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Research Article

ANTIDIABETIC AND HYPOLIPIDEMIC ACTIVITY IN STEM OF *JATROPHA GOSSYPIFOLIA* L.

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ABSTRACT

Objectives: The present study was carried out to investigate the antidiabetic and hypolipidemic efficacy of active fractions (chloroform fraction of 95 % ethanolic extract and butanol fraction of aqueous extract) of *J.gossypifolia* L.(stem) by measuring the status of blood glucose, serum insulin, carbohydrate metabolizing enzymes, lipid levels, renal and hepatic function markers of diabetic rats.

Methods: Chloroform fraction of 95% ethanolic extract and butanol fraction of aqueous extract of *J.gossypifolia* L. (stem) were administered to diabetic groups daily up to 15 days. Biochemical parameters notably fasting blood glucose, oral glucose tolerance, serum lipid profile, renal and hepatic function markers and enzymatic activities of carbohydrate metabolism were assessed.

Results: In single dose study, chloroform fraction of 95% ethanolic extract and butanol fraction of aqueous extract of *J.gossypifolia* L.(stem), significantly declined blood glucose levels of diabetic rats while in multiple dose studies, chloroform fraction of 95% ethanolic extract restored the elevated blood glucose profile, serum lipid level, renal and hepatic function markers to nearly normal level when compared with untreated group, however, butanol fraction of the aqueous extract did not show any marked effect.

Conclusion: The present study thus concludes that the antidiabetic efficacy of chloroform fractions of the 95 % ethanolic extract of *J.gossypifolia* L.(stem) have favourable effect in bringing down the severity of hyperglycemia, hyperlipidemia,, modulating effect on carbohydrate metabolizing enzymes, decline the increased level of renal and hepatic function markers and also improving glucose tolerance activity.

Keywords: Antidiabetic activity, Diabetes mellitus, *Jatropha gossypifolia* L., High fructose high fat diet fed Low-dosed STZ treated-rats, Neonatal STZ treated rats, Carbohydrate metabolizing enzymes.

Abbreviations: STZ- Streptozotocin, SEM- standard error mean, AUC- area under curve, n-STZ- Neonatally Streptozotocin induced diabetic rats, SD strain-Sprague Dawley strain, FBG-fasting blood glucose, OGTT-oral glucose tolerance test, LDL low-density lipoprotein and HDL high-density lipoprotein, TG-Triglycerides, TC-Total Cholesterol.

INTRODUCTION

Diabetes mellitus is a multifactorial disorder affecting carbohydrate, fat and protein metabolism resulting from increased hepatic glucose production, diminished insulin secretion and impaired insulin action. India continues to be the 'Diabetic Capital' of the world with 50.8 million diabetics and increasing their prevalence without any plateau.

Insulin resistance and impaired insulin secretion lead to hyperglycemia, hyperlipidemia and to an increase in hepatic glucose [1-2]. The chronic hyperglycemia and abnormalities in serum lipids of diabetes is associated with long-term damage, dysfunction and failure of various organs, especially the eyes, kidneys, nerves, heart and blood vessels [3-5]. The epidemic spread of type 2 diabetes and identification of new therapeutic avenues in the treatment of all pathological aspects of this disorder remain a major challenge for current biomedical research. Although wide varieties of pharmacological drugs are being used for T2DM treatment but due to addition to their adverse effects, drug treatments are not always satisfactory in maintaining normal level of blood glucose and avoiding late stage diabetic consequences [6,7], this leads to increasing demand for natural products with antidiabetic activity and less side effects [8,9].

The plant part selected for the present study is *J.gossypifolia L.* stem (Family: Euphorbiaceae) and was procured from Purba Midinapur, West Bengal. It is a bushy, gregarious shrub, up to 1.8m height. The field data of this plant is entered in Botany Division collection register vide serial number 8403. The plant has traditional uses such as emetic, emmengogue, blood purifier, anti-coagulant, antibacterial, anticancer, antiulcer [10-12]. The plant is known to possess various medicinal and pesticidal properties. However, no work has been done to assess the antidiabetic effect of *J.gossypifolia L.* stem in So this study aimed to evaluate the potential of the active fractions of *J.gossypifolia L.* stem on blood glucose, lipid profile, renal and hepatic function markers and insulin in rat model of fructose-induced insulin resistance, STZ treated rats and n-STZ treated rats and its modifying effect on carbohydrate metabolizing enzyme activities.

MATERIALS AND METHODS

Chemicals and Reagents

Metformin and STZ were purchased from Sigma Aldrich Co., USA. One touch glucometer and glucostrips was purchased from Roche Diagnostics India Ltd. All other chemicals unless otherwise mentioned were obtained from SRL, Mumbai.

Preparation of solvent extractions of *J. gossypifolia* stem

The stem of *J.gossypifolia* L. were cut into strands, shade dried and pulverized to a fine powder with the aid of an electric blender and extracted many times with 95% ethanol by percolation method allowed standing for 24 hours with occasional shaking using Soxhlet apparatus. Each percolate w ere collected , pooled, filtered and concentrated under vacuum to dryness using rotavapor at 40-45 ^{0}C. This extract is termed as 95% ethanolic extract. In the same manner the crude powder was extracted with 50% ethanol and distilled water, respectively and 50% ethanolic and aqueous extracts prepared.

Fractionation of 95% ethanolic, 50% ethanolic and aqueous extracts of *J.gossypifolia* stem

The 95% ethanolic, 50% ethanolic and aqueous extracts were further fractioned using column chromatography with solvents of increasing polarity viz. hexane, chloroform, butanol and water to obtain the respective fractions. Each extract was dried well and triturated with hexane and then concentrated under reduced pressure at 40°C.The insoluble portion was fractionated by chloroform and

concentrated under reduced pressure. Residue obtained after triturating with chloroform was then suspended in distilled water and then fractionated with butanol saturated with water. Butanol soluble fraction was concentrated under vacuum at 50˚C. Water soluble fraction was also concentrated under vacuum at 45-50˚C and finally dried under vacuum to get the final fractions.

Procurement and Selection of Animals

Male albino rats of Sprague Dawley strain (8 to 10 weeks of age: body weight range 160 ± 20 g) were procured from National Laboratory Animal Centre (NLAC) of the Institute. Research on animals was conducted in accordance with the guidelines of the committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) formed by the Government of India in 1964. Rats were housed in groups of five in polypropylene cages under controlled standard environmental conditions of temperature, humidity, air changes and 12 hr light-dark cycles. Prior to commencement of the experiment, all the rats were acclimatized to the new environmental condition for a period of one week. During the experimental period the rats were kept in a well ventilated animal house at room temperature of 25˚C and were supplied with standard pellets and fresh drinking water.

Induction of diabetes

A solution of STZ (60 mg/kg) in 100 mM citrate buffer (acidified saline solution), pH 4.5 was prepared and calculated amount of the fresh solution was immediately injected intraperitoneally to overnight fasted rats. After 48 hours when the diabetic state must have stabilised, the animals with blood glucose above 300 mg/dl were taken for the study. Blood glucose level measurements were always done by glucostrips (Roche) [13].

Single dose effect

Improvement on oral glucose tolerance of normal rats

The overnight fasted rats showing blood glucose level between 60 to 80 mg/dl was measured by glucometer using glucostrips were selected, divided into groups each consisted of five animals. Rats of experimental group were administered the suspension of the test sample orally prepared in 1.0 % gum acacia (vehicle) at pre-selected dose levels i.e. 250 mg/kg body weight in the case of extracts and 100 mg/kg in case of fractions. The standard antidiabetic drug i.e. Metformin was administered at 100 mg/kg dose levels. Animals of control group were given an equal amount of 1.0 % gum acacia and termed as sham treated control. An oral sucrose load of 10g/kg body weight was given to each animal exactly after 30 min post administration of the test sample/vehicle. Blood glucose of each rat was again determined from the tail vein at 30, 60, 90 and 120 min post administration of sucrose by glucostrips. The animals were kept unfed throughout the experiment [14].

