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Macrocyclic lactone based tri-model therapy to treat humpsore in cattle under humid tropical island ecosystem

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ABSTRACT

Humpsore is a chronic parasitic dermatitis in bovine species; induces severe economic losses to the dairy farmers. Various therapeutic methodologies were tried to treat humpsore in the past; however, they were partially effective against humpsore. Therefore, the present study was designed to assess the effect of tri-model therapy [macrocyclic lactone; Ivermectin, a piperazine derivative; diethylcarbamazine citrate (DECC) and an herbal ointment; Himax™] on treatment of humpsore in Andaman and Nicobar islands. Thirty-six cattle (n=36 affected) were selected and divided into Gr 1: control (infected without treatment; *n*=18) and Gr 2: treatment group (infected with treatment; ivermectin, DECC and Himax, *n*=18) in South Andaman district. In Gr 2, treatment to the ailing animals was given for 45 days. Physiological profiles, hematological profiles, biochemical profiles [total protein, TP; albumin, AL; globulin, GL; creatinine, CR; glucose, GLU; total cholesterol, CHO and urea, URE], liver functional enzymes [aspartate aminotransferase, AST; alanine aminotransferase, ALT and alkaline phosphatase, ALP], mineral profiles, oxidative stress profiles [total antioxidant capacity, TAC; superoxide dismutase, SOD; catalase, CAT and malondialdehyde, MDA] and cortisol were estimated on day 30 of post treatment. Lesion was significantly reduced in day 15 of post-treatment and completely healed on day 45 of post-treatment in Gr 2. Physiological profiles, liver functional enzymes, urea, total white blood cell count, differential cell counts, MDA and cortisol were reduced significantly and blood profiles, biochemical profiles, mineral profiles and antioxidant profiles were increased significantly in tri-model therapy treated animals. Therefore, it can be concluded that tri-model therapy is suitable to treat humpsore in dairy animals.

Keywords: Andaman and Nicobar islands, Cattle, Humpsore, Macrocyclic lactone, Piperazine derivative

Stephanofilariasis (humpsore) is caused by filarial worm *Stephanofilaria assamensis* and is transmitted by fly (*Musca conducens).* Humpsore is a common chronic skin disorder of cattle, endemic to the humid tropical climate of Indian subcontinent including Andaman and Nicobar islands (Rai *et al*. 2010, Perumal *et al.* 2021). Stephanofilariasis induces chronic pruritus, damage, loss of hair follicles and chronic dermatitis in cattle. Humpsore induces severe economic losses to the dairy farmers as this disease induces stress that leads to loss of milk production, poor growth rate, poor fertility rate and poor market value due to damaged skin. Humpsore typical lesions occur mostly in and around hump region (92.60%) and rarely in udder, sternal area and other parts of the body (Perumal *et al*. 2019, Perumal *et al.* 2021). Size of sore differs from a few cm to more than 30 cm. Higher prevalence of stephanofilariasis is observed in exotic and its crossbred compared to zebu cattle (Akter *et al.* 2016, Perumal *et al*. 2019), mostly prevalent in dark-colored cattle and dry season of the year due to abundance of fly population (Rai *et al*. 2010). Different researchers both in India and abroad attempted to treat the disease with different success rate with some of the therapeutic methodologies such as combination of antimony potassium tartrate and 4–8% phenothiazine ointment (Choudhury and Das 2012), tobacco ointment alone (Choudhury and Das 2012), injectable preparation of levamisole hydrochloride with zinc oxide ointment (Rai *et al*. 2010), combination of ivermectin, levamisole, and mastilep ointment (Puttalakshmamma *et al*. 2012) and ivermectin and topicure spray (Islam *et al*. 2018), etc. No effective therapeutic methods are available to treat this disease, and therefore we hypothesize an innovative "tri-model therapy" mixing of drugs as a macrocyclic lactone drug (Ivermectin), a piperazine derivative (DECC), and an herbal preparation (Himax™ ointment): the combination was very effective in controlling this disease in dairy animals. Ivermectin, a macrolide anthelmintic, is very effective against microfilaria and has less effect on adult filarial worm, whereas DECC is used for prophylaxis against filarial worm in canine (Sandhu 2016). Therefore, it is proposed in the current study that the combination of two FDA-approved medications with an antibacterial and

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fly-repellent ointment will have chemotherapeutic and chemoprophylactic effects against Stephanofilariasis in cattle.

