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Seroprevalence of *Sarcoptes scabiei var suis* infestation in swine population and its effect on haemato-biochemical and oxidative stress indices and its management with special reference to herbal ointmen

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ABSTRACT

The study was undertaken to know the sero-prevalence of sarcoptic infestation in pig in Mizoram and its effect on haemato-biochemical and oxidative stress indices and to develop cost effective herbal therapeutic management. Total 600 pigs of three district of Mizoram, Aizawl, Kolasib and Mamit were screened by using commercial indirect ELISA SARCOPTES 2001H Pig (AFOSA GmbH, Blankenfelde-Mahlow, Germany) to detect anti-Sarcoptic antibodies. The results cleared that out of 600 inspected pigs, 100 (16.66%) were found to be seropositive with sarcoptic mange. Geographically, pigs from Aizawl areas were more exposed to mange infestation (56/200, 28%) than Kolashib (32/200, 16%) and Mamit (12/200, 6%). The neutrophil and eosinophil was significantly ($P \leq 0.05$) increase and RBCs, Hb and PCV were significantly ($P \leq 0.05$) decreased in infested groups. There were significant ELISA SARCOPTES-ELISA 2001H Pig decrease in values of total protein, albumin, while there were significant ($P \leq 0.05$) increase in values of AST, ALT, ALP, BUN and creatinine In regard to oxidative stress, there was a significant ($P \leq 0.05$) increase in malonyldialdehyde (LPO) and NO and significant ($P \leq 0.05$) decrease in reduced GSH, SOD and TAA in infested group. In the present study, polyherb formulation showed similar efficacy to improve skin lesion, haemato-biochemical and oxidative stress indices with Ivermectin on 15th day of post therapeutic application against sarcoptic infestation in pigs. ELISA SARCOPTES-ELISA 2001H Pig represent a useful tool for population surveys and the plant extracts can be used as alternatives to drugs of synthetic origin.

Key words: Biochemical, ELISA, Haematology, Oxidative Stress, Pig, Polyherb formulation, Sarcoptic mange, Seroprevalence.

INTRODUCTION

Sarcoptic mange is a severe contagious disease and a major global health problem affecting humans and other mammalians, caused by the burrowing mite *Sarcoptes scabiei* (Walton *et al.,* 2008). Sarcoptic mange, caused by *Sarcoptes scabiei var. suis*, is of major economic importance in pig farming as it significantly reduces production efficiency and costs of acaricides used in its control are enormous and run into billions of dollars worldwide (Davies, 1995). This economic losses to the pig owners occurs may be due to decreased growth rate, decreased fertility and lower feed conversion ratio (Zimmermann and Kircher, 1998). Hence, mange infestation in pigs is very much important. However,their higher levels can result in metabolic dysfunction and biomolecular oxidative damage, which contributes to several pathological changes in the tissues (Valko *et al.,* 2007). Free radicals induce or contribute to in the pathogenesis of skin diseases expressed as erythema, edema, wrinkling, hypersensitivity, keratinization abnormalities and skin cancer (Bickers and Athar, 2006). This infestation may cause pig handlers itch also (Chakrabarti, 1990). Typically, mange infection results in anemia, poor nutritional status, and exhibited haemato-biochemical alteration which caused health problem (Perez *et al.,* 2006). Notwithstanding the economic impact and significance of *S. scabiei* infestation in animal populations, the pathogenesis to this disease is not well understood. In skin diseases, the body possesses an array of a potent antioxidant protection such as superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), GSH-peroxidase and the antioxidant vitamins A, E and C (Saleh *et al.,* 2011). Furthermore, Dimri *et al*. (2008) reported decrease in antioxidant enzyme activities and trace mineral concentrations in sarcoptic mange infestation, which suggested that sarcoptic mange is associated with compromise in antioxidant defence, and oxidative stress may play an important role in pathogenesis. A conclusive diagnosis is only possible by demonstrating the mite in skin scrapings. This is still the only diagnostic tool with a specificity of 100%. This method has, however, only a low

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sensitivity, depending on location and number of samples (Deckert *et al.*, 2000); but nonetheless it is very difficult to verify subclinical infections by skin scrapings (Jacobson *et al.*, 1999). A higher sensitivity for diagnosis in herds seems to be possible by the detection of antibodies to *Sarcoptes scabiei var. suis* in sera of infected pigs with enzyme-linked immunosorbent assays (ELISAs) (Bornstein and Wallgren, 1997).The treatment for mange usually depends on the administration of allopathic drugs, but these may carry a risk of toxicity to animals and people; thus, finding a non-allopathic treatment for mange remains a great challenge. In North Eastern Region, there is no any detail study was conducted. An epidemiological study on the disease will provide detail information on the prevalence and incidence of the disease in livestock reared in different States of NE region under different manage mental systems. Such information is a necessary prerequisite for designing appropriate strategies for control of the disease. The report of mange infestation in pigs from hilly region of India is not available. Keeping this in view, the study was undertaken for investigation of the sero-prevalence of sarcoptic infestation in pig in Mizoram.

MATERIALS AND METHODS

Selection of pig: Total 600 pigs were selected from different management system of three district of Mizoram, Aizawl, Kolasib and Mamit (Fig 1). The study included pigs of all age group revealing dermatological manifestations for instance itching, dermatitis especially in the ears, head, back, neck, shoulders and legs were examined.

Collection of Samples: From each selected animal two blood samples were collected by the ear vein puncture, one in a tube containing EDTA and the second in a tube without anticoagulant for subsequent serum collection. Blood samples collected in EDTA were used for hematological investigations and preparation of erythrocyte hemolysate.

