Hypoglycemic Potential and Safety of Aqueous Extract of Aspilia Mossambicensis in Alloxinised Diabetic Mice

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Abstract: *Aspilia mossambicensis* (Oliv.) Wild is ethnomedically used in management of numerous maladies, including diabetes mellitus. In spite of this, its efficacy and safety has not been scientifically validated. The aim of this study was to determine in vivo hypoglycemic activity and safety of the aqueous whole stem and leaf extracts of *Aspilia mossambicensis* (Oliv.) Wild in alloxanised diabetic swiss albino mice. The extract was assayed for hypoglycemic activity through oral administration at four doses of 50, 100, 200 and 300mg/kg body weight. The safety of the extract was evaluated by oral administration of the extract at doses of 450, 670 and 1000mg/kg body weight for twenty eight days and subsequently determining the body weights, normalized percent organ to body weight, hematological and biochemistry parameters. Phytochemicals composition was assayed through standard procedures. The extract showed strong dose independent hypoglycemic activity from the first to the sixth hour, after which diminishing activity was noted through to the 24th hour. Following oral administration of the extract in normal mice for 28 days, to assay for the safety of the extract, significantly lower rate of weekly weight gain was observed. Moreover, a significant increase in normalized percent organ to body weight of the liver and kidney was noted. A profile of hematological parameters revealed significant elevation in the level of packed cell volume and mean cell volume, lymphocytes and neutrophils count relevant to the normal control. Significant elevation in the level of AST, ALP, GGT and LDH were also noted, at higher doses of the extract. Hypoglycemic activity and the mild toxicity demonstrated by this plant at elevated doses may be due to the phytochemical composition of the plant extract. The study recommended continued use of the extract at the tested therapeutic doses.

WHO - World Health Organization, AST - aspartate transaminase, ALP - alkaline phosphatase, GGT - γ-glutamyltransferase) and LDH - lactate dehydrogenase.

Introduction

Diabetes mellitus is a chronic condition that results from deranged carbohydrate metabolism. The disease has varied etiology but characterized by chronic hyperglycemia either due to secretion of inadequate insulin or due to insulin resistance [1]. Prolonged hyperglycemia can cause renal and retinal complications. Diabetes leads to reduced life expectancy, vascular injury. Macro and micro vascular injury contributes to both morbidity and mortality [2]. WHO projected that by the number of diabetics may rise by more than two fold to 366 million by the year 2030 from an estimated 170 million in the year 2000 [1]. The WHO classified diabetes mellitus as Type one, otherwise known as Insulin Dependent Diabetes mellitus and type 2 known as Non Insulin Dependent Diabetes Mellitus depending on their etiology. Type I diabetes mellitus develops mainly due to destruction of the insulin secreting beta cells in the pancreas. Victims with this type are at a high risk of developing diabetic ketoacidosis in the face of uncontrolled hyperglycemia. The type is idiopathic and its development is thought to be linked to destruction of pancreatic beta cells. Type II diabetes on the other hand results from secretion of defective insulin that does not stimulate the body cells to regulate glucose even in the face of prolonged hyperglycemia [1]. Victims of diabetes mellitus normally present with symptoms like frequent urination, polydipsia, sudden unexplained weight loss, blurred vision (if prolonged)
and fatigue. If these symptoms are noted, random glucose blood sugar level above 11.00 mm/L is used as a reference value for diagnosing the disease. In absence of the above stated symptoms, the disease is diagnosed by determining the level of glycosylated haemoglobin (6.10-7.00 mm/L) and oral fasting glucose tolerance test ≥11.00mm/L on two separate occasions [3]. Conventional drugs used to manage diabetes mellitus have shown several drawbacks due to their adverse side effects. Sulphonylureas, a common oral hypoglycemic agent mode of action is by blocking the ATP dependent channel and this can lead to hypoglycemia, which can be fatal. Insulin, a common intraperitoneally administered hypoglycemic agent has been associated with reduction in the functionality of brain cells, poor appetite and fatty liver. Other unfavorable side effects associated with conventional hypoglycemic agents include lactic acidosis, neurological complications, light headedness and even death. In the face of this situation, it is imperative that safe, effective and cheap drugs are developed to manage this disease. Medicinal drugs come into play when all this factors are considered [4].

