Circulative Oxidative Stress Indices and Ameliorative Potential of S-Adenosyl Methionine in Canine Sarcoptes Infestation

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ABSTRACT

Present study describes the rhythm of oxidant/antioxidant status of dogs affected with sarcoptic mange and ameliorative effects of antioxidant (S-Adenosyl Methionine) supplementation on oxidant/antioxidant status of dogs affected with sarcoptic mange. Study was undertaken on twelve client owned dogs presented at TVCC, DUVASU, Mathura, confirmed to be suffering from sarcoptic mange, which were randomly divided in two groups (group 1 and 2) irrespective of age, sex and breed, comprising of six dogs each, while six healthy dogs were taken as control. Group 1 dogs were treated with only Doramectin @ 0.4 mg/kg body weight subcutaneous weekly for 5 treatments along with standard treatment of pyoderma, whereas group 2 were additionally given S-adenosylmethionine (SAMe) @ 20 mg/kg body weight orally for 28 days. In the present investigation dogs with sarcoptic mange were found in a state of oxidative stress as indicated by significantly elevated Total Oxidant Status (TOS) and Oxidative Stress Index (OSI) values and significantly reduced Total Antioxidant Capacity (T-AOC) values as compared to healthy dogs. The dogs of group 2 showed better clinical recovery and marked ameliorations in TOS, T-AOC and OSI values in comparison to group 1 at the end of therapy. On the basis of findings of present investigation, it is concluded that administration of SAMe in addition to standard therapy can mitigate these alterations expediting the clinical recovery of diseased dogs and therefore can be recommended as an adjunct therapy with miticides for management of canine sarcoptic mange.

HIGHLIGHTS

⦿ Significantly elevated total oxidant status and oxidative stress index and significantly reduced total antioxidant capacity values.
⦿ Administration of S-adenosylmethionine in addition to standard therapy can be recommended as an adjunct therapy in canine sarcoptic mange.

Keywords: Oxidative stress, S-Adenosyl Methionine, Sarcoptes, Canine

Oxidative stress arises either due to excess production of free radicals, or inadequate availability of antioxidants or a combination of both. Oxidative stress supervenes when generated free radicals exceeds the capacity of antioxidant defence of the body (Santra *et al*., 2000). Various substances known as reactive oxygen species (ROS) are constantly generated in vivo as an integral part of metabolism, as part of controlled inflammatory reactions and by exposure to environmental factors (Nemec *et al.,* 2000). Estimation of antioxidant enzymes activities and levels of endogenous antioxidants in blood are indirect but reliable methods for

assessment of free radicals activity and oxidative stress (Fang *et al*., 2002). Several available reports document compromise in endogenous antioxidant defence and increase in peroxidation products in blood indicating role of free radicals and oxidative stress in various infectious and inflammatory diseases in farm animals (Ranjan *et al*., 2005; Ranjan *et al*., 2006). In recent years there has been growing interest in understanding the role of free radicals in many diseases such as cancer, arteriosclerosis, aging, and parasitic infestation due to elevation of ROS or insufficient ROS detoxification and their prevention by antioxidants (Finkel and Holbrook, 2000).

Skin is considered as the mirror of health as there are numerous systemic states/diseases that may clinically be reflected by some skin abnormalities (Sharma *et al.,* 2009). Amidst these skin problems, genus *Sarcoptes*has a very broad host range, including more than 100 mammalian species belonging to 27 families and ten orders (Bornstein *et al.,* 2001; Pence and Ueckermann, 2002). Canine scabies (sarcoptic mange) is a highly contagious, intensely pruritic and potentially zoonotic skin condition caused by infestation of the skin by the mite, *Sarcoptesscabiei* var. *canis* (Anita and Peter, 2008), which burrows into its hosts` epidermis. Mechanical disruption and ingestion of cells and tissue fluids by the mites in the skin contribute to the pathogenesis of sarcoptic mange (Pence and Uckermann, 2002). Disease is characterized by severe pruritic papular eruptions, pinpoint crusts in combination with alopecia (Yathiraj *et al.,* 1990; Pin *et al*., 2006) lead by secondary self-inflicted traumatic excoriations. Several studies exhibited that a variety of inflammatory cells are activated which induce or activate various oxidant-generating enzymes to kill intra-cellular and extra-cellular parasites (Kocyigit *et al.,* 2005). *S. scabiei* infestation is often followed by proliferation of mast cells and resultant increase in chymase and tryptase activity is supposed to play an important role in development of skin lesions (Noviana *et al.,* 2004). It is known that inflammatory cells are increased as a result of inflammation in animals with mange; recruited neutrophils and macrophages produce reactive oxidants such as hydrogen peroxide, hypochlorite and oxygen radicals, and these reactive oxygen substances produced by cells of immune system show potent cytotoxic effects on parasites and as well as other pathogenic organisms. ROS trigger induction and maintenance of cutaneous inflammation (Trenam

