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Modulation Of Lipid Metabolism And Hepatic Oxidative Stress By Clerodendrum Infortunatum In Experimental Diabetes

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Abstract:
The prevalence of diabetes is growing at an alarming rate and is associated with variousmacro- and microvascular complications. Diabetic dyslipidemia along with aggravated oxidative stress can lead to the progression of cardiovascular diseases, which is the major cause of premature death in diabetes. Phytomedicines are gaining importance as it is cost-effective with a wide range of pharmacological potential. The present study evaluates the therapeutic potential of Clerodendrum infortunatum (CI) in the attenuation of lipid metabolism abnormalities and hepatic oxidative stress in experimental diabetes. The results showed that the supplementation of CI extract could significantly modulate the serum lipid profile in streptozotocin-induced diabetic rats. Correspondingly, CI could significantly regulate the activities of major lipogenic enzymes in the liver, supporting the potential hypolipidemic properties of CI to mitigate diabetic dyslipidemia. Furthermore, the antioxidant properties of the CI could significantly ameliorate hepatic oxidative stress by promoting antioxidant enzyme activities and reducing the level of lipid peroxidation product accumulation in diabetic liver. The results were comparable with the antidiabetic drug, glibenclamide. The present study provides pharmacological evidence supporting the antioxidant and hypolipidemic properties of CI in experimental diabetes. Hence, the study demonstrates the possible therapeutic application of CI in ameliorating dyslipidemia and associated comorbidities of diabetes which warrants further studies to explore the detailed molecular mechanism.

KeyWord*: Clerodendrum infortunatum; Dyslipidemia; Diabetes mellitus; lipid peroxidation; antioxidant enzymes.*

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I. Introduction

Diabetes Mellitus (DM) is a multifactorial metabolic syndrome and has been identified as a major growing health problem worldwide. Given the fact that the incidence and prevalence of DM have increased dramatically, it is estimated that approximately 9.3% of the world's population is suffering from DM and is projected to increase 10.2% by 2030 and 10.9% by 2045 [1,2]. DM is often associated with dyslipidemia because insulin regulates several key events in lipid and glucose metabolism[3,4,5]. These metabolic abnormalities of diabetes can increase reactive oxygen species production (ROS) and the resulting oxidative stress plays a pivotal role in the development of diabetes complications, both microvascular and cardiovascular[6]. Furthermore, dyslipidemia can be closely and causatively related to cardiovascular diseases (CVD) and can be considered as one of the most important modifiable risk factors[7,8,9]. It is estimated that 30-60% of diabetic patients have dyslipidemia[10, 11]. Of note, reports show that greater than 65% of diabetic death are due to CVD with hyperglycemia and hyperlipidemia being important risk factors [12,13].

Dyslipidemia characterized by lipid triad is a complex metabolic milieu associated with increased serum triglyceride (TG) levels, increased very low-density lipoprotein cholesterol VLDL and intermediate density lipoprotein cholesterol IDL, and decreased high-density lipoprotein cholesterol (HDL-C) levels[14,15]. Also, increased low-density lipoprotein cholesterol (LDL-C) and high levels of free fatty acids (FFAs) further stimulate the secretion of apolipoprotein B (ApoB) and VLDL[16,17]. Insulin resistance or lack of insulin inhibits lipolysis leading to increased FFAs generation and decreased lipoprotein lipase activity, which generates chylomicron-richTG and affects HDL-C metabolism[18,19]. Lipoprotein metabolism is also affected by

hyperglycemia-mediated increased glycosylation and oxidation, which further facilitates vascular compliance and may increase the risk of cardiovascular disease[20]. In addition, ROS can alter the composition of lipoproteins, producing oxidized low-density lipoproteins (Ox-LDL) inside the blood vessels. This, in turn favour increased expression of pro-inflammatory cytokines, adhesive molecules, and foam cell formation by the accumulation of cholesterol inside the macrophages that lead to the development of atheromatous plaques[21]. Thus the clustering of lipid abnormalities along with oxidative stress may aggravate therisk of cardiovascular complications in diabetic patients.

