Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2018.095

RESEARCH ARTICLE

Assessment of Toxicological Effect of Shogaol in Albino Mice

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ARTICLE HISTORY (18-013)

Received: January 09, 2018
Revised: September 07, 2018
Accepted: September 10, 2018
Published online: October 21, 2018

Key words:
6-Shogaol
Albino mice
Toxicological effects

ABSTRACT

Systemic studies dealing with 6-Shogaol ($C_{17}H_{24}O_3$) safety in experimental animals is lacking. Therefore, the aim of current study was to determine the safe dose of 6-Shogaol that can be used in mice model and to assess the potential toxicity that may be initiated by the higher concentrations of this substance by means of clinical observations and evaluation of histopathological changes in liver, kidney and spleen. Sixty mice were divided into 6 groups; groups 1 and 2 served as negative and vehicle control respectively, whereas animals of groups 3, 4, 5 and 6 were treated with 10, 20, 40, and 100 mg/kg b.w. Shogaol respectively. The animals were investigated daily for any sign indicative for activity alterations and toxicity along with their body weight measurement throughout experiment for 14 days. At the end of the experiment, the animals were euthanized and the liver, spleen and kidney were collected for histopathological examination. The results obtained from this study showed that the 10, 20 and 40 mg/kg b.w. doses of Shogaol were safe compared to the 100 mg/kg b.w. dose which resulted in mild clinical activity alterations, reduction in body weight gain and histopathological changes, probably attributed to Shogoal toxicity, in 30% of the total mice treated by this dose. No mortality was observed in all groups. These results may provide a new insight into the safe doses of this phenolic constituent of ginger in experimental animals and open the way for more future studies related to its medicinal benefits.

To Cite This Article: Snur Hassan MA and Hassan AH, 2018. Assessment of toxicological effect of shogaol in albino mice. Pak Vet J, 38(4): 377-383. http://dx.doi.org/10.29261/pakvetj/2018.095

INTRODUCTION

Plants are able to produce a large number of chemical compounds with significant biological effects and they have been used to manufacture a wide variety of medicines since the creation of man (Kennedy and Wightman, 2011; Cragg and Newman, 2013). Currently, about 100% of the human population all over the world depends on plant-derived medicines to meet their health requirements (Miara *et al.*, 2018). However, the majority of botanical preparations are used haphazardly without satisfactory knowledge about their safety or toxicity (Mbunde *et al.*, 2017). Therefore, in order to obtain appropriate information and directions related to these natural products, there is an urgent need for precise scientific certification on the safety/toxicity profile of these medicinal plants (Salasanti *et al.*, 2014).

Ginger (*Zingiber officinale* Roscoe), an indigenous plant in tropical Asia and probably in southern China and India, is a long life, cane-like plant with annual leafy stalks. The rhizomes of the plant have a powerful aroma and are extensively used as a spice for various foods and beverages and as medicine (Poudel *et al.*, 2015; Sharma *et al.*, 2016). In scientific studies concerned with ginger oleoresin samples, the most common bioactive composites that have been dealt with are Gingerol and Shogaol (Chen *et al.*, 2012; Liu *et al.*, 2015; Varakumar *et al.*, 2017). The Gingerols are thermally non-stable compounds which convert into Shogaol at high temperatures (Mashhadi *et al.*, 2013) and at time of preparation of dried ginger (Ok and Jeong, 2012; Wei *et al.*, 2017). The level of Gingerol was found to be reduced when the fresh ginger was cooked, desiccated and toasted, whereas the concentrations of Shogaol increase with such treatments (Semwal *et al.*, 2015).

It has been found that the 6-Shogaol ($C_{17}H_{24}O_3$), has *in vitro* and *in vivo* antibacterial, antifouling, anti-oxidative, analgesic, antipyretic and anti-inflammatory characteristics (Bak *et al.*, 2012; Mahluji *et al.*, 2013). Recent studies have demonstrated that 6-Shogaol, is more potent than 6-Gingerol in anti-inflammatory, antioxidant, and antiapoptotic effects (Peng *et al.*, 2012).

To our knowledge, systemic safety evaluation related to uses of 6-Shogaol in experimental animals is lacking; So, the present study aimed to determine the safe dose of 6-Shogaol that can be used in mice model and to evaluate the potential toxicity that may be initiated by the higher concentrations of this substance by means of clinical observations and evaluation of histopathological changes in the liver, kidney and spleen.

MATERIALS AND METHODS

Materials: Shogaol (≥90%) (SMB00311, and MW: 276.37) and pure olive oil (8873.1) were purchased from Sigma and Carlroth companies respectively.

