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THERAPEUTIC EFFECT OF ASHWAGANDHA (*Withania somnifera* L.) IN LIVER DYSFUNCTION OF OLD DOGS

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ABSTRACT

The present study was aimed to assess the hepatoprotective and anti-oxidant potential of hydro-alcoholic root extract of ashwagandha (*Withania somnifera*) against liver dysfunction in geriatric canine. Geriatric canine (50) with hepatic dysfunction were divided randomly into 2 equal groups. The treatment group animals received principal therapy in combination with hydro-alcoholic root extract of *W. somniferous* for 28 consecutive days, while control group received only principal therapy as per the cause of disease which resulted in hepatic derangement. The herbal treatment of *W. somnifera* root extract significantly improved serum ALT, AST, albumin, cholesterol and protein levels. SOD, GSH, LPO and catalase levels regained to normal levels in treatment group animals in post-treatment period as compared to pre-treatment period. The results suggest that crude hydro-alcoholic root extract of *W. somnifera* possesses biologically active principles that are hepatoprotective and anti-oxidant in nature. It provides an opportunity of alternative therapy with medicinal plant for small animals that are not able to use the allopathic drugs.

Key words: Ashwagandha, geriatrics, heopatoprotective, oxidative stress, principal therapy

INTRODUCTION

Geriatrics is naturally selected and genetically programmed in all the animals (Azkona *et al.,* 2009). This process is influenced by many factors which affect cells, tissues and body organs resulting in altered homeostasis (Barbieri *et al.,* 2001). Therefore, geriatric population is always at increased risk for the development of multiple organ abnormalities. The liver is uniquely placed between the portal and systemic circulation and the primary organ responsible for metabolism of endogenous and exogenous substances; hence there is progressive loss of liver function with ageing (Elberry *et al.,* 2010). With ageing, the common changes in hepatobilliary system decrease the number of hepatocytes and increase hepatic fibrosis (Cornelius, 1992). This distortion of liver architecture interferes in blood flow leading to liver inability to perform many functions (Brown *et al.,* 1997).

Ashwagandha (*Withania somnifera* L.) is known to have anti-inflammatory, immune-modulatory (Rasool and Varalakshmi, 2006), anti-arthritic (Ahmad *et al.,* 2010) and anti-ageing properties (Bhattacharya *et al.,* 1997). It has been proved to have hepatoprotective effect against radiation-induced (Mansour and Hafez, 2012) and iron-induced toxicity (Bhattacharya *et al.,* 2002).

However, protective activity of ashwagandha has not been investigated against naturally occurring cases of hepatic dysfunction in canines. The progression of hepatic abnormalities is dependent on many factors such as nutrition, exercise, life style and quality of life provided to companion animal which vary from country to country. The effect of ageing on progression of hepatic abnormalities is thus expected to vary in developing countries including India than in the developed countries. Hence, an attempt was made to assess the effects of alcoholic extract of *Withania somnifera* against naturally occurring cases of hepatic dysfunction in dogs under Indian conditions.

MATERIALS AND METHODS

Fresh roots of *Withania somnifera* were collected from natural habitat in and around Bareilly, UP (India) and the plant material got identified in the Department of Botany, Bareilly College, Bareilly (India). The roots were washed, dried in shade and ground in a mechanical grinder. The powdered material was extracted with 50% ethanol in soxlet apparatus upto 4 cycles. The extracted material was filtered through sterile muslin cloth and filtrate evaporated to dryness in a vacuum maintained at 38ºC. The dried powder was weighed and reconstituted in sterile phosphate buffer saline (PBS, pH 7.4 ± 0.1, 0.01 M) @ 200 mg extract per 5 mL PBS. The dose of extract was standardized in a pilot study by taking 5 cows in 2 batches having sub-clinical mastitis. The filtrate was filtered through a membrane filter (pore size 0.45 μm) and stored in a refrigerator until use.

The present study was conducted during 2012 and 2013 in the Division of Medicine, IVRI, Izatnagar (India). A total of 957 dogs were presented to Referral Veterinary Polyclinic, of which 251 were geriatric dogs [8-12 years]. The geriatric dogs were screened for liver failure. Canine having clinical signs, suggestive of liver failure with altered liver specific biochemical parameters and abnormal ultrasonographic findings of hepatic tissue, were selected for ameliorative study. Case control parallel design was adopted for assessing the ameliorative potential of ethanolic root extract of *W. somnifera* in 50 geriatric dogs having evidence of liver diseases. The dogs with hepatic insufficiency were randomly divided into 2 equal groups. The 1st group dogs received principal therapy as per the cause of disease [which resulted in hepatic derangement] and ethanolic root extract of *W. somnifera*, while 2nd group received principal therapy only. Sampling was done prior to the onset of treatment and then on day 7, 14 and 28 post-treatment of ethanolic root extract of *W. somnifera* [fed @100 mg kg^{-1} bw orally] and principal therapy as per cause *viz*., chemotherapy. Comparative hepato-ameliorative efficacy was assessed by observing the changes in clinical signs, liver ultrasonography, oxidative stress and restoration of blood biochemical indices to normality.

