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Histological and Biochemical Studies on Liver of Female Rats Treated with Different Concentrations of Ethanolic Extract of *Arum palaestinum*

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ABSTRACT

In literature, there is few reports available about *Arum palaestinum* effects on liver integrity. So, this study was aimed to investigate the histopathological and biochemical effects of ethanolic leave extract of *Arum palaestinum* on the liver of albino rats. Fifteen female adult rats were randomly divided into three groups. Group 1(control group), group 2 and 3 received daily dose of 250 and 500 mg/kg body weight (B W) respectively, for four weeks. Mortality was observed in rats treated with 500 mg/kg BW. Final body weight at both doses and liver weight at the dose of 500 mg/kg BW were significantly higher($P<0.05$) than the control. The extract caused a significant increase ($P<0.05$) of glucose, high density lipoprotein (HDL), albumin, Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Alkaline phosphatise (ALP) values, at the dose of 500 mg/kg BW compared to the control. On the other hand, a significant reduction ($P<0.05$) of low density lipoprotein (LDL), triglycerides and globulin was observed. More histological changes like aggregation of lymphocytes, steatosis, congestion and necrosis were observed in the livers of rats received the high dose of the extract. For the first time, our findings indicate that *Arum palaestinum* can have toxic effects on liver cells which may induce hepatocyte necrosis and liver fibrosis.

KEYWORDS: *Arum palaestinum*, Electron microscope, Histology, Light microscope, Liver, Rats

INTRODUCTION

Arum palaestinum Boiss. is a species of herbaceous plant belonging to the Araceae family [1,2]. It is traditionally used in the Mediterranean cuisine and folk medicine [1,3]. It is one of about 26 species of Arum, a genus native to northern Africa, Europe, western Asia and in the Mediterranean region including Jordan. [2,4,5,6, 7].

The use of wild medicinal plants is still practiced by Jordanians [8]. In Jordan, it is commonly known as "al-loof" and considered edible after being soaked in salty water or dried. The plant is also used in folk medicine to treat several diseases such as stomach acidity, cancer, diabetes, circulatory system disorders, internal bacterial infections and obesity [2,4,5,6,9], kidney infections [10], constipation, acne, and prostate disorders [11,12]. Moreover, it was reported that the plant possesses an inhibitory effect on smooth muscle contraction in rats [13].

Leaves of arum are used by people of Jordan as a food as well as folkloric medicine. Its use in several types of food toxicity and skin diseases was reported [14]despite their possible side effects on the organs. The plant can cause vomiting and swelling in the mouth and throat mucous membranes [14,15,16]. Moreover, symptoms include skin irritant, blister formation, cardiac arrhythmia, spasms, low body temperature, internal bleeding and gastro-intestinal tract disturbance [17].

To the best of our knowledge, few studies have been reported on this plant [18,19,20], and no reports exist in the literature on the possible histological changes in the internal organs. It is important, therefore, to know the possible harmful side effects. Hence, this study aimed to examine the possible histological changes in the liver of rats exposed to *Arum palaestinum* extract and the possible effects of the extract on some biochemical parameters.

MATERIALS AND METHODS

This experiment was carried out in the Department of Pathology, Faculty of Medicine, the University of Jordan, Amman during 2015.

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Plant materials: The leaves of *Arum palaestinum* were purchased from a Market in Ajloun, (North Jordan) and identified by a faculty member at the department of Plant production, Al-Balqa Applied University, Al-Salt, Jordan.

Preparation of plant extracts: The leaves of the plant were prepared and extracted according to Ferrigni *et al,*. [21]. Plant parts were separated and washed with distilled water to remove dirt , dried for 14 days in the shade at room temperature and then grind using blender. Each 30 g of powdered plant was extracted by refluxing with 100 ml of ethanol solvent for two weeks at room temperature with shaking at 150 rpm. All extracts were filtered through White canvas and filter paper. Thereafter, solvent was evaporated until dryness. The crude extracts weighed and dissolved in 0.05% dimethyl sulphoxide (DMSO) (Sigma, Germany) to have a stock of 1g/ml concentration. All extracts were purified by filtration through 0.22 µm filter units and kept at -20°C until use.

Experimental design: Adult female Albino rats, weighing between 190 and 210 grams, were used in this study. They were maintained and kept in rat cages at the Animal House of the Faculty of Medicine. They were fed with standard rat chow and allowed free access to clean fresh water in a bottle *ad libitum*. The rats received the doses orally via stomach tube and were grouped into three groups: Group 1 was the control group. Group 2 received 250 mg/kg BW of the ethanolic extract of *Arum palaestinum* leaves on a daily basis and group 3 was administered 500 mg/kg BW of the extract for duration of four weeks. The study was reviewed and accepted by the scientific research committee at Al-Balqa Applied University and animal procedures were performed in accordance with the recommendations of the National Institutes of Health [22].
At the end of the experimental period all rats were sacrificed and liver tissues were immediately taken from each animal for histopathological and biochemical analysis.