Animals and Treatments: Thirty male and thirty female mice (*Mus Muscula* species, *BALB/c* strain) weighing 25-30 gm were purchased from the Animal House at the College of Veterinary Medicine, University of Sulaimani (Sulaimaniyah, Iraq), accommodated in temperature and light-controlled environment and allowed consumption of tap water and standard food *ad libitum*. All of the *in vivo* experimentaions were performed humanely according to the Guide for the Care and Use of Laboratory Animals and the ethical approval that was obtained from the Ethics Committee at the College of Veterinary Medicine, University of Sulaimani.

The mice were divided into 6 groups and each group was subdivided according to gender into 2 subgroups (male and female subgroups, 5 mice each). Mice of group 1, served as negative control; mice of group 2, served as vehicle control, treated with 1 ml/Kg b.w. olive oil; whereas mice of groups 3, 4, 5 and 6 were treated with 10, 20, 40, and 100 mg/kg b.w. Shogaol respectively. Treatments of mice in groups 2, 3, 4, 5 and 6 were given as a single daily dose by oral gavages for 14 days (2 days treatment and 1 day interval).

Clinical observations, food consumption, and body weight measurements: The animals were investigated daily, 2 times a day throughout the experiment (for 14 days) for signs of acute toxicity such as diarrhea, itching, curved tail, falling of hair, mortality and any other sign indicative for activity alterations. The quantities of food consumption per mouse were recorded daily and the body weights of mice were recorded 3 times throughout the experiment (day 1, day 7 and day 14).

Tissue sampling and histopathological examination: At the end of the experimental period, the animals were euthanized with Ketamine and Xylazine. The liver, spleen and kidneys of the sacrificed mice were excised, cleaned by normal saline, trimmed into appropriate size, fixed in 10% neutral buffered formalin for 24 hours and undergone a series of histopathological processes to prepare 5 µm thick tissue sections which were fixed on glass slides, stained by hematoxylin and eosin dyes (Kiernan, 1996; Naseem *et al.*, 2018) and examined by different magnification powers of the light microscopy.

Statistical analysis: Statistical analysis was performed using the ANOVA (one-way), followed by Duncan's multiple comparism analyses of variance. Results were presented as mean±standard error (Mean±SE) and P values less than 0.05 were considered significant. All statistical explorations were accomplished using the SPSS software version 22 (SPSS Inc., USA).

RESULTS

Clinical observations: No mortality was observed in all groups. The animals were healthy in general with no clinical signs of toxicity. There were no unusual changes in behavior or in locomotor activity during the 14-day observation period. However, 3 mice (1 male and 2 female) out of the total 10 mice of group 6 (which were exposed daily to oral doses of 100 mg/kg b.w. Shogaol) showed weakness, reduced activity and rough body coat.

Food consumption: Shogaol treatment didn't show any significant variation ($P<0.05$) in food consumption by mice of the treatment groups in comparison with those of the control groups (Table 1). The average food intake per mouse was approximately equal to 4 gm/day for the male mice and 3.5 gm/day for the female mice.

Body weight measurements: Male and female mice of all groups showed significant increase ($P<0.05$) in their body weight measurements on day 7 and on day 14 in comparison with their initial body weights on day 1. However, no-significant variations ($P<0.05$) were seen in the body weight gain of the male and female mice in the treatment groups in comparison with that of male and female mice in the control groups except the female mice of group 6 (exposed daily to oral doses of 100 mg/kg b.w. Shogaol) which show significant decrease ($P<0.05$) in their body weight gain in comparison with that of the mice in the control negative group (Table 2).

Histopathological findings: The histopathological examination revealed no significant abnormalities in kidney, liver and spleen sections of mice belonging to the negative control and vehicle control groups (groups 1 and 2). In treatment groups, no significant lesions were seen in mice of group 3, 4, and 5 (treated respectively with 10, 20

Table 1: Means of food consumption (gram) in mice of all groups during the experiment

		Control groups		Shogaol treatment groups			
		Negative control	Vehicle control	10 mg/kg b.w.	20 mg/kg b.w.	40 mg/kg b.w.	100 mg/kg b.w.
Male mice	Day 1	4.0^a	3.5^a	3.6a	3.8^a	4.0^a	3.5^a
	Day 7	4.2^a	3.8^a	3.8^a	3.9^a	4.1a	3.6^a
	Day 14	4.5^a	3.9^a	4.0^a	4.1^a	4.3^a	3.9^a
Female mice	Day 1	3.9^a	3.8^a	3.5a	3.6^a	3.6^a	3.4^a
	Day 7	4.0^a	3.9^a	3.6^a	3.8^a	3.8^a	3.5^a
	Day 14	4.1a	4.0^a	3.8^a	3.9^a	4.0^a	3.6^a

The means of food consumption are expressed as gm/mouse/day. Within a row, the mean values with different small alphabetical superscripts vary from each other ($P<0.05$).

