

Research Article

Hypoglycemic Activity of *Curcuma longa* Linn Root Extracts on Alloxan Induced Diabetic RatsA. Mohammed^{1*}, A. M. Wudil², A. J. Alhassan², A.A. Imam², I. U. Muhammad², A. Idi²¹Department of Biochemistry, College of Medical Sciences, Abubakar Tafawa Balewa University, PMB 0248 Bauchi, Nigeria²Department of Biochemistry, Faculty of Basic Science, Bayero University, PMB 3011, Kano, Nigeria***Corresponding Author:**

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Abstract: Plants form the main ingredients of medicine in traditional system of healing and have been the source of inspiration for several major pharmaceutical drugs. In spite of great advances of modern scientific medicine, there is a rapid increase in the use of alternative medicine worldwide. To investigate the effect of the aqueous root extract, Methanol and n-Hexane root fractions of *Curcuma longa* Linn on Alloxan Induced Diabetic Rats. A total of thirty-six (36) rats were used and were grouped into six (6) groups of six (6) rats each. Group I served as normal control, group II served as diabetic control, Group III rats were induced with diabetes and administered with standard drug (Metformin, 100mg/kg) while Groups IV, V and VI were induced with diabetes and administered with crude aqueous extract, methanol and n-hexane fractions respectively at a dose of 400mg/kg body weight for four weeks. The research found that the methanol fraction showed highest potency with a significant ($p < 0.05$) decrease in blood glucose level when compared to diabetic control after few days of fraction administration, it was followed by the aqueous extract which also shows hypoglycemic activity a week after administration. N-hexane fraction showed a marked hypoglycemic activity only after about two weeks of administration with the fraction. The present study demonstrated that the methanol fraction possesses the highest hypoglycemic activity.

Keywords: Alloxan, Hypoglycemic activity, *Curcuma longa linn* and Metformin.

INTRODUCTION

Diabetes mellitus is a non-communicable disease which have been shown to improve with medicinal plants. It is a metabolic disorder characterized by chronic hyperglycemia with disturbances of carbohydrate, fats and protein metabolism resulting from defects in insulin secretion, insulin action or both [1]. Diabetes is also referred to as a syndrome of disorder in metabolism usually due to the combination of hereditary and environmental causes resulting in abnormally high blood sugar levels (hyperglycemia) [2]. Blood glucose levels are controlled by a complex interaction of multiple chemical and hormones in the body including the hormones insulin made in beta cells of the pancreas. Diabetes mellitus develops due to diminished production of insulin (in type I) or resistances to its effects (in type II and gestational), both leads to hyperglycemia, which largely causes the acute signs of diabetes and changes in energy metabolism [3]. As a result of the deficiency of insulin or inadequate insulin function there is an inadequate transfer of glucose into the cells; the utilization of glucose for energy and cellular products and its conversion to glycogen or fat

and storage as such are depressed, thereby leading to accumulation of glucose in the blood, causing hyperglycemia. Fat may be mobilized from adipose tissue and broken down to provide a source of energy, which is eventually withdrawn from the body by the liver and broken down to glycerol and fatty acids leading to oxidation by the hepatic cells to ketone bodies and metabolizes by cells to produced energy, carbon dioxide and water. Only a limited amount of ketone acids can be utilized by cells as such if ketogenesis proceeds rapidly, exceeding the rate at which they can be metabolized, the ketone acids accumulate in the blood causing ketosis or ketone acidosis [4]. Tissue protein may also be broken down to amino acids which are used in gluconeogenesis contributing to the hyperglycemia. Both the uptake of amino acids by the cells and body protein synthesis are decreased. Insulin-dependent diabetes mellitus (IDDM) usually has a sudden onset in a severe, acute form. In non-insulin-dependent diabetes mellitus (NIDDM) the onset is most often insidious going undetected and untreated for a considerable period of time.

Diabetes mellitus, a chronic non-communicable disease, is ranked 7th killer disease in the world with an estimated 382 million people affected, as reported by [5]. Conventional drugs used in the treatment of diabetes are sometimes inadequate, expensive and can have serious side effects. It is therefore imperative to search for alternative drugs of higher efficacy and safety to replace and/or support the currently used drugs for the treatment and/or management. The world health organization has also recommended the evaluation of the effectiveness and safety of plants used in traditional and complementary medicines [6].

