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Research Article

ANTIHYPERGLYCEMIC AND ANTIHYPERLIPIDEMIC ACTIVITY OF *JATROPHA GOSSYPIFOLIA* METHANOLIC EXTRACT IN STREPTOZOTOCIN-NICOTINAMIDE INDUCED DIABETIC RATS

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ABSTRACT

Objective: The objective of the present study is to explore the antihyperglycemic and antihyperlipidemic activities of *Jatropha gossypifolia* methanolic extract (ME) in streptozotocin (STZ)-nicotinamide (NIC) induced diabetic model.

Methods: Type II diabetes was induced by a single dose of NIC (110 mg/kg) and STZ (50 mg/kg b.w.) intraperitoneally. The diabetic animals were treated with ME (50 mg/kg and 100 mg/kg b.w.) of *J. gossypifolia*. At the end of experimental period, the effect of the ME on creatinine level, triglyceride (TG), total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL) and very LDL (VLDL) were analyzed. Liver function parameters such as glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT) were analyzed and liver glycogen content was estimated spectrophotometrically. After scarification of animals, the liver was collected and subjected to histopathology analysis. Glycogen content was estimated spectrophotometrically.

Results: The ME treated diabetic rats showed a significant increase in HDL level and a decrease in creatinine, TG, TC, and VLDL levels. The treated group showed a significant decrease in liver function parameters such as GOT and GPT levels and significantly increased the liver glycogen content.

Conclusion: These findings demonstrate that ME possess antihyperglycemic and antihyperlipidemic activity against STZ - NIC induced diabetic rats.

Keywords: *Jatropha gossypifolia,* Methanolic extract, Streptozotocin-nicotinamide induction, Antihyperglycemic, Antihyperlipidemic.

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INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder characterized by hyperglycemia arising as a consequence of relative or absolute deficiency of insulin secretion, resistance to insulin action or both [1]. The main pathophysiological defect of Type II diabetes is insulin resistance, a decrease in cellular response to insulin. This includes impairment in the insulin signaling pathway leading to a failure of the insulin-stimulated glucose uptake in targeted tissues such as muscle and fat [2].

The classic symptoms associated with diabetes are polyuria (condition of increased urination), polydypsia (increased thirst), and polyphagia (increased hunger). Other important mechanisms associated with Type II diabetes includes high glucagon levels in blood, increased breakdown of lipids within the fat cells, resistance to incretion and increased retention of salt and water by the kidneys [3,4] and hypercholesterolemia, a condition associated with an increased plasma concentration of LDL, VLDL, and elevated levels of HDL [5]. Obesity combined with insulin resistance is a major risk factor for the prevalence of non-insulin dependent diabetes mellitus [6]. Adipocyte is the only apparent link between diabetes and obesity, which stores excess glucose in the form of fat by changing dramatically in its size in accordance with metabolic needs [7].

Many medicinal herbs from Indian system of medicine have been shown to have hypoglycemic and hypolipidemic properties due to the presence of various secondary metabolites such as alkaloids, terpenoids, flavonoids, saponins, and tannins [8-10]. Glucose-lowering drugs including sulfonylureas, biguanides, alpha-glucosidase inhibitors, thiazolidinediones, and meglitinides have been shown for their effectiveness in governing diabetic signs, and most of them have been conveyed to pose one or other physiological complication or side effects on use [11]. *Jatropha gossypifolia* is a perennial herb belonging to the Euphorbiaceae family with the common name of bellyache bush. The plant has hairy leaves with three lobed fruit and flowers. Various parts of the plant such as leaves, roots, and stem bark are of medicinal importance [12]. Abreu *et al.* have reported that *J. gossypifolia* has antihypertensive activity [13]. Plant of this genus such as *Jatropha curcas* and *Jatropha tanjorensis* has been reported for antidiabetic activity [14,15]. Hence, this study was taken, to investigate the antihyperglycemic and antihyperlipidemic activities of the leaves of *J. gossypifolia* in streptozotocin (STZ)-nicotinamide (NIC) induced diabetic rats.

