Asian Science Bulletin

OPEN ACCESS

Phytochemical Profiling (GC-MS) of the Leaf Extract of *Mangifera indica* and its Hypolipidemic Effect on Serum Lipid Profile in Streptozotocin-Induced Diabetic Wistar Rats

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ABSTRACT

Background and Objective: Medicinal plants have been known to play a crucial role in the health system since time immemorial as plants contain natural compounds with medicinal properties. This research is aimed at profiling and investigating the hypolipidemic effect of ethanol extract of *Mangifera indica* in streptozotocin-induced diabetic Wistar rats. **Materials and Methods:** The albino Wistar rats weighing 110 to 242 g were randomly distributed into six groups of five rats each. Group I served as a normal control and was given feed and distilled water. Group II served as diabetic control. Groups III, IV, V and VI were induced with 40 mg/kgb. of streptozotocin. Group III was treated with 5 units/b.wt., of insulin. Group IV, V and VI were treated with 200, 300 and 400 mg/kg of *Mangifera indica* extract respectively. The duration of treatment was twenty one days. Extraction and biochemical analysis were carried out using standard laboratory techniques. The chemical components of the leaf extracts were characterized by GCMS analysis. **Results:** Administration of the extract resulted in a significant ($p<0.05$) decrease in TG, TC and LDL concentrations in all experimental groups when compared to the control group but an increase in HDL and VLDL concentrations. **Conclusion:** It may be concluded that the ethanol leaf extract of *Mangifera indica* may possess hypolipidemic properties and could be used to regulate lipid levels. The plant extract's GC-MS examination revealed that the most prevalent compounds were oleic acid, 9,12-octadecadienoic acid and 9,12-octadecadienal, etc.

KEYWORDS

Diabetes, hypolipidemic, medicinal plants, lipid profile, pharmacology

INTRODUCTION

Medicinal plants have been known to play a crucial role in the health system since time immemorial as plants contain natural compounds with medicinal properties. Medicinal plants are useful in the curing of human diseases and healing due to the presence of photochemical constituents[1].

Over the years, 80% of the population in developing countries continues to use medicinal plants and plant products in handling primary medical problems due to their accessibility, availability and affordability[2].

ISSN: 1813-0070 (Print)
https://doi.org/10.3923/asb.2024.17.23

Received: 03 Nov. 2023
Accepted: 05 Feb. 2024
Published: 20 Feb. 2024

In these countries, a variety of plants have been acclaimed to have properties such as anti-diabetic, anti-fungal, anti-inflammatory etc.[1] and they have been tested for such effects.

Mangifera indica also known as mango is one of the most popular tropical fruits. It is found in tropical and subtropical regions of the world (Asia). Mango fruit tree is usually large and perennial, its leaves are green and fruits vary in shape, size and color. There are more than a thousand varieties of mango trees all over the world. *Mangifera indica* has been an important herb in the Ayurvedic and indigenous medical systems for over 4000 years. Photochemical studies have shown that *Mangifera indica* leaves are very important sources of phenolic compounds and mangiferin[3]. Mangiferin possesses strong antioxidant, hypolipidemic, anti-inflammatory, cardiotonic, hypotensive, wound healing and antidiabetic activities. According to Ayurvedic medicine, various parts of the mango tree possess several medicinal properties. The root, bark, leaves, flowers, unripe and ripe fruit are acrid, cooling and astringent to the bowels. Different parts of *M. indica* have been used traditionally for the treatment of various ailments including gastrointestinal problems (dysentery, piles, stomach upset, biliousness, constipation), respiratory ailments (bronchitis, asthma, hiccup, throat problems), genitourinary problems (urinary discharges, leucorrhoea, vaginal problems) and ophthalmic complaints. It is also used as an aphrodisiac, tonic, appetizer, laxative, diuretic, stomachic and for tanning purposes in various parts of the world[4,5]. The aqueous extract of *M. indica* leaves has been reported to possess hypoglycemic activities.

