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Antioxidant activity of flavonoids isolated from *Dolichos biflorus* Linn. in rabbits fed high fat diet

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ABSTRACT

Flavonoids from *Dolichos biflorus* Linn. were isolated and screened for antioxidant activity on rabbits fed high fat diet (HFD). Methanolic extract of *Dolichos biflorus* was fractionated with hexane, chloroform, ethylacetate and methanol. Ethylacetate fraction which showed maximum flavonoid yield, were fractionated by column chromatography and estimated for flavonoids (total phenol) content. High fat diet rabbits 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, Catalase, Glutathione peroxidase, Glutathione-s-transferase, and non enzymatic antioxidant Glutathione showed enhanced activities on administration of isolated flavonoids from *Dolichos biflorus* in high fat diet rabbits. Hence it is concluded that flavonoids isolated from *Dolichos biflorus* were found to have protective action against high fat diet induced oxidative stress in different tissues in rabbits.

Key words : Antioxidant activity , *Dolichos biflorus*, flavonoids, high fat diet, rabbits

INTRODUCTION

Medicinal herbs are playing a key role in human health care. India is a gold mine of well recorded and traditionally well practiced knowledge of herbal medicine. These herbals therefore are considered to be useful means to prevent atherosclerosis. Among these herbal resources, the *Dolichos biflorus* Linn (horsegram) belonging to the family Fabaceae has been chosen for the present study. Hyperlipidemia is one of the risk factors for coronary heart disease[1]. Recently intake of flavonoids was shown to be inversely related to coronary heart disease mortality[2]. In traditional medicine, *Dolichos biflorus* is used for various diseases like antitumor [3], expectorant[4], abortion [5] and menstrural problems[6]. A high fat diet induces oxidative stress in the cell by producing reactive oxygen species[7]. In this study, the influence of the flavonoids rich fraction from *Dolichos biflorus* on high fat diet induced oxidative stress in rabbits has been investigated.

MATERIALS AND METHODS

Whole plants of *D.biflorus* were collected from Sankaran coil, Tirunelveli district of Tamilnadu, India and authenticated by the Botanical Survey of Medicinal Plant Unit, Siddha, Government of India, Palayamkottai, Tamilnadu. The plant material was pulverized by a mechanical grinder and passed through a 40 mesh sieve. The powdered materials were subjected to methanolic extraction by hot continuous percolation method in soxhlet apparatus [8] for 24 hours. After filtration through whatman filter paper no 40, the filtrate was evaporated to dryness in vaccum at 35^{0}C to 40^{0}C. The methanolic extract was fractionized with hexane, chloroform, ethyl acetate and methanol. These extracts were evaporated in vaccum and the percentage yield was

determined. Maximum yield was obtained from ethyl acetate fraction. This fraction was used for column chromatographic separation.

20g of Ethyl acetate fraction of methanolic extract *Dolichos biflorus* was adsorbed on slica gel [20g] and transferred to a column of silica gel equilibrated with hexane. Elution was performed with hexane, hexane: chloroform (90:10), hexane: chloroform (75:25), hexane: chloroform (50:50), hexane: chloroform (25:75), chloroform (100), chloroform: ethylacetate (75:25), chloroform : ethylacetate (50:50), chloroform : ethylacetate (25:75), ethyl acetate (100), ethyl acetate: methanol (75:25), ethyl acetate: methanol (50:50), ethyl acetate: methanol (25:75) and methanol (100). The flavonoid content was estimated [9] in all the fractions. Some solvent systems fractions gave rich flavonoids content are shown in Table 1. These fractions were filtered and concentrated by removing the solvent, mixed and used in the experiment.

Newzealand white rabbits of weighing 900-1100g were procured from the Central Animal House, Rajah Muthiah Medical College & Hospital, Annamalai University. The animals were kept in cages, 2 per cage, with12:12 hr light and dark cycle at 25º ± 2ºC.The animals were maintained on their respective diets and water *ad libitum*. Animals were divided into following 3 groups of 6 animals each:

Group I (Control) : Standard chow diet
Group II : High fat diet
Group III : High fat diet plus flavonoids from *D.biflorus* (10mg/kg body weight/ day)

Table 1: Yield of flavonoids from *Dolichos biflorus* in different solvent systems

Solvent systems	Flavonoids [Total phenol) S.E* (mg/g)
Chloroform : Ethyl acetate (75:25)	5.19 ± 0.04
Chloroform : Ethyl acetate (50:50)	10.23 ± 0.07
Chloroform : Ethyl acetate (25:75)	55.35 ± 0.34
Ethyl acetate (100%)	611.84 ± 5.80
Ethyl acetate : Methanol (75:25)	370.72 ± 3.14
Ethyl acetate : Methanol (50:50)	10.20 ± 0.06

* Average of three determinations

The compositions of the two diets were as follows:

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%.

