

## Acute toxicity study of alcoholic leaf extract of *Eruca sativa* in albino rats (*Rattus norvegicus*)

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### Abstract

*Eruca sativa* (Es) belongs to the family Brassicaceae (Cruciferae) commonly known as jarjeer have been used in traditional medicine for treatment of different diseases. The present study was performed to evaluate the acute toxicity of *Eruca sativa* ethanolic leaves extract (ESELE) in rats. For acute toxicity test, fifteen rats have been used and they were divided into three groups with five rats for each group, low dose of ESELE (2g/kg), high dose of ESELE (5 g/kg) and normal group. After 14 days all the animals were sacrificed by intraperitoneal injection with ketamine (100 mg/ml) and xylazine (100mg/ml) in a ratio 4:1 (v/v). The blood was collected for some liver function test such as [aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and total serum bilirubin (TSB)] and kidney function test such as (urea and creatinine). Then liver and kidney were fixed in formalin 10% for histopathological examination. Acute toxicity study with a higher dose of 5 g/kg did not manifest any toxicological signs in rats. There is no significant ( $P \leq 0.05$ ) difference between rats was given ESELE in all tested parameters levels as compared to their respective vehicle group. Histopathological examination of the organs did not reveal any abnormalities in all groups treated comparing with vehicle group. In conclusions, the present finding suggests that ESELE did not manifest any significant toxicity at any of the biochemical and histological examined parameters.

**Keywords:** *Eruca sativa*, Acute toxicity, Histopathology, Rats.

### دراسة السمية الحادة للمستخلص الكحولي لنبات الجرجير في الجرذان البيض

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### الخلاصة

نبات الجرجير (*Eruca sativa*) ينتمي إلى عائلة Brassicaceae (Cruciferae) استخدم في الطب البديل لعلاج مختلف الأمراض. تهدف الدراسة الحالية لتقييم المستخلص الكحولي لأوراق نبات الجرجير في السمية الحادة في الجرذان. وقد استخدم 15 جرذاً لتقييم السمية الحادة إذ قسمت الحيوانات على ثلاث مجاميع (مجموعة السيطرة، الجرعة الواطئة والجرعة العالية) بواقع 5 حيوانات لكل مجموعة، جرعت الحيوانات بالماء، (2 غم/كغم)، (5غم/ كغم) من المستخلص على التوالي. تم تخدير الحيوانات بعد 14 يوماً بواسطة حقن الكيتامين (100 ملغم/مل) والزيلازين (100 ملغم/مل) بنسبة 4:1 (حجم/حجم) تحت الجلد ومن ثم تم تضحيتهما. وبعد ذلك جمعت عينات الدم لتقييم بعض وظائف الكبد والكلى مثل AST، ALT، ALP، TSB، اليوريا والكرياتينين. تم تثبيت الكبد والكلى بالفورمالين 10% للفحوصات النسيجية. لم تظهر نتائج دراسة السمية الحادة بالجرعة العالية (5 ملغم/كغم) أيضاً أي علامات سمية في الحيوانات، كما أظهرت النتائج عدم وجود اختلافات معنوية في مستوى أنزيمات الكبد

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والبليروبين والكرياتينين بمستوى 0.05 بينما لوحظ زيادة معنوية في مستوى اليوريا للمجموعتين التي جرعت بالمستخلص مقارنة بمجموعة السيطرة بمستوى 0.05. أظهرت نتائج الفحوصات النسيجية عدم وجود علامات غير طبيعية في كلتا المجموعتين اللتين جرعتا بالمستخلص اذا ما قورنت بمجموعة السيطرة. يمكن الاستنتاج من الدراسة الحالية من ان المستخلص الكحولي لأوراق نبات الجرجير لم يظهر أي علامات سمية في المعايير الكيموحيوية والنسيجية المدروسة.

