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Acute toxicity of *Pistacia atlantica* green seeds on Sprague-Dawley rat model

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Abstract

The acute toxic effect of the aqueous extract of *Pistacia atlantica* green seeds was investigated in groups of Sprague-Dawley rats at oral doses of 200, 400, and 800 mg/kg body weight. After 14 days, there was no significant difference ($p>0.05$) in behavior, food and water consumption, body weight gain, and hematological and serum biochemical parameters between untreated control and extract-treated rats. There were lesions in the liver and kidney indicating that aqueous *P. atlantica* seed extract causes some hepatoxicity and nephrotoxicity in rats.

Introduction

Traditionally, wild plant species are being consumed as food and used to treat various ailments. Some of these plants may cause deleterious and disastrous health consequences. Thus, it is essential that toxicology trials are conducted on these plants and their products to determine their safety for human consumption.

Pistacia atlantica is a species of flowering trees from the family Anacardiaceae [1]. The plant is commonly known as wild pistachio, Mount Atlas mastic tree or turpentine tree [2]. In Kurdistan, it is known as *daraban* and the seed as *qazwan*. In Saudi Arabia, it is called *butum* [3] while in Iran it is known as *baneh* [2]. Wild pistachio is a deciduous tree that can reach 7m in height and approximately 1m in diameter. The plant is widely distributed, particularly in areas between the Irano-Turanian region and North Africa [3]. *P. atlantica* is economically valuable as it produces a resin that is used as chewing gum, mouth freshener, appetizer, laxative, the remedy for gastrointestinal disorders [1], wound healing, and to alleviate joint pains [4]. The ripe seeds are salted and consumed as nuts while the green seeds are eaten raw or used to flavor yogurt drinks. In medicine, the seeds are used as an antitussive and antidiarrheal, and to treat upset stomach [5-8].

Although the seeds of *P. atlantica* are widely used as food and alternative medicine, little is known about its toxicity. Hence, this study investigated the effect of *P. atlantica* green seeds' aqueous extract on

Sprague-Dawley rats. The aim of the study is to provide a better understanding of the possible deleterious effects following consumption of the plant seeds.

Materials and Methods

Preparation of plant extract

Fresh green *P. atlantica* seeds were purchased from a local market in Sulaimani province of Northern Iraq. The seeds were washed, ground using a juicer machine, and mixed with distilled water at 1:10 (w/v). The mixture was then continuously agitated for approximately 30 minutes before filtering with surgical gauze followed by Whatman no. 4 filter paper. The filtered mixture was freeze-dried and the resultant powdered extract stored in a tightly closed glass container at -20°C.

Laboratory animals

Twelve male and 12 female 7-8 weeks old Sprague-Dawley rats were used in the experiment. The rats were housed in plastic cages (2 rats/cage) under a 12 hours light/dark cycle at an ambient temperature of 26±2°C. The rats were acclimatized for 7 days before experimentation while provided with standard commercial pellet and clean water *ad libitum*.

The rats were randomly divided into four groups; Group 1 served as the control and was drenched with distilled water; Group 2 (T1), Group 3 (T2), and Group 4 (T3) were given 200, 400, and 800 mg/kg body weight, respectively, of freshly prepared aqueous *P. atlantica* seed extract for 14 days by intragastric gavage.

On day 15, the rats were anesthetized by intramuscular administration of 75 mg/kg body weight ketamine and 2 mg/kg body weight xylazine. The abdominal cavity was opened and blood collected from the caudal vena cava into heparinized tubes for hematological and plain tubes for biochemical analyses. After inspecting internal organs for pathological changes, the heart, liver, spleen, kidneys, brain, and lungs were collected, washed with saline solution and weighed. The organs were fixed in 10% neutral buffered formalin. Bone marrow samples were taken from the femur for microscopic examination.

Histopathological examination

Slides of preserved organ samples were stained with hematoxylin and eosin as described by Luna (1968) [9]. The histological slides were examined and the lesions scored using the blind study method. Ten random sections were taken from each slide and the lesions were scored as follows; 0: 0-25%, 1: 26-50%, 2: 51-75%, and 3: >75% of the organ sections abnormally affected [10].

