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Antidiabetic effects afforded by *Terminalia arjuna* in high fat-fed and streptozotocin-induced type 2 diabetic rats

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Abstract
The present study was design to evaluate the beneficial effects of *Terminalia arjuna* (TA) extract on hyperglycemia, lipid profile, renal damage markers and oxidative stress in the liver and pancreas of type 2 diabetes mellitus (T2DM) in rats. T2DM was induced by feeding rats with high-fat diet (HFD; 40%) for two weeks followed by single dose of streptozotocin (STZ; 40 mg/kg, intraperitoneally). Control and diabetic rats were treated with TA (500 mg/kg) for four weeks. After TA treatment, blood was drawn and rats were then sacrificed, and their liver and pancreas were dissected out for biochemical assays. The level of fasting blood glucose (FBG), glycated hemoglobin ($HbA1_C$), total cholesterol (TC), triglycerides (TG), low density lipoprotein-cholesterol (LDL-C) and very low density lipoprotein-cholesterol (VLDL-C) significantly ($P < 0.05$) increased while high density lipoprotein cholesterol (HDL-C) and hepatic glycogen decreased in the HFD/STZ group. TA treatment augmented these effects in the HFD/STZ + TA group. The HFD/STZ group showed elevated renal injury markers in serum, including blood urea nitrogen (BUN), serum creatinine (Scr) and alkaline phosphatase (ALP), which were decreased significantly ($P < 0.05$) by TA treatment. Moreover, treatment with TA significantly ($P < 0.05$) ameliorated thiobarbituric reactive substances (TBARS), malonaldehyde (MDA) and protein carbonyl (PC), and glutathione (GSH), glutathione-s-transferase (GST) and catalase (CAT) in liver and pancreas of HFD/STZ group. The study suggests that TA is effective in reducing hyperglycemia, hyperlipidemia and oxidative stress related to the risk of diabetes. Thus, it may have a therapeutic value for the treatment of T2DM.

Keywords: *Type 2 diabetes mellitus, Terminalia arjuna, Oxidative stress,Hyperglycemia; Lipid peroxidation*

Introduction

A disturbance in homeostatic phenomenon between generation of free radicals and the rate at which they are scavenged by enzymatic and nonenzymatic antioxidants is referred to as oxidative stress.[1] It is a well-established general mechanism for cell and tissue injury and primarily caused by free radicals. These radicals can bind with most normal cellular components including lipids, proteins, carbohydrates and nucleic acids.[2,3,4] Cells and biological fluids have an array of protective antioxidant mechanisms such as reduced glutathione (GSH), catalase (CAT) and glutathione-s-transferase (GST), for both preventing the production of free radicals and repairing oxidative damage.[5] It has been suggested that oxidative stress is one of the main contributory factor in the pathophysiology of many diseases, including type 2 diabetes mellitus (T2DM).[6,7,8] It is a group of "metabolic diseases" characterized by insulin resistance and relative, rather than absolute, insulin deficiency resulting hyperglycemia.[9,10,11] The pancreatic β-cell possesses the ability to respond to minor increases in the plasma glucose levels, thereby maintain the blood glucose level.[12] Progressive destruction of pancreatic β-cell leading to decreased insulin production and subsequent hyperglycemia that is observed in T2DM. In particular, liver is an insulin-sensitive tissue and plays a major role in maintaining glucose homeostasis by regulating the interaction between the glucose utilization and gluconeogenesis.[13] Therefore, damage to pancreas and liver is of decisive importance in the development and progression of diabetes. Current therapeutic opportunities for DM are mainly focused on the restoration of blood glucose within normal range. Efforts to discover antioxidants as useful drug candidates to combat diabetic complications are taking of great importance in research. Experimental and clinical research has confirmed the efficacy of several plants in the modulation of oxidative stress associated with DM due to their antioxidant property.[14,15]

Terminalia arjuna (TA) (Combretaceae), commonly known as *Arjuna*, has been used in Indian system of medicine (ISM) for over three centuries. The thick, white to pinkish gray bark has a long ethnomedicinal history,[16] including cancer treatment;[17] cardioprotective;[18] hypotensive, hypolipidemic[19] and wound healing activity.[20,21] Moreover, it plays a beneficial role in hepatic and renal disorders,[22] and has profound effects on hepatocellular carcinoma *in vivo* and *in vitro*.[23,24] Dwivedi and Jauhari[25] demonstrated that

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prolonged administration of TA did not show any adverse effect on renal, hepatic and hematological parameters.

Some preliminary studies have explored that TA enhance the antioxidant status in alloxan- induced diabetic rats.[26,27] The beneficial effects of TA on high fat diet (HFD) and streptozotocin (STZ)-induced diabetes have not been investigated previously. The present study was designed to evaluate the beneficial effects of TA on hyperglycemia, lipid profile, renal function markers and oxidative damage in liver and pancreas of HFD/STZ-induced T2DM in rats.

Materials and methods

Chemicals and reagents

Glutathione reduced (GSH), 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), thiobarbituric acid (TBA), and 2,4-dinitrophenyhydrazine (DNPH), Streptozotocin (STZ) were purchased from Sigma-Aldrich, Chemicals Pvt. Ltd, India. *Terminalia arjuna* bark extract powder was purchased from Saiba Industries, Mumbai, India.

Animals

Male Wistar rats weighing 180-200 g were used in the study. They were kept in the Central Animal House of Jamia Hamdard (Hamdard University) in colony cages at an ambient temperature of 25 ± 2 °C and relative humidity of 45-55% with 12 h light/dark cycles. They had free access to standard rodent pelleted diet (Hindustan Lever Ltd., Bombay, India) and water *ad libitum*, prior to the dietary manipulation. All procedures using animals were reviewed and approved by the Institutional Animal Ethical Committee that is fully accredited by the Committee for Purpose of Control and Supervision on Experiments on Animals (CPCSEA) Chennai, India.

