

Toxicological effect of *Parthenium hysterophorus* on kidney and its amelioration with *Prosopis cineraria* leaves in Wistar rats

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ABSTRACT

The present investigation was conducted to study *Parthenium hysterophorus* induced toxicity in the kidney and its amelioration with leaves of *Prosopis cineraria* in Wistar rats. A total of eighty albino rats were used. The toxicity was induced by oral feeding of ethanolic extract of *Parthenium* at 150, 300 and 450 mg/kg body weight in groups 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 weight along with 200 mg/kg body weight of methanolic extract of leaves of *Prosopis cineraria*. Group I served as a control while group VIII was kept as treatment control and fed only methanolic extract at 200 mg/kg body weight. *Parthenium* treatment leading to the generation of oxidative stress in rats by increasing LPO level and decreasing activity of GSH and SOD, which were ameliorated by *Prosopis* extract. Grossly on necropsy, mild to severe hemorrhage of the kidney in *Parthenium* treated groups II, III, and IV were observed and kidney sections showed haemorrhages, cloudy swelling, coagulative necrosis, hyalinization of glomeruli, heavy infiltration in the corticomedullary region and degeneration of tubular epithelium. The kidneys of groups V and VIII showed normal architecture details of the kidney, but in group VI showed normal glomeruli with mild pyknotic nucleus in degenerative tubules and in group VII showed dilated tubules in the medullary region with mild degenerative changes of the kidney on the 28th day, respectively.

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

Parthenium hysterophorus L. belongs to the family *Asteraceae*, is an invasive, foreign weed that has currently taken over practically all of India¹. Parthenin is a major toxic component, which is lethal to human beings and animals². 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 odour. Those animals can face rashes on their bodies and udders, alopecia, loss of skin pigmentation, allergic skin reactions, dermatitis, diarrhea, anorexia, pruritus, and death³⁻⁵. 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 skin⁶. The present work is 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.

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.

Parthenium was collected from the surrounding areas of 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). Five hundred gram of dried aerial

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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 ethanol for *Parthenium* and methanol for *Prosopis* for twelve hours and evaporated by using a rotary vacuum evaporator.

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 Prosopis @ 150 mg/kg b.wt and 200 mg/kg b.wt respectively; group-VI received 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 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 an ethanolic extract of *Parthenium hysterophorus* against rats was found to be 676.64 mg/kg body weight⁷.

About 500 mg of tissue (kidney) 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 -20°C until assayed for different oxidative stress-related biochemical parameters. A double beam UV-VIS spectrophotometer (CHINO, India) was used for recording the absorbance of the test sample.

After 28 days, all rats from each group were sacrificed using isoflurane inhalation anesthesia to study the pathological changes. The tissues of the kidney were collected in 10% neutral buffered formalin and embedded in paraffin wax. The processing of tissues was done by using acetone and benzene techniques⁸. The 4-to-5-micron thick sections were cut and stained with Haematoxylin and Eosin (H&E)⁹.

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 significant¹⁰.

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 kidney of rats treated with ethanolic extract of Parthenium and methanolic extract of Prosopis. A statistically significantly ($P \leq 0.05$) 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 kidney of rats. A statistically significantly ($P \leq 0.05$) 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-yl) 2, 5-diphenyl tetrazolium bromide] to its formazan in kidney of rats treated with ethanolic extract of Parthenium and methanolic extract of Prosopis. A statistically significantly ($P \leq 0.05$) 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

Table 1. Effect on lipid peroxidation in kidney of rats after 28 days oral administration of different dose of ethanolic extract of parthenium and its amelioration with methanolic extract of prosopis.

