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Phytochemical constituent, acute toxicity and hypoglycemic potential of *dichrostachys cinerea* methanol leaf extract

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ABSTRACT

Diabetes mellitus (DM) is a major public health problem worldwide, herbal derivatives have proved to be more efficient in the treatment of diabetes over synthetic drugs because of less side effects and adverse effects. The aim of this article is to investigate the phytochemical constituent, acute toxicity and hypoglycemic potential of *Dichrostachys cinerea* methanol leaf extract. Standard laboratory protocols were employed for phytochemical assay, fixed dose method was used to determine acute toxicity (LD_{50}). Alloxan was used to induce diabetes to experimental animals and fasting blood levels were recorded weekly using glucometer. Phytochemical analysis revealed the presence of numerous phytoconstituents. The result for acute toxicity of *D. cinerea* methanol leaf extract (DCME) showed that the LD_{50} is above 5000mg/kg. The body weight showed that there were no significant ($P>0.05$) difference in untreated control, standard control, DCME 100mg/kg, DCME 200mg/kg, DCME 400mg/kg and normal control initially. At week 1, 2 and 3, only group treated with standard drug and extract significantly ($P<0.05$) increases compared to both normal and untreated control. Before extract and standard drug administration (week 0), the fasting blood sugar of all the alloxan diabetes induced groups (i.e group 2, 3, 4, 5 and 6) significantly ($P<0.05$) increased compared to normal control group. After week 1, 2 and 3 of treatment there was no significant ($P>0.05$) difference between, extract treated groups compared to standard control. The present study revealed that *D. cinerea* methanol leaf extract contain numerous phytochemicals and the extract is practically not toxic at acute dose and with strong glucose lowering potentials.

Keywords: Phytochemicals, acute toxicity, hypoglycemic, diabetes mellitus, antioxidants, *Dichrostachys cinerea.*

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1. INTRODUCTION

Diabetes mellitus is a chronic medical condition and post a major public health problem (Sen et al., 2009). Type 1 diabetes also called insulin-dependent diabetes mellitus (IDDM) is caused by the autoimmune destruction of the pancreatic β- cell with no insulin Production (Ozougwu et al., 2013), while the major type of diabetes is Type 2 DM (T2DM), which is as a result of insufficient production of insulin or failure of insulin receptors to sense the presence of glucose as such limiting the entry of glucose into the cell (Bouche et al., 2004).

Herbal derivatives have proved to be more effective over synthetic drugs because of less side effects and adverse reaction (Mohammadi et al., 2020). Recently numerous medicinal plants have been documented to be essential in the management of diabetes globally and have been utilized empirically as anti diabetic and anti hyperlipidemic remedies (Patel et al., 2012). Recently, the use of natural products has gained more interest for treating diabetes and other ailments this is because of the compatibility to human biological system, through curing diseases and relieves physical sufferings in addition most people in developing country rely on herbal alternative because of their cultural acceptability, cost friendly and lesser side effects (Chowdhury et al., 2017).

Dichrostachys cinerea (L.) also known as Sickle bush, Bell mimosa, Chinese lantern tree or Kalahari Christmas tree is a semi-deciduous to deciduous fast growing tree, typically grows up to 7 meters in height (El-Sharawy et al., 2017). It characterized by strong alternate smooth spines (up to 8 cm long), dark grey-brown fractures on old branches and stems and bark on younger branches (Omoniwa et al., 2022). *Dichrostachys cinerea* has been reported to be used traditionally to treat many diseases including diabetes. Therefore, this article was carried out to provide scientific proof by investigating the hypoglycaemic, phytoconstituent and acute toxicity of the plant methanol extract.

2. MATERIALS AND METHODS

Collection and Identification of Plant Samples

Dichrostachys cinerea leaves were collected from Dolekaina town, Kamba Local Government Area of Kebbi State. The plant sample was authenticated by a Taxonomist from Department of Plant Science and Biotechnology, Kebbi State University of Science and Technology, Aleiro. A voucher specimen (KSUSTA/PSB/H/VOUCHER NO: 281A) is deposited in the same herbaruim.

