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Research Article

IN-VIVO ANTIOXIDANT AND LIPID PEROXIDATION EFFECT OF WHOLE PLANT OF *IONIDIUM SUFFRUTICOSUM* (Ging.) IN RATS FED WITH HIGH FAT DIET

D.SATHEESHKUMAR, A. KOTTAI MUTHU*AND R. MANAVALAN

Department of pharmacy, Annamalai University, Annamalai Nagar-608 002, India , **Email:** arthik03@yahoo.com

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ABSTRACT

The objective of the present investigation was to evaluate the *in vivo* antioxidant and lipid peroxidation effect of various extracts from whole plant of *Ionidium suffruticosum* (Ging.) in rat fed with high fat diet. Various extracts (Petroleum ether, Ethyl acetate and Methanol) of *Ionidium suffruticosum* (200 mg/kg body weight) was administered orally to rats fed with high-fat diet (HFD) daily for 9 weeks and the tissue (liver, heart and aorta) samples were collected at the end of experimental period. High fat diet rats showed significantly (P<0.001) reduced the levels of tissues enzymatic antioxidant and non enzymatic antioxidant (Glutathione). The level of thiobarbituric acid reactive substances (TBARS) are elevated in HFD rats (group II) when compared with control rats (group I). After administration of methanolic extract of *Ionidium suffruticosum* 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) and incresed the level of non enzymatic antioxidant Glutathione (GSH) when compared with HFD rats (Group II). The methanolic extract of *Ionidium suffruticosum* in high fat diet rats were found reduced the concentration of TBARS than that of HFD rats (group II). Similar result was not observed in other two extract treatment groups. Based on the results, we concluded that the methanolic extract of *Ionidium suffruticosum* is a significant source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses.

Keywords: *Ionidium* suffruticosum, antioxidant, high fat diet, rats.

INTRODUCTION

It is clearly established that long-term consumption of a high fat diet accelerates the development of Coronary Heart Disease (CHD). Dietary cholesterol can increase the level of serum cholesterol to levels which can place an individual at increased risk for the development or exacerbation of atherosclerosis[1, 2]. Therapeutic agents are control the levels of serum cholesterol have proven to be effective in the treatment of CHD[3, 4].

The high levels of free radicals in living systems are able to oxidize bio molecules, leading to tissue damage, cell death or various diseases such as cancer, cardiovascular diseases, arteriosclerosis, neural disorders, skin irritations and inflammations[5,6]. Antioxidant compounds can deactivate and scavenge the free radicals. Antioxidants can inhibit the effect of oxidants by donating hydrogen atom or chelating metals[7-9]. Synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are used as additives in foods to prevent oxidation of lipids[10-12]. Besides, BHA and BHT are restricted by legislative rules because of doubts over their toxic and carcinogenic effects. Therefore, there is a growing request and interest on natural and safer antioxidants in food applications, and a growing trend in consumer preferences for natural antioxidants[13, 14]. Natural antioxidants are studied extensively for their capacity to protect organisms and cells from damage induced by oxidative stress, the latter being considered a cause of ageing and degenerative diseases[15]. Recently, investigation of new sources of natural antioxidant became very important for human health. Natural antioxidants commonly exist on plants which contain polyphenolic compounds[16-18].

Ionidium suffruticosum(Ging.) (Syn: *Hybanthus enneaspermus*) it belongs to the family Violaceae known as Lakshmisheshta, Padmavati, Padmacharini or Purusharathna in Sanskrit, is an important plant in the Indian system of medicine. It is a small suffrutescent perennial herb found in the regions of former Madras Presidency in India, Ceylon, tropical Asia, Africa, and Australia. It grows 15-30 cm in height with many diffuse or ascending branches and is pubescent in nature[19].Traditionally the plant is used as an aphrodisiac, demulcent, tonic, diuretic, in urinary infections, diarrhoea, leucorrhoea, dysuria, and sterility[20]. Moreover, the plant is reported, in ancient ayurvedic literature, to cure conditions of "kapha" and "pitta", urinary calculi, strangury, painful dysentery, vomiting, burning sensation , wandering of the mind, urethral discharges, blood troubles, asthma, epilepsy, cough, and to give tone to the breasts[19]. In this study, we aimed to investigate the *in vivo* antioxidant and lipid peroxidation effect of various extracts from whole plant of *Ionidium suffruticosum* (Ging.) in rat fed with high fat diet.

