Antidiabetic, antihyperlipidemic and antioxidant activities of aqueous and ethanol extracts of leaves of Trewia nudiflora Linn in alloxan induced diabetic rats

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Abstract
The aim of this study was to investigate the antidiabetic, antihyperlipidemic and antioxidant activities of aqueous and ethanol extracts of leaves of Trewia nudiflora in alloxan (ALX) induced diabetic rats. Diabetes was confirmed after 5 days of single intraperitoneal injection of ALX (140 mg/kg) in albino Wister rats. Aqueous and ethanol extracts of leaves of Trewia nudiflora (100 and 200 mg/kg) and glibenclamide (10 mg/kg p.o.) orally administered daily for 15 days, blood was withdrawn for glucose determination on 0, 1, 10 and 15 days respectively. On the 15th day, overnight fasted rats were sacrificed and blood was collected for the determination of high density lipoproteins cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C), total cholesterol (TC), total glycerides (TG) and total proteins (TP). Aqueous and ethanol extracts of leaves of Trewia nudiflora at doses of 100 and 200 mg/kg showed significant reduction is blood glucose, lipid when compared to diabetic control group. In vitro DPPH radical scavenging activity of aqueous and ethanol extracts of leaves of Trewia nudiflora was also studied. We concluded that aqueous and ethanol extracts of leaves of Trewia nudiflora possess antidiabetic, antihyperlipidemic and antioxidant activities.

Keywords: Trewia nudiflora, Antihyperlipidemic, Antidiabetic, Antioxidant.

Introduction
Type 2 diabetes mellitus affected individuals more prone with cardiovascular diseases risk rather than individuals not affected with type 2 diabetes mellitus.[1] Diabetes also causes risk of blood pressure and LDL- cholesterol level. Globally diabetes has spread more frequently due to modern lifestyle and it also can be linked to an obesity and sedentary population.[2] Diabetes, an endocrine based disease have many complications as hyperglycemia, hyperlipidemia, vascular complications, such as atherosclerosis, diabetic
nephropathy and neuropathy.[3] The accelerated atherosclerosis and cardiovascular diseases in diabetes is likely to be multifactorial and therefore several therapeutic approaches can be considered [4] which also stimulating the search for new concepts and targets for the treatment of this incurable disease.

Alloxan (2, 4, 5, 6-pyrimidinetetron) is an oxygenated pyrimidine derivative and toxic glucose analogue. It is present as alloxan hydrate in aqueous solution.[5] The action of alloxan in the pancreas is preceded by its rapid uptake by the insulin-secreting β cells[6] and also due to autoimmune destruction of the β cells of the pancreas[7], when administered intravenously, intraperitoneal or subcutaneously to rodents and many other animal species.

Green plants are the source of many secondary metabolites, which are commercially important and find use in a number of pharmaceutical compound. Trewia nudiflora Linn. (Euphorbiaceae) commonly known as gutel is a small sized tree grows up to 5 meters in height. Leaves simple, Cordate, acuminate, both surface pubescent and long petiolate. Flowers arise from axilla or from terminal spikes.[8] Fruits hard, greenish yellow pods, which is staple food of Indian Rhinoceros. Trewia nudiflora Linn. (Euphorbiaceae) distributed in Madhya Pradesh, Uttarakhand, Punjab, Uttar Pradesh and Maharashtra. It’s root contains resinous matter and fat. Decoction of root is used as stomachic and alterative in flatulence, gout, rheumatism and malignancy especially leukemia and hepato- biliary affections etc.[9] A decoction of shoots and leaves of Trewia nudiflora is used as traditional medicine to relieve swelling and to treat flatulence, excessive bile and sputum. The leaves are applied on wounds to heal them with good efficiency.[10] On the basis of literature review and tribal information gathered from Kerakat, Jaunpur, Uttar Pradesh that the plant Trewia nudiflora (Gutel) have reported the use of the leaves for the management of diabetes mellitus. However, there is no scientific evidence to support this claim. Hence, the objective of this study was to ascertain the scientific basis for the use of Trewia nudiflora Linn. (Euphorbiaceae) in the management of diabetes using alloxan induced diabetic rats.

