Evaluation of the Antioxidative, Antidiabetic and Antilipidemic Effect of Bitter Melon Seeds (*Citrullus colocynthis*) Alcoholic Extract on Female Rats

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Abstract:

Background: Diabetes mellitus and hyperlipidemia are well-known risk factors for several illnesses including atherosclerosis, heart and vascular diseases and stroke. Herbal medicine is still the mainstay of about 75–80% of the world population, mainly in the developing countries, for primary health care because of better cultural acceptability, better compatibility with the human body and lesser side effects. Herbal extracts are introduced to the mankind since many centuries, several herbal extracts in different oral formulas have been recommended for diabetes mellitus and hyperlipidemia treatment all over the world. **Objective:** The present study was carried out to investigate the hypoglycemic, hypolipidemic and antioxidative effects of the extract of Bitter melon (*Citrullus colocynthis*) in young adult Sprague Dawley female rats for eight weeks. **Materials and Methods:** Forty female young adult Sprague Dawley female rats were divided into two sections. Section I (Normal animals) was S.C. injection with a vehicle (0.9% NaCl) and divided into two groups (10 rats / group), one of these served as control group and the second group was received extract of Bitter melon (*Citrullus colocynthis*) seed 50 mg/kg/day for 8 weeks and served as normal treated group. Group II was S.C. injection with Alloxan (diabetic rats) and divided into two group (10 rats each), one of these served as diabetic control group while the second group was received extract of *Citrullus colocynthis* (*C-colocynthis*) seed (50mg/kg/day) orally for 8 weeks and

served as diabetic treated group. After 8-weeks experiment was terminated and animals were sacrificed, heart blood was drawn and sera were separated for assessment of blood glucose, lipid profile, and lipid peroxidation value (MDA), reduced glutathione (GSH), lactate dehydrogenase (LDH), alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP) as well as total and direct bilirubin. **Results:** Both normal and diabetic rats showed a significant decrease in blood glucose, lipid peroxidation value (MDA), total cholesterol (TC), triglycerides (TG) as well as total and direct bilirubin. While, levels of reduced glutathione (GSH), lactate dehydrogenase (LDH), alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP) and total lipid were significantly increased in both normoglycemic and hyperglycaemic rats except ALT, AST and ALP in hyperglycaemic rats had no changes. **Conclusions:** The present study shed more of the light on the effect of Bitter melon (*Citrullus colocynthis*) extract and proves that this extract has antioxidant, hypoglycemic and hypolipidemic properties in the rats and may be used for treating diabetes mellitus.

Keywords: Bitter melon, blood glucose, lipid profile, liver functions, MDA, GSH, LDH.

Introduction:

Diabetes mellitus is a major endocrine disease, involving metabolic disorders of carbohydrate, fat and protein. Currently, more than 250 million people worldwide are living with the disease. It considered the 4th leading cause of global mortality **(Kaimal ,et. al., 2010)**. Since, Coronary heart disease (CHD) is common in people with diabetes mellitus (DM), the prevalence of angina was higher in people with than in those without diabetes **(Wingard & Barrett-Connor, 1995; Laakso & Lehto,1997)**. In the Multiple Risk Factor Intervention Trial, the age-adjusted incidence of CHD was four times greater in people with than in those without diabetes. In people with diabetes, CHD causes almost 60% of their deaths. They have a two to threefold increased risk for

CHD and two- to fourfold higher CHD morbidity and mortality rates. In people with diabetes, CHD occurs at a younger age, and women are affected as often as men **(Stamler, et.al., 1993).**