Experimental design and development of the HFD/STZ model of type 2 diabetes

Thirty two rats were divided separated into four groups of eight animals each: group I (control group) rats were fed standard diet (12% calories as fat) throughout the experiment; group II (control + TA) rats were fed standard diet throughout the experiment and given TA (500 mg/kg body wt., orally) for 4 weeks; group III (HFD/STZ group) rats were fed HFD (40% fat, 18% protein and 41% carbohydrate, as a percentage of total kcal) for 2 weeks[28] and then injected with STZ (40 mg/kg body wt., IP, in citrate buffer; pH 4.5); group IV (HFD/STZ + TA) rats were fed HFD for 2 weeks and then injected with STZ and then supplemented with TA for 4 weeks. The development of hyperglycemia in rats was confirmed by fasting blood glucose (FBG) estimation after 6 days of STZ injection. The animals that maintained fasting blood glucose higher than 140 mg/dl were considered diabetic and selected for studies. The TA treatment was started after diabetes was confirmed, and dose was determined from previous study.[27]

Oral Glucose Tolerance Test

In the last week of experiment, the Oral Glucose Tolerance Test (OGTT) was performed to assess the glucose tolerance. For this purpose, overnight fasted rats were fed orally 2 gm/kg body wt. glucose. Blood was collected at 0, 30, 60, and 120 min intervals from orbital sinus for glucose estimation. Animals were not anesthetized for this procedure.

Tissue preparation

At the end of experiment, rats were anesthetized by ether inhalation and blood was collected from the dorsal aorta. Approximately 0.2 ml of whole blood was taken in EDTA containing microtubes from the total blood of each animal, and immediately preserved in the refrigerator for subsequent analysis of glycated hemoglobin (HbA1c). Serum was separated by centrifugation at 1200 x g for 10 min and stored at -80 °C before analysis. Rats were then sacrificed, and their liver and pancreas tissues were excised immediately and perfused with ice-cold saline. For biochemical estimations, liver and pancreas tissues were homogenized at 4 °C with 10 times w/v 0.1 M phosphate-buffer PB, pH 7.4 containing protease inhibitors in a polytron homogenizer (Kinematica, AG, Switzerland). The homogenate was centrifuged at 800 x g for 5 min at 4 °C to separate the nuclear debris and was used for estimation of thiobarbituric reactive substances (TBARS) and malonaldehyde (MDA). The supernatant was further centrifuged at 10,000 x g for 20 min at 4 °C to get the post-mitochondrial supernatant (PMS), which was used for various biochemical assays.

Analytical procedures

Measurement of FBG

Fasting blood glucose was measured by glucose oxidase method using a commercial diagnostic kit from Span diagnostic Limited, Surat, India.

Determination of HbA1c level

HbA1c was assayed by cation-exchange method using a diagnostic kit from Crest Biosystem, Goa, India.

Estimation of liver glycogen content

Liver glycogen content was determined using the anthrone reagent method.[29] Immediately after excision from the animal, approximately 1 g of liver tissue is dropped in 3ml of 30% KOH. This tube was placed in a boiling water bath for 30 min, following this digest was cooled, transferred to a 50-ml volumetric flask and diluted to mark with water. Slowly, 5 ml of solution from the previous step was added to a test tube containing 10 ml of anthrone reagent, which was placed in cold water to prevent excessive heating. After thorough mixing, the tube was placed in a boiling water bath for exactly 10 min for the development of colour and cooled with running tap water. The optical density was read within 2 h in a spectrophotometer at 620 nm against a blank that was prepared by subjecting 5 ml of distilled water instead of sample to the same procedure.

Assay for lipid profile

Lipid profile [total-cholesterol (TC), triglycerides (TG), and HDL-cholesterol (HDL-C)] were estimated by using enzymatic kits procured from SPAN Diagnostics India, Ltd. (Surat, India); LDL-cholesterol (LDL-C) and VLDL-cholesterol (VLDL-C) were calculated by using Friedewald's equation.

Renal function markers in serum

Kidney damage during diabetes was evaluated by the

following markers in serum: serum creatinine (Scr) concentration, blood urea nitrogen (BUN) level and alkaline phosphatase (ALP) activity. Scr and BUN were estimated by using enzymatic kits procured from SPAN Diagnostics India, Ltd. (Surat, India). The calculations were obtained using the standard formula provided by the manufacturer's instructions and expressed as unit/dl. The activity of ALP was determined using p-nitrophenyl phosphate (PNPP) as a substrate.[30] The assay contained equal volumes of 0.1M glycine and 0.4% PNPP. The mixture was incubated for 30 min at 25 °C after adding 0.1ml serum. The absorbance was read at 410 nm. ALP activity was expressed as equnits/dl in serum.

Biochemical parameters in liver and pancreas

TBARS content

The method of Utley et al,[31] with some modification, was used to estimate the rate of LPO. Homogenate (0.25 ml) was pipetted into 15×100 mm test tubes and incubated at 37 °C in a metabolic shaker for 1 h. An equal volume of homogenate was pipetted into a centrifuge tube, placed at 0 °C and marked at 0 h incubation. After 1 h of incubation, 0.5 ml of 5% (w/v) chilled trichloroacetic acid (TCA), followed by 0.5 ml of 0.67% TBA (w/v) was added to each test tube and centrifuge tube, and centrifuged at 1000 × *g* for 15 min. Thereafter, the supernatant was transferred to other test tubes and was placed in a boiling water bath for 10 min. The absorbance of pink color produced was measured at 535 nm in a spectrophotometer (Shimadzu-1601, Japan). The TBARS content was calculated by using a molar extinction coefficient of 1.56 x 105 M^{-1} cm^{-1} and expressed as nmol of TBARS formed min^{-1} mg^{-1} of protein.

