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ARTICLES

Research Articles

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Antidiabetic potential of liquid-liquid partition fractions of ethanolic seed extract of Corchorus olitorious
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Antitrypanosomal activity of *Aristolochia ringens* against *Trypanosoma congolense* infection in mice

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The methanolic extract of *Aristolochia ringens* whole plant, commonly used in the traditional treatment of various diseases in humans and animal by some phytotherapist in Nigeria, was evaluated for antitrypanosomal efficacy in mice infected with *Trypanosoma congolense*. Following three days dose intraperitoneal administration, the extract produced antitrypanosomal effect at the three dosage levels of the four tested that is, 433.2, 288.8 and 144.8 mg/kg body weight through the complete suppression or delay in parasite establishment. There was a reduction in the level of parasitaemia as well as enhanced survival of the infected mice, although the plant extract did not significantly (P < 0.05) increase the survival period of the mice compared to the negative control (infected untreated). The results suggest that the use of the extracts traditionally has a pharmacological basis.

**Key words:** *Aristolochia ringens*, *Trypanosoma congolense*, anti-trypanosomal.

INTRODUCTION

Animal trypanosomosis is one the major constraints to livestock production, particularly in the subhumid and to a lesser extent in the wetter parts of the semiarid zone of Africa (Osho, 2005). Extraction of bioactive compounds from medicinal plants permits the demonstration of their physiological activity. It also facilitates pharmacology studies leading to synthesis of a more potent drug with reduced toxicity (Eban et al., 1991). Presently in the developing countries, synthetic drugs are not only expensive and inadequate for the treatment of diseases but are also often with adulterations and side effects (Shariff, 2001).

*Aristolochia ringens* is a plant that belongs to the family Aristolochiaceae (Watson and Dallwitz, 1992). The plant is commonly known in the south-western part of Nigeria as Akogun (Yoruba). It is an aromatic lianes, scramblers, climbing shrub or rhizomes. All species of *Aristolochia* have broad primary medullary rays. The herbal plant was known to contain alkaloids and aristolochic acids, (Mabberley, 1993). In the present investigation the *in vivo* activity of the medicinal plant (*A. ringens*) which have some medicinal values among local herds men for the treatment of both human and animal diseases is therefore evaluated.

MATERIALS AND METHODS

Drying of samples and extraction of plant specimens

The plant was purchased from the herbal sellers in an Akure market, Ondo state, and was identified in the Department of Forestry and Wood Technology, Federal University of Technology, Akure. The plant was identified by the chief technologist of the forestry and wood technology department in The Federal University of Technology Akure, Dr ogunnika. The whole plant was air dried for two weeks and crushed into smaller particles using mortar and pestle and later pulverized into fine powder in a pulverizing machine (Thomas-Willey milling machine). Thereafter, 100 g of the pulverized sample was measured and soaked in 300 to 500 ml of methanol.
and left for 72 h after which it was filtered into a clean flask using muslin cloth and then filtered through Whatman No 42 (125 mm) filter paper. The extract obtained was then concentrated in vacuo using rotary evaporator until the entire methanol was removed. The methanolic extracts preparations were freeze-dried after concentrated and stored in a freezer.

Inoculated parasite into mice

The Trypanosoma congolense inoculated was obtained from the National Institute of Trypanosomosis Research (NITR), Jos, Plateau State, Nigeria. The parasite was multiplied through animal passage.

Experimental animals

Blood was taken from the parasitised animals (the donor animal with parasite level at log 8.1 using “Rapid matching” method of Hebert and Lumsden (1976)). The donated blood was diluted in saline water to give log 6.9 before the mice weighing between 18.7 and 28.3 g were inoculated intraperitoneally.

Experimental design

Thirty mice were weighed and grouped randomly into six experimental units of each unit, with five experimental animals per group. Therapy were given of the plant extract based on calculation in milligram per kilogram of body weight for three days.

