



TANNINS OF *JATROPHA GOSSYPIFOLIA* EXERT ANTI-HYPERLIPIDEMIC EFFECT IN STREPTOZOTOCIN-NICOTINAMIDE INDUCED DIABETIC RATS

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Article Received on 01/12/2017

Article Revised on 25/12/2017

Article Accepted on 15/01/2018

ABSTRACT

Plants have assisted humankind since centuries as they are stockpiles of essential therapeutics and help to treat chronic diseases. The main purpose of the study is to evaluate the anti-diabetic and anti-hyperlipidemic activity of isolated tannins from methanolic extract of *Jatropha gossypifolia*. A total of 30 male wistar rats aged 2 to 3 months and weighing about 180-200 g were divided into six groups. Type II diabetes was induced by single intraperitoneal injection of 110 mg/kg b.w. nicotinamide and 50 mg/kg b.w. streptozotocin. Rats with marked hyperglycemia of fasting blood glucose level ≥ 250 mg/dL were selected for the study. Experimental animals were administered with 25 and 50 mg/kg b.w. of tannins. Bio chemical parameters such as creatinine level, triglyceride (TG), total cholesterol (TC), high density lipoprotein (HDL), low density lipoprotein (LDL) and very low density lipoprotein (VLDL) and liver function parameters such as Glutamate oxaloacetate transaminase (GOT), Glutamate pyruvate transaminase (GPT) were analysed. After sacrifice of animals, the liver was collected and subjected to histopathology analysis. Liver glycogen content was estimated spectrophotometrically. The experimental rats treated with 25 and 50 mg/kg b.w. of tannins showed significant increase in HDL level and liver glycogen content. The tannin treated rats exhibited a decrement in creatinine, TG, TC, VLDL, GOT and GPT levels. The isolated tannins from methanolic extract of *Jatropha gossypifolia* possesses anti-diabetic and anti-hyperlipidemic activity in streptozotocin-nicotinamide (STZ-NIC) induced diabetic rats.

KEYWORDS: *Jatropha gossypifolia*, Tannins, STZ-NIC induction, Biochemical parameters, Glycogen content, Anti-hyperlipidemic activity.

1. INTRODUCTION

Diabetes mellitus is a metabolic disorder which can be described by raised blood glucose level due to the imperfections in insulin production, insulin activity, or both which affects carbohydrate, fat and protein metabolism.^[1] Insulin resistance and delayed insulin release prompt hyperglycemia, hyperlipidemia and to an expansion in hepatic glucose. The constant hyperglycemia and variations from the norm in serum lipids of diabetes is related to damage in different organs, particularly the eyes, kidneys, nerves, heart and blood vessels.^[2, 3]

India keeps on being the 'Diabetic Capital' of the world with 50.8 million diabetics and expanding their pervasiveness with no level.^[4] The pathogenesis of diabetes mellitus and plausibility of its supervision by oral administration of antidiabetic agents, such as folk

medicines have stimulated a greater interest.^[5,6] India has a rich history of utilizing different medicinal herbs for treating diabetes. Numerous Indian plants have been examined for their useful in various sorts of diabetes and detailed reports of its activity has been reported in scientific journals.^[7-9]

The plant *Jatropha gossypifolia* (*J. gossypifolia*) (family: Euphorbiaceae) is a ragged gregarious perennial herb, found throughout India. It has noteworthy anticancer^[10], hepatoprotective^[11] and pesticidal activity.^[6] Plant tannins, which are beneficial towards human health are abundantly present in food and beverages with antioxidant polyphenols as its major constitution.^[12] Tannins are described to develop the glucose uptake through mediators of the insulin-signaling pathways, such as Phosphoinositide 3-Kinase and p38 Mitogen-Activated Protein Kinase activation, GLUT-4

translocation^[13-15] and antihyperglycemic agent in diabetic rats.^[16]

In our previous studies we have focussed on antidiabetic efficacy of *J. gossypifolia* tannins in L6 myotubes. HPLC analysis of tannins isolated from *J. gossypifolia* were carried out in reverse phase. The main constituents are known to be tannin derivatives and (–) epicatechin (unpublished data) and in methanolic extract of *J. gossypifolia* we have revealed anti-hyperglycemic and anti-hyperlipidemic activity in streptozotocin-nicotinamide induced diabetic rat.^[17] The present study focus on the anti-hyperlipidemic effect of isolated tannins from methanolic extract of *Jatropha gossypifolia* (ME) in STZ-NIC induced diabetic rats.

