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ANTIOXIDANT ACTIVITY OF *LIMONIA ACIDISSIMA* IN HIGH FAT DIET INDUCED HYPERLIPIDEMIC RATS

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ABSTRACT

To evaluate the *in-vivo* antioxidant potential of ethanolic leaf extract of *Limonia acidissima* against High Fat diet induced hyperlipidemia in rats. Ethanolic leaf extract of *Limonia acidissima* was administered orally (200 mg/kg, po) for 30 days, with HFD and the effect of extract on enzymatic antioxidants like Glutathione, superoxide dismutase (SOD), catalase (CAT) and peroxidase were estimated in liver homogenate. The ethanolic leaf extract of *Limonia acidissima* improved the glutathione, SOD, catalase, and peroxidase levels significantly as compared to control group. The present studies revealed that *Limonia acidissima* leaves have significant *in-vivo* antioxidant activity and can be used to protect tissue from oxidative stress.

Key words: *Limonia acidissima*, Antioxidant, Hyperlipidemia, Atorvastatin.

INTRODUCTION

Free radical induced oxidative damage has long been thought to be the most important consequence of the aging process (Harman, 1992). Such conditions are considered to be important causative factors in the development of diseases such as diabetes, stroke, arteriosclerosis, cancer, and cardiovascular diseases (Prior and Cao, 2000), (Yamaguchi *et al*., 2000). Studies show that these radicals also affect the equilibrium between pro-oxidants and antioxidants in biological systems, leading to modifications in genomes, proteins, carbohydrates, lipids and lipid peroxidation (Romero *et al*., 1998) thus inactivating antioxidant defenses. Plant and its products are rich sources of a phytochemicals and have been found to possess a variety of biological activities including antioxidant potential (Craig, 1970).

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Natural antioxidants are in high demand for application as nutraceuticals, bio-pharmaceuticals, as well as food additive because of consumer preference.

Limonia acidissima L. Swingle Syn. *Feronia elephantum* Correa, *Schinus Limonia* L. (Rutaceae), is a tropical plant species, indigenous to India and locally known as elephant apple. All the parts of *Limonia* are prescribed in indigenous system of medicine for the treatment of various ailments. Fruits are refrigerant, stomachic, stimulant, astringent, aphrodisiac, diuretic, cardiotonic, tonic to liver and lungs, cures cough, hiccup and good for asthma, consumption, tumours, opthalmia and leucorrhoea (Jadeja *et al*., 2005). Unripe fruit is astringent while seeds are used in heart diseases. The fruits are used as a substitute for bael (*Eagle marmelos*) in diarrhea and dysentery (Senthilkumar *et al*., 2010). The bark and leaves are used for vitiated conditions of vata and pitta (Anonymous, 1998). Leaves are astringent and carminative, good for vomiting, indigestions, hiccup and dysentery.

The leaves have hepatoprotective activity (Ilango and Chitra, 2009). The gum is demulcent and constipating, and is useful in diarrhoea, dysentery, gastropathy, haemorrhoids and diabetes (Nadkarni, 2006). The antihyperlipidemic activity of ethanolic leaf extract of *limonia acidissima* has reported. (Veda Vijaya *et al*., 2015). The present work has been, undertaken to study the *in vivo* antioxidant activity on the basis of pharmacological activities attributed to the plant.

MATERIALS AND METHODS

Plant Material

The leaves of *Limonia acidissima*, Linn. were collected from Ammapattai, Bhavani Taluk, Tamilnadu the outskirts of Bhavani, Erode District, Tamilnadu in the month of July 2014. The Plant was identified and authentified as *Limonia acidissima*, Linn. by Botanist Dr. Saravana Babu, Department of Botany, Chikkaiah Naicker College, Erode. The voucher specimen was kept in the laboratory (Specimen No: CNC/ERD/01/30/15) for future reference.

Preparation of Extract

The leaves were washed with water and dried in sunlight for one hour and then it was dried under shade. By the help of grinder the dried leaves were powered to get coarse. Dried course powders of the leaves were extracted with alcohol (90%) by using Soxhlet apparatus. The extracts were then concentrated, dried and stored in desiccators. Obtained dark green alcoholic extract were used for the pharmacological study.

