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EFFECT OF MANGE INFESTATION ON PARAOXONASE 1 ACTIVITY IN CAMEL

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ABSTRACT

Paraoxonase1 (PON1, aryldialkylphosphatase, EC 3.1.8.1.) is a calcium-dependent esterase enzyme, that play an important role in the protection of plasma lipoproteins and cell membranes from oxidative damage. The study was carried out on 40 clinically healthy camels and 10 camels suffered from clinical mange infestation. The serum PON 1 paraoxonase and arylesterase activity was determined by the method of hydrolysis of paraoxon and phenyl acetate, respectively. A significantly lower PON1 paraoxonase and arylesterase activity in mangy camels was seen as compared to their mean values in healthy camels. Serum glutathione peroxidase was significantly lower in mangy camels when compared to the clinically healthy ones. Serum MDL levels was significantly higher in mangy camels when compared to the clinically healthy ones. Serum HDL showed significant decrease while serum cholesterol and LDL was significantly increased in mangy camels, with no changes in TAG when compared to the clinically healthy ones. Total serum protein and albumin was decreased while AST and ALT was significantly increased in mangy she camels when compared to the clinically healthy ones.

Key words: HDL-cholesterol, mange, oxidative stress, paraoxonase1

Protection from oxidative stress in mammalian cells is due to a wide range of defence mechanisms, which include the activities of antioxidative enzymes, from these enzymes, Serum paraoxonase1 (PON1, aryldialkylphosphatase, E.C.3.1.8.1) which is a mammalian high-density lipoprotein associated enzyme, which catalyses hydrolyses of a broad spectrum of substrates, including organophosphorus compounds as well as oxidised lipids in the form of lipid hydroperoxides generated on low density lipoprotein (Mackness *et al,* 1998). The physiological substrates of paraoxonase are oxidised phospholipids of low-density lipoproteins (LDL) that appear during oxidative events caused by accumulating free oxygen radicals (Mackness *et al,* 1991a, 1993). The enzyme also hydrolyses phospholipid hydroperoxides and cholesterol ester hydroperoxides (esterase activity) and reduces lipid hydroperoxides to the respective hydroxides as well as degrades hydrogen peroxide (peroxidase activity) (Aviram *et al,* 2000). PON1 also protects HDL from peroxidation and improves reverse cholesterol transport to the liver (Aviram *et al,* 1998). It is also suggested that PON1 protects plasma membranes from free radical injury (Durrington *et al,* 2001). The enzyme degrades bioactive phospholipids, such as platelet-activating factor (PAF) (Rodrigo *et al,* 2001). Finally, it hydrolyses homocysteine thiolactone and prevents protein homocysteinylation, a process involved in atherogenesis (Jakubowski, 2000). Two closely related proteins: PON2 and PON3 have been identified. PON3 is also contained in HDL particles (Draganov *et al,* 2000; Reddy *et al,* 2001) whereas PON2 is absent in plasma but is expressed in many tissues (Ng *et al,* 2001). Both PON2 and PON3 possess antioxidant properties, but unlike PON1, they lack the paraoxon- or phenyl acetate-hydrolysing activity.

Anti-oxidative/anti-inflammatory activities of paraoxonase1 provide a relief from physiological oxidative stress as well as toxic environmental chemicals. Paraoxonase has been widely studied in human medicine, especially in relation to diseases which are characterised with increased oxidative stress such as coronary heart disease (McElveen *et al,* 1986), diabetes mellitus (Mackness *et al,* 1991b), uraemia (Juretic *et al,* 2001) and chronic liver damage (Ferré *et al,* 2002). Although the presence of paraoxonase in serum has been already confirmed in ruminants (Aldridge, 1953), and the first evidence on physical association of paraoxonase with lipoproteins was performed in cattle by Kitchen *et al* (1973) and Don *et al* (1975).

