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### Effect of some phytochemicals extracted from goat's rue (*Galega officinalis*) on some biochemical parameters in normal and alloxan-induced diabetic rats

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#### ABSTRACT

Effects of saponin, tannins and glycoside isolated from aqueous extract of *Galega officinalis* on some biochemical parameters in alloxan-induced diabetic rats was examined in this study. Phytochemical screening of the aqueous extract of *Galega officinalis* showed it contains saponins, alkaloid, flavonoid, tannin, cardiac glycoside, phenol, resin, terpenes and steroids, which were fractionated. 25 male rats weighing 180-200g were divided into 5 groups (i.e. A, B, C, D and E). Groups A, B respectively represent diabetic control and Normal control rats that were orally administered with distilled water while Groups C, D and E represent diabetic rats treated respectively with saponin, tannin and glycoside obtained from *Galega officinalis* to the dose of 400mg/kg body weight. The animals were treated for 21 days. Their blood was collected and analysed for blood glucose, Cholesterol, triglyceride, high density lipoprotein, low density lipoprotein concentrations and Serum alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase activities (ALT). There was a significant decrease ( $p < 0.05$ ) in blood glucose concentration for all the experimental animals administered with the phytochemicals when compared to the diabetic control rats. Total cholesterol, Triglyceride and low density lipoprotein levels also decreased significantly ( $p < 0.05$ ) in the rats treated with the phytochemicals while High Density Lipoprotein concentration increased significantly in the animals. ALP, AST and ALT activities in the serum significantly decreased in all the rats that received the phytochemicals. Results obtained in this study shows that the tested phytochemicals at 400mg/kg body weight may be effective in the management of diabetes.

**Keywords:** Diabetes; Toxicity; Alloxan, Hypolipidaemic, Aminotransferase

#### INTRODUCTION

Diabetes mellitus is one of the common metabolic disorders with micro-and macrovascular complications that results in significant morbidity and mortality. It is considered as one of the five leading causes of death in the world [1, 2]. Incidence and prevalence of type 2 diabetes are increasing globally; the World Health Organization estimated that in 2000, 171 million people had diabetes, representing 2.8% of the world's population, and predicts that this number will increase to 366 million (4.4%) by 2030 [3]. The quest to find a permanent cure to this disease is on and medicinal plants seem like the most viable prospects.

Medicinal plants are botanicals with chemopreventive and/or therapeutic effects on ailments [4]. The use of herbs/plants to treat disease is almost universal among non-industrialized societies [5]. These plants have been used to treat a wide range of ailments of which diabetes happens to be one. Many plants have been used for the treatment of diabetes mellitus in traditional systems of medicine throughout the world. Indeed, along with dietary measures, plant preparation formed the basis of the treatment of the disease until the introduction of insulin in 1922. A number

of review article have been published on the traditional use of plants in diabetes [6, 7] and on plants and phytochemicals whose reputed hypoglycaemic effects have been scientifically investigated [8, 9, 10, 11].

*Galega officinalis* (Goat's Rue) known in the old Herbals as *Herbarutaecaprariae*, is a leguminous or herbaceous plant in the faboidae subfamily. The plant has been extensively cultivated as a forage crop, an ornamental, a bee plant and as green manure. Its name derives from gale (milk) and ega (to bring on), as Gelega has been used as galactogogue in small domestic animals (hence the name "Goat's rue"). It is a hardy perennial that blows in the summer months. *Galega officinalis* is a bushy plant with lilac blue flowers that is native to Europe, Russia, and Iran, where it thrives in moist soils. It grows to a height of 3 feet with branching stems and oval, opposite leaves. The long flower stalk produces many light purple-to-pink-to-white flowers similar to those in the pea family. The plant is without scent, unless bruised, when it emits a disagreeable odour. This study sought to investigate the effect of phytochemicals isolated from *Galega officinalis* on some biochemical parameters in alloxan-induced diabetic rats.

