Extracts of *Gongronema latifolium* and *Vernonia amygdalina* improve glycaemia and histomorphology of testes of diabetic Wistar rats

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This study aimed to assess the histomorphological and glycaemic effects of the ethanolic leaf extracts of *Vernonia amygdalina* (VA) and *Gongronema latifolium* (GL) on the testis of alloxan-induced diabetic Wistar rats. Forty male Wistar rats were divided into two groups A and C; the former served as negative control group received normal saline, while the later was the diabetic group. This group was further divided into seven (C1 to C7) groups and treated as follows: C1 was diabetic positive control, C2 (150 mg/kg of GL), C3 (300 mg/kg of GL), C4 (150 mg/kg of VA), C5 (300 mg/kg of VA), C6 (100 mg/kg each of GL and VA) and C7 (Insulin 1 IU/kg), respectively. Alloxan (150 mg/kg) was used to induce diabetes and animals were anesthetized with chloroform and sacrificed. Blood glucose was recorded at the start, during and at the end of treatment. Testes were processed for histopathological examination. The groups treated with GL and VA extracts showed better cellular architecture upon histopathological examination than the diabetic control group that revealed marked necrosis and cellular hypoplasia. Blood glucose levels of the treated rats were reduced by GL and VA extracts with good leverage in organ and body weight indices. In conclusion, GL and VA have a protective effect on the histology of the testes and hypoglycemic activity in diabetic rats.

**Key words:** *Vernonia amygdalina, Gongronema latifolium, testis, histomorphology, diabetic rats.*

**INTRODUCTION**

Diabetes mellitus (DM) is a metabolic disorder of multiple etiology characterized by chronic hyperglycemia with disturbances of carbohydrates, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both (WHO, 1999). The number of people with DM is increasing due to population growth, aging, urbanization and increasing prevalence of obesity and physical inactivity (Wild et
This has led to the continued interest and research for various treatment options available to the repertoire of conventional medications (like insulin, glibenclamide, chlorpropamide, glucophage and many others) utilized by physicians. However, despite the availability of these known antidiabetic medicines, remedies from medicinal plants are used with success to treat DM (Bhattaram et al., 2002). To investigate the efficacy and potency thereof, animal models of experimental diabetes provide a considerable insight into the physiological and biochemical derangement of the diabetic state and thus many of the derangements have been characterized in hyperglycaemic animals (Odetola et al., 2006).

Many traditional plant treatments for DM and other diseases are used throughout the world, especially in developing countries of Africa (Saravanan and Prakash, 2004) due to their ready availability and more safety than the synthetic chemical drugs. Vernonia amygdalina (VA) (Del. Asteraceae) is a shrub commonly called "bitter leaf" in many parts of Nigeria and is widespread in both East and West Africa. It has petiolar green leaves of about 6 mm diameter with a characteristic odour and a bitter taste and can be 2 to 5 m tall (Singha, 1966). VA is drought resistant and hence grown in many homes in villages as fence post where it also serves as a nutritional source for soup condiment (Bonsi et al., 1995). Leaves of VA are used as vegetable in meals to stimulate the bowels and a therapy for fever. Profound ethnomedicinal and pharmacological properties have been reported in extracts of VA (Farombi, 2003). Leaf extracts of VA have both hypoglycemic and hypolipidaemic properties in experimental animals and so could be used in managing DM (Akah and Okafor, 1992).

Gongronema latifolium (GL) (Benth) (Asclepiadaceae) is a climber with woody hollow glaborous stems below and characterized by greenish yellow flowers (Anaso and Onochie, 1999). It is widespread in tropical Africa and can be found in rainforest, deciduous and secondary forests of many African countries like Senegal, Chad and Nigeria (where the South-eastern natives call the leaves 'Utasi' (Ugochukwu et al., 2003). The leaves serve as a leafy vegetable used as a spice for sauces, soups and salads (Anaso and Onochie, 1999) where it has been widely used in folk medicine for maintaining normal blood glucose levels (Okafor and Ham, 1999). Researchers have found the leaves very efficacious as an antidiarrheal and anti-tussive (Atangwho et al., 2009).

Although extracts from VA and GL have individually demonstrated antidiabetic action, recent evidence shows that antidiabetic efficacy of these extracts is enhanced when given in combination (Ebon et al., 2008; Atangwho et al., 2009). Antihyperglycemic activity of these plants are attributed to their ability to restore the function of pancreatic tissues by causing an increase in insulin release or inhibiting the intestinal absorption of glucose or due to facilitation of metabolites in insulin-dependent processes.

