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Amelioration of oxidative stress due to infection of *Demodex canis* in dogs with herbal extract of *Moringa oleifera*

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Abstract
Moringa oleifera is a well-known plant herb, rich in valuable phytochemicals with medicinal as well as dietary importance. The present study was therefore to specifically investigate the in-vitro and in-vivo antioxidant properties of methanolic (80%) and aqueous extracts of *M. olifera* in canine demodicosis. 18 dogs positive for demodex mites in deep skin scraping were selected for the proposed study. All the dogs were randomly divided into 3 equal groups (n=6), adopting different therapeutic regimen. Group A treated with Doramectin @600mg/kg bw. sc. weekly alone; group Btreated with methanolic extract of *M. oleifera* along with same miticidal therapy and group C treated withN-acetyl-l cysteine @70 mg/kg BW orally daily as standard antioxidant along with same miticidal therapy.Moreover, six apparently healthy dogs as negative control group D.The DPPH (1, 1-diphenyl-2-picrylhyrazyl) free radical scavenging activity of methanolic extract of *M. oleifera* and aqueous extract of *Moringa oleifera* and N-acetyl-l cysteine were 78.86%, 65.53% and93.98%respectively. Twelve peaks were detected during a run time of 1 hour as depicted in the chromatogram (HPLC). Among all the peaks detected, six peaks representing six different compounds were eluted in substantial amount, at retention times of 1.891, 2.238, 2.894, 3.213, 40.683 and 50.200 minutes. The mean value of oxidative indices like LPOs, SOD, catalase, and GSH were 8.02±0.32, 2.08±0.06, 45.10±2.66 and 0.26±0.01 revealed significantly ($p<0.05$) high value LPOs &SOD, low level of catalase and GSH, shows increasing of oxidative stress in demodicosis. After treatment mean values of LPOs, catalase, SOD close to healthy control animals.

Keywords: oxidative stress, herbal extract, demodicosis, *Moringa oleifera*

Introduction
Demodicosis is an inflammatory skin disease in dogs caused by tiny, cigar-shaped. eight-legged mites (Demodex mites. *Demodex canis* is the most common species of genus Demodex, besides this two other species *Demodex injai* and *Demodexcornei* also reported in dogs in different countries (Sivajothi *et al*., 2015) [13]. Loss of continuity of the skin defense barrier or immunosuppressive disease condition favor proliferation of mites in hair follicles and pilosebaceous gland resulting in clinical signs (Greve *et al*., 1966) [6].

Oxidative stress develops due to excess production of free radicals or insufficient availability of antioxidants or a combination of both. Lipid peroxidation by induced excess free radicals deteriorate cell membrane (Haliwell and Gutteridge (1999) [7]. Free radicals are responsible for erythema, inflammation, keratinization, hypersensitivity, photo-aging, wrinkling, auto immune reactions even skin cancer in human being (Trouba *et al*., 2002) [15].

Reactive species are believed to activate proliferative and cell survival signaling that can alter apoptotic pathways that may be involved in the pathogenesis of several skin disorders including dermatitis and some types of cutaneous diseases.

Treatment of canine demodicosis is multimodal, with effective acaricidal therapy, treatment of concurrent secondary infection of bacteria with broad spectrum antibiotics, effective drugs against endoparasite and underlying systemic disease must be undertaken to maximize the potential for successful treatment. Topical antiseptic therapy may be recommended to prevent or treatment of secondary bacterial skin infection (Scott *et al*., 2001) [11].

Despite a numbers of researches work were undertaken pertaining to pathophysiology and treatment of canine demodicosis, but still a lot of cases are refrain to hitherto available treatment module. Drug discovery is an ongoing requirement in order to find safe, effective, and affordable cures for an expanding spectrum of animal ailments. Many plants constitute a rich source of a wide variety of therapeutic molecules and therefore hold a great promise for new medicines. Plant materials remain an important resource to combat serious diseases in the world. Anwar *et al*., (2007) [1], reported various parts of *Moringa oleifera* are use as antioxidant, antitumor, anti-inflammatory, antiulcer, antihypertensive, cholesterol lowering agent, hepatoprotective, antidiabetics, antibacterial and antifungal activities due to presence of most important bioactive constituents which are alkaloids, tannin, flavonoids and phenolic compounds (Edeoga *et al*., 2005) [4].

Materials and methods

Eighteen dogs positive for demodex mites in deep skin scraping were selected for the proposed study. All the dogs were randomly divided into 3 equal groups (n=6) viz. group A, group B, and group C based on treatment regimen respectively irrespective of their sex, breed and age. Moreover, six apparently healthy dogs irrespective of sex, breed and age were taken as Group D, presented for routine health checkup and vaccination and had no skin lesions.

