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Toxicopathological evaluations of the ethanoic acid on liver tissues and blood parameters in albino rats

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Abstract The liver is affected by hazardous substances, exhibiting different degrees of toxicity. This study aimed to investigate the toxicopathological changes on liver tissues and blood parameters induced by ethanoic acid in rats. Female rats (n = 20) were divided randomly into two groups of 10: a group 1, control group and group 2, treatment group which were oraly administered the ethanoic acid (EA) at a dose of 1 ml/kg/day (10 % EA). Following fourteen days of treatment. There were no significant changes in the mean values of MCH, MCV and PLT, on the other hand, a significant lower in the mean values of RBC, HCT and Hb. Whereas, a significant increase in WBC, MCHC, AST and ALT were found in treated group when compared to control. Furthermore, the treated group showed bi-nucleated and apoptotic nuclei hepatocytes, loss of hepatic architecture and dilated sinusoidal areas. In conclusion EA consumption was found to have adverse effects on hepatic function in rats, hence the quantity of EA should be discouraged or reduced.
Key words: Toxicopathological, liver, Blood, Ethanoic Acid, Rat.

تقييم السمية المرضية لحمض الإيثانويك على أنسجة الكبد ومؤشرات الدم في الجرذان البيضاء

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الملخص يتأثر الكبد بالمواد الخطرة التي تظهر عليها درجات سمية مختلفة. حيث تهدف هذه الدراسة إلى دراسة التغيرات السمية المرضية على أنسجة الكبد ومؤشرات الدم الناجمة عن حمض الإيثانويك في الجرذان. تم استخدام عشرون جرذ، و تم تقسيمها إلى مجموعتين. استخدمت المجموعة الأولى كمجموعة سيطرة والمجموعة الثانية أعطت حمض الإيثانويك بجرعة 1 مل/ كغ/ يوم (10 ٪) عن طريق الفم لمدة أربعة عشر يوما. لا توجد فروق معنوية في القيم المتوسطة لكلا من متوسط كمية الهيموجلوبين لكرية الدم الحمراء ومتوسط حجم الكرية وعدد الصفائح الدموية، في حين لوحظ انخفاض ملحوظ في عدد الكريات الحمراء ومتوسط مكداس الدم وكمية الهيموجلوبين. كذلك وجد بأن هناك ازدياد معنوي احصائي في عدد الكريات البيضاء، ومتوسط تركيز الهيموجلوبين لكرية الدم الحمراء، وألانين أمينوترفراز والأسبارتيك أمينوتراز في مجموعة المعاملة عند مقارنتها بالمجموعة الضابطة. علاوة على ذلك، أظهرت مجموعة المعاملة خلايا كبد ثنائية النواة وموت خلوي، وفقدان بنية الخلايا الكبدية والمناطق الجيبية المتوسعة. في الخلاصة وجد بأن استهلاك حمض الإيثانويك له آثار سلبية على وظيفة الكبد في الجرذان، وبالتالي ينبغي التقليل من استخدام حمض الإيثانويك.
الكلمات المفتاحية: السمية المرضية، الكبد ، الدم ، حمض الإيثانويك ، الجرذ.

Introduction:

The liver is a target organ for toxicity because of its role in clearing and metabolizing chemicals through the process called detoxification. Drug and some chemicals induced liver disorders occurred frequently can be life threatening and mimic all forms of liver diseases [1]. In chronic liver injury, the injured cells release a number of cytokines and stimulate the kupffer cells to release more inflammatory mediators and various free radicals. Massive reactive oxygen species (ROS) production in the hepatic tissue induce oxidative stress, moreover, oxidative stress can induce many kinds of negative effects including membrane peroxidation, protein cleavage, and deoxyribonucleic acid (DNA) strand breakages, which could lead to cancer [2]. The precise mechanisms underlying drugs or chemicals induced hepatotoxicity and nephrotoxicity are gradually elucidated. However, there is still lack of effective therapeutic strategies or specific medicines for such diseases [3]. Moreover, chemicals induced toxicity is reported to be mediated by increased production of reactive oxygen species and free radicals. These ROS could interfere with the antioxidant defense system and can cause extensive tissue damage and cell dysfunction by reacting with macromolecules like proteins, membrane lipids and nucleic acids. However, oxidative stress could be a consequence of increased ROS generation and/or decreased antioxidant defense [4]. Superoxide dismutase (SOD) and catalase (CAT) provide major cellular defense against ROS and together they convert superoxide radicals first to H_2O_2 and then to

