ORIGINAL ARTICLE

Effect of ethanolic extract of *Euphorbia hirta* on chronic diabetes mellitus and associated cardiorenal damage in rats

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Abstract

Introduction: *Euphorbia hirta* is a traditional medicine having unique combination of possible therapeutic action including antidiabetic, antioxidant, and antiobesity. The present study focuses to evaluate the therapeutic potency of ethanolic extract of *E. hirta* against diabetes mellitus (DM) and associated cardiorenal injury. **Materials and Methods:** DM was induced by administration of double cycle of repetitive (single injection for 3 alternative days in one cycle) dose of streptozotocin (20 mg/kg/i.p) in high-fat diet animals. Fasting and postprandial blood glucose level, glycated hemoglobin A1c (HbA1c), C-peptide, and plasma insulin were measured to confirm the induction of DM. Serum lipid profile and thiobarbituric acid reactive substances (TBARS) were measured in diabetic animals. Diabetic animals were further evaluated for cardiorenal toxicity after 12 weeks by measuring creatine kinase MB, lactate dehydrogenase, cardiac troponin I, serum creatinine, and blood urea nitrogen, respectively. Structural alteration was observed by histological assessments of pancreatic β-cells and cardiorenal tissues. **Results:** A significant increased level of fasting and postprandial blood glucose, and HbA1c and decreased level of C-peptide and plasma insulin were observed in diabetic animals. Furthermore, diabetic animals showed abnormal lipid profile (dyslipidemia) and increase serum TBARS (oxidative stress). In addition, the increased level of cardiorenal biomarkers in diabetic animals confirmed the associated abnormalities. The treatment of *E. hirta* (400 mg/kg/p.o) significantly overcomes the perturbed level of hyperglycemic, lipid profile, oxidative stress, and cardiorenal biomarkers. Histological evaluation of pancreatic and cardiorenal tissue also validates the significant protection of *E. hirta* against DM and -induced cardiorenal toxicity. **Discussion and Conclusion:** Thus, *E. hirta*-induced cardiorenal protection may associate with its antihyperglycemic, antidyslipidemia, and antioxidant potential.

Key words: Cardiotoxicity, diabetes mellitus, dyslipidemia, *Euphorbia hirta*, high-fat diet, oxidative stress, renal abnormalities

INTRODUCTION

Diabetic metabolic disorder and associated vascular damages are always being the foremost cause of morbidity and mortality globally. It has been observed that 17.5 million individuals died due to cardiovascular diseases in 2012.[1] Millions of people have had a finding of coronary artery disease induced by diabetes mellitus (DM). These statistical facts seem to be increased in near future due to growing spectrum of this insidious metabolic disorder worldwide.[2] Chronic DM is an immense concerned metabolic disorder of 21st century which leads to several cardiorenal abnormalities as hyperglycemia is believed to induce coronary microvascular dysfunction and results to myocardial injury.[3] Yu *et al.* 2011 revealed the association of pro-inflammatory cytokine in micro- and macrovascular complications, triggered by hyperglycemia.[4] These metabolic challenges coupled with the generation of oxidative stress and dyslipidemia which further influenced

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the impairment of cardio and renal function.[5] In spite of the availability of several oral hypoglycemic agents, the fatal rate has been gradually increasing worldwide.[1] Integral part of research in diabetic and associated complications is to identify the potential therapeutic agent which can significantly recover the toxicity without affecting normal regulatory mechanism of body. Even several pathological signalings have been proposed by researcher, but the root cause of pathogenesis in DM-induced cardiorenal toxicity is predominantly focused on oxidative stress, lipid peroxidation, and reduced level of free radical scavenger.[6] Moreover, DM is a lifestyle disorder which more frequently develops in obese population as exuberant weight is always a settled risk element for DM. Epidemic reports revealed towering prevalence in the existence of obesity and DM.[7,8] The predominance of obesity contribute to DM is about 55% which is significantly high as the obese population is continuously rising throughout the world.[9]

Various natural compounds have potent antioxidant property and substantiate the protective mechanism against various pathological events. From the past one decade, herbal medicine would be a thrust area for finding their pharmacological and therapeutic values against DM.[10] Even, numerous plants and their extracts have been proved to be cardio and renal protective including *Centella asiatica,*[11] *Ephedra nebrodensis,*[12] *Punica granatum,*[13] *Pomegranate,*[14] and *Lagenaria siceraria standley*.[15,16] However, *E. hirta* (*Euphorbiaceae*), vernacularly called as Dudhi or Dudhanim, has unique combination of possible therapeutic action including antidiabetic, antioxidant, and antiobesity.[17-21] *E. hirta* extensively found in the Western Ghats and Northeastern banks of Tamil Nadu, India.[22] *E. hirta* has been proclaimed to hold alkaloids, tannins, saponins, and flavonoids. A list of active phytochemical constituents of *E. hirta* has been reported in recent investigation including alkaloids, flavanoids, phenol, tannins, saponnins, steroids, terpenoids, quinone, catechin, coumarins, sugars, proteins, and anthraquinone.[17,23-26] Moreover, the presence of numerous chief chemical constituents in *E. hirta* including quercetrin, polyphenolic flavonoid, and palmitic acid confirmed its antioxidant, anti-inflammatory, hepatoprotective, antiobesity, and antidiabetic potential of this folk medicine.[17-21] The antihyperglycemic activity can block the associated complications, and thus, the herbal extract of *E. hirta* may produce the pivotal action against DM and associated cardiorenal diseases. Indeed, the therapeutic potential of *E. hirta* against DM-associated cardiorenal toxicity is still unexplored; hence, this present study has designed to evaluate the antihyperglycemic and associated cardiorenal effect of *E. hirta* in diabetic rats.

