The Haryana Veterinarian (June, 2023) **62(1),** 109-113 ISSN 0033-4359 (P) **Research Article**

AMELIORATIVE EFFECT OF *PROSOPIS CINERARIA* EXTRACT ON GROSS, HISTOPATHOLOGICAL AND OXIDATIVE STRESS PARAMETERS AGAINST *PARTHENIUM HYSTEROPHORUS* INDUCED LIVER DAMAGE IN WISTAR ALBINO RATS

ANITA RATHORE*, ROHITASH DADHICH, ANIRUDH KHATRI[1], SARJNA MEENA[2] and HAKIM MANZER ALAM[3]
Department of Veterinary Pathology, College of Veterinary and Animal Science, Navania,Udaipur-313 601, India
[1]Department of Pharmaceutical Sciences, M.L.S. University, Udaipur-313 001
[2]Department of Veterinary Pathology, PGIVER, RAJUVAS, Jaipur-302 031
[3]Department of Veterinary Parasitology, College of Veterinary and Animal Science, Navania, Udaipur-313 601, RAJUVAS, Bikaner-334 001, India

Received: 21.08.2022; Accepted: 19.10.2022

ABSTRACT

The present investigation was conducted to study the *Parthenium hysterophorus* induced toxicity in liver and its amelioration with leaves of *Prosopis cineraria* in Wistar rats. A total of 80 rats were randomly divided into 8 groups (Group I, II, III, IV, V, VI, VII and VIII). The toxicity was induced by oral feeding of ethanolic extract of *Parthenium* at 150, 300 and 450 mg/kg body weight in group II, III and IV, respectively for 28 days. Group V, VI and VII were fed with ethanolic extract of *Parthenium* at 150, 300 and 450 mg/kg body along with 200 mg/kg body weight of methanolic extract of leaves of *Prosopis cineraria*. Group I served as control while group VIII was kept as treatment control and fed only methanolic extract of leaves of *Prosopis cineraria* at 200 mg/kg body weight. *Parthenium* treatment leading to generation of oxidative stress in rats by increased LPO level and decreased activity of GSH and SOD, which were ameliorated by *Prosopis* extract. Grossly on necropsy mild congestion, swollen and enlargement of liver in *Parthenium* treated group II, group III, group IV were observed and liver sections showed fatty changes, haemorrhages, and moderate to severe hepatocellular degeneration. The liver of group V, group VI, group VII, group VIII also showed near to normal, mild congestion and swelling, moderate congestion and normal appearance of liver on 28th day, respectively. It can be concluded that methanolic extract of *Prosopis* @ 200 mg/kg b.wt orally reduced the toxic change induced by *Parthenium hysterophorus* at dose rate of 150 mg/kg b.wt. to a satisfactory level.

Keywords: Amelioration, *Parthenium hysterophorus*, *Prosopis cineraria*, Wistar rats

How to cite: Rathore, A., Dadhich, R., Khatri, A., Meena, S. and Alam, H.M. (2023). Ameliorative effect of *Prosopis cineraria* extract on gross, histopathological and oxidative stress parameters against *Parthenium hysterophorus* induced liver damage in wistar albino rats. *The Haryana Veterinarian* **62(1)**: 109-113.

Parthenium hysterophorus L. belongs to the family Asteraceae, is an invasive, foreign weed that has currently taken over practically all of India (Adkins *et al.*, 1996). Parthenin is a major toxic component which is lethal to human beings and animals (Bezuneh, 2015). During times of scarcity of fodder cattle, sheep and goats are forced to eat parthenium, which can taint their meat and make dairy milk unpalatable due to its irritating odor. Those animals can face rashes on their bodies and udders, alopecia, loss of skin pigmentation, allergic skin reactions, dermatitis, diarrhea, anorexia, pruritus, and death. (Narsimhan *et al.*, 1977; Tudor *et al.*, 1982; Ahmed *et al.*, 1988; Ramita *et al.*, 2015). Leaf paste of *Prosopis cineraria* (L.) Druce, which is a state tree of Rajasthan, is applied on boils and blisters, including mouth ulcers in livestock and leaf infusion on open sores on the skin (Khatri *et al.*, 2010).

