

Short communication

Hypoglycemic effects of Corn steep liquor extracts in streptozotocin-induced diabetic rats

ABSTRACT

AIMS: The three plants presented in this study are *Citrullus colocynthis*, *Gladiolus psittacinus* and *Circuligo pilosa*. Their corn-steep fermenting liquor is traditionally used in preparing concoctions and decoctions for the treatment of various diseases. Our aim was to investigate the hypoglycemic effects of the extracts of these plants prepared with corn-steep fermenting liquor in diabetic rats.

METHODOLOGY: Diabetes was induced by single intraperitoneal administration of streptozotocin (45mg/kg). Normal as well as diabetic rats were divided into six groups of four rats each to receive different treatments for 15days. The effects of extracts were monitored on body weight, blood sugar and heamatological indices.

RESULTS: Qualitative phytochemical analysis of the extracts showed the presence of phenol, quinones, coumerin, saponins and flavonoids. Acute toxicity assay showed that *Gladiolus psittacinus* is toxic above 350mg/kg but other extracts did not show any death up to the dose of 2900mg/kg. The Corn steep liquor extracts were able to significantly ($p > 0.05$) reduce the elevated blood glucose level of diabetic rats. Also, body weight and heamatological indices in diabetic rats were improved by the administration of the extracts.

CONCLUSION: It can be concluded that corn steep fermenting liquor extracts can be used in the management of diabetes.

Keywords: Diabetes, Corn-steep fermenting liquor, *Citrullus colocynthis*, *Gladiolus psittacinus*, *Circuligo pilosa*

NTRODUCTION

Diabetes mellitus (DM) is a heterogenous metabolic disorder of carbohydrates, proteins and fat due to absolute or relative deficiency of insulin secretion with or without varying degree of insulin resistance (Oyedemi *et al.*, 2011). Patients with diabetes experience significant morbidity and mortality from microvascular (retinopathy, neuropathy, and nephropathy) and macrovascular (heart attack, stroke and peripheral vascular disease) complications. Due to these associated complications, management of diabetes mellitus is very difficult.

There are two major types of diabetes mellitus: type I and type II. Type 1 diabetes represents around 10% of all cases of diabetes, affecting approximately 20 million people worldwide (American Diabetes Association, 2009). It is a chronic autoimmune disease associated with selective destruction of insulin-producing β -cells of the pancreas. The onset of this disease

37 represents the end stage of β -cell destruction leading to type 1 diabetes mellitus. Type 2 diabetes is
38 the predominant form of diabetes and accounts for at least 90% of all cases of diabetes mellitus.

39 Diabetic mellitus is a major public health problem and has now become an epidemic with a worldwide
40 incidence of 5% in the general population. According to World Health Organization (WHO), more
41 than 360 million people are likely to be affected by diabetes mellitus by the year 2030. Most of the
42 orthodox drugs used for the treatment of diabetes are expensive, toxic and not cost effective
43 especially to low income population. Therefore, many people especially in the Sub Saharan Africa
44 especially Nigeria have turned to the use of traditional medicinal plants for the management of
45 diabetes. Although, the folkloric use of these plants is dated back to centuries, the scientific basis of
46 these indigenous claims has not been fully explored.

47 *Citrullus colocynthis* (CC), a plant commonly used in the management of diabetes in South West
48 Nigeria belongs to the family of *Cucurbitaceae* and it is otherwise known as the bitter apple, bitter
49 cucumber, desert gourd, vine of Sodom and 'egusi baara' (Yoruba, South west Nigeria). It resembles
50 a common watermelon (*citrullus lanatus*) vine but bears small, hard fruits with a bitter pulp. It
51 originally bore the scientific name *Colocynthis citrullus* (CO), but is now classified as *Citrullus*
52 *colocynthis*. In folkloric medicine, this plant has been utilized to treat constipation, diabetes, edema,
53 fever, jaundice, bacterial infections as well as cancer. It is also utilized as an abortifacient.

