Acute toxicity and antidiabetic activity of *Asystacia gangetica* leaf ethanol extract

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**Abstract**

**Purpose** – The purpose of this study is to examine the acute toxicity and antidiabetic activity of *Asystacia gangetica* leaf ethanol extract.

**Design/methodology/approach** – The study was designed as completely randomized *in vivo* experimental model. Where acute toxicity study was carried out using 30 albino mice, randomly assigned into six groups of five mice each. Toxicity signs and mortality were observed in the rats within a period of 24 h. The acute and sub-acute antidiabetic study was carried out using 50 rats, randomly assigned into five groups of 10 rats each. The rats’ blood glucose levels were determined and used to assess the acute and sub-acute antidiabetic activity of the extract.

**Findings** – Results obtained from the acute toxicity study indicated no death in any of the study groups, even at 5,000 mg/kg body weight and showed no signs of toxicity. The acute antidiabetic study showed that treatment with 400 mg/kg of the extract significantly (*p* = 0.01) lowered glucose level in the diabetic rats from 430.6 to 177.4 mg/dl while 800 mg/kg brought down glucose level from 370 to 144.2 mg/dl by the end of 6 h following administration when compared with the diabetic control group. It was observed that the effect of the extract mostly at 800 mg/kg also compared favorably with that of the standard drug (glibenclamide), which lowered glucose level in diabetic rats from 374.2 to 176.4 mg/dl. Furthermore, the significant reduction was evident from 4, 2 and 2h for 400 mg/kg extract, 800 mg/kg extract and 5 mg/kg glibenclamide, respectively. At sub-acute level the blood glucose was lowered from 155.6 to 127.2 mg/dl, 137 to 124.4 mg/dl and 151.8 to 121.8 mg/dl for diabetic rats treated with 400 mg/kg, 800 mg/kg and 5 mg/kg glibenclamide, respectively, when compared to the diabetic untreated rats, which ranged from 417.6 to 358.6 mg/dl. The biochemical profile, lipid profile and hematological examination were all positively restored near to normal with the herbal treatment at the different doses. At histopathology level, the liver of the rats treated with 400 mg/kg had moderate portal inflammation without interface or lobular hepatitis while that of 800 mg/kg showed severe portal inflammation with the interface and lobular hepatitis with extensive confluent necrosis. The pancreatic cells of the treated rat showed no significant difference in the β-cells of the islets of Langerhans with hyperplasia of the acinar cell when compared to the diabetic untreated.

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With a sense of deep humility, the authors appreciate Tertiary Education Trust Fund (TETFund), Nnamdi Azikiwe University Awka, Anambra State Nigeria for funding this research.
Research limitations/implications – The record of no death and signs of toxicity implies that the extract is safe for consumption even at a high dosage of 5,000 mg/kg body weight. The significant ($p = 0.01$) reduction in the plasma glucose level of the treated rats as compared to the control is an indication of blood glucose-lowering potential of the extract at two different doses. The significant reduction evident of the extract at different hours and days for the two doses implies that the extract rate of lowering potentials is dose-dependent. The evidence of moderate-severe portal inflammation with the interface and lobular hepatitis at 800 mg/kg treatment is an indication that the intake of this herb at high dosage for long period is not safe for the liver tissue.

Practical implications – The outcome of this study suggested that the *Asystacia gangetica* should also be used as a vegetable in-home food preparation and food processing to use its antidiabetic effect. The herbal extract could also be incorporated into a food product and processed into herbal tea bag for convenient. The subjection of this herbal plant to heat treatment during processing could be a possible avenue to make it safe.

Social implications – The economic burden and complications of diabetes mellitus management will be reduced if the practical implication of this research finding is implemented in foods as vegetable and in functional food production.

Originality/value – This study revealed that *Asystacia gangetica* leaf extract may be safe and effective for use at a low dose for acute management of diabetes mellitus. This research may be of value to those living with diabetes mellitus.

Keywords Acute toxicity, Antidiabetic activity, *Asystacia gangetica* leaf, Ethanol extract, Diabetes mellitus

Paper type Research paper

Introduction
Diabetes mellitus is a chronic metabolic disease with life-threatening complication. It is a chronic disorder in the metabolism of macro nutrients such as proteins, fats and carbohydrates (Osadebe et al., 2014). It was estimated by International Diabetes Federation (IDF) that 285 million people are living with diabetes, in 2010 showing that about 6.4 per cent of the world populace had diabetes and by 2030, the prevalence rate will increase to 439 million people amounting to about 7.7 per cent of the world population (Shaw et al., 2010). Over 90 per cent of the cases of diabetes are type 2 diabetes mellitus (T2D) (Boyle et al., 1999; Attele et al., 2002). This disease affects many people and is identified as the fifth leading causes of death in this twenty-first-century (Kazi, 2014; Wesam et al., 2016).

The management of diabetes mellitus is associated with a huge economic cost for the afflicted people and countries. In 2007, approximately 17.5 million people living with diabetes mellitus were reported to have spent about US$174bn in the management of diabetes mellitus (Cashen et al., 2008). However, in Nigeria, about 1 to 7 per cent of the populace is afflicted with diabetes mellitus (Wokoma, 2002; Fabiyi et al., 2002).

