

ISSN: 2320-9267 Indian Journal of Pharmaceutical and Biological Research (IJPBR)

Journal homepage: www.ijpbr.in

RESEARCH ARTICLE

Antidiabetic Activity of Fractions of the n-Hexane Extract of Leaves of *Eriosema psoraleoides* (Lam) G. Don (Leguminosae) on Alloxan-induced Diabetic Albino Rats

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ABSTRACT

Diabetes is a chronic disease that is raising health concerns. Eriosema psoraleoides is one of the medicinal plants reported to be used in managing this condition in Eastern Nigeria. This study investigated the effect of four fractions [(n-hexane; n-hexane:dichloromethane (50:50); dichloromethane; and dichloromethane: methanol (95:5)] of the n-hexane leaf extract of this plant on the blood glucose levels of alloxan-induced diabetic rats. The n-hexane extract was obtained by maceration, filtration, and evaporation using a rotary evaporator. Phytochemical screening of the extracts was carried out using standard methods. The extract was fractionated using column chromatography, and ten fractions were obtained. The fractions were pooled together into four fractions based on the similarity in their R_{e} values. A dose of 200 mg/kg of each of the fractions was given orally for 7 days to the alloxan-induced diabetic rats, and plasma blood glucose concentrations determined. Preliminary screening of the extract revealed the presence of flavonoids, steroids, reducing sugars, tannins, and combined anthraquinones. Two fractions, the n-hexane:dichloromethane (50:50) and the dichloromethane, produced significant (p < 0.05) decrease in blood glucose concentrations in their treated groups compared to that of glibenclamide (standard drug) treated, and the diabetic untreated groups of the diabetic rats. However, the n-hexane and the dichloromethane: methanol (95:5) fractions produced no significant (p < 0.05) decrease in blood glucose concentrations. The observed antidiabetic property of the n-hexane: dichloromethane (50:50) and the dichloromethane (100%) fractions suggests that E. psoraleoides could be used in the management of diabetes and this gives credence to its use in ethnomedicine for this purpose.

Keywords: Alloxan, Antidiabetic, *Eriosema Psoraleoides*, Glibenclamid.. Indian J. Pharm. Biol. Res. (2019): https://doi.org/10.30750/ijpbr.7.4.2

INTRODUCTION

Diabetes is a chronic insulin metabolic disease that is raising health concerns around the world. Diabetes mellitus (DM) is a metabolic disorder of multiple etiology characterized by hyperglycemia, often accompanied by glycosuria, polydipsia, and polyuria.^[1] Complications of this condition include retinopathy, nephropathy, and neuropathy with features of autonomic dysfunction. The global prevalence of diabetes among adults 18 years of age and above has risen from 4.7% in 1980 to 8.5% in 2014.^[2] Diabetes doubles a person's risk of death. From 2012–2014, diabetes is estimated to have resulted in 1.5-4.9 million deaths each year.^[2,3] Experts have projected that the incidence of diabetes is set to increase by 64% by 2025.^[4] Medicinal plants have found use in ethnomedicine in the management of this condition. The World Health Organisation (WHO),^[5] advocates the use of safe and efficacious herbal

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How to cite this article: Elechi NA, Ewelike FW. Antidiabetic activity of fractions of the n-hexane extract of leaves of *eriose-ma psoraleoides* (lam) g.don (leguminosae) on alloxan-induced diabetic albino rats. Indian J. Pharm. Biol. Res. 2019;7(4): 5-9.

Source of support: Nil

Conflict of interest: None.

Received: 28/09/2019 **Revised:** 30/10/2019 **Accepted:** 06/11/2019 **Published:** 31/12/2019

medicines in traditional medicine practice. *E. psoraleoides* (Leguminosae) is one of the medicinal plants reported to

be used in the management of diabetes in Eastern Nigeria. This plant is an erect herb. The leaves are 3-foliolate, with elliptic or oblong leaflets. The flowers are golden-yellow and hairy. The plant is commonly called Canary Pea in West Africa. Its synonyms include Crotalaria psoraleoides Lam, and Eriosema cajanoides (Guill. and Perr.) Hook. f. Some of its local uses include the treatment of venereal diseases, diarrhea and use as an oxytocic. Biological activities have been reported for some other species of the Eriosema genus. Five pyrano-isoflavones have been isolated from the roots of Eriosema kraussianum with penile relaxant activity.^[6] Chromones and phenolic compounds with antifungal activity have been isolated from the roots of E. tuberosu.^[7] An isoflavonoid glycoside with antiviral activity has also been isolated from E. tuberosum.^[8] The aqueous ethanol extracts of E. kraussianum have been reported to exhibit hypoglycaemic and vasorelaxant effects on laboratory rats.^[9] The purpose of this study is to investigate the antidiabetic potentials of the leaves of Eriosema psoraleoides to justify its use in ethnobotany.