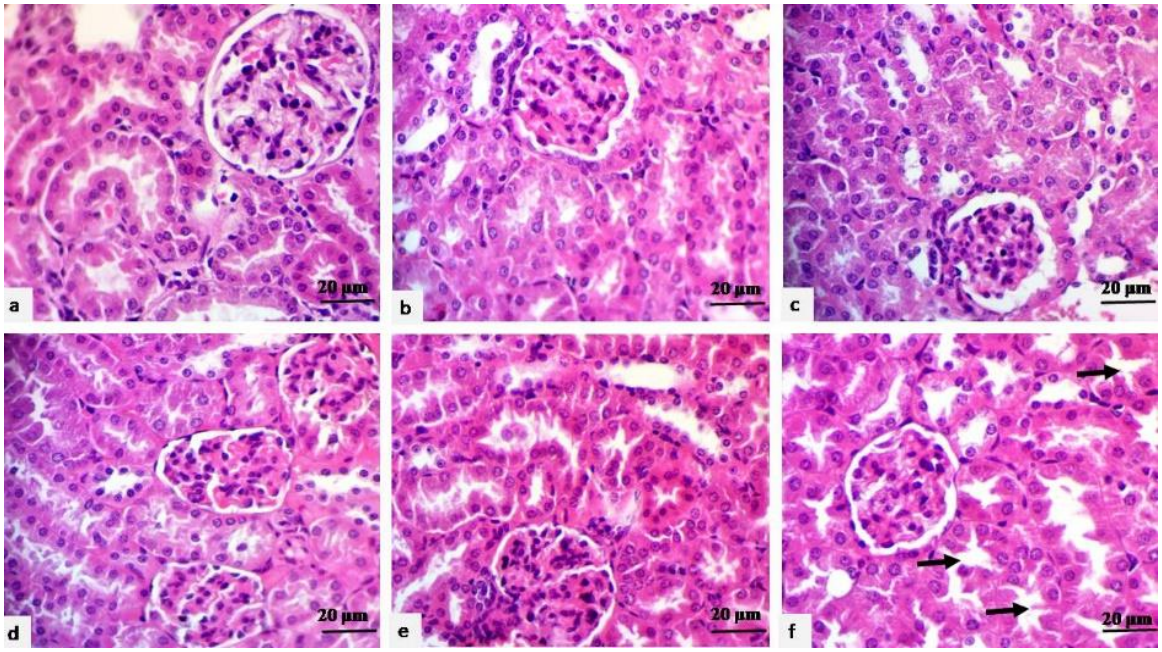

Fig. 1: Microscopic view of the kidney of the male mice in all groups. No significant abnormalities are evident in sections **a** (control negative), **b** (vehicle control "1 ml/kg b.w. olive oil treatment"), **c** (10 mg/kg b.w. Shogaol treatment), **d** (20 mg/kg b.w. Shogaol treatment) and **e** (40 mg/kg b.w. Shogaol treatment), whereas section **f** (100 mg/kg b.w. Shogaol treatment) shows swelling of the lining epithelial cells of the proximal and distal convoluted tubules resulting in star-shape appearance (black arrow) of their lumens. H&E stain, scale bar 20 µm.