Due to known toxicity of *Parthenium hysterophorus* in livestockas there is no appropriate antagonist in this field, the current study was formulated to study sub-acute toxicity of *Parthenium hysterophorus* L. in liver of Wistar albino rats and to evaluate the protective property of *Prosopis cineraria* (L.) Druce during *Parthenium* toxicity.

*Corresponding author: anni_vet11@rediffmail.com

MATERIALS AND METHODS

Experimental animals: Eighty (80) clinically healthy adult albino rats between 2 and 3 months of age of either sex, weighing about 100-150 g, were used in this study. The animals are housed in polypropylene cages for one week prior to the experiment to reduce non-specific stress, after which they are moved to the experimental lab under standard management conditions [at a temperature of 25° C (5° C), with a natural 12-hour light/12-hour dark cycle]. Standard rat feed and water were provided ad libitum throughout the experimental period. The necessary Institute Animal Ethical Committee approval was obtained.

Preparation of extract: *Parthenium* was collected from the surrounding areas of College of Veterinary and Animal Science (CVAS) Navania, Udaipur, and *Prosopis* from the desert area of the Shekhawati region (Rajasthan). Authentication (identification) of plant materials was done by the Botanical Survey of India, Jodhpur (Rajasthan). The voucher number isBSI/AZRC/Tech./2016-17- (Pl. Id.) /01 dated 03/04/2017 for *Parthenium hysterophorus* and BSI/AZRC/ I.12014/Tech./2017-18- (Pl. Id.) dated 16/10/2017 for *Prosopis cineraria*. Five hundred grammes

of dried aerial parts of the plant *Parthenium hysterophorus* and two hundred and fifty grammes of dried leaves of the plant *Prosopis cineraria* were grinded into fine powder and subjected to soxhlet extraction with 95% ethanol for *Parthenium* and 95% methanolfor *Prosopis* for twelve hours and evaporated by using a rotary vacuum evaporator to give amorphous solid masses.The ethanolic extract of the aerial part of *Parthenium hysterophorus* and methanolic extract of the leaves of *Prosopis cineraria* was subjected to qualitative phytochemical analysis according to the different identification tests (Rathore *et al.*, 2019).

Sub chronic treatment: A total of 80 rats were randomly divided into 8 groups (Groups I, II, III, IV, V, VI, VII and VIII). Group-I (n = 10) served as control in which 1% Tween 80 suspension (vehicle) administered. Treatment group II received ethanolic extract of *Parthenium*@ 150 mg/kg b.wt; group III received ethanolic extract of *Parthenium*@ 300 mg/kg b.wt; group IV received ethanolic extract of *Parthenium*@ 450 mg/kg b.wt; group V received ethanolic extract of *Parthenium* + methanolic extract of *Prosopis*@ 150 mg/kg b.wt and 200 mg/kg b.wt., respectively; group-VIreceived ethanolic extract of *Parthenium* + methanolic extract *Prosopis*@ 300 mg/kg b.wt and 200 mg/kg b.wt., respectively; group-VII received ethanolic extract of *Parthenium* + methanolic extract of *Prosopis*@ 450 mg/kg b.wt and 200 mg/kg b.wt and group -VIII served as treatment control and fed only methanolic extract *Prosopis* 200 mg/kg b.wt orally by gavage for 28 days. The oral LD50 of anethanolic extract of *Parthenium hysterophorus* against rats was found to be 676.64 mg/kg body weight (Maurya and Kushwaha, 2010). Blood was collected in dry sterilized vials containing an ethylene diamine tetraacetic acid (EDTA) from retro-orbital sinus of rats at the time of euthanasia for estimation of hematological parameters (Rathore *et al.*, 2019). Blood samples were collected in tubes, centrifuged at 2,500 rpm for 15 min and the serum separated and stored at -20º C for analysis. Serum samples was analyzed for determination of biochemical parameters (Rathore *et al.*, 2019).

