EVALUATION OF IN VIVO ANTIOXIDANT AND LIPID PEROXIDATION EFFECT OF VARIOUS EXTRACTS FROM WHOLE PLANT OF PAVETTA INDICA (LINN) IN RAT FED WITH HIGH FAT DIET

[1]ABDUL HAMEED THAYYIL, [2]ARUMUGAM KOTTAI MUTHU, [3]MOHAMMED IBRAHIM

[2]Department of Pharmacy, Annamalai University, Annamalai Nagar-608 002, India.
[1,3]Nizam Institute of Pharmacy & Research centre, Near Ramoji Film City, Deshmukhi, Hyderabad, Telugana. India
E-mail: [1]hameedthayyil@gmail.com.

Abstract- The study was carried out to determine the *in vivo* antioxidant and lipid peroxidation effect of various extracts of aerial parts of *Pavetta indica* (Linn). *in vivo* antioxidant and lipid peroxidation activity is screened by inducing hyperlipidemia with the help of High fat diet in wistar albino rats and tissue lipid peroxidation levels TBARS levels of antioxidant Enzymes such as Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPx), Glutathione reductase (GR),Glutathione S transferees (GST) and non enzymatic antioxidant Glutathione (GSH). High fat diet rats showed significantly ($P<0.001$) reduced the levels of tissues enzymatic antioxidant and non enzymatic antioxidant and enhanced the level of TBARS. The level of TBARS are elevated in HFD rats (group II) when compared with control group. Administration of methanolic extract of *Pavetta indica* in high fat diet rats were showed significantly ($p<0.001$) increased the levels of antioxidant Enzymes such as 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 groups.The methanol extract of *Pavetta indica* is a significant source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses.

Key words- *Pavetta indica* , High fat diet, Lipid peroxidation, Antioxidant.

I. 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 produced by radiation. Over production of ROS and inadequate antioxidant has been implicated in the pathogenesis and complication of some disease condition s like diabetes, Alzheimers disease, cancer, atherosclerosis, arthritis, neurodegenerative disease and aging process[2].

Oxidation is essential in many living organisms for the production of energy to fuel biological processes. However, the uncontrolled production of oxygen derived free radicals is involved in the onset of many diseases such as atherosclerosis, rheumatoid arthritis, and cancer as well as in degenerative processes associated with aging[3]. Almost all organisms are well protected against free radical damage by enzymes such as superoxide dismutase and catalase, 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. It is widely distributed from the Andaman Islands, India and the north-western Himalayas to southern China and southwards throughout Malaysia to northern Australia. The entire plant used medicinally as a bitter tonic, diuretic, inflammation, rheumatism, jaundice and ulcer [5]. In the indigenous system of medicine, it is reported that the decoction of the leaves are used to relieve haemorrhoidal pain, as a lotion for nose, analgesic, antipyretic, appetizer and the ulceration of mouth[6][7]. In literature, it has been reported as an antibacterial, antiviral and antimalarial [8]. *P. indica* leaves are used in the treatment of liver disease, pain from piles, urinary diseases and fever [9]. It is a medicinally important plant having antiinflammatory activities[10]and analgesic activity[11] , antidiabetic activity[12] , antimicrobial activity of leaf extract of *P. indica* [13]. Its root extract also have diuretic and purgative activity[14]. The leaves and roots are employed in the preparation of poultices for boils and itches; decoctions of leaves are used as a lotion for ulcerated nose and for heamorrhoids[15]. Root is used for anticephalagic. Leaf is used in haemorrhoidol pain and ulcerated nose. Wood is used as antirheumatic. Fruits are used as anthelmintic[16][17]. The phytochemicals produced by the plants for their self

protection have been demonstrated to protect human against a number of diseases. The leaves contain carbohydrate, glycosides, phytosterols, saponins, flavonoids and alkaloids. 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 various extract of aerial parts of *Pavetta indica* (Linn) in rat fed with high fat diet.

II. MATERIALS AND METHODS

Collection and Identification of Plant materials

The aerial parts of *Pavetta indica* (Linn), were collected 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.

Preparation of Extracts

The above powered materials were successively extracted with Petroleum 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 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 150-175g were obtained from the Central Animal House, Nizam Institute of Pharmacy & Research centre, Near Ramoji Film City, Deshmukhi, Hyderabad, A.P. India. 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.

Experimental Design

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

Group I : Standard chow diet

Group II : High Fat Diet

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

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

Group V : High fat diet + Methanol extract of *Pavetta indica* (Linn) (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[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 & 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%.