molecular oxygen and water. Another enzyme viz. glutathione peroxidase (GSH-Px) uses thiol reducing power of glutathione reduced (GSH) to reduce oxidized lipid and protein targets of ROS[5]. Ethanoic acid (EA) administered orally is immediately absorbed, uptake then occurs in liver and peripheral tissues. It is metabolized via acetyl-CoA in the tricarboxylic acid cycle in liver and skeletal muscle [6]. Hemolysis, disseminated intravascular coagulation, and liver dysfunction occurred after oral high concentration of EA poisoning in human [7]. Moreover, chronic ingestion of large amount of EA (7ml/kg/d (99%) in diet) caused hepatocellular degeneration with fibrous tissues, vascular congestion, necrosis, hypokalemia, hyperreninemia, osteoporosis and hepatocellular carcinoma [8,9]. The aim of the present study was to assessment the toxicopathological changes on liver tissues and blood parameters induced by ethanoic acid in female albino rats.

Material and Methods:

Chamicals:

Ethanoic acid (EA) (CH_3COOH) (99.8 %, Sigma-Aldrich). EA was obtained from the Omar Al-Mokhtar University.

Animals and experimental design:

The present study was conducted using 20 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 per-experimentation period to acclimatize to laboratory conditions in order to avoid any complications along the course of the experiment. The animals were acclimatized to standard laboratory conditions, with a temperature of 22 °C with standard light-darkness cycles of 12 hours. Rats were fed with laboratory diet and water *ad libitum* with fresh daily supplies. All rats were weighted weekly and the weight were recorded before the experimental procedures and at the end of the experiment. All rats were randomized into two groups 10 rats in each:

- Normal control group (NC): Rats were given orally distilled water for 14 days.
- Treated group (TG): Rats were given orally EA (10 %) by gavage at a dose of 1 ml/kg/b.w./day according to Pastrelo *et al.,* [10] for 14 days.

After the completion of treatment period, all rats were fasted for 24 hours [11] and animals were sacrificed and blood samples were collected from cutting the jugular vein for complete blood count (CBC) measurements, or liver functions tests then the liver was removed.

Haematological investigation:

Blood was collected in EDTA-coated tubes. The samples were kept at room temperature for maximally five hours before they were run on haemocytometer (Medical Electronic machine. Scopus-micro, Medical Centre) in Al-Razi Laboratory for Medical Analysis, El-Beida City. Various haematological parameters were performed including white blood cells (WBC), red blood cells (RBC), haematocrit (HCT), haemoglobin (Hb), the mean cell haemoglobin (MCH), the mean cell haemoglobin concentration (MCHC), the mean cell volume (MCV) and platelets count (PLT).

Determination of the liver function tests:

Blood samples were collected and left to clot, then centrifuged at 3000 rpm for 10 minutes and stored at -80 °C until biochemical analysis. Sera were used for the determination of biochemical analysis such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT), were evaluated to determine of the liver function tests by using spectrophotometer method according to Bergmeyer *et al.,* [12] in Al-Razi Laboratory for Medical Analysis, El-Beida City.

Histopathological examinations:

Tissue specimens from liver were taken and washed in normal saline, dried and placed in 10 % buffered formalin for histopathological examinations [13]. 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 °C 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 [14].