The plant Turmeric (*Curcuma longa*), also known as *Gangamau* in Hausa language is a rhizomatous herbaceous perennial plant of the ginger family (*Zingiberaceae*), *Zingiberaceae* grows 5 - 6 feet high in the tropical regions of Southern Asia, with trumpet-shaped, dull yellow flowers. Its roots are bulbs that also produce rhizomes, which then produce stems and roots for new plants. Turmeric has a bitter and somewhat sharp taste; individual plants are about 1m tall with long oblong leaves when exposed to temperatures between 20 °C and 30 °C (68 °F and 86 °F) and a considerable amount of annual rainfall. Plants are gathered annually for their rhizomes, and are reseeded from some of those rhizomes in the following season. The rhizome from which the turmeric is derived, is tuberous, with a rough and segmented skin which mature beneath the foliage in the ground, they are yellowish brown with a dull orange interior. The main rhizome is pointed or tapered at the distal end and measures 2.5–7.0 cm (1–3 inches) in length and 2.5 cm (1 inch) in diameter, with smaller tubers branching off. When the turmeric rhizome is dried, it can be ground to a yellow powder with a bitter, slightly acid, yet sweet, taste. The known active compounds in Turmeric include curcuminoids, a family of curcumin and related compounds and the volatile oil fraction, characterized by turmerones. Research is focusing on the whole herb and its extracts which are expected to be more effective than isolated curcumin [7].

Studies suggest Turmeric originated in Southern India which continues to be the world's largest producer. As a seedless plant, its movement to new locations throughout the tropics has been dependent upon people. By 800 AD Turmeric had spread across much of Asia, including China, and across Africa. By the 18th century Turmeric made its way to Jamaica and it is now cultivated throughout the tropics, including Hawaii and Costa Rica. In the ancient times, Ayurvedic medicine used Turmeric for the digestive, circulatory and respiratory systems. It was used in treating indigestion, cough, arthritis, diabetes and purifying blood. Similarly, Chinese medicine uses Turmeric for the treatment of epigastric and abdominal pain, various menstrual irregularities, swellings and trauma [8].

MATERIAL AND METHODS

Material

Study Animals

Male and female albino rats weighing about 100g and 120g were purchased from animal house of Biological Science Department, Bayero University, Kano. The animals were housed in well-ventilated cages in the animal house of Biological Science Department of Bayero University Kano. The rats were allowed to acclimatize for one week prior to the experiment and had access to food and clean water.

Plant Material

Tumeric (*Curcuma longa*) roots were collected from Toro local Government Area of Bauchi State-Nigeria. The plant was identified and authenticated at the Herbarium of Plant Biology Department, Bayero University Kano and was given a voucher number of (BUK/HAN/0188). The root was shade dried and ground to powder.

Methods

Extract Preparation

Crude Aqueous Extract Preparation

The dried plant was manually grounded using pestle and mortar and the powdered plant kept in air tight container until used. Exactly 500g of the dried sample was soaked in 1 litre of distilled water in a conical flask. The suspension was shaken vigorously and left to stand at room temperature for 24 hours with intermittent vigorous shaking. The extract was then after filtered with muslin cloth and then was filtered by passing through Whatman's Filter No.1. The filtrate thus obtained was concentrated by complete evaporation of solvent using rotary evaporator to yield the crude aqueous extract E1.

Methanol and n-hexane fractions preparation

The hundred gram of the powder was weighed and soaked in two litres of 1:1 methanol and n-hexane, the solution was shaken vigorously and left to stand at room temperature for 24 hours. The suspension was transferred into a separatory funnel for separation and the extracts were concentrated and evaporated using rotary evaporator and dried under water bath to yield methanol fraction F1 and n-hexane fraction F2.

The volume to be administered to animals was calculated according to the method of [9].

Volume to be administered in mls =

$$\frac{\text{weight of rats in (kg)} \times \text{Dose (mg/kg)}}{\text{Concentration of Extract (mg/ml)}}$$

Preparation Of Alloxan

One (1) gram of Alloxan Hydrate was dissolved in 10ml of distilled water and to give a concentration of 100mg/ml.

