

ORIGINAL RESEARCH ARTICLE

Antioxidant Effect Of *Citrullus Colocynthis* On Alloxan Induced Diabetic Rats

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ABSTRACT

Aqueous extract of *Citrullus colocynthis* was screened for its antioxidant effect in alloxan induced diabetic rats. Preliminary phytochemical screening of the plant shows the presence of large amount of terpenoids, phenolics and flavanoids. The presence of phenolic compound prompted us to evaluate its antioxidant effect. An appreciable decrease in peroxidation products was observed in *C. colocynthis* (300 mg/kg body wt.) treated diabetic rats. The decreased activities of key antioxidant enzymes such as SOD, CAT, GSH and Gpx in diabetic rats were brought back to near normal range upon *C. colocynthis* treatment.

Key Words: *Citrullus colocynthis*, alloxan, lipid peroxidation, antioxidant.

Abbreviations: SOD, Superoxide dismutase; LPO, lipid peroxidation; CAT, catalase; GSH, reduced glutathione; GPx, glutathione peroxidase; OH, hydroxyl radical; RO₂, peroxy radical; MeOH, methanol; EtOH, ethanol.

INTRODUCTION

Diabetes is a serious metabolic disorder with micro and macro vascular complications that result in a significant morbidity (or) mortality. The increasing proportion of the aging population, consumption of calorie rich diet, obesity and sedentary life style have led to a tremendous increase in the number of diabetes worldwide (vats et al., 2004). Diabetes is a disease of great concern to many all over the world and is known for its complications that include: diabetic nephropathy, neuropathy and retino- pathy (Zimmet et al., 2001). Several antioxidants of plant materials are experimentally proved and widely used as more effective agents against oxidative stress (Manonmani et al., 2002). Normal cells have a number of endogenous antioxidants which eliminates toxic oxymetabolites under normal conditions (Reilly et al., 1991). The antioxidant might protect a target at many different stages in oxidative sequences such as (1) Removing oxygen or decreasing local oxygen concentration, (2) removing catalytic metal ions and (3) scavenging the initiating

radicals such as OH, RO and RO₂. The *Citrullus colocynthis* also known as bitter apple, bitter cucumber and vine of Sodom, is a viny plant native to the Mediterranean Basin and Asia. They contain active substances like saponins, alkaloids and glycosides (Abdel-hasan *et al.*, 2000). They also have antidiabetic, anti hypersensitive (Ziyatt et al., 1997) and immunostimulant effect (Bendjeddo et al., 2003). The dried pulp of its fruit is used as a traditional medicine mostly for constipation (Arena and Drew, 1980). In East Africa, the seed tar was used by nomads in traditional medications applied to the skin. Many workers suggested that the fruits of *Colocynthis* posses anti tumour activity (Faust et al., 1958).

In United Arab Emirates, the seeds are used to treat diabetes. However, when the chloroform and MeOH extracts of seeds, EtOH extracts of leaves and pulp of *C. colocynthis* were investigated in normal and diabetic induced rats and isolated organs of rats and rabbits, seed extract had no effect on fasting glucose levels in diabetic rats and they had no effect in the oral

glucose tolerance test. This study is aimed at investigating the antioxidant effect of aqueous extract of the seeds of *C. colocynthis*.

MATERIALS AND METHODS

Plant material

The seeds of *C. colocynthis* were collected from Perambalur region. The plant was identified, authenticated by Botanist Dr. V.Kumaresan, Department of Biotechnology, Thanthai Hans Roever College, Perambalur, Tamilnadu, India. The seeds were dried and powdered in grinding mill.

Preparation of extracts

The seeds were pulverized in grinding. 200g of powdered seeds was dissolved in 400 ml of distilled water for 1h at room temperature with continuous shaking. The mixture was filtered using sterile gauze. A fresh filtrate was used for the treatment (Ghaithi *et al.*, 2004).

Animals

Male albino rats weighing about 150 - 200 g obtained from the Eaisma Institute, Karur, were used for the study. The rats were kept in the animal house at a room temperature of 15 - 30° C, fed with commercial food ad libitum and had free access to water. The experiments were designed and conducted in accordance with the ethical norms approved by the government.