Statistical analysis:

The data of results obtained were subjected to statistical analysis and expressed as mean ± SE. The data were statically analyzed by Student's t-test at $P < 0.05$. All statistical procedures were performed with the Minitab statistical analysis package program (Minitab version 17).

Results:

Haematological investigation:

From the inspection of the data recorded in table (1) a significant increase ($P < 0.05$) in the mean value of WBC in treated group (14.56 ± 2.17) as compared to control group (9.157 ± 0.489). Moreover, animals that reserved of (EA-10 %) showed, a significant increase in the mean value of MCHC (36.329 ± 0.356) as compared with control (34.50 ± 0.127). Whereas, a significant decline ($P < 0.05$) occurred in the mean value of RBC in treated group (7.223 ± 0.260) when compared with control group (8.131 ± 0.052). Also, a significant decrease in the mean value of HCT and Hb in treated rats (43.29 ± 1.15 and 14.829 ± 0.358) as compared to control rats (47.429 ± 0.975 and 16.357 ± 0.290). However, a non-significant change in the MCH, MCV and platelets was recorded for treated group as compared to control group.

Table 1: Averages of CBC parameters in control and treated group.

Parameter	Control (NG)	EA-10% (TG)
WBC ($10^3/mm^3$)	9.157±0.489^b	14.56±2.17^a
RBC ($10^6/mm^3$)	8.131±0.052^a	7.223±0.260^b
HCT %	47.429±0.975^a	43.29 ±1.15^b
Hb (g/dl)	16.357±0.290^a	14.829±0.358^b
MCH (pg/cell)	20.114±0.296^a	20.143±0.243^a
MCHC (g/dl)	34.50±0.127^b	36.329±0.356^a
MCV	58.31±1.04^a	56.657±0.261^a

(fl)		
PLT (10^3/µl)	972.9±56.9[a]	997.9±65.4[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-10% = ethanoic acid (10%). * NC = Normal control. TG= Treated group.

Determination the enzymatic activities of liver:

The mean values of aspartate transaminase (AST) and alanine transaminase (ALT) activities of control and treated group were presented in table (2). In the mean values of AST showed, a significant increase ($P < 0.05$) in TG (134.14 ± 3.12) as compared to NG (73.86 ± 1.42). In addition, there was a significant increase ($P < 0.05$) in the mean value of ALT between treated group (62.86 ±3.40) when compared with control group (22.71 ± 1.02).

Table 1: Averages of mean values of aspartate transaminase (AST) and alanine transaminase (ALT) activities in control and treated group**.**

Parameter	Control (NG)	EA-10% (TG)
AST (IU/L)	73.86±1.42[b]	134.14±3.12[a]
ALT(IU/L)	22.71±1.02[b]	98.14±2.73[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-10% = ethanoic acid (10%). * NC = Normal control. TG = Treated group.

Histopathological preparations:

Microscopically, in the control group the liver showed normal histological structure; normal central vein, sinusoidal capillary size with no evidence of congestion or narrowing and normal hepatocyte without any changes in their cytoplasm and nucleus (Fig. 1). The portal areas contained branches of the hepatic artery and bile duct, embedded in connective tissue (Fig. 2). Ethanoic acid treated group with EA-10 % showed necrotic hepatocytes, vacuolated hepatocytes, cytoplasm indicating hydropic degeneration and congestion of central veins. Moreover, vasodilatation and congestion of portal area, hemorrhage and cellular infiltration were appeared (figs. 3 and 4). Some hepatocytes were bi-nucleated while others contained apoptotic nuclei, loss of hepatic architecture with increased signs of necrosis and dilated sinusoidal areas. In addition, dilated blood vessels and increase of kupffer cells, degenerating hepatocytes, hydropic swelling, pale cytoplasm become vacuolated and appeared moth-eaten, karyolytic and kariorrhxsis nuclei in some cells, hypertrophied hepatocytes with deeply stained shrunken nuclei (Fig. 5), and corrugated central vein surrounded by fibrotic area were also observed (Fig. 6).

