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Research Article

AMELIORATIVE EFFECT OF *PROSOPIS CINERARIA* EXTRACT ONGROSS AND HISTOPATHOLOGICAL PARAMETERS AGAINST *PARTHENIUM HYSTEROPHORUS* INDUCED SKIN DAMAGE IN WISTAR ALBINO RATS

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ABSTRACT

The present investigation was conducted to study the *Parthenium hysterophorus* induced toxicity in skin 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 necropsyred, loss of hair at forehead of skin in *Parthenium* treated group-IV was observed and skin sections showed mild lymphocytic infiltration in dermal area and degeneration in dermis, infiltration of inflammatory cells around hair follicles of dermis were found. The skin of group-V and group-VIII showed near to normal, and group-VI, group-VII showed low ameliorations of skin 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*, Skin, Wistar rats

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Parthenium hysterophorus L. belongs to the family Asteraceae, is an invasive, foreign weed that has currently taken over practically all of India (Adkins and Shabbir, 2014). Parthenin is a major toxic component which is lethal to human beings and animals (Bezuneh, 2015). During times of scarcity of feed cattle, sheep and goats are forced to eat parthenium, which can taint their meat and make dairy milk unpalatable due to its irritating odor. These animals can face rashes on their bodies and udders, alopecia, loss of skin pigmentation, allergic skin reactions, dermatitis, diarrhea, anorexia and death (Narasimhan *et al.*, 1977; Tudor *et al.*, 1982 and Ahmed *et al.*, 1988). 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).

The toxicity of *Parthenium hysterophorus* in livestock is a major problem in Veterinary field, the current study was formulated to study sub-acute toxicity of *Parthenium hysterophorus* L. in skin of Wistar albino rats and to evaluate the protective property of *Prosopis cineraria* (L.) Druce during Parthenium toxicity.

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MATERIAL 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 were 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 collectedin the month of April, 2017 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 is BSI/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 grams of dried aerial parts of the plant *Parthenium hysterophorus* and two hundred and fifty grams 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% methanol for 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 (Khatri *et al.*, 2010).

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 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 LD_{50} of an ethanolic 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 -20° C for analysis. Serum samples were analyzed for determination of biochemical parameters (Rathore, 2019).

Histopathology

After 28 days, all rats from each group were sacrificed using isoflurane inhalation anesthesia to study the pathological changes. The tissues of the skin 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 (Luna, 1968).

Statistical analysis

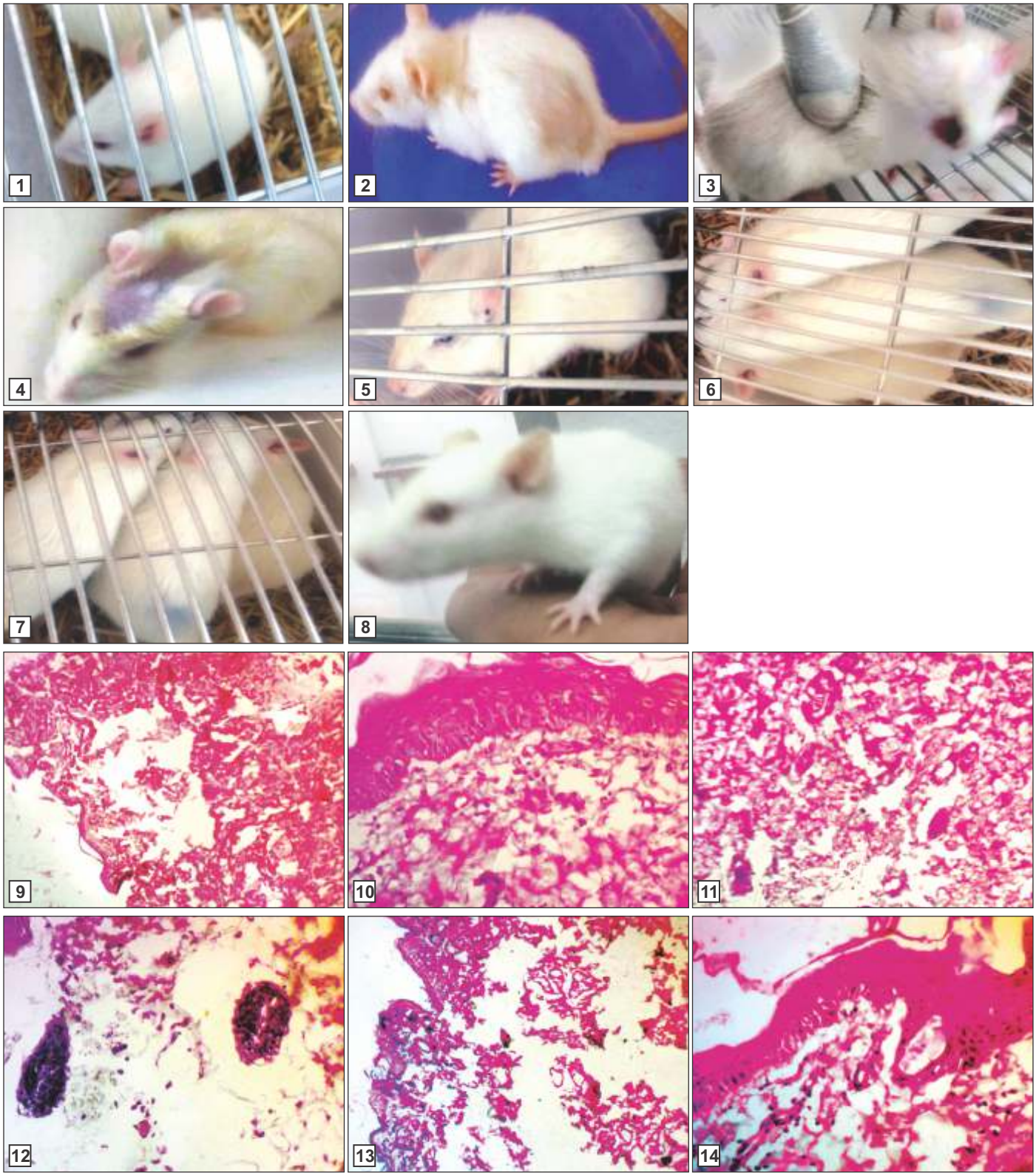
Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons post hoc test, using IBM SPSS software. P values <0.05 were considered to be significant (Snedecor and Cochran,1994).

