Physicochemical and Anti-Diabetic Effect of the Crude Leaf Extract of Mallotus oppositifolius (Euphorbiaceae) in Alloxan Induced Diabetic Mice

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Abstract

Background: Diabetes mellitus results when there is an imbalance in the production, metabolism and regulation of insulin and blood glucose respectively, in the human body. This study investigated the fresh leaf microscopy and chemomicroscopy, phytochemical, acute toxicity and the anti-diabetic potentials of Mallotus oppositifolius using the mice diabetic model.

Methods: Mallotus oppositifolius was extracted using the cold maceration method with absolute methanol. The acute toxicity (LD 50) of the crude extract was determined in mice using Lorke’s method. The anti-diabetic studies were conducted using the standard protocol of alloxan induced diabetic mice.

Results: The findings showed that no toxicity was observed up to 5000 mg/kg doses of the crude extract. The phytochemical screening revealed the presence glycosides, saponins, alkaloids, flavonoids and tannins, steroids and terpenoids. The crude extract of Mallotus oppositifolius decreased blood glucose level significantly (P<0.05) within 10 hours' acute treatment and 14 days long term treatment. Histological examination showed a consistent rejuvenation of the pancreatic β cells islet of diabetic mice treated with methanol leaf extract Mallotus oppositifolius. The result also showed abundance of manganese, magnesium, iron, calcium, zinc and potassium which are all implicated in secretion and metabolism of insulin.

Conclusion: Mallotus oppositifolius may have acted by restoring pancreatic beta-cell integrity through mopping of reactive oxygen species (ROS) associated with the diabetic state, and thereby improving pancreatic function and consequently, lowering of fasting blood glucose levels. These findings provide ample evidence to support the traditional use of Mallotus oppositifolius in the management of diabetes mellitus.

Keywords: Acute toxicity • Anti-diabetic • Mallotus oppositifolius • Blood glucose • Nutrient

Diabetes mellitus results when there is an imbalance in the production, metabolism and regulation of insulin and blood glucose respectively, in the human body. It is a metabolic disorder with huge economic implications. Diabetes mellitus is defined as a chronic disease that occurs when the pancreas cells does not produce enough insulin or when the body cannot effectively metabolize the insulin it produces [1] leading to impairment of insulin action thereby causing a metabolic leakage. It is usually caused by a combination of hereditary and environmental factors, resulting in abnormal high blood sugar level (hyperglycaemia) [2], polyuria, polydyspnea, weight loss, sometimes with polyphagia, and blurred vision [3]. It is a degenerative disease of the blood glucose system caused by imbalance in the function of the pancreatic beta cell resulting in beta cell’s inability to produce insulin thereby leading to hyperglycaemia associated with retinopathy, nephropathy, neuropathy and cardiovascular complications [4]. Hyperglycaemia by oxidative stress is pathologically linked to the onset and progression of diabetes mellitus and this leads to severe life complications if left untreated [5]. Pathophysiology of diabetes are associated with high levels of inflammatory cytokines, hyperactivity of leukocytes, increased tissue fibrosis [6] and accumulation of reactive oxygen species which leads to oxidative stress and sometimes increased damage to beta cells and biomolecules [7]. Though there is dearth of data on prevalence of diabetes in Nigeria, the number of people with diabetes rose from 108 million in 1980 to 422 million in 2014 globally [1] and demographic data indicates rising epidemiology of diabetes particularly in low- and middle-income countries than in high-income countries. Diabetes is projected to become the seventh leading cause of death globally by 2030 and total deaths from diabetes are estimated to rise by more than 80% in the next 10 years [8]. Therefore, the need to identify natural products with antidiabetic potentials that are safe, available, acceptable and efficacious for treatment of diabetes.

Available literatures have shown that Nigeria has a history of ancient, rich and diverse cultural traditions, associated with the use of medicinal plants for the treatment of different diseases. These plants contain both primary and secondary metabolites with therapeutic and nutraceutical values. Mallotus oppositifolius (Geiseler) Mull. Arg. (Euphorbiaceae) is one of the medicinal plants with antidiabetic potentials that are safe, available, acceptable and efficacious for treatment of diabetes.