Plant Preparation and Extraction

The leaves of *Dichrostachys cinerea* were washed with clean water and allowed to dry under shade for two weeks. They were then grinded to coarse powder using mortar and pestle. Five hundred grams (500g) of the powdered sample was soaked in 2500mls of methanol for 72 hrs (Dupont et al., 2002). They were then filtered using muslin cloth and the filtrates were evaporated using an oven set at 45^{o}C. The dried extracts were stored separately in an air tight container and kept in refrigerator at 4^{o}C. The percentage yield of the extract was calculated using the formula.

$$\text{Percentage yield} = \frac{\text{weight of extract}}{\text{weight of ground plant material}} \times \frac{100}{1}$$

Phytochemical Screening of *Dichrostachys cinerea*

The Phytochemical screening for the presence of saponins, tannins, alkaloids, flavonoids, tannins, steroids, saponins, glycosides, cardiac glycosides, saponin glycosides, balsams, anthraguines and volatile oil were carried out according to the methods described by Harbone, (1973), Trease and Evans, (1989) and Sofowora, (1983).

Acute Oral Toxicity Studies (LD_{50})

The acute oral toxicity studies of *Dichrostachys cinerea* methanol leaf extract were undertaken as per the Organization for Economic Co-operation and Development (OECD, 2008) guidelines for testing of chemicals by up-and-down procedure. The rats were fasted overnight and the weight of each rat used was recorded just before use. Animals were divided randomly into two treatment groups each group consisting of three Albino rats. The animals were administered with extract 3000mg/kg. Again a higher dose of 5000mg/kg of *Dichrostachys cinerea* methanol leaf extract was given to second group of rats. Animals were kept under close observation for 1hr, 4hrs, 6hrs and 12hrs after administering the extracts and then they were observed daily for 14 days for any change in general behavior and other physical activities.

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Induction of Diabetes

Diabetes was induced in the rats by intra peritoneal injection of Alloxan in a dose of 120mg/kg body weight in Normal Saline (Chougale et al., 2007). Diabetes was confirmed in the animal after 48 hours by estimation of fasting BGLs and only rats with blood glucose level above 150mg/dl were used for the study.

Fasting Blood Glucose Monitor

Fasting blood sugar was determined using Accu-check active glucometer by Roche Diagnostic according to the method of Marks and Dawson, (1965).

Experimental Design

The rats were randomly divided into 6 groups (n=4) and treated as follows:

Group 1	Normal control (untreated).
Group 2	Alloxan treated (diabetic)
Group 3	Alloxan induced-diabetic rats treated with glibenclamide (0.2mg/kg).
Group 4	Alloxan induced-diabetic rats treated with extract (100mg/kg).
Group 5	Alloxan induced-diabetic rats treated with extract (200mg/kg).
Group 6	Alloxan induced-diabetic rats treated with extract (400mg/kg).

The extract was administered to the animals orally. Body weight changes as well as fasting blood sugar levels were monitored weekly throughout the experimental period. The rats were sacrificed on the twenty-second day of the experiment.

Data Analysis

The data generated from the study are present as Mean ± Standard error of mean (SEM) and subjected to one-way analysis of variance (ANOVA) and statistical difference between means were separated using Duncan multiple comparison test using statistical package for social science (SPSS) version 20. Values are considered statistically significant at $P<0.05$.

3. RESULTS AND DISCUSSION

RESULTS

Percentage Yield

The extraction of *Dichrostachys cinerea* leaf with methanol yielded 32.37% (Appendix III). The extract easily dissolves in water and is black green in colour; with a sticky texture.

Results of Phytochemical Screening

The qualitative phytochemical screening of *Dichrostachys cinerea* leaf methanol extract is presented in Table 1.