Histological study

Light microscopy: Small pieces of the liver were taken immediately after the rats were sacrificed and fixed in 10% formaldehyde for 24 hours. The specimens were transferred into an automated processor where they were dehydrated in ascending different concentrations of ethanol and cleared by xylene. The dehydration step was as follows: 80% ethanol for 1 hour, 95% ethanol 3 changes for 1 hour each. Then100% ethanol 2 changes for 1 hour each. The tissues were then cleared in xylene 2 changes for 2 hours. After that the specimens were infiltrated in molten paraffin wax 2 changes for one hour and a half each. When the samples were processed they were taken and embedded in paraffin wax. Sections of 5µm thick were cut by rotary microtome and stained by Hematoxylin & Eosin (H & E) for routine histological examination. The tissue sections were evaluated for histological changes under Zewiss compound light microscope and photomicrographs were taken.

Electron microscopy: The scarified animals that were used for light microscopy investigations were used also for having specimens for transmission electron microscopy. The samples taken from the liver were cut into about $1mm^3$ specimens and immersed in 3% glutaraldehyde in 0.1 M sodium phosphate buffer, pH 7.3, for 24 hours at 4ºC to fix them and then routinely post fixed with 2% osmium tetroxide. After that, dehydration was done using a graded series of acetone(75%, 95% and 100% respectively). Then, the samples were put in propylene-oxide for 30 minutes. Finally, the samples were infiltrated and embedded in Araldite medium and left in for 24 hours. Ultrathin sections as well as semi thin sections were obtained using an ultramicrotome with glass knife. The ultrathin sections were mounted on copper grids. Then, these sections were stained with uranyl acetate and lead citrate. Finally, the stained sections on grids were studied under transmission electron microscope.

Blood collection and Biochemical analysis: Rats were sacrificed after 24 hours of the last dose of extract. Under anaesthetized with diethyl ether, blood samples were collected directly from the eye in plain test tubes then centrifuged at 2500 rpm for 5 minutes to separate the serum to be used for various parameters. Blood glucose was measured by glucometer. Laboratory analyses were performed at Jabal Al-Hussein Medical Laboratories, Amman, Jordan. Serum was used for all biochemical assays and parameters were measured using commercial kits according to the instructions of manufacturer.

Statistical analysis: All data were expressed as means ± SD. Student's t-test was used to compare the mean values of test groups and control. Differences in mean values were considered significant at $p < 0.05$.

RESULTS

Body weight and liver weight: Body weight gradually increased at both doses comparable with that of control. Final body weight at both doses was significantly higher ($P < 0.05$) than in the control (Table 1). There was no abnormal signs of toxicity or death observed after the 30 days of treatment at the dose of 250 mg/kg. Liver weight at the dose of 500 mg/kg was significantly higher than in the control; however there was no significant difference at the dose of 250 mg/kg compared to the control. Mortality was observed in rats received 500 mg/kg of ethanol leaves extract of arum after 15 days (Table 2).

Biochemical findings: As shown in Table 3, arum caused a significant increase ($P< 0.05$) of glucose, HDL, albumin, ALT, AST and ALP values, at the dose of 500 mg/kg compared to the control. On contrary, a significant reduction ($P< 0.05$) of LDL, triglycerides and globulin was observed. Total cholesterol and total protein remained unaltered. At the dose of 250 mg/kg, all the parameters except glucose and albumin significantly decreased ($P < 0.05$). Significant changes of most biochemical parameters between the two doses was also observed.

Histopathological findings

Light Microscopic Results: The normal histology of liver in control rats is shown in Figure 1. This figure shows normal hepatocytes, normal central vein and normal sinusoid. The prominent histological change in both animals treated with 250 mg/Kg body weight and 500 mg/Kg of extract was necrosis as shown in Figures 3 and 4. Large necrotic regions is recorded in rats treated with high dose of the extract compared with necrotic regions in animals treated with 250 mg/Kg body weight. Figure 2 shows lobular activity indicated by aggregation of lymphocytes in rats treated with 250 mg/Kg body weight.

Rats treated with both doses of the extract have congested liver compared to the control. The congestion was found in the blood vessels and in the sinusoids but it was more sever in rats treated with high dose of the extract as shown in Figures 2,3,4 and 6. Figure 5 shows fat deposition regions which resemble steatosis.

Transmission Electron Microscopic (TEM) Results: The liver normal histology and ultra structure of control rats is demonstrates in Figure 7. Congestion of RBCs that was reported under light microscope is also seen under the electron microscope as aggregation of RBCs. In Figure 8 less RBCs are aggregated in liver of animals treated with 250 mg/Kg of arum compared with numerous RBCs that are aggregated in liver of rats treated with 500 mg/Kg of the extract as illustrated in Figure 9.