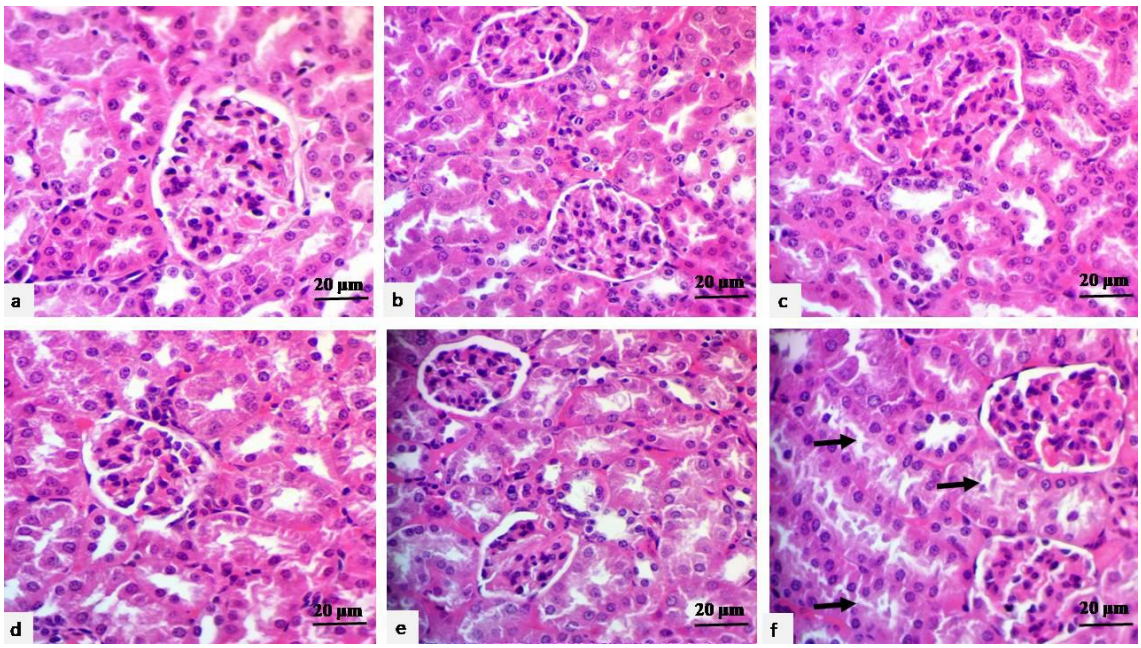

Fig. 2: Microscopic view of the kidney of the female mice in all groups. No significant abnormalities are evident in sections **a** (control negative), **b** (vehicle control "1 ml/kg b.w. olive oil treatment"), **c** (10 mg/kg b.w. Shogaol treatment), **d** (20 mg/kg b.w. Shogaol treatment) and **e** (40 mg/kg b.w. Shogaol treatment), whereas section **f** (100 mg/kg b.w. Shogaol treatment) shows swelling of the lining epithelial cells of the proximal and distal convoluted tubules resulting in star-shape appearance (black arrow) of their lumens. H&E stain, scale bar 20 µm.

Table 2: Body weight measurements and body weight gain (gram) in mice of all groups during the experiment

		Control groups		Shogaol treatment groups			
		Negative Control	Vehicle control	10 mg/kg b.w.	20 mg/kg b.w.	40 mg/kg b.w.	100 mg/kg b.w.
Male mice	Day 1	23.83±0.54^{a}	22.50±0.76^{a}	24.83±1.13^{a}	23.66±0.80^{a}	22.83±0.74^{a}	24.00±0.85^{a}
	Day 7	27.50±0.67^{b}	26.50±0.56^{b}	28.16±0.60^{b}	27.66±0.61^{b}	26.66±0.66^{b}	28.16±0.60^{b}
	Day 14	31.00±0.57^{c}	30.00±1.06^{c}	32.00±0.44^{c}	31.16±0.87^{c}	30.50±0.42^{c}	31.00±0.36^{c}
	Weight gain	7.17±0.03^{A}	7.5±0.0.3^{A}	7.17±0.69^{A}	7.5±0.07^{A}	7.67±0.32^{A}	7±0.49^{A}
Female mice	Day 1	25.50±0.42^{a}	24.50±0.34^{a}	24.33±0.49^{a}	24.83±0.47^{a}	25.50±0.34^{a}	24.66±0.42^{a}
	Day 7	28.66±0.49^{b}	27.50±0.50^{b}	27.50±0.42^{b}	27.83±0.65^{b}	28.50±0.34^{b}	27.66±0.84^{b}
	Day 14	32.33±0.42^{c}	30.83±0.40^{c}	30.66±0.49^{c}	31.00±0.63^{c}	31.66±0.76^{c}	30.33±0.33^{c}
	Weight gain	6.83±0.07^{A}	6.33±0.1^{AB}	6.33±0.00^{AB}	6.17±0.16^{AB}	6.16±0.42^{AB}	5.67±0.09^{B}

The body weights and body weight gains are expressed by mean ± standard error. Within a column, body weight values with different small alphabetical superscripts vary from each other (P<0.05). Within the weight gain's rows, weight gain values with different capital alphabetical superscripts vary from each other (P<0.05).

