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Hypoglycaemic effects of the methanolic extract of Aerial part of *Chrysanthellum indicum* in rats

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ABSTRACT

The preliminary phytochemical screening of methanolic extract of Chrysanthellum indicum revealed the presences of alkaloids, flavonoids, saponins, steroid, terpenoids and tannins. Also the LD_{50} of the extract is found to be 1131.4 mg/kg intraperitoneal. The effect of the methanolic extract on blood glucose levels of alloxan induced diabetes in Wistar rats was also investigated. Three doses of the extract (100, 200 and 400 mg/Kg) were administered intraperitoneally. The three doses administered there was no significant difference at 1 day of extract administration when compared to negative control. However, after 3, 5,7 and 9 days of extract administration there was a significant ($p<0.05$) decrease in blood glucose levels in all the three doses administered when compared to negative control (normal saline). As regard to the positive control (metformin) there was a significant ($P<0.05$) in the blood glucose levels when compared to negative control (normal saline). In conclusion, the three doses of the extract administered shown both significant ($p<0.05$) hypoglycemic and anti-hyperglycemic effects in Wistar rats.

Keywords-Hypoglycaemic *Chrysanthellum indicum* Diabetes mellitus.Blood glucose levels

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INTRODUCTION

Diabetes mellitus (DM) is a major health problem all over the world. Globally, the number of people that has been diagnosed with diabetes has exploded in the past two decades. In 2000, 151 million people in the world were diabetic. With the current rate of increase (6% per annum), it has been projected that 221 million people will be diabetic in 2010 and 324 million by 2025[1]. Several approaches were made to reduce the hyperglycemia, the hallmark of diabetes mellitus, with treatments such as sulfonylureas, which stimulates pancreatic islet cells to secrete insulin;

meteoric, which acts to reduce hepatic glucose production glucosidase inhibitors, which interfere with glucose adsorption and insulin itself, which suppresses glucose production and augments glucose utilization [2] . The growing public interest and awareness of natural medicines have led the pharmaceutical industry and academic researchers to pay more attention to medicinal plants [3]. The apparent reversal of trend from western to herbal medicine is partly due to the fact that synthetic drugs have always shown adverse reactions and other undesirable side effects. This has led to the belief that natural products are safer because they are more harmonious with biological systems. In addition, the cost of administering modern anti-diabetic drugs is beyond the reach of people in the low income group and those living in the rural areas. In Nigeria, hundreds of plants are used traditionally for the management of diabetes mellitus. To date, however, only a few of these medicinal plants have received scientific scrutiny, despite the fact that the World Health Organization has recommended that medical and scientific examinations of such plants should be undertaken [4] .

Chrysanthellum indicum Linn. Vatke (Compositae) is a faintly aromatic annual herb that is widely distributed in the tropics. The plant is commonly known in Hausa as dunkufe and in southern Nigeria as Oyigi or Abilere in Yoruba[5] . The plant is used for antifebrile, detoxification and hypotensive purposes [6] . A hot water extract of the plant is used as an Emmenagogue, abortifacient, for rheumatism and menstrual irregularities [7, 8]. In West Africa, the plant is used in treating maturing boils, fevers, jaundice and Gonorrhoea [9]. In Northern Nigeria and Ghana, it is used in the treatment of hepatitis (in combination with *Tamarindus indicus*) and heart problems [10] .

Pharmacological studies of the ethanolic extract of the plant revealed antihypertensive activity[11] , antibacterial and antifungal activities [12]. The defatted ethanolic extract also showed an anti-tumour activity [13]. The methanolic extract from the flowers of *Chrysanthellum indicum* exhibited strong inhibitory activity against xanthine oxidase and rat lens aldose reductase enzymes [14]. The antioxidant properties of the flavonoids isolated from the plant has been reported [9].[15] Reported the hypotensive and gastrointestinal stimulatory effects of the aqueous extract of the plant.

In the limelight of the above information the present study was undertaken to evaluate the effects of methanolic, extract of *Chrysanthellum indicum* on the blood glucose levels of alloxan-induced diabetic Wistar rats

MATERIALS AND METHODS

Plant Material

The whole plant *Chrysanthellum indicum* was collected from Kauran Wali Village, in Kudan Local Government Area, Kaduna State, Nigeria, in June, 2009. The plant was identified and authenticated by Malam M.Musa of the Herbarium Section Department of Biological Sciences, A.B.U., Zaria. A voucher specimen (NO. 2991) was deposited at the herbarium for future reference. The plant material was cleaned, air dried for two weeks and then crushed into coarse powder with a pestle and mortar. 150g of the powered plant was successively macerated with

methanol for 48 hours with occasional shaking. The solvent was evaporated to give an average yield of 19.1%.w/w.