RESULTS AND DISCUSSION

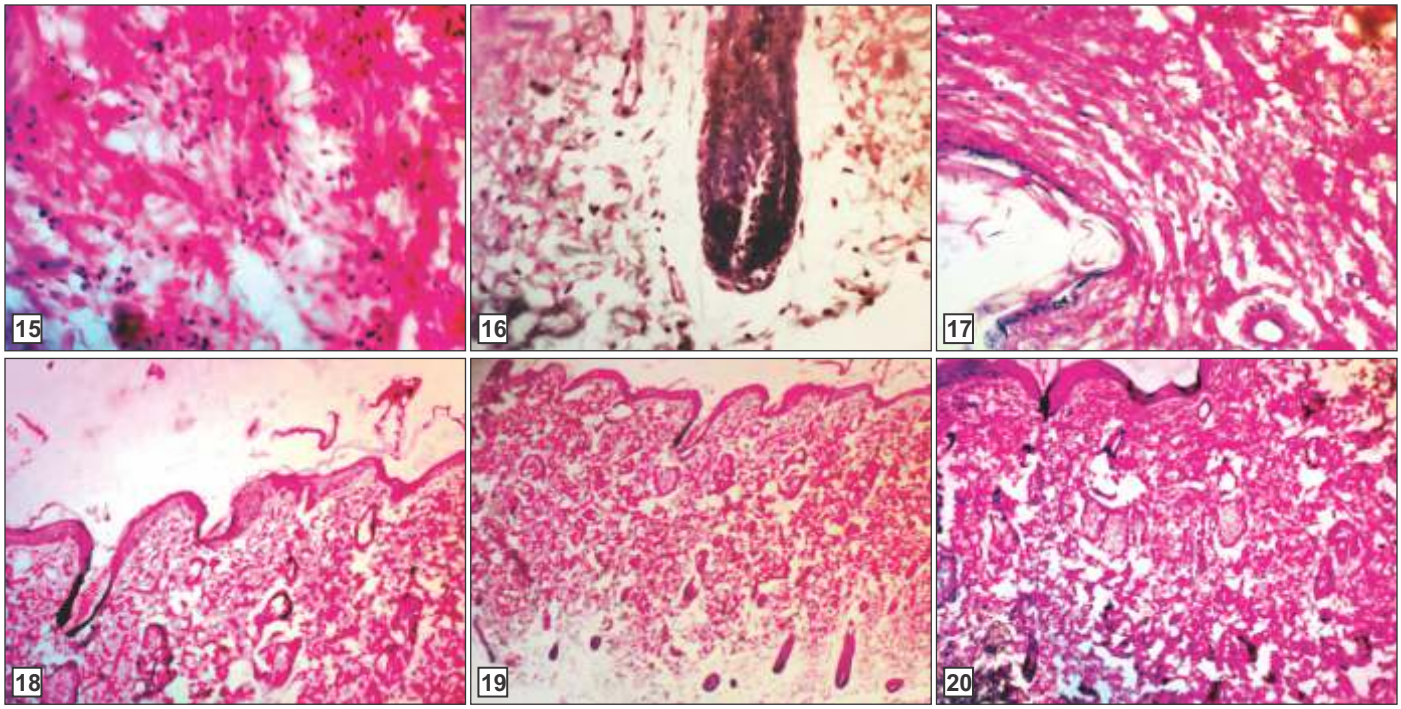
Parthenin (Major sesquiterpene lactone of Parthenium) may cause toxicity by glutathione and protein alkylation, resulting in increased oxidative stress and in functional changes of alkylated proteins (Amorin, 2013). Phytochemical analysis of *Prosopis cineraria* of the crude extracts revealed the presence 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, 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, group-II, III and group IV skin showed erythema and alopeciaon the 28th day of the experiment (Figs. 1-4). These observations were similar to the results of [Ahmed *et al.*, 1988; Garg, 2004; Narasimhan *et al.*, 1977; Ananda *et al.*, 2008; Singh and Gupta, 2006 and Enciso-Roca, 2017). These changes might be due to the toxic effects of Parthenin which is a photodynamic substance (Garg, 2004). It is a sesquiterpene lactone which may be responsible for dermatitic lesions. Group-V skin showed healed to normal appearance on 28th day of experiment, but in group-VI and VII no amelioration was recorded. Group-VIII showed normal appearance of skin compared to control group (Figs. 5-8).