Testicular function is impaired in DM. Perturbations in hormonal milieu (testosterone, follicle stimulating hormone, luteinising hormone, prolactin and growth hormone) have been reported by Hutson et al. (1983) and may be linked to alterations of Leydig cell functions (Benítez and Pérez Díaz, 1985). In spontaneously diabetic BB (biobreeding/Worcester) rats, decrease in testicular testosterone production and altered spermatogenesis have been reported indicating that this process is inherent to this disease (Hassan et al., 1993). These changes could be due to testicular dysfunction associated with sustained hyperglycaemia in diabetic rats (Cameron et al., 1990) with impairment in sexual function and thus may lead to infertility.

With conflicting report on VA indicating the presence of toxic phytochemicals that may exert toxicological potentials on some visceral organs, especially in diabetic state, the effect of VA and GL on glycaemia and testicular histomorphometry of alloxan-induced diabetic Wistar rats was investigated.

**MATERIALS AND METHODS**

Collection and preparation of plant extract of fresh mature leaves of *V. amygdalina* and *G. latifolium* were obtained from a local market and authenticated by a Plant Taxonomist in the Department of Botany and Ecological Studies, University of Uyo, Uyo. Voucher specimens have been deposited at the department for reference (UUH 2083 and UUH 2088, Uyo). The leaves were separately chopped into smaller bits with a knife and ground into powder with a manual grinder. 1.40 kg of GA and 1.83 kg of VA was macerated with 4 L of 80% ethanol. The mixtures were allowed for 72 h in the refrigerator for thorough extraction of the plant active components. After filtration, the solvent was removed under reduced pressure in a rotary evaporator at a temperature below 50°C and dried to a constant weight of 46.6 g (4.05%) and 36.4 g (3.47%) yield, respectively. The extracts were then stored in a refrigerator until required for use.

**Animals and experimental design**

Forty sexually mature male Wistar rats weighing 200 to 300 g were obtained from the animal house of the Department of Zoology and Environmental Biology, University of Calabar, Calabar. They were housed in cages (5 animals per cage), maintained on standard rat chow (Livestock Feeds, Nigeria Ltd) and provided with water at *ad libitum*. They were allowed to acclimatize for fourteen days in the animal holding room of the Department of Anatomy, Faculty of Basic Medical Sciences, University of Uyo, to the laboratory conditions before the commencement of the experiment. The use of the animals and the experimental protocol was approved by the Experimental Ethics Committee on Animals Use of the Faculty of Basic Medical Sciences, University of Uyo, Nigeria. The animals were weighed and grouped into non-diabetic control (group A) and the diabetic groups (C1 to C7). At the beginning of the experiment, blood glucose (BG) level of all
the rats were taken so as to confirm that they were not diabetic. All the values gotten were below 175 mg/dl. Thereafter, the BG readings were taken weekly for the four weeks of experimentation.

**Induction of experimental diabetes**

After overnight fast, diabetes was experimentally induced on the animals by intraperitoneal (i.p.) administration of alloxan monohydrate dissolved in normal saline (150 mg/kg) (Szukdelski, 2001). The blood sugar levels were monitored with a glucometer (Accu-Chek, Roche Diagnostics) after 72 h and the rats with plasma glucose level >250 mg/dl were considered as diabetic and were recruited for the study. All treatments were daily and lasted for 28 days as follows: Group A: Normal rats serving as negative control (treated with normal saline); Group C1: Diabetic rats (positive control) not treated with extracts; Group C2: Diabetic rats treated daily with GL 150 mg/kg body weight; Group C3: Diabetic rats treated daily with GL 300 mg/kg body weight; Group C4: Diabetic rats treated daily with VA 150 mg/kg body weight; Group C5: Diabetic rats treated daily with VA 300 mg/kg body weight; Group C6: Diabetic rats treated daily with 100 mg/kg body weight VA+GL; Group C7: Diabetic rats treated daily with insulin lente (Humulin® L) (1 IU/kg).