Induction

Alloxan monohydrate was used to induce diabetes mellitus in normoglycemic rats. Animals were allowed to fast for 16 h and were injected intraperitoneally with freshly prepared alloxan monohydrate in normal saline in a dose of 120 mg/kg body weight (Demerdash *et al.*, 2005). Blood and urine glucose levels of these rats were estimated 72 h after alloxan administration and the rats having hyperglycemia and glycosuria were selected for the experiment.

Experimental design

The animals were divided into five groups of six each.

Group I: Normal control

Group II: Normal control + plant treatment (300 mg/kg body wt)

Group III: Diabetic control

Group IV: Diabetic control + plant treatment

Group V: Diabetic control + glibenclamide treatment (0.5 mg/kg body wt).

Biochemical analysis

Fasting blood glucose level was determined by the method of Sasaki *et al.* (1972). The Hb level

was measured by Drabkin and Austin (1932). HbA1C was estimated by the methods of Nayak and Pattabiram (1981). Plasma insulin was estimated by using ELISA kit by Linco Research Inc. The level of lipid peroxidation was assayed by the method of Ohkawa *et al.* (1979). The GSH level was determined by the method of Ellman (1959). The activity of glutathione peroxidase was assayed by the method of Rotruck *et al.* (1973). The superoxide dismutase was assayed by the method of Misra and Fridovich (1972). Catalase was assayed according to the method of Takahara *et al.* (1960).

Statistical analysis

The values are expressed as mean \pm standard deviation (S.D) for 6 rats in each group. All other data were analyzed with SPSS/16.0 student software. Hypothesis testing method included one way analysis of variance (ANOVA) followed by post hoc testing performed with least significant difference (LSD) test. The p value of less than 0.05 was considered to indicate statistical significance.

RESULTS

(Table 1) shows the qualitative analysis of phytochemicals in the aqueous extract of *C. Colocynthis* seeds. From preliminary phytochemical screening, it was found that the extract showed a positive response for the presence of flavanoids, alkaloids, glycosides, saponins, phyto-sterols, steroids, proteins and triterpenoids.

Table-1: List of phytochemicals in aqueous extract of *Citrullus Colocynthis* seeds.

Phytochemicals	Influence
Flavanoids	+
Alkaloids	+
Glycosides	+
Saponins	+
Steroids	+
Triterpenoids	+
Tannins	+
Carbohydrate	+
Protein	+
Amino acids	+
Anthroquinones	-

+ = Present; - = absent.

Treatment with *C. colocynthis* aqueous extract and its effect on blood glucose level, insulin, Hb, and HbA1C are shown in (Table 2). The diabetic control rats showed a significant increase in the blood glucose and HbA1C level. The

administration of *C. colocynthis* and glibenclamide to diabetic rats restored the changes in glucose and HbA1C level. The insulin and Hb levels were reduced in diabetic control rats. In diabetic treated groups IV and V, the insulin and Hb levels increased.

(Table 3) and (Table 4) shows changes in the activities of LPO, SOD, CAT, Gpx and GSH in

serum and liver of normal and treated rats. The activities of SOD, CAT, GSH and Gpx levels in serum and liver decreased in diabetic control rats. After the treatment, the levels came back to near normal. In diabetic control rats, LPO level increased. In plant and drug treated rats, the levels reduced significantly.

Table 2. Effect of *C. colocynthis* (CC.Aqt) on blood glucose, protein, insulin Hb and HbA1C levels.