Induction Of Diabetes Mellitus With Alloxan

Rats induced with Diabetes mellitus were fasted overnight for a period of 12 hours; diabetes was induced by injecting alloxan hydrate intraperitoneally at dose of 100 mg/kg using a sterile 1ml syringe. The volume of the solution containing 100 mg/kg given to each experimental albino rat was determined by the following relationship;

$$\text{Volume to be administered in mls} = \frac{\text{weight of rats in (kg)} \times \text{Dose (mg/kg)}}{\text{Concentration of Extract (mg/ml)}}$$

Animals with fasting blood glucose ≥ 200 mg/dl after 48 hours of alloxan administration were considered to be diabetic and are used for the study [9].

Experimental Protocol

Thirty-six (36) rats were used and were grouped into six (6) groups of six (6) rats each.

Extracts were administered to the animals for the period of four weeks.

Group I: Normal Control

Group II: Diabetic Control

Group III: Standard drug (Metformin, 100mg/kg body weight)

Group IV: Diabetic, administered with 400mg/kg body weight of crude aqueous extract E1

Group V: Diabetic, administered with 400mg/kg body weight of methanol fraction F1

Group IV: Diabetic, administered with 400mg/kg body weight of n-hexane fraction F2

Fasting blood glucose concentrations of rats was determined at an interval of three days throughout the period of extract administration.

Effect of Extract on Weight, Feed and Water Intake of the Rats

Feed and water intakes were measured every day at the same hour during the experimental periods while the body weight of the animals were measured at zero day and every seventh day for the period of the experiment.

Statistical Analysis

Results were expressed as mean \pm standard deviation and analyzed using ANOVA, with p value < 0.05 considered significant followed by Tukey's post hoc test. A component of GraphPad InStat3 Software [10] version 3.05 by GraphPadInc was used to analyze the data.

RESULTS AND DISCUSSIONS

Results

Table 1 shows the effect of oral administration of the different solvents (aqueous crude extract E1, methanol fraction F1 and n-hexane fraction F2) on fasting blood glucose concentrations of diabetic rats taken at the interval of three (3) days over four weeks of treatment. At 48th hours after alloxan injection, the blood glucose levels of group II (diabetic control group) and test groups (groups III – VI) increase significantly ($p < 0.05$) compared to normal control (group I), thus confirming induction of diabetes in the diabetic control group and test groups. A significant decrease ($p < 0.05$) was observed in fasting blood glucose of diabetic rats in group III, group IV and group V treated with standard drug, aqueous crude extract E1 and methanol fraction F1 respectively following six (3), twelve (12), six (6) and fifteen (15) days of extracts administration.

Table 1: Blood glucose level (mg/dl) of rats before and after alloxan induction, and at three days' interval following treatment with 400mg/kg body weight of *Curcuma longa* linn Crude aqueous extract, methanol and n-Hexane fractions for 28 days

GROUP	No Alloxan	48 Hrs	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18	Day 21	Day 24	Day 27
Normal	85.25 ±7.63	82.00 ^{a,b,c,d,e} ±4.30	79.80 ±8.32	83.40 ±2.97	88.40 ±4.98	87.40 ±3.44	86.00 ±4.00	83.40 ±1.95	81.00 ±1.73	82.00 ±0.71	80.40 ±1.14
Diabetic	87.25 ±4.43	215.60 ^a ±56.08	230.80 ^{a,b} ±25.28	279.80 ^{a,b} ±21.66	307.60 ^{a,b,c,d} ±21.43	310.20 ^{a,b,c,d} ±9.06	354.60 ^{a,b,c,d} ±7.60	363.20 ^{a,b,c,d} ±5.22	370.60 ^{a,b,c,d} ±5.64	379.80 ^{a,b,c,d} ±5.23	385.20 ^{a,b,c,d} ±5.54
Metformin	83.25 ±7.27	196.60 ^b ±25.20	118.40 ^a ±13.74	120.20 ^a ±26.93	95.40 ^a ±12.26	83.40 ^a ±4.93	82.60 ^a ±2.30	87.20 ^a ±5.02	88.20 ^a ±10.92	86.20 ^a ±3.70	80.40 ^a ±6.45
C. Aqueous	81.60 ±4.56	245.40 ^c ±25.67	205.00 ±11.36	180.80 ±20.56	154.40 ^b ±20.88	120.20 ^b ±14.31	108.00 ^b ±12.71	88.20 ^b ±4.15	84.60 ^b ±3.44	83.40 ^b ±2.07	81.40 ^b ±1.14
Methanol	80.40 ±7.79	212.20 ^d ±22.12	159.20 ^b ±30.68	118.00 ^b ±3.13	108.40 ^c ±10.88	98.20 ^c ±5.59	89.80 ^c ±5.02	84.00 ^c ±3.08	83.2 ^c ±4.60	81.60 ^c ±2.61	79.60 ^c ±0.89
n-Hexane	83.80 ±6.34	247.2 ^e ±41.78	207.00 ±29.18	196.80 ±16.75	170.00 ^d ±21.86	153.80 ^d ±18.82	120.60 ^d ±10.04	117.60 ^d ±7.72	114.00 ^d ±6.29	111.60 ^c ±5.39	98.60 ^d ±4.60

