Phytochemical Studies of Different Fractions of *Galega officinalis* Extract and Their Effects on Some Biochemical Parameters in Alloxan-Induced Diabetic Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Authors CDL and GIA designed the study and prepared the manuscript. Author GI managed the literature searches and prepared rat application. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Galega officinalis Linn (Goat's rue) is popularly called *sakpani* in Hausa speaking part of Nigeria. The Plant belongs to the family Leguminoseae. *G. officinalis* is chiefly used as an antidiabetic plant because of its ability to reduce blood sugar levels. The plant has positive effect on breast-milk production. Aqueous, methanolic, ethanolic and acetone extracts of the plant *Galega officinalis Linn* (Leguminoseae) and fractionations of the methanolic extracts were carried out in stages using standard procedures. Alkaloids, glycosides and flavonoids from the methanolic extracts of *G. officinalis* at 400 mg/kg body weight significantly ($p<0.05$) reduced the plasma glucose concentration in diabetic treated rats at the 3rd and 28th day of treatment when compared to diabetic untreated rats with alkaloids, glycosides and flavonoids producing 81.61%, 27.20% and 21.76% decreases in glucose concentrations. Also alkaloids from the methanolic extracts of *G. officinalis* improved lipid profile and reverse the adverse effects of induced diabetic on selected marker

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enzymes. Methanolic fractions F_1, F_2, F_3 and F_6 produced remarkable effect by decreasing glucose concentration in diabetic treated rats compared to diabetic untreated rats. Methanolic extract of *G. officinalis* proved to be a rich source of phytochemicals with potent hypoglycaemic and hypolipidemic effects.

1. INTRODUCTION

Plants as medicines have being practiced by man for the cure of several diseases for centuries [1,2,3]. At present, despite the abundance and advancement of synthetic drugs, a significant proportion of the population of developing countries still depend on traditional medicines for their health care needs [3]. According to World Health Organization 2001, 60% of the world's population depend on traditional medicine, and 80% of the population in developing countries depend almost entirely on traditional medical practices, in particular, herbal medicine for their primary health care needs [4,5]. In Traditional Chinese Medicine (TCM), for instance, the disease has been referred to as "Xiaokezheng" or "Xiaodanzheng," both of which mean diabetes [6]. In the Sri Lankan traditional medicinal system, the disease has been stated as "Madhumeha" [7]. Plants have always been an exemplary source of drugs and have directly or indirectly yielded many important medicines in the past. For instance, the discovery of the widely used hypoglycemic drug, Metformin, came from the traditional approach of using *Galega officinalis* [3]. Overall, herbal remedies are gaining popularity because of several advantages such as a comparatively lower incidence of side-effects at recommended dosages, better patient tolerance, relatively low cost, and acceptance due to a long history of use. From the perspective of diabetes, the more important cause is that herbal medicines provide rational means for remedying the disease condition as well as many other ailments which are obstinate and incurable in more Western systems of medicine. Medicinal plants continue to contribute significantly to modern prescription of drugs by providing lead compounds upon which the synthesis of new drugs are made. [2] reported that 60% of the anticancer drugs and 75% of the anti-infectious disease drugs approved from 1981-2002, could be traced to natural origins. In addition, 61% of all new chemical entities introduced worldwide as drugs during the same period could be traced to or were inspired by natural products [8].

Galega officinalis Linn (Goat's rue) is popularly called *sakpani* in Hausa speaking part of Nigeria. The Plant belongs to the family Leguminoseae. *G. officinalis* is chiefly used as an antidiabetic plant because of its ability to reduce blood sugar levels. The plant has positive effect on breast-milk production [9]. With over 800 herbal medicinal plants in use for the treatment of Diabetics [10], the search for active biomolecules from medicinal plants with anti-diabetic properties is still on.

2. MATERIALS AND METHODS

2.1 Plant Material

The plant *G. officinalis Linn* (Goat's rue, whole plant) was collected at Naraguta village, University of Jos permanent site, Plateau State, Nigeria. The plant was identified and authenticated at the Department of Plant Science and Technology, University of Jos, Nigeria. A Voucher sample was then deposited at the Federal College of Forestry, Jos, Plateau State with the voucher Number FHJ 101.