MATERIALS AND METHODS

Plant materials

The whole plant of *Ionidium suffruticosum* (Ging), were collected from Kilikulam, Tirunelveli District of Tamil Nadu, India. Taxonomic identification was made from Botanical Survey of Medicinal Plants Unit Siddha, Government of India. Palayamkottai. The whole plant of *Ionidium suffruticosum* (Ging), were dried under shade, segregated, pulverized by a mechanical grinder and passed through a 40 mesh sieve. All the three extract were stored in screw cap vial at 4ºC until further use.

Preparation of Extracts

The above powdered materials were successively extracted with Petroleum ether (40-60ºC) by hot continuous percolation method in Soxhlet apparatus[21] for 24 hours. Then the marc was subjected to Ethyl acetate (76-78ºC) for 24 hrs and then marc was subjected to Methanol for 24 hours. The extracts were concentrated by using a rotary evaporator and subjected to freeze drying in a lyophilizer till dry powder was obtained.

Animals

Thirty six adult male Wistar rats, weighing approximately 150-180g were obtained from Central Animal House, Rajah Muthiah Medical College, Annamalai University. The animals were kept in cages, 2 per cage, with relative humidity (55%) in a 12 hour light/dark cycle at $25^0 \pm 2^0$C. They were given access to water and a commercial diet *ad libitum*. The experiment were carried out as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India, and approved by the Institutional Animal Ethics Committee (IAEC), Annamalai University (Approved number: 160/1999/CPCSEA/745).

Diets

The compositions of the two diets were used as follows[22]:

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%.

Experimental Design

A total number of 36 rats were divided into six groups of six rats each:

Group I : Standard chow diet (Control).

Group II : High Fat Diet (HFD).

Group III : HFD + Pet.ether extract of *Ionidium suffruticosum* (200mg/kg B.Wt)

Group IV : HFD + Ethyl acetate extract of *Ionidium suffruticosum* (200mg/kg B.Wt)

Group V : HFD + Methanolic extract of *Ionidium suffruticosum* (200mg/kg B.Wt)

Group VI : HFD + standard drug atorvastatin (1.2 mg/kg body weight)

Testing of *in vivo* antioxidant and lipid peroxidation

Rats of group III, IV and V were orally fed with the various extracts of *Ionidium suffruticosum* (200mg/kg body weight) and rats of group VI were fed with standard drug atorvastatin (1.2 mg/kg body weight). The dose was fixed as per the OECD guidelines. All the three extracts as well as standard drug atorvastatin were suspended in 2% tween 80[23] separately and fed to the respective rats by oral intubation. At the end of 9 weeks all the rats 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*[24]. It was used for the estimation of thiobarbituric acid reactive substances[25] (TBARS). Another portion of the tissues was homogenized with phosphate buffer saline and used for the estimation of reduced Glutathione[26] (GSH), Superoxide dismutase[27] (SOD), Catalase[28] (CAT), Glutathione peroxidase [29](GPx) and Glutathione reductase[30] (GR).

Statistical Analysis

Data obtained from experiment animals were expressed as mean standard error (± SEM). Statistical differences between the control and experimental groups were evaluated by one-way ANOVA and Duncan multiple comparison tests. A difference in the mean values of $p<0.05$ was considered to be significant.

