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Research Article

INSIGHT OF METHANOLIC EXTRACT OF *VENTILAGO MADERASPATANA* LEAVES ON HEPATOPROTECTIVE ACTIVITY UNDER STREPTOZOTOCIN-INDUCED DIABETIC RATS

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ABSTRACT

Objective: The methanolic leaf extract of *Ventilago maderaspatana* (MEVML, 200 mg/kg body weight [b.w.]) was investigate to hepatoprotective activity under streptozotocin-induced (45 mg/kg b.w.) diabetic (Di) rats.

Methods: In the present study, we determined the blood glucose levels, b.w., insulin, creatinine, and bilirubin levels in normal, Di, Di treated with MEVML and positive control rats.

Results: The Di rats shown adverse changes in blood glucose levels, b.w., insulin, creatinine, and bilirubin levels when compared to other group rats. Reverse the adverse changes in the above parameters when treated the Di rats with MEVML

Conclusion: The MEVML shown anti-diabetic activity and reverse the adverse changes in the above parameters in liver so that the MEVML supported the traditional claim of the hepatoprotective activity under Di condition.

Keywords: Diabetes mellitus, Methanolic leaf extract of *Ventilago maderaspatana*, Hyperglycemia, Bilirubin.

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INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder, in which hyperglycemia was take place as result of lack of insulin or insulin resistance [1,2]. However, Worldwide 25% of population was affected by DM and it was predicted to be increased 552 million by 2030 [3]. Hyperglycemic condition for long period could generate a high amount of free radicals that can ensure diabetic (Di) complications by the malfunction or damage of insulin dependent organs [4,5].

In DM, etiology of liver diseases is an important incidence. Liver is insulin dependent organ which plays a major role in carbohydrates and lipid metabolism. Consequently, liver induced the hypertriglyceridemia and hypercholesterolemia under hypoinsulinemia activity. High amount of transaminases was found frequently under Di condition due to hepatocytes which were oxidatively damaged by the generation of elevated levels of free radicals and subsequently develop the micro- and macro-vascular diseases which are the major causes of morbidity and death [6-9]. Thus, the need of anti-diabetic drugs which are more effective and safer. At present, numbers of anti-diabetic drugs were used in the Di management, including biguanide, thiazolidinedione, and sulfonylurea. However, these drugs were restricted due to several considerable side effects that were raised when used these agents [10]. Now, the turn has been taken in diabetes management which was herbal medicine. However, from hundreds of years throughout the World, many herbal medicinal plants and their bioactive constituents were used to diabetes and its complications [11]. The plants which have shown anti-diabetic activity may be due to it contains carotenoids, flavonoids, phenols, tannins, terpenoids, alkaloids, and glycosides [12,13]. *Ventilago maderaspatana* is widely grown in South Greece, India, Indonesia, Myanmar, and Sri Lanka forests of low elevations. It is commonly known as a red creeper. Bark is dark gray in color with vertical cracks exposing the vermillion inner bark surface [14,15]. Conventionally, *V. maderaspatana* was used to treat many disorders such as skin problems, fever, and diabetes and also used as digestive carminative [16]. The bark, root, leaf, hole plant, and other parts of different extracts of *V. maderaspatana* have shown different medicinal values such as antimicrobial activity, anti-Di activity, hepatoprotective, cardioprotective respectively [17-20].

However, to the best of our knowledge, hepatoprotective activity under Di condition by MEVML remains to be determined. To obtain more knowledge about *V. maderaspatana*, this study was made to attempt an investigation on the therapeutic potentiality of MEVML against streptozotocin (STZ)-induced liver damage in rats.

METHODS

Chemicals

STZ was purchased from Sigma Aldrich, Ultra-sensitive ELISA kit for rat insulin (Linco Research, Inc., St. Charles, MO), blood glucose, bilirubin, and creatinine test kits were purchased from Recon Diagnostics, Ltd., Vadodara, India. All other chemicals used in the study were of analytical grade and were obtained from standard commercial suppliers.

Plant material

Leaves of *V. maderaspatana* were collected from Seshachalam Forest, Tirupati, Chittoor, and Andhra Pradesh, India. The identity of the plant was confirmed by Taxonomist of the Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh, India and deposited a voucher specimen (No. 456) in the same department.

Animals

Male Wistar rats (160±40 g) were purchased from the Indian Institute of Science, Bengaluru, India. The study was carried out according to the principles guided for the care and use of experimental animals as per CPCSEA (Resolution No: 09/(i)/a/CPCSEA/IAEC/SVU/ZOOL/KSR dt.08.07.2012). The animals received a standard pellet diet and water *ad libitum* and were maintained under laboratory environmental conditions (23±2°C, 40–60% relative humidity and 12 h of light-dark cycle).