الكلمات المفتاحية: نبات الجرجير، السمية الحادة، أمراض نسيجية، الجردان

## Introduction

Acute toxicity test gives clues on the range of doses that could be toxic to the animal; it could also be used to estimate the therapeutic index (LD50/ED50) of drugs and Xenobiotics (1). According to the Food and Drug Administration (FDA) panel of experts, as stated in the Guidelines for toxicity tests (2000), acute toxicity tests can provide preliminary information on the toxic nature of a material for which no other toxicology information is available. The more subtle and chronic forms of toxicity, such as carcinogenicity, mutagenicity, and hepatotoxicity, may well have been overlooked by previous generations and it is these types of toxicity that are of most concern when assessing the safety of herbal remedies (2, 3). Safety should be the overriding criterion in the selection of medicinal plants for use in health care systems (3). As with all forms of self-treatment, the use of herbal medicinal products also presents a potential risk to human health. Herbal toxicity can be evaluated by [1] observing human or animal populations exposed to the plant material, [2] administering the plant medicine to animals under controlled conditions and observing the effects (*in vivo*) and [3] exposing cells, sub-cellular fractions or single-celled organisms to the plant material (*in vitro*) (4). As usual, all initial acute toxicity tests are performed on either rats or mice because of the low cost, the availability of the animals, and the fact that abundant reference toxicologic data for many compounds in these species are available (5). In addition, these animals generally metabolize compounds in a similar manner to humans and the compounds (including metabolites) may have similar pharmacodynamics in the animals and humans. Before the experiment is performed, a total number of animals of similar body weight and same sex, or equal numbers of both sexes, are selected and randomly assigned to test (treatment) and control groups (5). Here among Kurdish and Arabian people rocket leaf, *Eruca sativa* (Es) belongs to the family Brassicaceae (Cruciferae) (6) locally known as Jarjeer, is widely used in salads. Further, Greek medicine is used Es leaf in diuretic, stimulant, and in the treatment of stomach disorders and scurvy (7). The seeds and tender leaves are known in Arabian countries to increase sexual desire and are considered to be an aphrodisiac. It is also used as a carminative and to alleviate abdominal discomfort and improve digestion. Despite the flavour and medicinal value of plant resources, research has proven that some of the plants are toxic to human and animals due to the presence of certain compounds (8). Therefore in the present study Es leaf has been selected for evaluating potential toxicity in laboratory rats.

## Materials and Methods

- **Plant material and preparation of extract:** Fresh *Eruca sativa* leaves were purchased from a local vegetable market in Erbil, and the identity of these leaves were confirmed by Dr. Abdullah Shakor taxonomist of the Department of Biology College of Education University of Salahaddin-Erbil-Iraq. After identification of Es, the leaf part of the plant were cleaned, dried in the shaded place for 7-10 days then were finely powdered using electrical blender. The powdered leaves were stored in dark glass flasks to protect them from light and moldiness. The amount of 100 g of

coarsely pulverized rocket leaves were placed in a glass percolator with 1000 ml of ethanol and were allowed to stand at room temperature for about 72 h. After 3 days the mixture was filtered using a fine muslin cloth followed by filter paper (Whatman No. 1). The mixture was then distilled under reduced pressure in a rotary evaporator (RE 200B/UK) (9). The plant extract was then dissolved in Tween 20 (10% v/v) and administered orally to rats in concentrations of different doses (10).