Materials and Methods

Collection of plant material and extraction

The leaves of Trewia nudiflora were collected from the local area of Kerakat, Jaunpur District, Uttar Pradesh, India in the month of November 2012 and authenticated at Department of Botany, Safia College, Bhopal, Madhya Pradesh. The voucher specimen (436/Bot/Saifia/13) has been preserved in our laboratory for further collection and reference.

The leaves was dried under shade, powdered with a mechanical grinder and passed through a 40-mesh sieve. The successive solvent cold extraction method used to obtain various extracts including petroleum ether, chloroform, ethyl acetate, ethanol and aqueous extracts. The solvents were removed from the extracts under reduced pressure by using a rotary vacuum evaporator (Buchi model, Jyoti Lab, Gwalior, India). The percentage yield of extracts was noted. The greenish brown extract was obtained and is dissolved in their respective solvents for pharmacological studies.

Preliminary phytochemical screening

The aqueous and ethanol extract of Trewia nudiflora Linn. was screened for the presence of various phytoconstituents like steroids, alkaloids, glycosides, flavonoids, carbohydrates, amino acids, proteins and phenolic compounds. [11,12]

Animals

Healthy, adult Albino Wistar rats (180-200gm) of either sex were purchased from the National Center for Laboratory Animal sciences, Hyderabad used for study. Housed individually in polypropylene cages, maintained under standard conditions (12 h light; and 12 h dark cycle; 23±2º C, 50± 5%, relative humidity), they were fed with standard rat pellet diet (Hindustan Lever Ltd; Mumbai, India) and were ad libitum. The Institutional Animal Ethics Committee (TIT/IAEC/831/PCol/2013/17) approved the study.

Acute toxicity study

The acute oral toxicity study has to be carried out as per the guidelines set by OECD, revised draft guidelines 423, received from CPCSEA, ministry of social justice and empowerment, Govt. of India. [13]
The animals are randomly selected, marked to permit individual identification, and kept in their cages for at least 5 days prior to dosing to allow for acclimatization to the laboratory conditions. The test substance is administered in a single dose by gavage using a stomach tube or a suitable intubation canula. In the unusual circumstance that a single dose is not possible, the dose may be given in smaller fractions over a period not exceeding 24 hours.

Three animals are used for each step. The dose level to be used as the starting dose is selected from one of four fixed levels, 5, 50, 300 and 2000 mg/kg body weight. The starting dose level should be that which is most likely to produce mortality in some of the dosed animals.

**Induction of diabetes**

The animals were fasted for 12 h prior to the induction of diabetes.[14] ALX freshly prepared in 0.5% Tween 80 was administered intraperitoneally (i.p.) at single dose of 140 mg/kg. Development of diabetes was confirmed by measuring blood glucose concentration 5 days after the administration of Alloxan. Rats with blood glucose level of above 200 mg/dl were considered to be diabetic and used for the studies.

**Experimental design**

The rats were randomized into seven groups comprising of six animals in each groups as given below. Solvent/ aqueous and ethanol extracts (100 and 200 mg/kg)/ glibenclamide (GLB) was administered orally using an intra-gastric tube once daily for 15 days.