Results are expressed as mean ± SD (n=5). Values in the same column bearing similar superscripts are significantly different at P<0.05.

Figure 1 and 2 showed that the feed and water intake of the diabetic rats were increased throughout the study period as compared with the normal control group. However, after extract administration the feed

and water intake was markedly reduced as compared with the diabetic untreated rats. The effect of oral administration of extracts markedly increased the body weight of the animals as shown in Figure 3.

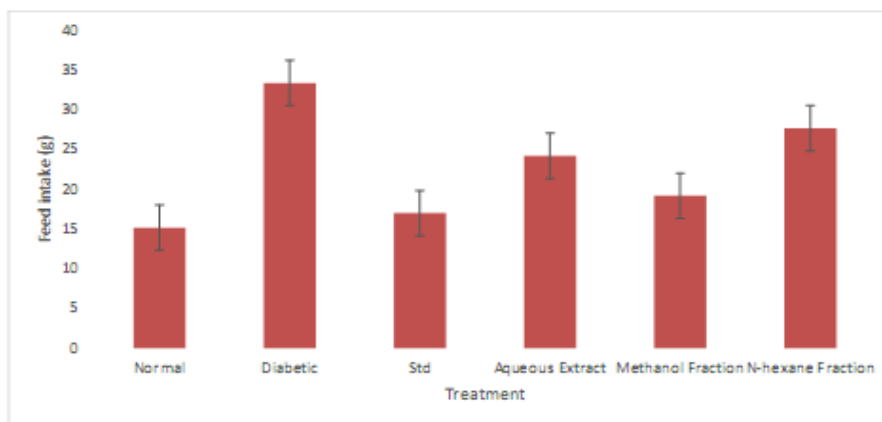


Fig-1: Effect of oral administration of 400mg/kg body weight of *Curcuma longa* linn Crude aqueous extract, methanol and n-Hexane fractions on feed intake
Results are expressed as mean \pm SD (n=5).

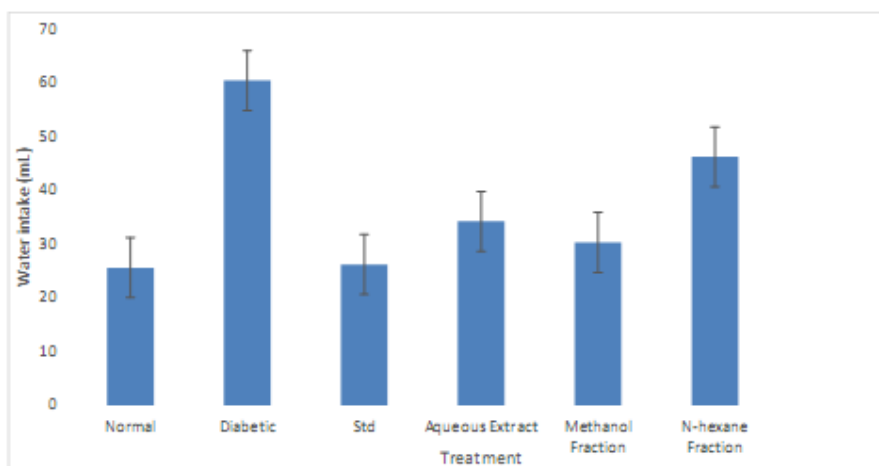


Fig-2: Effect of oral administration of 400mg/kg body weight of *Curcuma longa* linn Crude aqueous extract, methanol and n-Hexane fractions on water intake
Results are expressed as mean \pm SD (n=5).

