Ovozoa 12 No. 3, December 2023 p-ISSN 2302-6464 e-ISSN 2722-967X
Available at https://e-journal.unair.ac.id/OVZ/index

Original article

Effect of *Peganum harmala* seeds extract on the hepatic tissue structure and fetus of mice

Samira Musa Sasi[1], Nagia Musa Alghoul[2], Nuri Awayn[3], Ahmed Elghoul[4], Ragil Angga Prastiya[5]

[1] Zoology Department, Faculty of Science, University of Tripoli, Libya
[2] Cell and tissue culture department, Biotechnology Center, Tripoli, Libya
[3] Department of Chemistry, Faculty of Science, University of Tripoli, Tripoli, Libya
[4] Chemistry Department, Ministry of Interior, Tripoli, Libya
[5] Department of Veterinary Reproduction, School of Health and Life Science, Universitas Airlangga, Indonesia

[*] Corresponding author, e-mail: Samira_18_3@yahoo.com
Open access under CC BY – SA license, DOI: 10.20473/ovz.v12i3.2023.141-147
Received August 31 2023, Revised November 18 2023, Accepted November 23 2023
Published online December 2023

ABSTRACT

Peganum harmala is one of the most used plants for the treatment of many diseases. Its effective compounds have pharmaceutical and medicinal properties. This study aims to determine the effect of aqueous extract of harmala plant seeds on body and liver weight, aspartate aminotransferase (AST), alanine transaminase (ALT), and the histological structure of liver of mice, as well as the size of the fetuses sired by treated mice. Sixteen adult male mice were divided into two groups of eight. The first group (control) was given distilled water orally, while the second group received the aqueous extract of harmala seeds at a dose of 300 mg/kg bw for three weeks. three untreated females were housed with one treated male for mating. At the end of the treatment, six male mice of each group were weighed and killed. Liver was extracted, weighed and its enzymes were measured. Also, sections of the liver were prepared for histological examination. The results showed a decrease in the body weight of the treated mice and a significant increase in the average weight of the fetuses compared with the control group, as well as marked changes in the hepatic tissue structure. There was no impact of the extract on fetal body length, liver weight and hepatic enzymes (AST and ALT) of treated mice. Further studies should be conducted to determine a safe dose that does not affect any organ in the body, so that it can be used for the treatment of many diseases.

Keywords: fetus, liver enzymes, liver histopathology, male mice, *Peganum harmala*

INTRODUCTION

Medicinal plants are considered an important source of chemical substances with potential therapeutic effects; therefore, these types of plants have increased in use in recent years in traditional medicine especially among people in developing countries. *Peganum harmala* is one of the most significant medicinal plants used in traditional medicine for the treatment of several chronic heart disorders in China (Liu *et al*., 2013) and Spain (Bremner *et al*., 2009). The harmala plant belongs to the Zygophyllaceae family, grows in

How to cite this article: Sasi SM, Alghoul NM, Awayn N, Elghoul A, Prastiya RA. 2023. Effect of *Peganum harmala* seeds extract on the hepatic tissue structure and fetus of mice. Ovozoa: Journal of Animal Reproduction. 12: 141-147.

sandy soils, steppe areas, and semi-arid regions (Nafisi *et al*., 2010), as well as spread across North Africa, Central Asia, Middle East, America and Australia (Mahmoudian, 2002). In addition, it is an important source of natural products such as tannins, alkaloids, coumarins, flavonoids, terpenoids, sterols and essential oils (Benbott *et al*., 2013), these compounds are of great importance in pharmacology (Benbott *et al*., 2018). Previous studies have shown that this plant extracts exhibits various biological activities such as antiviral, antioxidant, antibacterial, antitumor, cardio-protective, antidiabetic, cerebral-protective, anti-proliferative (Oodi *et al*., 2017; Abderrahim *et al*., 2019) and anti-inflammatory (Akhtar *et al*., 2022). In addition, they have antileishmanial (Madah *et al*., 2020) and diuretic effects (Al-Saikhan and Ansari, 2016).

