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Evaluation of *In Vivo* Antioxidant and Lipid Peroxidation Activities of Different Extracts of Aerial Parts of *Pavetta indica* (Linn)

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Abstract : The present investigation was to evaluate the *in vivo* antioxidant and lipid peroxidation effect of different extracts of aerial parts of *Pavetta indica* (Linn). High fat diet rats showed significantly (P<0.001) reduction the levels of tissues enzymatic antioxidant and non enzymatic antioxidant and incresed the level of Thiobarbuuric acid reactive substance. The level of thiobarbuuric acid reactive substance are elevated in HFD rats (group II) when compared with control group. Treatment of methanol extract of *Pavetta indica* in high fat diet rats were showed significantly (p<0.001) increment the levels of Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPx), Glutathione reductase (GR),Glutathione S transferees (GST) and non enzymatic antioxidant Glutathione (GSH) when compared with HFD rats (Group II). The methanolic extract of *P. indica* in high fat diet rats were found lowered the concentration of TBARS when compared with HFD rats (Group II). In comparison of all the three extract treated group with standard group, the methanol extract of *Pavetta indica was* showed significant result than that of other extracts treated groups. The methanol extract of *Pavetta indica* is a significant source of natural antioxidant, which might be useful in preventing the progress of different oxidative stresses.
Key words : *Pavetta indica ,* High fat diet, Lipid peroxidation, Antioxidant.

Introduction

Antioxidant act as a defence mechanism that orotect against deleterious effects of oxidative reaction produced by reactive oxygen species in a biological system[1].Reactive oxygen species not only are produced naturally in cell following stress or respiration but also have been reported to be formed by radiation. Over production of ROS and inadequate antioxidant has been implicated in the pathogenesis and complication of some disease conditions like diabetes, Alzheimers disease, cancer, atherosclerosis, arthritis, neurodegenerative disease and aging process[2]. Oxidation is necessary in many living cells for the construction of energy to fuel biological processes. But, the uncontrolled construction of oxygen derived free radicals is involved in the start of many diseases such as atherosclerosis, rheumatoid arthritis, cancer and aging[3]. Almost all organisms are well protected against free radical damage by enzymes such as SOD and CAT, or compounds such as ascorbic acid, tocopherols and glutathione[4]. When the mechanism of antioxidant protection becomes unbalanced by factors such as aging, deterioration of physiological functions may occur resulting in diseases and accelerated aging.

However, the antioxidants present in human diet are of great interest as possible protective agents to help the human bodies reduce oxidative damage.

Pavetta indica Linn. belongs to the family Rubiaceae.The entire plant used as a bitter tonic, diuretic, inflammation, rheumatism, jaundice and ulcer[5]. The decoction of the leaves are used to relieve haemorrhoidal pain, treatment of analgesic, antipyretic, appetizer and the ulceration of mouth[6,7]. This plant is used as antibacterial, antiviral and antimalarial[8]. *P. indica* leaves are used in the treatment of liver disease, pain from piles, urinary diseases and fever[9] and used as antiinflammatory activities[10], analgesic activity[11] and antidiabetic activity[12].*P.indica* leaves extracts are used as antimicrobial activity[13]. Its root extracts are used diuretic and purgative activity[14]. Leaves are used as a lotion for ulcerated nose and for heamorrhoids[15]. Root is used for anticephalagic. Wood is used as antirheumatic. Fruits are used as anthelmintic[16,17]. However, no data are available in the literature on the antioxidant activity of aerial parts of *Pavetta indica* (Linn). Hence, the aim of the present investigation was to evaluate the *in vivo* antioxidant and lipid peroxidation effect of different extract of aerial parts of *Pavetta indica* (Linn).

Materials and Methods

Collection and Identification of Plant materials

The aerial parts of *P. indica* (Linn), were gathered form kalakkadu, Tirunelveli District , Tamil Nadu, India. Taxonomic identification was made from Botanical Survey of Medical Plants Unit Siddha, Government of India. Palayamkottai. The aerial parts of *Pavetta indica* (Linn), were dried under shade, segregated, pulverized by a mechanical grinder and passed through a 40 mesh sieve.