Blood glucose lowering effect of STZ induced diabetic rats

The STZ-induced diabetic animals having their blood glucose level between 300 to 400 mg/dl were separated, randomized into groups each consisted of five animals each and sham treated control group received orally vehicle (1.0% gum acacia). Rats of experimental groups were orally administered suspension of the desired test samples and standard antidiabetic drug Metformin, respectively. The blood glucose levels of each rat were measured at 0, 30, 60, 90, 120, 180, 240 and 300 min post administration of test sample/vehicle/standard antidiabetic drug. Food but not water was withheld from the cages during 0 to 300 min [15].

Multiple dose effect

Antidiabetic, hypolipidemic and protective effect on renal and hepatic function markers of STZ-induced diabetic rats

STZ-induced diabetic rats were showing stable or increasing blood glucose level (between 250-350mg/dl) after 7 days were screened and finally selected and randomized into groups of 5 animals in each. One group was considered as sham treated control group while the others as experimental groups. The test sample i.e. chloroform fraction of 95% ethanolic extract and butanol fraction of aqueous extract of *J.gossypifolia* stem and standard drug Metformin was administered to experimental groups, respectively at a dose of 100mg/kg body weight daily for 15 consecutive days. Fasting blood glucose levels, OGTT, lipid, renal and hepatic functions parameters were followed at preselected time intervals. At the end of experiment, the blood was withdrawn from jugular vein, serum separated and estimated for total lipid profiles and renal and hepatic function markers [16].

Oral Glucose Tolerance Test

For the oral glucose tolerance test, glucose (3.0 g/kg,) was administered to rats that had been fasted for 16 h. Blood samples were collected from a tail vein at 0, 30, 60, 90 and 120 min. The results of OGTT are expressed as integrated area under the curve over the 2 h after glucose administration [17].

Antidiabetic, hypolipidemic and protective effect on renal and hepatic function markers of high fructose-high fat fed-low dosed STZ-induced diabetic rats

Male rats weighing 160–200 g were used in the study. For induction of type 2 diabetes, the rats were fed with high fructose high fat diet (diet containing: 60 % fructose, 21 % casein, 13 % saturated fat, 1.0 % salt mixture, and trace amount vital minerals and vitamins) for 6 weeks. After this period blood was withdrawn from the retro-orbital plexus of each rat for the estimation of the lipid profiles. The rats showing their serum triglyceride ≥ 300 mg/dl and total-cholesterol ≥ 150 mg/dl were considered as hyperlipidemic. These hyperlipidemic rats were injected STZ intraperitoneally at the dose of 45mg/kg [18]. Two days after injection, these STZ-treated hyperlipidemic rats were fasted and fasting blood glucose levels were estimated; rats having glucose levels ≥280 mg/dl were considered diabetic and taken for further studies. The rats were fed with high fructose diet throughout the experiment. These were divided into four groups. The rats of Group I were treated with 1.0 % gum acacia, Group II were treated with chloroform fractions of 95% ethanolic extract of *J. gossypifolia* at 100 mg/kg b.w, Group III was treated with butanol fraction of aqueous extract at 100mg/kg b.w. Group IV was treated with standard drug Metformin at 100mg/kg b.w.. The treatments were continued for 15 consecutive days. The oral glucose tolerance of each animal was followed on day 7 and 14 respectively whereas the serum lipid profiles, renal and hepatic profile were followed on day 8 and 15 post treatment [19].

Effect of oral glucose tolerance test on Neonatally-streptozotocin treated diabetic rats

Healthy SD rats were kept for breeding. The animals were maintained in a temperature and humidity regulated room. To induce type 2 diabetes, STZ (90 mg/kg, i.p.) was administered to 2-day-old male pups [20]. The pups were weaned when 4 weeks old and selected for screening by oral glucose tolerance test with glucose level ≥ 200 mg/dl at 2 h also in accordance with the type-II diabetic model. 8 weeks after the STZ injection and rats were considered to be diabetic and later included in the research. The animals were fed with laboratory pellet and water ad libitum. These rats were divided into three groups each consisted of 5 animals. The animals of Group I received vehicle i.e.1% gum acacia every day, whereas animals of Group II and III were treated with test sample i.e. chloroform fractions of 95% ethanolic extract of *J.gossypifolia* stem and standard antidiabetic drug Metformin at 100 mg/kg b.w, respectively for 14 days. The fasting blood glucose level and OGTT post glucose load of each rat was performed at selected time intervals i.e. on day 0, 7 and 14 post treatment. Each group of animals was treated daily over 14 days orally [21].

Blood biochemical examination of serum lipids and insulin levels

Total cholesterol, Total triglycerides, LDL-cholesterol, HDL-cholesterol, AST, ALT, Creatinine, Total Bilirubin, urea and uric acid in serum of each animal were measured on an automated analyzer (Cobas-Integra 400) employing the respective assay kits and instructions as provided by the manufacturer. Serum insulin level

was measured employing the kit and instructions as provided by Mercodia (Uppsala Sweden).

Enzymatic Analysis

At the end of the experiment, animals were sacrificed after an overnight starvation and livers were quickly excised for activity determination of key enzymes of carbohydrate metabolism. A 10% homogenate of each was prepared in 150 mM KCl (w/v) using Potter Elevejhem glass homogenizer fitted with Teflon pestle. The homogenates were centrifuged at 3000× rpm for 15 min at 4°C; supernatant was stored and used as enzyme source. Protein content of the supernatant was determined by the method of Lowry et al. [22] using bovine serum albumin as the standard.

Glucose-6-phosphatase

Glucose-6-phosphatase activity was measured according to the method of Hubscher and West [23]. The 1.0 mL assay system contained 0.3 M citrate buffer (pH 6.0), 28 mM EDTA, 14 mM NaF, 200 mM glucose-6-phosphate and appropriate amount of enzyme protein. Tubes were incubated at 37°C for 30 min after which the reaction was terminated by addition of 1.0 ml of 10% TCA. Estimation of inorganic phosphate (Pi) in protein-free supernatant was done according to the method of Taussky and Shorr [24].

Glycogen Phosphorylase

Glycogen phosphorylase activity was measured according to the method of Barthet et al. [25]. The 1.0 ml assay mixture contained 0.2 ml mixture A [glycogen 57 mg, glucose-1-phosphate 188 mg, NaF 42 mg and 5' AMP (4 mM) in 10 ml distilled water] and 0.1 ml mixture B, enzyme protein. Tubes were incubated at 37°C for 30 min after which the reaction was terminated by addition of 0.1 ml of 10% TCA; 0.4 ml sodium acetate (100 mM) was then added to prevent the spontaneous hydrolysis of glucose-1-phosphate present in the reaction mixture. Estimation of inorganic phosphate in protein-free supernatant was done according to the method of Taussky and Shorr.

Alpha –glucosidase

This was done according to a slight modification of the procedure reported by Cogoli et al.[26] 100µl of the purified α -glucosidase (0.1 mg/ml) were added to the assay system containing 100µl of 67 mM phosphate buffer (pH 6.8) and 25µl of glutathione (1.0 mg ml) and the volume was made 1 ml by using 775 µl of TDW. The reaction mixture was incubated at room temperature for 10 min with the 10 µl test sample (100 lM) dissolved 100% DMSO. Reaction was started by the addition of 50 µl p-nitrophenyl-a- D-glucopyranoside (1 M) and increase in absorbance was recorded at 405 nm.

Statistical Analysis

Each biochemical parameter was expressed as Mean ± SD. Quantitative glucose tolerance of each animal was calculated by AUC method (Prism Software). The average fall in AUC of experimental group compared to control group was termed as % activity. Analysis of statistical significance of differences in measurements between samples was done by using Dunnett's test following one way ANOVA. It is denoted by p values. Statistically Significance difference was set at following levels * represents p< 0.05, ** represents p< 0.01 and *** represents p< 0.001.