MATERIALS AND METHODS

Therapeutic agents: Ivermectin injection (HITEK™) was purchased from Kachhela Medex Pvt. Ltd., Nagpur, India. Diethylcarbamazine citrate (Hetrazan™) and Himax™ ointment were procured from Wyeth India, Mumbai, India, and Indian Herbs Overseas, Uttar Pradesh, India, respectively.

Animals and experimental design: The present study was conducted at the organized cattle dairy farms, South Andaman District, Andaman and Nicobar islands, India (11.6060°N, 92.7058°E) from Januray 2021 to December, 2022. The investigation was carried out in the crossbred cattle (5–7 years old; Jersey, Holtesin and Friesian in indigenous cattle). Humpsore affected cattle with a single irregular or circular shaped, large/chronic humpsore wound (>12 cm diameter) were selected for the present investigation. Humpsore was confirmed by detection of microfilaria in skin scraping of the infected animals. A total of thirty-six affected cattle were chosen and were randomly divided into two groups, namely, Gr 1: control (infected without treatment) and Gr 2: treatment group (infected with tri-model therapy treatment) with eighteen animals in each group. Experimental animals were maintained under uniform management with semi-intensive system of rearing where they were allowed for grazing from 7:00 AM to 12:00 PM. Watering and feeding were executed as per the farm routine. *Ad lib.* clean drinking water was available. The details of the groups and treatment protocol areas are:

Group 1(Gr1): Control (infected animals without treatment);

Group 2(Gr1): Treatment (infected animals with treatment with tri-model therapy).

Humpsore was cleaned with a mild liquid soap (Lifebuoy liquid soap, Hindustan Unilever Ltd., Mumbai, India) and crusts were removed. Papaya (*Carica papaya*) mist was applied on the wound for about 30 min on first two days. Injection of ivermectin @ 200 µg/kg body weight was administered three times by subcutaneous route at 15-day interval (day 0, 15 and 30). Herbal antiseptic and fly-repellent ointment (Himax ointment) was applied daily on the wound for 45 days. DECC 6 mg/kg body weight was administered orally daily for 45 days.

Hematological analysis: Blood samples were collected from the experimental animals and its profiles were analysed by using veterinary fully automatic hematological analyzer (Prokan, PE-6800) within 30 min after samples were collected. Blood parameters: total red blood cells (TRBC), haemoglobin (Hb), erythrocyte sedimentation rate (ESR), packed cell volume (PCV), total white blood cell (TWBC), differential leucocyte count (DLC: lymphocytes, monocytes, neutrophils, eosinophils) and platelets were estimated. Remaining blood samples were centrifuged at 1200 × g for 15 min at 4°C and separated plasma was preserved at -20°C for further analysis.

Biochemical profiles: TP, AL, GL, CR, GLU, CHO, URE, AST, ALT, ALP, Ca, P, Mg, Fe, Cu and Zn were estimated with commercially available diagnostic kits (ERBA Mannheim, Germany) using biochemistry analyzer (Automated Clinical Chemistry Analyser EM200, ERBA Diagnostics Mannheim GmbH, Germany).

Oxidative stress and hormone profiles: TAC (709001), SOD (706002) and catalase (707002) were estimated in blood plasma by using Cayman's Catalase assay kit (Cayman Chemical Company, USA) as per the manufacturer's guidelines. MDA was estimated by using TBA (Thiobarbituric acid)-TCA (Trichloro acetic acid) method. Blood cortisol was estimated using a commercially available ELISA kit (500360, Cayman Chemical Company, USA). All these antioxidants, oxidative free radical and cortisol were estimated in a 96-well clear polypropylene microplate using Alere Microplate Reader (Alere Medical Pvt Ltd, India, AM 2100).

Statistical analysis: In the present study, in order to determine any possible differences in the observed experimental parameters with respect to control and treatment groups, student t-test was applied using SAS Software (SAS, Version 9.3.1; SAS Institute, Inc., Cary, NC, 2011). Figures represents the non-transformed data. The mean values were expressed as mean±SEM. Differences were considered significant if $p<0.05$.

