WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 6.647
Volume 6, Issue 10, 63-75 Research Article ISSN 2278 – 4357

IN-VITRO ANTIOXIDANT ACTIVITY AND ANTI-HYPERLIPIDEMIC EFFECT OF ETHANOL EXTRACT OF *MANGIFERA INDICA* LEAVES AND BARK ON WISTAR ALBINO RATS

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Article Received on
30 July 2017,

Revised on 20 August 2017,
Accepted on 10 Sept. 2017,

DOI: 10.20959/wjpps201710-10009

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ABSTRACT

The Anti-lipidemic activity of *Mangifera indica* leave and bark on rat model and in vitro antioxidant activity of the plant bark was studied. The antioxidant activity was determined using nitric oxide, 1,1-diphenyl-2-picrylhydrazyl (DPPH) and anti-lipid peroxidation free radical scavenging activities. The plant extract showed highest inhibition in nitric oxide (93.5 ± 0.71) at the highest concentration 200mg/ml used. This is higher compared to lipid peroxidation and DPPH % scavenger of the plant extract which are (81.5 ± 3.54) and (62 ± 5.66) respectively at the same concentration of 200mg/ml. the % inhibition of the plant bark extract showed low inhibition concentration as against 50% (IC_{50}). Serum lipid assay (total cholesterol, high density lipoprotein, low density lipoprotein, triglycerides and very low density lipoprotein) of the rats were measured to assess the anti-lipidemic activity of both the plant leave and bark extract. The result shows non-significant changes in all the parameters between the control groups and the rats administered with the plant leave and bark extract. It can be deduced from the lipid assay that the administration of the ethanol plant extracts did not cause accumulation of lipid in the serum level of the rats. The LDL-c level was also on the normal range. Therefore this plant can be used as an anti-lipidemic agent.

KEYWORDS: Anti-lipidemic, antioxidant, Mangifera indica, lipid peroxidation, DPPH and nitric oxide.

INTRODUCTION

Medicinal plants have been identified and used throughout human history.[1]

Mangifera indica, also known as mango has been an important herb and indigenous medicine for over 4000 years. Mangoes belong to the genus *mangifera* which consists of about 30 species of tropical fruiting trees in the flowering plant family Anacaridiaceae. According to ayurveda, varied medicinal properties are attributed to different parts of the mango tree. Mangoes are very low in saturated fat, cholesterol and sodium. They are also an excellent source of dietary fiber and vitamin B6, as well as a good source of vitamin A and vitamin C. Mangoes are rich in minerals like potassium, magnesium and copper and they are one of the best sources of quercetin, beta carotene and astragalin. These powerful antioxidants have the power to neutralize free radicals throughout the body. Ailments like heart disease, premature aging, cancer and degenerative diseases are due to these free radicals that damage the cells.

The tonic made from mangoes in herbal medicine is used to treat bleeding gums, anemia, cough, constipation, nausea, fever, sea sickness and as a cure for weak digestion.[2]

Mangoes have an impressive vitamin content that assures overall health. They are rich in potassium (4% in 156 mg) and magnesium (2% in 9 mg) and mangoes are great natural remedies for high blood pressure patients. They also contain selenium, calcium, iron and phosphorus. Mangoes are said to be vitamin powerhouses as they are rich in riboflavin, vitamin B6, A, C, E, K, niacin, folate, thiamin and panthothenic acid. These components help you to avoid a host of diseases that can come from deficiencies of these vitamins and minerals.[3]

Oxidative stress reflects an imbalance between the systemic manifestation of reactive oxygen species and biological system's ability to detoxify the reactive intermediates or repair the resulting damage. Disturbances in the normal redox state of cells can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including proteins, lipids and DNA. In humans, oxidative stress is thought to be involved in the development of cancer[2], Parkinson's disease[3], Alzheimer's disease and atherosclerosis[4], heart failure[5], myocardial infarction[6,7], fragile X syndrome[8], sickle cell disease[9], linchen planus.[10]

An antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons or hydrogen from a substance to an oxidizing agent. Oxidation reactions can produce free radicals. In turn, these radicals can start chain reactions. When the chain reaction occurs in a cell, it can cause damage or death to the cell. Antioxidants terminate these chain reactions by removing free radical intermediates and inhibit other oxidation reactions. They do this by being oxidized themselves, so antioxidants are often reducing agents such as thiols, ascorbic acid, or polyphenols.[11]

