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Research Article

EVALUATION OF HYPOGLYCAEMIC EFFECT OF ETHANOL SEED EXTRACT OF *Citrullus colocynthis* ON AN ALLOXAN INDUCED DIABETIC RATS

Wazis CH [1]*, Timothy SY [1], Yesufu HB [2], Mashi JS [3], Kirawa BM [1]

[1]Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Maiduguri, Maiduguri, Nigeria

[2]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Maiduguri, Maiduguri, Nigeria

[3]Department of Pharmaceutical Services, Federal Medical Centre Gombe, Nigeria

*Corresponding Author Email: chamawazis@yahoo.com

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ABSTRACT

Plants, since ancient time, are used for health benefits by all cultures as well as source of medicines. It has been estimated that about 80-85% of global population rely on traditional medicines for their primarily health care needs and it is assumed that a major part of traditional therapy involves the use of plant extracts or their active principles. One such traditionally used medicinal plant is *Citrullus colocynthis* commonly known as 'bitter apple.' In this present study we have evaluated the hypoglycaemic effects of *Citrullus colocynthis* seeds extract in alloxan-induced diabetic rats. The seeds were collected, dried, powdered, defatted with hexane and then extracted with 95% ethanol which was used for the phytochemical and antidiabetic screenings. The phytochemical screening of the *Citrullus colocynthis* ethanol seeds extract revealed the presence of alkaloids, flavonoids, tannins, carbohydrates, phenols, saponins and glycosides. The effects of oral administration of *Citrullus colocynthis seeds* extract on the blood glucose level of diabetic rats were evaluated. It was observed that the ethanol seed extract of this plant has some antidiabetic properties in rats. The altered glucose level in the diabetic rats was significantly reverted back to a considerable value by the administration of *Citrullus colocynthis seeds* extract. The phytochemicals present in the *Citrullus colocynthis seeds* extract may account for the observed hypoglycaemic effect.

Keywords: *Citrullus colocynthis*, antidiabetic, alloxan.

INTRODUCTION

Plants, since ancient time, are used for health benefits by all cultures as well as source of medicines. It has been estimated that about 80-85% of global population rely on traditional medicines for their primary health care needs and it is assumed that a major part of traditional therapy involves the use of plant extracts or their active principles [1]. Traditional medicine is a blending together of dynamic medical knowledge solidly founded on ancestral experience [2]. Plants based drugs have been used World Wide in traditional medicine. Traditionally, plants are reliable sources of treatment of diseases in different parts of the World. Their use contributes significantly to primary health care delivery as they are regarded as invaluable sources of pharmaceutical products. Many medicinal plant species have spread globally both via intentional and carefully planned transfers and as the unintentional outcome of people's movements. Globally, medicinal plants have been unique source of medicines and constituted the most common human use of biodiversity [3].

A traditional application of trees as medicine is not uncommon in Africa. For example, of the many species of rural Hausa land of Nigeria, 254 species are used (in 1854 remedies) for illnesses that are manifested physiologically such as fevers, vomiting, pain, and so on and 215 (overlapping) species are used (in 452 remedies) for conditions that may include tangible signs but are managed through the mediation of spirits, witches, and sorcery [3]. Plants are reputed in the indigenous systems of medicine for the treatment of various diseases. Phyto-chemicals isolated from plant sources are used for the prevention and treatment of several medical problems including diabetes mellitus. There are more than 800 plants species showing a hypoglycemic activity. The World Health Organization [4] has also recommended the evaluation of the effectiveness of plants in conditions where safe modern drugs are lacked [5].

One such traditionally used medicinal plant is *Citrullus colocynthis*. *Citrullus colocynthis* (Cucurbitaceous), commonly known as 'bitter apple'. The plant has been used to treat constipation, oedema, fever, jaundice, leukaemia, bacterial infections, cancer and used as an abortifacient [6]. *Citrullus colocynthis* possess a wide range of pharmacological activities such as antimicrobial activity, anti-inflammatory activity, anesthetic activity antihypertensive and including dermatological problems and gynaecological, urinary and pulmonary infections [6, 7]. Usable part of this plant is its fruit which is yellowish, apple-sized, and extremely bitter mainly used as a strong laxative in treatment of acute and chronic constipation [8]. The leaves of this herb are used to treat asthma and jaundice, whereas the root is a traditional treatment for amenorrhea, breast inflammation, arthralgias and ophthalmic diseases [9]. *Citrullus colocynthis* is considered as a mixed blessing plant agent as the plant was reported to have some medical effects as well as it was reported to have toxic and even lethal effects [10].