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following activities of M. oppositifolius: antifungal and antibacterial properties of the plant [10,13], antitrypanosomal and anti-helminthic activity in vitro, anti-inflammatory activity [11] and antioxidant activity [14,15]. This study investigated the fresh leaf chemomicroscopy, phytochemical, acute toxicity and the anti-diabetic potentials of Mallotus oppositifolius using the mice diabetic model.

**Materials and Methods**

### Apparatus and Equipment

Apparatus and equipment used in this study include: glass column, flasks, beakers, test tubes, measuring cylinders, analytical weighing balance (Mettler H3, Switzerland), blood glucose meter (Accu Answer, USA), water bath (Techmel & Techmel, Texas, USA), refrigerator, mortar and pestle, milling machine, vacuum pump and VLC column, lancet, glucometer. glucose strips.

### Chemicals, Reagents and Drugs

Alloxan monohydrate, metformin, distilled water, methanol was purchased from Juhel Pharmaceuticals Limited.

### Collection, Identification and Preparation of Plant Materials

Fresh leaves of Mallotus oppositifolius were collected before sunrise from Amawbia and was properly identified by a plant taxonomist Mr. Felix Nwafor at the Department of Pharmacognosy and Environmental Medicine, University of Nigeria, Nsukka. A voucher number PCG/UNN/0377/Euphorbiaceae was obtained.

### Animals, Procurement and Housing

A total of 36 (only males to avoid pregnancy) albino mice, (weighing between 18-35 g) were procured from the Laboratory Animal Facility of the Department of Pharmacology and Toxicology, University of Nigeria, Nsukka and transferred to the animal House of the Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University where the animals were used for the experiment. The animals were handled in compliance ethical guideline for handling of animals according to [18].

### Methods

#### Phytochemical analysis

Tests were carried out according to the procedures outlined by [17,18]

#### Determination of minerals

The mineral analysis was done by the method of [19] as follows: The sample was first ashed in the oven at 600°C and 2g of the samples were analysed for the selected nutrients (manganese, sodium, Iron phosphate, sulphate and calcium) using standard protocol according to [19].

#### Extraction of plant material

The dry powder (600g) of Mallotus oppositifolius leaves were cold macerated in 6000 ml of methanol (200 g of powdered sample in 2,000ml of methanol in three different glass jars) for 48 hours. The mixture was sieved using Muslim cloth and it was further filtered with Whatman filter paper (number 1). The crude extract was allowed to evaporate to dryness at room temperature.

#### LD<sub>50</sub> determination

The acute toxicity test to determine the LD<sub>50</sub> of M. oppositifolius leaf extracts was carried out using the modified method of [20] as reported by Onyeka et al. [21] briefly, nine animals (rats) were randomly allocated into 3 groups of 3 rats each. Animals in groups 1, 2, and 3 were given 10, 100, and 1000 mg/kg of the crude extracts respectively orally. Animals were therefore monitored for signs of toxicity and mortality for 48 hours. All the animals survived, and were further subjected to acute toxicity test with higher doses in the second trial. In the second trial, 4 animals were randomly allocated to 4 groups of one animal each. Animals in groups 1, 2, 3, and 4 were given 1200, 1600, 2900, and 5000 mg/kg of the crude extracts, respectively.

### Induction of experimental diabetes

Alloxan monohydrate was used to induce diabetes in the experimental mice using the method described by [22] Animals were fasted for 24 hours, followed by the injection of single dose of 120 mg/kg body weight of alloxan monohydrate intraperitoneally. The alloxanized mice were kept for 3 days with free access to food and water for hyperglycaemia to develop. It is reported that a single dose of alloxan (120 mg/kg) produces a decrease in insulin level, hyperglycemia, elevated total lipids, triglycerides, and cholesterol and decreased the high-density lipoproteins [22]. Baseline fasting blood glucose levels were determined using Accu Answer Glucometer (USA).