**Collection of blood samples for analysis**

At the end of the four (4) weeks, food was withdrawn from the rats and they were fasted overnight and serial blood glucose levels were withdrawn. After which the rats were then euthanized under chloroform vapour and sacrificed. Laparotomy was performed on the animals and the testis was carefully removed, weighed using an electronic balance. The tissues were then suspended in 10% buffered formalin for fixation preparatory to histological processing for light microscopy using the H&E method. This procedure involved dehydration of the testes with graded ethanol concentrations (50, 70, 90 and 100%, respectively), clearing in xylene, followed by infiltration in paraffin wax for 2 h at 56°C and embedding in paraffin wax for 48 h. Sections (5 μm thick) were then obtained, using a rotary microtome, subjected to haematoxylin and eosin (H&E) staining procedure and examined under a light microscope. The histopathological slides were independently read by a histopathologist blinded to the study.

**Statistical analysis**

All data were collected and analyzed using the Student’s t-test and levels of significance considered was p<0.05. All data are expressed as mean ± standard deviation.

**RESULTS**

**Mortality**

There were no recorded deaths in the treated-groups during the 28 day period. A 0.4% decline in body weight in the diabetic control group was observed with other groups recording a marginal increase in this parameter (insulin group and GL 100 mg/kg groups) almost having similar values to the negative control (normal saline) (Table 1).

![Figure 1](image)

The pre-diabetic BG of all the groups was below 175 mg/dl. However, BG reading at termination of experiment showed significant different (P≤0.001) between normal control (group A) and the experimental diabetic groups (C1, C2, C3, C4, C5, C6 and C7). Also statistically significant is the comparison showing diabetic control C1 with C2, C3 and C4; (b) when C5 compares with A, C2, C3 and C4); (c) when C6 compares with A, C2, C3 and C4); (d) when C7 compares with A, C3 and C2) (Table 1).

Weekly BG monitoring (Table 2) also revealed interesting fluctuations in this parameter across the groups. Testicular weight readings for the groups indicated a decline in groups C6 and C7, but this was not significantly so (Figure 1).

**Histopathological observations**

Histopathological outcomes from the experiment are represented in Plates A to C7 at magnifications of ×100 and 400 for each group. The histoarchitecture of the normal control (normal saline) group were essentially normal with layered spermatogenic cells in the lumen of the seminiferous tubules. The interstitial spaces were populated with Leydig cells. Similar observations are seen in Plates C2 and C7. However, the cytoarchitecture of diabetic control showed remarkable necrosis and hypocellularity in some seminiferous tubules with loss of spermatocytes. Focal areas of hypocellularity were seen in some seminiferous tubules of group C3, while some tubules in group C4 where hypoplastic (Plates A to C7).

**DISCUSSION**

Although DM is known to cause many systemic complications; hypogonadism is not widely recognized to be one of them (Vignera et al., 2012). In addition, attention has been focused on glycaemic control and cytology of seminal fluid with information on the histoarchitectural perturbations of the testes seldom looked at and still need further investigations. Therefore, the present study was carried out to evaluate the glycaemic and histopathological findings in protocol involving treatment with VA and GL on diabetic rats. By 2020, there will be an estimated 250 million patients with DM in the world. In addition, patients with diabetes have high risk of blindness, renal failure and myocardial infarction. Although genetic factors are likely to have a role in this disease; increases in insulin resistance play an important role in its pathogenesis. Though, insulin is presently one of the most important therapeutic agents...
Table 1. Body weight (BW) and blood glucose (BG) changes in the experimental groups.

<table>
<thead>
<tr>
<th>Groups (n=5)</th>
<th>Initial BW (g)</th>
<th>Final BW (g)</th>
<th>Change (%)</th>
<th>Initial BG (mg/dl)</th>
<th>Final BG (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>240±24.49</td>
<td>283±24.17</td>
<td>43 (18)</td>
<td>54.8</td>
<td>81.8± 2.52</td>
</tr>
<tr>
<td>C1</td>
<td>294±6.00a</td>
<td>282±24.17</td>
<td>12 (0.4)</td>
<td>68.8±7.86a</td>
<td>478.4±29.08a</td>
</tr>
<tr>
<td>C2</td>
<td>245±16.51</td>
<td>285±24.49</td>
<td>40 (16)</td>
<td>68±2.03</td>
<td>165.8±79.63</td>
</tr>
<tr>
<td>C3</td>
<td>261±13.08</td>
<td>268±24.37</td>
<td>7 (0.3)</td>
<td>68±10.06b</td>
<td>119.4±66.24</td>
</tr>
<tr>
<td>C4</td>
<td>284±16.00</td>
<td>315±17.75</td>
<td>31 (11)</td>
<td>39±2.93</td>
<td>239.8±64.46</td>
</tr>
<tr>
<td>C5</td>
<td>237±7.35</td>
<td>242±7.18</td>
<td>5 (0.2)</td>
<td>42.4±4.27</td>
<td>426±14.35b</td>
</tr>
<tr>
<td>C6</td>
<td>219±11.00</td>
<td>236±13.55</td>
<td>17 (0.8)</td>
<td>32.8±2.69</td>
<td>430.6±45.09c</td>
</tr>
<tr>
<td>C7</td>
<td>258±16.55</td>
<td>302±14.28</td>
<td>44 (17)</td>
<td>60.8±5.55c</td>
<td>340±12.25d</td>
</tr>
</tbody>
</table>