The biochemical parameters assayed were serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) (Rivers *et al.,* 1996), urea (Wybenga *et al.,* 1971) and serum cholesterol (Lopes-Virella *et al.,* 1977). Lipid peroxidation was assessed in erythrocyte homogenate in terms of erythrocytic catalase (Aebi, 1983), superoxide dismutase activity [Madesh and Balasubramanium, 1998] and reduced glutathione (GSH) by estimating free-SH groups, using 5-5' dithiobis 2-nitrobenzoic acid (DTNB) method [Sedlak and Lindsay, 1968]. The data was analyzed by one-way analysis of variance followed by Tukey test for multiple comparisons using SPSS version 17.0 software (SPSS Inc., Chicago, USA). Mann-Whitney U test was used for micronucleus frequencies comparisons. Differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

The dogs with liver disease showed clinical signs of anorexia, depression, ascites and jaundice. Significantly lower serum total protein (6.36 ±1.31 mg dL^{-1}) and albumin (1.68 ± 0.21 mg dL^{-1}) levels were found in geriatric dogs with liver disorders. Significant increase in mean AST (244.95 ±

58.2 IU L^{-1}) and ALT (280.77 ± 43.39 IU L^{-1}) and total billirubin (13.22 ± 2.14 mg d L^{-1}) was seen in geriatric dogs with liver disorders. The rise in serum AST and ALT have been attributed to their cytoplasmic location which is released into circulation after cellular damage (Panda and Kar, 1997). Although other organs like kidney, heart muscle, skeletal muscles and pancreas also contribute to the rise in ALT activities (Kaileh *et al.*, 2007), the simultaneous increase in ALT and AST levels in our study suggest a hepatic disorder as the ecostructure of liver and gall bladder revealed thickened wall of gall-bladder, cholecystitis and hepatomagaly. The other marker of liver cellular injury was significantly altered in geriatric dogs with liver disorder. Sallie (1992) attributed increased serum levels of liver specific biochemical parameters to the damage in liver membrane.

Serum ALT, AST, albumin, cholesterol and protein improved significantly in treatment group. The restoration of AST and ALT to their normal levels was seen in treatment group on 14^{th} day of treatment. In control a non-significant decrease in AST and ALT was seen on 28^{th} day (Table 1). The results showed that *W. somnifera* extract attenuated extensive changes in dogs with hepatic dysfunction. Somasundaram *et al.* (1983) attributed hepato-protective action of *W. somnifera* to membrane stabilizing activity due to active principles sitoindosides VIIX and withaferin-A which suppress increase in serum AST and ALT. GGT and cholesterol in treatment group decreased on 14^{th} and 28^{th} day of treatment. In control GGT was reduced on 28^{th} day of treatment. Ziauddin *et al.* (1996) attributed this activity of *W. somnifera* roots to withanolides (withaferin A, D and withanolide G) compounds which are steroidal and act as hormone precursors that can convert into physiologic hormones when needed. The serum protein and albumin showed significant rise after 14^{th} day of treatment in treated group, while control showed insignificant rise in protein and albumin. Singh *et al.* (2010) attributed increases in total protein and albumin to the stimulation of stem cell proliferation in hepatic tissues due to *W. somnifera* extract treatment. Mishra (2000) reported that ashwagandha root extract possess withanolides which show anti-inflammatory property and suggested that cyclooxygenase inhibition may be involved as a mechanism of action in *W. somnifera.*

SOD, CAT and GPx activities serve as index of antioxidant status of tissues. Significantly low SOD, CAT and GPx activities were observed in erythrocyte homogenate of dogs suffering from hepatic dysfunction (Table 2). It has been hypothesized that oxidative stress is involved in the etiology of hepatic complications in aged human (Austad, 1997). Lipid peroxidation is one of the characteristic features of increased oxidative stress associated with hepatic failure (Bhattacharya *et al.*, 2002). The reduction in SOD activity in dogs with hepatic dysfunction may be due to the

Table 1: Effect of *Withania somniferous* alcoholic root extract on serum biochemical parameters in dogs with hepatic dysfunction