Hematological parameters

Hematological parameters analyzed were erythrocyte, thrombocyte, and total and differential leukocyte counts, hemoglobin concentration, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC).

Serum biochemistry

Blood samples in plain tubes were centrifuged at 3500 rpm to collect serum. Serum total protein, albumin, glucose, cholesterol, bilirubin, creatinine, urea concentrations and alanine transaminase (ALT), aspartate transaminase (AST), γ-glutamyl transaminase (GGT), and lactate dehydrogenase (LDH) activities were determined spectrophotometrically.

Statistical analysis

Data were analyzed using one-way ANOVA, followed by the Duncan *post hoc* test. A probability value <5% was considered statistically significant.

Results

Animal weight and behavioral changes

All rats treated with aqueous *P. atlantica* seed extract showed normal feed and water intake. The treatment did not cause any behavioral change or mortality in the rats. Also, there was no sign of illness in the rats throughout the course of treatment.

There was no significant difference (p>0.05) in weight gain between the control and *P. atlantica* extract-treated rats (Table 1).

Table 1. Weight of rats treated with *Pistacia atlantica* extract

Group	Initial weight (g)	Weight after treatment (g)	Weight gain (g)	Weight gain (%)
Control	150.20±4.06	168.53±3.12	18.33±1.03	12.23±0.98
T1	148.88±3.35	168.20±3.57	19.32±1.29	12.98±0.90
T2	149.22±2.97	167.18±4.92	17.97±2.91	12.03±1.90
T3	149.92±2.97	167.55±2.19	17.63±0.86	11.78±0.80

Values represent the mean of group ± SD (n=6). Control group administered distilled water. Group T1, T2, and T3 treated with 200, 400 and 800 mg/kg body weight aqueous *P. atlantica* seed extract, respectively. No significant difference (p>0.05) was detected among means within column. Statistical analysis: one-way ANOVA with *post hoc* Duncan test.

Postmortem findings and organ weights

The organ weights and the relative organ-to-body weights of heart, liver, spleen, kidneys, brain, and lungs of the rats are shown in Table 2. After 14 days of treatment, the internal organs did not show any abnormality at autopsy. The weight of all organs examined did not differ significantly (p>0.05) between control and treatment groups. However, treated rats were observed to show slightly lower average total kidney and higher total lung weights than the untreated control.

Table 2. Organ and relative organ-to-body weights of rats treated with *Pistacia atlantica* extract

Parameter		Group			
		Control	T1	T2	T3
Organ weight (g)	Heart	0.58±0.05	0.58±0.05	0.55±0.03	0.54±0.03
	Liver	5.42±0.10	5.43±0.27	5.30±0.17	5.40±0.24
	Spleen	0.47±0.08	0.45±0.05	0.50±0.09	0.50±0.09
	Kidneys	1.07±0.08	1.05±0.10	1.02±0.08	1.00±0.09
	Brain	1.25±0.05	1.23±0.08	1.20±0.09	1.22±0.08
	Lungs	1.03±0.08	1.07±0.10	1.08±0.08	1.10±0.06
Relative organ to body weight (%)	Heart	0.34±0.02	0.34±0.03	0.33±0.02	0.32±0.02
	Liver	3.22±0.09	3.23±0.13	3.17±0.10	3.22±0.11
	Spleen	0.28±0.04	0.27±0.03	0.30±0.05	0.30±0.05
	Kidney*	0.63±0.04	0.62±0.06	0.61±0.04	0.60±0.05
	Brain	0.74±0.03	0.73±0.04	0.72±0.04	0.73±0.04
	Lungs	0.61±0.04	0.63±0.05	0.65±0.04	0.66±0.04

*total weight of right and left kidneys. Values represent mean of group ± SD (n=6). Group T1, T2, and T3 treated with 200, 400 and 800 mg/kg body weight aqueous *P. atlantica* seed extract, respectively. No significant difference (p>0.05) among means within row. Statistical analysis: one-way ANOVA with *post hoc* Duncan test.