Groups	Treatment	LPO (nmole/g) Kidney
I	Control (Vehicle)	23.21±0.60 ^e
II	Ethanolic extract of Parthenium @ 150 mg/kg b.wt	38.04±0.47 ^c
III	Ethanolic extract of Parthenium @ 300 mg/kg b.wt	44.53±0.54 ^b
IV	Ethanolic extract of Parthenium @ 450 mg/kg b.wt	53.43±0.57 ^a
V	Ethanolic extract of Parthenium + Methanolic extract of Prosopis @ 150 mg/kg b.wt and 200 mg/kg b.wt respectively	30.73±0.42 ^d
VI	Ethanolic extract of Parthenium + Methanolic extract of Prosopis @ 300 mg/kg b.wt and 200 mg/kg b.wt respectively	40.25±0.57 ^c
VII	Ethanolic extract of Parthenium + Methanolic extract of Prosopis @ 450 mg/kg b.wt and 200 mg/kg b.wt respectively	52.02±0.60 ^a
VIII	Methanolic extract of Prosopis @ 200 mg/kg b.wt	22.92±0.48 ^e

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.

Table 2. Effect on reduced glutathione in kidney of rats after 28 days oral administration of different dose of ethanolic extract of parthenium and its amelioration with methanolic extract of prosopis.

Groups	Treatment	GSH (mM/g) Kidney
I	Control (Vehicle)	1.98±0.02 ^a
II	Ethanolic extract of Parthenium @ 150 mg/kg b.wt	1.67±0.02 ^b
III	Ethanolic extract of Parthenium @ 300 mg/kg b.wt	1.32±0.01 ^c
IV	Ethanolic extract of Parthenium @ 450 mg/kg b.wt	1.01±0.03 ^d
V	Ethanolic extract of Parthenium + Methanolic extract of Prosopis @ 150 mg/kg b.wt and 200 mg/kg b.wt respectively	1.79±0.02 ^b
VI	Ethanolic extract of Parthenium + Methanolic extract of Prosopis @ 300 mg/kg b.wt and 200 mg/kg b.wt respectively	1.36±0.02 ^c
VII	Ethanolic extract of Parthenium + Methanolic extract of Prosopis @ 450 mg/kg b.wt and 200 mg/kg b.wt respectively	1.02±0.03 ^d
VIII	Methanolic extract of Prosopis @ 200 mg/kg b.wt	1.99±0.03 ^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≤0.05 in Tukey's multiple comparison post hoc test.

Table 3. Effect on superoxide dismutase in kidney of rats after 28 days oral administration of different dose of ethanolic extract of parthenium and its amelioration with methanolic extract of prosopis.

Groups	Treatment	SOD (unit/mg) Kidney
I	Control (Vehicle)	132.74±1.41 ^a
II	Ethanolic extract of Parthenium @ 150 mg/kg b.wt	113.97±2.23 ^c
III	Ethanolic extract of Parthenium @ 300 mg/kg b.wt	88.04±1.80 ^e
IV	Ethanolic extract of Parthenium @ 450 mg/kg b.wt	51.08±1.11 ^f
V	Ethanolic extract of Parthenium + Methanolic extract of Prosopis @ 150 mg/kg b.wt and 200 mg/kg b.wt respectively	125.07±1.25 ^b
VI	Ethanolic extract of Parthenium + Methanolic extract of Prosopis @ 300 mg/kg b.wt and 200 mg/kg b.wt respectively	96.49±1.56 ^d
VII	Ethanolic extract of Parthenium + Methanolic extract of Prosopis @ 450 mg/kg b.wt and 200 mg/kg b.wt respectively	51.41±0.91 ^f
VIII	Methanolic extract of Prosopis @ 200 mg/kg b.wt	133.02±1.75 ^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≤0.05 in Tukey's multiple comparison post hoc test.

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 oxidative stress and in functional changes of alkylated proteins¹¹. 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 wine¹². 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 oxidase¹³.

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 kidney showed hemorrhage on the 28th day of the experiment (Fig. 1-3). These findings might be due to the Parthenium induced toxic effects on kidneys. Similar findings were also observed by³. Group V, VI and VII kidneys on 28th day revealed near to normal, mild swollen and enlargement. These changes could be due to interaction of Parthenium and *Prosopis cineraria* (Fig. 4-5). In group VIII Prosopis treated rat showed normal appearance of kidney due to antioxidant activity of plant extractas compared to control group I (Fig. 6).