Insufficient levels of antioxidants, or inhibition of the antioxidant enzymes, cause oxidative stress and may damage or kill cells. Oxidative stress is damage to cell structure and cell function by overly reactive oxygen-containing molecules and chronic excessive inflammation. Oxidative stress seems to play a significant role in many human diseases, including cancers.[12] The use of antioxidants in pharmacology is intensively studied, particularly as treatments for stroke and neurodegenerative diseases. For these reasons, oxidative stress can be considered to be both the cause and the consequence of some diseases. Antioxidants are found in certain foods and may prevent some of the damage caused by free radicals by neutralising them. These include the nutrient antioxidants, vitamins A, C and E and the minerals, copper, zinc and selenium. Other dietary food compounds, such as the phytochemicals in plants, are believed to have greater antioxidant effects than vitamins or minerals. These are called the non-nutrient antioxidants and include phytochemicals, such as lycopenes in tomatoes and anthocyanins found in cranberries.[7]

Hyperlipidemia, hyperlipoproteinemia, or involves abnormally elevated levels of any or all lipids and/or lipoproteins in the blood. It is the most common form of dyslipidemia (which includes any abnormal lipid levels). Hyperlipidemias are divided into primary and secondary subtypes. Primary hyperlipidemia is usually due to genetic causes (such as a mutation in a receptor protein), while secondary hyperlipidemia arises due to other underlying causes such as diabetes. Lipid and lipoprotein abnormalities are common in the general population, and are regarded as a modifiable risk factor for cardiovascular disease due to their influence on atherosclerosis. In addition, some forms may predispose to acute pancreatitis.

In medicine, combined hyperlipidemia is a commonly occurring form of hypercholesterolemia characterized by increased LDL and triglyceride concentrations, often accompanied by decreased HDL.[13]

The essence of this work is to bring back the consciousness of the usefulness of the mango tree as a medicinal plant. In these era, the use of traditional medicine is going extinct as a result of the introduction of modern medicines whose main ingredient are also the major constituents of the plant. The plant kingdom has untapped medicinal potentials and this has led to a resurgence of interest in ethno-medicine, ethno-botany and ethno-pharmacology. Only about 1% of tropical species have been studied for pharmaceutical potentials.[14] Therefore this work was designed to evaluate the *In-Vitro* Antioxidant Activity and anti-Hyperlipidemic effect of ethanol extract of *Mangifera indica* leaves and bark on wistar albino rats.

MATERIALS AND METHODS

Collection of plant materials

The plant samples of *Mangifera indica* leaves and bark were gotten from the residential area of the National root crop research institute Umudike, Abia state, situated in the south-eastern geo-political zone of Nigeria and was identified by Dr. Garuba Omosun of the department of plant science and biotechnology, Michael Okpara University of Agriculture, Umudike. A voucher specimen (Herbarium No. 1331) was deposited in the Herbarium of the department of plant science and biotechnology, College of Natural sciences.

Preparation of plant extract

The leaves and bark of *Mangifera indica* plant was oven dried using carbolite electric oven England at 52^{o}c at the biochemistry laboratory National Root Crop Research Institute for 3 days and was pulverized by a pulverizing machine at the soil science laboratory of the same institute.

50g of each of the samples was soaked in 200ml of 95% ethanol for a period of 72 hours. The resulting aqueous mixture was filtered with whatman number one filter paper to obtain the filtrate. The filtrate was then concentrated by heating in a water bath. An approximate value of 3.5g was obtained for the leave extract and 5.0g for the bark extract and was stored at room temperature.

Experimental animals

Twenty-four wistar albino rats of both sexes weighing between 180g to 200g and swiss mices were obtained from the biochemistry department of the University of Nigeria Nsukka Enugu state and was housed at the animal house of the department of biochemistry Michael Okpara

University of Agriculture Umudike were it was acclimatized for a period of two (2) weeks. They were allowed free access to pelleted vital feed and clean water.

EXPERIMENTAL DESIGN

Acute toxicity test

The acute toxicity test was done using 20 Swiss mice of both sexes. The mice were divided into five groups, each having four (4) mice.

Group A mice received 1000mg/ml while group B, C, D and E received 500mg/ml, 250mg/ml, 125mg/ml and 62.5mg/ml respectively. The route of administration of the extract was through intra peritonea route of administration as described by Miller and Kinter 1994.The number of death in each group was recorded within a period of 72 hours. The lethal dose 50 was calculated using the following formula.[15] [16]

$LD_{50}\sqrt{\text{conc. with the highest death} \times \text{ lowest conc. without death.}}$

$LD_{50}\sqrt{1000 \times 250}$

LD_{50} =500mg/ml.

