International Journal of Multidisciplinary Sciences and Advanced Technology
Journal home page: http://www.ijmsat.com

Histological and physiological changes in kidney tissues induced by ethanoic acid in albino rats

Eda M. A. Alshailabi*[1], Salma F. Majeed[1] and Ahmed S. H. Ahmeedah[2]

1- Zoology Department, Omar Al-Moukhtar University, El Bayda, Libya.

2- Zoology Department, Tobruk University, Tobruk, Libya.

E-Mail: qtuby2014@gmail.com

Received 15February 2021, Accepted 10March 2021, Available online 30 March 2021

Original article

ABSTRACT

Ethanoic acid (EA) is a widely used organic acid with corrosive properties. A small amount of its concentration may cause serious poisoning with a fatal outcome. This study aimed to investigate the histological and physiological changes on kidney tissues induced by EA in female albino rats. Thirty female rats were used, they were divided into three groups, one group serves as control group and the other groups were administered the EA as follows: Treated group (1); animals received EA (1 ml/ kg/ day) with concentration of 5 % and treated group (2); animals received EA (1 ml/ kg/ day) with concentration of 10 % orally by gavage for fourteen days. The mean values of creatinine observed in treated animals do not differ significantly from the corresponding values observed in controls. Whereas, the results showed a significant increase in urea levels in rats that received EA-10% as compared to EA-5 % rats and control rats. Histological observations in kidney tissues showed degeneration of renal glomerulus with widening of Bowman's space, hyalinization of glomerular tuft and tubular basement membrane degeneration in glomerulus, indicating dilated tubule in EA-5% rats. Moreover, the EA-10% groups showed degenerative lesions in glomerulus with lymphocytic infiltration cells, severe dilatation and vascular congestion with clogging of RBCs. In conclusion, ethanoic acid consumption have adverse effects on renal function in rats, hence quantity of ethanoic acid should be discouraged or reduced in making of meals.

Key words: Histological, Physiological, Kidney, Ethanoic Acid, Rat.

1. Introduction

Kidney as the excretory and filtration unit of the body is highly exposed to the heavy free radical load and therefore may lead to various anomalies in the normal functioning of its own physiology [1]. Moreover, the cell membrane which is normally selectively permeable, now becomes permeable to any molecule, because of the free radicals leading to numerous oxidative cellular damage of the biological membrane. Such injury to cell membrane changes the functions of the cells and ultimately affects the internal cellular environment and finally leading to cellular death [2]. Furthermore, the chain of free radicals generated from cells weakens/down regulates the antioxidative enzyme system thus making them unable to scavenge these radicals. Glutathione reduced (GSH) which is primary antioxidant system plays a crucial role in the defense of cells from reactive free radicals and other oxidants species. Further, superoxide dismutase (SOD) and catalase (CAT) are powerful antioxidant molecules which always remain active during their reduced state in order to be compatible to scavenge free radical [3, 4]. Various literatures support heavy free radical production and physiological stress as a major causative agent of ethanoic acid. Such physiological stresses during EA condition may impair the renal functions due to hyperproduction of urea, uric acid and creatinine in blood serum which constitutes the basic parameters for renal function analysis [5]. On the other hand, a progressive concentration of toxicants along the nephron may result in intraluminal precipitation of relatively insoluble compounds, causing acute renal failure

secondary to tubular obstruction. Finally, renal transport, accumulation and metabolism of chemicals and drugs contribute significantly to the susceptibility of the kidney to toxic injury [6]. Ethanoic acid (EA) is a widely used organic acid with corrosive properties that depend on its concentration. At concentrations between 2 % and 3 % it is mainly used as an antifungal or antibacterial agent; between 5 % and 8 % it is mainly found in vinegar and is safe for consumption as a vegetable preservative or condiment; and between 30 % and 90 % it is used as an antiseptic or a household cleaning agent. In some countries 80 % EA is used in the preparation of pickled food [7]. Moreover, ethanoic acid is a commonly used seasoning. Foods such as sushi and marinated meats and vegetables that are prepared with EA contain 0.2-1.5 ml of ethanoic acid/ 100 g. Besides, ethanoic acid is also used traditionally as a folk medicine and is believed to have several effects such as improving appetite [8]. Furthermore, EA caused acute and chronic poisoning of hemolysis, acute renal failure, acute liver failure and coagulopathy in human [7]. They found chronic ingestion of large amount of 5 % EA caused hypokalemia, hyperreninemia, osteoporosis and acute poisoning of hepatocellular carcinoma after oral ingestion of EA.

