

Effect of Hot Aqueous Extract of *Citrullus colocynthis* L. Fruit on some Biochemical and Haematological Parameters in Alloxan – Induced Diabetic Rats

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Abstract

The present study deals with the effect of the aqueous extract of *Citrullus colocynthis* L. fruit on some biochemical and haematological values in alloxan-induced diabetic rats, including: serum glucose, total cholesterol, triglycerides, total protein, total urea, serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), alkaline phosphatase (ALP), haemoglobin (Hb), packed cell volume (PCV) and white blood cell (WBC) were determined after oral administration of the extract to diabetic rats in a dose of 300mg/Kg body weight for 3 weeks.

A total of 12 rats were involved in this study and were divided into three experimental groups: control, diabetic and *C.colocynthis*-treated diabetic. It was found that *C.colocynthis* treatment decrease the elevated serum glucose, cholesterol, triglycerides, ALP and ALT in diabetic rats. Other parameters including: total protein, urea, AST, Hb, PCV and WBC were found to be unchanged.

It is concluded that oral *C. colocynthis* treatment might normalize some abnormal biochemical parameters in diabetic rats but it has no effect on the haematological parameters.

Introduction

Diabetes mellitus (DM) is syndrome, initially characterized by a loss of glucose homeostasis resulting from defects in insulin secretion, insulin action both resulting impaired metabolism of glucose and other energy- yielding fuels such as lipids and proteins (Scheen,1997). It is one of the most common metabolic disorders, with a world wide prevalence estimated to be between 1% and 5%. The increasing prevalence of DM in the world is a cause of concern . DM leads to abnormalities in carbohydrates ,protein and lipid metabolism (Sacks,1997).It was found that the red and white blood cell (RBC and WBC) counts lower, in type diabetes than in non-diabetic rabbits (Meral *et al*;2004).

Experimental diabetes in animals has provided considerable insight into physiological and biochemical derangement of the diabetic state. Many of derangement have been characterized in hyperglycemic animals (Saravanan and Pari,2005). In spite of the presence of known antidiabetic medicine in the pharmaceutical market, remedies from medicinal plants are used with success to treat this disease (Bhattaram *et al*,2002). Many traditional plant treatments for diabetes are used throughout the world. *Citrullus colocynthis* L. a plant belonging to the family Cucurbitaceae, it is a climbing annual medicinal herb in the desert area distributed in African and Arabian countries and India. The fruits of *C.colocynthis* have been commonly used as antidiabetic medication in tropical and subtropical countries

(Diwan *et al.*,2000).This plant has insulintropic effects (Nmila *et al.*,2000) , mild immunostimulating effects (Beneddou *et al.*,2003), antioxidant activity (Kumar *et al.*,2008) and anticancer activity (Yoshikawa *et al.*,2007). Phytochemical screening revealed that *C. colocynthis* extract contains triterpene , flavonoids, tertiary and quaternary alkaloids, glycosides and saponin compounds (Abdel-hassan *et al.*,2000). Benmehdi *et al.* (2008) reported a significant reduction of blood sugar level in diabetic rats that treated with aqueous extract of *C. colocynthis* seeds.

Although DM has become a widely spread disease in Iraq, only few reports addressed the problem. The present investigation was undertaken to study the effect of *Citrullus colocynthis* aqueous extract on some biochemical and haematological parameters in alloxan diabetic rats.

Materials and Methods

Preparation of Extract:

The fruit of *C.colocynthis* L. were purchased from local herb store. The fruit were dried and ground to powder, then the powder (18 g) was extracted with (600 ml) of hot water by magnetic stirrer for 1 hour, the extract left overnight then filtered through surgical gauze and filter paper Whitman No.1 to obtain the aqueous extract which used for the experiment (Alabadi and Alali,2008).

Animals and Induction of Diabetes:

Male albino rats were the chosen experimental animals for the study. Animals were normal and healthy. They were housed in well aerated cages under normal environmental conditions of temperature and humidity. Animals were fed on commercial balanced diet and tap water was offered *ad libitum* all over the experimental period.

The rats were divided into 3 groups of 4 rats each, group 1: normal treated rats, group 2: diabetic rats, group 3: diabetic rats given aqueous extract of *C. colocynthis* (300 mg/Kg body weight) daily using an intragastric tube for 3 weeks. The rats of group 2 and 3 were injected intraperitoneally with alloxan monohydrate dissolved in normal saline at a dose of 150 mg/Kg body weight (Saravanan and Pari,2005). Rats with moderate diabetes having hyperglycemia (blood glucose level>200 mg/dl) were used for the experiment.