METHODS

Chemicals and reagents

Metformin, NIC were obtained from Himedia, India. STZ was purchased from Sigma-Aldrich, St. Louis, USA. Organic solvents were of the highest analytical grade. All other fine chemicals, organic solvents, and reagents used were of higher analytical grade and purchased from Himedia, India.

Collection of plant material, extraction, and phytochemical constitution

J. gossypifolia leaves were collected from Chennai, Tamil Nadu, India. The collected material was authenticated in a plant anatomy research institute (PARC/2015/3142), Chennai, Tamil Nadu, India. Leaves were shade dried, powdered and packed in the Soxhlet apparatus and extracted with methanol. The extraction was carried out for 72 hrs at 60°C. The extract was filtered and concentrated in a rotary evaporator.

The extract was stored at room temperature for further analysis. Preliminary phytochemical screening for the presence of flavonoids was done by colorimetric aluminum chloride method [16], total phenolic content [17], and tannin content was done by Folin–Ciocalteu method [18,19].

Experimental group

The *in vivo* study was in accordance to the animal Institutional Ethical Committee (ethical clearance no 090/835/IAEC-2014). Male Wistar rats, of 6 to 8 was old and weighing 150-200g, were used for the study. Totally, 45 animals were purchased from Tamil Nadu Veterinary and Animal Sciences University, Chennai, India, and were acclimatized in the Center for Animal House, SRM University, Chennai, India. All animals were acclimatized to laboratory conditions for one week and allowed free access to sterilized water and pellet diet. The rats were maintained in sterilized polypropylene cages with sterile paddy husk as bedding and maintained in 12 hrs light+12 hrs dark cycle at temperature ±25°C.

Development of insulin resistant animal model

Diabetes was induced in male Wistar rats (aged 2-3 months and 180-200 g body weight) by intraperitoneal administration of NIC (110 mg/kg b.w.) and STZ (single dose of 50 mg/kg b.w.) dissolved in freshly prepared 0.01 M citrate buffer, pH 4.5. STZ was injected after 15 minutes from the administration of NIC. NIC was freshly prepared in saline [20]. After checking the blood glucose levels at 3rd day and 7th day of induction, the rats with marked hyperglycemia (FBG≥250 mg/dl) were selected and used for the study [21].

Animal grouping and drug administration

A total of 45 rats (30 diabetic rats and 15 normal rats) were divided into six groups with five rats per group:

Group 1: Control rats (0.5% CMC); n=5

Group 2: Control rats treated with methanolic extract (ME) (100 mg/kg b.w.); n=5

Group 3: Diabetic rats (STZ+NIC); n=5

Group 4: Diabetic rats treated with ME (50 mg/kg b.w.); n=5

Group 5: Diabetic rats treated with ME (100 mg/kg b.w.); n=5

Group 6: Diabetic rats treated with metformin (500 mg/kg b.w.); n=5.

All experiments were carried out in overnight fasted rats.

Estimation of biochemical parameters

The blood samples were collected using retro orbital plexus on 0th, 7th, 14th, 21st, 28th, and 35th day. Blood samples were allowed to clot for 30 minutes and centrifuged at 3000 rpm for 15 minutes. The serum was collected and used for biochemical parameters such as creatinine level by Jaffe method. Total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL) and very LDL (VLDL) estimated according to enzymatic GPO, trinder, and endpoint method. Liver function parameters such as glutamate oxaloacetate transaminase, glutamate pyruvate transaminase were done by International Federation of Clinical Chemistry method. All the biochemical parameters were carried out using commercially available test kits from Robonik prietest, India [22,23].

Histopathological investigation

At the end of the study period, control and treated group animals were sacrificed, and liver was dissected out for histopathological studies. After washing the samples with saline, they were stored in 10% formalin. The tissues were fixed in paraffin, and thin section (5 mm) of samples were taken and stained with hematoxylin and eosin for microscopic assessment [24].