RESULTS

Effect of crude powder, 95% ethanolic, 50% ethanolic and aqueous extracts of *J.gossypifolia* stem and standard drug metformin on the improvement of glucose tolerance post sucrose load in normal rats and blood glucose lowering on STZ-induced diabetic rats

Table 1 presents the average blood glucose profile of crude powder, 95% ethanolic, aqueous and 50 % ethanolic extracts of *J.gossypifolia* stem on the improvement of oral glucose tolerance post sucrose load in normal rats. Crude powder of *J.gossypifolia* stem produced 10.5% improvement. Out of 3 extracts the 95% ethanolic extract and aqueous extract showed significant reduction on OSTT to the tune of 26.2% (p<0.01) and 26.5% (p<0.01) while the standard antidiabetic drug Metformin showed improvement on OSTT by 27.3% (p<0.01) at 100 mg/kg in normal rats.

The mean blood glucose concentration of control and crude powder, 95% ethanolic extract, 50% ethanolic extract and aqueous extract of *J.gossypifolia* stem on STZ-induced diabetic rats are shown in table 1. The results obtained from the variance analysis showed that crude powder of *J.gossypifolia* stem produced 14.8%(p<0.05) lowering whereas among the three extracts i.e., 95% ethanolic, 50% ethanolic and aqueous extracts prepared from the crude powder of *J.gossypifolia* stem, only 95% ethanolic extract and aqueous extract significantly inhibited the postprandial rise in blood glucose level of STZ-induced diabetic rats and the average antihyperglycemic activity of the 95% ethanolic extract and aqueous extracts inhibited the rise in postprandial hyperglycemia to the tune of 18.0 %(p<0.01) and 15.5% (p<0.05)respectively during the period 0-300 min. The standard drug Metformin showed 23.3(p < 0.01) blood glucose inhibition effect after 5 h intervals.

Table 1: Effect of crude powder, 95% ethanolic, 50% ethanolic and aqueous extracts of *J.gossypifolia* stem and standard drug metformin on the improvement of glucose tolerance post sucrose load in normal rats and blood glucose lowering on STZ-induced diabetic rats

Test Samples	Dose (mg/kg)	% Activity	
		Normoglycemic rats (0-120min AUC)	STZ induced diabetic rats (0-300 min AUC)
Control		14979±105	130134±2146
Crude Powder	250	13356±280 (10.5)	110844±4527 (14.8*)
95% Ethanolic extract	250	11082±422 (26.2**)	106581±2037 (18.0**)
50% Ethanolic extract	250	14454±401 (3.50)	113091±1931 (12.8)
Aqueous extract	250	11000±239 (26.5**)	109866±2742 (15.5*)
Metformin	100	10887±427 (27.3**)	99684±2244 (23.3**)

Values are mean ±S.E. of three independent experiments.

Effect of fractions of 95% ethanolic, 50% ethanolic and aqueous extracts of crude powder of *J.gossypifolia* stem on the improvement of glucose tolerance post sucrose load in normal rats and blood glucose lowering on STZ-induced diabetic rats

All the extracts were fractionated and were subjected to antihyperglycemic studies at dose level of 100mg/kg on oral glucose tolerance post sucrose load in normoglycemic rats (Table2). Based on the result, those fractions which found active in normoglycemic rat model i.e. chloroform fraction of 95% ethanolic extract, chloroform and butanol fractions of aqueous extract and butanol and aqueous fractions of 50% ethanolic extract showed 19.6 % (p<0.01), 13.4% (p<0.05), 15.9% (p<0.05),18.5% (p<0.01) and 10.0 % (p<0.05) improvement on glucose tolerance post sucrose load in normal rats were further confirmed for their antihyperglycemic potential in STZ induced diabetic rats.

Table 2 also shows the antihyperglycemic activity profile of different fractions of plant *J. gossypifolia* at 100 mg/kg of dose on STZ induced diabetic rats as per the standardized procedure. Out of which chloroform fraction of 95% ethanolic extract, butanol fractions of aqueous extract and aqueous fraction of 50% ethanol extract showed significant antihyperglycemic potential. An average antihyperglycemic effect were calculated to be around 21.5 % (p<0.01), 20.4 % (p<0.01) and 23.6 % (p<0.01) during 300min respectively on STZ induced diabetic rats.

Table 2: Effect of fractions of 95% ethanolic, 50% ethanolic and aqueous extracts of crude powder of *J.gossypifolia* stem on the improvement of glucose tolerance post sucrose load in normal rats and blood glucose lowering on STZ-induced diabetic rats

S. No.	Extract	Fractions	% Activity	
			Normoglycaemic rats (0-120min AUC)	STZ induced diabetic rats (0-300 min AUC)
1	95% Ethanolic	Control	14844±547	134360 ±1120
		Hexane	13386±207 (10.9)	116880 ±1379 (13.0)
		Chloroform	11942±247 (19.6 **)	105466 ±2819 (21.5**)
		Butanol	13410±318 (9.71)	ND
		Aqueous	13208±174 (7.45)	ND
2	Aqueous	Control	14844±547	
		Hexane	14586±206 (1.65)	ND
		Chloroform	12848±221 (13.4*)	113900±2818 (15.2)
		Butanol	12472±247 (15.9*)	106880±2352 (20.4**)
		Aqueous	14318±239 (3.62)	ND
3	50% Ethanolic	Control	14898±518	
		Hexane	14054±213 (5.56)	ND
		Chloroform	13296±286 (9.71)	ND
		Butanol	12140±216 (18.5 **)	115120 ±2325 (14.3)
		Aqueous	13336±107 (10.0 *)	102530 ±3114 (23.6**)

Values are mean ±S.E. of three independent experiments. ND-Not done

Antidiabetic, hypolipidemic and protective effects on renal and hepatic function markers of STZ-induced diabetic rats

Table-3 represents the effect of long term treatment with chloroform fraction of 95% ethanolic extract and butanol fractions of aqueous extract of *J.gossypifolia* stem and standard drug metformin on fasting blood glucose level, improvement on OGTT, serum insulin and lipid levels of STZ-induced diabetic rats.

Effect on FBG and OGTT of STZ treated diabetic rats

The FBG got decreased by 24.9, 30.1 % on day7 and 30.3, 17.2 % on day 14 post treatment of chloroform fraction of 95% ethanolic extract and butanol fraction of aqueous extract *of J. gossypifolia* stem respectively whereas standard drug metformin decreased FBG by 20.0% after day 7 and 43.9% after day 14whereas when the area under the curve was compared between groups, the chloroform fraction of 95% ethanolic extract showed a significant fall of 27.0% ($p< 0.01$) and 36.6% ($p<0.01$) and metformin showed 32.1% ($p<0.01$) and 42.8% ($p<0.001$) improvement in glucose tolerance on 7th and 14th day respectively as compared to that in the control group. Whereas butanol fraction of aqueous extract caused mild decline in blood glucose levels (table-3).

Effect on serum- insulin level of STZ treated diabetic rats

The serum insulin levels in the chloroform fraction of 95% ethanolic extracts of *J.gossypifolia* stem treated groups were also increased.

Table 3: Effect of chloroform fraction of 95% ethanolic extracts and butanol fraction of aqueous extract of *J.gossypifolia* stem and standard drug metformin on fasting blood glucose, improvement in OGTT, serum insulin level and Lipid profile of STZ induced diabetic rats