MATERIALS AND METHODS

Source of Plant Materials, Collection and Identification

The part of the plant used in this project research is the seed of *Citrullus colocynthis* which was collected behind Faculty of Veterinary Medicine and Centre for Arid Zone studies of the University of Maiduguri campus, Jere local Government of Borno State, Nigeria. The plant was identified by a plant

taxonomist, Prof. S.S Sanusi of Biological Sciences Department, Faculty of Science, University of Maiduduri.

Removal of the Seeds from the Fruit Pulp

The fruits were divided into halves using knife and the seeds were removed by hand. Mature black seeds were selected, dried and powdered mechanically using wooden pestle and mortar. The powdered plant material was stored in an air tight container prior to extraction.

Defatting and Extraction

About 1 kg of the powdered plant material was soaked in n-hexane for 48 hours, after which it was filtered. About 748 g of the residue was macerated with ethanol for 24 hours and filtered appropriately. The solvent was recovered using rotary evaporator and the marc dried in a decicator.

Phytochemical Screening

A little quantity of the extract was subjected to phytochemical screening to test for the presence of the following secondary metabolites: alkaloids, carbohydrate, flavonoids, saponins, tannins, glycosides, (cardiac, steroidal), terpenes/terpenoids, fatty acids and resins [11-17].

Experimental Animal and Acclimatization

These rats were purchased from the Animal House section of the Faculty of Pharmacy, University of Maiduguri, Borno State. The animals were maintained in standard wire meshed plastic cages in Pharmacology and Toxicology Laboratory of the Faculty of Pharmacy.

The animals were kept in plastic cages at standard condition of temperature and light and humidity for a period of two weeks to allow them acclimatize to laboratory condition. They were allowed free access to drinking water and standard livestock feed (Grand Cereals and Oil Mills Ltd.) Bukuru, Jos, Plateau State, Nigeria. Adult albino rats of both sexes weighing 90-160 g were used for both the acute toxicity studies (LD_{50} determination) and the hypoglycaemic effect amounting to the total number of 32 rats in all.

Acute Toxicity Studies (LD_{50} Determination)

The acute toxicity of the ethanol seed extract of *Citrullus colocynthis* was determined using standard conventional procedure described by Lorke [18]. In this study, 2 route of administration were considered, that is, oral and intraperitoneal route. This comprises 2 phases which include:

Phase I: The rats were divided into three groups of three rats for each route; they were then treated with the ethanol seed extract of *Citrullus colocynthis* at doses of 10 mg/kg, 100 mg/kg and 1000 mg/kg body weight orally and another group intraperitoneally and were observed for signs of acute toxicity and mortalities for 24 hours.

Phase II: Three dose levels were used based on the result of phase I after 24 hours for the oral route. Three rats were given the dose of 1600 mg/kg, 2900 mg/kg and 5000 mg/kg respectively. These rats were then observed for 24 hours for signs of toxicity and death after which the LD_{50} (acute toxicity) was calculated.

Statistical analysis

The data generated during the study were expressed as Mean ± Standard Error of Mean (SEM) and analyzed using student t-test (GraphPad Prism 6 Demo). $p<0.05$ was considered significant.

