Research Article

ANTIHYPERLIPIDEMIC AND ANTIOXIDANT ACTIVITIES OF *MANGIFERA INDICA LINN.*, IN ALLOXAN INDUCED RATS

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ABSTRACT

Present investigation was undertaken to evaluate antihyperlipidemic and antioxidant activities of ethanolic extract of *Mangifera indica* leaves in alloxan induced hyperlipidemic rats. Alloxan produced a significant increase in cholesterol, triglycerides, VLDL, LDL levels. Treatment with *Mangifera indica* extract produced decrease in alloxan induced lipid levels. There was a significant decrease in GSH, SOD, CAT, GP_X, GR and HDL. Administration of *Mangifera indica* to hyperlipidemic rats reduced the effect of lipid peroxidation and increased the activities of antioxidant enzymes. The results suggest *Mangifera indica* to be beneficial for the treatment of hyperlipidemia.

Keywords: Ethanolic extract, Alloxan, Hypo lipidemic effect, *Mangifera indica.*

INTRODUCTION

Hyperlipidemia is an important risk factor in the initiation and progression of atherosclerosis and coronary heart disease. These are the most common form of heart diseases of lipoprotein disorder and the single most important causes of premature death in the developed world. In the UK one in four men and one in 5 women die from this disease. It was estimated that 3,00,000 people have myocardial infarction. Each year approximately 1.7 million people have angina. The death rats from coronary heart disease in the UK are among the highest in the world more than 1.500,000 people died from coronary heart disease but are falling unfortunately the incidence increased in Eastern Europe and many developing countries[1]. Hyperlipidemia and associated lipid disorders are considered to cause the atherosclerotic cardiovascular disease[2].

Alloxan selectively kills the insulin producing beta cells found in the pancreas, it is used to induce diabetes in laboratory animals. This occurs most likely because of selective uptake of the compound due to its structural similarity to glucose[3]. However, alloxan is not toxic to the human beta cell, even in very high doses, probably due to differing glucose uptake mechanism in humans and rodents.It is however toxic to the liver and the kidney in high doses[4]. In the experimental animals alloxan produces hyperglycemia, hyperlipidemia and oxidative stress.

Cells have developed a comprehensive set of antioxidant defense mechanisms to prevent free radicals[5]. The principle enzymatic antioxidants are SOD, CAT and GP_X. Enhanced lipid peroxidation associated with depletion of antioxidants in tissues is a characteristic observation in alloxan treated rats[6]. Alloxan induces oxidative stress. Several studies have shown that alloxan alter the activity of antioxidant enzymes like SOD, CAT, GP_X, GR, and antioxidant molecules like GSH in animals[7] and human beings[8].

Phytochemical research from different parts of *M. indica* has demonstrated the presence of phenolic constituents, triterpenes, flavonoids, phytosterol, and polyphenols[9-13]. This species is purposed to possess numerous therapeutic properties including analgesic, anti inflammatory[14], immunostimulant[15], antioxidant[16,17], spasmolytic, anti diarrhoea[18], antilipidemic[19], antidiabetic[20], antiamebic[21] anthelminthic, antiallergic[22] and anti bacterial applications.

MATERIALS AND METHODS

Collection of plant materials: Fresh leaves of *Mangifera indica* were collected during March-April 2011, from Mahadhevapattinam, Thiruvarur District, Tamilnadu, India and botanically identified. The leaves were washed with distilled water, shade dried, powdered, and stored in an air tight container until future use.

Preparation of ethanolic extract: Preparation of plant extract was done according to the previously described procedure. The collected fresh leaves were thoroughly cleaned with distilled water, dried well and powdered. It was soaked in absolute ethanol in cold (72 hrs). After three days, the extract was filtered, and then it was evaporated at 40°C in cylindrical water bath for the elimination of solvent. A semisolid extract (40g) was obtained after complete elimination of alcohol under reduced pressure. It was stored in refrigerator until used.