Groups	Glucose (mg/dl)	Insulin (mg/dl)	Hb (g/dl)	HbA ₁ C (%)	Urine sugars
Normal	89.33 ± 2.16	92.16 ± 6.49	11.3 ± 0.96	4.15 ± 0.15	+
Normal + CC.Aqt	93.50 ± 5.57 ^a	87 ± 19.43 ^a	10.01 ± 0.48 ^a	4.23 ± 0.20 ^a	+
Diabetic Control	268.60 ± 5.66 ^{b*}	48.18 ± 12.64 ^{b*}	6.73 ± 0.57 ^{b*}	10.23 ± 0.38 ^{b*}	+++
Diabetic + CC.Aqt	109.17 ± 4.25 ^{cd*}	83.56 ± 2.39 ^{cd*}	9.8 ± 0.29 ^{cd*}	5.90 ± 0.88 ^{cd*}	+
Diabetic + Glibenclamide	72.60 ± 2.16 ^{c*}	91.66 ± 6.25 ^{c*}	12.6 ± 1.65 ^{c*}	4.95 ± 0.15 ^{c*}	+

Table 3. Effect of *C. colocynthis* extracts (CC.Aqt) on the levels of SOD, CAT, GSH, GPX and LPO in serum of alloxan diabetic rats.

Group	SOD (IU/L)	CAT(IU/L)	GSH (IU/L)	GPX (IU/L)	LPO (IU/L)
Normal	198.17 ± 12.52	21.66 ± 4.50	107.50 ± 12.51	157 ± 10.71	92.41 ± 16.52
Normal + CC.Aqt	201.60 ± 13.66 ^a	22.83 ± 4.79 ^a	113 ± 17.64 ^a	159.17 ± 10.85 ^a	95.16 ± 18.14 ^a
Diabetic Control	153.50 ± 8.89 ^{b*}	10.65 ± 2.49 ^{b*}	165.67 ± 11.05 ^{b*}	127.90 ± 9.59 ^{b*}	158.13 ± 17.75 ^{b*}
Diabetic + CC.Aqt	193.83 ± 11.63 ^{cd*}	22.80 ± 5.67 ^{cd*}	107.50 ± 9.48 ^{cd*}	152.33 ± 8.86 ^{cd*}	102.83 ± 15.13 ^{cd*}
Diabetic + Glibenclamide	191.67 ± 11.41 ^{c*}	22.31 ± 6.08 ^{c*}	120.83 ± 7.60 ^{c*}	160.17 ± 8.49 ^{c*}	94.67 ± 14.20 ^{c*}

Table 4. Effect of *C. colocynthis* extracts (CC.Aqt) on the levels of SOD, CAT, GSH, GPX and LPO in liver of alloxan diabetic rats.

Group	SOD (IU/L)	CAT(IU/L)	GSH (IU/L)	GPX (IU/L)	LPO (IU/L)
Normal	197.1 ± 12.52	20.63 ± 4.15	104.67 ± 13.41	157.67 ± 10.32	95 ± 16.16
Normal + CC.Aqt	201.06 ± 14.40 ^a	21.83 ± 4.97 ^a	112.17 ± 17.50 ^a	151.67 ± 10.42 ^a	94.50 ± 18.06 ^a
Diabetic Control	124.3 ± 29.20 ^{b*}	8.33 ± 2.94 ^{b*}	208.17 ± 46.91 ^{b*}	105 ± 13 ^{b*}	188.50 ± 66.34 ^{b*}
Diabetic + CC.Aqt	198 ± 14.80 ^{cd*}	23.66 ± 6.62 ^{cd*}	110.67 ± 15.35 ^{cd*}	154.83 ± 13.93 ^{cd*}	106.67 ± 29.70 ^{cd*}
Diabetic + Glibenclamide	178.8 ± 37.12 ^{c*}	20.16 ± 5.11 ^{c*}	116.17 ± 10.79 ^{c*}	158 ± 10.29 ^{c*}	102.50 ± 15.08 ^{c*}

Values are given as mean ± SD of six rats from each group; Values are statistically significant at *P<0.05.