Estimation of glycogen content

Per g of liver, samples were rinsed with ice-cold buffer (saline) and incubated with 30% KOH at 55°C for 30 min with occasional shaking. 0.2 ml of sodium sulfate was added, and glycogen was precipitated by the addition of ethanol (5 ml). The precipitate was removed and dissolved in 10 ml of water. To this, 1 ml of 1.2 mol/l HCl (1 ml) was added and boiled for 2 hrs and neutralized with 0.5 mol/l NaOH and OD was taken at 620 nm [25,26].

Statistical analysis

The results were expressed as mean±standard deviation (SD). One-way analysis of variance (ANOVA) was used for data analysis, followed by Duncan's multiple range test (DMRT) using a Statistical Package for the Social Sciences (SPSS) software Version 17.0 for windows. The significance level was considered as $p<0.05$.

RESULTS AND DISCUSSION

Phytochemical constitution of *J. gossypifolia* ME

Phytochemical screening of ME of *J. gossypifolia* showed the presence of phytochemical constituents such as tannins, flavonoids, and phenolic contents. The ME yield was found to be 9.6% (w/w). Tannin content was found to be 1.732±0.001 mg/ml, phenol content was 0.892±0.001 mg/ml, and flavonoid content was found to be 1.3125±0.0015 mg/ml. Previous reports have shown that the higher tannin, flavonoid, and phenol content have been extracted using methanol from various plant extracts due to its increased polarity [27-31].

Effect of ME on creatinine level

The effects of ME on serum creatinine level in control and experimental group rats are given in Table 1. In diabetic rats, the increased serum creatinine level was observed throughout the study which represents liver and renal damage to the diabetic rats from initial day 2.547±0.08 (0th day) until the end of the experiment 2.773±0.12 (35th day). Oral administration of ME (50 and 100 mg/kg b.w.) to the experimental groups significantly reduced the creatinine level. ME low dose (LD) showed a reduction in creatinine level from 2.513±0.36 (0th day - before treatment) to 1.584±0.06 (35th day - after treatment) whereas, high dose (HD) of ME exhibited a decrement from 2.515±0.38 to 1.462±0.21 in creatinine level. Administration of metformin also showed a decrease from 2.546±0.34 to 1.398±0.28 in creatinine level. The reduction in the creatinine level by ME supports its shielding nature on the liver and renal damage. Similarly, upsurge in creatinine level was decreased by administration of ethanolic extract (200 and 400 mg/kg b.w.) of *Allium cepa* Linn from 4.512±0.19 to 2.475±0.147 [32].

Effect of ME in TG and TC

Table 2 illustrates the effect of ME on the level of serum TG and TC in control and experimental groups. The levels of TG (248.11±3.98 mg/dL) and TC (279.420±3.99 mg/dL) were significantly increased in the diabetic group rats until end of the study whereas administration of LD and HD of ME decreased the level of TG from 205.44±3.26 to 129.38±2.97 mg/dL and 203.22±3.49 to 117.07±2.94 mg/dL. A significant decrease in the level of TC was observed at the end of the experiment (195.116±2.92 in LD ME and 178.801±2.25 mg/dL in HD ME). Diabetic mellitus is characterized by an elevation in cholesterol and TG levels which are

Table 1: Effect of ME on serum creatinine in STZ-NIC induced diabetic rats (n=5)

Groups	Creatinine mg/dL	
	Before treatment	After treatment
Control	1.248±0.06	1.206±0.07
Control+*J. gossypifolia*	1.224±0.07	1.209±0.06
Diabetic group	2.547±0.08$^{\#}$	2.773±0.12$^{\#}$
Diabetic+*J. gossypifolia* ME (LD)	2.513±0.36	1.584±0.06*
Diabetic+*J. gossypifolia* ME (HD)	2.515±0.38	1.462±0.21*
Diabetic+metformin	2.546±0.34	1.398±0.28*

Values are expressed as means±SD (n=5) from three independent experiments. Statistical evaluation was done by one-way ANOVA followed by DMRT. *$p<0.05$ as compared with diabetic group, $^{\#}p<0.05$ as compared with control group. *J. gossypifolia: Jatropha gossypifolia*, LD: Low dose, HD: High dose, STZ-NIC: Streptozotocin-nicotinamide, ME: Methanolic extract

termed as hyperlipidemia. The results clearly showed that ME brought TG and TC levels to normal due to increase in the level of insulin secretion which inhibits lipases and decreases the fat deposition and upturn the consumption of glucose.

