



Effect of Aqueous Leaf Extract of *Eriosema psoraleoides* on Antihyperglycemic and Hypolipidemic Potentials in Alloxan-Induced Diabetic Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Author NFO carried out the bench work, performed the statistical analysis and wrote and monitored the first draft of the manuscript, author OVN managed and supervised the experimental protocol, authors NJO, NCB and AMB managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This study was targeted at valuing a claim by traditional herbal practitioners that the leaf of *Eriosema psoraleoides* possesses a hypoglycemic and hypolipidemic property by assessing the effect of aqueous leaf extract of *Eriosema psoraleoides* on antihyperglycemic and hypolipidemic potentials in alloxan-induced diabetic rats.

Methodology: Thirty male albino rats weighing 180-220 g were divided into 5 experimental groups of six rats each; control, diabetic untreated, diabetic treated with glibenclamide and diabetic treated with *Eriosema psoraleoides*. Diabetes was induced by 130 mg/kg body weight (b.wt) of alloxan monohydrates. The control and diabetic groups received normal saline while the diabetic treated

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groups were administered with 0.3 mg/kg body weight glibenclamide, and 200 mg/kg, 400 mg/kg body weight of aqueous leaf extract of *Eriosema psoraleoides* respectively. The experiment period was 7 days, with the determination of their glucose level and body weight every two days. At the end of the experimental period, the animal's blood samples were taken from the animals for the determination of total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL) and low-density lipoprotein (LDL). Statistical comparisons were performed by one-way analysis of variance with repeated measures and one-way analysis of variance followed by Duncan's multiple range tests.

Results: The results of the study show a significant reduction in blood glucose. The result also showed that in diabetic rats, body weight was decreased but the application of the extract increased ($P < 0.05$, $n = 6$). Similarly, the result showed a significant decrease in total cholesterol, TG and LDL level of the diabetic group when compared with the control, glibenclamide and extract treated diabetic groups (with the highest performance at 400 mg/kg). Also, *Eriosema psoraleoides* aqueous leaf extract treated diabetic rat's shows a significant increase in HDL levels compared to the diabetic control.

Conclusion: The study indicates that *Eriosema psoraleoides* possess hypolipidemic and antihyperglycemic potentials.

Keywords: Alloxan; antihyperglycemic; *Eriosema psoraleoides*; glibenclamide; hypolipidemic.

1. INTRODUCTION

In the past decades, research has been focused on the scientific evaluation of traditional drugs of plant origin and the screening of plants material for more effective and safe use has continued to gain more medicinal importance. The medicinal values of many plants cannot overemphasize in the light of oral traditions and folklore from the distant past that have continued to extol the healing virtues of these plants and their extracts. Unfortunately, only a few of such medicinal plants have been validated [1]. One of the plants commonly employed in ethnomedicine in the management of some disease conditions such as diabetes mellitus is *Eriosema psoraleoides*. The active principles found in *Eriosema psoraleoides* (Lam) G. Don (Leguminosae) fruits, leaves, and roots are mostly used traditionally for the treatment/management and prevention of some diseases and infections such as cutaneous and subcutaneous parasitic infections, diabetes, diarrhea, and pulmonary troubles. The phytochemical analysis of the aqueous extract of *Eriosema psoraleoides* indicated the presence of some bioactive components such as flavonoids, alkaloids, glycosides, steroids, reducing sugars, resins, tannins and saponins [2]; which possess medicinal properties that are harnessed for the treatment of different diseases. The active ingredients for a vast number of pharmaceutically derived medications contain components originating from phytochemicals in seeds and leaf of plant materials. In a report, *Eriosema psoraleoides* is said to be used as a chewing stick for the cure of dental problems in