MATERIALS AND METHODS

Animals

Animals were approved by the Institutional Animal Ethics Committee as per the guidelines of CPCSEA, New Delhi, India (Protocol No.: CPCSEA\AIP\2013\004). Adult Wistar albino rats (200–220 g) of either sex were used in this study which were primarily acclimatized in departmental animal house at standard laboratory conditions (temperature: 25°C ± 1°C, relative humidity: 45–55%, and 12:12 h light:dark cycle) and allowed to have rat chow and water *ad libitum*.

Chemicals

High-fat diet (HFD) was prepared by mixing of 5% mixture of Vanaspati ghee and coconut oil (2:1) and 2.5% cholesterol to normal pellet diet. Streptozotocin (STZ) was purchased from HiMedia Laboratories Pvt. Ltd., Mumbai, India. Enzymatic kits for lipid profile estimation (total cholesterol [TC], high-density lipoprotein [HDL], low-density lipoprotein [LDL], and triglycerides [TG]) were purchased from Erba Diagnostics, Inc., USA. Glycated hemoglobin A1c (HbA1c) ELISA kit was purchased from Genxbio Health Sciences Pvt. Ltd. Delhi, India, and rat C-Peptide ELISA kit and rat insulin ELISA kit were procured from RayBiotech, Norcross, GA. Estimation of cardiac troponin I (cTnI), creatine kinase MB (CKMB), and lactate dehydrogenase (LDH) was done using commercially available kit purchased from Logotech India Pvt. Ltd. (Delhi, India) and Transasia Bio-Medicals Ltd., Solan, India, respectively. Serum creatinine and blood urea nitrogen (BUN) were measured for estimating the renal damage using commercially available kits purchased from Sigma-Aldrich, US. All other chemicals employed in the present study were of analytical grade.

Experimental Protocol

To induce chronic or late stage, a small dose of STZ was given in repeated manner at two different stages in HFD-fed animals. Animal was divided into four groups including normal control, disease control, drug *per se* and treatment group.

Group 1: Normal control group received vehicle injections (sodium citrate buffer, 2%), normal food pellets, and water *ad libitum*.

Group 2: Diseases control group received HFD from 21 days prior and continue to whole study duration at *ad libitum*. After 21 days exposure of HFD, the animals were exposed with single intraperitoneal injection of STZ (20 mg/kg/day) for consecutive 3 days (one cycle of challenge). The same challenge of STZ was repeated for second cycle of challenge after 15 days and thus the model is defined as double cycle of repetitive dose (DCRD).

Group 3: Drug *per se* group received only ethanolic extract of *E. hirta* leaves (400 mg/kg/p.o)[20,27] throughout the study.

Group 4: Animals of treatment group were treated with ethanolic extract of *E. hirta* leaves (400 mg/kg/p.o) after induction of diabetes against DCRD model of diabetic animals as mentioned in Group 2. All biochemical estimations for measuring the

induction and confirmation of T2DM and *E. hirta* effect were evaluated after 12 weeks of last STZ dose administration.

Measurement of Chronic Hyperglycemia and Oxidative Stress

All biochemical estimations were assessed after 12 weeks (from the last dose of STZ) which confirm the chronic stage of DM and association of cardiorenal toxicity. Blood glucose was measured from overnight fasted and oral glucose-administered animals (postprandial blood glucose). Postprandial blood glucose was measured in animals having pre-treatment of glucose solution (2 g/kg/p.o) before 120 min of analysis.[28] The plasma glucose level was estimated using commercially available kits. The level of HbA1c, serum insulin, and C-peptide was measured by commercially available ELISA kit. In addition, the oxidative stress was measured by estimating serum thiobarbituric acid reactive substances (TBARS) as mentioned in the previous study.[29]

Measurement Of Beta Cell Destruction

Destruction of pancreatic beta cells represents the conditions of chronic DM. Animals from each group were sacrificed and isolate the pancreas immediately. The isolated pancreas was fixed in paraffin wax blocks, and sections of about 5 µm thickness were cut. Hematoxylin-eosin (H and E) staining was applied, and histological photomicrographs were evaluated under a microscope.

Measurement of Lipid Profile (TC, TG, HDL, and LDL)

Different markers for serum lipid profile including TC, HDL, LDL, and TG were measured using commercially available kit as appraised by Sharma *et al*. 2016.[29]

Measurement of Cardiorenal Toxicity

After induction of DM, animals were further allowed to induce cardiorenal disorders after 12 weeks. Serum sample of each group was further evaluated for the estimation of various biomarkers associated to cardiac and renal damage including cTnI and CKMB, LDH and serum creatinine, and BUN, respectively, using commercially available kit.[29] Furthermore, the structural alteration in these vital organs was estimated by measuring histological changes. Thus, section of cardiorenal tissues of each group was stained by H and E staining, and histological photomicrographs were evaluated under microscope.[29]

Statistical Analysis

All values were expressed as mean±standard deviation. All biochemical data originating from each group of rats were analyzed by one-way ANOVA followed by Tukey's multiple comparison test using Sigma Plot version 11.0, from Systat Software, Inc., San Jose California USA. A $P < 0.05$ was considered statistically significant.

