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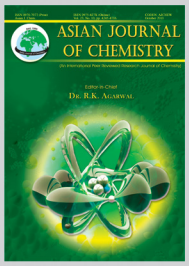

Lipid Peroxidation and *in vivo* Antioxidant Effect of Various Extracts of Whole Plant of *Bridelia scandens* in CCl_4 Induced Hepatotoxicity Rats

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The objective of the present investigation was to evaluate the lipid peroxidation and *in vivo* antioxidant effect of various extracts of whole plant of *Bridelia scandens* in CCl_4 induced rats. Carbon tetrachloride treated rats were showed significantly ($P < 0.001$) reduced the levels of tissues enzymatic antioxidant and enhanced the level of non enzymatic antioxidant (glutathione). The level of TBARS are elevated in CCl_4 teated rats (group II) when compared with group I (control). The methanolic extract of *Bridelia scandens* in CCl_4 treated rats were found lowered the concentration of TBARS when compared with CCl_4 treated rats. Carbon tetrachloride induces the oxidative stress in cell by producing reactive oxygen species. After administration of methanolic extract of *Bridelia scandens* in CCl_4 treated rats were showed significantly ($P < 0.001$) increased the levels of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR) and reduced the level of non enzymatic antioxidant glutathione (GSH) when compared with CCl_4 induced rats (group II). Based on the results, it was concluded that the methanolic extract of *Bridelia scandens* is a significant source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses.

Key Words: *Bridelia scandens*, CCl_4, Enzymatic and non enzymatic antioxidants.

INTRODUCTION

Oxidative stress induced by reactive oxygen species (ROS) is implicated in the pathogenesis of a variety of vascular diseases, including atherosclerosis, hypertension and coronary artery diseases[1]. There is extensive evidence to implicate free radicals in the development of degenerative diseases[2]. It is suggested that free radical damage to cells leads to the pathological changes associated with aging[3]. Radical reactions are also important in the development of chronic diseases that are life limiting like cancers, hypertension and cardiac infarction, atherosclerosis, rheumatism and also in cataract[4]. Free radical induced oxidative stress, which involve preventive mechanisms, repair mechanism, physical defenses and antioxidant defenses[5]. It is commonly recognized that antioxidants radicals can neutralize potentially harmful reactive free radicals in body cells before they cause lipid and protein oxidation and may reduce potential mutation and therefore, help prevent cancer or heart diseases[6].

Bridelia scandens belongs to the family Euphorbiaceae. It is distributed in the warm regions of India and Southeast Asia. This plant used as antimicrobial activity[7]. The bark decoction has been used in the traditional medicine for the treatment of asthma, intestinal worms and cough and leaves are used against colics. Tannins were isolated from the bark. The fatty alcohol with the m.f. $C_{22}H_{46}O$, named bridelyl alcohol besides fatty acids and a phlobatannin were isolated from the leaves of *Bridelia scandens*[8]. Taraxenone was isolated from hexane extract of roots[9]. Based on the literature survey it was revealed that lack of scientific report regarding antioxidant activity of the whole plant of *Bridelia scandens* (Roxb) Willd. Hence the aim of the present study was to evaluate the lipid peroxidation and *in vivo* antioxidant effect of various extracts of *Bridelia scandens* CCl_4 induced rats.

EXPERIMENTAL

Collection and identification of plant materials: The whole plant of *Bridelia scandens* (Roxb) Willd, were collected from Kilikulam, Tirunelveli District of Tamil Nadu, India. Taxonomic identification was made from Botanical Survey of Medical Plants Unit Siddha, Government of India. Palayamkottai. The whole plant of *Bridelia scandens* (Roxb) Willd, were dried under shade, segregated, pulverized by a mechanical grinder and passed through a 40 mesh sieve.