The flavonoids from *Dolichos biflorus* were suspended in 2% tween 80 fed to the group III rabbits by oral intubation in addition to their respective diets. At the end of 9 weeks all the animals were sacrificed by cervical decapitation after overnight fasting. Animals were given enough care as per the Animal Ethical Committee's recommendations. (approval number - 154 -160/ 1999/CPCSEA) Portion of the tissues like liver, heart and aorta were homogenized with phosphate buffer saline and used for the estimation of Superoxide dismutase (SOD)[10], Catalase (CAT[11], Glutathione peroxidase (GPX) [12], Glutathione – s – transferase (GST)[13] and Glutathione[14].

Results were expressed as mean ± SE of 6 rabbits in each group. One way analysis of

variance (ANOVA) with Scheffe's multiple comparisons test were used to determine the statistical significance. P< 0.05 was considered significant.

RESULTS AND DISCUSSION

All the fractions were collected in tubes and flavonoid contents were estimated. Some solvent systems fractions gave rich flavonoids contents are shown in Table 1. Other solvent system fractions were found to have negligible amount of flavonoids. Flavonoids fractions were filtered and concentrated by removing the solvent, mixed and used in the experiment.

The average body weight change and weight gain are shown in Table 2. The average body weight

Table - 2: Effect of flavonoids from *D.biflorus* on HFD fed rabbits body weight changes

Groups	[Values are mean ± SE of 6 rabbits] Average weight per group Initial Weight (g)	Final Weight(g)	Weight gain (g)
Group I	953.33±1.5	1057.16±2.66	104.33 ± 2.51
Group II	950.16±2.19a^{NS}	1120.01±0.56a*	169.66 ± 2.45
Group III	956.16±1.68a^{NS},b^{NS}	1097.58±1.26a*,b*	141.33 ± 2.57
P values	: *< 0.001, **< 0.05		
NS	: Non Significant		
Group I	: Control		
Group II	: High Fat Diet (HFD)		
Group III	: HFD + Flavonoid from *Dolichos biflorus* (10 mg/kg body wt/day)		

a → group I compared with groups II, III.
b → group II compared with group III.

Table - 3: Effect of flavonoids from *D.biflorus* on tissues SOD and CAT in HFD rabbits

Groups	[Values are mean (SE of 6 rabbits] SOD (unit min/mg/protein) Liver	Heart	Aorta	CAT ((moles of H2O2 consumed min/mg/protein) Liver	Heart	Aorta
Group I	11.72 (0.5	14.36 (0.24	21.07 (0.07	22.98 (0.07	14.08 (0.18	13.05 (0.25)
Group II	7.30 (0.19a*	6.39 (0.15a*	11.03 (0.11a*	12.69 (0.15a*	8.22 (0.7a*	6.35 (0.11a*
Group III	10.56 ±0.14a**,b*	12.61 ±0.18a**,b*	20.39 ±0.17	18.80 ±0.164a*,b*	11.00 ±0.20a*,b*	10.38 ±0.15a*,b
P value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
F ratio	170.06	548.40	1599.20	1868.20	312.23	319.16
p values	: *< 0.001, ** < 0.05					
NS	: Non Significant					

Details of groups I – III are same as in Table 2.
a → group I compared with groups II, III
b → group II compared with groups I, III

gain increased in HFD rabbits (169.66g) compared with control rabbits (104.33g). Administration of flavonoids from *D.biflorus* were found to decrease the weight gain (141.33g).

Effect of flavonoids from *Dolichos biflorus* on liver, heart and aorta SOD and CAT activities results is shown in Table 3.