Induction of diabetes

After 1 week, acclimatization of healthy animals was fasted overnight and diabetes was induced by a single intraperitoneal injection of freshly prepared STZ (45 mg/kg body weights [b.w]) in 0.1 M cold citrate buffer (pH 4.5). When their blood glucose levels reached above 250 mg/dL, those animals were considered as Di on the 3rd day after STZ injection.

Grouping of animals

The rats were divided into five groups of six rats each and the oral treatment was given every day for 1 month.

- Group I, normal control (NC): Rats received 0.9% saline and fed with normal diet.
- Group II, Di control (DC): STZ (STZ 45 mg/kg b.w.) was given intraperitoneally for the induction of diabetes.
- Group III, MEVML treatment (MEVML): Rats received MEVML (200 mg/kg b.w.) orally for a period of the month.
- Group IV, Di+Glibenclamide treatment (Di+Glb): Di rats received Glb (20 mg/kg b.w.) for 1 month treatment.
- Group V Di+MEVML treatment (Di + MEVML): Di rats received MEVML, as described in Group III, for 1 month treatment.

Collection of blood and analysis

After completion of the treatment period, the blood was collected into vials from over-night fasted animals by retro-orbital sinus puncture under mild chloroform anesthesia. Serum was obtained and stored at −20°C until used for biochemical assays.

B.W. measurement and estimation of blood glucose levels

Blood glucose was measured using a one-touch glucometer (Accu-Chek). After 24 h of the last dose, blood glucose levels were measured from overnight fasted rats in each group. Bodyweights of all the animals were recorded prior to the treatment and after treatment.

Estimation of insulin levels in serum

Insulin levels were analyzed of all groups before and after the treatment. Insulin was measured by ELISA assay (Linco, Millipore).

Estimation of bilirubin levels in serum

Serum bilirubin levels were measured by the method described [21], using BioVision assay kit. Briefly, 50 µl of sample were added to sodium nitrite and sulfanilic acid solutions. Pink colored azo compound was read at 546 nm in ultraviolet–visible spectrophotometer.

Estimation of creatinine levels in serum

Creatinine levels were analyzed in serum of all groups using kit.

RESULTS

Effect of MEVML on glucose levels

The effect of MEVML on blood glucose levels in normal and Di rats is represented in Fig. 1. The blood glucose of NC rats increased in 1 month treatment period; however, the blood glucose levels of DC rats were significantly increased when compared with NC rats after injection with STZ. MEVML did not alter the blood glucose levels of compared to normal rats alone, while treated the Di rats with MEVML significantly decreased the blood glucose levels compared to DC rats.

Effect of MEVML on blood insulin and b.w.

As shown in Fig. 2, the serum insulin levels in Di rats were decreased significantly compared with normal rats. After 1 month treatment with MEVML (200 mg/kg), serum insulin levels of Di rats were significantly increased compared with DC rats. In normal treated rats, there was no increase in insulin levels after the treatment with MEVML.

The MEVML was examined on b.w. As shown in Fig. 3, the b.w. of DC group was decreased significantly ($p<0.01^{abcde}$) after the injection of STZ compared with NC rats. Administration of MEVML significantly increased the b.w. in Di rats compared with DC rats, while in normal rats, there was no effect on the b.w. when treated with MEVML.

Effect of MEVML on bilirubin and creatinine

The serum bilirubin and creatinine levels in Di group were significantly increased compared to NC group, as shown in Figs. 4 and 5. A significant decrease in bilirubin levels was observed in Di group when treated with MEVML, while MEVML treatment to the normal rats was not shown any significant changes on bilirubin and creatinine levels which were compared with the NC rats.

DISCUSSION

STZ is a chemical that was synthesized in *Streptomyces achromogenes*. It has cytotoxic activity and potentially damaged the pancreas, especially islet of beta cell. Consequently, it has induced type 1 diabetes in animals, especially in rats. [22]. The anti-diabetic drug and Glb are regularly used in STZ-induced diabetes to compare the efficacy of a variety of hypoglycemic drugs in experimental animal studies [23].

The development of anti-diabetic drugs with safe and low cost for Di patients is still a challenge for researchers working in this area [24]. The previous experimental studies on herbal medication can enlighten the efficacy of herbal medicine on diseases and can offer new functional that leads to reduce toxicity, time, and money [25]. Thus, require modern method for development of modern treatment by using animals as experimental model which mimic the range of pathophysiological changes those were visualized in Di humans [24].

