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Aqueous ethanolic extract of *Acalypha inferno* accelerates the clearance of glucose in normoglycaemic rats

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Abstract
Glucose tolerance refers to the body's ability to metabolise glucose within a stipulated time interval. Failure of the body to use glucose results in hyperglycaemia and progressively to diabetes. *Acalypha inferno* (family Euphorbiaceae) is an ornamental plant found widely in the tropics of Africa and known for its phytoremediating properties. The study was aimed at investigating the effects of aqueous ethanolic extract of *Acalypha inferno* on oral glucose tolerance in normoglycaemic rats. A 50% hydro-ethanolic extract of the leaves was prepared, and the oral glucose tolerance effect of the plant assessed for 14 days in normoglycaemic rats at doses of 100mg, 250mg and 500mg/kg body weight. Glibenclamide (10mg/kg) was used as a standard drug. The effect of treatment on body weight, OGTT at day 7 and 14, lipid profile and kidney function were assessed. On day 7, FBG level increased from 4.93±0.29mmol/l to 25.03±0.93mmol/l after 1 hour and 17.53±4.50mmol/l after 3 hours in normal group. Extract and drug treated groups prevented such increases at all doses (p<0.001). FBG levels were restored to basal levels after 8 hours. Total cholesterol increased in the 250 mg group with a significant increase in LDL levels in the 100mg, 250mg and 500mg group compared to the normal. There was no significant difference in relative kidney weight, urea and creatinine levels. The hydro-ethanolic extract of *Acalypha inferno* possesses glucose tolerance abilities in normoglycaemic rats.

Keywords: Oral glucose tolerance, *Acalypha inferno*, Normo-glycaemic rats, Hyperglycaemia

Introduction
Our world today has contributed to the changing lifestyles of many people across the globe. Our diet and the way we live have been associated with many diseases of which diabetes is no exception. Diabetes mellitus is a metabolic disorder caused by the inadequate secretion of insulin, leading to an elevation in plasma glucose levels. [1] Diabetes is not a single disease but rather a heterogeneous group of syndrome characterized by glycosuria, hyperlipidaemia, and hyperglycaemia and glucose intolerance due to relative or absolute insulin deficiency. [2] Glucose intolerance has been reclassified by the World Health Organization (WHO), incorporating a new class known as impaired fasting glycaemia. [3] Accordingly, WHO defines glucose intolerance as a fasting blood glucose level of above 6.0 mmol/L or over 7.8 mmol/L two hours after consuming 75 g of glucose. [4] This classification includes people with either impaired fasting glucose (IFG) and impaired glucose tolerance (IGT). From epidemiological studies conducted by the National Institute for Health and Care Excellence (NICE), the prevalence of impaired glucose tolerance increases linearly from about 15% in middle age to 35-40% in the elderly. [5] Also, the International Diabetes Federation Atlas [6] indicated that the population of individuals with impaired glucose tolerance stood at 415 million. It was estimated that 5 million people died in 2015 from diabetes and associated ill-health which was equivalent to one death every six seconds. [7]

Screening guidelines by the American Diabetes Association [6] outlines two tests used to diagnose glucose intolerance namely the fasting plasma glucose test and oral glucose tolerance test (OGTT). The oral glucose tolerance test (OGTT) is a diagnostic test that measures the body's ability to use glucose. It is not often used as a diagnostic test for Type 2 diabetes because it does not give a direct measure of insulin impairment. However, it is extensively used as a sensitive indicator of gestational diabetes. The test is based on oral administration of glucose and subsequently following plasma glucose and insulin levels over time. An elevation (>120 min after administration) in both plasma glucose and insulin constitutes impaired glucose tolerance. In OGTT, glucose level more than 11.1 mmol/L suggests diabetes. [6] The OGTT is influenced by many factors including age, diet, state of health, GI function and emotional state.

According to research, many plants have been found to possess antidiabetic and hence glucose tolerance properties. [8] Notable amongst them are *Acalypha indica* and *Allium sativum. Acalypha inferno* (L, family Euphorbiaceae) is known as flame copperleaf and originated from the South Pacific Islands. [9] It is a sun-loving perennial shrub and known for its aesthetic value. *Acalypha inferno* has also been found to be a potent phytoremediator of zinc. [10] Phytochemical analyses on hydroethanolic leaves extract of Ghanaian cultivars has shown the presence of triterpenoids, sterols, alkaloids, coumarins, flavonoids, saponins, glycosides and hydrolysable tannins with significant free radical scavenging activity and antimicrobial effect. [11] It is also safe in rodents. [12] The main aim of this study was to investigate the oral glucose tolerance effects of hydroethanolic extract of *Acalypha inferno* on normoglycaemic rats.