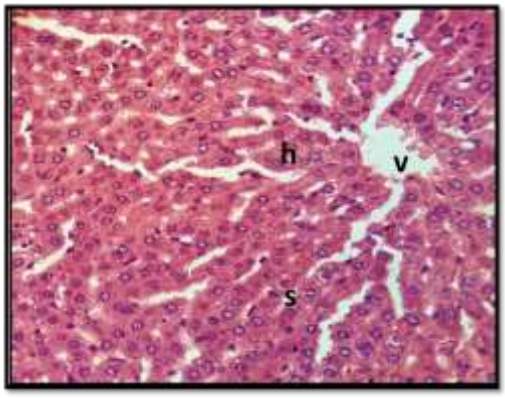

Figure 1: Photomicrograph of the liver section of control female rats showing, normal central vein (v), sinusoidal capillary (s) and hepatocytes structures (h) (H & E stain, X400).

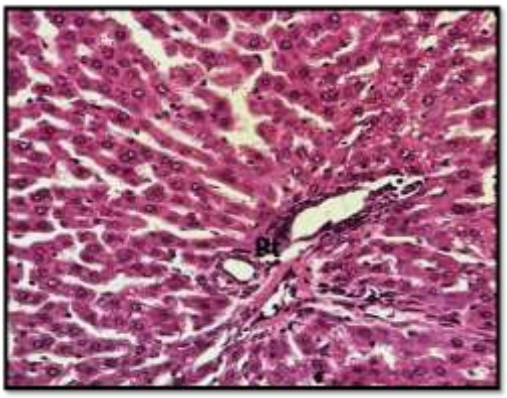

Figure 2: Photomicrograph of the liver section of control female rats showing, normal portal area (pt) (H & E stain, X400).

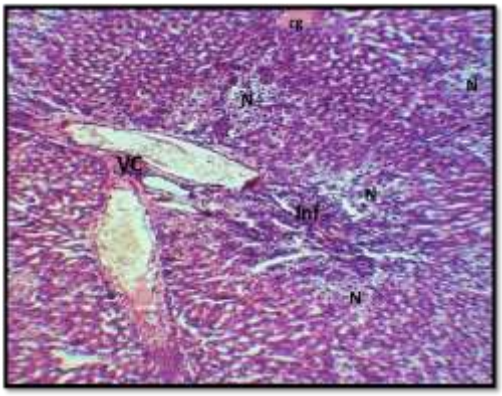

Figure 3: Photomicrograph of the liver section of

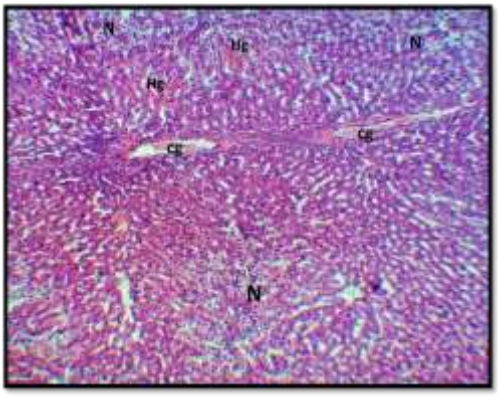

Figure 4: Photomicrograph of the liver section of

female rats treated with ethanoic acid 10 % showing, congestion (cg) in the central vein, inflammatory cell infiltration (Inf), vasodilatation and congestion (VC) of portal area and necrotic areas (N) (H & E stain, X100).