By the year 2025 the incidence diabetes is expected to increase to over 380 million in both developed and developing countries **(Roglic, 2005).** It is characterized by hyperglycaemia together with biochemical alterations of glucose and lipid metabolism. Insufficient insulin and increased oxidative stress along with hyperlipidaemia has been suggested in the pathogenesis and progression of diabetic complications such as atherosclerosis, myocardial infarction, neuropathy, nephropathy, retinopathy, micro and macro vascular damage and poor wound healing **(Halliwell & Gutterid, 1989)**. Living organisms use a great variety of antioxidant compounds and produce antioxidant enzymes responsible for deactivating reactive intermediaries of oxygen. But antioxidant substances and enzymes are not wholly effective in preventing oxidative damage especially in conditions like diabetes mellitus, where free radicals are produced in excess. Oxidative stress occurs when there is an imbalance between free radical production and antioxidant defenses, resulting in deregulation of cellular functions **(Ravi, et. al., 2004).** Hyperglycemia is involved in the generation of oxygen-free radicals. Biological antioxidants are compounds that protect biological systems against the potentially harmful effects of processes or reactions that can cause excessive oxidations. They could also be referred to as scavengers **(Salvemini &Botting, 1993 and Lee, et. al., 2002).** *Citrullus colocynthis* seeds contained protein (particular were rich in methionine and cystine), oil (Oleic, Linoleic, Linolenic), ash, crude fiber, nitrogen free extract (NFE), potassium, saponins, phosphorous and iron . The plant showed the presence of large amounts of phenolics and flavonoids that have antioxidant activity **(Sawaya, et.al., 1986)** . *Citrullus colocynthis* had a beneficial effect on improving the glycemic profile without severe adverse effects in type II diabetic patients**(Kumar,et. al., 2008).** It was

used as purgative, anthelmentic, antipyritic, carmanative, cures tumours, leucoderma, asthma, jaundice, enlargment of spleen, tuberculous glands of the neck, elephantiases and ulcers, also reported that fresh fruit and seeds are eaten as an laxative and removing kidney stones **(Huseini, et.al., 2009)**. It was possess cardiac depressent and smooth muscle relaxant effects and cytotoxic activities and used locally for **(Lavie, et.al., 1959; Bolous, 1983 and Shah,et.al., 1989)** stimulation to hair growth and its cucrbitacin content **(Hussein, 1985)** had an anticancer effects and anti-hepatotoxic activity . Many of the modern purgative pills contain the solid extract of *colocynth* in small doses it is expectorants, so root is useful in cough and asthmatic attacks in childern, jaundice, urinary disease, rheumatism and for abdominal enlargement and have **(Ageel, et.al., 1987)** inhabitory effect for prostaglandin formation. This effect was accompanied by significant induction of COX-2 protein expression **(Ching-jang and Mei-Chiao , 2002)** . The present study was carried out to investigate the hypoglycemic, hypolipidemic and antioxidative effects of the extract of Bitter melon (*Citrullus colocynthis*) in young adult Sprague Dawley female rats for eight weeks.

Materials and Methods:

Forty female young adult Sprague Dawley female rats were divided into two sections. Section I (Normal animals) was subcutaneously (s.c.) injection with (0·9% (w/v) NaCl) as vehicle and divided into two groups (10 rats / group), one of these served as control group and the second group was received ethanol extract of Bitter melon (*Citrullus colocynthis*) seed 50 mg/kg/day for 8 weeks and served as normal treated group (50mg/kg/day was more effective in lowering fasting blood glucose and chosen according to **(Grover, et.al., 2002)**. Group II was fasting for 24 h then given a single s.c. injection of freshly prepared alloxan solution using saline (0·9% (w/v) NaCl) as vehicle, at a dose of 12 mg alloxan/100 g body weight **(Bahnak & Gold 1982).** Rats then divided into two groups (10 rats each), one of these served as diabetic control group and