The results indicated that activities of both superoxide dismutase (SOD) and catalase (CAT) were significantly reduced in HFD rabbits (group II) when compared with control rabbits (group I). High fat diet can cause the formation of toxic intermediates that can inhibit the activity of antioxidant enzymes [15] and the accumulation of O_2^- and H_2O_2 which in turn forms hydroxyl radicals[16]. Treatment of flavonoids from *Dolichos biflorus* improved the activities of SOD and CAT in HFD fed rabbits (group III) when compared with HFD rabbits (group II). The improved enzyme activities might be due to the removal of toxic free radical species (or) might be due to the direct activation of SOD and CAT enzymes by the flavonids obtained from *D.biflorus*.

Effect of flavonoids from *Dolichos biflorus* on tissues glutathione peroxidase (GPx) and Glutathione-s-transferase activities results is shown in Table 4.

Table - 4: Effect of flavonoids from *D. biflorus* on tissues glutathione peroxidase and glutathione s-transferase in HFD rabbits

Groups	[Values are mean ± SE of 6 rabbits] Glutathione Peroxidase (GPx) (mg of GSH consumed/ min/ mg/ protein)			Glutathione S-transferase (GST)mmole of CDNB – GSH – conjugate to min/mg/protein)		
	Liver	Heart	Aorta	Liver	Heart	Aorta
Group I	14.51 ±0.15	15.89 ±0.20	21.80 ±0.16	24.91 ±0.14	18.83 ±0.18	16.95 ±0.19
Group II	7.73 ±0.19a*	8.55 ±0.16a*	11.84 ±0.27a*	10.61 ±0.19a*	10.81 ±0.24a*	9.01 ±0.16a*
Group III	11.60 ±0.18a*,b*	12.01 ±0.17a*,b*	16.13 ±0.22a*,b*	16.49 ±0.24a*,b*	15.20 ±0.18a*,b*	12.54 ±0.06a*,b*
P value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
F ratio	294.10	271.12	754.05	1415.18	518.68	390.71
p values	: *< 0.001, ** < 0.05					
NS	: Non Significant					

Details of groups I – III are same as in Table 2.
a → group I compared with groups II, III b → group II compared with groups I, III

The results indicated that the activities of glutathione peroxidase (GPx) and Glutathione-S-transferase were significantly decreased in tissues such as liver, heart and aorta of rabbits fed HFD (group II) in comparison to control rabbits (group I). High fat diet decreased the ratio of oxidixed glutathione/reduced glutathione in tissue[17]. Administration of flavonoids from *Dolichos biflorus* in high fat diet rabbits restored the activities of Glutathione peroxidase and glutathione-s-transferase in the tissues when compared with rabbits fed HFD rabbits [group II]. This effect might be due to detoxification of H_2O_2 and reduction of a variety of hydroperoxides such as phospholipid hydroperoxides, fatty acid hydroperoxides by flavonoids.

Effect of flavonoids from *Dolichos biflorus* on liver, heart and aorta of glutathione content in rabbits fed HFD results are shown in Table 5.

Table 5 : Effect of flavonoids from *D. biflorus* on tissues glutathione in HFD rabbits

Group	[Values are mean ± SE of 6 rabbits] Glutathione (mg/g tissue)		
	Liver	**Heart**	**Aorta**
Group I	17.23 ± 0.20	19.81 ± 0.39	14.54 ± 0.14
Group II	7.67 ± 0.21a*	11.23 ± 0.14a*	8.37 ± 0.13a*
Group III	14.93 ± 0.24a*,b*	17.84 ± 0.17a*,b*	11.9 ± 0.28a*,b*
P value	<0.001	<0.001	<0.001
F ratio	6424.2	255.92	212.38
p values	: *< 0.001, ** < 0.05		
NS	: Non Significant		

Details of groups I – III are same as in Table 2.
a → group I compared with groups II, III b → group II compared with groups I, III

The activities of glutathione concentration in tissues were significantly decreased in high fat diet rabbits [group II] as compared to the control rabbits [group I]. Significant decrease of the GSH in high fat diet group suggests the enhanced oxidative stress in hyperlipidemic state as reported by the earlier studies[18]. Treatment of flavonoids from *Dolichos biflorus* in high fat diet fed rabbits significantly increased the GSH levels. The increased GSH concentration may be due to enhanced the synthesis of glutathione. Hence it is concluded that administration of flavonoids from *Dolichos biflorus* manifests protective action against high fat diet induced oxidative stress in different tissues like liver, heart and aorta in rabbits.

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