54 *Gladilous psittascinus* (GP) belongs to *Iridaceae*, an herbaceous plant that is commonly known as
55 Maid of the mist, dragon's head lily and 'Baaka' (Yoruba, South west Nigeria). It occurs virtually
56 throughout the grasslands, savannas and woodlands of sub-Saharan Africa. Traditionally, it is used
57 as remedy for cold, dysentery, asthma, gonorrhea and infections (Adjanohoun *et al.*, 1991), also it
58 methanolic extract had also been investigated in alloxan induced type 2 diabetic rats (Adediwura and
59 Kio, 2008).

60 *Curculigo pilosa* Schum and Thonn (CP) is a member of the family *Hypoxidaceae*. The rhizomes of
61 *Curculigo pilosa* Schum and Thonn, was the first African species to be described of the *Curculigo*
62 genus (Palazzino *et al.*, 2000) and is commonly known as 'Epakun' in Nigeria. In the traditional
63 medicine of Southwestern Nigeria, CP rhizome is used as a purgative as well as for the
64 management and treatment of hernia, infertility, diabetes, genital infections and sexually transmitted
65 infections (Burkill, 1994). It is also used in the manufacture of infant food and sorghum beer
66 (Sofodiya *et al.*, 2011). Extract of CP has been reported to show a dose dependent vaso-
67 constricting effect on rabbit aorta (Palazzino *et al.*, 2000), antimicrobial and anticandidal activities
68 (Gbadamosi and Egunyomi, 2010). Two benzyl benzoate diglucosides, piloside A and piloside
69 B, and glucosyl-fused nor- lignan, pilosidine, nyasicoside, curculigine, curculigoside and
70 pilosidine were reported isolated from the rhizome of this plant (Palazzino *et al.*, 2000).

71 Corn steep liquor (CSL) is a waste product of wet corn milling, usually a filtrate in the production of
72 palp. It is an important solvent in tradition medicine of southwestern Nigeria, where it is used to form
73 infusion in treatment of diabetes (Soladoye *et al.*, 2012).

The aim of this research therefore is to investigate the hypoglycemic effects of corn steep liquor extracts of CC, GP and CP in streptozotocin-induced diabetic rats, this is with a view to scientifically substantiate the indigenous use of these plants as antidiabetic herbs.

MATERIALS AND METHODS

Preparation of corn steep liquor (CSL)

This was prepared by steeping corn (maize) using a method modified from Omidiji *et al.*, 1997. Well-washed healthy, dried maize grains (1000g) were soaked in 3L hot water (100°C) for 72h, milled using a well-washed blender and filtered using muslin cloth. The filtrate was then allowed to settle for 24h. The resultant supernatant (corn steep liquor) was decanted and used in the extraction of the three plants.

Plant materials

Citrullus colocynthis fruit, *Circuligo pilosa* rhizome, and *Gladiolus psittacinus* corm were obtained from Bodija Market, Ibadan, Oyo State, Nigeria and authenticated in Botany Department, University of Ibadan.

Extraction

Citrullus colocynthis pulp was freeze dried while *Circuligo pilosa* rhizome and *Gladiolus psittacinus* corm were air-dried. The dried plant materials were blended using an electric blender. Then, 100g of powdered plant material(s) were macerated with 1.5L of corn steep liquor. After 72h, the extracts were filtered using muslin cloth and the filtrates were quickly frozen and concentrated using rotary evaporator. The extracts were subsequently kept in refrigerator until use.

Experimental animals

Healthy Wistar albino rats (100-120g) were obtained from Physiology animal house, College of Medicine, University of Ibadan. They were fed with standard pellet diet (Ladokun feeds) and water was given *ad libitum*. They were kept under a constant 12-h light and dark cycle. The experimental protocols were conducted in accordance with internationally accepted guidelines for care and use of laboratory animals.

Phytochemical screening

Phytochemical screening was carried out for all the extracts according to method of Sofowora (1993) and Harborne (1973).

Acute toxicity

The study was conducted in two phases according to the method of Lorke, 1983. In the first phase, three groups of three fasted rats each were administered with the extract at respective oral doses of 10mg, 100mg, and 1000mg per kg body weight. The rats were observed for signs of toxicity and possible deaths for 24hr. In the second phase, another three groups of one fasted rat each were administered respective doses of 1500mg, 2900mg and 5000mg per kg body weight of the extract. They were equally monitored as in phase one for signs of toxicity and deaths. LD₅₀ was

determined as the square root of the geometrical mean of highest non lethal dose and the lowest lethal dose.

