EFFECT OF ETHANOL LEAF EXTRACT OF MANGIFERA INDICA ON THE LIPID PROFILE OF AN ALLOXAN INDUCED DIABETIC ALBINO RATS.

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ABSTRACT

The effect of ethanol leaf extract of Mangifera indica on lipid profile was conducted using alloxan induced Diabetic albino rats. The albino rats were divided into four groups (A, B, C and D) containing five rats each. Groups A and B were administered Mangifera indica ethanol leaf-extract by oral intubation at the doses of 200 and 400 mg/kg body weights respectively for two weeks. Animals in group C served as the positive control (diabetic untreated rats) while the animals in group D (non-diabetic untreated group) served as the negative control. The serum levels (mg/dl) of total cholesterol, LDL-cholesterol, HDL-cholesterol and triacylglycerol were studied spectrophotometrically. Total cholesterol, triacylglycerol, LDL-cholesterol and glucose levels were significantly (p<0.05) reduced by the extract while levels of HDL-Cholesterol were increased significantly in diabetic treated rats when compared with the control group rats. The results showed that the leaf-extract of Mangifera indica possess anti-diabetic and anti-hyperlipidemie properties thus suggesting its beneficial effect in the treatment of diabetes mellitus associated with hyperlipidemia and related cardiovascular complications.

Keywords: Mangifera indica, Diabetes and lipid profile.

INTRODUCTION

Diabetes is a chronic metabolic disease characterized by derangements in carbohydrates, fat and protein metabolism caused by defective insulin secretion or action, resulting in long term multi-organ complications [1, 2, 3, 4]. Chronic
hyperglycemia causes damage to different organs of the body such as eyes, heart, kidneys, nerves and blood vessels. Two major types are recognized chemically; type 1 and type 2. Type 1 diabetes mellitus usually appear in childhood or in the teens as a result of very low insulin secretion caused by defective beta cell function, the result of an autoimmune process [5, 6, 7, 8, 9]. Types 1 is characterized by hyperglycemia and hypertriglyceridemia, and episodes of severe ketoacidosis. The hyperglycemia results from the inability of the insulin dependent tissues to take up glucose and from accelerated hepatic gluconeogenesis from amino acids derived from muscle proteins. Type 2 diabetes mellitus occurs in middle-aged to older obese people and is characterized by hyperglycemia, often with hypertriglyceridemia and other features of the metabolic syndrome [10, 11, 12].

Lipid profile or lipid panel, is the collective term given to the estimation of typically total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density Lipoprotein cholesterol (LDL-C) and triglycerides (TG). This is used to identify hyperlipidemia (various disturbances of cholesterol and triglyceride levels), many form of which are recognized risk factors for cardiovascular disease and sometimes pancreatitis. High-density lipoprotein (a combination of fat and protein) formed in blood is called good cholesterol because it removes excess cholesterol from the blood and takes it to the liver where they are detoxified and hence is related to lower risk of heart and blood vessel disease, while low-density lipoprotein (a combination of fat and protein) formed in blood is called bad cholesterol because it picks up cholesterol from the blood and circulates it to the cells, hence a high LDL level is related to a higher risk of heart and blood vessel disease [13, 14, 15, 16].

Medicinal plants are the great importance to the health of individual and community. More than a thousand of these plants have been claimed to possess medicinal or curative properties, of which mango leaves has been suggested to be among the marry species to have been identified as having medicinal properties and therapeutic use. The most important bioactive substances which makes them perform such active functions like anti-diabetic, antibacterial are alkaloids, tannin, flavonoid, saponin and phonolic compounds [18, 19, 20, 21]. Mango leaves have
been claimed to be one of those plants that possesses the medicinal values, which possess some chemical active substances that produce a definite physiological action on the human body and animal health [22,23, 24, 25].