RESULTS ANALYSIS

Phytochemistry

The results of phytochemistry of *Citrullus colosynthis* indicate the presence of alkaloids, flavonoids, tannins, steroids and carbohydrates **(**Table 1**).**

Table 1: Phytochemistry of ethanol seed extract of *Citrullus colosynthis*

Test	Results
Alkaloid	+
Flavonoid	+
Cardiac glycoside	–
Saponins	+
Tannins	+
Anthraquinone glycoside	–
Steroid	+
Phlobatannins	–
Carbohydrate	+

+ = Present, -= Absent

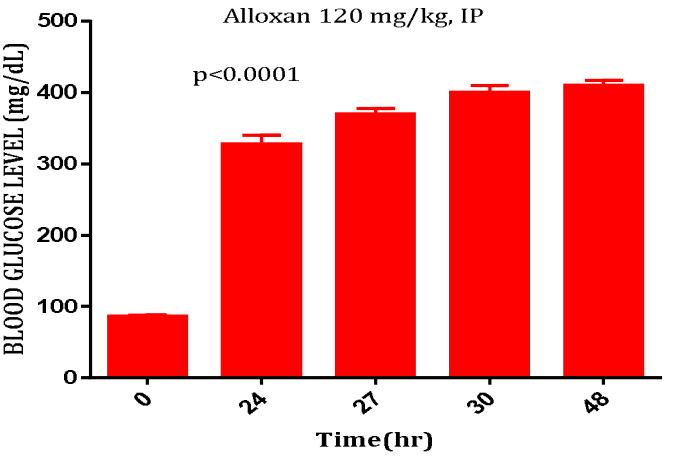

Figure 1: **A study showing time-dependent induction of diabetes mellitus with alloxan monohydrate (120 mg/kg, IP) in rats (n=5)**

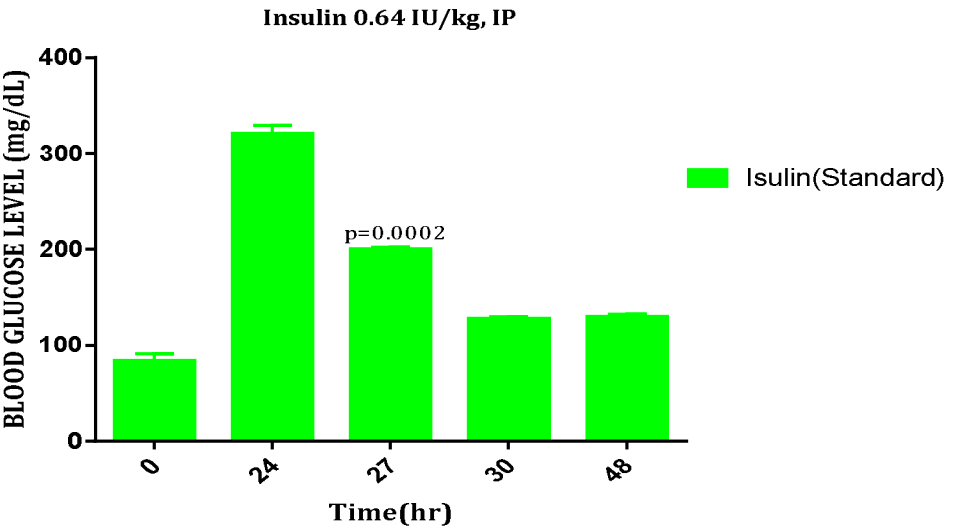

Figure 2: Effect of insulin (0.64 IU/kg, IP) on Alloxan-induced diabetic rats (n=5).