Similarly, TG and TC level was found to be significantly ($p<0.001$) lesser than the diabetic group in various fractions of *Annona reticulata* leaf. The TG levels of various groups at the end of the experimental day were 195.49±10.038 mg/dL to methanolic fraction treated group and 198.32±15.827 mg/dL ethyl acetate fraction treated group and cholesterol level were found to be 114.45±4.531 mg/dL in methanolic fraction treated group and 61.65±5.255 mg/dL for ethyl acetate fraction treated group [33].

Effect of ME on lipid profile

STZ-NIC induced diabetic group rats showed increased level of LDL (209.07±3.48 mg/dL) which increases the coronary risk factor and decreased the level of HDL (21.72±1.07 mg/dL) which are shown to have a cardiovascular risk factor. The increase in the level of TG and TC is concomitant with increased level of LDL, VLDL, and decrease the level of HDL. Treatment to the STZ-NIC induced diabetic groups with two doses of (50 and 100 mg/kg b.w.) ME significantly reduced the level of LDL and VLDL level and increased HDL level as compared with the diabetic rats (Table 3). The lipid profile level of various groups at 35th day for HDL was 49.83±1.96 and 54.38±1.08 mg/dL and LDL level at the final day was 120.17±2.24 and 101.31±2.04 mg/dL followed by VLDL at 25.87±1.32 and 23.40±1.37 mg/dL corresponding to LD and HD of ME, respectively. The result suggests that the ME has considerably dropped the blood lipid irregularities. Previous studies with *Achatina fulica* (0.5 mg) ME dissolved in 1% gum acacia in alloxan - induced diabetic mice showed antihyperlipidemic activities, which supports our data [34].

Liver enzyme parameters

Table 4 indicates the decrease in serum aspartate transaminase (AST) and alanine transaminase (ALT) levels up on oral administration of ME (LD and HD) showed 38.236±1.29 and 36.488±1.26 U/L AST level and 40.236±2.27 and 37.688±1.24 U/L ALT levels, respectively. A significant increase in the serum AST (191.314±2.82 U/L) and ALT (204.914±2.20 U/L) level in diabetic control rats is a sign of hepatic injury due to leakage of these enzymes from liver cytosol to the blood stream. From the result, it is evident that the ME maintains the AST and ALT level indicating the hepatoprotective role in thwarting diabetic complications. Oral doses of mistletoe *Loranthus micranthus* extracts to Wistar rats at 551 mg/kg b.w. and 827 mg/kg b.w. had significantly lower ALT activities to 15.96 and 14.24 U/L and mild variations in the AST activities of 31.99 and 34.30 U/L [35].

Table 2: Effect of ME on TG and TC in STZ-NIC induced diabetic rats (n=5)

Groups	TG (mg/dL)		TC (mg/dL)	
	Before treatment	After treatment	Before treatment	After treatment
Control	95.36±1.94	94.92±1.75	154.94±2.25	154.388±2.23
Control+*J. gossypifolia*	95.76±1.86	95.92±1.96	156.952±2.28	160.168±2.25
Diabetic group	207.21±3.15$^{\#}$	248.11±3.98$^{\#}$	246.542±3.24$^{\#}$	279.420±3.99$^{\#}$
Diabetic+*J. gossypifolia* ME (LD)	205.44±3.26	129.38±2.97*	248.602±3.27	195.116±2.92*
Diabetic+*J. gossypifolia* ME (HD)	203.22±3.49	117.07±2.94*	246.32±3.29	178.801±2.25*
Diabetic+metformin	201.72±3.18	107.84±2.18*	243.436±3.87	166.802±2.14*