ethnomedicine [3,4]. In another report, *Eriosema psoraleoides* is used in a polyherbal preparation to treat malaria in Tanzania; and that the stem bark of *Sapium ellipticum*, and the root of *Vernonia amygdalina* are included in this preparation [5]. An earlier published report had indicated that *E. psoraleoides* are used both as antimalarial and aphrodisiac [6]. Recently, a scientific report has shown that the aqueous extract of *Eriosema psoraleoides* demonstrated antidiabetic effect by lowering blood glucose in alloxan-induced diabetic rats [2]. However, there is no known scientific report carried out on the effect of such treatment on antihyperglycemic and hypolipidemic potentials in alloxan-induced diabetic rats to the best of our knowledge. It is in view of this, that this study was designed to investigate the treatment effect of *Eriosema psoraleoides* aqueous extract on antihyperglycemic and hypolipidemic potentials in alloxan-induced diabetic rats.

2. MATERIALS AND METHODS

2.1 Collection of Plant Materials

Fresh leaves of *Eriosema psoraleoides* were collected from Eha-Ndiagu in Nsukka Local Government Area of Enugu State, Nigeria. Botanical identification and authentication were performed by Mr. Ozioko of the International Center for Ethnomedicine and Drug Development Nsukka, Enugu State, Nigeria, where a herbarium sample with voucher specimen number Intercedd/16170 was prepared and deposited.

2.1.1 Extraction of the active agents of *Eriosema psoraleoides*

The leaves of *Eriosema psoraleoides* were air-dried separately at room temperature, then into a powdery form using an electrical grinding machine. From the ground samples, 200 g were macerated in 800 ml of aqueous solvent for 24h with intermittent shaking. Following filtration through Whatman No. 1 filter paper, the filtrate (that is the active agents of the extract) concentrated to solid matter using rotary evaporators, which then become the stock sample of the aqueous leaf extract which was used for the analysis. These extracts were stored in the refrigerator compartment to prevent microbial growth. The extract was reconstituted in distilled water in appropriate concentration before administration.

2.2 Animals

Adult male Wistar albino rats of 16 - 20 weeks and an average weight of 180 to 220 g were obtained from the Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Enugu State, Nigeria. The animals were acclimatized for the duration of seven days under standard environmental conditions with 12 h light/dark cycle maintained on regular feed (vital feed) and water *ad libitum*.

2.3 Chemicals/Reagents/Samples

All the chemicals and reagent used in this study were of analytical grade and purchased commercially.

2.4 Experimental Design

2.4.1 Grouping of animals/treatment

Thirty male Albino Wistar rats were acclimatized at the same conditions of temperature and pressure and the same animal feeds were used for all the rats. The rats were divided into five groups of six rats each as shown below:

Group	Title
Group 1	Normal rats treated with normal saline (Control)
Group 2	Diabetic rats, no treatment (Positive control)
Group 3	Diabetic rats treated with the first dose of extract (200 mg kg ⁻¹ b.wt)
Group 4	Diabetic rats treated with the second dose of extract (400 mg kg ⁻¹ b.wt)
Group 5	Diabetic rats treated with the standard drug: Glibenclamide (0.3 mg kg ⁻¹ b.wt)

After the experiment, the animals were sacrificed and blood was collected and used for biochemical analysis.

2.4.1.1 Preparation of glibenclamide sample

The stock concentration of Glibenclamide was prepared by dissolving 5 mg of the standard drug in a solution of 20 ml of 9% normal saline bringing the stock concentration to 0.1 ml. The dose used was 0.3 mg/kg body weight.

2.4.1.2 Induction of experimental diabetes mellitus

The animals were fasted for 16 to 18 h with free access to water prior to the induction of diabetes. Induction of diabetes was carried out by single intraperitoneal injection of alloxan monohydrate (Sigma St Louis, M.O., USA) dissolved in 0.9% v/v normal saline solution at a dose of 130 mg/kg body weight. The diabetes was assessed in alloxan-induced rats by determining the blood glucose concentration 72 h after injection of alloxan monohydrate. The rats with a blood glucose level above 200 mg/dl were then selected for the study.