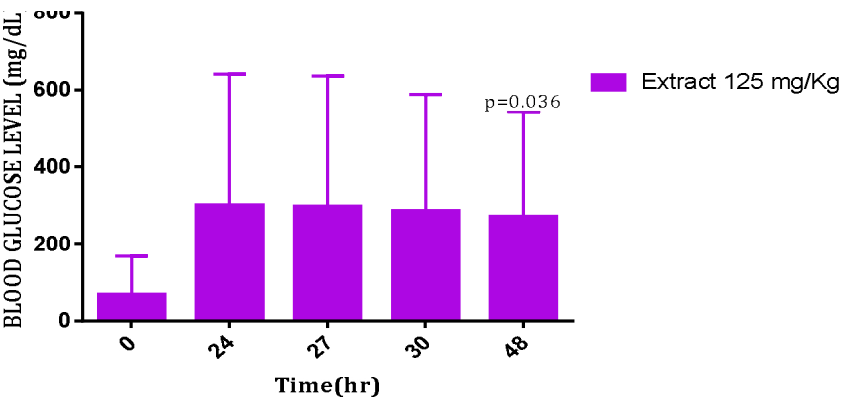

Figure 3: Hypoglycaemic effect of 125 mg/kg, PO of the extract

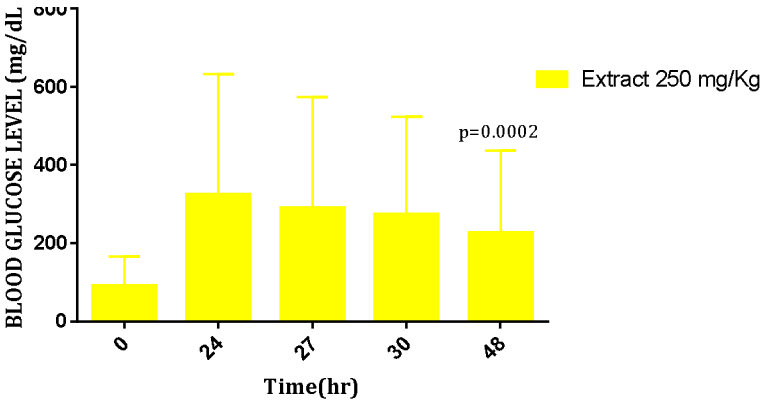

Figure 4: Hypoglycaemic effect of 250 mg/kg, PO of the extract

Fig 1.5 Hypoglycaemic effect of 500 mg/kg, PO of the extract

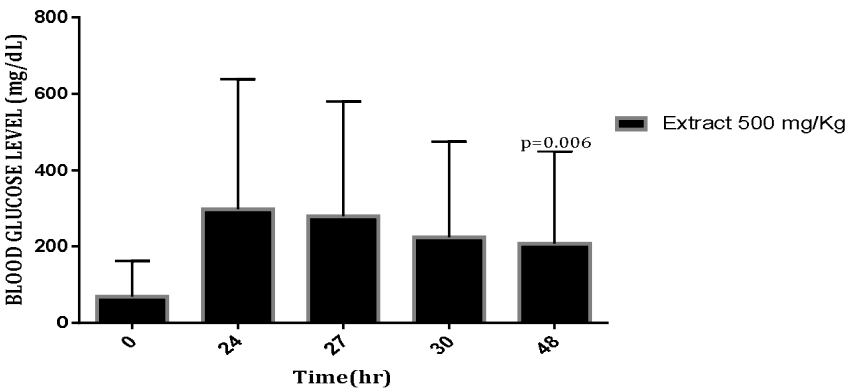

Figure 5: Hypoglycaemic effect of 500 mg/kg, PO of the extract

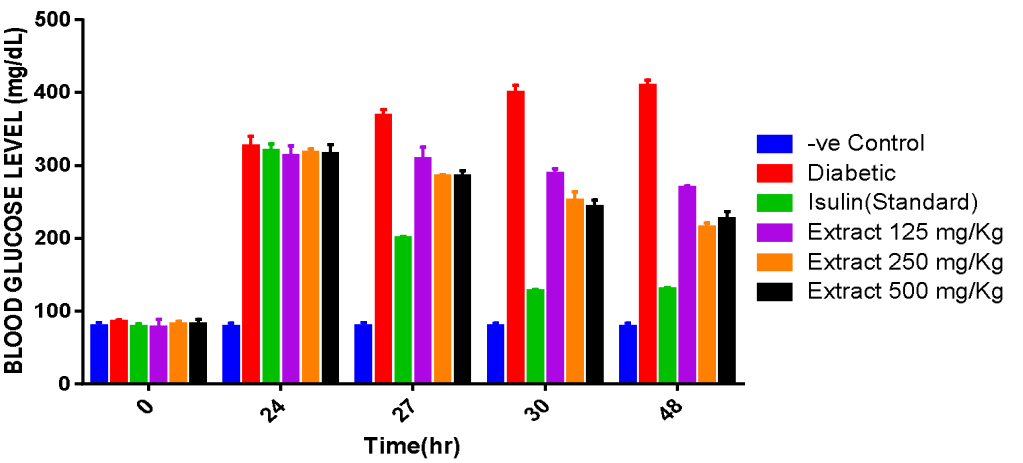

Figure 6: Effects of Insulin (0.64 IU/kg, IP), *C. colocynthis* (PO) on Alloxan-induced Diabetic rats.