The striking necrotic effect of the two doses of the extract on the hepatocytes is confirmed when the liver samples are studied under the electron microscope. Partially disrupted nucleus is obvious in Figure 8 confirming the mild necrosis of hepatocytes in rats treated with low dose of the extract. Adding to that sever necrotic hepatocyte of high dose treated rats is appeared as sequential disruption of mitochondria (Figure 9) and partially disrupted mitochondria as well as partially disrupted nucleus (Figure 10).

Steatosis is only reported in the cytoplasm of hepatocytes when high dose of the extract is administered. The accumulation of lipid droplets lead to this effect is illustrated in Figure 9.

Table 1: Body weight of rats with different treatments of *Arum palaestinum* extract

Body weight	Control	Treated rats	
		Dose 250 mg/kg BW	Dose 500 mg/kg BW
Initial weight (g)	196.0^{a} ±4.90	211.0^{ab} ±10.20	210.0^{ab} ±12.65
Final weight (g)	202.6^{a} ±15.59	223.2^{b} ±1.83	219.8^{b} ±12.25
Difference (g)	6.00	12.00	9.00
Difference %	3.06	5.69	4.29
Weight gain (g/day)	0.21	0.43	0.32

Data are expressed as means ± Standard Deviation of five rats.
$^{a, b}$:Different letters indicate statistically significant differences in the same row ($P < 0.05$).

Table 2: Mean (± SD) values of liver weight of rats with different treatments of *Arum palaestinum*

Parameters	Control	Treated rats	
		Dose 250 mg/kg BW	Dose 500 mg/kg BW
Liver weight (g)	8.24^{a}±1.32	8.94±0.73	9.42^{b}±0.51
Liver index	4.07	4.07	4.22
Lethality	0	0	2/5
Remarks	No significant change was observed		Two rats died on day15

Data are expressed as means ± Standard Deviation of five rats.
$^{a, b}$: Different letters indicate statistically significant differences in the same row ($P < 0.05$).
(The liver index was calculated as liver weight / body weight × 100)

Table 3: Biochemical parameters of rats under normal and experimental conditions

Biochemical parameters	Control	Treated rats	
		Dose 250 mg/kg BW	Dose 500 mg/kg BW
Glucose (mg/dl)	95.20^{a}±10.303	125.40^{b}±17.895	120.20^{b}±8.704
Total cholesterol (mg/dl)	69.80^{a}±8.084	63.00^{b}±5.404	71.60^{a}±12.467
Triglycerides (mg/dl)	85.40^{a}±26.097	83.40^{b}±14.094	84.20^{b}±13.644
HDL (mg/dl)	22.60^{a}±2.278	19.38^{b}±2.224	25.78^{c}±2.977
LDL (mg/dl)	33.56^{a}±7.876	26.38^{b} ±1.395	28.98^{bc}±10.316
Total Protein (g/dl)	8.02^{a}±0.261	7.64^{b}±0.269	8.02^{a}± 0.132
Albumin (g/dl)	2.88^{a}±0.375	3.06^{b}±0.056	3.12^{c}±0.413
Globulin (g/dl)	5.14^{a}±0.357	4.58^{b}±0.230	4.91^{c}± 0.472
ALT (U/L)	90.60^{a}±31.260	71.40^{b}±4.079	150.40^{c}±76.828
AST (U/L)	169.80^{a}±46.279	131.00^{b}±13.690	237.20^{c}± 85.412
ALP (U/L)	228.00^{a}±66.995	152.64^{b}±18.518	320.80^{c}± 104.667

Data are expressed as means ± Standard Deviation of five rats.
$^{a, b, c}$: Different letters indicate statistically significant differences in the same row ($P < 0.05$).

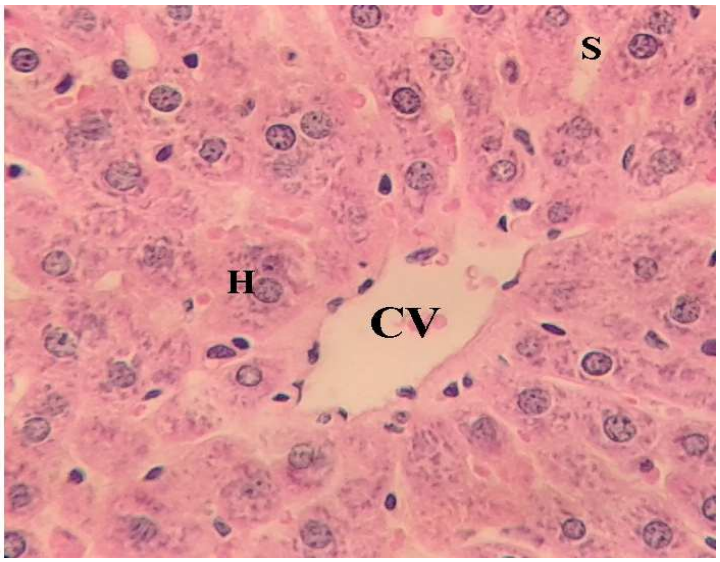

Fig. 1: Liver of control rats showing normal hepatocytes (H), normal central vein (CV), sinusoid (S). (H & E stain, 400X)