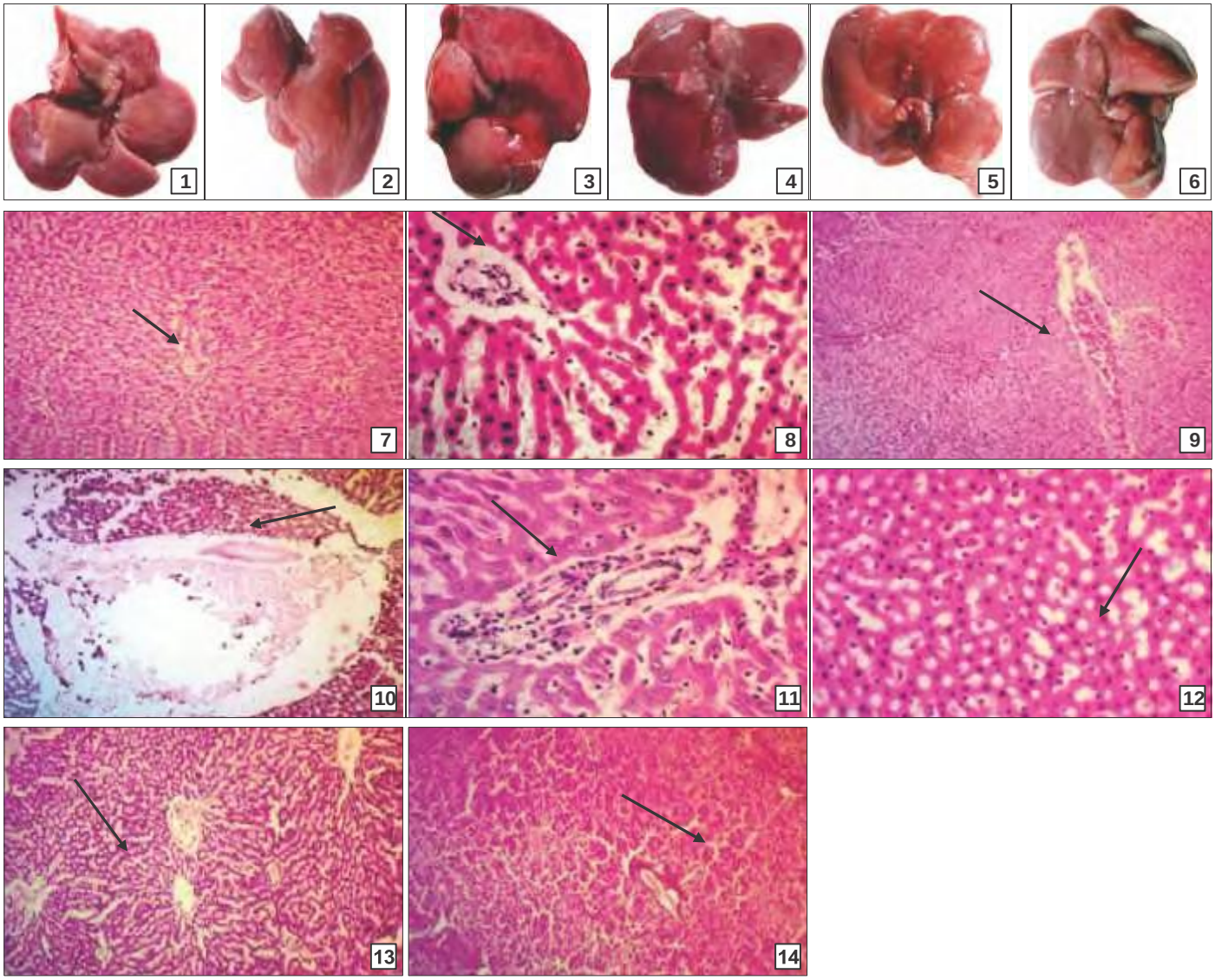
Oxidative stress analysis: About 500 mg of tissue (liver) was weighed and taken in 5 ml of ice-cold PBS (pH 7.4). Another 100 mg of sample was weighed separately and put into 1 ml of 0.02 M ethylene diamine tetraacetic acid (EDTA) solution for reduced glutathione (GSH) estimation. The homogenates (10%) prepared with the IKA Homogenizer under ice-cold conditions were centrifuged for 10 min at 3000 rpm. The supernatant was stored at -20º C until assayed for different oxidative stress-related biochemical parameters. Adouble beam UV-VIS spectrophotometer (CHINO, India) was used for recording the absorbance at 535 nm for Lipid peroxidation (LPO) estimation, at 412 nm for Reduced glutathione (GSH) estimation and at 570 nm for Superoxide dismutase (SOD) estimationof the test sample.