2. Material and methods:

Materials:

Experimental animals :

The present study was conducted using healthy female albino rats (*Rattus norvegicus)* with an average weight of 180-225 g. Animals were obtained from the Zoology Department, Faculty of Science, University of Omar Al-Mokhtar, El-Beida, Libya. All animals were allowed two weeks pre-experimentation period to acclimatize to laboratory conditions in order to avoid any complications along the course of the experiment. They were housed in cages at room temperature. Rats were fed with laboratory diet and water *ad libitum* with fresh daily supplies. All rats were weighted weekly and the weights were recorded before the experimental procedures and at the end of the experiment.

Experimental chemicals:

Ethanoic acid (CH_3COOH) (99.8 %, Sigma-Aldrich). Animals were given EA orally by gavage at a dose of 1 ml/ kg/ b. w./ day for 14 days. EA was given to animals in this study at two concentrations (5 % EA and 10 % EA).

Methods:

Experimental design:

In the present study a total number of 30 female albino rats were used. All rats were randomized into three groups 10 rats in each:

1- Normal control group (NC): Rats were given orally distilled water for 14 days.

2- Treated group 1 (TG1): Rats were given orally EA (5 %) according to [9] by gavage at a dose of 1 ml/ kg/ b. w./ day for 14 days.

3- Treated group 2 (TG2): Rats were given orally EA (10 %) according to [10] by gavage at a dose of 1 ml/ kg/ b. w./ day for 14 days.

After the completion of treatment period, all rats were fasted for 24 hours and animals were sacrificed and blood samples were collected from cutting the jugular vein for kidney functions tests then the kidneys were removed.

Determination of the kidney function tests:

Blood samples were collected and left to clot, then centrifuged at 3000 rpm for 10 minutes and stored at -80 ºC until biochemical analysis. Sera were used for the determination of biochemical analysis such as urea and creatinine, to determine the kidney function tests of the control groups and the experimental groups by using spectrophotometer method according to [11] in Al-Razi Laboratory for Medical Analysis, El-Beida City.

Histological examinations:

Small pieces of kidney were taken and washed in normal saline, dried and placed in 10 % buffered formalin for histological and histochemical examinations [12]. Dehydration of fixed tissues was carried out using ascending grades of ethyl alcohol (70 %, 90 % and 100 %), then cleared with xylene. Infiltration with paraffin wax at 60 ºC was followed by embedding. Paraffin blocks were cut at 5 microns from all specimens, using a Cambridge Rocking Microtome, and affixed to slides and stained with Haematoxylin and Eosin for general histological examination [13].

Statistical analysis:

The data of results obtained were subjected to statistical analysis and expressed as mean ± SE. The data were statically analyzed by one way analysis of variance ANOVA. Means were separated using Tukey's test at $P < 0.05$. The student's T test also used for comparitions between two means. All statistical procedures were performed with the Minitab statistical analysis package program (Minitab version 17).

3. Results:

Determination of the kidney function tests:

The data on measuring serum urea and creatinine levels, are tabulated in table (1). A significant increase ($P < 0.05$) found in urea levels in rats that received EA-10 % (43.14 ± 3.60) as compared to EA-5 % (29.57 ± 2.57) and control rats (25.43 ± 1.13). Although, no significant changes found between EA-5 % group and control group. Whereas, rats that reserved EA at 10 % showed, a significant increase ($P < 0.05$) in the mean value of creatinine (1.043 ± 0.092) as compared with EA-5 % rats (0.786 ± 0.026). Moreover, no remarkable changes found in creatinine levels between treated rats with EA and controls.

Table 1: Averages of mean values of urea and creatinine levels in control and experimental groups.

Parameter	Control (NC)	EA-5% (TG1)	EA-10% (TG2)
Urea (mg/dl)	٢٥,٤٣±1.13^{b}	٢٩,٥٧±2.57^{b}	٤٣,١٤±3.60^{a}
Creatinine (mg/dl)	٠,٨٤٣±0.03^{a,b}	٠,٧٨٦±0.026^{b}	١,٠٤٣±0.092^{a}

Data are expressed as mean ± SE of rat within each row, means with different superscript (a & b) were significantly different at $P < 0.05$, were means superscripts with the same letters mean that there is no significant difference ($P < 0.05$).

*EA-5% = Ethanoic acid (5 %). * EA-10% = Ethanoic acid (١٠ %).

* NC = Normal control. TG1= Treated group 1. TG2= Treated group 2.