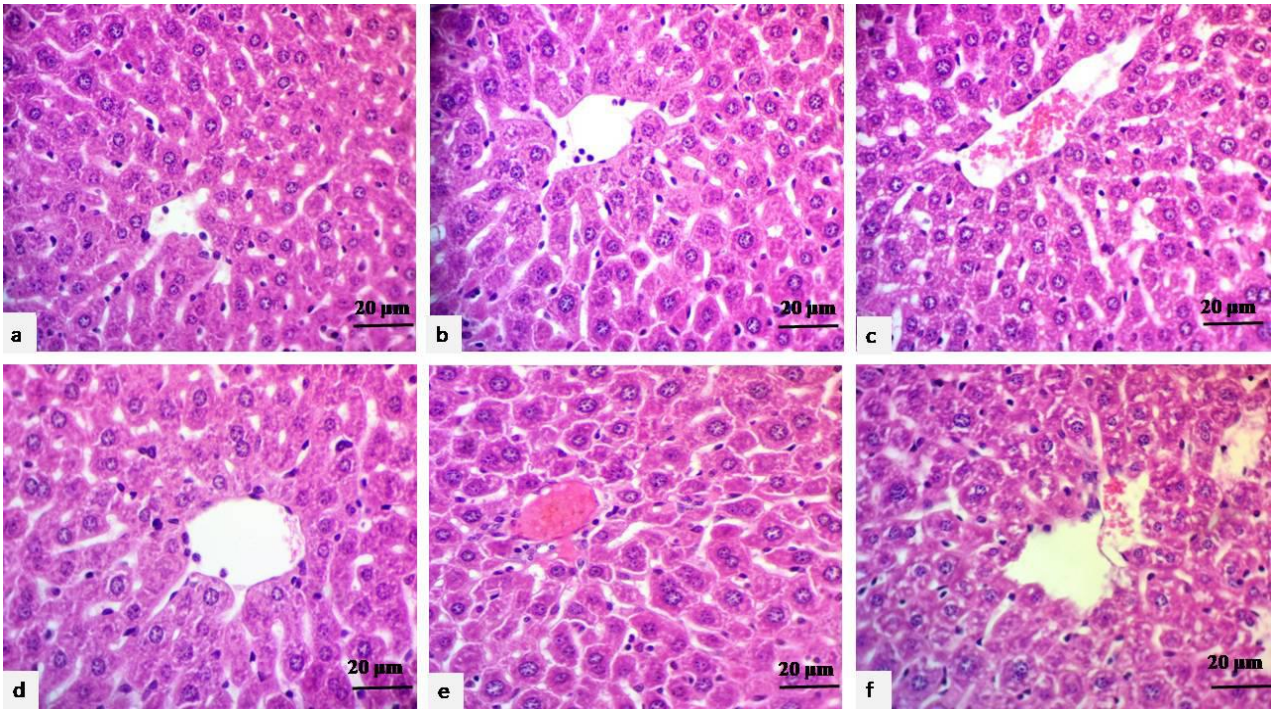

Fig. 3: Microscopic view of the liver of the male mice in all groups. No significant abnormalities are evident in sections **a** (control negative), **b** (vehicle control "1 ml/kg b.w. olive oil treatment"), **c** (10 mg/kg b.w. Shogaol treatment) and **d** (20 mg/kg b.w. Shogaol treatment). Section **e** (40 mg/kg b.w. Shogaol treatment) shows central vein congestion and section **f** (100 mg/kg b.w. Shogaol treatment) shows swelling of the hepatocytes resulting in narrowing of the sinusoids. H&E stain, scale bar 20 µm.