BW: Body weight; BG: blood glucose levels; *a,b,c p<0.001.

Table 2. Effect of Gongronema latifolium and Vernonia amygdalina on serial weekly blood glucose levels of rats.

<table>
<thead>
<tr>
<th>Treatment-dose (mg/kg)</th>
<th>Blood glucose level (mg/dl) (Mean±SD)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
</tr>
<tr>
<td>A - Non-diabetic control</td>
<td>54.80±1.24</td>
</tr>
<tr>
<td>C1 - Diabetic control</td>
<td>68.00±7.86</td>
</tr>
<tr>
<td>C2 - GL150 mg/kg</td>
<td>46.80±2.03</td>
</tr>
<tr>
<td>C3 - GL300 mg/kg</td>
<td>68.00±10.6</td>
</tr>
<tr>
<td>C4 - VA150 mg/kg</td>
<td>39.40±2.93</td>
</tr>
<tr>
<td>C5 - VA300 mg/kg</td>
<td>42.40±4.27</td>
</tr>
<tr>
<td>C6 - GL+VA (100 mg/kg)</td>
<td>32.80±2.69</td>
</tr>
<tr>
<td>C7 - Insulin 1 IU/kg</td>
<td>60.80±5.55</td>
</tr>
</tbody>
</table>

*p<0.001.

Known to medicine, efforts have continued to seek for insulin substitutes from synthetic or plant sources for the treatment of diabetes (Erenmemisoglu et al., 1995; Azu et al., 2005).

In our study, microscopic examination of testes of alloxan-induced diabetic rats revealed widening of interstitial spaces with evidence of necrosis in some seminiferous tubules suggestive of germinal epithelial changes. These contrasted markedly from the normal architecture in normal control and groups treated with GL, combined mixture of GL and VA as well as insulin accordingly. These results are more or less consistent with previous studies on testes of streptozotocin diabetic rats by Ballester et al. (2004) and Asuquo et al. (2010) which reported alterations, vaculations and distortion of both seminiferous epithelium and peritubular tissue. While we did not report stereological data in this study, these observations suggest that significant reduction of the thickness of seminiferous epithelium is very likely and could plausibly be attributable to unfavourable hormonal perturbations, particularly FSH. Elbastawisy et al. (2012) showed that changes in seminiferous tubular alterations in offspring of STZ-diabetic mothers were due to decreased FSH level. Putatively, declines in FSH levels cause a decrease in the tubular FSH receptors which in turn diminishes significantly the response of the tubular epithelium to FSH stimulation. It could also be attributable to a decrease in the expression of the insulin receptors which would lead to loss of insulin mediated cell proliferation in seminiferous tubules.

Though, there was weight loss (0.4%) recorded in diabetic animals (C1); all other groups showed a marginal increase. Weight loss is one of the symptoms of diabetes mellitus occurring especially when glycaemic control is poor (Akpaso et al., 2011). This appreciation in weight indicates that the treatment with VA and GL allowed the tissues to access the glucose both to supply energy and spared some to build tissue materials required for growth. The results showed that GL and VA at lower dose of 150 mg/kg remarkably restored this parameter to almost normal control levels just as the group treated with insulin (Table 1). This closely mirrors changes in blood glucose levels in the protocol with significant reduction in the final blood glucose level of the groups (C2, C3, C4, C5, C6) treated with the extracts following the induction of diabetes. This was in agreement with previous report by Nwanjo (2005).
Figure 1. Testes weight of the control group and experimental groups.