RESULTS

Evaluation of Mortality, Body Weight, and Serum Glucose Level

Assessment of all parameters was carried at the end of 12 weeks (after last dose of STZ) as mentioned in protocol. No mortality was found in any group, but significant body weight reduction has observed in diabetic rats as compared to normal control [Table 1].

Assessment of Chronic Hyperglycemia and Oxidative Stress

Level of fasting and postprandial blood glucose was significantly increased in disease control group (DCRD groups) as compared to normal control group, whereas the no disturbance in drug *per se* group was observed for fasting and postprandial blood glucose level. Treatment of *E. hirta* extract (400 mg/kg/p.o) significantly reverses the increased level of hyperglycemia in diabetic animals [Table 1]. In addition, the other biomarkers of hyperglycemia including HbA1c were measured to assess increased level in the diabetic group. STZ treated or diseases control (DCRD) group has a substantial raised level of HbA1c as compared to normal control group. Treatment with *E. hirta* significantly reduced the HbA1c level in the treated group as shown in Figure 1a.

Moreover, serum insulin and C-peptide were substantially decreased in disease control group as compared to normal control group, whereas no reduction in serum insulin and C-peptide was found in drug *per se* group. *E. hirta* significantly upsurges the level of both serum insulin and C-peptide against diseases control group [Figure 1b and 1c, respectively]. Chronic diabetes significantly increased the oxidative stress which was measured by the increased level of serum TBARS in diabetic animals as compared to normal control animal. The treatment of *E. hirta* extract has shown the substantial reduction in oxidative stress marked by a reduced level of serum TBARS in diabetic animals as compared to diseases control group [Figure 1d].

Assessment of Lipid Profile

Abnormality in cholesterol metabolism leads to dyslipidemia. The significant increase in TC, LDL, TG, and reduced level of HDL was measured in diabetes animals as compared to normal control group which endorses the lipid metabolism disorders. However, no such deflection of lipid profile was measured in drug *per se* group. Whereas, the treatment of

Table 1: Effect of ethanolic extract of *E. hirta* leaves against HFD and STZ (20 mg/kg/once for DCRD) on total mortality, body weight, and hyperglycemia including fasting and postprandial glucose level

Evaluating parameters	Normal control	HFD STZ (20 mg/kg/once for 3+3 days)	*E. hirta* (400 mg/kg/*p.o*)	HFD+STZ+*E. hirta*
Total mortality (%, up to 12 weeks)	0	0	0	0
Body weight (g) after last dose of STZ	210.57±12.48	143.68±10.58#	211.55±9.32	169.58±11.47*
Fasting blood glucose (mg/dL)	92.65±7.83	254.56±3.38#	93.34±6.52	121.45±5.74*
Post prandial glucose level (mg/dL)	185.87±7.6	473.57±6.4#	187.73±6.8	231.63±8.5*

All data are expressed as mean±SD for n=6, #represent P<0.05 as compared with normal control group and *represent P<0.05 as compared with disease control group. *E. hirta: Euphorbia hirta,* HFD: High-fat diet, DCRD: Double-cycle of repetitive dose, STZ: Streptozotocin, SD: Standard deviation

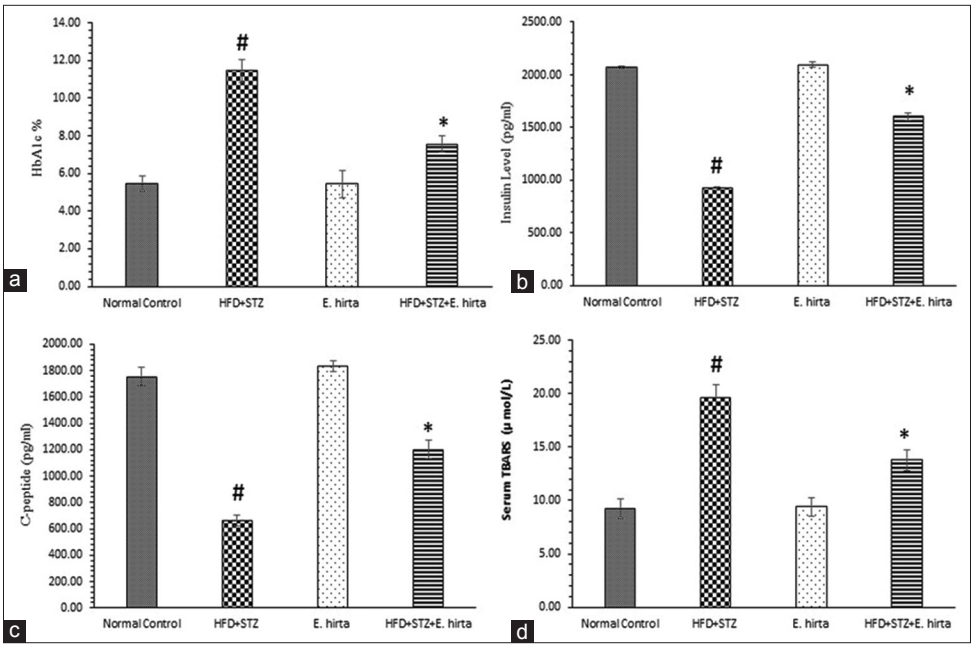

Figure 1: The effect of ethanolic extract of *Euphorbia hirta* leaves on key biomarkers (glycated hemoglobin A1c, serum insulin, and C-peptide) of diabetes mellitus induced by high-fat diet and streptozotocin (20 mg/kg/once for double-cycle of repetitive dose) and oxidative stress (thiobarbituric acid reactive substances). All data are expressed as mean ± standard deviation for n = 6, # represents P < 0.05 as compared with normal control group, and *represents P < 0.05 as compared with disease control group

E. hirta against diabetes-induced dyslipidemia significantly reduced the level of TC, LDL, and TG and increase HDL level as shown in Table 2.