2.2 Preparation of Extracts

The plant material wasair dried under shade until constant weight was obtained and grinded to fine powdered form using a blender. 100 g of the powdered forms was weighed and dissolved in 1000 ml of distilled water and boiled for 15 minutes, allowed to cool and then filtered using Whatman Filter paper Grade No. 1. The filtrate was then allowed to dry in a water bath (50ºC). The extract was stored in an air tight container and kept until required. The methanolic, ethanolic and acetone extracts were prepared using similar methods except that 100 g of the fine powder was dissolved in 400 ml of the solvent respectively and allowed to stand for 3 h. Filtrates were dried at room temperature as against water bath in aqueous extraction.

Fig. 1. Image of *Galega officinalis Linn* (Goat's rue) grown in Northern Nigeria

2.3 Experimental Animals

Male albino rats (Wistar Strain) weighing between 180-250 g used in this study were purchased from the animal house of the University of Jos, Nigeria and the National Veterinary Research Institute Vom, Nigeria. The animals were maintained under standard conditions, had free access to food (Grand Cereal, Oils and Mills Products, Jos, Nigeria) and water *ad libitum*. The animals were monitored with care and all the experimental procedure with the animals was in accordance with the internationally accepted principles for laboratory animal use and the experimental protocols were duly approved by the ethical committee of Animal House of Pharmacology Department University of Jos, Jos.

2.4 Induction of Diabetics

Diabetics mellitus was induced in animals by single intraperitonial injection of 150 mg/kg body weight of alloxan monohydrate (Sigma, St. Louis, USA) suspended in normal saline, after an overnight fasting. 48 h later diabetics were confirmed using One Touch Glucometre. Animal with fasting blood glucose level ≥250 mg/dL were considered diabetic and included in the study.

2.5 Experimental Design

The study was conducted in stages; Stage I: Phytochemical studies from different solvents, Stage II: Diabetic studies of crude methanolic extract, Stage III: Phytochemical studies of crude methanolic extracts, Stage IV: Lipid profile studies of crude alkaloids from methanolic extract, Stage V: Fractionation of crude methanolic extract.

Stage I:

Crude extraction and phytochemical studies of different solvents extracts from *G. officinalis Linn*. using standard procedures. Phytochemicals screening was conducted using methods described by Harborne [11], Trease and Evans [12] and Sofowora [13]. Alkaloids, flavonoids, glycosides and saponins were also extracted from methanolic extracts of *G. officinalis* using standard procedures.

Stage II:

Twenty five albino rats were randomly assigned into five groups of five rats each and acclimatized for 7 days. Group A served as control and received 0.5 ml distilled water, orally per day. Group B - E were diabetic induced rats administered 0.5 ml distilled water, methanolic extract of *G. officinalis*, Garlic oil and Metformin (14.2 mg/kg bw), daily for 28 days respectively. Glucose concentrations were determined at the end of the study period.

Stage III:

Thirty albino rats were randomly assigned into six groups of five rats each and acclimatized for 7 days. Group A served as control and received 0.5 ml distilled water, orally per day. Group B - F were diabetic induced rats administered 0.5 ml distilled water, alkaloids, flavonoids, glycosides and saponins from methanolic extract of *G. officinalis* respectively and glucose concentrations determined at 3rd and 28th day post treatment.

Stage IV:

Fifteen albino rats were randomly assigned into three groups of five rats each and acclimatized for 7 days. Group A served as control and received 0.5 ml distilled water, orally per day. Group B and C were diabetic induced rats administered 0.5 ml distilled water and alkaloids from methanolic extract of *G. officinalis* respectively. Animals were sacrificed at the 28th day. Lipid profile and selected marker enzymes were assayed from prepared sera.

Stage V:

Fractions obtained from methanolic extracts of *G. officinalis* numbering seven ($F_1 - F_7$) were administered to experimental rats for 28 days, with plasma glucose determinations at 3rd and 28th day. The grouping was as follows: Forty five albino rats were randomly assigned into nine groups of five rats each and acclimatized for 7 days. Group A served as control and received 0.5 ml distilled water, orally per day. Group Bwere diabetic induced rats administered 0.5 ml distilled water per day while groups C – I were diabetic rats administer Fractions $F_1 - F_7$ respectively.