Despite considerable progress in scientific studies on T2D and research and development of antidiabetic agents, yet the cause is not completely understood. Though, mounting facts from epidemiological research finding suggests that the primary causes of T2D remain environmental and genetic factors. Both factors are the contributing factor to insulin resistance and loss of beta cells function that result in impairment in insulin action, insulin production or both. The occurrence of hyperglycemia in diabetes mellitus is due to impairment in insulin action (Laakso, 2001). Such hyperglycemia results to glucotoxicity, which affects the cells and peripheral tissues, which are clinically important in the cause of diabetes-related complications such as cardiovascular disease, nephropathy, retinal blindness, neuropathy and peripheral gangrene (Clements and Bell, 1985). Therefore, the most effective therapy for people living with diabetes mellitus is the maintenance of glycemic homeostasis.
Glycemic control is the most accepted approach for T2D treatments in an attempt to lessen complications and death. Apart from drugs, lifestyle and diet are very significant approach in the management of diabetes mellitus and should not be neglected. The lack of efficiency and undesirable side effect make the current antidiabetic drugs unsuitable and require alternative (Howlett and Bailey, 1999). In other to ensure the safety of the people living with diabetes mellitus, it becomes a necessity to develop alternative antidiabetic medicine that will be devoid of the above-stated challenges with suitable efficiency. Sonia et al. (2018) stated that most herbal extract/formulation are known to have a permanent cure, no adverse effect, cheap, eco-friendly and safe in the management of diabetes mellitus.

WHO reported that about 80 per cent of the world’s populations are dependent on traditional medicine, particularly plant drugs for primary health care? Herbs were used to treat different types of disease conditions before the birth of traditional Western medicine (Basch et al., 2003). A number of plants have shown varying degrees of hypoglycemia and antidiabetic activities (Onoagbe et al., 1999), and 100s of such plants are in common use. However, so many of them have not been scientifically proven to have antidiabetic activities or either been checked on its dosage-dependent effect.

Asystacia gangetica commonly known as Ganges primrose, Chinese violet and akpu-arachi in Nigeria from the family of Acanthaceae is an herb (Adeyemi et al., 2011). It is called Ganges because it is obtained from the river named Gange where it is assumed that the species exist. It is an herbaceous ground cover plant that grows to the height of 30 to 60 cm. It has a colored flower with tessellated purple mark on the palate that makes the plants very attractive. It also grows fast and spreads easily with dark green leaves. Over a long period of time, the flowers are produced followed by brown seeds capsules (Ramesar et al., 2008). This plant is widely distributed across South Asia, tropical America, sub-Saharan Africa, Oceania and Nigeria. The edible parts include the tender leaves and stems, which are eaten fresh, stir-fried or boiled.

The scientific mind will not be satisfied by mere claims no matter and from whatever source they originate unless corroborated by experimental and clinical evidence. As it is evident that plant are a treasure house for many potent medicines, it is important to scientifically evaluate the traditional practices and upgrade the existing knowledge and make it accessible to the universal public. A good number of popularly used Nigerian plants have not been scientifically-scrutinized for their efficacy and antidiabetes properties (Onoagbe et al., 1999). Thus, these were the drive to this study of the acute toxicity and dose-dependent antidiabetic activity of Asystacia gangetica leaf extract.

**Methodology**

**Experimental design**

The study was designed as completely randomized in vivo experimental model.

**Collection, identification and preparation of plant material**

The Asystacia gangetica leaves were collected in March from Nnamdi Azikiwe University Awka environment and were identified and authenticated by a taxonomist with a voucher specimen Number: MOUAU/VPP/16/016. The fresh leaves were picked from the plants and washed with distilled water. According to the method of Tosan et al. (2014), the herb was dried at room temperature until a constant weight was
obtained then pulverized into a fine powder and stored in air tight glass container till further use.

Preparation of plant extract
The plant extraction was carried out according to Tosan et al. (2014). In this method, 100 g of powdered leaves of *Asystacia gangetica* was soaked in 1,000 ml ethanol (95 per cent) for 72 h in a beaker and the mixture was stirred every 18 h using a sterile glass rod. The mixture was filtered with a fine muslin cloth and then by Whatman no 1 filter paper. The filtrate was evaporated to solid under reduced pressure in a rotary evaporator at 40°C. The concentrated ethanol residue obtained was stored in air tight container at 4°C till further use.

Ethical permit for use of laboratory animal, animal collection and preparation
The Research Ethics Committee, constituted under the guidelines of National Institute of Health, Department of Physiology, Pharmacology, Biochemistry and Animal Health College of Veterinary Medicine, Micheal Okpara University of Agriculture Umudike, Abia State, Nigeria, approved all the animal experimental protocols (Registered Number: CVM/VPP/EP/16/012) prior to the commencement of this study.

A total of 30 mice (30 to 40 g) and 50 adult rats (90 to 110 g) were obtained from animal production unit of College of Veterinary, Michael Okpara University of Agriculture, Umudike. The mice were used for the acute toxicity determination of the extract while the rats were used for the antidiabetic study. The rats were kept in aluminum cages, allowed access to feed and water ad libitum but were fasted for 12 h before the commencement of the experiment.

For the antidiabetic study, 50 rats were assigned to 5 groups of 10 rats each. Group 1 served as the normal control while the animals in Groups 2 to 5 were made diabetic via the administration of a single intraperitoneal dose of alloxan monohydrate (160 mg/kg). The animals were confirmed diabetic after five days via the determination of their serum glucose levels on a single touch glucose meter (accu check active). Animals with glucose levels of 190 mg/kg and above were used.

Acute toxicity study
The acute toxicity test was done according to Kaber’s method, as was modified and described by Ijioma et al. (2015). A total of 30 mice of both sexes weighing 30 to 40 g were divided into 6 groups of 5 mice each. Groups were assigned graded oral doses of the extract in the order of 500, 1,000, 2,000, 3,000, 4,000 and 5,000 mg/kg body weight.

Acute and sub-acute antidiabetic study of the plant extract:
The rats were grouped and assigned treatment according to the order below:
Group 1 Normal control rats, which received an oral dose of normal saline.
Group 2 Diabetic control rats, which were given no treatment.
Group 3 Diabetic rats treated with low dose (400 mg/kg) of plant extract.
Group 4 Diabetic rats treated with high dose (800 mg/kg) of plant extract.
Group 5 Diabetic rats treated with glibenclamide (5 mg/kg).