Assay for MDA

MDA which is a measure of the end product of lipid peroxidation was measured.[32] Briefly, the reagents acetic acid 1.5 ml (20%), pH 3.5; 1.5 ml TBA (0.8%), and 0.2 ml sodium dodecyl sulfate (8.1%) were added to 0.1 ml of processed tissue sample. The mixture was then heated at 100 °C for 1 h. The mixture was cooled with tap water and 5 ml of n-butanol:pyridine (15:1% v/v) 1 ml of distilled water was added. The mixture was shaken vigorously. After centrifugation at 4000 rpm for 10 min, the organic layer was withdrawn, and absorbance was measured at 532 nm using a spectrophotometer (Shimadzu-1601, Japan). The amounts of MDA formed in each of the samples were expressed as the nmol MDA formed/h/mg protein by using a molar extinction coefficient of 1.56 x 105 M^{-1} cm^{-1}.

Assay for protein carbonyl (PC)

PC level was measured by the method of Levine et al.[33] The PMS (0.5 ml) was treated with an equal volume of 20% TCA for protein precipitation. After centrifugation, the pellet was resuspended in 0.5 ml of 10 mM DNPH in 2 M HCl and vortexed repeatedly at 10 min intervals for 1 h in dark. This mixture was treated with 0.5 ml of 20% TCA. After centrifugation at 10, 000 × g at 4 °C for 3 min, the precipitate was extracted three times with 0.5 ml of 10% TCA and dissolved in 2.0 ml of NaOH at 37 °C. Absorbance was recorded at 360 nm in a spectrophotometer (Shimadzu-1601, Japan). PC level was expressed as nmol carbonyl mg^{-1} protein, using a molar extinction coefficient of 22 x 104 M^{-1} cm^{-1}.

Assay for GSH

Reduced GSH content was determined by the method of Jollow et al,[34] with slight modification. PMS was mixed with 4.0% sulfosalisylic acid (w/v) in a 1:1 ratio (v/v). The samples were incubated at 4 °C for 1 h, and later centrifuged at 1200 × g for 15 min at 4 °C. The assay mixture contained 0.1 ml of supernatant, 1.0 mM DTNB, and 0.1M PB (pH 7.4) in a total volume of 1.0 ml. The yellow colour that developed was read immediately at 412 nm in a spectrophotometer (Shimadzu-1601, Japan). The GSH content was calculated as mmol GSH mg^{-1} of protein, using a molar extinction coefficient of 13.6 x103 M^{-1} cm^{-1}.

Assay for GST

The activity of GST was measured by the method of Habig et al.[35] The reaction mixture consisted of 1.0 mM GSH, 1.0 mM CDNB, 0.1 M phosphate buffer (pH 7.4) and 0.1 ml of PMS in a total volume of 3.0 ml. The change in absorbance was recorded at 340 nm by using Shimadzu spectrophotometer UV-1601 and enzyme activity was calculated as nmol of CDNB conjugate formed min^{-1} mg^{-1} protein using molar extinction coefficient of 9.6 x 103 M^{-1} cm^{-1}.

Assay for CAT

Catalase activity was assayed by the method of Claiborne.[36] Briefly, the assay mixture consisted of 0.05 M phosphate buffer (pH 7.0, 0.019 M) hydrogen peroxide (H2O2), and 0.05 ml PMS in a total volume of 3.0 ml. Changes in absorbance were recorded at 240 nm. Catalase activity was expressed as nmol H_2O_2 consumed min^{-1} mg^{-1} protein.

Total protein content

Total protein content was determined using bovine serum albumin (BSA) as a standard. [37]

Statistical analysis

Results are expressed as mean ± S.E.M. (n = 8). Statistical analysis of the data was obtained via analysis of variance (ANOVA), followed by Tukey's test. $P < 0.05$ was considered statistically significant.

Results

Effect of TA on OGTT in the HFD/STZ-induced rat model of diabetes

Blood glucose level of the control, the HFD/STZ group and the HFD/STZ + TA groups at different time points (0, 30, 60 and 120 min) after oral administration of glucose (2 g/kg) shown in Figure 1. In the HFD/STZ group, the peak increase in blood glucose level was observed after 60 min and remained high over next 60 min. TA treatment significant ($P < 0.05$) decreased blood glucose level at 60 and 120 min in the HFD/STZ + TA group compared to the HFD/STZ group.

Effect of TA on FBG in HFD/STZ group

Table 1 shows the effect of TA on the FBG level. A significant ($P < 0.05$) increase in FBG was observed in

Table 1: Effect of TA treatment on FBG, hepatic glycogen and HbA1c in the HFD/STZ induced rat model of diabetes

Parameters/groups	Control (C)	C + TA	HFD/STZ	HFD/STZ + TA
FBG (mg/dl)	107.76±5.2	109.32±4.7	328.87±6.7[a]	225.82±6.1[b]
		(+ 1.44%)	(+ 205.18%)	(- 31.33%)
Hepatic glycogen	365.27±4.7	367.04±3.43	169.86±1.21[a]	230.06±2.6[b]
(µg)		(+ 0.48%)	(- 53.49%)	(+ 35.44%)
HbA1c (%)	5.55±0.29	5.82±0.92	11.18±0.67[a]	8.81±0.77[b]
		(+ 4.86%)	(+ 101.44%)	(- 21.19%)

Values are expressed as mean ± S.E.M. (n=8). The HFD/STZ group showed a significant decrease in hepatic glycogen and significant increase in FBG and HbA1c compared to the control group ([a]P < 0.05 HFDSTZ vs. control group). TA treatment significantly ameliorated these parameters in the HFD/STZ + TA group compared to the HFD/STZ group ([b]P < 0.05 HFD/STZ vs. HFD/STZ + TA group). Values in parentheses indicate percentage increase (+) or decrease (-) as compared to the control or HFD/STZ group.