RESULTS AND DISCUSSION

Adverse effect of HFD on the health of humans and animal species has previously been emphasized by several researchers[31, 32]. It has been reported that high levels of fat increase fat-mediated oxidative stress and decrease antioxidative enzyme activity[33] therefore, many chronic health problems that are attributed to HFD are known to be the consequences of oxidative damage[34]. On this account, there are various reports indicating the beneficial effects of antioxidant supplementation in preventing dyslipidemia and cardiovascular disease[35-37].

Increase lipid peroxidation refers an imbalance between intracellular free radical production, cellular defence mechanisms and melandialdehyde as one of the most important lipid peroxidation markers[38]. As shown in Table 1, HFD rats significantly increased TBARS levels in liver, heart and aorta compared to control group. However, the methanolic extract of *Ionidium suffruticosum* significantly ($p<0.001$) decreased tissues (Liver, heart and aorta) TBARS levels in methanolic extract plus HFD group compared to HFD rats (group II). The similar result was not found in other two extract treatment groups. This result indicates that methanolic extract of *Ionidium suffruticosum* decreases lipid peroxidation and eventually may have a role in reducing the hazardous effects of high-fat diet.

Table 1: Effect of various extract of *Ionidium suffruticosum* 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	23.80 ± 0.20^{b*}	40.58 ± 0.16^{b*}	17.61 ± 0.16b*	4.81 ± 0.05^{b*}	7.54 ± 0.09 ^{b*}	6.51 ± 0.01 ^{b*}
Group II	70.56 ± 0.19^{a*}	80.72 ± 0.17^{a*}	69.25 ± 0.09^{a*}	1.29 ± 0.06^{a*}	3.67 ± 0.15 ^{a*}	3.45 ± 0.03 ^{a*}
Group III	49.08 ± 0.29^{a**,b*}	60.26 ± 0.29 ^{a**,b**}	38.31 ± 0.35 ^{a**,b*}	2.60 ± 0.20^{a**,b*}	5.59 ± 0.02$^{a**, b**}$	4.21 ± 0.04 ^{a**,b*}
Group IV	39.97 ± 0.25 ^{a*,b*}	51.77 ± 0.18 ^{a**,b**}	31.26 ± 0.41 ^{a*,b**}	3.44 ± 0.11^{a**,b**}	6.21 ± 0.01 ^{a*,b**}	5.05 ± 0.03 ^{a**,b*}
Group V	30.27 ± 0.26 ^{a**,b*}	45.39 ± 0.61 $^{a*, b**}$	22.30 ± 0.07 ^{a**,b*}	4.13 ± 0.10 ^{a*,b*}	7.14 ± 0.03 ^{a*,b*}	5.50 ± 0.03 ^{a*,b*}
Group VI	25.20 ± 0.30 ^{a*,b*}	40.61 ± 0.13 ^{b*}	17.17 ± 0.13 ^{a*,b*}	4.79 ± 0.01 ^{a*,b*}	6.82 ± 0.03 ^{a*,b*}	5.81 ± 0.05 ^{a*,b*}

Data 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 (HFD) ; Group III : High fat diet+Pet.ether extract of *Ionidium suffruticosum* (200mg/kg B.wt) ; Group IV : High fat diet+Ethyl acetate extract of *Ionidium suffruticosum* (200mg/kg B.wt) ; Group V : High fat diet + Methanol extract of *Ionidium suffruticosum* (200mg/kg B.wt) ; Group VI : High fat diet + Standard drug atorvastatin (1.2 mg/kg B.wt)

Glutathione (GSH) is essential for the cellular antioxidant defense response and acts as an essential cofactor for antioxidant enzymes[39]. As shown in Table 1. A significant ($p<0.001$) decrease in liver, heart and aorta GSH levels in HFD rats compared to control rats (group I), while increase in liver, heart and aorta GSH levels in methanolic extract plus HFD group compared to HFD (group II) and other extracts treatment groups (III &IV). Under the oxidative stress conditions, GSH is consumed by the GSH related enzymes to detoxify peroxides produced due to increased lipid peroxidation[40]. In HFD group, significant raise in lipid peroxidation and concomitant GSH activity may be a consequence of depleted glutathione stores.

