POLYHERBAL EXTRACTS FROM GONGRONEMA LATIFOLIUM AND OCIMUM GRATISSIMUM IMPAIRED LIVER FUNCTION IN ALLOXAN INDUCED DIABETIC RATS

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ABSTRACTS
The effect of four weeks co-administration of gongronema latifolium and ocimum gratissimum was studied in alloxan induced diabetic rats.

At the end of the period of extract administration, results showed that extracts caused a slight decrease in blood glucose, alkaline phosphatase, total bilirubin and albumin levels, although not significant compared to normal control (P > 0.05), but caused an elevated AST and ALT levels (P < 0.05). Data suggest that co-administration of these extracts may be harmful to the liver.

KEYWORDS: gongronema latifolium, ocimum gratissimum, hepatic enzymes, bilirubin, albumin. Alloxan.

INTRODUCTION
Gongronema latifolium (Asclepiadaceae) is locally called “Utazi” by Igbos and “Arokeke” by the Yorubas in Nigeria is a herbaceous shrub with yellow flower and stem yields milky exudates when cut. It is an edible rainforest plant native to the South Eastern part of Nigeria, has been widely used in folk medicine as a spice and vegetable [1,2]. The leaf extract has the ability to inhibit subcutaneous phycomycosis [3] Several studies have reported that aqueous and ethanol extract of the plant exhibited hypolipidemic, anti-lipid peroxidative, hypoglycemic [2,4], antidiabetic [5], hepatoprotective [6-9]. Ocimum gratissimum is a perennial plant that is woody at the base with an average height of 1-3 m high. The leaves are broad and narrowly ovate, usually 5-13 cm long and 3-9 cm wide. It is a scented shrub with lime-green fuzzy leaves [10]. In Nigeria, this plant is called “effinrin-nia” by the Yoruba’s,
“Nchumou” in Igbo and “diadoya” in Hausa \[^{11}\]. It is used extensively used throughout West Africa as a febrifuge, antimalarial and anti-convulsant \[^{12}\]. Administration of aqueous leaf extract caused a statistically significant reduction in plasma glucose\[^{13}\] and liver enzymes. \[^{13},\ 14\] Alloxan is mainly used to induce diabetes in experimental animals owing to the fact that it causes severe necrosis of pancreatic \(\beta\)-cells with consequent lack of insulin secretion\[^{15}\]. The liver is the most effective organ for regulating glucose metabolism by assimilating increased glucose through gluconeogenesis \[^{16}\]. Enzymes directly associated with the conversion of amino acid to keto acids are alanine aminotransferase (ALT) and aspartate aminotransferase (AST). ALT and AST activities are used as the indicators of hepatocytes damage \[^{17}\]. ALT and AST are the two of the most reliable markers of hepatocellular injury or necrosis. Their levels can be elevated in a variety of hepatic disorders. Of the two, ALT is thought to be more specific for hepatic injury because it is present mainly in the cytosol of the liver and in low concentrations elsewhere. AST has cytosolic and mitochondrial forms and is present in tissues of the liver, heart, skeletal muscle, kidneys, brain, pancreas and lungs, and in white and red blood cells, \[^{18}\] whereas alkaline phosphatase (ALP) is a membrane bound enzyme in the liver and bone. Bilirubin is produced by abnormal breakdown of pigment-containing proteins, especially hemoglobin from senescent red blood cells and myoglobin from muscle breakdown. Bilirubin released from such sources, tightly albumin-bound is delivered to the liver where it is efficiently extracted and conjugated by hepatic glucuronidation and sulfation. Conjugated bilirubin is rapidly excreted into bile and removed from the body through the gut. Elevated levels of conjugated bilirubin imply liver disease. \[^{19}\] Hypoalbuminemia is noted in various liver disorders. Hepatic synthesis of albumin is decreased in patients with cirrhosis and liver cancer which with portal hypertension. Albumin leaks off the liver surface into the peritoneal cavity. The redistribution may contribute to production ascites by increasing oncotic pressure in the peritoneal cavity. \[^{20}\] Since several studies \[^{6-9,13-14}\] have reported that extracts of *gongronema latifolium* or *ocimum gratissimum* reduced liver enzymes, total bilirubin and albumin only at low dose concentration, therefore, the present study investigates the synergistic effects of both extracts in alloxan induced male diabetic rats.

