

## Phytochemical Screening and Blood Glucose-Lowering Activity of Methanol Extract of *Icacina Trichantha* (Oliv.) Tuber

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### Abstract

Diabetes mellitus (DM) is a group of metabolic disorders characterized by high blood sugar, insulin resistance, and/or relative lack of insulin. The use of plants in the treatment of diabetes is pertinent to address and remedy the shortcomings and side effects of conventional drugs. *Icacina trichantha* (Oliv.) of the Icacinaceae family is reportedly used traditionally as medicine for the treatment of soft tumours and diabetes, and as an aphrodisiac, the tuber being the most widely used part. Here, we report the blood glucose-lowering activity of *Icacina trichantha* tubers using an *invivo* rat model. The air-dried tubers were pulverised and macerated in methanol for 72 h. The resulting extract was concentrated *in vacuo* and used for both the phytochemical screening and the treatment of alloxan-induced diabetes (80 mg/kg body weight) in albino Wistar rats via a single intraperitoneal injection. Blood glucose level was monitored every other day for seven (7) days using a Finetest glucometer. The phytochemical screening results showed the presence of saponins, tannins, alkaloids and phenols in abundance while flavonoids, steroids and glycosides were in moderate amounts in the extract. Our results showed a significant ( $p < 0.05$ ) peak blood glucose reduction of 76.00 % observed on day 7 of treatment with extract (1000 mg/kg). The result compared favourably with 81.76 % observed for glibenclamide (2.5 mg/kg) on the same day, justifying the ethnobotanical use of *Icacina trichantha* tuber in the management of type 2 diabetes.

### Introduction

Diabetes mellitus which is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both (American Diabetes Association, 2009) has been a very important public health problem with a steady increase in both the number of cases and its prevalence over the past few decades (NCD-RisC, 2016). The symptoms of marked hyperglycemia include polydipsia, polyuria, weight loss, sometimes with polyphagia, and blurred vision (American Diabetes Association 2009).

**Keywords:** *Icacina trichantha*, diabetes, hyperglycemia, *in vivo*, animal models

*Icacina trichantha* (Oliv.), commonly called 'false yam,' in English and 'Gbegbe' by the Yoruba-speaking tribe of Western Nigeria (Dalziel, 1937) is a small perennial shrub that is drought-resistant. The plant belongs to the *Icacinaceae* family and is indigenous to West and Central Africa (Buhkill, 1985). The plant is extensively used in rural areas and thus regarded as a major handy household medicine in an emergency; hence, virtually all households have the macerated tuber in ethanol stored in a corked bottle. The tuber (the most widely used part of the plant) is sometimes as large as yam and eaten during famine or dried and pounded to a white powder which is used in soup or added to food while the leaves are

used for wrapping processed oil bean seeds known as 'ugba' in Igbo (Buhkill, 1985).

*Icacina trichantha* tuber is commonly used in both Ayurveda and homoeopathic medicine, as well as by many traditional healers for the treatment of diabetes, constipation, poisoning, rheumatism, toothache, yellowness of eye which is usually associated with malaria, to induce emesis and abortion, while the juice of the tuber can be used to treat mumps (Asuzu and Ugwueze, 1990; Asuzu and Abubakar, 1995; Ariwado *et al.*, 2012). The tuber and the leaves are used as aphrodisiacs (Burkill, 1994), while the crushed leaves and the seeds, macerated in local gin are used for the treatment of hypertension and asthma (Ajibesin *et al.*, 2008).

This study is designed to investigate the anti-hyperglycaemic activity of *Icacina trichantha* tuber methanol extract using rats.

## Materials and Methods

### Plant Material

*Icacina trichantha* tubers were harvested in the Mariam area, Ago-Iwoye Ogun State, Nigeria, and identified and authenticated at the Forest Herbarium Ibadan (FHI) of the Forestry Research Institute of Nigeria (FRIN) Ibadan, where a voucher specimen with number FHI 110445 was deposited.

### Extract Preparation

*Icacina trichantha* tubers peeled and cut into small pieces, were air-dried and pulverized with an industrial blender. The crude methanol extract was prepared by maceration of 900 g of the pulverized tubers in methanol at room temperature for 72 h. The solvent was decanted and filtered every 24 h, and replaced with fresh portion throughout the extraction. The filtrates collected were pooled and concentrated using a Buchi Rotary Evaporator at 37 °C, weighed and stored in a desiccator until ready for analysis.

### Phytochemical Screening

The methanol extract of *Icacina trichantha* was subjected to phytochemical screening for the

presence of secondary plant metabolites using standard methods (AOAC, 1990).