Table 1 Phytochemical Constituents of *D. cinerea* Leaf Methanol Extract

PHYTOCHEMICALS	OBSERVATION
Alkaloids	+
Flavonoids	+
Tannins	+
Saponin	+
Glycoside	+
Cardiac glycoside	+
Anthraquinones	-
Phenols	+
Terpenoids	+

KEY: + = Present, - = Not detected

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Acute Toxicity (LD_{50}) Profile of D. cinerea Methanol Leaf Extract

The results of acute oral administration of the methanol leaf extracts in fixed dose indicated no mortality up to 14 days after treatment. There was also no sign of toxicity. Hence the LD_{50} is above 5000mg/kg

Effect of D. cinerea Methanol Leaf Extract on Body Weight (g) of diabetic Rats

The effect of *D. cinerea* methanol leaf extract on body weight of albino rats treated for 21 days is presented in Figure 1. Before administration of extract (week 0) there were no significant (P>0.05) difference in the bodyweight of group untreated control, standard control, DCME 100mg/kg, DCME 200mg/kg, DCME 400mg/kg and normal control. At week 1and week 2, only group treated with standard drug significantly (P<0.05) increases compared to both normal and untreated control. Also at group week 3 only group treated with standard drug significantly (P<0.05) increases compared to both normal and untreated control.

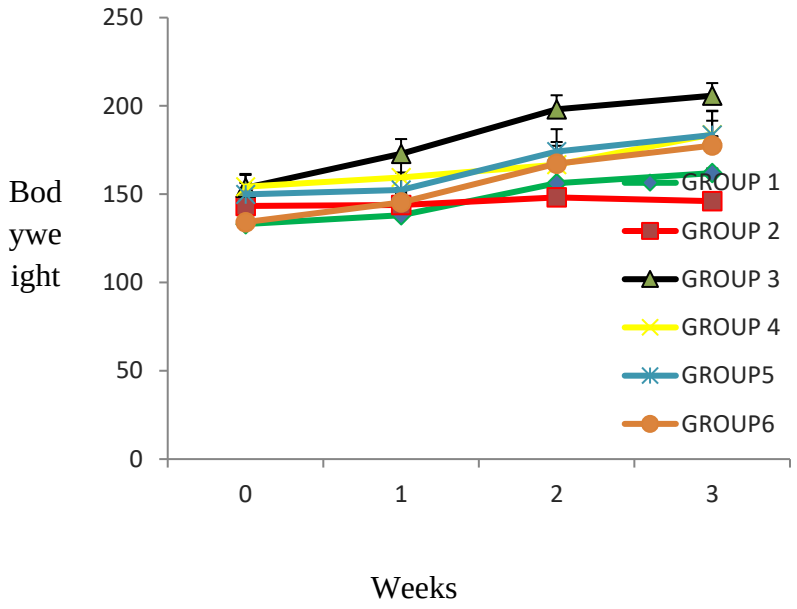

Figure 1 Bodyweight of Diabetic Animals Administered with *D. cinerea* Leaf Methanol Extract.
{Group 1 (normal control), group 2 (negative control), group 3 (positive control), group 4 (100mg/kg b.w), group 4 (200mg/kg b.w) and group 5 (400mg/kg b.w)}.

Effect of D. cinerea Methanol Leaf Extract on Fasting Blood Sugar

Before extract and standard drug administration (week 0), the fasting blood sugar of all the alloxan diabetes induced groups (i.e group 2, 3, 4, 5 and 6) significantly increased (P>0.05) compared to the normal control group (Table 2). After week 1, 2 and 3 of treatment there was no significant (P<0.05) difference between, DCME 100mg/kg, DCME 200mg/kg and DCME 400mg/kg when compared to standard control. At week 2 and 3 of the experiment there was a promising non-significant deference (P>0.05) between all the extract treatment groups DCME 100mg/kg, DCME 200mg/kg and DCME 400mg/kg when compared to normal control. However there was significant (P>0.05) increase in fasting blood sugar of diabetic control at week 1, 2 and 3 compared normal control respectively.