A cholesterol-rich diet brings about remarkable modifications in antioxidant defense mechanisms. In addition to, recently report shown that hypercholesterolemia diminishes the antioxidant defense system and decreases the activities of Superoxide dismutase (SOD) and Catalase (CAT), elevating the lipid peroxide content[41]. As 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[42] and the accumulation of O_2^- and H_2O_2 which in turn forms hydroxyl radicals[43].After administration of methanolic extract of *Ionidium suffruticosum* 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 various extract of *Ionidium suffruticosum* 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	3.67 ± 0.01 ^{b*}	1.74 ± 0.03 ^{b*}	2.87 ± 0.02^{b*}	28.45 ± 0.22 ^{b*}	49.30 ± 0.16 ^{b*}	30.85 ± 0.27 ^{b*}
Group II	1.84 ± 0.02 ^{a*}	0.89 ± 0.01 ^{a*}	1.43 ± 0.06 ^{a*}	16.78 ± 0.20 ^{a*}	30.53 ± 0.14 ^{a*}	21.79 ± 0.17 ^{a*}
Group III	2.41 ± 0.02 ^{a**,bNS}	1.26 ± 0.02 ^{a**,b**}	2.38 ± 0.02 ^{a**,b*}	22.32 ± 0.09 ^{a**,b*}	40.25 ± 0.17 ^{a**,b*}	23.53 ± 0.18 ^{a*,b**}
Group IV	2.76 ± 0.03 ^{a*,b**}	1.48 ± 0.02 ^{a*,b*}	2.51 ± 0.01 ^{a*,b*}	23.78 ± 0.08 ^{a*,b*}	43.76 ± 0.09 ^{a*,b**}	26.63 ± 0.10 ^{a**,b**}
Group V	3.30 ± 0.01 ^{a*,b*}	1.61 ± 0.01 $^{a*, b*}$	2.68 ± 0.03 ^{a*,b*}	26.98 ± 0.23 ^{a*,b*}	47.46 ± 0.22 ^{a*,b*}	29.61 ± 0.15 ^{a*,b*}
Group VI	3.63 ± 0.01 ^{a*,b*}	1.68 ± 0.02 ^{a*,b*}	2.85 ± 0.01 ^{a*,b*}	29.01 ± 0.07 ^{a*,b*}	47.99 ± 0.21 ^{a*,b*}	31.12 ± 0.06 ^{a*,b*}

Data 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.

Glutathione peroxidase (GPx) is more important than catalase for detoxification of hydrogen peroxide in brain, because the brain contains small amounts of catalase and GPx can also interact directly with lipid peroxides[44, 45]. As shown in Table 3. Tissues glutathione peroxidase and reductase levels were significantly (p<0.001) decreased 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[46]. Administration of methanolic extract of *Ionidium suffruticosum* along with the HFD significantly (p<0.001) enhanced the levels of glutathione peroxidase and glutathione reductase in all the tissues as compared with HFD rats. Similar result was not observed in other two extracts treatment groups. A standard drug atorvastatin administered rats also showed elevated level of glutathione peroxidase and glutathione reductase.