received saline vehicle only. While the second group was received ethanol extract of *Citrullus colocynthis* (*C-colocynthis*) seed (50mg/kg/day) orally for 8 weeks and served as diabetic treated group. The diabetic state was ascertained in terms of loss of body weight, polyuria, glycosuria, polydipsia, polyphagia and blood glucose levels **(Nerurkar *et al.* 1988).** Symptoms of diabetes were observed within a week of Alloxan injection. The last dose of Bitter melon (*Citrullus colocynthis*) was given 12 h prior to killing the animals and during this time animals were fasted. At the end of 8 weeks (2 month) experiment was terminated and animals were sacrificed, heart blood was drawn each sample was collected into 2 tubes, heparinized and non-heparinized. The non heparinized blood samples were allowed to coagulate and then centrifuged at 3000 x *g* for 15 min at 4°C. The separated sera were used for the estimation of serum level of glucose, activities of ALT, AST, ALP, and LDH, total and direct bilirubin, MDA and lipid profile.The heparinized blood samples were haemolyzed using bidistilled water and the haemolysate of each sample was deproteinized with meta-phosphoric acid and the clear supernatant was used for the estimation of GSH level. Glucose was determined spectrophotometrically as described by **(Trinder, 1969)**. End products of lipid **peroxidation**, specifically malondialdehyde (**MDA**) was determined spectrophotometrically as described by **(Jain, 1989 and Janero, 1990)**. Determination of serum ALT and AST activity was done using a test reagent kit according to the method described by **(Reitman and Frankel, 1957)**. Determination of serum ALP activity was carried out using a test reagent kit according to the method of **(Kind and King, 1954)**. LDH was determined in serum kinetically using a test reagent kit according to the method of **(Buhl and Jackson, 1978)**. GSH in blood was determined according to the method described by Chanarin . Determination of serum total and direct bilirubin was carried out using a test reagent kit base on method of **(Fevery *et. al.,* 1976).** The concentration of total lipids was determined by colorimetric method **(Zollner and Kisch, 1962)**. The serum

triglycerides of were measured by colorimetric methods **(Weingand, 1988)**. The serum total cholesterol was measured using the method of Roschlay and coworkers **(Roschlay, *et. al.*, 1975)**. The high density lipoproteins-cholesterol (HDL-C) was determined using the method of **(Jacobs, *et. al.*,1990)**. The cholesterol in very low density lipoprotein (vLDL-C) and low density lipoprotein particles(LDL-C) were calculated using Friedewald's equations **(Friedewald, *et.al.*, 1972)**. All measurements were performed in triplicate, at least three times. All specimens were kept as aliquots until they could be tested at the same time. Data collected were analyzed by one-way ANOVA utilizing computerized statistical program (InStat).

Results:

Citrullus colocynthis seed ethanol extract administrated orally to normal and diabetic rats for eight weeks induced significant decrease ($P < 0.001$) in blood glucose levels, total bilirubin, direct bilirubin and lipid peroxidase value as compared with normal and diabetic controls. While, *Citrullus colocynthis* seed extract induced significant increase ($P < 0.001$) in the levels of lactate dehydrogenase, reduced glutathione, transaminases and alkaline phosphatase as shown in table 1.

Table (1): Blood glucose, total bilirubin, direct bilirubin, lipid peroxidation value (MDA), lactate dehydrogenase (LDH), reduced glutathione (GSH), serum transaminases (ALT & AST) and Alkaline phosphatase (ALP) of diabetic and treated rats of both normal and diabetic for 8-weeks.

Parameters	Normal		Diabetic	
	Control group	Normal Rats +*C. colocynthis* Extract	Diabetic group	Diabetic Rats + *C. colocynthis* extract
Glucose	91.44 ± 3.61	73.28* ± 1.22	178.61 ± 1.29	119.12* ± 1.29
	--	- 19.86 %	--	- 33.30 %
Total Bilirubin	0.82 ± 0.03	0.69* ± 0.04	1.61 ± 0.27	1.22* ± 0.05
	--	- 15.85 %	--	- 24.22 %
Direct Bilirubin	0.29 ± 0.01	0.17* ± 0.09	0.43 ± 0.01	0.28* ± 0.03
	--	- 41.37 %	--	- 34.88 %