### Experimental design

A total of 30 diabetic mice were randomized into 6 groups of 5 mice each: group 1 received distilled water (1 ml), group 2 received metformin (500mg/kg), group 3 received methanol extract at 250 mg/kg, group 4 received methanol extract at 500 mg/kg while group 5 served as untreated control.

### Blood Glucose determination

Baseline pretreatment blood glucose level (zero hour) of animals was taken, and the diabetic animals were treated with the given doses. Blood glucose levels were measured at 2, 4, 6, 8 and 10 hours post treatment using blood glucose meter [23].

Daily administration of metformin, and fractions were carried out for a period of 14 days (2 weeks) on the same animals. Blood glucose levels were measured on day 2, 4, 6, 8, 10, 12 and 14. After administration of the last dose, animals were fasted overnight and final blood glucose level was taken. Animals were sacrificed using chloroform anesthesia and organs for histology were harvested [23].

### Histopathology procedure

The histopathological studies were done according to the methods [24].

### Statistical Analysis

Data obtained from the study were analyzed using Statistical Package for Social Sciences (SPSS-20). Results were presented as mean ± Standard error of mean (SEM) of sample replicates. Raw data were subjected to one-way analyses of variance (ANOVA) followed by post hoc turkey’s test. p<0.05 was considered to be statistically significant.

### Results

#### Phytoconstituents

The result of the qualitative phytochemicals is presented in Table 1. The result showed the presence of Alkaloids, Glycosides, Flavonoids, Tannins, Phlobatanins, Saponins, Steroids, Anthocyanins, and Terpenoids in M. oppositifolius and this result is similar to the findings [10] (Table 1).

#### Mineral Content of M. oppositifolius

The result of the mineral profiling of M. oppositifolius is presented in Table 2. The result showed that M. oppositifolius leaf richly contained chlorine (159.7 mg), calcium (80 mg), phosphorus (59.7 mg), magnesium (7.8 mg), manganese (0.7 mg), zinc (0.5 mg), sulphate (0.2 mg) and iron (0.1 mg) when compared to P. officinalis, which had the highest content of calcium (159.7 mg). This implied that the leaf of M. oppositifolius is rich in mineral elements which is of great nutraceutical importance. (Table 2)

#### Acute Toxicity of Mallotus Oppositifolius

The result of the acute toxicity studies of the methanol leaf extracts of M.
oppostifollous showed that the LD₅₀ is above 5000 mg/kg because no death was recorded when administered orally and this implied that there was no toxicity associated with its consumption at a dosage less than or equal to 5,000 mg/kg and this was in agreement with previous research studies.

Effect of Mallotus Oppostifollous on Blood Glucose Level (Acute or Hourly Study)

The result of the effect of methanol crude extract of Mallotus oppositifollous on blood glucose level at hourly acute administration is presented in Table 3. The methanol crude extract significantly (P<0.05) reduced the fasting blood glucose levels in alloxan induced diabetic mice. The two doses (250 and 500 mg/kg) of the methanol extract and metformin 500 mg/kg caused a significant (P<0.05) reduction in the fasting blood glucose levels from 0 hour to the 10th hour of treatment. The methanol extract displayed dose dependent reduction in blood sugar level with respect to time. The result showed that the crude methanolic extract had over 80% reduction of blood glucose at 10 hours administration of 500mg/kg of M. oppositifolius and this found to be the most significant reduction of blood glucose level (Table 3).

Table 1. The Qualitative Phytoconstituents of M. oppositifolius, D. velutinum, S. nodiflora and Honey.

<table>
<thead>
<tr>
<th>S/N</th>
<th>Bioactive compound</th>
<th>M. oppositifolius</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>Present</td>
</tr>
<tr>
<td>2</td>
<td>Glycosides</td>
<td>Present</td>
</tr>
<tr>
<td>3</td>
<td>Flavonoids</td>
<td>Present</td>
</tr>
<tr>
<td>4</td>
<td>Tannins</td>
<td>Present</td>
</tr>
<tr>
<td>5</td>
<td>Phlobatanins</td>
<td>Present</td>
</tr>
<tr>
<td>6</td>
<td>Saponins</td>
<td>Present</td>
</tr>
<tr>
<td>7</td>
<td>Steroid</td>
<td>Present</td>
</tr>
<tr>
<td>8</td>
<td>Terpenoids</td>
<td>Present</td>
</tr>
<tr>
<td>9</td>
<td>Anthocyanins</td>
<td>Present</td>
</tr>
</tbody>
</table>

Table 2. The Mineral Components of M. oppositifolius.