2. MATERIALS AND METHODS

2.1. Chemicals and Reagents

Streptozotocin was purchased from Sigma-Aldrich, St. Louis, USA. Metformin, Nicotinamide were obtained from Himedia, India. Blood glucose level was checked using Accu-Chek® Active Blood Glucose Meter. 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.

2.2. Plant material collection and extraction

Leaves of *J. gossypifolia* were collected from Chennai, Tamilnadu, India. Authentication of the plant material was obtained in plant anatomy research institute (PARC/2015/3142), Chennai, Tamilnadu, India. The collected leaves were shade dried, powdered and packed in the soxhlet apparatus and extracted with hexane, dichloromethane, ethyl acetate and methanol. The extraction was carried out for 72 h at 60°C. The extract was filtered and concentrated in rotary evaporator.^[18] Tannins were isolated from ME using gelatin precipitation method.^[14]

2.3. Determination of tannin content

Tannin content is determined according to the method of Porter *et al.*, 1998.^[19] Briefly, 0.50 ml of plant extract / isolated tannins was diluted with 70% acetone and 0.3 ml of butanol-HCl reagent (95:5 v/v) and 0.1 ml of ferric reagent (2% Ferric ammonium sulphate in 2N HCl) is added and vortexed. The content was kept in a boiling water bath for 97 to 100°C for 60 min and cooled. The absorbance was measured at 550 nm and tannic acid is used as a standard for this experiment.

2.4. Validation of Tannins by Ferric chloride test and Thin Layer Chromatography (TLC)

The tannins in the extracts were qualitatively validated by ferric chloride test. The extracts were incubated with 2.5% ferric chloride solution for 10 min at room temperature for the formation of precipitate.^[20] The extracts were subjected to Thin Layer Chromatography with chloroform: methanol (1:2) as mobile phase and 2.5% Ferric chloride as spray reagent for detection. The

extract were dropped on the silver-coated TLC sheet and kept undisturbed till the extracts reach the top. The sheet was then dried, sprayed and followed by a gentle heating on a hot plate.^[21]

2.5. Isolation of tannins from ME

Tannins were extracted using gelatin precipitation method.^[14] Briefly, ME (100 mg/ml) was prepared and centrifuged at 4000 rpm for 10 min and filtered using 0.2 µ filter. To the filtrate, 40 mg/ml of gelatin was added in the ratio of 1:4 and centrifuged for 10 min at 4000 rpm. The supernatant and pellet (tannins + gelatin) was collected separately. To the collected supernatant 40 mg/ml of gelatin was added in the ratio of 1:4 and centrifuged at 4000 rpm for 10 min. The above step was repeated to ensure the complete removal of tannins from the supernatant. The pellets obtained during the various stages of centrifugation were pooled together using ethanol and the tubes were then centrifuged at 4000 rpm for 10 min. The resultant supernatant was transferred to a fresh tube, air dried and reconstituted with methanol (0.01 %).

2.6. Experimental groups

The *in vivo* study was in accordance to animal institutional ethical committee (Ethical clearance no 090/835/IAEC-2014). 6 to 8 weeks old male wistar rats and weighing 150-200g were used for the study. Totally, 30 animals were acquired from Tamilnadu veterinary and animal science university, Chennai, India and were accustomed in the center for animal house, SRM Institute of Science and Technology, Chennai, India. All animals were acclimatized to laboratory circumstances for one week and permitted free admittance to sterilized water and pellet diet. The rats were housed in Sterilized polypropylene cages with sterile paddy husk as bedding and maintained in 12hr light + 12hr dark cycle at temperature ± 25°C.^[22]

2.7. Development of insulin resistant animal model

In male wistar rats, diabetes was induced by intraperitoneal administration of Nicotinamide 110 mg/kg b.w. and single dose of 50 mg/kg b.w. STZ dissolved in freshly prepared 0.01M citrate buffer, pH 4.5. STZ was injected after 15 minutes from the administration of Nicotinamide. Nicotinamide was freshly prepared in saline. Streptozotocin was prepared freshly in citrate buffer.^[23] After checking the blood glucose levels using Accu-Chek® Active Blood Glucose Meter at 3rd and 7th day of induction, the rats with marked hyperglycemia (FBG ≥250 mg/dl) were selected and used for the study.^[24]

2.8. Animal grouping and drug administration

A total of 30 rats (20 diabetic rats and 10 normal rats) were divided into six groups with five rats per group. All experiments were carried out in overnight fasted rats.

