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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 labouratory 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 Leila 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' administerded 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.

Parameter	Powdered Seed	Ethanol extract	Hex Frac	Chlo Frac	Ethyl Frac	But Frac	Aqu Frac
Alkaloids	+++	+++	++	++	++	++	-
Tannins	+	++	+	++	-	++	-
Flavonoids	+++	+++	-	+++	++	+++	-
Glycosides	++	+++	-	+++	+	+++	++
Saponins	++	+	-	++	-	++	-
Saponin glycoside	-	-	-	-	-	-	-
Steroids	++	+++	-	-	-	-	-
Cardiac glycoside	++	++	+++	++	++	++	-
Anthraquinone	+	-	-	-	-	-	-
Volatile oil	+++	+++	+	++	+++	+	++
Balsams	+	+	-	-	-	+	+

+++ 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.

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

Table 2. Contd.

Aqueous fraction	500	300.6±145.91	216.8±122.28 (27.9)	200.8±119.93 (33.2)	173.0±114.32 (42.4)	172.0±114.65 (42.8)
	250	318.6±93.52	248.8±119.61 (21.9)	228.6±136.77 (28.2)	199.0±138.07 (37.5)	210.8±133.49 (33.8)

Values are mean ± SD (n=5). *Significant difference ($p < 0.05$). (%) Reduction in blood sugar = untreated FBS - treated FBS/untreated FBS ×100.

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

Treatment	Dose (mg/kg)	Blood sugar change			
		1 h	2 h	3 h	4 h
Control	-	-6.4±28.08	2.2±30.85	12.6±28.75	21.2±25.40
Glibenclamide	0.2	-32.8±23.18	14.4±36.95	78.4±45.46	132.4±67.96
Hexane fraction	500	74.2±63.11	107±49.95	82.4±64.12	90.6±55.98
	250	16.8±51.46	46.8±40.81	41.2±33.85	40.0±30.27
Chloroform fraction	500	66.2±74.23	92.4±84.54	147.2±*62.27	159.2±*66.16
	250	52.6±50.59$^{\#}$	63.2±70.71	104.6±127.53	97.4±146.28
Ethyl acetate fraction	500	107.8±94.39$^{\#}$	122.6±68.99*	134.4±*60.95	101.8±77.8
	250	19.6±33.78	46.8±32.82	54.6±28.6	63.8±45.35
Butanol fraction	500	46.2±39.02	90.2±51.39	119±69.58	130.2±72.33
	250	10.0±9.90	14.6±4.67	24.6±9.26	40.6±31.33
Aqueous fraction	500	83.8±64.58	99.8±64.57	127.6±66.39	128.6±63.02
	250	69.8±61.64$^{\#\#}$	90.0±82.90	119.6±91.47	107.8±85.78

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

Table 4. Calculated percentage reduction in blood sugar due to fractions.

Treatment	Dose (mg/kg)	% reduction in blood sugar			
		1 h	2 h	3 h	4 h
Control	-	-1.9	0.7	3.8	5.2
Glibenclamide	0.2	-7.7	3.4	18.8	31.7
Hexane fraction	500	18	25.0	19.9	21.9
	250	4.6	12.8	11.3	10.9
Chloroform fraction	500	16.5	23.1	36.7	39.7
	250	13.0	15.6	25.9	24.1
Ethyl acetate fraction	500	24.1	27.4	30.1	22.8
	250	5.5	13.1	15.3	17.9
Butanol fraction	500	12.9	25.7	33.0	36.3
	250	3.0	4.3	7.3	12.0
Aqueous fraction	500	27.9	33.2	42.4	42.8
	250	21.9	28.2	37.5	33.8

% reduction in blood sugar=untreated FBS- treated FBS /untreated FBS ×100.

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 *Corchorus 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 mucronata* possess hypoglycemic activity instreptozocin 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, flavanoids (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 (Ahad et al., 2011; Ocho-Anin et al., 2010; Atangwho et al., 2009).

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

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 phytotochemicals 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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