LIPID PROFILE OF ALLOXAN-INDUCED DIABETIC WISTAR RATS TREATED WITH ETHANOLIC LEAF EXTRACT OF ERIOSEMA PSORALEOIDES

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Authors' contributions
This work was carried out in collaboration between 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, author NJO, NCB, OIO and OES managed the literature searches.

Abstract
Aim: This study was carried out to assess the lipid profile of alloxan-induced diabetic wistar rats treated with ethanolic leaf extract of *Eriosema psoraleoides.*
Methodology: Thirty male albino rats weighing 180-220 g were used in this study. Eriosema psoraleoides leaves were extracted by soaking 200 g of the powdered leaves in 5 liters of 95% ethanol with repeated stirring for two days. The crude extract was filtered and recovered filtrate was concentrated at 60^{o}C using a rotary evaporator. The semi-solid extract was further dried to a constant weight by evaporation in a water bath at 60^{o}C. Diabetes wasthe induced using alloxan monohydrates (130 mg/kg I.P). Group 1 were normal control group given normal saline, group 2 (positive control) rats were diabetic untreated, group 3 and 4 were administered with 200 and 400 mg/kg of ethanolic extract after induction with alloxan, group 5 were diabetic group given 0.3 mg/kg glibenclamide. Treatment was continued daily and orally for 7 days. Blood glucose levels and body weight (b.wt) were monitored every two days. The lipid profile was determined on the 7th day of administration. 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 extract significantly ($p < 0.05$) reduces blood glucose level at 200 and 400 mg/kg notably from the 5th day to the 7th day. The effect of the extract compared well with that of glibenclamide which also produced significant reduction ($p < 0.05$) in blood glucose level in the diabetic rats from the 5th day onwards. The extract significantly increased ($p < 0.05$) the body weight of the treated groups but a reduction was seen in the diabetic on treated group when compared to the normal group. Similarly, the result showed a significant reduction 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 400mg/kg). Also, Eriosema psoraleoides ethanolic 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* have potential hypoglycemic and hypolipidemic effect and could be a useful source of anti-diabetic agent..

Keywords: Diabetes, *Eriosema psoraleoides*, Glibenclamide, Hypoglycemic effect, Lipid profile

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1. Introduction

Diabetes mellitus is a chronic disease caused by inherited or acquired deficiency in insulin secretion and by decreased responsiveness of the organs to secrete insulin. It has been characterized by increased levels of oxidative stress, hyperglycemia, and hyperlipidemia which are implicated in the development of diabetic complications[1]. Diabetes complication includes dysfunction and disturbances of various organs, especially the heart, kidneys, nerves, eyes and blood vessels[2] and often associated with symptoms such as fatigue, increase thirst, loss of weights, urination, nausea, at times vomiting and weight loss in spite increased appetite which ultimately complicates in macrovascular, microvascular, and neuropathic disorders[3,4].

Diabetes has now become an epidemic with a worldwide incidence of about 9% in the general population[5], making it one of the most common non-communicable diseases[6]. In the year 2012, about 1.5 million deaths were caused by diabetes directly and 80% of these deaths occur in low and middle-income countries[7]. Diabetes is projected to be the 7th cause of death by 2030[8,9]. This is because none of the antidiabetic drugs could give a long term glycaemic control without causing any adverse side effects[10,11].

Plants are the basis for the development of modern drugs and medicinal plants have been used in many years in daily life to treat diseases all over the world[12,13]. Plants with an antioxidant property still remain a major source for drug discovery in spite of the great development of synthetic molecules which are used for the management and/or control in the complication of diseases such as diabetes mellitus. In diabetes, the causes and sites of intervention in the biochemical process are diverse and high serum total triglyceride level has been implicated. Differences in the lipid profile of diabetic and non-diabetic individuals are now apparent and lipid abnormalities are common in patients with diabetes mellitus[14]. Unfortunately, only a few of such medicinal plants have been scientifically validated[15,16].