Mango (Mangifera indica) leaves are a rich source of phenol compounds with strong antioxidant power, particularly mangiferin, a spatial xanthenes commonly called as “super- antioxidant” because of their potent capacity and other phenol compounds like quercetin, widely student by its pharmacological properties [26, 27, 28,29, 30].

The leaves of Mangifera indica are used as an anti diabetic agent in Nigerian folk medicine, although when aqueous extract given orally altered blood glucose level because Mangifera indica possess hypoglycemic activity as a result of an intestinal reduction of the absorption of glucose [31,32,33,34,35,36,37,38,39,40].

AIM AND OBJECTIVES

To determine the effects of ethanol extract of mango (mangifera indica) leaf on lipid profile of alloxan induced Diabetic albino rats.

MATERIALS AND METHODS

Collection of Biological Materials

The male and female rats of 8 to 12 weeks old with the average weight of 100 to 200g were sourced from the Department of veterinary medicine, University of Nigeria Nsukka (UNN) Enugu State, Nigeria and acclimatized for two weeks in the animal house of the Department of Biochemistry, Ebonyi State University (EBSU), Abakaliki. The rats were housed and fed in cages at room temperature with a 12-12hours light-darks cycle in the animal house. The leaves (Mangifera indica) were obtained from number 25 Mbam-agbo Street, Abakaliki, Ebonyi State, at Mbam-Agbo's Compound. It was authenticated by Dr. (Mrs) Nnamani at the Applied Biology Department of EBSU, Nigeria. The leaves were air-dried at room temperature of 29°C,
after which it was ground with grounding machine. Then proximate composition and phytochemical test commenced.

**Preparation of Plant Extract**

The leaves of the plant (*Mangifera indica*) were air-dried at room temperature for two weeks and then ground with grounding machine. The leaves powder 200g was soaked with absolute aqueous ethanol (98%) for three days. The crude extract was filtered with Whitman filter paper 4, and the filtrate was concentrated and dried via evaporation. The dried extract was kept in refrigerator at room temperature till its use.

**Phytochemical Tests**

The phytochemicals and proximate analysis of *Mangifera indica* leaves were carried out using the procedures outlined by Harborne (1989) and Trease and Evans (2002).

**Experimental Design**

At the end of two weeks of acclimatization period, diabetes was induced by intraperitoneal (IP) injection of alloxan (150mg/kg body weight). Three grams (3g) of alloxan was dissolved in 30ml of normal saline. Mbaka *et al.* (2009) [41] after 72hours blood was taken from the lateral veins of the tail and the blood sugar levels were monitored with a glucometer for estimation of blood glucose level. The animals with blood sugar level more than 200mg/dl were considered diabetic and included in the experiment (Lenzen *et al.*, 2008).

**Animal Grouping and Administration**

The animals were randomly assigned into four groups namely A, B, C and D with group containing five animals each and they as follows:
Group A: Alloxan-induced and treated with 200mg/kg body weight of *Mangifera indica* extract.

Group B: Alloxan-induced and treated with 400mg/kg body weight of *Mangifera indica* extract.

Group C: Alloxan-induced and untreated was given 5ml of normal saline which served as a negative control.

Group D: Animals not induced and untreated but were given 5ml of normal saline throughout the experimental period and served as positive control.

**Blood Collection from the Animal**

At the end of three weeks, twenty-four (24hrs) after the last dose administration, they were anaesthetized with chloroform and blood obtained via cardiac puncture into a plane test tube bottle. The blood was later spun in a centrifuge at 5000-revolution for 10minutes. Serum was separated and pipette into another clean plane test tube bottle and stored in the refrigerator for measurement of the biochemical parameters (Wasan *et al.*, 2001).

**Lipid Profile Test**

**Determination of Total Serum Cholesterol**

The evaluation of total cholesterol was done by the method as detailed by Allain and Roschlain (1979).