Hematological parameters

Aqueous *P. atlantica* seed extract treatment did not affect the hematological parameters (Table 3) of rats because no difference in the hematological parameter values was observed among treatment groups or between control and treatment groups. However, the average erythrocyte counts and hemoglobin concentrations were slightly higher in the control and T1 than the T2 and T3 groups, but these differences were not statistically significant (p>0.05). Other hematological parameters did not differ among groups of

rats. These results indicate aqueous *P. atlantica* seed extract treatment does not cause an adverse effect on the blood parameters of rats.

Table 3. Hematological parameters of rats treated with *Pistacia atlantica* extract

Parameter	Group			
	Control	T1	T2	T3
Erythrocytes ($\times10^{12}$/L)	7.09±0.17	7.09±0.27	6.99±0.23	7.03±0.16
Hematocrit (L/L)	40.50±0.54	39.83±1.47	39.66±1.50	40.50±1.04
Hemoglobin (g/L)	135.00±2.09	131.50±2.58	132.50±3.33	131.83±2.85
MCH (pg)	19.03±0.64	18.55±0.79	18.98±0.61	18.95±0.37
MCHC (g/L)	33.35±0.48	33.06±1.05	33.50±0.58	32.70±0.59
MCV (fL)	57.00±1.41	56.33±1.86	56.67±2.25	57.67±1.50
Total Leukocytes ($\times10^{9}$/L)	9.78±0.99	9.53±2.16	8.85±2.21	9.40±2.26
Neutrophils ($\times10^{9}$/L)	2.46±0.52	2.42±0.75	2.23±0.75	2.44±0.54
Lymphocytes ($\times10^{9}$/L)	6.37±0.56	6.11±1.30	5.66±1.40	5.98±1.76
Monocytes ($\times10^{9}$/L)	0.59±0.17	0.64±0.22	0.58±0.28	0.60±0.16
Eosinophils ($\times10^{9}$/L)	0.35±0.11	0.35±0.07	0.37±0.12	0.35±0.14
Basophils ($\times10^{9}$/L)	0	0	0	0
Thrombocytes ($\times10^{9}$/L)	1230.0±60.3	1236.7±53.2	1240.0±62.3	1253.3±65.6

Values represent mean of group ± SD (n=6). Group T1, T2, and T3 were treated with 200, 400 and 800 mg/kg body weight aqueous *P. atlantica* seed extract, respectively. No significant difference ($p>0.05$) was recorded among means within row. Statistical analysis: one-way ANOVA with *post hoc* Duncan test. MCH = mean corpuscular hemoglobin. MCV = mean corpuscular volume. MCHC = mean corpuscular hemoglobin concentration.

Serum biochemistry

The AST activity of rats treated with aqueous *P. atlantica* seed extract was observed to be generally lower than the untreated control group (Table 4). The serum cholesterol concentration was highest in rats treated with 800 mg/kg body weight aqueous *P. atlantica* seed extract, which was the highest dose used in this study. However, the difference in these values among groups was not significant ($p>0.05$).

Table 4. Serological tests of rats treated with *Pistacia atlantica* extract

Parameter	Group			
	Control	T1	T2	T3
Albumin (g/L)	45.53±3.56	45.93±1.74	46.30±2.12	46.05±1.60
ALT (U/L)	52.61± 5.07	51.65±4.55	53.65±5.88	50.85±5.74
AST (U/L)	121.01±13.57	117.83±17.41	114.75±15.91	112.45±16.83
Cholesterol (mmol/L)	1.71±0.25	1.74±0.08	1.75±0.21	1.80±0.14
Creatinine (µmol/L)	69.33±6.53	70.83±5.84	71.33±3.83	69.50±3.93
GGT (U/L)	<3.00	<3.00	<3.00	<3.00
Glucose (mmol/L)	9.41±0.76	9.45±0.56	9.75±0.51	9.43±0.45
LDH (U/L)	1076±176	1190±111	1183±123	1158±160
Total bilirubin (µmol/L)	0.36±0.10	0.31±0.11	0.30±0.08	0.36±0.12
Total protein (g/L)	69.73±3.79	69.53±3.16	70.03±1.99	69.81±3.43
Urea (mmol/L)	7.11±0.41	7.31±0.27	6.95±0.22	7.28±0.33