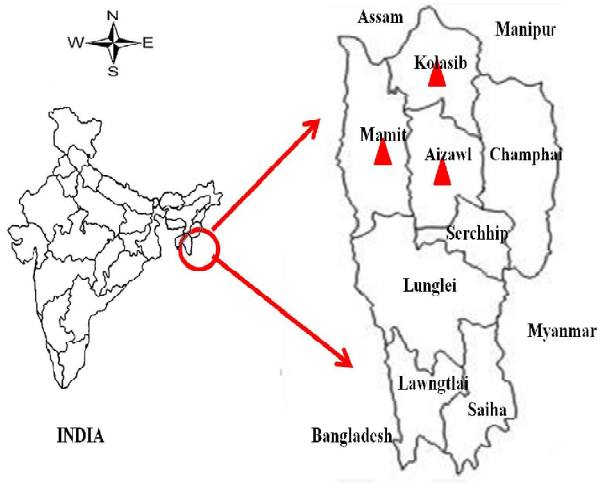

Fig 1: Map of Mizoram, showing area (shaded) where samples were collected.

Blood without anticoagulant was centrifuged at 1,000×g for 10 min. Serum was collected and stored at -80°C until processing for ELISA, biochemical and oxidative stress indices estimation.

Parasitological Examination:

Skin Scraping: Skin-scraping samples were collected in test tubes containing 5 mL of 10% potassium hydroxide solution. After gentle heating for 2–3 min, centrifuged the samples at 1500rpm for 2mints and discard the supernatant. Then a drop of the precipitation of this mixture was put on a glass slide and examined under microscope for presence or absence of the mite *S. scabiei*. The mite was identified by the third and fourth pairs of legs, which did not project beyond the margin of the body and by other morphological characteristics of the species

ELISA test: The commercial indirect ELISA SARCOPTES-ELISA 2001H Pig (AFOSA GmbH, Blankenfelde-Mahlow, Germany) was used according to the manufacturer's instructions to detect anti-Sarcoptic antibodies. OD was measured at 450 nm with ELISA reader. The results are expressed as antibody units which were calculated as subtract the optical density of negative control serum NC (OD_{NC}) from the optical density of positive control serum PC (OD_{PC}) as well as from the optical density of tested serum samples (OD_{sample}).

$$OD_{PC,\ corr} = OD_{PC} - OD_{NC}$$

ODsample, corr = ODsample – OD_{NC}

Calculated the percentage of optical density of samples (= test results, TR) from the follow-ing formula:

$$TR = \frac{OD_{sample,\ corr} \times 100}{OD_{PC,\ corr}}$$

Interpretation of test results was interpreted as TR <16 negative, TR 16-24 unequivocal and TR >24 positive.

Hematological Parameters: The evaluated hematological parameters included estimation of red blood cell count (RBCs), white blood cells (WBC), hemoglobin concentration (Hb), packed cell volume (PCV) and differential leukocytic count (DLC). These parameters were analysis with the help of blood cell counter (MS4, Natherlands).

Biochemical and Oxidative Stress Biomarkers: Serum were evaluated for the concentrations of total protein (TP), albumin (Alb), Total globulin (determined by subtracting albumin from serum total protein), blood urea nitrogen (BUN), creatinine and serum enzymatic activities of alanin aminotransferase (ALT) aspartateaminotransferase (AST), and alkaline phosphatase (ALP). All these parameters were determined by spectrophotometric method using commercially available test kits supplied by Biomed diagnostics (Germany) and following the manufacturer's instructions. Nitric oxide (NO•) was determined in serum as

nitrite concentration after reduction of nitrate to nitrite with the Griess reagent. The reaction was measure at 550 nm (Ding *et al.*, 1988).

Oxidative and antioxidative status was evaluated by measuring Lipid peroxide level (LPO), reduced glutathione (GSH), superoxide dismutase (SOD) and Total antioxidant activity. All these parameters were determined by spectrophotometric method using commercial kits (Cayman chemical, USA).

Collection of herbal plant and preparation of medicine: The plants to be evaluated in this study *Azadirachta indica (Neem)* leave, *Citrus limon* (lemon leave), *Allium cepa* (Onion) and *Allium sativum* (garlic) cloves and *Curcuma longa* (Turmeric) were collected from their natural habitat and identified by taxonomists using standard flora and voucher specimens was deposited in the national herbarium, Regional Office, Botanical Survey of India (BSI), Shillong, Meghalaya, India. The selected plant materials were washed with tap water and allow to shade dried for about 2-3 weeks. The dried materials were made powder form by electric blender and kept in tightly closed bottle in refrigerator until used for efficacy study. The plant materials were used on the basis of published literature. The powdered plant materials were individually extracted by using decoction method for 30 min (plant: water 1:20 w/v). The extracts were filtered and concentrated under reduced pressure (concentration ratio 100:5). For therapeutic trial, Polyherbal ointment (PHO) was prepared by using of each aqueous extracts of *Azadirachta indica* (*Neem*) leave, *Citrus limon* (lemon) leave , *Allium cepa* (Onion) and *Allium sativum* (garlic) cloves and *Curcuma longa* (Turmeric) in the white petrolatum oleaginous base. Briefly, the oily phase consisted of white petrolatum was heated in a beaker to about 70°C using a water bath. After melting, all the aqueous extracts heated to the same temperature as the oleaginous components, were added to the oily phase and mixed with a stirrer at 500 rpm. The mixture was slowly cooled and stirred for 30 min until congealed.

