Antidiabetic and antihyperlipidemic activity of *Euphorbia thymifolia* L. extracts on streptozotocin-nicotinamide induced type 2 diabetic rats

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**ABSTRACT**

The present investigation is aimed to elucidate the hypoglycaemic and hypolipidemic effect of the aqueous and methanol extract of *Euphorbia thymifolia* (ET) aerial parts in streptozotocin-nicotinamide (STZ-NA) induced diabetic rats. Diabetes was induced by administration of STZ (65 mg/kg, i.p.) followed by NA (110 mg/kg i.p.) 15 min later. The diabetic rats were treated with aqueous and methanol extract from ET (250 and 500 mg/kg, p.o., respectively) for 28 days. Fasting blood glucose level, plasma insulin, HbA1c, urea, serum creatinine, lipid profile, liver glycogen, ALP, total protein and histopathology of pancreas were carried out to assess antidiabetic and antihyperlipidemic effect. After treatment with ET extracts fasting blood glucose, HbA1c levels, urea, serum creatinine and total protein were significantly decrease in diabetic rats. However, serum insulin level and liver glycogen were significantly increased in diabetic rats. The extracts also exhibited a significant hypolipidemic effect as evident from fall in total cholesterol, triglycerides, low-density lipoproteins level. The histopathological studies of the pancreas in extract treated diabetic groups revealed almost normal appearance. This study supports the traditional proclamations on *E. thymifolia* aerial parts.

**INTRODUCTION**

Type 2 diabetes mellitus (DM) is a chronic metabolic syndrome categorized by increased blood sugar level (hyperglycaemia) consequentially progressive diminution in the discharge of insulin, insulin activity or both (Akpan, et al., 2007). The DM is associated with hyperinsulinemia, hyperglycaemia, insulin resistance, hyperlipidemia, and hypertension (Taylor, et al., 1994). The present scenario for the treatment of DM employed regimen, workout, oral hypoglycemic agent and injectable insulin. However, oral antidiabetic drugs and insulin have characteristic adverse effects. This encouraged the quest of novel medicines which may work systematically distinctive way compared to the current drug therapy (Palsamy and Subramanian, 2008). Consequently, research is meant to conventional medicinal plants which are used as a part of practices and also the discovery of new molecules from phytoconstituents with lesser adverse effects (Chandramohan, et al., 2008). Ayurveda, a traditional system of medicine comprises a diverse range from ancient to modern approaches for the treatment and prevention of illness. A traditional Indian system of medicine has represented a few medications from the traditional indigenous plants in the treatment of liver disease, diabetes, inflammation and cardiac disease (Kemper and Lester, 1999). The genus *euphorbia* is unique in that it contains highly reputed plants useful in various diseases. This genus has diverse chemical entities with a lot of structural variation sources. Such plants like *Amla* (*Emblica officinalis*), *Bhoiamli* (*Phyllanthusfractus*), *Arendmul* (*Racinoscommunis*) etc. are medicinally valuable in the treatment of some chronic disease such as diabetes, liver disease, asthma, inflammation etc., (Mwine and Van Damme, 2011).

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**Euphorbia thymifolia** (ET) belong to family Euphorbiaceae, known as *Nanidudheli*, is traditionally used as a blood purifier, sedative haemostatic, bronchial asthma, and inflammation (Lee, et al., 1990). In view of above medicinal properties of *Euphorbia thymifolia*, it is of interest to undertake a systematic study as their hypoglycaemic activity for such plant. Thus present study was aimed to investigate the antidiabetic and anti-hyperlipidaemic activities of aqueous and methanol extract of ET aerial part on streptozotocin-nicotinamide induced DM in rats.

**MATERIAL AND METHODS**

**Drugs and chemicals**

Glibenclamide (ZydusCadila, Ahmedabad, India) was obtained as gift sample. Streptozotocin (STZ) and nicotinamide (NA) were purchased from Himedia, Mumbai, India. All standard kits were bought from Span Diagnostic lab, Surat, India. All other reagents and chemical used in the experiment were of analytical grade purchased from SD Fine Chemical, Mumbai, India.

**Plant material**

Fresh aerial parts of ET were collected during September 2014 from Amargadh village, Taluka, district of Rajkot, Gujarat, India. The plant was identified and authenticated by CSIR-NISCAIR, New Delhi, India. Voucher specimens (DP/SVU/PHCOG/Herb/03) of same have been deposited in SumandeeVidyapeeth University, Vadodara for future reference.