## MATERIALS AND METHODS

Twenty-five male albino rats of norvegicus strain weighing between 180 - 200g were obtained from the Animal Holding of University of Jos, Jos, Nigeria. *Galega officinalis* plant was also obtained from Jos, Plateau State, Nigeria. Assay Kits for Alkaline phosphatase, Aspartate aminotransferase and Alanine aminotransferase were products of Randox Laboratories, United Kingdom. All other reagents used were of analytical grade and were all prepared in all glass distilled water.

### Experimental Design

25 male albino rats were allowed 7 days acclimatization period in standard metal cages before commencement of experiment. They were also allowed access to normal rat chow and tap water throughout the experiment. All animal procedures were in strict accordance with the NIH guide for the care and use of laboratory animals. The rats were divided into 5 groups (i.e. A, B, C, D, and E). Groups A, B respectively represent diabetic control and Normal control rats that were orally administered with distilled water while Groups C, D and E represent diabetic rats treated respectively with saponin, tannin and glycoside obtained from *Galega officinalis* to the dose of 400mg/kg body weight. Treatment lasted for 21 days after which rats were sacrificed 24 hours after last extract administration.

### Induction of Diabetes

Diabetes was induced by a single intraperitoneal injection of freshly prepared alloxan solution at 150mg/kg body weight dose after fasting the animals for 12hours. The animals were returned to their cages after injection and allowed free access to food and water. After 4 days, the fasting blood glucose concentrations were measured from tail blood samples by using a OneTouch Ultra® glucometer (Lifescan; Johnson & Johnson, Milpitas, CA, USA). Animals with blood glucose concentrations above 8mmol/L were taken as diabetic and used for the experiment.

### Blood Collection

Blood samples for glucose level determination were collected from the tail tip of the rats. To obtain serum for Liver function indices analysis, the rats were anaesthetized in a jar containing cotton wool soaked in chloroform, they were then sacrificed by jugular puncture and their blood collected in an unheparinized bottle and allowed to stand for 10 minutes to clot. Serum was then collected using a Pasteur pipette.

### Enzyme, Protein and Liver Function indices Measurements

Serum triglycerides, total cholesterol and HDL were estimated by enzymatic colorimetric end point methods using Span diagnostic reagent kit. LDL and VLDL were obtained by calculations using the formula provided in cholesterol diagnostic kit booklet. Aspartate aminotransferase (AST) (EC 2.6.1.1) and Alanine aminotransferase (ALT) (EC 2.6.1.2) activities were assayed at 546nm [12]. Serum Total Protein concentration was determined at 540nm using the Biuret method [13]. Serum total and conjugated bilirubin were analysed at 540nm [14]. Serum albumin determination was done using the method of [15] at 639nm. The blood glucose levels were determined for all the samples by the glucose-oxidase method [16]. All measurements were done using spectronic 21 digital Spectrophotometer (Bausch and Lomb, Rochester NY).

### Statistical analysis

The data were subjected to statistical analysis. All significant differences were determined by ANOVA and were complemented by Duncan's multiple range test.

## RESULTS

Table 1 shows the result of qualitative phytochemical analysis of aqueous extract of *Galega officinalis* whole plant. The result revealed the presence of alkaloid, flavonoids, Tannins, saponin, cardiac glycoside, phenols, resins, terpene and steroids.

There was a significant decrease ( $p < 0.05$ ) in blood glucose concentration for all the experimental animals administered with the various phytochemicals when compared to the diabetic control animals (Table 2).

Lipid profile analysis showed a significant increase ( $p < 0.05$ ) in Total cholesterol, Triglyceride and Low Density Lipoprotein and a significant decrease ( $p < 0.05$ ) in High Density Lipoprotein for all the experimental animals when compared with the diabetic control group (Table 3). The results obtained for High Density Lipoprotein concentration for animals that were treated with the phytochemicals were not significantly different from the normal control group. The effect of the phytochemicals on serum ALP, ALT and AST activities is as presented in Table 4. All the animals administered with the various phytochemicals gave a significant decrease in Serum ALP, ALT and AST activities when compared to the diabetic control animals.