The use of *Eriosema psoraleoides* in the treatment of Diabetes mellitus is not common, however, there is a claim by traditional medicine users that the plants' leaves have hemopoeitic properties. Based on this knowledge, the ethanolic extract of *Eriosema psoraleoides* is selected to evaluate hyoglycemic and hypolipidemic activities in alloxan-induced diabetic rats.

2. Materials and Methods

2.1 Plant

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 ethanolic extract of *Eriosema psoraleoides* leaves was prepared by soaking 200 g of the powdered leaves in 5 liters of 95% ethanol (BDH, England) with repeated stirring for two days. The crude extract was filtered and recovered filtrate was concentrated at 60^{o}C using a rotary evaporator. The semi-solid extract was further dried to a constant weight by evaporation in a water bath at 60^{o}C and the yield determined was 5.68%.

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 a duration of seven days under standard environmental conditions with 12h light/dark cycle maintained on regular feed (vital feed) and water *ad libitum*.

2.3 Chemicals/reagents/samples

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

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

2.4.1.2 Induction of experimental diabetes mellitus

A freshly prepared solution 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. Induction of diabetes was carried out by single intraperitoneal injection of alloxan monohydrate to overnight fasted rats. Blood glucose was measured after 72 hrs of alloxanisation by Accu-Chek glucometer (Roche Diagnostics, Germany), and it was confirmed by testing for glucosuria using glucose indicator sticks. Rats showing fasting blood glucose (FBG) levels > 200 mg/dl were selected for the study.

2.4.1.3 Blood glucose determination

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 12hours before each glucose determination, which was repeated every 48hrs 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[17].

2.4.1.5 Low density lipoprotein- cholesterol concentration (LDL): The Low-density lipoprotein- cholesterol concentration was determined according to the method[18].

2.4.1.6 High density lipoprotein-cholesterol concentration (HDL): The high-density lipoproteins- cholesterol concentration was determined according to the method[19].

2.4.1.7 Estimation of triacylglycerol concentration (TG): The triacylglycerol concentration was determined according to the method[20].

2.5 Statistical analysis

All the data are expressed as mean ± standard error of thw 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[21]. The results were considered statistically significant if the values are 0.05 higher or lower.

3. Results

Table 1: Effect of ethanolic 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 EE (200mg/kg)	Group 4 Diabetes treated with EE (400mg/kg)	Group 5 Diabetes treated with Glibenclamide (0.3mg/kg)
Day 1	182.10±38.37	191.15±48.54	186.62±49.87	190.40±48.21	191.17±40.97
Day 3	183.08±38.23	187.73±47.15	189.28±50.45	191.05±48.12	191.40±40.43
Day 5	187.20±37.42	184.50±46.68	189.40±49.80	192.15±48.43	193.93±40.59
Day 7	190.23±36.70	178.73±45.98	191.90±48.61	193.75±48.77	195.15±40.58

EE: Ethanolic extract; *EE*: *Eriosema psoraleoides*. 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 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: Effect of administration of ethanolic 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 EE (200mg/kg)	Group 4 Diabetes treated with EE (400mg/kg)	Group 5 Diabetes treated with Glibenclamide (0.3mg/kg)
Day 1	86.25±8.26	86.25±6.02	94.75±16.40	100.75±10.63	90.75±14.41
Day 3	92.50±12.58	365.00±8.44	378.75±8.83	252.75±8.07	313.25±9.29
Day 5	93.50±6.45	369.00±5.52	315.50±9.54	326.50±9.91	351.75±11.70
Day 7	92.75±11.62	358.49±5.12	181.50±9.33*	192.25±5.74*	193.00±6.98*

EE: Ethanolic extract; *EE*: *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 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 seperation 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 seperation of days.