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Excess production of free radicals beyond the endogenous counteracting mechanism has been reported in various infections and inflammatory conditions (Ranjan *et al,* 2006). It was hypothesised that hepatic changes observed in sarcoptic mange may be associated with altered balance between pro-oxidant and antioxidant defense in hepatic tissues. Moreover, the understanding of paraoxonase1 in veterinary research is still poor. Therefore, the present study was designed to study the effect of mange infestation on paraoxonase activity and other biochemical parameters in order to evaluate the status of antioxidant defense in serum of camels with sarcoptic mange.

Materials and Methods

Animals

A total number of 50 Egyptian camels (*Camelus dromedaries*) were used in this study. Faecal samples from all camels under study shows negative for parasitic eggs. Animals showing skin lesions suggestive of mange were subjected to skin scraping examination. Skin lesions include severe itching dermatitis, alopecia, scabs, thickening and wrinkling of skin of the inner surface of thighs, underside of the neck and brisket and around root of the tail. Animals found positive for mange (10 camel) were classified as clinical sarcoptic mange group and clinically healthy camel showing no skin lesions (40 camel) and negative for mites in skin scraping examination served as healthy control group.

Collection of blood samples

At early morning, fasting blood samples (10 ml) were collected from jugular vein from all camels in clean dry labeled test tubes. The separated clear non haemolysed serum were collected in sterile clean Eppindorff tubes and stored at -20°C until analysis.

PON1 paraoxonase activity

PON1 activity toward paraoxon was determined according to the method described by Beltowaski *et al* (2002) by measuring the initial rate of substrate hydrolysis to p-nitrophenol, whose absorbance was monitored at 405 nm in the assay mixture (1 ml) containing 1.0 mM paraoxon, 1.0 mM $CaCl_2$ and 50 µl of serum in 100 mM Tris/HCl buffer (pH 8.0). The enzyme activity was calculated from extinction coefficient of p-nitrophenol (18290 M-1. cm-1) and was expressed in U/ml; where 1 U of enzyme hydrolyses 1 nmol of paraoxon/min.

PON1 arylesterase activity

PON1 activity toward phenylacetate was determined according to the method described by Beltowaski *et al* (2002) by measuring the initial rate of substrate hydrolysis to phenol, whose absorbance was monitored at 270 nm in the assay mixture (1.05 ml) containing 2mM substrate, 2mM $CaCl_2$ and 5µl of serum in 100mM Tris/HCl (pH 8.0). The enzyme activity was calculated from extinction coefficient of phenol (1310M-1. cm-1) and was expressed in U/ml; where 1 U of enzyme hydrolyses 1µmol of phenylacetate/min.

Other biochemical parameters

The serum triacylglycerol, cholesterol and HDL-cholesterol were measured using commercial reagent kit from Biosystems Co, France. Serum glutathione peroxidase activity (GSH-Px) was measured according to Chiu *et al* (1976). Lipid peroxides as malondialdehyde was assayed according to Placer *et al* (1966).

Statistical analysis

Statistical analysis was performed using the SPSS 9.0 statistical software for Windows. The Scheffe test was used to calculate ANOVA, and the Pearson coefficient was used to calculate correlation. Statistical analysis was done by SAS (1996).

Results

Table (1) showed that serum PON1 paraoxonase and arylesterase activities in mangy camels (7.75±0.54 and 3.97±0.44 U/ml, respectively) was significantly decreased ($P \geq 0.01$) compared to their values in clinically healthy ones (25.02±1.32 U/ml and 11.78±0.23 U/ml, respectively). On the other hand, table (1) revealed that serum glutathione peroxidase activity and MDA levels are significantly decreased ($P \geq 0.01$) in mangy camels compared to their medium values in clinically healthy ones. There was significant increase in LDL and total cholesterol levels as well as a significant decrease in HDL levels in mangy camels compared to their medium values in clinically healthy ones. While triacylglycerol levels in parasitic camels were non significantly different from their medium values in clinically healthy ones. Moreover, aspartate aminotransferase and alanine aminotransferase activities was significantly increased in mangy camels in comparison to clinically healthy ones while total protein and albumin levels was significantly decreased in mangy camels compared to clinically healthy ones (but not reach the value of difference $P \geq 0.01$).