PHARMACOLOGICAL STUDY

Animals

Healthy male Sprague – Dawley rats weighing between 200 – 250 gm were used for this study. The animals were obtained from animal house, IRT Perundurai Medical College, Erode, Tamilnadu, India. On arrival, the animals were placed at random and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24±2^{o}C and relative humidity of 30 – 70 %. A 12:12 light: day cycle was followed. All animals were allowed to free access to water and fed with standard commercial pelleted rat chaw (M/s. Hindustan Lever Ltd, Mumbai). All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethics Committee (688/2/C-CPCSEA) and were in accordance with the Institutional ethical guidelines.

High Fat Diet induced Hyperlipidemic Model – Antioxidant Study

The rats were divided into 5 groups of 6 animals each. The animals of all the groups except normal group were given a high cholesterol diet consisting of 2% cholesterol, 1% cholic acid and 2 ml coconut oil (Rumi Ghosh *et al*., 2010) with standard pellet diet for 30 days. The first group served as normal control received 0.5% Carboxy Methyl Cellulose (CMC) orally for 30 days. The second group served as hyperlipidemic control, was given High cholesterol diet while the third group was treated with ethanolic extract of *Limonia acidissima* leaves (200 mg/kg, p.o.), once a day for 30 days. The fourth group was treated with Atorvastatin suspension prepared with 0.5% CMC (10mg/kg; p.o.), once a day for 30 days.

On 31st day the animals were sacrificed and the liver was rapidly excised, rinsed in ice-cold saline, and a 10% w/v homogenate was prepared using 0.15M KCI, centrifuged at 800 g for 10 min at 4°C. The supernatant obtained was used for the estimation of antioxidants like Glutathione (Buetler *et al*., 1963), superoxide dismutase (Kono, 1978), Catalase (Luck, 1971) and Peroxidase (Alexander, 1962).

Statistical Analysis

Results were expressed as mean ± SEM. The data were analyzed by using one way analysis of variance (ANOVA) followed by Dunnet's t test using GraphPad version 3. P values < 0.05 were considered as significant.

RESULTS

The effects of ethanolic leaf extract of *Limonia acidissima* on antioxidants in high fat diet induced hyperlipidemic rats were shown in the Table. No. 1. The result showed that the activities of glutathione, SOD, catalase and peroxidase in group treated with High Fat Diet declined significantly than that of normal group. Co-administration of ethanolic leaf extract of *Limonia acidissima* at a dose of 200 mg/kg for 30 days markedly prevented these High Fat Diet induced alteration and maintained enzymes level near to normal values. Standard (Atorvastatin) treated group also significantly increased the level of glutathione, SOD, Catalase and peroxidase in High Fat Diet induced hyperlipidemic animals.

DISCUSSION AND CONCLUSION

The antioxidant activity of ethanolic leaf extract of *Limonia acidissima* (200mg/kg) was evaluated to validate the scientific proof on the basis of ethnobotanical information for its antihyperlipidemic activity.

The Result of present study revealed that the oral administration of ethanolic leaf extract of *Limonia acidissima* (200mg/kg) restored the antioxidant enzymes in rats by maintaining the Glutathione, SOD, Catalase and Peroxidase in liver homogenate. This finding provides that the antihyperlipidemic activity reported by the *Limonia acidissima* leaves may be due to its antioxidant property.

Table 1. The effect of ethanolic leaf extract of *Limonia acidissima* on Antioxidant in high fat diet induced hyperlipidemic rats

S.No	Drug Treatment	Antioxidant Parameters			
		Glutathione (µmol/mg of Protein)	**SOD** (Units/mg of Protein)	**Catalase** (µmol/minute/mg of protein.)	**Peroxidase** (Units/mg of Protein)
1	Group I Normal Control 0.5% CMC	18.88±1.21***	31.54±2.71***	11.57±0.98***	83.24±3.59***
2	Group II Hyperlipidemic Control (HFD)	8.6±0.45	17.44±1.27	1.26±0.08	14.97±1.11
3	Group III Reference Control Atorvastatin (10mg/Kg) + HFD	15.98±1.06**	29.67±1.69***	9.74±0.87***	68.22±2.85***
4	Group IV *Limonia acidissima* (200mg/Kg) + HFD	14.79±0.62**	21.68±2.32**	9.12±0.33***	59.52±2.80***

The values were expressed as Mean ± SEM (n=6) *P<0.05,**P<0.001 & ***P<0.001 Vs Hyperlipidemic Control

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