Drugs extracted from plants have been used to treat various maladies since the advent of civilization and diabetes mellitus is not an exception. This has been done over the years with nothing but the belief that they are safe and effective. The trend is very common in developing countries where it is reported that about 80% of the population relies on these drugs to treat different diseases [5] Aspilia mossambicensis (Oliv.) Wild has been used by Mbeere people of Eastern Kenya in managing diabetes and its associated complications. Locally, the plant is known as muti (Mbeere and Kamba). The plant belongs to the family Asteraceae. It grows to a height of about 1.5 metres with characteristic yellow flowers. It is widely distributed in Eastern and Southern part of Africa and Madagascar. In a study conducted by Musyimi et al [6] leaves of the plant have been found to exhibit strong bactericidal and bacteriostatic activity against Staphylococcus aureus and Escherichia coli [6].

To date, no study has been conducted and published to validate the safety and efficacy of the plant in question. With this fact in mind, the study was meticulously planned and executed to come up with concrete and scientifically verifiable basis to validate its prolonged use. This was executed by orally administering aqueous extracts of Aspilia mossambicensis (Oliv.) Wild on mice.

**Study site**

The study was planned, set up and executed to completion at the laboratories in the Department of Biochemistry and Biotechnology in the School of Pure and Applied Sciences in Kenyatta University, Kenya.

**Collection and identification of plant materials for the study**

Stems of Aspilia mossambicensis (Oliv.) Wild with leaves were collected in the month of September 2015 from Gatongu village Siakago division, Mbeere North sub County in Embu County, Kenya. Consultations were done with traditional medical practitioners on the parts of the plant that are used. Scientific validation and identification was sought from a plant taxonomist; at East African Herbarium, National Museums of Kenya. Voucher specimens were deposited at the museum for future reference.

**Preparation of the aqueous plant extracts**

The fresh whole stems with leaves collected were chopped in to tiny pieces, dried under a shade for two week still constant weight. The dried plant samples were ground into a fine powder using an electric mill, packed in plastic sachets and stored in a dark cabinet. 100g of the powder was extracted in a liter of double distilled water in a water bath set at 60°C for six hours. The extract obtained was filtered in another conical flask and concentrated by evaporation. The concentrated filtrate was freeze dried in 200 ml portions in a Modulyo freeze drier (Edward England) for 48 hours. The dark brown powder obtained was taken and stored in sealed airtight bags and kept refrigerated in a freezer at -20°C ready for the bioassay.

**Animals for the experiment**

White male swiss albino mice of 3-4 weeks of age weighing 23-25g were used. They were obtained from the Department of Zoology in the College of Pure and Applied Pure Sciences, Chiromo Campus of the University of Nairobi. The animals were fed on standard approved rodent pellets and allowed free access to clean water. The animals were housed at standard conditions of 25°C with twelve hours of light and corresponding numbers of darkness within the twenty four hour period at the departmental animal house. Before executing the study, permission was sought and obtained from the host Institutions’ Ethics Committee on Handling and Care of Animals.

**Induction of hypoglycemia**

Hypoglycemia was experimentally induced in mice by injecting a single dose of 186.9mg/kg body weight of freshly prepared alloxan monohydrate. Forty eight
hours after administration of alloxa monohydrate, blood glucose concentration was determined using glucose analyzer model On-call plus-ACON LAB Inc-U.S.A. Animals with blood glucose level above 11.00mm/L were considered diabetic and therefore fit for the study. Before commencement of the in vivo bioassay, the animals were fasted for 8-12 hours [7].

Experimental plan

Thirty five mice involved in the bioassay were divided randomly into seven groups of five mice per group. Group I consisted of normal unmanipulated mice while Group II consisted of diabetic mice (both Groups were treated with 0.1 ml of normal saline). Group III consisted of diabetic mice treated with 3 mg/kg body weight glibenclamide dissolved in 0.1 ml normal saline. Group IV, VI, VI and VII consisted of diabetic mice treated with 50, 100, 200 and 300mg/kg body weight dissolved in 0.1 ml normal saline.

Assaying of blood glucose

The assay of blood glucose concentration levels was done by first sterilizing the tail with 10% alcohol and nipping the tail to draw blood. Sugar levels were established at 1, 2, 3, 4, 6 and 24 hours using glucometer model and On-call plus-ACON LAB Inc-U.S.A. .