Induction of diabetes mellitus

Diabetes was induced in overnight fasted experimental rats by a single intraperitoneal injection of streptozotocin (45 mg/kg body weight) dissolved in freshly prepared cold citrate buffer (0.1 M, pH 4.5). After 72h, plasma glucose was measured and those rats with fasting blood glucose greater than 10.2mmol/l were considered diabetic and used in the present study.

Experimental design

After the successful induction of experimental diabetes, the rats were divided into six groups (n = 4) and treated as follow:

Control group treated with distilled water

Diabetic control rats

Diabetic rats treated with GP (150mg/kg)

Diabetic rats treated with CC (300mg/kg)

Diabetic rats treated with CP (300mg/kg)

Diabetic rats treated with Metformin (150mg/kg).

The dose of the extracts was determined from acute toxicity studies and they were administered orally for 15 days using intragastric tube. Body weight and plasma glucose level measurements were conducted periodically. Plasma glucose level was measured using one-touch glucometer during the experimental period. The rats were fasted overnight and sacrificed by cervical decapitation after 15days. The blood was collected into EDTA sample bottles.

Hematological indices determination

After two weeks treatment with the three extracts, blood samples were obtained through retro-orbital puncture from the rats for the determination of the blood parameters: Red blood cells (RBC), packed cell volume (PCV), hemoglobin concentration (Hb), white blood cell count (WBC) and its differential counts using the method of Dacie and Lewis (1995).

Statistical analysis

Data were mean of 3 replicates $\pm$ standard Error (SE). Statistical analysis was carried out using SPSS version 20. One way analysis of variance was adopted for comparison of means, and the results were subject to post hoc test using least square deviation (LSD). The data were expressed as mean $\pm$ standard error. $P < 0.05$ was considered significant

RESULTS

Phytochemical screening

The phytochemical screening of the extracts revealed the presence of phenol, quinones, coumarin steroid, phlobatannin, saponin, terpenoid and flavonoid in at least one of the extracts (Table 1)

148 Table 1: Phytochemical screening of the Corn steep liquor extracts of CC, GP and CP

	CC	GP	CP
Phenol	Present	Present	Present
Phlobatannin	Absent	Absent	Present
Quinone	Present	Present	Absent
Coumarin	Present	Present	Present
Saponin	Absent	Present	Present
Steroid	Present	Present	Present
Flavonoid	Present	Absent	Absent
Terpenoid	Absent	Present	Present

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150 Acute toxicity

151 The Corn steep liquor extract of *Gladiolus psittacinus* started showing signs of toxicity like difficulty
 152 breathing and slow response as from the dose of 100mg/kg but did not produced any mortality until
 153 1000mg/kg was administered. But *Citrullus colocynthis* and *Curculigo pilosa* did not show any sign of
 154 toxicity until the dose of 5000mg/kg although no mortality was recorded for *Citrullus colocynthis* even
 155 when the animals were monitored for further 7 days. LD₅₀ of *Citrullus colocynthis*, *Gladiolus*
 156 *psittacinus* and *Curculigo pilosa* was evaluated by geometric mean of the highest non lethal dose and
 157 the lowest lethal dose and they were calculated to be 3807mg/kg, 316mg/kg and 2828 mg/kg
 158 respectively. Therefore CC and CP can be considered to be relatively safe while GP is considered
 159 toxic (Lorke, 1983).