Group 1: Infected untreated animals in this group did not receive any drug and served as (negative control).
Group 2: Animals of group 6 were given the standard antitrypanosomal drug that is, Diminal® at 10 mg/kg intraperitoneally at manufacturer recommendation dosage for only one day .and this served as the positive control.
Group 3: Infected and treated with 433.22 mg/kg/day.
Group 4: Infected and treated with 288.8 mg/kg/day.
Group 5: Infected and treated with 144.8 mg/kg/day.
Group 6: Infected and treated with 69.7 mg/kg.

These doses were chosen for administration based on the calculated values of 75 to 12.5% of the obtained LD₅₀ for the plant in a separate preliminary toxicity experiment.

Stages of the experiments

In vivo evaluation of antitrypanosomal activity

The inoculated mice were left for two days for the parasite to get established, and after the appearance of parasitaemia in 48 h post inoculation attaining log value of 5.4, treatment commenced with the extracts at different dose levels .

Reconstitution and administration of extract

All extracts were freshly reconstituted in saline solution prepared with distilled water and administered at the various doses. Animals of group 2, 3, 4, 5 and 6 were administered the test extract consecutively for three days on the basis of calculated dose range levels derived from LD₅₀ previously determined by intraperitoneal route. Mice were checked daily during and after the treatment to estimate the number of trypanosomes from blood at their tail in a wet blood film preparation.

Determination of parasitaemia

After 24 h of administration of the extract, a drop of blood from tail of each mouse was taken for examination under microscope for determination of level of parasitaemia as described by Hebert and Lumsden (1976) to check for effect of the extract on the parasite. The absolute number of parasites per millilitre of blood was calculated as log using the rapid matching method for estimating the host’s parasitaemia according to Herbert and Lumsden (1976). Briefly, higher levels of infection, parasite levels was measured by matching microscopic fields of a wet blood film against charts and when fewer parasites were present, by counting the number of trypanosomes in 5, 10 or 20 such microscope fields.

Assessment of extract efficacy

For the assessment of the antitrypanosomal activity of the extracts, the level of parasitaemia (expressed as log of the absolute number of parasites per millilitre of blood) in treated animals was compared with that in the untreated control animals.

Statistical analysis

The therapeutic effects were assessed by subjecting the parasitological data of treated and control animals to one way analysis of variance (ANOVA) using statistical package for social sciences (SPSS) version 17 with Duncan multiple range test, and the P-values of < 0.05 considered as significant and those > 0.05 as insignificant.

RESULTS

Effects of therapy of methalonic stem of A. ringens

The effect of the efficacy of A. ringens on the level of parasitemia in mice infected with T. congolense are shown on Table 1. The response on the first day post therapy showed that there was no significant difference (P > 0.05) in the effect observed on the mean level of parasitemia in all the extract levels of concentration and both positive and negative control. Visible effects were seen on the second day, where the positive control reduced (P < 0.05) the parasites in the mice. There was no significant difference (P > 0.05) in the reduction level of parasitemia of all levels of the extract concentration on the same day. However, on the third day post therapy the extract of concentrations of 433.2, 288.8 and 144.8 mg/kg were able to reduce the level of parasitemia and the pronounced effect of the cumulative therapy was seen. On the 4th day, when all grades of extract concentration further reduced, level of parasitemia in the infected mice showed no significant difference (P > 0.05) between the positive control and all three higher levels of the concentration of the extract used. A more noticeable effect was seen in the highest level of concentration of extract (433.2 mg/kg) as the cumulative plasma level completely reduced the parasites and appeared; the whole parasites were totally eliminated on the 4th day, however on the 5th day there was a recrudescence of
parasitemia in some of the mice. The plant extract did not significantly increase the survival period of the mice compared to the negative control (Table 2). However some were able to stay longer than the negative control group. The maximum days obtained for the doses of 288.80 and 433.22 mg/kg were higher than the negative control.