Sub-acute toxicity

Twenty four (24) wistar albino rats were used for the study. The experimental animals were divided into six (6) groups of four (4) rats in each treatment of 1, 2, 3, 4, 5 and 6. Treatment 1 was given the extract in a concentration of 400mg/ kg, treatment 2 was given 300mg/kg, treatment 3 received 200mg/kg, treatment 4 received 100mg/kg, treatment 5 received 50mg/kg and treatment 6 which is the control group was given normal saline all at the volume of 0.5ml, two times daily for 14 days. The extract was given orally using gavage. The animals were sacrificed and the blood collected through cardiac puncture. The collected blood samples were centrifuged for 15 minutes at 2500rpm and plasma separated for lipid profile analysis.

Biochemical assessment of lipid profile

The biochemical parameters for the lipid profile notably total cholesterol (TC), triglycerides (TAG) and High-density lipoprotein cholesterol (HDL-C) level in the blood serum were measured by enzymatic method using Randox Diagnostic Kits Spectrophotometrically. Low density lipoprotein was calculated as per Fried Wald's equation.[17]

VLDL = TG/5, LDL = TC – (HDL+ VLDL).

Antioxidant assay

DPPH radical scavenging assay

The free radical scavenging capacity of M. indica extracts were determined using DPPH. The DPPH (5 mg) was weighed accurately using a pre-calibrated weighing balance and transferred to a 5 mL volumetric flask, dissolved (by sonication) and diluted with methanol to achieve 1 mg mL-1 strength (stock I). The stock I was diluted with methanol to obtain 0.3 mM DPPH solution. The control reaction mixture consisted of 100 µL of 0.3 mM DPPH solution and 3.9 ml of methanol whereas test reaction mixture consisted of additional 100 µL of 1 mg mL-1 extracts of M. indica. After an incubation period of 30 min, reduction of a DPPH free radical was measured by recording the absorbance (abs) of test and control reaction mixtures at 517 nm. The % scavenges and absorbances were obtained for the concentrations at 200mg, 100mg, 50mg, 25mg, vitamin C at 100mg and the control. The percentage of the DPPH radical scavenging is calculated using the equation.

$$\% \text{ inhibition of DPPH radical} = \frac{\text{Abs. of control} - \text{Abs of test sample}}{\text{Abs of control}} \times 100$$

Nitric Oxide Scavenging Activity

The compound sodium nitroprusside is known to decompose in aqueous solution at physiological pH (7.2) producing NO under aerobic conditions, NO reacts with oxygen to produce stable products (nitrate and nitrite) the quantities can be determined using Griess reagent.[16] Two (2)ml of 10mM sodium nitroprusside dissolved in 0.5ml phosphate buffer saline (pH 7.4) was mixed with 0.5ml of sample at various concentrations (0.2-0.8mg/mL). The mixture was then incubated at 25oC. After 150min of incubation, 0.5mL of the incubated solution is withdrawn and mixed with 0.5mL of Griess reagent [(1.0mL sulfanilic acid reagent (0.33% in 20% glacial acetic acid at room temperature for 5min with 1ml of naphtylenediaemine dichloride) 0.1%w/v)]. The mixture is then incubated at room temperature for 30mins and its absorbance pouring into a cuvette is measured at 546nm. The concentrations used is 200mg, 100mg, 50mg, 25mg vitamin C 100mg. The amount of nitric oxide radical is calculated as:

$$\% \text{ inhibition of NO radical} = \frac{\text{Abs of control} - \text{Abs of test sample}}{\text{Abs of control}} \times 100$$

Lipid Peroxidation (LPO) Assay

Lipid peroxidation is an autocatalytic process, which is acommon consequence of cell death. This process may cause peroxidative tissue damage in inflammation, cancer and toxicity of

xenobiotics and aging. Malondialdehyde (MDA) is one of the end products in the lipid peroxidation process. LPO in this homogenate is determined by measuring the amounts of malonaldehyde produced primarily. Tissue homogenate (0.2ml), 0.2ml of 8.1% sodium dodecyl sulfate (SDS) 1.5mL of 20% acetic acid and 1.5mL of 8% CBA are added. The volume of the mixture is made up to 4mL with distilled water and then heated at 95oC on a water bath for 60 min using glass ball as condenser. After incubation, the tubes are cooled to room temperature and final volume was made to 5ml in each tube. Five ml of butanol extract:pyridine (15:1) mixture is added and the contents are vortexed thoroughly for 2 mins. After centrifugation at 3000rpm for 10mins, the upper organic layer is taken and its OD is taken at 532nm against an appropriate blank without the sample. The levels of lipid peroxides can be expressed as n moles of thiobarbituric acid reactive substances (TBARS)/mg protein using an extinction coefficient of 1.56× 105 ml cm-1.s.