Several in vitro studies reported that the harmala plant had an impact on rat fetus led to fetal death, abortion (Mahmoudian, 2002) and reduction in fetal weight (Mahmoudian, 2002; Adaay, 2014). The seeds were the most important part of this plant, having abortive (Sasi *et al*., 2013; Abbas *et al*., 2021) anti-cancer, anti-rheumatism, anti-asthmatic and antispasmodic effects as well as reducing irritation in larynx and increasing milk flow (Abbas *et al*., 2021). Apart from that, it was useful for treating epilepsy, memory loss, kidney stone and jaundice (Niroumand *et al*., 2015). Several studies showed that the alkaloids in the seeds had antibacterial (Nenaah, 2010; Shaheen and Issa, 2020), antitumor (Bournine *et al*., 2017), antiproliferative (Habli *et al*., 2017), antioxidant (Dalimunthe *et al*., 2018), antidepressant (Hamid *et al*., 2017) activities. The aim of the current study was to evaluate the effect of aqueous extract of harmala plant seeds on body and liver weight, aspartate aminotransferase (AST), alanine transaminase (ALT), and the histological structure of liver of mice, as well as the size of the fetuses sired by treated mice.

MATERIALS AND METHODS

Animals

This study was performed on 16 male albino mice, aged between 6-7 weeks and body weight between 25-30 grams. They were housed in animal cage of the Department of Zoology, Faculty of Science, University of Tripoli. Mice were exposed to a 12-hour light-dark cycle and maintained at laboratory temperature ranged between 23-25°C. They were given pellet and drinking water ad libitum.

Ethical approval

Ethical approval for dealing with mice in this study was obtained from the Ethics Committee of the University of Tripoli (Ref No; SREC 18-2022).

Plant collection and preparation of harmala plant seeds extract

This plant was collected from western mountain in Libya and was classified at Department of Botany, University of Tripoli, Libya. The seeds of the harmala plant were separated, cleaned, air-dried and ground into a powder using a grinding machine. Extract was made by adding a limited amount of the seeds powder to a limited amount of distilled water. The mixture was boiled, cooled to room temperature and then used for treatment.

Experimental design

Mice were divided equally into two groups. The first group (control) received distilled water orally and the second group was given harmala seeds extract at a dose of 300 mg/kg bw for three weeks. At the end of the dosing period, sex mice were weighed, killed and dissected. Livers were excised, weighed and stored in 10% formalin solution for histological studies. The remaining mice in each group was used for mating to obtain fetuses.

Blood analysis

At the end of treatment, blood samples were collected from the facial veins of all mice and centrifuged at 3000 rpm for 30 min to obtained the serum, which was stored at - 20°C. After that, liver functions were evaluated by measuring AST and ALT. The assay was performed according to the manufacturer's standard protocol.

How to cite this article: Sasi SM, Alghoul NM, Awayn N, Elghoul A, Prastiya RA. 2023. Effect of *Peganum harmala* seeds extract on the hepatic tissue structure and fetus of mice. Ovozoa Journal of Animal Reproduction. 12: 141-147.

Histopathological analysis

Liver tissue was processed through a graded ethanol series, cleared in xylene and embedded in paraffin blocks. Sections were cut to a thickness of 5 µm. After that, the sections were stained with hematoxylin and eosin, and examined under a light microscope.

Fetal weight and length

Each of the three untreated females were housed with one treated male; females were then checked every 24 hours for the presence of a vaginal plug. Once the vaginal plug was formed and observed, that day was considered day zero of gestation. On the 18th day of pregnancy, the pregnant females were weighed and killed by cervical dislocation. The fetuses were removed and their weight and length were recorded.

Data Analysis

Statistical analysis was carried out using SPSS software (version 20). Data obtained from experiments were expressed as mean ± standard deviation (mean ± SD). One-way analysis of variance was applied to determine the level of significance between the treated and control group. The difference was considered significant at $p < 0.05$.

RESULTS

Effect on body weight and liver weight

The body weight of male mice treated with aqueous extract of harmala plant seeds was significantly lower than that of mice in the control group (Table 1).

Table 1 Body and liver weight (grams, means ± SD) of mice were given aqueous extract of *Peganum harmala* seeds

	body weight (g)	liver weight (g)
control	29.6 ± 1.14 ^{b}	1.37 ± 1.11
treated	25.2 ± 0.55 ^{a}	1.33 ± 1.14

Different superscript in a same column indicated significant difference ($p < 0.05$); control group: mice were given distilled water; treated group: mice were given 300 mg/kg bw aqueous extract of *Peganum harmala* seeds; treatment was given orally for three weeks.

Effect on liver enzymes

The current study showed that aqueous extract of harmala plant seeds resulted in non-significant changes ($p > 0.05$) in liver enzymes (AST and ALT) of treated mice compared to the control group (Table 2).