In vivo triple dose toxicity study

This essay utilized 20 unmanipulated normal animals which were randomly separated into four groups each consisting of five animals each. Group I was administered orally with 0.1 ml physiological saline, Group II, III and IV were orally administered with 450, 670 and 1000 mg/kg body weight dissolved in 0.1 ml physiological saline. Administration of the extract to the normal mice was done daily for 28 days and the animals were keenly observed daily for any mortality or behavioral changes throughout the whole period. The animals were allowed free access to water and food.

Determination of body and organ weights

Body weight of animals used in the bioassay was determined once prior to administration of the extract of Aspilia mossambicensis (Oliv.) Wild, once a week during the period of administration of the extract and also on the day of sacrifice. On the twenty eighth day, all the animals were euthanised after which the liver, kidney, brain, spleen and the testes were meticulously dissected. The weights of the named organs were taken, recorded and the organs preserved in 10% formaldehyde.

Determination of haematological parameters

Standard procedures as outlined by [8] were observed in assaying of the haematological parameters. Parameters determined were red blood cells, haemoglobin, mean cell volume, mean corpuscular haemoglobin concentration, packed cell volume and platelets. White blood cells and associated differentials like neutrophils, lymphocytes, eosinophils, basophils and monocytes were also determined.

Determination of biochemistry parameters

Blood for biochemical assay was collected in separate EDTA free vials and allowed to stand for three hours for complete clotting. After clotting, the samples were centrifuged for ten minutes to obtain a clear serum which was frozen at -20°C ready for assaying. Biochemical parameters assayed were alanine transaminase, aspartate transaminase, gamma glutamyltransferase, alkaline phosphatase, lactate dehydrogenase, creatinine, creatinine kinase and amylase. The tests were performed based on the standard procedures.

Quantitative determination of phytochemicals of the extracts

Standard procedures were employed to quantitatively determined the levels of phytochemicals .The following bioactives were profiled; alkaloids, saponins, tannins, terpenoids and total phenols with strict adherence to. Two grams of the freeze dried extract of Aspilia mossambicensis (Oliv.) Wild was first soaked in 100 ml diethyl ether in a Soxhlet apparatus for two hours to de-fat it. Total phenols were measured observing standard procedures outlined by [9], tannins by [10], alkaloids by [11] and flavonoids by [12].

Data processing and statistical analysis

The data obtained from the assay was recorded in excel Spreadsheet cleaned and exported to statistical package Minitab version 18. The results were recorded as Mean ± Standard Deviation of the number of animals used per assay. Data analysis was done using ANOVA and Tukey’s pairwise comparison. The level of significance was set at ρ ≤ 0.05.

Results

In vivo hypoglycemic activity of Aspilia mossambicensis (Oliv.) Wild

The aqueous leaf extracts of A. mossambicensis (Oliv.) Wild yielded 8.5% (w/w) brown powder. The four test doses of 50mg/kg, 100mg/kg, 200mg/kg and 300mg/kg of body weight
demonstrates varying level of hypoglycemic activity when administered orally to diabetic mice. In the first hour following administration of the extract at 50 mg/kg of body weight lowered blood glucose to 69.5%, 100mg/kg to 68.3% and 200mg/kg to 83.5% and 300mg/kg body weight to 82.0%. Glibenclamide on the other hand lowered blood glucose to 69.4% within the same duration. By the sixth hour, glibenclamide had lowered blood glucose to 32.2% whereas the dose of 50mg/kg of the extract to 26.8, 100mg/kg to 32.5, 200mg/kg to 35.0 and 300mg/kg body weight to 37.9% (p ≤ 0.05) (Table 1 Figure 1).