Preparation of extracts: The above powdered materials were successively extracted with petroleum ether (40 - 60 ºC) by hot continuous percolation method in Soxhlet apparatus[10] for 24 h. Then the marc was dried and then subjected to ethyl acetate (76-78 ºC) for 24 h, then marc was dried and then it was subjected to methanol for 24 h. The extracts were concen-

TABLE-1
EFFECT OF VARIOUS EXTRACTS OF *Bridelia scandens* ON TISSUE TBARS AND GLUTATHIONE (GSH) IN CCl_4 TREATED RATS

Groups	TBARS (n mol of MDA formed/g tissue)		GSH (mg/g tissue)	
	Liver	Kidney	Liver	Kidney
Group I	1.29 ± 0.09b*	1.33 ± 0.02b*	1.18 ± 0.06b**	1.30 ± 0.02 b*
Group II	2.67 ± 0.04a*	2.82 ± 0.01a*	0.46 ± 0.02a**	0.42 ± 0.01 a*
Group III	2.55 ± 0.038a**,b**	2.75 ± 0.01 a**,b*	0.50 ± 0.03a**,b*	0.51 ± 0.02 a**,b*
Group IV	2.37 ± 0.03 a**,b*	2.38 ± 0.04 a*,b*	0.59 ± 0.02a**,b*	0.62 ± 0.01 a*,b*
Group V	1.35 ± 0.02 a*,b*	1.65 ± 0.03 a*,b*	0.98 ± 0.03 b*	0.99 ± 0.01 b*
Group VI	1.40 ± 0.01 a*,b*	1.51 ± 0.03 a*,b*	0.85 ± 0.02 b*	0.88 ± 0.03 b*

Values are mean ± SE of 6 rats: *P* values: * < 0.001, ** < 0.05; NS : non significant; a → group I compared with groups II, III, IV, V, VI; b → group II compared with groups III, IV, V, VI; Group I : control; Group II : CCl_4 + LP; Group III: CCl_4 + Pet. ether extract of *Bridelia scandens* (300 mg/kg B. wt); Group IV: CCl_4 + ethyl acetate extract of *Bridelia scandens* (300 mg/kg B. wt); Group V: CCl_4 + methanol extract of *Bridelia scandens* (300 mg/kg B. wt); Group VI: CCl_4 + standard drug silymarin (100 mg/kg B.wt)

TABLE-2
EFFECT OF VARIOUS EXTRACTS OF *Bridelia scandens* ON TISSUE SUPEROXIDE DISMUTASE (SOD) AND CATALASE (CAT) IN CCl_4 TREATED RATS

Groups	SOD (unit min/mg/protein)		CAT (μ moles of H_2O_2 consumed min/mg/protein)	
	Liver	Kidney	Liver	Kidney
Group I	6.16 ± 0.01 b*	5.51 ± 0.03 b*	3.16 ± 0.05 b*	3.34 ± 0.02 b*
Group II	3.72 ± 0.04 a*	4.16 ± 0.02 a*	1.14 ± 0.07 a*	1.33 ± 0.01 a*
Group III	3.78 ± 0.07 a**,b*	4.26 ± 0.02 a**,b**	1.29 ± 0.02 a**,b*	1.43 ± 0.04 a**,b**
Group IV	4.26 ± 0.02 a**,b*	4.44 ± 0.02 a**,b*	1.65 ± 0.03 a**,b*	1.83 ± 0.03 a**,b*
Group V	5.84 ± 0.04 a*,b*	5.17 ± 0.03 a*,b*	2.93 ± 0.03 a*,b*	2.97 ± 0.03 a*,b*
Group VI	4.85 ± 0.07 a*,b*	4.94 ± 0.03 a*,b*	2.85 ± 0.02 a*,b*	2.97 ± 0.03 a*,b*

Values are expressed as mean ± SE (n=6 rats); *P* values: * < 0.001, ** < 0.05; NS : non significant; a → Group I compared with groups II, III, IV, V, VI; b → Group II compared with groups III, IV, V, VI; Details of group I-VI are same as in Table-1

trated by using a rotary evaporator and subjected to freeze drying in a lyophilizer till dry powder was obtained. The extracts were suspended in 2 % Tween 80[11].

Animals and treatment: Male Wister rats of 16-19 weeks age, weighing 150-175 g were procured from the Central Animal House, Rajah Muthiah Medical College, Annamalai University. The animals were kept in cages, 2 per cage, with 12:12 h light and dark cycle at 25 ± 2 ºC. The animals were maintained on their respective diets and water *ad libitum*. Animal Ethical Committee's clearance was obtained for the study. Animals were divided into following 6 groups of 6 animals each: Group I (Control) : liquid paraffin (LP) (3 mL/kg body weight); Group II : CCl_4 + LP (1 mL CCl_4/kg body weight); Group III : CCl_4 + Pet. ether extract of *Bridelia scandens* (300 mg/kg body weight); Group IV : CCl_4 + ethyl acetate extract of *Bridelia scandens* (300 mg/kg body weight); Group V : CCl_4 + methanol extract of *Bridelia scandens* (300 mg/kg body weight); Group VI : CCl_4 + standard silymarin (100 mg/kg body weight)