2.6 Collection of Samples

At 3rd and 28th day of treatments, whole blood samples were collected from animals' tails. Using a One Touch® Glucometerkit, the blood glucose concentrations were determined. Prior to the 29th day, the rats were subjected to an overnight fast. They were anesthetized at the time of sacrifice by been placed in a seal cotton wool soaked in diethyl ether inhalation jar. Blood samples were collected into centrifuge tubes and allowed to clot for about 45 minutes, after which they were spun at 5000 rpm for 5 minutes, the serum collected were transferred into bijou bottle using pasture pipette and kept for analysis.

2.7 Biochemical Parameters

Biochemical parameters assayed; Total cholesterol concentration, serum HDL-cholesterol and triacylglyceride were done using Randox diagnostic reagent kits. LDL-cholesterol was estimated using Friedewald formula [14]. Serum enzymes to include ALT and AST were determined by methods of Reitman and Frankel [15], while ALP was determined using King-Armstrong [16].

2.8 Statistical Analysis

Data were presented as Mean ± Standard Deviation (SD) of 5 replicates and were analyzed using DMRT following one-way analysis of variance (ANOVA) using SPSS 16.0 computer software package (SPSS Inc., Chicago, U.S.A). Differences at $p < 0.05$ were considered significant.

3. RESULTS

The phytochemicals screening of the raw *G. officinalis Linn* using selected solvents reveal that alkaloids, flavonoids, tannins, cardiac glycosides, terpenes and steroids were present in all four (Aqueous, Methanolic, Ethanolic and Acetone) extracts (Table 1). However, saponnis and resins were not detected in ethanolic and acetone extracts but present in aqueous and methanolic extracts of the plant while phenols and balsam were detected in only ethanolic and acetone extracts (Table 1).

Garlic oil administered to the diabetic rats showed a better result in lowering glucose levels in diabetic treated rats compared to metformin and methanolic extracts of *G. officinalis Linn*. However, all three treated groups had significantly ($p < 0.05$) decreased glucose levels when compared to diabetic untreated rats (Table 2).

Selected phytochemicals from methanolic extracts of *G. officinalis Linn*; alkaloids, flavonoids, glycosides and saponins administered at 400 mg/kgbwt/day for 28th days, showed varying effects on glucose concentrations in diabetic rats. Alkaloids, flavonoids and glycosides expressed significant ($p < 0.05$) decrease in glucose concentrations at 3rd Day (28.98%, 35.94%, 18.84%) and 28th Day (81.61%, 21.76%, 27.20%) respectively. Saponins obtained from the plant could not

express similar effect throughout the duration of the study (Table 3).

Total Cholesterol, TG, and LDL significantly ($p<0.05$) decrease in diabetic rats treated with alkaloids from *G. officinalis Linn.* When compared to diabetic untreated rats with a corresponding significant ($p<0.05$) increase in HDL of treated rats (Table 4).

The serum levels of marker enzymes (AST, ALT and ALP) in diabetic control (Untreated) rats increased significantly ($p<0.05$) when compared to the normal control rats. Alkaloid extracts significantly reverse these changes in the marker enzymes when compared to diabetic untreated rats (Table 5).

Different fractions from methanolic extract of *G. officinalis Linn* were screened for phytochemicals

and results presented in Table 6. Fraction 3 (F_3) retained more of its phytochemicals with alkaloids, flavonoids, resins, terpenes and steroids present. This is closely followed by Fractions 2 (F_2) while the least detected with a single phytochemical (cardiac glycosides) present is Fraction 1 (F_1) (Table 6).

Various fractions from *G. officinalis Linn* methanolic extract showed varying effect on serum glucose levels in alloxan-induced diabetic rats (Table 7). At Day 3, all fractions had significant ($p<0.05$) decrease in glucose level when compared to diabetic control. At Day 28, glucose levels were further reduced in diabetic treated rats especially fractions 1, 2, 3 and 6 with 75.68%, 60.14%, 66.55% and 72.64% decrease in glucose concentration (Table 7).