Most people in developing countries (including Nigeria) consume herbal medicines without adequate knowledge of the pharmacologic actions and side effects of such medications, hence the further need to evaluate the effect of these plants to advise patients especially diabetic patients on the intake of herbal medications. The ethanolic extract of immature leaves has been assessed for favorable hypolipidemic and hepatoprotective activities[6]. With the paucity of literature on the effect of *Mangifera indica* on serum lipid profile, therefore, this present study aims to scientifically investigate the effects of administration of the ethanol extract of mature *Mangifera indica* on lipid profile.

MATERIALS AND METHODS

Study area: The study was conducted in the Department of Biochemistry, Veritas University, Bwari, Abuja, Nigeria. The study was carried out from June 2023 to July 2023.

Materials: The following materials were used for this work: Fresh leaves of *magnifera indica*, albino Wistar rats, rubber cages, feed plates, distilled water, cotton wool, Whatman filter paper and plain sample bottles.

Chemicals and reagents: Several chemicals and analytical grade reagents were purchased and used for this research which includes: Ethanol (98%), which was obtained from the Department of Biochemistry Laboratory, Veritas University, Abuja, Nigeria. The beta cell destructive agent, streptozotocin (STZ) was obtained from Sigma, St. Louis, Missouri, USA.

Kits for high-density lipoprotein, total cholesterol, triacylglycerol, chloride, calcium and phosphorus were obtained from Dialab Production Austria. Insulin injection (NPH, Humulin), needles and other syringes used were obtained from Nazel Pharmacy, Bwari, Abuja, while EDTA tubes were obtained from the Department of Biochemistry laboratory, Veritas University, Abuja, Nigeria.

Equipments and glasswares: The Following types of equipment and glassware were used in the course of this research:

- Water bath, electric blender, refrigerator, deep freezer, table centrifuge (B. Bran Scientific and Instrument Company, England), rotary evaporator (RE-52A, Shangai Ya Rong Biochemistry Instrument Company, England), visible spectrophotometer (SP-300 optima, Japan) automated micropipettes (0.5-50 and 100-1000 µL), automated multi-channel micropipette (0.5-250 µL), glass pipettes (0.5-25 mL), Beakers (10-1000 mL) and test tubes

- Generator/alternate power supply (Honda, 3500Kva)
- Sysmex® Automated Haematology AnalyzerKX-21N, Sysmex corporation, Kobe, Japan
- Carbolite electric oven, England. Jenway digital spectrophotometer, France
- Sartorius digital weighing balance, Germany
- Soxhlet apparatus, company, Excello, England
- Semi-micro Kjedahl apparatus Excello, England
- NAAFCO Fume cupboard, Nigeria, Cliffton electric hot plate stirrer, USA
- Gallenkamp muffle furnace, England, General laboratory glass wares, materials and consumables

Methods

Identification and preparation of plant materials: Fresh leaves of 2.5 g *Mangifera indica* were obtained from a local garden in Bwari Local Government Area Abuja, Nigeria. The plant specimen was identified and authenticated by a botanist. The leaves were washed with tap water to remove dust particles and debris and allowed to completely drain. The dried leaves were crushed into a fine powder and the sample weighing 1000 g was soaked in 3000 mL of 80% ethanol for 72 hrs after which it was sieved and filtered using filter paper. The filtrate was subjected to concentration using a rotary evaporator to obtain a concentrated extract. The concentrate was stored in sterile bottles and kept in the refrigerator until -40°C until the time of use. Appropriate concentration of the extract was subsequently made by dilution with distilled water and administered to the animals.

Gas chromatography-mass spectroscopy of *Mangifera indica*: The extract of *Mangifera indica* was dissolved in DMSO and subjected to GC-MS analysis according to the method explained by Edet *et al*.[7].

Handling and treatment of animals: Thirty adult male rats weighing between 150-245 g were acquired from an animal house in Plateau State, Nigeria and kept in the animal house of the department. The rats were divided into six groups of five rats each (Table 1). The rats were allowed to acclimatize in the experimental animal house for one week before the commencement of the experiment. The animals were kept in rubber cages under standard conditions (ambient temperature, 28.0±2.0°C and humidity, 46%, with 12 hrs light/dark cycle), fed with growers' feed. All the rats in both test and control groups were allowed free access to feed and water throughout the experimental period. Good hygiene was maintained by constant cleaning and removal of faeces and spilt feed from cages daily.