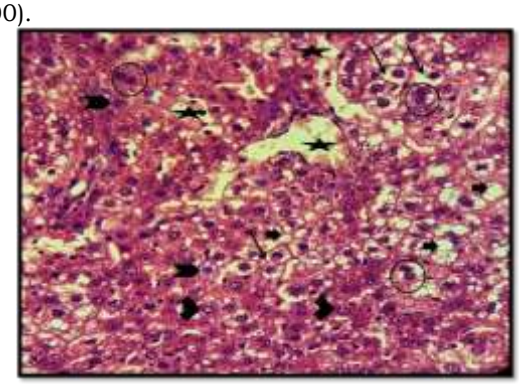

Figure 5: Photomicrograph of the liver section of female rats treated with ethanoic acid 10 % showing, bi-nucleated hepatocytes (circles), loss of hepatic architecture with degenerating hepatocytes and hydropic swelling, pale cytoplasm become vacuolated (long arrows), dilated blood vessels (stars), necrosis cells (short arrows), karyolytic nuclei (long head arrows) and kariorrhxsis nuclei in some cells (short head arrows) (H & E stain, X400).

female rats treated with ethanoic acid 10 % showing, dilated congested (cg) blood vessels, haemorrhage (Hg) and necrotic areas (N) (H & E stain, X100).

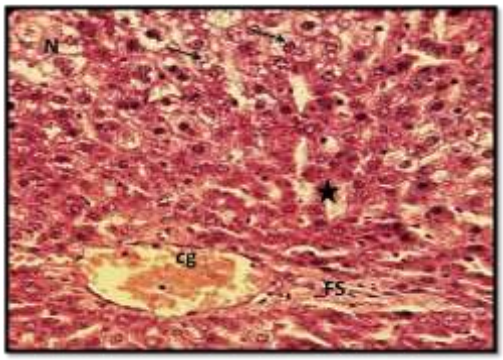

Figure 6: Photomicrograph of the liver section of female rats treated with ethanoic acid 10 % showing, congestion (cg) in the central vein surrounded by fibrotic area (FS), dilated sinusoidal areas (star), necrosis cells (N) and degenerating hepatocytes (long arrows) (H & E stain, X400).

Discussion:

The results of this study showed no a significant difference was noted in the MCH, MCV, and PLT between treated group and control. These results were supported by [15,16] who reported that no marked changes in the MCH and MCV parameters between EA groups and control; this is in agreement with the findings. Furthermore, a significant increase in the mean value of WBC and MCHC in treated group as compared to control group. Additionally, the mean values of RBC, HCT and Hb showed a significant decrease occurred in treated rats when compared with control group. These findings were supported by the findings in [17], they revealed that ethanoic acid caused decreasing of RBC and Hb with increasing of WBC in rats when compared with control. They supposed that, alteration in hematological parameters due to tissue damage is an important clinical manifestation of inflammatory diseases. Also they suggested that EA caused reduction in RBC with subsequent decline in Hb concentration. The observed decrease in the number of RBCs, accompanied by a decreased Hb, seems to confirm that ulcers probably caused excessive blood loss as a result of serious gastrointestinal tract bleeding, hemolysis of red blood cell and poor absorption of iron in the intestine. Nevertheless, the increase in WBC may be due to the inhibition of prostaglandin synthesis through cyclooxygenase enzyme and enhances haematopoisis, because of prostaglandin E2 (PGE2) increases intracellular cyclic adenosine monophosphate (AMP) levels in target cells [18]. In addition to, cyclic AMP and PGE2 block neutrophils recruitment and ethanoic acid may be enhanced by two fold neutrophils recruitment [19]. The significant reduction in the red blood cell and heamoglobin may have resulted from the suppression of circulating hormone, erythropoietin (a glycoprotein which stimulates the process of erythropoiesis). Reduction in blood concentration of erythropoietin may result in a normochromic, normocytic anemia [20].

In the present study, there was the AST and ALT activities showed a significant increase in treated group as compared to normal control group. This is accompanied with [9,21,22] they found that the administration of ethanoic acid to rats caused of increasing in AST and ALT activities. Moreover, liver is the center of biotransformation and detoxification of foreign compounds and is the most vulnerable to the chemical assaults such as ethanoic acid poisoning [21]. Raised activities of these enzymes indicate cell damage which might have resulted from several mechanisms. Peters *et al.* [22] demonstrated that the increase activities of AST and ALT are generally a result of liver disease associated with some degree of hepatic necrosis such as cirrhosis, carcinoma, viral or toxic hepatitis, drugs and obstructive jaundice. Previous studies have reported the serum AST and ALT are considered to be among the most sensitive markers employed in the diagnosis of hepatotoxicity [23,24]. Liver injury indicated by marked elevation in serum activities of AST and ALT enzymes associated with markedly histopathological changes. These changes may be due to the diffuse vacuolar degeneration, fat vacuoles and necrosis of hepatocytes and markedly focal fibrosis which causes increases in the permeability of cell for liver enzymes [25,26,27].