Table 3: Effect of 7 days administration of ethanolic 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 EE (200mg/kg)	Group 4 Diabetes treated with EE (400mg/kg)	Group 5 Diabetes treated with Glibenclamide (0.3mg/kg)
TC	3.96 ± 0.20^{bc}	4.25 ± 0.53^{b}	3.38 ± 0.37^{ab}	3.21 ± 0.06^{a}	3.77 ± 0.18^{abc}
TG	1.43 ± 0.13^{ab}	1.66 ± 0.12^{cd}	1.49 ± 0.11^{ab}	1.48 ± 0.48^{ab}	1.75 ± 0.00^{cd}
HDL	1.53 ± 0.06^{ab}	1.35 ± 0.24^{c}	2.10 ± 0.14^{ab}	1.90 ± 0.14^{ab}	1.90 ± 0.14^{ab}
LDL	1.67 ± 0.15^{a}	2.63 ± 0.59^{b}	1.50 ± 0.28^{a}	1.55 ± 0.07^{a}	1.95 ± 0.07^{a}

EE- Ethanolic 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.

Table 3 shows it is significant (P<0.05) difference in HDL and LDL concentration in diabetic rats treated with 200 mg/kg ethanolic extract when compared with diabetic untreated rats. Also, diabetic rats treated with 400 mg/kg ethanolic extract shows significant (P<0.05) difference in TC and LDL concentrations. Glibenclamide treated rats also showed significant (P<0.05) difference in LDL level when compared with diabetic untreated rats.

4. Discussion

Diabetes mellitus is characterized by elevated level of oxidative stress, decreased level of antioxidant defences, haematology parameters and serum lipid profile abnormalities[22]. Recent available hypoglycemic agents produce some serious side effects like hypoglycemic coma[23] and hepatorenal disturbances[24]. Apart from the side effects, their costs are relatively high for management of diabetes and hypertension; as such alternatives are needed for better management of diabetes and cardiovascular complications. Hence, the search for safer and more effective anti-diabetic and anti-hypertension agents has continued. Also, there is WHO's recommendation for search on the beneficial use of medicinal plants in the management of diabetes mellitus[25]. Investigation of hypoglycemic agents derived from plants origin has also gained relevance over decades. Compounds from plant origin can provide an alternative treatment for diabetes. An Example of such plant is *Eriosema psoraleoides*, it has been noted that its extracts contain some bioactive compounds such as flavonoids, alkaloids, glycosides, steroids, reducing sugars, resins, tannins, and saponins[11,16]. The presence of flavonoids, tannins, and saponins explains why *Eriosema psoraleoides* is used for diabetes treatment because the constituents are used ethnopharmacologically to treat diabetes and hyperglycemia. In this way, compounds such as flavonoids, steroids, terpenoids, and phenolic acids has been reported to be bioactive anti-diabetic components[26].

Alloxan induces diabetes by damaging the insulin secreting cells of the pancreas leading to hyperglycaemia. Hyperglycaemia in diabetic patients is associated with alterations in glucose and lipid metabolism[1]. Alloxan induces damage and death of pancreatic islet-cells in several experimental animal models, thus causing diabetic mellitus and decreasing the secretion of insulin. The cytotoxic action of alloxan is mediated by reactive oxygen species (ROS). Alloxan has been reported to be a specific β-cytotoxic drug and acts by complexing with the metal ions in the islets[27]. The dosage of 130 mg/kg body weight of alloxan used in this study caused moderate diabetes[28]. 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.

Dehydration and loss of body weight have been associated with diabetes mellitus[29]. The body weights of the experimental animals were measured and represented in Table 1. The initial body weights were similar in diabetic and diabetic treated groups. The diabetic untreated group showed a significant reduction in body weight on days 3, 5 and 7 when compared to the treated and normal control groups. The progressive increase in weight in the extracts treated groups suggests that *Eriosema psoraleoides* ethanolic extract has the ability to reduce hyperglycemia. It might be possible that treatment with the extract can lead to better utilization of nutrients in the diet and thus

a gain in weight. This may also be due to the protective effect of the extract in controlling muscle wasting (reversal of gluconeogenesis).