- **Experimental animals:** In this experiment fifteen healthy adult male *Rattus norvegicus* rats were obtained from the Experimental Animal House, College of Medicine/Haweler Medical University. The rats (6-8 weeks old) weighed between 150g – 200g were kept at room temperature in humidity rooms on a standard light/dark cycle (12 h light; 12 h dark cycle) at (22±3C°). Each rat was placed individually in separate plastic cages (56 × 39 × 19) bedded with wooden chips in the animal house of Biology Department/ College of Education/ Salahadin University-Erbil. The rats were fed with standard rat diet chow and tap water (11). They were kept under observation for about two weeks before the initiation of the experiment. All the procedures described were reviewed and approved by Institutional Animal Ethical Committee. Throughout the experiments, all animals received human care according to the criteria outlined in the “Guide for the Care and Use of laboratory Animals” prepared by the National Academy of Sciences and published by the national Institute of health.
- **Acute toxicity test:** The acute toxic study was performed to determine a safe dose for the Es extract. Fifteen *Rattus norvegicus* male rats were assigned equally into 3 groups as shown in the (Table 1). The animals were fasted overnight (food but not water) prior dosing. Food was withheld for a further 3 to 4 hours after dosing. The animals were observed for 30 min and 2, 4, 8, 24 and 48 h after the administration for the onset of clinical or toxicological symptoms. Mortality, if any was observed over a period of 2 weeks. On 15th day, all the animals were killed ethically. Histological and physiological parameters were determined following standard methods (12, 13).

**Table (1) Distribution of rats in their experimental groups**

Groups of animals	Number of rats	Treatment	Dose (mg/kg)	Duration
Control (Normal)	5	Water	—	14 days
Low dose	5	ESELE	2	14 days
High dose	5	ESELE	5	14 days

- **Behavioural observation and mortality:** Throughout the study period, all animals were observed for behavioural signs of toxicity, morbidity and mortality. Mortality checks were made twice daily and determination of behavioural signs was observed daily for all animals. Detailed observations of the individual animals were made weekly in comparison with the normal group animals. Observations included gross evaluations of the skin for any sign of respiration (dyspnoea), salivation, exophthalmia, convulsion and any changes in locomotion such as whether the animals tend to stay quietly or actively moving in their cage.
- **Body weight changes:** The body weights of each rat in all groups were measured at the initiation of treatment, after 5 days, after 10 days and on the day of scheduled termination.
- **Sacrificing of the laboratory animals:** The animals were fasted overnight and anaesthetized by intraperitoneal injection with ketamine (100 mg/ml) and xylazine (100mg/ml) in a ratio 4:1 (v/v) (HIKMA pharmaceuticals, Amman-Jordan). The blood were collected and the liver and kidney were surgically removed immediately from the sacrificed animals, organs were weight, cut into smaller pieces approximately 0.5 cm in thickness and then fixed in formalin fixative 10% (Approximately 20 times the volume of fixative used to the volume of the tissues to obtain optimal fixation) for histological studies.

- **Gross necropsy and histopathology:** At scheduled termination, all surviving animals were anaesthetized by intraperitoneal injection with ketamine (100 mg/ml) and xylazine (100mg/ml) in a ratio 4:1 (v/v). Gross post-mortem examinations were performed on all terminated animals. Liver and kidney from each animal were routinely processed and embedded in paraffin. After sectioning and staining with haematoxylin and eosin (H&E) stain method, all slides were observed under microscope (AmScoop microscope eyepiece camera. China).
- **Serum biochemical tests:** Blood samples were taken from the rats through cardiac puncture then transmitted into centrifuge tubes without ethylene diamine tetraacetic acid (EDTA), kept at room temperature for (20-30) minute, centrifuge for (15) minute at (3000) rpm for 15 minutes (14).
- **Liver and kidney functions:** For liver and kidney functions analysis, blood was collected into clot activator tube. All samples were analysed immediately by Cobas c 311- Roche Diagnostics in the Clinical Diagnostic Laboratory. Liver function parameters analysed were aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase and total bilirubin. Kidney function parameters analysed were urea and creatinine. The results were compared to that of the rats' respective control group.
- **Histological preparation:** The liver and kidney were fixed in 10% of buffered formalin solution. The tissue process (Dehydration, Cleaning and Infiltration) were done automatically using Automated Tissue Processing Leica (TP1020). Then, the tissues were embedded in paraffin wax using Leedo HISTOEMBEDDER. The embedded tissues were sectioned with microtome to produce 5 µm paraffin wax tissue sections. Then, the sections were stained with haematoxylin & eosin followed by mounting with DPX mounting media. Next, the mounted sections were evaluated for microscopic examination using light microscope (Carl Zeiss, Japan).
- **Statistical analysis:** All the data were analysed by Statistical Package Social Science (SPSS) version 17.0. One-way ANOVA is used to show the mean differences between all samples (\* p<0.05).