Table 2 AST and ALT (U/L, means ± SD) of mice were given aqueous extract of *Peganum harmala* seeds

	AST (U/L)	ALT (U/L)
control	90.10 ± 3.60	71.65 ± 4.20
treated	101.20 ± 8.33	72.30 ± 3.40

control group: mice were given distilled water; treated group: mice were given 300 mg/kg bw aqueous extract of *Peganum harmala* seeds; treatment was given orally for three weeks.

Effect on fetus

The findings in (Table 3) showed that harmala plant seeds extract at a dose of 300 mg/kg bw for three weeks led to a significant increase in the body weight of fetus of the treated group compared to the control group ($p < 0.05$). No significant changes were observed in the length of the fetus in the treated group as compared to the control group ($p > 0.05$).

Table 3 Body weight and body length (means ± SD) of the fetus sired by male mice were given aqueous extract of *Peganum harmala* seeds

	body weight (g)	body length (cm)
control	0.85 ± 0.14 ^{a}	1.75 ± 0.16
treated	1.06 ± 0.16 ^{b}	1.64 ± 0.19

Different superscript in a same column indicates significant difference ($p < 0.05$); control group: mice were given distilled water; treated group: mice were given 300 mg/kg bw aqueous extract of *Peganum harmala* seeds; treatment was given orally for three weeks; no. of females: 6 for each group; female mice were untreated.

Effect on the liver tissue structure

Histological examination of the liver

How to cite this article: Sasi SM, Alghoul NM, Awayn N, Elghoul A, Prastiya RA. 2023. Effect of *Peganum harmala* seeds extract on the hepatic tissue structure and fetus of mice. Ovozoa Journal of Animal Reproduction. 12: 141-147.

tissues from control group showed a normal histological morphology of the liver (Figure 1A). On the other hand, mice that were given 300 mg/kg bw of harmala seeds extract demonstrated microvacuoles in the cytoplasm of some hepatocytes and loss of the cellular details in some others (Figure 1B).

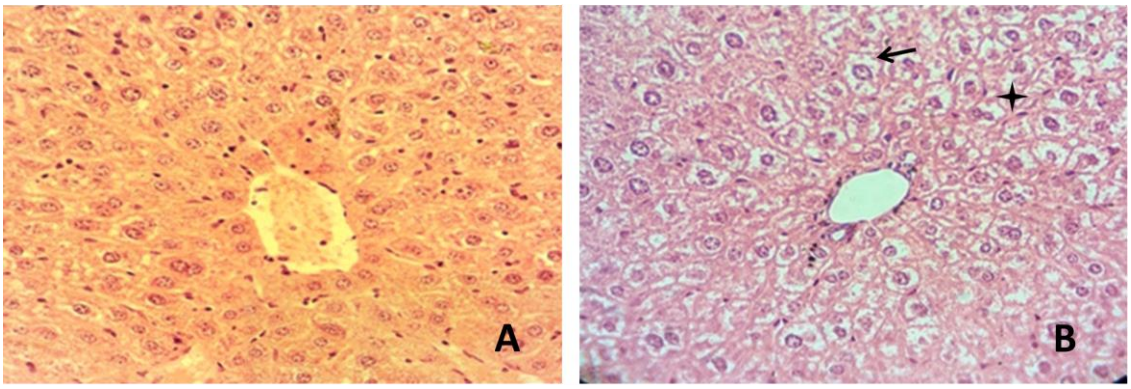

Figure 1 Photomicrograph of mice liver section of control group (A) showing a normal structure of the liver and treated group (received 300 mg/kg bw *Peganum harmala* seed extract) (B) showing vacuoles in the cytoplasm (black arrow) and loss of cellular details of some the hepatic cells (star).

DISCUSSION

Harmala seeds extract exposure reduced body weight but not liver weight

The aqueous extract of harmala plant seeds reduced body weight in male mice. This finding was in line with other studies (El-Dwairi and Banihani, 2007; Benbott *et al*., 2022) who reported that aqueous extract of the harmala plant given to rats caused weight loss. In addition, the results of this study are in accordance with previous research findings which revealed that alcoholic extract of harmala seeds at a dose of 3000 mg/kg for 2 weeks caused a significant reduction in body weight in mice (Al-Jborrey and Al-Shahwany, 2017). Rezzagui *et al*. (2020) and Mollashahi and Kazerani (2020) reported that oral administration of methanolic extract of harmala seeds to mice did not cause any significant changes in the body weight. This weight loss might be caused by loss of appetite. No significant changes were observed in liver weight of treated mice. Similar result was obtained by Rezzagui *et al*. (2020) who showed that oral administration of crude aqueous extract of harmala seeds to mice for 28 consecutive days did not have any effect on the liver weight of the treated mice. However, other studies have reported that harmala extract caused an increase in the liver weight of treated rats (Adeeb, 2015; Benbott *et al*., 2022). Differences in results between studies may be due to treatment period or dose or extraction method.