Treatment was done daily via the oral route. The glucose levels were determined for every rat after 0, 2, 4 and 6 h to assess acute antidiabetic activity while for sub-acute antidiabetic was determined for every seven days for 21 days.
The percentage fall in glucose level
This was calculated using the formula:

\[
\text{% fall in glucose level} = \frac{\text{Initial plasma glucose level} - \text{Final plasma glucose level} \times 100}{\text{Initial plasma glucose level}}
\]

Biochemical profile determination
The biochemical parameters [serum creatinine (Bowers and Wong, 1980), serum urea, aspartate aminotransferase (Reitman and Frankel, 1957), alanine transferase (Reitman and Frankel, 1957), alkaline phosphate (King and Kind, 1957), serum total bilirubin, total protein and serum albumin concentration (Doumas et al., 1971)] were analyzed spectrophotometrically using the standard diagnostic kits (Randox laboratories limited, UK).

Lipid profile determination
The lipid profile, which includes serum cholesterol, triacylglycerol and high density lipoprotein (HDL)-cholesterol were analyzed spectrophotometrically using the standard diagnostic kits (Agape diagnostic, Switzerland) while LDL-cholesterol and VLDL-cholesterol were calculated by the following formulas, respectively:

\[
\begin{align*}
\text{LDLC} &= \text{TC} - (\text{HDL-C} + \text{triglycerides}) \\
\text{VLDLC} &= \frac{\text{triglycerides}}{5}
\end{align*}
\]

where:

- LDLC = Low density lipoprotein cholesterol.
- HDLC = High density lipoprotein cholesterol.
- VLDLC = Very low-density lipoprotein cholesterol.

Hematology profile study
The hematology profile, which included red blood cell count (RBCC), packed cell volume (PCV), haemoglobin concentration (HC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cell count (WBCC) and platelets (PLT) count were obtained at once using an automated analyzer (Mindray company, China), following the procedures as described in the instrument manual. The blood obtained from the experimental rats after 21 days of treatment via cardiac puncture.

Histopathology examination of the liver and pancreatic tissues
Histological examination of the harvested liver and pancreas of the studied rats were prepared using the methods of Arsad et al. (2014). The tissues were dehydrated in graded levels of ethanol, cleared in xylene, and embedded in paraffin wax for sectioning. The 5 \( \mu \text{m} \) thick sections were cut, mounted on glass slides and stained with hematoxylin and eosin for light microscopy. The photomicrographs of slides were observed under the microscope with magnifications of \( \times 400 \) and \( \times 100 \). Selected images were captured using a moticam 2.0 digital camera attached to a computer.

Statistical analysis
All data generated in triplicate from this study was subjected to analysis of variance (ANOVA) and Duncan's multiple range test at the 95 per cent probability level to determine Acute toxicity and antidiabetic activity.

Acute toxicity and antidiabetic activity
the difference among treatment levels. Statistical package for social sciences (SPSS Version 23) was used.

Results and discussion

Acute toxicity study

The result of the acute toxicity study of *Asystasia gangetica* leaf showed that no mortality was observed in all test groups within 24 h of the study. The animals were observed further for 7 days and still showed no mortality. The ethanol extract of *Asystacia gangetica* was found to be safe at the tested dose level of 5,000 mg/kg body weight. This result is similar to the findings of Mukesh and Patill (2010), reported that the extract of *Pongamia pinnata* leaf showed no sign of toxicity at the dose level of 5,000 mg/kg body weight while Chandana *et al.* (2013) reported that *Alternanthera brasiliana* Kuntze leaf extract was found to be safe up to 2,000 mg/kg body weight. Also, Karuppasamy *et al.* (2014) reported that the ethanol extract of *Melastoma malabathricum* Linn leaf in alloxan-induced diabetic rats was safe up to a dose of 2,000 mg/kg body weight.

Acute antidiabetic study

The acute antidiabetic study of *Asystasia gangetica* leaf ethanolic extract is shown in Table I. There was significant ($p = 0.01$) increase in the blood glucose level of the induced diabetic rats from 94.8 to 456.8 mg/dl when compared to the normal control rats at zero hour for the plant extract. The increase in the blood glucose level after alloxan administration may be due to insulin deficiency or resistance state in diabetic rats (Karuppasamy *et al.*, 2014).

The rats treated with 400 mg/kg extract at zero hour showed no significant ($p = 0.7$) reduction in blood glucose level when compared to the diabetic control rats while at 2, 4 and 6 h there was significant reduction in the blood glucose level from 414.8 to 327.2, 358.6 to 225.4 and 338.4 to 177.4 mg/dl, respectively, when compared to the diabetic control rats.

The diabetic rats treated with 800 mg/kg extract and 5 mg/kg glibenclamide showed significant ($p = 0.01$) reduction in the blood glucose level at 0, 2, 4 and 6 h when compared to the diabetic control rats. Interestingly, the treatment at 800 mg/kg extract showed no significant ($p = 0.7$) reduction in the blood glucose level when compared to the diabetic rats treated with 5 mg/kg glibenclamide (standard drug) at 0, 2, 4 and 6 h when compared to diabetic control rats. This means that the extract at the dose of 800 mg/kg compared favorably with that of standard drug (5 mg/kg glibenclamide) treatment.

At 4 and 6 h, the treatment with 400 mg/kg extract, 800 mg/kg extract and 5 mg/kg glibenclamide showed no significant reduction in the blood glucose level from 358.60 to 225.4, 225.4 to 218.2 and 218.2 to 202.6 mg/dl, respectively.