Values represent mean of group ± SD (n=6). Group T1, T2, and T3 treated with 200, 400 and 800 mg/kg body weight aqueous *P. atlantica* seed extract, respectively. No significant difference ($p>0.05$) among means within row. Statistical analysis: one-way ANOVA with *post hoc* Duncan test. ALT = alanine transaminase; AST = aspartate transaminase; GGT = gamma-glutamyl transpeptidase; LDH = lactate dehydrogenase**.**

Histopathological changes

The spleen, heart, brain, lungs, and bone marrow did not show any histological abnormality in all groups of rats (photomicrographs not included). However, the liver parenchyma showed central vein dilation and narrowing of the sinusoids in the treatment groups, unlike the normal liver architecture of the untreated control (Figure 1). Focal centrilobular leukocytic infiltration, hydropic degeneration, and ballooning hepatocyte degeneration were observed in the livers of the T2 and T3 rats.

The kidneys of T1 rats exhibited glomerular capillary congestion with focal segmental glomerulosclerosis. There was dilation of the Bowman's space and swelling of the epithelial lining of the proximal and distal convoluted tubules and loops of Henle (Figure 2). Interstitial tissue hemorrhage was also evident in the kidneys of T1 rats. The T2 rats showed lobulation of the glomerular tuft, and moderate collecting tubular degeneration with accumulation of necrotic debris in the lumen of the tubules. The T3 rats showed marked tubular and loop of Henle degeneration and dilated Bowman's space.