Harmala seeds extract had no effect on liver enzymes

The aqueous extract of harmala plant seeds had no effect on hepatic enzymes (AST and ALT) in mice. These results were similar to a study conducted by Rezzagui *et al* (2020) which reported that oral administration of methanolic extract of harmala seeds did not show significant differences in ALT and AST levels. Previous studies reported that harmala seeds extract caused significant changes in AST and ALT levels in rats (Adeeb, 2015; Benbott *et al*., 2022). Moghadam *et al*., (2021) found that mice treated with harmaline (active ingredient in *Peganum harmala*) intraperitoneally at the doses of 50 and 100 mg/kg for 4 weeks caused a significant reduction in ALT and AST levels. Another study demonstrated that intraperitoneal injection of harmala seeds alkaloids female rats caused a significant decrease in liver enzymes (ALT and AST) (Mahdeb *et al*., 2020). These contradictions between different studies may be

How to cite this article: Sasi SM, Alghoul NM, Awayn N, Elghoul A, Prastiya RA. 2023. Effect of *Peganum harmala* seeds extract on the hepatic tissue structure and fetus of mice. Ovozoa Journal of Animal Reproduction. 12: 141-147.

attributed to differences in dose, treatment period, method of administration, or method of extraction.

Aqueous extract of harmala plant seeds caused marked changes on liver tissue

The histological results of this study are in accordance with previous studies which found that repeated administration of 150 mg/kg of the alcoholic extract of harmala plant twice a week for a month caused vesiculation in the cytoplasm, severe damage to hepatic cells and pyknotic nuclei (Mohamed *et al*., 2013). Similar results were obtained by Kal-Taie (2019) and Benbott *et al*. (2022) who reported that administration of harmala extract caused histological disorders in the liver tissue of treated animals. Also, Qazan (2009) indicated that chicks fed food containing 10 % harmala plant leaves for two weeks resulted in histological changes in liver tissue. However, other studies revealed that harmala plant had a protective effect against thiourea (Hamden *et al*., 2008) and CCL4 (Ahmed *et al*., 2013) induced liver damage in rats. The recurrent histological lesions in liver tissue may be caused by alkaloids in the seeds of the harmala plant (Diaz, 2015).

Effect of harmala seeds extract on fetus

Harmala plant seeds extract increased fetal weight. However, this extract had no effect on fetal length. Previous studies showed that harmala plant caused a decrease in fetal weight (Kermanian *et al*., 2003; Adaay, 2014) and body length (Kermanian *et al*., 2003). Another study showed that administering boiled harmala plant seeds orally to pregnant mice at varying doses at 7-12 days of gestation caused a reduction in fetal weight (Sasi *et al*., 2017). This controversy in results between different studies may be due to the period of treatment or dosage or the gender of animals.

CONCLUSION

The results of this research revealed that administration of harmala seeds extract for male mice at a dose of 300 mg/kg bw for three weeks resulted in a significant reduction in body weight and an increase in fetal weight, as well as marked histological changes in the liver. There was no effect of the extract on liver weight, fetal body length and the hepatic enzymes, the AST and ALT.

ACKNOWLEDGEMENTS

The authors are grateful to Department of Zoology, Faculty of Science, University of Tripoli and cell & Tissue Culture Department, Libyan center of Biotechnologies for providing the laboratories facilities for this investigation.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

SMS and NMA planed the experimental design of this study. SMS, NMA and RAP performed drafting, revision and wrote the final manuscript. All other authors read and approved the final manuscript.

FUNDING INFORMATION

This study was funded by the authors.

REFERENCES

Abderrahim LA, Taibi K, Abderrahim CA. 2019. Assessment of the antimicrobial and antioxidant activities of Ziziphus lotus and *Peganum harmala*. Iran J Sci Technol Trans A Sci. 43: 409-14.