Values are expressed as means±SD (n=5) from three independent experiments. Statistical evaluation was done by one-way ANOVA followed by DMRT. *p<0.05 as compared with diabetic group, $^{\#}$p<0.05 as compared with control group. *J. gossypifolia: Jatropha gossypifolia,* LD: Low dose, HD: High dose, STZ-NIC: Streptozotocin-nicotinamide, ME: Methanolic extract, TG: Triglyceride, TC: Total cholesterol

Table 3: Effect of ME on lipid profile in STZ-NIC induced diabetic rats (n=5)

Groups	HDL (mg/dL)		LDL (mg/dL)		VLDL (mg/dL)	
	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Control	58.99±1.92	58.22±1.81	76.87±1.57	77.18±1.42	19.07±1.08	18.98±1.05
Control+*J. gossypifolia*	59.14±1.21	58.84±1.63	78.67±1.81	82.12±1.97	19.15±1.04	19.19±1.06
Diabetic group	35.04±1.23$^{\#}$	21.72±1.07$^{\#}$	170.08±2.85$^{\#}$	209.07±3.48$^{\#}$	41.44±1.69$^{\#}$	49.62±1.89$^{\#}$
Diabetic+*J. gossypifolia* ME (LD)	35.38±1.11	49.83±1.96*	172.94±2.54	120.17±2.24*	41.08±1.93	25.87±1.32*
Diabetic+*J. gossypifolia* ME (HD)	35.88±1.21	54.38±1.08*	169.79±2.86	101.31±2.04*	40.64±1.75	23.40±1.37*
Diabetic+metformin	36.28±1.62	56.68±1.07*	166.83±2.64	88.62±1.59*	40.35±1.95	21.56±1.12*

Values are expressed as means±SD (n=5) from three independent experiments. Statistical evaluation was done by one-way ANOVA followed by DMRT. *p<0.05 as compared with diabetic group, $^{\#}$p<0.05 as compared with control group. *J. gossypifolia: Jatropha gossypifolia*, LD: Low dose, HD: High dose, STZ-NIC: Streptozotocin-nicotinamide, ME: Methanolic extract, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, VLDL: Very low-density lipoprotein

Table 4: Effect of ME on AST and ALT in STZ-NIC induced diabetic rats (n=5)

Groups	AST (U/L)		ALT (U/L)	
	Before treatment	After treatment	Before treatment	After treatment
Control	33.778±1.21	33.59±1.72	34.960±1.60	34.720±1.03
Control+*J. gossypifolia.*	34.732±1.25	34.704±1.39	35.370±1.43	34.980±1.58
Diabetic group	145.734±2.36$^{\#}$	191.314±2.82$^{\#}$	179.340±2.50$^{\#}$	204.914±2.20$^{\#}$
Diabetic+*J. gossypifolia* ME (LD)	145.366±2.27	38.236±1.29*	179.286±2.48	40.236±2.27*
Diabetic+*J. gossypifolia* ME (HD)	144.79±2.45	36.488±1.26*	179.550±2.42	37.688±1.24*
Diabetic+metformin	146.132±2.72	31.28±1.58*	180.172±2.29	35.880±1.86*

Values are expressed as means±SD (n=5) from three independent experiments. Statistical evaluation was done by one-way ANOVA followed by DMRT. *p<0.05 as compared with diabetic group, $^{\#}$p<0.05 as compared with control group. *J. gossypifolia: Jatropha gossypifolia*, LD: Low dose, HD: High dose, STZ-NIC: Streptozotocin-nicotinamide, ME: Methanolic extract, AST: Aspartate transaminase, ALT: Alanine transaminase