Statistical Analysis

The results are presented as mean ± SEM (Standard Error of Mean). It was statistically analyzed using one-way Analysis of Variance (ANOVA). The data for the plant extracts were correlated using Pearson Correlation Coefficient and it was considered statistically significant at the value of $p \leq 0.05$.

RESULTS

Table 1: Antioxidant Result for Nitric Oxide Inhibition Activity of *Mangifera indica* Bark Extract at P ≤ 0.05.

Concentration (mg/ml)	% Inhibition Activity	
	% Inhibition	Standard (IC_{50})
200	93.5± 0.71	
100	74.0.± 4.24	
50	50.0± 3.31	91.4 ±0.29
25	19.0± 1.41	

NITRIC OXIDE

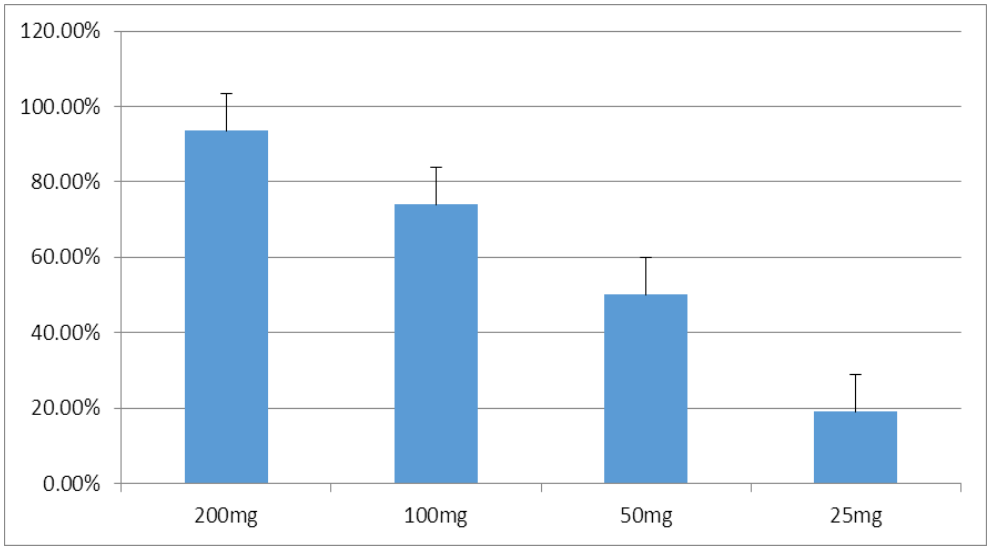

Figure 1; % Inhibition of Nitric Oxide by Mangifera indica Bark extract.

Table 2. Anti-lipid Peroxidation Activity on Mangifera indica Ethanol Extract at P ≤ 0.05.

Concentration (mg/ml)	% Inhibition Activity	
	% Inhibition	Standard (IC_{50})
200	81.5± 3.54	
100	57± 7.07	
50	43.5± 4.95	94.6±0.20
25	16± 5.65	