Table 1. Phytochemical screening of *G. officinalis Linn* using selected solvents

Phytochemicals	Aqueous	Methanol	Ethanol	Acetone
Alkaloids	+	+	+	+
Flavonoids	+	+	+	+
Tannins	+	+	+	+
Saponins	+	+	-	-
Cardiac glycosides	+	+	+	+
Terpenes and steroids	+	+	+	+
Resins	+	+	-	-
Phenols	-	-	+	+
Balsam	-	-	+	+

Key:+ = Detected; - = Not detected

Table 2. Weight variation in normal and diabetic rats

Group	Initial weight (g)	Final weight (g)	Differential weight (g)	Percentage weight (%)
N C	165	184	+ 19	+ 10.32
D C	175	154	-22	-14.28
D + E	160	164	+ 4	+ 2.44
D + Std drug	180	171	+ 9	+ 5.26

Table 3. Effect of *G. officinalis Linn* methanolic extract, garlic oil and metformin on glucose level in alloxan-Induced diabetic rats

Group	Glucose concentration (mg/dl)	% Decrease in Glucose Conc.
Normal control	77.4 ± 1.2	NA
Diabetic control	255.4 ± 2.3^b	NA
Diabetic + *G. officinalis*	90.0 ± 1.7^c	64.84
Diabetic + Garlic oil	81.0 ± 1.2^a	68.29
Diabetic + Metformin	91.8 ± 2.0^c	64.06

Values are mean ± SD except for % Decrease in Glucose Concentration, n=5
[a-c]Values within a column with no common superscripts are significantly different ($p<0.05$)

Table 4. Effect of some phytochemicals isolated from *G. officinalis Linn* methanolic extract on glucose level in alloxan-Induced diabetic rats

Groups	Glucose concentration (mg/dl)		% Decrease in Glucose Conc.	
	3rd day	28th day	3rd day	28th day
Normal control	86.00 ± 2.45^a	81.00 ± 2.23^a	NA	NA
Diabetic control	345.00 ± 4.12^d	386.00 ± 2.56^d	NA	NA
Diabetic + alkaloids	245.00 ± 1.44^b	71.00 ± 2.55^a	28.98	81.61
Diabetic + flavonoids	221.00 ± 2.55^b	302.00 ± 1.58^c	35.94	21.76
Diabetic + glycosides	280.00 ± 6.08^c	281.00 ± 1.58^b	18.84	27.20
Diabetic + saponins	366.00 ± 1.42^e	412.00 ± 2.45^e	-6.09	-6.74

Values are mean±SD except for % Decrease in Glucose Concentration, n=5
[a-e] Values within a column with no common superscripts are significantly different (p<0.05)

Table 5. Effect of alkaloid from *G. officinalis Linn* methanolic extract on lipid profile in alloxan-induced diabetic rats

Groups	Lipid profile (Mmol/L)			
	Total Chol.	TG	HDL	LDL
Normal control	0.50 ± 0.01^a	0.90 ± 0.05^a	2.20 ± 0.02^a	0.80 ± 0.08^a
Diabetic control	2.40 ± 0.08^b	2.70 ± 0.08^b	0.67 ± 0.05^b	2.94 ± 0.01^b
Diabetic + Alkaloid	0.90 ± 0.10^c	1.10 ± 0.50^a	2.51 ± 0.10^c	1.24 ± 0.06^c

Values are Mean ± S.D, n=5
[a-c] Values within a column with no common superscripts are significantly different (p<0.05)

Table 6. Effect of alkaloid from *G. officinalis Linn* methanolic extract on some serum enzymes in alloxan-Induced diabetic rat

Groups	Selected marker enzymes (Mmol/L)		
	AST	ALT	ALP
Normal control	274.33 ± 1.10^a	103.33 ± 1.10^a	208.00 ± 0.82^a
Diabetic control	471.64 ± 2.25^b	133.33 ± 1.20^b	363.33 ± 2.0^b
Diabetic + Alkaloid	277.50 ± 2.01^a	106.20 ± 1.10^a	210.10 ± 1.50^a