**Table 1: Qualitative Phytochemical screening of aqueous extract of *Galega officinalis***

S/No	Phytochemicals	Result
1.	Alkaloid	+
2.	Flavonoid	+
3.	Tannins	+
4.	Saponins	+
5.	Cardiac glycoside	+
6.	Terpenes and Steroids	+
7.	Phenol	+
8.	Resin	+

Key: + = Present  
- = Absent

**Table 2: Effect of Saponin, tannin and glycoside extracted from *Galega officinalis* on serum glucose levels of normal and alloxan- induced diabetic rats**

	Diabetic Control	Normal Control	Saponins 400mg/kg	Tannin 400mg/kg	Glycoside 400mg/kg
Glucose (mmol/L)	8.70±0.09 <sup>a</sup>	5.23±0.05 <sup>b</sup>	4.23±0.08 <sup>c</sup>	3.37±0.03 <sup>d</sup>	5.70±0.08 <sup>c</sup>

$n=5 \pm SEM$ ; values carrying superscripts different from the diabetic control are significantly different ( $P < 0.05$ ). Concentrations are expressed in mMol/L.

**Table 3: Effect of Saponin, Tannin and Glycoside extracted from *Galega officinalis* on lipid profile parameters in normal and alloxan- induced diabetic rats**

	Diabetic control	Normal control	Saponins 400mg/kg	Tannin 400mg/kg	Glycoside 400mg/kg
T.Chol	2.50±0.05 <sup>a</sup>	2.20±0.05 <sup>b</sup>	2.03±0.03 <sup>c</sup>	2.03±0.03 <sup>d</sup>	2.30±0.05 <sup>b</sup>
HDL	0.69±0.44 <sup>a</sup>	1.50±0.05 <sup>b</sup>	1.37±0.03 <sup>b</sup>	1.45±0.05 <sup>b</sup>	1.40±0.46 <sup>b</sup>
TG	2.70±0.05 <sup>a</sup>	0.90±0.05 <sup>b</sup>	2.03±0.03 <sup>c</sup>	2.10±0.05 <sup>c</sup>	2.27±0.03 <sup>d</sup>
LDL	1.29±0.01 <sup>a</sup>	0.52±0.05 <sup>b</sup>	0.25±0.01 <sup>c</sup>	0.16±0.01 <sup>d</sup>	0.45±0.03 <sup>b</sup>

$n=5 \pm SEM$ ; values carrying superscripts different from the diabetic control are significantly different ( $P < 0.05$ ). Concentrations are expressed in mMol/L.

**Table 4: Effect of Saponin, Tannin and Glycoside extracted from *Galega officinalis* on some serum enzyme activities in normal and alloxan- induced diabetic rats**

	Diabetic control	Normal Control	Saponins 400mg/kg	Tannin 400mg/kg	Glycoside 400mg/kg
ALP	363.33±0.72 <sup>a</sup>	218.00±0.47 <sup>b</sup>	335.67±0.26 <sup>c</sup>	223.03±0.03 <sup>d</sup>	162.00±0.47 <sup>e</sup>
ALT	133.33±0.72 <sup>a</sup>	123.33±0.72 <sup>b</sup>	123.00±0.94 <sup>b</sup>	62.67±0.27 <sup>d</sup>	99.33±0.27 <sup>e</sup>
AST	471.67±0.72 <sup>a</sup>	274.33±0.72 <sup>b</sup>	292.00±0.47 <sup>c</sup>	223.00±0.47 <sup>d</sup>	332.33±0.27 <sup>e</sup>

$n=5 \pm SEM$ ; values carrying superscripts different from the diabetic control are significantly different ( $P < 0.05$ ). Enzyme activities are expressed in UI.

## DISCUSSION

The qualitative phytochemical analysis of aqueous extract of *Galega officinalis* whole plant shows the presence of alkaloid, flavonoids, Tannins, saponin, glycoside, phenols, resins, terpene and steroids. Many kinds of natural products, such as terpenoids, alkaloids, flavonoids, phenolics, and some others, have shown antidiabetic potential [17- 19]. The presence of tannins may also be responsible for antidiabetic properties [20].

A major serum abnormality in Diabetes Mellitus is hyperglycemia, which was induced in the diabetic rats in the present study. This is as a result of the pancreatic  $\beta$ -cell destruction with intraperitoneal injection of alloxan [21]. However, the blood glucose concentration was significantly reduced in the diabetic animals that were treated with saponin, tannins and glycoside.

