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# Antidiabetic Activity of ethanol extract of *Citrus medica* L. peels in streptozotocin induced diabetic rats

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## ABSTRACT

Diabetes causes increased oxidative stress, which is thought to play an important role in the pathogenesis of various diabetic complications. The antioxidant actions are keys to preventing or reversing diabetes and its complications. Thus the aim of the present study was to evaluate the antioxidant potential of ethanolic extract of *Citrus medica* L. peels (ECMP) extract on Thiobarbituric Acid Reactive Substances (TBARS) as index of lipid peroxidation and on the glycemic control in streptozotocin induced diabetic rats. Effect of oral administration of ECMP (200 and 400 mg/kg) on the level of blood glucose, glycosylated hemoglobin, on TBARS levels were estimated. Glibenclamide was used as a standard drug. The elevated level of blood glucose, glycosylated hemoglobin, TBARS observed in diabetic rats were significantly decreased after treatment with ECMP for 8 weeks in diabetic rats. From the results it suggest that the ethanolic extract of *Citrus medica* L. peels possessed potent antioxidant and antidiabetic properties which may be due to the presence of biological active ingredient such as alkaloids, flavonoid, triterpenoids and phenolic compounds.

**Key words:** diabetes, streptozotocin, oxidative stress, *Citrus medica* L.

## INTRODUCTION

Oxidative stress in cells results from the increased generation of reactive oxygen species and/or from decreases in antioxidant defense potential.[1] Several hypotheses have been put forth to explain the genesis of free radicals in diabetes. These include autoxidation processes of glucose, the non-enzymatic and progressive glycation of proteins with the consequently increased formation of glucose-derived advanced glycosylation end products (AGEs), and enhanced glucose flux through the polyol pathway.[2,3] World Health Organization has recommended that medicinal plant research warrant attention. [4,5] Plants have been the major source of drugs in Indian system of medicine. Earliest description of curative properties of medicinal plants was found in Rig Veda (2500- 1800 BC), Charaka Samhita and Sushruta Samhita give extensive description on various medicinal herbs. [6] Diabetes mellitus has been shown to be a state of increased free radicals formation. Oxidative stress may increase in diabetes owing to a higher production of reactive oxygen species results into deficiency in antioxidant defense systems. [7] Thus the antioxidant actions are keys to preventing or reversing diabetes and its complications [8] Thus the aim of the present study was to evaluate the protective antioxidant effects of ethanolic extract of *C. medica* L. peels on the glycemic control in streptozotocin induced diabetic animals.

## MATERIALS AND METHODS

### Animals

All the experiments were carried out in Wistar rat (180-220 g) of either sex. The animals had free access to food and water, and they were housed in a natural light–dark cycle. The animals were acclimatized to the laboratory conditions for at least two week before experiments. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) and the laboratory animals were taken care according to the guidelines of CPCSEA, Ministry of Forests and Environment, Government of India (registration number 729/02/a/ CPCSEA).

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### Chemicals

Glibenclamide as a standard (Themis Pvt.Ltd, Mumbai), Streptozotocin (purchased from Spectrochem Labs, Mumbai), glucose estimation kit (Beacon Diagnostics Pvt. Ltd, Navasari) and glycosylated haemoglobin estimation kit (Quality Biomedicals, Mumbai) All the reagents and chemicals used in present study were of analytical grade.

### Plant material

The fresh fruits of *Citrus medica L*. were collected from its natural habitat at Hadgaon in Nanded region, Maharashtra, India. The plant was authenticated by Dr. Miss. A. Chaturvedi, Post Graduate Teaching Department of Botany, Rashtra Santa Tukadoji Maharaj Nagpur University, Nagpur (Voucher specimen no. 9844).

### Preparation of plant extract

From the collected fruits of *Citrus medica L*. peels were removed manually and dried under shade and undergone crushing in electric blender to form powdered and subjected to extraction by using Soxhlet extractor using distilled ethanol as a solvent in ratio of 1:5 (50 g powder with 250 ml solvent). The extracts were concentrated by evaporation at room temperature and were used for pharmacological studies. The percent yield for ethanolic extract of *Citrus medica L*. peels was found to 24.49 % w/w.

### Phytochemical screening

Preliminary phytochemical screening for the presence of alkaloids, glycosides, tannins, saponins, flavonoids, triterpenoids, phenols, volatile oils was carried out using standard test procedures.[9]

### Administration of extract

Suspension of ethanolic extract was prepared in 0.5% carboxymethyl cellulose using Tween 20 (0.2% v/v) as a suspending agent. The extract was administered in a dose of 200 and 400 mg/kg respectively to streptozotocin induced diabetic Wistar rats. Control groups were given only 0.5% carboxymethyl cellulose with Tween 20 (0.2% v/v).