Therapeutic evaluation: The 24 positive cases of manage infested pigs were divided into two groups as Gr II which was treated with ivermectin inj. @ 200 µg/kg body weight (Das *et al*. 2010) SC ly on 0day and 15day and Gr III were treated with Poly-herb preparation applied locally for 7days. The 12 healthy pigs were grouped as Gr.I for comparison of therapeutic efficacy. Therapeutic evaluation was done with the help of improvement of clinical signs, blood haemato-biochemical and oxidative stress indices. The skin scraping and blood samples was carried out on 0 day and 15days post therapy in both the treated groups to see the mange load and haemato-biochemical and oxidative stress indices to observe the efficacy of therapeutic regiment. All the examination was done as per the method mentioned above.

Statistical analysis: The values were expressed as mean ± SE and data were analyzed by t-test and one way analysis of variance followed by the post Hoc Duncan test using statistical software package, SPSS 16.0 (2007). The level of statistical significance for all comparison was established at $P \leq 0.05$.

RESULTS AND DISCUSSION

The results cleared that out of 600 inspected pigs, 100 (16.66%) were found to be seropositive with sarcoptic mange of sexes, ranging in age from 7 months to 3years. When compared the result of ELISA test with skin scraping, the result showed 100 sera positive to ELISA from 100 sera (100%) in comparison to skin scraping which showed 40% positive. The commercial indirect ELISA SARCOPTES-ELISA 2001H Pig (AFOSA GmbH, Blankenfelde-Mahlow, Germany) was used according to the manufacturer's instructions to detect anti- Sarcoptic antibodies. Product specification indicates a sensitivity of 94% and a specificity of 97% in domestic pigs. In this study, sensitivity of the test defined as the proportion of ELISA-positive samples from animals with macroscopically visible MLLs and demonstrated mite infestation by skin scraping at the time of sample collection (truly infected, n=40) and specificity as the proportion of samples from animals without MLLs (truly non-infected, n=560) that were found negative by skin scraping. Considering the 600 samples from animals with either absence of MLLs or confirmed mite infestation, we obtained a sensitivity of 100% and a specificity of 89.29 % (Table 1). Considering all analysed samples, the percentage of antibody-positive samples increased with the chronicity of lesions.

Clinical signs associated with mite infestation revealed inappetance, alopecia, pruritus with marked crusting of the skin. Of 40 pigs with lesions, 12 were classified as medium infestation while 28 pigs showed extensive lesion. The results of the physical observation of the conditions of the animals showed that the eyes, muzzle, ears, neck, face, shoulder and back were the area's most frequently affected. Examination of the skin scrapping under the microscope showed numerous different developmental stages of *Sarcoptic* mites as eggs, larvae, nymphs and adult mites were seen (Fig 2. The mites were identified as *Sarcoptes scabiei* which are oval to round in shape, with dorsally convex tortoise-like body that is covered with spines and triangular scales with only the first two pairs of legs protruding beyond

Table 1: Estimation of the sensitivity and specificity of a comm--ercial indirect enzyme-linked immunosorbent assay (ELISA; SARCOPTES-ELISA 2001H Pig).

ELISA results	MLLs and mites (n=40)	No MLLs (n=560)
Positive (%)	40 (100)	60(10.71)
Negative (%)	-	500(89.29)

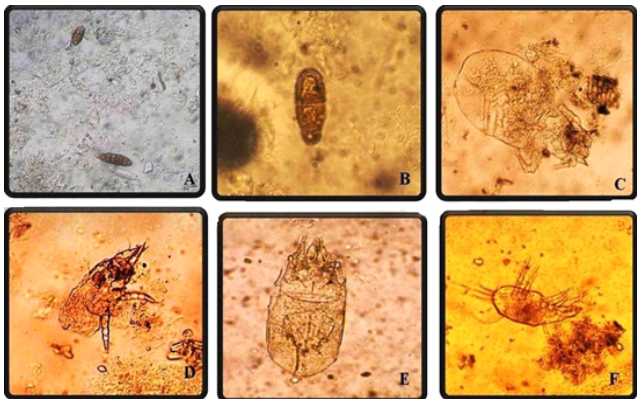

Fig 2: Different stages of *Sarcoptic scabiei var suis* A. Egg, B. Nymph, C, D, E& F. Adult.

the body margin. Geographically, pigs from Aizawl areas were more exposed to mange infestation (56/200, 28%) than Kolashib (32/200, 16%) and Mamit (12/200, 6%).

In order to successfully treat an infectious disease, a good understanding of the biology of the organism is very important, because a lot of different organisms can cause similar clinical signs on an animal. As seen in this case, the tentative diagnosis was made based on the clinical presentation of the disease and the areas affected, which is in line with Mercks (2011). Smets and Vercruysse (2000) conducted a large prevalence survey on mange in Belgium pig farms, and they found that clinical scoring of lesions (Average Dermatitis Score, ADS) was the most useful for diagnosis of mange in herds, but importantly, only 45% of ADS positive animals were positive for mites in ear scrapings. Such results highlight the present difficulty in diagnosing mange in individual animals. An alternative diagnostic method for scabies may be immunodiagnostic ELISA utilizing *S. scabiei* recombinant antigens (Jayaraj *et al.*, 2011). The objective of this study was to utilize ELISA; SARCOPTES-ELISA 2001H Pig diagnostic sensitivity over the course of infection. The sensitivity (100%) of the SARCOPTES-ELISA 2001 Pig for diagnosis of sarcoptic mange in pigs were higher than indicated by the manufacturer for domestic pigs (sensitivity 94%) and lower specificity (89.29%) than manufacturer (specificity, 97%). Similar result was also found by Nimmervoll *et al.*, (2013). Increases sensitivity (100%), indicating a role of the dynamics of the immune response in the success of antibody detection. Positive results for 60 ‘‘truly noninfected’’ animals might be due to cross reactions with other mites (Dockmann, 2004), subclinical *S. scabiei* infestations (Ippen *et al.*, 1995), or a lack of detection of mild skin lesions. In this study, the relationship between microscopical examination of skin scraping and ELISA test was discussed. Ochs *et al.*, (2001) described that these animals have antibodies without any clinical signs due to latent phase of disease with negative result in microscopic examination. Identification of all life stages on the examined animals suggests that the mites found on the pigs were an established *S. scabiei type suis* population. Infestations generally appeared as papular eruptions with erythema, pruritis and hair loss. With the progress of lesions affected areas become thickened, with crusts or exudates and consequently infected by secondary microbial invaders after excoriation of the skin due to scratching and rubbing (Gary and Durden, 2009). The present study revealed 16.66% seroprevalence of Sarcoptic mange infestation which is agreement with Das *et al.*, (2010) and Rajkhowa *et al.* (2012) who reported 10.71% prevalence in Maghalaya and 23.61% in Assam.