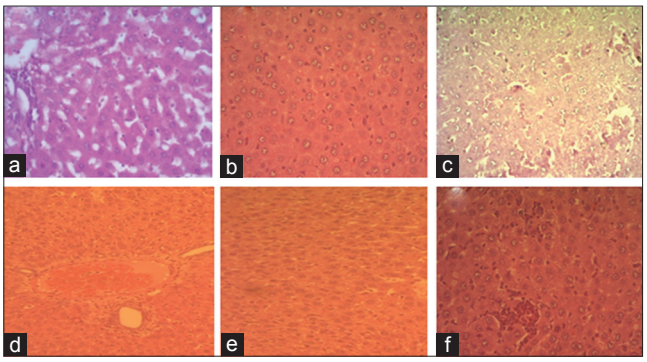

Fig. 1: Histopathology of liver in streptozotocin-nicotinamide induced diabetic rats after 35 days of the treatment with methanolic extract (ME). (a) Control rats (b) control+ME rats showing normal hepatic cells. (c) Diabetic rats showing hepatocellular necrosis. (d and e) ME 50 and 100 mg/kg b.w., respectively, showing normal hepatocellular with normal cytoplasm and nucleus. (f) Diabetic+metformin (500 mg/kg) showing distinct hepatic layer with normal cytoplasm and nucleus

Table 5: Effect of ME on liver glycogen in STZ-NIC induced diabetic rats (n=5)

Groups	Liver glycogen mg/g
Control	56.14±1.37
Control+*J. gossypifolia.*	53.42±1.52
Diabetic group	18.74±0.97#
Diabetic+*J. gossypifolia* ME (LD)	49.23±1.20*
Diabetic+*J. gossypifolia* ME (HD)	55.58±1.47*
Diabetic+metformin	59.95±1.68*

Values are expressed as means±SD (n=5). Statistical evaluation was done by one-way ANOVA followed by DMRT. *p<0.05 as compared with diabetic group, #p<0.05 as compared with control group. *J. gossypifolia: Jatropha gossypifolia*, LD: Low dose, HD: High dose, STZ-NIC: Streptozotocin-nicotinamide, ME: Methanolic extract

Effect of ME on glycogen content in STZ-NIC induced diabetic rats

The liver plays an important role in postprandial hyperglycemia which is involved in the synthesis of glycogen. Glycogen synthase is activated by synthase phosphatase resulting in glycogenesis. Oral administration of ME (50 and 100 mg/kg b.w.) significantly increased the liver glycogen level of 49.23±1.20 and 55.58±1.47 mg/g, respectively, when compared with the diabetic rats which showed 18.74±0.97 mg/g of glycogen. *Helianthus annuus* L., seed ethanolic extract 250 and 500 mg/kg b.w. for 21 days in STZ-NIC induced diabetic rats resulted in significant increase in liver glycogen of 12.65±0.32 and 13.32±0.28 mg/g, respectively [36]. The diabetic rats treated with ME fetched back liver glycogen to normal levels, and this may be due to the improved secretion of insulin, resulting in the augmentation of glycogenesis (Table 5).

Histopathological studies

Fig. 1 summarizes the histology of liver in control and experimental rats. Control rats (Fig. 1a and b) showing normal hepatic cells. Diabetic control rats (Fig. 1c) showing hepatocellular necrosis and fading nuclei in STZ-NIC induced rats. The experimental group rats which received ME 50 and 100 mg/kg b.w. (Fig. 1d and e) indicated the section of liver showing hepatocellular manner with normal nucleus and cytoplasm. Diabetic rats treated with metformin (500 mg/kg b.w.) showed separate hepatic layer with normal nucleus and cytoplasm. A similar result was observed in *Ficus amplissima* smith, bark extracts (50, 100, and 150 mg/kg b.w.) in STZ induced diabetic rats showing normal hepatocellular architecture in experiment groups and liver was necrotized in diabetic control rats [37]. Histopathological study of the liver revealed that the ME significantly enhanced central vein and lobular architecture.

CONCLUSION

From the above results, it is concluded that *J. gossypifolia* ME has exhibited significant hypoglycemic and hypolipidemic potential. In this study, we have demonstrated that ME have a favorable effect on creatinine, TG, TC, HDL, LDL, VLDL, ALP, and AST bringing the levels close to normal level. This indicates that the ME of *J. gossypifolia* may possibly be used for the development of a pharmaceutical drug for treating diabetes and associated complications.

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