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Ameliorative effect on oxidative stress induced by high fat diet in rats by *Averrhoa bilimbi*

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Abstract
Hyperlipidemia causes overproduction of oxygen free radicals which leads to oxidative stress. Lipid lowering drugs for treating hyperlipidemia is associated with many adverse effects. So, bioactive components with hypolipidemic and free radical-scavenging activities have received much attention as potential, nontoxic alternative. The present study was aimed to evaluate the ameliorative effect of *Averrhoa bilimbi* fruit powder in oxidative stress induced by high fat fed diet in liver as well as kidney. Forty eight rats were divided into six groups of eight animals each. Hyperlipidemia was induced by giving HFD for 15 days for all groups except normal control. Rats were fed with *A. bilimbi* fruit powder (125, 250 and 500 mg/kg body weight) and with rosuvastatin (10mg/kg body weight) orally from 16th day along with HFD for 30 days. The animals were sacrificed on day 45 and the liver as well as kidney tissues were dissected out for the assessment of antioxidant status. Antioxidant enzymes like SOD, GSH were found to be increased along with a decrease in LPO after the treatment with *A. bilimbi.* The result indicated the potential of *A. bilimbi* fruit powder in lowering the high fat diet induced oxidative stress in rat liver and kidney.

Keywords: *Averrhoa bilimbi*, high fat diet, oxidative stress, superoxide dismutase, reduced glutathione

Introduction
Normal cellular function depends on a balance between the reactive oxygen species produced and the antioxidant defense mechanisms available in the cell. Hyperlipidemia leads to an increase in free radical production leading to increased lipid peroxidation (Harrison *et al*., 2003) [1] and the oxidative injury could be attributed to the development of hyperlipidemia complications (Stocker, 2004) [2]. The physical properties of cellular membranes are altered during hyperlipidemia which leads to the escape of free radicals. Oxidative modification of LDL is an early event in the pathogenesis of atherosclerosis (Engelmann *et al*., 1992) [3].

The increased oxidative stress linked to hyperlipidemia raises the levels of oxidised low density lipoproteins (ox-LDL) thereby leading to the pathogenesis of atherosclerosis. Studies have shown that the risk of hyperlipidemia was reduced by consumption of plant based foods rich in natural antioxidants (Kris Etherton *et al*., 2002) [4]. Thus, natural remedies which combine antioxidant and hypocholesterolemic activities are expected to be effective in reducing the lipid peroxidation rate, restore the body's antioxidant capacity and prevent the initiation and progression of atherosclerosis in hyperlipidemic patients.

Averrhoa bilimbi is a small-sized tropical tree belonging to oxalidaceae family and its fruits were used traditionally to treat diabetes in India. It is known to possess antibacterial, antiscorbutic and astringent properties. *A. bilimbi* has been widely used in the treatment of fever, mumps, pimples, itches, boils, rheumatism, cough, syphilis, scurvy, whooping cough and hypertension (Goh *et al*., 1995) [5]. This study aims to investigate the antioxidant potential of *A. bilimbi* fruit powder in rats fed with high fat diet (HFD).

Material and Methods
Experimental animals
Forty eight male wistar albino rats weighing between 150-200 g were used for the study. They were acclimatised to the laboratory conditions for 1 week before starting of the experimental work.

Plant materials / chemicals
A. bilimbi fruits were collected from Thrissur district of Kerala and were authenticated. Cholesterol as well as cholic acid were purchased from SRL (Sisco Research Laboratories private Limited, Mumbai). The reference drug used for the study was rosuvastatin (Rosulip 10 mg, Cipla)

Induction of Hyperlipidemia

The high fat diet was prepared by mixing 77 per cent standard diet, 20 per cent coconut oil, 2 per cent cholesterol, 1 per cent cholic acid and 1ml coconut oil supplemented with egg (Hassarajani *et al*., 2007) [6] and was given for 15 days.