Materials and Methods

1. Plant Preparation and Extraction

Leaves of *Acalypha inferno* were collected from the forecourts of the Biochemistry Annex, KNUST. The plant material was authenticated at the Department of Herbal Medicine, KNUST-Kumasi by Dr. George Sam (taxonomist) and a voucher specimen deposited at the herbarium for reference (KNUST/HM/2017/L018). They were destemmed, washed, shade dried for 2 weeks and milled. A 50% aqueous-ethanolic extraction was carried out by suspending 100g of the powdered extract in 1000ml of 50% ethanol (50:50, ethanol: water, v/v). The mixture was allowed to stand for 24 hours at room temperature on a mechanical shaker (YLK, Japan). The mixture was then filtered using a cotton wool and concentrated using a Heidolph rotary evaporator (Germany) under reduced pressure. The extract was transferred into sterile zip-lock bags, frozen and freeze-dried to obtain the hydroethanolic extract of *Acalypha inferno* (AIE). Respective doses for the study were prepared by reconstituting the extract in distilled water.

2. Preparation of Standard Drug

Glibenclamide (Diabenol, Thailand) tablets, an oral hypoglycaemic drug was used as standard drug. The tablets were dissolved in freshly distilled water to form a suspension and administered at a dose of 10 mg/kg body weight (b.wt) orally.

3. Experimental Animals

Female Wistar rats weighing 115-150g were obtained from the University of Ghana Medical School, Legon. They were acclimatized for 14 days in an animal holding facility of the Department of Biochemistry and Biotechnology, KNUST, Kumasi. The animals were housed in aluminium cages under standard husbandry conditions (12hrs. light/dark cycle) and were provided freshly prepared distilled water and standard laboratory food (mash) during the study period. All animal experiments were performed according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiment on Animals (CPCSEA) and Guide for Care and Use of Laboratory Animals. [13] Animals were handled humanely throughout the duration of the experiment.

4. Experimental Design

The animals were divided randomly into five groups by similar body weights with 4 rats in each group and treated as follows for 14 days. Doses of AIE were selected based on safety data. [12]

Group 1: Normoglycaemic rats administered 1.0 ml distilled water per day orally.
Group 2: Normoglycaemic rats administered 10 mg/kg b.wt glibenclamide per day orally.
Group 3: Normoglycaemic rats administered 100 mg/kg b.wt AIE per day orally.
Group 4: Normoglycaemic rats administered 250 mg/kg b.wt AIE per day orally.
Group 5: Normoglycaemic rats administered 500 mg/kg b.wt AIE per day orally.

5. Determination of Oral Glucose Tolerance

The fasting blood glucose (FBG) levels of the animals were determined after every 7 days of the extract administration. The FBG levels were determined by tail puncture at intervals of 0 (before glucose administration), 1, 4, 8 and 24 hours after glucose administration on the 7th day and 14th day using the OneTouch Select Glucometer and test strips. The results were reported in mmol/L. Prior to the test, the animals were fasted overnight and administered 10 mg/kg b.wt glucose solution before their FBG levels assessed.

6. Effect of Treatment on Body Weight

The weights of the animals were measured every four days till the 14th day using a mass balance. The percentage change in body weights were calculated by measuring the initial body weight and the body weight at each day interval given by the formula:

$$\%\ Change\ in\ Body\ Weight = \frac{Weight_n - Weight_{initial}}{Weight_{initial}} \times 100$$

Where Weight $_n$ is the body weight on Day 4, D8…D14 and Weight $_{initial}$ is the body weight on D0

7. Determination of Biochemical Parameters

After 14 days of treatment, the animals were sacrificed by ether anaesthesia after an overnight fast. Incisions were quickly made in the cervical regions of sacrificed animals using sterile blade and blood collected into Serum Separator Tubes and centrifuged at 3000rpm for 15 minutes. The sera were separated into Eppendorf tubes and stored at 4^{o}C prior to analyses. Biochemical parameters such as Total cholesterol, triglycerides, high density lipoproteins (HDL) and low density lipoproteins (LDL) were measured. Serum creatinine and urea levels were also measured using the Flexor Chemistry Analyser (Japan).

8. Effect of Treatment on Relative Organ Weight

The organs of the experimental animals, liver and kidneys were excised, cleaned with buffered saline and weighed to obtain the absolute organ weight (AOW). The relative organ weights were calculated using the formula:

$$ROW = \frac{AOW}{Weight\ at\ Sacrifice} \times 100$$

9. Data Analysis

The GraphPad Prism 5.0 was used to analyze the data. The data was subjected to two-way ANOVA to compare the differences in mean values between the groups. This was followed by Bonferroni Post hoc multiple comparison test to assess the actual differences existing between each experimental group. The data was run at a 95% confidence and P values <0.05 was considered statistically significant.

