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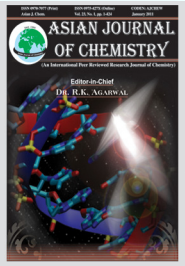

In vivo Antioxidant and Lipid Peroxidation Effect of Various Extracts of Whole Plant of *Mucuna pruriens* (Linn) in Rat Fed With High Fat Diet

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The present study was conducted to evaluate the *in vivo* antioxidant and lipid peroxidation effect of various extracts of whole plant of *Mucuna pruriens* (Linn). High fat diet rats showed significant decreased activities of tissue enzymatic and non enzymatic antioxidant. High fat diet induces the oxidative stress in cell by producing reactive oxygen species. Antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), non enzymatic antioxidant glutathione (GSH) and thiobarbituric acid reactive substance levels were increased in the administration of methanolic extract of *Mucuna pruriens* in high fat diet rats when compared to other two extracts. Hence it is concluded that methanolic extract of *Mucuna pruriens* were found to have protective action against high fat diet induced oxidative stress in different tissues in rats.

Key Words: High fat diet, *Mucuna pruriens*, Antioxidant activity, Rats.

INTRODUCTION

A common theme which underlies etiology of several degenerative disorders is free radical stress. The production of free radicals is inextricably linked to the inflammatory process. Free radicals prime the immune response, recruit inflammatory cells and are innately bactericidal[1,2].

Reactive oxygen and nitrogen species play key roles in normal physiological processes, including cellular life/death processes, protection from pathogens, various cellular signaling pathways and regulation of vascular tone[3]. Oxidative stress is caused by an insufficient capacity of biological systems to neutralize excessive free radical production, which can contribute to human diseases and ageing[4], including cardiovascular disease[5], neurodegenerative disease and age-related cognitive decline[6], obesity and insulin resistance[7], as well as immune system dysfunction[8]. Oxidative stress also contributes to the accumulation of damaged macromolecules and organelles, including mitochondria[6,9]. Ethnomedical literature contains a large number of plants that can be used against diseases, in which reactive oxygen species and free radical play important role. There is a plethora of plants that have been found to possess strong antioxidant activity[10]. Recent reports indicate that there is an inverse relationship between the dietary intake of antioxidant-rich foods and the incidence of human diseases[11]. 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.

Mucuna pruriens Linn belongs to the family fabaceae, commonly known as cowhage plant or kapikacho or kevach in Hindi, is the most popular drug in Ayurvedic system of medicine[12]. Traditionally, in India, the seeds of *Mucuna pruriens* are used as a tonic and aphrodisiac for male virility. It has been reported to be antidiabetic[13]. Its different preparations (from seeds) are used for the management of several free radical-mediated diseases such as ageing, rheumatoid arthritis, diabetes, atherosclerosis, male infertility and nervous disorders. It is also used as an aphrodisiac and in the management of Parkinsonism, as it is a good source of L-dopa[14]. The antiepileptic and antineoplastic activity of methanol extract of *Mucuna pruriens* has been reported[15]. It had been reported analgesic and antiinflammatory[16]. It is also used as a fertility agent in men[17].

However, so far no study has been reported to evaluation of *in vivo* antioxidant and lipid peroxidation effect of the whole plant of *Mucuna pruriens* (Linn.). Hence the objective of the present research work is to investigate the *in vivo* antioxidant and lipid peroxidation effect of various extracts of the whole plant of *Mucuna pruriens* (Linn) in rat fed with high fat diet.

EXPERIMENTAL

The whole plant of *Mucuna pruriens* (Linn), were collected from Neiyur dam, Kanyakumari 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 *Mucuna pruriens* (Linn), 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[18] 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 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[19].

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) : standard chow diet. Group II: high fat diet. Group III: High fat diet + Pet. ether extract of *Mucuna pruriens* (200 mg/kg B.wt). Group IV: High fat diet + ethyl acetate extract of *Mucua pruriens* (200 mg/kg B.wt). Group V : High fat diet + methanol extract of *Mucuna pruriens* (200 mg/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[20]:

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 and 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 and choline mixture 0.5 %, cholesterol 0.4 %.

Rats of groups III, IV and V were orally fed with the various extracts of *Mucuna pruriens* (pet. ether ethyl acetate and methanol) and rats of group VI were fed with standard drug atorvastatin. Both the *Mucuna pruriens* 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*.[21]. It was used for the estimation of thiobarbituric acid reactive substances[22] (TBARS). Another portion of the tissues was homogenized with phosphate buffer saline and used for the estimation of reduced glutathione[23] (GSH), glutathione peroxidase[24] (GPx), glutathione reductase[25] (GR), catalase[26] (CAT) and superoxide dismutase[27] (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 activities of TBARS 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 significant decline in the level of TBARS, in rats administered with methanolic extract of *Mucuna pruriens* along with HFD when compared with other two extracts.

Glutathione (GSH), a tripeptide present in all the cells is an important antioxidant[28]. GSH also functions as free radical scavenger in the repair of radical caused biological damage[29]. The activities of glutathione (GSH) in HFD rats are shown in Table-1. 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 *Mucuna pruriens* along with HFD rats increased the levels of glutathione when compared with other two extracts.