Discussion

Oxidative stress has been implicated to play important roles in aetiopathogenesis of various

infectious, inflammatory and degenerative diseases (Evans and Halliwell, 2001 and Irshad and Chaudhuri, 2002). It supervenes when generated free radicals exceeds the capacity of antioxidant defense of the body (Santra *et al,* 2000). In the present study, the serum paraoxonase1 activity, as a part of the antioxidative system, was significantly decreased in camels suffering from mange in which serum PON1 paraoxonase and arylesterase activity in mangy camels than serum PON1 paraoxonase and arylesterase activity in clinically healthy ones (Table 1). This result was confirmed by Dimri *et al* (2008) who noticed significantly ($P < 0.05$) lower superoxide dismutase and catalase activities in Indian water buffaloes suffering from sarcoptic mange in comparison to healthy buffaloes. Ehab (2004) recorded a significantly decreased serum and liver paraoxonase and arylesterase activities in mice infected with *Schistosoma mansoni* after 8 and 12 weeks as compared with control.

The obtained lower serum PON1 activity in mangy camels could be considered as a result of 2 possibilities, firstly deficiency of serum calcium occurring in mange infestation (Parmar *et al,* 2005 and Shanthkumar and Suryanarayana, 1995) which is necessary for PON1 stability and activity. The decrease in serum calcium level in mange infestation may contribute to dermatitis, keratinisation and thickening of skin in many parts of diseased camel's body which may affect the conversion of 7-dehydrocholesterol (precursor of Vitamin D_3) which present under skin into active calcitriol hormone (1, 25 $(OH)_2$-D_3) which stimulate intestinal absorption of calcium against concentration The second possibility is that alteration in hepatic functions occurring in mange infestation (Dimri and Sharma, 2004a) which is the main site for paraoxonase1 secretion. Since all antioxidants act in concert, lower serum paraoxonase1 activity, as an enzymatic component of the antioxidative system, could contribute to an overall reduction of effectiveness and the total capacity of the antioxidative system in camels infested with sarcoptic mange.

The data recorded in table (1) showed a significant decrease of glutathione peroxidase activity in mangy camels when compared with clinically healthy ones. These results agree with that of Dimri *et al* (2008) who reported significantly ($P < 0.05$) lower superoxide dismutase and catalase activities in Indian water buffaloes suffering from sarcoptic mange in comparison to healthy buffaloes.

Our study showed a significant increase in MDA level in mangy camels when compared with clinically healthy camels. This result comes in agreement with results obtained by Dimri *et al* (2008) who reported significantly ($P < 0.05$) increased MDA level in Indian water buffaloes suffering from sarcoptic mange in comparison to healthy buffaloes. As part of the antioxidative defence against lipid peroxidation, lower PON1 activity contributes to an increased risk of oxidative stress in camels with clinical sarcoptic mange. Significantly higher level of MDA and reduced activities of serum paraoxonase1 and glutathione peroxidase observed in the present study indicated decrease in antioxidant defense and oxidative damage to hepatic tissues in animals with clinical sarcoptic mange.

The present work reported a significant increase in total and LDL cholesterol and a significant decrease in HDL cholesterol with no differences in TAG levels in mangy camels when compared with clinically healthy ones.

The results of our study indicated that total protein and albumin levels were significantly decreased in mangy camels as compared to healthy ones but did not reach the value of difference ($P \geq 0.01$). Lower values of total protein and albumin in mange affected camels were reported by (El-

Table 1. Effect of mange infestation on serum paraoxonase1 (paraoxonase and arylesterase activities), glutathione peroxidase activities and Malondialdehyde level.