Table 2 Effect of *D. cinerea* Methanol Leaf Extract on Fasting Blood Sugar

Treatment	Week 0	Week 1	Week 2	Week 3
Normal control	73.50±7.42[a]	78.25±9.44[a]	87.00±15.37[a]	87.00±4.74[a]
Diabetic control	223.75±24.17[b]	260.00±15.53[b]	288.50±36.76[b]	293.50±37.57[b]
Standard drug (0.2mg/kg)	317.00±73.36[b]	74.00±6.65[a]	95.25±14.19[a]	98.50±9.07[a]
DCMLE (100mg/kg)	202.00±27.04[b]	97.00±3.70[a]	151.50±77.81[a]	107.75±7.19[a]
DCMLE (200mg/kg)	249.75±34.80[b]	72.00±4.30[a]	70.50±14.97[a]	90.25±10.69[a]

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DCMLE (400mg/kg)	296.75±41.43[b]	114.00±45.71[a]	113.00±19.46[a]	107.25±14.67[a]

Values are presented as mean ± SEM (n = 4) value having same superscript are not significantly different at (P>0.05) analysed using One-Way ANOVA, followed by Duncan multiple comparison test with SPSS version 20.0.

4. DISCUSSION

Many indigenous plants have been reported to contain numerous constituents belonging to different chemical classes of secondary metabolites such as alkaloids, terpenoids, essential oils, glycosides, steroids, phenolic constituents, aliphatic compounds and polysaccharides. Leaves, stem and roots of majority of these plants are a rich source of proteins, flavonoids, alkaloids and glycosides (Hussein and El-Anssary, 2019). These active compounds have been exposed to several biological activities, including antiseptic, anti-inflammatory, anti-cancer, antimicrobial and antidiabetic activities (Dhama et al., 2021). Tran and Le, (2020) reported that plant-derived secondary metabolites are small molecules or macromolecules biosynthesized in plants including steroids, alkaloids, phenolic, lignans, carbohydrates and glycosides, etc. that possess a diversity of biological properties beneficial to humans, such as their antiallergic, anticancer, antimicrobial, anti-inflammatory, antidiabetic and antioxidant activities. In the present study, the antidiabetic activity observed might be due to the presence of these phytoconstituents in *D. cinerea* methanol leaf extract.

Acute toxicity describes the adverse effects of a substance that result either from a single exposure or from multiple exposures in a short period of time **(**usually less than 24 hours**)**. Acute toxicity tests in animals (i.e, rat) use mortality as the main observational endpoint in order to derive a LD_{50} (Reinert et al., 2002). Substances with LD_{50} below 5mg/kg are classified to be highly toxic, 5-50mg/kg highly toxic, 50-500mg/kg moderately toxic, 500-5000mg/kg slightly toxic, 5000-15000mg/kg practically non-toxic, while substances with LD_{50} above 15,000 mg/kg are termed relatively harmless (Schrage et al., 2011). In the present study, the LD_{50} of *D. cinerea* methanol leaf extract was found to be above 5000mg/kg, suggesting that the extract is relatively non-toxic at acute dose.

Diabetes results to insufficient insulin availability within the biological system, thus preventing blood glucose from entering into cells to use as energy. When this occurs, the body starts burning fat and muscle for energy, causing a reduction in overall body weight, however antidiabetic drugs plays vital role in reviving weight lost (Bullon et al., 2014). In the present study, the effect of *D. cinerea* methanol leaf extract on maintaining bodyweight of animals might be attributed to the plant anti diabetic potential.

According to Ayrle et al., (2016) herbal medicines and plant components with insignificant toxicity and no side effects are notable therapeutic options for the treatment of diabetes globally. Most tests have demonstrated the benefits of medicinal plants containing hypoglycemic properties in diabetes management. The most common herbal active ingredients used in treating diabetes are flavonoids, tannins, phenolic, and alkaloids (Krishnaiah et al., 2009). The existence of these compounds implies the importance of the anti-diabetic properties of many plants as observed the present study. Hence the glucose lowering effect of *D. cinerea* methanol leaf extract in alloxan induced diabetic animals might be due to the presence of these phytochemicals.

5. CONCLUSION

In conclusion, the present study revealed that *D. cinerea* methanol leaf extract contain numerous pharmacologically active phytochemicals and the extract is practically not toxic at acute dose, the extract also revealed strong glucose lowering potentials thereby validating the traditional utilization of this plant in the management of diabetes.

Conflict of Interest

The authors declare that there are no conflicts of interests.

Ethical approval

The Animal ethical guidelines are followed in the study for experimentation. The ethical guidelines for plants & plant materials are followed in the study.

Funding

The study has not received any external funding.

Data and materials availability:

All data associated with this study are present in the paper.

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