Since the beginning of human civilization, plants have been used to alleviate various human pathologies. As a cultural heritage of various tribes, herbal medicines play a significant role in health care for a large proportion of the world's population[22]. The medicinal plant-derived pharmacological agents are relatively safe with fewer side effects. Hence, there is a dire need to explore medicinal plants for their pharmacological potential. *Clerodendrum infortunatum* (CI) is a small shrub occurring throughout the plains of India, and possesses antihyperglycemic, anti-inflammatory, anti-microbial, antioxidant, anticonvulsant, analgesic, hepatoprotective, wound healing, anticancer and nootropic properties[23]. It is widely used as an ingredient in various traditional systems of medicine. The free radical scavenging and antioxidant activity of various parts of CI has been previously studied[24-30]. Evidence suggests that leaf extract of CI possesses antidiabetic properties due to the phytochemical, pheophytin, contained in it[31]. In addition, preclinical antihyperglycemic and antioxidant activity of leaf extract of CI has been studied in streptozotocin (STZ)-induced diabetic rats[32]. Another study reported the potential of CI for reducing testicular damage in diabetic rats[33]. The previous study conducted in our laboratory has shown the hypoglycemic and hepatoprotective role of CI in STZ-induced diabetic rats[34]. In light of these reports, the present study aims to evaluate the effect of CI on diabetic dyslipidemia and hepatic oxidative stress in experimental diabetes.

II. Material And Methods

Chemicals

The chemicals used in the study were of analytical grade, purchased from Sigma Aldrich, USA and SRL Pvt Ltd. Mumbai, India.

Plant material and extraction

The botanical identity of CI whole plant, collected from Pandalam, Kerala, was confirmed by Dr. Mathew Dan, Scientist E1, Plant Genetic Resource Division, Jawaharlal Nehru Tropical Botanic Garden and Research Institute. A voucher specimen (No.60694) has been deposited in JNTBGRI, Palode, Thiruvananthapuram. The whole plant of CI was washed properly, shade dried and coarse powdered. Then the powder was used to prepare water extract and hexane extract. The preliminary studies conducted based on qualitative and quantitative phytochemical analysis showed that water extract is more potent than hexane extract. So we selected water extract of CI (200mg and 400mg/body weight) for further detailed study in experimental diabetic animal models.

Animals and experimental design

Male albino rats (Wistar) (200-250 g), were provided with laboratory chow (Hindustan Lever Lab diet, India) and water ad libitum throughout the experimental period. The rats were housed in a room with a temperature maintained at 23 ± 1^0C and 12 hours of light and dark cycles. The relative humidity of 50 ± 10% and ventilation frequency of 10-30 times per hour were maintained. The animals were acclimatized under laboratory conditions for two weeks before the experiments. Institutional guidelines were strictly followed throughout the study for animal experimentation and handling in conformity with the directions given by the Government of India for the use and care of laboratory animals (Approved by Institutional Animal Ethics Committee CKL/TOX/IAEC/40-2014)

Rats were made diabetic by giving a single intraperitoneal injection of STZ (40 mg/kg body weight in 0.1M citrate buffer – pH 4.5). The animals were allowed to drink 5% glucose solution overnight to overcome the drug-induced hypoglycemia. Three days after streptozotocin administration, animals with fasting blood glucose between 200-250 mg/dl, were considered diabetic and were selected for the study. Animals were divided into 5 groups. Group I - Normal rats; Group II – STZ-induced diabetic rats; Group III - STZ-induced diabetic rats supplemented with Glibenclamide (600 µg/kg body weight); Group IV and Group V – STZ-induced diabetic rats supplemented with CI water extract at the doses 200mg and 400mg/kg body weight respectively. The experimental duration was 40 days. At the end of the experimental period, animals were sacrificed, blood and tissues were collected for further analysis.

Biochemical parameters

Estimation of serum total cholesterol and HDL cholesterol were performed by the methods of Allain et al.[35] and Pisani et al.[36] respectively. The serum LDL+VLDL Cholesterol was calculated from the values of total cholesterol and HDL. Serum triglycerides were measured by the method of Rifai et al.[37]. Assay of β-Hydroxy methyl glutaryl Co-A reductase (HMG CoA reductase) was done by Rao and Ramakrishnan[38]. Isocitrate dehydrogenase (ICD) activity was estimated by the method of Kornberg[39]. The activity of malic enzyme (ME) was assayed by the method of Ochoa[40]. The liver tissue homogenate was used for the assay of antioxidant enzymes and lipid peroxidation products according to the respective protocols. Catalase (CAT) was assayed by the method of Maehly and Chance[41] and superoxide dismutase (SOD) was assayed by the method described by Kakkar et al.[42]. Glutathione peroxidase (GPx) activity was estimated by the method of Agerguard and Jence[43], glutathione reductase (GRd) activity by the procedure of David and Richard[44], and the glutathione content (GSH) by the procedure of Patterson and Lazarow[45].Thiobarbituric acid reactive substances (TBARS) were described by the method described by Okhawa et al.[46]. Hydroperoxides (HP) and conjugated dienes (CD) were estimated by the method of John and Steven[47].