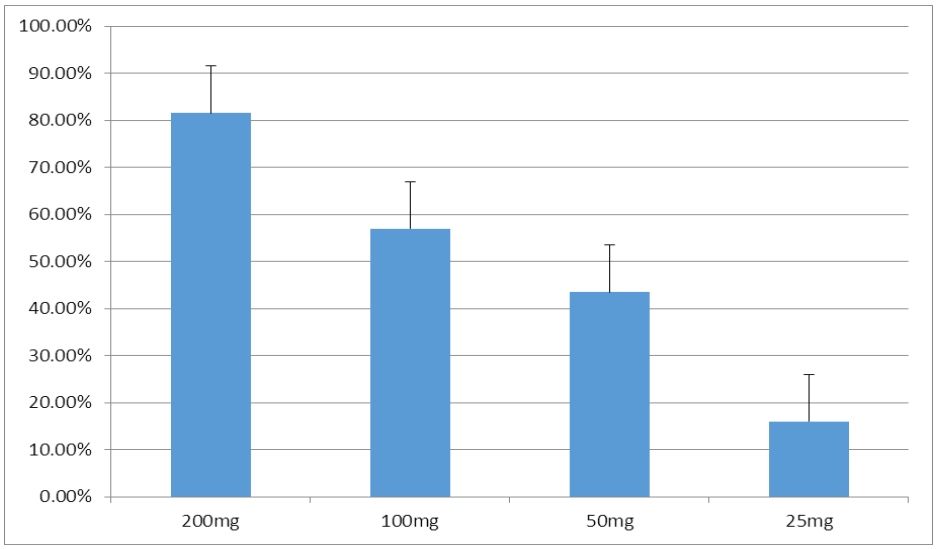

Figure 2: % Inhibition of Anti Lipid Peroxidation Activity by Mangifera Indica Bark extract.

Table 3; DPPH Result for % Scavenging Activity on Mangifera indica Bark Extract at P≤ 0.05.

Concentration (mg/ml)	% Inhibition Activity	
	% Inhibition	Standard (IC_{50})
200	62± 5.66	
100	41.50± 2.21	
50	23.5± 2.21	96.50 ±0.29
25	6.5± 2.12	

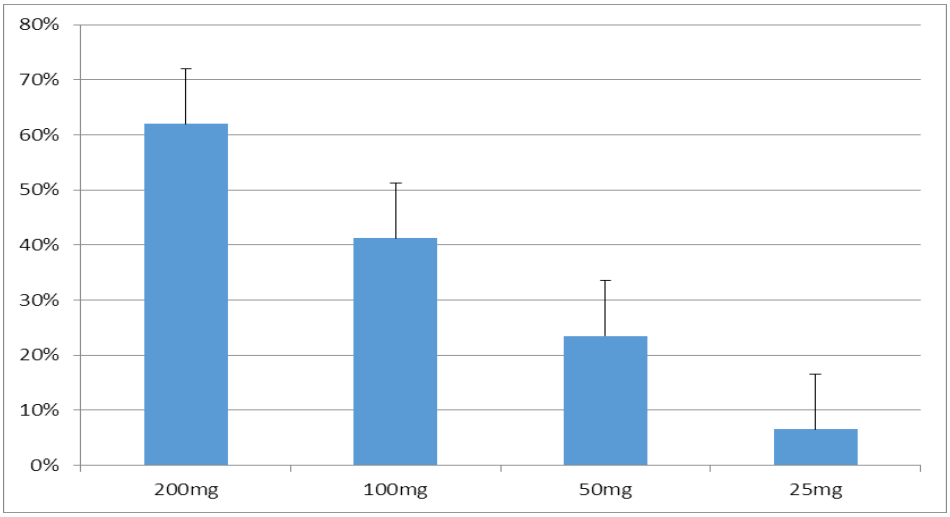

Figure 3; Represents in Vitro Concentration Dependent Percentage Inhibition of DPPH Scavenging Activity by Mangifera indica Bark.

Table 4: Result of Anti-lipidemic Activity of Leave Extract at p≤ 0.05.

GROUPS (mg/kg)	TC (mmol/l)	TAG (mmol/l)	HDL (mmol/l)	LDL (mmol/l)	VLDL (mmol/l)
400	4.6 ± 0.42	1.5 ± 0.42	1.45 ± 0.07	2.05 ± 0.21	0.30 ± 0.08
300	4.3 ± 0.14	1.3 ± 0.14	2.0 ± 0.14	2.45 ± 0.07	0.26 ± 0.04
200	4.35 ± 0.35	1.45 ± 0.07	2.9 ± 0.14	1.15 ± 0.05	0.29 ± 0.01
100	3.53 ± 0.78	1.35 ± 0.07	1.65 ± 0.07	1.7 ± 0.28	0.36 ± 0.03
50	4.65 ± 0.21	1.43 ± 0.21	1.95 ± 0.35	2.25 ± 0.09	0.29 ± 0.05
0.5 normal saline	3.35 ± 0.21	1.45 ± 0.06	1.15 ± 0.06	2.1 ± 0.30	0.32 ± 0.02

Table 5. Result of Anti-lipidemic Activity of Mangifera indica Bark at p ≤ 0.05.