Clinical diagnosis and scoring of clinical symptoms 0 to 5 based on of demodectic mange was based on physical examination like intense pruritus, popular, eruption, crusting, excoriations, erythema and alopecia. All the scales were added to express as demodicosis induced skin lesion (DISL). The standard method to diagnose demodicosis is microscopic evaluation of material obtained by deep skin scraping (Muller *et al*., 2000 and Scott *et al*., 2001) [10, 11].

Plant Materials and Preparation Extract

The powder of shade dried leaves of M. *oleifera* was subjected to extraction using two different solvent, 80% methanolic and aqueous (100% Distilled water) by cold maceration method as described by Handa *et al*., (2008) [8] with slight modification at room temperature. The properly dissolved mixture was filtered by double layer muslin in Buchner funnel and again filtered with whatman filter (42). The residue was again macerated with fresh solvent similarly the process was repeated thrice. The filtrates were placed in evaporating trays for evaporation hot air circulating oven at 40 °C. The remnant/extract (coal tar like) have collected after complete evaporation and preserved in air tight glass containers at -20 °C in deep freezer till further use.

Oxidative Stress Indices

Assessment of oxidative stress induced due to demodectic mange pre-treatment and post-treatment based on antioxidant parameter like SOD (superoxide dismutase), GSH (Reduced Glutathione) as well as oxidative stress indices like LPO (Lipid peroxidase) (MDA) and Catalase.

Therapeutic module

There were three groups in which different combination of antioxidant added along with acaricides specific and supportive therapy. Standard therapy consisted of doramectin @ 600 µg/kg SC weekly. The standard supportive therapy was antihistaminic as per need for the reduction of pruritus as well as inflammations, Salmon oil rich in omega fatty acid therapy for better recovery of lesions and support dermal condition in group each.

Group A animals were treated with Doramectin @600 µg/kg SC and supportive therapy as mentioned above. Group B animals were treated with Doramectin @600 µg/kg SC weekly and Moringa leaf extract @ 250mg/kg orally daily as antioxidant along with supportive therapy. Group C animals were treated with Doramectin @600 µg/kg SC weekly and standard antioxidant N- acetyl-l-cysteine @ 70 mg/kg OD orally daily ($C_5H_9NO_3S$) Himedia Laboratories Pvt Ltd Mumbai, India) along with supportive therapy. The data obtained was compiled and statistical analysis was carried out as described by Snedecor and Cochran (1994) [14].

Results and discussions

The DPPH activity of methanolic (80%) extract of *Moringa oleifera* and aqueous extract of *Moringa oleifera* was 78.86% and 65.53% respectively. From the above observation it can be say that the antioxidant property of methanolic extract is more than aqueous extract of *Moringa oleifera*, because extract obtained in solvent 80% methanolic solution have both water soluble as well as alcohol soluble antioxidant compounds from *Moringa olifera* leaf powder.Twelve peaks were detected during a run time of 1 hour as depicted in the chromatogram (Fig. 1). Among all the peaks detected, six peaks representing six compounds were eluted in significant percentage at retention times of 1.891, 2.238, 2.894, 3.213, 40.683 and 50.200 minutes. Siddhuraju and Becker, (2003) [12] reported the presence of flavonoids, quercetin and kaempferol in aqueous, methanolic and ethanolic leaf extracts of *M. oleifera.* Vongsak *et al.,* (2014) [14] also detected the presence of three major components (crypto-chlorogenic acid, isoquercetin and astragalin) in leaf extracts. These studies support the findings of the experiment. The most common site of body involved was back 55.55%, followed by forelimb 44.22%, periorbital 33.33%, and hindlimbs and calvaria 27.77, chin and neck 22.22%, and the least affected was perioral, abdomen and anus 16.66%while, Elsheika *et al.* (2011) [5] observed 97% alopecia and 61.5% pruritus in juvenile demodicosis.