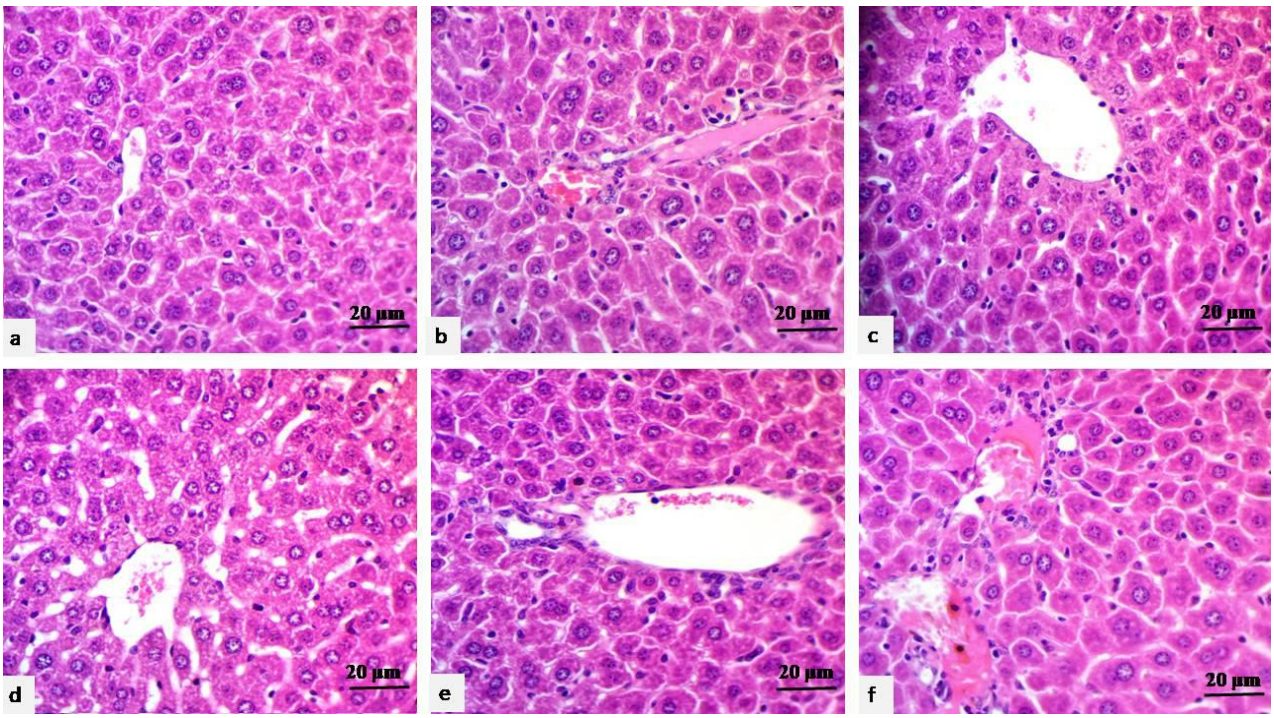

Fig. 4: Microscopic view of the liver of the female mice in all groups. No significant abnormalities are evident in sections **a** (control negative), **b** (vehicle control "1 ml/kg b.w. olive oil treatment"), **c** (10 mg/kg b.w. Shogaol treatment), **d** (20 mg/kg b.w. Shogaol treatment) and **e** (40 mg/kg b.w Shogaol treatment), whereas section **f** (100 mg/kg b.w. Shogaol treatment) shows central vein congestion and swelling of the hepatocytes (black arrow) resulting in narrowing of the sinusoids. H&E stain, scale bar 20 µm.

and 40 mg/kg b.w. Shogaol), whereas group 6 (treated with 100 mg/kg body weight Shogaol) showed mild changes in one male and two female mice (the same that showed clinical activity alterations) represented by swelling of the lining epithelial cells of the proximal and distal convoluted renal tubules resulting in narrowing of their lumina (Fig. 1 and 2), central vein congestion in the liver associated with swelling of hepatocytes (Fig. 3 and 4) and congestion of the venous sinuses of the splenic red pulp (Fig. 5 and 6).