Histological preparations:

Microscopically, control kidney section showed normal histological structure of glomeruli and renal tubules, the glomerular basement membrane was thin and delicate, mesangial cellularity and matrix were within normal limits with no evidence of shrinkage or swelling. The tubules which were lined by cuboidal epithelial cells had a normal luminous appearance (Figs. 1 and 2). The kidney of female rats treated with 5 % of EA showed hyalinization of glomerular tuft and tubular basement membrane degeneration in glomerulus indicating dilated tubule and peritubular dilatation with architectural loss of tubules, severe hemorrhage was observed in glomerulus, desquamated epithelial cells and hyaline casts, visceral epithelial sheet with intratubular hemorrhage (Fig. 3). Also, noticed vacuolated cytoplasm, necrotic changes with pyknotic nuclei were noticed (Fig. 4). Moreover, injury with desquamated epithelial cells hydropic swelling, pale cytoplasm, hyalinization and degeneration of the renal tubules and necrotic areas were observed (Fig. 5). Treated rats with EA-10 % showed atrophied glomerulus with collapsed tuft, wide Bowman's space, degenerative lesions in renal tubules, marked dilatation and congestion of blood vessels, vacuolated tubules with degenerated epithelial cells, necrotic changes in tubules with pyknotic nuclei (Fig. 6). On the other hand, degenerative lesions in glomerulus with lymphocytic infiltration cells, wide Bowman's space, degeneration in renal tubules, and necrotic areas were showed in figure (7). Moreover, severe dilatation and vascular congestion with clogging of RBCs and hyalinization of renal tubules (Fig. 8).

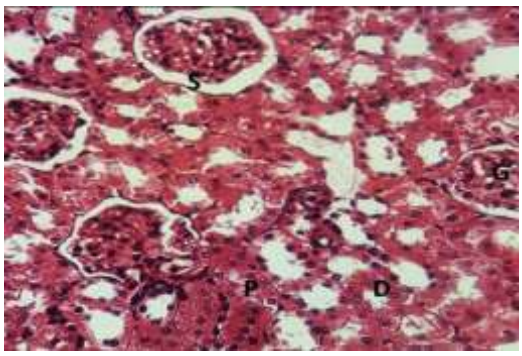

Figure 1: Photomicrograph of the kidney section of control female rats showing, normal histological structure of tubules (Proximal convoluted tubules (P) and distal convoluted tubules (D)), Bowman's space (S) and glomeruli (G) (H & E stain, X400).

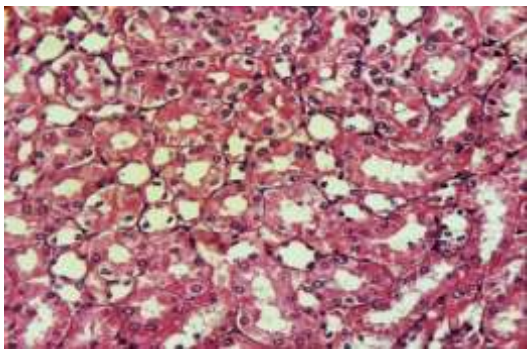

Figure 2: Photomicrograph of the kidney section of control female rats showed normal histological structure of tubules (H & E stain, X400).

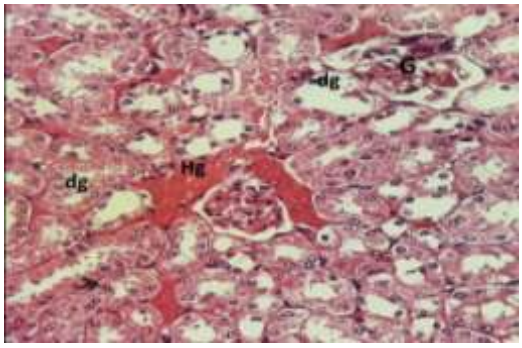

Figure 3: Photomicrograph of the kidney section of female rats treated with ethanoic acid 5 % showing, hyalinization of glomerular (G), tuft and tubular basement membrane, degeneration (dg) and dilated tubules, desquamated epithelial cells, visceral epithelial sheet with interatubular hemorrhage (Hg) (H & E stain, X400).

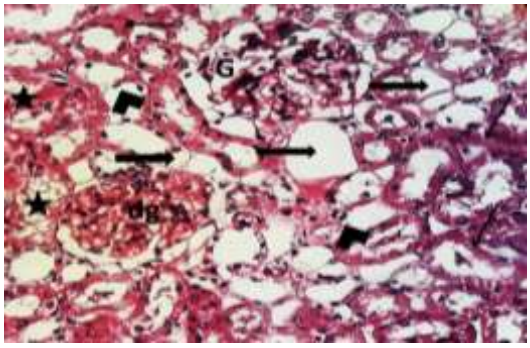

Figure 4: Photomicrograph of the kidney section of female rats treated with ethanoic acid 5 % showing, hyalinization of renal tubules and tubular basement membrane (long arrow), degeneration (dg) in glomerulus (G), vacuolated tubules with degenerated epithelial cells (stars), necrotic changes in tubules (thick long arrows) with pyknotic nuclei (head arrows) (H & E stain, X400).