Biochemical estimation: Rats of groups III, IV and V were orally fed with the various extracts of *Bridelia scandens* (pet.ether, ethyl acetate and methanol) and rats of group VI were fed with standard drug silymarin. Both the *Bridelia scandens* extracts and silymarin were suspended in 2 % Tween 80 separately and fed to the respective rats by oral intubation. At the end of 14th day all the animals were sacrificed by cervical decapitation after overnight fasting. Liver and kidney were isolated and weighed accurately and used for the preparation of homogenate. Animals were given enough care as per the Animal Ethical Committee's recommendations. Portions of the tissues from liver and kidney were blotted, weighed and homogenized with methanol (3 volumes). The lipid extract obtained by the method of Folch *et al.*[12]. It was used for the estimation of thiobarbituric acid reactive substances[13] (TBARS). Another portion of the tissues was homogenized with phosphate buffer saline and used for the estimation of reduced glutathione[14] (GSH), superoxide dismutase[15] (SOD). Catalase[16] (CAT), glutathione peroxidase[17] (GPx) and glutathione-S-transferase[18] (GST).

Statistical analysis: Results were expressed as mean ± SE of 6 rats in each group. One way analysis of variance (ANOVA) test was used to determine the statistical significance. Significance level was fixed at 0.05.

RESULTS AND DISCUSSION

Table-1 was summarized that the activities of tissues TBARS and glutathione levels in CCl_4 treated rats. Elevated level of TBARS levels were found in liver and kidney of group II rats when compared with group I. The concentration of TBARS was significantly reduced by methanolic extract of *Bridelia scandens* with CCl_4 treated rats. Glutathione is a major non-protein thiol in living organisms, which plays a central role in coordinating the body's antioxidant defense process[19,20]. Decline in the glutathione content in the liver of CCl_4 intoxicated rats and its subsequent return towards the near normalcy in CCl_4 plus methanolic extract of *Bridelia scandens* administered group also reveal antilipid peroxidative effect of *Bridelia scandens*.

Table-2 showed that the effect of methanolic extract of *Bridelia scandens* on tissues Superoxide dismutase and catalase enzyme levels in HFD rats. Superoxide dismutase (SOD) is one of the important intracellular antioxidant enzymes, present

TABLE-3
EFFECT OF VARIOUS EXTRACTS OF *Bridelia scandens* ON TISSUE GLUTATHIONE PEROXIDASE (GPx) AND GLUTATHIONE-S-TRANSFERASE (GST) IN CCl_4 TREATED RATS

Groups	GPx (mg of GSH consumed/min/mg protein)		GST (μ mole of CDNB – GSH – Conjugate to /min/mg protein)	
	Liver	Kidney	Liver	Kidney
Group I	20.87 ± 0.02b*	22.54 ± 0.04b*	28.80 ± 0.02 b*	30.59 ± 0.22 b*
Group II	9.52 ± 0.02a*	10.32 ± 0.03a*	11.32 ± 0.10 a*	12.34 ± 0.02 a*
Group III	10.32 ± 0.02a*,b**	11.58 ± 0.02 **,b**	11.68 ± 0.032 a**,b**	13.82 ± 0.01 a**,b*
Group IV	11.79 ± 0.05 a**,b**	12.48 ± 0.03a**,b**	14.55 ± 0.04 a**,b*	16.76 ± 0.01 a*,b*
Group V	17. 48± 0.03 a*,b*	16.50± 0.03a*,b*	21.35 ± 0.02 a*,b*	24.44 ± 0.03 a*,b*
Group VI	17.32 ± 0.02 a*,b*	15.43 ± 0.04 a*,b*	20.38 ± 0.04 a*,b*	24.58 ± 0.04 a*,b*

Values are expressed as mean ± SE (n=6 rats); *P* values: * < 0.001, ** < 0.05; NS: Non Significant; a → Group I compared with groups II, III, IV, V, VI; b → Group II compared with groups III, IV, V, VI; Details of group I-VI are same as in Table-1

in all aerobic cells has an antitoxic effect against superoxide anion[21]. Catalase is a haemoprotein and it protects cells from the accumulation of H_2O_2 by dismutating it to form H_2O and O_2 or by using it as an oxidant in which it works as peroxidase[22]. The activities of SOD and catalase in the tissue like liver and kidney were significantly ($P < 0.001$) lowered in group II rats than that of group I. This effect may be due to clear manifestation of excessive formation of free radicals and activation of lipid peroxidation system resulting in tissue damages[23]. After administration of methanolic extract of *Bridelia scandens* with CCl_4 treated rats were showed significantly increases the levels of SOD and catalase when compared with group II.