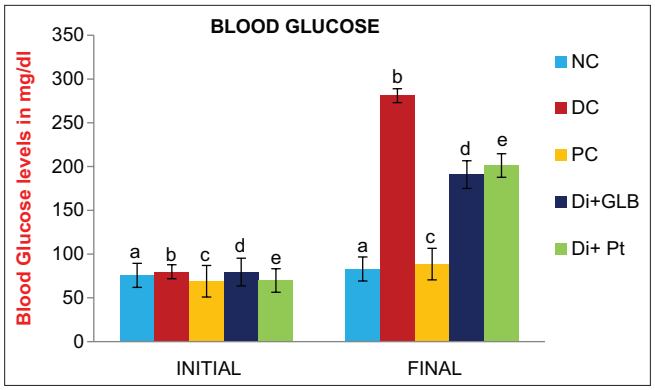

Fig. 1: Effect of methanolic leaf extract of *Ventilago maderaspatana* on glucose levels in serum under diabetic condition. The groups which not have same alphabet letter are significant ($p<0.01^{abcde}$) to the control. Values are means±S.D for six rats per group

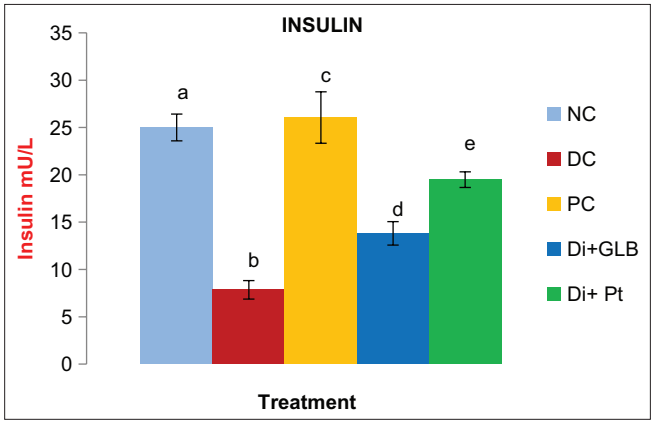

Fig. 2: Effect of methanolic leaf extract of *Ventilago maderaspatana* on insulin levels in serum under diabetic condition. The groups which not have same alphabet letter are significant ($p<0.01^{abcde}$) to the control. Values are means±S.D for six rats per group

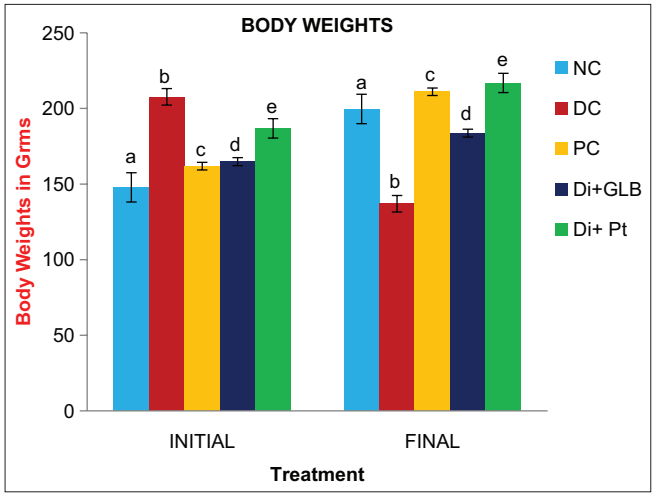

Fig. 3: Effect of methanolic leaf extract of *Ventilago maderaspatana* on body weight under diabetic condition. The groups which not have same alphabet letter are significant ($p<0.01^{abcde}$) to the control. Values are means±S.D for six rats per group

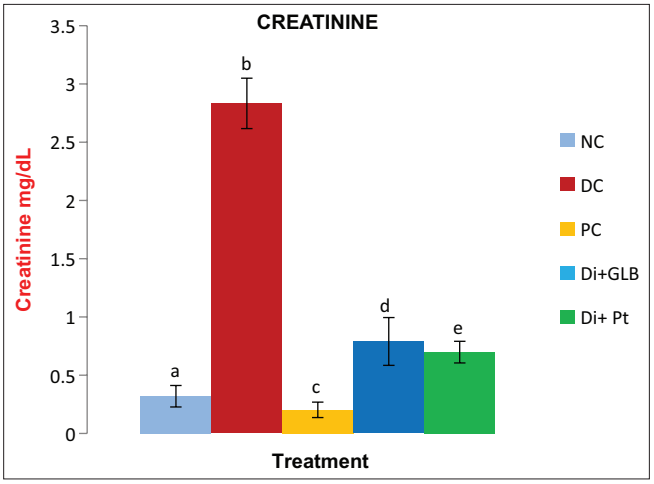

Fig. 5: Effect of methanolic leaf extract of *Ventilago maderaspatana* on creatinine levels in serum under diabetic condition. The groups which not have same alphabet letter are significant (p<0.01) to the control. Values are means±S.D for six rats per group

