Evaluation of anti-diabetic activity of *Trianthema portulacastrum* Linn in Dithizone induced diabetes in Wistar rats.

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**ABSTRACT**

*Trianthema portulacastrum* Linn. Whole plant is an extensively used plant for medication in India for the treatment of various health issues. This study was focused to prefigure the antidiabetic potency of Chloroform extract of the *Trianthema portulacastrum* (CETP) whole plant administered at two doses (100mg/kg and 200 mg/kg) for the duration of 21days in Dithizone - induced diabetic rats. The rats were separated into five groups, each group containing six animals. All Groups except Group I were made diabetic by intraperitoneal administration of Dithizone(50mg/kg). Group I served as control group, Group II served as diabetic control received Dithizone(50mg/kg), Group III rats were administered with glibenclamide (10mg/kg), a standard oral hypoglycemic agent while Group IV and Group V diabetic rats were served with 100 mg/kg and 200 mg/kg of CETP whole plant respectively. Potency of the plant extract as an antidiabetic was assessed in dithizone induced diabetic models by comparing biochemical parameters like blood glucose level, Glucose tolerance, lipid profiles along with the liver antioxidant enzymes, glycogen content and Glycogenic enzymes which were quantified using standard experimental procedures. There exists a significant reduction in levels of blood glucose, raise in glucose tolerance, and improved imbalance in lipid metabolism in diabetic rats after administration of the extract at 100mg/kg and 200mg/kg. *T.portulacastrum* with 200mg/kg of the extract showed the best hypoglycemic action by comparing favorably well with Glibenclamide. This investigation clearly showed that, the extract is endowed with hypoglycemic activity, *T.portulacastrum* may also defend the deterioration of liver due to diabetes.

**INTRODUCTION**

WHO reports Diabetes is a major endocrinial disorder which will affect a near population of 10% worldwide in the year 2020 (*King et al.*, 1998). Diabetes mellitus is a group of metabolic disorders with hypoglycemia where there is a rise in blood sugar level in an individual due to either reduced insulin production or no utilization the insulin by cells that are produced in. This raised blood sugar associated with the classical signs like polyphagia, polydipsia and polyuria. According to the traditional Indian system of medicine (Ayurveda), a men-
tion was made on a good number of plants for the cure of diabetes or ‘madhumeha’ and some of them have been experimentally screened and the active principles have been isolated and identified (Grover et al., 2002). It is crucial to induce diabetes in experimental animals which open window for the researcher to develop a new therapy in order to terminate the side effects including patient factors and environmental parameters that nullifies a clinical investigation (Potenza et al., 2011). However, search for the new anti-diabetic drugs continues. The current study was developed to evaluate the anti-diabetic potency of chloroform extract of Trianthema portulacastrum in dithizone induced diabetic rats. The potency was comparable with Glibenclamide (a standard hypoglycemic drug).

MATERIALS AND METHODS

Plant material and Extraction

The whole plant of Trianthema portulacastrum was collected from the forests of Maisammaguda, Secunderabad situated in the state of Telangana (India) and shade dried. The plant specimen was authenticated by a botanist of Osmania University and authenticated voucher specimen Number 145 of the plant has been potted in the department for future reference. The plant material was shade dried, then milled to coarse powder mechanically and successively extracted with Petroleum ether, Chloroform, Ethyl acetate and Methanol using Soxhlet-extractor. Method of maceration was followed for water for 72 hours. The rotary evaporator was used for concentrating the extracts, dried in vacuum desicators, properly labelled and weighed, stored thereafter in the refrigerator until further use. Preliminary Phytochemical screening for the above plant extracts was conducted (Divya, 2020). Based on the presence of phytochemical constituents, chloroform extract was considered for the evaluation of anti-diabetic potential.

Animals

Ethical approval for this experimental study was obtained from the Institutional Animal Ethical Committee with an Approval no: CPCSEA/IAEC/JLS/11/11/19/14. Wistar albino rats with average body weight from 150g to 250 g were utilized in this study. They were procured from Sanzyme Bio-analytical lab, Plot no. 8 Sys.No.542, Kothur(V), Shameerpet, R.R. Dist. The rats were caged in polypropylene cages and maintained under standard conditions (12 h light and dark cycles at 25 ± three °C and 35-60 % humidity). Standard pellet feed and tap water were provided ad-libitum.