MATERIALS

The plant material was collected from Nsukka, Nigeria, and identified as *E. psoraleoides* (Leguminosae) by Mr. A. Ozioko of the Biodiversity and Conservation Organisation (BDC), Nsukka, with Herbarium number: INTERCEDD 968. Healthy Wistar Albino rats of both sexes with a weight range of 120–200g were selected for this study. They were obtained from the animal house of the Department of Pharmacology, Faculty of Pharmaceutical Sciences, University of Port Harcourt. The rats were fed with standard feed and water throughout the study.

METHODS

Phytochemical screening of the powdered leaves was carried out using standard methods.^[10-12]

Extraction of Plant Materials

A 2kg of the dried and powdered leaves of *E. psoraleoides* was extracted with n-hexane in a maceration jar, for a period of 3 days. The solution was then filtered, and the filtrate evaporated to dryness on a rotary evaporator. A black n-hexane extract was obtained to be used for the study. For the fractionation of the n-hexane extract, the column chromatographic method used was dry packing, with silica gel as the stationary phase. The four mobile phases used were n-hexane; n-hexane:dichloromethane (50:50); dichloromethane; dichloromethane:methanol (95:5). The eluents were spotted on thin layer chromatography (TLC) plates, and those with similar spots were bulked together and dried as different fractions.

Alloxan-induced Diabetic Assay

Diabetes was induced in the albino rats through intraperitoneal administration of 120 mg of alloxan monohydrate/kg body weight of the animals.^[13] After 4 days, the animals that showed blood glucose levels above 200 mg/dL were considered diabetic and used for the study. The diabetic animals were arranged into 6 groups of 5 animals each. A 7th group consisted of normal rats not induced with alloxan, to serve as normal control.

Treatment Schedule

Rats in group 1 were treated with glibenclamide (4 mg/kg body weight), group 2 were diabetic untreated, group 3 were normal control. Rats in groups 4–7 were each treated with 200 mg/kg b.w. of the four fractions, respectively. Animals were fasted overnight but allowed access to water before oral treatment. Basal blood glucose levels at zero time were determined (with a glucometer) before oral treatment. On day 1, the blood glucose levels were determined at intervals of 60 minutes for the next 240 minutes.^[14] On days 2–7, the blood glucose levels were determined 2 hours after the treatment, daily. But, rats in groups 2 (diabetic untreated) and 3 (normal control) received no treatment throughout the study. However, they had their blood glucose levels determined daily also. This study was carried out in accordance with the research ethics guidelines.

Statistical Analysis

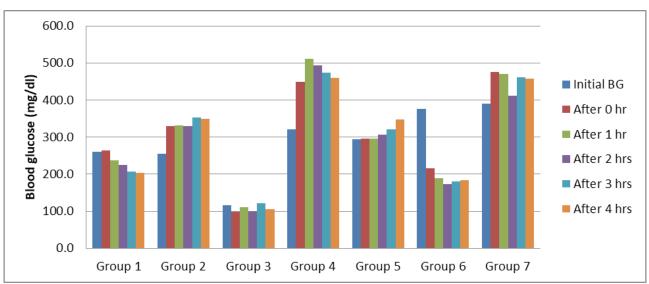
Readings were obtained from the five animals per group, and the mean determined. All data obtained were expressed as mean \pm SEM. Significant changes in the treatment data were obtained by comparison with the diabetic untreated, where p <0.05 is considered significant. Comparisons were by means of ANOVA, and data analysis by Graph Pad Prism.^[15]

RESULTS

Results of phytochemical tests showed the presence of flavonoids, combined anthraquinones, tannins, carbohydrates, reducing sugar, and steroidal nucleus. However, saponins, alkaloids, unsaturated lactone ring, and deoxy sugar were absent. Figures 1 and 2, below, show the mean blood glucose levels on day 1 and days 2–7, respectively. Tables 1 and 2 show the corresponding percent reductions in glucose levels.

DISCUSSION

It could be seen from Figure 1 and Table 1 that the hexane:dichloromethane (DCM) (50:50) and the DCM fractions caused slight decreases in the blood glucose levels, compared with the untreated diabetic group.