Table 4.1: Effects of orally administered aqueous leaf extract of Aspilia mossambicensis (Oliv.) Wild on blood glucose levels in alloxan induced diabetic mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>0hr</th>
<th>1hr</th>
<th>2hr</th>
<th>3hr</th>
<th>4hr</th>
<th>6hr</th>
<th>24hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control/Saline</td>
<td>5.12±0.14aA</td>
<td>5.00±0.20aA</td>
<td>4.96±0.25abc</td>
<td>4.94±0.25abc</td>
<td>4.98±0.19abc</td>
<td>5.16±0.13abc</td>
<td>5.38±0.25abc</td>
</tr>
<tr>
<td>Diabetic/Saline</td>
<td>17.14±1.99abc</td>
<td>18.72±2.10abc</td>
<td>20.66±2.50bcd</td>
<td>22.48±2.47abcd</td>
<td>24.20±1.95abc</td>
<td>25.66±1.91abc</td>
<td>27.75±1.11abc</td>
</tr>
<tr>
<td>Diabetic/gliben</td>
<td>15.38±2.45abc</td>
<td>10.68±2.15abc</td>
<td>8.30±0.87abc</td>
<td>7.02±0.71abc</td>
<td>6.16±0.76abc</td>
<td>4.96±0.35abc</td>
<td>8.04±0.68abc</td>
</tr>
<tr>
<td>50(mg/kgbw)</td>
<td>13.26±2.17abc</td>
<td>9.22±1.97abc</td>
<td>7.00±1.04abc</td>
<td>5.82±1.08abcd</td>
<td>4.72±0.32abcd</td>
<td>3.86±0.23abcd</td>
<td>8.54±0.73abcd</td>
</tr>
<tr>
<td>100(mg/kgbw)</td>
<td>14.80±3.18abc</td>
<td>10.12±2.55abc</td>
<td>7.80±1.34abc</td>
<td>6.52±1.14abc</td>
<td>5.46±0.67abc</td>
<td>4.82±0.24abc</td>
<td>7.90±1.02abc</td>
</tr>
<tr>
<td>200(mg/kgbw)</td>
<td>15.10±4.24abc</td>
<td>12.62±3.05abc</td>
<td>9.74±2.24abc</td>
<td>7.24±1.14abc</td>
<td>6.14±0.68abc</td>
<td>5.30±0.43abc</td>
<td>10.26±2.07abc</td>
</tr>
<tr>
<td>300(mg/kgbw)</td>
<td>14.02±2.52abc</td>
<td>11.50±2.04abc</td>
<td>8.58±1.06abc</td>
<td>7.24±1.03abc</td>
<td>5.96±0.87abc</td>
<td>5.32±0.82abc</td>
<td>9.20±1.02abc</td>
</tr>
</tbody>
</table>

Results are expressed as Means ± SD for five mice per group. Values followed by the same lower case superscript are not statistically different (p≤0.05) along a column whereas values marked with the same upper case superscript along a row are not significantly different at (p≤0.05) ; analysed by ANOVA followed by Tukey’s pairwise comparison.

Figure 1: Mean percentage change in blood glucose levels of A. mossambicensis (Oliv.) Wild administered orally in swiss albino alloxanised diabetic male mice.

Effect of aqueous plant extracts of A. mossambicensis on rate of body weight gain and relative percent organ to body weight

Effects of oral administration of 450, 670 and 1000mg/kg body weight of aqueous plant extracts of A. mossambicensis (Oliv.) Wild on body weight gain and relative percent organ to body weight on normal male mice are recorded in Table 3. Throughout the experimental period, there was an observed, dose dependent, significantly lower, weekly weight gain in test doses, relative to the control. Administration of the extract resulted in statistically significant increase in the relative percent organ to body weights of the liver and
kidney at 450, 670, 1000mg/kg body weight However, there was no significant change in relative percent organ to body weight of the lungs, spleen, heart, testes and brain (ρ > 0.05) (Table 2).

<table>
<thead>
<tr>
<th>Treatment (mg/kgbw)</th>
<th>Weekly weight of normal male swiss albino mice (g)</th>
<th>Δ Weight/Week (g/Week)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>23.58±1.34a</td>
<td>25.34±1.19a</td>
</tr>
<tr>
<td>450</td>
<td>23.58±1.68a</td>
<td>24.92±1.76a</td>
</tr>
<tr>
<td>670</td>
<td>23.50±0.98a</td>
<td>24.64±1.07a</td>
</tr>
<tr>
<td>1000</td>
<td>23.31±1.33a</td>
<td>23.99±1.32a</td>
</tr>
</tbody>
</table>

Table 2: Effect of oral administration of aqueous leaf extract of Aspilia mossambicensis (Oliv.) Wild for 28 days on body weight of normal male swiss albino mice

Results are expressed as Mean± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at ρ ≤ 0.05; analysed by ANOVA followed by Tukey’s pairwise comparison. Key - A-represents change in

Table 3: Effect of oral administration of aqueous extract of A. mossambicensis (Oliv.) Wild for 28 days on relative percent organ to body weights of normal male swiss albino mice

Results are expressed as Mean± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at ρ ≤ 0.05; analysed by ANOVA followed by Tukey’s pairwise comparison.