et al., 1992). To minimize oxidative damage, organisms developed antioxidative mechanisms triggered by an increased ROS production. The free radicals play a key role in host defence against the invading parasite (Hicks and Elston, 2009), but when generated at high levels they can result in metabolic dysfunction and biomolecular oxidative damage, which contribute to pathological changes in the tissues (Trouba *et al.,* 2002; Valko *et al.,* 2007). Oxidants such as ROS are balanced against this antioxidative defence system that consists of enzymes and metabolites in all subcellular compartments (Halliwell, 2006). This oxidative stress is mostly in the form of damage to lipids, DNA, carbohydrates and proteins (Bargaglia *et al.,* 2009). Response of individual against detrimental consequences of free radicals is through antioxidant defence system, comprising of antioxidant enzymes, like Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), and glutathione reductase; endogenous antioxidants, such as GSH and ubiquinone and nutritional antioxidants such as vitamins C and E, zinc, folate, selenium, and carotenoids.

S-Adenosylmethionine (SAMe) is a co-substrate formed naturally by healthy liver and involved in production of glutathione – an essential antioxidant that protects liver cells, detoxifies liver, enhances immune system, and neutralizes damaging elements called free radicals. S-adenosylmethionine (SAMe) is generated from L-methionine and ATP in a two-step reaction catalysed by methionine adenosyltransferase (MAT) (Mato and Lu, 2007; Hanje *et al.,* 2006).

Macrocyclic lactones (MLs) are a large family of broad-spectrum antiparasitic drugs widely used for the treatment of arthropods and nematodes parasites in veterinary and human medicine (Hennessy and Alvinerie, 2002) and includes two distinct chemical families milbemycins and avermectins. Doramectin a novel avermectin produced by fermentation of *Streptomyces avermitilis*, has a wide antihelmenthic and ectoparasiticidal spectrum of activity (Goudie *et al.,* 1993). It is approved for use in domestic animals at the dosage of $0.2\,mg\,kg^{-1}$ of bodyweight (Logan *et al.,* 1993; Reinmeyer and Courteny, 2001). Hence, the present study was conducted to determine the alteration in circulative oxidative stress indices as well as to estimate ameliorative potential of S-Adenosyl Methionine along with doramectin in canine sarcoptes infestation.

MATERIALS AND METHODS

Ethical approval

The guidelines for animal experimentation followed in this study was approved by Institutional Animal Ethics Committee and the ethical guidelines of UP Pt. Deen Dayal Upadhyaya Pashu Chikitsa Vigyan Viswavidyalaya evam Go-Anusandhan Sansthan (DUVASU), Mathura were followed during all the experiments.

Selection of dogs

The present study was undertaken on twelve client owned dogs confirmed to be suffering from sarcoptic mange irrespective of age, sex and breed presented at TVCC, DUVASU, Mathura, while six healthy dogs were taken as control. The most prominent clinical signs observed were persistent and intense pruritus, alopecia, crust formations, excoriations and irritability in all clinical cases. The lesions were observed on head, pinnae, legs (elbows and hocks) and ventrum. Confirmatory diagnosis of sarcoptic mange was based on the presence of Sarcoptes scabiei mites or mites with their developmental stages on microscopic examination of skin scrapping material. Faecal samples were negative for eggs of internal parasites and dogs were free from any concurrent disease in study group.