Histopathology: After 28 days, all rats from each group were sacrificed using isoflurane inhalation anesthesia to study the pathological changes. The tissues of the liver were collected in 10% neutral buffered formalin and embedded in paraffin wax.The processing of tissues was done by using acetone and benzene techniques (Lillie, 1965). The 4-to-5-micron thick sections were cut and stained with Haematoxylin and Eosin (H&E). (Luna, 1968).

Statistical analysis: Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons post hoc test, using IBM SPSS software. P values <0.05 were considered to be significant. (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

The oxidative damage produced by free radicals is referred to as oxidative stress and has been associated with several degenerative diseases. Lipid peroxidation (LPO) was measured in terms of malondialdehyde (MDA) produced in the liver of rats treated with ethanolic extract of *Parthenium* and methanolic extract of *Prosopis*. A statistically significantly (P≤0.05) increased level of LPO was observed in the *Parthenium* treated group as compared to control animals. This alteration was significantly restored by *Parthenium* co- administered with *Prosopis* extract at low dose toxicity in the rats, as shown in Table 1. Reduced glutathione (GSH) was measured by estimating free-SH groups, using 5, 5'-dithio-bis-2-nitrobenzoic acid (DTNB) in the liver of rats. A statistically significantly (P≤0.05) decreased level of GSH was observed in the *Parthenium* treated group as compared to control animals. This alteration was significantly restored by *Parthenium* co-administered with *Prosopis* extract at low dose toxicity in the rats, as shown in Table 2. Superoxide dismutase (SOD) was measured by generation of superoxide by pyrogallol autooxidation and the inhibition of superoxide dependent reduction of the tetrazolium dye MTT [3-(4-5 dimethyl thiazol 2-xl) 2, 5-diphenyl tetrazolium bromide] to its to its formazan in liver of rats treated with ethanolic extract of *Parthenium* and methanolic extract of *Prosopis*. A statistically significantly (P≤0.05) decreased level of SOD was observed in the *Parthenium* treated group as compared to control animals. This alteration was significantly restored by *Parthenium* co- administered with *Prosopis* extract at low dose toxicity in the rats, as shown in Table 3. Parthenin (Major sesquiterpene lactone of *Parthenium*) may cause toxicity by glutathione and protein alkylation, resulting in increased



Figs. 1-14. **(1)** Parthenium treated rat (G-II) Liver on 28th day showing mild congestion; **(2)** Parthenium treated rat (G-III) Liver on 28th day showing congestion and swollen appearance; **(3)** Parthenium treated rat (G-IV) Liver on 28th day showing severe congestion and enlargement; **(4)** Parthenium and Prosopis treated rat (G-VI) Liver on 28th day showing mild congestion and swelling; **(5)** Parthenium and Prosopis treated rat (G-VII) Liver on 28th day showing moderate congestion and swelling; **(6)** Prosopis treated rat (G-VIII) Liver on 28th day showing normal appearance; **(7)** Photomicrograph of liver showing mild haemorrhages, congested CV with necrotic hepatocyte (Group II day 28) H&E, 100X. **(8)** Photomicrograph of liver showing infiltration of leukocytes between hepatic cord and central vein (Group II day 28) H&E, 400X. **(9)** Photomicrograph of liver showing congested blood vessels with few pyknotic nucleus in hepatocytes (Group III day 28) H&E, 100X. **(10)** Photomicrograph of liver showing dilated central vein with degenerated hepatocytes (Group III day 28) H&E, 100X. **(11)** Photomicrograph of liver showing bile duct hyperplasia with infiltration of lymphocytes (Group IV day 28) H&E, 400X. **(12)** Photomicrograph of liver showing fatty change in hepatocytes and congestion in central vein (Group IV day 28) H&E, 400X. **(13)** Photomicrograph of liver showing sinusoidal dilatation (Group IV day 28) H&E, 100X. **(14)** Photomicrograph of liver showing severe hepatocellular degeneration (Group IV day 28) H&E, 100X.