Table 2: Effect of TA treatment on serum lipid profile

Parameters/groups	Control (C)	C + TA	HFD/STZ	HFD/STZ + TA
TC (mg/dl)	154.24±2.5	156.91±2.6	299.44±2.0[a]	205.44±2.3[b]
		(+1.73%)	(+94.13%)	(- 31.39%)
TG (mg/dl)	106.26±2.9	107.33±3.1	256.57±2.4[a]	187.76±3.5[b]
		(+1.00%)	(+141.45%)	(- 26.81%)
HDL-C (mg/dl)	49.3±0.92	48.11±1.2	20.98±0.87[a]	28.91±0.79[b]
		(- 2.41%)	(- 57.44%)	(- 26.38%)
LDL-C (mg/dl)	83.67±1.4	85.65±1.6	227.13±0.94[a]	138.98±0.82[b]
		(+ 2.366%)	(+ 171.45%)	(- 38.81%)
VLDL-C (mg/dl)	21.25±1.2	22.02±0.98	50.91±1.5[a]	38.25±0.88[b]
		(+3.62%)	(+ 139.57%)	(- 24.86%)

Values are expressed as mean ± S.E.M. (n=8). The HFD/STZ group showed a significant decrease in HDL-C with the significant increase in TC, TG, LDL-C and VLDL-C in HFD/STZ group compared to the control group ([a]P < 0.05 HFD vs. control group). TA treatment reversed these effects in the HFD/STZ + TA group compared to the HFD/STZ group ([b]P < 0.05 HFD/STZ vs. HFD/STZ + TA group). Values in parentheses indicate percentage increase (+) or decrease (-) as compared with the control or HFD/STZ group.

Table 3: Effect of TA treatment on renal function markers (BUN, Scr and ALP) in serum of the HFD/STZ induced rat model of diabetes

Parameters/groups	Control (C)	C + TA	HFD/STZ	HFD/STZ + TA
BUN (mg/dl)	19.00 ±1.32	20.14 ±2.1	51.02±1.5[a]	30.54 ±0.79[b]
		(+5.98%)	(+168.50%)	(- 40.13%)
Scr (mg/dl)	1.09 ±0.04	1.16 ±0.06	2.65 ±0.03[a]	1.91 ±0.08[b]
		(+6.17%)	(+142.87%)	(- 27.92%)
ALP (units/dl)	26.32±0.69	27.12 ±0.72	44.65±0.83[a]	32.87±0.56[b]
		(+3.03%)	(+ 69.64%)	(- 26.38%)

Values are expressed as mean ± S.E.M. (n=8). The HFD/STZ group showed a significant increase in renal function markers (BUN, Scr and ALP) in serum of the HFD/STZ group compared to the control group ([a]P < 0.05 HF/DSTZ vs. control group). TA treatment significantly modulated these parameters in the HFD/STZ + TA group compared to the HFD/STZ group ([b]P < 0.05 HFD/STZ vs. HFD/STZ + TA group). Values in parentheses indicate percentage increase (+) or decrease (-) as compared with the control or HFD/STZ group

HFD/STZ group compared to the control rats. TA treatment significantly (P < 0.05) reduced FBG in the HFD/STZ + TA group compared to the HFD/STZ group. Only TA treatment did not show any significant change in the FBG compared to the control rats. The four-week treatment with TA resulted in significant (P < 0.05) antihyperglycemic effect.

Effect of TA on HbA1c in the HFD/STZ-induced rat model of diabetes

Effect of TA on HbA1c level in the HFD/STZ is shown in Table 1. A significant (P < 0.05) increase in HbA1c level was observed in the HFD/STZ group compared to the control rats. TA treatment in the HFD/STZ + TA group significantly (P < 0.05) decreased the HbA1c level. There was no significant change in HbA1c level in TA treatment in the control + TA treated rats when compared to control rats.

Effect of TA on hepatic glycogen content in the HFD/STZ-induced rat model of diabetes

The HFD/STZ group showed the decreased levels of glycogen content compared to the control group (Table 1).

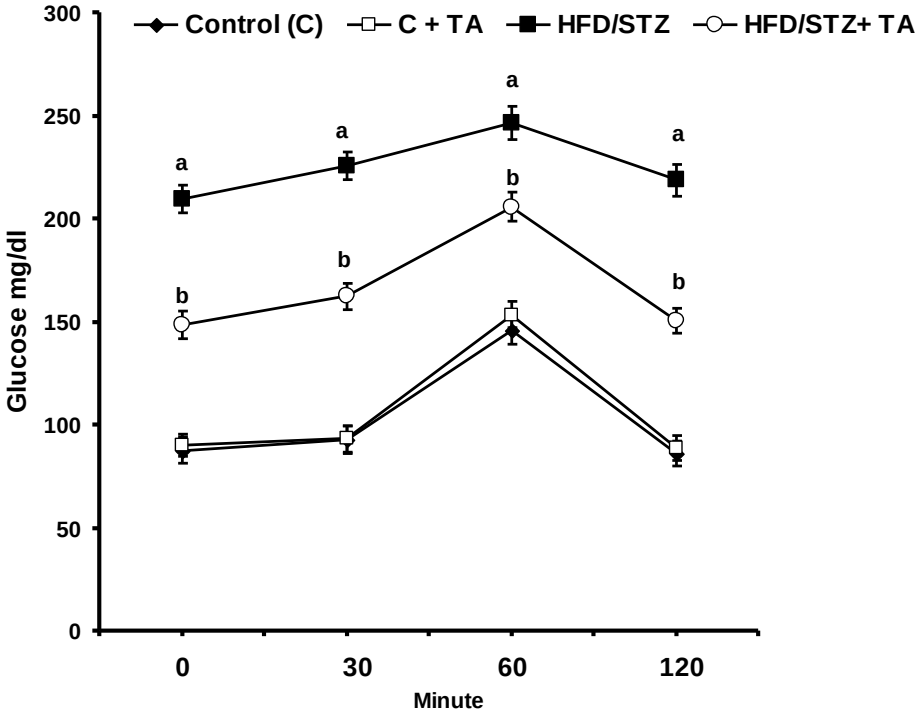


Figure 1. Effect of TA treatment on oral glucose tolerance. Values are expressed as mean ± S.E.M. (n = 8). Glucose tolerance was evaluated from the time course of serum glucose after administration of glucose solution (2 g/kg, p.o.) in OGTT. The HFD/STZ group showed the increase in blood glucose level at 60 min and remained high over next 60 min compared to the control group ($^{a}P < 0.05$ HFD/STZ vs. control group). TA treatment decreased the blood glucose at both time points in the HFD/STZ + TA group compared to the HFD/STZ group ($^{b}P < 0.05$ HFD/STZ + TA vs. HFD/STZ group).