Table 3: Effect of various extract of *Ionidium suffruticosum* 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	8.95 ± 0.04^{b*}	12.59 ± 0.12^{b*}	15.51 ± 0.10^{b*}	1.46 ± 0.01 ^{b*}	2.68 ± 0.01 ^{b*}	1.68 ± 0.01 ^{b*}
Group II	5.59 ± 0.07^{a*}	8.54 ± 0.07 ^{a*}	8.54 ± 0.07^{a*}	0.73 ± 0.02^{a*}	1.30 ± 0.01^{a*}	0.79 ± 0.02^{a*}
Group III	7.07 ± 0.05^{a*,b**}	9.82 ± 0.12^{a**,b*}	8.99 ± 0.11 ^{a**,b*}	1.03 ± 0.03 ^{a**,b**}	2.12 ± 0.01 ^{a*,b**}	1.18 ± 0.01 $^{aNS, b*}$
Group IV	7.61 ± 0.09 ^{a**,b**}	13.44 ± 0.11 ^{a*,b**}	10.10± 0.08 ^{a**,b**}	1.13 ± 0.01 ^{a*,b**}	2.30 ± 0.01 ^{a*,b**}	1.30 ± 0.01 ^{a**,b*}
Group V	8.55 ± 0.12 ^{a*,b*}	10.59 ± 0.13 ^{a*,b*}	11.56 ± 0.04 ^{a**,b*}	1.36 ± 0.01 ^{a**,b*}	2.44 ± 0.02 ^{a*,b*}	1.61 ± 0.01 ^{a*,b*}
Group VI	8.95 ± 0.09 ^{a*,b*}	11.12 ± 0.09 ^{a*,b*}	11.29 ± 0.08 ^{a*,b*}	1.41 ± 0.01 ^{a**,b*}	2.65 ± 0.01 ^{a*,b*}	1.70 ± 0.01 ^{a*,b*}

Data 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.

CONCLUSION

On the basis of the results obtained in the present study, we conclude that the methanolic extract of whole plant *Ionidium suffruticosum* had significant *in vivo* antioxidant and lipid peroxidation activity. These *in vivo* antioxidant 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 studies are required to again more insight in to the possible mechanism of action.

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REFERENCES

1. Onyeneke EC, Adebisi KE, Eriyamremu GE, Ojeaburu SI, Asagba SO, Oluba OM. Effect of lipid-based diet on some lipid-metabolizing enzymes. *J. Med. Sci* 2007; 7(8): 1283-1289.
2. Oluba OM, Adeyemi O, Ojieh GC, Adebisi KE, Isiosio IO, Aboluwoye CO. Effect of dietary cholesterol on some serum enzymes. *J. Med. Sci* 2008a; 8(4): 390-394.
3. Bays H and Stein EA. Pharmacotherapy for atherogenic dyslipidaemia - Current therapies and future agents. *Expert Opin. Pharmacother* 2003; 4: 1901-1938.
4. Linsel-Nitschke P and Tall AR. HDL as a target in the treatment of atherosclerotic cardiovascular disease. *Nat. Rev. Drug Discov* 2005; 4: 193-205.