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

Group 2: Control rats treated with tannins of *J. gossypifolia* (50 mg/kg b.w.); *n* = 5

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

Group 4: Diabetic rats treated with low dose of tannins of *J. gossypifolia* (LD)

(25 mg/kg b.w.); $n = 5$

Group 5: Diabetic rats treated with high dose of tannins of *J. gossypifolia* (HD)

(50 mg/kg b.w.); $n = 5$

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

2.9. In vivo biochemical parameters

Blood samples from the control and experimental group rats were collected using retro orbital plexus on 0th, 7th, 14th, 21st, 28th and 35th days. Collected blood samples were allowed for 30 min to clot and centrifuged at 3000 rpm for 15 min. The serum was collected separately and used for biochemical parameters such as creatinine level by Jaffe method.^[25] Total cholesterol (TC), triglyceride (TG), high density lipoprotein (HDL), low density lipoprotein (LDL) and very low density lipoprotein (VLDL) estimated according to enzymatic GPO, trinder, end-point method.^[26] Liver function parameters such as Glutamate oxaloacetate transaminase (GOT), Glutamate pyruvate transaminase (GPT) was done by IFCC method.^[27] All the biochemical parameters were carried out using commercially available test kits from Robonik prietest, India.^[28, 29]

2.10. Histopathological investigation of liver sample

At the end of the study period (35th day), animals were sacrificed and liver was dissected out for histopathological studies. Control and experimental group liver samples were washed with saline and 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.^[30]

2.11. Estimation of glycogen content

Liver samples (per gram) were rinsed with ice-cold buffer (saline) (pH 7.4) and incubated with 30% KOH at 55°C for 30 min with occasional shaking. Sodium sulphate (0.2 ml) was added and glycogen was precipitated by the addition of 5 ml ethanol. The precipitate was removed and dissolved in water (10 ml). To this, 1ml of 1.2 mol/l HCl was added and boiled for 2 h and neutralized with 0.5 mol/l NaOH and OD was taken at 620 nm.^[31, 32]

2.12. Statistical analysis

The results were expressed as mean $\pm$ SD. One way analysis of variance (ANOVA) was used for data analysis, followed by Duncan's multiple range test (DMRT) by using statistical package of social science (SPSS) software version 17.0 for windows. The significance level was considered as $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Tannin estimation and extraction

The dried and powdered leaves of *J. gossypifolia* were extracted using methanol which yielded 9.6% (w/w) respectively. The tannin content in the extracts were determined according to the method of Porter *et al.*, 1998.^[19] The Ferric chloride binds to the polyphenol group present in the extract thereby detects the presence of tannin. Tannin content was found to be significantly higher in ME of *J. gossypifolia* (9.95 mg/ml) as shown in Figure 1. This has been corroborated with higher tannin content that has been extracted using methanol from various other plant extracts.^[33]

The tannins were extracted from ME of *J. gossypifolia* using gelatin precipitation technique. The tannin extract was estimated to contain 4.584 mg/ml of tannin which was less compared to the concentration in the ME (9.95 mg/ml). This loss in tannins may be due to the fact that some of the tannins may be left in the gelatin polymer during the separation process.

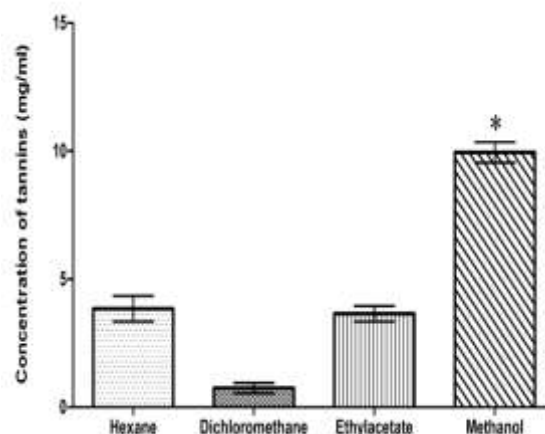


Figure 1: Estimation of tannin content in extracts. Values are expressed as means $\pm$ SD from three independent experiment in triplicates. Statistical evaluation was done by one way ANOVA followed by Duncan's multiple range test (DMRT). * $p < 0.05$ as compared with other groups.