2.4.1.3 Determination of blood glucose level

Fasting blood glucose was determined by the glucose oxidase method using Accu chek glucometer (Roche Diagnostics, Germany). The tail of the rat was cut swiftly with a sterile scalpel and a drop of blood was squeezed onto the test area of strip inserted into the glucometer. The animals have fasted for 12 hours before each glucose determination, which was repeated every 48 hrs until the end of the experiment (7 days). Body weights of all groups of rats were assessed on the same days that blood glucose levels were measured.

2.4.1.4 Total Cholesterol concentration (TC)

The total cholesterol concentration was determined according to the method [7].

2.4.1.5 Low-density lipoprotein-cholesterol concentration (LDL)

The Low-density lipoprotein-cholesterol concentration was determined according to the method [8]

2.4.1.6 High-density Lipoprotein-cholesterol concentration (HDL)

The high-density lipoproteins-cholesterol concentration was determined according to the method [9].

2.4.1.7 Estimation of Triacylglycerol Concentration (TG)

The triacylglycerol concentration was determined according to the method [10].

2.5 Statistical Analysis

All the data are expressed as mean ± standard error of the mean (SEM). Statistical comparisons were performed by one-way analysis of variance (ANOVA) with repeated measures and one-way ANOVA followed by Duncan’s multiple range tests [11]. The results were considered statistically significant if the values are 0.05 higher or lower.

3. RESULTS

Table 1 shows that the extract of *Eriosema psoraleoides* and glibenclamide do not have significant ($P>0.05$) effect on the body weights of alloxan-induced diabetic rats. This can be inferred from the value of the LSD for groups’ means separation. No mean difference between any pair of groups in this table exceeds 63.003.

Table 2 shows that the extract and glibenclamide produced significant ($P<0.05$) decrease in blood glucose levels of the alloxan –induced diabetic rats on day 7 when compared to group 2. The concerned mean difference are all greater than the LSD (52.60) for mean separation of groups. The mean differences between group 1 and that of groups 3 and 4 do not exceed the LSD (52.60) on day 7. The table also shows that in the group 3, 4 and 5 there is significant ($P<0.05$) decrease in the blood glucose levels of the rats on day 7 when compared to day 5 as can be inferred from the LCD (21.41) for mean separation of days.

Table 1. Effect of aqueous leaf extract of *Eriosema psoraleoides* on body weight in alloxan-induced diabetic rats for seven days

	Group 1 normal control	Group 2 diabetic untreated	Group 3 diabetes treated with AE (200 mg/kg)	Group 4 diabetes treated with AE (400 mg/kg)	Group 5 diabetes treated with Glibenclamide (0.3 mg/kg)
Day 1	182.10±38.37	191.15±48.54	187.95±53.01	186.80±49.54	191.17±40.97
Day 3	183.08±38.23	187.73±47.15	189.20±54.40	187.70±49.18	191.40±40.43
Day 5	187.20±37.42	184.50±46.68	194.50±41.57	192.18±48.58	193.93±40.59
Day 7	190.23±36.70	178.73±45.98	197.55±40.62	198.50±48.04	195.15±40.58

Mean difference between any pair of groups on any day greater than LSD of 63.003 implies that the pair shows ($P<0.05$) difference. In all the groups $n = 6$

Table 2. Effect of administration of aqueous leaf extract of *Eriosema psoraleoides* on blood glucose level (mg/dl) of alloxan-induced diabetic rats for seven days

	Group 1 normal control	Group 2 diabetic untreated	Group 3 diabetes treated with AE (200 mg/kg)	Group 4 diabetes treated with AE (400 mg/kg)	Group 5 diabetes treated with glibenclamide (0.3 mg/kg)
Day 1	86.25±8.26	86.25±6.02	83.75±9.81	89.00±6.38	90.75±14.41
Day 3	92.50±12.58	365.00±8.44	360.75±5.63	320.25±6.26	313.25±9.29
Day 5	93.50±6.45	369.00±5.52	347.00±9.02	334.00±10.00	351.75±11.70
Day 7	92.75±11.62	358.49±5.12	121.75±13.57*	128.75±13.98*	193.00±6.98*