oxidative stress and in functional changes of alkylated proteins (Amorin *et al.*, 2013). Phytochemical analysis of the crude extracts revealed the presence of flavonoids among the other chemical constituents within them. Flavonoids are plant polyphenolic antioxidants found in many fruits, vegetables and beverages such as tea and wine (Rice-Evans *et al.*, 1996). The antioxidant property (scavenging reactive oxygen species) of flavonoids is due to their ability to chelate free radicals immediately by donating a hydrogen atom or by single-electron transfer. Flavonoids can also act through inhibition of free radical generating enzymes such as xanthine oxidase, lipoxygenase, protein kinase C, cyclooxygenase, microsomal monooxygenase, mitochondrial succinoxidase, and NADPH oxidase (Banjarnahor and Artanti, 2014).

The rats were sacrificed on the 28th day of the experiment, and gross abnormalities were recorded at the end of the trial. Grossly, the group II, III and IV liver showed congestion, swollen and enlargement on the 28th day of the experiment (Figs. 1-3). These observations were similar to the results of Narsimhan *et al.* (1977) and More, 1979. These changes might be due to the toxic effects of *Parthenium hysterophorus* on the liver. Group V showed near to normal appearance on

Table 1. Effect on oxidative stress-related biochemical parametersin liver of rats

Groups	Treatment	LPO (nmole/g) Liver	GSH (mM/g) Liver	SOD (unit/mg) Liver
I	Control (Vehicle)	35.89±0.58^{e}	3.65±0.03^{a}	165.09±2.20^{a}
II	Ethanolic extract of Parthenium@ 150 mg/kg b.wt	49.01±0.76^{c}	3.18±0.03^{c}	140.91±1.31^{c}
III	Ethanolic extract of Parthenium@ 300 mg/kg b.wt	54.26±0.47^{b}	2.76±0.02^{d}	116.70±2.36^{d}
IV	Ethanolic extract of Parthenium@ 450 mg/kg b.wt	61.90±0.60^{a}	2.15±0.03^{e}	81.03±2.28^{e}
V	Ethanolic extract of Parthenium+Methanolic extract of Prosopis@ 150 mg/kg b.wt and 200 mg/kg b.wt., respectively	41.18±0.71^{d}	3.37±0.02^{b}	153.88±1.32^{b}
VI	Ethanolic extract of Parthenium+Methanolic extract of Prosopis@ 300 mg/kg b.wt and 200 mg/kg b.wt., respectively	50.12±0.62^{c}	2.85±0.02^{d}	124.47±2.06^{d}
VII	Ethanolic extract of Parthenium + Methanolic extract of Prosopis@ 450 mg/kg b.wt and 200 mg/kg b.wt.	60.31±0.56^{a}	2.16±0.01^{e}	81.72±1.85^{e}
VIII	Methanolic extract of Prosopis@ 200 mg/kg b.wt	35.12±0.28^{e}	3.66±0.03^{a}	166.22±1.82^{a}

All values are represented Mean ± SEM; n=10 in each group; values bearing different superscript in the same column differ significantly between groups at$P \leq 0.05$ in Tukey's multiple comparison post hoc test.

the 28th day due to low dose of *Parthenium* and *Prosopis* ameliorating at initial level. In groups VI and VII, the liver showed mild congestion and swelling, moderate congestion may be due to *Parthenium* and *Prosopis* interaction not ameliorating at the proper level due to higher dose of *Parthenium* (Figs. 4-5). In group VIII, on the 28th day, the normal appearances as compared to control groupI (Fig. 6).