RESULTS AND DISCUSSION

Tri-model therapy reduced the abnormally higher physiological profiles indiced by humpsore [RT; 6.04%, RR; 14.23%, PR; 11.65% and ST; 12.75%] in the treated cows (Fig. 1). Similarly, the blood profiles [TRBC; 16.91%, Hb; 22.02%, ESR; 24.72% and PCV; 14.84%] were higher and TWBC (14.56%), DLC [neutrophil; 22.25%, lymphocyte; 11.36%, monocyte; 25.83%, eosinophil; 54.54%] and platelet (9.84%) were lower significantly ($p<0.05$) in tri-model treated cows compared to those in control group (Fig. 2). TP (24.80%), ALB (28.76%), GLO (21.37%), ALB/GLO ratio (28.35%), GLU (15.89%) and CHO (8.34%) were higher and CR (12.54%) and URE (14.28%)

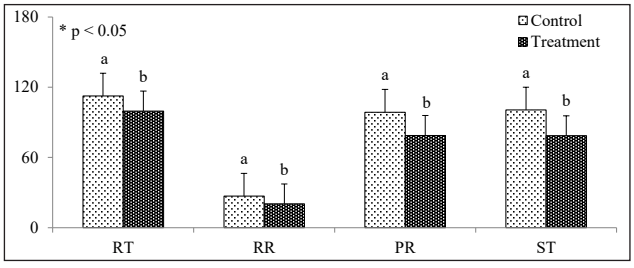

Fig. 1. Trimodel treatment on physiological profiles in humpsore affected animals (mean±SEM). Vertical bar on each point represents standard error of mean. Vertical bar with small letters (a, b) indicates significant ($p<0.05$) difference between the control and treatment cows. RT: Rectal temperature (°F), RR: Respiration rate (bpm), PR: Pulse rate (bpm), ST: Skin temperature (°F). *indicates $p<0.05$. n=6 cows for control and treatment cows.

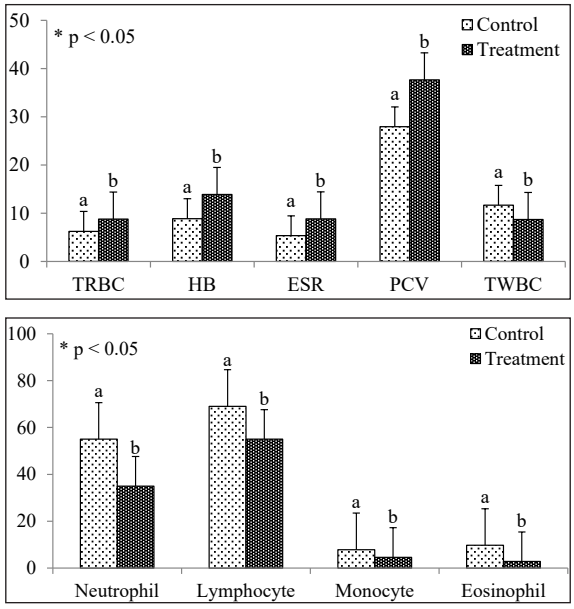

Fig. 2. Trimodel treatment on hematological profiles in humpsore affected animals (mean±SEM). Vertical bar on each point represents standard error of mean. Vertical bar with small letters (a, b) indicates significant (p<0.05) difference between the control and treatment cows. TRBC: Total red blood cell (×10^6/mm^3), HB: Haemoglobin (g/dl), ESR: Erythrocyte sedimentation rate (mm/hr), PCV: Packed cell volume (%) and TWBC: Total white blood cell (×10^3/mm^3). *indicates p<0.05. n= 6 cows for control and treatment cows.

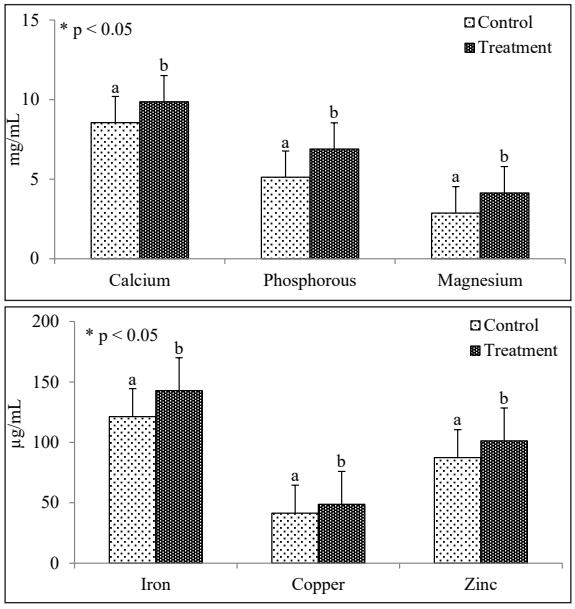

Fig. 4. Trimodel treatment on blood mineral profiles in humpsore affected animals (mean±SEM). Vertical bar on each point represents standard error of mean. Vertical bar with small letters (a, b) indicates significant (p<0.05) difference between the control and treatment cows. *indicates p<0.05. n=6 cows for control and treatment cows.