Groups	Fasting blood glucose (mg/dl)			Oral Glucose tolerance (0-120 min AUC)			Insulin (µg/l)		TG (mg/dl)			TC (mg/dl)			LDL (mg/dl)			HDL (mg/dl)		
	Day 0	Day 7	Day 14	Day 0	Day 7	Day 14	Day 0	Day 15	Day 0	Day 8	Day 15	Day 0	Day 8	Day 15	Day 0	Day 8	Day 15	Day 0	Day 8	Day 15
Sham treated Control (1.0 % gum acacia)	281± 20.3	269± 22.2	214.2 ± 15.7	44179 ± 2008	50910 ± 2623	57903 ± 2052	78.0± 2.99	86.0± 1.10	172.6 ±1.26	177.0 ±1.22	183.8± 1.64	88.1 ± 0.77	92.2± 3.18	95.7± 1.98	68.8± 0.94	70.6± 2.54	73.8± 0.99	29.2± 0.71	27.8± 2.70	25.8± 1.20
95% Eth-chloroform Fraction Treated (100 mg/kg)	272± 12.6	202± 31.4 ** (24.9)	149± 10.9 *** (30.3)	48699 ± 2344	**37125** ±1785 ** (27.0)	36669 ± 1314* * (36.6)	80.0± 9.80	112.0± 3.40 (+30.2)	171.2 ±0.89	162.4 ±2.99 (8.24)	154.6± 1.22* (15.8)	82.7 ± 0.92	80.6± 2.23* (12.5)	77.7± 1.43* (18.8)	65.1± 0.85	59.1± 4.78* (16.2)	57.6± 1.54* * (21.9)	27.1± 0.54	32.4± 3.17* (+16.5)	36.4± 1.34** (+41.0)
Aqs- Butanol Fraction Treated (100 mg/kg)	277± 12.0	188.8 ± 9.7** (30.1)	177.8 ± 22.0 * (17.2)	45379 ± 1709	44298 ±2537 (12.9)	47211 ± 1792* * (18.4)	86.5± 23.8	92.0± 4.91* (+6.97)	173.1 ±1.37	171.2 ±0.56 (3.27)	170.7± 1.43 (7.12)	85.6 ± 1.84	83.8± 1.49 (4.88)	78.8± 1.39* (17.6)	60.1± 1.54	65.3± 3.99 (7.50)	69.9± 1.34 (5.28)	25.7± 0.92	28.32± 1.99 (+1.79)	28.2± 0.58 (+9.30)
Metformin Treated (100 mg/kg)	264± 11.9	215 ± 24.5 ** (20.0)	120.2 ± 4.0 ** (43.9)	47759 ± 1667	34527 ± 1527* ** (32.1)	33102 ± 1555* ** (42.8)	83.8± 22.9	129.0± 15.2* (+50.0)	169.4 ±0.71	156.9 ±1.96 (11.3)	149.9± 3.59** (18.6)	88.6 ± 0.83	78.8± 2.23* (14.5)	70.2± 1.76 ** (26.6)	66.1± 0.66	56.0± 1.56 ** (20.6)	41.5± 0.40 *** (43.7)	28.2± 0.94	34.0± 3.71** (+22.3)	37.0± 0.61** (+43.4)

Changes in lipid level of STZ treated diabetic rats

It was intended to assess the effect of long term treatment on associated abnormal lipid profile in STZ induced diabetic rats. The effect of repeated oral administration of chloroform fraction of 95% ethanolic extract and butanol fraction of aqueous extract of *J. gossypifolia* stem on abnormal lipid profile in STZ rats were estimated before and after 8 and 15 days of treatment is shown in Table 3. The elevated levels of TG, TC, and LDL cholesterol were brought down significantly after 8 and 15 days treatment period. A fall of 8.24 %, 11.3 % on day 8 and 15.8% and 18.6% on day 15 in TG level whereas 12.5 %, 14.5% on day 8 and 18.8% and 26.6% on day 15 in TC level of chloroform fraction of 95% ethanolic extract of *J. gossypifolia* stem and standard drug metformin treated group respectively. LDL cholesterol to be calculated around 16.2%, 20.6% on day 8 and 21.9% and 43.7% were observed on day 15 of chloroform fraction of 95% ethanolic extract of *J. gossypifolia* stem and standard drug metformin treated diabetic rats respectively. There was also increase of 16.5%, 22.3% on day8 and 41.0% and 43.4% on day 15 in HDL cholesterol of chloroform fraction of 95% ethanolic extract of *J. gossypifolia* stem and standard drug metformin treated diabetic rats respectively. While no significant reduction in lipid level was observed in butanol fraction of aqueous extracts-treated diabetic rats.

Biochemical parameter level restoration of STZ treated diabetic rats

In order to examine the effect of chloroform fraction of 95% ethanolic extract and butanol fraction of aqueous extract of *J.gossypifolia* stem supplementation on the regulation of biochemical parameters of the diabetic rats, serum ALT, AST and T-bil levels were determined to evaluate the hepatic functions, while uric acid, creatinine and urea concentrations were studied to assess the renal functions (Table 4). Treatment with chloroform of fraction 95% ethanolic extract of *J.gossypifolia* stem and metformin caused marked decreased on Serum urea, uric acid and creatinine levels in STZ-diabetic rats when compared to the control rats whereas efficacy of the effect of chloroform fraction of the 95% ethanolic extract of *J.gossypifolia* stem on hepatic markers caused an improvement in AST, ALT and bilirubin levels in the diabetic rats. Similar effects were observed with metformin, but they were less in magnitude when compared with those of butanol fraction of aqueous extracts.

Table 4: Effect of chloroform fraction of 95% ethanolic extracts and butanol fraction of aqueous extract of *J.gossypifolia* stem and standard drug metformin on hepatic and renal function markers of STZ-induced diabetic rats

Groups	Hepatic function markers									Renal Functions markers								
	Serum T-bilrubin (mg/dl)			Serum –AST (U/l)			Serum-ALT (U/l)			Serum-Urea (mg/dl)			Serum-Uric acid (mg/dl)			Serum-Creatinine (mg/dl)		
	Day 0	Day 8	Day 15	Day 0	Day 8	Day 15	Day 0	Day 8	Day 15	Day 0	Day 8	Day 15	Day 0	Day 8	Day 15	Day 0	Day 8	Day 15
Sham treated Control (1.0 % gum acacia)	0.32 ± 0.01	0.36 ± 0.9	0.37± 0.23	101.9 ±1.22	108.4 ± 2.67	112.4 ± 3.90	77.0± 1.34	83.0± 0.93	87.1 ± 3.90	111.0 ± 2.0	116.4 ± 0.4	121.2 ± 3.5	3.98± 0.85	4.07± 0.80	4.09± 0.60	0.75± 0.40	0.84± 0.50	0.91±0.43
95% Eth-Chloroform fraction treated (100 mg/kg)	0.35± 0.02	0.28± 0.05** (22.2)	0.24± 0.01** (35.1)	98.3 ±3.16	88.4 ± 2.77* (18.4)	74.3 ± 4.60** (33.8)	76.2± 2.90	66.6± 1.99* (19.7)	62.6 ± 3.00** (31.2)	115.9 ± 1.1	99.9± 2.0* (14.1)	91.9± 2.5** (24.1)	3.71± 0.98	3.31± 1.20* (18.6)	2.87± 0.32** (29.8)	0.81± 0.40	0.77± 0.02 (8.33)	0.70± 0.38** (23.0)
Aqs-Butanol Fraction Treated (100 mg/kg)	0.34 ± 0.02	0.33 ± 0.05 (8.33)	0.33± 0.88* (10.8)	109.2 ±1.90	94.8 ± 1.00 (12.5)	93.2 ± 4.00 * (17.0)	72.1± 2.33	73.1± 3.13* (11.9)	77.0± 1.20 * (11.5)	114.1 ± 2.9	102.0 ± 2.7* (12.3)	108.1 ± 3.0 (10.8)	3.82± 0.39	3.79± 0.11 (6.87)	3.82± 0.39 (6.06)	0.78± 0.80	0.72± 0.56* (14.2)	**0.**71± 0.25* (21.9)
Metformin treated (100 mg/kg)	0.33 ± 0.01	0.26 ± 0.10 ** (27.7)	0.21± 0.18** (43.2)	96.8 ±2.00	77.9 ± 3.16 ** (28.1)	60.4 ± 5.12** * (46.2)	70.0± 0.48	62.0± 2.67 ** (25.3)	56.5± 6.09 *** (35.1)	109.1 ± 3.0	94.4 ± 3.5 ** (18.9)	81.1 ± 3.6*** (33.0)	3.69± 0.60	3.26± 0.29 * (19.9)	2.74± 0.62** (33.0)	0.81± 0.34	0.64± 0.08** (23.8)	0.60± 0.08*** (34.0)

Antidiabetic, hypolipidemic and protective effects on renal and hepatic function markers of High fructose high fat diet fed-low dosed STZ-induced diabetic rats (HFD-STZ)

Table 5 represents the effect of repeated oral administration of chloroform fraction of 95% ethanolic extract and butanol fractions of aqueous extract of *J.gossypifolia* stem on fasting blood glucose (FBG), glucose tolerance, serum insulin level and lipid profile of high fructose high fat diet fed low dosed STZ-induced diabetic rats for 15 consecutive days.