Extractions

The above plant materials were progressively extracted with Pet.ether (40-60^{0}C) by hot continuous percolation method in Soxhlet apparatus[18] for 24 hrs. Then the marc was subjected to ethyl acetate (76-78^{0}C) for 24 hrs and then mark was subjected to Methanol for 24 hrs. The extracts were concentrated by use 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 17-19 weeks age, weighing 150-175g were obtained from the Animal House, Nizam Institute of Pharmacy & Research centre, Near Ramoji Film City, Deshmukhi, Hyderabad, A.P. India. The rats were set aside in cages, 2 per cage, with 12:12 hr light and dark cycle at 25^0±2^{0}C. The rats were maintained on their particular diets and water *ad libitum.* Animal Ethical Committee's clearance was obtained for the present investigation.

Experimental Design

Rats were divided into following 6 groups of 6 rats each:

Group I : Standard chow diet
Group II : High Fat Diet(HFD)
Group III : HFD plus Pet.ether extract of *P. indica* (Linn) (200mg/kg B.wt)
Group IV : HFD plus ethyl acetate extract of *P. indica* (Linn) (200mg/kg B.wt)
Group V : HFD plus methanolic extract of *P. indica* (Linn) (200mg/kg B.wt)
Group VI : HFD plus standard drug atorvastatin (1.2 mg/kg B.wt)

Animal diet

The ingredients of the two diets were as follows[20] .

Normal diet:

Wheat flour 22.5%, powder of simmered bengal gram 60%,powder of skimmed milk 5%, casein powder 4%, refined oil 4%, starch with blended salt 4% and vitamin and choline blend 0.5%. ***High fat diet:*** normal diet with coconut oil 9% and cholesterol 0.4%.

Assessment of *in vivo* antioxidant and lipid peroxidation

Treatment group III, IV and V rats were orally fed with the different extracts of *Pavetta indica* and group VI rats were fed with atorvastatin. All the extracts and standard medication were suspended in 2% tween 80 alone and fed to the individual rats by oral intubation. The study end of 63 days all the animals were sacrificed by cervical dislocation after overnight fasting. Liver, heart and aorta were cleared of adhering fat, weighed exactly and used for the preparation of homogenate. Parts of the tissues from liver, heart and aorta were blotted, weighed and homogenized with methanol (3 volumes). The lipid extract was extracted by the method of Folch et al.(1957)[21]. It was used for the estimation of thiobarbituric acid reactive substances (TBARS)[22] . Another piece of the tissues was homogenized with phosphate buffer saline and utilized for the estimation of reduced Glutathione (GSH)[23] , Superoxide dismutase (SOD)[24] , Catalase (CAT)[25], and Glutathione Peroxidase (GPx)[26], Glutathione Reductase (GR) [27].

Statistical analysis

Results were mentioned as mean ± SE of 6 rats in each group. The statistical significance among the groups was determined by using one way analysis of variance (ANOVA), followed by Dunnet's multiple comparison test. Significance level was set at 0.05.

Results and Discussion

As shown in Table 1 and 2. The TBARS and conjugated dienes levels were increment in liver, heart and aorta in IIgroup rats are a clear reasonable sign of too much formation of free radical and beginning of lipid peroxidation. The HFD is known to induce oxidative stress in the cells by producing ROS[28]. This results in increased lipid peroxidation leading to elevated concentration of TBARS and conjugated dienes[29]. The significantly reduced the level of Thiobarbuituric reactive substance and conjugated dienes in rats administered with methanol extract of *P.indica* to that of high fat diet rats (II group). This activity may possibly presence of phytoconstituents, flavonoids in the *Pavetta indica* .

Table 1. Activity of different extracts of aerial parts of *P. indica* on tissues TBARS in HFD rats

Groups	**TBARS (n mol of MDA formed/g tissue)**		
	Liver	Heart	Aorta
Group I	$24.80 \pm 0.25^{b*}$	$45.65 \pm 0.21^{b*}$	17.76 ± 0.54b*
Group II	$76.87 \pm 0.43^{a*}$	$85.52 \pm 0.32^{a*}$	$64.22 \pm 0.12^{a*}$
Group III	$70.28 \pm 0.20^{a**,b*}$	$80.26 \pm 0.20^{a**,b**}$	$58.05 \pm 0.21^{a**,b*}$
Group IV	$39.97 \pm 0.13^{a*,b*}$	$65.95 \pm 0.27^{a**,b**}$	$41.07 \pm 0.43^{a*,b**}$
Group V	$32.06 \pm 0.22^{a**,b*}$	$45.10 \pm 0.14^{a*,b**}$	$24.07 \pm 0.18^{a**,b*}$
Group VI	$27.42 \pm 0.31^{a*,b*}$	$43.61 \pm 0.16^{b*}$	$19.65 \pm 0.73^{a*,b*}$