DISCUSSION

The demonstrable effect of the *A. rigens* on the level of parasitemia in the mice showed evidently from the results that the extract had antitrypanosomal activity *in vivo* at 433.288.8 and 144.8 (the dosage levels tested). Even at its crude status, the exhibited trypanocidal effect on the fourth day is an indication of high antitrypanosomal principle. This effect may be associated with level of some bioactive molecules (alkaloid) revealed as one the component. Hoet et al. (2003) reviewed that the quinoline alkaloids were from Cinchona bark (Rubiaceae). The observed action could also be attributed to some other phytochemical components present in the plant probably working in synergy. The relapse observed on day 5 for the highest dose which earlier caused the total clearance of the parasite might be due to reduced level of the extract in the plasma. The plant extract may also possess some valued antitrypanosomal principles which were responsible in prolonging some survival period of infected mice.

Conclusion

*A. rigens* demonstrated antitrypanosomal action and requires further elucidation and exploratory investigation.

REFERENCES


Full Length Research Paper

Antidiabetic potential of liquid-liquid partition fractions of ethanolic seed extract of Corchorus olitorious

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The Corchorus olitorius seeds were pulverized (grounded) to powder. The powdered seed (200 g) was extracted with 500 ml of ethanol (99.9%) within a period of 24 h and the procedure repeated 3 times using the same powdered extract. Extraction and fractionation were carried out with some modification in the choice of primary solvent (water) and partitioning (separating) solvents (hexane, chloroform, ethyl acetate and butanol). The fractions obtained (hexane, chloroform, ethyl acetate, saturated butanol and last remaining aqueous) were tested for antidiabetic and phytochemical properties. Two doses were employed while testing in diabetic rats, 500 and 250 mg/kg body weight. Diabetes was induced by a single intraperitoneal injection of 150 mg/kg body wt alloxan (Sigma) in saline. Animals with a blood glucose level ≥ 150 mg/dl were considered diabetics. All the fractions had some bioactivity in alloxan induced diabetic rats. The activity being better with the 500 mg doses than the 250 mg. Statistical significance (p < 0.05) in bioactivity (blood sugar change) was only seen with the aqueous fraction (1 h post treatment), chloroform fraction (1, 3 and 4 h post treatment) and the ethyl acetate fraction (2 and 3 h post treatment). The action of the seed extract can be attributed to phytochemical content of the extract. Of these flavanoids, alkaloids, saponins have been reported to have hypoglycaemic effect.

Key words: Corchorus olitorius (CO), alloxan induced diabetic rat, fractionation, antidiabetic, phytochemical

INTRODUCTION

The therapeutic cure for diabetes mellitus has remained elusive despite the discovery of an array of medications that can ameliorate the outcome of the disease (Holman, 2013). Plants have remained a veritable source for drug discovery the world over (Etuk, 2006). The leaves extract of Corchorus olitorius (CO) had been reported to possess hypoglycaemic effect (Abo et al., 2008) and high antibacterial activity (Adegoke and Adebayo, 2009). The crude ethanolic extract of the seed has been evaluated in our laboratory for antidiabetic properties in experimental animals (In Press). The current effort is aimed at fractionating the ethanolic seed extract of the plant and assessing the antidiabetic effect of each fraction in alloxan induced diabetic rats. The outcome may stimulate the development of an antidiabetic drug from the plant extract.

The experimental model of a disease aids not only the understanding of the pathophysiology of the disease but also the development of drugs for its treatment (Etuk, 2010). Alloxan is a well known diabetogenic agent widely used to induce type II diabetes mellitus (DM) in animals (Viana et al., 2004). Alloxan causes selective necrosis of pancreatic islet β-cells producing different grades of the severity of DM by varying dose used. The simplistic argument made against the use of alloxan to induce type II DM is that alloxan produces β-cells damage thus

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leading to type I rather than type II DM. But studies showed that there are no differential response to hypoglycemic agents by alloxan and glucose loading hyperglycemic (with intact pancreatic cells) rats (Etuk, 2010). The best known drug induced DM is the alloxan induced, capable of inducing both type I and type II DM with proper dosage selection (Etuk, 2010). These suffice its use in this study.