MDA	2.64 ± 0.21	1.51* ± 0.04	7.85 ± 0.30	3.61* ± 0.14
		- 42.80 %	--	- 54.01 %
LDH	132.90 ± 2.41	183.15* ± 2.32	108.34 ± 1.29	129.11* ± 2.21
	--	+ 37.81 %	--	+ 19.17 %
GSH	39.95 ± 1.55	52.32* ± 1.39	33.16 ± 1.31	42.25* ± 1.14
	--	+ 30.96 %	--	+ 27.41 %
ALT	27.19 ± 1.32	41.25* ± 1.13	52.32 ± 1.43	61.41* ± 1.31
	--	+ 34.08 %	--	+ 14.80 %
AST	44.12 ± 1.18	55.32* ± 1.24	71.68 ± 1.91	83.44* ± 1.16
	--	+ 25.38 %	--	+ 14.09 %
ALP	78.50 ± 1.23	93.22* ± 2.16	112.23 ± 2.72	121.51ns ± 2.67
	--	+ 15.79 %	--	+ 7.63 %

Data expressed as Mead ±SD. ALT**:** Alanine aminotransferase and AST: aspartate aminotransferase. ALP: alkaline phosphatase, and LDH: lactate dehydrogenase; MDA: malondialdehyde

Referring to hypolipidemic effect of *Citrullus colocynthis* seed ethanol extract administrated orally to normal and diabetic rats for eight weeks, the obtained data showed decrease in serum total lipids, triglycerides, total cholesterol, low density lipoproteins and very low density lipoproteins. Also, significant increase in high density lipoprotein levels was reported. These changes were statistically highly significant ($P < 0.001$) when compared to normal and diabetic controls.

Table (2): Serum lipid profiles of both normal and diabetic rats treated with *C.colocynthis* extract for 8-weeks.

Parameters	Normal		Diabetic	
	Control group	Normal rats +*C. colocynthis* extract	Diabetic group	Diabetic +*C. colocynthis* extract
TL	285.14 ± 2.93	239.25* ± 1.14	301.52 ± 1.93	254.23* ± 1.28
	--	- 16.09 %	--	- 15.68 %
TG	46.60 ± 1.71	41.31* ± 0.11	62.43 ± 1.16	43.86* ± 2.31
	--	- 11.35 %	--	- 29.74 %
TC	125.12 ± 1.24	98.66* ± 1.13	132.25 ± 1.62	93.56* ± 1.64
	--	- 21.14 %	--	- 29.25 %
HDL-C	55.14 ± 1.82	53.39ns ± 1.23	39.10 ± 0.28	46.45* ± 0.33
	--	- 03.17 %	--	+ 18.79 %
LDL-C	49.70 ± 1.50	31.44* ± 1.82	84.24 ± 1.26	62.14* ± 1.82
	--	- 36.74 %	--	- 26.23 %
vLDL-C	10.45 ± 1.51	8.33* ± 0.32	12.98 ± 0.82	9.13* ± 0.12
	--	- 20.28 %	--	- 29.66 %

Data expressed as Mead ±SD. TL: total lipids; TG: triglycerides; TC: total cholesterol; HDL-C: high density lipoproteins-cholesterol; vLDL-C: very low density lipoprotein and LDL-C: low density lipoprotein particles.

Discussion:

Since ancient times, plant remedies have been used to help to relieve diabetes. In the 6th century B.C., one of the Indian physician at this time classifying diabetes as a urinary disorder recommended plant remedies for its treatment **(Shanmugasundaram, et. al., 1990).** Also, prior to the development of insulin injection therapy in 1921, diabetes was managed entirely with indigenous medicinal plants **(Ahmad, et al 1999).** In the present study, oral administration of *Citrullus colocynthis* seed ethanol extract to female adult control and diabetic rats induced hypoglycemic and hypolipidemic effects. The antidiabetic properties of *Citrullus colocynthis* is probably due to enhanced insulin secretion or due to increase in peripheral glucose uptake, decreases gluconeogensis and inhibited release of counter-regulatory hormones **(Abd El-Baky, et al, 2009).** Also, **Berbecaru-Iovan et. al., 2009** explained that the hypoglycemic effect may be due to the stimulation of insulin biosynthesis and secretion or to the increase in the levels of glucose transporters and stimulation of peripheral glucose uptake and utilization. Also, the components of bitter melon (*C-colocynthis*) extract appear to have structural similarities to animal insulin (**Lee-Huang, et. al., 2000; Al-Ghaithi, et. al., 2004 and Dhanasekar & Sorimuthu, 2005**).