Induction of diabetes: Prior to induction, the animals were subjected to a 12 hrs overnight fast and induction was done by intraperitoneal injection of 40 mg/kg b.wt., with streptozotocin using sodium citrate (0.5M) reconstituted buffer. After three days' diabetes was confirmed with a fasting blood glucose concentration of $\geq$200 mg/dL. This was done using an accu check glucometer with blood obtained from the tail vein of the rats. The extract was administered for 21 days.

Collection of blood for analysis: Animals were anaesthetized in chloroform vapor, 24 hrs after the last day of extract administration and dissected for blood collection. Blood sample was collected by cardiac puncture into a set of plain sample bottles and allowed to clot for 2 hours after which serum was obtained by centrifugation at 3000 rpm for 10 min. The serum was transferred into sterile plain tubes using a sterile pipette and stored under frozen conditions for biochemical analysis.

Biochemical estimations: Biochemical analysis of lipid parameters: Total cholesterol, HDL-c, LDL-c and triglycerides was carried out under standard laboratory techniques as reported.

Statistical analysis: Results obtained from this study were analyzed by One-way Analysis of Variance (ANOVA), followed by Student's t-test to evaluate the significance of the difference ($p<0.05$) between the mean value of the measured parameters in the respective test and control groups using SPSS windows 22.0.

Table 1: Experimental animal groupings of both the control and test groups

Group	Number of rats	Group title	Treatment administered
Group I	5	Normal control (NC)	Distilled water and feed
Group II	5	Diabetic control (DC)	Distilled water and feed
Group III	5	Insulin group (IG)	5 unit/kg b.wt., of insulin
Group IV	5	Treatment group IV (TGIV)	200 mg/kg of *Mangifera indica*
Group V	5	Treatment group V (TGV)	300 mg/kg of *Mangifera indica*
Group VI	5	Treatment group VI (TGVI)	400 mg/kg of *Mangifera indica*

Ethical consideration: Standard ethical procedures were strictly adhered. All the animal experiments were carried out in accordance with the guidelines of the Institution's Animal Ethical Committee.

RESULTS AND DISCUSSION

Results and interpretation of results

The effect of ethanol extract of *Mangifera Indica* on serum lipid profile concentrations of treated rats: Table 2 presents the lipid profile level of rats treated with extracts of *Mangifera Indica*. Triglyceride levels were significantly different among the treatment groups (<0.05). The highest triglyceride level was observed in treatment group IV (136.24±6.05) followed by DG (132.06±3.30), TGV (116.04±2.19), IG (57.33±2.09) with the lowest value observed in TGVI (50.27±1.89). Total cholesterol also showed a significant difference between the treatment groups (<0.05). The highest level was found in NC group (456.82±15.74) followed by DG, IG and TGIV. However, rats in treatment group V and group VI of the extract had significantly lower total cholesterol concentrations (79.05±4.15 and 117.54±13.03, respectively). Untreated rats (DG) had significantly lower HDL concentrations compared to all other groups. However, a dose-dependent increase in HDL concentrations was observed for the extract-treated groups which were comparable to the normal control (26.24±2.92) and insulin (23.31±0.66) administered groups. The LDL levels were significantly different across the groups. Untreated diabetic rats had significantly higher LDL concentrations (247.09±8.59) compared to the other groups. Rats in normal control had the lowest LDL concentration followed by insulin administered group. Nevertheless, the rats in group VI had similar LDL concentrations as the standard drug (insulin) group. The VLDL concentration was significantly ($p<0.05$) lower in the normal group (2.28±0.19) followed by group VI (10.50±0.80) but higher in insulin group (55.05±1.29).

Administration of the extract resulted in a significant ($p<0.05$) decrease in TG, TC and LDL concentrations in all experimental groups when compared to the control group but an increase in HDL and VLDL concentrations.

Lipids are important components of the homeostatic function of the body. They also contribute to important processes occurring in the body[8]. Abnormalities in lipids play a central role in the genesis and progression of atherosclerosis[9], which is a risk factor for the development of cardiovascular diseases.