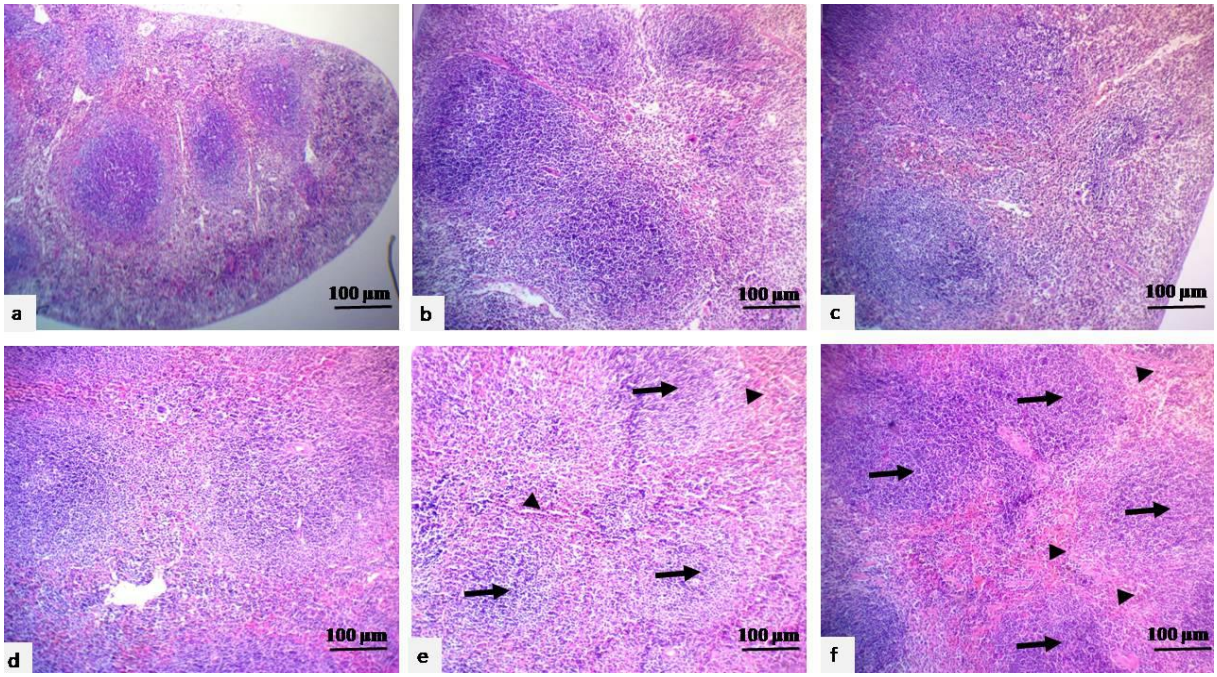

Fig. 5: Microscopic view of the spleen of the male mice in all groups. No significant abnormalities are evident in sections **a** (control negative), **b** (vehicle control "1 ml/kg olive oil treatment"), **c** (10 mg/kg b.w. Shogaol treatment), **d** (20 mg/kg b.w. Shogaol treatment) and **e** (40 mg/kg b.w. Shogaol treatment),whereas section **f** (100 mg/kg b.w. Shogaol treatment) shows lymphocytic hyperplasia (black arrows) in the white pulp regions and congestion of the red pulp sinusoids (black arrow heads) resulting in narrowing of the sinusoids. H&E stain, scale bar 100 µm.