Experimental Design

The rats were split into two sets, each comprising five groups (n = 6 in each group): Former one for the evaluation of anti-diabetic/toxicity studies and the latter for the evaluation of glucose tolerance. Diabetes was induced in all groups other than Group-I by injecting Dithizone at 50mg/kg b/w intraperitoneally. Development of diabetes was allowed for 3 days. Group-I regarded as control received normal saline (1ml/kg p.o.) as a vehicle for a period of 21 days. Group-II regarded as diabetic control, were administered with Dithizone (50mg/kg). Group-III received Glibenclamide (10mg/kg). Groups IV and V were administered with 100mg/kg and 200 mg/kg body weight/day of Chloroform Extract of Trianthema portulacastrum (CETP).

Estimation of Blood Glucose level

At the end of the study, the experimental rats were subjected to overnight fasting. On the 22nd day, Blood samples (3ml) were withdrawn from the tail vein and subjected for the estimation of blood glucose level using a glucometer (Accu-Chek, Roche Products (Pty) Ltd., South Africa) at 0, 1/2, 1, 2, 4, 6, 8hr.

Estimation of Lipid parameters and Total Protein

Parameters like triglycerides, total cholesterol, HDL cholesterol, and LDL-cholesterol concentrations in serum were determined by automatic analyser technique (Beckman Coulter Inc., Ireland). Total protein in the serum was estimated using bovine serum albumin as a standard (Henry et al., 1974).

Liver Function Tests

The concentrations of hepatic markers like total bilirubin (Malloy and Evelyn, 1937), alkaline phosphatase (ALP) (Wright et al., 1972), aspartate and alanine transaminases (AST and ALT) (Gad-Elkareem et al., 2019) were determined in the serum using Randox Assay kits.

Biochemical estimation of markers of oxidative stress

At the end of the study, on 22nd day, animals were sacrificed and the liver tissue from all the experimental groups of animals was removed carefully followed by washing thoroughly with ice-cold saline, Wet tissue of 0.5 gms was taken exactly and allowed for homogenization in 0.1M Tris–Hydrochloric acid buffer at pH 7.4, the temperature of 4°C in a Remi homogenizer with a Teflon pestle rotated at 600 rpm for 30 min. The homogenate was allowed for centrifugation at 2500 rpm for 10 min at 4°C using a refrigerated centrifuge. The resultant supernatant
was utilised for performing the assay of lipid peroxidation products and antioxidant enzymes such as malondialdehyde (MDA) (Uchiyama and Mihara, 1978), reduced glutathione (GSH) (Sedlak and Lindsay, 1968), superoxide dismutase (SOD) (Richard et al., 1976), Catalase (CAT) (Aebi, 1984).

**Oral Glucose Tolerance Test**

On day 22, the rats from groups I to V of the latter set were received glucose orally (2 g/kg body weight) after 30 min from the administration of the extract/drug (Joy and Kuttan, 1999). Blood samples were withdrawn from the tail vein prior to glucose administration and at 30, 60, and 90 min after glucose loading for immediate measurement of blood glucose levels.

**Estimation of Glycogen content and Gluconeogenic enzymes**

Hepatic glycogen content was estimated by the method of Carroll et al., (Carroll et al., 1956), hepatic gluconeogenic enzymes like glucose-6-phosphatase was estimated by the method described by Koide and Oda (Hikaru and Toshitsugu, 1959), succinate dehyrogenase was estimated by the method proposed by Slater EC et al., (Slater and Bonner, 1952).

**Statistical Analysis**

Data were expressed as mean ± SEM of six replicates and subjected to one-way analysis of variance (ANOVA) followed by Duncan’s multiple range test to determine significant differences in all the parameters. Values were considered statistically significant at P < 0.05.

**RESULTS AND DISCUSSION**

**Estimation of Blood Glucose Level**

On the last day of experiment, it was proven that there existed a significant fall in blood glucose levels when the rats were administered continuously with chloroform extract of *T. portulacastrum* in diabetic rats (Table 1 and Figure 1). The effect was more noticeable in the rats treated with 200 mg/kg body weight of the plant extract and compared fairly well with rats received glibenclamide. The results from the current study revealed that the Chloroform extract (200 mg/kg) of *Trianthema portulacastrum* whole plant exhibited significant antihyperglycemic effect in Dithizone-induced diabetic rats by fall in the fasting blood glucose level. The marked reduction in the levels of fasting blood glucose in diabetic rats treated with the Chloroform Extract Of *Trianthema portulacastrum* (CETP) may be either due to the increased secretion of insulin from beta cells of pancreas or stimulation of the residual pancreatic mechanism, probably by increasing peripheral utilization of glucose.