- **Group I:** normal control rats were given 0.5% Tween 80 for 15 days.
- **Group II:** Diabetic controls have been given 0.5% Tween 80 for 15 days, 5 days after alloxan (140 mg/kg, i.p.) treatment.
- **Group III:** Rats have been given Glibenclamide (10 mg/kg/day, p.o.) for 15 days, 5 days after alloxan (140 mg/kg, i.p.) treatment.
- **Group IV:** Test rats have been given ethanol extract of *Trevisia nudiflora* (100 mg/kg, p.o.) for 15 days, 5 days after alloxan (140 mg/kg, i.p.) treatment.
- **Group V:** Test rats have been given ethanol extract of *Trevisia nudiflora* (200 mg/kg, p.o.) for 15 days, 5 days after alloxan (140 mg/kg, i.p.) treatment.
- **Group VI:** Test rats have been given aqueous extract of *Trevisia nudiflora* (100 mg/kg, p.o.) for 15 days, 5 days after alloxan (140 mg/kg, i.p.) treatment.
- **Group VII:** Test rats have been given aqueous extract of *Trevisia nudiflora* (200 mg/kg, p.o.) for 15 days, 5 days after alloxan (140 mg/kg, i.p.) treatment.

Blood samples were collected from retro-orbital plexus of each rat under mild anesthesia at 0, 1, 2 and 3 h after solvent/ethanol and aqueous extracts of leaves of *Trevisia nudiflora* (100 and 200 mg/kg)/ glibenclamide administration and serum glucose was estimated by enzymatic glucose oxidase method. Percent reduction in serum glucose was calculated with respect to the initial level. Five days before the termination of the experiment, the oral glucose tolerance test (OGTT) was performed to assess the glucose tolerance. For this purpose, overnight fasted rats were fed glucose (2 g/kg) orally and blood was collected at 0, 30, 60 and 120 min interval from orbital sinus for glucose estimation. On 15th day of the study, blood samples were collected for biochemical estimations. Later animals were sacrificed and liver was removed, cleaned and washed in ice-cold normal saline for biochemical study.

**Biochemical analysis**

Serum total cholesterol [15], total glycerides [16], LDL-c, VLDL-c [17] and HDL-c [18] were estimated using standard enzymatic kits (ERBA diagnostic Mannheim GMBH, Germany) spectrometrically. Total protein was estimated by using bovine serum albumin as a standard. [19]

**Determination of DPPH radical scavenging activity**

The free radical scavenging activity of ethanol and aqueous extracts of leaves of *Trevisia nudiflora* and ascorbic acid were measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH.[20] DPPH solution (0.1 mM) in ethanol was prepared and 1 mL of this solution was added to 3 mL of extract solution in water at different concentrations (100-1000 μg mL⁻¹). After 35 min, the absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicated
higher free radical scavenging activity. The capability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH scavenged(\%)} = \frac{A_{\text{const}} - A_{\text{test}} \times 100}{A_{\text{const}}}$$

where, $A_{\text{const}}$ is the absorbance of the control reaction and $A_{\text{test}}$ is the absorbance in the presence of the sample of the extracts.

Statistical analysis: The amount of extracts needed to inhibit free radicals concentration by 50% (IC$_{50}$) was graphically estimated using linear regression lines.[20]

**Histopathological studies:**

The histopathology study of pancreas was also performed which showed hypoglycemic effect.[21]

**Statistical analysis**

1. **Antioxidant activity:** The amount of extracts needed to inhibit free radicals concentration by 50% (IC$_{50}$) was graphically estimated using linear regression lines.

2. **Oral glucose tolerance test:** The data was represented as mean ± SEM. Results was analyzed by one way ANOVA followed by Dunnett’s multiple comparison tests using Graph pad in stat 3.0 software.

3. **Anti-diabetic activity:** The data was represented as mean ± SEM. Results was analyzed by one way ANOVA followed by Dunnett’s multiple comparison tests using Graph pad in stat 3.0 software. Results were expressed as the mean ± S.E.M. for statistical analysis of the data group means, were compared by one-way analysis of variance (ANOVA) followed by Tukey’s post-test for multiple comparisons. $p < 0.01$ was considered to be statistically significant.

**Results**

Preliminary study was performed on the aqueous and ethanol extracts of the leaves of *Trewia nudiflora* and the presence of various phytocconstituents such as alkaloids, glycosides, flavonoids, steroids, fixed oils, Phenolics and tannins were determined.