Values are Mean ± S.D, n=5
[a-c] Values within a column with no common superscripts are significantly different (p<0.05)

Table 7. Phytochemical screening of the fractions of *G. officinalis Linn* methanolic extracts

Phytochemicals	Fractions						
	F$_1$	F$_2$	F$_3$	F$_4$	F$_5$	F$_6$	F$_7$
Alkaloids	-	+	+	-	-	-	-
Flavonoids	-	+	+	-	-	-	-
Tannins	-	-	-	-	+	+	+
Saponins	-	-	-	-	-	+	+
Cardiac glycosides	+	-	-	-	-	-	-
Terpenes and steroids	-	+	+	+	-	-	-
Resins	-	-	+	+	+	-	-

Key: + = Detected - = Not detected

4. DISCUSSION

Diabetes Mellitus (DM) describes a metabolic disorder of multiple aetiology, it is characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. DM is a common endocrine disorder in which there occur increased food and water intake [17], it is a growing health concern worldwide [18]. The effects of diabetes mellitus include long-term damage, dysfunction and failure of various organs. Diabetes mellitus may present with characteristic symptoms such as thirst, polyuria, blurring of vision, and weight loss [19,20]. The present study wish to contribute to

Table 8. Effect of different fractions of *G. officinalis Linn* methanolic extract on glucose level in alloxan-Induced diabetic rats

Fractions	Glucose concentration (mg/dl)		% Decrease in glucose Conc.	
	3rd day	28th day	3rd day	28th day
Normal control	85 ± 1.12^a	87 ± 1.10^a	NA	NA
Diabetic control	258 ± 1.23^b	296 ± 2.11^d	NA	NA
Diabetic + F_1	175 ± 1.35^c	72 ± 1.10^a	32.17	75.68
Diabetic + F_2	198 ± 1.32^e	118 ± 1.21^c	23.26	60.14
Diabetic + F_3	197 ± 1.33^e	99 ± 1.22^b	23.64	66.55
Diabetic + F_4	168 ± 1.10^d	216 ± 2.41^e	34.88	27.03
Diabetic + F_5	148 ± 1.22^c	119 ± 1.33^c	42.63	32.77
Diabetic + F_6	151 ± 1.12^c	81 ± 1.22^a	41.47	72.64
Diabetic + F_7	201 ± 2.32^e	212 ± 2.22^e	22.09	28.38

Values are mean ± SD except for % Decrease in Glucose Concentration, n=5
[a-e] Values within a column with no common superscripts are significantly different (p<0.05)

the knowledge of *G. officinalis* in treatment of DM by a bio-guided fractionation of crude extracts of the plant.

The phytochemicals screening of the raw *G. officinalis Linn* using selected solvents reveals that alkaloids, flavonoids, tannins, cardiac glycosides, terpenes and steroids were present in all four (Aqueous, Methanolic, Ethanolic and Acetone) extracts (Table 1). Several phytochemicals including alkaloids, flavonoids, glycosides, glycolipid, steroids, carbohydrates, glycopeptides, terpenoids, amino acids, saponins and inorganic ions are known to affect various metabolic pathways, which in turn directly or indirectly affect the level of glucose in the human body [21,22]. *G. officinalis* prove to be rich in such phytochemicals which may produce potent hypoglycemic, anti-hyperglycemic and glucose suppressive activities.

Sharp difference was observed after induction of diabetes in the experimental rats as shown in Table 2. The normal control showed a significant increase in body weight whiles the diabetic control group, treatment groups showed a decrease in body weight.