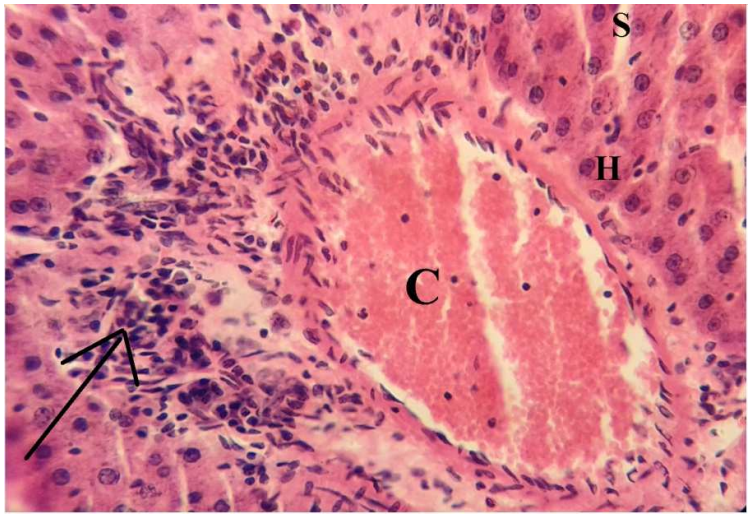

Fig.2: Liver of treated rats with dose 250 mg/kg BW showing lobular activity i.e., aggregation of lymphocytes (→) and congestion in blood vessels (C). (H & E stain, 400X)

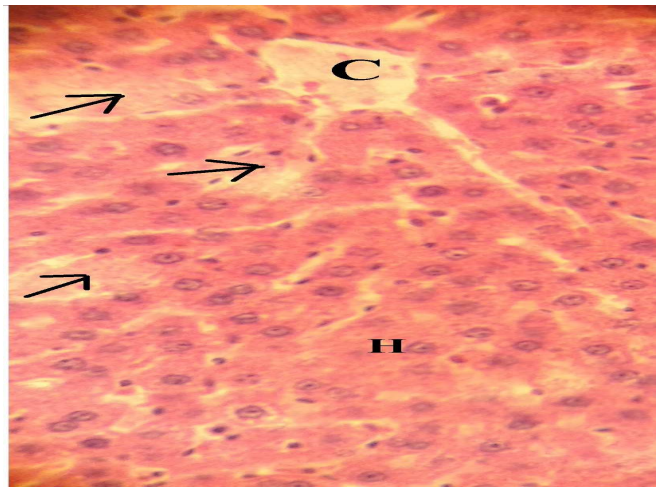

Fig. 3: Liver of treated rats with dose 250 mg/kg BW showing necrosis(→), congestion in blood vessels (C) and hepatocytes (H). (H & E stain, 400X)

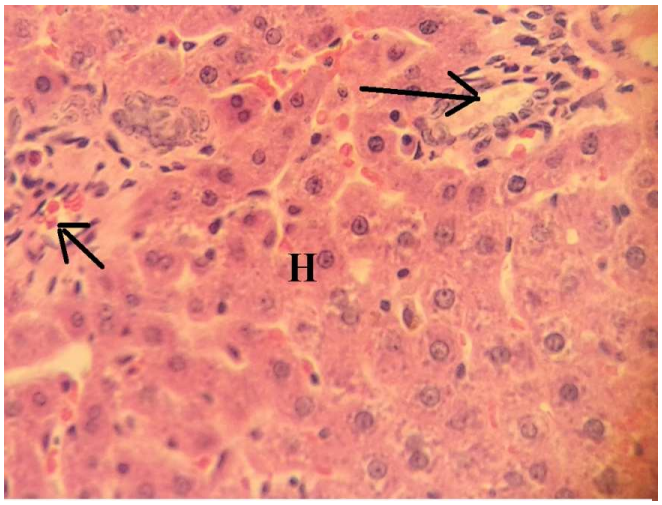

Fig. 4: Liver of treated rats with dose 250 mg/kg BW showing abnormal blood vessel (large arrow), congestion in sinusoid (small arrow) and hepatocytes (H). (H & E stain, 400X)