**Preparation of extracts**

The collected leaves were subjected to the sun dried, pulverized and sieved through mesh size 40. The cold maceration method was utilized to prepare aqueous and methanol extracts of *E. thymifolia* (ETW and ETM respectively) for 72 h. The final extract was concentrated using a rotary evaporator (Mack, Ahmedabad) under reduced pressure at a temperature of 60°C and subsequently lyophilized and stored in a desiccator and used for further studies. The yield of ETM and ETW extracts was 16.34% and 6.25% (w/w) respectively.

**Experimental animals**

Healthy adult male Wistar albino rats (180-200 g, 12-13 week old) obtained from Zydus Research Centre, Gujarat, India, were allow access to water and food *ad libitum*, and maintained under constant (25 ± 1°C), humidity (65 ± 10%) and 12h light/dark cycle. The study was sanctioned by CPCSEA and IAEc approved the protocols (Approval No.: SVU/DPIAEc/2014/03/14).

**Acute oral toxicity study**

Healthy overnight starved Wistar rats of either sex weighing 180-200g were utilized to determine acute oral toxicity. The rats were allocated into groups (n = 3) and orally fed with methanol and aqueous extracts of ET in ascending doses as 500, 1000, 2500, 3000, 4000 and 5000 mg/kg body weight (OECD, 2001). They were observed continuously for gross behaviour up to 14 days.

**Oral glucose tolerance test (OGTT)**

The overnight starved normal healthy Wistar rats were utilized to perform OGTT (Bonner-Weir, 1988). The rats were allocated into six groups (n=6). Group I to VI administered orally with 0.2% Carboxymethylcellulose (CMC) solution, aqueous and methanol extracts of EUT (500 mg/kg suspended in 0.2% CMC) and standard drug glibenclamide (5 mg/kg suspended in 0.2% CMC), respectively. After treatment with extracts blood samples were withdrawn at 0, 30, 60 and 120 min by retro-orbital puncture under diethyl-ether anaesthesia. The fasting blood glucose (FBG) concentration was measured by diagnostic strips (Accu-check, Roche Diagnosis, USA).

**Chemically induced type 2 Diabetes**

The DM was induced in overnight starved normal healthy Wistar rats by administration of STZ (65 mg/kg, i.p.) followed by NA (110 mg/kg i.p.) 15 min later (Masiello, et al., 1998). The elevated blood glucose level (hyperglycaemia) was confirmed on the 5th and 7th day after administration of STZ-NA, with the help of diagnostic strips (Accu-check, Roche Diagnosis, USA). The rats having FBG level >200 mg/dl along with glycosuria were chosen for the experimental design.

**Design of experiment**

The diabetic rats were allocated into seven groups (n=6) as per Table 1. Normal control rats and diabetic control rats received 0.2% CMC orally while standard group received glibenclamide (suspended in 0.2% CMC) orally up to 28 days. The treatment groups were received extracts (suspended in 0.2% CMC) orally up to 28 days orally. All aforementioned treatments were started one week after injection of STZ-NA. All treatments were given daily to the respective group of animals for 28 days. On 28th day blood samples were withdrawn by puncturing the retro-orbital under diethyl-ether anesthesia and stored with or without EDTA tubes. For separation of serum, blood was allowed to clot for 15 min, and it was then centrifuged at 5000 rpm for 20 min. The serum was stored at -20°C until further biochemical analysis.

**Biochemical parameters**

The fasting plasma glucose level was estimated by diagnostic strips (Accu-check, Roche Diagnosis, USA). Glycosylated hemoglobin (HbA1c) was estimated from whole blood (Ohkawa, et al., 1979). Serum insulin was estimated using available commercial ELISA kit (Andersen, et al., 1993). Liver glycogen was measured from serum (Carroll, et al., 1956). Total protein, serum creatinine, and urea were estimated by available commercial kits (Lowry, et al., 1951, Owen, et al., 1954, Varley, 1954).

Serum alkaline phosphatase (ALP) was measured by the method of King and Armstrong (1934). Reitman and Frankel (1957) were suggested method for estimation of hepatic amino transferase enzymes which includes aspartate amino transferase (AST or SGOT) and alanine aminotransferase (ALT or SGPT). The serum total cholesterol (TC), triglyceride (TG), low density
lipoproteins (LDL) and high density lipoproteins (HDL) concentrations were determined using commercial kits by enzymatic photocolorimetric methods (Das, et al., 2015).