**Experimental Design**

**Induction of diabetes in rats**

Overnight fasted animals were injected with STZ (60mg/kg dissolved in 0.1M citrate buffer pH 4.5) intraperitoneally (i.p). After 48 hours only those rats which showed plasma glucose levels > 250 mg/dl were classified as diabetic and were included in the study. The animals were allowed to drink 5% glucose solution overnight for prevention of mortality due to hypoglycemic convulsing reaction. After 10 days of STZ administration the remaining survival animals considered as diabetics were used for experimentation. [10]

**Antidiabetic activity in streptozotocin induced diabetic rats**

10 days after streptozotocin induction, control and survival diabetic rats were randomly divided in five groups, each consisting of five animals. Group one as a normal vehicle control received 0.5% sodium CMC with twin 20 (0.2%v/v). Group 2 as a diabetic control and received vehicle only. Group 3 diabetic animals received glibenclamide (10mg/kg, p.o.) Group 4 and 5 diabetic animals received 200 and 400 mg/kg of ethanolic extract of *C. medica* L peel respectively. After the 4 and 8 weeks of above treatments schedule animal's blood was withdrawal by retro-orbital plexus for determination of plasma glucose, glycosylated haemoglobin and determination of thiobarbituric acid reactive substance was carried out after 8 weeks.

**Determination of blood glucose, and glycosylated hemoglobin**

Plasma blood glucose was estimated by glucose estimation kit by GOD-POD method while glycosylated hemoglobin was assayed according to reported method by using colorimetric kit using a colorimetric method, based on the phenol sulphuric acid reaction of carbohydrates. [11]

**Determination of thiobarbituric acid reactive substance (TBARS):**

Biological specimen contains a mixture of thiobarbituric acid reactive substances (TBARs) including lipid peroxide and aldehyde, which increase as a result of oxidative stress and determined spectrophotometrically. In practice TBARs are expressed in terms of MDA equivalent. [12]

**Statistical analysis**

All the results were expressed as mean ± SD. (n=5) The Statistical significance between means was analyzed using one-way analysis of variance (ANOVA) followed by Dunnet's multiple comparison post-test using Graph **Pad software.** *P-values < 0.01* were considered as more significant.

**RESULT**

The result of the phytochemical screening revealed the presence of alkaloids, flavonoids, triterpenoids, steroids and volatile oil. Table 1 shows the plasma glucose values, before and after 4 and 8 weeks treatment in normal, diabetic untreated and diabetic treated rats with ethanolic extract of *C.medica* L. peels (ECMP). Once daily oral administration of extract (200 and 400 mg/kg p.o) and glibenclamide (10 mg/kg) produced significant ($p < 0.01$) decrease in plasma glucose levels after 4 and 8 weeks which demonstrate antidiabetic activity in the extract.

Table 2 shows the values of glycosylated haemoglobin for normal, diabetic untreated and diabetic treated rats. ECMP treated groups dose of 200mg/kg and 400mg/kg showed significant ($p< 0.01$) decrease in the glycosylated hemoglobin after 4 and 8 weeks treatment.

However oral administration of ethanolic extract of *C. medica* L. significantiy ($p < 0.01$) reduces the level of TBARS in diabetic rats as compared to diabetic control groups of rats as shown in Table 3. These findings suggest that the ethanolic extract of *C. medica* prevents the formation of glycosylated haemoglobin which ultimately protect from the formation of reactive oxygen species and induction of lipid peroxidation by STZ in diabetic rats.

**Table 1: Effect EECM on blood glucose level of Streptozotocin induced diabetic rats**

| Group | Treatment | Dose p.o. | 0 Week (Pre-treatment) (mg/dl) | 4 Weeks (Post-Treatment) (mg/dl) | 8 Weeks (Post-Treatment) (mg/dl) |
|---|---|---|---|---|---|
| I | Normal Control | - | 115±6.55 | 116.2±4.91 | 119.8±7.59 |
| II | Diabetic Control | - | 368.6±38.68 | 429.2±25.05[a] | 485±23.57[a] |
| III | Standard Glibenclamide | 10 mg/kg | 389.8±58.67 | 186.8±19.90[b] | 132.4±8.87[b] |
| IV | EECM | 200 mg/kg | 371.4±49.47 | 330.20±48.59[b] | 200.2±23.86[b] |
| V | EECM | 400 mg/kg | 408.4±46.52 | 297±35.48[b] | 133.4±10.94[b] |

*The values are given as MEAN±SEM of five rats in each group. [a] indicates significant ($p < 0.01$) when diabetic control group compare to normal control group at 0 week.*

*[b] indicates significant ($p < 0.01$) when treatment group compared with diabetic control animals group. One-way ANOVA followed by Dunnet's multiple comparison test.*

**Table 2: Effect of EECM on glycosylated hemoglobin in Streptozotocin induced diabetic rats**

| Group | Treatment | Dose p.o. | 0 Week (Pre-treatment) (mg/dl) | 4 Weeks (Post-Treatment) (mg/dl) | 8 Weeks (Post-Treatment) (mg/dl) |
|---|---|---|---|---|---|
| I | Normal Control | - | 7.13±0.98 | 7.17±1.42 | 7.20±1.14 |
| II | DiabeticControl | - | 13.8±1.26[a] | 14.62±0.65[a] | 15.87±0.26[a] |
| III | Standard Glibenclamide | 10 mg/kg | 14.37±0.43 | 10.72±1.56[b] | 8.19±0.68[b] |
| IV | ECMP | 200 mg/kg | 13.47±0.67 | 12.10±1.04[b] | 10.04±0.68[b] |
| V | ECMP | 400 mg/kg | 14.54±0.12 | 12.08±0.43[b] | 8.32±0.37[b] |