were lower significantly in tri-model therapy treated compared to those in control group (Fig. 3). Similarly, liver functional enzymes [AST; 14.76%, ALT; 31.21% and ALP; 64.05%] were lower in tri-model treated compared to those in control group (Fig. 3). Calcium (7.11%), phosphorous (14.73%), magnesium (18.62%), iron (8.18%), copper (8.16%) and zinc (7.33%) were significantly (p<0.05) higher in tri-model treated as compared to those in control group (Fig. 4). Antioxidants (TAC; 12.64%, SOD; 18.73% and CAT; 22.31%) were higher and MDA (32.63%) was lower in tri-model therapy treated as compared to those in control group at day 30 (Fig. 5). Similarly, cortisol was significantly decreased in treated as compared to those in control group (Fig. 5). Blood parameters expressed significant (p<0.05) positive correlation with TP, AL, GL, GLU, CHO, minerals and antioxidants whereas significant (p<0.05) negative correlation with TWBC, DLC, CR, URE, liver functional enzymes and MDA in tri-model treated cows.

Various treatment regimen were attempted to cure humpsore with varied success rate. However, in our study, tri-model therapy (papaya washing + DECC tablet + Ivermectin injection + Himax ointment) was used to treat humpsore which was successfully cured within 3 weeks of therapy. This treatment improved the health and wellbeing, milk production and fertility rate in crossbred cattle of ANI.

Papaya latex contains enzymes [papain, chymopapain, caricain, glycyl endopeptidase and papaya lipase], which have antiviral, antibacterial, antiprotozoal, antifungal, anti-inflammatory and wound healing properties (Vij and Prashar 2015). It desloughs the necrotic tissue and prevents wound infection (Kong *et al*. 2021). Papaya is also used as an ulcer protector. Papain is a cysteine proteinase that digests necrotic tissue by liquefying eschar, thus,

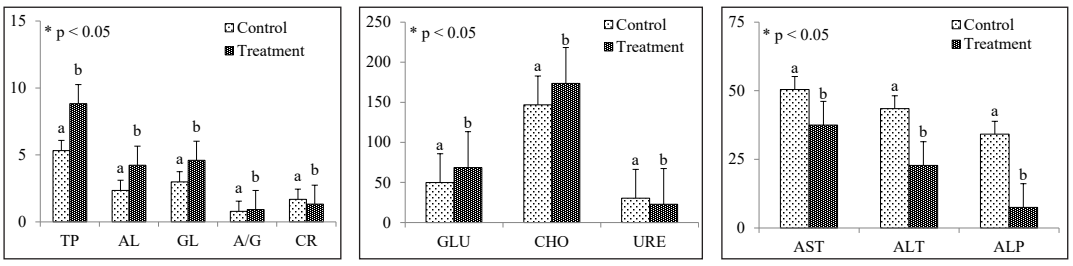

Fig. 3. Trimodel treatment on blood biochemical profiles in humpsore affected animals (mean±SEM). Vertical bar on each point represents standard error of mean. Vertical bar with small letters (a, b) indicates significant (p<0.05) difference between the control and treatment cows. TP: Total protein (g/dL), AL: Albumin (g/dL), GL: Globulin (g/dL), A/G: Albumin/Globulin ratio, CR: Creatinine (mg/dL), GLU: Glucose (mg/dL), CHO: Cholesterol (mg/dL), URE: Urea (mg/dL), AST: Aspartate aminotransferase (IU/L), ALT: Alanine aminotransferase (IU/L) and ALP: Alkaline phosphatase (IU/L). *indicates p<0.05. n= 6 cows for control and treatment cows.