160 Table 2: Phase I of acute toxicity test of Corn steep liquor extracts of CC, GP and CP

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Extract	Dose(mg/kgbw)	Mortality after 24 hour
Control	-	0/3
CC	10	0/3
	100	0/3
	1000	0/3
	1000	0/3
CP	10	0/3
	100	0/3
	1000	0/3
	1000	0/3
GP	10	0/3
	100	0/3
	1000	0/3
	1000	2/3

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166 Table 3: Phase II of acute toxicity test of Corn steep liquor extracts of CC, GP and CP
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Extract	Dose(mg/kgbw)	Mortality after 24 hour
Control		
CC	1600	0/1
	2900	0/1
	5000	0/1
CP	1600	0/1
	2900	0/1
	5000	1/1
GP	1600	1/1
	2900	1/1
	5000	1/1

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173 Effect of the extracts on the blood sugar and body weight

174 CSL extracts of CC, GP and CP were able to lower the elevated blood sugar level of the diabetic rats,
175 the reduction in the elevated blood sugar level after 14th day of treatment was significant ($p > 0.05$)
176 (Table 4). Also changes in body weight of the control and experimental rats were showed in Table 5.
177 They was a significant ($p > 0.05$) decrease in the body weight of diabetic group when compared with
178 the control whereas the diabetic rats treated with extracts as well as metformin showed significant
179 ($p > 0.05$) increase in body weight when compared with the diabetic untreated group.

180 Table 4: Effect of CSL extract of CC, GP and CP on blood sugar (mmol) of the streptozotocin induced
181 diabetic rats

	Initial	7 day	14 day
Control	4.27 ± 0.07	4.33 ± 0.03	4.22 ± 0.06
Diabetic Control	11.57 ± 0.32 ^a	13.57 ± 0.61 ^a	15.93 ± 0.23 ^a
GP	11.60 ± 0.12 ^a	9.87 ± 0.24 ^{ab}	8.70 ± 0.61 ^{ab}
CP	12.23 ± 0.55 ^a	11.03 ± 0.20 ^{ab}	9.57 ± 0.39 ^{ab}
CC	11.77 ± 0.14 ^a	10.83 ± 0.22 ^{ab}	9.00 ± 0.45 ^{ab}
Metformin	13.47 ± 0.80 ^a	10.07 ± 0.24 ^{ab}	8.10 ± 0.26 ^{ab}

182 The data are expressed as mean ± S.E n= 4 animals per group

183 a when compared with control $p > 0.05$, b when compared with diabetic untreated $p > 0.05$

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Table 5: Effect of CSL extract of CC, GP and CP on body weight (g) of the streptozotocin induced diabetic rats

	Initial	7 day	14 day
Control	128.30 ± 1.99	142.33 ± 1.51	152.40 ± 0.87
Diabetic Control	122.47 ± 0.58 ^a	116.00 ± 3.51 ^a	109.20 ± 2.02 ^a
GP	124.87 ± 0.69 ^a	130.20 ± 1.49 ^{ab}	135.07 ± 1.11 ^{ab}
CP	125.77 ± 0.73 ^a	132.57 ± 1.31 ^{ab}	138.63 ± 0.94 ^{ab}
CC	122.40 ± 1.13 ^a	128.60 ± 0.91 ^{ab}	136.99 ± 0.60 ^{ab}
Metformin	118.30 ± 1.53 ^a	130.47 ± 0.84 ^{ab}	140.63 ± 1.13 ^{ab}

The data are expressed as mean± S.E n= 4 animals per group

a when compared with control p > 0.05, b when compared with diabetic untreated p > 0.05

Effect of CSL extracts on heamatological indices

The effect of CSL extracts of CC, GP and CP is shown in Table 6. The induction of diabetes mellitus lead to significant alteration (p > 0.05) in the values of heamatological indices assayed, there was significant decrease in packed cell volume (PCV), heamoglobin (Hb), red blood cell (RBC) and lymphocytes count while white blood cell and neutrophil count were significantly increased. The administration of the extracts was able to improve the heamatological indices to near the values of the control rats (non-diabetic).

Table 6: Effect of CSL extract of CC, GP and CP on heamatological indices of the streptozotocin induced diabetic rats