Results

1. Effect of Treatment on Percentage Change in Body Weight

Figure 1 shows the effect of treatment on the percentage change in body weight of the rats. The body weights were measured from day 0 to day 14 at a four-day interval. A varied effect was observed.

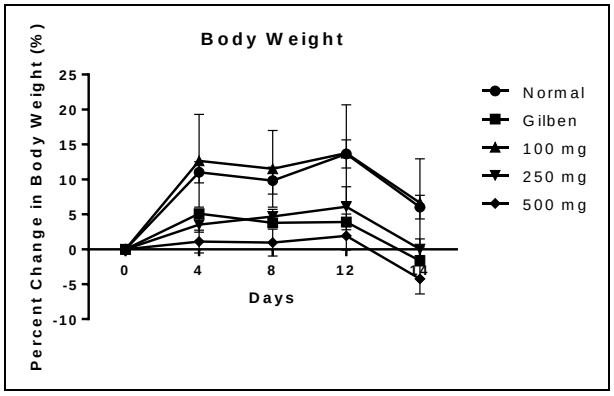

Fig 1: Effect of treatment on body weight of the rats. Each point represents a mean ±SEM of 4 animals

2. Effects of Treatments on Glucose Tolerance on Day 7

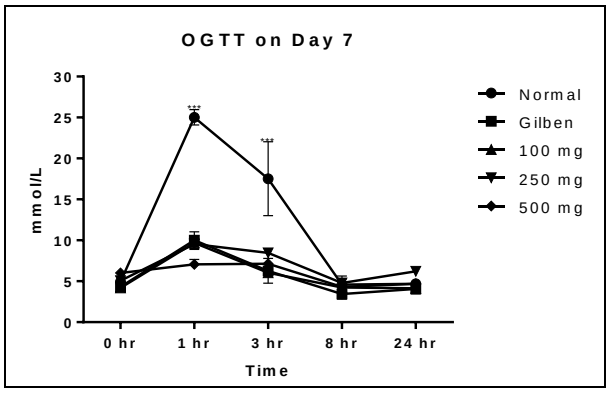

Fig 2: Effect of treatment on oral glucose tolerance (OGT) on day 7. Each point represents the mean ±SEM of 4 animals.

Figure 2 shows a time series of the effect of the treatment on glucose tolerance on day 7. A sharp increase in plasma glucose levels from the baseline readings (4.93±0.29 mmol/L) in the normal group in the 1st hour (25.03±0.93 mmol/L) was observed. Blood glucose levels decreased in the 3rd hour with a further decline in glucose level in 8th hour to the 24th hour. A decrease in plasma glucose levels in the 500mg group in the 1st hour compared to the glibenclamide group was also observed.

3. Effects of Treatments on Glucose Tolerance on Day 4

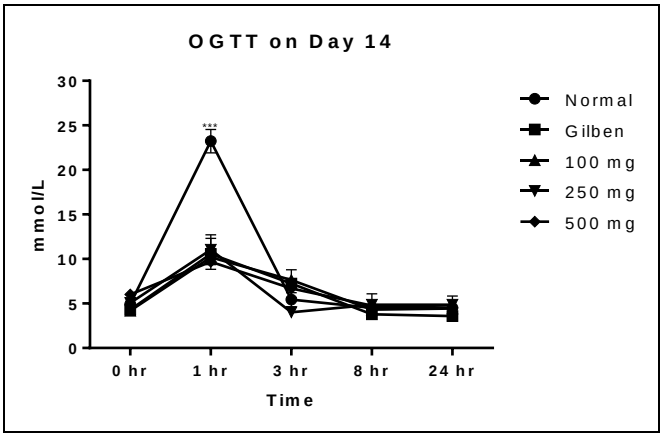

Fig 3: Effects of treatment on OGT ont day 14. Each point represents a mean ±SEM of 4 animals.

Fig 3 shows a time series of the effect of the treatments on OGTT on day 14. A similar trend observed where there was increase plasma glucose levels from the baseline in the normal group in the 1st hour followed by a sharp decline in the 3rd, 8th and 24th hour. All drug treated groups showed normal glucose levels.

4. Effects of Treatment on Relative Organ Weight (ROW)

Table 1 shows the effect of the treatment on the relative weights of the liver and kidneys. There was no signficant difference in weights of the organs in all groups compared to the normal.

Table 1: Effect of treatment on relative organ weight

	Normal	Glibe	100 mg	250 mg	500 mg
Liver	2.42±0.07	2.46±0.10	2.40±0.10	2.74±0.10	2.38±0.09
Kidney	0.64±0.03	0.58±0.03	0.63±0.03	0.65±0.02	0.63±0.02

Mean ±SEM, n=4, **p*<0.05 compared with Normal**.**

5. Effects of Some Biochemical Indices

Table 2: Effects of treatment on lipid profile.