In Table 3, numerous chemicals were present, with varying retention times and percentage abundances (area%), according to the GC-MS spectra results. The eluted chemicals were subjected to mass spectrometry (MS) analysis at different times to determine their nature, chemical structures and retention durations. Large molecules broke up into smaller pieces, which is why the peaks at various m/z ratios appeared. The mass spectra identified from the MassHunter data library system represent the compound's fingerprint. The plant extract's GC-MS examination revealed that the most prevalent compounds were oleic acid, 9,12-octadecadienoic acid and 9,12-0ctadecadienal, etc.

In this study, diabetic albino rats were subjected to treatment with ethanol leaf extract of *Mangifera indica* to observe its hypolipodemic effects and results from the study on the lipid profile of the rats treated with *Mangifera indica* suggest that the different treatments significantly affected the measured lipid profile

Table 2: Effect of ethanol extract of *Mangifera indica* on serum lipid profile concentrations in treated rats

Group	TG	TC	HDL-c	LDL-c	VLDL-c
NC	13.16±0.63^{a}	456.82±15.74^{e}	26.24±2.92^{b}	23.46±1.72^{a}	2.28±0.19^{a}
DG	132.06±3.30^{d}	285.77±8.07^{d}	13.68±2.00^{a}	247.09±8.59^{e}	18.87±1.27^{c}
IG	57.33±2.07^{b}	169.52±29.93^{c}	23.31±0.66^{b}	52.83±3.29^{b}	55.05±1.29^{f}
TGIV	136.24±6.05^{d}	194.21±3.37^{c}	24.61±2.07^{b}	166.15±3.91^{d}	41.45±1.87^{e}
TGV	116.04±2.19^{c}	79.05±4.15^{a}	29.93±2.76^{b}	82.72±24.43^{c}	27.25±1.21^{d}
TGVI	50.27±1.89^{b}	117.54±13.03^{ab}	40.27±2.67^{c}	56.47±3.17^{b}	10.50±0.80^{b}

Values are presented as Mean±SEM of five determinations and values with different superscripted alphabet along a column are significantly different at $p<0.05$

Table 3: GC-MS analysis expressing the active compounds present in *Mangifera indica* leaves extract

S/no.	Retention time (min)	Area (%)	Names of compounds
1	5.6395	0.1614	2-Hexyn-1-ol
2	5.8575	0.9748	2-Hexyn-1-ol
3	6.1887	0.1526	1-(p-Toluidino)-1-deoxy-.beta.-d-Idopyranose
4	6.3593	0.283	Eicosyl propyl ether
5	7.0018	0.3839	Morpholine, 4-methyl-, 4-oxide
6	7.4801	0.7945	2-Hexyne
7	7.7528	2.1343	Dodecanoic acid
8	8.0478	0.9236	9,12-Octadecadienal
9	8.2897	1.6572	9,12-Octadecadienal
10	8.6533	5.5199	9,12-Octadecadienal
11	13.064	12.1682	9-Oxabicyclo[6.1.0] nonane
12	13.3407	3.0178	2-Methyl-E,E-3,13-octadecadien-1-ol
13	13.6912	2.7701	9,17-Octadecadienal, (Z)-
14	14.1224	4.3057	9,12-Octadecadienal
15	14.2963	2.2704	9,12-Octadecadienoic acid, methyl ester, (E, E)-
16	14.5514	5.8698	Oleic acid
17	14.9751	2.0471	9,12-Octadecadienal
18	15.174	1.8319	9,12-Octadecadienal
19	15.3986	1.7522	Oleic acid
20	16.367	4.6382	9,12-Octadecadienoic acid, methyl ester, (E, E)-
21	17.1285	2.833	Oleic acid
22	17.8908	1.2885	9,12-Octadecadienal
23	18.2009	2.8251	9,12-Octadecadienal
24	18.473	1.1179	Oleic acid
25	19.5623	4.3558	9-Oxabicyclo [6.1.0] nonane
26	23.2022	0.7322	9-Oxabicyclo[6.1.0] nonane, cis-
27	23.3099	0.5021	9-Oxabicyclo[6.1.0] nonane
28	23.459	1.2027	9,12-Octadecadienal
29	23.8257	1.1491	cis-13-Octadecenoic acid
30	25.0103	7.5301	9,12-Octadecadienal
31	25.6198	12.6493	9,12-Octadecadienal
32	26.1518	1.7604	9,12-Octadeca dienoic acid (Z,Z)-
33	26.4306	1.0297	1,14-Tetradecanediol