Animals

Adult Wister rats (200-250g) of either sex obtained from the Animal House Department of Pharmacology and Clinical Pharmacy, Ahmadu Bello University, Zaria were used. The animals were maintained in a well ventilated room, fed on Excel feeds (Feed Masters, Ilorin) and water *ad libitum.*

Phytochemical screening of plant fraction

Preliminary phytochemical screening of the two extracts were performed for the presence of secondary metabolites using the following reagents and chemicals: alkaloids with Mayer's and Dragendorf [16,17]Flavonoids with the use of Mg and HCl[18,19] Tannins with 1% gelatin and 10% NaCl solutions and saponins with ability to produce- d[19] .

Acute toxicity studies rats (LD_{50})

LD_{50} determination was conducted using the method of [20] . In the initial phase, 3 groups of three animals each were treated with the methanolic extract of the plant at doses of 10,100 and 1000mg/kg body weight *i.p*. and observed for 24 hours. In the second phase, 4 groups of one animal each were injected with the methanolic extract at doses of 200, 400, 800 and 1600mg/kg for rats. The LD_{50} values were determined by calculating the geometric mean of the doses for which 0/1and 1/1 were found.

Induction of experimental diabetes mellitus

The animals were fasted for 16–18 hours 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%$^{v/v}$ cold normal saline solution at a dose of 150 mg/kg body weight [21]. Since alloxan is capable of producing fatal hypoglycemia as a result of massive pancreatic insulin release, rats were treated with 20 % glucose solution intraperitoneally after 6h. The rats were then kept for the next 24h on 5 % glucose solution bottles in their cages to prevent hypoglycemia[22] . The diabetes was assessed in alloxan-induced rats by determining the blood glucose concentration 72 hours after injection of alloxan. The rats with blood glucose level above 200mg/dl were then selected for the study.

Experimental design

The Alloxan induced diabetic Wistar rats were randomly assigned into five groups (A-E) of five rats (n=5) each as follows:

Group A-was used as the negative control group and were treated with normal saline (i.p)
Group B- was used as the positive control group and were treated with metformin (250mg/dl) (orally)

Group C-received 100mg/kg of body weight of the *Chrysanthellum indicum* extract (i.p)
Group D- received 200mg/kg of body weight of the *Chrysanthellum indicum* extract (i.p)
Group E- received 400mg/kg of body weight of the *Chrysanthellum indicum* extract (i.p)

Determination of blood glucose levels

The blood samples were collected by cutting the tail artery of the rats. Blood samples for blood glucose determination were collected from the tail at intervals of 0, 1, 3, 5, 7 and 9 days respectively. Determination of the blood glucose level was done by the glucose oxidase principle[23] (Beach and Turner 1958) using the one touch glucometer strips and reported as mg/dl.

Statistical Analysis

All the data are expressed as mean ± SEM. Statistical comparisons were performed by one way analysis of variance (ANOVA) followed by Duncan's multiple range tests [24] (Duncan *et al*, 1977). The results were considered statistically significant if the p values were 0.05 or less. The data were analyzed using SPSS vision 17.0.

RESULTS

Preliminary Phytochemical Screening

The results from the preliminary phytochemical analysis of the aerial part of *Chrysanthellum indicum* extract revealed the presence of alkaloids, flavonoids, saponins, steroids/terpenoids, tannins as shown in table 1 below.