Diabetic studies of crude methanolic extract of *G. officinalis* revealed the antidiabetic potentials of the plant. Methanolic extract from *G. officinalis* lowered the high blood glucose in diabetic rats in a similar manner with the reference drug metformin (Table 3). Garlic oil however, significantly expressed a better effect. A similar result of the effect of garlic oil on some biochemical parameters in diabetic induced rats has also been documented [23,24]. The above effects achieved by methanolic extract of *G. officinalis* is either by increasing serum levels of

insulin or increasing the production of insulin from pancreatic β-cells, inhibit glucose absorption in the gut, stimulate glycogenesis in liver or increase glucose utilization by the body [21,22,25]. *G. officinalis* (Goat's rue) contains an alkaloid called Galegin which is key to its extraordinary anti diabetic effects. Galegin is chemically similar to Guanidine, the standard compound from which many conventional diabetic drugs are manufactured, such as metformin [26,27]. Ward, [28] also reported that the chemical benefits of *G. officinalis* on diabetic model are similar to that of metformin.

Alkaloids, flavonoids, glycosides and saponins were further extracts from methanolic extract of *G. officinalis*. Administration of these phytochemicals at a dosage of 400 mg/kg bwt./day for 28th days, showed varying effects on glucose concentrations in diabetic rats (Table 4). Alkaloids, flavonoids and glycosides expressed significant (p<0.05) decrease in plasma glucose concentrations at Day 3 and Day 28. Flavonoids present a 35.94% and 21.76% decrease respectively, expressing their natural hypoglycemic properties. It has been reported that some flavonoids possess hypoglycemic properties because they improve altered glucose and oxidative metabolism of the diabetic states [29]. However, at day 28, alkaloids produced 81.61% decrease in glucose concentration. These promising result increase our curiosity in investigating the effects of alkaloids from Methanolic extract of *G. officinalis* on lipid profile and selected marker enzymes in alloxan-induced diabetic rats (Table 5 and Table 6).

Alterations in serum lipid profile in diabetes are likely to increase the risk of coronary heart disease [30]. Significant (p<0.05) reduction in

serum lipids; total cholesterol, triglyceride and low density lipoprotein level in diabetic rats treated with alkaloids from methanolic extract of *G. officinalis Linn* should be considered as beneficial in long term prognosis of diabetes. Alterations in lipid metabolism; hypertriacyl-glycerolemia and hypercholesterolemia, as observed in diabetic untreated rats usually contribute to the pathogenesis of vascular complications in diabetes [31,32]. Alkaloids from methanolic extracts of *G. officinalis Linn* significantly ($p < 0.05$) reverse these alterations in lipid metabolism and also restore the protective role of HDL- cholesterol (Table 4).

The levels of AST, ALT and ALP were significantly ($p < 0.05$) increased in alloxan-induced diabetic untreated rats (Table 6) as previously reported [33,34]. Alkaloid extracts from *G. officinalis Linn* significantly reverse these changes in the marker enzymes when compared to diabetic untreated rats.

The last stage of this study involves the fractionation of methanolic extracts of *G. officinalis Linn* resulting in seven (7) fractions which were subjected to phytochemical screening and hypoglycaemic studies (Table 7 and Table 8). Fraction 3 (F_3) retained more of its phytochemicals with alkaloids, flavonoids, resins, terpenes and steroids present and produced 66.55% decrease in glucose concentrations at day 28. These compounds (phytochemicals) may be responsible for the hypolipidemic effect and restoration of enzymatic functions [35] as supported by the data obtained. Alkaloids produced 81.61% reduction in glucose while crude methanolic extract produced 64.84% reduction in glucose when compared with diabetic untreated rats. Alkaloids from the methanolic extract could be responsible for the hypoglycemic properties of *G. officinalis.* None of the fractions could produce a greater effect as alkaloids from the methanolic extract at the 28th day of treatment.

5. CONCLUSION

G. officinalis proved to be a rich source of phytochemicals and also revealed its antidiabetic potentials in experimental rats. Water, methanol, ethanol and acetone were able to extract a quit number of phytochemicals from the plant with the methanolic extract expressing a similar effect as metformin in diabetic treated rats. Furthermore, the methanolic extracts significantly improved the lipid profile and reverse adverse effect of

diabetics on serum enzymes (AST, ALT and ALP). At day 28, fractions F_1, F_2, F_3 and F_6obtained from the methanolic extract of *G. officinalis* produced remarkable effect by decreasing glucose concentration in diabetic treated rats.

CONSENT

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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