The glycogen levels were significantly increased by TA treatment the HFD/STZ + TA compared to the HFD/STZ group. The results obtained for the glycogen content indicates that there was no significant difference in glycogen content between the control and control + TA group.

Effect of TA on serum lipid profile in the HFD/STZ-induced rat model of diabetes

The effects of TA on lipid profile in the HFD/STZ rats are shown in Table 2. HFD/STZ group showed a significant ($P < 0.05$) increment in serum TC, TG, LDL-C and a significant ($P < 0.05$) decrement in serum HDL-C compared to the control group. Treatment of TA in the HFD/STZ + TA group significantly ($P < 0.05$) restored all the changes in the lipid profile. TA treatment did not show any significant changes in the lipid profile when compared to the control group.

Effect of TA on renal function markers in the HFD/STZ-induced rat model of diabetes

Effects of TA on renal markers (BUN, Scr and ALP) were measured to demonstrate renal function in serum of the HFD/STZ group. There were no significant changed in BUN, Scr, and ALP in the control + TA group while these factors were significantly ($P < 0.05$) increased in the HFD/STZ group compared to the control group. TA treatment significantly ($P < 0.05$) decreased these markers in the HFD/STZ + TA group compared to the HFD/STZ group (Table 3).

Effect on oxidative damage in the liver

Effect of TA on TBARS and MDA contents in the HFD/STZ-induced rat model of diabetes

There were no significant changed in TBARS and MDA contents in control + TA treated group compared to the control group. These parameters were significantly ($P < 0.05$) increased in the HFD/STZ group compared to the control group. Levels of TBARS and MDA in the HFD/STZ group decreased significantly ($P < 0.05$) with TA treatment (Figures 2A and 2B.).

Effect of TA on PC in the HFD/STZ-induced rat model of diabetes

PC content did not change by TA treatment in the control + TA group compared to the control group. PC content was significantly ($P < 0.05$) increased in the HFD/STZ group compared to the control alone. TA treatment significantly ($P < 0.05$) decreased PC content in the HFD/STZ + TA group compared to the HFD/STZ group alone (Figure 3A).

Effect of TA on GSH in the HFD/STZ-induced rat model of diabetes

Level of GSH did not affect by TA treatment in the control + TA treated group compared to the control group. However, a significant ($P < 0.05$) depletion in GSH was observed in the HFD/STZ group compared to the control group. TA treatment significantly ($P < 0.05$) augmented GSH level in the HFD/STZ + TA group compared to the HFD/STZ group (Figure 3B).

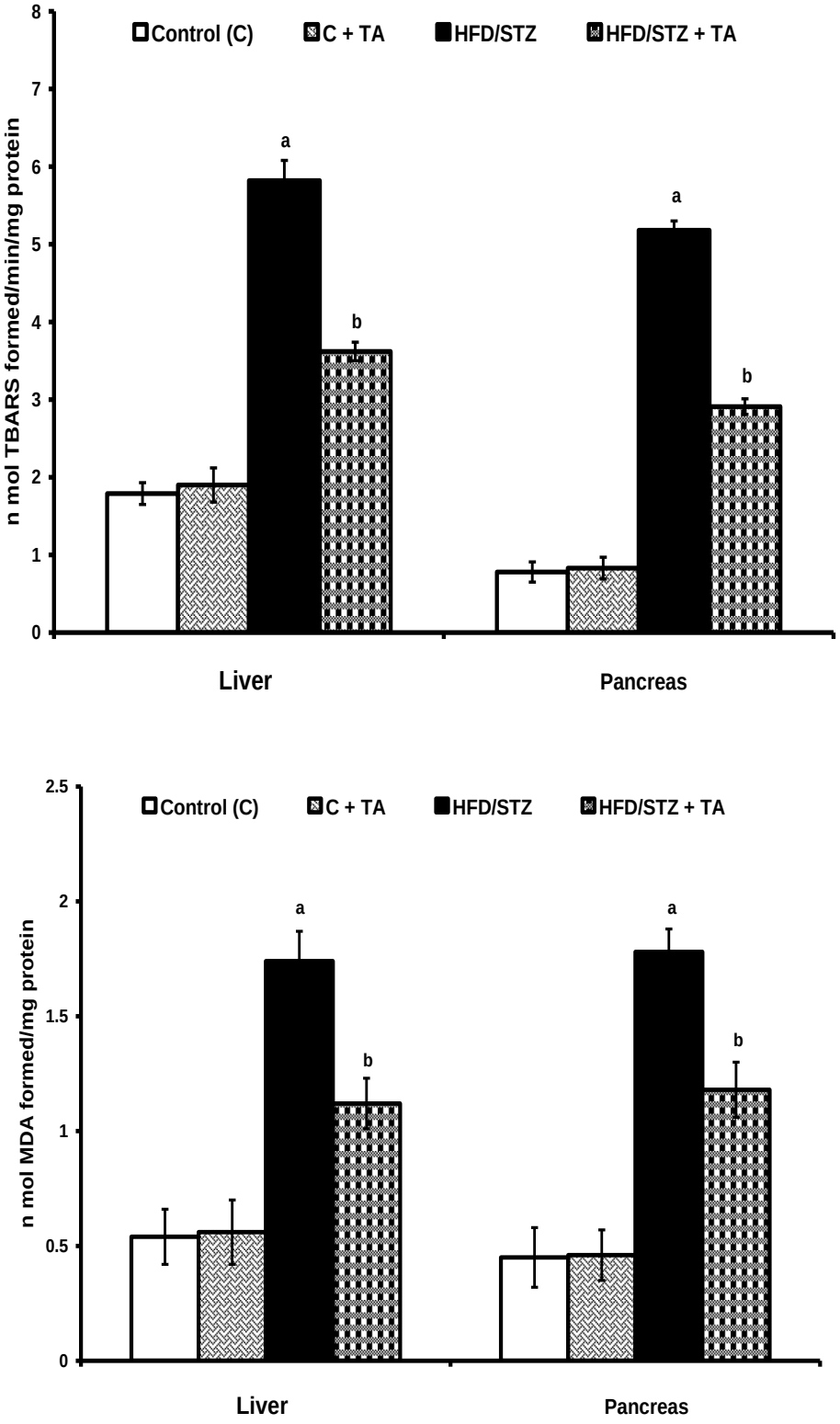
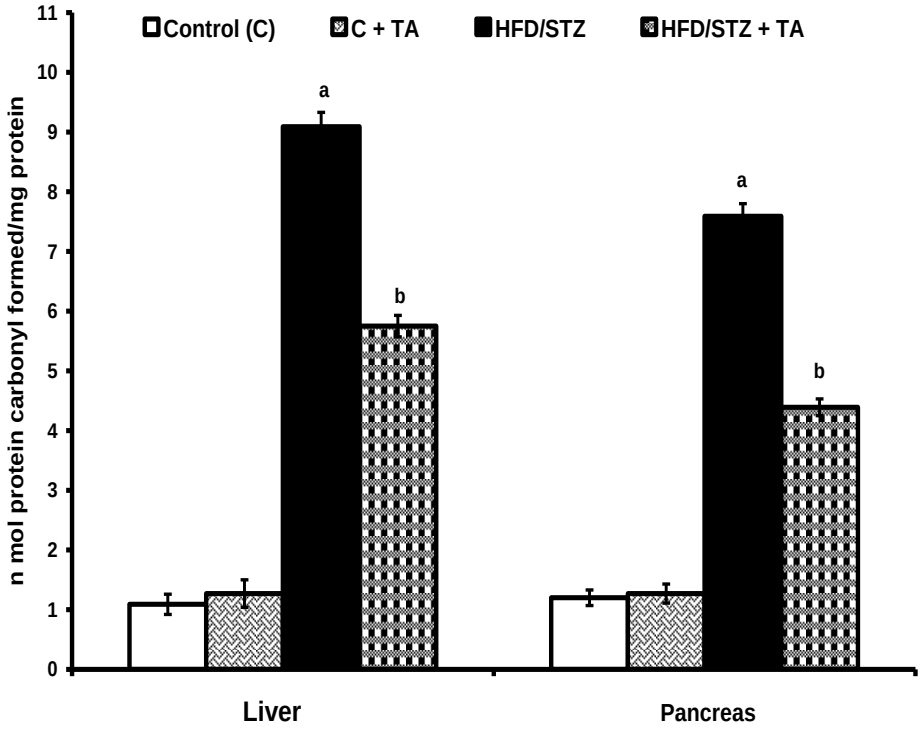