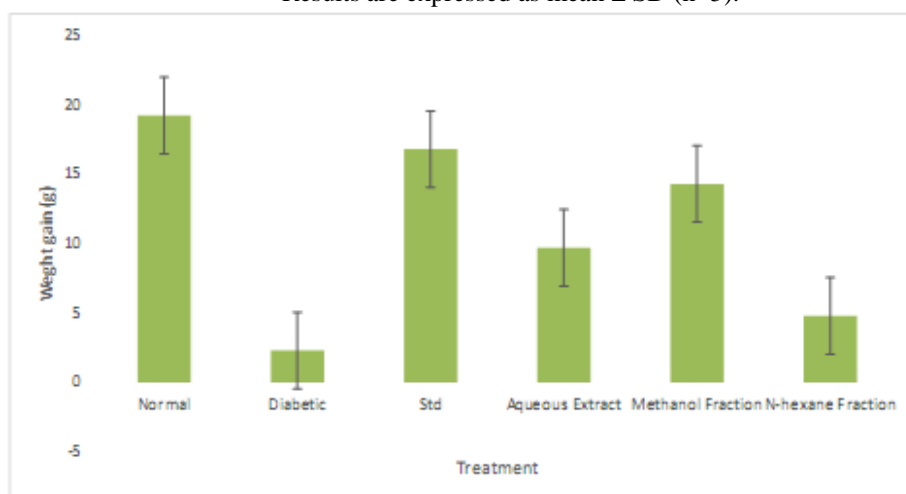


Fig-3: Effect of oral administration of 400mg/kg body weight of *Curcuma longa* linn Crude aqueous extract, methanol and n-Hexane fractions on weight gain
Results are expressed as mean \pm SD (n=5).