Blood sample collection

With the informed oral assent of the dog owners, about 5 ml of blood from each diseased dog was collected from the recurrent tarsal or radial vein using disposable syringe and needle. Blood samples were acquired from all exploratory canines on days 0, 14 and 28 during the study period. Blood was taken in plane vials without any anticoagulant and the vials were kept in a slanted position for 25-30 minutes at normal room temperature, followed by subjected to be centrifuged @2000 rpm for 5 minutes for harvesting of serum for the study of biochemical panels, total antioxidant capacity and total oxidant status. Similarly, blood samples were also procured from the healthy dogs and were used as reference value (Control Group).

Mite counts

For parasitological examination, a dulled scalpel blade was held perpendicular to the skin and used with modest pressure to scrape in the direction of hair growth. Skin scraping samples of 5 cm × 5 cm were taken from five affected sites. Scraping samples of the dogs from the study group were transferred into test tube, and 2-3 ml of 10% KOH was added; samples were mildly heated over spirit lamp until for 1–2 bumps were seen and then centrifuged on 1500 rpm for 5 min. Obtained precipitates were examined under microscope, and mites were identified according to morphological features. The average mite count was determined by averaging the mite count from five fields and was performed on days 0, 14 and 28 to assess the laboratory-based recovery (Parasitological cure).

Clinical recovery score analysis

For assessment of clinical recovery seven clinical symptoms (pruritus, erythema, papules, excoriations, crusts (scabs), alopecia and pyoderma lesions) were assessed and rated on a scale from absent (0) to severe (3). The seven scores were added up and expressed as a Sarcoptes-induced skin lesions score (SSLS) that could have values between 0 and 21 (Krieger *et al.,* 2005). Percent clinical recovery was calculated by using formula e.g., % Clinical recovery = [(Day 0 SSLS – SSLS at day post therapy) / Day 0 SSLS] × 100.

Assay of canine total oxidant status (TOS) and canine total antioxidant capacity (T-AOC)

The Biocodon Sandwich Elisa kits were used for quantitative estimation of canine total oxidant status (TOS) and canine total antioxidant capacity (T-AOC) following manufactures guidelines. The TOS to T-AOCratio was regarded as the OSI which was analysed as per method suggested by Camkerten *et al*. (2009).

Treatment plan

The dogs affected with sarcoptic acariasis were allocated into two groups, each comprises of six dogs and the design of study was as follows:

Treatment Group 1: Dogs with sarcoptic acariasis were treated with Inj. Doramectin @ 0.4 mg/kg body weight subcutaneous every week for 5 treatments (Tilley *et al.,* 2007) along with standard treatment of pyoderma if

any with Tab. Cephalexin @ 15-20 mg/kg body weight orally twice daily for 7-10 days and Shampoo containing chlorhexidine gluconate 2.1% and ketoconazole 1% applied at weekly intervals.

Treatment Group 2: Dogs with sarcoptic acariasis were treated with Inj. Doramectin @ 0.4 mg/kg body weight subcutaneous every week for 5 treatments (Tilley *et al.,* 2007) along with Tab. S-adenosylmethionine (SAMe) @ 20 mg/kg body weight orally once daily for 28 days. (Webster and Cooper, 2009). Additionally, standard treatment of pyoderma if any similar to group 1.

Control Group: Comprised of minimum six healthy dogs of any age, breed and sex. Healthy status was confirmed by thorough clinical examination and application of same laboratory tests, which were utilized for treatment group.

Statistical analysis

The values of various parameters were expressed as mean ± S.E. and Data were analysed by one-way (ANOVA) Analysis of Variance followed by the Post-Hoc tukey HSD test using Statistical Package for the Social Sciences (SPSS 16.0). The level of statistical significance for all comparisons was established at (P<0.05).