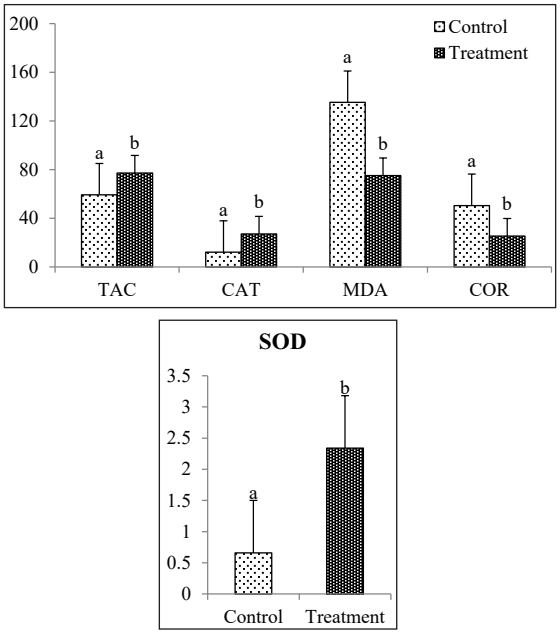

Fig. 5. Trimodel treatment on liver functional enzymatic profiles in humpsore affected animals (mean±SEM). Vertical bar on each point represents standard error of mean. Vertical bar with small letters (a, b) indicates significant ($p<0.05$) difference between the control and treatment cows. TAC: Total antioxidant capacity (µM/L), CAT: Catalase (nM/min/mL) and MDA: Malondialdehyde (µM/L), COR: Cortisol (ng/mL) and SOD: Superoxide dismutase (U/mL). *indicates $p<0.05$. n=6 cows for control and treatment cows.

facilitating the migration of viable cells from the wound edge into the wound cavity (Yang *et al*. 2021). Papain also helps in reducing the bacterial burden, decreasing exudates and increasing granulation tissue formation. Papain isolated from the latex of unripe papaya pulp is one of the earliest substances used in wound care and chronic skin ulcer therapy as it has anti-bacterial and fibrinolytic properties (Ezike *et al*. 2009).

DECC is drug of choice to treat the filariasis. It kills both microfilariae and adult worms (Dreyer *et al*. 2006). DECC is well absorbed following oral administration. It reaches all parts of the body within 25 min after its intake, with peak plasma concentrations within 1-2 h. The plasma half-life varies from 6.1 to 8.1 h. The standard DECC treatment regime is 6 mg/kg per day over a 10 to 20-day period in filariasis treatment. The elimination half life ranges from 10-12 h. If the urine is alkalinized, renal excretion of unchanged drug is prevented and the half life of the drug increases. It has an antagonistic action of the metabolic enzymes that metabolize arachidonic acid indicates minimizes the inflammatory condition. On the other side, DECC involves two processes; In first process, the filarial muscular activity decreases, secondary to hyperpolarization of membranes by the piperazine moiety of DECC and in the second process, the microfilarial surface membranes are altered by making them more susceptible to host defenses (Kuhlmann and Fleckenstein 2017). Therefore, DECC was administered at 24 h interval to the cattle to treat humpsore for 20-30 days depending upon the degree of the humpsore.

Ivermectin is universally used anti-filarial medicine and it is a macrocyclic lactone with wide-range of activity on filarial parasites. Ivermectin interacts with postsynaptic glutamate-gated chloride channels (GluCl), in turn increases influx of Cl-ions via the cell membrane by incitement of specific ivermectin-sensitive ion channels, resulting in muscle hyperpolarization causes paralysis of the microfilarias (Geary and Moreno 2012). Ivermectin acts quickly on the microfilariae and with long-lasting effect, inhibits adult female worms to release additional microfilariae. Dermal microfilarial loads are generally decreased by 78% within two days and by some 98% within two weeks of Ivermectin treatment (Tan *et al*. 2022). Elimination half-life of ivermectin in cattle is 2.0, 3.7, and 8.3 days with aqueous, aqueous: glycerol-formal (50:50 v/v), and propylene glycol: glycerol-formal (60:40 v/v) formulations, respectively (Foo *et al*. 2022) whereas its metabolites may persist for more days. Reduced concentrations of dermal microfilariae obtained after this timespan, suggests that ivermectin affected microfilariae all are not eliminated in the first few days. This is supported by reports that microfilariae migrate into deeper dermal layers, sub-cutaneous fat, connective tissue and lymph nodes following administration of the ivermectin (Ngwewondo *et al*. 2021). Ivermectin literally impedes with the capability of microfilariae to steer clear of the immune system, proceeding in the host's own immune feedback being capable to conquer the immature worms and therefore kills those microfilariae (Pessanha de Carvalho *et al*. 2021). It is well known that ivermectin administered through SC route has persistent for 10-21 days (O'Shaughnessy *et al*. 2019). Therefore, ivermectin was administered at the 15-day interval (day 0, 15 and 30) in the present study to treat humpsore.