**Serum Lipid Profile and Total Protein**

From the experimental study, it was observed that there was an elevation in the levels of serum cholesterol, triglycerides, and LDL and reduced HDL and protein concentrations in diabetic rats when compared with the control group (Table 2). The chloroform extract of *T.portulacastrum* and Glibenclamide significantly reduced the levels of serum cholesterol, triglycerides, and LDL and raised HDL to near normal, as observed in control after 21 days of treatment. An increase in the concentrations of total cholesterol and LDL-cholesterol, Hypertriglyceridemia and reduced HDL-cholesterol as observed during diabetes were associated with a raised risk of myocardial infarction (Mediene-Benchekor et al., 2001). Elevated HDL-cholesterol, reduced LDL-cholesterol Levels and prevented elevation in the levels of triglycerides in experimental diabetic rats by the treatment with *T.portulacastrum* extract were indications of reduced risk of myocardial infarction.

**Figure 1:** Effect of oral administration of *Trianthema portulacastrum* whole plant Chloroform extract on blood glucose levels in diabetic rats (*n* = 6, mean ± SEM)

**Figure 2:** Effect of oral administration of *Trianthema portulacastrum* Chloroform extract on blood sugar levels in glucose-loaded diabetic rats (*n* = 6, mean ± SEM).

**Measurement of Liver Function Parameters**

The Group-II diabetic rats exhibited a marked increase in levels of ALT, ALP, AST and bilirubin concentrations in serum when compared with the control (group I) (Table 3). Continuous administration of chloroform extract of *T.portulacastrum* to diabetic rats for 21 days was able to restore all the liver function indices back to normalcy.
Table 1: Effect of oral administration of Trianthema portulacastrum whole plant Chloroform extract on blood glucose levels in diabetic rats (n= 6, mean ± SEM)

| Groups                  | Blood glucose level (mmol/L) | 0hr | 1/2 hr | 1 Hr | 2 Hr | 4 Hr | 6 Hr | 8Hr |
|------------------------|-------------------------------|-----|--------|------|------|------|------|-----|
| Control                |                               | 88.2| ± 2.4  | 87.8 | 87.5 | 2.7  | 87.4 | 2.5 |
| Diabetic control       |                               | 286.6| ± 3.4  | 285.5| 285.1| ± 3.5| 283.8| 2.9 |
| Diabetic               |                               | 287.7| ± 5.2  | 272.8| 260.8| 224.6| 200.2| 190.2|
| + Glibenclamide        |                               | 289.3| ± 2.4  | 275.2| 263.4| ± 252.8| 223.5| 229.4|
| Diabetic + TP extract (100mg/kg) |                   | 288.8| ± 2.3  | 272.5| 261.6| 239.5| 219.4| 209.8|
| Diabetic + TP extract (200mg/kg) |                     | 289.3| ± 2.4  | 275.2| 263.4| ± 252.8| 223.5| 229.4|

Table 2: Effect of oral administration of T. portulacastrum whole plant Chloroform extract on serum lipid profile and total protein in diabetic rats (n = 6, mean ± SEM)

| Groups                  | Cholesterol (mg / dL) | Triglycerides (mg / dL) | HDL (mg/dL) | LDL (mg/dL) | Total protein (g/L) |
|------------------------|-----------------------|-------------------------|-------------|-------------|---------------------|
| Control                | 109.26±0.78           | 85.1±0.86               | 35.5±0.98   | 43.19±0.01  | 88.60±0.34          |
| Diabetic control       | 136.03±0.70#          | 132.1±0.50#             | 24.5±0.49#  | 58.19±0.40# | 76.60±0.69#         |
| Diabetic + Glibenclamide | 112.9±0.170***87.2±0.158*** | 32.8±0.42***         | 49.8±0.54*** | 83.4±0.21***   |
| Diabetic + TP extract (100mg/kg) | 130.5±0.21*           | 109.2±0.56*             | 29.4±0.51*  | 53.9±0.28*   | 79.3±0.13*          |
| Diabetic + TP extract (200mg/kg) | 122.3±0.35**          | 95.4±0.12**             | 31.4±0.12** | 51.10±0.15** | 82.4±0.18**         |

Table 3: Effect of oral administration of Trianthema portulacastrum Chloroform whole plant extract on some liver function parameters of diabetic rats (n = 6 ± SEM).