Haemato-biochemical changes and Oxidative stress indices in *Sarcoptes scabiei var suis* infested pig: Due to the effect of mange infection, there were significantly ($P<0.05$) decrease the level of hemoglobin (8.82±0.12 g/dl), hematocrit (28.46±0.28%), neutrophils (30.88±0.99%) and eosinophil (11.89±0.23%) in comparison to healthy group (12.74±0.44gm/dl, 44.67±0.92% 30.88±0.99% and 1.50±0. 34% respectively). Similarly, BUN (38.41±0.61mg/dl), creatinine (3.69±0.06mg/dl), AST (76.49±1.44 U/L) and ALP (259.63±8.10 U/L) were significantly ($P<0.05$) increased in infected pigs whereas proteins (4.68 ±0.04g/dl), albumin (2.69±0.03g/dl) and globulin (1.99±0.05g/dl) were decreased significantly ($P<0.05$) in comparison to healthy ones (18.17±1.45mg/dl, 1.10±0.063mg/dl, 48.33±2.53 U/L,153.67±22.53 U/l, 6.13±0.11g/dl,3.33±0.11g/dl and 2.80±0.12 g/dl respectively).

Blood LPO level (8.24±0.09µmol/L) and NO level (25.59±0.58µmol/L) in pigs with mange was significantly higher ($P\leq 0.05$) than that in healthy pigs (3.73±0.13µmol/L and 15.84±2.28µmol/L respectively). Pigs infested with mange showed significantly lower ($P\leq 0.05$) value of GSH level (0.22 ± 0.02 µM GSH/mg Hb), Total anti-oxidant (TAA) (0.62±0.01 mmol/L) and SOD (0.61±0.01 U/mg Hb) as compared with the healthy ones (0.33±0.02 µM GSH/mg Hb, 1.08±0.02 mmol/L and 1.22±0.03 U/mg Hb respectively).

Alterations in haematological values as seen in this study may be as a result of the lymph sucking activities of the mites as earlier described by Christensen (2005). The lower level of RBCs coupled with low Hb values for mange infested pigs might attribute to decrease food intake and anemia caused by parasitic infestation. Similar finding noticed by (Adejinmi *et al.*, 2004) who referred that RBCs are responsible for carrying oxygen to the body’s tissue, and fewer RBCs in the body results in anemia. Early reports suggested that anemia in mangy animals is a result of feeding behavior of mites on blood or suppression of erythropoiesis due to the effect of toxic substances secreted by mites (Deloach and Wright, 1981).The neutrophilia may be a result of increased phagocytic mechanism against the salivary toxins of the mites which may carry some microorganisms.

Eosinophilia exhibited by mange infested pigs is probably due to allergic reactions caused by the mites or activation of immune system. The decrease in serum protein, albumin and globulin level observed is probably due to the fact that *Sarcopte scabi var suis* continuously seep and suck fluid from mange infested animals. In the present investigation, raised serum creatinine, blood urea nitrogen, AST, ALP level as well as hypoalbuminemia were noted in infected groups as compared to control healthy pigs. A variety of parasitic diseases led to change in serum biochemical parameters in pigs. Altered value of various biochemical indicators have also been reported from severe mite infection in calves (Fisher, 1982). It seems that alteration of such biochemical parameters may be associated with compromised organ functions from severely scabies-infected pigs.

Apart from dermatological symptoms and alteration of haemato-biochemical alteration, scabies infection is also associated with oxidative stress changes. Oxidative stress is an important indicator to adjudge the degree of tissue damage in host system. Free radicals and oxidative stress play a pivotal role in pathogenesis of various allergic and inflammatory skin diseases (Okayama, 2005). Many pro-inflammatory cytokines induce synthesis of NO, which protects host through direct parasite killing or by limiting parasite growth (Youfang *et al*., 2008). It has been seen that scabies antigen exhibits up-regulated production and secretion of many pro-inflammatory cytokines from various skin cells (Mullins *et al.,* 2009). Based on current findings, it could be hypothesized that elevated serum nitrate status in mite- infected pigs might be due to excess cytokine expression or evolved to limit further multiplication of mites. Increased levels of lipid peroxides may be implicated in the pathology of skin lesions induced by Sarcoptic mites. Lipid hydroperoxides are by-products of lipid peroxidation and increased levels of lipid peroxidation products are associated with parasitic infestations (Dimri *et al.,* 2010). In the present study, enhanced oxidative stress i.e. lipid peroxidation and Nitric oxide and reduced antioxidant status *viz*. GSH, SOD and TAA activity were observed in pigs with mite infection. Results of the present study are in agreement with the previous scientific reports demonstrating increased LPO levels to be associated with ecto-parasitic infestations in various species of animals including sheep with sarcoptic mange (Yaralıoglu Gurgoze *et al.,* 2003), with psoroptic mange (Dimri *et al.,* 2010), dogs with sarcoptic mange (Singh *et al.,* 2012), with demodectic mange (Dimri *et al.,* 2008) and buffaloes with sarcoptic mange (Dimri *et al.,* 2008). In the present study, we found that the pigs with sarcoptic mange showed the exhaustion of these enzymes due to consistent free radical attack. The decreased level of the body antioxidant (GSH) and reduced activities of the antioxidant enzymes for instance GSH, SOD, TAA imply that *S. scabiei var. suis* infested pigs are in a state of significant oxidative stress and an altered antioxidant defense mechanism is under operation.

