IN VIVO ANTIOXIDANT AND LIPID PEROXIDATION EFFECT OF METHANOLIC EXTRACT OF TUBEROUS ROOT OF *Ipomoea digitata* (Linn) IN RAT FED WITH HIGH FAT DIET

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ABSTRACT: The study was carried out to determine the *in vivo* antioxidant and lipid peroxidation effect of methanolic extract of tuberous root of *Ipomoea digitata* (Linn). High fat diet rats 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 HFD rats (group II) when compared with control group. High fat diet induces the oxidative stress in cell by producing reactive oxygen species. Administration of methanolic extract of *Ipomoea digitata* in high fat diet rats were showed 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 HFD rats (Group II). The methanolic extract *Ipomoea digitata* in high fat diet rats were found lowered the concentration of TBARS when compared with HFD rats. Based on the results, we concluded that the methanolic extract of *Ipomoea digitata* is a significant source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses.

Key words: High fat diet, *Ipomoea digitata,* Antioxidant activity, rats.

INTRODUCTION

Recently there has been an upsurge of interest in the therapeutic potential of medicinal plants as antioxidants in reducing such free radical-induced tissue injury[1]. Reactive oxygen species can easily initiate the lipids causing damage of the cell membrane constituent i.e. phospholipids, lipoproteins by propagating a reaction cycle[2]. Considerable evidence have accumulated to implicate cellular damage arising from reactive oxygen species (ROS), at least in part, in the etiology and pathophysiology of human diseases such as neurodegenerative disorders (e.g. Alzheimer disease, Parkinson disease, multiple sclerosis, Down's syndrome), inflammation, viral infections, autoimmune pathologies, and digestive system disorders such as gastrointestinal inflammation and ulcer[3-5].

Oxygen free radicals are formed in tissue cells by many endogenous and exogenous causes such as metabolism, chemicals, and ionizing radiation[6]. Oxygen free radicals may attack lipids and DNA giving rise to a large number of damaged products[7]. Antioxidants are important in the prevention of human diseases. Antioxidant compounds may function as free radical scavengers, complexes of pro-oxidant metals, reducing agents and quenchers of singlet oxygen formation [8].

The tuberous root of *Ipomoea digitata* (linn) is belongs to the convolvolaceae family. The root large ovoid or elongated tuber ous roots. The root is regarded as a diuretic, leprosy, burning sensation, vomiting and disease of blood, anthelmintic, syphilis and spleen disease[9]. The plant used as Aphrodisiac activity[10] and anti microbial activity[11]. Resin glycoside was isolated from leaves and stems of *Ipomoea digitata*[12]. Therefore, the present investigation focused to evaluate the *in vivo* antioxidant and lipid peroxidation effect of methanolic extract of tuberous root of *Ipomoea digitata*.

MATERIAL AND METHODS

1. Collection and identification of plant materials

The tuberous root of *Ipomoea digitata* (Linn), 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 tuberous root of *Ipomoea digitata* (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 methanol by hot continuous percolation method in Soxhlet apparatus[13] 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[14].

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 $25^0 \pm 2^0$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 4 groups of 6 animals each:

Group I (Control) : Standard chow diet : Group II : High Fat Diet
Group III : High fat diet + Methanol extract of *Ipomoea digitata* (300mg/kg B.wt)
Group IV : High fat diet + standard drug atorvastatin (1.2 mg/kg B.wt)

Animal diet

The compositions of the two diets were as follows[15]:

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 group III were orally fed with the methanolic extract of *Ipomoea digitata* and rats of group IV were fed with standard drug atorvastatin. Both the methanolic extract *Ipomoea digitata* 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*[16]. It was used for the estimation of thiobarbituric acid reactive substances[17] (TBARS). Another portion of the tissues was homogenized with phosphate buffer saline and used for the estimation of reduced Glutathione[18] (GSH), Superoxide dismutase[19] (SOD), Catalase[20] (CAT), and Glutathione peroxidase[21] (GPx), Glutathione reductase[22] (GR).

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 illustrates the activities of tissues TBARS levels in HFD rats. TBARS levels were increased 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 reduced the levels of TBARS, in rats administered with methanolic extract of *Ipomoea digitata* along with HFD.

Table 1: Effect of methanolic extract of *Ipomoea digitata* on tissues 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	22.46 ± 0.47b*	40.28 ± 0.26b*	17.61 ± 0.16b*	4.59 ± 0.15b*	8.60 ± 0.08 b*	5.50 ±0.09 b**
Group-II	76.64 ± 0.18a*	80.94 ± 0.31a*	65.31 ± 0.27a*	1.92 ± 0.05a*	4.31 ±0.08 a*	3.12 ± 0.04 a*
Group-III	34.24 ± 0.29a**,b*	50.04 ±0.24 a*,b*	27.88 ± 0.18 a*,b*	3.66 ± 0.10a**,b*	6.31 ± 0.07a*,b*	4.99 ± 0.05 a*,b*
Group-IV	28.23 ± 0.20 a*,b*	44.02 ± 0.23 b*	17.99 ± 0.23 a*,b*	4.26 ± 0.03a*,b*	7.37 ±0.06b*	5.44 ± 0.09 a*,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. : b →group II compared with groups III, IV.