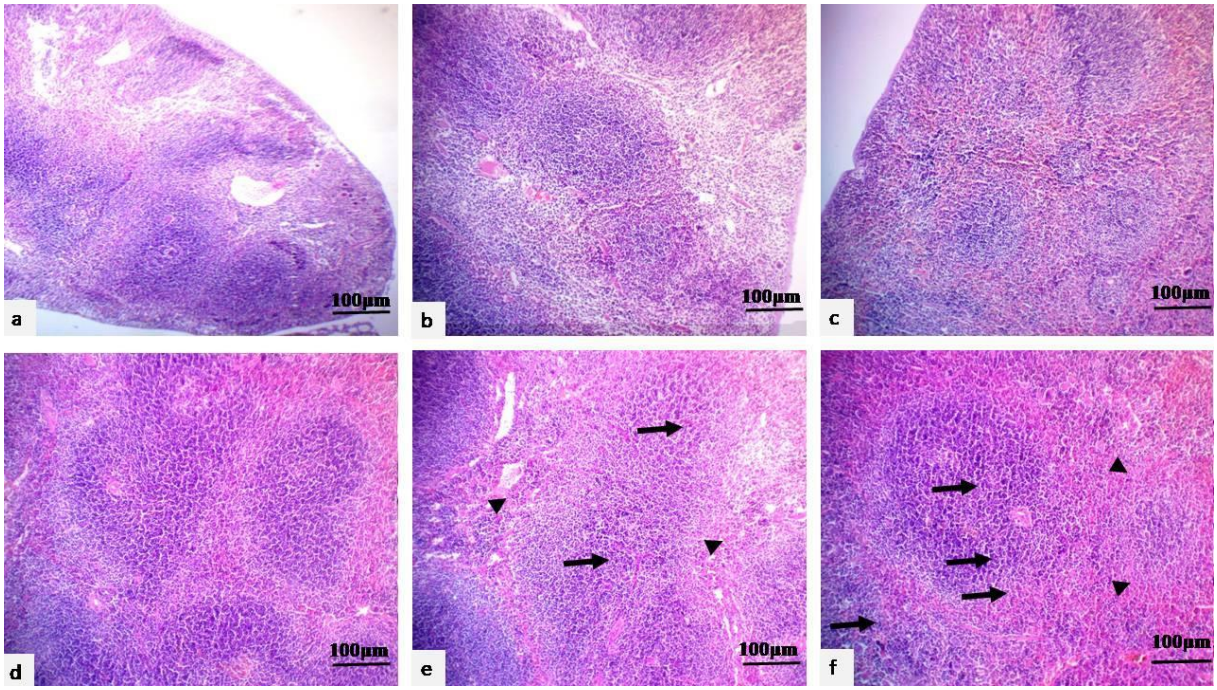

Fig. 6: Microscopic view of the spleen of the female mice in all groups. No significant abnormalities are evident in sections **a** (control negative), **b** (vehicle control "1 ml/kg olive oil treatment"), **c** (10 mg/kg b.w. Shogaol treatment), **d** (20 mg/kg BW Shogaol treatment) and **e** (40 mg/kg b.w. Shogaol treatment), whereas section **f** (100 mg/kg b.w. Shogaol treatment) shows lymphocytic hyperplasia (black arrows) in the white pulp regions and congestion of the red pulp sinusoids (black arrow heads) resulting in narrowing of the sinusoids. H&E stain, scale bar 100 µm.

DISCUSSION

Medicinal plants and herbal products are widely used in traditional medicine; however, some of these plants and products didn't receive adequate scientific studies related to their safety and efficacy and there is an urgent need for precise scientific studies on their safety/toxicity profile (Pan *et al.*, 2013). The study of acute toxicity of traditional medicine and herbal products in small animal models is an essential manner to determine their safety and efficacy (Elufioye and Onoja, 2015). Ginger has been used as a medicinal plant a long time ago; however, systemic studies dealing with its safety in experimental animals is lacking (Jeena *et al.*, 2011; Chang *et al.*, 2012) with special reference to the Shogaol component which, in contrast to the other components of ginger, received no test studies regarding its acute toxicity in experimental animals.

Therefore, this study aimed to determine the safe dose of Shogaol to be used in mice and the obtained results revealed that the 10, 20, and 40mg/kg b.w. doses were not associated with any mortalities or significant abnormalities in clinical activities and histopathological changes in the liver, kidney and spleen. Whereas the 100 mg/kg b.w. dose (given to mice of group 6) has resulted in a significant decrease in body weight gain in the female mice and slight alterations in clinical activities represented by diminished activity and rough body coat in 30% of mice (1 male and 2 females out of the total 10) treated by this dose. This difference between male and female mice response to the 100 mg/kg b.w. dose indicates that the Shogoal exhibits gender-based variation in its pharmaceutical efficacy and toxicity due to some hormonal effects which are believed to be responsible for the majority of differences in adverse drug reactions observed between the genders (Nicolson *et al.*, 2010) .

The animal activity, food consumption and body weight gain results obtained from this study are, in general, constant with the results of other studies that revealed that using of the ginger extract in experimental animals did not result in any disturbances in clinical activities or body weight loss (Rong *et al.*, 2009; Rahman *et al.*, 2014). However, the significant decrease in body weight gain in the female mice and the decreased activity and rough body coat observed in the 30% of mice in group 6 can be attributed to some adverse reactions induced the higher doses of Shagoal such as stomach discomfort, irritation and a significant increase in exfoliation of gastric epithelial cells (Terry *et al*., 2011; Mills and Bone, 2013).

Slight histopathological changes, represented by swelling of the epithelial cells of the renal tubules, central vein congestion in the liver associated with swelling of hepatocytes and congestion of the venous sinuses of the splenic red pulp, were observed on the same 3 mice that showed clinical activity alterations. These changes might be attributed to possible adverse effects of the 100 mg/kg dose of Shogaol and they are in accordance with the results of other authors who stated that using of high doses of ginger extracts for short duration or low to moderate doses for prolonged duration in murine rodents resulted in degeneration and proliferation of hepatocytes, splenic damages associated with lymphoid hyperplasia and tubular degeneration associated with glomerulo-sclerosis and hypertrophy of glomeruli in the kidney (Modaresi *et al.*, 2011; Amer *et al.*, 2013; Udo-Affah *et al.*, 2014).

Conclusions: The results of the current study revealed that the oral administration of 40 mg/kg b.w. Shogaol for 14 days is safe for the mice model and not associated with any unusual changes in behavior, locomotor activity, food consumption, body weight measurements and histopathological findings; whereas the 100 mg/kg b.w. dose was associated with slight harmful effects represented by a decrease in body weight gain of the female mice and weakness, reduced activity, rough body coat & mild histopathological changes in 30% of the mice exposed to this dose.

Authors contribution: The practical part and result & discussion writing were achieved by SMAH, whereas the general writing of the manuscript and reviewing of the result & discussion were achieved by AHH.

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