Table 2: Therapeutic evaluation of herbal medicine in comparison with Ivermectin on haemato-biochemical parameters in *Sarcoptes scabiei var suis* infestated pig.

Parameters	Group	Day 0	Day 15
Hb	Gr. I (n=6)	12.78 ± 0.44^{Ab}	12.12 ± 0.40^{b}
(gm/dl)	Gr. II (n=6)	9.12 ± 0.41^{Ba}	11.48 ± 0.41^{a}
	Gr. III(n=6)	$8.55\pm0.25^{B\,a}$	10.80 ± 0.34^{a}
PCV (%)	Gr. I (n=6)	$44.67\pm0.92^{A\,a}$	45.67 ± 0.92^{a}
	Gr. II (n=6)	$29.33\pm0.67^{B\,a}$	41.33 ± 3.02^{b}
	Gr. III(n=6)	29.17 ± 0.79^{Ba}	39.33 ± 2.14^{b}
TEC	Gr. I (n=6)	6.15 ± 0.14^{Ab}	5.95 ± 0.14^{b}
($\times10^6$/c mm)	Gr. II (n=6)	$4.47\pm0.14^{B\,a}$	5.88 ± 0.06^{b}
	Gr. III(n=6)	4.23 ± 0.10^{Ba}	5.45 ± 0.16^{b}
TLC	Gr. I (n=6)	12.83 ± 0.86^{a}	12.13 ± 0.83^{a}
($x10^3$/C mm)	Gr. II (n=6)	12.83 ± 0.86^{a}	10.37 ± 0.23^{a}
	Gr. III(n=6)	12.47 ± 0.75^{a}	10.50 ± 0.35^{a}
Neutrophils	Gr. I (n=6)	46.50 ± 3.60^{Ab}	43.50 ± 3.60^{a}
(%)	Gr. II (n=6)	29.83 ± 4.58^{Ba}	47.33 ± 3.59^{b}
	Gr. III(n=6)	33.17 ± 2.10^{Ba}	44.83 ± 2.79^{b}
Lymphocytes	Gr. I (n=6)	50.67 ± 3.61^{a}	50.97 ± 3.61^{a}
(%)	Gr. II (n=6)	54.83 ± 4.36^{a}	48.50 ± 3.42^{a}
	Gr. III(n=6)	53.33 ± 1.76^{a}	50.17 ± 2.34^{a}
Monocyte	Gr. I (n=6)	1.33 ± 0.42^{a}	1.33 ± 0.42^{a}
(%)	Gr. II (n=6)	1.33 ± 0.42^{a}	1.33 ± 0.42^{a}
	Gr. III(n=6)	1.33 ± 0.42^{a}	1.33 ± 0.42^{a}
Eosinophil	Gr. I (n=6)	1.50 ± 0.34^{Aa}	1.20 ± 0.31^{a}
(%)	Gr. II (n=6)	11.66 ± 0.56^{Ba}	2.83 ± 0.31^{b}
	Gr. III(n=6)	12.17 ± 0.31^{Ba}	3.67 ± 0.33^{b}
Total Protein	Gr. I (n=6)	6.13 ± 0.11^{Ab}	6.07 ± 0.07^{b}
(gm/dl)	Gr. II (n=6)	$4.77\pm0.10^{B\,a}$	5.72 ± 0.17^{a}
	Gr. III(n=6)	4.58 ± 0.17^{Ba}	5.43 ± 0.18^{a}
Albumin	Gr. I (n=6)	3.33 ± 0.11^{a}	3.18 ± 0.08^{a}
(gm/dl)	Gr. II (n=6)	2.75 ± 0.17^{a}	2.98 ± 0.18^{a}
	Gr. III(n=6)	2.90 ± 0.19^{a}	2.83 ± 0.07^{a}
Globulin	Gr. I (n=6)	2.80 ± 0.12^{a}	2.88 ± 0.142^{a}
(gm/dl)	Gr. II (n=6)	2.02 ± 0.18^{a}	2.73 ± 0.19^{a}
	Gr. III(n=6)	1.68 ± 0.17^{a}	2.60 ± 0.14^{b}
ALT (U/L)	Gr. I (n=6)	$24.17\pm1.45^{A\,a}$	24.66 ± 1.76^{Aa}
	Gr. II (n=6)	56.33 ± 4.21^{Ba}	37.00 ± 2.17^{Ab}
	Gr. III(n=6)	60.17 ± 4.58^{Ba}	44.33 ± 3.05^{Ab}
AST(U/L)	Gr. I (n=6)	48.33 ± 2.53^{Aa}	49.83 ± 2.57^{a}
	Gr. II(n=6)	90.67 ± 1.89^{Ba}	59.33 ± 3.76^{b}
	Gr. III(n=6)	89.83 ± 2.81^{Ba}	63.17 ± 5.29^{b}
ALP (U/L)	Gr. I(n=6)	153.67 ± 22.53^{Aa}	154.83 ± 22.29^{Aa}
	Gr. II(n=6)	426.67 ± 23.83^{Ba}	214.50 ± 30.46^{Bb}
	Gr. III(n=6)	432.33 ± 23.14^{Ba}	$248.00\pm28.71^{B\,b}$
BUN (mg/dl)	Gr. I(n=6)	18.17 ± 1.45^{Aa}	18.50 ± 1.48^{a}
	Gr. II(n=6)	37.00 ± 1.31^{Ba}	20.83 ± 2.70^{b}
	Gr. III(n=6)	39.17 ± 1.45^{Ba}	23.67 ± 1.93^{b}
Creatinine	Gr. I(n=6)	1.10 ± 0.06^{Aa}	1.17 ± 0.08^{Aa}
(mg/dl)	Gr. II(n=6)	3.98 ± 0.16^{Ba}	1.43 ± 0.18^{Bb}
	Gr. III(n=6)	4.13 ± 0.15^{Ba}	$1.73\pm0.25^{B\,b}$