*The values are given as MEAN±SEM of five rats in each group. [a] indicates significant ($p < 0.01$) when diabetic control group compare to normal control group at 0 week.*

*[b] indicates significant ($p < 0.01$) when treatment group compared with diabetic control animals group. One-way ANOVA followed by Dunnet's multiple comparison test.*

**Table 3: Effect of EECM on Lipid Peroxidation in Streptozotocin induced diabetic rats**

| Group | Treatment | Dose p.o. | 8 Weeks (Post-Treatment) |
|---|---|---|---|
| I | Normal Control | - | 0.2323±0.01 |
| II | Diabetic Control | - | 0.6074±0.09[a] |
| III | Standard Glibenclamide | 10 mg/kg | 0.2741±0.08[b] |
| IV | EECM | 200 mg/kg | 0.2777±0.05[b] |
| V | EECM | 400 mg/kg | 0.1577±0.05[b] |

*The values are given as MEAN±SEM of five rats in each group. [a] indicates significant ($p < 0.01$) when diabetic control group compare to normal control group at 0 week.*

*[b] indicates significant ($p < 0.01$) when treatment group compared with diabetic control animals group. One-way ANOVA followed by Dunnet's multiple comparison test*

## DISCUSSION

Diabetes mellitus is a clinical syndrome characterized by inappropriate hyperglycemia caused by a relative or absolute deficiency of insulin or by a resistance to the action of insulin at the cellular level. It is the most common endocrine disorder.[13,14] The glucose stays in blood instead and its level then gets too high, causing diabetes. Current medications can have serious side effects including nausea, diarrhea, skin rash, weight gain, respiratory infections, liver damage, hypertension, neuropathy and headaches and meager problem of tolerance to medicines.[15,16] Herbal drugs play a role in the diseases, most of the them speed up the natural healing process. STZ-induced experimental diabetes is a valuable model for induction of diabetes. Further, the STZ diabetic animals may exhibit most of the diabetic complications, namely, myocardial cardiovascular, gastrointestinal, nervous, vas deferens, kidney, and urinary bladder dysfunctions through oxidative stress.[17]

The aim of present study is investigation of antidiabetic activity of ethanolic extract of Citrus medica on blood glucose in STZ-induced diabetic rats. This was clearly evidenced by the increased level of insulin in diabetic rats treated with extract. It has been reported that Finger citron i.e. *Citrus medica* L. var. Sarcodactylis posses the insulin secretagogue bioactivity.[18] The most of all chemical constituents were present in ECMP includes steroids, phenolics, flavonoids, alkaloids and volatile oil. In our study, we have observed that there is a decrease in the plasma blood glucose level when Streptozotocin diabetic rats were treated with our ethanolic extract of Citrus medica L. This may be due to potentiation of the insulin effect of plasma by increasing the pancreatic secretion of insulin from existing ß-cells of islets of Langerhans or it release from bound insulin or may be due to its antioxidant activity.

During diabetes, the excess glucose present in the blood reacts with hemoglobin to form glycosylated hemoglobin. It has been reported that various proteins, including hemoglobin, albumin, collagen, LDL, or crystalline proteins undergo non-enzymatic glycation in diabetes.[19] The rate of glycation is proportional to the concentration of blood glucose.[20] Glycosylated hemoglobin has been found to be increased over a long period of time in diabetes.[21] There is an evidence that glycation itself may induce the formation of oxygen-derived free radicals in diabetic condition. Therefore, the measurement of glycosylated hemoglobin is supposed to be a very sensitive index for glycemic control.[22]In the present study, the diabetic rats had shown higher levels of glycosylated hemoglobin compared to those in normal rats indicating their poor glycemic control; while after 4 and 8 weeks of ECMP treatment, the glycosylated hemoglobin significantly decreased as comparable to standard glibenclamide, indicating a decrease in the status of glycation.[23]

Lipid peroxidation is one of the characteristic features of chronic diabetes. The increased free radicals produced may react with polyunsaturated fatty acids in cell membranes leading to lipid peroxidation. Lipid peroxidation will in turn result in the elevated production of free radicals.[24]In the present study, we have observed that ECMP reduced oxidative stress by increasing antioxidant defense and reducing free radical induced lipid peroxidation.

## CONCLUSION

In conclusion, our study demonstrated beneficial effects of Citrus medica L. (400 mg/kg), in STZ induced diabetic rats. It decreases blood glucose level and has potential to decrease TBARS levels by inhibiting the lipid peroxidation. These findings would be helpful in diabetic patient for prevention of diabetic complications related to level of oxidative stress.

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