Effects on red blood cells and related parameters after administration of the aqueous extracts to normal male swiss albino mice

Effects of oral administration of the aqueous extracts of Aspilia mossambicensis (Oliv.) Wild on normal male swiss albino mice at doses of 450, 670 and 1000mg/kg body weight on hematological parameters are recorded in Table 4. A significant rise in the level PCV at 670 and 1000mg/kg and MCH at 450, 670 and 1000mg/kg body weight in a dose dependent pattern was noted (ρ ≤ 0.05). However, there was no significant change in levels Hb, RBC, MCV, MCHC, PLT and MPV across the treatments (ρ > 0.05).

Table 4: Effect of oral administration of aqueous leaf extract of A. mossambicensis (Oliv.) Wild for 28 days on platelets, RBC and related parameters in normal male swiss albino mice

Results are expressed as Mean± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at ρ ≤ 0.05; analysed by ANOVA followed by Tukey’s pairwise comparison. RBC = red blood cell count; Hb = haemoglobin; PCV = packed red cell volume; MCH = mean corpuscular haemoglobin; MCHC = mean corpuscular haemoglobin concentration; MCV = mean corpuscular volume, PLT = platelets; MPV = mean platelet volume.

The effects on leucocytes differentials following oral administration of the aqueous extracts to normal male albino mice at 450, 670 and 1000mg/kg body weight of A. mossambicensis (Oliv.) Wild for 28 days are shown in Table 5. Administration of the extract resulted in a significant rise (ρ ≤ 0.05) in NEU at 670 and
Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at ρ ≤ 0.05; analysed by ANOVA followed by Tukey’s pairwise comparison. WBC = white blood cell count; NEU = Neutrophils; EOS = eosinophils; BAS = basophils; MON = monocytes; LYM = lymphocytes.

Administration of the extract at the three test doses had no significant effect on the levels of ALT, AST/ALT. However, the extract caused a significant increase in the level of AST, CK after 28 days on lipid profiles and glucose levels in normal male swiss albino mice relevant to the control (ρ ≤ 0.05).

Results in this table are expressed as Mean ± SD for five experimental models per group. Means that do not share a letter (superscript) are significantly different from each other at ρ ≤ 0.05; analysed by ANOVA followed by Tukey’s pairwise comparison. AST = alanine transaminase; ALT = aspartate transaminase; ALP = alkaline phosphatase; GGT = γ-glutamyltransferase; LDH = lactate dehydrogenase; CK = creatine kinase; α-AMYL = α-amylase; AST/ALT = the ratio of the activity of aspartate transaminase to alanine transaminase.

**Table 6: Effects of oral administration of aqueous leaf extracts of *A. mossambicensis* (Oliv.) Wild for 28 days on enzyme markers of liver and the kidney**

<table>
<thead>
<tr>
<th>Treatment (mg/kgbw)</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
<th>ALP (U/L)</th>
<th>GGT (U/L)</th>
<th>LDH (U/L)</th>
<th>CK (U/L)</th>
<th>α-AMYL (U/L)</th>
<th>AST/ALT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>60.0±5.61a</td>
<td>420.2±22.5b</td>
<td>2.20±1.09b</td>
<td>1.80±0.44b</td>
<td>608.7±30.8a</td>
<td>288.0±8.46</td>
<td>407.2±33.4ab</td>
<td>7.50±0.26a</td>
</tr>
<tr>
<td>450</td>
<td>59.0±4.24a</td>
<td>414.0±21.1b</td>
<td>2.60±0.54b</td>
<td>2.20±0.44b</td>
<td>626.4±27.4a</td>
<td>287.1±9.17</td>
<td>463.0±43.9ab</td>
<td>7.22±0.98a</td>
</tr>
<tr>
<td>670</td>
<td>62.6±4.88a</td>
<td>433.4±26.6b</td>
<td>3.40±1.14b</td>
<td>4.40±1.34ab</td>
<td>609.6±29.8a</td>
<td>296.5±8.5b</td>
<td>669.0±40.0a</td>
<td>6.30±0.81a</td>
</tr>
<tr>
<td>1000</td>
<td>63.6±3.51a</td>
<td>475.8±21.30b</td>
<td>3.90±1.00b</td>
<td>5.30±1.34b</td>
<td>726.0±48.3a</td>
<td>343.8±9.5a</td>
<td>720.4±26.0a</td>
<td>6.74±1.31a</td>
</tr>
</tbody>
</table>

Results in this table are expressed as Mean ± SD for a group of five experimental mice. Means that do not share a letter (superscript) are significantly different from each other at ρ ≤ 0.05; analysed by ANOVA followed by Tukey’s pairwise comparison. ALT = alanine transaminase; AST = aspartate transaminase; ALP = alkaline phosphatase; GGT = γ-glutamyltransferase; LDH = lactate dehydrogenase; CK = creatine kinase; α-AMYL = α-amylase; AST/ALT = the ratio of the activity of aspartate transaminase to alanine transaminase.