Table I.
The effect of acute study of *Asystasia gangetica* leaf ethanol extract on the blood glucose level of the studied rats (mg/dl)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>0 h</th>
<th>2 h</th>
<th>4 h</th>
<th>6 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>94.8±3.9</td>
<td>87.6±4.5</td>
<td>83.0±4.4</td>
<td>78.4±7.0</td>
</tr>
<tr>
<td>Diabetic untreated</td>
<td>456.8±42.5</td>
<td>414.8±55.5</td>
<td>358.6±31.3</td>
<td>338.4±49.7</td>
</tr>
<tr>
<td>Diabetic treated with 400 mg/kg extract</td>
<td>430.6±40.9</td>
<td>327.2±47.7</td>
<td>225.4±55.2</td>
<td>177.4±19.1</td>
</tr>
<tr>
<td>Diabetic treated with 800 mg/kg extract</td>
<td>370.0±26.6</td>
<td>273.8±42.8</td>
<td>218.2±11.7</td>
<td>144.2±11.4</td>
</tr>
<tr>
<td>Diabetic treated with 5 mg/kg glibenclamide</td>
<td>374.2±72.6</td>
<td>252.0±39.6</td>
<td>202.6±6.2</td>
<td>176.4±19.2</td>
</tr>
</tbody>
</table>

Notes: Values are means ± SD, $n = 10$, data were analyzed by one-way ANOVA followed by Duncan multiple range tests using SPSS. Data in the same columns bearing different superscript are significantly different at 95 per cent probability level as compared with normal and diabetic untreated control group.
The significant decrease in the blood glucose level may be attributed to the stimulation of the residual pancreatic mechanism or to a probable increase in the peripheral use of glucose (Sachdewa and Khemni, 2003). This result is in agreement with the findings of Karuppassamy et al. (2014), which reported a significant dose-dependent decrease in blood glucose levels of the diabetic treated group after the treatment with Melastomamalabathricum Linn ethanol extract at a dose of 150 and 300 mg/kg. Ismail et al. (2015) also reported the same decreasing trend in blood glucose level of the diabetic rats treated with methanolic extracts of Nepenthes bicalcarata hook F. within 60 min of treatments.

Percentage fall in blood glucose level in acute antidiabetic study
The percentage fall in blood glucose level in acute antidiabetic study of the ethanolic extract of Asystacia gangetica is shown in Table II. The normal control rats had 7.5, 5.2 and 5.5 per cent blood glucose fall at 2, 4 and 6 h, respectively. The diabetic untreated control rats had 9.1, 13.5 and 5.6 per cent blood glucose fall at 2, 4 and 6 h, respectively. The diabetic rats treated with 400 mg/kg extract had 24.0, 31.1 and 21.2 per cent blood glucose fall at 2, 4 and 6 h, respectively. Diabetic rats treated with 800 mg/kg extract had 26, 20.3 and 33.9 per cent while with 5 mg/kg glibenclamide had 32.6, 19.6 and 12.9 per cent blood glucose fall at 2, 4 and 6 h, respectively. It was observed that there was no significant (p = 0.7) different between the diabetic rats treated with 400 mg/kg extract when compared with diabetic untreated. This implied that this dosage had no significant effect on lowering the blood glucose level. The treatment with 800 mg/kg extract showed a significant difference in the blood glucose level of the diabetic rats when compared with the untreated diabetic rats. The treatment with 800 mg/kg extract lowered the blood glucose level of the diabetic rats near to normal. The trend shown by the diabetic rats treated with 800 mg/kg extract indicates that it is more effective on the blood glucose reduction as the time increased, which means that the action is more effective for long time treatment while that of 5 mg/kg glibenclamide showed opposite trend when compared to that of 800 mg/kg extract. This means that the treatment with standard drug has an acute effect and its potential decreases with increase in time. These connotate that effective treatment of diabetes mellitus with the extract of Asystacia gangetica is dose and time-dependent.

At 6 h the treatment with 800 mg/kg extract (33.9 per cent) did better than the treatment with a standard drug (12.9 per cent). This simply means that the extract could replace the standard drug in the management of diabetes mellitus.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>2 h</th>
<th>4 h</th>
<th>6 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>7.5\pm 0.0</td>
<td>5.2\pm 0.0</td>
<td>5.5\pm 0.0</td>
</tr>
<tr>
<td>Diabetic untreated</td>
<td>9.1\pm 0.0</td>
<td>13.5\pm 0.0</td>
<td>5.6\pm 0.0</td>
</tr>
<tr>
<td>Diabetic treated with 400 mg/kg extract</td>
<td>24.0\pm 0.0</td>
<td>31.1\pm 0.0</td>
<td>21.2\pm 0.0</td>
</tr>
<tr>
<td>Diabetic treated with 800 mg/kg extract</td>
<td>26.0\pm 0.0</td>
<td>20.3\pm 0.0</td>
<td>33.9\pm 0.0</td>
</tr>
<tr>
<td>Diabetic treated with 5 mg/kg glibenclamide</td>
<td>32.6\pm 0.0</td>
<td>19.6\pm 0.0</td>
<td>12.9\pm 0.0</td>
</tr>
</tbody>
</table>

Notes: Values are means±SD, n = 10, data were analyzed by one-way ANOVA followed by Duncan multiple range tests using SPSS. Data in the same columns bearing different superscript are significantly different at 95 per cent probability level as compared with normal and diabetic untreated control group.
Sub-acute antidiabetic study