Parameter	Healthy camel N = 15	Mangy camel N = 9
PON1paraoxonase activity (U/ml)	25.02 ± 1.32^{A}	7.75 ± 0.54^{B}
PON1arylesterase activity (U/ml)	11.78 ± 0.23^{A}	3.97 ± 0.44^{B}
Glutathione peroxidase (U/ml)	1.86 ± 0.11^{A}	1.25 ± 0.07^{B}
Malondialdehyde (nmol/ml)	16.74 ± 1.23^{B}	47.89 ± 2.17^{A}
Triglycerides (mg/dl)	96.31 ± 2.26^{A}	105.16 ± 6.43^{A}
Total cholesterol (mg/dl)	98.45 ± 3.42^{B}	111.73 ± 4.60^{A}
High density lipoprotein (mg/dl)	43.65 ± 1.99^{A}	34.20 ± 1.39^{B}
Low density lipoprotein (mg/dl)	35.11 ± 3.21^{B}	56.47 ± 3.55^{A}
Total protein (g/dl)	7.01 ± 0.31^{A}	6.11 ± 0.33^{A}
Albumin (g/dl)	3.93 ± 0.16^{A}	2.93 ± 0.25^{A}
Aspartate aminotransferase (U/l)	17.37 ± 1.06^{B}	41.56 ± 2.21^{A}
Alanine aminotransferase (U/l)	8.57 ± 0.87^{B}	19.00 ± 1.74^{A}

Means within the same raw carrying different letters differ significantly ($P \geq 0.01$).

Magawry, 1983 and Singh *et al*, 2003) who reported a significant decrease in the values of serum total protein and albumin in camels infested with mange. They observed that decrease in total protein can be attributed to the state of anorexia and to skin damage due to disease condition which leads to protein breakdown with consequent change in the plasma protein level. In addition, these can be attributed to the loss of appetite and itching conditions in diseased cases. Low level of albumin in affected camels of the present study could be due to loss of albumin as a result of dermatitis or decreased synthesis due to hepatic dysfunction.

Moreover, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were significantly increased in mangy camels as compared to healthy ones. These results were in agreement with results obtained by Gammaz *et al* (1993). Fisher and Crookshank (1982) reported damage of hepatic tissues in cattle calves infected with *Psoroptes ovis*. In addition, Dimri and Sharma (2004a) revealed a degenerative changes in camel liver in cases of sarcoptic mange. The significant increase in the activities of AST and ALT may be attributed to impairment in hepatic functions due to altered balance between prooxidant and antioxidant defense in hepatic tissues or due to effect of toxin produced by parasites or secondary bacterial invasion of the skin.

Result of present study indicate that serum PON1 activity in camels was significantly decreased in case of sarcoptic mange infestation possibly due to decrease in antioxidant defense system in camels with clinical sarcoptic mange. Furthermore, it can be suggested that depletion of antioxidant defense mechanisms in mangy camels may, in part, play a role in impairment of cellular functions and render hepatocytes more susceptible to the lethal effects of endogenous or exogenous peroxides. Oxidative stress, at least in a part, may be responsible for various biochemical changes as observed in serum of camels suffering from sarcoptic mange.

References

Aldridge WN (1953). Serum esterases. I. Two types of esterase (A and B) hydrolysing p-nitrophenyl acetate, propionate and butyrate, and a method for their determination. Journal of Biochemistry 53:110-117.

Aviram M, Hardak E, Vaya J, Mahmood S, Milo S, Hoffman A, Billicke S, Draganov D and Rosenblat M (2000). Human serum paraoxonases (PON1) Q and R selectively decrease lipid peroxides in human coronary and carotid atherosclerotic lesions. PON1 esterase and peroxidase-like activities. Circulation 101: 2510-2517.

Aviram M, Rosenblat M, Bisgaier CL, Newton RS, Primo-Parmo SL and La Du BN (1998). Paraoxonase inhibits high-density lipoprotein oxidation and preserves its functions. A possible peroxidative role for paraoxonase. The Journal of Clinical Investigation 101:1581-1590.

Beltowski J, Wójcicka G and Marciniak A (2002). Species- and substrate-specific stimulation of human plasma paraoxonase 1 (PON1) activity by high chloride concentration. Acta Biochimica Polonica 49:927-936.

Chiu D, Fredrick H and Tappel AL (1976). Purification and properties of rat lung and soluble glutathione peroxidase. Biochemica et Biophysica Acta 445:558-566.