The histopathological examination of liver tissues of ethanoic acid at a dose of 10 % showed necrotic hepatocytes, vacuolated hepatocytes, cytoplasm indicating hydropic degeneration and congestion of central veins. Moreover, vasodilatation and congestion of portal area, hemorrhage, cellular infiltration, loss of hepatic architecture with

increased of necrosis and dilated sinusoidal areas. In addition, dilated blood vessels, degenerating hepatocytes, hydropic swelling, pale cytoplasm become vacuolated and appeared moth-eaten, karyolytic and kariorrhxsis nuclei in some cells. These findings were supported by the findings in [28,29], they revealed that changes in liver sections such as small focal areas in hepatocyte, infiltration of the lymphocytes in the portal spaces, focal-type necrosis, and fibrosis focal area in male and female guinea pigs. In addition, hepatocellular degeneration with fibrous tissues, vascular congestion, necrosis, infiltration of the lymphocytes around the central vein, few inflammatory cells infiltration with moderate fibrosis and the presence of microvesicular steatosis in rats treated groups with ethanoic acid [9]. The damage in liver in the present study appeared in the form of an inflammatory cells infiltration, widening of the sinusoids, increasing of Kupffer cells, loss of normal hepatic tissue architecture and disappearance of normal organization. Similar results were obtained by Sakr *et al.* [30] who found that an inflammatory response occurred when Kupffer cells were activated by the free radicals and secretes cytokines that attract and activate neutrophils thereby enhancing the liver injury. The degeneration of liver tissues and cellular metabolism could be degenerative changes in the cytoplasm, where integrity of cytoplasm is quite important for maintenance of cellular vital functions. Moreover, integrity of mitochondrial membrane is important for maintanence of vital functions and determination of apoptotic process. So these degenerations in mitochondrial membranes and crista structure could have negatively affect oxidative metabolism and vital balance limits of the cell [31].

In conclusion ethanoic acid (EA) consumption have adverse effects on hepatic function in rats, hence the quantity of EA should be discouraged or reduced.

References:

[1]- El-Kordy, E. A. and Makhlouf, M. M. (2014). Possible protective role of ginger extract on diclofenac induced hepatotoxicity in adult male albino rats (Histological and ultrastructural studies). *Life Science Journal.* **11** (8): 248-258.

[2]- Eid, J. I. Eissa, S.M. and El-Ghor, A. A. (2015). Bisphenol A induces oxidative stress and DNA damage in hepatic tissue of female rat offspring. *The Journal of Basic and Applied Zoology*. **71**: 10-19.

[3]- Hasan, M. N. Khan, R. A. Nasiruddin, M. and Khan, A. A. (2016). Ameliorative effect of *Nigella sativa* oil against paracetamol induced hepatic and renal damages in rats. *Journal of Pharmaceutical Research International*. **13** (3): 1-10.

[4]- Rai, S. and Halder, C. (2003). Pineal control of immune status and hematological changes in blood and bone marrow of male squirrels (*Funambulus pennanti*) during their reproductively active phase. *Comparative Biochemistry and Physiology Part C.* **136**: 319-328.

[5]- Farooqui, Z. Afsara, M. Rizwana, S. Khanb, A. A. and Khana, F. (2016). Oral administration of *Nigella sativa* oil ameliorates the effect of cisplatin on membrane enzymes, carbohydrate metabolism and oxidative damage in rat liver. *Toxicology Reports.* **3**: 328-335.