Effect on FBG and OGTT of HFD-STZ treated diabetic rats

The chloroform fraction of 95% ethanolic extract and butanol fractions of aqueous extract of *J.gossypifolia* stem significantly decreased pre-prandial blood glucose level on 7^{th,} by 15.4,% and 20.8% and 14th day by 21.9% and 26.7% respectively and chloroform fraction of 95% ethanolic extract also decreased the postprandial blood glucose level by 12.3% on day 7 and 21.0% on day 14 but butanol fractions of aqueous extract of *J.gossypifolia* decreased blood glucose by 13.7 on day 7 but showed very mild decreased i.e. 6.39% on day 14 as compared to that in the high-fructose high-fat fed control group while standard drug Metformin decreased both pre-prandial and postprandial blood glucose level by caused 30.1% improvement after 7 day and 22.7% improvement after 14 day and. 15.0% on day 7 and 22.6 % on day 14 respectively (table 5).

Effect on serum insulin level HFD-STZ treated diabetic rats

Chloroform fraction of 95% ethanolic extract and butanol fractions of aqueous extract of *J.gossypifolia* stem treatment did not caused any marked decrease in the serum insulin level.

Lipid profile changes in HFD- STZ treated diabetic rats

Abnormalities in lipid profile are one of the most common complications in diabetes mellitus. High fructose high fat diet fed -low dosed Streptozotocin-induced diabetic rats showed elevated serum cholesterol, triglycerides and LDL-C levels and lowered HDL-cholesterol level (Table 5). The various parameters of blood lipid profile of severely diabetic rats were estimated before and after 8 and 15 days of treatment. Treatment with chloroform fraction of 95% ethanolic extract of *J. gossypifolia* stem declined their serum triglycerides, cholesterol and LDL levels by around 27.7% , 17.9 % and 16.2% respectively, and increased the level of HDL around 28.5% on day 15th post treatment.

Table 5: Effect of chloroform fraction of 95% ethanolic extracts and butanol fraction of aqueous extract of *J.gossypifolia* stem and Standard drug Metformin on FBG, improvement in OGTT, serum insulin level and lipid profile of high fructose high fat diet fed -low dosed STZ induced diabetic rats

Groups	Fasting blood glucose (mg/dl)			Oral Glucose tolerance (0-120 min AUC)			Insulin (µg/l)			TG (mg/dl)			TC (mg/dl)			LDL (mg/dl)			HDL (mg/dl)		
	Day 0	Day 7	Day 14	Day 0	Day 7	Day 14	Day 0	Day 15	Day 0	Day 8	Day 15	Day 0	Day 8	Day 15	Day 0	Day 8	Day 15	Day 0	Day 8	Day 15
Sham treated Control (1.0 % gum acacia)	222.2 ± 11.2	226.8± 12.8	245.6± 13.9	50668 ±2345	50766± 1470	54603± 1550	269.9 ±28.5	273.9 ±32.9	315 ± 64.0	329 ±76.8	339±7 8.6	145± 19.0	156 ± 18.5	167 ±18.2	72.5± 6.3	73.1± 3.2	74.7 ± 3.4	44.1 ± 5.4	40.1 ± 1.6	38.9 ± 2.7
95% Eth-chloroform Fraction Treated (100 mg/kg)	219.8 ± 30.9	191.8± 26.5* (15.4)	191.6± 17.6** (21.9)	52131 ±1345	44520± 834* (12.3)	43086± 1757** (21.0)	278.0 ±23.9	250.8 ±22.1 (8.43)	311± 22.6	273± 42.2* (17.0)	245 ± 58.0* (27.7)	158 ± 8.0	143 ± 3.7 (8.33)	137 ± 3.8* (17.9)	70.0 ±7.2	65.0± 7.3 (10.9)	62.0 ± 9.0* (16.2)	45.0 ± 5.0	46.7 ± 2.0 (+16.4)	50.0 ± 3.9** (+28.5)
Aqs- Butanol Fraction Treated (100 mg/kg)	199.6 ± 23.1	179.6± 25.1** (20.8)	180.0± 28.5** * (26.7)	51079 ±1885	43800± 1697* (13.7)	51111± 1487 (6.39)	280.8 ±12.0	274.9 ±21.5 (0.36)	312± 37.0	304± 29.6 (7.59)	289 ± 22.2 * (14.7)	166 ± 3.9	152± 5.6 (2.56)	145 ± 3.7 (13.1)	73.0 ±1.9	69.9± 9.9 (4.37)	67.0 ± 3.6 (9.45)	40.0 ± 3.6	42.0 ± 3.9 (+5.00)	44.0 ± 2.0* (+15.7)
Metformin Treated (100 mg/kg)	218.8 ± 15.7	158.4± 19.0** * (30.1)	189.8± 20.5** * (22.7)	49077 ±2340	43125± 1259* (15.0)	42258± 3308** (22.6)	273.2 ±19.1	199.8 ±12.6 (27.0)	320± 62.1	328± 73.3 (0.30)	311 ± 52.1 (8.25)	167± 11.3	161 ±13.2 (3.20)	159 ±14.2 (4.79)	72.4 ± 2.1	72.9± 4.0 (0.27)	74.3 ± 2.2 (0.53)	44.1 ± 0.19	43.2 ±0.17 (+7.73)	43.5 ± 0.13 (+11.8)

Table 6: Effect of chloroform fraction of 95% ethanolic extracts and butanol fraction of aqueous extract of *J.gossypifolia* stem and standard drug metformin on hepatic and renal functions level of high fructose high fat diet fed-low dosed STZ induced diabetic rats

Groups	Hepatic function Markers									Renal Functions Markers								
	Serum T-bilrubin (mg/dl)			Serum-AST (U/L)			Serum-ALT (U/L)			Serum-Urea (mg/dl)			Serum-Uric acid (mg/dl)			Serum-Creatinine (mg/dl)		
	Day 0	Day 8	Day 15	Day 0	Day 8	Day 15	Day 0	Day 8	Day 15	Day 0	Day 8	Day 15	Day 0	Day 8	Day 15	Day 0	Day 8	Day 15
Sham treated Control (1.0 % gum acacia)	2.45± 12.6	2.56± 1.26	2.84 ± 0.08	170.1± 1.22	178.0 ±1.55	186.3 ± 1.69	76.8 ± 2.50	79.9± 0.23	82.0± 0.90	78.6 ± 1.32	81.6 ± 6.80	84.9± 2.03	4.70± 11.2	4.81± 1.22	4.85 ±0.11	3.30 ± 1.67	3.60 ± 3.78	3.78 ± 0.29
95% Eth-Chloroform Fraction treated (100 mg/kg)	2.31± 13.6	1.89± 1.23** (26.1)	1.56 ± 0.04*** (45.0)	179.0± 2.77	160.9 ±2.00 (9.60)	156.2 ± 0.34* (16.1)	78.0± 1.21	66.3± 4.00* (17.0)	58.2 ± 3.00* * (29.0)	78.0 ± 0.45	70.0 ± 0.22* (14.2)	62.6± 0.29** (26.2)	4.88± 2.90	4.75± 0.07 (1.24)	4.62± 1.02 (4.74)	3.40 ± 2.45	3.11 ± 3.77* (13.6)	2.90 ± 0.16** (23.2)
Aqs-Butanol Fraction Treated (100 mg/kg)	2.47± 12.8	2.17± 0.28 * (15.2)	1.86 ± 0.03*** (34.5)	174.0± 1.00	175.0 ±1.09 (1.68)	178.1±2 .99 (4.40)	75.0± 2.23	72.6± 0.67 (9.13)	68.6 ± 0.56* (16.3)	72.9 ± 2.31	70.5 ± 5.43* (13.6)	70.0± 1.88** (17.5)	4.94± 1.19	4.86± 1.03 (1.03)	4.82 ±0.30 (0.61)	3.42 ± 2.90	3.40 ± 1.55 (5.55)	3.32 ± 0.20* (12.1)
Metformin treated (100 mg/kg)	2.37± 2.33	1.80 ± 0.03** (29.6)	1.45± 0.28*** (48.9)	176.6± 0.04	161.7 ±2.21 (9.15)	158.1±2 .22* (15.1)	69.0± 0.06	61.0± 0.18** (23.6)	58.1 ± 1.00* * (29.1)	74.5 ± 0.19	68.9 ± 2.70** (15.5)	60.0± 1.22** (29.3)	4.82± 0.09	4.72± 1.11 (1.87)	4.69 ±0.18 (3.29)	3.34 ± 4.0	3.00 ± 4.99* (16.6)	2.89 ± 3.15** (23.5)