Assessment of Associated Cardiovascular and Renal Damage

A significant increase in cardiac damage biomarker including CKMB, LDH, and cTnI was observed in diabetic animals as compared to normal control that confirmed the presence of cardiac injury induced by chronic diabetes [Figure 2a-c, respectively]. However, the treatment of ethanolic extract of *E. hirta* significantly reduced the cardiac damage biomarker in diabetic animals.

Moreover, the renal abnormalities were measured by the increase levels of BUN and serum creatinine in diabetic animals as compared to normal control group [Figure 3a and b, respectively]. The drug *per se* group did not show any abnormality in cardio and renal biomarkers. However, the treatment of *E. hirta* extract against diabetic induced renal damage was significantly reduced by measuring the decreased level of BUN and serum creatinine as compared to diseases control group [Figure 3a and b, respectively].

Histological Assessment of Pancreas and Cardiorenal Damage

The pancreas is composed of exocrine and endocrine cells where islets of Langerhans are tightly packed by surrounding

Table 2: Effect of ethanolic extract of *E. hirta* leaves against HFD and STZ (20 mg/kg/once for DCRD) induced dyslipidemia measured by total cholesterol, high-density lipoprotein, low-density lipoprotein, and triglyceride level

Evaluating parameters	Normal control	HFD+STZ (20 mg/kg/once for 3+3 days)	*E. hirta* (400 mg/kg/p.o)	HFD+STZ+*E. hirta*
Total cholesterol (mg/dL)	57.79±6.33	276.49±7.59#	58.04±8.58	107.49±8.39*
High-density cholesterol (mg/dL)	32.13±5.44	24.51±2.14#	31.83±2.22	29.55±3.92
Low-density cholesterol (mg/dL)	15.66±4.21	62.54±5.62#	15.27±3.35	35.22±3.54*
Triglyceride level (mg/dL)	67.76±3.12	242.44±5.77#	67.57±4.45	178.63±4.35*

All data are expressed as mean±SD for n=6, #represents P<0.05 as compared with normal control group and *represents P<0.05 as compared with disease control group. *E. hirta: Euphorbia hirta,* HFD: High fat diet, DCRD: Double-cycle of repetitive dose, STZ: Streptozotocin, SD: Standard deviation

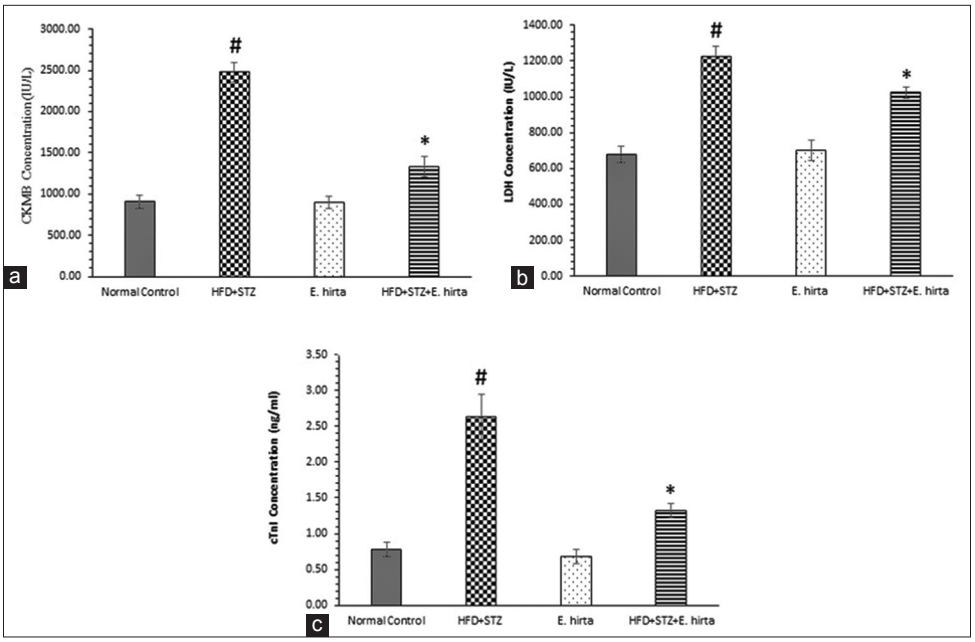

Figure 2: Effect of ethanolic extract of *Euphorbia hirta* leaves on cardiac biomarkers including creatine kinase MB, lactate dehydrogenase, and cardiac troponin I in high-fat diet and streptozotocin (20 mg/kg/once for the double-cycle of repetitive dose)-induced diabetes animals. All data are expressed as mean ± standard deviation for n = 6, #represents P < 0.05 as compared with normal control group and *represents P < 0.05 as compared with disease control group