Histopathology of the kidney revealed changes like hemorrhage between glomeruli and tubules and cloudy swelling of tubules in group II on the 28th day of the experiment (Fig. 7-8). Group III showed shrinkage

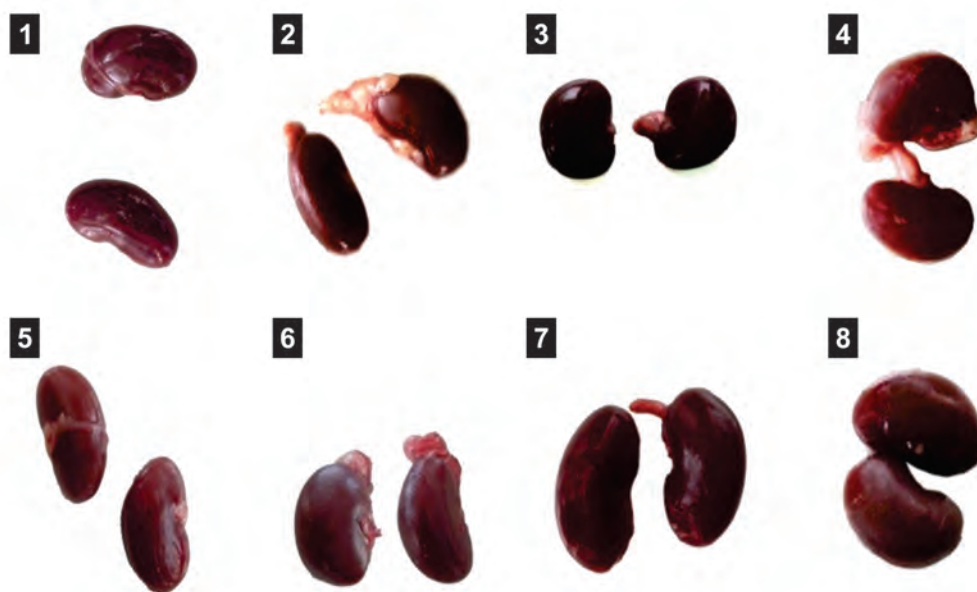


Fig. 1. Control group rat (G-I) kidney on 28th day showing normal appearance; **Fig. 2.** Parthenium treated group rat (II) kidney on 28th day showing mild haemorrhage; **Fig. 3.** Parthenium treated group rat (III) kidney on 28th day showing dark moderate haemorrhage; **Fig. 4.** Parthenium treated group rat (IV) kidney on 28th day showing severe haemorrhage; **Fig. 5.** Parthenium and Prosopis treated group rat (V) kidney on 28th day showing near to normal appearance; **Fig. 6.** Parthenium and Prosopis treated group rat (VI) kidney on 28th day showing mild swollen in appearance; **Fig. 7.** Parthenium and Prosopis treated group rat (VII) kidney on 28th day showing mild enlargement; **Fig. 8.** Prosopis treated group rat (VIII) kidney on 28th day showing normal appearance.

of glomeruli and swelling and coagulative necrosis of kidney tubules on the 28th day of the experiment (Fig. 9-10). In group IV the kidney showed hyalinization of glomeruli, sloughing of tip of papillary tubules and heavy infiltration in corticomedullary region with necrosed

tubular epithelium on the 28th day of the experiment (Fig. 11-14). Similar changes were observed by^{5,3,14-17}. In group V and group VIII of the kidney showed normal architecture details on the 28th day of the experiment. In group VI and VII were also similar to that of group II,