The prevalence of diabetes for all age-groups worldwide was estimated to be 2.8% in 2000 and 4.4% in 2030. The total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030. The prevalence of diabetes is higher in men than women, but there are more women with diabetes than men. The urban population in developing countries is projected to double between 2000 and 2030 (Sarah et al., 2004). In Africa, the prevalence of DM is estimated at about 2.4%, in Nigeria, at about 3.1% (Gill et al., 2009).

MATERIALS AND METHODS

Laboratory animals

Male albino rats from the Biological Sciences Department of Usmanu Danfodiyo University (UDUS) were used for the study. The rats were housed in metal cages in the laboratory at temperature between 30 to 37°C; 12 h/12 h light/dark cycle and maintained with free access to standard rat feeds and water, for 7 days before experimentation. 12 h before experimentation, food was withdrawn but water available ad libitum.

Extraction and fractionation procedure

Extraction and fractionation were according to Gandhi et al. (2003) and Leilaf et al. (2007) with some modification in the choice of primary solvent (water) and partitioning (separating) solvents (hexane, chloroform, ethyl acetate and butanol). The powdered seed (200 g) was extracted with 500 ml of ethanol (99.9%) within a period of 24 h and the procedure repeated 3 times using the same powdered extract. The solvent was removed at 45°C under vacuum. The ethanol extract residue obtained was dissolved in water (500 ml) and exhaustively extracted by consecutive liquid/liquid partition with hexane (500 ml), chloroform (500 ml), ethyl acetate (500 ml) and saturated butanol (500 ml) using a separating funnel (1000 ml). The hexane, chloroform, ethyl acetate, saturated butanol and last remaining aqueous fractions was evaporated to obtain fractions (Gandhi et al., 2003). The fractions obtained (hexane, chloroform, ethyl acetate, saturated butanol and last remaining aqueous) were tested to evaluate the antidiabetic and phytochemical properties.

Phytochemical analysis

The phytochemical constituents of the CO fractions were conducted using methods outlined by Odebiyi and Sofowora (1979).

Antidiabetic studies

Induction of diabetes in rats

Diabetes was induced by a single intraperitoneal injection of 150 mg/kg body wt alloxan (Sigma) in saline. Animals with a blood glucose level ≥ 150 mg/dl were considered diabetic (Diniz et al., 2008).

The normal control was injected intraperitoneally with normal saline (2 ml/1 kg). A commercial available Glucometer (Accu Chek Active, Roche Diagnostics GmbH, D-68298 Germany) was used to determine blood glucose level in the animals (Glucose dye oxidoreductase mediator reaction method). Blood glucose was measured through tail tipping blood technique (Karl-Heinz et al., 2001).

Hypoglycemic activity in alloxan induced diabetic rats

In this experiment, seven major groups of rats consisted of 5 alloxan induced diabetic rats each. A group without any form of treatment but 10% tween 20 in normal saline was used as diluents in the treatment groups (Gowthamarajan and Sachin, 2010). Another consisted of alloxan induced diabetic rats administered 0.2 mg/kg glibenclamide orally and groups 3 to 7 consisted of 2 dosage groups (250 and 500 mg/kg) each with 5 alloxan induced diabetic rats’ administered the fractions (hexane, chloroform, ethyl acetate, butanol and aqueous fractions). Glucose levels were measured just prior to and 1, 2 and 4 h after extract/drug administration (adm) (t = 0 min). Results were calculated as percentage decrease of the initial value (by the difference between the glucose level at time t = 0 min and at the respective hours) (Cunha et al., 2008).