**Measurement of Serum Triacylglycerols Concentration**

The method of Tietz (1990) was applied to assay for the triacylglycerol quantities in the blood serum.
**HDL Determination**

High density lipoprotein cholesterol fraction was separated by the Precipitation techniques of (Jacob et al., 1990) and the cholesterol content was determined by the Method of (Allain et al., 1974).

**LDL Cholesterol Determination**

LDL Cholesterol was measured using the calculation below

\[
LDL = \text{Total Cholesterol} - \text{Triacylglycerides} - \text{HDL Cholesterol in mg/dl}
\]
RESULTS

RESULTS OF PHYTOCHEMICALS ANALYSIS

Table 1: Results of phytochemical constituents of mango (*mangifera indica*) leaves.

<table>
<thead>
<tr>
<th>Phytochemical Constituents</th>
<th>Concentrations (mg/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenol</td>
<td>52.12 ± 0.00</td>
</tr>
<tr>
<td>Reducing sugar</td>
<td>28.59 ± 0.01</td>
</tr>
<tr>
<td>Tannin</td>
<td>20.72 ± 0.58</td>
</tr>
<tr>
<td>Flavonoid</td>
<td>15.70 ± 0.00</td>
</tr>
<tr>
<td>Alkaloid</td>
<td>59.48 ± 0.01</td>
</tr>
<tr>
<td>Terpenoid</td>
<td>51.48 ± 0.01</td>
</tr>
<tr>
<td>Soluble carbohydrate</td>
<td>10.18 ± 0.01</td>
</tr>
<tr>
<td>Glycoside</td>
<td>4.54 ± 0.01</td>
</tr>
<tr>
<td>Steroid</td>
<td>3.32 ± 0.01</td>
</tr>
<tr>
<td>Saponin</td>
<td>2.46 ± 0.00</td>
</tr>
<tr>
<td>Hydrogen cyanide</td>
<td>1.50 ± 0.01</td>
</tr>
</tbody>
</table>

Values are expressed in mean ± standard.
RESULT OF PROXIMATE ANALYSIS

Table 2: Results of proximate composition of mango (*Mangifera indica*) leaves.

<table>
<thead>
<tr>
<th>Proximate Contents</th>
<th>Values (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>6.67 ± 0.01</td>
</tr>
<tr>
<td>Ash</td>
<td>3.19 ± 0.12</td>
</tr>
<tr>
<td>Fats</td>
<td>1.80 ± 0.11</td>
</tr>
<tr>
<td>Protein</td>
<td>9.81 ± 0.01</td>
</tr>
<tr>
<td>Fibre</td>
<td>2.37 ± 0.01</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>76.16 ± 0.02</td>
</tr>
</tbody>
</table>

The values are expressed in mean ± standard deviation.

RESULTS OF ACUTE TOXICITY

At the end of 72 hours there was no significant change observed in the behavioral or autonomic responses in the experimental animals after treatment with different doses of *Mangifera indica* leaf extract. There was also no mortality in these animals during the observational period of the experiment.

RESULT OF GLUCOSE LEVELS OF ALBINO RATS.

The result of glucose concentrations of both diabetic and control animals are shown in figure 1 below.
Figure 1: The effects of ethanol extract of *Mangifera indica* leaf on glucose levels of alloxan induced Diabetic albino rats. The result showed a significant (P<0.05) decrease in the glucose level of rats in the treated groups (A and B) compared to induced and untreated group (C). There was also significant (P<0.05) increase in glucose levels of induced and treated groups (A and B) compared to the control group (D).