**In-vitro antioxidant activity**

Table 1 results revealed that the investigated aqueous and ethanol extracts of leaves of *Trewia nudiflora* presented that the DPPH free radical scavenging activity and IC$_{50}$ value of ethanol and aqueous extracts of leaves were found to be 581.80 and 714.29 μg/ml. The IC$_{50}$ value of ascorbic acid was found to be 537.63 μg/ml.

**Oral glucose tolerance test (OGTT)**

Table 2 shows the effect of doses of ethanol and aqueous extract of leaves of *Trewia nudiflora* on diabetic rats. After 120 min of glucose administration the fall observed with the ethanol extract of dose of 100mg/kg and of 200mg/kg simultaneously, the fall has also been observed in aqueous extract of dose of 100mg/kg and of 200mg/kg that is compared with the standard drug Glibenclamide in diabetic rats.

**Antidiabetic effect of aqueous and ethanol extracts of leaves of *Trewia nudiflora***

Table 3 shows the anti-hyperglycemic effect of ethanol and aqueous extracts of leaves of *Trewia nudiflora* at doses of 100 and 200mg/kg. It has been noted that the effect of treatment of the extracts shows significant reduction on blood glucose levels of diabetic rats, on first day it was found to be 130.83±0.6009 and on 15th day it was found to be 94.5 ± 0.2236, whereas the standard drug glibenclamide shows the anti-hyperglycemic effect as on 1st day and on 15th day was found to be 135 ± 0.7303 and 85.5 ± 0.5627 respectively.

**Antihyperlipidemic effect of aqueous and ethanol extracts of leaves of *Trewia nudiflora***

Table 4 shows the effect of extracts on serum lipid profile, in diabetic rats, a decrease in the serum triglycerides, total cholesterol, LDL (low density lipids) and VLDL (very low density lipids) levels, and an increase in the HDL (high density lipids) cholesterol levels were observed. Alloxan treatment
resulted in elevation of TG, TC, VLDL-C, LDL-C, and reduction of HDL-C levels as compared to the normal control rats. Aqueous and ethanol extracts of leaves of *Trewia nudiflora* (100 and 200 mg/kg) and GLB (10 mg/kg) reduction in elevated serum TG, TC, VLDL-c, LDL-c, TC/HDL-c and LDL-c/HDL-c and HDL-c level was restored respectively when compared to diabetic control.

**Histopathological Study**
The histopathology study of pancreas was also performed which showed hypoglycemic effect.

**Table: 1** IC₅₀ values of ethanol and aqueous extracts of leaves of *Trewia nudiflora* along with the standard ascorbic acid

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Test sample</th>
<th>IC₅₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ascorbic acid</td>
<td>537.63</td>
</tr>
<tr>
<td>2</td>
<td>Ethanol leaves</td>
<td>581.36</td>
</tr>
<tr>
<td>3</td>
<td>Aqueous leaves</td>
<td>714.29</td>
</tr>
</tbody>
</table>

**Fig 1** The reducing power of ethanol and aqueous extracts of leaves of *Trewia nudiflora* compared with that of ascorbic acid
Fig 2 The percentage inhibition of ethanol and aqueous extracts of leaves of *Trewia nudiflora* compared with that of ascorbic acid.