Histopathology of the liver revealed changes like mild haemorrhages, congested central vein with necrotic hepatocytes and infiltration of leucocytes between the hepatic cord and central vein in group II on the 28th day of the experiment (Figs. 7-8). Group III showed congested blood vessels with few pyknotic nucleuses in hepatocytes and dilated central vein with degenerated hepatocytes on the 28th day of the experiment (Figs. 9-10). In group IV, the liver show edbile duct hyperplasia with infiltration of few lymphocytes, fatty change in hepatocytes, sinusoidal dilation, and severe hepatocellular degeneration on the 28th day of the experiment (Figs. 11-14). These changes are due to the limited detoxifying mechanism of the liver, which were observed by Narsimhan *et al.* (1977), Qureshi *et al.* (1980), Ahmed *et al.* (1988), Garg, 2004, Ananda *et al.* (2008) and Thoolen *et al.* (2010). In group V, the liver showed normal central vein and portal triad on the 28th day of the experiment. In group VI, the liver showed fatty change with mild congestion. In group VII, the liver showed congested portal vein with moderate hepatic degeneration these mild to moderate changes might be due to toxic action of *Parthenium* and insufficient ameliorating action of *Prosopis*. In group VIII liver revealed normal hepatocytes and central vein on the 28th day of the experiment.This could be due to the administration of *Prosopis* extract as a foreign substance.

From the above findings, it can be concluded that methanolic extract of leaves of *Prosopis* @ 200 mg/kg b.wt orally reduced the toxic change induced by *Parthenium hysterophorus* at dose rate of 150 mg/kg b.wt. to a satisfactory level, but at higher doses of *Parthenium hysterophorus* i.e., 300 and 450 mg/kg b.wt., the ameliorations were not to a satisfactory level. Hence, for obtaining better and more satisfactory results, more research is required to separate purified, specific molecules present in *Prosopis cineraria* which can be used as an ameliorative agent against *Parthenium hysterophorus* intoxication.

REFERENCES

Adkins, S., and Shabbir, A. (2014). Biology, ecology and management of the invasive parthenium weed (*Parthenium hysterophorus* L.). *Pest Manage. Sci.* **70**: 1023-1029.

Ahmed, M.N., Rao, P.R. and Mahendar, M. (1988). Experimental introduction of acute toxicity in buffalo calves by feeding *Parthenium hysterophorus* Linn. *Indian J. Anim. Sci.* **58**: 731-734.

Amorin, M.H.R., Costa, R.M.G.D., Lopes, C. and Bastos, M.S.M. (2013). Sesquiterpene lactones: Adverse health effects and toxicity mechanisms. *Crit. Rev. Toxicol.* **43(7)**: 559-579.

Ananda, K.J., Ansar Kamran, C., D'Souza, P.E., Yathiraj, S. and Prathiush, P.R. (2008). *Parthenium hysterophorus* toxicity in dogs. *Indian Vet. J.* **85**: 1337.

Banjarnahor, S.D.S. and Artanti, N. (2014). Antioxidant properties of flavonoids. *Med. J. Indones.* **23(4)**: 239-244.

Bezuneh, T.T. (2015). Phytochemistry and antimicrobial activity of *Parthenium hysterophorus* L.: A review. *Sci. J. Anal. Chem.* **3(3)**: 30-38.

Garg, S. (2004). Veterinary Toxicology. CBS Publishers and distributors, New Delhi. pp. 118-120.

Khatri, A., Rathore, A. and Patil, U.K. (2010). *Prosopis cineraria* (L.) Druce: A boon plant of desert- An overview. *Int. J. Biomed. Adv. Res.* **4(2)**: 27-29.

Lillie, R.D. (1965). Histopathologic technique and practical histochemistry. McGraw Hill Book Co. New York and London. p. 176.

