Indian Journal of Traditional Knowledge
Vol 23(2), February 2024, pp 154-161
DOI: 10.56042/ijtk.v23i2.8723

Management of seasonal dermatitis in horses by using leaf extract of *Aerva javanica*

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Received 03 February 2021; revised 04 December 2023; accepted 02 February 2024

Occurrence of seasonal allergic dermatitis, especially insect bite hypersensitivity and atopic dermatitis are very common in horses. At present there is no satisfactory treatment is available for management of these skin allergies. Plant flavonoids such as quercetin, kaempferol and their glycoside derivatives have been reported for their anti allergic and anti-inflammatory properties. In present study we have prepared an extract from the leaves of *Aerva javanica*, which was found effective to manage clinical cases of seasonal allergic dermatitis in horses. Clinical signs of the allergic dermatitis in horses were alopecia, thickening of skin and itching. On histopathological examination of skin biopsy samples taken from the clinical cases epidermal hyperplasia, orthokeratotic and parakeratotic hyperkeratosis, spongiosis, occasional trichomalacia, multifocal areas of aggregation of lymphocytes with or without infiltration of eosinophils were observed. Mass spectrometer analysis of more purified extract suggested a glycoside of kaempferol may be an active ingredient of the extract.

Keywords: *Aerva javanica*, Alopecia, Dermatitis, Flavonoid, Horse

IPC Code: Int Cl.[24]: A61K 36/00

Seasonal allergic dermatitis in horses usually develops due to hypersensitivity to the environmental allergens such as insects, bedding, feeds and pollen grains[1-2]. Clinical signs of atopic allergic dermatitis includes wheezing, puritis, hair loss and orthokeratotic and parakeratotic hyperkeratosis[3-5]. Insect bite hypersensitivity (IBH) commonly called summer itch/sweat itch, is also a hypersensitivity reaction against culicoides in horses and have worldwide prevalence[6-8]. During our clinical practice in Northwestern part of India we have observed occurrence of such cases remains at peak from May to September months. After end of rainy season clinical condition improves in most of the horses, whereas in some horses lesions prevail throughout the year. Most affected areas of the horse body are mane, neck, face and tail. At present there is no satisfactory treatment exists against sweet itch/summer itch in horses[7]. Itching can be controlled temporarily by the application of corticosteroids. Effect of steroids occurs for short duration and steroids have many side effects in horses such as development of laminitis. Involvement of large area of body and prolonged use of topical or parental steroids make the treatment impractical. Treatment by using vaccines against IL5 has been also used at experimental level with limited success[9]. At present management of insect bite hypersensitivity is done by avoiding exposure of horses to culicoides midges use of corticosteroids and antihistamines[10-11]. Following identification of allergen, allergen specific immunotherapy can also be effective[11]. Use of advanced technologies like identification of specific allergen is a costly affair for the horse farmers of middle and poor income countries. So there is immediate need to develop cost-effective management for the seasonal allergic dermatitis in horses.

Herbal medicines for skin ailments are being used traditionally since ancient time for the treatment of skin ailments and cosmetic purpose in India[12,13]. Spray of essential oils of some plants also reported to have beneficial effects against insect bite hypersensitivity in horses[14]. Many plant flavanoids have strong anti-inflammatory and mast cell stabilizing activities[15-17]. Flavonoids such as quercetin and kaempferol have been reported for their anti-allergic and mast cell-

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stabilizing property[18,19]. Kaempferol inhibits airway thickening by allergic reaction in asthma model[20]. Mast cell stabilizers are recommended to prevent allergic reactions[21]. Quercetin and kaempferol both prevent Ig E-mediated hypersensitivity[19]. Insect bite hypersensitivity in horses is an IgE mediated allergic reaction and atopic dermatitis is associated with skin barrier dysfunction[22]. Quercetin, isorhamnetin and other flavonoids constituents are reported to improve the skin barrier function[23]. Use of *Aerva javanica* has been reported for many ailments diuretics, demulcents, purgatives, emetics, tinder, arthritis, proud flesh, toxoplasma in traditional medicines[24]. The decoction, juice, and paste of the leaves of genus *Aerva* have been utilized by the local people of the desert areas for the treatment of skin diseases and wound healing[25-27]. In India we came to know from the inhabitants of Thar desert of Rajasthan that their ancestors were using decoction of this plant for the amelioration of skin diseases in human beings and animals prior to availability of modern allopathic medicines. In our recent study[28] also, we have found that two kaempferol derivatives obtained from the leaves of *A. javanica* have shown antiproliferative property without any side effect. So we hypothesized that these kaempferol derivatives of the *A. javanica* extract can be used therapeutically against seasonal dermatitis, especially insect bit hypersensitivity and atopic dermatitis in horses.