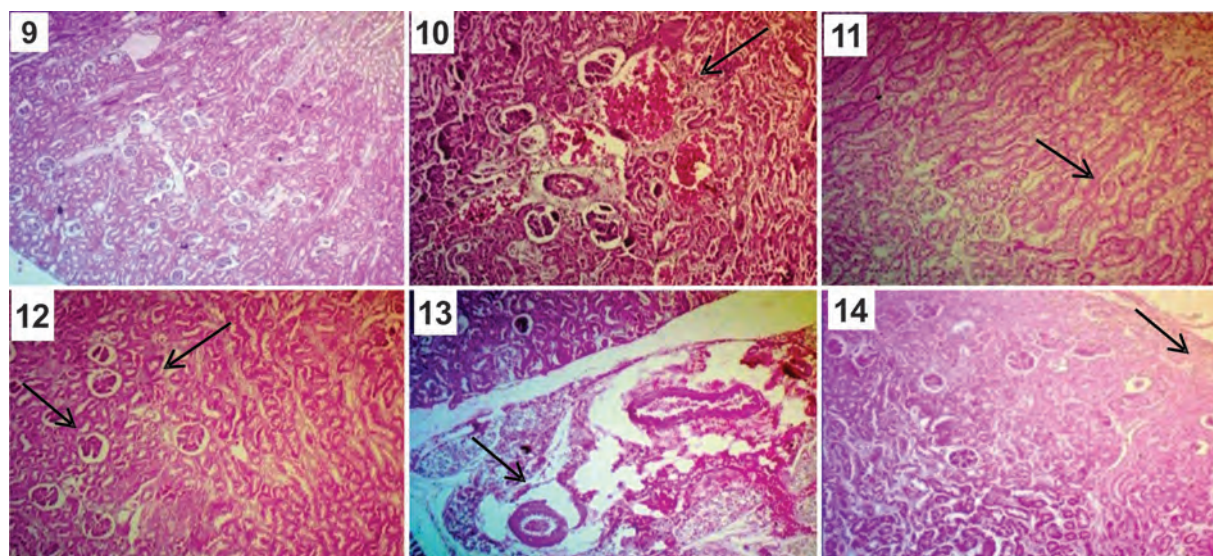


Fig. 9. Photomicrograph of kidney showing normal glomeruli and tubules (Group I, 28th Day) H&E 40X; **Fig. 10.** Photomicrograph of kidney showing haemorrhage between glomeruli and tubules (Group II, 28th Day) H&E 100X; **Fig. 11.** Photomicrograph of kidney showing cloudy swelling of tubules (Group II, 28th Day) H&E 100X; **Fig. 12.** Photomicrograph of kidney showing mild intertubular haemorrhage with swelling and shrinkage of glomeruli (Group III, 28th Day) H&E 100X; **Fig. 13.** Photomicrograph of kidney showing congested and dilated blood vessels in capsules (Group III, 28th Day) H&E 100X; **Fig. 14.** Photomicrograph of kidney showing coagulative necrosis in tubules (Group III, 28th Day) H&E 100X.