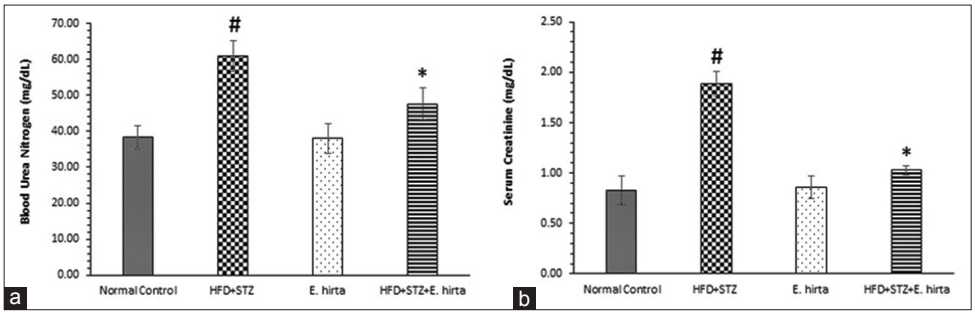

Figure 3: Effect of ethanolic extract of *Euphorbia hirta* leaves on renal biomarkers including blood urea nitrogen and serum creatinine in high-fat diet and streptozotocin (20 mg/kg/once for double cycle of repetitive dose)-induced diabetes animals. All data are expressed as mean ± standard deviation for n = 6, #represents P < 0.05 as compared with normal control group and *represents P < 0.05 as compared with disease control group

acinar cells of the exocrine portion of the pancreas.[30,31] Pancreatic structure is also divided by intact intralobular and interlobular connective tissue septa.[30,31] Histological section of normal control and drug *per se* group showed the normal architecture of islets of Langerhans surrounding by acinar cells and intact intralobular and interlobular connective tissue septa. Whereas, in diabetic pancreas shown, the significant inflammation in acinar cells represents with small vacuoles in exocrine components and the portion of islets of Langerhans was entirely lost or shrunk [Figure 4].

Moreover, the interlobular ducts were completely flattened with epithelium which confirms the complete destruction of β-cells or islets of Langerhans in HFD- and STZ-treated diabetic animals. The treatment group showed the significant protection in pancreatic damage by showing the reduced inflammation of acinar cells, protected portion of islets of Langerhans, and interlobular ducts [Figure 4].

In addition, the histology section of normal renal tissue shown structural damages in glomerulus including glomerular capsular wall distortion, mesangial cell expansion, and myofibrillar loss as compared to the renal histology of normal control animals [Figure 4]. The associated complication was further investigated in the treated group having treatment of *E. hirta* extract in diabetic animals. The consequences revealed the decreased glomerulus damage, and tubular necrosis has observed in the treatment group as compared to diseases control group. Furthermore, the section of normal control group shows normal histology of the heart section with no histological lesions. Whereas, the diabetic group showed the measurable histological lesions including thrombus formation, cytoplasmic vacuolization, and myofibrillar loss which indicates the induction of diabetes-induced cardiac toxicity. The treatment of *E. hirta* extract significantly reduced the myocardial lesions as compared to diseases control group [Figure 4].

DISCUSSION

The present study investigates the therapeutic potential of ethanolic extract of *E. hirta* leaves against most realistic animal model of chronic DM and associated cardiorenal injury. Chronic or late-stage diabetes is an insidious metabolic disorder having towering prevalence and a root cause of cardiac and renal abnormalities which make this metabolic complication more fatal.[1-6] The current outcomes

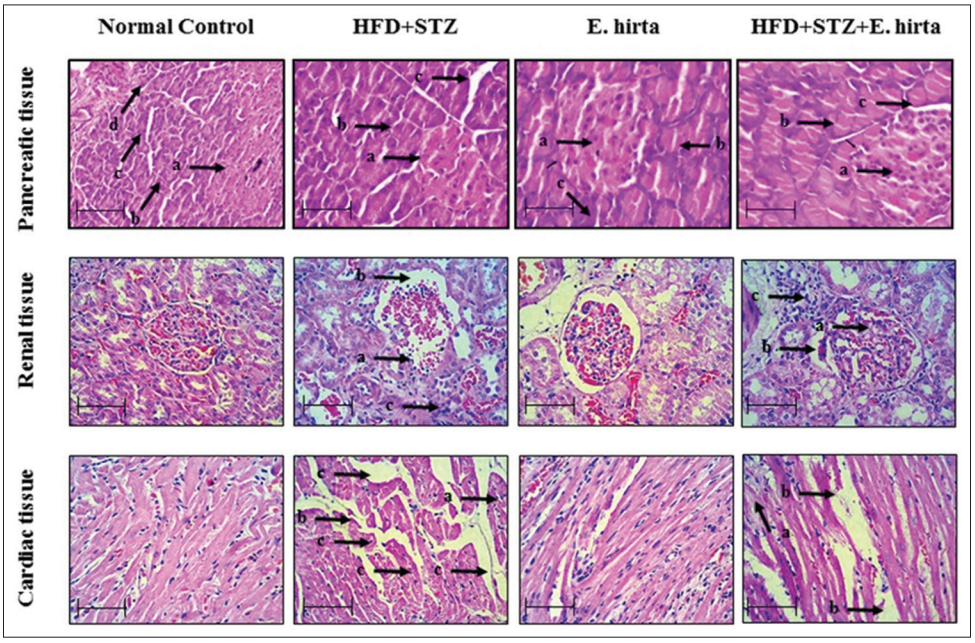

Figure 4: The histological examination of pancreatic and cardiorenal tissue using inverted microscope (Cosmo Laboratory Equipment) at 40× (scale bar = 100 μm). Histological architecture of normal control and drug *per se* groups shows the circular shape of islets of Langerhans represented by "a," acinar cells represented by "b." Pancreatic lobules represented by "c" and interlobular connective tissue septa represented by "d." Whereas, diabetic pancreas shows significant damage of islets of Langerhans "a," swelled acinar cells "b" and flattened interlobular ducts represented by "c." *Euphorbia hirta*-treated group shows mild damage of islets of Langerhans "a", no swelling in acinar cells "b," and less flattened interlobular ducts represented by "c." Histological sections of myocardial tissue including normal control verse diabetic group that showing thrombus formation "a," cytoplasmic vacuolization "b," and myofibrillar loss "c." Whereas, treatment group shows less thrombus formation "a," cytoplasmic vacuolization "b," and myofibrillar loss "c" as compared to diabetic group. Moreover, histological examination renal tissue was assessed by glomerulus damage including glomerular capsular wall distortion "a," mesangial cell expansion "b," and microvascular condensation "c" in diabetic group as compared to normal control group. However, the treatment of *E. hirta* extract shows the mild damage of glomerular capsular wall "a," mesangial cell expansion "b," and microvascular condensation "c" as compared to diabetic group