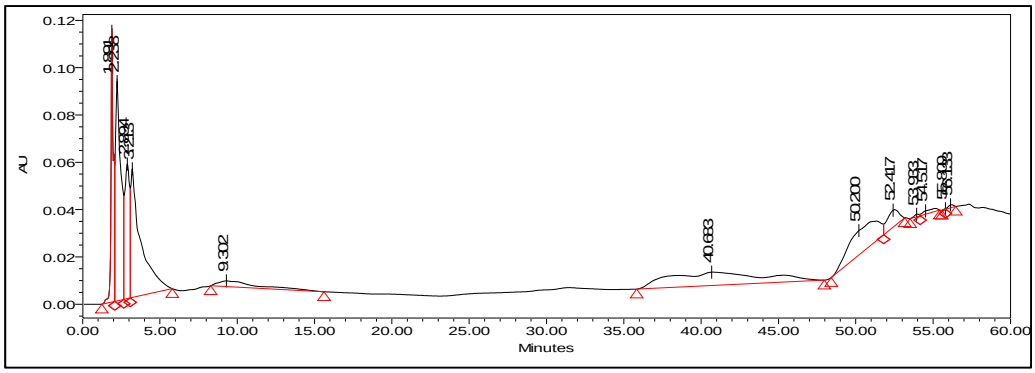

Fig 1: Chromatogram of methanolic leaf extract of *Moringa oleifera*

These clinical signs in demodicosis might be due to inflammation response against migratory and feeding behaviors of the developing mites (Elsheika *et al.*, 2011) [5] and thus draining tracts formed might be due to rupture of hair follicles in severe cases (Horne, 2010) [9].

Oxidative Stress Indices

Lipid Peroxidase (LPO)

In pretreatment groups, the mean LPOs value of infected groups were significantly (p<0.05) higher in compare to healthy control group D. The mean value of LPOs in infected group A, B and C were 8.06±0.67, 7.14±0.52 and 8.84±0.42 respectively which were quite high in compare to negative healthy group 1.09±0.23. After treatment, marked reduction in LPO were found in all treated groups and mean LPO values for these groups vary significantly (p<0.05) comparing with normal healthy groups. However, more reduction in LPO in treated group B and C as compare to treated group A. the marked reduction in LPO occurs because of scavenging activity of demodectic mange induced free radicals by supplementation of methanolic extract of moringa in group B and N-acetyl-l-cysteine in group C as antioxidant. Elevated LPO, in all Demodex spp. infected groups might be due to enhanced oxidative damage to tissues, either due to compromise in antioxidant defense or excess production of free radicals. The degree of he peroxidative damage appears to vary with severity of the disease, similar justification also made by Dimri *et al*., (2008) [3].

Catalase

The mean value of infected group A, B and C were 43.43±5.08, 46.41±5.55 and 45.46±3.82 respectively which were very low in compare to negative healthy group 95.99±9.95. After treatment, marked increased in catalase value were found in all treated groups and mean catalase values significantly (p<0.05) increased towards normal healthy groups

Superoxide Dismutase (SOD)

The mean value of infected group A, B and C were 2.02±0.15, 2.18±0.11 and 2.05±0.16respectively which were higher in compare to negative healthy group 2.18±0.11. After treatment, marked reduction in SOD were found in all treated groups and mean SOD values significantly (p<0.05) increased towards normal healthy groups showed positive response to treatment in each trial groups. Reduction of SOD activity in group B and C were more as compare to group A, reveals moringa extract may be alternative to conventional chemical antioxidant.

Elevation in SOD activity in demodicosis might be attributed to up-regulation in its synthesis to counteract free radicals. When the risk of oxidative damage increases, endogenous antioxidant protection also increases suggested by Basha and Rani 2003 [2].

Reduced Glutathione (GSH)

The mean value of infected group A, B and C were 0.25±0.02, 0.27±0.03 and 0.27±0.01 respectively which were very low in compare to negative healthy group 0.55±0.03. However, the increased level of GSH was more in group B and C as compare to group A. Group B and C were treated with doramectin weekly subcutaneously along with methanolic moringa extract and N-acetyl-l-cystine respectively. Polyphenolic and flavonoids having methanolic extract of *Moringa oleifera* has excellent antioxidant properties.

Therapeutic regimen

In present study, weekly subcutaneous administration of doramectin @600 µg/kg body weight as miticidal for demodex in dogs along with different antioxidant combination were given in group A, B and C. Evaluation of these therapeutic regimens was assessed on the basis of resolution of clinical lesions, reduction of mites counts in successive skin scraping on 0 day, 14th day, 28th day and 56th day in group A, B and C.

Summary and conclusions

Study of oxidative indices reveals high value of LPOs and SOD, low level of catalase and GSH, shows increasing of oxidative stress in demodicosis. The therapeutic regimen of subcutaneous miticides used i.e. doramectin with oral supplementation of extract of *Moringa oleifera* in group B, indicating marginal superiority over doramectin alone. Hence, from the present study it may be concluded that use of weekly doramectin along with extract of moringa was found to be effective in terms of rapid amelioration of clinical lesions in comparison to weekly doramectin alone.

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