Hydrophilic, lipophilic and enzymatic antioxidants are significantly higher in epidermis than in dermis (Quiles *et al*. 2022). Ascorbate, urate and glutathione were more than four times higher, ubiquinol-10 was nine times higher and catalase was seven times higher in epidermis indicated that the epidermis is as a biological barrier to prevent the oxidative damage. Normal bacterial flora in the skin generates the ROS. Mechanical injury, abnormal humidity, immunodeficiency or metabolic disorders can result in increased bacterial infection which could result in increased bacterial ROS production. This could act synergistically with ROS from host phagocyte cells to increase the generation of pro-inflammatory agents causing tissue damage (Zaninelli *et al.* 2021). ROS are centrally involved in all wound healing processes as low concentrations of ROS generation are required to fight against invading microorganisms and cell surviving signaling (Dunnill *et al*. 2017). In line, antioxidant and anti-inflammatory properties of several antioxidant strategies have proven beneficial to improve the non-healing state (Kunkemoeller and Kyriakides 2017). Ongoing oxidative stress, associated lipid peroxidation, protein modification and DNA damage

have impaired the wound healing processes via higher cell apoptosis and senescence (Bryan *et al*. 2012). Clinical investigations indicate that the non-healing wounds are conserved in highly oxidizing condition, which in turn lead to weakened wound repair. Clinical environment like tissue hypoxia due to camouflage of the worm on the flow of the blood vessels is commonly correlated with highly oxidizing environments. In the current study, therefore, considerably higher concentration of oxidative free radicals was obtained in the humpsore diseased cattle than in normal unaffected cattle.

Glutathione/glutathione disulphide ratio suggests the level of the oxidation rate and this oxidation rate is increased gradually throughout the life and at the same time, glutathione antioxidant defense system is reduced as age advanced (Galli *et al*. 2022). Such conditions favour to develop ulcerated wound or delay the wound healing as it is occurred in adult or aged animals. Normally, during wound healing process, concentration of tissue enzymatic and non-enzymatic antioxidants is reduced as these antioxidants are needed for healing processes (Lopez *et al*. 2022). It is not possible to supply antioxidants due to aging for the process of wound healing leads to higher production of ROS which further imposes the animals to vulnerability to the oxidative stress (Barber *et al.* 2023). It would appear that a physiologically significant contribution to the lipophilic antioxidants of skin layers and surface is derived from sebaceous gland secretion (Pincemail and Meziane 2022). Similarly, in the present study result revealed that the unaffected animals had significantly higher level of antioxidants than the affected animal groups. The treatment quickens the healing process and significantly enhanced the concentration of antioxidants and declined the level of the oxidative stress profile in the affected animals.

Rectal temperature is an indicative marker to assess the stress in domestic livestock species (Sejian *et al*. 2010). In caprine and bovine species, similar report was found, where RT was higher as the animal suffered diseases/stresses (Koczura *et al.* 2020). Based on the previous literature in other animal species, it was suggested that the livestock suffered diseases and severe stress as evident from the physiological parameters. ST was significantly higher in the disease stressed than healthy animal group.