GROUPS (mg/kg)	TC (mmol/l)	TAG (mmol/l)	HDL (mmol/l)	LDL (mmol/l)	VLDL (mmol/l)
400	4.45 ± 0.35	1.4 ± 0.14	1.55 ± 0.07	2.55 ± 0.21	0.28 ± 0.02
300	4.45 ± 0.07	1.4 ± 0.28	1.50 ± 0.14	2.35 ± 0.04	0.28 ± 0.05
200	4.30 ± 0.42	1.2 ± 0.14	1.75 ± 0.21	2.15 ± 0.05	0.24 ± 0.03
100	3.95 ± 0.07	1.75 ± 0.07	1.95 ± 0.03	1.7 ± 0.28	0.35 ± 0.01
50	4.70 ± 0.14	1.65 ± 0.35	1.60 ± 0.14	2.45 ± 0.07	0.33 ± 0.07
0.5ml normal saline	3.70 ± 0.14	1.40 ± 0.14	1.30 ± 0.14	2.10 ± 0.25	0.28 ± 0.03

DISCUSSION

Table 1 presents the results obtained for % inhibition of nitric oxide antioxidant activity of *Mangifera indica* bark extract. Table 2 presents the % scavenging activity of lipid peroxidation and table 3, the % scavenging activity of DPPH. Figure 1, 2 and 3 represents the nitric oxide inhibitory effect, anti-lipid peroxidation activity and DPPH scavenging activity respectively.

From table 1, 2 and 3, it was observed that nitric oxide has the highest inhibitory effect at all concentrations when compared to the scavenging activity of anti-lipid peroxidation and DPPH scavenger. It is therefore seen that nitric oxide has a better scavenging ability on *Mangifera indica* bark than anti-lipid peroxidation and DPPH scavenger. The antioxidant assay shows it to be concentration dependent. Based on the inhibitory concentration of a substance, ethanolic extract of *Mangifera indica* bark will have to contain as much quantity of antioxidant compounds as equivalent of vitamin C to effectively reduce the oxidants in the reaction. Antioxidant capacity of vitamin C has been used as a reference standard from which plant extracts with potential activity are compared.[20] Nitric oxide radicals play important roles in various types of inflammatory conditions including juvenile diabetes, multiple sclerosis, arthritis and ulcerative colitis.[18] The antioxidant effect of plant products is mainly due to radical scavenging activity of phenolic compounds such as flavonoids, polyphenols and tannins.[21] The antioxidant effect of this plant extract agrees with the work[22] on the standard drug vitamin C as it scavenges free radicals and increases as concentration increases. The plant extract possesses antioxidant activity.[23]

From table 4 and 5 oral administration of *Mangifera indica* leave and bark extracts in 400mg/kg, 300mg/kg, 200mg/kg, 100mg/kg and 50mg/kg concentration for 14 days showed non-significance ($p<0.05$) changes in the serum total cholesterol (TC), triglycerides (TG), low density lipoprotein cholesterol (LDL-c) and very low density lipoprotein (VLDL-c) of the rats compared to the control. HDL–cholesterol was significantly increased in the rats treated with the extracts when compared to those in the control group. HDL cholesterol levels have a protective role in coronary artery diseases.[24] HDL is reported to have a protective function against atherogenesis since an independent inverse relationship between bloods HDL-c levels and cardiovascular risk incidence has been reported.[25] During blood circulation, HDL-c mediates the transfer of excess cholesterol from the peripheral cells to the liver for its catabolism by a pathway termed as “reverse cholesterol transport” hence

increased serum HDL-c levels may prove beneficial in lipid disorders and might also serve as a cardioprotective factor to prevent the gradual initiation of atherosclerotic process. Apart from wide usage of mango as antidiabetic it can also be used for the treatment of hyperlipidemia. The mango extract at a dose of 400 mg/kg orally showed significant anti-hyperlipidemic activity which may be attributed due to presence of flavonoids, saponins, glycosides, tannins, phenolics. The slight increase in HDL-c on administration of the plant samples is an indicator of the plant as a good antihyperlipidemic agent.

CONCLUSION

The result obtained from this study have led to the conclusion that ethanolic extract of the leave and bark of *mangifera indica* has significant antioxidant and anti-hyperlipidemic activity and hence can be exploited as an anti-hyperlipidemic therapeutic agent.

ETHICAL ISSUES

All animal experiments were in compliance with the National Institute of Health Guide for care and use of laboratory animals (Pub. No. : 85-23, revised 1985). An approval for the use of animals' experimental protocols was secured from the university committee of ethical and appropriate use of animals.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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