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The Effects of Ethanolic Extract of Handal (*Citrullus colocynthis* L.) Fruit Pulp on Blood Glucose level in Normal Fasting Rats

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Abstract

Background: *Citrullus colocynthis* L. is a member of the family Cucurbitaceae. It is widely available in Sudan and traditionally used for treatment of skin infections, diabetes and constipation worldwide.

Objectives: This study aimed to investigate the effects of ethanolic extract of *C. colocynthis* fruit pulp on blood glucose level in normal fasting rats.

Method: Two groups of rats, each of twenty one were used to study the effects of the extract (300 mg/kg/ml) on fasting blood glucose level (BGL) and glucose tolerance test (GTT).

Results: The oral administration of ethanolic extract of *C. colocynthis* fruit pulp resulted in a sudden drop in BGL of normal fasting rats after four hours. This was accompanied by some toxic effects appeared as severe diarrhea, followed by deaths of some rats (2/7). Moreover, the administration of the extract did not exhibit a remarkable reduction or improvement in the induced hyperglycemia.

Conclusion: This study showed that, ethanolic extract of *C. colocynthis* fruit pulp reduced the BGL without significant hypoglycemic effect and the extract appeared to be highly toxic..

Keywords: *Citrullus colocynthis*, fruit pulp, fasting blood glucose, glucose tolerance test.

Introduction

Traditional use of medicines is recognized as a way to learn about potential future medicines. By the year 2001, researchers identified 122 compounds used in mainstream medicine, which were derived from ethnomedical plant sources; 80% of these compounds were used in the same or related manner as the traditional ethnomedical use [1]. Many medicinal plants synthesize substances that are useful to the maintenance of health in humans and other animals. These include, for example, alkaloids and phenolic compounds that serve as plant defense mechanisms against predation by microorganisms, insects, and

herbivores [2-3]. Although many consumers believe that herbal medicines are safe, because they are natural, herbal medicines and synthetic drugs may interact, causing toxicity and adverse effects to the patient [4]. Furthermore, adulteration, inappropriate formulation, or lack of understanding of plant and drug interactions have led to adverse reactions that are sometimes life threatening or lethal [5]. Thus, proper trials and studies are needed to determine the safety and efficacy of each plant before they can be recommended for medical use [6].

Citrullus colcynthis L. Schrad is a member of the family Cucurbitaceae. This plant commonly known as colocynth, bitter apple, bitter cucumber, egusi or vine of Sodom. It is native to dry areas of North Africa, being common throughout the Sahara, areas of Morocco, Egypt and Sudan, eastward through Iran to India and other parts of tropical Asia [7]. The different parts of the plant (seeds, leaves, fruit pulp and fruit rind) had been studied, and the main active constituents were identified. The seeds are rich in fatty acids such as myristic, palmitic, stearic, oleic, linoleic and linolenic acid. The main active phytoconstituent present in the pulp is phytosterol glycoside (citrullol). The other glucoside cucurbitacins including elaterinide and cucurbitacine E, cucurbitacine B, other glycosides liberating cucurbitacines I and L, alkanes, aliphatic alcohols, alkaloids and choline base. The bitter substances are colocynthin and colocynthetin [8].

The dried pulp of *C. colcynthis* fruit was used as a traditional medicine in the Sudan, mostly for skin infections, edema and, in some instances, for diabetes. It has been also used for constipation worldwide [9] however, the ingestion of the cathartic fruit can have many undesired effects, including bloody diarrhea and even true acute toxic colitis and changes in the colon similar to these seen with other laxative abuse [10-12]. In East Africa, the seed tar was used by nomads in traditional medications applied to the skin [13]. In some Arab countries (United Arab Emirates and Morocco) the seeds are used to treat diabetes. However, when the chloroform and methanol extracts of seeds and ethanol extracts of leaves and pulp of *C. colocynthis* were investigated in normal and diabetic streptozotocin- induced rats, neither the seeds extract nor the leaf extract had any effect on fasting glucose levels in normal or diabetic rats, and they had no effect in the oral glucose tolerance test [14]. Many workers suggested that the fruits of *C. colcynthis* possess antitumor activity [15].