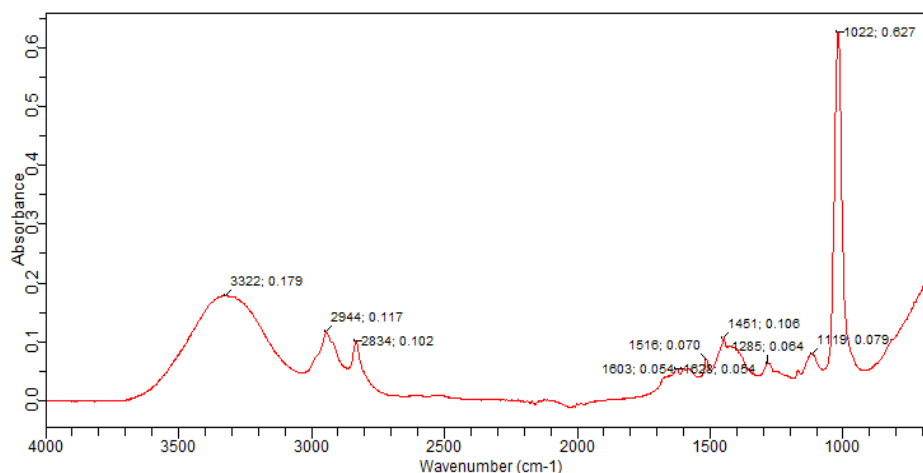


Fig-4: FTIR Analysis of methanol fraction of *Curcuma longa linn* root

DISCUSSION

The pancreas is an endocrine organ in vertebrates containing α -cells, β -cells, δ -cells and γ -cells; secreting glucagon, insulin, somatostatin and peptide proteins respectively. It also functions as an exocrine organ producing α -amylase, lipases, peptidases and ribonuclease which catalyze the hydrolysis of starch, fats, peptides and ribonucleic acids in the duodenum [11]. Alloxan is a toxic glucose analogue which selectively destroy β -cells of pancreas when administered to animals. This causes an insulin dependent diabetes mellitus known as alloxan-induced diabetes in the animals, which is characteristically similar to Type 1 diabetes in humans. Alloxan is selectively toxic to insulin producing pancreatic β -cells because it preferentially accumulates in β -cells through uptake via glucose transporter-2 (GLUT2) [12]. For all animals a single dose of alloxan, 140 – 180 mg/kg is administered to induce diabetes [13]. In this study, a milder approach to induce diabetes was adopted using a dose of 100mg/kg body weight of alloxan was used to achieve induction of diabetes to rats within a period of 48 hours before the commencement of extract administration. The result showed slow but steady induction of diabetes within 48 hours, this might be as a result of low dose used. This finding is in accordance with the research of [9,14] also reported the induction of diabetes using 120mg of alloxan but commenced treatment after five days of diabetes induction.

Administration of the extract for three and six days shows a significant decrease in blood glucose in groups administered with standard drug (Metformin, group III) and methanol fraction (Group V), but it was unable to reverse the marked hyperglycemia in group IV and group VI treated with crude aqueous extract and n-hexane fraction. Considerable normalcy in glucose levels in group IV and group VI treated with crude aqueous extract and n-hexane fractions became obvious on the 12th and 15th day of extract administration. The methanol fraction has the highest efficacy in decreasing glucose levels among all test groups, however both the

crude aqueous extract and n-hexane fraction produce substantial decrease in blood glucose level following fifteen days of extract administration (Fig 1). This is in accordance with the findings of [9] who reported "that aqueous extract of *Khaya senegalensis* stem bark possesses anti-hyperglycemic activity on alloxan induced diabetic rats".

The hypoglycemic ability exhibited by the extracts may not be unconnected with the presence of phytochemicals. The results suggest that there is difference in the potency (methanol fraction >> crude aqueous extract >> hexane fraction) of phytochemical constituents that mediate the hypoglycemic ability of the plant. The potent action of the methanol fraction could be ascribed to the synergistic effects of terpenoids and flavanoids that serves as antioxidants which scavenge free radical species generated by alloxan, thus preventing the destruction of pancreas beta cells and to maintain physiological functions of body organs [15]. It may also act by inhibiting α -glucosidase and α -amylase activities and/or stimulating insulin release from beta cells; this is because the diabetic produced from this instance was moderate rather than severe.

The feed and water intake of the diabetic rats were significantly increased as compared with the normal rats. These symptoms are well known markers of type 2 diabetes in both human and animal models which are direct consequence of insulin deficiency [16]. The feed intake was significantly reduced after administration of 400mg/kg methanol fraction followed by 400mg/kg crude aqueous extract then n-hexane fraction. The water intake of diabetic animals was also significantly higher than the diabetic treated rats. Also, administration of 400 mg/kg of methanol fraction significantly lower the water intake than the groups treated with 400 mg/kg of crude aqueous extract and n-hexane fraction. These results were similar to the report of [16] who demonstrated the effect of *A. africana* in controlling the desire for food and water intake under diabetic condition. A significant decrease in the body

weights of diabetic animals was observed 10 days after induction of alloxan into the animals. The loss in the body weight of the diseased animals agrees with the finding of [16] who observed similar effect on diabetic animals induced with streptozotocin. This reduction has been linked to degradation of structural proteins and muscle wasting [11]. Oral administration of plant extracts was able to improve the body weight of the animals. The result indicated that methanol fraction F1 possessed the ability of managing glucose level as well as controlling muscle wasting and induced adipogenesis [17].

FTIR result of the methanol fraction which is the most active fraction revealed the presence of compounds containing carboxylic acids, alcohols and/or phenolic functional groups, with absorbance at 3322 cm^{-1} .

ETHICAL APPROVAL

All authors hereby declare that Principle of laboratory animal care [18] and ethical guidelines for investigation of experimental pain in conscious animals [19] were observed during experimentation.

CONCLUSION

The research concludes that the methanol fraction was found to possess the highest hypoglycemic activity. Hence, further studies that aim to isolate the active compound (s) or elucidating the possible mechanism of action should use the fraction.

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