Biochemical parameter level restoration of HFD-STZ treated diabetic rats

Table 6 presents the effect of chloroform fraction of 95% ethanolic extract and butanol fraction of aqueous extract of *J.gossypifolia* stem on renal and hepatic functions levels in serum of high fructose high fat diet fed–low dosed STZ induced diabetic rats. Kidney damage during diabetes was evaluated by the following markers in serum: serum creatinine, urea and uric acid were measured to demonstrate renal function in serum of the HFD-STZ group. Treatment with chloroform fraction of 95% ethanolic extract of *J.gossypifolia* stem and Metformin caused marked decreased on Serum urea, uric acid and creatinine levels whereas diabetic rats also showed elevated activities of hepatic (T-BIL, AST and ALT) functional markers. In the 15 days of treatment. Treatment with chloroform fraction of 95% ethanolic extract of *J. gossypifolia* stem declined their serum triglycerides, cholesterol and LDL levels by around 27.7% , 17.9 % and 16.2% respectively, and increased the level of HDL around 28.5% on day 15th post treatment.fraction of 95% ethanolic extract treated group. Similar effects were observed with metformin. But there were no significant changed in renal and hepatic markers in butanol fraction of aqueous extract treated group.

Effect on improvement in glucose tolerance on neonatal-Streptozotocin induced diabetic rats

Effect on FBG and OGTT of n-STZ treated rats

n- STZ-induced diabetic rats showed abnormal glucose tolerance after 8 weeks. Table 7 presents prolonged treatment of chloroform fraction of 95% ethanolic extract of *J.gossypifolia* stem and standard drug Metformin (100mg/kg/d) in neonatally-STZ induced diabetic rats on 7th and 14th days post treatment. Chloroform fraction of 95%ethanolic extracts did not cause any significant effect on FBG of day 7 but significant antihyperglycemic activity was found after 14 days of treatment in diabetic rats. It produced 18.3% ($P < 0.05$) fall in FBG levels compared to the initial FBG levels prior to the treatment. Metformin produced 29.9% decrease on day 14 on FBG levels in diabetic rats. The chloroform fraction treated group showed improvement on OGTT i.e., 21.7% ($p<0.01$) on day 7 and around 33.9% ($p <0.001$) on day 14 whereas metformin showed improvement on OGTT to the tune of around 27.5% ($p<0.001$) on day 7 and 29.5% ($p <0.001$) on day 14th day, respectively, as compared to that of the sham treated control group.

Table 7: Effect of chloroform fraction of 95% ethanolic extracts of *J.gossypifolia* stem and standard drug metformin on fasting blood glucose and improvement in OGTT on n- STZ induced diabetic rats

Group	Fasting blood glucose			Oral Glucose tolerance (0-120 min AUC)		
	Day 0	Day 7	Day 14	Day 0	Day 7	Day 14
Sham treated Control (1.0 % gum acacia)	245.7±19.9	241.2±5.7	227.2±17.2	49891.3±2123	51158±2210	54383±1487
95% Eth-Chloroform fraction treated (100 mg/kg)	230.2±246	247.2±19.0 (2.48)	185.5±28.1* (18.3)	45709±2358	40035±2631 ** (21.7)	35906±2806*** (33.9)
Metformin Treated (100 mg/kg)	247.2±10.1	230.2±24.0 (4.56)	159.2±28.1 ** (29.9)	43845.3±2154	37043±1511*** (27.5)	38340±2526*** (29.5)

Table 8: Effect of chloroform fraction of 95% ethanolic extracts and butanol fraction of aqueous extract of *J.gossypifolia* stem and Standard drugs on some key regulatory enzymes of glucose homeostasis

Group	% Inhibition		
	α Glucosidase	Glucose-6-phosphatase	Glycogen phosphorylase
Eth-Chloroform fraction treated (100 µg/ml)	80.6***	32.5**	19.5*
Aqs-Butanol fraction treated (100 µg/ml)	53.4***	12.1	13.8
Acarbose(25 µg/ml)	68.0***	-	-
Sodium-ortho-vanadate treated (25 µg/ml)	-	49.0**	-

Effect on some key regulatory enzymes of Carbohydrate metabolism

Table-8 shows the percent inhibitory activity of chloroform fraction of 95% ethanolic extract and butanol fraction of aqueous extract of *J.gossypifolia* stem and standard drug Acarbose on α-glucosidase enzyme, result showed the inhibitory effect of Chloroform fraction of 95% ethanolic extract and butanol fraction of aqueous extract of *J.gossypifolia* stem around 80.6% and 53.4% respectively at the concentration of 100 µg/ml compared to the acarbose which showed around 68% inhibition on alpha-glucosidase at the concentration of 25µg/ml. Table 8 also shows the inhibitory effect of Chloroform fraction of 95% ethanolic extract and Butanol fraction of aqueous extract of *J. gossypifolia* stem on Glucose-6-phosphatase and Glycogen phosphorylase. Chloroform fraction of 95% ethanolic extract of *J.gossypifolia* stem inhibited glucose 6-phosphatase enzyme significantly to the extent of 32.5% and 12.1% respectively while the standard drug sodium ortho-vanadate inhibited to the extent of 49% and % inhibition for enzyme Glycogen phosphorylase for Chloroform fraction of 95% ethanolic extract and Butanol fraction of aqueous extract of *J.gossypifolia* stem calculated to be around 19.5% and 13.8% respectively.

DISCUSSION

The pathogenesis of diabetes mellitus and the possibility of its management by existing therapeutic agents without any side effects have stimulated great interest in recent years. The present study was undertaken to investigate the anti-diabetic and hypolipidemic effects of stem of *J.gossypifolia* in diabetic rat's model with the aim to find out the active fractions have an insulin-like rapid onset of action to the moderate to severe diabetic state.

Antihyperglycemic activity assessed in sucrose-loaded normoglycemic rat model which is usually due to insulin secretogogues, insulin sensitizing, insulin mimetic activities, etc. In the present study we have investigated the efficacy of the plant extracts and fractions of *J. gossypifolia* stem on normoglycemic rats. 95% Ethanolic extract and its chloroform fraction and aqueous extract and its butanol fraction of *J.gossypifolia* stem were found effective with significant lowering in blood glucose level as evidenced from the fact the extracts/fractions probably have insulin secretaceous activity as its compares with that of Metformin, which is insulin secretaceous[27].

STZ induced hyperglycaemic has been described as a useful experimental model to study the activity of hypoglycemic agents. It is the confirmatory animal model for the screening of antidiabetic compounds for type 1 diabetes mellitus. STZ selectively destroys the pancreatic insulin secreting β-cells, leaving less active cells and results in Type I Diabetes mellitus and produces hyperglycemia [28, 29]. Single dose treatment of 95% ethanolic extract and chloroform fraction of 95% ethanolic extract and aqueous extract and its butanol fraction of *J.gossypifolia* stem were tested for antihyperglycemic activity and found effective with significant

lowering in blood glucose level to STZ induced diabetic rats and significantly improve the glucose tolerance more than that of standard drug Metformin showed. However, it was expected that the hypoglycaemia principle in 95% ethanol extracts of *J.gossypifolia* stem can act indirectly by stimulating the release of insulin into the bloodstream.