Dimri U and Sharma MC (2004a). Effects of Sarcoptic mange and its control with oil of *Cedrus deodara, Pongamia glabra, Jatropha curcas* and benzyl benzoate, both with and without ascorbic acid on growing sheep: epidemiology; assessment of clinical, haematological, cellmediated and humoral immune responses and pathology. Journal of Veterinary Medicine 51:71-78.

Dimri U and Sharma MC (2004b). Effects of Sarcoptic mange and its control with oil of *Cedrus deodara, Pongamia glabra, Jatropha curcas* and benzyl benzoate, both with and without ascorbic acid on growing sheep: assessment of weight gain, liver function, nutrient digestibility, wool production and meat quality. Journal of Veterinary Medicine 51:79-84.

Dimri U, Sharma MC, 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.

Don MM, Masters CJ and Winzor DJ (1975). Further evidence for the concept of bovine plasma arylesterase as a lipoprotein. Journal of Biochemistry 151:625-630.

Draganov DI, Stetson PL, Watson CE, Billecke SS and La Du BN (2000). Rabbit serum paraoxonase 3 (PON3) is a high density lipoprotein-associated lactonase and protects low density lipoprotein against oxidation. The Journal of Biological Chemistry 275:33435-33442.

Durrington PN, Mackness B and Mackness MI (2001). Paraoxonase and atherosclerosis. Arteriosclerosis, Thrombosis, and Vascular Biology 21:473-480.

Ehab MMA (2004). Arylesterase and paraoxonase in serum and liver of mice infected with *Schistosoma Mansoni:* effect of zinc administration. The Egyptian Journal of Biochemistry 22(2):123-138.

El-Magawary SM (1983). Parameters of some blood constituents in normal and diseased camels. Ph.D.Thesis, Faculty of Veterinary Medicine, Zagazig University, Egypt.

Evans P and Halliwell B (2001). Micronutrients: Oxidant/ antioxidant status. Journal of Nutrition 121:324-338.

Ferré N, Camps J, Fernandez-Ballart J, Arija V, Murphy MM, Ceruleo S, Biarnes E, Vilella E, Tous M and Joven J (2003). Regulation of serum paraoxonase activity by genetic, nutritional and lifestyle factors in the general population. Clinical Chemistry 49(9):1491-1497.

Fisher WF and Crookshank HR (1982). Effects of *Psoroptes ovis* (Acarina: Psoroptidae) on certain biochemical

constituents of cattle serum. Veterinary Parasitology 11:241-251.

Gammaz HA, Osama AA, Mohamed OB and Magdi SA (1993). Effect of Diazinon spray on some haematological and biochemical parameters of camels. Zagazig Veterinary Journal 21(3):572-579.

Irshad M and Chaudhuri PS (2002). Oxidant-antioxidant system: role and significance in human body. Indian Journal of Experimental Biology 40:1233-1239.

Jakubowski H (2000). Calcium-dependent human serum homocysteine thiolactone hydrolase. The Journal of Biological Chemistry 275:3957-3962.

Juretic D, Tadijanovic M and Rekic B (2001). Serum paraoxonase activities in hemodialysed uremic patients: cohort study. Croatian Medical Journal 42:146-150.

Kitchen BJ, Masters CJ and Winzor DJ (1973). Effects of lipid removal on the molecular size and kinetic properties of bovine plasma arylesterase. Journal of Biochemistry 135:93-99.

Mackness MI, Arrol S and Durrington PN (1993). Protection of low-density lipoprotein against oxidative modification by high-density lipoprotein associated paraoxonase. Atherosclerosis 104:129-135.

Mackness B, Mackness MI, Arrol S, Turkie W and Durrington PN (1998). Effect of the human serum paraoxonase 55 and 192 genetic polymorphisms on the protection by high density lipoprotein against low density lipoprotein oxidative modification. FEBS Letters 423:57-60.

Mackness MI, Arrol S and Durrington PN (1991a). Paraoxonase prevents accumulation of lipoperoxides in low-density lipoprotein. FEBS Letters 286:152-154.

Mackness MI, Harty B, Bhatnagar D, Wincour PH, Arrol S, Ishola M and Durrington PN (1991b). Serum Paraoxonase activity in familial hypercholesterolemia and insulin dependent diabetes mellitus. Atherosclerosis 86:193-199.