Group I : standard chow diet. (Control) : Group II : High Fat Diet.
Group III : High fat diet + Methanol extract of *Ipomoea digitata* (300mg/kg B.wt)
Group IV : High fat diet + standard drug atorvastatin (1.2 mg/kg B.wt)

Glutathione, an endogenous antioxidant defense, is found in liver at high concentration. It plays a central role in the defense against free radicals, peroxides and a wide range of xenobiotics and carcinogens[23]. Table 1 demonstrates the levels of Glutathione (GSH) in HFD rats. The significant fall in the levels of tissues Glutathione were observed in high fat diet rats (group II) as compared to the control rats (group I). Administration of methanolic extract of *Ipomoea digitata* along with HFD rats substantially enhanced the levels of glutathione when compared with HFD rats (group II).

Table 2 shows that the effect of methanolic extract 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[24] and the accumulation of O_2^- and H_2O_2 which in turn forms hydroxyl radicals[25].After administration of methanolic extract of *Ipomoea digitata* along with HFD significantly increases the activities of SOD and CAT in tissues of rats when compared with high fat diet rats (group II).

Table 2: Effect of methanolic extract of *Ipomoea digitata* 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	3.46 ± 0.06 b*	1.77 ±0.03 b*	2.44 ±0.11b*	27.96 ± 0.18 b*	46.09 ± 0.19b*	31.26 ± 0.16 b*
Group-II	1.59 ± 0.08 a*	0.80 ± 0.03 a*	1.53 ± 0.02 a*	15.73 ± 0.10 a*	30.98 ± 0.22 a*	21.38 ± 0.31 a*
Group-III	3.06 ±0.03 a*,b*	1.42 ±0.02 a*,b*	2.25 ± 0.05 a*,b*	23.88 ± 0.15 a**,b*	43.78 ±0.15 a**,b*	26.52 ± 0.19 a*,b*
Group-IV	3.64 ±0.03 b*	1.72 ±0.03 a*,b*	2.62 ± 0.01 a*,b*	27.29 ±0.18 b*	45.40 ±0.16 a*,b*	29.97±0.07 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: b → group II compared with groups III, IV.
Details of group I-IV are same as in Table 1.

The activities of tissues glutathione peroxidase (GPx) and glutathione reductase (GR) in HFD rats were presented in Tables 3. Tissues glutathione peroxidase and reductase levels were significantly decremented in rats fed with HFD (group II) as compared to the control rats (group I). High fat diet decreased the ratio of oxidized glutathione/ reduced glutathione in tissue[26]. Administration of methanolic extract *Ipomoea digitata* along with the HFD enhanced the levels of glutathione peroxidase and glutathione reductase in all the tissues as compared with HFD rats. A standard drug atorvastatin administered rats also showed elevated level of glutathione peroxidase and glutathione reductase.

Table 3: Effect of methanolic extract of *Ipomoea digitata* 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	8.58 ± 0.10b*	14.80 ± 0.20b*	13.18 ± 0.11b*	1.56 ± 0.04 b*	2.90 ± 0.10 b*	1.84 ± 0.03 b*
Group-II	5.38 ± 0.07a*	7.55 ± 0.07a*	7.01 ± 0.03a*	0.73 ± 0.01 a*	1.47 ± 0.05 a*	0.85 ± 0.03 a*
Group-III	8.16 ± 0.03a*,b*	13.38 ± 0.12 a**,b*	11.96 ± 0.07 a**,b*	1.20 ± 0.02 a*,b*	2.24 ± 0.02 a*,b*	1.35 ± 0.02 a*,b*
Group-IV	8.55 ±0.10 a*,b*	14.36 ±0.10 a*,b*	13.18 ± 0.05 a*,b*	1.47 ± 0.02 a**,b*	2.80 ± 0.04 a*,b*	1.72 ± 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.
b →group II compared with groups III, IV. Details of group I-IV are same as in Table 1.

CONCLUSION

The results of the above study clearly demonstrated that the methanolic extract of *Ipomoea digitata* had significant *in vivo* antioxidant and lipid peroxidation activity. These *in vivo* study indicate that this plant extracts is a significant source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses. Therefore, further investigations need to be carried out to isolate and identify the antioxidant compounds present in the plant extract.

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