Figure 2 (A,B): Effect of TA treatment on TBARS levels. Values are expressed as mean ± S.E.M. (n = 8). The HFD/STZ group showed a significant increase in TBARS levels compared to the control group ($^{a}P < 0.05$ HFD/STZ vs. control group). TA treatment significantly decreased TBARS levels in the HFD/STZ + TA group compared to the HFD/STZ group ($^{b}P < 0.05$ HFD/STZ +TA vs. HFD/STZ group). (B). Effect of TA treatment on MDA levels. Values are expressed as mean ± S.E.M. (n = 8). The HFD/STZ group showed a significant increase in MDA levels compared to the control group ($^{a}P < 0.05$ HFD/STZ vs. control group). TA treatment significantly decreased MDA in the HFD/STZ + TA group compared to the HFD/STZ group ($^{b}P < 0.05$ HFD/STZ + TA vs. HFD/STZ group).



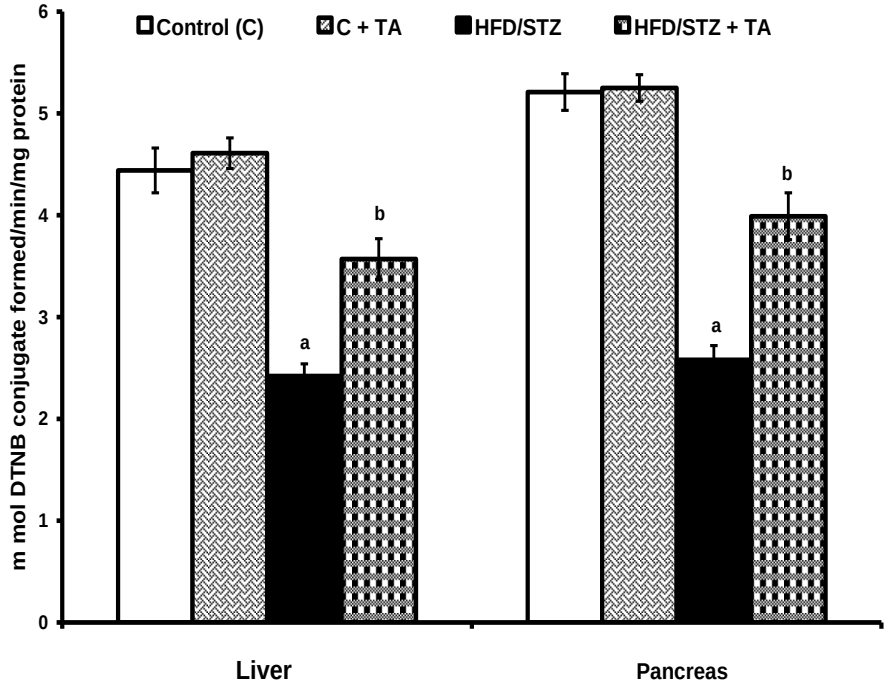


Figure 3 (A, B): Effect of TA treatment on PC content. Values are expressed as mean ± S.E.M. (n = 8). The HFD/STZ group showed a significant increase in PC content compared to the control group ($^{a}P < 0.05$ HFD/STZ vs. control group). TA treatment significantly decreased PC content in the HFD/STZ + TA group compared to the HFD/STZ group ($^{b}P < 0.05$ HFD/STZ + TA vs. HFD/STZ group). **(B).** Effect of TA treatment on GSH content. Values are expressed as mean ± S.E.M. (n = 8). The HFD/STZ group showed a significant decrease in GSH content compared to the control group ($^{a}P < 0.05$ HFD/STZ vs. control group). TA treatment significantly increased GSH content in the HFD/STZ + TA group compared to the HFD/STZ induced diabetic group ($^{b}P < 0.05$ HFD/STZ +TA vs. HFD/STZ group).