### Results

- **Acute toxicity study:** The acute toxicity of 95% ethanolic extract of Es via oral administration was evaluated in male rats. Two different doses of plant extract were used and the results were compared to the control group to demonstrate if any changes occur during 14 days of the experiment. Different parameters were tested to determine the toxicity of 95% ethanolic extract of Es.
- **Behavioural observation and mortality:** The animals pre-treated with a dose of 2 g/kg and 5 g/kg of ESELE were kept under observation for 14 days. All the animals remain alive and did not manifest any significant visible of toxicity at these doses of plant extract within short- and long-term outcome. There were no abnormal signs, behavioral changes, or macroscopic finding at any time of observation. There was no mortality in the above mentioned doses at the end of 14 days of observation as shown in (Table 2).

**Table (2) The observation data for toxicology study of ESELE**

Group	Occurrence of mortality					
	10 min	30 min	3 h	24 h	48 h	14days
Normal	0/5	0/5	0/5	0/5	0/5	0/5
2 g/kg	0/5	0/5	0/5	0/5	0/5	0/5
5 g/kg	0/5	0/5	0/5	0/5	0/5	0/5

- **Analysis of body weight:** The changes of body weight of animals throughout the 14 days were recorded. Table (3) illustrated that there is no any significant changes observed in any of groups pre-treated with ESELE when compared to their respective normal group. The percentage increase in body weight of treatment rats measured every 5 day and the increasing was about 11gm.

**Table (3) Body weight analysis of acute toxicity test after pre-treated with 95% ethanolic extract of Es in male rats**

Dose	Body weight (g) Day 0	Body weight (g) Day 5	Body weight (g) Day 10	Body weight (g) Day 15
Normal	162.5±3.8	169±3.2	180.5±2.9	191±4.9
2 g/kg (LD)	165.2±6.88	172.5±8.6	181.4±3.8	192±8.9
5 g/kg (HD)	167.3±2.9	175±4.4	182.5±4.4	192.8±1.5

LD: low dose; HD: high dose. Each column expresses the mean ± SEM of n=5 using one way ANOVA. \*P≤0.05 Vs normal.

- **Weight of organs:** Table (4) shows the effects of the ethanol extract on the weights of some vital body organs in rats. ESELE administration did not cause a significant difference in the organ weights of rats in both control and test groups.

**Table (4) liver weight analysis of acute toxicity test after pre-treated with 95% ethanolic extract of Es in male rats**

Groups	Treatment	Liver (gm)	Kidney (right) (gm)	Kidney (left) (gm)
Normal (Control)	water	8.2±0.41	0.89±0.12	0.88±0.10
LD	2 gm/kg b.wt	8.34±0.55	0.9±0.18	0.88±0.04
HD	5 g/kg b.wt	8.22±0.65	0.88±0.13	0.9±0.1

Each column expresses the mean ± SEM of n=5 using one way ANOVA. \* P≤0.05 Vs normal.

- **Serum biochemistry.**
- **Liver function test:** For serum biochemistry analysis, different parameters have been tested to investigate the liver function test in male rats pre-treated with 95% ethanolic extract of Es such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and total serum bilirubin (TSB) as shown in (Table 5). The results showed that there were no significant differences (P≤0.05) among all the groups.

**Table (5) Liver function test in acute toxicity test after pre-treatment with 95% ethanolic extract of Es in male rats**

Groups	Treatment	AST (IU/L)	ALT (IU/L)	ALP(IU/L)	TSB(mg/dl)
Normal	water	77.16±10.8	244.86±4.47	142.6±1.12	0.1±0.0
LD	2 g/kg	74.56±7.88	262.6±4.26	143.2±0.91	0.1±0.0
HD	5 g/kg	74.24±4.44	268.7±13.4	145±0.94	0.1±0.0

AST: aspartate aminotransferase; ALT: alanine transaminase; ALP: alkaline phosphatase and TSB: Total Bilirubin. Each column expresses the mean ± SEM of n=5 using one way ANOVA. \* P≤0.05 Vs normal.