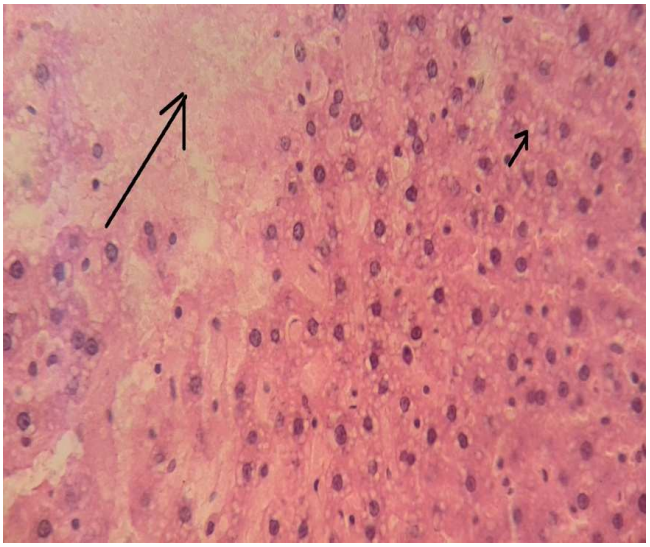

Fig. 5: Liver of treated rats with dose 500 mg/kg BW showing sever necrosis (large arrow) and steatosis (small arrow). (H & E stain, 400X)

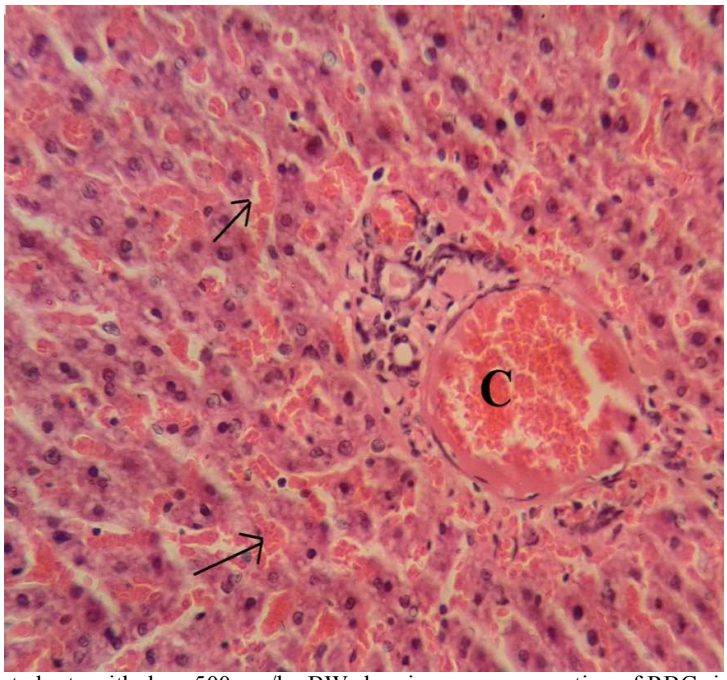

Fig. 6: Liver of treated rats with dose 500 mg/kg BW showing sever congestion of RBCs in blood vessels (C) and in sinusoids (arrow). (H & E stain, 400X)