3.2. Tannins validation by ferric chloride test and thin layer chromatography

Ferric chloride is specific for tannin detection as it forms a complex by binding with the phenolic groups in the extracts. The test also detects the type of tannin based on the colour of the precipitate. The presence of tannin is detected by the formation of a coloured precipitate. The type of the tannins could be detected by blue, black and green precipitate formation.^[34] ME of *J. gossypifolia* (Figure 2a) showed the presence of tannin content as green precipitate. The isolated tannins from ME of *J. gossypifolia* by gelatin precipitation method (Figure 2b) showed dark green precipitation which confirmed the presence of tannin content. Phytochemical screening of extracts of the leaves of *Ajuga remota* Benth indicated

the presence of tannin content by ferric chloride test which supports our data.^[35]

Thin layer chromatography of ME and tannin for qualitative analysis of tannin were performed with 10 μ l of 1 mg/ml stock^[21] which showed presence of tannin by appearance of green (Fig. 2c) or blue (Fig. 2d) color in the TLC sheet. Similar presence of tannin in Ferric Chloride reagent was observed in ethanolic extract of polyherbal formulation ADJG.^[36]

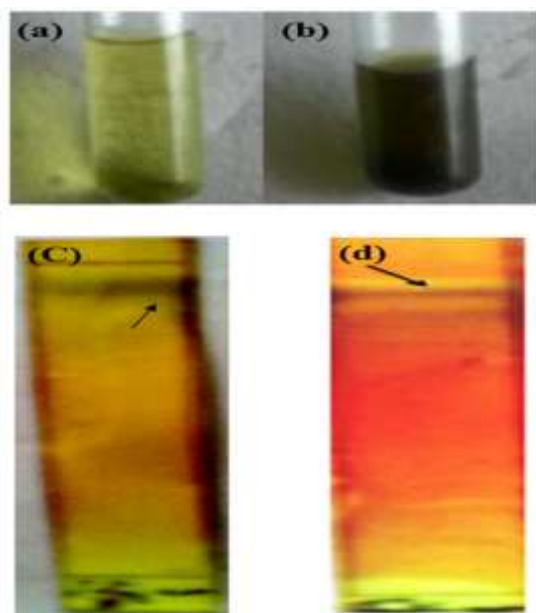


Figure 2: Validation of tannin content by ferric chloride test and thin layer chromatography in ME extract and tannin from three independent experiments.

Table 1: Effect of tannins on serum creatinine in STZ-NIC induced diabetic rats (n=5) after 35 days of treatment.

Groups	Creatinine mg/dL	
	Before treatment	After treatment
Control	1.248 $\pm$ 0.06	1.206 $\pm$ 0.07
Control + Tannin	1.283 $\pm$ 0.07	1.268 $\pm$ 0.07
Diabetic group	2.547 $\pm$ 0.08 [#]	2.773 $\pm$ 0.12 [#]
Diabetic + Tannin (LD)	2.538 $\pm$ 0.37	1.496 $\pm$ 0.08 [*]
Diabetic + Tannin (HD)	2.565 $\pm$ 0.34	1.423 $\pm$ 0.21 [*]
Diabetic + Metformin	2.546 $\pm$ 0.34	1.398 $\pm$ 0.28 [*]

Values are expressed as means $\pm$ SD (n =5) from three independent experiments. Statistical evaluation was done by one way ANOVA followed by Duncan's multiple range test (DMRT). *p < 0.05 as compared with diabetic group, #p < 0.05 as compared with control group.