RESULTS AND DISCUSSION

In present study the most prominent and characteristics signs observed were persistent and intense pruritus, alopecia, crust formations, excoriations and irritability in all 12 clinical cases suffering from sarcoptic mange. The pinnal-pedal scratch reflex was found to be positive in 75 % of sarcoptes infested dogs. Bourdeau *et al.* (2004) reported that approximately 50 % of scabetic dogs have a positive pinnal-pedal reflex, while others showed rates between 75 % and 90 % (Griffin, 1993; Mueller *et al.,* 2001). Keratinization was noticed in about 50 % cases and erythema was found in most of the dogs. Typically, lesions were observed on the head and the pinnae, the legs (particularly the elbows and the hocks) and the ventrum.

Serum biochemistry

Biochemical parameters of healthy dogs and dogs suffering from Canine Sarcoptic Acariasis are depicted in Table 1-4. The mean values of biochemical parameters viz. aspartate aminotransferase (AST), alanine aminotransferase (ALT) were significantly higher in scabetic dogs than in control while glucose, cholesterol, total protein, albumin, albumin/globulin ratio and blood urea nitrogen showed significantly lower mean values in scabetic dogs than in control. Significant amelioration in various biochemical parameters were recorded on day 28 post therapy in the treatment group. However, biochemical parameters like AST, ALT and cholesterol levels could not attain comparable values to that of healthy controls at day 28 post therapy in treatment group 1 while treatment group 2 revealed remarkable amelioration in these panels on day 28 post therapy which could be attributed to ameliorative potentials of SAMe in this group.

In the present study the mean blood glucose levels in sarcoptic dogs was significantly lower than in control indicating hypoglycaemia, which is in agreement with previous findings (Sakina and Mandial, 2013; Narang *et al.,* 2015; Beigh *et al.,* 2016). Hypoglycaemia in scabies might be due to increased need of skin, during inflammatory reactions for glucose (Sharma, 2006).The serum glucose level in both the diseased group increased towards normalcy at day 28 post therapy. The mean serum cholesterol levels in sarcoptic dogs was significantly lower than in control. Similarly, Beigh *et al.* (2016) reported decreased cholesterol level in severely scabies-infected dogs which could be attributed to decreased synthesis by the liver. The estimated panels in group 2 attained comparable values to that of healthy controls at day 28 post therapy which could be due to the ameliorative effects of SAMe supplementation on liver.

A significant decrease in mean serum total protein level in sarcoptic dogs as compared to the healthy group indicated hypoproteinaemia, which was in agreement with the observations of Solanki *et al.* (2007) and Sakina and Mandial (2013). A significant decrease in mean serum albumin level in sarcoptic dogs as compared to the healthy group indicated hypoalbuminemia. Similar findings of reduced total protein and albumin were also reported by Lodh *et al.* (2012) and Reddy *et al.* (2014) in their study. A significant decrease in albumin-globulin ratio level in sarcoptic dogs as compared to the healthy group was also noticed in our study similar to findings of Behera *et al.* (2011). In the present study, lower albumin level would have resulted in decreased total protein and A/G ratio. Present findings were in agreement with the

earlier findings of decreased total protein and albumin in canine scabies (Chandy *et al.,* 2000) and sarcoptic mange in sheep (Hirudkar *et al.,* 1997). As the reduction in total serum protein was observed in affected dogs because albumin that is released leading to hypoproteinemia condition and that may be due to burrowing nature of the mite leading to leaching of protein fractions from the body fluids (Benjamin, 1978). In the present study the decreased albumin levels might either be due to malnutrition or parasitism which sufficiently showed that scabies affects feed conversion efficiency and growth weights. Decrease in albumin levels might be attributed to continuous loss of plasma protein through internal bleeding and subsequent haemodilution as a result of mobilization of fluid from pruritis which attributed to hypoalbuminemia (Benjamin, 1978). The estimated panels of total protein and albumin in both the treatment groups achieved a comparable value to that of healthy animals at day 28 post therapy. In the present study nonsignificant difference in mean serum globulin level in sarcoptic dogs was recorded as compared to the healthy group, which is in agreement with the findings of Lodh *et al.* (2012). However, Sakina and Mandial (2013) and Beigh *et al.* (2016) recorded hyperglobinemia in sarcoptic infested dogs.