Results obtained from table 2 show that all doses of *Eriosema psoraleoides* ethanolic extract significantly ($P<0.05$) lowered blood glucose levels within the 7 days study. The action of *Eriosema psoraleoides* on blood glucose in diabetic rats is similar to that of Glibenclamide (0.3mg/kg), a potent hypoglycaemic agent, and suggested that *Eriosema psoraleoides* ethanolic extract contain active principles with the potent hypoglycaemic property. The extract may have achieved this hypoglycaemic property via increased insulin secretion or release of glucagon, increased peripheral utilization of glucose, inhibition of endogenous glucose production or by inhibition of intestinal glucose absorption as reported in existing literatures[30,31,32]. The extract may also have potentiated pancreatic secretion of insulin from existing residual Beta cells of islets of Langerhans.

In diabetes, increase in plasma lipids represents a risk factor for coronary heart disease. Also, an increase in total cholesterol and LDL concentration was observed in alloxan-induced diabetic rats. The abnormally high concentration of plasma lipids in diabetes is mainly due to the increase in the mobilization of free fatty acids from the peripheral depots since insulin inhibits the hormone-sensitive lipase[33]. In this study, orally administered of *Eriosema psoraleoides* ethanolic extract to diabetic rats (for 7-days) reduced significantly the serum TC, LDL, TG levels and increase HDL concentrations in the diabetic treated group compared to the diabetic control and this reduction was dose-dependent manner. Thus, the normalization of lipids in diabetic rats treated with extract may be due to its stimulatory effect on insulin secretion from pancreatic â cells[34]. This could account for its use in traditional medicine for the treatment of diabetes and hypertension. High levels of triglycerides, LDL, VLDL has been reported to be associated with heart disease, insulin resistance, and diabetes mellitus[14] and the increase in blood sugar level in diabetes is accompanied by a marked increase in cholesterol, triglycerides, LDL, VLDL and reduction in HDL. HDL helps clear excess cholesterol from the body in a process of inverse cholesterol transport; results from this study shown an increase in HDL indicate its function in excretion. The underlying mechanism of lipid-lowering effect of *Eriosema psoraleoides* ethanolic extract (especially at 400mg/kg) could be by inhibition of lipid absorption due to the presence of saponin and tannin in *Eriosema psoraleoides* ethanolic extract as earlier reported[11]; that the extracts contain rich phytochemical constistuents or this could be by inhibition of cholesterol esterase, activation of fatty acid synthase, acetyl-CoA carboxylase and production of triglyceride precursors such as acetyl-CoA and glycerol phosphate[35]. Another mechanism of lipid-lowering effect of *Eriosema psoraleoides* ethanolic extract could be modulated by the flavonoid content[11]. Flavonoids (capable of decreasing the triacylglycerols and total cholesterol in the blood of rats) from plants have been reported for its various implications in the reduction of lipids by inhibiting hepatic HMG-CoA reductase[36,37]. Flavonoids decreased the triacylglycerols and total cholesterol in the blood of rats[38].

Studies have shown that increased in the risk factor of cardiovascular disease correlate with an increase in TC, TG, LDL, atherogenic index level and a decrease in HDL concentrations[39]. HDL is the smallest of the lipoprotein species containing approximately 20% cholesterol ester and very little triglyceride[1]. Low HDL-level is an important predictor of cardiovascular disease and high HDL level (in dose-dependent manner) in the treated groups may be an indication that the extract may play an important role in protecting against cardiovascular disease. In addition, it has been reported that non-enzymatic glycosylation of HDL accelerates its catabolism and the same

mechanism may be responsible for the low level of HDL observed in alloxan-diabetic rats[36,40]. LDL-cholesterol concentration has strongly and positively been linked to risk of atherosclerosis and other cardiovascular diseases[36,1].

Conclusion

In this study, the significant reduction of the high blood glucose in diabetic extract treated the group to the values of the diabetic control and glibenclamide treated group indicates anti-hyperglycaemic activity of ethanolic leaf extract of *Eriosema psoraleoides.* This study also revealed that ethanolic leaf extract of *Eriosema psoraleoides* exerts an antidiabetic effect by lowering serum lipids in alloxan-induced diabetic rats and if used as a hypoglycemic agent, may also reverse dyslipidemia associated with diabetes and reduce cardiovascular complications that are very prevalent in diabetic patients.

Conflict of Interest

The authors report no conflict of interest. The authors alone are responsible for the conduct and writing of this manuscript.

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