Values bearing different superscript (a, b and c) in a row differ significantly ($P< 0.05$) and (A,B,C) in a column differ significantly ($P< 0.05$).

Therapeutic response of poly-herb formulation against mange infested pigs: Clinical signs and vital parameters were improved after 15 days of treatment in all the groups. Based on grade codes revealed higher recovery rates in the both the treated group on day15 applications. In treated groups, the size of lesions on affected parts was reduced considerably after the 15th day of post therapies. Examination of skin scrapings after the first application revealed a remarkable decrease in all stages of mites. Also, the animals' condition improved and itching and rubbing almost disappeared. In the poly-herb group (Gr.III), the mites were almost nil in skin scrapings after the 15th day of post therapy application, against the seventh in the Ivermectin group (Gr.II). Recovery in the Gr.II was quick as compared to the Gr.III as shown by shrinkage of affected parts and distinct hair growth after the 15th day of post application.

Analysis of variance revealed significant effects of treatment on haemato-biochemical values ($P < 0.05$). Overall all the haemato-biochemical parameters are significantly ($P<0.05$) improved in both the treated groups on day 15th of post therapy in compare to healthy group (Table 2). The critical analysis revealed that poly-herb ointment has similar effect on improving haemato-biochemical parameters which were altered due to mange infestation as compare to Ivermectin therapy.

Similarly oxidative stress indices were also significantly ($P<0.05$) improved in both the treated groups as compare to healthy group (Gr.I) (Table 3). Oxidant markers *viz*. LPO and NO significantly ($P<0.05$) decreased in Gr.II and Gr.III and there were no significant difference with Gr.I. Similarly all the antioxidant parameters were significantly ($P<0.05$) increased on 15th day of post therapy in both Gr.II and Gr.III and there were no significant ($P<0.05$) with Gr.I on day 15th of post therapy.

With an emerging concept of non-chemical, non-toxic and environmentally congenial acaricides, the use of herbal products for controlling various ectoparasites is needed in modem research. Medicinal herbs have reportedly been used worldwide to treat mange and other skin infections of animals, with varied efficacy (Viegi *et al*. 2003). Barring a few reports, the herbal drugs have hardly been tried as acaricides in animals, though the acaricidal effect of *Azurdiruchta indica* is well established. Kale and Panchegaonkar (1969) successfully treated 67 goats suffering from sarcoptic mange with oil of Karanj (*Pongamia pinnutru*). Srivastava and Chhabra (1971) used oil of *Erucustivu* (Tara) with sulfur in sarcoptic mange on buffaloes and found it 100% effective in killing the mites. Chhabra *et al*., (1994) used Dermocept (herbal) cream against mange in camels and buffaloes with complete cure within 20 days of the start of treatment. A herbal preparation consisting of garlic, onion lemon extracts, turmeric powder and camphor in karanj oil applied once daily for 5 consecutive days eliminated *Sarcoptic scabi* infestation in piglets within 5 days of application (Dwivedi and Sharma, 1986). Polyherb ointment eliminated mite on 15th day of post application and improved skin lesion and all the haemato-biochemical along with oxidative stress and antioxidant status. It might be due to presence of acaricidal activity of different herbal component present in the polyherb formulation.

Table 3: Therapeutic evaluation of herbal medicine in comparison with Ivermectin on oxidative stress indices in *Sarcoptic scabieversuis* infestated pig.

Parameters	Group	Day 0	Day 15
LPO(µmol/L)	Gr. I (n=6)	3.73±0.13 ^{Aa}	3.61±0.19 ^{Aa}
	Gr. II (n=6)	8.75±0.33 ^{Ba}	4.17±0.29 ^{Ba}
	Gr. III(n=6)	8.59±0.27 ^{Ba}	4.69±0.23 ^{Bb}
GSH(mol/L)	Gr. I (n=6)	0.33±0.02 ^{Aa}	0.32±0.02 ^{a}
	Gr. II (n=6)	0.16±0.018 ^{Ba}	0.37±0.04 ^{b}
	Gr. III(n=6)	0.17±0.02 ^{Ba}	0.41±0.04 ^{b}
SDO(U/mg Hb)	Gr. I (n=6)	1.08±0.02 ^{Aa}	1.09±0.017 ^{a}
	Gr. II (n=6)	0.59±0.02 $^{B a}$	0.97±0.03 ^{b}
	Gr. III(n=6)	0.58±0.02 ^{Ba}	1.02±0.03 ^{a}
TAA(mmol/L)	Gr. I (n=6)	1.22±0.03 ^{Aa}	1.23±0.03 ^{a}
	Gr. II (n=6)	0.61±0.02 ^{Ba}	1.11±0.06 ^{b}
	Gr. III(n=6)	0.63±0.03 ^{Ba}	1.22±0.03 ^{b}
NO(µmol/L)	Gr. I (n=6)	15.84±2.28 ^{a}	14.71±2.29 ^{a}
	Gr. II (n=6)	23.83±2.28 ^{a}	15.91±1.52 ^{a}
	Gr. III(n=6)	24.73±2.35 ^{a}	16.43±1.513 ^{a}

Values bearing different superscript (a, b and c) in a row differ significantly ($P < 0.05$) and (A,B,C) in a column differ significantly($P<0.05$).