**MATERIALS AND METHODS**

**Animal Models**

Twenty-five male albino wistar rats weighing (160-250 g) were used in the study. The animals were obtained from the Animal House of Department of Pharmacology, College of Medicine and Health Sciences, University of Port Harcourt, Nigeria. They were kept under
standard laboratory condition and fed with commercial Growers mesh (Top Feeds Ltd.) and water *ad libitum*. The animals were kept in plastic cages and allowed to acclimatize for 2 weeks. The rats were divided into five groups namely groups I, II, III, IV and V. Twenty overnight fasted rats from groups II, III, IV and V rats were made diabetic using single intraperitoneal injection (i.p.) of freshly prepared solution of alloxan monohydrate (100 mg/kg body weight) dissolved in physiological solution. The alloxanized rats were kept for two days with free access to food and water. The rats were fasted on the 3rd day for 12 hours and their blood glucose levels were determined using Finetest glucometer and its corresponding strips. The rats that exhibited glucose level above 200 mg/dl were used for the study.

**Extraction of Plant Material**

*Gongronema latifolium* and *ocimum gratissimum* were purchased from the local market in Elele, Rivers State. The fresh leaves were washed ad sundried for 7 days. The dried leaves were grounded into fine powder and packed separately. About 200 g of the fine powder of the two leaves each were extracted with 1000 ml of ethanol by cold maceration for 48 hours and filtered. The preparation was filtered using Whatman No. 1 filter paper and the filtrate was dried in a hot air oven to obtain the ethanol extract (100 g). This method was used in the extract of the two plants respectively. From the stock solution appropriate volumes were taken.

**Study Protocol**

The extracts of *ocimum gratissimum* and *gongronema latifolium* was administered twice daily by gavage. Group I (5 rats) were used as controls. Group II (5 rats) received food and water only. Group III (5 rats) received 200 mg/kg of *ocimum gratissimum* extract. Group IV (5 rats) received 200 mg/kg of *gongronema latifolium* extract. Group V (5 rats) received 100 mg/kg of *ocimum gratissimum* and *gongronema latifolium* respectively.

**Blood Sample Collection and Analysis**

Blood glucose level was monitored simultaneously as the administration of extract progressed throughout the duration of the experiment. At the end of the experiment, the rats were anaesthetized under chloroform and sacrificed. 5 ml of blood was collected via cardiac puncture from each rat and put into EDTA container.
Serum Biochemistry
Whole blood was separated with high speed macrocentrifuge at 3,500 rev/minute for 10 minutes and serum was separated by Pasteur pipette for analysis of the following biochemical assays; blood glucose estimation by glucose oxidase method, \(^{21}\) alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were performed based on Randox diagnostic kit on the principles of Reitman and Frankel, \(^{22}\) alkaline phosphatase (ALP) was determined by the method of Bessey et al., \(^{23}\) total bilirubin was estimated using the colorimetric method as described by Jendrassik and Grof. \(^{24}\) Serum albumin was estimated using the photometric colorimetric test (BCG-method). \(^{25}\)

Statistical Analysis
The data obtained was analyzed using the Statistical Package for Social Sciences (SPSS version 16.0 for windows). Analysis of variance (ANOVA) was used to compare means, and values were considered significant at P < 0.05.

RESULTS
Table 1 shows the blood glucose level before and after administration of alloxan induced diabetes mellitus. The experimental groups II, III, IV and V exhibited blood glucose levels (P < 0.05) above 200 mg/dl respectively. Table 2 shows that blood glucose level of groups III, IV and V was significantly reduced (P < 0.05) at the 3rd week of experiment compared to group II. There was significant difference (P < 0.05) in the blood glucose at the 3rd week between group I compared to group III, IV and V respectively.

Table 1: Blood glucose level before and after administration of diabetes mellitus.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Blood glucose before induction of diabetes (mg/dl)</th>
<th>Blood glucose after induction of diabetes (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I Control</td>
<td>77.25 ± 8.70</td>
<td>77.25 ± 8.70</td>
</tr>
<tr>
<td>II Diabetic Control</td>
<td>63.50 ± 4.09</td>
<td>245.50 ± 20.99</td>
</tr>
<tr>
<td>III 200mg/kg O.G.</td>
<td>78.75 ± 6.66</td>
<td>308.75 ± 53.07</td>
</tr>
<tr>
<td>IV 200mg/kg G.L.</td>
<td>86.25 ± 2.56</td>
<td>290.75 ± 43.67</td>
</tr>
<tr>
<td>V 400mg/kg of O.G + G. L</td>
<td>78.25 ± 3.61</td>
<td>382.00 ± 27.36</td>
</tr>
</tbody>
</table>

Table 2: Blood glucose level at different weeks and at the end of experiment after treatment with *ocimum gratissimum* (O. G) and *gongronema latifolium* (G. L).