At the end of the experiment period (21 days), the animals in all three group were fasted for 12 hours and blood samples were taken under anesthesia.

Biochemical Analysis:

Blood samples which collected in 10 ml plain tube were used for serum preparation. Clear serum samples were separated by centrifugation at 3000 rpm for 20 min.

Serum glucose concentration was measured immediately by the glucose oxidase method (Trinder, 1960). Serum total protein was determined according to Armstrong and Carr (1964), serum cholesterol was assayed by Meiattini method (1978). Urea determination is based upon the cleavage of urea with urease (Berthelot's reaction) according to Fawcett and Scott(1960). Triglyceride was measured using Fossati and

Prenciple method(1982).The activities of serum enzymes AST, ALT and ALP were determined using Reitman and Frankel method (1957).

Haematological Analysis:

A blood which collected in tubes containing (EDTA) were used to assess haematological changes which include: white blood cell (WBC), packed cell volume (PCV) and haemoglobin (Hb) (Coles, 1986; Schalm *et al.*,1975).

Statistical Analysis:

Statistical comparisons of data were made by one way ANOVA and means of student's t-test ; $P < 0.05$ was regarded as significant(Alabadi and Alali,2008)

Results

The effect of *C. colocynthis* on some biochemical parameters of alloxan-induced diabetic rats is shown in table 1. Serum glucose and triglyceride concentration were increased significantly ($P < 0.05$)in diabetic rats. Treatment with *C. colocynthis* decreased the elevated glucose and triglycerides levels significantly in treated diabetic rats (group 3) to a level didn't differ than those of normal rats (group 1).Serum cholesterol levels in diabetic exhibited significant increase compared to normal rats. However, *C. colocynthis* treated animals showed levels of cholesterol significantly less than those of group 1 and group 2.

There was a non significant difference of both total protein and urea concentrations in all experimental groups.

Table 1. Effect of aqueous extract of *C. colocynthis* on some biochemical parameters of alloxan-induced diabetic rats

Parameters	Group 1	Group 2	Group 3
Glucose (mg/dl)	90.33±7.5 ^b	201±30.5 ^a	90±4 ^b
Cholesterol (mg/dl)	161.67±11.06 ^b	183.67±4.04 ^a	146.33±9.07 ^c
Triglycerides (mg/dl)	94±12.12 ^b	138.33±27.1 ^a	95.67±8.33 ^b
Total Protein (mg/dl)	63.33±4.5	59.33±6.35	60.33±7.64
Urea (mg/dl)	25.66±5	24.33±6.65	23.66±2.89

*Means in the same row with different superscripts significantly differ ($P < 0.05$).

*Values are expressed as mean ±SD.

As shown below, table 2 represent the activity of serum marker enzymes (AST,ALT and ALP) in normal and experimental rats. It was found a significant increasing in ALP activity in diabetic (group 2) compared to normal group , *C. colocynthis* treatment decreased the elevated ALP level of diabetic rats to a level less

than that of normal group. In addition, treatment with *C. colocynthis* decreased the level of ALT activity significantly compared to diabetic and normal rats. However, there was non significant difference in AST level for all three groups ($P < 0.05$).

Table 2. Effect of aqueous extract of *C. colocynthis* on some serum enzymes (AST, ALT and ALP) of alloxan-induced diabetic rats

Serum Enzymes	Group 1	Group 2	Group 3
AST (U/L)	21.67±7.1	22±12	9.67±1.5
ALT (U/L)	31.33±1.15 ^a	24.67±12.66 ^a	9±1 ^b
ALP (U/L)	219.33±50.85 ^b	475.67±39.8 ^a	57.33±6.5 ^c

*Means in the same row with different superscripts significantly differ ($P < 0.05$).

*Values are expressed as mean ±SD.

It is observed in table 3, that Hb conc., PCV percentage and WBC count were found significantly unchanged in all three groups ($P < 0.05$).

Table 3. Effect of aqueous extract of *C. colocynthis* on some haematological parameters of alloxan-induced diabetic rats

Parameters	Group 1	Group 2	Group 3
Hb (g/dl)	10.82±1.8	11.26±1.1	10.02±0.95
PCV (%)	33.47±5.45	34.8±3.34	31.07±2.85
WBC (cell/mm ³)	1555.6±527	1333.3±300	1533±632

*Means in the same row with different superscripts significantly differ ($P < 0.05$).

*Values are expressed as mean ±SD.

Discussion

Alloxan, a beta cytotoxin, induces "chemical diabetes" in a wide variety of animal species by damaging the insulin secreting pancreatic β -cell, resulting in decrease in endogenous insulin release, which paves the ways for the decreased utilization of glucose by the tissues (Omamoto *et al.*, 1981).