TABLE 1: Showing the preliminary phytochemical constituents of *Chrysanthellum Indicum*

Constituent/Test	Inference
Alkaloids	+
Flavonoids	+
Saponins	+
Steroids/terpenoids	+
Tannins	+

Key : + =present

Acute Toxicity Studies

The LD_{50} of methanolic extract of aerial part of *Chrysanthellum indicum* in Wistar rats was found to be 1131.4mg/kg body weight intraperitoneal

TABLE.2: Showing the effect of Chrysanthellum *Indicum* on The Alloxan-Induced Diabetes Wistar Rats

Treatments	0	1 day	3 days	5 days	7 days	9 days
Group A Normal saline	382.8±36.4	539.0±36.8	418.6±52.7	412.2±30.4	342.4±21.2	309.4±36.4
Group B metformin	389.4±55.6	266.2±37.8^{a}	197.2±54.4^{a}	133.0±45.2^{a}	94.0±5.9^{a}	90.4±10.8^{a}
Group C 100mg/kg	396.2±62.7	324.0±51.3^{ns}	177.2±25.5^{a}	160.8±36.0^{a}	162.4±29.3^{a}	112.4±9.8^{a}
Group D 200mg/kg	383.6± 69.9	382.6±87.1^{ns}	293.8±41.5^{a}	246.6±40.3^{a}	222.2±39.6^{a}	85.2±12.3^{a}
Group E 400mg/kg	381.6±57.6	366.8±44.3^{ns}	206.0± 44.9^{a}	103.4±22.0^{a}	89.6±7.3^{a}	70.0±11.2^{a}

Value are given as mean ±SD for 5 rats in each group; experimental groups are compared with diabetic control.(normal saline)
Values are statistically significant at ^{a}-P<005 $^{ns=}$ Not significant

The effect of the different doses (100, 200 and 400mg/kg) of the extract of *Chrysanthellum indicum* and the control groups (metformin and normal saline treated groups) in alloxan-induced diabetes wistar rats as shown in table 2 above.

The result show a significant decrease ($P<0.05$) in the blood glucose level with 400 and 100mg/kg after 1day of the extract treatment when compared with the normal saline treated group, while 200mg/kg group shows a significant decrease ($P<0.05$) in the blood glucose levels after 2 day of extract treatment when compared with the normal saline treated group. The reference drug (metformin) shows a significant decrease ($P<0.05$) in the blood glucose levels from day 1 of the extract administration when compared with the normal saline group.

DISCUSSION AND CONCLUSION

Alloxan monohydrate is one of the chemical agents used to induce diabetes mellitus. It induces diabetes by partial destruction of the cells of Islets of Langerhan's [25] . This results in decreased insulin levels and hyperglycemia leading to type 1 diabetes mellitus. However, animal models of diabetes differ significantly from each other and none can be taken, without reservation, to reproduce the essentials of human diabetes[26] .

In the alloxan-induced diabetic groups, the effect of three doses (100mg/Kg, 200mg/Kg and 400mg/Kg) of *Chrysanthellum indicum*, metformin and control groups were evaluated. The dose of metformin (250 mg/kg) as a positive control showed a significant decrease after 1 day of administration while the three doses of the extract there was no significant decrease in the blood glucose levels when compared to negative control (normal saline) group. However, after 3 days there was a significant ($p<0.05$) decrease in the blood glucose levels in all the treated groups when compared to negative control group. The highest activity resides at the lowest dose of the extract administered that is 100mg/kg. Also at 5, 7 and 9 days of extract administration there was also a significant ($p<0.05$) decrease in the blood glucose levels when compared to negative control group. The highest activity resides at the highest dose of the extract administered that is the dose of 400mg/kg.

The intraperitoneal LD_{50} of the extract was found to be 1131.4mg/kg body weight in rats. The preliminary phytochemical screening of the extract revealed the presences of alkaloids, steroids, terpenoids ,tannins, saponins and flavonoids. Flavonoid and tannins isolated from the other antidiabetic medicinal plants has been found to stimulate secretion or possess an insulin like-effect [27]. Effect of the flavonoids quercetin and ferulic acid on pancreatic β-cells leading to their proliferation and secretion of more insulin have been proposed by [28,29] and Sri-Balasubashini, *et al*., (2004) as the mechanism by which they reduced hyperglycaemia caused by alloxan-diabetic rats. The flavonoids present in *may* also be acting similarly thereby decreasing the high blood glucose levels of alloxan-diabetic Wistar rats. The extract might possess Insulin like effect on peripheral tissues either by promoting glucose uptake and metabolism or inhibiting hepatic gluconeogenesis.

In conclusion, the results of the present study confirm the hypoglycemic effect of *Chrysanthellum indicum* extract when administered to alloxan -induced diabetic animal model

which suggest the presence of biologically active components which may be worth further investigation and elucidation. Further studies are in fact currently under way to isolate and characterize the active principle (s) of the crude extract.

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