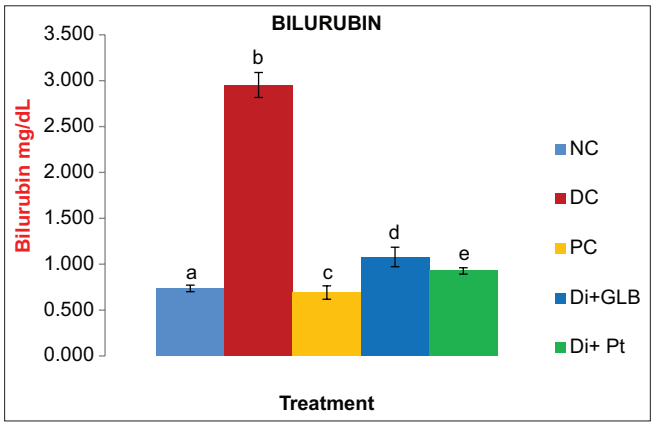

Fig. 4: Effect of methanolic leaf extract of *Ventilago maderaspatana* on bilirubin levels in serum under diabetic condition. The groups which not have same alphabet letter are significant (p<0.01) to the control. Values are means±S.D for six rats per group

Persistent chronic high glucose level in the blood was a sign of diabetes [13] which was observed in the present study in STZ-induced Di rats when compared with normal rats. Reduction of the blood glucose level in the blood is the main strategy in diabetes management [26]. However, treatment with MEVML considerably reduced the blood glucose levels in Di rats compared with DC rats, as shown in Fig. 1 conceivably due to potentiating of insulin secretion from existing β cells in pancreas or may enhance the conversion of blood glucose to glycogen by the liver [27]. Thus, the MEVML potentially had shown hypoglycemic activity under Di condition.

The other important diabetes management is to secretion and levels of insulin in the blood. Improvement of insulin secretion and insulin sensitivity of the tissue was the main strategy in diabetes treatment as a consequence of insulin levels declined due to the malfunction of pancreas in diabetes [28]. In the present study, obtained results shown in Figs. 2 and 3, serum insulin levels and b.w. were significantly decreased in Di rats compared with normal rats, while serum insulin levels and b.w. of Di rats considerably enhanced when treated with MEVML by asset of its hyper insulin activity to lift the insulin secretion by stimulation or regeneration of β cells of the pancreas [29].

Several of the earlier reports have demonstrated that diabetes was associated with liver damage, caused by elevation of liver biomarkers such as aspartate aminotransferase, alanine aminotransferase, gamma-glutamyltransferase, bilirubin, and alkaline phosphatase in serum [6]. Over insulinization accompanied with hyperglycemia is consequences to develop liver disease. However, the activities of serum enzyme can be used as useful biomarkers for monitoring the cytotoxicity of xenobiotics, including STZ [30]. In our study, STZ induces hepatic tissue damage which is one of the typical changes in diabetes as evidenced by elevation of serum bilirubin and creatinine levels in Di rats compared with NC rats, as shown in Figs. 4 and 5. Conversely, treatment of the Di rats with MEVML caused reduction in the levels of bilirubin and creatinine in serum compared with the DC rats. In accordance with this report, other workers have been confirmed that anti-diabetic activity of medicinal plants, especially polyphenols antioxidant activity, could protect the liver from oxidative injury initiated by hyperglycemic-induced free radicals [31,32]. Thus, MEVML administration to Di group of rats significantly declined liver biomarkers suggesting its hepatoprotective nature in Di condition.

CONCLUSION

Nowadays, pharmacologists and pharmaceutical companies were interest to develop new drugs from the natural medicinal plant without any adverse effects; previously, *V. maderaspatana* (Red Creeper) has been used for curing of various diseases. This plant contains a number of phytochemical compounds from various parts such as roots, leaves, bark, and seeds, which may have shown various medicinal properties. In our study, the MEVML shown anti-diabetic activity by decreased the adverse effect that was induced under STZ-induced Di condition. A very few pharmacological studies on this plant were carried out; further studies are need to develop as an anti-diabetic drug.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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AUTHORS' CONTRIBUTIONS

Venugopal Reddy B and Ramana Reddy M contributed equally to this work and should be considered co-first authors. Remaining third author helping to us consider as co-author fourth author consider as corresponding author because we did the work under his guidance.

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