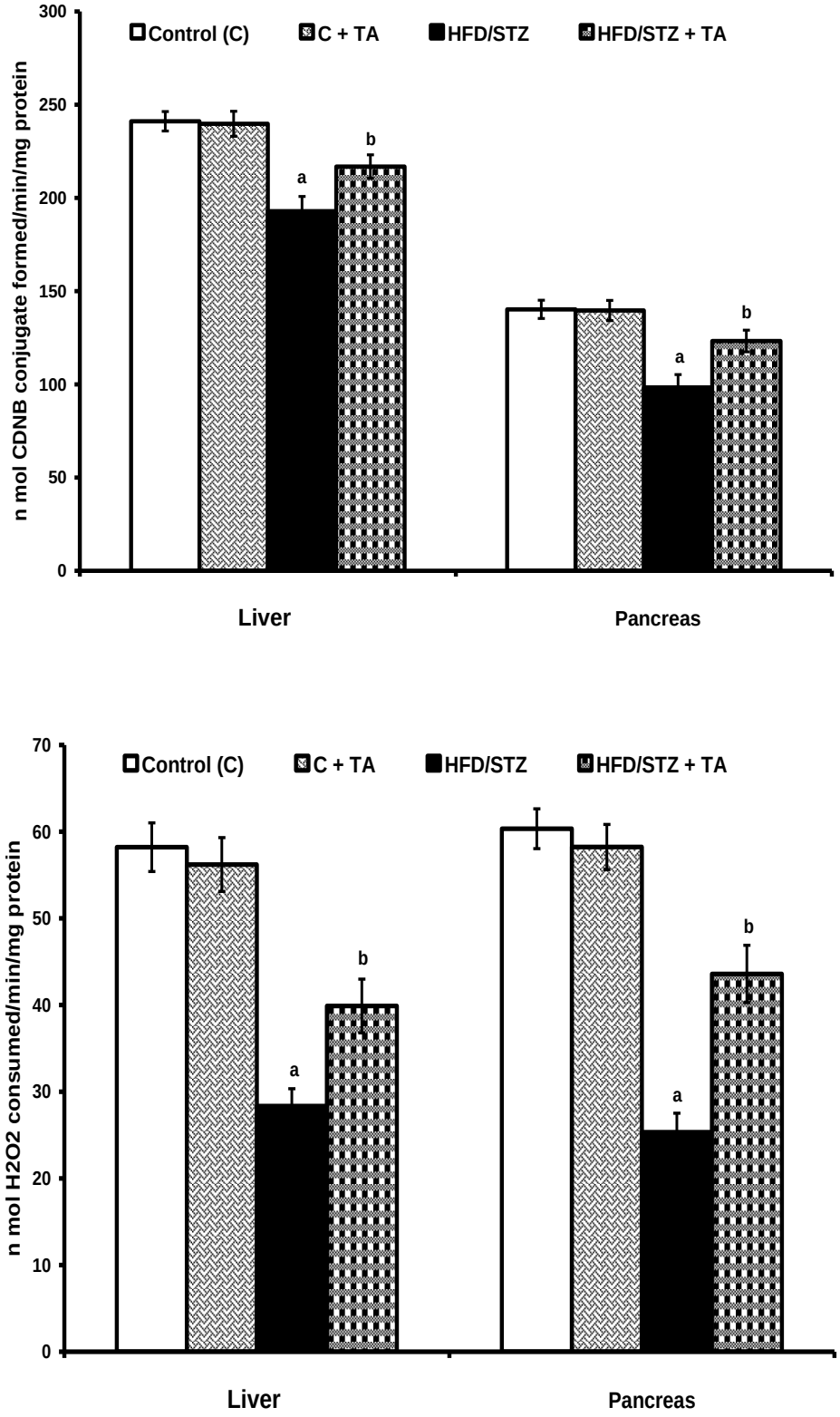


Figure 4 (A,B): Effect of TA treatment on GST activity. Values are expressed as mean ± S.E.M. (n = 8). The HFD/STZ group showed a significant decrease in GST activity compared to the control group ($^{a}P < 0.05$ HFD/STZ *vs.* control group). TA treatment significantly increased its activity in the HFD/STZ + TA group compared to the HFD/STZ group ($^{b}P < 0.05$ HFD/STZ + TA *vs.* HFD/STZ group). **(B).** Effect of TA treatment on CAT activity. Values are expressed as mean ± S.E.M. (n = 8). The HFD/STZ group showed a significant decrease in CAT activity compared to the control group ($^{a}P < 0.05$ HFD/STZ *vs.* control group). TA treatment significantly increased enzyme activity in the HFD/STZ + TA group compared to the HFD/STZ group ($^{b}P < 0.05$ HFD/STZ + TA *vs.* HFD/STZ group).

Effect of TA on GST and CAT activity in the HFD/STZ-induced rat model of diabetes

Effects of TA on the activity of GST and CAT in the HFD/STZ group and control groups are shown in Figures 4A and 4B). The activity of GST and CAT in the control + TA group was attenuated but the elevation was not significant compared to the control group. On the other hand, the activity of GST and CAT was depleted significantly ($P< 0.05$) in the HFD/STZ group compared to the control group. TA treatment significantly ($P < 0.05$) restored the activity of these enzymes in the HFD/STZ + TA group compared to the HFD/STZ group.