The activities of tissues glutathione peroxidase (GPx) and glutathione-s-transferase (GST) in CCl_4 treated rats were presented in Table-3. Tissues glutathione peroxidase and reductase levels were significantly falls in group II rats as compared to the group I rats. Administration of methanolic extract of *Bridelia scandens* with CCl_4 treated rats enhanced the levels of glutathione peroxidase and glutathione-s-transferase in tissues like liver and kidney as compared with group II rats. A standard drug silymarin administered rats (group VI) also showed elevated level of glutathione peroxidase and glutathione-s-transferase.

Conclusion

On the basis of the results obtained in the present study, we conclude that the methanolic extract of *Bridelia scandens* had significant *in vivo* antioxidant and lipid peroxidation activity when compared with other extracts. The phytoconstituents may be responsible for the inhibition of lipid peroxidation and enhance the antioxidant activities of methanolic extract of *Bridelia scandens*. Further studies are needed to isolate the active components from this plant.

REFERENCES

1. M. Okuda, N. Inoue, H. Azumi, T. Seno, Y. Sumi and K. Hirata, *Arterio Sci. Thromb. Vasc. Biol.*, **21**, 1483 (2001).
2. C.E. Cross, *Ann. Internal Med.*, **107**, 526 (1987).
3. K.B. Beckman and B.W. Ames, *Physiol. Rev.*, **78**, 547 (1998).
4. B. Ostrowska and Z. Rzemykowska, *Herba. Pol.*, **44**, 417 (1998).
5. S.S. Ali, N. Kasoju, A. Luthra, A. Singh, H. Sharanabasava, A. Sahu and U. Bora, *Food Res. Int*., **41**, 1 (2008).
6. K. Dastmalchi, H.J.D. Dorman, M. Kosar and R. Hiltunen, *Food Sci. Technol.*, **9**, 239 (2007).
7. S. Kotigadde, S. Jose, A. Zachariah, P. Premanathan and K.G. Bhat, *J. Commun. Dis*., **37**, 135 (2005).
8. P. Sengupta and B.N. Ghosh, *J. Indian Chem. Soc.*, **40**, 247 (1963).
9. H.K. Desai, D.H. Gawad and T.R. Govindachari, *Indian J. Chem.*, **14**, 473 (1976).
10. J.B. Harborne, Phytochemical Methods, In Chapman & Hall, New York, edn. 11 (1984).
11. B.H. Waynforth, Injection Techniques, Academic Press, London (1980).
12. J. Folch, M. Lees and G.H. Sloane, *J. Biol. Chem.*, **226**, 497 (1957).
13. W.H. Nichans and B. Samulelson, *Eur. J. Biochem.*, **6**, 126 (1968).
14. G.L. Ellman, *Arch. Biochem. Biophy.*, **82**, 70 (1959).
15. J.T. Rotruck, A.L. Pope, H.E. Ganther, A.B. Swanson, D.G. Hafeman and W.G. Hoekstra, *Science*, **179**, 588 (1973).
16. R.D. Mavis and E. Stellwagen, *J. Biol. Chem*., **243**, 809 (1968).
17. A.K. Sinha, *Anal. Biochem*., **47**, 389 (1972).
18. W.H. Habig, *J. Biol. Chem*., **249**, 7130 (1974).
19. R. Campos, A. Garrido, R. Guerra and A. Valenzuela, *Planta Med.*, **55**, 417 (1989).
20. A. Valenzuela, C. Lagos, K. Schmidt and L.A. Videla, *Biochem. Pharmacol.*, **34**, 2209 (1985).
21. T. Rajmohan and A.L. Loki, *Indian J. Biochem. Biophys*., **40**, 354 (2003).
22. D. Bhakta, M.R. Lowe and W.J. Groh, *J. Ethanopharmacol.*, **66**, 227 (1999).
23. G.C. Kuriakose and G.M. Kurup, *Indian J. Exp. Biol.*, **46**, 52 (2008).