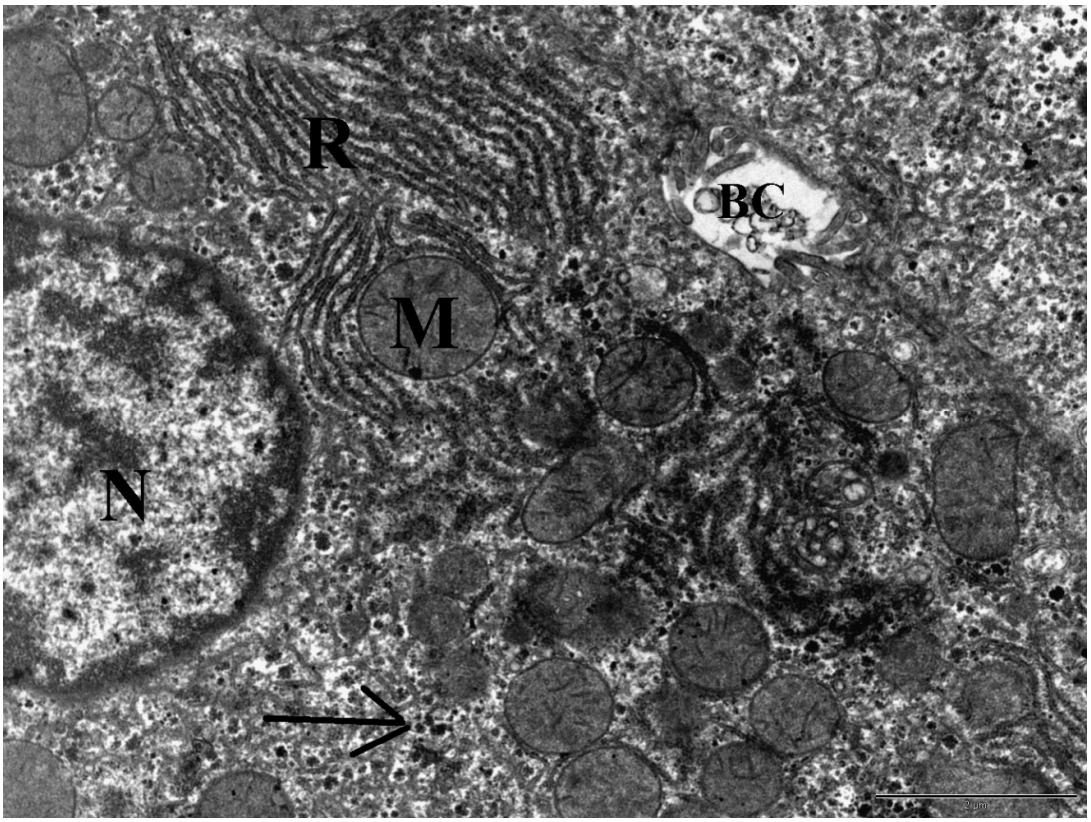

Fig. 7: Electron micrograph of control rat showing normal ultra-structure of the liver. N: nucleus ; M: mitochondria ; R: Rough Endoplasmic Reticulum ;
BC: bile canalculus; →: glycogen granules. (mag. 8900X).

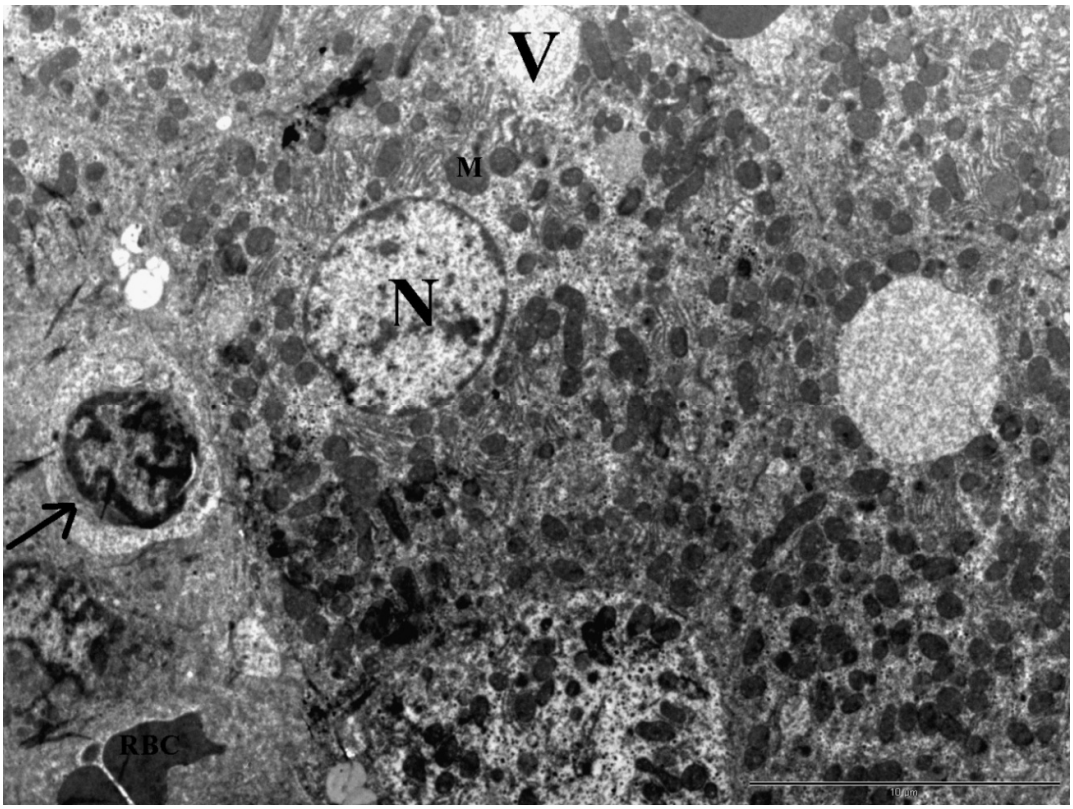

Fig. 8: Electron micrograph of low dose (250 mg/kg BW) treated rat showing abnormal ultra-structure of the liver. N: nucleus; RBC: red blood cell; M: mitochondria; V: vacuole arrow: partially disrupted nucleus. (mag. 4400X).