Statistical analysis

All analyses were performed by using the statistical package SPSS/PC +, Version 17 (SPSS Inc, Chicago, IL, USA). Data were analyzed by one-way analysis of variance (ANOVA). All the results were expressed as mean value ± SD. Pair-fed comparison between the groups was made by Duncan's multiple range test. 'p' Values of 0.05 or less were considered significant.

III. Result

Effect of CI on serum lipid profile

Mounting evidence suggests that dyslipidemia and diabetes are closely associated, and hyperglycemia along with an altered lipid profile can complicate the pathophysiology of the disease[48]. So in the present study, the effect of CI administration on the serum lipid profile of diabetic rats was analysed. The level of serum cholesterol was significantly high in STZ-induced diabetic rats compared to the normal rats (Fig 1). But treatment with glibenclamide and water extract at a dose of 400 mg showed significantly decreased cholesterol levels compared to the diabetic control group. However, the level of serum HDL-Cholesterol was significantly low in STZ-induced diabetic rats compared to the normal rats (Fig 2). Treatment with glibenclamide and 400 mg water extract of CI showed significantly increased HDL-Cholesterol levels compared to the diabetic control group When we calculated the level of serum LDL+VLDL Cholesterol, STZ-induced diabetic rats showed a significantly high value as compared to the normal group (Table 1). Supplementation of glibenclamide and 400 mg water extract of CI showed a significant decrease in serum LDL+VLDL level compared to the diabetic control group. In addition, as shown in figure 3, STZ-induced diabetic rats showed significantly increased triglycerides level compared to the normal rats. Supplementation with glibenclamide and 400 mg water extract of CI showed significantly decreased level of triglycerides compared to the diabetic control group.