This study was designed to investigate the effects of ethanolic extract of *C. colocynthis* fruit pulp on blood glucose levels in normal fasting rats.

Materials and Methods:

Plant material

Fresh ripe fruits of *C. colocynthis* were purchased from the local market in Wad Medani city, Gezira State, Central Sudan, in April, 2010. The species was botanically authenticated and herbarium specimens were deposited at the National Herbarium, Medicinal and Aromatic Plants Research Institute, National Centre for Researches (NCR), Khartoum, Sudan.

The ripe fruits were peeled to separate the rind (epicarp and outer most layer of mesocarp). Mature black seeds were separated manually from the pulp. Then, the pulp was dried and minced with a grinder into a powder.

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Extraction of the plant material

The pulp powder of *C. colocynthis* (250 g) was macerated in 1.5 L ethanol (99%) and stirred continuously for 48 hours at room temperature. The mixture was filtered using Bukhner fennel under vacuum, then the extract was evaporated to dryness at room temperature in a large porcelain dish to give yellowish material (yield=51.7 g). The residue was dissolved in distilled water to obtain the desired concentration.

The effects of ethanolic extract of *C. colocynthis* fruit pulp (300 mg/kg/ml) on BGL in normal fasting rats

Experimental animals

The experiment was carried out according to the method developed by Tayyba Zia *et al.*, 2001[16]. Animals used were twenty one Albino rats weighing 170 – 200g, housed in a clean animal house and subjected to especial nutritional program. Animals were acclimatized for a period of 7 days under standard environmental conditions. They were divided into three groups each of seven rats and submitted to fasting for 18 hours before the study. Group I (negative control) administered water (1 ml), group II (positive control) received glibenclamide (5 mg/kg/ml) and group III (test group) given the ethanolic extract of *C. colocynthis* fruit pulp (300 mg/kg/ml). The water, glibenclamide water suspension and the extract were administered to the rats using intragastric tubes.

Determination of fasting blood glucose levels

After treatment, blood samples were collected from the tail vein of each rat separately. BGLs were determined using an electronic glucometer, Glucose plus (Halpern, Quebec, Canada) at 0, 1, 2, and 4 hours (Table 1).

Oral glucose tolerance test of ethanolic extract of *C. colocynthis* (300 mg/kg/ml) in normal fasting rats

Oral glucose tolerance test (OGTT) was performed according to the method developed by Wasfi, 1994[14]. Twenty one Albino rats were divided into three groups, each of seven. Basal BGLs were determined from blood samples taken from tail-tips of each rat. Then glucose load of 1 g/kg body weight was given first to the three groups of rats. This was followed by water (1 ml) for group I (negative control), glibenclamide (5 mg/kg/ml) for group II (positive control) and ethanolic extract of *C. colocynthis* (300 mg/kg/ml) for group III (test group). BGLs were determined at 0, 1, 2 and 4 hours using an electronic glucometer.

Data analysis

The obtained data were expressed as means ± standard error of means (SEM) and analyzed using analysis of variance (ANOVA). Comparisons with the control groups were made using One-way ANOVA. Differences were considered significant, if P-value <0.05.

Results and Discussion:

There is an increasing tendency for traditional medicine in the world. Many people prefer to take herbal products instead of chemical medicines. However, over consumption of herbal medicines has led to many

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unpredictable side effects. One of these traditional medicines is *C. colocynthis*, which is used by diabetic patients as a hypoglycemic agent. This study was conducted to study the possible hypoglycemic effects of ethanolic extract of *C. colocynthis* fruit pulp (300 mg/kg/ml) on BGLs in normal fasting rats.