3.4. Effect of tannins on TG and TC level

Table 2 shows the serum level of TG and TC in normal and experimental group rats. The diabetic untreated groups have a significant elevation in TG (248.11 $\pm$ 3.98 mg/dL) and TC (279.420 $\pm$ 3.99 mg/dL) levels and a significant reduction of TG and TC was observed in diabetic rats treated with 25 and 50 mg/kg b.w. of tannins from 204.92 $\pm$ 3.51 to 110.35 $\pm$ 2.28 mg/dL and 203.58 $\pm$ 3.25 to 90.36 $\pm$ 1.84 mg/dL in TG and 249.908 $\pm$ 3.85 to 184.262 $\pm$ 2.34 mg/dL and 248.764 $\pm$ 3.34 to

3.3. Effect of tannins on creatinine level

Decrease in serum protein and increase in serum creatinine levels are the indication of kidney dysfunction. Increase in creatinine level was observed in diabetic group and experimental group rats before (0th day) and after the treatment period (35th day) as shown in Table 1. There was a significant decrease in the serum creatinine level when orally administrated with 25 and 50 mg/kg b.w. of tannins (1.496 $\pm$ 0.08 mg/dL and 1.423 $\pm$ 0.21 mg/dL). Metformin (500 mg/kg b.w.) was used as a standard antidiabetic drug, which showed 1.398 $\pm$ 0.28 mg/dL creatinine level. Reduction in creatinine level shows the protective nature of tannins on kidney dysfunction. *Terminalia catappa* Linn fruits showed significant decrease in serum creatinine level when administered with petroleum ether (0.70 $\pm$ 0.1 mg/dL), methanolic (0.52 $\pm$ 0.1 mg/dL) and aqueous extract (0.63 $\pm$ 0.1 mg/dL).^[37]

173.704 $\pm$ 2.95 mg/dL in TC. Reduction in TG and TC validates that isolated tannins are forestalling dyslipidemia and lipotoxicity. Some of the synthetic antidiabetic do not prevent dyslipidemia, consequently raising the danger of cardiovascular difficulties. Subsequently, these outcomes can be viewed as in contradiction of diabetic dyslipidemia. These results are also in accordance with previously reports in *Symplocos cochinchinensis* (Lour.) S. Moore hexane (250 and 500 mg/kg) extract.^[38]

Table 2: Effect of tannins on serum TG and TC in STZ-NIC induced diabetic rats (n=5) after 35 days of treatment

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 + Tannin	95.75 ± 1.75	95.48 ± 1.85	154.434 ± 2.24	157.876 ± 2.26
Diabetic group	207.21 ± 3.15 [#]	248.11 ± 3.98 [#]	246.542 ± 3.24 [#]	279.420 ± 3.99 [#]
Diabetic + Tannin (LD)	204.92 ± 3.51	110.35 ± 2.28 [*]	249.908 ± 3.85	184.262 ± 2.34 [*]
Diabetic + Tannin (HD)	203.58 ± 3.25	90.36 ± 1.84 [*]	248.764 ± 3.34	173.704 ± 2.95 [*]
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 Duncan's multiple range test (DMRT). *p < 0.05 as compared with diabetic group, #p < 0.05 as compared with control group.

3.5. Effect of tannins on lipid profile

Diabetes is furthermore connected with hypercholesterolemia because of irregular metabolism of lipids. The serum lipid and lipoprotein profile are additionally raised that pulls diabetes to high danger of coronary disease, in diabetes mellitus. STZ-NIC induced diabetic rats showed increased LDL (209.07 ± 3.48 mg/dL), VLDL (49.62 ± 1.89 mg/dL) levels and decreased HDL (21.72 ± 1.07 mg/dL) level (Table 3). Oral administration of tannins (25 and 50 mg/kg b.w.) for 35 days significantly reduced LDL, VLDL and increased HDL levels as compared with the diabetic

group rats. The level of lipid profile of experimental rats at 35th d for HDL was 52.46 ± 1.02 and 56.16 ± 1.09 (mg/dL) and 107.60 ± 2.18 and 96.26 ± 1.37 (mg/dL) were observed in LDL and followed by VLDL at 20.48 ± 1.24 and 17.06 ± 1.05 (mg/dL) corresponding to LD and HD of tannins respectively. The downturn of the unusual lipid profile on treatment with the tannin extracts concludes plausible stimulation of the lipoprotein lipase enzyme. Similarly, *Cyclea peltata* ethanolic root extract in (400 mg/kg b.w.) streptozotocin induced diabetic rats reversed the abnormal lipid profiles, which supports our data.^[30]

Table 3: Effect of tannins on serum lipid profile in STZ-NIC induced diabetic rats (n=5) after 35 days of treatment.