	Control	Diabetic Control	GP	CP	CC	Metformin
PCV (%)	46.00±2.08	34.00±2.01 _a	40.67±0.6 _a ^b	40.33±0.88 _{ab}	41.00±1.88 _b ^a	42.00±1.15 ^b
Hb(g/dl)	14.17±0.90	10.87±0.29 _a	11.30±0.36 _{ab}	12.23±0.75 _{ab}	12.80±0.87 _{ab} ^a	11.30±0.24 _b ^a
RBC(10⁶μl)	6.46 ±0.70	5.27±0.70 _a ^a	6.27±0.82 _b ^b	5.45±0.19 _a ^a	5.58±0.35 ^a	5.70±0.40 ^a
WBC(10³μl)	8.12± 0.96	10.08±0.37 _a	17.35±0.88 _{ab}	8.10±0.39 _b ^b	8.65±1.46 ^b	7.58±0.92 ^b
Lymp.(%)	68.33±3.71	60.00±4.16 _a	59.00±1.54 _a	60.33±2.40 _a	61.33± 2.24 ^a	62.00±2.00 ^a
Neutro.(%)	27.00±4.04	34.33±3.84 _a	33.00±1.72 _a	35.67±2.09 _a	32.67± 2.4 ^a	32.33±1.88 _a

The data are expressed as mean± S.E n= 4 animals per group

a when compared with control p > 0.05, b when compared with diabetic untreated p > 0.05

DISCUSSION

Streptozotocin (STZ) is a drug commonly used to induce diabetes mellitus by selective cytotoxicity effect on pancreatic beta cells, thereby damaging the pancreatic beta cells and disrupt insulin actions. Streptozotocin-induced diabetic animals displayed polyuria, increased water intake, dehydration, weight loss and muscle wasting, excessive hair loss and scaling, diarrhea, cataracts and increased food intake (Wei *et al.*, 2003). This research work aimed at studying the hypoglycemic effects of the corn steep liquor extract of CC, GP and CP in STZ-induced diabetes rats, especially to ascertain the toxicity and the safety dose of the extracts. The result of the phytochemicals screening show that the extracts are rich in phytochemicals and result have shown that phytochemicals present in fruits and vegetables may prevent or cure some diseases due to their antioxidant, anti-inflammatory, anticancer and antimicrobial properties (Sucupira *et al.*, 2012). Also according to Lorke 1993, the result of acute toxicity shows that CC and CP could be considered relatively safe while GP is toxic, the toxicity of *Gladiolus psittacinus* is further confirmed by the extremely high value of white blood cell counts in GP treated rats. The administration of the extracts to diabetic animals resulted in improved glycemic control which prevented the loss of body weight, polyuria and excess of food and fluid intake. The glucose lowering mechanism of the extracts might be through stimulation of either surviving β cells or regenerated β cells of islets of Langerhans to release more insulin from the pancreas or it can be correlated with reminiscent effect of hypoglycemic sulphonylurea that promotes insulin secretion by the closure of K^+ -ATP channels, membrane depolarization and stimulation of Ca^{2+} influx, an initial key step in insulin secretion from the remnant β cells or from regenerated β cells of pancreas (Sundaram *et al.*, 2012).

The assessment of haematological parameters is used to reveal the deleterious effect of diabetes mellitus in animal and man. The occurrence of anaemia in diabetes mellitus has been reported due to the increased non-enzymatic glycosylation of RBC membrane proteins (Oyedemi *et al.*, 2011). In this research work, the extracts were able to bring the levels of the haematological indices back to near normalcy, this might be due to the fact that the extracts may contain some phytochemicals/nutrients that can stimulate the formation or secretion of erythropoietin in the stem cells of the animals. Erythropoietin is a glycoprotein hormone which stimulates stem cells in the bone marrow to produce red blood cells (Ohlsson and Aher, 2006). The stimulation of this hormone enhances rapid synthesis of RBC (Abu-Zaiton, 2010). The increase in the WBC count of the

animals could be as a result of the anaemia that was induced in the animals. The animal's system may have assumed the cause of the anaemia to be a result of infection or a disease condition. The system may therefore have concentrated on the production of white blood cells to combat the assumed infections (Okonkwo *et al.*, 2013). White blood cells have been known to increase rapidly following foreign attack by pathogens and the systems normal physiologic response will be to boost the body's defense mechanisms (Eyong *et al.*, 2004).

CONCLUSION

It can be concluded that all the extracts at the dose administered exhibits potent antidiabetic effect and shows improvement in the heamatological indices of STZ-induced diabetics rats however further investigation is still needed to find out its mechanism of action and to establish its therapeutic potential in the treatment of diabetes and diabetic complications.

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