In the present study the mean aspartate transferees (AST) andalanine tranferese (ALT) levels in sarcoptic dogs was significantly higher than in control. Similarly, Beigh *et al.* (2016) reported elevated AST & ALT level in severely scabies-infected dogs which points toward compromised liver functions. Elevated levels of ALT, AST and decreased levels of albumin, glucose and cholesterol points toward compromised liver functions in severely scabies-infected dogs. As liver is the main organ for glycogenolysis, gluconeogenesis and for albumin and cholesterol synthesis decreased glucose, albumin and cholesterol concentration observed could be attributed to decreased synthesis by the liver. Similar changes in biochemical parameters have also been observed in sarcoptic mange affected goats (De and Dey, 2010) and dogs (Sharma *et al.,* 2011). During infestation the *S. scabiei* mite burrows in the stratum corneum of the skin and resides within the skin of the host during entire life cycle process (Willis *et al.,* 2006). As the infestation progresses, the number of mites increases in the skin and it releases sufficient antigenic material which are able to increase pro-inflammatory cytokines level in blood and skin cells (Arlian *et al.,* 2003; Mullins

et al., 2009) and the pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and other cytokines have been found to play a significant role in the onset of liver disease (Thurman *et al.,* 1999). Thus, the probable cause of liver dysfunction could be due to adverse effects of pro-inflammatory cytokines and/or secretion of some toxins by mites that affects the vital organs. The estimated panels of AST and ALT values in group 2 attained comparable values to that of healthy controls at day 28 post therapy which could be attributed to the effects of S-Adenosylmethionine, which is an essential antioxidant that protects liver cells, detoxifies liver, enhances immune system, and neutralizes damaging elements called free radicals.

In present study significantly decreased BUN values were recorded at day 0 & 14 in diseased dogs as compared to the values of healthy dogs, while no significant changes were observed in creatinine values. Allaam *et al.* (2014) reported significantly low urea and creatinine in Egyptian Buffaloes (*Bubalus bubalis*) infested by sarcoptic mangeand attributed that decrease of urea should be linked with loss of appetite. The estimated panels attained comparable values to that of healthy controls at day 28 post therapy in both the treatment groups.

Total oxidant status (TOS), total antioxidant capacity (T-AOC) and oxidative stress index (OSI)

Apart from dermatological symptoms, scabies infection is also associated with physiological changes and oxidative stress is an important indicator to adjudge the degree of tissue damage in host system and it plays an important role in aetio-pathogenesis of various infectious, inflammatory and degenerative diseases (Ewans and Halliwell, 2001), including sarcoptic dermatitis (Saleh *et al.,* 2010; Beigh *et al.,* 2013b). Total Oxidant Status (TOS), Total Antioxidant Capacity (T-AOC) and Oxidative Stress Index (OSI) of healthy dogs and dogs suffering from Canine Sarcoptic Acariasis are depicted in Table 5.

In the present investigation dogs with sarcoptic mange were found in a state of oxidative stress as indicated by significantly elevated concentration of Total oxidant status (TOS) and Oxidative Stress Index (OSI) and significantly reduced concentration of Total antioxidant capacity (T-AOC) as compared to healthy dogs. In present study TOS and OSI values in group 2 attains comparable values

Table 1: Alteration in serum glucose, cholesterol and triglyceride of healthy dogs and dogs suffering from Canine Sarcoptic Acariasis.

Groups	Glucose(mg/dL)			Cholesterol (mg/dL)			Triglyceride (mg/dL)		
	DAY 0	DAY 14	DAY28	DAY 0	DAY 14	DAY 28	DAY 0	DAY 14	DAY 28
Healthy	70.33±3.16[b]	70.50±2.99[b]	70.66±2.99	222.83±11.89[b]	222.66±11.20[b]	222.83±12.09[b]	53.83±5.32	54.00±4.71	54.00±4.92
Gr 1	46.83±1.57[aA]	57.66±2.20[aB]	68.83±2.79[C]	164.50±5.15[a]	171.33±4.16[a]	174.16±4.21[a]	48.50±5.34	56.83±8.21	61.50±6.71
Gr 2	47.66±2.96[aA]	58.33±2.66[aB]	69.33±2.65[C]	164±6.59[aA]	190.50±5.03[aB]	220.66±9.18[bC]	48.00±5.47	55.83±8.79	62.16±7.61

Mean with different superscript (a, b, c) in columns are differing significantly in between the groups, otherwise non-significant.
Mean with different superscript (A, B, C) in rows are differing significantly in between the intervals, otherwise non-significant.