Experimental design

For all groups excluding normal control (Group I) hyperlipidemia was induced by giving high fat diet (HFD) for 15 days. Group II remained hyperlipidemic throughout the experiment. Groups III, IV and V were fed with *A. bilimbi* fruit powder (125, 250 and 500 mg/kg body weight respectively) and Group VI with rosuvastatin (10mg/kg body weight) orally from 16th day along with HFD for 30 days. The animals were sacrificed on day 45 and the liver and kidney tissues were dissected out for the assessment of antioxidant status.

Assay of antioxidant parameters

The animals were sacrificed on day 45 and the liver and kidney tissues were dissected out for the assessment of antioxidant status. SOD was estimated according to the method of Madesh and Balasubramanian (1998) [7]. Level of reduced glutathione was determined by the method of Ellman (1959) [8].The levels of lipid peroxidation was determined by the method of Fraga *et al.* (1988) [9].

Statistical analysis

Data were subjected to statistical analysis using One way analysis of variance followed by Duncan Multiple range test (DMRT) and the results were expressed as Mean ± Standard error (SE) of eight rats in each group. Statistical analysis was done by using the statistical software SPSS Version 21.0.

Results and Discussion

The mean values of superoxide dismutase (SOD), reduced glutathione (GSH) and lipid peroxidation (LPO) levels of liver for all the groups (G_I to G_{VI}) on day 45 are depicted in table 1.

Superoxide dismutase (SOD) level

Superoxide dismutase catalyzes the dismutation of superoxide anion (O^{2-}) which is generated from aerobic metabolic reactions into hygrogen peroxide (H_2O_2) and plays a critical role in the defence of cells against the reactive oxygen species (ROS) induced oxidative damage and is inevitable for survival in all oxygen-metabolizing cells (McCord and Fridovich, 1969) [10]. Superoxide dismutase also inhibits oxidative inactivation of nitric oxide to peroxynitrite formation thereby preventing endothelial dysfunction.

In the present study, the normal control group showed mean SOD value of 9.05 ± 0.09 units/mg of protein in liver. The hyperlipidemic control group showed significantly decreased SOD values in liver and kidney compared to G_I. This is in accordance with the findings of Abreu *et al*. (2014) [11] who obtained a decreased SOD activity in the liver of rats fed with a hypercholesterolemic diet containing 25 per cent soybean oil and 1per cent cholesterol. SOD values of G_{VI} treated with reference drug rosuvastatin was found similar to normal control (G_I) group.

Treatment with *A. bilimbi* fruit powder at the dose of 125, 250 and 500 mg/kg body weight resulted in a dose dependant increase in liver as well as kidney SOD levels. These results are in line with the findings of Thamizhselvam *et al*. (2015) [12] who observed that *A. bilimbi* fresh juice significantly increased the SOD levels in liver, kidney and blood in paracetamol intoxicated experimental rats. The presence polyphenolic compounds like flavanoids and tannins may be the reason for the increased antioxidant status in animals (Thamizhselvam *et al*., 2015) [13].

Reduced glutathione (GSH) level

Reduced glutathione (GSH), a tripeptide composed of glutamate, cysteine and glycine amino acid residues is the most abundant non-protein thiol in animal cells. GSH plays a role in the detoxification of hydrogen peroxide, superoxide anion, hydroxyl radicals and electrophilic compounds via catalysis by glutathione S transferases (GST) and glutathione peroxidases (GPx) (Townsend *et al*., 2003) [14].

In the present investigation, the normal control group showed mean GSH value of 17.89 ± 0.47 and 8.27 ± 0.12 µg / mg of tissue in liver and kidney respectively. The hyperlipidemic control group showed significantly decreased GSH values compared to G_I which is in accordance with the findings of Devi *et al*. (2014) [15].

A. bilimbi treated groups showed a dose dependant increase in the levels of GSH in liver as well as kidney indicating the protection against oxidative stress by enhancing antioxidant enzyme levels. This is in agreement with the results of Thamizhselvam *et al*. (2015) [13] who also observed an increase in the GSH activity in liver, kidney and blood after the treatment with *A. bilimbi* fresh juice in paracetamol intoxicated experimental rats. Nagmoti *et al*. (2010) [16] observed methanolic extract of *A. bilimbi* significantly enhanced hepatic GSH levels in CCl_4 intoxicated rats.