CONCLUSION

The evaluated commercial ELISA for the detection of *Sarcoptes scabiei var. suis* in domestic pigs was successfully applied. This study demonstrated that both ivermectin and polyherb formulation are effective and safe for clearance of clinical signs and reduce the mange microscopically. It became evident that the plant extracts may be tested further in practice as alternatives to drugs of synthetic origin.

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REFERENCES

Adejinmi, J. O., Sadiq, N. A., Fashanu, S. O., Lasisi, O. T. and Ekundayo, S. (2004). Studies on blood parasites of sheep in Ibadan, Nigeria. *African Journal of Biomedical Research*. **7**:41 – 43.

Bickers, D. R. and Athar, M. (2006). Oxidative stress in the pathogenesis of skin disease. *Journal of Investigative Dermatology*. **126**: 2565–2575.

Bornstein, S. and Zakrisson, G. (1993). Clinical picture and antibody response in pigs infected by Sarcoptes scabiei var. suis. *Vet. Dermatol*. **4** (3):123–131.

Chakrabarti M, A. (1990). Pig handler's itch. *International Journal of Dermatology*. **29**(3):205-206.

Chhabra, M. B., Rajinder, K., Gupta, S. K. and Kumar, R. (1994). Efficacy of Dermocept (herbal) cream against mange in camel and buffalo. *Indian Veterinary Journal*. **71**: 167-169.

Christensen, K. (2005). The biology of goat. External Parasites. http://www.goatbiology.com/ mites.html

Das, M., Laha, R., Devi, P., Bordoloi, R.K. and Naskar, S. (2010). Sarcoptic mange infestation in pigs in a hilly region of Meghalaya. *Tropical Animal Health and Production*. **42**: 1009–1011 doi: 10.1007/s11250-009-9523-4

Davies, P.R. (1995). Sarcoptic mange and production performance of swine: a review of the literature and studies of associations between mite infestation, growth rate and measures of mange severity in growing pigs. *Veterinary Parasitology*. **60**(3-4):249–264.

Deckert, A., Nixon, R., Daigenault, J., Pentney, P. and Dewey, C. (2000). The evaluation of the Bommeli ELISA SARCOPTEST for Sarcoptes scabiei var.suis and the prevalence of mange in Ontario,Canada.In:Proceedings of the 16th IPVS Congress. Melbourne, Australia, 17–20 September 2000, p. 268.

Deloach, J.R. and Wright, F.C. (1981). Ingestion of rabbit erythrocytes containing 51Cr-labeled hemoglobin bypsoroptes species,that originated in cattle, mountain sheep or rabbits. *Journal of Medical Entomology*. **18**:345–348.

Dimri, U., Ranjan, R., Kumar, N., Sharma, M.C., Swarup, D., Sharma, B. and Kataria, M. (2008). Changes in oxidative stress indices, zinc and copper concentrations in blood in canine demodicosis. *Veterinary Parasitology*. **154**: 98–102 doi: 10.1016/ j.vetpar.2008.03.001.

Dimri, U., Sharma, M.C., Swarup, D., Ranjan, R. and Kataria, M. (2008). Alterations in hepatic lipid peroxides and antioxidant profile in Indian water buffaloes suffering from sarcoptic mange. *Research in Veterinary Science*. **85**:101–105 doi: 10.1016/j.rvsc. 2007.07.006. [PubMed] [Cross Ref]

Dimri, U., Sharma, M.C., Yamdagni, A., Ranjan, R. and Zama, M.M. (2010). Psoroptic mange infestation increases oxidative stress and decreases antioxidant status in sheep. *Veterinary Parasitology*. **168**: 318–322.

Ding, A.H., Nathan, C.F. and Stuehr, D.J. (1988). Release of reactive nitrogen intermediates and reactive oxygen intermediates from mouse peritoneal macrophages: Comparison of activating cytokines and evidence for independent production. *Journal of Immunology*. **141**:2407–2412.

Dockmann, J. (2004). Serologische Untersuchungen auf Antiko ¨rper gegen Sarcoptes scabiei v. suis in sauenhaltenden Betrieben mit unterschie- dlichen Behandlungsstrategien gegen Ektopar- asiten. PhD Thesis, Veterinary Medicine, Hannover University, Hannover, Germany, 159 pp.

Dwivedi. S.K. and Sharma, M.C. (1986). Studies on a herbal preparation against scabies in indigenous pigs. *Indian Journal Veterinary Medicine*. **6**:51-.

Fisher, W.F. (1982). Effects of Psoroptes ovis (Acarina: Psoroptidae) on certain biochemical constituents of cattle serum. *Veterinary Parasitology*. **11**: 241–251 doi: 10.1016/0304-4017(82)90047-4

Gary, R.M. and Durden (2009). Medical and Veterinary Entomology, 2nd ed. Elsevier Publication, Amsterdam. p. 469-470.