<table>
<thead>
<tr>
<th>S/N</th>
<th>Parameter</th>
<th>Values (mg/100 g) (Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Magnesium</td>
<td>7.820 ± 0.17</td>
</tr>
<tr>
<td>2</td>
<td>Calcium</td>
<td>79.947 ± 0.10</td>
</tr>
<tr>
<td>3</td>
<td>Chlorine</td>
<td>159.730 ± 0.02</td>
</tr>
<tr>
<td>4</td>
<td>Sodium</td>
<td>0.067 ± 0.02</td>
</tr>
<tr>
<td>5</td>
<td>Sulphate</td>
<td>0.223 ± 0.01</td>
</tr>
<tr>
<td>6</td>
<td>Iron</td>
<td>0.107 ± 0.01</td>
</tr>
<tr>
<td>7</td>
<td>Phosphorous</td>
<td>59.703 ± 0.14</td>
</tr>
<tr>
<td>8</td>
<td>Manganese</td>
<td>0.703 ± 0.00</td>
</tr>
<tr>
<td>9</td>
<td>Zinc</td>
<td>0.587 ± 0.01</td>
</tr>
<tr>
<td>10</td>
<td>Potassium</td>
<td>0.087 ± 0.03</td>
</tr>
<tr>
<td>11</td>
<td>Cadmium</td>
<td>0.0157 ± 0.00</td>
</tr>
</tbody>
</table>

Table 3. Effect of various treatments on blood glucose level (Hourly study).

<table>
<thead>
<tr>
<th>Dose</th>
<th>FBS (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 hr</td>
</tr>
<tr>
<td>Normal control</td>
<td>81.4 ± 2.07</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>258 ± 3.00</td>
</tr>
<tr>
<td>Metformin 500 mg/kg</td>
<td>419.2 ± 0.00</td>
</tr>
<tr>
<td>Methanol Extract 250 mg/kg</td>
<td>394.8 ± 2.09</td>
</tr>
<tr>
<td>500 mg/kg</td>
<td>453.00 ± 0.77</td>
</tr>
</tbody>
</table>

Values are presented as mean ± Standard error of mean (SEM), n=5.

Effect of Mallotus Oppostifollous on Blood Glucose Level (Acute or Hourly Study)

The result of the effect of methanol crude extract of Mallotus oppositifollous on blood glucose level at hourly acute administration is presented in Table 3. The methanol crude extract significantly (P<0.05) reduced the fasting blood glucose levels in alloxan induced diabetic mice. The two doses (250 and 500 mg/kg) of the methanol extract and metformin 500 mg/kg caused a significant (P<0.05) reduction in the fasting blood glucose levels from 0 hour to the 10th hour of treatment. The methanol extract displayed dose dependent reduction in blood sugar level with respect to time. The result showed that the crude methanolic extract had over 80% reduction of blood glucose at 10 hours administration of 500mg/kg of M. oppositifolius and this found to be the most significant reduction of blood glucose level (Table 3).

The highest reduction rate was found in 500 mg/kg (34.44 %) while 500 mg/kg (28.9 %) (Table 3).