**Histopathological study**

After scarification of rodent, the pancreas was immediately dissected out and washed instantly with saline and impregnate in formalin (10% v/v) solution. Furthermore, the finely cut sections were stained with Haematoxylin and Eosin (H&E) and monitored in the microscope (Olympus BX10, Tokyo, Japan) for the occurrence of histopathological changes. The histopathological changes were observed blindly in normal and treated animal groups.

**Statistical analysis**

All the values are stated as mean ± SEM. Control group and treatment groups are statistically tested using one-way ANOVA followed by post hoc Bonferroni multiple comparisons in Prism 5, GraphPad Software, Inc. The significance level was set at P < 0.05 for all tests.

**RESULTS**

**Acute oral toxicity studies in rats**

Aqueous and methanol extracts of ET did not show any acute toxicity symptoms i.e. mortality, morbidity, as well as no significance, changes observed in the general behaviour of animals, up to the dose of 2500 to 5000 mg/kg. Therefore, 1/10th dose of extracts was selected as a therapeutic dose for the present study.

**The effects of ET extracts on OGTT**

The tolerant elevation in postprandial blood glucose level was observed within 30 min of administration of oral glucose, which was maximum at 60 min in all rats. The treatment with ETM 500 mg/kg and ETW 500 mg/kg showed significant (P< 0.01) reduction in the blood glucose level in diabetic rats while ETM and ETW 250 mg/kg showed moderate reduction in blood glucose level (Fig.1).

**The effects of ET extracts on the fasting blood sugar level in diabetic rats.**

The FBG of the normal and experimental animals was estimated before and at days 0, 7, 14, 21 and 28 from treatment. In normal control rats, FBG level was practically similar throughout the study. On contrary, the STZ-NA induced diabetic rats demonstrated a significant increment in the level of FBG as compared to normal control rats. The diabetic rats treated with ET extracts at a dose of 250 mg/kg and 500 mg/kg showed a significant (P < 0.05) decrease in the FBG level (Table 1).

![Fig. 1: The effect of ET extracts on OGTT level in rats. All Values are expressed as mean ± SEM (n=6). ###p< 0.05 vs. Normal, *p< 0.05 vs. Diabetic and **p < 0.01 vs. Diabetic (One-way ANOVA followed by Bonferroni post hoc test).](image)

**Table 1: Effect of ET extracts on FBG of diabetic rats.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>0 Day</th>
<th>7&lt;sup&gt;th&lt;/sup&gt; Day</th>
<th>14&lt;sup&gt;th&lt;/sup&gt; Day</th>
<th>21&lt;sup&gt;st&lt;/sup&gt; Day</th>
<th>28&lt;sup&gt;th&lt;/sup&gt; Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>96.33 ± 1.116</td>
<td>96.66 ± 1.626</td>
<td>95.33 ± 1.667</td>
<td>99.00 ± 2.757</td>
<td>95.667 ± 1.054</td>
</tr>
<tr>
<td>Diabetic</td>
<td>270.33 ± 22.630</td>
<td>293.16 ± 17.990</td>
<td>302.50 ± 15.115</td>
<td>306.16 ± 14.934</td>
<td>341.50 ± 15.050**</td>
</tr>
<tr>
<td>Glibenclamide 5 mg/kg</td>
<td>257.50 ± 16.329</td>
<td>240.50 ± 13.099</td>
<td>165.833 ± 4.902</td>
<td>169.667 ± 4.104</td>
<td>136.667 ± 2.917</td>
</tr>
<tr>
<td>ETW 250 mg/kg</td>
<td>269.33 ± 6.936</td>
<td>230.50 ± 8.160</td>
<td>196.50 ± 4.395</td>
<td>175.66 ± 4.447</td>
<td>156.00 ± 2.082*</td>
</tr>
<tr>
<td>ETW 500 mg/kg</td>
<td>247.83 ± 14.925</td>
<td>212.66 ± 9.982</td>
<td>184.83 ± 4.881</td>
<td>169.00 ± 3.327</td>
<td>149.66 ± 1.585*</td>
</tr>
<tr>
<td>ETM 250 mg/kg</td>
<td>251.83 ± 11.140</td>
<td>217.50 ± 8.539</td>
<td>191.50 ± 4.822</td>
<td>177.83 ± 3.525</td>
<td>162.167 ± 2.040b</td>
</tr>
<tr>
<td>ETM 500 mg/kg</td>
<td>235.66 ± 11.896</td>
<td>208.83 ± 9.680</td>
<td>188.00 ± 5.663</td>
<td>168.16 ± 3.953</td>
<td>152.16 ± 2.455c</td>
</tr>
</tbody>
</table>