5. Oktay M, Gulcin I, Kufrevioglu OI. Determination of in vitro antioxidant activity of fennel (*Foeniculum vulgare*) seed extracts. *Lebensm. Wissen. Technol* 2003; 36: 263-271.
6. Gulcin I, Elias R, Gepdiremen A, Taoubi K, Köksal E. Antioxidant secoiridoids from fringe tree (*Chionanthus virginicus* L.). *Wood Sci. Technol* 2009; 43: 195-212.
7. Sen CK, Packer L, Hanninen O. Handbook of oxidants and antioxidants in exercise (1st edn). Elsevier Science B.V. Amsterdam, 2000.
8. Gulcin I, Buyukokuroglu ME, Oktay M, Kufrevioglu OI. Antioxidant and analgesic activities of turpentine of Pinus nigra Arn. Subsp. pallsiana (Lamb.) Holmboe. J. *Ethnopharmacol* 2003a; 86: 51-58.
9. Prakash D, Upadhyay G, Singh BN, Singh HB. Antioxidant and free radical-scavenging activities of seeds and agri-wastes of some varieties of soybean (*Glycine max*). *Food Chem* 2007; 104: 783-790.
10. Gulcin I, Oktay M, Kufrevioglu OI, Aslan A. Determinations of antioxidant activity of lichen *Cetraria islandica* (L) Ach. *J. Ethnopharmacol* 2002a; 79: 325-329.
11. Gulcin I, Mshvildadze V, Gepdiremen A, Elias R. Antioxidant activity of a triterpenoid glycoside isolated from the berries of Hedera colchica: 3-O-(α-D-glucopyranosyl)-hederagenin. *Phytother. Res* 2006a; 20: 130-134.
12. Gulcin I, Elias R, Gepdiremen A, Boyer L. Antioxidant activity of lignans from fringe tree (*Chionanthus virginicus* L.). *Eur. Food Res. Technol* 2006b; 223: 759-767.
13. Elmastas M, Gülçin I, Öztürk L, Gökçe I. Investigation of antioxidant properties of spearmint (Mentha spicata L.). *Asian J. Chem* 2005; 17: 137-148.
14. Elmastas M, Türkekul I, Öztürk L, Gülçin I, Isıldak Ö, Aboul-Enein HY. The antioxidant activity of two wild edible mushrooms (*Morchella vulgaris* and *Morchella esculanta*). *Comb. Chem. High T. Scr* 2006a; 9: 443-448.
15. Elmastas M, Gülçin I, Beydemir S, Küfrevioglu ÖI, Aboul-Enein HY. A study on the in vitro antioxidant activity of juniper (*Juniperus communis* L.) seeds extracts. *Anal. Lett* 2006b; 39: 47-65.
16. Gülçin I, Büyükokuroglu ME, Oktay M, Küfrevioglu ÖI. On the in vitro antioxidant properties of melatonin. *J. Pineal. Res* 2002b; 33: 167-171.
17. Gülçin I, Elmastas M, Aboul-Enein HY. Determination of antioxidant and radical scavenging activity of basil (*Ocimum basilicum*) assayed by different methodologies. *Phytother. Res* 2007; 21: 354-361.
18. Stoilova I, Krastanov A, Stoyanova A, Denev P, Gargova S.Antioxidant activity of a ginger extract (*Zingiber officinale*). *Food Chem* 2007; 102: 764-770.
19. Kirtikar KR and Basu BD. In: Indian Medicinal Plants, Vol I, 2nd ed., Periodical Experts Book Agency, Delhi.1991: 212-213.
20. Yoganarasimhan SN. In: Medicinal Plants of India-Tamilnadu, Vol II, Cyber Media, Bangalore. 2000: 276.
21. Harborne JB. Phytochemical methods 11 Edn.In Chapman &, Hall.New York, 1984; 4-5.
22. Kottai Muthu A, Sethupathy S, Manavalan R and Karar PK. Hypolipidemic effect of methanolic extract of *Dolichos biflorus* Linn in high fat diet fed rats. *Ind.J.Exp.Biol* 2005; 43:522-525.
23. Waynforth BH. Injection techniques. Experimental and surgical techniques in the rats, Academic Press, London, 1980; 3.
24. Folch J, Lees M and Sloane GH. A simple method for the isolation and purification of total lipids from animals tissues. *J Biol Chem* 1957; 226: 497.
25. Nichans WH, Samulelson B. Formation of malondialdehyde from phospholipid arachidonate during microsomal lipid peroxidation. *Eur J Biochem* 1968; 6:126-30.
26. Ellman GL. Tissue sulfhydroyl groups. *Arch.Biochem.Biophy* 1959; 82:70.