Effect of ethanolic extract of *C. colocynthis* (300 mg/kg/ml) on the BGLs in normal fasting rats

The ethanolic extract of *C. colocynthis* resulted in significant (P=0.001) but abrupt reduction in the fasting BGL of normal rats by 50.33 mg/dl. However, this reduction was abnormal in that, it was sudden and occurred only after four hours. When this reduction was compared to that produced by glibenclamide, it was found that the reduction obtained by glibenclamide was significant (P=0.001), normal and showing gradual manner of reduction (42.6 mg/dl), four hours later (Table 1). It was also noted that, the same dose (300 mg/kg) that produced this abrupt reduction in the BGL, appeared to be extremely toxic, developing severe diarrhoea, dullness and ruffled hair. Diarrhoea was a prominent sign of *C. colocynthis* poisoning [17]. Some of the treated rats in the test group were died (2/7) within the four hours of the experiment.

Table1: Effects of oral administration of water (1 ml), glibenclamide (5 mg/kg/ml) and ethanolic extract of *C. colocynthis* (300 mg/kg/ml) on the blood glucose levels in normal fasting rats

Treatment	BGLs (mg/dL, mean±S.E.M)				Reduction of Glycemia (mg/dL)	P. value
	0h	1h	2h	4h		
Water	**86±2.25**	**84.33±3.67**	**79.83±1.52**	**80.5±3.01**	5.5	0.339
Glibenclamide	87.8±**4.03**	62±**6.15**	50.17±**3.17**	45.2±**3.12**	42.6	0.001
C. colocynthis	98±**3.5**	96±**2.6**	81.67±**2.16**	47.67±1.5	50.33	0.001

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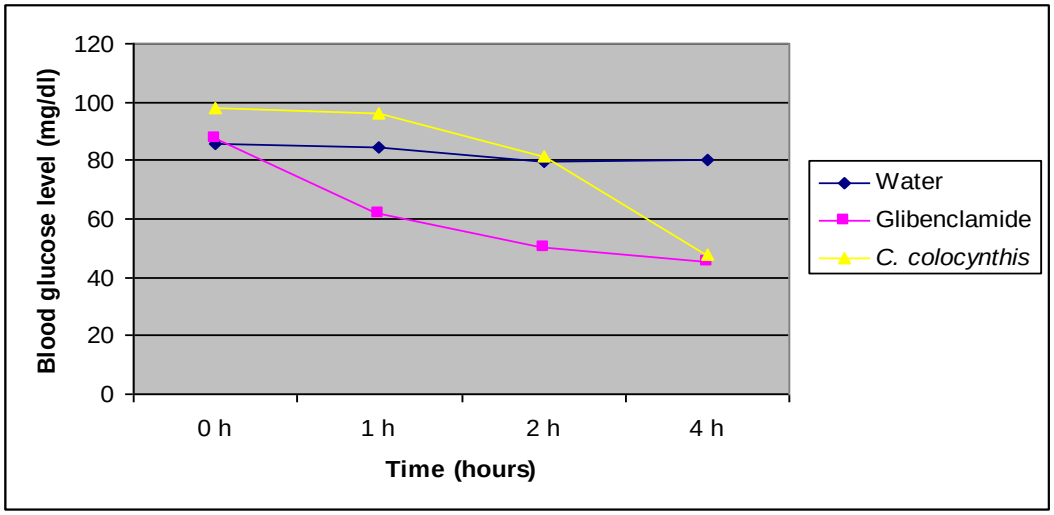

Figure 1: Effects of oral administration of water (1 ml), glibenclamide (5 mg/kg/ml) and ethanolic extract of *C. colocynthis* (300 mg/kg/ml) on the blood glucose levels in normal fasting rats

Glucose tolerance test of ethanolic extract of *C. colocynthis* (300 mg/kg/ml) in normal fasting rats

Results obtained showed that the administration of 1 g/kg glucose caused an increase in BGLs (hyperglycemic peak) in normal fasting rats in the first hour. It was also found that the administration of *C. colocynthis* extract did not exhibit a remarkable reduction or improvement in the induced hyperglycemia and a slight reduction (26.33 mg/dl) was observed in the first three hours (Table 2).