Table III shows the effect of the sub-acute study of ethanol extract of *Asystacia gangetica* leaf on blood glucose level of the studied rats. The blood glucose of rats treated with 400 mg/kg extract decreased from 155.60 to 127.20 mg/dl while that of 800 mg/kg decreased from 137 to 124.40 mg/dl. The rats treated with 400 and 800 mg/kg extract at 7 to 21 days showed significant ($p = 0.004$) difference when compared with the diabetic untreated rats. Interestingly, the rats treated with 400 and 800 mg/kg extract had no significant ($p = 0.6$) difference when compared with the rats treated with 5 mg/kg glibenclamide (standard drug). This means that the extract at the dose of 400 and 800 mg/kg compared favorably with that of the standard drug (glibenclamide) at 5 mg/kg treatment. The trend of this result is in line with that of Ismail *et al.* (2015), who reported decreasing trend in blood glucose level of the diabetic rats treated with methanolic extracts of *Nepenthes bicalcarata* hook F. Also, Wesam *et al.* (2016) reported that treatment of streptozotocin (STZ)-induced diabetic rats with 200 mg/kg of chloroform extract of Acacia Arabica bark for two weeks significantly reduced the level of serum glucose. STZ-induced diabetic rats treated with 1,000 mg/kg of ethanolic extract of *Achyranthes aspera* leaves had a significant reduction in their serum glucose level. More so, Peace and Precious (2018) reported a significant decrease ($p < 0.05$) in the serum glucose levels of alloxan-induced diabetic rats from 454.7 and 569.7 mg/dl to 149.3 and 297.3 mg/dl, respectively, after 14 days treatment with 300 and 600 mg/kg maxima fruit juice, respectively. Nevertheless, Widodo *et al.* (2019) reported that diabetic rats treated with milk fermented using *Lactobacillus casei* strain AP had a decreased in the serum glucose level from 172.4 ± 2.1 to 147.2 ± 6.0 mg/dl ($p < 0.05$) after 15 days of treatment. This may be due to intestine inhibition of glucose absorption or due to an increase in glucose transport from the blood (Kumar *et al.*, 2011).

### Percentage fall in blood glucose level in sub-acute antidiabetic study

The percentage fall in blood glucose level of normal and alloxan-induced diabetic rats after 21 days of treatment with *Asystacia gangetica* leaf ethanol extract is shown in Table IV. It was observed that the treatment with 5 mg/kg of glibenclamide (19.8 per cent) showed significant ($p = 0.004$) difference when compared with other levels of treatment, followed by 400 mg/kg extract treatment (18.2 per cent). This showed that the treatment with 400 mg/kg extract gave a higher percentage fall in blood glucose when compared to 800 mg/kg extract, which gave 9.1 per cent. This connotes that the intake of the herbal extract at a low dosage of 400 mg/kg may be more efficient in bringing down blood glucose level. The trend of percentage fall treatment with herbal extract was also reported by Mohammed *et al.* (2010) and Mukesh and Patill (2010). This indicates the efficacy of this herbal extract in glycemic control.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>84.8±3.4</td>
<td>79.8±1.4</td>
<td>77.0±2.5</td>
</tr>
<tr>
<td>Diabetic untreated</td>
<td>417.6±48.4</td>
<td>385.4±69.7</td>
<td>358.6±58.1</td>
</tr>
<tr>
<td>Diabetic + 400 mg/kg extract</td>
<td>155.6±13.8</td>
<td>140.8±19.7</td>
<td>127.2±13.1</td>
</tr>
<tr>
<td>Diabetic + 800 mg/kg extract</td>
<td>137.0±14.9</td>
<td>133.6±13.2</td>
<td>124.4±11.2</td>
</tr>
<tr>
<td>Diabetic + 5 mg/kg glibenclamide</td>
<td>151.8±22.5</td>
<td>136.0±14.6</td>
<td>121.8±7.8</td>
</tr>
</tbody>
</table>

**Notes:** Values are means±SD, $n = 10$, data were analyzed by one-way ANOVA followed by Duncan multiple range tests using SPSS. Data in the same columns bearing different superscript are significantly different at 95 per cent probability level as compared with normal and diabetic untreated control group.

### Table III.
The effect of sub-acute study of ethanol extract of *Asystacia gangetica* leaf on blood glucose level of the studied rats
Biochemical profile of the sub-acute antidiabetic study
The effect of sub-acute study of Asystacia gangetica leaf ethanol extract on the biochemical profile of studied rats is shown in Table V. Table V showed that the normal rats serum level of aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), serum total bilirubin (STB), serum urea (SU) and creatinine increased from 20.2 to 30.8 IU/L, 13.2 to 26.4 IU/L, 65.4 to 67.2 IU/L, 0.8 to 0.9 mg/dl, 15.2 to 15.8 mg/dl and 0.6 to 0.7 mg/dl, respectively, when induced with diabetes mellitus. The induction of the rats with diabetes leads to a decrease in the serum level of total protein (TP) and serum albumin from 7.0 to 5.56 g/dl and 3.6 to 2.9 g/dl, respectively. The observed increase in SU and creatinine level in diabetic rats when compared to normal control rats could be due to renal damage caused by abnormal glucose regulation and glycosylated protein tissue levels (Karuppasamy et al., 2014). This is an indication of impaired renal function in diabetic rats. Malfunctioning of the kidney is due to hyperglycemia, which causes an increase in metabolic waste products in the blood (Catalina et al., 2018). Also, Catalina et al. (2018) reported that treatment with 150 mg/kg of methanolic extracts of Hamelia patens resulted to the best kidney protective effect in relation to serum creatinine.

The increase in ALP, AST, ALT and STB levels may be due to liver dysfunction. This may have occurred by leakage of enzymes from the liver cytosol into the blood stream; it represents the toxicity of alloxan on the liver (Karuppasamy et al., 2014). In diabetic condition, the occurrence of reduction of TP and SA may be due to proteinuria, albuminuria or increased protein catabolism, which are clinical markers in diabetic nephropathy (Karuppasamy et al., 2014). This result trend is in agreement with the report of Karuppasamy et al. (2014) on the antidiabetic activity of ethanol extract of Melastoma malabathricum Linn leaf in alloxan diabetic rats.