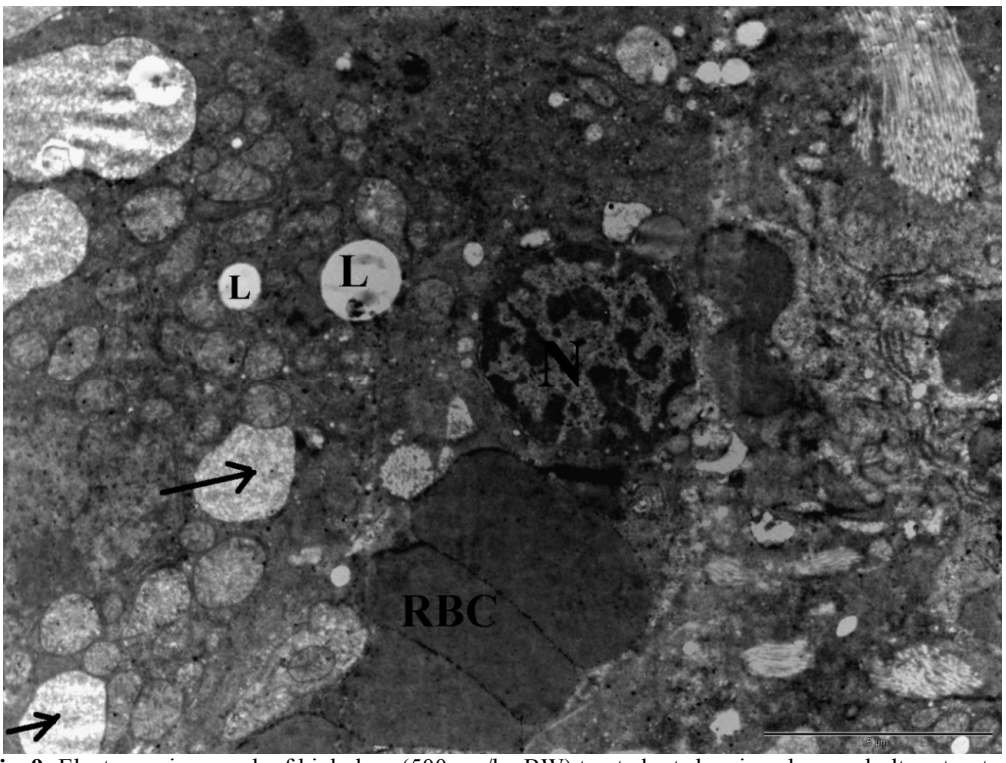

Fig. 9: Electron micrograph of high dose (500 mg/kg BW) treated rat showing abnormal ultra-structure of the liver. N: nucleus; RBC: red blood cell; L: lipid droplet; →: sequential disruption of mitochondria. (mag. 4400X).