RESULTS

Phytochemicals of fractions

The phytochemicals isolated in the raw powdered seed were also seen in the ethanol extract, with exception of anthraquinone which was absent in the ethanol extract. All the fractions of the ethanolic seed extract were noticed to have volatile oil present. Also, all the fractions except the aqueous fraction contained alkaloids and cardiac glycosides. All the fractions, except the hexane fraction contained glycosides. The fractions were noticed to have phytochemicals in different combinations and proportions. While the aqueous fraction had the least, containing 3 (glycosides, volatile oil and balsams), hexane fraction contained 4 (alkaloids, tannins, cardiac glycosides and volatile oil), ethyl acetate had 5 (alkaloids, flavanoids, glycosides, cardiac glycoside and volatile oil), chloroform had 7 (alkaloids, tannins, flavanoids, glycosides, saponins, cardiac glycoside and volatile oil) and butanolic fraction had the highest 8 (alkaloids, tannins, flavanoids, glycosides, saponins, cardiac glycoside, volatile oil and balsams). All the fractions lacked steroids and anthraquinone present in the powdered seed (Table 1).

Bioassay of fractions in alloxan induced diabetic rats

The bioassay were carried out using two doses, 500 and 250 mg/kg and these showed the fractions all had some bioactivity in alloxan induced diabetic rats (Tables 2 to 4). The activity was better with the 500 mg doses than the
Table 1. Phytochemicals of fractions.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Powdered Seed</th>
<th>Ethanol extract</th>
<th>Hex Frac</th>
<th>Chlo Frac</th>
<th>Ethyl Frac</th>
<th>But Frac</th>
<th>Aqu Frac</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+++</td>
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<td>++</td>
<td>-</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+++</td>
<td>+++</td>
<td>-</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td>Glycosides</td>
<td>++</td>
<td>+++</td>
<td>-</td>
<td>+++</td>
<td>+</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>Saponins</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Saponin glycoside</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Steroids</td>
<td>++</td>
<td>+++</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cardiac glycoside</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>Anthraquinone</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Volatile oil</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Balsams</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+++ means high concentration; ++ means medium concentration and + means low concentration.

Table 2. Effect of CO on blood glucose level of alloxan-induced diabetic rats/ reduction% in blood sugar.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/dl)</th>
<th>Pre-treatment</th>
<th>1 h</th>
<th>2 h</th>
<th>3 h</th>
<th>4 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>331.6±54.99</td>
<td>338.0±55.26 (-1.9)</td>
<td>329.4±56.19 (0.7)</td>
<td>319.0±5826 (3.8)</td>
<td>314.2±54.87 (5.2)</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>0.2</td>
<td>417.6±22.85</td>
<td>450.0±111.08 (-7.8)</td>
<td>403.2±112.0 (3.4)</td>
<td>339.2±116.45 (18.8)</td>
<td>285.2±112.71 (31.7)</td>
</tr>
<tr>
<td>Hexane fraction</td>
<td>500</td>
<td>413.2±116.78</td>
<td>339.0±132.14 (18)</td>
<td>306.2±127.94 (25.9)</td>
<td>330.8±147.03 (19.9)</td>
<td>322.6±140.96 (21.9)</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>365.6±149.61</td>
<td>348.8±131.63 (4.6)</td>
<td>318.8±121.64 (12.8)</td>
<td>324.4±120.30 (11.3)</td>
<td>325.6±127.64 (10.9)</td>
</tr>
<tr>
<td>Chloroform fraction</td>
<td>500</td>
<td>400.6±81.05</td>
<td>334.4±111.3 (16.5)</td>
<td>308.2±122.74 (23.1)</td>
<td>253.4±89.99 (36.7)</td>
<td>241.4±100.87 (39.7)</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>404.6±158.02</td>
<td>352.0±143.33 (13)</td>
<td>341.4±139.88 (15.6)</td>
<td>300.0±135.59 (25.9)</td>
<td>307.2±141.78 (24.1)</td>
</tr>
<tr>
<td>Ethyl acetate fraction</td>
<td>500</td>
<td>446.8±143.89</td>
<td>339.0±156.78 (24.1)</td>
<td>324.6±126.45 (27.4)</td>
<td>312.4±97.20 (30.1)</td>
<td>345.0±126.11 (22.8)</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>356.0±136.26</td>
<td>336.4±137.42 (5.5)</td>
<td>309.2±150.50 (13.1)</td>
<td>301.4±142.04 (15.3)</td>
<td>292.2±137.68 (17.9)</td>
</tr>
<tr>
<td>Butanol fraction</td>
<td>500</td>
<td>358.6±48.54</td>
<td>312.4±79.22 (12.9)</td>
<td>268.4±91.24 (25.2)</td>
<td>240.4±102.4 (33)</td>
<td>228.4±103.60 (36.3)</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>337.8±120.20</td>
<td>327.8±121.78 (3)</td>
<td>323.2±118.69 (4.3)</td>
<td>313.2±120.26 (7.3)</td>
<td>297.2±120.40 (12)</td>
</tr>
</tbody>
</table>
Table 2. Contd.