Methodology

A. javanica grows abundantly in barren lands of desert Thar. It is used for treatment of skin conditions by tribal and local people of the desert, but its uses for the treatment of different skin affections are not well documented. This is the first report of successful use of *A. javanica* leaf extract for the treatment of seasonal dermatitis in horses. In the present study, clinical cases of dermatitis in horses were diagnosed by the help of case history, clinical signs of itching, dermatitis and alopecia. Histopathology was carried out to make differential diagnosis from mange and other skin conditions. Qualitative improvement of clinical condition of the skin was used as marker for the therapeutic efficacy of the herbal extract.

Materials and Methods

Extract 1

Extract was prepared by the method described for proud flesh from the *A. javanica* with slight modifications[28,29]. Leaves of *A. javanica* were collected and dried at room temperature under shade for 3-5 days. The leaves are grounded to a powdered form using a mixer grinder. Grounded leave powder 100 g was added to 1000 mL of water in a covered flask and kept at room temperature for 3 days with intermittent shaking.

This mixture was sonicated with water at 70% amplitude for 10 min on a 20 KHz capacity sonicator, about 1500 mL of extra water was added during sonication of 100 g leaf powder. The insoluble components (debris) were removed by filtration through thin cotton cloth. The filtrate was then evaporated on rotary evaporator (1 L capacity, bath temperature 85°C at 400 mbar vacuum, rotation speed 50-250 rotations per minute), till complete drying. At this stage, the extract starts sticking on the walls of the flask.

The dried plant extract was then dissolved in 900-1100 mL of methanol and kept at room temperature for 24 h to dissolve methanol soluble materials in the methanol. Thereafter methanol soluble fraction (MSF) was dried by using rotary evaporator (Bath temperature 60°C at 200 to 400 mbar vacuum).

Said dried extract was then poured on a glass column (30-centimeter-long and 18 mm wide) filled with silica (60-120 mesh) up to 25 cm and about 1 to 3 g of MSF was poured on top of it. Then the column was eluted with absolute ethanol till the colored compound eluted. EEF (ethanol eluted fraction**)** was collected and rotary dried it was named WEM1.

Extract 1

Saturated aqueous solution of the collected extract was used as topical spray @ 2 mg/ mL, on the affected areas of skin of the horses for 18-21 days.

Extract 2

This was prepared from extract 1 by further purification by chloroform and acetone washings in sequence. Dried extract 1 (about 1 to 2 g) was added to 250 mL chloroform and kept for one hours. After removing chloroform, the remaining extract was mixed with 250 mL acetone and kept for 1 h and acetone soluble fraction was collected and discarded twice. This fraction was named WEM1F2 and was applied topically at 0.10 mg/mL in water once daily on affected area of skin for 15-20 days.

Identification of active ingredients

Mass spectrometer analysis of extract 2 (WEM1F2) was conducted at Indian Institute of Technology,

Ropar (Punjab) using time of flight mass spectrometer positive electrospray method as outsourcing[28].

Clinical cases

Study was ethically approved by the Institute Animal Ethics Committee of ICAR NRCE Hisar (NRCE/CPCSEA/2018-19/SN12). Horses suffering from allergic dermatitis were belonging to the different stud farms of Rajasthan and Punjab states of India. All the cases were presented and treated between months of May to October. Occurrence of dermatitis and alopecia in summer season is very common in horses. We have seen 21 cases of summer itch during this period, all these horses were earlier treated by ivermectin for suspecting mange by local veterinarians or farmers but no improvement was reported. Due to limitation of laboratory scale production of extract we can treat only 11 horses by using extracts. Alopecia, itching and thickening of skin were the most common clinical signs. Extract 1 (WEM1) was used in 7 cases while Extract 2 (WEM1F2) was used in 4 cases. Both the extracts were applied topically as spray for 20 to 30 days. All the cases treated by using extract 1 and extract 2 showed visible clinical improvements.

Diagnosis

For differential diagnosis of clinical cases of dermatitis, skin biopsy samples were collected in 10% buffered formalin from all the clinical cases and processed for histopathology using paraffin embedding and hematoxylin and eosin (H & E) staining method. The cases were suspected for atopic dermatitis (AD) or insect bite hypersensitivity (IBH) based on their correlation with clinical history, distribution and nature of lesions, and histopathological findings. Clinical cases of dermatitis where ectoparasites (mange) were found on microscopic examination were excluded from the present study.