parameters of the rats. The treatment with the plant extract resulted in to decrease in triglycerides, total cholesterol and LDL levels with an increase in HDL levels. Results show that the extract may have a beneficial effect on triglycerides and total cholesterol levels. Treatment group IV and DG were associated with higher levels of triglycerides and total cholesterol. Higher levels of serum lipid may be a result of the disturbance in the regulation of the activity of the hormone-sensitive enzyme, lipase, by insulin due to its deficiency or resistance caused by streptozotocin destruction of beta islet cells[10]. Lipase is inhibited by insulin in the adipose tissues and in the absence of insulin, the plasma level of free fatty acids increases[11]. In the liver, the free fatty acid is converted to acetyl CoA and the excess acetyl CoA is further converted to triglycerides and cholesterol.

Most findings from different studies were in agreement with these results[12,13]. The low triglyceride and total cholesterol levels observed in the group treated with 400 mg/kg (TGVI) of the extract suggested that the extract may have a lipid-lowering effect and may help lower the risks of cardiovascular diseases. This

effect could be due to the presence of the bioactive compounds present in the extract. Diabetic group (DG) had lower levels of HDL compared to other treatment groups and a dose-dependent increase was observed in the treatment groups with the group administered 400 mg/kg having the highest levels of HDL. This indicates that *Mangifera indica* increases HDL levels, lowering the risks of cardiovascular diseases and atherosclerosis. Lower LDL levels were also observed in rats administered (400 mg/kg) of the extract (TGVI) compared to the diabetic group which had the highest LDL level. This suggested that the treatment with *Mangifera indica* extract may have a beneficial effect on LDL cholesterol levels and may help to reduce the risks of atherosclerosis and cardiovascular diseases associated with high levels of LDL cholesterol. The high level of LDL in the diabetic group could be due to an increase in the transport of free fatty acids from the peripheral depots. However, according to the findings of this study, treatment with 400 mg/kg (TGVI) of the *Mangifera indica* extract had the highest levels of VLDL cholesterol compared to the diabetic control other treatment groups. This suggests that the treatment with *Mangifera indica* extract may have a negative effect on VLDL cholesterol levels, which may increase the risk of cardiovascular diseases associated with high levels of VLDL cholesterol.

The HDL and LDL cholesterol are the two major lipoproteins involved in lipid metabolism that play important roles in the transport of lipids (cholesterol, cholesterol esters and triglycerides), abnormal levels determine risk factors for cardiovascular diseases. The HDL promotes the removal of excess cholesterol from peripheral tissues to the liver where it can be excreted into the small intestine because of this HDL is regarded as good cholesterol and high levels are associated with a decreased risk of cardiovascular diseases. The LDL on the other hand promotes the uptake of cholesterol from the liver to peripheral tissues for cellular use. Higher levels can result in formation of plaques on the walls of the arteries leading to conditions such as stroke, heart attack, hypertension and coronary heart disease.

Dyslipidemia normally associated with diabetes may occur due to insulin deficiency or resistance because insulin has an inhibitory action on HMG-COA reductase, a key enzyme which is responsible for the metabolism of LDL particles rich in cholesterol[2].

CONCLUSION

This study showed that the administration of ethanol leaf extract of *Mangifera indica* may suggest that treatment with the extract may have varying effects on lipid metabolism depending on the dose and presence of other factors. Beneficial effects particularly reducing triglycerides, total cholesterol and LDL cholesterol levels while increasing HDL cholesterol levels have been observed. Therefore, the plant extract of *Mangifera indica* may possess hypolipidemic properties.

SIGNIFICANCE STATEMENT

Diabetes is a health condition of major concern that is widespread due to the prevalence of obesity and inactive life habits. lipids are important components of the homeostatic function of the human body. Abnormalities in blood lipid levels can result in the development of atherosclerosis and eventually cardiovascular diseases which is the leading cause of mortality in diabetes. Globally, diabetes has affected millions of persons, especially in developing countries and there is increased mortality due to lack of proper management and high cost of conventional medications. Therefore, there's a need to provide an alternative source of treatment using herbal plants which possess medicinal properties with easy availability and affordability.

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