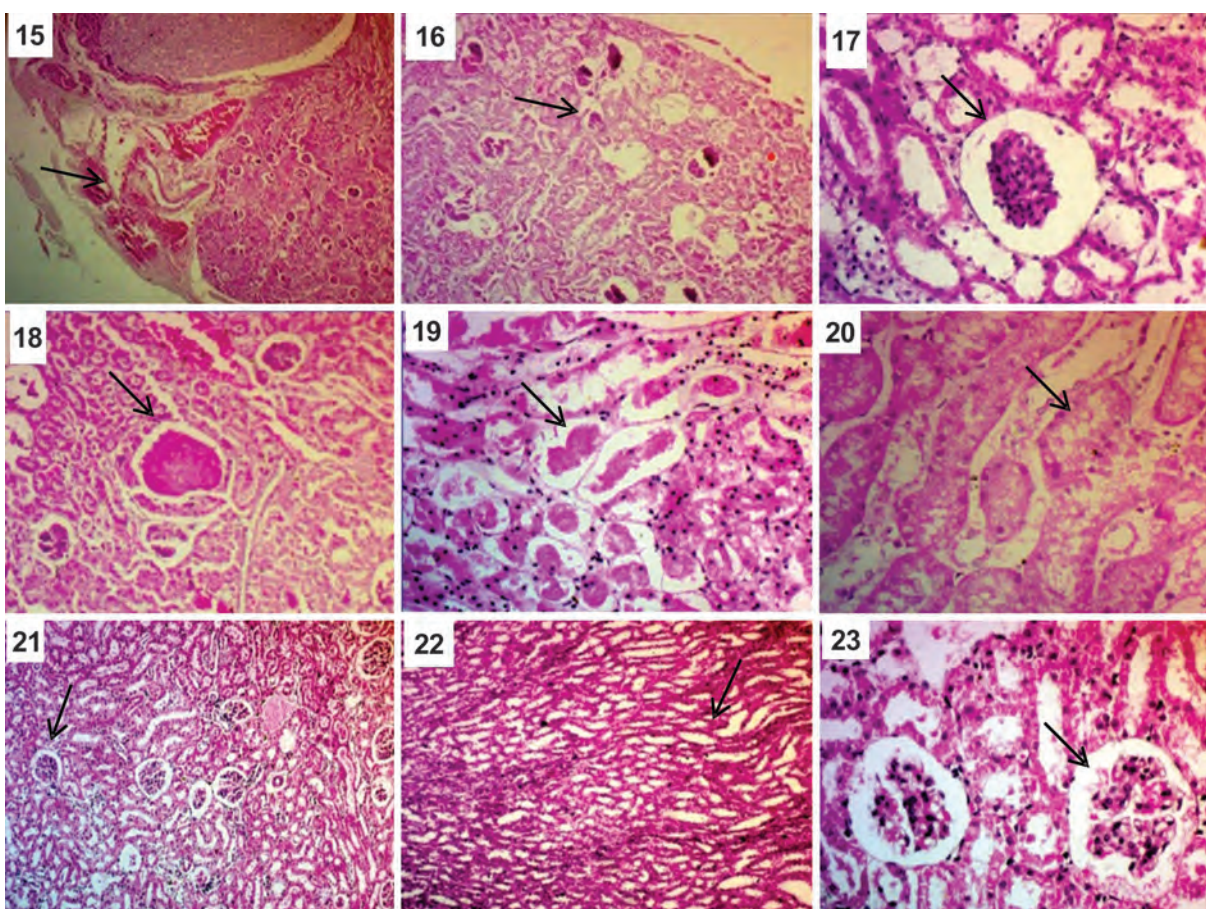


Fig. 15. Photomicrograph of kidney showing sub capsular haemorrhage (Group IV, 28th Day) H&E 400X; **Fig. 16.** Photomicrograph of kidney showing degeneration of glomeruli (Group IV, 28th Day) H&E 100X; **Fig. 17.** Photomicrograph of kidney showing shrinkage of glomeruli and increase bowmans space (Group IV, 28th Day) H&E 400X; **Fig. 18.** Photomicrograph of kidney showing hyalinization of glomeruli and degeneration of tubular epithelium (Group IV, 28th Day) H&E 100X; **Fig. 19.** Photomicrograph of kidney showing tubular epithelial necrosis (Group IV, 28th Day) H&E 400X; **Fig. 20.** Photomicrograph of kidney showing vacuole formation in tubules (Group IV, 28th Day) H&E 400X; **Fig. 21.** Photomicrograph of kidney showing normal glomeruli with pyknotic nucleus in degenerated tubules (Group VI, 28th Day) H&E 100X; **Fig. 22.** Photomicrograph of kidney dilated tubules in medullary region with moderate degenerative changes (Group VII, 28th Day) H&E 100X; **Fig. 23.** Photomicrograph of kidney showing normal appearance with mild changes in epithelium of tubules (Group VIII, 28th Day) H&E 400X.

group III and group IV except mild pyknotic nucleus in degenerative tubules on 28th day of the experiment.

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.

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