Values expressed as mean ± SEM (n=6). **p< 0.05 vs. Normal, "p< 0.05 vs. Diabetic, *p< 0.01 vs. Diabetic and **p< 0.001 vs. Diabetic.
Nevertheless, a higher level of liver glycogen, SGOT, SGPT, ALP and total protein in control, diabetic induced and drug treated rats were significant (P < 0.05) decrease in the levels of serum creatinine and urea as compared to normal control rats. After the treatment with extracts of ET at a dose of 250 mg/kg and 500 mg/kg, there was a significant increase in the levels of serum creatinine and urea as compared to diabetic induced rats. Similar, HDL level decrease in STZ-NA induced diabetic rats as compared to normal rats. Treatment with glibanclamide and extracts of ET were showed a significant (P < 0.001) elevation in the level of plasma insulin at a dose of 250 mg/kg and 500 mg/kg.

The data summarized in table 3 reveal that, all the extracts shows a marked protective effect on these biochemical parameters in treatment groups. However, treatment with ETM (500 mg/kg) shows a marked effect as compared to other extracts in diabetic rats.

The effect of ET extracts on serum lipid profile

The results in table 4 represented the serum lipid profile includes TC, TG, LDL and HDL levels in STZ-NA induced type 2 diabetes in rats. In diabetic rats, there was a significant (P < 0.05) increase in lipid profile except HDL as compared to normal control rats. Treatment with glibenclamide and extracts of ET were showed significant (P < 0.001) reduction in TC, TG and LDL level.

Values expressed as *mean ± SEM (n=6), **p< 0.05 vs. Normal, ***p< 0.05 vs. Diabetic, *p< 0.01 vs. Diabetic and *p< 0.001 vs. Diabetic.*
The histopathological study of the pancreas

Histopathology studies indifferent treated groups showed normal physiology in normal control rats. Treatment with extracts from ET resulted in the refurbishment of pancreatic islets to near normal construction. This suggests a possible influence of extracts of ET to regeneration or repair of the cells of islets of Langerhans in STZ-NA treated group (Fig. 2A-G).

![Histological study of the pancreas](image)

Fig. 2: Histological study of the pancreas (representative H&E-stained): (A) Normal group, (B) Diabetic group, (C) Glibenclamide 5 mg/kg, (D) ETM 250 mg/kg treated, (E) ETM 500 mg/kg treated, (F) ETW 250 mg/kg treated and (G) ETW 500 mg/kg.

DISCUSSION

The principle underlying hyperglycaemia in DM involves decreased use of glucose by the tissues as well as over production of glucose via excessive hepatic glycogenolysis and gluconeogenesis (Latner, 1958). Persistent hyperglycaemia, the common feature of DM can result in complications. Thus, the purpose of the treatment was to control the elevated blood glucose level (Association, 1997). Traditionally Euphorbia species has been utilized in the management of DM (Alarcon-Aguilara, et al., 1998). In the present investigation, OGTT was supported that methanol and aqueous extracts of ET have the capability to reduce blood sugar levels. Hence, it is of interest to undertake a systematic study as their pharmacological activity like anti diabetic and antihyperlipidemic activity of extracts on STZ-NA induced DM in rats.

Antidiabetic studies reveal that aqueous and methanol extracts of ET have sub-chronic antidiabetic activity. The higher potential of ETM (500 mg/kg) over others extracts, which contains a polyphenol, triterpenoids, flavonoids and saponins that may result in increased antidiabetic potential (Verma, et al., 2013). The DM in rats was manifested via intraperitoneal administration of STZ-NA. The vital foundation of DM occurs in rats was due to damage of β-cells of Langerhans (Ranjna, 1999), which leads to the excessive breakdown of liver glycogen to glucose-6-phosphate along with a reduction in sugar consumption via tissue. The STZ oral administration alone might bring about hyperglycaemia via fatal devastation of Langerhans of β-cells. Adjacent to this in a current experimental model combination of STZ-NA exhibit hyperglycaemia and reduce glucose resistance may cause DNA strand breaks which may lead to the progression of type-II diabetes (Lukić, et al., 1998).