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Blood glucose level after first week of treatment (mg/dl)</th>
<th>Blood glucose level after second week of treatment (mg/dl)</th>
<th>Blood glucose level at the third week of experiment (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I Control</td>
<td>69.50 ± 4.21</td>
<td>79.25 ± 2.29</td>
<td>72.50 ± 2.02</td>
</tr>
</tbody>
</table>
Data represented as mean + SEM; (*) P < 0.05 significant difference between control (b) P < 0.05 significant difference between diabetic control

### Effect Of With Ocimum Gratissimum (O. G) and Gongronema Latifolium (G. L) On Liver Function

Table 4 shows the effect of *ocimum gratissimum* and *gongronema latifolium* extract on liver function. The table shows that treatment of group III, IV and V respectively caused a significant decrease (P < 0.05) in total bilirubin compared to group II. There was no statistically significant difference (P > 0.05) in total bilirubin between group III compared with group I. There was statistically significant difference (P < 0.05) in albumin, ALT and ALP between group III, IV and V respectively compared to group II. There was also statistically significant difference (P < 0.05) in albumin, ALT and ALP between group III, IV and V respectively compared to group I. Table 4 also shows statistically significant difference (P < 0.05) in AST between group III, IV and V respectively compared to group I. There was statistically significant difference (P < 0.05) in AST between group III and IV compared to group II; however, there was no statistically significant difference (P > 0.05) between group V compared with group II.

### DISCUSSION

There was significant reduction in the blood glucose level after treatment with *ocimum gratissimum* in agreement with previous reports in rats and *gongronema latifolium* observed in this study agrees with previous reports in rats, [12, 26-27] whereas significant reduction was
greater after treatment with *gongronema latifolium* extract. [4, 28] Co-administration of extracts did not produce much statistically significant difference as expected, suggesting that hypoglycemic effect was mediated mainly by *gongronema latifolium* extract. Data have shown that *gongronema latifolium* extract possessed insulin-like activity. [28] The best known function of insulin is the hypoglycemic effect. [27, 29]

The impairment in the liver results in increased activities of AST, ALT and ALP and these serum activities are roughly proportional to the extent of tissue damage. [30-32] ALT and AST are the two of the most reliable markers of hepatocellular injury or necrosis. [18] Evaluation of the transaminases and alkaline phosphatase was carried out in order to determine liver impairment and also to investigate the effects of both extracts. Results (table 3) showed a decrease in the levels of AST, ALT and ALP although not significant compared to normal control. Several studies have showed that *gongronema latifolium* extract significantly reduced liver enzymes at low dose concentration (200-300 mg/kg) in diabetic rats [33, 34] and in acetaminophen induced hepatic toxicity. [35] Low dose concentration (11-88 mg/kg) of *ocimum gratissimum* extract did not cause significant effect on the serum levels of ALP, AST and ALT [36] and elevation in values of AST, ALP and ALT tend to be dose dependent, in such, that at a high dose (> 400mg/kg) significantly reduced these hepatic enzymes. [37] Therefore, we can suggest that co-administration of extracts increased hepatic enzymes in a dose related pattern in the present study. Bilirubin is produced by abnormal breakdown of pigment-containing proteins, especially hemoglobin from senescent red blood cells and myoglobin from muscle breakdown. Bilirubin released from such sources, tightly albumin-bound is delivered to the liver where it is efficiently extracted and conjugated by hepatic glucorinadation and sulfation. [19] Result (table 3) showed that total bilirubin increased significantly which could be as a result of hemolysis. Our recent study [38] showed that *gongronema latifolium* decreased MCH an MCHC levels in alloxan induced diabetic rats which may be due to hemolysis resulting to abnormal breakdown of hemoglobin thereby causing increased level of bilirubin. This hemolytic action of *gongronema latifolium* extract may also have produced increase in the AST observed in the present study since AST is present in red blood cells. [18] It is not surprising that level of albumin increased (table 3) because albumin is tightly bound to bilirubin resulting to an increase in serum albumin. Therefore, it can be concluded that effect of co-administration of *gongronema latifolium* and *ocimum gratissimum* extracts on liver function is dose related that could be harmful to the liver.
REFERENCE