Elechi et al./ Indian J. Pharm. Biol. Res., 2019;7(4):5-9

Figure 1: Mean blood glucose levels (mg/dL) in hours on day 1

Group 1 = Glibenclamide (4 mg/kg); Group 2 = Diabetic untreated; Group 3 = Normal control; Group 4 = n-Hexane (200 mg/kg); Group 5 = Hexane: DCM, 50:50 (200 mg/kg); Group 6 = DCM; Group 7 = DCM: Methanol, 95:5 (200 mg/kg)

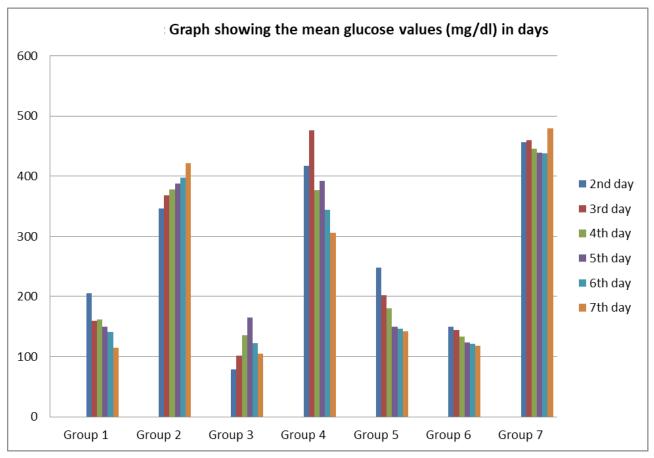


Figure 2: Mean blood glucose levels (mg/dl) on Days 2–7 Group 1 = Glibenclamide (4 mg/kg); Group 2 = Diabetic untreated; Group 3 = Normal control; Group 4 = n-Hexane (200 mg/kg); Group 5 = Hexane: DCM, 50:50 (200 mg/kg); Group 6 = DCM; and Group 7 = DCM: Methanol, 95:5 (200 mg/kg)

Elechi et al./ Indian J. Pharm. Biol. Res., 2019;7(4):5-9

Treatment group	1-hour	2 hours	3 hours	4 hours				
Glibenclamide (4 mg/kg) body weight	9.23%*	13.71%*	20.26%*	22.05%*				
Normal control	3.67%	13.17%*	-4.97%	8.86%*				
n-hexane (100%)	-59.17%	-53.57%	-47.56%	-43.11%				
n-hexane: dichloromethane (50:50)	-0.68%	-4.20%	-9.30%	-18.62%				
Dichloromethane (100%)	49.28%*	53.81%*	51.94%*	51.42%*				
Dichloromethane:methanol (95:5)	-20.77%	-5.39%	-18.38%	-17.52%				

Table 1: Percentage reduction in blood sugar on day 1

*Significant percentage reduction (p < 0.05), compared to the diabetic untreated group

	Table 2: Percentage reduction	in blood sugar levels from day 2 to 7
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Treatment group	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Glibenclamide	55.5%*	57.8%*	59.1%*	65.2%*	71.2%*	74.8%*
Normal control	31.7%*	11.8%	-17.0%	-42.9%	-6.26%	9.50%
n-hexane (100%)	-29.7%	-47.8%	-17.2%	-21.7%	-6.83%	4.77%
n-hexane: dichloromethane (50:50)	15.4%	31.1%*	38.4%*	48.8%*	50.0%*	51.4%*
Dichloromethane (100%)	60.0%*	61.59%*	64.51%*	67.18%*	67.69%*	68.50%*
Dichloromethane: methanol (95:5)	-17.01%	-17.95%	-14.36%	-12.48%	-12.39%	-23.00%

*Significant percentage reduction (p < 0.05) compared to the untreated diabetic group

In Figure 2 and Table 2, showing glucose levels from Day 2–7, the decreases caused by these two fractions were significant (p <0.05), and were comparable to that of glibenclamide. However, the n-hexane, and then the DCM: methanol (95:5) fractions produced no significant (p <0.05) reductions in glucose levels. Medicinal plants are used throughout the world for a range of diabetic complications. Several investigations have been conducted, and many plants have shown positive activity.^[16] Secondary plant metabolites like coumarins flavonoids and terpenoids have been reported to have hypoglycaemic effects in various experimental animal models.^[17,18] Effect of flavonoids on pancreatic β -cells leading to their proliferation and secretion of more insulin has been proposed.^[19]

Synthesis, release, an increase of insulin sensitivity, or the insulin-like activity of the plant extract have also been proposed.^[20]

The presence of similar phytochemicals in this plant, and any of the previously proposed mechanisms could be responsible for its observed antidiabetic activity.

CONCLUSION

The results of this study have shown that the n-hexane: dichloromethane (50:50), and the dichloromethane fractions of the n-hexane leaf extract of *E. psoraleoides* possess antidiabetic activities. This tends to lend credence to its use in ethnomedicine for the management of diabetes mellitus.

Further Work

Further work is on-going to isolate and characterize the active principles responsible for this reported activity.

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