EVALUATION OF *IN VIVO* ANTIOXIDANT AND LIPID PEROXIDATION EFFECT OF VARIOUS EXTRACTS OF THE WHOLE PLANT OF *BORRERIA HISPIDA* (LINN) ON RAT FED WITH HIGH FAT DIET

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ABSTRACT

The present investigation was to evaluate the *in vivo* antioxidant and lipid peroxidation effect of various extracts of whole plant of *Borreria hispida* (Linn). High fat diet rats showed significant decreased activities of tissue enzymatic, non enzymatic antioxidant and elevated levels of TBARS. High fat diet induces the oxidative stress in cell by producing reactive oxygen species. Administration of methanolic extract of *Borreria hispida* in high fat diet rats were showed enhanced activities of antioxidant Enzymes such as Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPx), Glutathione reductase (GR), non enzymatic antioxidant Glutathione (GSH) and lowered the concentration of TBARS when compared with other two extracts. It is concluded that the methanolic extract of *Borreria hispida* could be used as a potential preventive intervention for free radical-mediated diseases.

Keywords: High fat diet, *Borreria hispida,* Antioxidant activity, lipid peroxidation.

INTRODUCTION

Reactive oxygen and nitrogen species play key roles in normal physiological process, including cellular life/death process, protection from pathogens, various cellular signaling pathways, and regulation of vascular tone[1]. Oxidative stress is caused by an insufficient capacity of biological systems to neutralize excessive free radical production, which can contribute to human diseases and aging [2], including cardiovascular disease[3], neurodegerative disease and age-related cognitive decline[4], obesity and insulin resistance[5], as well as immune system dysfunction[6]. Oxidative stress also contributes to the accumulation of damaged macromolecules and organelles, including mitochondria[4, 7]. Recent reports indicate that there is an inverse relationship between the dietary intake of antioxidant-rich foods and the incidence of human diseases[8]. So, many researchers have focused on natural antioxidants and in the plant kingdom numerous crude extracts and pure natural compounds were previously reported to have antioxidant properties.

Borreria hispida is belongs to the family Rubiaceae. It is widely distributed in throughout India, up to 900m in hills and on all dry lands as a weed. The seed of *Borreria hispida* is used as PPAR-alpha gene expression, antioxidant redox status, protein metabolism in STZ diabetic rats. Potential role of *Borreria hispida* in ameliorating cardiovascular risk factor (Vasanthi HR, 2009)[9]. Literature survey revealed that there is a lack enough scientific report regarding *in-vivo* antioxidant and lipid peroxidation effect of the whole plant of *Borreria hispida* (Linn.).

Hence the aim of the present study was to examine the *in-vivo* antioxidant and lipid peroxidation effect of various extracts of the whole plant of *Borreria hispida* (Linn) in rat fed with high fat diet.

MATERIALS AND METHODS

1. Collection and identification of plant materials

The whole plant of *Borreria hispida* (Linn), were collected from Naserath, Tuticorin 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 *Borreria hispida* (Linn)*,* were dried under shade, segregated, pulverized by a mechanical grinder and passed through a 40 mesh sieve.

2. Preparation of extracts

The above powdered materials were successively extracted with petroleum ether (40-60^{0}C) by hot continuous percolation method in Soxhlet apparatus[10] for 24 hrs. Then the marc was dried and then subjected to ethyl acetate (76-78^{0}C) for 24 hrs, then marc was dried and then it was subjected to methanol for 24 hrs. The extracts were concentrated 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].

3. Animals and treatment

Male Wister rats of 16-19 weeks age, weighing 150-175g 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 hr light and dark cycle at 25^0±2^{0}C. 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): Standard chow diet

Group II : High Fat Diet

Group III: High fat diet + Pet.ether extract of *Borreria hispida* (200mg/kg B.wt)

Group IV: High fat diet +Ethyl acetate extract of *Borreria hispida* (200mg/kg B.wt)

Group V: High fat diet + Methanol extract of *Borreria hispida* (200mg/kg B.wt)

Group VI: High fat diet + standard drug atorvastatin (1.2 mg/kg B.wt)

Animal diet

The compositions of the two diets were as follows[12]:

Control diet: Wheat flour 22.5%, roasted bengal gram powder 60%, skimmed milk powder 5%, casein 4%, refined oil 4%, salt mixture with starch 4% and vitamin & choline mixture 0.5%.