**Effect of oral administration of aqueous leaf extract on lipid profiles and glucose**

The effects of oral administration of 450, 670 and 1000mg/kg body weight of aqueous extract of *A. mossambicensis* (Oliv.) Wild in normal male swiss albino mice for 28 days on lipid profiles and glucose are indicated in Table 7. Oral administration of the extract demonstrated an insignificant change in the level of D-BIL, TC, HDL-C, and LDL but caused significant reduction in the level of glucose at 1000mg/kg body weight and TG at 670 and 1000mg/kg body weight in comparison to the control (ρ ≤ 0.05).

**Table 7: Effect of oral administration of aqueous leaf extract of *A. mossambicensis* (Oliv.) Wild for 28 days on lipid profiles and glucose levels in normal male swiss mice**

<table>
<thead>
<tr>
<th>Treatment (mg/kgbw)</th>
<th>Lipid profiles and glucose levels</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T-BIL (μM)</td>
</tr>
<tr>
<td>Control</td>
<td>2.94±0.23a</td>
</tr>
<tr>
<td>450</td>
<td>2.96±0.32a</td>
</tr>
<tr>
<td>670</td>
<td>2.94±0.11a</td>
</tr>
<tr>
<td>1000</td>
<td>3.35±0.22a</td>
</tr>
</tbody>
</table>

Results are given as Mean ± SD for five experimental models per group. Means that do not share a letter (superscript) are significantly different from each other at ρ ≤ 0.05 analysed by ANOVA followed by Tukey’s pairwise comparison. T-BIL = total bilirubin; D-BIL = direct bilirubin; I-BIL = indirect bilirubin;.
TG = triacylglycerols; TC = total cholesterol; HDL-C = high density lipoprotein cholesterol; LDL-C = low density lipoprotein cholesterol; GLU = glucose.

Table 8: Phytochemical composition of aqueous extracts of the whole stems and leaves of *Aspilia mossambicensis* (Oliv.) Wild

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Phytochemical Content(mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tannins</td>
</tr>
<tr>
<td><em>A. mossambicensis</em> (Oliv.) Wild</td>
<td>29.11±0.02</td>
</tr>
</tbody>
</table>

Quantitative analysis of phytochemical composition of the aqueous extracts of *Aspilia mossambicensis* (Oliv.)

Discussion

Intraperitoneal administration of alloxan monohydrate to induce diabetes is widely used in research involving animals. The compound leads to elevated levels of reactive oxygen species which causes pancreatic beta cells’ necrosis resulting to inadequate secretion of insulin making the study animal models diabetic [13]. Oral administration of *Aspilia mossambicensis* (Oliv.) Wild whole stems and leaves extracts to diabetic mice showed strong dose independent hypoglycemic activity. Dose independent hypoglycemic activity demonstrated in this study may have been due to uptake of the extracts through active transport.