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 + Tannin	58.42 ± 1.36	59.53 ± 1.58	76.86 ± 1.62	77.68 ± 1.59	19.15 ± 1.05	19.05 ± 1.04
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 + Tannin (LD)	35.84 ± 1.52	52.46 ± 1.02 [*]	173.23 ± 2.35	107.60 ± 2.18 [*]	40.85 ± 1.67	20.48 ± 1.24 [*]
Diabetic + Tannin (HD)	35.98 ± 1.64	56.16 ± 1.09 [*]	172.28 ± 2.84	96.26 ± 1.37 [*]	40.71 ± 1.86	17.06 ± 1.05 [*]
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 Duncan's multiple range test (DMRT). *p < 0.05 as compared with diabetic group, #p < 0.05 as compared with control group.

3.6. Tannin activity on liver enzyme parameter

Serum enzymes such as AST and ALT are exploited as a part of the assessment of hepatic disorders. An escalation in these enzymes reflects active liver damage. STZ-NIC induced diabetic rats showed increase in AST (191.314 ± 2.82 U/L) and ALT (204.914 ± 2.20 U/L) levels indicating hepatic disorders due to outflow of AST and ALT enzymes from liver cytosol to blood stream. Significant decrease in the serum AST and ALT levels were observed in tannin (25 and 50 mg/kg b.w.) treated rats after the treatment. The liver enzyme levels of various groups at 35th day for AST was 37.472 ± 1.49 and 33.314 ± 1.47 U/L and ALT level at the final day was 38.688 ± 1.38 and 36.012 ± 1.46 U/L corresponding

to LD and HD of tannins respectively (Table 4). Similar findings were reported with Mistletoe *Loranthus micranthus* extract that significantly lowered ALT (15.96 and 14.24 U/L) and AST (31.99 and 34.30 U/L) (level upon oral administration at 551 mg/kg b.w. and 827 mg/kg b.w.).^[39]

Table 4: Effect of tannins on liver enzyme in STZ-NIC induced diabetic rats (*n*=5) after 35 days of treatment

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 + Tannin	34.768 ± 1.27	34.714 ± 1.31	36.058 ± 1.18	35.344 ± 1.34
Diabetic group	145.734 ± 2.36 [#]	191.314 ± 2.82 [#]	179.340 ± 2.50 [#]	204.914 ± 2.20 [#]
Diabetic + Tannin (LD)	147.668 ± 2.31	37.472 ± 1.49 [*]	179.688 ± 2.37	38.688 ± 1.38 [*]
Diabetic + Tannin (HD)	145.714 ± 2.79	33.314 ± 1.47 [*]	180.762 ± 2.15	36.012 ± 1.46 [*]
Diabetic + Metformin	146.132 ± 2.72	31.281 ± 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 Duncan's multiple range test (DMRT). **p* < 0.05 as compared with diabetic group, #*p* < 0.05 as compared with control group.

3.7. Effect of tannins on liver glycogen content in STZ-NIC induced diabetic rats

Liver plays an important role in glycogen synthesis. Glycogenesis results in the activation of glycogen synthase enzyme by synthase phosphatase. STZ-NIC induced diabetic rats showed reduced glycogen content level (18.74 ± 0.97 mg/g) until the end of the study indicating the improper secretion of insulin in glycogenesis. Oral administration of tannins (25 and 50 mg/kg b.w.) showed 52.68 ± 1.65 and 57.23 ± 1.54 mg/g of liver glycogen content corresponding to LD and HD of tannins indicating the improved insulin secretion resulting in the augmentation of glycogenesis. Similarly, ethanolic extract (250 and 500 mg/kg b.w.) of *Helianthus annuus* L., seed resulted in increased liver glycogen 12.65 ± 0.32 and 13.32 ± 0.32 mg/g respectively.^[40]

Table 5: Effect of tannins on liver glycogen level in STZ-NIC induced diabetic rats (*n*=5) after 35 days of treatment.