The present study indicates that oral administration of *C. colocynthis* extract decreases serum glucose, cholesterol and triglycerides in alloxan diabetic rats. The hypoglycemic effect of extract could be attributed for more extent to the presence of saponins and glycosidic components in the plant (Abdel-hassan *et al.*, 2000). The possible mechanism of action may be correlated with insulinotropic effect of *C. colocynthis* fruits (Nmila *et al.*, 2000). Insulin is potent inhibitor of lipolysis, since it inhibits the activity of the hormone sensitive lipases and suppresses the release of free fatty acids (Locci *et al.*, 1994). During diabetes, enhanced activity of this enzyme

increases lipolysis and releases more free fatty acids into circulation (Agardh *et al.*,1999). Increased fatty acids conc. also increases the β -oxidation of fatty acids, producing more acetyl CoA and cholesterol during diabetes. In normal condition, insulin increases the receptor-mediated removal of LDL-cholesterol and decreased activity of insulin during diabetes causes hypercholesterolemia and hypertriglyceridemia (Bopanna *et al.*,1997).

Blood total protein and urea has been shown to be unchanged in normal and diabetic rats, these results are coincide with the findings of Khouri *et al.*(2007).

Elevation of alkaline phosphatase (ALP) in current study is a useful marker for the damage of liver which could be primarily due to increase in blood glucose levels, which upon autoxidation generate free radicals and secondarily due to the effects of diabetogenic agent alloxan (Szkudelski,2001 ; Israeli and Bogin, 1986). A significant decrease in blood levels of both ALP and ALT in *C. colocynthis* treated animals were found. These results agree with Chaturvedi *et al.*(2003) and Khouri *et al.*(2007). So , it seems that *C. colocynthis* has a hepatoprotective activity. Haematological parameters (Hb, PCV and WBC) were found not significantly changed in diabetic rats compared with the values of normal group even when treated with *C.colocynthis*. However, some studies showed that PCV and WBC counts lower in diabetes than in non-diabetic individuals (Palmieri *et al.*,2001). It is concluded that oral *C. colocynthis* treatment might normalize some biochemical parameters, but it has no effect on the haematological parameters under study. Thus, the present study provides a scientific evidence for the use *Citrullus colocynthis* as anti-diabetic and anti-hepatotoxicity agent.

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تأثير المستخلص المائي الساخن لثمار الحنظل *Citrullus colocynthis* L. على بعض المعايير
البايوكيميائية والدموية في الجرذان المصابة بداء السكري بتأثير الالوكسان

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الخلاصة

تناولت الدراسة الحالية تأثير مستخلص ثمار نبات الحنظل *Citrullus colocynthis* L. على بعض القيم البايوكيميائية والدموية في الجرذان المصابة بداء السكري المستحث بالالوكسان. إذ تم تحديد القيم التالية: كلوكوز المصل والكوليستيرول الكلي والكليسيريدات الثلاثية والبروتين الكلي، اليوريا و انزيم اسبارتات امينوترانسفيريز و انزيم الانين امينوترانسفيريز و انزيم الفوسفاتيز القاعدي و الهيموغلوبين وحجم الخلايا المتراسة وعدد كريات الدم البيضاء، وذلك بعد تجريع جرذان مصابة بداء السكري بجرع من المستخلص مقدارها 300 ملغم/ كغم من وزن الجسم ولمدة 3 اسابيع. أجريت هذه الدراسة على 12 جرذا والتي قسمت الى ثلاث مجاميع مختبرية هي: المجموعة الضابطة والمجموعة المصابة بداء السكري والمجموعة المصابة بداء السكري ومعامله بمستخلص الحنظل. لقد وجد ان المعاملة بمستخلص ثمار الحنظل قد خفضت من المستويات العالية لكل من كلوكوز المصل والكوليستيرول والكليسيريدات الثلاثية وانزيمي الانين امينوترانسفيريز والفوسفاتيز القاعدي في الجرذان المصابة بداء السكري، بينما لم تتغير المعايير الاخرى وهي البروتين الكلي واليوريا و انزيم اسبارتات امينوترانسفيريز والهيموغلوبين وحجم الخلايا المتراسة وعدد كريات الدم البيضاء. يستنتج من الدراسة ان التجريع الفموي لمستخلص الحنظل يمكن ان يعيد بعض المعايير البايوكيميائية الشاذة الى حالتها الطبيعية في الجرذان المصابة بداء السكري بينما لم يكن له تأثير على المعايير الدموية.