AE: Aqueous extract; E. *Psoraleoides*: *Eriosema psoraleoides*. In all the groups $n=6$. Mean difference between any pair of days in a group greater than LSD value of 21.41 is significant at 5% level. Mean difference between any pair of groups on any day greater than LSD of 52.60 is significant at 5% level. p -value < 0.05 shows significant difference from Group 2.

Table 3 shows it is significant ($P < 0.05$) difference in TG concentration in diabetic rats treated with 200 mg/kg aqueous extract when compared with diabetic untreated rats. A significant ($P < 0.05$) difference is also observed in LDL level in diabetic rats treated with 400 mg/kg aqueous extract when compared with diabetic untreated rats. Glibenclamide treated rats also showed significant ($P < 0.05$) difference in LDL level when compared with diabetic untreated rats.

4. DISCUSSION

Plants have served as a source of medicinal agents for decades, and a remarkable number of modern drugs have been isolated from plants, many of which are based on their use in ethnomedicine. These plant-based ethnomedicine systems have continued to play a significant role in health care; with about 80% of the world's populations mainly depending on ethnomedicines for their primary health care [12]. Plant products also have an important role in the health care systems of the remaining 20%, who live in developed countries like America, Europe [13]. Many studies have revealed that many plants extract effectively lowered blood glucose level in alloxan-induced diabetic animals [14,15].

The use of alloxan to induce diabetes in rats represents a well-established animal model of type I insulin dependent diabetes mellitus characteristically similar to type I diabetes in human [16]. The alloxan-treated rats, therefore, appear to represent a good laboratory model for experimental diabetes state, with residual or remnant insulin production by the pancreatic beta-cells. There is a possibility for the survival of few beta-cells and this has been proven by several workers who observed antihyperglycemic activity with oral hypoglycemic agents like glibenclamide, tolbutamide etc, in alloxan-induced diabetic rats [17,18].

Glibenclamide was used as a reference drug mimicking several insulin actions in-vivo which include suppressing hepatic glucose production, increasing insulin sensitivity of extrapancreatic tissue, stimulation of lipogenesis and inhibition of lipolysis, enhancing peripheral glucose uptake, decreasing hepatic glycogenolysis and gluconeogenesis and as well as absorption of glucose from the gastrointestinal tract [19,20].

Diabetes mellitus causes failure to use glucose for energy that leads to increased utilization and decreased storage of protein responsible for the

reduction of body weight essentially by depletion of the body proteins [21]. As shown in Table 1, oral administration of aqueous extracts of *Eriosema psoraleoides* at doses of 200 mg/kg and 400 mg/kg body weight, as well as glibenclamide do not have significant ($P > 0.05$) effect on body weights of the alloxanized rats. The reduction could be linked to dehydration, degradation of structural proteins and muscle wasting. The loss in body weight of diabetic subjects agrees with the findings of [22] who observed a similar effect on diabetic animals induced with alloxan. The result indicated that aqueous extracts may possess the ability to spare dietary proteins to prevent muscle wasting and induced adipogenesis. Generally, oxidative stress could lead to a loss in weight because the body resorts to the use of proteins for energy generation in the absence of glucose.

In this present study, intraperitoneal injection of 130 mg/kg body weight of alloxan caused a significant increase in the level of blood glucose indicating the establishment of a diabetic state. However, treatment of diabetic animals with 200 and 400 mg/kg body weight. Extracts significantly reduced the plasma blood glucose level. However, the mechanism of hypoglycaemic effect of *Eriosema psoraleoides* is not known yet, but it could be that the extract facilitates glucose utilization by peripheral tissues or by decreasing hepatic glycogenolysis and gluconeogenesis or it could also stimulate increased insulin production from possibly regenerating pancreatic beta cells, however, further study could be carried out to measure insulin level and liver glycogen in order to know its mechanisms of action.