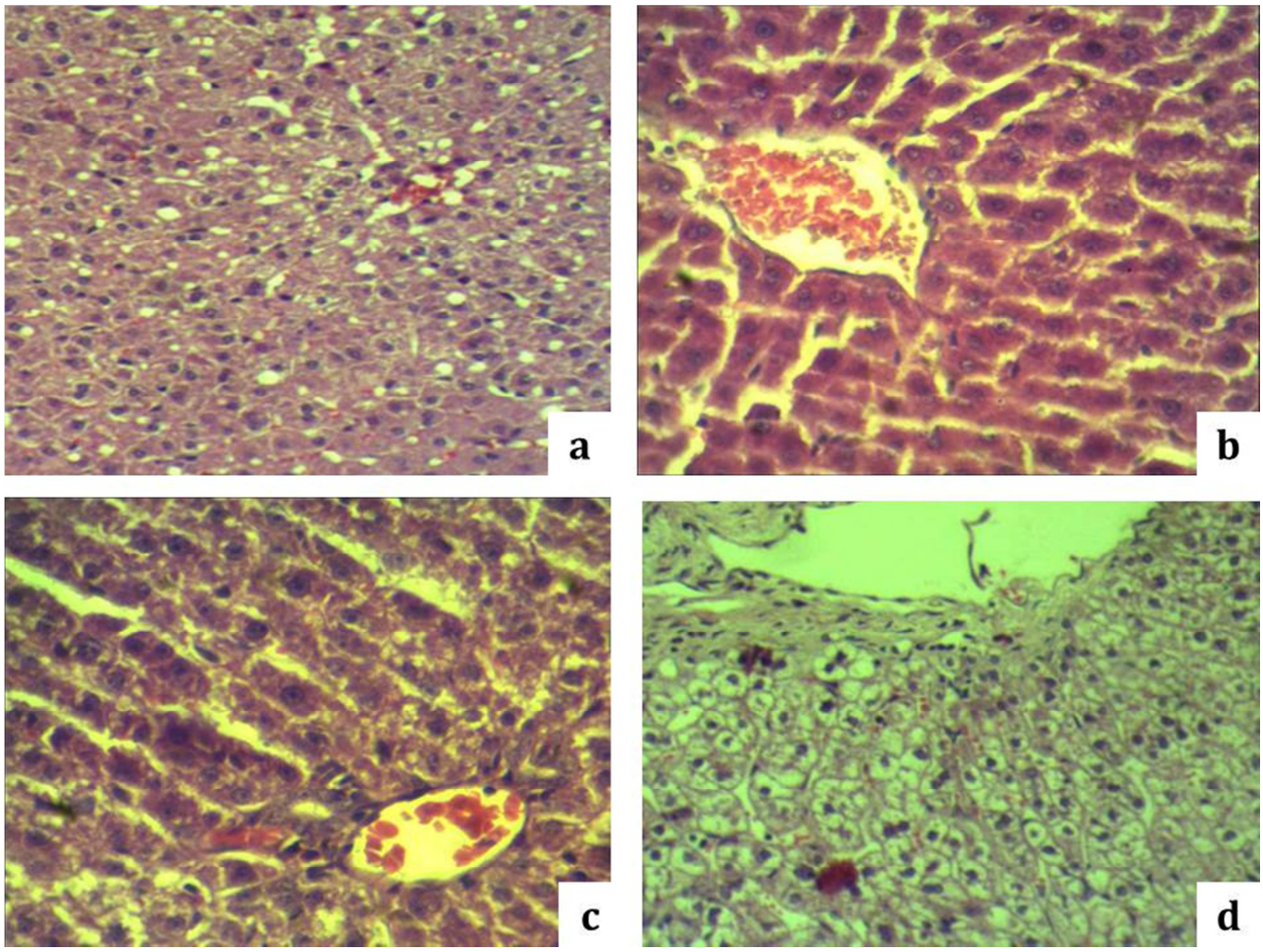

Figure 1. Liver sections of rats treated with aqueous *Pistacia atlantica* seed extract. a: Control group with normal liver parenchyma (100X). b: Group T1 with central vein dilation, swollen hepatocytes, and narrowing of sinusoidal capillaries (400X). c: Group T2 with central vein dilation and hepatocyte hydropic degeneration (400X). d: Group T3 with central vein dilation, focal centrilobular leukocytic infiltration, and ballooning hepatocyte degeneration (ballooned hepatocytes are markedly enlarged with diluted cytoplasm) (400X). Control group was given sterile water. Group T1, T2, and T3 treated with 200, 400, and 800 mg/kg body weight of *P. atlantica* extract, respectively. H&E stain.