Discussion

In the present study we demonstrated that HFD/STZ-induced diabetes in rats causes hyperglycemia, hyperlipidemia, renal function deficits and oxidative damage in liver and pancreas. Moreover, treatment with TA, by virtue of its anti-oxidant potential, significantly ameliorated HFD/STZ-induced alterations. This shows that TA possess a phytochemical reservoir of heuristic therapeutic value[16, 17, 18, 19, 20, 21, 22] and exhibits hypoglycemic and high anti-oxidant potential.[26, 27]

Elevated level of free radicals causes imbalance in homeostatic phenomena between oxidants and antioxidants in the body. The increase in free radicals in diabetic animals may be due to the auto-oxidation of glucose.[38] Antioxidants, which can scavenge free radicals against damage and decay, have an important role in biological system and may be helpful in the prevention of these diseases. In the present study, during OGTT, blood glucose level were found to be increased in HD/STZ group with time and were maintained until 120 min in diabetic rats. TA treatment significantly improved glucose tolerance, as indicated by a reduction in peak blood glucose level at 60 and 120 min in the HFD/STZ + TA group. Moreover, the HFD/STZ-induced diabetic group exhibited increased FBG concentration while TA treatment to HFD/STZ + TA group reduced its concentration thereby showing its antihyperglycemic activity. Anti-hyperglycemic effects of TA are reporting due to increased peripheral glucose utilization.[27] In our study, HbA1c level was also found to be significantly increased in HFD/STZ group compared to control rats while TA treatment decreased the level of HbA1c. This beneficial effect of TA may occur due to its free radical scavenging property inhibit oxidative reactions associated with protein glycation.[39] A decrease in blood glucose level might also contribute to decreased level of glycated hemoglobin in TA treated HFD/STZ group. It is clearly described that glycogen deposition from glucose is impaired in diabetic animals.[40] In our study, the hepatic glycogen content in HFD/STZ group decreased when compared to HFD/STZ + TA treated and control rats. These results were corroborated with previous studies in diabetic animals.[41] TA might enhance glucose utilization by peripheral tissues and increase the glycogen stores in the liver.

It has been demonstrated that during DM a variety of derangements in metabolic and regulatory processes occurs, which in turn leads to hyperlipidemic condition in diabetic people.[42] In our study, the HFD/STZ-model of diabetes exhibited abnormalities in lipid metabolism as evidenced from the significant elevation of serum TC, TG, LDL-C, VLDL-C and reduction of HDL-C levels. Treatment with TA for four weeks significantly reduced the TC, TG, LDL-C, VLDL-C level and significantly increased HDL-C levels in HFD/STZ rats. These results are in accordance with previous report.[19]

In addition, recent evidence suggests that diabetic condition is associated with changes in morphology and eventually function alteration in kidney.[43, 44] Previous research has reported increased activity of these biomarkers during renal damage.[45] Our work clearly show that increased levels of kidney functional markers in serum (namely, BUN, Scr, and ALP) of HFD/STZ group. In contrast, the HFD/STZ + TA treated rats showed significant reduction in these markers, thus showing its ability to protect against diabetes-induced kidney damage.

LPO (lipid peroxidation), is often used as an index of oxidative tissue damage which causes free radical damage to membrane components of the cell and resulting cell necrosis and inflammation.[46] The increase oxidative stress might have induced the peroxidation of polyunsaturated fatty acids and lead to the formation of TBARS and MDA, as byproducts of LPO. Increased TBARS and MDA production played an important role in the progression of diabetes.[47] Free radicals can also lead to the formation of carbonyl groups which are the end products of protein oxidation. Their levels in tissues serve as relatively stable markers of oxidative damage.[48] In the present study, TBARS, MDA and PC formation increased significantly in the liver and pancreas of HFD/STZ group. Treatment with TA significantly modulated these parameters.

In body endogenous system glutathione, acts as an antioxidant by scavenging free radicals.[6] It has been described that low glutathione levels in diabetes is an indicator of increased oxidative stress.[49] Decreased level of GSH in diabetic rats may increase susceptibility to tissue oxidative damage. In the present study, we also observed a significant decrease in GSH content in the liver and pancreas of HFD/STZ. TA treatment increased GSH content, is possibly due to less production of free radicals or its scavenging property. The decreased level of GSH is also contributed to lower activity of GST antioxidant enzyme, because GSH is required as a substrate for GST activity. Catalase is also another important antioxidant enzyme that involved in the removal of free radicals by the detoxification of H_2O_2. It breaks H_2O_2, a toxic compound, into water and oxygen. In the present study, we observed antioxidant enzymes (GST and CAT) activity decreased significantly in HFD/STZ group. Treatment with TA significantly improved these antioxidant enzymes activities in HFD/STZ + TA group.

The antioxidant activity of TA bark extract is contributed due to rich concentration of its active constituents include tannins, triterpenoid saponins (arjunic acid, arjunolic acid, arjungenin, arjunglycosides), flavonoids (arjunone, arjunolone, luteolin), gallic acid, ellagic acid, oligomeric

proanthocyanidins (OPCs), phytosterols, calcium, magnesium, zinc, and copper.[50, 51] It is thought that the flavonoids and OPCs provide free radicals scavenging activity and enhance the antioxidant status in body. Antidiabetic activity of TA may be due to the stimulation of the β- cells of the pancreatic islets.[27]

In conclusion, our results showed that TA is a potent antidiabetic agent and beneficial in the control of diabetes related abnormalities in serum lipid profile, renal markers and oxidative damage in liver and pancreas of HFD/STZ-induced rat model of T2DM. Further studies regarding the mechanism underlying the protective action against oxidation, the lipid-lowering action, and the hypoglycemic action of TA extract need to be defined whether the TA extract can offer an alternate treatment for the patients of T2DM.

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