Table 2 Effect of ethanol and aqueous extract of leaves of *Trewia nudiflora* on OGTT of diabetic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment / mg/kg</th>
<th>Blood glucose levels (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 min</td>
</tr>
<tr>
<td>I</td>
<td>Normal control</td>
<td>80.83 ± 0.4773**</td>
</tr>
<tr>
<td>II</td>
<td>Glibenclamide, 10 mg/kg</td>
<td>89.66 ± 0.4944**</td>
</tr>
<tr>
<td>III</td>
<td>Control, 0.5% Tween 80</td>
<td>126.5 ± 0.7638</td>
</tr>
<tr>
<td>IV</td>
<td>Ethanol extract 100 mg/kg</td>
<td>113.5 ± 0.7638**</td>
</tr>
<tr>
<td>V</td>
<td>Ethanol extract 200 mg/kg</td>
<td>103.67 ± 0.6667**</td>
</tr>
<tr>
<td>VI</td>
<td>Aqueous extract 100 mg/kg</td>
<td>111.17 ± 0.6009**</td>
</tr>
<tr>
<td>VII</td>
<td>Aqueous extract 200 mg/kg</td>
<td>104 ± 0.3651**</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM for (n=6) rats in each group, when compared to control **p<0.01, *p<0.05 and ns p>0.05.
**Table 3** Effect of ethanol and aqueous extracts of leaves of *Trewia nudiflora* on blood glucose level in alloxan induced diabetic rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment / Dose</th>
<th>Blood glucose level (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 day</td>
</tr>
<tr>
<td>I</td>
<td>Normal control</td>
<td>82.33 ± 1.358**</td>
</tr>
<tr>
<td>II</td>
<td>Glibenclamide 10 mg/kg</td>
<td>134 ± 0.7303**</td>
</tr>
<tr>
<td>III</td>
<td>Control 0.5% Tween 80</td>
<td>124 ± 0.3651</td>
</tr>
<tr>
<td>IV</td>
<td>Ethanol extract 100 mg/kg</td>
<td>125.33 ± 0.8819**</td>
</tr>
<tr>
<td>V</td>
<td>Ethanol extract 200 mg/kg</td>
<td>130.83 ± 0.6009**</td>
</tr>
<tr>
<td>VI</td>
<td>Aqueous extract 100 mg/kg</td>
<td>133.83 ± 0.7923**</td>
</tr>
<tr>
<td>VII</td>
<td>Aqueous extract 200 mg/kg</td>
<td>140.5 ± 0.5627**</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM for (n=6) rats in each group, when compared to control **p<0.01 and ns p>0.05.

**Table 4** Effect of ethanol and aqueous extracts of leaves of *Trewia nudiflora* on lipid profiles and total proteins in diabetic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Biochemical parameters (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TC</td>
</tr>
<tr>
<td>Normal control</td>
<td>118.5 ± 0.2236**</td>
</tr>
<tr>
<td>Glibenclamide 10 mg/kg</td>
<td>122.5 ± 0.2236**</td>
</tr>
<tr>
<td>Diabetic Control</td>
<td>140.5 ± 0.2236</td>
</tr>
<tr>
<td>Ethanol extract 100 mg/kg</td>
<td>120.5 ± 0.2236**</td>
</tr>
<tr>
<td>Ethanol extract 200 mg/kg</td>
<td>115.5 ± 0.2236**</td>
</tr>
<tr>
<td>Aqueous extract 100 mg/kg</td>
<td>125.5 ± 0.2236**</td>
</tr>
<tr>
<td>Aqueous extract 200 mg/kg</td>
<td>117.5 ± 0.2236**</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM for (n=6) rats in each group, when compared to control **p<0.01 and ns p>0.05.
Histopathological studies

**Fig 3** Shows normal acini, and normal cellular population in the islet of Langerhans in pancreas of vehicle treated rats (normal control rats)

**Fig 4** Shows the lobule with regenerated islets in Glibenclamide (10mg/kg/day, p.o.) treated rats

**Fig 5** Shows extensive damage to the islet of Langerhans and reduced dimensions of islet in diabetic control rats (toxic control rats)

**Fig 6** Restoration of normal cellular population size of islet with hyperplasia by *Trewia nudiflora* ethanol extract 200 mg/kg

**Fig 7** Partial restoration of normal cellular population and enlarged size of β cells with hyperplasia by *Trewia nudiflora* ethanol extract 100 mg/kg.