DISCUSSION

Diabetes mellitus arises from the irreversible destruction of the pancreatic beta cells causing degranulation and reduction of insulin secretion (Junod et al., 1969). The use of traditional medicine and medicinal plants in most developing countries, as a normative basis for the maintenance of good health, has been widely observed (Bhattaram et al., 2002)

This study was therefore undertaken to assess the antioxidant effect of *C. Colocynthis* in alloxan induced rats. A variety of herbs and herbal extracts contain different phytochemicals with biological activity that can have valuable therapeutic index. Most of the protective effect of fruits and vegetables has been attributed to phytochemicals, which are non-nutrient plant

compounds such as carotenoids, flavanoids, isoflavanoids and phenol acids. Most of the phytochemicals have the ability to inhibit lipid peroxidation and also possess hypoglycemic and hypolipidemic properties. (Johnson et al., 1993)

Flavanoids extend the activity of vitamin C and act as antioxidants that protect LDL cholesterol from oxidation inhibit platelet aggregation and act as anti-inflammatory agent. Rupasinghe et al. (2003) have reported that saponins possess hypocholesterolemic, antidiabetic, antitumour, antiviral, antioxidative, anticarcinogenic and hepatoprotective properties. Phenolic compounds have cholesterol lowering effect (Leontowicz et al., 2002). It has also been found that plant alkaloids have the tendency to release insulin

from pancreatic beta cells (Ignachimuthu and Amalraj, 1998). The aqueous extract of *C. colocynthis* produces significant blood glucose lowering effect in alloxan induced diabetic rats. The possible mechanism includes the stimulation of beta cells and subsequent release of insulin and activation of insulin receptors. A number of other plants have also been reported to have antidiabetic and insulin release stimulatory effect (Prince *et al.*, 1998; Pari and Uma, 1999). HbA1C was found to increase in patients with diabetes mellitus to about 16% (Koeing *et al.*, 1976) and the increment is directly proportional to the fasting blood sugar level. The HbA1C level reduced after the treatment of plant extract and drug.

The involvement of free radicals in diabetes and the role of these toxic species in LPO and the antioxidant defense system have been studied. Hypoinsulinemia in diabetes increases the activity of the enzyme fatty acetyl CoA oxidase which initiates beta oxidation of fatty acids, resulting in lipid peroxidation (Horie *et al.*, 1981). Increased lipid peroxidation impairs membrane function by decreasing membrane fluidity and changing the activity of membrane bound enzyme and receptors (Acworth *et al.*, 1997). Our present study showed the significant elevation of LPO in diabetic rats. The increased LPO content suggest that peroxidative injury may be involved in the development of diabetic complications.

Lipid peroxide mediated tissue damages have been observed in the development of Type 1 and 2 diabetes mellitus (Feillet-Coudray *et al.*, 1999). Previous studies have reported that there was an increased LPO in liver and kidney of diabetic rats (Latha and Pari 2002; Venkateswaran and Pari, 2002).

SOD and CAT are the two scavenging enzymes that remove the toxic free radicals (Wohaieb and Godin, 1987). Previous studies have reported that the activity of SOD is low in diabetes mellitus (Vucic *et al.*, 1997). Reduced activities of SOD and CAT in liver and kidney have been observed during diabetes, and this may result in a number of deleterious effects due to the accumulation of oxygen radicals and hydrogen peroxide (Searle and Wilson, 1980). Administration of *C. colocynthis* increases the activity of these enzymes and may help to control the free radical, as the plant is known to be rich in flavanoids and triterpenoids, well

known antioxidants which scavenge the free radicals generated during diabetes.

Reduced glutathione (GSH), a key antioxidant, is an important constituent of intracellular protective mechanisms against oxidative stress (Ross, 1998). We have observed a significant decrease in GSH levels in liver and serum during diabetes. This represents increased utilization due to oxidative stress (Anuradha and Selvam, 1993). The decreased GSH content contributes to the pathogenesis of complications associated with chronic diabetic state. Depression in GPx activity was also observed in liver and serum during diabetes. GPx has been shown to be an important adoptive response to conditions of increased peroxidative stress (Matkovics *et al.*, 1982).

The result of the SOD, CAT and GPx activity suggest that *C. colocynthis* contains a free radical scavenging activity, which could exert a beneficial action against pathological alterations caused by the presence of super oxide and hydrogen peroxide. This action could involve mechanisms related to scavenging activity.

In conclusion, the present investigation shows that aqueous extract of *C. colocynthis* possess an antidiabetic effect in addition to antioxidant activity.

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