Values are given as mean ± SE (n=6 rats)
P values : $^{*} < 0.001$, $^{**} < 0.05$
a → groups II, III, IV, V & VI compared with group I.
b →groups I, III, IV, V & VI compared with group II.
I group : standard chow pellet. (Control)
II group : High Fat Diet.
III group : HFD + PE extract of *P. indica* (200mg/kg B.wt)
IV group : HFD + EA extract of *P. indica* (200mg/kg B.wt)
V group : HFD + Methanol extract of *P. indica* (200mg/kg B.wt)
VI Group : HFD + Atorvastatin (1.2 mg/kg B.wt)

Table 2. Activity of different extract of aerial parts of *Pavetta indica* on tissues conjugated diene in HFD rats

Groups	Conjugated diene (μ moles /g tissue)		
	Liver	Heart	Aorta
Group I	174.82 ± 0.21[b*]	164.68 ± 0.12[b*]	471.65 ± 0.11b*
Group II	290.44 ± 0.15[a*]	270.43 ± 0.34[a*]	736.45 ± 0.11[a*]
Group III	280.90 ± 0.75[a**,b*]	254.75 ±0.38[a**,b**]	720.38 ± 0.31[a**,b*]
Group IV	241.71 ± 0.26[a*,b*]	231.41 ± 0.37[a**,b**]	658.76 ±0.41[a*,b**]
Group V	195.88 ± 0.42[a**,b*]	179.75 ± 0.42[a*, b**]	480.84 ± 0.59[a**,b*]
Group VI	187.26 ± 0.34[a*,b*]	170.66 ± 0.16[b*]	469.34 ± 0.23[a*,b*]

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

The activity of different extracts of *Pavetta indica* on tissues superoxide dismutase and catalase enzyme levels in HFD rats were presented in Table 3 and 4. The activities of superoxide dismutase and catalase in the tissue like liver, heart and aorta significantly (P<0.001) reduced in II groups of animal. HFD can origin the development of toxic intermediates that can slow down the activity of antioxidant enzymes[30] and the gathering of O_2^- and H_2O_2 which in turn forms OH radicals[31] . Catalase decomposes hydrogen peroxide and helps to protect the tissues from highly reactive OH radicals. Treatment of with methanol extract of *P. indica (Linn)* along with HFD significantly enhanced the activities of SOD and CAT in tissues of rats to that of other extracts treated groups.

Table 3. Activity of different extracts aerial parts of *Pavetta indica* on tissues SOD in HFD rats

Groups	Superoxide dismutase (unit min/mg protein)		
	Liver	Heart	Aorta
Group I	3.74 ± 0.04[b*]	1.86 ± 0.03[b*]	2.88 ± 0.01b*
Group II	1.76 ± 0.02[a*]	0.77 ± 0.02[a*]	1.47 ± 0.02[a*]
Group III	1.97 ± 0.02[a**,b*]	0.96 ±0.02[a**,b**]	1.65 ± 0.02[a*,b*]
Group IV	2.38 ± 0.06[a*,b*]	1.25 ± 0.02[a**,b**]	2.32 ±0.04[a*,b*]
Group V	3.42 ± 0.06[a**,b*]	1.68 ± 0.02[a*, b**]	2.70 ± 0.02[a**,b*]
Group VI	3.73 ± 0.30[a*,b*]	1.85 ± 0.02[b*]	2.80 ± 0.02[a*,b*]

Values are expressed as mean ± SE (n=6 rats)
P values : * < 0.001, ** < 0.05
a → groups II, III, IV, V & VI compared with group I.
b → groups I, III, IV, V & VI compared with group II.
Group I-VI details are similar as in Table 1.