The treatment with the ethanol extract significantly (p = 0.001) increased TP of diabetic rats from 5.56 to 6.94, 7.0 and 6.92 g/dl for 400, 800 and 5 mg/kg, respectively, when compared to the normal rats (7.0 g/dl). It was observed that treatment with 800 mg/kg extract restored the TP level to normal more than the standard drug. This implied that the treatment could be dose-dependent. The SA was significantly restored to normal from 2.91 to 3.8, 4.0 and 3.6 g/dl for 400, 800 and 5 mg/kg extract and drug, respectively. However, the treatment with 800 mg/kg extract restored TP and SA toward normal more than the standard drug. The restoration of TP and SA to normal after treatment with the plant extract could be possible through the increase in the insulin-mediated amino uptake, enhancement of protein synthesis and/or inhibition of protein degradation (Ramachandra et al., 2012). This result trend complies with the report of Karuppasamy et al. (2014).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>21 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>9.1d±0.1</td>
</tr>
<tr>
<td>Diabetic untreated</td>
<td>14.1±0.0</td>
</tr>
<tr>
<td>Diabetic treated with 400 mg/kg extract</td>
<td>18.2±0.0</td>
</tr>
<tr>
<td>Diabetic treated with 800 mg/kg extract</td>
<td>9.2d±0.1</td>
</tr>
<tr>
<td>Diabetic treated with 5 mg/kg glibenclamide</td>
<td>19.8±0.0</td>
</tr>
</tbody>
</table>

**Notes:** Values are means±SD, n = 10, data were analyzed by one-way ANOVA followed by Duncan multiple range tests using SPSS. Data in the same columns bearing different superscript are significantly different at 95 per cent probability level as compared with normal and diabetic untreated control group.

Asystasia gangetica leaf ethanol extract
<table>
<thead>
<tr>
<th>Treatment</th>
<th>TP (g/dl)</th>
<th>SA (g/dl)</th>
<th>AST (U/L)</th>
<th>ALT (IU/L)</th>
<th>ALP (IU/L)</th>
<th>STB (mg/dl)</th>
<th>SU (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>7.0±0.1</td>
<td>3.6±0.2</td>
<td>66.4±3.8</td>
<td>13.2±1.2</td>
<td>0.9±0.0</td>
<td>15.0±0.5</td>
<td>0.9±0.0</td>
<td>0.7±0.0</td>
</tr>
<tr>
<td>Diabetic Untreated</td>
<td>5.5±0.3</td>
<td>2.9±0.2</td>
<td>26.4±1.9</td>
<td>21.2±2.1</td>
<td>0.9±0.0</td>
<td>15.9±0.8</td>
<td>0.9±0.0</td>
<td>1.5±0.1</td>
</tr>
<tr>
<td>Diabetic + 400 mg/kg extract</td>
<td>6.9±0.3</td>
<td>3.8±0.2</td>
<td>21.6±3.3</td>
<td>24.6±3.4</td>
<td>0.9±0.0</td>
<td>17.4±1.8</td>
<td>0.9±0.0</td>
<td>0.7±0.0</td>
</tr>
<tr>
<td>Diabetic + 800 mg/kg extract</td>
<td>6.9±0.8</td>
<td>4.0±0.4</td>
<td>24.0±3.3</td>
<td>26.4±3.3</td>
<td>0.9±0.0</td>
<td>17.4±1.8</td>
<td>0.9±0.0</td>
<td>0.7±0.0</td>
</tr>
<tr>
<td>Diabetic + 5 mg/kg Glibenclamide</td>
<td>6.9±0.8</td>
<td>3.6±0.2</td>
<td>30.8±3.1</td>
<td>33.4±3.3</td>
<td>0.9±0.0</td>
<td>17.4±1.8</td>
<td>0.9±0.0</td>
<td>0.7±0.0</td>
</tr>
</tbody>
</table>

**Notes:** Values are means±SD, n = 10. Data were analyzed by one-way ANOVA followed by Duncan multiple range tests using SPSS. Data in the same columns bearing different superscript are significantly different at 95 per cent (p<0.05) probability level as compared to normal and diabetic untreated control group. TP = total protein; SA = serum albumin; AST = aspartate amino transferase; ALT = alanine amino transferase; ALP = alkaline phosphatase; STB = serum total bilirubin; and SU = serum urea.

**Table V.**
The effect of sub-acute study of *Asystasia gangetica* leaf ethanol extract on the biochemical profile of studied rats.
Diabetic rats treated with ethanol extract significantly reduced the AST, ALP, STB and creatinine from the range of 30.8 to 26.4 IU/L, 67.2 to 65.4 IU/L, 0.8 to 0.7 mg/dl and 0.7 to 0.6 mg/dl while ALP and SU reduced from 26.4 to 22.2 IU/L and 15.8 to 15.5 mg/dl, respectively.

At both doses, the extract effect on liver biomarkers (AST, ALP and Creatinine) efficiently competed with that of the 5 mg/kg of glibenclamide when compared with the normal rats because no significant ($p = 0.8$) existed between them. The reduction in these liver biomarkers represents the protective action of the ethanol extract of this plant in diabetic condition thus improving renal and hepatic functions. This observation is consistent with an earlier report on hepatoprotective potentials of leaf extracts of *V. amygdalina* in mice (Iwalokun et al., 2006).

