects of Hapalosiphon aureus proteins can be classified as antimicrobial, antioxidative, antithrombotic, antihypertensive or immuno-modulator (19).

V. Conclusion

Our results demonstrate that paracetamol is capable of inducing marked alterations in biochemical parameters, and inhibiting the function of antioxidant enzymes. Hapalosiphon aureus, administered after paracetamol exposure, minimized paracetamol-associated hazards. Therefore, giving alkaloid compound isolated from Hapalosiphon aureus could be beneficial for alleviating paracetamol toxicity. Further studies are required, using a human population, to confirm theses protective effects.

Table 1: Levels of ALT, AST, Cholesterol, Triglyceride and Albumin in serum of control and experimental male rats.

Biochemical Parameters (MEAN ± SD)						
Groups	n	ALT(U/L)	AST(U/L)	Cholesterol(mg/dL)	Triglyceride (mg/dL)	Albumin (g/L)
Group(I)	6	137.88±3.17^{c}	147.52±4.24^{c}	58.13±6.23^{b}	36.92±1.92^{b}	46.91±0.74^{a}
Group(II)	6	138.19±3.36^{c}	151.29±7.85^{c}	56.28±6.95^{b}	37.65±3.39^{b}	46.56±0.97^{a}
Group(III)	6	156.00±5.53^{a}	172.82±7.85^{a}	71.64±8.33^{a}	53.22±3.51^{a}	37.46±2.93^{c}
Group(IV)	6	148.83±5.83^{b}	164.35±2.75^{b}	61.09±5.86^{b}	40.85±6.22^{b}	43.59±2.38^{b}

- Each value represents mean ±SD values with non identical superscript (a , b or c …etc) were considered significantly differences (P <0.05).
- Group I: was control group , received orally a daily dose of(1 ml /kg) normal saline for fourteen days
- Group II : was given orally a daily dose of (1ml/kg) alkaloid compound isolated from Hapalosiphon aureus for two week.
- Group III : was given two doses of paracetamol intraperitoneally (1ml/kg), first dose at the 1st day of experimental and the second dose at the 4th day of experimental for one week.
- Group IV: was given two doses of paracetamol intraperitoneally (1 ml /kg), first dose at the 1st day of experimental and the second dose at the 4th day of experimental for one week(1 ml/kg) of alkaloid compound isolated from Hapalosiphon aureus daily oral intake for one week .

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