revealed the significant cardio and renal toxicity in diabetes group. Chronic hyperglycemia and increased oxidative stress may be the possible pathology for diabetes-induced cardiorenal damage.[5,6] Indeed, cardiac muscle is more susceptible to free-radical injury, since it contains low levels of detoxifying enzymes/molecules for free-radical including superoxide dismutase, glutathione catalase, and lipid peroxidation.[32] In clinical relevance, very few antioxidant agents are available which may protect diabetic-associated cardiorenal complications. The progressive augmentation in cardiorenal diseases demands a need of potent therapeutic agents which may guard the sinister state. *E. hirta* is belonging to *Euphorbiaeceae* family and known for its anti-inflammatory, antioxidant, anticancer, antifertility, antimicrobial, antiarthritic, antipyretic, and hepatoprotective potential.[17-21,27] Recent reports also revealed the antidiabetic potential of *E. hirta* in laboratory animals.[27] A recent study suggests that the flavonoid content present in *E. hirta* may responsible for its α-amylase and α-glucosidase inhibitory activities.[33]

The present study illustrates that HFD and DCRD of STZ (20 mg/kg/i.p/day) treatment leads to chronic or late-stage diabetes and associated cardio-renal diseases. The consequences from diabetic animal models signify the fact that low dose of STZ in repetitive manner can significantly produce chronic and sustain hyperglycemia as compared to single high dose of STZ. Moreover, instead of fast induction of β-cell death induced by a single dose of STZ, multi-low doses of STZ (as shown in DCRD model) can induce inflammation-mediated destruction of β-cells as reported by Zhang *et al*. (2008).[34] Furthermore, the diabetic rats from the current study have shown the significant decrease in body weight which may due to the severe catabolic metabolism influenced by compensatory hyperinsulinemia against chronic hyperglycemia and by a late reduction in insulin synthesis due to partial destruction and exhaustion of β-cell.[35] Treatment with ethanolic extract of *E. hirta* significantly blocks the continuous reduction of body weight in diabetic animals. The severity of metabolic disorder was further confirmed by the hyperglycemia, increased HbA1c, TBARS, and decreased serum insulin and C-peptide level diabetic animals. Indeed, the increase in fasting and postprandial blood glucose indicates the overall hyperglycemia. Recent studies have clearly mentioned a strong association between increased postprandial hyperglycemia and cardiovascular risk, oxidative stress, and endothelial dysfunction.[36,37] In addition, HbA1c is another unique biomarker to assess the overall glucose as increased blood glucose reacts with hemoglobin and formed HbA1c which further reflects the harmful glycation sequelae (retinopathy and nephropathy).[38] Furthermore, the increased non-enzymatic glycosylation in diabetic patients is key marker of a possible association between hyperglycemia and vascular complications.[38] C-peptide and insulin are the endogenous pancreatic peptide that secretin equimolar concentration. Estimation of C-peptide is more accurate analysis of insulin measurement as it has longer half-life as compared to insulin.[39] Release of insulin and C-peptide are interlinked as the source of release is same for both peptides. Decreased level of serum insulin and C-peptide represents the marked β-cell destruction.[39,40]

Chronic diabetes extensively abrupts the lipid profile (dyslipidemia) which is another most pathological event that causes vascular complications including atherosclerosis, endothelial dysfunction, and cardio and renal damage.[6] The presence of dyslipidemia was confirmed by an increased level of TC, LDL, and TG and a decreased level of HDL.

Furthermore, the alternated biochemical parameters including CKMB, LDH, cTnI, BUN, and serum creatinine level confirmed the induction of associated cardiorenal damage as reported in previous reports.[6] It is widely reported that free-radical generation triggers membrane peroxidation and disruption of cardiac myocytes, which can lead to increased release of CKMB, LDH, cTnI, BUN, and serum creatinine.[41] Ethanolic extract of *E. hirta* was found to inhibit the fasting and postprandial blood glucose, HbA1c, and TBARS and upsurge the level of serum insulin and C-peptide in diabetic animals. Treatment of *E. hirta* also reduced the diabetes-associated increase level of CKMB, LDH, cTnI, BUN, and serum creatinine. As mentioned in the previous study, the increased level of reactive oxygen species can destruct the redox potential of the cells destabilize the variety of cell functions including maintenance of cell integrity and protection of cells against toxins.[6,41] The present study shows the increased level of oxidative stress marker in rat heart, whereas *E. hirta* significantly reduces the TBARS concentration which revealed the antioxidant potential of *E. hirta* extract.