However, β-cells are still able to release insulin in the presence of sugar, which is likely to be same as to type II DM in human. The oral treatment with extracts exhibited a significant decrease in the elevated FBG levels and increase in serum insulin level. The mechanism behind the restoration in elevated FBG in diabetic rats was due to the enhancement of the activity of plasma insulin via either release from already existing β-cells or from its bound forms.

Excessive glucose present in the blood bound to the hemoglobin and increase significantly the percentage of HbA1c in diabetic rats which may be due to the anabolic effect of insulin as well as the protein synthesis (Jarald, et al., 2013). Consequently, diabetic rats showed a significant increase in HbA1c and decrease in hemoglobin levels in STZ-NA induced diabetic rats. The oral treatment with ET extracts showed a significant reduction in HbA1c level. The ability of methanol and water extracts of E. thymifolia to reduce HbA1c levels in diabetic rats showed its potential effect in prevention of the complication associated with diabetes mellitus. Urea is a chief molecule in the metabolism of protein. The deamination of amino acid in the liver produces ammonia which is converted to urea and excreted out through urinary system. However, some of urea is bound to hemoglobin and found higher in RBC than plasma (Ranjna, 1999). The significant (P<0.001) decrease in urea observed with diabetic rats treated with E. thymifolia (250 mg/kg and 500 mg/kg body weight) is clearly evident the potential effect of ET extracts which may not be due to the diminishing in urea cycle or decrease in glomerular filtration as in renal disease. Creatinine is waste product generated through muscle and protein diet. The higher concentration of creatinine in blood is clearly evident for the impairment of kidney functions. The present result reveals that, the treatment with extracts of ET aerial part have potential effect on STZ-NA induced increase in serum creatinine level. The earlier report stated that the liver glycogen levels has been decreased during DM (Grover,
et al., 2000), this because of unavailability of insulin in DM which consequence into the inactivation of glycogen synthase (Huang et al., 2000). The treatment with extracts of ET displayed a significant alteration in the hepatic glycogen level in rats as compared to the diabetic control group, which may be due to the induction of the glycogen synthase enzyme via enhancement of glycogenesis (Salahuddin and Jalalpure, 2010). Table 3 summarized the effect of STZ-NA on the activity of hepatic enzymes in serum which are served as marker in diabetes and obesity. In present investigation, liver enzymes such as SGPT, SGOT and ALP levels were increased significantly (P<0.005) in STZ-NA induced diabetic rats as compared to normal rats. This event was supported by evident that there is a leaking out of these enzymes from the liver cytosol and migrating into the circulation due to toxicity of STZ (Shokeen et al., 2008). The elevated enzyme levels were significantly restored to normal after treatment with ET extracts for 28 days support the antidiabetic effect. Furthermore, restoration in level of liver enzyme also indicates normal function of liver.

The DM is showed significant changes in serum lipid profile may lead to hyperlipidaemia characterized by the elevation of TC, TG, and LDL levels. However, there was a decreased in levels of HDL (Verma, et al., 2012). In the meantime insulin has an inhibitory action on key enzyme 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMG-CoA reductase), in de novo synthesis of cholesterol. Insulin resistance or deficiency results in activation of lipoprotein lipase, this consequence in hydrolyse TG which is associated with hyperlipidaemia in diabetic rats (Jarald, et al., 2008). In the present study, treatment with extracts of ET significantly reversed dyslipidaemia by the significant decrease in TC, TG and LDL coupled to increase in HDL. The mechanism behind antihyperlipidemic activities of plants would be a lesser generation of cholesterol, via decreasing the activity of HMG-CoA reductase enzyme (Sharma et al., 2003). A histopathology study shows the partial destruction of the pancreatic cells in DM control animals as compared to normal control animals. The histopathology study of treated animals showed restoration of pancreatic cells near to normal in the extracts treated animals.

CONCLUSION

Euphorbia thymifolia control elevated blood glucose level in diabetic rats but also lower the lipid profile associated with this metabolic syndrome. The higher potential of ETM (500 mg/kg) over aqueous extracts, which contains a polyphenol, triterpenoids, flavonoids and saponins (Parmar and Pundarikakshudu, 2017) that may result in increased antidiabetic and antihyperlipidemic potential. This investigation is evidently corroborating the proclamations of the traditional system of medicine for Euphorbia thymifolia aerial parts as a regimen in the treatment of diabetes and obesity.

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