- **Kidney function test:** The parameters of kidney functions that have been tested were urea and creatinine. The levels of all groups were analyzed as indication of kidney functions and compared to their normal group. As illustrated in (Table 6), there were significant (p≤0.05) increase in level of urea among HD and LD compared to normal group but there were no significant differences in level of creatinine among HD, LD and normal group.

**Table (6) Effect of ESELE on the kidney function in rats**

Groups	Treatment	Urea	Creatinine
Normal (control)	water	38.64±0.41	0.42±0.37
LD	2 g/kg	47.38±1.82*	0.34±0.25
HD	5 g/kg	49.66±1.51*	0.34±0.2

Each column expresses the mean ± SEM of n=5 using one way ANOVA. \* P≤0.05 Vs normal.

- **Histological examination of liver:** Histopathological examination of the liver did not reveal any abnormalities in all groups treated compared to normal group where showed healthy hepatocyte except HD group that show very few number of dead

hepatocytes that considered normal as showed in (Fig. 1). There is no any degenerated or necrotic hepatocyte, vacuolated hepatocyte, fragmented nucleus and inflammatory cell that usually occur in hepatotoxicity.

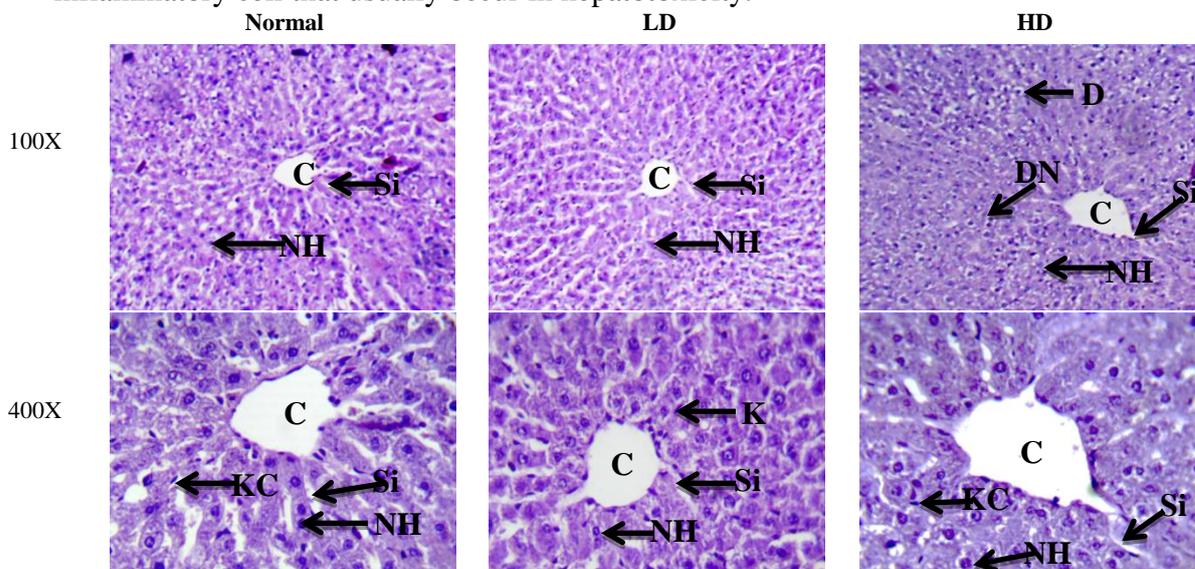


Fig. (1) Histological section of liver in rats. Normal group showing normal hepatocyte (NH), central vein (C), sinusoid (Si) and kupffer cell (KC). Low Dose (LD) treated with 2 g/kg of ESELE showed normal structural appearance. High Dose (HD) treated with 5 g/kg of ESELE showed normal structural appearance with some dead cells (DC) and double nucleated cell (DN).