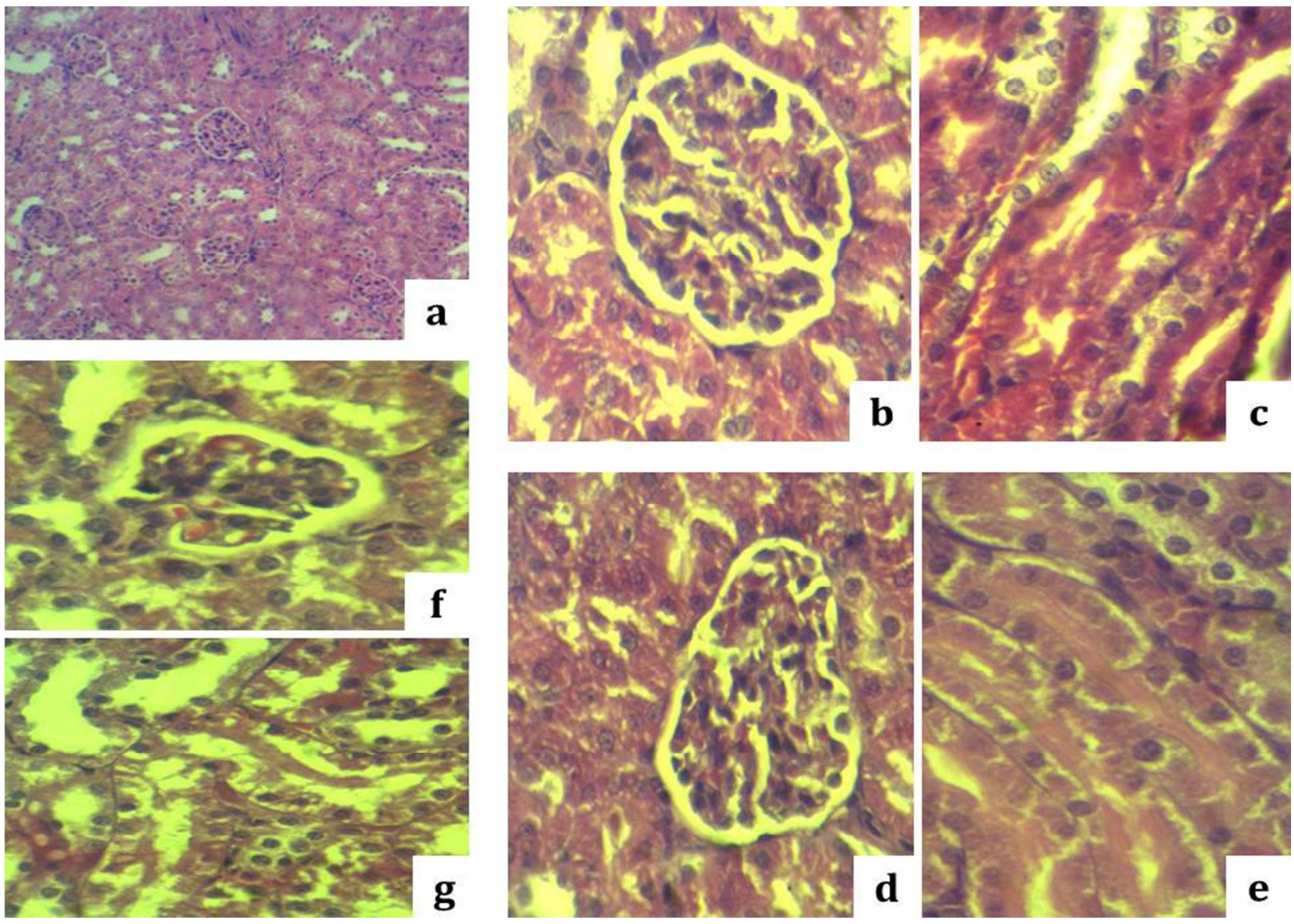

Figure 2. Kidney sections of rats treated with aqueous *Pistacia atlantica* seed extract. a: Control with normal kidney structures (40X). b: Group T1 with glomerular capillary congestion, dilated Bowman's space, and variable swelling of the proximal and distal convoluted tubular epithelial lining. c: Group T1 with interstitial tissue hemorrhage, a swollen loop of Henle epithelial lining, blurry cytoplasm with a star-shaped lumen (400X). d: Group T2 with lobulation of the glomerular tuft. e: Group T2 with a moderate loop of Henle degeneration of and presence of necrotic debris in the lumen of the loop (400X). f: Group T3 with marked proximal and distal convoluted tubular degeneration, and dilated Bowman's space. g: Group T3 with a marked loop of Henle degeneration (400X). Control group was given sterile water. Groups T1, T2, and T3 were treated with 200, 400, and 800 mg/kg body weight *P. atlantica* extract, respectively. H&E stain.

Discussion

The study examined the acute toxic effects of aqueous *P. atlantica* seed extract in rats. Among signs of toxicity of administered compounds are morbidity, mortality, and pharmacological or toxicological abnormalities [11]. Behavioral changes in toxicity are usually the result of neurological disorders and autonomic dysfunctions. In this study, the aqueous *P. atlantica* seed extract did not cause mortality or behavioral changes and the microscopic appearance of the brain tissue of these rats was normal, indicating that the plant product has no adverse effects on nervous tissue integrity.

Body weight and body weight gain are also used to determine the toxic effects of administered compounds and chemicals [12]. In fact, the Society of Toxicologic Pathology recommends the inclusion of organ weight data in all toxicity studies, whether acute or chronic [13], to determine if compounds intended for therapeutic purposes are detrimental to health [14]. Aqueous *P. atlantica* seed extract did not affect the body weight, body weight gain, organ weight of rats in this study.