<table>
<thead>
<tr>
<th>Aqueous fraction</th>
<th>500</th>
<th>300.6±145.91</th>
<th>216.8±122.28 (27.9)</th>
<th>200.8±119.93 (33.2)</th>
<th>173.0±114.32 (42.4)</th>
<th>172.0±114.65 (42.8)</th>
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</thead>
<tbody>
<tr>
<td>250</td>
<td>318.6±93.52</td>
<td>248.8±119.61 (21.9)</td>
<td>228.6±136.77 (28.2)</td>
<td>199.0±138.07 (37.5)</td>
<td>210.8±133.49 (33.8)</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± SD (n=5). *Significant difference (p < 0.05). (%) Reduction in blood sugar = untreated FBS - treated FBS/untreated FBS x100.

Table 3. Blood sugar change due to treatment with CO fractions.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Blood sugar change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 h</td>
<td>2 h</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>-5.4±28.08</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>0.2</td>
<td>-32.8±23.18</td>
</tr>
<tr>
<td>Hexane fraction</td>
<td>500</td>
<td>74.2±63.11</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>16.8±51.46</td>
</tr>
<tr>
<td>Chloroform fraction</td>
<td>500</td>
<td>66.2±74.23</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>52.6±50.59*</td>
</tr>
<tr>
<td>Ethyl acetate fraction</td>
<td>500</td>
<td>107.8±94.39*</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>19.6±33.78</td>
</tr>
<tr>
<td>Butanol fraction</td>
<td>500</td>
<td>46.2±39.02</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>10.0±9.90</td>
</tr>
<tr>
<td>Aqueous fraction</td>
<td>500</td>
<td>83.8±64.58</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>69.8±61.64##</td>
</tr>
</tbody>
</table>

Values are mean ± SD (n=5). *significant difference (p<0.05) with respect to control. #significant difference (p<0.05) with respect to glibenclamide and ## p<0.01.

250 mg. Statistical significance in bioactivity (blood sugar change) was only seen with the aqueous fraction (1 h post treatment), chloroform fraction (1, 3 and 4 h post treatment) and the ethyl acetate fraction (2 and 3 h post treatment) (Table 3). The calculated percentage reduction in blood sugar due to fractions (Table 4) showed that the aqueous fraction had the best bioactivity, followed by chloroform, butanol, ethyl acetate and hexane fractions in that order, respectively.