Besides biochemical markers, the histological changes were observed to confirm the β-cells destruction and cardiorenal damage in diabetic and treatment groups. Instead of the presence of other pancreatic cells (alpha-cells, delta-cells, and polypeptides), the islets of Langerhans contain β-cell which is responsible to secrete insulin and destruction of β-cell leads to down secretion of insulin.[30,42] The consequences were supported by the present investigation where the acinar cells were swelled and the islets of Langerhans was entirely lost or shrunk which confirm the β-cell destruction. The treatment of *E. hirta* in diabetic animals showed the significantly protection in pancreatic cell destruction which indicates the anti-inflammatory and antioxidant potential of *E. hirta*. Moreover, the association of cardiovascular damage and renal injury was also observed in histological assessment as represented in previous reports.[6,41] The altered biochemical marker and histological variation endorsed the late-stage diabetes-induced micro- and macrovascular alteration. However, the administration of *E. hirta* to diabetic animals significantly protects the cardiorenal damage which was induced by chronic diabetic condition.

Conclusively, the ethanolic extract of *E. hirta* leaves significantly controls the glucose level, dyslipidemia, reduced

oxidative stress which explores the anti-hyperglycemic, antidyslipidemia and antioxidant potential of our investigation plant extract. Moreover, the protection of cardiorenal tissue from diabetes-associated injury significantly revealed the cardio and renal protective potential of *E. hirta* against chronic diabetes-induced micro- and macrovascular abnormalities.

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REFERENCES

1. Mendis S. Global progress in prevention of cardiovascular disease. Cardiovasc Diagn Ther 2017;7:32-8.
2. Leon BM, Maddox TM. Diabetes and cardiovascular disease: Epidemiology, biological mechanisms, treatment recommendations and future research. World J Diabetes 2015;6:1246-58.
3. Kibel A, Selthofer-Relatic K, Drenjancevic I, Bacun T, Bosnjak I, Kibel D, *et al*. Coronary microvascular dysfunction in diabetes mellitus. J Int Med Res 2017;45:1901-29.
4. Yu XY, Chen HM, Liang JL, Lin QX, Tan HH, Fu YH, *et al*. Hyperglycemic myocardial damage is mediated by proinflammatory cytokine: macrophage migration inhibitory factor. PLoS One 2011;6:16239.
5. Chen SC, Tseng CH. Dyslipidemia, kidney disease, and cardiovascular disease in diabetic patients. Rev Diabet Stud 2013;10:88-100.
6. Sharma AK, Khanna D, Balakumar P. Low-dose dipyridamole treatment partially prevents diabetes mellitus-induced vascular endothelial and renal abnormalities in rats. Int J Cardiol 2014;172:530-2.
7. van Belle TL, Coppieters KT, von Herrath MG. Type 1 diabetes: Etiology, immunology, and therapeutic strategies. Physiol Rev 2011;91:79-118.
8. Al-Goblan AS, Al-Alfi MA, Khan MZ. Mechanism linking diabetes mellitus and obesity. Diabetes Metab Syndr Obes 2014;7:587-91.
9. Olokoba AB, Obateru OA, Olokoba LB. Type 2 diabetes mellitus: A review of current trends. Oman Med J 2012;27:269-73.
10. Rupeshkumar M, Kavitha K, Haldar PK. Role of herbal plants in the diabetes mellitus therapy: An overview. Int J App Pharm 2014;6:1-3.
11. Kumari S, Deori M, Elancheran R, Kotoky J, Devi R. *In vitro* and *in vivo* Antioxidant, anti-hyperlipidemic properties and chemical characterization of *Centella asiatica* (L.) extract. Front Pharmacol 2016;7:400.
12. Ballero M, Foddis C, Sanna C, Scartezzini P, Poli F, Petitto V, *et al*. Pharmacological activities on *Ephedra nebrodensis* Tineo. Nat Prod Res 2010;24:1115-24.
13. Sharifiyan F, Movahedian-Attar A, Nili N, Asgary S. Study of pomegranate (*Punica granatum* L.) peel extract containing anthocyanins on fatty streak formation in the renal arteries in hypercholesterolemic rabbits. Adv Biomed Res 2016;5:8.
14. Sreekumar S, Sithul H, Muraleedharan P, Azeez JM, Sreeharshan S. Pomegranate fruit as a rich source of biologically active compounds. BioMed Res Int 2014;2014:686921.
15. Upaganlawar A, Balaraman R. Cardioprotective effects of *Lagenaria siceraria* fruit juice on isoproterenol-induced myocardial infarction in wistar rats: A biochemical and histoarchitecture study. J Young Pharm 2011;3:297-303.
16. Takawale RV, Mali VR, Kapase CU, Bodhankar SL. Effect of *Lagenaria siceraria* fruit powder on sodium oxalate induced urolithiasis in wistar rats. J Ayurveda Integr Med 2012;3:75-9.
17. Asha S, Deevika B, Sadiq Am. *Euphorbia hirta* linn-a review on traditional uses, phytochemistry and pharmacology. World J Pharm Res 2015;3:180-205.
18. Kumar S, Malhotra R, Kumar D. *Euphorbia hirta*: Its chemistry, traditional and medicinal uses, and pharmacological activities. Pharmacog Rev 2010;4:58-61.
19. Subramanian SP, Bhuvaneshwari S, Prasath GS. Antidiabetic and antioxidant potentials of *Euphorbia hirta* leaves extract studied in streptozotocin-induced experimental diabetes in rats. Gen Physiol Biophys 2011;30:278-85.
20. Tuhin RH, Begum MM, Rahman MS, Karim R, Begum T, Ahmed SU, *et al.* Wound healing effect of *Euphorbia hirta* linn. (*Euphorbiaceae*) in alloxan induced diabetic rats. BMC Complement Altern Med 2017;17:423.
21. Gupta SS, Azmi L, Mohapatra PK, Rao CV. Flavonoids from whole plant of *Euphorbia hirta* and their evaluation against experimentally induced gastroesophageal reflux disease in rats. Pharmacog Mag 2017;13:127-34.
22. Patil SB, Naikwade NS, Magdum CS. Review on phytochemistry and pharmacological aspects of *Euphorbia hirta* Linn. J Pharm Res Health Care 2009;1:113-33.
23. Bhagwat GG, Chaulang G, Ghodke D, Suresh PK, Govindrao YP, Onkar GR. Pharmacognostic study of plant *Euphorbia hirta* L. J Pharm Res 2008;1:39-43.
24. Santana KC, Pinangé DS, Vasconcelos S, Oliveira AR, Brasileiro-Vidal AC, Alves MV, *et al.* Unraveling the karyotype structure of the spurges *Euphorbia hirta* Linnaeus, 1753 and *E. hyssopifolia* Linnaeus, 1753 (*Euphorbiaceae*) using genome size estimation and heterochromatin differentiation. Comp Cytogenet 2016;10:657-69.
25. Kausar J, Muthumani D, Hedina A, Anand V. Review of the phytochemical and pharmacological activities of *Euphorbia hirta* Linn. Pharmacogn J 2016;8:310-3.
26. Selvakumar P, Kaniakumari D, Loganathan V.