Parameters	Group*	Days after treatment		
		0	14	28
Gamma-glutamyl transferase (UL^{-1})	Treatment	22.58 ± 2.68	17.87 ± 2.34*	9.23 ± 2.12*
	Control	24.56 ± 2.98	21.23 ± 2.13	17.56 ± 2.66*
Cholesterol ($mgdL^{-1}$)	Treatment	262.00 ± 14.17	233.90 ± 18.58*	217.12 ± 18.41*
	Control	237.43 ± 26.86	245.16 ±51.87	199.58 ± 49.35
Total protein (gdL^{-1})	Treatment	5.25 ± 0.41	5.00 ± 0.47	6.84 ± 0.37*
	Control	5.66 ± 0.83	5.02 ± 0.71	5.36 ± 0.84
Albumin (gdL^{-1})	Treatment	2.19 ± 0.29	2.85 ± 0.30 *	3.00 ± 0.03*
	Control	2.72 ± 0.41	2.78 ± 0.67	3.04 ± 0.62
Globulin (gdL^{-1})	Treatment	2.05 ± 0.25	2.16 ± 0.40	2.28 ± 0.30
	Control	1.93 ± 0.50	1.98 ± 1.01	2.58 ± 0.82
Aspartate amino-transferase (UL^{-1})	Treatment	185.66 ± 15.86	163.1 ± 15.40*	86.49 ± 13.91*
	Control	161.78 ± 16.38	152.84 ± 15.06	143.68 ± 18.26
Alanine amino-transferase (UL^{-1})	Treatment	137.94 ± 17.70	117.64 ± 19.80*	64.40 ± 15.50*
	Control	125.70 ± 14.80	128.32 ± 13.80	119.60 ± 15.23

Treatment group: *Withania somniferous* alcoholic root extract @ 100 mg kg^{-1} body weight and principle therapy. Control group: Principle therapy. Values with superscript* differ significantly between the group

Table 2: Effect of *Withania somniferous* root extract on oxidative stress (for 28 days) in dogs with hepatic dysfunction

Groups	Interval (days)	LPO (nm mL^{-1} mg^{-1} Hb)	CAT (U mg^{-1} Hb)	GSH (mm mL^{-1})	SOD (U mg^{-1} Hb)
W. somniferous	0	2.11 ± 0.34	5.38 ± 0.40	0.31± 0.07	4.75 ± 0.61
Treatment	7	2.05 ± 0.24	5.63 ± 0.28	0.41± 0.07	4.98 ± 0.89
	14	1.95 ± 0.42*	6.80 ± 0.66*	0.34 ± 0.06*	5.01 ± 0.59*
	28	1.71 ± 0.40*	6.72 ± 0.68*	0.39 ± 0.08*	5.34 ± 0.69*
Control	0	1.94 ± 0.17	5.93 ± 0.45	0.158 ± 0.02	4.88 ± 0.87
	7	2.28 ± 0.23	5.58 ± 0.95	0.159 ± 0.05*	4.31 ± 0.65
	14	1.85 ± 0.17	5.87 ± 1.21	0.113 ± 0.01	4.66 ± 0.54
	28	1.79 ± 0.37	5.75 ± 0.83	0.22 ± 0.04*	4.56 ± 0.56

Treatment group: *Withania somniferous* alcoholic root extract @ 100 mg kg^{-1} body weight and principle therapy. Control group: Principle therapy. Values with superscript* differ significantly between the group

enhanced production of superoxide radical anions (Chaudhary *et al.*, 2003). Dogs suffering from hepatic dysfunction had significantly decreased SOD, CAT and GPx levels in erythrocyte homogenate (4.75 ± 0.61 U mg^{-1} Hb, 5.38 ± 0.40 U mg^{-1} Hb, 0.31 ± 0.07 mm mL^{-1}, respectively) as compared to post-treatment values (5.34 ± 0.69 U mg^{-1} Hb, 6.72 ± 0.68 U mg^{-1} Hb, L^{-1} 0.39 ± 0.081 mm mL^{-1}, respectively). However, no significant change was observed in control. Bhattacharya *et al.* (2002) reported that ethanolic extract of *W. somnifera* is a good source of natural antioxidants such as flavonoids and polyphenols and consumption of *W. somnifera* or its products may contribute substantial amounts of antioxidants to the diet. Evidences show the importance of medicinal plants in treatment of oxidative stress-induced cell death (Chaudhary *et al.*, 2003). The LPO lowering effect may be due to withanolide, the ingredients known for antioxidant activity (Bhattacharya *et al.*, 2002). *W. somnifera* reportedly protects liver against hepatic injury by inhibition of lipid peroxidation and restoration of antioxidant enzymes in diabetic dogs (Ali *et al.*, 2001). The study demonstrates the anti-peroxidative and antioxidant effects of *W. somnifera* extract. The study revealed that ashwagandha root extract has some role in dogs with hepatic dysfunction which reflects its hepatoprotective effect. The root extract significantly reduced lipid peroxidation and increased SOD and catalase activities, so possess free radical scavenging property.

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Competing interests The authors declare that they have no competing interests.

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