### Lipid profile of the sub-acute antidiabetic study of the studied rats

The levels of total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) for ethanol extract of *Asystasia gangetica* leaf sample is shown in Table VI. The serum of alloxan-induced diabetic rats showed significant ($p = 0.00$) increase in all the lipid profile parameters except the HDL, which decreased significantly when compared to the normal rats. The ethanol extracts of *Asystasia gangetica* leaf treated rats showed a significant ($p = 0.00$) decrease from the range of 118.8 to 90.9 mg/dl, 137.5 to 124.6 mg/dl, 27.5 to 24.93 and 48.1 to 14.1 mg/dl for TC, TAG, LDL and VLDL, respectively, except the HDL, which significantly increased from 43.6 to 50.4 mg/dl when compared to the normal control rats. Interestingly, it was noted that the diabetic rats treated with the two doses of extract competed favorably in restoring the HDL level better than the normal control rats when compared with the standard drug (5 mg/kg glibenclamide). It was also observed that both administration of 400 and 5 mg/kg glibenclamide to the diabetic rats competed favorably in bringing down the levels of TAG and LDL while the levels of TC and VLDLP were restored close to normal with the 800 mg/kg treatment when compared to the normal control rats.

The pattern of this result finding is similar to the research by Karuppasamy et al. (2014) on ethanol extract of *Melastoma malabathricum* Linn leaf in alloxan-induced diabetic rats. Also, Pawan et al. (2010) reported a significant ($p < 0.05$) increase in serum triglycerides, cholesterol and decrease in HDL in diabetic rats while these parameters decrease except for HDL that increased with the STZ-induced diabetic rats administered with chloroform fraction of *Abutilon indicum* leaf.

The abnormal high concentrations of serum lipid in diabetic animals are mainly due to an increased mobilization of free fatty acids from peripheral fat depots (Al-Logman and Zari, 2009).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>TC</th>
<th>HDL</th>
<th>TAG</th>
<th>LDL</th>
<th>VLDLP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>83.7±2.4</td>
<td>48.5±3.1</td>
<td>117.2±6.4</td>
<td>23.4±1.2</td>
<td>11.7±1.3</td>
</tr>
<tr>
<td>Diabetic Untreated</td>
<td>118.8±3.4</td>
<td>43.6±3.2</td>
<td>137.5±4.2</td>
<td>27.5±0.8</td>
<td>48.1±5.4</td>
</tr>
<tr>
<td>Diabetic + 400 mg/kg extract</td>
<td>106.7±5.5</td>
<td>50.4±2.2</td>
<td>124.6±4.1</td>
<td>24.9±0.8</td>
<td>31.3±4.0</td>
</tr>
<tr>
<td>Diabetic + 800 mg/kg extract</td>
<td>90.9±1.1</td>
<td>49.8±1.2</td>
<td>133.2±6.0</td>
<td>26.6±1.2</td>
<td>14.1±1.9</td>
</tr>
<tr>
<td>Diabetic + 5 mg/kg Glibenclamide</td>
<td>102.9±5.1</td>
<td>50.4±2.2</td>
<td>126.1±5.4</td>
<td>25.2±1.0</td>
<td>27.1±5.0</td>
</tr>
</tbody>
</table>

**Notes:** Values are means±SD, $n = 10$, data were analyzed by one-way ANOVA followed by Duncan multiple range test using SPSS. Data in the same columns bearing different superscript are significantly different at 95 per cent ($p < 0.05$) probability level as compared with normal and diabetic untreated control group. TC = total cholesterol; HDL = high density lipoprotein; TAG = triglycerides; LDL = low density lipoprotein; and VLDL = very low density lipoprotein.

**Table VI.** Lipid profile of sub-acute study of *Asystasia gangetica* leaf ethanol extract of the studied rats in mg/dl.
The immediate stated reasons could be the rationale for the significant increase in the level of serum TC, TG, LDL and VLDL, as well as a marked reduction in the level of HDL in diabetic rats. The result of Mukesh and Patill (2010) revealed that the dose of 500 mg/kg of *Pongamia pinnata* leaf extract in alloxan-induced diabetic rats not only lowered TC, TG and LDL but also enhanced the cardioprotective lipid HDL.

Increased level of HDL was also reported by Karuppasamy et al. (2014) in alloxan-induced diabetic rats from 26.36 to 36.39 mg/dl. Additionally, Peace and Precious (2018) reported a significant decrease ($p < 0.05$) in the cholesterol and triglyceride levels of alloxan-induced diabetic rats treated with 600 mg/kg maxima fruit juice for 14 days while the high-density lipoprotein levels increased ($p < 0.05$).

The observed hypolipidemic effect may be because of decreased cholesterogenesis and fatty acid synthesis (Pawan et al., 2010). The above action could be beneficial in preventing diabetic complications such as coronary heart diseases and atherosclerosis in diabetic condition.

**Hematology profile of the sub-acute antidiabetic study**

Hematology profile (RBC, hemoglobin = Hb, PCV, white blood cell count = WBC, MCH, MCV, MCHC and PLT) of sub-acute anti-diabetic study of *Asystacia gangetica* leaf ethanol extract on studied rats is shown in Table VII. There was a significant ($p = 0.003$) reduction in RBC, Hb, PCV, MCH, MCV, MCHC and PLT with increased WBC values in the alloxan-induced diabetic rats when compared to normal control rats.

The result agrees with the existing literature that anemia is a common pathophysiology associated with diabetes mellitus (Akindele et al., 2012). Colak et al. (2012) also reported that diabetes mellitus causes the development of hypochromic anemia due to a fall in the iron content of the body resulting from oxidative stress associated with the condition.

The ethanol extract of *Asystacia gangetica* leaf, however, elevated these parameters (RBC, Hb, PCV, MCH, MCV, MCHC and PLT) and decreased WBC values in the alloxan-induced diabetic rats after 21 days of treatment. This suggested that the extracts have anti-anemic activity, may be attributed to its high iron content (Saliu et al., 2012) and ability to improve bone marrow functions, a major site for erythropoiesis (Orhue et al., 2008).