Table 2: Alteration in serum total protein, albumin and globulin of healthy dogs and dogs suffering from Canine Sarcoptic Acariasis

Groups	Total protein (gm/dL)			Albumin (gm/dL)			Globulin (gm/dL)		
	DAY 0	DAY 14	DAY 28	DAY 0	DAY 14	DAY 28	DAY 0	DAY14	DAY 28
Healthy	6.06±0.06[b]	6.08±0.06[b]	6.10±0.05	3.21±0.06[b]	3.25±0.05[b]	3.26±0.06	2.85±0.03	2.81±0.03	2.83±0.03
Gr1	4.46±0.11[aA]	5.01±0.04[aB]	5.95±0.03[C]	1.70±0.06[aA]	2.35±0.03[aB]	3.13±0.04[C]	2.76±0.06	2.76±0.05	2.81±0.03
Gr2	4.45±0.08[aA]	4.85±0.16[aB]	5.98±0.06[C]	1.68±0.04[aA]	2.16±0.10[aB]	3.15±0.04[C]	2.76±0.04	2.68±0.07	2.83±0.03

Mean with different superscript (a, b, c) in columns are differing significantly in between the groups, otherwise non-significant.
Mean with different superscript (A, B, C) in rows are differing significantly in between the intervals, otherwise non-significant.

Table 3: Alteration in serum A/G ratio, AST and ALT of healthy dogs and dogs suffering from Canine Sarcoptic Acariasis

Groups	A/G ratio			AST (µ/L)			ALT (µ/L)		
	DAY 0	DAY 14	DAY 28	DAY 0	DAY 14	DAY 28	DAY 0	DAY 14	DAY 28
Healthy	1.12±0.02[b]	1.13±0.03[b]	1.15±0.03	21.49±1.20[a]	21.72±1.43[a]	21.84±1.66[a]	28.33±3.72[a]	28.43±2.65[a]	28.18±3.09[a]
Gr1	0.64±0.04[aA]	0.84±0.00[aB]	1.10±0.02[C]	30.14±0.60[b]	29.07±0.49[c]	28.66±0.57[b]	38.01±1.02[b]	36.66±0.97[b]	35.15±0.83[b]
Gr2	0.64±0.03[aA]	0.80±0.02[aB]	1.10±0.01[C]	30.00±0.30[bC]	25.67±0.22[bB]	21.96±0.19[aA]	38.16±1.16[bC]	34.08±0.84[abB]	28.88±0.50[aA]

Mean with different superscript (a, b, c) in columns are differing significantly in between the groups, otherwise non-significant.
Mean with different superscript (A, B, C) in rows are differing significantly in between the intervals, otherwise non-significant.

Table 4: Alteration in serum BUN, ALP and creatinine of healthy dogs and dogs suffering from Canine Sarcoptic Acariasis

Groups	BUN (mg/dL)			ALP (µ/L)			CREATININE (mg/dL)		
	DAY 0	DAY 14	DAY 28	DAY 0	DAY 14	DAY 28	DAY 0	DAY 14	DAY 28
Healthy	14.13±0.43[b]	14.66±0.35[b]	14.08±0.39	98.16±19.29	98.50±18.99	98.66±18.75	0.59±0.02	0.57±0.02	0.58±0.03
Gr1	12.21±0.41[a]	13.16±0.36[a]	13.50±0.32	93.83±29.38	97.16±16.71	96.66±17.47	0.52±0.12	0.48±0.06	0.58±0.06
Gr2	12.50±0.40[a]	13.20±0.40[a]	13.66±0.24	93.66±32.88	98.83±18.21	96.50±17.25	0.50±0.12	0.46±0.06	0.54±0.02

Mean with different superscript (a, b, c) in columns are differing significantly in between the groups, otherwise non-significant.
Mean with different superscript (A, B, C) in rows are differing significantly in between the intervals, otherwise non-significant.