High fat diet: Wheat flour 20.5%, roasted bengal gram 52.6%, skimmed milk powder 5%, casein 4%, refined oil 4%, coconut oil 9%, salt mixture with starch 4% and vitamin & choline mixture 0.5%, cholesterol 0.4%.

Rats of groups III, IV and V were orally fed with the various extracts of *Borreria hispida* (pet.ether, ethyl acetate and methanol) and rats of group VI were fed with standard drug atorvastatin. Both the *Borreria hispida* extracts and atorvastatin were suspended in 2% tween 80 separately and fed to the respective rats by oral intubation. At the end of 9 weeks all the animals were sacrificed by cervical decapitation after overnight fasting. Liver, heart and aorta were cleared of adhering fat, 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, heart and aorta were blotted, weighed and homogenized with methanol (3 volumes). The lipid extract obtained by the method of Folch *et al*[13]. It was used for the estimation of thiobarbituric acid reactive substances[14] (TBARS). Another portion of the tissues was homogenized with phosphate buffer saline and used for the estimation of reduced Glutathione[15] (GSH), Glutathione peroxidase[16] (GPx), Glutathione reductase[17] (GR), Catalase[18] (CAT), and Superoxide dismutase[19] (SOD).

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

The concentration of tissues TBARS levels in HFD rats are shown in Table 1. Elevated levels of TBARS in liver, heart and aorta in group II rats are a clear manifestation of excessive formation of free radical and activation of lipid peroxidation. The significantly lowered the concentration of TBARS, in rats administered with methanolic extract of *Borreria hispida* along with HFD when compared with other two extracts.

Effect of various extracts of *Borreria* hispida on tissues Glutathione (GSH) content in rats fed HFD results are shown in Table 1. GSH also functions as free radical scavenger in the repair of radical caused biological damage[20]. The activities of glutathione concentration in tissues were significantly decreased in high fat diet rats (group II) as compared to the control rats (group I). Administration of methanolic extract of *Borreria hispida* along with HFD rats increased the levels of glutathione when compared with other two extracts.

Table 1: Effect of various extracts of *Borreria hispida* on tissue TBARS and Glutathione (GSH) in rats fed HFD

Groups	TBARS(n mol of MDA formed/g tissue)			GSH(mg/g tissue)		
	Liver	Heart	Aorta	Liver	Heart	Aorta
Group I	24.93 ± 2.05b*	42.39 ± 2.97b*	17.38 ± 1.22b**	4.28 ± 0.36b**	7.52 ± 0.49 b*	5.49 ± 0.32 b*
Group II	78.35 ± 6.27a*	85.42 ± 5.27a*	66.17 ± 4.48a*	1.84 ± 0.31a**	4.11 ± 0.26 a*	2.88 ± 0.17 a*
Group III	42.61 ± 3.14a**,b**	58.43 ± 4.14 a**,b*	30.14 ± 3.92 a**,b*	3.68 ± 0.24a**,b*	6.10 ± 0.34 a**,b**	4.46 ± 0.36 a*,b*
Group IV	38.47 ± 4.53 a**,b*	51.19 ± 3.68 a*,b*	27.21 ± 4.57 a**,b*	3.85 ± 0.19a**,b*	6.58 ± 0.29 a*,b*	5.01 ± 0.12 a**,b**
Group V	31.04 ± 4.11 a*,b*	48.12 ± 4.07 a*,b*	24.18 ± 3.26 a*,b*	3.98 ± 0.17 b*	7.01 ± 0.38 b*	5.19 ± 0.14 b*
Group VI	24.15 ± 1.09 a*,b*	41.92 ± 2.86 a*,b*	16.97 ± 1.49 a*,b*	4.30 ± 0.26 b*	7.60 ± 0.34 b*	5.56 ± 0.29 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.

Table 2: Effect of various extracts of *Borreria hispida* on tissue Superoxide dismutase (SOD) and Catalase (CAT) in rats fed HFD