When the absorbency thresh hold was exceeded, the unabsorbed fraction was excreted from the body mainly through the kidneys. After cellular uptake, the extract may have effectuated glucose lowering action through enhancement of insulin secretion, release of bound insulin or even limiting absorption of glucose from the alimentary tract [14,15,16,17], accelerated conversion of glucose to glycogen in the liver and stimulating glucose metabolism in the muscle cells [18,19] Quantitative assaying of phytochemical composition in aqueous extracts of *Aspilia mossambicensis* (Oliv.) Wild revealed presence of saponins alkaloids, tannins, total phenols and flavonoids in varied concentration. Flavonoids and tannins from ethanolic leaf extracts of *Lantana camara* Linn. have been demonstrated to exhibit hypoglycemic activity on mice [20]. Myricetin, a polyhydroxylated flavanol demonstrated strong insulin like activity and also accelerated the transfer of glucose into adipocytic cells resulting to hypoglycemia [19]. Alkaloids, which are found in the aqueous extracts of the aqueous plant extract under study, have been found to show strong blood glucose lowering effect [20]. Epigllo-catechin-3-gallate which is a tannic compound was found to demonstrate strong antidiabetic activity [21]. Phenols demonstrated from *Ficus carica* L. fruits demonstrated strong anti diabetic activity [22]. Phenols extracted from *Olea europea* L. effect were found to play a big role in revitalization of damaged cells in diabetic mice [23]. Toxicity studies for any drug should be carried out after its efficacy is scientifically validated. Adverse toxicity renders the drug unsuitable for therapeutic purposes. To achieve this, an aqueous extracts of *Aspilia mossambicensis* (Oliv.) Wild was administered at three high doses of 450, 670 and 1000mg/kg body weight for twenty eight days in mice. The three groups of animals administered with the three doses of the extract recorded relatively lower weight gain in a dose dependent version. The extract administered had phytococonstituents that accelerated the process of degradation of proteins in the skeletal muscles resulting to growth slowdown in the animals [24]. Alkaloids, tannins, saponins and flavonoids have been found to be toxic. For example, saponins cause erythrocytic cellular haemolysis and renal hemorrhage due to renal tubular injuries. Very high levels of saponins have been reported to cause cardiac arrest and sudden hypoglycemia which can be fatal [25]. Alkaloids cause injury to the biliary tract, nuclear enlargement of liver cells (magalocytosis) and benign hepatic tumor [26] though it is important to note that these deleterious effects were not noted in this investigation. Solanine, radicaline and solanine are some classic examples of phytoalkaloids. Animals exposed prolonged administrations of phenols have been reported to present with renal and liver injjury [27]. Tannins suppress feeds intake through inducing astringency and a slow down absorption of digestion of food in the gastrointestinal tract. Salivary mucins like salivary glycoproteins play a big role in protection of the buccal cavity mucosa [27]; but it is important to note that this protective role is nullified by tannins through formation of stable complexes with the salivary glycoproteins hence contributing further to astringency, low palatability of food and suppressed digestion of food. Tannins are classified into

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hydrolysable and condensed tannins (protoanthocyanidins). After ingestion, hydrolysable tannins are converted into short chain metabolites like tannic acid which are capable of inducing gastro enteric bleeding which further leads to impaired absorption of digested food, renal damage and severe liver injury [21]. Condensed tannins on the other hand cause decelerated growth by inducing decelerated growth through suppression of appetite and breakdown of food into simple soluble absorbable molecules. The unabsorbed tannins further cause more injury to the intestinal mucosa hence interfering with the absorption of food [21]. This can result in accelerated excretion of proteins that result in protein malnutrition.

Oral administration of aqueous extracts of Aspilia mossambicensis (Oliv.) Wild resulted to elevation in the level of paced cell volume of mean haemoglobin concentration. This may be linked to impaired folic acid absorption due to presence of alkaloids in the administered extracts which have been reported to cause elevation in the level of erythron parameters in question [28]. Decreased feed intake due to depressed appetite may also have contributed to reduced appetite. Elevation in the level of lymphocytes and neutrophils perhaps may due to the phytoconstituents in the extracts enhancing the immunity of the animals. Phosphocreatine which serves as an immediate source of ATP generation is rapidly exhausted hence interfering with transmembrane ionic pump mechanism. This results to swelling of the cells making the intracellular components to leak to the intercellular space resulting to their elevation after they find their way into the normal circulatory system [28]. This swelling is supported by the elevation in the level of AST (aspartate transaminase), ALP (alkaline phosphatase) GGT (γ-glutamyltransferase) and LDH (lactate dehydrogenase). The fact that the levels of direct and indirect bilirubin were not affected shows that the functionality of the organs in question was not adversely affected.

Conclusion,

Aspilia mossambicensis (Oliv.) Wild used in diabetes management demonstrated significant activity at the three therapeutic doses administered orally. Higher doses of 670 and 1000mg/kg body weight produced mild toxic effects through reduction in body weight gain, increase in organ weights of the liver and kidney, PCV and MCV, lymphocytes and neutrophils, AST, ALP, GGT and LDH. Hypoglycemic activity and the toxicity demonstrated by this plant at elevated doses may be due to the phytochemical composition in the extract.

Reference