Table 2: Effects of oral administration of water (1 ml), glibenclamide (5 mg/kg/ml) and ethanolic extract of *C. colocynthis* (300 mg/kg/ml) on glucose tolerance test in normal fasting rats

Preparation	BGLs (mg/dL, mean)				Reduction of Glycemia (mg/dL)
	0h	1h	2h	3h	
Water	62±2.22	115±3.7	59±1.5	66±2.12	49
Glibenclamide	78±4.03	89±5-15	71±3.17	64±3.12	25
C. colocynthis	87±1.7	107.33±2.2	84±2.1	81±1.9	26.33

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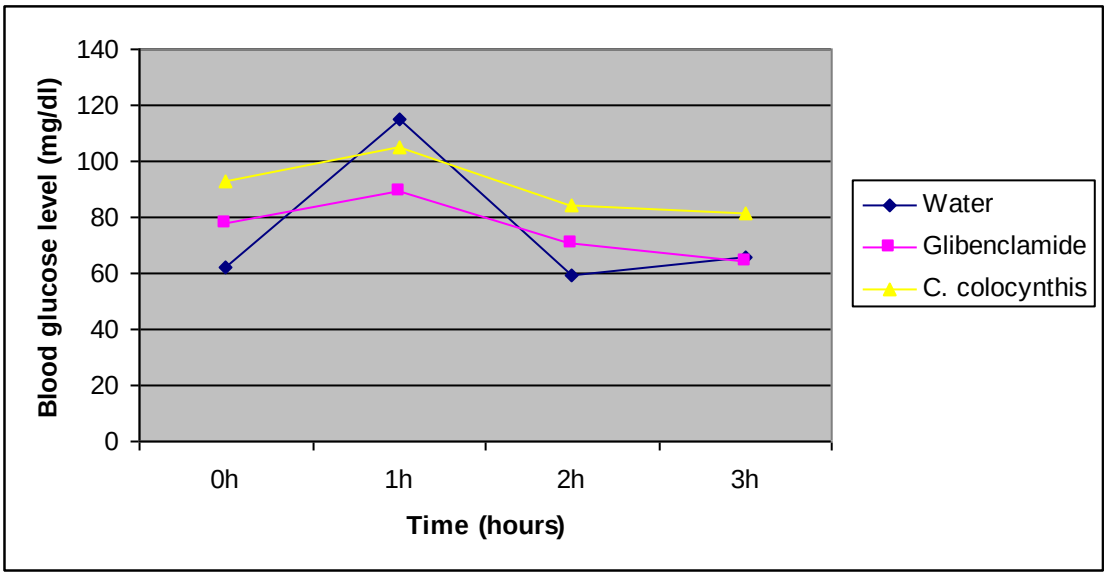

Figure 2: Effects of oral administration of water (1 ml), glibenclamide (5 mg/kg/ml) and ethanolic extract of *C. colocynthis* (300 mg/kg/ml) on glucose tolerance test in normal fasting rats.

The present study demonstrated a reduction in BGL followed the oral administration of the ethanolic extract of *C. colocynthis* fruit pulp. However, the pattern of the reduction was not normal, compared to the standard drug, glibenclamide, because it was accompanied by severe diarrhea and deaths of some animals (2 out of 7). The occurrence of these toxicities may reflect that, the reduction in BGL may be considered as a part of the toxic effects of the plant and not an advantage.

It is known that traditional medicine and ethnobotanical information play an important role today as subjects for scientific research. The results of such assessment can provide a number of plants, which can claim priority to be investigated for a selected biological activity or efficacy against selected disorders or diseases. Thus, phytochemical screening is needed to separate phytoconstituents responsible for beneficial and/or toxic effects.