Plates A I and II: Photomicrograph of testes of control animals, H&E x100 and x400. L is seminiferous tubular lumen; I is interstitial between seminiferous tubules; Lc is Leydig cell; Sp is spermatocyte; St is spermatids; yellow arrow indicates the basement membrane of seminiferous tubule. All architecture are essentially normal with layered spermatogenic cell series.

The extracts of leaves of GL and VA when combined produced reduction in glucose levels similar in extent to insulin treatment. Plant extracts are known to contain phytochemicals including tannins, saponins, polyphenols and alkaloids and dietary fibers which are said to contribute to their blood glucose lowering effect (Tiwari and Rao, 2002). Although the present findings confirm the antiglycaemic potential in the VA and GL, the precise mechanism of its hypoglycemic action may be attributed to their ability to restore the function of pancreatic tissues by causing an increase in insulin output and/or inhibit the intestinal absorption of glucose or to facilitation of metabolites in insulin-dependent processes. Synergistic actions of VA and GL could also be exerted via oxidative stress attenuation, insulin mimetic action and beta cell regeneration as earlier
Plate C1 I and II. Photomicrograph of testes of diabetic control animals, H&E x100 and x400. L is seminiferous tubular lumen; I is interstitial between seminiferous tubules; yellow arrow indicates the basement membrane of seminiferous tubule; red arrow indicates degenerating spermatocytes. Note the distorted cellular patterns with evidence of necrosis in the seminiferous tubules with loss of spermatogenic integrity.

Plates C2 I and II. Photomicrograph of testes of animals treated with GL150mg/kg, H&E x100 and x400. L is seminiferous tubular lumen; I is interstitial between seminiferous tubules; yellow arrow indicates the spermatogonium in basement membrane of seminiferous tubule; red arrow indicates spermatocyte.

postulated by Gbolade et al. (2009). It is however interesting to note that the extract was more effective when used in lower doses than that in higher doses applied. The reason(s) for this discordance may be connected with possible phytochemical contents in the plants as previously reported by Azu et al. (2005).

The obtained results revealed that VA and GL are very important plants with beneficial bioactive ingredients that can be exploited for cytoprotective purposes. Previous findings have shown that V. amygdalina has a strong antioxidant activity corresponding to mitigation of the generation of hydroxyl radicals (Battell et al., 1999; Yeh et al., 2003); hence, it is postulated that this antioxidant activity may provide possible rationale for the observed...
**Plates C3 I and II.** Photomicrograph of testes of animals treated with GL300mg/kg, H&E x100 and x400. L is seminiferous tubular lumen; I is interstitial between seminiferous tubules; yellow arrow indicates the spermatogonium in basement membrane of seminiferous tubule; red arrow indicates spermatocyte. Note focal hypocellularity in some tubules.

**Plates C4 I and II.** Photomicrograph of testes of animals treated with VA150mg/kg, H&E x100 and x400. L is seminiferous tubular lumen; I is interstitial between seminiferous tubules; Sp is spermatocyte; St is spermatids; Sg is spermatogonium. Note the hypoplastic tubule.
**Plates C5 I and II.** Photomicrograph of testes of animals treated with VA 300mg/kg, H&E ×100 and ×400. L is seminiferous tubular lumen; I is interstitial between seminiferous tubules; Bv is blood vessel; Sg is spermatogonium (red arrow). Note necrotic spermatocyte (yellow arrow).

**Plates C6 I and II.** Photomicrograph of testes of animals treated with VA+GL 100mg/kg, H&E ×100 and ×400. L is seminiferous tubular lumen; I is interstitial between seminiferous tubules; Bv is blood vessel; red arrow indicates blood vessel in I. Histology is essentially normal.
Plates C7 I and II. Photomicrograph of testes of animals treated with insulin lente (1 IU/kg), H&E x100 and x400. L is seminiferous tubular lumen; I is interstitial between seminiferous tubules; red arrow is spermatocyte. Architecture is essentially normal.

therapeutic effects of *V. amygdalina*.

**Conclusion**

The extracts of leaves of *V. amygdalina* and *G. latifolium* have a protective effect on the histology of testes and significantly reduce blood sugar levels in diabetic rats. The study suggests that *V. amygdalina* and *G. latifolium* may be beneficial for diabetic patients suffering from low reproductive efficiency.

**Conflict of Interest**

Authors declare that there is no conflict of interest.

**REFERENCES**