Testing of *in vivo* antioxidant and lipid peroxidation

Rats of group III, IV and V were orally fed with the various extracts of *Pavetta indica* and rats of group VI were fed with standard drug atorvastatin. Both the 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 dislocation 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.(1957)[21]. It was used for the estimation of thiobarbituric acid reactive substances (TBARS)[22] . Another portion of the tissues was homogenized with phosphate buffer saline and used 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 expressed as mean ± SE of 6 rats in each group. The statistical significance between the groups was analyzed by using one way analysis of variance (ANOVA), followed by Dunnet's multiple comparison test. Significance level was fixed at 0.05.

III. RESULTS AND DISCUSSION

As shown in Table 1 and 2. The TBARS and conjugated dienes levels were increment in liver, heart and aorta in group II rats are a clear reasonable sign of excessive formation of free radical and initiation of lipid peroxidation. The high fat diet is known to induce oxidative stress in the cells by producing reactive oxygen species (ROS)[28]. This results in increased lipid peroxidation leading to elevated concentration of TBARS and conjugated dienes[29]. The significant decline in the level of TBARS and conjugated dienes in rats administered with methanolic extract of *Pavetta indica.* when compared to HFD rats (II group). This effect may be due to phytoconstituents, flavonoids present in the *Pavetta indica* .

Table 1. Effect of various extracts of aerial parts of *Pavetta indica* on tissues TBARS in High fat diet 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 expressed as mean ± SE (n=6 rats)
P values
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 : High fat diet + Petroleum ether extract of *Pavetta indica* (200mg/kg B.wt)
IV group : High fat diet + ethyl acetate extract of *Pavetta indica* (200mg/kg B.wt)
V group : High fat diet + Methanolic extract of *Pavetta indica* (200mg/kg B.wt)
VI Group : High fat diet + standard drug atorvastatin (1.2 mg/kg B.wt)

Table 2. Effect of various extract of aerial parts of *Pavetta indica* on tissues conjugated diene in High fat diet rats

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

Values are expressed 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 same as in Table 1.

The effect of various extracts of *Pavetta indica* on tissues SOD and CAT enzyme levels in HF diet rats were presented in Table 3 and 4. The activities of SOD and CAT in the tissue like liver, heart and aorta significantly (P<0.001) lowered in rats fed with high fat diet (groupII).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 radicals[31] . Catalase decomposes hydrogen peroxide and helps to protect the tissues from highly reactive hydroxyl radicals. Treatment of with methanolic extract of *Pavetta indica (Linn)* along with high fat diet significantly increased the activities of SOD and CAT in tissues of rats when compared to other extracts treated groups.

Table 3. Effect of various extracts aerial parts of *Pavetta indica* on tissues SOD in High fat diet rats

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

: * < 0.001, ** < 0.0

Values are expressed as mean ± SE (n=6 rats)
P values
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 same as in Table 1.

Table 4. Effect of various extracts from aerial parts of *Pavetta indica* on tissues CAT in High fat diet rats

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

Values are expressed as mean ± SE (n=6 rats)
P values
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 same as in Table 1.

The activities of tissues glutathione reductase (GR), glutathione peroxidase (GPx) and glutathione-S-transferese in HFD rats were shown in Tables 5 ,6 and 7. The results indicated that the concentration of glutathione peroxidase (GPX), glutathione reductase and glutathione-s transferase significantly decreased in tissues (aorta, heart and liver) of rats fed with high fat diet. High fat diet decreased the ratio of oxidized glutathione/ reduced glutathione in tissue [32]. Administration of methanolic extract of *Pavetta indica* along with the high fat diet increased the activities of glutathione peroxidase, glutathione reductase and glutathione S-transferase in all the tissues. It might be due to help to propagation of biological membranes found to be associated with increase in the activities 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. Effect of various extracts of aerial parts of *Pavetta indica* on tissues Glutathione Peroxidase in High fat diet 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

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 same as in Table 1.

Table 6. Effect of various extracts of aerial parts of *Pavetta indica* on tissues Glutathione Reductase in High fat diet 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

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 same as in Table 1.

Table 7. Effect of various extracts of aerial parts of *Pavetta indica* on tissues Glutathione S-Transferase in High fat diet rats

Groups	Glutathione – S – transferase (GST) (μ mole of CDNB – GSH – Conjugate to /min/mg protein)		
	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

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 same as in Table 1.

As shown in Table 8. The significant ($p<0.001$) fall in the levels of tissues Glutathione (GSH) were seen in high fat diet rats (II group) when compared with the control rats (I group). 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 high fat diet might be due to the increase in the activity of the enzyme glutathione reductase which catalyses the conversion of oxidized glutathione to reduced glutathione in liver (or) might be due to enhanced synthesis of GSH [35].

Table 8. Effect of various extracts of aerial parts of *Pavetta indica* on tissues glutathione in High fat diet 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 expressed as mean ± SE (n=6 rats)

* < 0.001, ** < 0.001, ** < 0

P values

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 same as in Table 1.

CONCLUSION

The present study demonstrated that high fat diet-induced hyperlipidemia was associated with an increase 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 phytoconstituents 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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