In conclusion some toxic effects *C. colocynthis*, rather than hypoglycemic effects were evident.

Because of the popularity of this plant in traditional medicine for treatment of some diseases such as diabetes mellitus, people should be informed of the potential toxic effects. In addition, further investigations are required to elucidate the mechanisms through which the toxic effects and the reduction in BGL occurred.

References:

1. Fabricant DS, and Farnsworth NR (2001). The value of plants used in traditional medicine for drug discovery 109: 69-75. Fact sheet no. 134: *Traditional medicine*". World Health Organization, 2008-12-01. Retrieved 2009-05-02.

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2. Lai PK, and Roy J (2004). Antimicrobial and chemopreventive properties of herbs and spices 11 (11): 1451-1460.

3. Tapsell LC, Hemphill I, and Cobiac L, (2006). Health benefits of herbs and spices: the past, the present, the future 185.

4. Talalay P. and Talalay P. (2001), The Importance of Using Scientific Principles in the Development of Medicinal Agents from Plants, *Academic Medicine*, 76 (3):238.

5. Elvin-Lewis M. (2001), Should we be concerned about herbal remedies,"*Journal of Ethnopharmacology* 75, 141-164.

6. Ernst E. (2007). "Herbal medicines: balancing benefits and risks". *Novartis Found. Symptoms.* 282: 154–167; 167–172, 212–8

7. Trease G. and Evans W. (2002). *Text Book of Pharmacognasy*. Tindall and Cassell, London, Baillere,: 210-234.

8. Duke JA (1978). The quest for tolerant germplasm.In: ASA Special Symposium 32, Crop tolerance to suboptimal land conditions. American Society for Agronomy Madison, Wisconcin. P: 1-61

9. Arena J.M. and Drew R.H., (1980). Poisoning: Toxicology, Symptoms, Treatment, 5th Edn. Thomas, Springfield, II.252-256.

10. Goldfain D., Lauergne A., Galian A., and Chauvenic, L. (1989). Pecular acute toxic colitis after ingestion of colocynthi; a dinicopathological study of three cases. *Gut* 30: 1412– 1418.

11. Al Faraj S. (1995). Haemorrhagic colitis induced by *Citrullus colocynthis*. *Annual Tropen Parasitology* 89 (6): 695– 696.

12. Habs M., Jahn S.A. and Schmahi, D. (1984). Carcinogenic activity of condensate from coloquint seeds (*Citrullus colocynthis*) after chronic epicttaneous administration to mice. *Journal of Cancer Research and Clinical Oncology* 108 (1): 154–156.

13. Ziyyat A., Legssyer A. and Mekhfi, H. (1997). Phytotherapy of hypertension and diabetes in oriental Morocco. *Journal of Ethnopharmacology* 58 (1): 45–54.

14. Wasfi I. A. (1994). Some pharmacological studies on *Citrullus colocynthis*. *Journal of Herbs, Spices and Medicinal Plants* 212: 65–79.

15. Faust R.E., Gwaline G.E., Ramsted E.J., Whiting D.A. and Yousif N.J. (1989). Cucurbitacin glycosides from *Citrullus colocynthis*. *Phytochemistry* and *Pharmaceutical Association* 47(1): 325-330.

16. Tayyaba Zia S., Nazrul Hasnain S. and Hasan K. (2001). Evaluation of the oral hypoglycaemic effect of *Trigonella foenum graecum* L. (methi) in normal mice. *Journal of Ethnopharmacology*, 75 (23), :191-195.

17. M.A. Al-Yahya , A.H. AL-Farhan and S.E.I. Adam (2000). Preliminary toxicity study on the individual and combined effects of *Citrullus colocynthis* and *Nerium oleander* in rats. *Science Direct Fitoterapia*, 71(41): 385-391