A study showing time-dependent induction of diabetes mellitus with alloxan monohydrate (120 mg/kg, ip) in rats

The average basal blood glucose was 85.37±2.46 mg/dL and increased to 326.20±13.61 mg/dL (3.8-fold increase; p<0.0001) 24 hours post alloxan administration, then 368.30±8.91 (4.3-fold increase p=0.061), 399.47±10.42 mg/dL (4.7-fold increase P=0.085), 409.00±8.43 mg/dL (4.8-fold increase P=0.52), after 27, 30 and 48 hours post alloxan, respectively. These times were selected to cover the short, intermediate, and long-term actions of antidiabetic drugs (Figure 1).

Effect of insulin (0.64 IU/kg, IP) on Alloxan-induced diabetic rats

The average basal glucose level in this set of control rats was determined to be 78.53±3.75 mg/dL. Administration of alloxan (120mg/kg, IP) increased glucose level to 319.97±9.32 mg/dL, (4-fold increase, p<0.0001) was observed after 24 hours. Following three hours administration of insulin at 0.64 IU/kg, IP, there was a significant decrease in glucose level to 199.87±2.67 mg/dL (38% decrease, p=0.0002) at 27 hours post alloxan injection. Similarly there was a significant decrease in glucose level to 127.07±2.74 mg/dL (60.3% decrease, p<0.0001) at 30 hours post alloxan injection. The blood glucose returned to an insignificant level of 129.33±3.23 mg/dL (p=0.62) at 48 hours post alloxan injection compared with alloxan only treated animals. To evaluate the efficacy and folkloric use of the extracts in the management of diabetes mellitus, the rats were treated with the seed extract after 24 hours of alloxan administration and blood glucose levels were determined after 27, 30 and 48 hours (Figure 2).

The effect of ethanol seed extract of *Citrullus colocynthis* (125 mg/kg, PO) on Alloxan (120 mg/kg) induced diabetic rats

The average basal glucose level was 76.90±11.62 mg/dL. On the administration of Alloxan (120 mg/kg, IP) the glucose level increased to 313.23±13.89 mg/dL (4-fold increase p<0.0001) at 24 hours. Following the administration of *C. colocynthis* ethanol seed extract (125 mg/kg, PO), there was an insignificant decrease in glucose level to 308.73±16.19 mg/dL (1.4% decrease, p=0.84) three hour after administration of the extract, (i.e 27 hours post alloxan injection). There was also non significant decrease in glucose levels to 288.37±7.00 mg/dL (7.9% decrease, p=0.31) at six hours after administration of extract, (30 hours post Alloxan injection). Then the blood glucose continues to drop to 269.00±2.91 mg/dL at twenty four hours after administration of extract, (48 hours post alloxan injection) (Figure 3).

The effect of ethanol seed extract of *Citrullus colocynthis* (250 mg/kg, PO) on Alloxan (120 mg/kg) induced diabetic rats

The average basal glucose level was 81.80±4.69 mg/dL. On administration of Alloxan (120 mg/kg, IP) the glucose level increased to 317.33±4.85 mg/dL (3.9-fold increase) at 24 hours. Following the administration of *Citrullus colocynthis* seed extract (250 mg/kg, PO), there was an insignificant decrease in glucose level to 284.50±2.92 mg/dL (10% decrease, p=0.004) at three hours after administration of the extract, (27 hours post alloxan injection). Similarly there was an insignificant decrease in glucose levels to 251.60±12.05 mg/dL (20.7% decrease, p=0.057) at six hours after administration of extract, (30 hours post alloxan injection). And insignificantly reduced to 214.90±5.88 mg/dL (p=0.052) after twenty four hours of extract administration, (48 hours post Alloxan) (Figure 4).