Luna, L.G. (1968). Manual of histologic staining method of armed forces institute of pathology. (3rd Edn.), Mc. Grow. Hill book company, New York. pp. 111-112.

Maurya, S. and Kushwaha, V.B. (2010). Effect of ethanolic extract of *Parthenium hysterophorous* on haematological parameters in rat. *Bioscan.* **5(3)**: 437-440.

More, P.R. (1979). Pharmacological and toxicological investigations on *Parthenium hysterophorus* Linn. M.V.Sc. Thesis Marathwara Agricultural University, Parbhani, Maharashtra (India).

Narasimhan, T.R., Ananth, M., Swamy, M.N., Babu, M.R., Mangala, A. and Rao, P.V.S. (1977). Toxicity of *Parthenium hysterophorus* L. to cattle and buffaloes. *Experientia.* **33(10)**: 1358-1359.

Qureshi, M.I., Vadlamudi, V.P. and Wagh, K. R. (1980). A study on sub-acute toxicity of *Parthenium hysterophorus* Linn. in goats. *Livest. Adv.* **5(12)**: 39-40.

Ramita, K., Raipat, B.S. and Sinha, M.P. (2015). Impact of methanolic extract of *Parthenium hysterophorus* L. on liver profile of Wistar albino rats. *Int. J. Exc. Knowl.* **2(1)**: 56-66.

Rathore, A., Dadhich, R., Purohit, K., Sharma, S.K., Vaishnava, C.S., Joseph, B. and Khatri, A. (2019). Phytochemical screening and total phenolic and flavonoid content in leaves of *Prosopis cineraria* (L.) Druce. *Int. J. Chem. Stud.* **7(3)**: 1853-1855.

Rathore, A., Dadhich, R., Purohit, K., Sharma, S.K., Vaishnava, C.S., Joseph, B. and Khatri, A. (2019). Impact of *Prosopis cineraria* (L.) Druce leaves on hematological parameters against induced sub-acute toxicity of *Parthenium hysterophorous* L. in wister albino rats. *Int. J. Chem. Stud.* **7(1)**: 294-296.

Rathore, A., Dadhich, R., Purohit, K., Sharma, S.K., Vaishnava, C.S., Joseph, B. and Khatri, A. (2019). Biochemical effect of induced subacute toxicity of *Parthenium hysterophorus* L. and its amelioration with *Prosopis cineraria* (L.) Druce leaves in wistar albino rats. *Vet. Pract.* **20(2)**: 188-190.

Rice-Evans, C.A., Miller, N.J. and Paganga, G. (1996). Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biol. Med.* **20**: 933-956.

Snedecor, G.W. and Cochran, W.G. (1994). Statistical methods. Ch. 12 & 13. (8th Edn.), Oxford and IBH Publications, New Delhi.

Thoolen, R., Maronpot, R.R., Harada, T., Nyska, A., Rousseaux, C. and Nolte, T. (2010). Proliferative and nonproliferative lesions of the rat and mouse hepatobiliary system. *Toxicol. Pathol.* **38**: 5S-81S.

Tudor, G.D., Ford, A.L., Armstrong, T.R. and Bromage, E.K. (1982). Taints in meat from sheep grazing *Parthenium hysterophorus*. *Aust. J. Exp. Agric. Husb.* **22(115)**: 43-46.

CONTRIBUTORS MAY NOTE

- Research/Clinical articles are invited for next issue from the Scientists/Veterinarians engaged in Veterinary Profession.
- Please follow strictly the format of 'The Haryana Veterinarian' for manuscript writing/submission.
- Please pay processing fee of Rs. 1000/- online in the account of Dean, College of Veterinary Sciences, along with each article.
- After revision, please return the revised manuscript and rebuttal at the earliest.
- Please mention your article reference number in all correspondence for a quick response.
- We solicit your co-operation.
- All correspondence should be addressed to 'The Editor', Haryana Veterinarian, Department of Veterinary Parasitology, College of Veterinary Sciences, LUVAS, Hisar-125004.

Editors