Experimental animals: In the present study, healthy, pathogen free, albino rats (both sexes) of Swiss strain weighing 150-200g were purchased from Rainbow institute, Bangalore, Karnataka, India, and housed under standard husbandry conditions (30°C±2°C, 60-70% relative humidity and 12h : 12h day- night cycle), supplied

with standard rat feed (Sai Durga feed and food, Bangalore) and water *ad libitum.*

Glass wares and chemicals: All the glass wares used for analytical purpose were Borosil make, and ethanol used for the extraction of plant leaves was obtained from Scientific chemicals, Chennai. Alloxan was purchased from Pvt. Ltd., Cochin. All other chemicals and reagents used in this study were procured from Qualigen and Ranbaxy fine chemicals Pvt. Ltd., Mumbai. All chemicals used were of analytical grade.

Experimental design: Animals were divided into five groups of six animals each. Group I served as control which received standard feed and water. Group II Disease induced by intraperitoneal injection of alloxan (2mg /100g bodyweight) daily for 20 days. Group III Co treated with alloxan (2mg/100g b.w., intraperitoneally) and ethanolic extract of *Mangifera indica* (300mg/kg b.w.,) orally. Group – IV Received herbal extract in a dosage of (300mg / kg b.w.,) orally.

Study protocol: The test formulations were administered for 20 days, once in a day. At the end of experiment rats were sacrificed by cervical decapitation. Blood was collected and centrifuged for serum separation. The tissues were dissected out, weighed and washed using ice cold saline solution and dried between the folds of filter papers, weighed and homogenized using standard phosphate buffer in glass homogenizer with teflon pestle. The homogenate was then centrifuged at 1000 rpm for 5 minutes and the supernatant was used for various biochemical assay.

Statistical Analysis: The values are expressed as mean ± S.D. The statistical comparison was performed by one way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT), using SPSS version 12 for windows (SPSS Inc. Chicago; http: www.spss.com). The values considered statistically significant if the p value was less than 0.05.

RESULTS AND DISCUSSION

In the present investigation alloxan induced hyperlipidemic rats were found with increase in triglycerides, total cholesterol, LDL cholesterol, VLDL cholesterol levels (Table 1) which correlates with earlier findings that an increase in lipid levels is observed not only in diabetic animals but also in diabetic patients[23]. The HDL cholesterol is involved in transport of cholesterol from peripheral tissues to liver and thereby it act as a protective factor. In the present study also, level of HDL cholesterol was found to be decreased in disease induced rats. The level of HDL cholesterol was increased in alloxan induced diabetic rats when treated with *Mangifera indica*. This indicates that *Mangifera indica* may help to transport peripheral tissue cholesterol to liver and thereby decreased the blood cholesterol.

In the present study, dyslipidemia observed in alloxan induced hyperlipidemic rats is in accordance with the previous research findings[24,25]. The diabetes induced hyperlipidemia may be due to excess mobilization of fat from adipose tissue because of underutilization of glucose[26]. The hypolipidemic action of *Mangifera indica* may be due to its potential to resist lipid peroxidation[27,28].

Administration of ethanolic extract of Mangifera indica led to significant reduction in TG, TC and LDL cholesterol with a concomitant elevation in HDL cholesterol. Improvement in serum lipid profile following treatment with the extract suggested its role as a lipid lowering agent

Table 1: The effect of *Mangifera indica* extract on Lipid profile

Groups Treatment	Triglycerides (mg/dL)	Total cholesterol (mg/dL)	VLDL (mg/dL)	LDL (mg/ dL)	HDL (mg/dL)
Group I Control	63±2.4	85.25±1.3	13.07± 2.6	12.05± 1.5	37.01±2.1
Group II Alloxan alone	130.8±3.4^{+}	105± 2.9^{+}	27.29±2.5^{+}	32.9±2.4^{+}	20.27±2.3^{+}
Group III Alloxan+MIELEt (300mg/ kg b.wt)	70.6±4.3*	90.56±1.8*	24.05±3.1*	25.88±1.4*	32.20±2.0*
Group IV MIELEt (300mg/ kg b.wt)	62.31±2.6*	83.0± 1.6*	12.09± 1.9*	11.25±1.2*	39.3±1.3*

Values are mean ± SD (n=6); MIELEt – *Mangifera indica* ethanolic leaves extract; $^{+}p < 0.05$ compared to normal control rats; $^{*}p < 0.05$ compared to diseases control rats