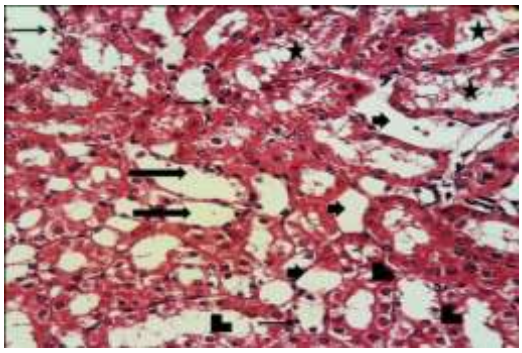

Figure 5: Photomicrograph of the kidney section of female rats treated with ethanoic acid 5 % showing, injury with desquamated epithelial cells (thick long arrows) and pyknotic nuclei (long arrows), hydropic swelling, pale cytoplasm become vacuolated (head arrows), hyalinization and degeneration of the renal tubules (stars) and necrotic areas (short arrows) (H & E stain, X400).

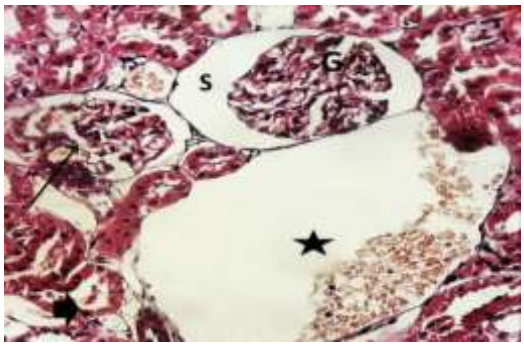

Figure 6: Photomicrograph of the kidney section of female rats treated with ethanoic acid 10 % showing, degeneration (long arrow) of renal glomerulus (G) with widening of Bowman's space (S), dilatation and vascular congestion (star) and pyknotic nuclei (head arrow) (H & E stain, X400).

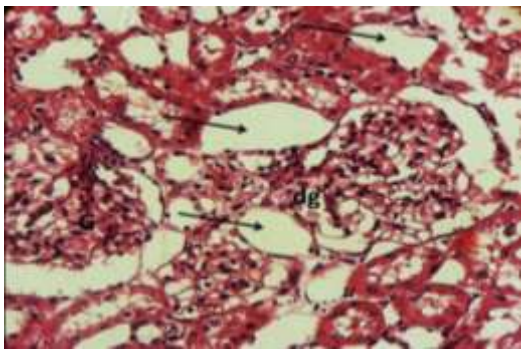

Figure 7**:** Photomicrograph of the kidney section of female rats treated with ethanoic acid 10 % showing, degenerative lesions (dg) in glomerulus (G) with lymphocytic infiltration cells, wide Bowman's space, degeneration in renal tubules, and necrotic areas (long arrow) (H & E stain, X400).

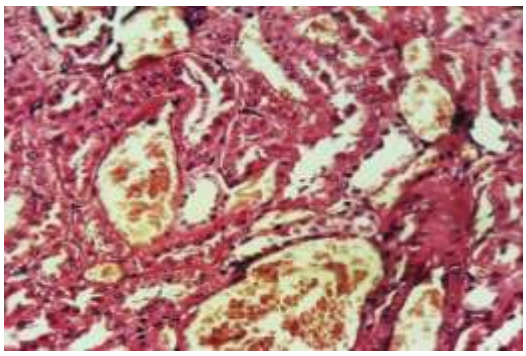

Figure 8: Photomicrograph of the kidney section of female rats treated with ethanoic acid 10 % showing, severe dilatation and vascular congestion with clogging of RBCs and hyalinization of renal tubules and tubular basement membrane (H & E stain, X400).