As STZ destructs the beta cells resulting in insufficient insulin secretion causing diabetes[30]. It is widely accepted animal model and reported to resemble human hyperglycemic non ketotic diabetes mellitus [31] and this was evident from marked depletion of serum insulin levels in STZ treated group which ultimately led to hyperglycemia condition. It could be envisaged that the chloroform fraction of 95% ethanolic extract of *J. gossypifolia* was found to improve glucose tolerance significantly and markedly elevates the serum insulin level of diabetic rats and may contain some biomolecule that may sensitize the insulin receptor to insulin or stimulates the β-cells of islets of langerhans to release insulin which may finally lead to improvement of carbohydrate metabolizing enzymes towards the re-establishment of normal blood glucose level.

The disturbances in metabolisms together with oxidative stress are likely to affect hepatic and renal functions as serum enzymes such as AST and ALT were used in the evaluation of hepatic damage have been related with increased gluconeogenesis and ketogenesis [32] and elevated levels of serum urea and creatinine were observed in diabetic rats, which are considered as significant markers of renal dysfunction[33]. Oral administration of chloroform fraction of 95% ethanolic extract in diabetic rats resulted in reduction in the activities of these enzymes in serum compared to the diabetic untreated group. It indicates the prevention of renal and hepatic damage in diabetic rats. Chloroform fraction of 95% ethanolic extract of *J. gossypifolia* also significantly reduced the TC, TG, LDL-C levels with an increase of HDL-C in treated diabetic rats compared to untreated diabetic rats and this may be due to the insulinotropic effect or insulin secretagogue activity of this fraction.

In High fructose-high fat diet fed- low dose STZ model can be used to develop obese diabetic rats that mimic human diabetes in terms of obesity and impaired insulin sensitivity which plays a pivotal role in the development of diabetes. They are fed with a High fructose high-fat diet for 6 weeks and then injected with STZ (45 mg/kg).Before STZ injection; rats fed with high fructose fat diet, have increasing prevalence of obesity, diabetes mellitus, and non-alcoholic fatty liver disease characterized by an impaired glucose tolerance test. Fructose-induced insulin resistant states are commonly characterized by a profound metabolic dyslipidemia, which appears to result from hepatic and intestinal overproduction of atherogenic lipoprotein particles. A high flux of fructose to the liver, perturbs glucose metabolism and glucose uptake pathways, and leads to a significantly enhanced rate of lipogenesis and triglyceride synthesis, resulting in high flux of glycerol and acyl portions of triglyceride molecules from fructose catabolism, creating the known dyslipedemic profile and cause insulin resistance[34]. STZ injection elevates glucose, insulin, lipid concentrations. Fructose fed fat-fed-STZ rats are not insulin deficient compared to normal diet-fed rats but display hyperglycemia associated with reduced insulin stimulated adipocyte glucose clearance. These rats exhibit hyperinsulinemia, hyperglycemia and obesity [35].

Insulin resistance and compensatory hyperinsulinaemia have been shown to predict the development of type 2 diabetes. Hence, measurements of biochemical parameters are necessary to prevent cardiac complications in diabetes condition. In this study, chloroform fraction of 95% ethanolic extract of *J. gossypifolia* stem showed significant reduction in TC, TG, LDL levels and increased level of HDL in type 2 diabetic model rats. Our study was also focused to know the protective activity of chloroform fraction of 95% ethanolic extract and butanol fraction of aqueous extract of *J. gossypifolia* stem against hepatic and renal damage caused by diabetes. In diabetic rats, administration of chloroform fraction of 95% ethanolic extract at the doses of 100mg/kg and Metformin significantly ($p<0.01$) brought back the activities of these renal and hepatic markers to near normal levels.

The neonatal-STZ treated rats are suitable model of type II diabetes as it exhibiting the various stages of Type 2 diabetes mellitus such as impaired glucose tolerance and hyperglycemia. The n-STZ rat exhibit slightly lowered serum insulin, slightly elevated plasma glucose levels and lowered pancreatic insulin content [36]. The b cells in the n-STZ rats bear a resemblance to the insulin secretory characteristics found in Type 2 diabetic patients. After 14 days of treatment with chloroform fraction of 95% ethanolic extract of *J. gossypifolia* stem improved glycemic control. These data indicate that one of the putative mechanisms of the antidiabetic action of chloroform fraction of 95% ethanolic extracts is the induction of glucose-inducible insulin secretion in diabetic rats.

We have confirmed the enzymatic activity of chloroform fraction of 95% ethanolic extract and butanol fraction of aqueous extract of *J.gossypifolia* stem affects the activities of key enzymes in the liver of STZ induced diabetic rats. These enzymes include the gluconeogenic enzyme glucose-6-phosphatase, glycogenic enzyme glycogen phosphorylases and carbohydrate digesting enzymes α -glucosidase which played a very important role in homeostasis regulation of blood glucose levels. Whereas the gluconeogenic enzyme glucose- 6-phosphatase is a crucial enzyme of glucose homeostasis because it catalyses the ultimate biochemical reaction of both glycogenolysis and Gluconeogenesis [37]. These seem to be the consequence of the high glucose- 6- phosphatase activities and this increased activity may be due to insulin insufficiency in a diabetic state [38,39]. In Chloroform fraction of 95% ethanolic extract and butanol fraction of aqueous extract of *J.gossypifolia* stem and sodium ortho vanadate treated rats, the activity of glucose-6-phosphatase were found to be decreased and it may be due inhibition of gluconeogenesis [40] While inhibition of hepatic glycogen phosphorylase is a promising treatment for attenuating hyperglycemia in Type 2 diabetes. The oral administration of Chloroform fraction of 95% ethanolic extract and butanol fraction of aqueous extract of *J.gossypifolia* stem was significantly decreasing enzymes activities in STZ-induced diabetic rats by inhibiting glycogenolysis , that improve glycemic control, based on patients with hepatic glycogen storage diseases, where episodic hypoglycemia is observed [41,42].

The inhibition of α-glucosidase enzymes involved in the digestion of carbohydrates. Dietary polysaccharides are hydrolysed by alpha glucosidase enzymes to produce glucose and other monosaccharide and are absorbed through the small intestine into the hepatic portal vein. This results in elevation of the postprandial blood glucose level; it can be an important target in the management of blood glucose level. The inhibitors of α-glucosidase, in consequence, could retard the use of carbohydrates to suppress the postprandial hyperglycemia. α-glucosidase inhibitors like acarbose is known to reduce postprandial hyperglycemia by interfering with the digestion of dietary carbohydrates [43, 44]

CONCLUSION

In conclusion, the present study demonstrated that chloroform fraction of 95% ethanolic extract of *J. gossypifolia* stem, is a valuable candidate for the search of antidiabetic ingredients from natural sources and as a possible candidate in the treatment of type 2 diabetes associated with hyperlipidemia.

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Conflict of interest statement

We declare that we have no conflict of interest.