- **Histological examination of kidney:** As shown in (Fig. 2), all groups showed normal structural appearance and distribution of glomerulus with healthy proximal and distal convoluted tubules. Further, no congestion, inflammation and dilatations of convoluted tubules have been observed that usually occur in case of kidney toxicity.

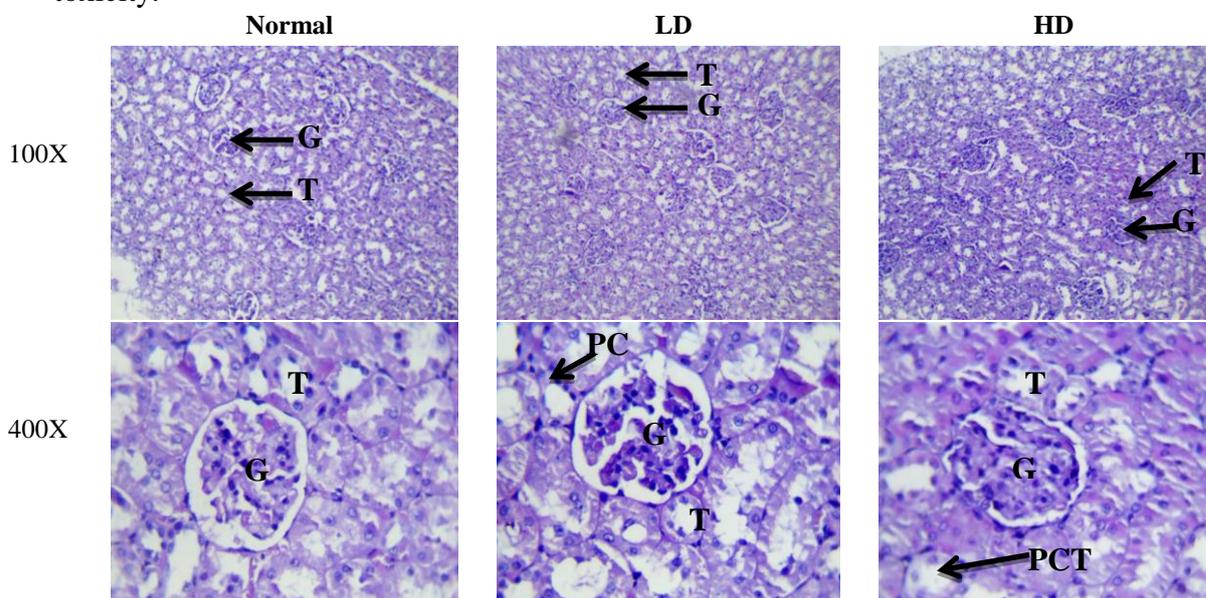


Fig. (2) Histological section of liver in rats. Normal group showing normal glomerulus (G) and kidney tubule (T). LD treated with 2 g/kg of ESELE showed normal structural appearance of glomerulus (G) and proximal convoluted tubules (PCT). HD treated with 5 g/kg of ESELE showed normal structural appearance of glomerulus (G) and proximal convoluted tubules (PCT).