At the dose of 800 mg/kg, the extract competed favorably with better efficacy more than the standard drug in the restoration of these parameters.

**Histopathology examination of the liver and pancreatic tissues**

The histopathology examination of liver tissues of the studied rats is shown in Figure 1 (normal control rat), Figure 2 (diabetic untreated rat), Figure 3 (diabetic rat treated with 5 mg/kg glibenclamide), Figure 4 (diabetic rat treated with 400 mg/kg extract) and Figure 5 (diabetic rat treated with 800 mg/kg). At histopathology level, it was observed that the diabetic rat treated with 400 mg/kg extract had moderate portal inflammation without interface or lobular hepatitis when compared to normal control rat while at 800 mg/kg there was severe portal inflammation with the interface and extensive confluent necrosis. The occurrence of portal inflammation of the liver organ implies that this leaf extract may likely not be safe for consumption especially at a high dose for prolong study and should be discouraged from being used traditionally as antidiabetic herbs because of the likely risk it would impose to the consumer.

The histopathology examination of pancreas tissues of the studied rats is shown in Figure 6 (normal control rat), Figure 7 (diabetic untreated rat), Figure 8 (diabetic rat treated with 5 mg/kg
<table>
<thead>
<tr>
<th>Treatment</th>
<th>RBC (X 10^12/L)</th>
<th>PCV (%)</th>
<th>Hgb (g/dl)</th>
<th>WBC X 10^9/l</th>
<th>PLT (x 10^9/l)</th>
<th>MCV (FL)</th>
<th>MCH (Pg)</th>
<th>MCHC (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>7.4±0.3</td>
<td>49.7b±0.9</td>
<td>14.0b±0.6</td>
<td>8.1b±0.8</td>
<td>1.023a±60.0</td>
<td>70.6a±1.2</td>
<td>19.1b±0.6</td>
<td>281.5b±8.9</td>
</tr>
<tr>
<td>Diabetic Untreated</td>
<td>6.5±0.4</td>
<td>46.2d±2.1</td>
<td>12.6±0.6</td>
<td>9.2a±0.6</td>
<td>815.2d±32.7</td>
<td>66.8d±1.6</td>
<td>18.8d±0.4</td>
<td>273.2d±7.0</td>
</tr>
<tr>
<td>Diabetic + 400 mg/kg extract</td>
<td>7.0±0.2</td>
<td>48.5a±1.3</td>
<td>14.2a±0.6</td>
<td>9.0a±0.6</td>
<td>914.4b±15.4</td>
<td>68.4d±1.5</td>
<td>20.0d±0.4</td>
<td>291.8d±6.7</td>
</tr>
<tr>
<td>Diabetic + 800 mg/kg extract</td>
<td>7.3ab±0.4</td>
<td>51.8a±2.4</td>
<td>14.7a±0.5</td>
<td>8.7ab±0.6</td>
<td>914.6b±35.3</td>
<td>70.8d±0.9</td>
<td>20.1b±0.68</td>
<td>281.5b±6.9</td>
</tr>
<tr>
<td>Diabetic + 5 mg/kg Glibenclamide</td>
<td>6.8cd±0.3</td>
<td>48.1d±1.0</td>
<td>13.6d±0.7</td>
<td>8.6d±0.5</td>
<td>865.6c±22.6</td>
<td>70.5d±2.8</td>
<td>19.9d±0.2</td>
<td>282.9d±11.9</td>
</tr>
</tbody>
</table>

**Notes:** Values are means±SD, n=10, data were analyzed by one-way ANOVA followed by Duncan multiple range test using SPSS. Data in the same columns bearing different superscript are significantly different at 95 per cent (p < 0.05) probability level as compared with normal and diabetic untreated control group. RBC = red blood cell; PCV = packed cell volume; Hgb = hemoglobin; WBC = white blood cell; PLT = platelet count; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; and MCHC = mean corpuscular hemoglobin concentration.
Figure 1. Histopathology of liver slide for normal control rat

Figure 2. Histopathology of liver slide of diabetic untreated rat

Figure 3. Histopathology of liver slide for diabetic rats treated with 5 mg/kg glibenclamide

Figure 4. Histopathology of liver slide for diabetic rats treated with 400 mg/kg Asystasia gangetica leaf extract
glibenclamide), Figure 9 (diabetic rat treated with 400 mg/kg extract) and Figure 10 (diabetic rat treated with 800 mg/kg). It was observed that the pancreatic tissues of diabetic rats treated with 400 and 800 mg/kg extract showed no different in the $\beta$-cells of the islets of Langerhans. This connotes that the two doses of the extract did not restore the beta-cell atrophy to near normal. This means that the blood-glucose-lowering potential of this herb may be due to enhanced glucose utilization by peripheral tissues. This result disagrees with the report of Han et al. (2014) that Cornus Officinalis extract caused reduction of blood glucose level in diabetic rats by elevation of insulin level via improving Langerhans's islet damage, a number of insulin-releasing $\beta$-cells.

Acute toxicity and antidiabetic activity
Limitations and future research
The results of this study needs to be confirmed by carrying out the phytochemical and anti-nutrient composition of this herbal plant to ascertain the reason for the portal inflammation of liver tissue at histopathology level. This will help to know the treatment that could be given to this plant to make it safe for consumption.

Conclusion
The findings of this study confirmed that ethanol extract of *Asystacia gangetica* has an antidiabetic activity but may not be safe due to its portal inflammation effect on the liver tissue. Thus, *Asystacia gangetica* leaf extract may be discouraged
from being used as a traditional antidiabetic herb in the management of diabetes mellitus.

References


Further reading


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