Preliminary phytochemical investigation of extract of leaves and stem of *Euphorbia hirta*. Int J Curr Sci 2012;4:48-51.
27. Maurya AK, Tripathi S, Ahmed Z, Sahu RK. Antidiabetic and antihyperlipidemic effect of *Euphorbia hirta* in streptozotocin induced diabetic rats. Pharm Lett 2012;4:703-7.
28. Nagalakshmi K, Sujatha S. Nanoencapsulation augments release efficacy and glucose tolerance of 14-deoxy, 11, 12-didehydro andrographolide loaded polycaprolactone nanoparticles in streptozotocin-nicotinamide induced Type 2 diabetes. Int J Appl Pharm 2017;9:51-3.
29. Sharma AK, Kumar A, Taneja G, Nagaich U, Deep A, Rajput SK. Synthesis and preliminary therapeutic evaluation of copper nanoparticles against diabetes mellitus and -induced micro (renal) and macro vascular (vascular endothelial and cardiovascular) abnormalities in rats. RSC Adv 2016;6:36870-80.
30. Tsuchitani M, Sato J, Kokoshima H. A comparison of the anatomical structure of the pancreas in experimental animals. J Toxicol Pathol 2016;29:147-54.
31. Nurdiana S, Goh YM, Ahmad H, Dom S, Ebrahimi M. Changes in pancreatic histology, insulin secretion and oxidative status in diabetic rats following treatment with *Ficus deltoidea* and vitexin. BMC Complementary Altern Med 2017;17:290.
32. Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods: Impact on human health. Pharmacogn Rev 2010;4:118-26.
33. Sheliya MA, Begum R, Pillai KK, Aeri V, Mir SR, Ali A, *et al*. *In vitro* α-glucosidase and α-amylase inhibition by aqueous, hydroalcoholic, and alcoholic extract of *Euphorbia hirta* L. Drug Dev Ther 2016;7:26-30.
34. Zhang M, Lv XY, Li J, Xu ZG, Chen L. The characterization of high-fat diet and multiple low-dose streptozotocin induced Type 2 diabetes rat model. Exp Diabetes Res 2008;2008:704045.
35. Srinivasan K. Viswanad B, Asrat L, Kaul CL, Ramarao P. Combination of high-fat diet-fed and low-dose streptozotocin-treated rat: A model for Type 2 diabetes and pharmacological screening. Pharmacol Res 2005;52:313-20.
36. Wang YJ, Xie XS, Feng SG, Long QX, Ai N, Wang BF. Causes of death in STZ-induced rat models of diabetes mellitus. Sichuan Da Xue Xue Bao Yi Xue Ban 2014;45:691-5.
37. Ketema EB, Kibret KT. Correlation of fasting and postprandial plasma glucose with HbA1c in assessing glycemic control; Systematic review and meta-analysis. Arch Public Health 2015;73:43.
38. Sherwani SI, Khan HA, Ekhzaimy A, Masood A, Sakharkar MK. Significance of HbA1c test in diagnosis and prognosis of diabetic patients. Biomarker Insights 2016;11:95-104.
39. Yosten GL, Maric-Bilkan C, Luppi P, Wahren J. Physiological effects and therapeutic potential of proinsulin C-peptide. Am J Physiol Endocrinol Metab 2014;307:955-68.
40. Kuhtreiber WM, Washer SL, Hsu E, Zhao M, Reinhold P 3rd, Burger D, *et al.* Low levels of C-peptide have clinical significance for established Type 1 diabetes. Diabet Med 2015;32:1346-53.
41. Beysel S, Unsal IO, Kizilgul M, Caliskan M, Ucan B, Cakal E. The effects of metformin in Type 1 diabetes mellitus. BMC Endocr Disord 2018;18:1.
42. Sheliya MA, Rayhana B, Ali A, Pillai KK, Aeri V, Sharma M, *et al*. Inhibition of α–glucosidase by new prenylated flavonoids from *Euphorbia hirta* L. herb. J Ethnopharmacol 2015;176:1-8.

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