[6]- Fushimi, T. Tayama, K. Fukaya, M. Kitakoshi, K. Nakai, N. Tsukamoto, Y. and Sato, Y. (2001). Acetic acid feeding enhances glycogen repletion in liver and skeletal muscle of rats. *Nutrient Metabolism.* **131** (7): 1973-1977.

[7]- Kamijo, Y. Soma, K. Iwabuchi, K. and Ohwada, T. (2000). Massive noninflammatory periportal liver necrosis following concentrated acetic acid ingestion. *Archives of Pathology and Laboratory Medicine.* **124** (1): 127-129.

[8]- Chibishev, A. Sikole, A. Pereska, Z. Chibisheva, V. Simonovska, N. and Orovchanec, N. (2013). Severe renal function impairment in adult patients acutely poisoned with concentrated acetic acid. *Archives of Industrial Hygiene and Toxicology.* **64** (1): 153-158.

[9]- Bouazza, A. Bitam, A. Amiali, M. Bounihi, A. Yargui, L. and Koceir, E. A. (2016). Effect of fruit vinegars on liver damage and oxidative stress in high-fat-fed rats. *Pharmaceutical Biology.* 54 (2): 260-265.

[10]- Pastrelo, M. M. Ribeiro, C. C. D. Duarte, J. W. Gollücke, A. P. B. Artigiani-Neto, R. Ribeiro, D. A. Miszputen, S. J. Oshima, C. T. F. and Paiotti, A. P. R. (2017). Effect of concentrated apple extract on aexperimental colitis induced by acetic acid. *International journal of molecular and cellular medicine.* **6** (1): 38-49

[11]- El-Shinnawy, N. A. Abd-Elmageid, S. A. and Alshailabi, E. M. A. (2014). Evaluation of antiulcer activity of indole-3-carbinol and/or omeprazole on aspirin-induced gastric ulcer in rats. *Toxicology and industrial health.* **30** (4): 357-375

[12]- [12] Bergmeyer, H. U. Scheibe, F. and Wahlefeld, A. W. (1978). Optimization of methods for aspartate aminotransferase and alanine aminotransferase. *Clinical Chemistry.* **24**: 58-73.

[13]- Lillie, R. D. (1954). Histopathological techniques and practical histochemistry. Mc Graw-Hill U.S.A.

[14]- Harris, H. F. (1990). After Bruce Casselman, W. C. (1959): Histochemical Technique, By Methuen And Co. Ltd.

[15]- Mahmoodi, M. Hosseini-zijoud, S. M. Nabati, S. Modarresi, M. Mehrabian, M. Sayyadi, A. and Hajizadeh, M. (2013). The effect of white vinegar on some blood biochemical factors in type 2 diabetic patients. *Journal of Diabetes and Endocrinology.* **4** (1): 1-5.

[16]- Ur Rehman, Z. Ul Haq, A.,Akram, N. Abd El-Hack, M. E. Saeed, M. Rehman, S. U. Meng, C. Alagawany, M. Sayab, M. Dhama, K. and Ding, C. (2016). Growth performance,

intestinal histomorphology, blood hematology and serum metabolites of broilers chickens fed diet supplemented with graded levels of acetic acid. *International Journal of Pharmacology.* **12** (8): 874-883.

[17]- Olamilosoye, K. P. Akomolafe, R. O. Akinsomisoye, O. S. Adefisayo, M. A. and Alabi, Q. A. (2018). The aqueous extract of *Ocimum gratissimum* leaves ameliorates acetic acid-induced colitis via improving antioxidant status and hematological parameters in male Wistar rats. *Egyptian Journal of Basic and Applied Sciences.* **5** (3): 220-227.

[18]- Daud, A. N. Silva, K. I. D. Deng, J. Gamelli, R. L. Jones, S. B. and Shanker, R. (2003). Prostaglandin E2 mediates growth arrest in NFS-60 cells by down-regulating interleukin-6 receptor expression. *Biochemical Journal.* **1** (370): 315-321.