Results

Cases of allergic dermatitis showed alopecia with dry or scaly lesions distributed in face, mane, neck, chest, back and tail of the affected horses. On histopathological examination of skin biopsy samples taken epidermal hyperplasia, orthokeratotic and parakeratotic hyperkeratosis, spongiosis, occasional trichomalacia and multifocal areas of aggregation of lymphocytes, histiocytes and fibroblasts in dermis (Fig. 1-3) were observed. Occasional foci of neutrophil and eosinophil infiltration suggesting pyoderma was also seen in epidermis of one case (Fig. 4).

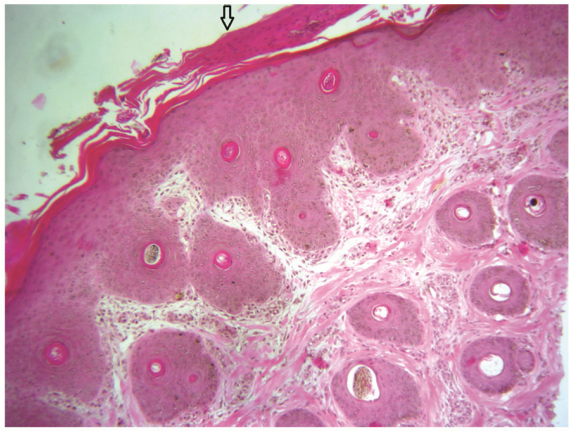

Fig. 1 — Parakeratotic hyperkeratosis (arrow), epidermal hyperplasia and trichomalacia in AD. HE X 100

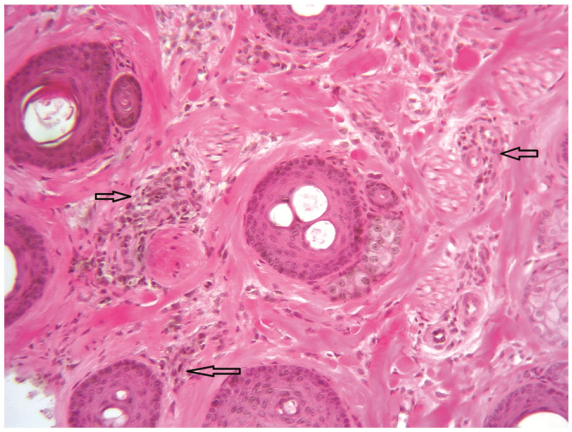

Fig. 2 — Multifocal areas of lymphocyte, histiocytes and fibroblast accumulation (arrow) in dermis. Also note distorted follicles and perifollicular fibrosis

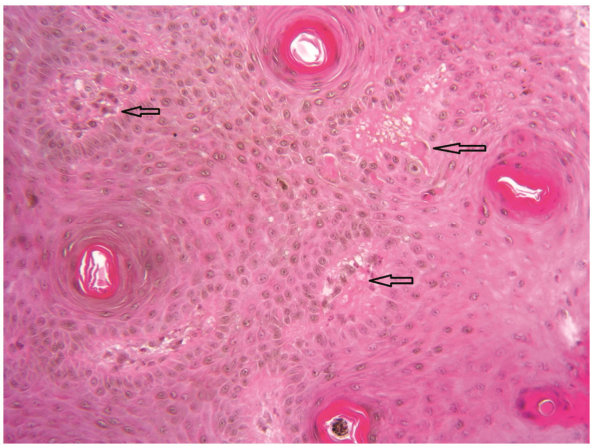

Fig. 3 — Epidermis showing spongiosis (arrow) and trichomalacia. HE X 200

In 6 cases out of 7 cases where extract 1 was applied intensity of itching decreased within minutes of topical application of spray. Appearance of hairs on the site of alopecia were observed in around 10 to 12 days, lot of hairs were observed on 20th day and skin become normal within 30 days of the topical application of the extract 1 (Fig. 5-7). Similar trend of recovery were observed on topical application of extract 2 in the applied 4 clinical cases of dermatitis. Complete recovery could not be achieved in one clinical case (Fig. 8), however visible improvement was observed in that case too. Rest untreated 10 cases did not showed any improvement during the study period.