## Discussion

The acute toxicity profile of ESELE could be considered favorable judging from the absence of adverse clinical manifestations in experimental animals after 14 days of observation. Blood is an important index of physiological and pathological status in man and animals and the parameters of clinical biochemistry usually measured include AST, ALT, ALP, TSB, urea, and creatinine. The normal range of these parameters can be altered by the ingestion of some toxic plants. Clinical biochemistry values were within the range of the control animals tested and similar to some of the control reference values published by other researchers (15). The 14 days observation period was recommended by the OECD guidelines for observation of any incidences of delayed toxicity or death, or to observe for appearance and then disappearance of toxic effects. In this study, no rats exhibited any behavioral signs of toxicity (such as changes in locomotor activity; bizarre reactions; sensitivity to pain, sound and touch; aggressive behavior; convulsions; paralysis, etc.) or mortality after a single oral administration of ethanolic extract at high and low dose of 5 and 2 g/ kg/b.wt, respectively. Thus, indicating that the extract does not produce acute oral toxicity. The increase in body weight of both group every 5 day were about 11g, which considered normal and gradually as observed in rats of similar age group in the previous studies (16). Hence, it was suggested that the extracts had no effect on food intake or causes weight loss in the animals. ESELE administration did not cause a significant difference in the organ weights of rats in both control and test groups. This suggests that the extract did not interfere with the organs. Parameters for liver function evaluation such as AST, ALT, ALP and TSB were analyzed. It is known that many toxic plants compounds accumulate in the liver where they are detoxified (17). A study of liver function tests may therefore prove useful in assessing especially the toxic effects of medicinal plants on the liver. These tests involve mainly determination of AST and ALT and any marked necrosis of the liver cells lead to a significant rise of these enzymes in the blood serum (18). The lack of this effect on these liver enzymes (significant rise) shows that the extract is non-toxic on the hepatocytes. Also, an increase in the level of serum alkaline phosphatase and secrete large amount into plasma which indicated a severe histological change in the liver (19). (20) indicated that the main medicinal and therapeutic properties of this plant include antigentoxic effect against human hepatoma (HePG2) cells which is attributed to the presence of erucin and erysolin compounds in the plant extract (20). For the evaluation of renal function, two different parameters have been analyzed such as urea and creatinine (21). The effects of ESELE on blood chemistry were reported and there was no significant ( $p \leq 0.05$ ) difference in level of creatinine comparing with control group. As creatinine considered a more sensitive indicator for kidney damage that means ESELE is not toxic to kidney even in a high concentration. This finding was in accordance with (22) who reported that the alcoholic extract of *Es* possesses a potent free radical scavenging, antioxidant and nephroprotective activities. In another hand, there were significant ( $p \leq 0.05$ ) increases in level of urea with ESELE treated group compared to control group. In fact, medicinally it has been reported that urea dose not considered as a high sensitive indicator of kidney damage. Many factors are accompanied to increase urea in blood such as diet rich in protein, kidney tube obstruction and age as well (23). The liver and kidney are two organs which were taken for histopathological studies. Because of using the oral route of administration and presence of any sign of toxicity would be most evident in those organs. In this study, the present examinations showed normal structural appearance in liver as well as in kidney. The absence of gross and histopathological lesions in the organs could suggest the level of safety of the aqueous extract on the animals. The result of this investigation was

agreement with studies (24) that had used Es for treatment role against oxidative stress produced by ethanol by increasing/maintaining the levels of antioxidant molecules and antioxidant enzymes, but different as compared to other studies (25) that had used the Es for its effect on the histological structure of some organs in mice where shows hypertrophy of the hepatic cells with the accumulation of glycoprotein granules while sections of the kidney did not show a remarkable changes. Many factors could be influenced the result which includes: different extraction methods used, the different animal used, plant materials sources and the part of the plant used for extraction can affect toxicity properties. Previous studies show that ethanolic extract of Es was subjected to chemical analysis and results indicated that alkaloids, terpenes, flavonoids, saponins, glycosides and steroids were present in the extract (26), this components possesses potential antioxidant and renal protective activity and preclude oxidative damage inflicted to the kidney (22). This finding is in accordance with that reported by (24) on rats, in which all treated rats did not produce any sign of toxicity or mortality when they were given ethanolic extract of *Eruca sativa* leaves. Thus, it was observed that ESELE was safe at doses up to 5 g/ kg/b.wt.

Conclusions, ESELE did not manifest any significant toxicity at any of the biochemical and histological examined parameters. Therefore, it can be concluded that ESELE can be used upon human up to 5 g/kg b.wt.

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