4. Discussion:

The results in the present study showed high marked changes found in urea levels in animals treated with EA-10 % in comparison with other groups. Whereas, non-significant difference in creatinine level between treated animals and controls is in agreement with the results of [14, 15, 16] who suggested that ethanoic acid administration showed no significant change in creatinine levels between treated animals and controls. On the other hand, the results in this study showed a significant increase in EA-10 % group in the mean values of creatinine when compared with EA-5 % group. These results were supported by the findings in [17]. Urea is the major end product of protein catabolism and is primarily produced in the liver and secreted by the kidneys. It is the primary vehicle for removal of toxic ammonia from the body [18]. Urea and creatinine enzymes are very sensitive markers employed in the diagnosis of kidney diseases. Serum urea and creatinine levels may be indicators of acute tubular necrosis caused by toxicity [19]. Nevertheless, the significant increase in serum urea concentration may be attributed to damage of the urea cycle leading to increase production of the metabolic product. Consequently, increase in urea levels may be due to defects in urea synthesis that may result in ammonia intoxication [20]. Creatinine is commonly measured as an index of glomerular function [21]. It is excreted exclusively through the kidney. Therefore, damage to the kidney will make the kidney inefficient to excrete both urea and creatinine and causes their accumulation in the blood. Therefore, the high level of blood urea and creatinine will indicate kidney damage [22]. Such physiological stresses during ethanoic acid condition may impair the renal functions due to hyperproduction of urea, uric acid and creatinine in blood serum which constitutes the basic parameters for renal function analysis [5]. Moreover, Benhelima *et al.* [23] stated that the markedly high serum rates of urea and creatinine in rats were indicative of marked necrosis of kidney epithelium and dilatation of proximal tubules with interstitial inflammation, damage in the last part of nephron and collecting system. The histopathological examination of kidney tissues of ethanoic acid at a dose of 5 % group in the present study showed degeneration of renal glomerulus with widening of Bowman's space, some focal areas of degenerated vacuolated cells, dilatation and vascular congestion and dilated of blood vessels, atrophied glomerulus with collapsed tuft, wide Bowman's space, degenerative lesions in renal tubules, vacuolated tubules with degenerated epithelial cells, necrotic changes in tubules with pyknotic nuclei. On the other hand, degenerative lesions in glomerulus with lymphocytic infiltration cells, wide Bowman's space and degeneration in renal tubules, severe dilatation and vascular congestion with clogging of RBCs, hyalinization of renal tubules detachment of many tubular epithelial cells from their basement membrane, tubular degeneration, inflammatory cells, dilatation and vascular congestion with fibrosis. These changes may be in response to nephrotoxic by drugs as proximal convoluted tubules are the most common site of toxicant induced renal injury. The reason for this relate in part to the selective accumulation of xenobiotics into this segment of nephron. Maintenance of tubular integrity depends on cell to cell and cell to matrix adhesions, after a chemical insult adhesion of nonlethally damaged cells to basement membrane is compromised, leading to their detachment from basement membrane and later on it may lead to sloughing of cells and formation of interatubular casts. Loss of brush border in proximal convoluted tubules can result from toxicant induced alterations in membrane integrity and cytoskeleton component [24]. In the present study infiltration of inflammatory cells i.e. lymphocytes were observed in the interstitium of kidney of EA rats, this infiltration of lymphocytes was suggestive of interstitial nephritis and may be attributed to hypersensitivity after exposure to toxic drug [6]. On the other hand, hypercellularity in glomeruli was

suggestive of proliferative glomerular nephritis which may be because of proliferation of endothelial cells, mesangial cells or infiltration of inflammatory cells [25]. Additionally, tubular epithelial swelling may be attributed to disruption of cell volume and ion homeostasis by toxicants, thus increasing ion permeability and inhibiting energy production, resulting into ATP depletion. Following ATP depletion Na^+, K^+, and ATP activity decreases resulting in K^+ efflux, Na^+ and Ca^- influx, cell swelling and ultimately cell membrane rupture. This influx may be a trigger for cell swelling and cell death [6]. Another elucidation of nephrotoxicity induced by chemicals the activation of inflammatory process shown by elevated pro-inflammatory cytokine renal tumor necrosis factor-α (TNF-α) level. This was proved before that some chemicals could upshot reactive oxygen species (ROS) generation, induce the protein kinase B (PKB), nuclear factor-kappa B (NF-kB), and mitogen-Activated protein kinase (MAPK) pathways beside elevation of cytokines, including TNF-α and interleukin-1α (IL-1α) levels [26]. In general, most of the tubular and glomerular damages occur during the reperfusion phase following ischemia, and generation of ROS. Reactive oxygen species are capable of reacting with lipids, proteins and nucleic acids leading to lipid peroxidation, impairments of enzymatic processes and DNA damages. The accumulation of ROS and reduction in antioxidant enzyme activities lead to damage in cellular components such as lipids and proteins [27].

5. Conclusion:

The present findings clearly demonstrate that ethanoic acid is capable of inducing dose dependent histological and physiological changes in the kidney tissues of the experimental rats. These effects may decrease the protective factors of animals. Hence quantity of ethanoic acid should be discouraged or reduced.

6. References:

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