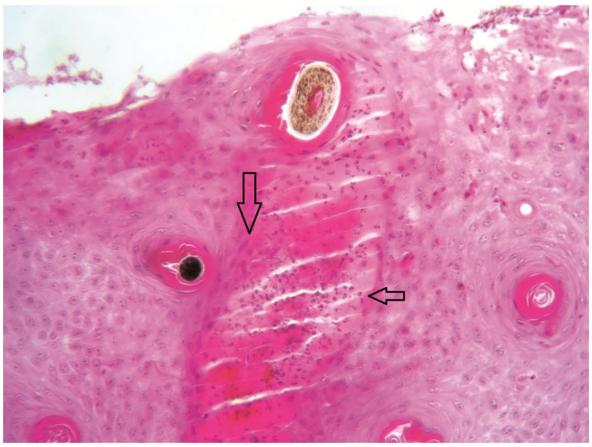

Fig. 4 — Pyoderma in epidermis characterized by focal accumulation of neutrophils and eosinophils (arrow) in case of AD. HE X 200

On mass spectrometer analysis of WEM1F2, mass to charge ration (m/z) peaks were observed at 287, 331, 375, 419, 463, 507, 551, 595, 639, 683, 727, 815 and 903. There was a similar difference of 44 (m/z) in series was observed from m/z 287 to m/z 903.

Discussions

This report is the first report for the management of allergic dermatitis in horses by using extract obtained

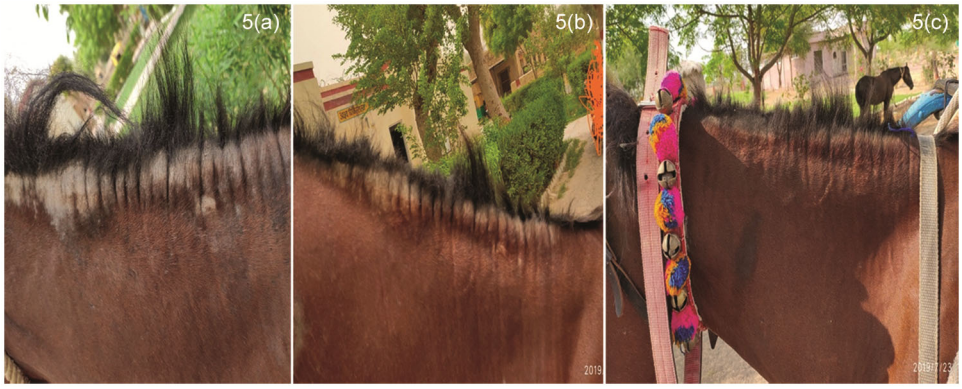

Fig. 5 — (A) Area of dermatitis and alopecia on mane before start of treatment (B). Improvement noticed after 8 days of application of extract. (C) Complete healing of skin and appearance of hair on 17th day of application of herbal extract

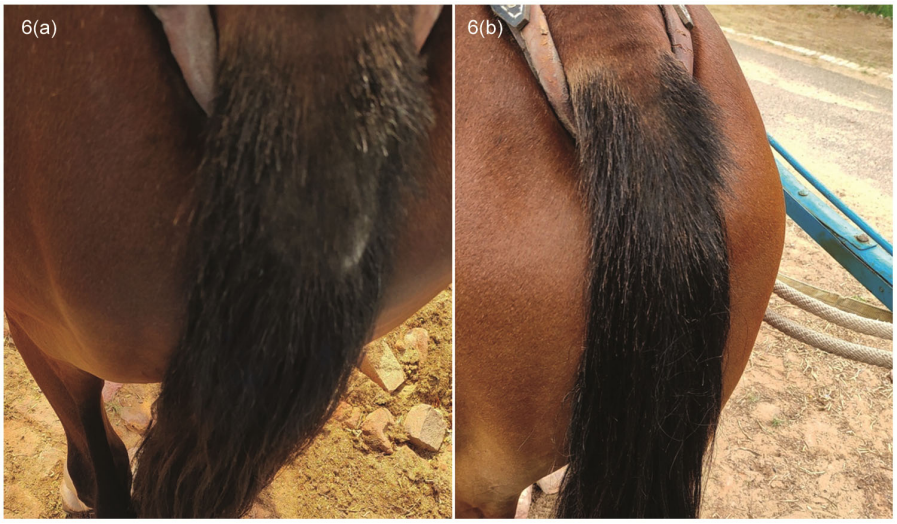

Fig. 6 — (A) Alopecia at tail head on 0 day of application of herb (B) Hair growth on 21st day of extract application on tail head