Table 4. Activity of different extracts from aerial parts of *Pavetta indica* on tissues CAT in HFD rats

Groups	CAT (µ moles of H_2O_2, consumed min/mg protein)		
	Liver	Heart	Aorta
Group I	28.80 ± 0.21^{b*}	45.58 ± 0.23^{b*}	30.61 ± 0.06b*
Group II	15.56 ± 0.09^{a*}	30.72 ± 0.04^{a*}	20.69 ± 0.06^{a*}
Group III	17.02 ± 0.09^{a**,b*}	33.31 ±0.10^{a**,b**}	22.92 ± 0.20^{a**,b*}
Group IV	19.41 ± 0.08^{a*,b*}	37.27 ± 0.03^{a**,b**}	24.44 ±0.08^{a*,b**}
Group V	27.48± 0.08^{a**,b*}	46.34 ± 0.05$^{a*, b**}$	28.46 ± 0.06^{a**,b*}
Group VI	27.20 ± 0.20^{a*,b*}	46.61 ± 0.09^{b*}	28.17 ± 0.02^{a*,b*}

Values are given as mean ± SE (n=6 rats)
P values : $^{*} < 0.001$, $^{**} < 0.05$
a →groups II, III, IV, V & VI compared with group I.
b →groups I, III, IV, V & VI compared with group II.
Group I-VI details are similar as in Table 1.

The effect of tissues glutathione reductase (GR), glutathione peroxidase (GPx) and glutathione-S-transferese in high fat diet animals were shown in Tables 5 ,6 and 7. The results indicated that the concentration of glutathione peroxidase (GPX), glutathione reductase and glutathione-s transferase significant reduction in aorta, heart and liver of rats fed with HFD. High fat diet lowered the ratio of oxidized glutathione/ reduced glutathione in tissue[32]. Treatment of methanol extract of *Pavetta indica* along with the HFD enhanced the activities of glutathione peroxidase, glutathione reductase and glutathione S-transferase in aorta, heart and liver. It may be due to facilitate to circulation of biological membranes found to be linked with raise in the performance of GPX. Glutathione peroxidase (GPX) mainly detoxifies H_2O_2[33] and is involved in the reduction of a variety of hydroperoxides such as phospholipid hydroperoxides, fatty acid hydroperoxides.

Table 5. Activity of different extracts of aerial parts of *Pavetta indica* on tissues Glutathione Peroxidase in HFD rats

Groups	GPx (mg of GSH consumed/min/mg protein)		
	Liver	Heart	Aorta
Group I	8.90 ± 0.02^{b*}	15.58 ± 0.01^{b*}	14.51 ± 0.06b*
Group II	5..56 ± 0.09^{a*}	7.43 ± 0.07^{a*}	6.25 ± 0.02^{a*}
Group III	5.99 ± 0.04^{a**,b*}	7.90 ±0.05^{a**,b**}	7.19 ± 0.04^{a**,b*}
Group IV	6.85 ± 0.07^{a*,b*}	10.22 ± 0.03^{a**,b**}	9.25 ±0.03^{a*,b**}
Group V	8.06 ± 0.02^{a**,b*}	14.97 ± 0.06$^{a*, b**}$	14.29 ± 0.04^{a**,b*}
Group VI	8.48 ± 0.03^{a*,b*}	15.18 ± 0.03^{b*}	15.24 ± 0.04^{a*,b*}

Values are expressed as mean ± SE (n=6 rats)
P values : * < 0.001, ** < 0.05
a →groups II, III, IV, V & VI compared with group I.
b →groups I, III, IV, V & VI compared with group II.
Group I-VI details are similar as in Table 1.

Table 6. Activity of different extracts of aerial parts of *Pavetta indica* on tissues Glutathione Reductase in HFD rats

Groups	GR (mg of GSH consumed/min/mg protein)		
	Liver	**Heart**	**Aorta**
Group I	1.58 ± 0.04^{b*}	2.72 ± 0.04^{b*}	1.78 ± 0.01b*
Group II	0.63 ± 0.12^{a*}	1.17 ± 0.03^{a*}	0.84 ± 0.02^{a*}
Group III	0.79 ± 0.01^{a**,b*}	1.66 ±0.02^{a**,b**}	0.92 ± 0.01^{a**,b*}
Group IV	1.16 ± 0.18^{a*,b*}	1.96 ± 0.02^{a**,b**}	1.07 ±0.03^{a*,b**}
Group V	1.45 ± 0.02^{a**,b*}	2.61 ± 0.02$^{a*, b**}$	1.68 ± 0.01^{a**,b*}
Group VI	1.62 ± 0.03^{a*,b*}	2.80 ± 0.02^{b*}	1.77 ± 0.01^{a*,b*}