| Groups                  | Total bilirubin (µmol / L) | ALP (U/L) | ALT (U/L) | AST (U/L) |
|------------------------|----------------------------|-----------|-----------|-----------|
| Control                | 0.48 ± 0.11                | 12.7 ± 0.21| 16.4 ± 0.12| 12.2 ± 0.13|
| Diabetic control       | 1.38 ±0.46#                | 45.4 ±0.19# | 34.3 ±0.17# | 24.4 ±0.14# |
| Diabetic + Glibenclamide | 0.65 ±0.31***              | 27.2 ±0.15*** | 21.6 ±0.34*** | 17.2 ±0.26*** |
| Diabetic + TP extract (100mg/kg) | 0.98 ±0.5*              | 33.4 ±0.18*  | 26.2 ±0.61*  | 19.2 ±0.15*  |
| Diabetic + TP extract (200mg/kg) | 0.83 ±0.8**              | 30.7 ±0.22** | 23.7 ±0.14** | 18.5 ±0.23** |
Table 4: Effect of oral administration of Trianthema portulacastrum Chloroform extract on antioxidant profiles.

| Groups                        | MDA(μM/100g wet tissue) | GSH(μM/g wet tissue) | CAT(Units/mg protein) | SOD(Units/mg protein) |
|-------------------------------|-------------------------|----------------------|-----------------------|------------------------|
| Control                       | 0.85 ± 0.03             | 22.4 ± 1.2           | 8.42 ± 0.6            | 9.2 ± 0.5              |
| Diabetic control              | 1.38 ± 0.5#             | 13.5 ± 1.4#          | 4.98 ± 0.7#           | 5.3 ± 0.7#             |
| Diabetic + Glibenclamide      | 0.91 ± 0.05***          | 18.6 ± 1.3***        | 7.81 ± 0.5***         | 8.8 ± 0.4***           |
| Diabetic + T.P extract (100mg/kg) | 0.98 ± 0.05*          | 15.24 ± 1.4*         | 6.15 ± 0.4*           | 7.2 ± 0.5*             |
| Diabetic + T.P extract (200mg/kg) | 0.94 ± 0.03**          | 17.2 ± 1.6**         | 7.12 ± 0.3**          | 8.1 ± 0.3**            |

Table 5: Effect of oral administration of Trianthema portulacastrum Chloroform extract on blood sugar levels in glucose-loaded diabetic rats (n = 6, mean ± SEM).

| Groups                        | Blood glucose level (mmol/L) |
|-------------------------------|-------------------------------|
|                              | 0 min | 30 min | 60 min | 90 min |
| Control                       | 88.2 ± 2.4 | 247.8 ± 3.2 | 280.8 ± 3.2 | 128.5 ± 2.7 |
| Diabetic control              | 286.6 ± 3.4 | 336.1 ± 3.2 | 315.5 ± 3.8 | 310.1 ± 3.5 |
| Diabetic + Glibenclamide      | 267.7 ± 5.2 | 302.8 ± 3.8 | 260.8 ± 2.6 | 228.6 ± 2.6 |
| Diabetic + T.P extract (100mg/kg) | 294.3 ± 2.4 | 325.1 ± 3.4 | 300.4 ± 3.5 | 282.8 ± 3.6 |
| Diabetic + T.P extract (200mg/kg) | 288.8 ± 2.3 | 292.5 ± 3.8 | 261.6 ± 3.5 | 249.5 ± 3.7 |

Table 6: Effect of Trianthema portulacastrum on Glycogen content and Gluconeogenic enzymes.

| Groups                        | Glycogen (mg/g wet tissue) | Glucose -6- phosphatase(nmol NADP+ reduced/min/mg protein) | Succinate dehydrogenase(nmol Pot. Ferricyanide reduced/min/mg protein) |
|-------------------------------|-----------------------------|-------------------------------------------------------------|------------------------------------------------------------------------|
| Control                       | 4.85 ± 0.76                 | 19.33 ± 1.29                                               | 5.16 ± 0.8                                                            |
| Diabetic control              | 2.64 ± 0.6#                 | 14.53 ± 1.09#                                             | 2.92 ± 0.56#                                                          |
| Diabetic + Glibenclamide      | 4.58 ± 0.42***              | 17.96 ± 0.54***                                           | 4.74 ± 0.4***                                                          |
| Diabetic + T.P extract (100mg/kg) | 3.8 ± 0.53*                | 15.46 ± 0.76*                                             | 3.98 ± 0.23*                                                          |
| Diabetic + T.P extract (200mg/kg) | 4.15 ± 0.75**              | 17.36 ± 0.81**                                           | 4.25 ± 0.41**                                                          |
Biochemical estimation of markers of oxidative stress