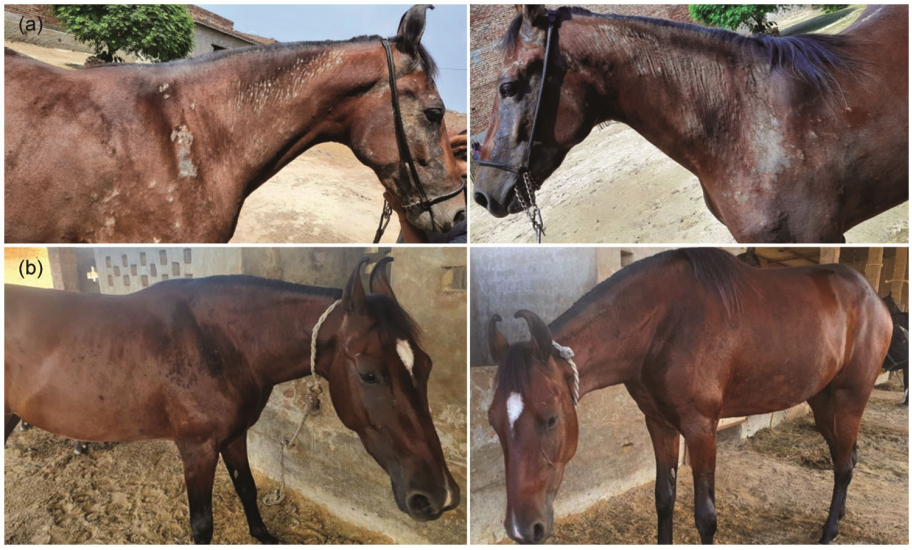

Fig. 7 — (A) Dermatitis and alopecia over neck, shoulders and face, before application of herb (B) Clinical improvement after 20 days of application of herb

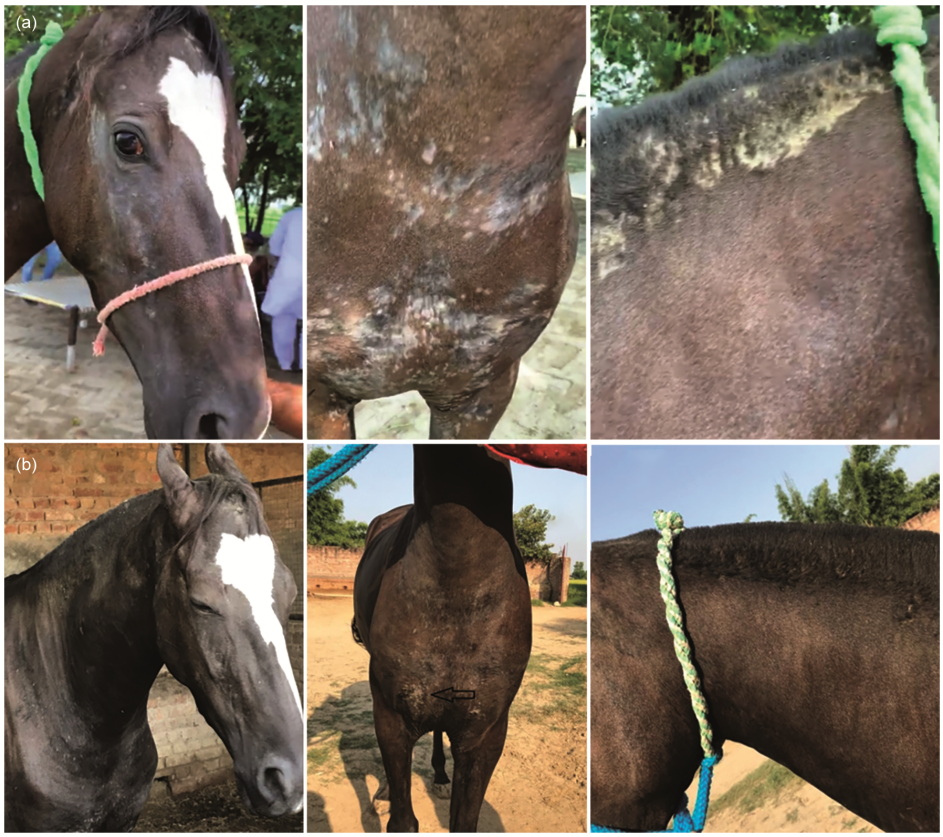

Fig. 8 — (A) Clinical signs before application of herb (B) on 20th day of application, some lesions left (arrow) probably associated bacterial infection

from the leaves of *A. javanica*. It is a desert plant found abundantly in barren lands of subtropical desert climatic conditions of Rajasthan (India) and many other countries.