Saponins from a variety of sources have been shown to have hypoglycemic activity [22, 23], although the detailed molecular mechanism of this activity is still unclear. [24] reported that recent clinical trials have also found that combined therapy of total saponins with sulfonylureas, a class of antidiabetic drugs, could lower the blood glucose level and ameliorate clinical symptoms in the treatment of type II diabetic patients whose blood glucose levels were not well controlled by oral hypoglycemic agents [25].

It has also been reported by [26] that condensed tannin extracts of amaranth grain, finger millet, field bean, sunflower seeds, drumstick, and amaranth leaves showed promising antidiabetic effects with potential  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibition activities of 23% to 44% and 58% to 88%, respectively. Furthermore, Tannic acid which is a main component of hydrolyzable tannin has been reported to possess glucose transport-stimulatory and adipocyte differentiation-inhibitory activities and determination of the concentration-activity relationship of tannic acid-induced glucose transport revealed a dose-response curve similar in shape to that of insulin which implies a potential similarity in the working mechanisms of the molecules [27].

Glycosides have hypoglycemic activities [28]. Leucopelargonidin, a glycoside isolated from the bark of *Ficus bengalensis* Linn demonstrated significant hypoglycemic, hypolipidemic and serum insulin raising effects in moderately diabetic rats [29]. Single dose treatment of another glycoside, dimethoxy derivative of perlargonidin 3-O- $\alpha$ -L rhamnoside (250mg/kg), isolated from the bark of *Ficus bengalensis* Linn also decreased fasting blood glucose by 19% and improved glucose tolerance by 29%. On one-month treatment (at 100mg/kg), the fasting blood glucose levels went down almost to half of the pretreatment levels and glucose tolerance improved by 15% [30]. The mechanism of action of saponin, tannin and glycoside isolated from *Galega officinalis* whole plant was not checked in this study but results obtained may agree with the earlier similar researches done on the phytochemicals [25-30]. A principal action of insulin in adipose tissue is to inhibit the activity of hormone-sensitive lipase, reducing the release of free fatty acid and glycerol [31]. In diabetes mellitus, insulin deficiency causes excessive mobilization of chylomicrons and VLDL leading to hypertriacylglycerolemia [31]. In this study, the concentrations of serum total cholesterol, triglycerides and LDL significantly increased while that of HDL significantly decreased in alloxan-induced diabetic rats. This result agrees with the one published by [32]. However, on administration of the phytochemicals, serum total cholesterol, triglycerides, and LDL concentrations decreased significantly with a significant increase in HDL concentration. The results obtained in this study is in harmony with what those obtained in previous similar studies [33- 35]. This may be attributed to the hypolipidemic and/or insulin-mimetic potential of saponin, tannin and glycoside isolated from *Galega officinalis*. It should however be noted that the exact phytochemicals and their mechanisms of action were not elucidated in this study and therefore leaves room for further research.

The liver is prone to xenobiotic-induced injury because of its central role in the metabolism of foreign compounds and its portal location within the circulatory system [36]. Alloxan, a xenobiotic, acts by destroying the insulin-producing beta cells of the islets of Langerhans in the pancreas of the Liver [37]. In this study, there was a significant increase in serum ALP, AST and ALT concentration in the diabetic animals which supports that diabetes may be induced due to liver dysfunction [38]. The increase in the activities of serum ALP, AST and ALT therefore may be due to the leakage of these enzymes from the liver cytosol into the blood stream [39, 40], which gives an indication on the hepatotoxic effect of Alloxan. However, administration of Saponin, Tannin and Glycoside from *Galega officinalis* brought about a significant reduction in the activities of the enzymes. The mechanism with which these phytochemicals elicit this effect is not known yet but may be attributed to their inhibitory activity of alloxan-induced liver damage.

## CONCLUSION

In conclusion, the reduction in the levels of glucose, total cholesterol, triglyceride and LDL on administration of the phytochemicals has proven that these phytochemicals have hypoglycemic and hypolipidemic properties and could be used in the management of diabetes mellitus.

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