Table 5: Total oxidant status (TOS), total antioxidant capacity (T-AOC) and oxidative stress index (OSI) of healthy dogs and dogs suffering from Canine Sarcoptic Acariasis

Groups	TOS (pg/ml)			T-AOC (U/ml)			OSI		
	DAY 0	DAY 14	DAY 28	DAY 0	DAY 14	DAY 28	DAY 0	DAY 14	DAY 28
Healthy	13.30±0.42[a]	13.15±0.38[a]	13.33±0.40[a]	5.13±0.35[b]	5.16±0.25[b]	5.14±0.22[b]	2.63±0.15[a]	2.56±0.12[a]	2.6±0.09[a]
Gr1	42.63±2.29[bB]	36.32±1.60[cAB]	26.74±1.49[bA]	2.59±0.17[aA]	2.91±0.21[aAB]	3.51±0.13[aB]	16.95±1.72[bB]	12.96±0.93[cB]	7.69±0.65[bA]
Gr2	43.19±2.55[bC]	26.04±1.47[bB]	14.72±0.99[aA]	2.54±0.14[aA]	5.04±0.12[bB]	7.11±0.29[cC]	17.18±1.32[bC]	5.18±0.34[bB]	2.09±0.18[aA]

Mean with different superscript (a, b, c) in columns are differing significantly in between the groups, otherwise non-significant.
Mean with different superscript (A, B, C) in rows are differing significantly in between the intervals, otherwise non-significant.

to that of healthy controls at day 28 post therapy and even a significantly increased value of T-AOCthan that of healthy controls at day 28 post therapy was observed. This condition could be attributed to the ameliorative/antioxidant effect of S-Adenosylmethionine (SAMe) supplementation in this group. S-Adenosylmethionine (SAMe) is a co-substrate formed naturally by healthy liver and involved in production of glutathione which is an essential antioxidant that protects liver cells, detoxifies liver, enhances immune system, and neutralizes damaging elements called free radicals. Ara *et al.* (2008) reported that reactive oxygen and nitrogen species inactivate methionine adenosyltransferase (MAT) secondary to oxidation or nitrosylation of a cysteine residue on the enzyme. SAMe is involved in synthesis and metabolism of several vital compounds, such as phospholipids, nucleic acids, methyl-accepting proteins, hormones and neurotransmitters through its transmethylation reactions. In intracellular trans-sulphuration pathway, SAMe represents an important precursor of cysteine, taurine and glutathione (GSH), the major intracellular antioxidant. SAMe increases the intracellular availability of cysteine, it increases hepatic GSH levels and has antioxidant activity. (Lieber, 2002; Martinez *et al.,* 2002; Avila *et al.,* 2002; Hanje *et al.,* 2006). Several studies have demonstrated that SAMe can alleviate signs of oxidative and nitrosative stress (Lieber, 2002; Hanje *et al.,* 2006). The cytoprotective benefits of SAMe in hepatobiliary disease include augmentation of hepatocyte GSH levels, improvement in membrane fluidity, modification of cytokine expression, alterations in DNA/histone methylation and modulation of apoptosis (Mato and Lu, 2007; Martinez *et al.,* 2002; Avila *et al*., 2002).

In parallel to the findings of present study, previously Camkerten *et al.* (2009) and Beigh *et al.* (2016) reported that the oxidant/antioxidant balance is shifted towards oxidative in dogs with sarcoptic mange. Further, Behera *et al.* (2011) reported significantly elevated parameters of oxidative stress get normalize with ivermectin + vitamin E-selenium treated group, but not in ivermectin treated group, in sarcoptic mange of dogs.