**DISCUSSION**

In diabetic rats, the bioassay of fractions were carried out using two doses, 500 and 250 mg/kg and this showed the fractions all had some...
bioactivity in alloxan induced diabetic rats (Tables 2 to 4). The activity was better with the 500 mg doses than the 250 mg. Statistical significance (p < 0.05) in bioactivity (blood sugar change) was only seen with the aqueous fraction (1 h post treatment), chloroform fraction (1, 3 and 4 h post treatment) and the ethyl acetate fraction (2 and 3 h post treatment) (Table 3). The Calculated percentage reduction in blood sugar due to fractions (Table 4) showed the aqueous fraction having the best bioactivity, followed by chloroform, butanol, ethyl acetate and hexane fractions in that order. Using the calculated percentage reduction in blood sugar (Table 4), in the 1st hour, all the fractions were noticed to have a better sugar control to glibenclamide in the following order; aqueous fraction, ethyl acetate, hexane, chloroform and butanol fractions. In the 2nd hour, the fractions had a better control to glibenclamide in this order; aqueous, ethyl acetate, hexane, butanol and chloroform. In the 3rd hour, the order was aqueous, chloroform, butanol, ethyl acetate, hexane and lastly glibenclamide. In the 4th hour, the order was aqueous, chloroform, butanol, glibenclamide, ethyl acetate and hexane. These findings suggested the different liquid-liquid partition fractions of the ethanolic seed extract of Cichorius olitorius had different efficacy, onset of action and period of action as an antidiabetic.

There are a number of other plants with acclaimed antidiabetic activity. Among these are Treculia africana and Bryophyllum pinnatum in the management of diabetes and heart disease (Ogbonnia et al., 2008), there is also report that ethanol leaves extract of Cissampelos muconata possesses hypoglycemic activity in streptozocin induced diabetic rats. Gynostemma pentaphyllum Tea was found to improve insulin sensitivity in Type 2 diabetic patients (Huyen et al., 2013). Aqueous extract of Ganoderma lucidum has shown significant hypoglycemic effects in alloxan induced diabetic wistar rats (Mohammed et al., 2007). Aerial parts of Phyllanthus niruri have great potentials as anti-diabetic remedy (Nwanjo, 2007). Aqueous extract of Ficus religiosa bark possess significant anti diabetic activity (Rucha et al., 2010). Oral administration of Boerhaavia diffusa and Ocimum sanctum possess anti-hyperglycemic activity (Dwividendra et al., 2013).

Hypoglycemic activity of Fumaria parviflora in the treatment of diabetes has been validated (Fatemeh et al., 2013). The action of the liquid-liquid partition fractions of the seed extract can be attributed to phytochemical content of the extract. Of these phytochemicals, flavonoids (Taoying et al., 2009; Kaku et al., 2004), alkaloids (Day et al., 1990), saponins (George et al., 2002) have been reported to have hypoglycaemic effect. Several researchers have reported plant extracts (hypoglycaemic agents) with several combinations of phytochemicals to which the reported phytochemicals (Table 1) belong (Anin et al., 2011; Ocho-Anin et al., 2010; Atangwo et al., 2009).

Adeneye and Adeyemi (2009) reported the phytochemicals, alkaloids, flavonoids, tannins and glycosides of Hunteria umbellata to have hypoglycaemic effects in

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>% reduction in blood sugar</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 h</td>
<td>2 h</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>-1.9</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>0.2</td>
<td>-7.7</td>
</tr>
<tr>
<td>Hexane fraction</td>
<td>500</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>4.6</td>
</tr>
<tr>
<td>Chloroform fraction</td>
<td>500</td>
<td>16.5</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>13.0</td>
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<tr>
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<td>500</td>
<td>24.1</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>5.5</td>
</tr>
<tr>
<td>Butanol fraction</td>
<td>500</td>
<td>12.9</td>
</tr>
<tr>
<td></td>
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<td>3.0</td>
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<td>500</td>
<td>27.9</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>21.9</td>
</tr>
</tbody>
</table>

% reduction in blood sugar=untreated FBS- treated FBS /untreated FBS × 100.
normoglycaemic, glucose and nicotine-induced hyperglycaemic rats. It therefore would mean that the hypoglycaemic action of the fractions of the seed extract of CO could be due to the phytochemicals present singly or in combination. This study stimulated further research (ongoing) on the most active fraction in the bid to isolate and structurally elucidate the active antidiabetic agent/agents.

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Journal of Pharmacognosy and Phytotherapy

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