Abbas MW, Hussian M, Qama M, Ali S, Shafiq Z, Wilairatana P, Mubarak MS. 2021. Antioxidant and anti-inflammatory effects of *Peganum harmala* extracts: An in vitro and in vivo Study. Molecules 26: 6084.

Adaay MH. 2014. Embryotoxicity of harmaline and harmalol hydrochloride on pregnant rats. J Mater Environ Sci. 5: 127-32.

Adeeb L. 2015. Histological study of effect of *Peganum harmala* aqueous extract on spleen, liver enzymes and parameter of blood in albino rats. Int J Sci Res. 4: 2296-300.

Ahmed H, El-Zahab HA, Alswiai G. 2013.

How to cite this article: Sasi SM, Alghoul NM, Awayn N, Elghoul A, Prastiya RA. 2023. Effect of *Peganum harmala* seeds extract on the hepatic tissue structure and fetus of mice. Ovozoa Journal of Animal Reproduction. 12: 141-147.

Purification of antioxidant protein isolated from *Peganum harmala* and its protective effect against CCL4 toxicity in rats. Turk J Biol. 37: 39-48.

Akhtar MF, Raza SA, Saleem A, Hamid I, Ashraf Baig MMF, Sharif A, Sohail K, Javaid Z, Saleem U, Rasul A. 2022. Appraisal of anti-arthritic and anti-inflammatory potential of folkloric medicinal plant *Peganum harmala*. Endocr Metab Immune Disord Drug targets 22: 49-63.

Al-Jborrey MH, Al-Shahwany AW. 2017. Histopathological effect of *Peganum harmala* alcoholic extract of seeds on liver and kidney in mice. Biomed Biotech. 5: 1-5.

Al-Saikhan FI, Ansari MN. 2016. Evaluation of the diuretic and urinary electrolyte effects of methanolic extract of *Peganum harmala* L. in Wistar albino rats. Saudi J Biol Sci. 23: 749-53.

Benbott A, Bahri L, Boubendir A, Yahia A. 2013. Study of the chemical components of *Peganum harmala* and evaluation of acute toxicity of alkaloids extracted in the Wistar albino mice. J Mater Environ Sci. 4: 558-65.

Benbott A, Mahdi D, Derouiche K, Saida K, Zellagui A. 2018. Nephrotoxicity of crude alkaloids extract of *Peganum harmala* seeds in rats. World J Environ Biosci. 7: 63-6.

Benbott A, Mosbeh C, Karouche S, Hamadouche N, Mahdi D. 2022. Subacute hepatotoxicity of alkaloids extracts of *Peganum harmala. L* seeds in Wister albino rats. Not Sci Biol. 14: 1-12.

Bournine L, Bensalem S, Fatmi S, Bedjou F, Mathieu V, Iguer-Ouada M, Kiss R, Duez P. 2017. Evaluation of the cytotoxic and cytostatic activities of alkaloid extracts from different parts of *Peganum harmala* L. (Zygophyllaceae). Eur J Integr Med. 9: 91-6.

Bremner P, Rivera D, Calzado MA, Obon C, Inocencio C, Beckwith C, Fiebich BL, Munoz E, Heinrich M. 2009. Assessing medicinal plants from South-Eastern Spain for potential anti-inflammatory effects targeting nuclear factor-kappa B and other pro-inflammatory mediators. J Ethnopharmacol. 124: 295-305.

Dalimunthe A, Hasibuan PAZ, Silalahi J, Satria D. 2018. Antioxidant activity of alkaloids fractions of *Litsea cubeba* Lour. fruits. Asian J Pharm Clin Res. 11: 31.

Diaz GJ. 2015. Toxicosis by plant alkaloids in human and animals in Colombia. Toxins (Basel) 7: 5408-16.

El-Dwairi QA, Banihani SM. 2007. Histo-functional effects of *Peganum harmala* on male rat's spermatogenesis and fertility. Neuroendocrinology Letters. 28: 305-10.

Habli Z, Toumieh G, Fatfat M, Rahal ON, Gali-Muhtasib H. 2017. Emerging cytotoxic alkaloids in the battle against cancer: Overview of molecular mechanisms. Molecules 22: 250.

Hamden K, Masmoudi H, EIlouz F, EIfeki A, Carreau S. 2008. Protective effects of *Peganum harmala* extracts on thiourea induced diseases in adult male rat. J Environ Biol. 29: 73-7.