### Biological Assay

Adult albino Wister rats of both sexes weighing between 150 and 200 g obtained from the animal house, Babcock University, Ilishan Ogun State, Nigeria were used for the experiment. The rats were housed in cages at room temperature, under a naturally illuminated environment of 12 h light and 12 h dark cycle, were fed on standard pelletized feed and allowed water *ad libitum*. The animals were cared for under the ethical guidelines and care of laboratory animals and *in vivo* animal studies of Olabisi Onabanjo University.

Diabetes was induced in overnight fasted rats intraperitoneally using freshly prepared alloxan monohydrate (80 mg/kg) in normal saline (Abdel-Barry *et al.*, 1997) and allowed to rest for three days to stabilize their blood glucose level. 72 h after induction, rats with blood glucose levels greater than 200 mg/dL were considered hyperglycemic and selected for the study.

Four groups of rats in cages labelled A, B, C and D containing 5 rats each were used for the experiment. Cage A contained the rats which were neither induced nor treated but were allowed food and water *ad libitum* and served as the normal control group. The alloxan-induced diabetic rats were separated into three groups in Cages B, C, and D. Cage B contained the diabetic untreated rats, which served as the negative control group; they were induced but untreated with neither the extract nor glibenclamide the standard drug. Cages C and D contained diabetic rats treated with the methanol crude extract (1000 mg/kg body weight, dissolved in methanol and distilled water), and glibenclamide (2.5 mg/kg) respectively serving as the test group and the positive control group.

All treatments were administered orally thrice daily throughout the test period using an oral cannula.

### Determination of Blood Glucose Level

All blood samples were collected by the tail-tipping method. The blood glucose levels were measured using a Finetest Glucometer and test strips on days 1 (at 0 min, 1 h, 2 h, 3 h), 3, 5 and 7 of the study. The test was carried out 2 h after the last dosage had been administered for each of the animals per group.

### Statistical Analysis

The data obtained from the experiment were analysed using a one-way analysis of variance (ANOVA). Inter-group comparisons were by LSD and Duncan post-hoc test (SPSS version 14). The level of significance was taken to be  $p < 0.05$  and values were expressed as Mean  $\pm$  SEM.

### Results and Discussion

#### Phytochemical Screening Results

The *Icacina trichantha* tuber extract contained saponins in high amounts; tannins, Alkaloids and phenols were moderately present while flavonoids, steroids and glycosides were also found in trace amounts but anthraquinones were absent as shown in the phytochemical screening results in Table 1.

**Table 1:** Phytochemical screening result of the methanol extract of *Icacina trichantha* tuber

Phytochemical Test	Result
Saponins	+++
Tannins	++
Alkaloids	++
Phenols	++
Flavonoids	+
Steroids	+
Glycosides	+
Anthraquinones	-

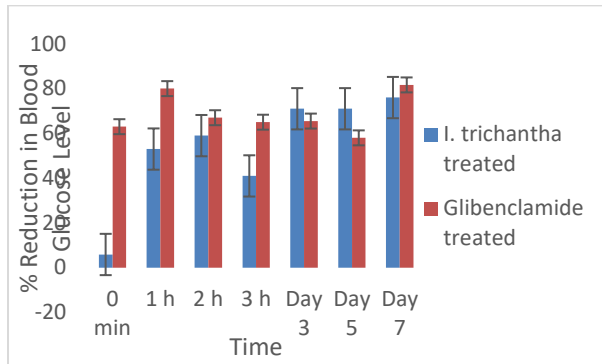
**Key:** +++ =High amount, ++ = moderate amount, + = Trace amount, - = Absent

The mean $\pm$ SEM values of the blood glucose concentrations obtained for each group of animals are presented in Table 2. Peak reduction in blood glucose levels of 77.26 mg/dL and 60.00 mg/dL were observed for rats in groups C and D treated with *I. trichantha* extract and glibenclamide respectively on day 7 which were significantly different from the diabetic untreated group B at  $p < 0.05$ .

**Table 2:** Blood Glucose levels (mg/dL) of Alloxan Induced Diabetic Rats Treated with *I. trichantha* tuber extract (1000 mg/kg) and Glibenclamide (2.5 mg/kg)

Cages	Day 1				Day 3	Day 5	Day 7
	0 min	1 h	2 h	3 h			
A Normal rats	94.50 $\pm 1.60$	100.74 $\pm 1.07$	95.50 $\pm 0.74$	102.76 $\pm 0.87$	109.30 $\pm 1.04$	96.06 $\pm 1.12$	80.00 $\pm 1.87$
B Diabetic Untreated	405.76 $\pm 7.13$	409.40 $\pm 6.79$	380.76 $\pm 6.35$	358.32 $\pm 6.07$	361.76 $\pm 7.61$	291.3 $\pm 45.22$	327.0 $\pm 13.22$
C <i>I. trichantha</i> tuber extract (1000 mg/kg)	326.26* $\pm 8.82$	192.40* $\pm 16.20$	154.50* $\pm 4.92$	158.50* $\pm 12.08$	103.04* $\pm 0.51$	82.66* $\pm 0.58$	77.26* $\pm 2.82$
D Glibenclamide (2.5 mg/kg)	153.50* $\pm 4.44$	129.26* $\pm 6.51$	123.26* $\pm 2.22$	136.76* $\pm 7.60$	124.54* $\pm 2.75$	120.32* $\pm 13.96$	60.00* $\pm 0.89$