Parasitological cure efficacy

Microscopic examination of skin scrapings of dogs with CSA revealed presence of high numbers of eggs, developing stages and adult mites per microscopic field before start of the therapy. *Sarcoptes*-infested dogs were examined for skin scrapping on days 0, 14 and 28 of the study. The parasitological cure rate was calculated as the proportion of dogs negative for *S. scabiei* mites on the basis of microscopic examination of deep skin scrapings obtained on day 14 and 28 post therapy. In Sarcoptes-infested dogs, the parasitological cure rate on day 14 was 40% in both the treatment groups. A 100% parasitological cure rate was noticed in both the treatment groups at day 28 post therapy. The numbers of dogs parasitologically cured from *Sarcoptes* infestation on various days of therapy are depicted in Table 6.

Table 6: Parasitological cure efficacy of various days of therapy in various groups of dogs suffering from Canine Sarcoptic Acariasis

Parasitological cure efficacy of various days of therapy			
Groups	Day 0	Day 14	Day 28
1	0/5	2/5 (40%)	5/5 (100 %)
2	0/5	2/5 (40%)	5/5 (100%)

Clinical cure efficacy

Pro-inflammatory cytokine production appears to be the effector key in the pathogenesis of scabies (Walton *et al.,* 2008). These cytokines can also be generated from the inflammation of the skin itself caused by physical stimulation of the burrowing mites (Portugal *et al.,* 2007). Triggering of this pro-inflammatory cascade lead to excessive generation of the reactive oxidants, free radicals which include reactive oxygen species (ROS) such as hydroperoxide radical (OH), superoxide anion radical (O_2) and reactive nitrogen species (RNS) such as nitric oxide radical (NO) in the biological system (Bickers and Athar, 2006). Free radicals induce or contribute to adverse effects on the skin, including edema, erythema, wrinkling, inflammation, autoimmune reaction, hypersensitivity and keratinization abnormalities (Bickers and Athar, 2006). ROS, in addition to imparting oxidative insult by damaging structural and functional components of cellular systems, is also able to induce apoptosis (Sarafian and Bredesen, 1994; Czaja, 2002). Sarcoptes-induced skin lesion score (SSLS) and clinical recovery score analysis (% improvements in SSLS) of various days of therapy in

Table 7: Sarcoptes-induced Skin Lesion Score (SSLS) and (%) Improvement SSLS in various groups of dogs suffering from Canine Sarcoptic Acariasis

| Groups | Sarcoptes-induced Skin Lesion Score (SSLS) | | | (%) Improvement SSLS | |
	DAY 0	DAY 14	DAY 28	DAY 14	DAY 28
1	15.48±0.47[C]	9.20±0.24[bB]	5.88±0.20[bA]	40.37±1.93[aA]	61.84±1.66[aB]
2	15.73±0.64[C]	4.80±0.32[aB]	1.46±0.11[aA]	69.50±1.47[bA]	90.68±0.57[bB]

Mean with different superscript (a, b) in columns are differing significantly in between the groups, otherwise non-significant.

Mean with different superscript (A, B) in rows are differing significantly in between the intervals, otherwise non-significant.

various groups of dogs suffering from Canine Sarcoptic Acariasis are depicted in Table 7.

In the present study group 2 revealed significantly better % improvement in SSLS at day 14 and 28 post therapy compared to group 1 at similar days. The difference in rates of clinical recovery from sarcoptic mange in treated groups could be attributed to the ameliorative/antioxidant effect of SAMe which enhances immune system, and neutralizes damaging elements called free radicals. Several studies have demonstrated that SAMe can alleviate signs of oxidative and nitrosative stress (Lieber, 2002; Hanje *et al.,* 2006).

CONCLUSION

On the basis of findings of present investigation, it is concluded that *Sarcoptes scabiei* infestation induces alterations in various biochemical parameters in dogs and lead to a state of significant oxidant-antioxidant imbalance. Administration of SAMe in addition to standard therapy can mitigate these alterations expediting the clinical recovery of diseased dogs and therefore can be recommended as an adjunct therapy with miticides for management of canine sarcoptic mange.

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