From the results obtained on the last of the experimental study (on 22nd day of the experimental study) were listed in Table 4. Declined levels of malondialdehyde (MDA) in the standard drug-treated group shown statistical significance in comparison with the control group. In a similar way, with 100mg/kg dose and 200 mg/kg of chloroform extracts of the plant were shown a reduction with statistical significance compared with the control group. The enzymatic levels of CAT, GSH and SOD, were lowered significantly in diabetic rats when compared with normal control rats. The standard drug, the test extracts (100 mg/kg and 200 mg/kg ) showed elevated levels of these enzymes with statistical significance. Diabetes and experimental animal models exhibit high oxidative stress due to unremitting and chronic hyperglycemia, which thereby lessen the activity of the antioxidative defense system and thus pop in the free radicals generation. The present investigation reports a significant increase in hepatic MDA of diabetic control rats which suggest that peroxidative injury may be involved in the development of diabetes. The extract treated diabetic animals showed a significant fall in MDA, which indicate that the Chloroform Extract Of Trianthema portulacastrum (CETP) is having. Potential to inhibit the oxidative damage of hepatic tissues. In the current study, Chloroform Extract of this plant shown a significant increase in hepatic GSH levels, which may be the contributing property of the Tanacetum parthenium chloroform extract to exerts its anti-diabetic potential. An enzymatic antioxidant such as SOD and CAT were considered as preliminary enzymes since they are involved in the direct elimination of reactive oxygen species (ROS) (Arulsevan and Subramanian, 2007). ROS causes non-enzymatic glycosylation and oxidation resulting in the inactivation and inhibition of antioxidant enzymes such as CAT and SOD (Al-Azzawie and Alhamdani, 2006). In the current study, it was observed that long term treatment with the extract reverses the activities of these enzymatic antioxidants (SOD, CAT), by significantly increasing the activity of such enzymes.

Oral Glucose Tolerance Test

Table 5 and Figure 2 represents the blood glucose levels of the experimental rats after glucose intake (oral). The blood glucose level in the control rats raised to the high level after 30 min of glucose intake and decreased to near normalcy at 90min. In the diabetic control group (Group II), the blood glucose concentration was high after 30 min of glucose intake and remained high for the next 60 min. Trianthema portulacastrum (Group IV and V) and drug (glibenclamide) treated diabetic rats (Group III) were shown a significant decrease in blood glucose level in comparison to diabetic control rats (Group II).

Analysis of Glycogen content and Gluconeogenic enzymes

Table 6 represents the effect of Trianthema portulacastrum on Glycogen content and Gluconeogenic enzymes. Hepatic glycogen content in diabetic rats (group II) was found to be significantly reduced when compared with the control (group I). Treatment with the standard drug (group III), Trianthema portulacastrum (group IV and V) enhanced the glycogen storage efficiency of the liver of diabetic rats (treated) in comparison with diabetic control animals. In the fasting stage, the Glycogen content of normal animals was slightly higher than diabetic animals and this may be due to breakdown of glycogen to retain the normal blood glucose levels, whereas glycogen levels in diabetics were found to be very low against high blood glucose levels, is maybe possibly due to lower levels of glycogen synthase activity. The activity of glucose-6-phosphatase, the regulatory enzyme of hexose monophosphate shunt (HMP shunt pathway) was found to be lowered in diabetic animals and increased in drug (Glibenclamide), Trianthema portulacastrum chloroform extract-treated animals. The activity of this enzyme was higher in comparison to untreated diabetic animals, indicating an improvement in glucose utilization by this (HMP shunt) pathway. Succinate dehydrogenase activity was decreased in diabetic control animals (Group II), was found to be increased in standard (Glibenclamide), Trianthema portulacastrum treated animals (Group IV and V). Increase in succinate dehydrogenase activities in treated animals (Groups III, IV and V) indicates better utilization of energy-yielding intermediates by Krebs cycle.

CONCLUSIONS

It is evidenced that oral administration of chloroform extract of Trianthema portulacastrum exerted marked hypoglycemic activity in Dithizone-induced diabetes using Glibenclamide as standard in experimental Wistar rats. The rise lipid parameters like total cholesterol, total bilirubin and hypoproteineemia were also critical parameters taken into consideration in case of liver damage. The results also revealed the beneficial effects of this medicinal plant in improving the imbalance in lipid metabolism experienced during diabetes, as evi-
enced by antioxidant defence properties. It can, therefore, be concluded from this study that the chloroform extract of *Trianthema portulacastrum*, besides its hypoglycemic action, could protect the liver against impairment due to diabetes.

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**Conflict Of Interest**

The authors declare that they have no conflict of interest for this study.

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