Hematological profiles are important in the determination of physiological changes occurring in animals (Velayudhan *et al.* 2022). Hematological parameters such as PCV, RBC, MCV, MCHC and MCH are used to evaluate the stress/disease status of animals (Perumal *et al*. 2019). Hb and TWBC are also indicators of adaptation to adverse disease stress conditions (Velayudhan *et al.* 2022). Moreover, Hb value is used to assess the degree of stress and wellbeing of animals. The blood parameters were significantly affected in disease/ stress induced goat as indicated that these animals were suffered with severe haemo-concentration. The higher value in disease affected animal was due to adaptive mechanism exhibited by the animals. Hematological profiles were significantly reduced in diseased than in healthy animals and this was due to the following reasons: (1) higher nuisance and stress and higher body temperature lead to rise in water intake and reduced feed intake (Polat *et al.* 2020) resulting in deficiency of essential minerals and protein which are important components for Hb synthesis, insufficient levels leads to reduced erythropoiesis, (2) higher body temperature in diseased stage which in turn induces the rostral cooling centre of the hypothalamus to stimulate the medial satiety centre, which in turn inhibits the appetite centre, resulting in reduced feed intake (Okuyucu *et al.* 2023), (3) at high ambient temperature, peripheral vasodilatation and redistribution of cardiac output are associated with expansion of blood volume and hemodilution (Hempstead *et al*. 2021) and (4) destruction of more number of RBC (Żarczyńska *et al*. 2021). In general, the oxygen consumption rate is increased when the animal suffering diseases/stress in summer season. Moreover, the animals are moving long distance in the forest to search the feed especially in summer seasons. This leads to higher oxygen demand to meet the muscular activity. This increases the RBC production and hemoglobin concentration in livestock. Further, in the experiment, the animals were allowed to drink water or feed the fodder that lead to hemodilution in summer season cousing lower blood parameters particularly RBC, HB, PCV and ESR (Sejian *et al*. 2012). Generally during thermal or disease stress, severe dehydration had been reported in livestock which ultimately led to higher level of Hb and PCV (Ruiz-Ortega *et al*. 2022). Further, severe water deprivation in these livestock species when exposed to summer season and disease condition could have aggravated the condition. In addition, PCV and Hb are considered to be the indices of the organic response to disease stress in livestock species (Sejian *et al*. 2010). Similarly, PCV value decreased in diseased condition due to excessive RBC destruction and/or hemodilution. Therefore, significantly lower erythrocytic indices were observed in the diseased animals in the present study. This study revealed higher TWBC and neutrophils in diseased than in healthy animals. This could be due to release of corticosteroids due to humpsore disease which in turn increased leukocyte count and accelerated mobilization of mature neutrophils from bone marrow storage pool (Brooks *et al.* 2022). It revealed that during the disease period, lymphocyte count could also be increased. Livestock might be affected by sub-clinical parasitic infection in summer which in turn increased leucocyte profiles (Underwood *et al.* 2015). Monocyte count was also higher during the disease condition which could be associated with increased cortisol secretion (Brooks *et al.* 2022)]. Therefore, TWBC was higher during diseased stage. Similar reports were available in literature regarding higher TWBC in bovine species (Brahmbhatt *et al.* 2021) during diseased state. Negative energy balance and higher blood cortisol concentration in diseased animal leads to immune-suppression which in turn increases susceptibility towards infectious diseases (Rainard *et al*.

2022). Therefore, TWBC production was increased in humpsore disease affected cattle in Andaman and Nicobar islands.

Animals with ulcerated wound as like humpsore may be catabolic and metabolically compromised (Kumar *et al.* 2021). Protein and energy substrates are important in maintenance of immuno system and are as active components of several antioxidants (Moazzen *et al.* 2022). Under conditions of metabolic stress/humpsore disease stress, intake of these micronutrients is important to maintain or restore the tissue viability. However, in many studies, the patients suffered from the ulcerated wound are deficient of the antioxidants. In present study, similar report was observed as deficiency of these antioxidants and enhanced oxidative stress profile leads to humpsore condition in cattle. Therefore, supplementation of these antioxidants either through feed or injectable or implant form may reduce the incidence of the humpsore along with other treatment protocols.

Healing process of the epidermis naturally presumes migration of keratinocytes from the wound edge which sustain proliferation, differentiation and apoptosis. Histopathological studies revealed hyper proliferative stem cells and actively proliferating keratinocytes at the ulcerated wound margin (Hosseini Mansoub 2021). Despite the presence of hyper proliferative keratinocytes, healing of the ulcerated wound was slow, suggesting that the problems actually lie with distorted organization of the wound bed. In these patients, it may have been caused by infection and impaired nutritional supply, which impairs keratinocytes migration. ROS may be implicated in impairment of early stages of healing in the wound bed in ulcerated wounds through inflammatory process, with an imbalance of ROS and antioxidant process. Therefore, in the present study, we observed the humpsore wound healing was quicker and faster in the DEC and doramectin treated group than in untreated group.

The study concluded that trimodel therapy cured the humpsore lesions by controlling the microfilaria as well as adult filarial worm population, besides, improved antioxidant level and reduced oxidative stress profiles and cortisol concentration. This therapy considerably reduced the stress and improved the health and wellbeing. Therefore, milk yield and fertility rate has been improved considerably in the trimodel therapy treated animals.

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