Effect of Mallotus Oppostifollous on the Blood Glucose Level (Daily Study)

The result of the effect of methanol crude extract of Mallotus oppositifollous on blood glucose level at daily acute administration is presented in Figure 1 and Appendix 1. The methanol crude extract significantly (P<0.05) reduced the fasting blood glucose levels in alloxan induced diabetic mice. The two doses (250 and 500 mg/kg) of the methanol extract and metformin 500 mg/kg caused a significant (P<0.05) reduction in the fasting blood glucose levels from day 1 to day 14 of treatment. The methanol extract displayed dose dependent reduction in blood sugar level with respect to time. The result showed that the crude methanolic extract had over 80% reduction of blood glucose at 14 days administration of 500 mg/kg of M. oppositifollous and this found to be the most significant reduction of blood glucose level. The highest reduction rate was found in 500 mg/kg (80.40 ± 10.00/80.85). (Figure 1)
Effect of Crude Methanol Extract on the Body Weight of the alloxan induced diabetic mice

The result of the effect of crude methanol extract on body weight is presented in Fig 1. The result showed significant reduction in the body weight of the animals induced with alloxan monohydrate and this was statistically different when compared with the treated group. The result showed a marked increase in body weight after treatment with various extracts and metformin. Weight increase was more in normal control and the treated group when compared to diabetic control. (Figure 2) showed remarkable recovery rate by the treated group in body weight of mice. Therefore, the treatment with of diabetic mice with M. oppositifolius showed significant weight recovery of mice while the negative control had reduced weight and did not recover in the body throughout the study period (Figure 2).

Histopathology Result of the pancreatic

The histopathology result is presented in Plate A to D. The pancreas of the diabetic mice (Group 1), without treatment were severely degenerated and this was the reason for continuous destruction of the group beta cells as seen in plate 3. Photomicrograph of a pancreatic section of metformin treated diabetic mice shows normal histoarchitecture with an increase in the number of islet cells when compared to the untreated group that were severely degenerated. Diabetic group 4 and 3 received 500 mg/kg and 250 mg/kg respectively and showed remarkable protection of the pancreatic cells with mildly despaired architecture of the pancreatic β cells islet, when compared to the untreated group that were severely degenerated. The findings showed that crude extract had excellent antidiabetic activity as seen in plate D and F when compared with negative control of severely damaged pancreas. (Figure 3)

Discussion

Alloxan-monohydrate induced in male mice avails a functional and physiological model for the study of hypoglycemic agents. The alloxan-induced diabetic mice had a three to four-fold increase in blood glucose (100 mg/dl to 200 mg/dl) relative to the normal control mice. The phytochemical analysis of the crude extract revealed the presence of cardiac glycosides, tannins, saponins, alkaloids, steroids and flavonoids and this is in agreement with the findings of [25] who reported the presence of alkaloids, phenols,
flavonoids, anthraquinones, and cardenolides. The crude leaf extract of *Mallotus oppostifollous* had no toxicity effect of up to 5000 mg/kg and these findings are in agreement with the findings and report of [26]. The presence of alkaloids, flavonoids, saponins and tannins indicates the presence of phenolic compounds which has been linked and reported to have antihyperglycemic activity [27]. These antidiabetic activities are attributed to the presence of phenolic phytochemicals which has been reported to possess potent antidiabetic effects in through diverse mechanisms like anti-inflammatory, antioxidant and anti-carcinogenic properties as reported by [27]. These hypoglycemic constituents could be the alkaloids, flavonoids, saponins, tannins, and steroids present in these plants [28], among others flavonoids, alkaloids, tannins, terpenoids, and sterols have been associated with hypoglycemic activity [29,30]. Also, as reported by [31] flavonoids like myricetin, a polyhydroxylated flavonols, has insulin mimetic properties and stimulates lipogenesis and glucose transport in the adipocytes, hence lowering blood sugar [29]. The alkaloid promotes the regeneration of pancreatic islets following destruction of the beta cells, hence restoring the secretion of insulin and thus correcting hyperglycemia [29]. The lowering of blood glucose levels by in the same manner regardless of the dosage might suggest that the extracts may reflect uptake of the active constituents through saturable active transport; it may also reflect maximum hypoglycemic activity at the lowest dose used (250 mg/kg body weight). The methanol leaf extracts (250 and 500 mg/kg) of *Mallotus oppostifollous* as well as metformin produced significant (P<0.05) percentage reduction in the fasting blood glucose levels when compared with the untreated control from day 0 to 14th day of treatment and this implied that *M. oppostifollous* had significant anti-diabetic activities against alloxan induced diabetic in mice and hence supports the use folklorically for the treatment of diabetes mellitus. The result showed significant recovery of the body weight after treatment with both the crude extract and metformin. Induction of diabetes with alloxan at 120mg/
kg initiates hyperglycemia by causing oxidative death of the pancreatic beta cells as it preferentially accumulates in the beta cells as glucose analogues thereby leading to severe loss in body weight. The recovery in body weight indicates the rejuvenation of the death cells and regulatory function of the pancreatic beta cells leading to restoration of equilibrium in the regulation and metabolism of blood glucose level. The observation that both the leaf extracts of Mallotus oppositifolius in various dosage modes lowered blood glucose indicates that these extracts have hypoglycemic constituents.