Our results are in agreement with who reported a beneficial effect on improving the glycemic profile without severe adverse effects in type II diabetic patients. This hypoglycemic effect may be due to the interference of the constituents contained in *Citrullus colocynthis* with the carbohydrate metabolism leading to the depletion of hepatic glycogen or its oil may have a beneficial effect by restoring pancreatic beta-cell mass in diabetic rats **(Elawad, et.al., 1984; Parmar and Kar, 2008 and Huseini, et.al., 2009)**

In this study ethanol extract of *Citrullus colocynthis* exert antioxidant effect represented by of significant decrease in lipid peroxide and enhancing the GSH activities in both normal and diabetic rats this in particular induced additional protection against radicals and electrophilic compounds, which in agreement with **(Haider and Zhao, 1996; Gebhardt, R., 2003; Kumar, et.al., 2008; Sebbagh, et.al., 2009).**

In this study, the obtained data demonstrated that *Citrullus colocynthis* extract exert an elevation of LDH and AST. Since Bitter melon extract administration result in glucokinase, glucose- 6-phosphate and phosphofructokinase values and a decrease in hexokinase value so the elevations in the activities of LDH and AST are attributed for these reasons and this in agreement with **(Bu-Abbas, et.al.,1998; Adam, et.al., 2001 and Debersac, et.al., 2001 and Rathi et. al., 2002).**

In the present work *Citrullus colocynthis* treated rats showed a significant elevating tendency in the serum ALT and ALP, which in agreement with the report of **(Wasfi, I.A., 1994; Bakhiet and El-Adam, 1995 and Ethan, 2003)** who found that significant increases in ã-glutamyltransferase and alkaline phosphatase have been observed in experimental animals after oral administration of bitter melon fruit juice and seed extract . In this study, ethanol extract of *Citrullus colocynthis* induced favorable effects on serum lipid parameters in normal and diabetic rats which in accordance with several investigators reported concerning the hypolipidemic ability of *Citrullus colocynthis.* This was attributed to the defatted part of the seeds, which is rich in fibers and contains steroid saponins **(Valette, et.al., 1984)**. The fiber-rich fraction induced a hypocholesterolemic effect, and the saponin and protein rich fraction was showen to reduce plasma cholesterol and triacyglycerol levels (**Ribes and Costa, 1987)**. Saponins were shown to be implicated in the hypocholestrolemic effect of seeds **(Sauvaire,et.al., 1991)**. Saponins have also been identified as the hypocholesterolemic component of seeds, interacting with bile salts in the

digestive tract (**Stark & Madar, 1993**). In addition, the lowering in the cholesterol level may be attributed to the enhancing effect of *Citrullus colocynthis* extract and its oil on cholesterol secretion in the bile (**Enomoto,et.al., 2001**) . *Citrullus colocynthis* possesses active hypolipidaemic constituents **(Daradka,et.al., 2007)**. The results showed both total and direct bilirubin significantly decreased in *Citrullus colocynthis* treated rats in agreement with **(Adam, *et. al.*,2001)** who indicated that there were changes in concentrations of bilirubin after ingestion *Citrullus colocynthis* extract. Also, the results demonstrated that an increase in feces lipid content in rats treated with the *Citrullus colocynthis* extract, in confirm with **(Salama, 1973)** who reported that steroidal saponins was reacted with fat diet in the digestive tract which may lead to the decrease in the absorption of cholesterol leading to a significant increased in feces lipid content in rats treated with the extract.

Conclusions: We conclude that bitter melon (*Ccolocynthis*) exert hypoglycemic, hypolipidaemic and antioxidative influence on both normoglycemic and hyperglycemic diabetic rats. Phytotherapy offers a valuable opportunity to discover new natural compounds with beneficial effects on glucose and lipids homeostasis and with the possibility of developing a few useful drugs from medicinal plants with a long history of human use. Also, further studies will carried out to confirm the mechanism and mode of action of this extract on the pancreatic and endothelial cells including histochemical and immunolocalization studies.

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