**RESULTS OF LIPID PROFILES**

The results of lipid profile are shown in figures 2, 3, 3 and 4 below.
Figure 2: The effects of ethanol extract of *Mangifera indica* leaf on total cholesterol of alloxan induced Diabetic albino rats. The result showed a significant increase (P<0.05) of serum total cholesterol level in the experimental groups A and B compared to the control group (D). The induced and untreated group (C) had highest levels of serum total cholesterol.
Figure 3: The effects of ethanol extract of *Mangifera indica* leaf on HDL-Cholesterol of alloxan induced Diabetic albino rats. The result showed a significant increase (P<0.05) in the serum HDL-C levels of A and B compared to control group D. There is also no significant change at (P>0.05) in serum HDL of the experimental group C (induced and untreated) compare to control group (D).
Figure 4: The effects of ethanol extract of *Mangifera indica* leaf on LDL-Cholesterol of alloxan induced Diabetic albino rats. The result showed a significant increase (P>0.05) in the serum LDL levels in the induced and untreated group compared to the group (A and B). The experimental groups (A and B) showed significant increase at P<0.05 compared to the control group (D).
Figure 5: The effects of ethanol extract of *Mangifera indica* leaf on triglyceride of alloxan induced Diabetic albino rats. The result showed a significant increase (P<0.05) in serum triglyceride levels of the experimental groups (A and B) compared to the control group (D). The induced and untreated group (C) had the highest level of triglyceride.
DISCUSSION AND CONCLUSION

The result of phytochemical analysis revealed the presence of tannins, flavonoids, phenols, saponins, glycosides, alkaloids, terpenoids, steroids, reducing-sugar and hydrogen cyanide. The plant had high amounts of alkaloids, phenols, terpenoids, reducing sugar and tannins, moderate levels of flavonoids and soluble carbohydrate with low concentration of glycoside, steroids, saponins and hydrogen cyanide. Alkaloids and its synthetic derivatives has been reported as basic medicinal agents for their analgesic, antispasmodic and bactericidal effects by inhibiting certain mammalian enzymatic activities such as those of phosphodiesterase, thereby prolonging the action of cyclic AMP [42, 43, 44, 45, 46, 47, 48, 49, 50]. The high amount of phenol in this study is in accordance with the research of El-Sheik, (2012) [23] which suggests that different parts of Mangifera indica have demonstrated the presence of phenolic constituents, triterpenes, flavonoids, phytosterols and polyphenols, which are known to possess antioxidant properties.

The proximate analysis of Mangifera indica leaves showed high amounts of carbohydrate, moderate amounts of protein and moisture with minute amounts of ash, fat, and fibre. The result of this study is in agreement with Joona et al., (2013) [31] on the same leaf extract of mangifera indica. The high amount of carbohydrate and protein showed its usefulness as energy source and body building as reported by [51, 52, 53, 54]. The result of acute toxicity of Mangifera indica leaves revealed that the plant is non-toxic even at the dose of 5000mg/kg body weight. This suggested that the Mangifera indica leaf is safe for human consumption in treatment of ailments and as food.

There was a significant increase (P>0.05) on the glucose levels of the induced and untreated animals compare to the both experimental groups (A and B) and control group (D). The increase in the glucose levels indicated that the animals are diabetic which is due to administration of 150 mg/kg body weight of alloxan. Also, there was a significant decrease at P<0.05 in the animals given 200 mg/kg body weight of the extract compare to animals given 400 mg/kg and the reduction in the
glucose levels compare favorably to that of the animals that was given normal saline only (group D). This may be as a result of high amount of tannins as showed by phytochemical analysis, tannins inhibits glucose transport across the intestine through inhibition of sodium-glucose co-transporter-1 thereby suppressing hyperglycemic effects [55, 56, 57, 58, 59, 60]. The higher reduction at 200 mg/kg of the extract showed that the effect is not dose dependent. The result of anti-diabetic effect of this plant is in agreement with the report of Reda et al. (2010) who posited that 30, 50 and 70 mg/kg of aqueous leaf extract of mango the decreased blood glucose levels to 31.20%, 37.78% and 43.29% respectively. This may also be attributed to the dietary tannins which are said to reduce feed efficiency and weight gain in chicks by forming complexes with metal ions and with macro-molecules such as protein and carbohydrates [61, 62, 63, 64, 65, 66]. However, the report of the same work is dose dependent which disagreed with our result. Similarly, the result of this study is in line with the research of Ajah et al. (2015) on anti-diabetic effect of leaf extract of Pterocarpus santalinoides.