[19]- Neha, J. Arun, R. K. and Vinoy, S. K. (2011). The effect of acetylsalicylic acid on hematological and biochemical parameters in female albino rats. *International Journal of Biological and Pharmaceutical Technology.* **2** (3): 412-418.

[20]- Sunday, O. Otimenyin, M. O. Uguru, E. A. and Ochigbo, E. A. (2009). The effect of aqueous extracts of *Momordica balsamina* on haematological and biochemical parameters in rats. *Asian Journal of Pharmacology and Clinical Research.* **2** (1): 21-25.

[21]- Soltan, S. S. and Shehata, M. M. E. M. (2012). Antidiabetic and hypocholesrolemic effect of different types of vinegar in rats. *Life Science Journal.* **9** (4): 2141-2151.

[22]- Périco, L. L. Rodrigues, V. P. Ohara, R. Bueno, G. Nunes, V. V. A. Dos Santos, R. C. Camargo, A. C. L. Júnior, L. A. J. De Andrade, S.F. Steimbach, V. M. B. and Da Silva, L. M. (2018). Sex-specific effects of *Eugenia punicifolia* extract on gastric ulcer healing in rats. *World Journal of Gastroenterology.* **24** (38): 4369-4418.

[23]- Peters, D. E. Uwakwe, A. A. and Monago, C. C. (2014). Effect of antiretroviral drug (arved) on hepatic enzymes in albino rats. *Journal of Applied Sciences and Environmental Management.* **18** (2): 351-357.

[24]- Saafi, E. B. Ouedi, M. L. Elfekc, A. Zakhama, A. M. F. Najjar, Hammami, M. and Achour, L. (2011). Protective effect of date palm fruit extract (*Phoenix dactylifera* L.) on DM induced oxidative stress in rat liver. *Experimental and Toxicologic Pathology.* **63**: 433-441.

[25]- Hasan, K. M. M. Tamanna, N. and Haque, M. A. (2019). Biochemical and histopathological profiling of Wistar rats treated with *Brassica napus* as a supplementary feed. *Journal of Nutrition and Food Sciences.* **7** (1): 77-82.

[26]- Pourbakhsh, H. Taghiabadi, E. Abnous, K. Hariri, A. T. Hosseini, S. M. and Hosseinzadeh, H. (2014). Effect of *Nigella sativa* fixed oil on ethanol toxicity in rats. *Iranian Journal of Basic Medical Sciences*. **17** (12): 1020-1031.

[27]- Al-Ahdab, M. A. (2015). Effect of Sesame oil, *Nigella sativa* L oil and their mixtures on lipid profile and liver enzymes in hypercholesterolemic rats. *Journal of American Science.* **11** (12): 66-73.

[28]- Dardouri, K. Haouem, S. Gharbi, I. Sriha, B. Haouas, Z. El Hani, A. and Hammami, M. (2016). Combined effects of Cd and Hg on liver and kidney histology and function in Wistar rats. *Journal of Agricultural Chemistry and Environment.* **5** (4): 159-169.

[29]- Saad-Hossne, R. Hossne, W. S. and Montenegro, M. R. G. (2007). Effects of phenol, glycerin and acetic acid on the liver of guinea pigs. *Acta Cirurgica Brasileira.* **22** (6): 436-440.

[30]- Sakr, S. A. Abd-ElSamie, H. and Sheir, R. A. (2007). Effect of chromium on carbon tetrachloride-induced hepatotoxicity in mice: Histological and histochemical studies. *Egypt Journal of Experimental Biology. (Zool.).* **3**: 91 - 100.

[31]- Bakar, E. and Aktac, T. (2014). Effects of sodium benzoate and citric acid on serum, liver and kidney tissue total sialic acid levels: An ultrastructural study. *Journal of Applied Biology and Biotechnology.* **8** (2): 9-15.