In diabetes, there is hyperlipidemia which may be linked to insulin deficiency; in this state (hyperglycemia) fatty acids are mobilized from adipose tissue causing accumulation of excess fatty acids in the liver which are then converted to triglyceride [23]. Administration of aqueous leaf extract of *Eriosema psoraleoides* produced significant beneficial effects in the lipid profile of the treated diabetic rats, significantly reduce total cholesterol, triglycerides, and low-density lipoprotein level at higher dose whereas high-density lipoprotein was significantly increased in diabetic rats. This is in line with previous studies where the hypolipidemic activity of some medicinal plants in alloxan-induced diabetic rats have been documented [24,25,26]. The observed hypolipidemic effect recorded in this study could be due to the presence of some phytochemical

Table 3. Effect of 7 days administration of aqueous leaf extract of *Eriosema psoraleoides* on lipid profile in alloxan-induced diabetic rats

	Group 1 normal control	Group 2 diabetic untreated	Group 3 diabetes treated with AE (200 mg/kg)	Group 4 diabetes treated with AE (400 mg/kg)	Group 5 diabetes treated with Gliben- clamide (0.3 mg/kg)
TC	3.96±0.20 ^{bc}	4.25±0.53 ^b	3.58±0.33 ^{abc}	3.29±0.33 ^{ab}	3.77±0.18 ^{abc}
TG	1.43±0.13 ^{ab}	1.66±0.12 ^{cd}	1.24±0.48 ^a	1.48±0.08 ^{bc}	1.75±0.00 ^{cd}
HDL	1.53±0.06 ^{ab}	1.35±0.24 ^c	1.93±0.21 ^{ab}	1.93±0.32 ^{ab}	1.90±0.14 ^{ab}
LDL	1.67±0.15 ^a	2.63±0.59 ^b	2.08±0.28 ^{ab}	1.63±0.06 ^a	1.95±0.07 ^a

AE-Aqueous extract; TC- Total cholesterol; TG-Triacylglycerol; HDL-High-density lipoprotein; LDL- Low-density lipoprotein. Groups with different superscript(s) are significantly different at 5% level. In all the groups n=6

compounds which include alkaloids, flavonoids, saponins and tannins present in the extract [2]. All these compounds are known to reduce serum lipid level in animal especially alkaloid which is known to normalize lipogenesis due to its insulinogenic effect on lipid metabolism while flavonoids cause the decrease in the activity of HMG-CoA reductase in the liver. Thus an excess fatty acid in the plasma produced by alloxan-induced diabetes promotes the conversion of excess fatty acids into phospholipids and cholesterol in the liver. These two substances along with excess TAGs formed in the liver may be discharged from the liver in the form of lipoproteins. The HDL is an anti-atherogenic lipoprotein which transports cholesterol from peripheral tissues into the liver, thereby acting as a protective factor against coronary heart disease [27]. It is well known that LDL plays an important role in arteriosclerosis and hyper-cholesterolemia [26]. The decrease in cholesterol, TAGs and LDL levels achieved by the administration of aqueous leaf extract of *Eriosema psoraleoides* to diabetic rats serve possible protection against hypercholesterolemia which demonstrates an important finding of this experiment and support the traditional use of the plant in the management of diabetes.

5. CONCLUSION

The findings of this study have demonstrated that aqueous leaf extract of *Eriosema psoraleoides* possess anti-hyperglycemic effect close to that of the standard drug (glibenclamide) and effectively maintained the lipid profile within an acceptable level. This suggests that the extract could be efficiently used to treat diabetes mellitus and prevent most of the health complications associated with the condition.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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