The results of the total cholesterol levels showed a significant increase at P<0.05 in the induced and untreated animals when compare to others. Also, there was a significant reduction at P<0.05 of the animals given 400 mg/kg body weight compare to those given 200 mg/kg. It was found that there was a significant decrease in total cholesterol which agrees with the work done by Shah et al., (2010) on the leaf extract of mango. Treatment with aqueous extract of Mangifera indica leaves significantly decreased total serum cholesterol, and this may be as a result of the presence of phytoconstituents such as phenols alkaloids, and flavonoids. This agrees with the report of Aja et al., (2010) on Talinum triangulare. Flavonoids can help to prevent atherosclerosis, a disease characterized by accumulation of fats inside the arterial wall (Okwu, 2001). Flavonoids also prevent oxidative cell damage because of its free radical scavengers and potent water soluble anti-oxidant property Nwali, et al., (2014) as a result can help lower the risk of coronary heart diseases. This study also agrees with Reda, et al., (2010) on water extract of the bark of mango plant and Ficus bengalensis linn aerial roots and barks.
The result showed a significant decrease (P<0.05) in the serum HDL-C levels of the induced and untreated group (C) compared to other groups. There is also no significant change at P>0.05 in serum HDL of the experimental groups compared to control group (D). This is in accordance with the result of Parvez, (2016) who reviewed that treatment with aqueous extract of mango leaves showed significant decrease in elevated total cholesterol, triglyceride, low density lipoprotein and very low density lipoprotein, with significant increase in high density lipoprotein. It could also be observed that total cholesterol, LDL-cholesterol and VLDL cholesterol levels increased significantly in diabetic rats compared with the negative control rats as a result of diabetes which is also in line with Reda, et al., (2010). The decrease may be due to increased inhibition of intestinal absorption of cholesterol, interference with lipoprotein production, increased expression of hepatic LDL receptors and their protection by saponin activities [67, 68, 69, 70]. Saponin decreases the uptake of certain nutrients such as glucose and cholesterol at the gut through intraluminal physicochemical interaction [71, 72, 73, 74].

The result showed a significant increase (P<0.05) in the serum LDL levels in the induced and untreated group compared to others. The experimental groups A and B showed significant increase at P<0.05 compared to the control group (D) while between the experimental groups A and B there is a significant increase at P<0.05 in group A compared to B. This is in accordance with the work done by Khyati et al., (2010). This may be attributed to saponin which has been reported by Pachkore, et al., (2012) to possess the property of precipitating, coagulating red blood cells, formation of foams in aqueous solutions, and in cholesterol binding. The result is also in agreement with the work done by Parvez, (2016) on aqueous extract of mango leaves (200 mg/kg body weight) which showed significant decrease in elevated total cholesterol, triglyceride, low density lipoprotein (LDL-C) with significant increase in high density lipoprotein (HDL-C).

The result showed a significant increase (P<0.05) in serum triglyceride levels of the experimental groups A and B compared to the control group (D). The induced and untreated group (C) recorded the highest level of triglyceride while the control
group (D) had the least levels of triglycerides. This is in disagreement with the study of Reda et al., (2010) on water extract of the bark of mango plant and Ficus bengalensis linn aerial roots and barks which decreased triacylglycerol by 54% and LDL+VLDL- cholesterol by 60% compared with control rats.

CONCLUSION

The findings of this research indicated that Mangifera indica leaf extract possess anti-diabetic and anti-hyperlipidemic properties thus suggesting its beneficial effect in the treatment of diabetes mellitus and prevention of cardiovascular complications. The actions of aqueous extract of leaves of Mangifera indica may be attributed due to presence of some bioactive compounds such as flavonoids, saponins, glycosides, tannins, and phenolics contained in the plant.

REFERENCES


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