Groups	Liver glycogen mg/g
Control	56.14 ± 1.37
Control + Tannin	54.83 ± 1.85
Diabetic group	18.74 ± 0.97 [#]
Diabetic + Tannin (LD)	52.68 ± 1.65 [*]
Diabetic + Tannin (HD)	57.23 ± 1.54 [*]
Diabetic + Metformin	59.95 ± 1.68 [*]

Values are expressed as means ± SD (*n* =5) from three independent experiments. Statistical evaluation was done by one way ANOVA followed by Duncan's multiple range test (DMRT). **p* < 0.05 as compared with diabetic group, #*p* < 0.05 as compared with control group.

3.8. Histopathological studies

Figure 3 represents the histology of liver in control and experimental rats. (a-b) control rats showed normal hepatic cells. STZ-NIC induced diabetic rats (c) showed hepatocellular necrosis and fading nuclei. Oral administration of tannins 50 and 100 mg/kg b.w. (d and e) liver sections showed normal nucleus and cytoplasm indicating that the tannins significantly enhanced central vein and lobular architecture. (f) Diabetic rats treated with standard drug metformin (500 mg/kg b.w.) showed hepatocellular manner with normal nucleus and cytoplasm. Similar results in liver histopathological studies in *Ficus amplissima* smith bark extract, which supports our data.^[41]

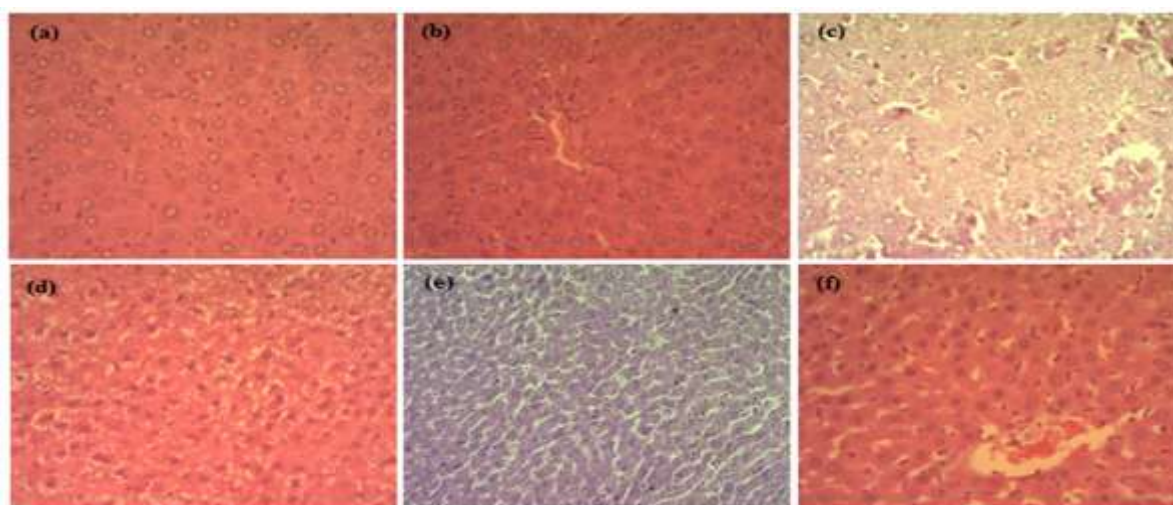


Figure 3: Histopathology of Liver in STZ-NIC induced diabetic rats after 35 days of treatment with tannins. (a) Control rats (b) control + tannin 50 mg/kg b.w. rats showing normal hepatic cells. (c) Diabetic rats showing

hepatocellular necrosis. (d and e) tannin (LD) 25 and (HD) 50 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.

CONCLUSION

All these productive impacts of tannins from *J. gossypifolia* are particularly helpful in forestalling hyperglycemia, cardiovascular and hepatic diseases. Taking everything into account, this examination has without a doubt gave reasonable affirmation and proof to the protective nature of tannins from *J. gossypifolia* in the treatment of diabetes and for the development of pharmaceutical drug for diabetes.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ACKNOWLEDGEMENT

The authors sincerely thank the Center for animal house, SRM Medical College, Chennai, India for providing facility to carry out the *in vivo* studies.

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