Ippen R., Nickel, S., Schroder H.D. (1995). Krankheiten des jagdbaren Wildes. Deutscher Landwirtschaftsverlag, Berlin,Germany, 189–195.

Jacobson, M., Bornstein, S. and Wallgren, P. (1999). The efficacy of simplified eradication strategies against sarcoptic mange mite infections in swine herds monitored by an ELISA. *Vet. Parasitol*. **81**: 249–258.

Jayaraj, R., Hales, B., Viberg, L., Pizzuto, S., Holt, D., Rolland, J.M., O'Hehir, R.E., Currie, B.J. and Walton, S.F. (2011). A diagnostic test for scabies: IgE specificity for a recombinant allergen of Sarcoptes scabiei. *Diagnostic Microbiology and Infectious Disease*. **71**:403–407.

Kale, S.M. and Panchegaonkar, M.R. (1969). Treatment of sarcoptic mange in goats with oil of Karanj. *Indian Veterinari Journal*. **46**:622-625.

Mercks (2011). Mange in sheep and goats. Merck sharp and Dohme corp., a subsidiary of Merck and Co. Inc., Whitehouse Station, NJ USA.

Mullins, J.S., Arlian, L. G. and Morgan, M.S. (2009). Extracts of Sarcoptes scabiei De Geer down modulate secretion of IL-8 by skin keratinocytes and fibroblasts and of GM-CSF by fibroblasts in presence of proinflammatory cytokines. *Journal of Medical Entomology*. **46**: 845-851.

Nimmervoll, H., Hoby, S., Robert, N., Lommano, E., Welle, M. and Ryser-Degiorgis, M.P. (2013). Pathology of sarcoptic mange in red foxes (*Vulpes vulpes*): Macroscopic and histologic characterization of three disease stages. *Journal of Wildlife Disease*. **49**:91–102.

Ochs, H., Lonneux, J.F., Losson, B.J. and Deplazes, P. (2001). Diagnosis of psoroptic sheep scab with an improved enzyme-linked immunosorbent assay. *Veterinary Parasitology*. **96** : 233–242.

Okayama, Y. (2005). Oxidative stress in allergic and inflammatory skin diseases. *Curr Drug Targets Inflammation and Allergy*. **4**:517–519. doi: 10.2174/1568010054526386

Perez, J.M., Gonzalez, F.J., Serrano, E., Granados, J.E., Fandos, P., Carro, F. and Soriguer, R.C. (2006). Is blood collected from shot Iberian ibex (*Capra pyrenaica*) useful for monitoring their physiological status? *European Journal of Wildlife Research*. **52**:125-131.

Rajkhowa, S., Das, A., Baruah, R.K., Kalita, C. and Das, J.P. (2012). Prevalence of mange mite infestation in an organized pig farm and its management. In Compendium of XXIII National Congress of Veterinary Parasitology and National Symposium on "Parasitology Today: From Environmental and Social Impact to the application of Geo informatics and Modern Biotechnology" held in the Department of Parasitology, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati-781 022, Assam from 12th Dec. to 14th. Dec. 2012. p. 55.

Saleh, M.A., Mahran, O.M. and Al-Salahy, M.B. (2011). Circulating Oxidative Stress Status in Dromedary Camels Infested with Sarcoptic Mange. *Veterinary Research Communication*. **35**:35–45.

Singh, S.K., Dimri, U., Sharma, M.C., Swarup, D., Kumar, M. and Tiwary, D. (2012). Psoroptes cuniculi induced oxidative imbalance in rabbits and its alleviation by using vitamins A, D3, E, and H as adjunctive remedial. *Tropical Animal Health and Production*. **44**:43–48. doi: 10.1007/s11250-011-9884-3.

Smets, K. and Vercruysse, J. (2000). Evaluation of different methods for the diagnosis of scabies in swine. *Veterinary Parasitology*. **90**:137–145.

Srivastava, G.C. and Chhabra, R.C. (1971). Treatment of sarcoptic mange in buffaloes with indigenous remedies. *Indian Veterinary Journal*. **48**: 196-199.

Valko, M., Leibfritz, D., Moncol, J., Cronin, M.T.D., Mazur, M. and Telser, J. (2007). Free radicals and antioxidants in normal physiological functions and human disease. *Int. J. Biochem. Cell Biol.* **39**: 44–84.

Viegi, L., Pieroni, A., Gurrera, P. M. and Vangelisti, R. (2003). A review of plants used in folk veterinary medicine in Italy as basis for a databank. *Journal of Ethno pharmacology*. **89**: 221-244.

Walton, S., Beroukas, D., Roberts-Thomson, P. and Currie, B. (2008). New insights into disease pathogenesis in crusted (Norwegian) scabies: the skin immune response in crusted scabies. *British Journal of Dermatology*. **158**: 1247-1255.

Youfang, G. U., Bingyun, W., Hongying, Z., Xinzhi, M. and Yonglin, S. (2008). Cytokines level changes in goats infected with Fasciola hepatica. *Frontiers of Agriculture in China*. **2**:242–244.

Yaralıoglu-Gurgoze, S., Sahin, T., Sevgili, M., O zkutlu, Z., Temizer-Ozan, S.(2003). The effects of ivermectin or doramectin treatment on some antioxidant enzymes and the level of lipid peroxidation in sheep with natural sarcoptic scap. *J. Fac. Vet. Med. YYU*. **14**: 30–34.

Zimmermann, W. and Kircher, P. (1998). Serologische Bestandesuntersuchung und Sanierungsuberwachung der Sarcoptes scabiei var. suis Infektion: erste vorlaufige *Resultate. Schweiz Arch Tierheilkd*. **140**: 513–517.