Lipid peroxidation (LPO) level

Lipid peroxidation is a self-propagating reaction generated mainly by the attack of ROS on polyunsaturated fatty acids (PUFA) that involves hydrogen abstraction or addition of an oxygen radical resulting in lipid peroxyl radicals and hydroperoxides (Yin *et al*., 2011) [17]. Malondialdehyde (MDA) is one of the most mutagenic secondary products formed during lipid peroxidation (Esterbauer, 1990) [18] and is a widely used biomarker for oxidative stress.

The mean LPO values of normal control group was found to be 56.71 ± 1.02 n M MDA/g and 77.74 ± 0.59 nM MDA/g of tissue in liver and kidney tissues respectively. The hyperlipidemic control group showed significantly elevated TBARS values compared to G_I. This is in accordance with the findings of Abreu *et al*. (2014) [11] and Devi *et al*. (2014) [15] who observed that consumption of the hypercholesterolemic diet increased LPO in liver, indicating increased oxidative stress.

The present study revealed a decrease in lipid peroxidation after the treatment with *A. bilimbi*. The result is in line with treatment with Nagmoti *et al*. (2010) [16] who observed methanolic extract of *A. bilimbi* significantly reversed the increased MDA levels caused by CCl_4. Tan *et al*. (2005) [19] also reported that ethanolic extract and aqueous fraction of *A. bilimbi* resulted in reduction of TBARS formation in streptozotocin (STZ) induced diabetic rats.

Table 1: Effect of *A. bilimbi* fruit on SOD, GSH and LPO in liver of rats fed on a high fat diet. (Mean ± SE, n=8)

Groups	Superoxide dismutase (Units/ mg of protein)	Reduced glutathione (µg / mg of tissue)	Lipid peroxidation (nM MDA/g of tissue)
G_I	9.05 ± 0.09^{a}	17.89 ± 0.47^{e}	56.71 ± 1.02^{c}
G_{I1}	3.52 ± 0.09^{f}	10.27 ± 0.20^{f}	127.75 ± 1.60^{a}
G_{I11}	5.84 ± 0.10^{e}	23.07 ± 0.59^{c}	74.76 ± 1.08^{b}
G_{IV}	6.35 ± 0.11^{d}	24.48 ± 0.12^{b}	73.82 ± 0.90^{b}
G_V	7.72 ± 0.09^{c}	30.19 ± 0.35^{a}	62.46 ± 0.66^{b}
G_{VI}	8.39 ± 0.09^{b}	19.31 ± 0.48^{d}	63.29 ± 0.56^{b}

Table 2: Effect of *A. bilimbi* fruit powder on SOD, GSH and LPO levels in kidney of rats fed on a high fat diet. (Mean ± SE, n=8)

Groups	Superoxide dismutase (Units/ mg of protein)	Reduced glutathione (µg / mg of tissue)	Lipid peroxidation (nM MDA/g of tissue)
G_I	12.26 ± 0.16^{a}	8.27 ± 0.12^{e}	77.74 ± 0.59^{c}
G_{I1}	5.34 ± 0.14^{e}	5.29 ± 0.14^{f}	163.59 ± 1.37^{a}
G_{I11}	7.30 ± 0.11^{d}	14.01 ± 0.31^{d}	96.05 ± 0.95^{b}
G_{IV}	9.25 ± 0.078^{c}	14.57 ± 0.17^{c}	76.40 ± 0.91^{cd}
G_V	10.81 ± 0.54^{b}	15.42 ± 0.14^{b}	76.48 ± 0.08^{cd}
G_{VI}	11.69 ± 0.08^{a}	16.71 ± 0.09^{a}	74.56 ± 0.81^{d}

Conclusion

The present study evaluated the ameliorative effect of oral administration of *A bilimbi* fruit powder on oxidative stress induced by high fat fed diet in liver. Administration of *A. bilimbi* fruit powder at doses of 125, 250 and 500 mg/kg body weight resulted in significant ($p<0.001$) lowering of oxidative stress in hyperlipidemic rats. The results suggest that *A. bilimbi* fruit powder could be a promising agent for the prevention and control of hyperlipidemic complications like oxidative stress.

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