Hamid HA, Ramli ANM, Yusoff MM. 2017. Indole alkaloids from plants as potential leads for antidepressant drugs: A mini review. Front Pharmacol. 8: 96.

Kal-Taie MA. 2009. Effect of seed alkaloids of *Peganum harmala* on histological liver and some blood parameters in male mice. Biochem cell. Arch. 19: 2883-9.

Kermanian F, Joghataei MT, Mahdizadeh M. 2003. To Study Teratogenic Effect of *Peganum harmala* plant on skeletal system and mice embryo growth rate using alizarin red. J Iran Anat Sci. 1: 35-9.

Liu L, Zhao T, Cheng XM, Wang CH, Wang ZT. 2013. Characterization and determination of trace alkaloids in seeds extracts from *Peganum harmala* Linn. using LC-ESI-MS and HPLC. Acta Chromatogr. 25: 221-40.

Madah M, Haddad S, Khazem M. 2020. Evaluation of the effect of *Peganum harmala* extracts on the in vitro viability of *Leishmania tropica* promastigoes in comparison to Glucantime. J Parasit Dis.

How to cite this article: Sasi SM, Alghoul NM, Awayn N, Elghoul A, Prastiya RA. 2023. Effect of *Peganum harmala* seeds extract on the hepatic tissue structure and fetus of mice. Ovozoa Journal of Animal Reproduction. 12: 141-147.

44: 858-63.
Mahdeb N, Bettihi S, Ghedjati N, Bouzidi A. 2020. Evaluation of acute toxicity of the alkaloids of *Peganum harmala L* seeds in Albino wistar female rats. Adv Pharmacol Pharm. 8: 24-30.
Mahmoudian M, Jalilpour H, Salehian P. 2002. Toxicity of *Peganum harmala*: Review and a case report. Iran J Pharmacol Ther. 1: 1-4.
Moghadam TK, Jafary H, Yaghmaei P. 2021. Evaluation of the effect of harmaline on the serum level of liver index enzymes in a model of non-alcoholic fatty liver of male NMRI mice. J Anim Biol. 14: 143-52.
Mohamed AHS, Al-Jammali SMJ, Naki ZJ. 2013. Effect of repeated administration of *Peganum harmala* alcoholic extract on liver and kidney in albio mice: A histo-pathological study. J Sci Innov Res. 2: 585-97.
Mollashahi E, Kazerani HR. 2020. Metabolic effects of methanol extract of harmala (*Peganum harmala*) in rats fed with normal diet. Intern Med Today 26: 414-31.
Nafisi S, Bonsaii M, Maali P, Khalilzadeh MA, Manouchehri F. 2010. Beta-carboline alkaloids bind DNA. J Photochem Photobiol B: Biol. 100: 84-91.
Nenaah G. 2010. Antibacterial and antifungal activities of beta-carboline alkaloids of *Peganum harmala* (L) seeds and their combination effects. Fitoterapia 81: 779-82.
Niroumand M C., Farzaei M.H., Amin, G. 2015. Medicinal properties of *Peganum harmala* L. in traditional Iranian medicine and modren phytotherapy: Review. J Tradit Chin Med. 35: 104-9.
Qazan WS. 2009. The effect of low level of dietary *Peganum harmala L*. and Ballota undulate or their mixture on chicks. Anim Vet Adv. 8: 1535-8.
Rezzagui A, Merghem M, Derafa I, Dahamna S. 2020. Acute and sub-acute toxic effects of Algerian *Peganum harmala L*. crude extract. J Drug Deliv Ther. 10: 115-21.
Sasi NM, Sasi SM, Alfelah A. 2017. Effect of boiled *Peganum harmala* seeds on embryos of experimental laboratory mice. J Pure Appl Sci. 16: 77-81.
Sasi SM, Sasi NM, Dawd DS. 2013. Effect of boiled *Peganum harmala* seeds on pregnant female mice. J Basic Appl Sci. 19: 68-73.
Shaheen H, Issa MY. 2020. In vitro and in vivo activity of *Peganum harmala L*. alkaloids against phytopathogenic bacteria. Sci Hortic. 264: 108940.

How to cite this article: Sasi SM, Alghoul NM, Awayn N, Elghoul A, Prastiya RA. 2023. Effect of *Peganum harmala* seeds extract on the hepatic tissue structure and fetus of mice. Ovozoa Journal of Animal Reproduction. 12: 141-147.