Hematopoiesis is a dynamic process that results in a fast turnover of blood cells. The hematopoietic system is also prone to the toxic effect of xenobiotics [15]. Thus, changes in hematological parameters are among the first to occur in toxicity [11]. In our study, administration of aqueous *P. atlantica* seed extract did

not cause a significant change in the hematological parameters. At the same time, the bone marrow tissue did not show any abnormality that could be attributed to the extract.

The liver and kidneys are clearing organs that are among the first to show the effects of toxicity. In cases where toxic compounds cause hypertrophy with increased secretion of liver enzymes, the liver weight may increase [16]. However, with aqueous *P. atlantica* seed extract treatment, the rat liver weights did not vary from that of the untreated control, suggesting the chemical components of the extract did not cause hepatic hypertrophy. On the other hand, the histopathology of the liver of treated rats showed some evidence of toxicity. This was reflected by hepatocyte degeneration, central vein dilation, and focal leukocyte infiltrations. These findings suggest that the aqueous *P. atlantica* seed extract contains chemical components that cause inflammation of the liver and subsequent liver damage.

Changes in serum renal function parameters and liver enzymes are often not commensurate with kidney or liver tissue changes. Gross or microscopic abnormalities may manifest first before biochemical parameters associated the organ becomes abnormal, or *vice versa*. This is particularly true in the acute phase of nephrotoxicity and hepatoxicity. However, with prolonged toxicity, these organs may show morphological changes with simultaneous serum biochemical abnormalities. In our study, the serum ALT, AST, LDH, and GGT activities and albumin concentration were determined in rats treated with aqueous *P. atlantica* seed extract for 14 days, as a measure of the acute toxic effect of the compound on the liver [17]. The serum bilirubin concentration was also determined, although this parameter does change as early as the leakage of serum enzymes, ALT, and AST, in acute liver diseases and damage. GGT can be found at high activity in renal tubular and bile epithelium and surface of hepatocytes. However, serum GGT is generally of hepatic origin. Serum albumin reflects the protein production function of the liver [17, 18]. Liver diseases may cause hypoalbuminemia [19]. In rats treated, although histopathology showed some lesions in the liver that may be caused by the aqueous *P. atlantica* seed extract, the serum liver biochemicals remained relatively normal. The liver has an enormous ability to detoxify noxious compounds while compensating for the loss of function. It is possible that the lesions caused by aqueo*us P. atlantica* seed extract after 14 days of treatment are not severe enough to cause hepatocyte damage associated with elevated serum enzyme and bilirubin concentrations. This is also a testament to the compensatory capacity of the liver [20].

The serum urea and creatinine concentrations, a measure of glomerular function, were normal in the aqueou*s P. atlantica* seed extract-treated rats, although histopathological examination showed some lesions associated with renal tissue damage. This phenomenon is presumed to be similar to that seen the liver, where tissue changes precede serum biochemical abnormalities. There is also the possibility that in acute toxicity, the extract causes renal tissue damage without immediately affecting glomerular filtration. Thus, renal tissue lesions may manifest from the toxic effect of the aqueous *P. atlantica* seed extract while clearance of urea and creatinine remain relatively unaffected.

Myocardial hypertrophy may be reflected as increased heart weight; however, this change is difficult to identify microscopically [21]. In this study, aqueous *P. atlantica* seed extract did not cause a change in heart weight or its relative organ-to-body weight, suggesting the extract has no adverse effect on the heart.

Conclusion

The aqueous extract of *P. atlantica* seed does not cause any abnormality in hematological and serological parameters, growth rate, and relative organ weights of rats. However, the extract can cause hepatocellular and renal tissue injuries. *P. atlantica*, which is commonly used in traditional medicine and as a flavoring agent, has not been investigated for its toxic effects. The aqueous *P. atlantica* seed extract prepared from the fresh green seeds did not cause abnormal changes to the growth, heart, liver, spleen, brain, or lungs, or serum biochemical parameters of rats. However, the extract caused histopathological changes in the liver and kidneys showing that it is hepatotoxic and nephrotoxic.

Conflict of interest

Authors declared that there is no conflict of interest.

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