The effect of ethanol seed extract of *Citrullus colocynthis* (500 mg/kg, PO) on Alloxan (120 mg/kg) induced diabetic rats

The average basal glucose level was 82.10±6.62 mg/dL. On administration of Alloxan (120 mg/kg, IP) the glucose level increased to 315.90±12.95 mg/dL (3.8-fold increase) at 24 hours. Following the administration of *Citrullus colocynthis* seed extract (500 mg/kg, PO), there was an insignificant decrease in glucose level to 285.13±7.57 mg/dL (9.7% decrease, p=0.11) at three hours after administration of the extract, (27 hours post alloxan injection). Similarly there was a significant decrease in glucose levels to 243.03±9.70 mg/dL (23% decrease, P=0.027) at six hours after administration of extract, (30 hours post alloxan injection). And insignificantly reduced to 226.37±10.05 mg/dL (p=0.30) after twenty four hours of extract administration, (48 hours post alloxan) (Figure 5).

Effects of Insulin (0.64 IU/kg, IP), *C. colocynthis* (125 mg/kg 250 mg/kg and 500mg/kg, PO) on Alloxan-induced Diabetic rats

This is to evaluate the efficacy and folkoric use of the extract in the management of diabetes mellitus, the rats were treated with the extract at 24 hours of alloxan injection and readings were taken after 24, 27, 30 and 48 hours respectively (Figure 6).

DISCUSSION

The phytochemical components of the ethanol seed extract of *Citrullus colocynthis* that revealed the presence of alkaloids, flavonoids, saponins, tannins, and carbohydrates agrees with several literature reports in which similar constituents were detected [6, 15]. These chemical compounds were speculated to account for the observed pharmacological effect of the extract.

Animal models of diabetes such as genetically derived, nutritionally and chemically induced have been used extensively in diabetes research. Alloxan, a β-cytotoxin, induces diabetes through selective destruction of pancreatic beta cells which results in a decrease of insulin secretion.

In recent years, various plant extracts have been claimed to be useful for the treatment of diabetes mellitus. Earlier reports suggests that the plant extracts cause antihyperglycemic effect by promoting regeneration of beta cells or by protecting the pancreas from destruction, by restricting glucose as well as by promoting unrestricted endogenous insulin action or its effect on beta cells to release insulin and activate the insulin receptors to absorb blood sugar. Lakshmi and Sendrayaperumal [6] reported that, oral administration of *Citrullus colocynthis* pulp extract to diabetic rats restored the level of glycogen by means of decreasing the activity of glycogen phosphorylase and increasing the activity of glycogen synthase.

The diabetogenic effect of alloxan monohydrate (120 mg/kg) which supports the report of Etuk [19], where it was noted that, although chemical induction of diabetes mellitus with streptozotocin was the most widely used procedure; alloxan was the best known drug that induces type 1 diabetes. Alloxan is affordable and readily available. Additional effect that was seen following the administration of high dose is diarrhoea. Hanaa [20] reported that the administration of *C. colocynthis* extract did not exhibit a remarkable reduction or improvement in the

induced hyperglycemia and a slight reduction was observed in the first three hours. However, the pattern of the reduction was not normal, compared to the standard drug, glibenclamide, because it was accompanied by severe diarrhoea and deaths of some animals (2 out of 7). In the present study, *C. colocynthis* (250mg/kg) reduced alloxan- induced hyperglycemia by 10% and 20.7% 3 hours and 6 hours post administration, respectively. Insulin which was used as a standard antidiabetic drug reduced blood glucose level by 38% and 60% 3 hours and 6 hours post administration, respectively.

Therefore the result of this study is of significance because it revealed the hypoglycemic activity of ethanol seed extract of the *Citrullus colocynthis* which to the best of our knowledge has not been reported elsewhere. It has also paved a way for further studies on this and other species of *Citrullus*.

CONCLUSION

The ethanol seed extract of *Citrullus colocynthis* contain a lot of phytochemical constituents which may be responsible for the observed hypoglycaemic activity. This amply justify the local use of this plant in the management of hyperglycaemia.

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