The activities of SOD and CAT in HFD rats are shown in Table-2. 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[30] and the accumulation of O_2^- and H_2O_2 which in turn forms hydroxyl

TABLE-1
EFFECT OF VARIOUS EXTRACTS OF *Mucuna pruriens* 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	43.12 ± 4.21a**,b**	59.12 ± 3.48a**,b*	31.82 ± 3.41a**,b*	3.62 ± 0.19a**,b**	5.93 ± 0.42a**,b**	4.21 ± 0.28a**,b**
Group IV	39.52 ± 5.16a**,b*	52.33 ± 4.21a**,b*	29.63 ± 2.81a**,b*	3.73 ± 0.24a**,b**	6.25 ± 0.31a**,b*	4.92 ± 0.16a**,b*
Group V	31.18 ± 4.96 a*,b*	49.29 ± 3.82 a*,b*	26.49 ± 3.54a*,b*	3.94 ± 0.13 b*	6.87 ± 0.28b*	5.12 ± 0.08 b*
Group VI	24.15 ± 1.09 a*,b*	41.92 ± 2.86 a*,b*	16.97 ± 1.49a*,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. Group I: standard chow diet. (control). Group II: High fat diet. Group III: High fat diet + Pet. ether extract of *Mucuna pruriens* (200 mg/kg B.wt). Group IV: High fat diet + ethyl acetate extract of *Mucuna pruriens* (200 mg/kg B.wt). Group V: High fat diet + methanol extract of *Mucuna pruriens* (200 mg/kg B.wt), Group VI: High fat diet + standard drug atorvastatin (1.2 mg/kg B.wt).

TABLE-2
EFFECT OF VARIOUS EXTRACTS OF *Mucuna pruriens* 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 ± 0.23b*	1.76 ± 0.13b*	2.86 ± 0.19b*	28.43 ± 1.19b*	47.43 ± 3.82b*	30.89 ± 2.64b*
Group II	1.73 ± 0.24a*	0.84 ± 0.06a*	1.59 ± 0.12a*	16.89 ± 1.56a*	31.24 ± 1.64a*	21.34 ± 2.09a*
Group III	2.48 ± 0.19a**,b**	1.21 ± 0.09a**,b**	2.31 ± 0.18a**,b*	21.27 ± 2.41a**,b**	39.84 ± 3.61a**,b*	25.16 ± 1.94a**,b**
Group IV	2.61 ± 0.29a**,b*	1.38 ± 0.17a**,b*	2.42 ± 0.24a**,b**	23.18 ± 2.09a**,b*	42.12 ± 2.91a**,b*	26.72 ± 2.01a**,b*
Group V	3.18 ± 0.31a*,b*	1.52 ± 0.12a*,b*	2.61 ± 0.19a*,b*	25.21 ± 1.51a*,b*	45.31 ± 2.34a*,b*	28.16 ± 1.93a*,b*
Group VI	3.70 ± 0.19a*,b*	1.77 ± 0.18a*,b*	2.87 ± 0.16a*,b*	29.14 ± 1.63a*,b*	48.13 ± 2.94a*,b*	31.14 ± 2.93a*,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.

TABLE-3
EFFECT OF VARIOUS EXTRACTS OF *Mucuna pruriens* 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 ± 0.19b*	1.76 ± 0.16b*
Group II	5.36 ± 0.49a*	7.16 ± 0.51a*	6.92 ± 0.08a*	0.69 ± 0.08 a*	1.36 ± 0.07a*	0.82 ± 0.09a*
Group III	7.42 ± 0.65a**,b**	12.41 ± 0.32a**,b**	10.21 ± 0.13a**,b**	1.13 ± 0.03a**,b**	2.11 ± 0.03a**,b**	1.26 ± 0.11a**,b**
Group IV	7.89 ± 0.52a**,b*	12.89 ± 0.47a**,b*	11.56 ± 0.10a**,b*	1.19 ± 0.07a**,b*	2.23 ± 0.11a**,b*	1.37 ± 0.13a**,b*
Group V	8.23 ± 0.49a*,b*	13.62 ± 0.27a*,b*	12± 3.54a*,b*	1.23 ± 0.11a*,b*	2.34 ± 0.10a*,b*	1.44 ± 0.08a*,b*
Group VI	8.80 ± 0.59a*,b*	14.70 ± 0.38a*,b*	16.97 ± 1.49a*,b*	1.42 ± 0.12a*,b*	2.79 ± 0.15a*,b*	1.79 ± 0.14a*,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.

radicals[31]. Catalase decomposes hydrogen peroxide and helps protect the tissues from highly reactive hydroxyl radicals[32]. After administration of methanolic extract of *Mucuna pruriens* along with HFD significantly increases the activities of SOD and CAT in tissues of rats when compared with other extracts.

The activities of glutathione peroxidase and glutathione reductase in HFD rats are shown in Tables-3. The activities 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[33]. Administration of methanolic extract of *Mucuna pruriens* 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

Because free radicals are implicated in many diseases and age-related conditions, the antioxidant dependent actions of methanolic extract of *Mucuna pruriens* may have a wide range of beneficial effects. Therefore, further investigations need to be carried out to isolate and identify the antioxidant compounds present in the plant extract.

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