Histopathology of the skin revealed changes like erosion and degeneration of epidermis and dermis, mild infiltration in dermis, degenerated hair follicle in dermis, mononuclear cell infiltration around hair follicle and degeneration in dermisin group-II and III on the 28th day of the experiment (Figs. 9-12). In group-IV, the skin showed degeneration of epidermis and dermis, multi focal, mononuclear cell infiltration in the dermis, infiltration around hair follicles, severe mononuclear cell infiltration in the dermison the 28th day of the experiment (Figs. 13-



Figs. 1-14. **(1)** Normal appearance of skin **(2)** Parthenium treated rat (G-2) skin on 28th day showing dermatitis **(3)** Parthenium treated rat (G-3) skin on 28th day showing mild patches of dermatitis at forelimb **(4)** Parthenium treated rat (G-4) skin on 28th day showing red, loss of hair at forehead **(5)** Parthenium treated rat (G-5) skinon 28th day showing normal appearance **(6)** Parthenium treated rat (G-6) skin on 28th day showing mild loss of hair **(7)** Parthenium treated rat (G-7) skin on 28th day showing mild recovery of skin **(8)** Parthenium treated rat (G-8) skin on 28th day showing normal appearance of skin **(9)** Photomicrograph of skin showing erosion and degeneration of epidermis and dermis (Group-II) H& E 100X **(10)** Photomicrograph of skin showing mild inflammatory cell in dermis (Group-II) H& E 400X **(11)** Photomicrograph of skin showing degenerated hair follicle in dermis (Group-III) H& E 100X **(12)** Photomicrograph of skin showing mononuclear cell infiltration around hair follicle (Group-III) H& E 400X **(13)** Photomicrograph of skin showing degeneration of epidermis and dermis (Group-IV) H& E 100X **(14)** Photomicrograph of skin showing dermatitis in which multi focal, mononuclear cell infiltration in the dermis (Group-IV) H& E 400X



Figs. 15-20. **(15)** Photomicrograph of skin showing severe mononuclear cell infiltration in the dermis (Group-IV) H& E 400X **(16)** Photomicrograph of skin showing infiltration around hair follicles of dermis (Group-IV) H& E 400X **(17)** Photomicrograph of skin showing near to normal (Group-V) H& E 100X **(18)** Photomicrograph of skin showing mild degeneration of stratum corneum (Group-VI) H& E 100X **(19)** Photomicrograph of skin showing moderate degeneration of dermis (Group-VII) H& E 40X **(20)** Photomicrograph of skin showing normal epidermis sebaceous gland and hair follicle (Group-VIII) H& E 100X

16). similar changes were documented by (Ahmed *et al.*, 1988 and Prakash *et al.*, 2002). These changes might be due to accumulation of Parthenin photodynamic substance in skin (Garg, 2004). Toxicity of parthenium extract causes morphological changes and cell death exhibited cytotoxicity (Narasimhan, 1984). In group-V, the skin showed near to normal appearance of epidermis (Fig. 17). Similar observations were recorded by (Mecklenburg *et al.*, 2013). In group-VI showed mild degeneration of stratum corneum (Fig. 18). In group-VII showed moderate degeneration of dermis (Fig. 19). The amelioration which may be low due to potential toxic action of Parthenium and insufficient amelioration (antioxidant compounds) achievement of methanolic extract of Prosopis. Group-VIII showed normal epidermis, sebaceous gland and hair follicle on the 28th day of the experiment (Fig. 20).

CONCLUSION

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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