Groups	SOD (unit min/mg/protein)			CAT (µ moles of H_2O_2 consumed min/mg/protein)		
	Liver	Heart	Aorta	Liver	Heart	Aorta
Group I	3.69 ± 0.23 b*	1.76 ± 0.13 b*	2.86 ± 0.19b*	28.43 ± 1.19 b*	47.43 ± 3.82 b*	30.89 ± 2.64 b*
Group II	1.73 ± 0.24 a*	0.84 ± 0.06 a*	1.59 ± 0.12 a*	16.89 ± 1.56 a*	31.24 ± 1.64 a*	21.34 ± 2.09 a*
Group III	2.49 ± 0.21 a**,b*	1.31 ± 0.21 a**,b**	2.45 ± 0.21 a**,b*	22.43 ± 1.82 a**,b*	40.18 ± 1.93 a**,b**	26.18 ± 2.14 a**,b*
Group IV	2.83 ± 0.24 a**,b*	1.43 ± 0.18 a**,b*	2.57 ± 0.19 a**,b*	23.56 ± 2.63 a**,b*	43.21 ± 2.16 a**,b*	27.58 ± 2.63 a**,b*
Group V	3.21 ± 0.20 a*,b*	1.59 ± 0.20 a*,b*	2.63 ± 0.23 a*,b*	26.32 ± 1.92 a*,b*	44.93 ± 2.78 a*,b*	28.68 ± 1.97 a*,b*
Group VI	3.70 ± 0.19 a*,b*	1.77 ± 0.18 a*,b*	2.87 ± 0.16 a*,b*	29.14 ± 1.63 a*,b*	48.13 ± 2.94 a*,b*	31.14 ± 2.93 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.

Table 3: Effect of various extracts of *Borreria hispida* on tissue Glutathione peroxidase (GPx) and Glutathione reductase (GR) in rats fed HFD

Groups	GPx (mg of GSH consumed/min/mg protein)			GR (mg of GSH consumed/min/mg protein)		
	Liver	Heart	Aorta	Liver	Heart	Aorta
Group I	8.71 ± 0.53b*	14.69 ± 1.52b*	13.19 ± 1.21b*	1.41 ± 0.13 b*	2.72 ± 0.19 b*	1.76 ± 0.16 b*
Group II	5.36 ± 0.49a*	7.16 ± 0.51a*	6.92 ± 0.08a*	0.69 ± 0.08 a*	1.36 ± 0.07 a*	0.82 ± 0.09 a*
Group III	7.64 ± 0.38a*,b**	12.68 ± 0.26 a**,b*	10.18 ± 0.19 a**,b**	1.18 ± 0.10 a**,b**	2.18 ± 0.14 a**,b**	1.32 ± 0.10 a**,b*
Group IV	7.92 ± 0.24 a**,b**	13.82 ± 0.31 a*,b*	11.92 ± 0.13 a**,b**	1.21 ± 0.09 a**,b*	2.37 ± 0.02 a*,b*	1.41 ± 0.09 a*,b*
Group V	8.37 ± 0.27 a*,b*	14.01 ± 0.25 a*,b*	12.61± 0.12 a*,b*	1.25 ± 0.04 a*,b*	2.41 ± 0.07 a*,b*	1.52 ± 0.11 a*,b*
Group VI	8.80 ± 0.59 a*,b*	14.70 ± 0.38 a*,b*	16.97 ± 1.49 a*,b*	1.42 ± 0.12 a*,b*	2.79 ± 0.15 a*,b*	1.79 ± 0.14 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.

Table 2 shows that the effect of various extracts of *Borreria* hispida on tissues SOD and CAT enzyme levels in HFD rats. The activities of SOD and CAT in the tissue like liver, heart and aorta were significantly (P<0.001) lowered in rats fed with high fat diet (group II) than control group animals. High fat diet can cause the formation of toxic intermediates that can inhibit the activity of antioxidant enzymes[21] and the accumulation of O_2^- and H_2O_2 which in turn forms hydroxyl radicals[22]. After administration of methanolic extract of *Borreria hispida* along with HFD significantly increases the activities of SOD and CAT in tissues of rats when compared with other extracts.

Effect of various extracts of *Borreria* hispida on tissues glutathione peroxidase (GPx) and glutathione reductase (GR) in HFD rats are shown in Tables 3. The effect of glutathione peroxidase and reductase was also significantly decreased in tissues of rats fed with HFD as compared to the control rats. High fat diet decreased the ratio of oxidized glutathione/ reduced glutathione in tissue[23]. Administration of methanolic extract *Borreria hispida* along with the HFD increased the activities of glutathione peroxidase and glutathione reductase in all the tissues as compared with HFD. A standard drug atorvastatin administered rats also showed elevated level of glutathione peroxidase and glutathione reductase.

CONCLUSION

On the basis of the results obtained in the present study, we conclude that the methanolic extract of *Borreria hispida* 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 *Borreria hispida*. Further studies are needed to isolate the active components from this plant.

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