On histopathological examination of skin biopsy samples epidermal hyperplasia, orthokeratotic and parakeratotic hyperkeratosis, spongiosis, occasional trichomalacia and multifocal areas of aggregation of

lymphocytes, histiocytes and fibroblasts in dermis were observed (Fig. 1-3). Occasional foci of neutrophil and eosinophil infiltration suggesting pyoderma were also seen in epidermis in 2 cases (Fig. 4). Similar to findings of present study IBH in horses is characterized by proliferation of fibroblasts, infiltration of lymphocytes and eosinophils[8].

Presence of peaks at m/z 287, 463 and 595 were suggestive for a kaempferol derivative and difference of m/z 44 each time in a sequence was suggestive for a single compound which might have been added on ionization[28]. Extract 2 was used in 4 cases of dermatitis, intensity of itching was decreased from the first day of application. Hair growth was noticed on 10-12th days of application and complete healing of skin and appearance of full hair growth was completed from 20 to 30 days. Kaempferol and its derivatives are reported for their anti-inflammatory, anti-allergic and skin barrier protecting activities. Mast cells are known for immunoglobulin E triggered degranulation in allergic reactions, various cytokines, growth factors and proteioglycones are released from mast cells which further leads to proliferation of fibroblasts and fibrosis[30] as observed in most of cases of allergic dermatitis in present cases. Kaempferol and its derivatives are reported for their mast cell stabilizing activity and inhibitory effects on fibroblast collagen synthesis and fibrinolytic activity[31-35]. In histopathological findings in present study, lymphocytic infiltration in dermis was observed in all the cases (Fig. 1-3). Keratinocytes produces and stores IL1, a proinflammatory cytokine. IL1 is released after injury to the keratinocytes. IL1 initiate inflammation and attracts neutrophils and macrophases[36-38]. Kaempferol and its glycosides are reported to block IL1-induced inflammation[39].

It is also reported that kaempferol blocks lymphocyte proliferation[40]. Various herbs containing kaempferol are reported for their hair growth promoting efficacy[41-42] as observed in present study. A total of 7 horses were treated with extract 1 and all the horses showed improvement in clinical signs, complete recovery could not be achieved in one case (Fig. 8), where presence of foci of neutrophil and eosinophil infiltration was suggesting for pyoderma in epidermis (Fig. 4). It suggested that the case might have required antibacterial treatment for pyoderma along with the extract applied in the present study.

In comparative to the glycosides, aglycones of flavonoids show comparatively more skin deposition and protective effect on skin barrier function[43]. Fermentation converts flavonol glycosides to intermediate glycosides and finally to aglycones[44,45]. Aglycones of the flavonoids are less polar and their polarity increases as the sugar contents are added to them and glycosides of flavonoids are formed[46]. For a compound to work on skin there is need of solubility in polar and non polar solvent both[43]. To cross the upper most keratinized layer of epidermis, compound should be lipid soluble, while to cross the inner layers of cells of dermis compound should be water soluble. Fermentation which might have occurred on 3-4 days shocking in water might have converted glycosides of flavonoid into less complex glycosides or aglycone of the flavonoid. This might have played a role in improving biopolar nature of present extract to become effective for the management of dermatitis in horses. During the study, no side effect of the extract was observed in any horse.

Conclusion

Extract prepared from the leaves of *Aerva javanica* can be used to manage seasonal dermatitis in horses.

Acknowledgements

Authors are very thankful to Dr. S.C. Mehta Principal Scientist and Incharge Equine Production Center Bikaner, Dr. T. Rao Senior Scientist, Dr. R. K. Sawal Director NRCC Bikaner, Shri Brij Lal, Shri S. N. Paswan and Shri Chaturbhuj for providing help during the research.

Conflicts of Interest

There was no conflict of interest.

Funding

Present research was carried out under institute funded research project of ICAR National Research Center on Equines, India.

Author Contributions

RKD: Planning of the project, extraction from the plant, topical application of the extract to animals, monitoring of the clinical cases, Preparation of the manuscript. NK: cytotoxicity studies of the extract fraction, Preparation of the manuscript. SDN: Histopathology of the biopsy samples. JS: Topical application in horses and monitoring of the progress in horses. RAL: Collection of biopsy sample and monitoring of the clinical cases in horses. YP: Editing and final approval of the article.

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