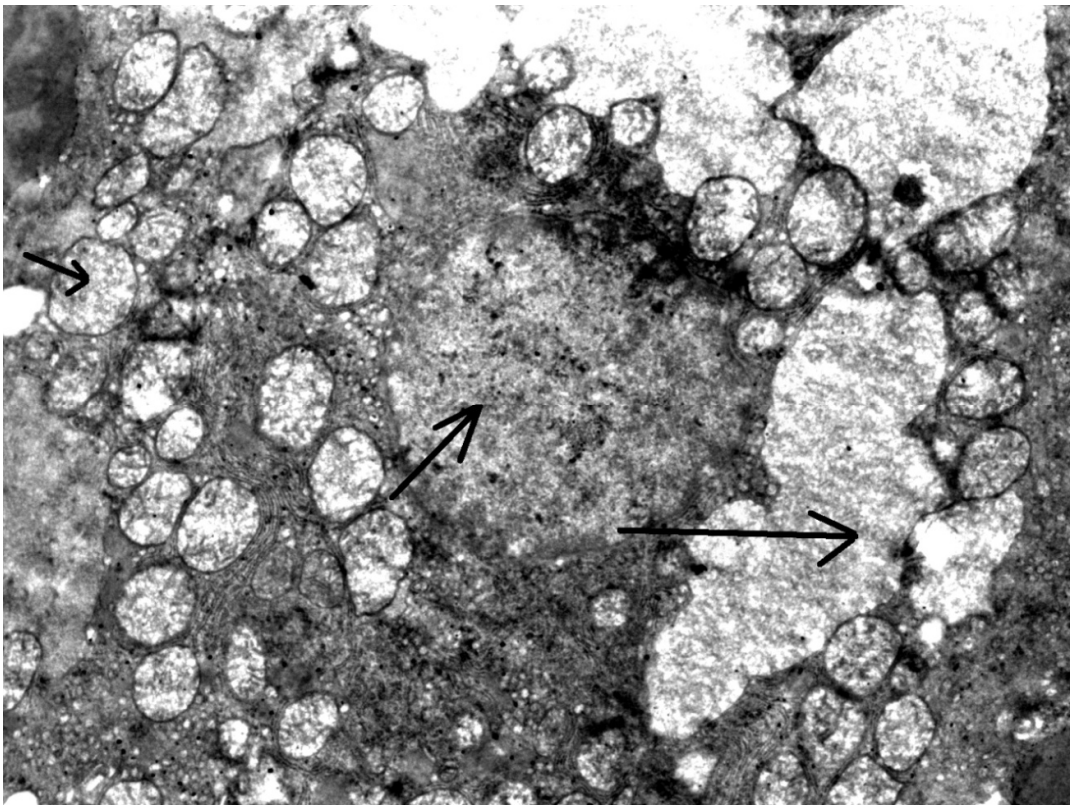

Fig. 10: Electron micrograph of high dose (500 mg/kg BW) treated rat showing abnormal ultra-structure of the liver. Small arrow: partially disrupted mitochondrion; Medium arrow: partially disrupted nucleus; Large arrow: disrupted mitochondria. (mag. 4400X).

DISCUSSION

Liver is a vital organ involved in regulation of many functions in the body, such as detoxification and protein synthesis [23]. A recent study on mice performed to examine the effect of *Arum palaestinum* Boiss on growth of tumor, demonstrated an effect of the plant extract on mice but without signs of toxicity; the effect was on suppressing prostate cancer cells and prostate tumors [24].

To the best of our knowledge, this is the first publication that studies the possible histological changes on the liver of rats exposed to *Arum palaestinum* extract. The results of this study showed abnormalities in sections of the liver rats treated with two doses for 30-days when compared with the control group. Severe abnormalities were observed in sections from rats treated with the high dose (500 mg/kg/day). No similar reports were found in literature to be compared with the histological effect of *Arum palaestinum* extract and therefore, the results of this study were compared to the histological effect for other plants.

Elevation of AST, ALT and ALP level in serum of treated rats at the dose of 500 mg/kg/day observed in the current study, indicates that plant extract induced liver impairment. This agree with earlier studies where elevated hepatic markers were reportedly [25,26,27] . This elevated levels of AST, ALT and ALP The elevation of liver enzyme levels in serum could be due to a damage in parts of liver tissue that may affect the cell membrane of hepatocytes leading to loss in its functional integrity as well as cellular leakage [28 , 29]

The results of a study conducted to evaluate the effects of the leaf extract of Lawsonia inermis plant (Henna), showed that, at a dose of 200 mg, no pathological changes were evident for liver sections of male rats. However, at a dose of 1000 mg significant pathological changes were observed in the liver [30]. In another study, rats were administered with 300 mg/kg of the aqueous extract of Kohautia grandiflora for one week. The extract had no significant effect on the body weight of the rats. A significant increase in the levels of AST, ALT and ALP was observed. Histopathological evaluation of the liver sections showed necrosis, congestion of the venous sinusoids and mononuclear cell infiltration [31].

The histological structure of liver and some biochemical parameters in experimental groups of rats injected with G. glabra aqueous extract at different doses for 30 days, shows a decrease in diameter of both hepatocytes and hepatocytes nucleus. Also, serum levels of ALP, AST and ALT revealed a significant increase in 200 mg/kg extract received animals [9]. Harizal *et al*. [32] reported that the methanolic extract of Mitragyna speciosa in all treated groups had some abnormal morphology characteristics and induces severe hepato-toxicity in rat liver.

At cellular and subcellular level the changes in nuclei, mitochondria and cytoplasm of treated rats' hepatocytes could be a result of plant extract metabolism in the liver in the form of increasing metabolic activity. Adding to that, necrosis may be due to incapability of liver to regenerate and renew hepatocytes and this in correlation with the detoxification process to get rid of toxicants [33 , 34] .

At tissue level, the changes that extends from congestion in blood vessels to congestion in sinusoids could be attributed to destruction in blood vessels since liver tissue is highly vascularised.

The active compounds in *Arum palaestinum* and related species, are aroin, cyanogenic glucosides, saponins, Ca++ oxalate raphides. As a mode of action , raphides penetrate mucosal cells, facilitate the entry of toxins. Toxins, interfere with central functions in an animal leading to liver disturbances[17]. Recently, a qualitative identification of 180 phytochemical metabolites in *Arum Palaestinum* leaves has been established, highlighting it as an abundant source of antioxidant phenolics and phytochemicals [1].

CONCLUSION

To the best of our knowledge, this is the first publication about the possible histological changes in the internal organs of rats exposed to *Arum palaestinum* extract. In conclusion, *A. palaestinum* can have toxic effects on liver cells which may induce hepatocyte necrosis and liver fibrosis. This study has shown that the studied plant has toxic potentials. Caution should therefore be taken in their use for medicinal purposes. However; more research is needed to clarify the issue. A long-term study using a different animal model and different doses should be performed to validate the findings of the present study.

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