27. Kakkar P, Das B, Visvanathan PN. A modified spectrophotometric assay of SOD. *Indian J Biochem Biophys* 1984; 21:130-2.
28. Sinha AK. Colorimetric assay of catalase. *Anal.Biochem* 1972; 47: 389.
29. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hatman DG and Hoekstra WG, Selenium; Biochemical roles as a component of glutathione peroxidise. *Science* 1973; 179: 588.
30. Mavis RD and Stellwagen E. *Journal of Biological Chemistry* 1968; 243: 809-814.
31. Steinberg D.Antioxidants and atherosclerosis: a current assessment. *Circulation* 1991; 84: 1420–1425.
32. Ghosh P, Bitsanis D, Ghebremeskel K, Crawford MA, Poston L. Abnormal aortic fatty acid composition and small artery function in offspring of rats fed a high-fat diet in pregnancy. *Journal of Physiology* 2001; 533: 815–822.
33. Slim RM, Toborek M, Watkins BA, Boissonneault GA, Hennig B. Susceptibility to hepatic oxidative stress in rabbits fed different animal and plant fats. *Journal of the American College of Nutrition* 1996; 15: 289–294.
34. Hamelet J, Demuth K, Paul JL, Delabar JM, Janel, N. Hyperhomocysteinemia due to cystathionine beta synthase deficiency induces dysregulation of genes involved in hepatic lipid homeostasis in mice. *Journal of Hepatology* 2007; 46: 151–159.
35. Mary N, Achuthan C, Babu B, Padikkala J. In vitro antioxidant and antithrombotic activity of Hemidesmus indicus (L) R. Br. *Journal of Ethnopharmacology* 2003; 87: 187–191.
36. Minhajuddin M, Beg ZH, Iqbal J. Hypolipidemic and antioxidant properties of tocotrienol rich fraction isolated from rice bran oil in experimentally induced hyperlipidemic rats. *Food and Chemical Toxicology* 2005; 43: 747–753.
37. Kupeli AE, Avcı G, Kucukkurt I, Keles H, Tamer U, Ince S, Yesilada E. Cholesterol-reducer, antioxidant and liver protective effects of Thymbra spicata L. var. spicata. *Journal of Ethnopharmacology* 2009; 126: 314–319.
38. Ogur R, Coskun O, Korkmaz A, Oter S, Yaren H, Hadse M. High nitrate intake impairs liver functions and morphology in rats; protective effects of α-tocopherol. *Environmental Toxicology and Pharmacology* 2005; 20: 161–166.
39. Hayes JD, Flanagan JU, Jowsey IR. Glutathione transferases. *Annual Reviews of Pharmacology and Toxicology* 2005; 45: 51–88.
40. Cathcart RF. Vitamin C: the nontoxic, nonrate-limited, antioxidant free radical scavenger. *Medical Hypotheses* 1985; 18: 61–77.
41. Anila L, Vijayalakshmi NR. Antioxidant action of flavonoids from Mangifera indica and Emblica officinalis in hypercholesterolemic rats. *Food Chem* 2003; 83: 569–574.
42. Thampi HBS, Manoj G, Leelamma S and Menon VG. Dietary fibre and lipid peroxidation: effects of dietary fibre on levels of lipids and lipid peroxides in high fat diet. *Ind. J. Exp. Biol* 1991; 29: 563.
43. Batra S, Singh SP and Srivasta VML. Xanthine oxidase, Superoxide dismutase, Catalase and lipid peroxidation in mastomys nataensis effect of dipentalonema viteae infection. *Indian J. Exp. Biol* 1989; 27:1067.
44. Adams JDJR, Klaindman LK, Odunze IN, Shen HC, Miller CA. Alzheimer's and Parkinson's disease. Brain levels of glutathione, glutathione disulfide and vitamin E. *Mol. Chem.Neuropathol* 1991; 14: 213–226.
45. Jones DP, Eklöw L, Thor H, Orrenius S. Metabolism of hydrogen peroxide in isolated hepatocytes: Relative contributions of catalase and glutathione peroxidase in decomposition of endogenously generated H_2O_2. *Arch. Biochem. Biophys* 1993; 210: 505–506.
46. De La Cruz JP, Quintero L, Villalobos MA and Sanchez.de.la Cuesta F. Lipid peroxidation and glutathione system in hyperlipedemic rabbits influence of olive oil administration. *Biochem. Biophys. Acta* 2000; 1485: 36.