Table 2: The effect of *Mangifera indica* extract on Antioxidant parameters

Groups Treatment	GP_X (U/L)	GR(U/L)	SOD(U/L)	CAT(U/L)	GSH(mg/dL)
Group I Control	66.6±4.4	31.0±2.5	14.7±1.3	8.5±1.0	21.7±2.19
Group II Alloxan alone	36.8±3.2^{+}	22.42±1.3^{+}	8.3±0.6^{+}	5.9±0.8^{+}	18.5±1.8^{+}
Group III Alloxan+MIELEt (300mg/kg b.wt)	48.6±4.3*	29.42±1.43*	12.9±1.1*	6.7±0.35*	20.3±1.33*
Group IV MIELEt (300mg/kg b .wt)	68.23±4.5*	32.02± 3.1*	15.0±1.6*	9.7±0.45*	22.5±1.87*

Values are mean ± SD (n=6); MIELEt – *Mangifera indica* ethanolic leaves extract; $^{+}p < 0.05$ compared to normal control rats; $^{*}p < 0.05$ compared to diseases control rats

Diabetic dyslipidemia has long been shown to have a strong relation with coronary heart disease (CHD). CHD is the most dangerous and life threatening complication of diabetes and the risk for CHD is diabetes increase two or more fold[29]. Increased TG and TC levels and decreased HDL cholesterol represent a deranged lipid profile known as atherogenic profile which leads to the development of CHD. As favourable effect on lipid profile was observed following treatment with extract, this indicated that extract might help to prevent the progression of cardiovascular diseases. In addition, several atherogenic indices such as TG/HDL cholesterol, TC/HDL cholesterol and LDL cholesterol /HDL cholesterol have been used to predict CHD risk[30,31]. Reduction of these indices in *Mangifera indica* treatment strongly supports to reduce the risk of developing heart diseases.

Exogenous chemicals and endogenous metabolic processes in the human body or in food system might produce highly reactive free radicals, especially oxygen derived radicals, which are capable of oxidizing biomolecules, resulting in cell death or tissue damage. These oxidative damages play a significantly pathological role in human diseases. Free radicals lead to cellular necrosis, which is implicated in some pathophysiological conditions including nephrotoxicity, atherosclerosis, rheumatoid arthritis as well as toxicity of many xenobiotics, ageing, inflammatory syndrome, respiratory diseases, liver diseases, cancer and AIDS.

Antioxidants in herbal plants protect cells against the damaging effects of reactive oxygen species (ROS), such as singlet oxygen, superoxide, peroxyl radical, hydroxyl radicals and peroxynitrite. They can also neutralize the side effects of free radicals by scavenging or chain breaking. These antioxidants must be constantly replenished since they are 'used up' in the process of neutralizing free radicals[32].

The balance between oxidants and antioxidant is crucial for the maintenance of the biological integrity of the tissues[33]. The depletion of SOD, CAT, GR, GP_X and GSH (Table 2) appears to be an early and necessary event occurring in alloxan induced diabetes mellitus and subsequent toxicity.

SOD is the major attractive metalloprotein in the antioxidant family. Increased synthesis of superoxide dismutase against superoxide anion radicals (O_2^-) production is an adaptive response of cells through the stimulation of gene transcription. The first line defense that the body has against superoxide free radicals is the enzyme known as "superoxide Dismutase" or (SOD), The importance of SOD is so paramount for the protection of our cells, that it SOD keeps oxygen under control[34]. In the process of removing superoxide free radicals, SOD requires catalase to remove hydrogen peroxide molecules which are by – products of the reactions created by SOD.

Reduction in SOD, CAT, GPx, GR and G-SH levels were found in disease induced group. Treatment with *M.indica* extract brought back the levels of anti oxidants to near normal. In the present study, treatment with *M.indica* reverted the altered lipid profile and anti oxidant levels.

CONCLUSION

The data of our studies suggests that *Mangifera indica* is more beneficial in hyper lipidemic and its associated complications, holding hope of the new generation anti hyper lipidemic drug.

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