Values are mean  $\pm$  SEM, n=5, \* significantly different from control at  $p < 0.05$



Values are Mean  $\pm$  SEM n=5

**Figure 1:** Percentage reduction in blood glucose level of alloxan-induced diabetic rats treated with *I. trichantha* extract and glibenclamide in groups C and D respectively.

There are different classes of diabetes but the majority of cases are classified as either type 1 or type 2 diabetes (American Diabetes Association, 2011). Also, there are quite many synthetic drugs or groups of drugs employed in the management of the disease but undesirable side effects are sometimes experienced with their usage. Medicinal plants are being focused on for possible use in the treatment of diabetes because of their phytochemical constituents that may give definite pharmacological actions in the human body (Akinmoladun *et al.*, 2007). The phytochemical screening of methanol extract of *Icacina trichantha* tuber shows the presence of saponins, alkaloids and tannins, steroids and glycosides (Table 1). The results are in agreement with other reports by Timothy and Idu, 2011; Shagal *et al.*, 2014 and Edori and Ekpete, 2015, while some other studies reported the presence of carbohydrates. The anti-hyperglycemic effect of the tuber could be related to the abundance of saponins in it. This is because saponins have been reported to cause a reduction in blood glucose levels by inhibiting the enzymes responsible for the breakdown of disaccharides into monosaccharides (Oishi *et al.*, 2007). The presence of flavonoids in the tuber extract is being reported here. This study shows that there is a relative abundance of phenols in the tubers and various phenolic phytochemicals have shown prospective beneficial effectiveness

against diabetes mellitus by moderating metabolic and signalling pathways at various levels including gene expressions, epigenetic regulation, protein expressions, and enzyme activities such as *in vitro*  $\alpha$ -glucosidase and  $\alpha$ -amylase activities (Kim *et al.*, 2000; Bennick, 2002; Hoda *et al.*, 2019). Table 2 shows the potential of the methanol extract of *Icacina trichantha* tuber (1000 mg/kg) to reduce blood glucose levels in experimentally-induced diabetic rats from 192.40 mg/dL at the first hour of treatment on day 1 to 77.26 mg/dL on day-7 while glibenclamide caused a reduction from 129.26 mg/dL to 60.00 mg/dL within the same period. The percentage reduction in blood glucose level caused by the extract increased steadily from day 1-7 (5.90%-76.00%). These results are in tandem with that of a similar work by Onakpa and Asuzu (2013), in which a dose-dependent reduction in fasting blood glucose levels and improved serum biochemical parameters were reported. The *I. trochanter*-treated group was seen to exert an anti-hyperglycemic effect almost as potent as the glibenclamide-treated group. This is because glibenclamide a highly purified drug, like any other sulphonylurea, is effective in mild diabetic states and ineffective in severe diabetic animals where pancreatic  $\beta$ -cells are destroyed (Qamar *et al.*, 2011). The statistical analysis showed that there was no significant difference between the normal (positive control) and glibenclamide groups but a statistically significant difference between the treated groups and the diabetic untreated group (Table 2). The extract of *Icacina trichantha* tuber also showed a normo-glycemic effect with blood glucose level reducing from 326.26 mg/dL on day 1 to 77.26 mg/dL on day 7 of treatment (Table 2). Thus, *I. trichantha* tuber methanol extract was able to reduce the blood glucose concentrations of the animals treated to within normal levels. It is pertinent to mention that the *Icacina trichantha* tuber extract used in this study is the crude extract thus, its effect on blood glucose concentration could be improved on purification.

## Conclusion

In conclusion, this study shows that oral administration of the methanol tuber extract of *Icacina trichantha* reduces blood glucose levels in alloxan-induced rats therefore suggesting that the extract has anti-hyperglycemic activity. The tuber extract has been used traditionally in the management of type 2 diabetes and our finding adds to the scientific data validating this use. The presence of secondary metabolites such as saponins, alkaloids, tannins and phenols no doubt contributes to the observed anti-hyperglycemic activity by their inhibitive actions on the enzymes responsible for the breakdown of disaccharides into monosaccharides and their moderation of metabolic and signalling pathways. Further work is recommended to purify and isolate the compound(s) responsible for the blood glucose-lowering effect observed and to establish the mechanism by which this is done.

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