**Fig 8** Restoration of normal cellular population size of islets with hyperplasia by *Trewia nudiflora* aqueous extract 200 mg/kg.
Discussion

Phytochemical studies have revealed the presence of several phytochemicals including alkaloids, glycosides, flavonoids, steroids, phenolic compounds and tannins. The percentage yield of aqueous and ethanol extracts of leaves of *Trewia nudiflora* were found to be more than the other extracts. Polyphenol are the major plant compounds with high level of antioxidant activity due to their ability to absorb, neutralize and to quench free radicals as well as their redox properties presence of conjugated ring structures and carboxylic group which have been reported to inhibit lipid peroxidation. Results obtained in the present study revealed that the levels of these phenolic compounds in the aqueous and ethanol extracts of leaves of *Trewia nudiflora* were considerable.

DPPH is frequently used to determine radical scavenging activity of natural compounds and its radical form absorbs at 517 nm due to the antioxidant activity, the absorption decreases may be the formation of its non radical form such as DPPH–H. Hence, the radical scavenging activity in the presence of a hydrogen donating antioxidant can be monitored as a decrease in absorbance of DPPH solution. The DPPH free radical scavenging activity of the ethanol and aqueous extracts of leaves of *Trewia nudiflora* and ascorbic acid showed at different concentrations. The investigated extracts demonstrated that the DPPH free radical scavenging activity and IC$_{50}$ value of ethanol and aqueous extracts of leaves were found to be 581.80 and 714.29 μg/ml, respectively. The IC$_{50}$ value of ascorbic acid was found to be 537.63 μg/ml. The diabetogenic agent Alloxan is a hydrophilic and chemically unstable pyrimidine derivative which is toxic to pancreatic β cells because it can generate toxic free oxygen radicals during redox cycling in the presence of reducing agents such as glutathione and cysteine. The increase in oxygen free radicals in diabetes could be due to increase in blood glucose levels, which generates free radicals due to auto oxidation. In the present work, involvement of free radicals in progression of disease and protective effects of *Trewia nudiflora* has been examined. Administration of ethanol and aqueous extracts of *Trewia nudiflora* for 15 days showed significant antidiabetic, antihyperlipidemic and antioxidant activities in Alloxan induced diabetic rats. Hyperlipidemia is one of the major cardiovascular risk factors. It has been demonstrated that insulin
deficiency in diabetes mellitus leads to a variety of derangements in metabolic and regulatory processes, which in turns leads to accumulation of lipids such as Total Glycerides and total cholesterol in diabetic patient, diabetes mellitus alters the normal metabolism of cholesterol and triglycerides showed an increase in alloxan induced diabetic rats. Under normal conditions, the enzyme lipoprotein lipase hydrolyses triglycerides. Diabetes mellitus results in failure to activate this enzyme thereby causing hypertriglyceridemia. Our data showed in line with notion as the Alloxan (140mg/kg, i.p.) treated diabetic rats exhibited clear cut abnormalities in lipid metabolism as evidenced from the significant elevation of serum TG, TC, LDL-C, VLDL-C and reduction of HDL-C levels. Treatment with ethanol and aqueous extracts of *Trewia nudiflora* for 15 days was sufficient to produce a significant reduction in the TG, TC, LDL-C, VLDL-C and significant increase in HDL-C levels in diabetic rats. These results indicate that ethanol and aqueous extracts of *Trewia nudiflora* has a lipid lowering effect on the diabetic rats. The findings of the present study shows a number of positive effects of *Trewia nudiflora* on rats with Alloxan induced disturbances in glucose tolerance and lipoprotein profile. Thus, ethanol and aqueous extracts of leaves of *Trewia nudiflora* is beneficial in the control of diabetes and abnormalities in lipid profiles. These beneficial effects of *Trewia nudiflora* are specially promising in the light of preventing lifestyle disease of the cardiovascular systems. The histopathology study of pancreas was also performed which showed hypoglycemic effect. The study reveals that in glucose-fed rats, the maximum hypoglycemic effect was produced within one hour during glucose tolerance test, this indicates that it takes about one hour for the active ingredient(s) or its (their) metabolites in the ethanol and aqueous extracts of leaves of *Trewia nudiflora* to enter into the circulation and target tissues to bring about hypoglycemic effect.

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References