	Normal	Glibe	100 mg	250 mg	500 mg
TChol	1.35±0.15	1.43±0.06	1.57±0.03	1.70±0.10	1.48±0.08
TG	0.85±0.05	0.70±0.06	0.87±0.12	0.90±0.06	0.93±0.05
HDL	0.90±0.04	0.83±0.05	0.70±0.06	0.93±0.09	0.65±0.06
LDL	0.28±0.12	0.46±0.03	0.69±0.07	0.59±0.04	0.64±0.13
Urea (mmol/L)	8.70±0.68	8.53±0.77	9.93±0.66	7.80±0.35	8.05±0.29
Creat (μmol/L)	51.75±5.15	44.75±3.42	48.00±2.65	45.33±4.33	46.25±1.03

Mean±SEM, n=4

Table 2 shows the effects of the treatments on some biochemical parameters (lipid and kidney profile). Treatments had no significant effect on the assessed parameter

Discussion

The use of orthodox medications such as sulphonyl urea in the treatment of diabetes have become common compared to the use of plant based drugs in our world today. These orthodox medications have over the years left many undesirable effects such as lactic acidosis, unexplained weight loss, amongst others on its users. [14] The use of organic or plant based drugs in the management of diabetes are therefore in high demand today compared to their synthetic forms since they are considered less toxic and free from side effects. The oral glucose tolerance ability of hydroethanolic extract of *Acalypha inferno* was investigated. Results obtained from the effects of treatments of the extract on the percentage change in body weight as showed in Figure 1. It is known that in individuals with lower glucose tolerance abilities or diabetes, glucose in circulation is not made readily available to cells to be used as energy. The body therefore relies on sources such breaking down fat stores and proteins to release energy which contributes to the drastic weight loss in hyperglycaemic or diabetic individuals. [15] The decline can also be attributable to effect of extract (especially at 500 mg) on the appetite of animals and it resultant effect of energy metabolism. [12]

Ad isitration of glucose in the OGTT resulted in signifcant increase in circulating glucose levels in animals in the first 3 hours. With time, plasma glucose levels were observed to return to basal levels. However, the adminsitration of a hypoglycemic agent could result in glucose levels returning basal levels faster than normal.

Glibenclamide belongs to the class of sulphonyl ureas and it acts by increasing the sensitivity and responsiveness of the beta cells of the pancreatic islets so that more insulin would be produced to lower the amount of glucose in circulation. [16] This accounted for the decline in glucose levels with time. It was observed that AIE at all doses resulted in significant decline in glucose levels from the 1st hour after administration. It could mean that the extract possessed a similar mechanism like the standard drug and as well increase the permeability of glucose into the cells by increasing the activity of glucose transporters to take up more glucose into the cells. [17] A similar trend was observed in the OGTT performed at day 14 in Fig 3 and hence the same mechanism of action of the standard drug and extract accounted for the decrease in glucose levels in the rats at their respective doses. The observed effect can be attributed to the phytochemical detected in AIE. [11] Flavonoids have been found to inhibit advanced glycation end product and also act as aldose reductase inhibitors. [18] According to research, hyperglycaemia causes the autooxidation of glucose to reactive oxygen species (ROS) or free radicals. As a result of this, diabetic individuals tend to have elevated amounts of the enzyme aldose reductase. [18] This enzyme is a key enzyme in the polyol pathway and catalyzes the NADP dependent reduction of glucose to sorbitol leading to the excessive accumulation of intracellular reactive oxygen species in the tissues and organs of diabetics. Flavonoids inhibit this enzyme and hence conversion of glucose to sorbitol is inhibited and this is expected as extract has been demonstrated to possess in vitro antioxidant activity. [11] Saponins have also been found to regulate glucose and lipid metabolism hence controlling hyperlipidaemia and hyperglycaemia. [17]

No significant change observed in the relative liver and kidney weights, which indicated that the extract at any dose was non- toxic to these organs. This was supported by the observed kidney function assay results (Table 2).

Table 2 showed the effects of the treatments on lipid profile. A non-significant change in the lipids were observed in all treated groups. Lipid profile is usually performed and used as part of a cardiac risk assessment to determine an individual's risk of cardiovascular diseases. [19] Higher amounts of total cholesterol and LDL are used linked to increased coronary risks. [20] Hyperglycaemia and diabetes are often characterised by hyperdyslipidaemia and hypertriglyceridemia. [21] It is therefore suggested that the use of Acalypha inferno as a hypoglycaemic agent also has lipid modulation effect to prevent dyslipidaemia associated with diabetes.

Conclusion

Acalypha inferno has been shown to possess oral glucose tolerance activity. Normo-glycemic rats administered 100 mg, 250 mg and 500 mg/kg bwt of the extract showed decreased levels of plasma glucose during the OGTT, giving experimental proof that the extract up to 500 mg could reduce plasma glucose levels.

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