Mackness MI, Mackness B, Durrington PN, Connelly PW and Hegele RA (1996). Paraoxonase: biochemistry, genetics and relationship to plasma lipoproteins. Current Opinion in Lipidology 7:69-76.

McElveen J, Mackness MI, Colley CM, Peard T, Warner S and Walker CH (1986). Distribution of paraoxon hydrolytic activity in the serum of patients after myocardial infarction. Clinical Chemistry 32(4):671-673.

Naresh R, Swarup D, Sharma MC and Ranjan R (2005). Clinical management of sarcoptic mange in Indian buffalo calves with a botanical ointment. Veterinary Record 156:684-685.

Ng CJ, Waldeigh DJ, Gangopadhyay A, Hama S, Grijalva VR, Navab M, Fogelman AM and Reddy S (2001). Paraoxonase-2 is a ubiquitously expressed protein with antioxidant properties and is capable of preventing cell-mediated oxidative modification of low density lipoprotein. The Journal of Biological Chemistry 276 (48):44444-44449.

Noviana D, Harjanti D, Otsuka Y and Horii Y (2004). Proliferation of protease-enriched mast cells in sarcoptic skin lesions of racoon dogs. Journal of Comparative Pathology 131:28-37.

Parmar AJ, Singh V, Chaudhary SS, Prajapati BH and Sengar YS (2005). Haematobiochemical studies on sarcoptic mange in camel (*Camelus dromedarius*) in Banaskantha district (North Gujarat). Journal of Parasitic Diseases 29(1):71-73.

Patel JS, Patel RR and Panchasara HH (2002). Economic losses due to sarcoptic mange in camel calves. Veterinary Practitioner 3:186-189.

Patel JS, Patel RR, Panchasara HH and Brahmaxtri KG (2003). Epizootiology of sarcoptic mange in buffalo calves. Indian Veterinary Journal 80:972-974.

Placer ZA, Crushman L and Johnson BC (1966). Estimation of product of lipid peroxidation (Malondialdehyde) in biochemical systems. Analytical Biochemistry 16:359-364.

Ranjan R, Naresh R, Patra RC and Swarup D (2006). Erythrocyte lipid peroxides and blood zinc and copper concentrations in acute undifferentiated diarrhoea in calves. Veterinary Research Communications 30:249-254.

Reddy ST, Wadleigh DJ, Grijalva V, Ng C, Hama S, Gangopadhyay A, Shih DM, Lusis AJ, Navab M and Fogelman AM (2001). Human paraoxonase-3 is an HDL-associated enzyme with biological activity similar to paraoxonase-1 protein but is not regulated by oxidized lipids. Arteriosclerosis, Thrombosis, and Vascular Biology 21:542-547.

Rodrigo L, Mackness B, Durrington PN, Hernandez A and Mackness MI (2001). Hydrolysis of platelet-activating factor by human serum paraoxonase. Journal of Biochemistry 354:1-7.

Said MS (1946). Mange in Egyptian camels, the morphology, life history and biochemics of *Sarcoptis scabiei*. Var. Cameli with an outline of the history, pathology and treatment. Ph.D. Thesis, Faculty of Veterinary Medicine, Cairo University, Egypt.

Santra A, Maiti A and Chowdhury A (2000). Oxidative stress in liver of mice exposed to arsenic contaminated water. Indian Journal of Gastroenterology 19:112-115.

SAS (1996). Statistical Analysis System. Users Guide Statistics, SAS Institute Cary, North Carolina.

Shanthkumar G and Suryanarayana C (1995). Clinico-biochemical and therapeutic studies on mange in buffalo calves. Indian Veterinary Journal 72:77-79.

Singh I, Khurana R and Khokhar RS (2003). Serum biochemical alterations in mangy camels. Haryana Veterinarian 42:48-50.

Singh OV, Singh JL, Prasad S and Dabas YPS (1999). Haemato-biochemical studies in concurrent infestation of Setaria and mange in buffaloes. Indian Veterinary Journal 76:934-935.