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

Table 7. Activity of different extracts of aerial parts of *Pavetta indica* on tissues Glutathione S-Transferase in HFD rats

Groups	Glutathione – S – transferase (GST)		
	Liver	**Heart**	**Aorta**
Group I	24.82 ± 0.02^{b*}	20.18 ± 0.06^{b*}	17.68 ± 0.04b*
Group II	10.54 ± 0.04^{a*}	8.78 ± 0.02^{a*}	7.56 ± 0.09^{a*}
Group III	11.79 ± 0.05^{a**,b*}	9.74 ±0.09^{a**,b**}	8.21 ± 0.05^{a**,b*}
Group IV	13.39 ± 0.05^{a*,b*}	10.85 ± 0.02^{a**,b**}	9..26 ±0.01^{a*,b**}
Group V	20.35 ± 0.08^{a**,b*}	16.68 ± 0.07$^{a*, b**}$	15.05 ± 0.04^{a**,b*}
Group VI	22.10 ± 0.03^{a*,b*}	18.46 ± 0.02^{b*}	15.17 ± 0.06^{a*,b*}

Values are expressed as mean ± SE (n=6 rats)
P values : *< 0.001, **< 0.05
a → groups II, III, IV, V & VI compared with group I.
b → groups I, III, IV, V & VI compared with group I.
Group I-VI details are similar as in Table 1.

Activity of different extracts of aerial parts of *Pavetta indica* on tissues glutathione in HFD rats as shown in Table 8. The significant (p<0.001) reduced the levels of tissues Glutathione (GSH) were seen in HFD animals (II group) to compared with I groups of animals. GSH also functions as free radical scavenger in the repair of radical caused biological damage. The reduced levels may be an attempt by the tissue to counteract the increased formation of lipid peroxides that are handled by antioxidant enzymes such as Glutathione peroxidase which scavenges H_2O_2 utilizing GSH as substrate[34]. Increase in glutathione concentration in *Pavetta indica* methanolic extract treated rats with HFD might be due to the increase in the concentration of glutathione reductase which catalyses the conversion of oxidized glutathione to reduced glutathione in liver (or) might be due to increase the production of GSH[35].

Table 8. Activity of different extracts of aerial parts of *Pavetta indica* on tissues GSH in HFD rats

Groups	Glutathione		
	Liver	**Heart**	**Aorta**
Group I	4.45 ± 0.04[b*]	7.70 ± 0.06[b*]	5.76 ± 0.05b*
Group II	1.78 ± 0.03[a*]	4.24 ± 0.07[a*]	2.82 ± 0.04[a*]
Group III	1.98 ± 0.02[a**,b*]	4.74 ±0.02[a**,b**]	3.03 ± 0.03[a**,b*]
Group IV	2.35 ± 0.08[a*,b*]	5.09 ± 0.02[a**,b**]	3.93 ±0.02[a*,b**]
Group V	3.91 ± 0.02[a**,b*]	7.19 ± 0.04[a*, b**]	5.11 ± 0.07[a**,b*]
Group VI	4.25 ± 0.03[a*,b*]	7.76 ± 0.03[b*]	5.74 ± 0.04[a*,b*]

Values are given as mean ± SE (n=6 rats)
P values : * < 0.001, ** < 0.05
a → groups II, III, IV, V & VI compared with group I.
b → groups I, III, IV, V & VI compared with group I.
Group I-VI details are similar as in Table 1.

Conclusion

The present investigation demonstrated that high fat diet-induced hyperlipidemia was related with an enhance in the oxidative stress and that after administration of the methanol extract of aerial parts of *Pavetta indica* had significant reduction of oxidative stress and protection against high fat/cholesterol diet-induced damage to the cardiac tissues possibly through positive modulation of the cardiac antioxidant system. The phytochemicals may be responsible for the inhibition of lipid peroxidation and enhance the antioxidant activities of methanol extract of *Pavetta indica*. The findings therefore support the ethano medicinal use of the *Pavetta indica* in the management of cardiovascular complication like atherosclerosis.

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