The result showed abundance of zinc, magnesium, phosphorus, iron, sulphate, chloride, calcium and magnesium and traces of sodium, potassium and cadmium and this should be included in the standardization of these drugs. Zinc, manganese, iron, calcium, magnesium and potassium all affects the insulin secretion, metabolism and regulation of blood glucose level.

Magnesium is involved in various pathway of insulin secretion and utilization such as in insulin secretion, binding, activity, as cofactor in various pathway involved in glucose oxidation, utilization and transportation [32]. Magnesium is also reported in glucose phosphorylation and its metabolism and its deficiency has been implicated in insulin resistance, carbohydrate intolerance, dyslipidemia and complications of diabetes [33].

Manganese is important for human health where it functions as a key constituent of metallo-enzymes activator in cellular biochemical reactions [34]. It also activates an antioxidant enzyme known as manganese superoxide dismutase (MnSOD) that protects the cell membranes and tissues from disruption and degeneration. It is reported to be involved in the modulation of glucose transport across cell membranes [35, 36] and its deficiency may lead to alteration in lipid metabolism.

Zinc is involved in all aspect of insulin secretion, assimilation and metabolism [37] it plays a key role in the regulation of insulin production by pancreatic tissues and glucose utilization by muscles and fat cells [38]. Zinc plays a role in the protection of β-cell against destruction and has an anti-viral effect. Diabetics consequently leads to excretion of excessive amounts of zinc in the urine and therefore require supplementation [35] which is found abundant in M. oppositifolious. Deficiency of intracellular zinc increases β-cell beta cell vulnerability to free radical attack. Restoring zinc levels in diabetic patients would counteract the deleterious effects of oxidative stress. In view of the positive role of zinc in insulin and beta cells, the anti-diabetic effect M. oppositifolious in the treatment of diabetes mellitus may be attributed to considerable amounts of zinc present in it and this is in agreement with the findings of [39].

Calcium is reported to improve insulin sensitivity in some type 2 diabetic populations [39]. The increase in the concentration of ionized cytosolic Ca ions directly mediates the effect of glucose to stimulate insulin release from rat islet of Langerhans [40] and its alterations in calcium flux can have adverse effects on β-cell secretory function [40].

Potassium has been reported to yields improved insulin sensitivity, responsiveness and secretion [41] insulin administration induces a loss of potassium; and a high potassium intake reduces the risk of heart disease, atherosclerosis, and cancer [40,42]. Potassium deficiency can result in adverse effects on calcium and saponins. The positive role of manganese, magnesium, zinc, calcium and potassium in metabolism, secretion and utilization of insulin therefore suggest they should be included in the standardization and use of M. oppositifolious for the treatment of diabetes mellitus.

Mallotus oppositifolious which may have acted by restoring pancreatic beta-cell integrity through mapping of reactive oxygen species (ROS) associated with the diabetic state, and thereby improving pancreatic function and consequently, lowering of fasting blood glucose levels. These findings provide ample evidence to support the traditional use of Mallotus oppositifolious in the management of diabetes mellitus, and therefore, I recommend its continuous use for treatment of diabetes mellitus. This study findings, have validated the assertion, inclusion and adoption of plant-based drugs for anti-diabetic activity due to their perceived effectiveness in the treatment anti-diabetics.

References