REFERENCES

1. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care 2008; 30: 42-47.
2. Reaven GM. Banting Lecture: Role of insulin - resistance in human diseases. Diabetes 1988; 37: 1597-1607.
3. Settin D Jr, Boxer GE. Studies in carbohydrate metabolism III. Metabolic defects in alloxan diabetes. Journal of Biological Chemistry 1944; 156:217.
4. Brandy RO, Gurin S. Biosynthesis of labeled fatty acids and cholesterol in experimental diabetes. Journal of Biological Chemistry 1950; 187:589-596.
5. Randle PJ, Garland PB, Hales CN, Newsholme EA. The glucose fatty acid cycle: its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. Lancet 1963; 1(7285):785-789.
6. U.K Prospective Diabetes Study Group: Overview of six years therapy of type 2 diabetes: a progressive disease: diabetes, 1995; 44: 1249-1258.
7. Moller DE: New drug targets for type 2 diabetes and the metabolic syndrome. Nature 2001; 414(6865): 821-827.
8. Kirtikar KR, Basu BD. Indian Medicinal Plants, III, International Book Distributors, Allahabad. 1996; 2247.
9. Banerji J, Das B, Bose P, Chakabarti R and Chaterjee A.Traditional Medicine. Oxford and IBH Publishing Co. Pvt, Ltd, New Delhi. 1993; 352.
10. Oduola T, Popoola GB, Avwioro OG, Oduola TA, Ademosun AA and Lawal MO. Use of Jatropha gosssypifolia stem latex as a haemostatic agent: how safe is it? Journal of Medicinal Plant Research 2007; 1(1): 14-17.
11. Ravindranath N, Venkataiah B, Ramesh C, Jayaprakash P, Das B. Jatrophenone, a Novel Macrocyclic Bioactive Diterpene From Jatropha gossypifolia. Chemical and Pharmaceutical Bulletin 2003; 51(7): 870-871.
12. Kupchan SM, Sigel CW, Matz MJ, Renauld JAS , Haltiwanger RC, Bryan RF. Jatrophone, a Novel Macrocyclic Diterpenoid Tumor Inhibitor from Jatropha gossypifolia. Journal of the American Chemical Society 1970; 92(14): 4476-4477.
13. Nyunai N, Njikam N, Mounier C, Pastoureau P. Blood glucose lowering effect of aqueous leaf extract of Ageratum conyzoides in rats. African Journal of Traditional,complementary and alternative medicine 2006;3(3):76-79.
14. Tiwari P, Rahuja N, Kumar R, Lakshmi V, Srivastava MN, Agarwal SC et al.. Search for antihyperglycemic activity in few marine flora and fauna. Indian Journal of Science and Technology 2008; 1(5): 1- 5.
15. Varshney K, Gupta S, Rahuja N, Rawat AK, Singh N, Tamrakar AK, et al, Synthesis, Structure–Activity Relationship and Docking Studies of Substituted Aryl Thiazolyl Phenylsulfonamides as Potential Protein Tyrosine Phosphatase 1B Inhibitor. ChemMedChem 2012; 7:1185-1190.
16. Srivastava SP, Mishra A, Bhatia V, Narender T, Srivastava AK. Acacia catechu hard wood: potential anti-diabetic cum anti-dyslipidemic. Medicinal Chemistry Research 2011; 20(9):1732–1739.
17. Choi JH, Hwang IK, Yi SS, Yoo KS, Lee CH, Shin HC, et al. Effects of streptozotocin-induced type 1 diabetes on cell proliferation and neuronal differentiation in the dentate gyrus; Correlation with memory impairment. The Korean Journal of Anatomy 2009; 42(1):41-48.
18. Tanja YR. Diet-induced models for obesity and type 2 diabetes. Drug Discovery Today: Disease Models 2007;4(1): 3–8.
19. Mlinar B, Marc J, Janez A, Pfeifer M. Molecular mechanism of insulin resistance and associated diseases. Clinica Chimica Acta 2007; 375:20–35.
20. Chakravarthy BK, Gupta S, Gambhir SS, Gode KD. Pancreatic beta cell regeneration- a novel antidiabetic mechanism of Pterocarpus marsupium Roxb. Indian Journal of Pharmacology 1980;12:123-127.
21. Andrade-Cetto A, Revilla-Monsalve C., Wiedenfeld H. Hypoglycemic effect of Tournefortia hirsutissima L., on n-streptozotocin diabetic rats. Journal of Ethnopharmacology 2007; 11:96-100.
22. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. Journal of Biological Chemistry 1951; 193:265-275.
23. Hubscher G, West GR. Specific assays of some phosphatase in subcellular fractions of small intestinal mucosa. Nature 1965; 205:799-800.
24. Taussky HH, Shorr E. A microcolorimetric method for the determination of inorganic phosphorus. Journal of Biological Chemistry 1953; 202:675-685.
25. Barthet J, Rall TW, Sutherland EW. The relationship of epinephrine and glucagon to liver phosphorylase IV. Effect of epinephrine and glucagon on the reactivity of phosphorylase in liver homogenates. Journal of Biological Chemistry 1957; 224:463-475
26. Cogoli, A.; Mosimann, H.; Vock, C.; Balthazar, A. K. V.; Semenza, G. Eur. Journal of Biochemistry 1972, 30,7.
27. Lamela M, Cadavid I, Gato A, Calleja JM. Effects of Lythrum salicaria in normoglycemic rats. Journal of Ethanopharmacology 1985; 14:83–91
28. Szkudelski T. The mechanism of Alloxan and streptozotocin Action in β cells of the rats pancreas. Physiological Research 2001; 50:537 – 546.
29. Skyler JS .Prediction and prevention of type I diabetes: progress, problem and prospects. Clinical Pharmacology and Therapeutics 2007; 81(5):768-771.
30. Gomes A, Vedasiromoni JR, Das M, Sharma RM, Ganguly DK. Antihyperglycemic effect of black tea (Camellia sinensis) in rat. Journal of Ethnopharmacology 1995;45(3):223-226.
31. Weir GC, Clore ET, Zmachinski CJ, Bonner-Weir S. Islet secretion in a new experiment model for non-insulin dependent diabetes. Diabetes 1981; 30:590–595.
32. Felig P, Marliss E, Ohman J, Cahill JF. Plasma amino acid levels in diabetic keto acidosis. Diabetes 1970; 19:727– 728.
33. Busserolles J, Gueux E, Rock E, Mazur A, Rayssiguier Y. Substituting honey for refined carbohydrates protects rats from hypertriglyceridemic and prooxidative effects of fructose. Journal of Nutrition 2002; 132:3379-3382.
34. Atlas of End Stage Renal Disease in the United States, National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases: Annual data report. Bethesda; 2011.
35. Kazumi T, Odaka H, Hozumi T, Ishida Y, Amano N, Yoshino G. Effects of dietary fructose or glucose on triglyceride production and lipogenic enzyme activities in the liver of Wistar fatty rats, an animal model of NIDDM. Endocrine Journal 1997; 44: 239-245.
36. Arulmozhi DK, Veeranjaneyulu A, Bodhankar SL. Neonatal streptozotocin-induced rat model of Type 2 diabetes mellitus: a glance. Indian Journal of Pharmacology 2004; 36(4): 217–221.
37. Mithieux G, Vidal G, Zitoun C, Daniele N, Minassian C. Glucose- 6- phosphatase mRNA and activity are increased to the same extent in liver and kidney of diabetic rats. Diabetes 1996; 45: 891- 896.
38. Guignot L, Mithieux G. Mechanisms by which insulin associated or not with glucose, may inhibit hepatic glucose production in the rat. American Journal of Physiology: Endocrinology and Metabolism 1999; 277: 984 – 989.
39. Arathi G, Sachdanandam. Therapeutic effect of Semecarpus and Cardium Linn nut milk extract on carbohydrate metabolizing and mitochondrial TCA cycle and respiratory chain enzymes in mammary carcinoma rats. Journal of Pharmacy and Pharmacology 2003; 55: 1283-1290.
40. Gupta D, Raju J, Baquer NZ. Modulation of some gluconeogenic enzyme activities in diabetic rat liver and kidney: effect of antidiabetic compounds. Indian Journal of Experimental Biology 1999; 37:196-199.
41. Murphy ED and Anderson JW. Tissue Glycolytic and Gluconeogenic Enzyme Activities in Mildly and Moderately

Diabetic Rats: Influence of Tolbutamide Administration. Endocrinology 1974; 94(1), 27-34.

42. Kumari KS, Immanuel G, Dhanya BS, Raj A. Evaluation of the effect of tigogenin on the activities of certain key enzymes of carbohydrate metabolism in Streptozotocin induced diabetic rats. International Journal of Biological & Medical 2012; 3(1): 1242-1247.
43. Bischoff H. Pharmacology of α -Glucosidase inhibition. European Journal of Clinical Investigation 1994;24(3): 3–10,
44. Amin R, Abdul-Ghani AS, Suleiman MS. Effect of Trigonella foenum graecum on intestinal absorption. Proc. of the 47th Annual Meeting of the American Diabetes Association (Indianapolis U.S.A.) Diabetes 1987; 36(1): 211A.