Figure 1. Level of serum cholesterol

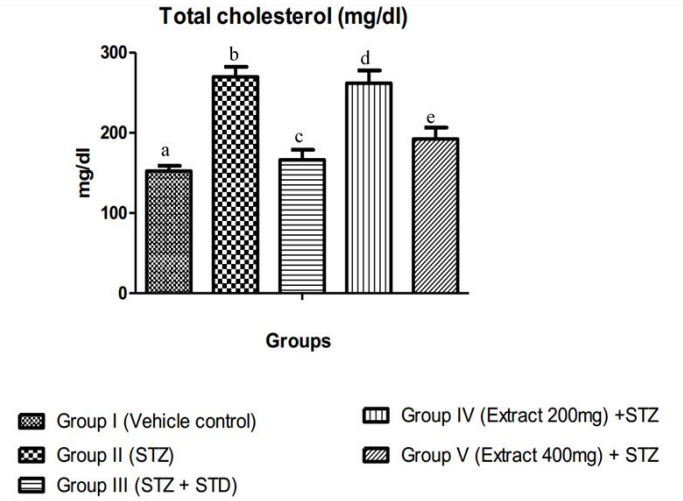

Figure 2. Level of serum HDL-Cholesterol

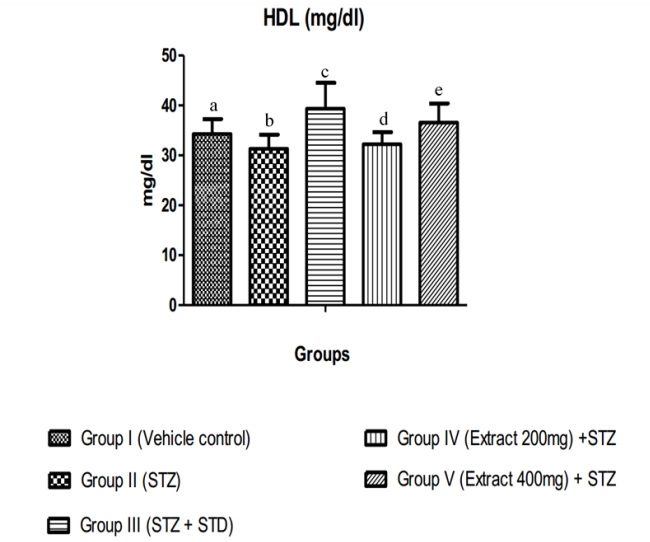

Figure 3. Level of serum triglycerides

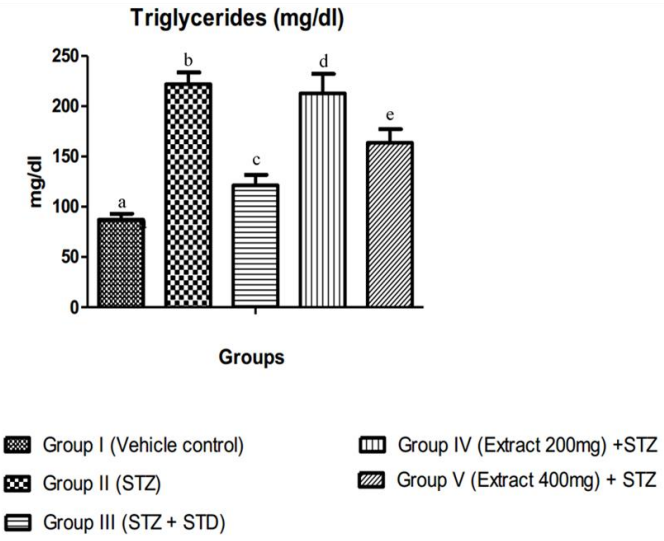

Table 1. Level of serum LDL+VLDL

Groups	LDL+VLDL (mg/dl)
Group I	118.047
Group II	238.43
Group III	127.00
Group IV	229.52
Group V	155.92

Results are expressed as mean ±SD. Values with the same superscript do not differsignificantly. Significance accepted at $p<0.05$

Effect of CI on lipogenic enzymes in liver

Next, the activity of major lipogenic enzymes in the liver of both diabetic and treated rats were evaluated. The activity of HMG CoA Reductase was significantly increased in STZ-induced diabetic rats because of the low HMG CoA/Mevalonate ratio in diabetic conditions compared to normal. Treatment with glibenclamide and 400 mg water extract of CI significantly decreased the activity of HMG CoA Reductase compared to the diabetic control group. Also, the activities of major lipogenic enzymes, ICD and ME, were significantly increased in diabetic rats compared to normal rats. Supplementation with glibenclamide and water extract of CI at a dose of 400 mg significantly decreased the activities of both ICD and ME compared to the untreated diabetic group. The results are detailed in table 2.

Table 2. Activities of lipogenic enzymes in liver

Groups	HMG CoA/Mevalonate	ICD (U/mg protein)	ME (U/mg protein)
Group I	5.15±0.52[a]	35.18±2.89[a]	31.23±0.27[a]
Group II	1.15±0.17[b]	77.75±7.13[b]	75.15±0.71[b]
Group III	4.21±0.39[c]	47.23±4.25[c]	42.15±0.38[c]
Group IV	2.56±0.20[d]	63.18±0.59[d]	65.29±0.61[d]
Group V	3.88±0.38[e]	55.39±0.50[e]	51.57±0.49[e]

Results are expressed as mean ±SD. Values with the same superscript do not differ significantly. Significance accepted at $p<0.05$

Effect of CI on antioxidant status in liver

As oxidative stress plays a pivotal role in the development of diabetes complications, the level of antioxidant status in the liver has a significant role in diabetic progression[49]. So the activities of major antioxidant enzymes such as Catalase, SOD, GPx and GRd in the liver were evaluated. The activities of these enzymes were significantly reduced in STZ-induced diabetic rats compared to normal rats (Table 3). But the administration of CI and glibenclamide significantly increased the activities of these antioxidant enzymes as compared to the diabetic group. The superior effect was shown by CI when compared to glibenclamide. In addition, the level of the major endogenous antioxidant, GSH, in the liver was significantly decreased in STZ-induced diabetic rats compared with the normal rats (Table 4). Glibenclamide and CI (400 mg/kg body weight) administration significantly increased the GSH level compared to the diabetic control group. A more significant effect was shown by CI at a dose of 400 mg when compared to glibenclamide.

Table 3: Activities of antioxidant enzymes in liver

Groups	CAT (U/mg protein)	SOD (U/mg protein)	GPx (U/mg protein)	GRd (U/mg protein)
Group I	11.15±0.93[a]	58.13±0.52[a]	79.13±7.11[a]	35.74±2.94[a]
Group II	3.12±0.25[b]	12.85±0.93[b]	31.78±2.73[b]	13.55±1.28[b]
Group III	6.15±0.57[c]	28.15±2.11[c]	42.99±3.79[c]	20.16±1.91[c]
Group IV	6.23±0.58[d]	35.32±2.99[d]	50.84±4.56[d]	24.89±2.03[d]
Group V	8.73±0.73[e]	42.13±3.60[e]	63.48±5.66e	29.37±2.21[e]

Results are expressed as mean ±SD. Values with the same superscript do not differ significantly. Significance accepted at $p<0.05$

Table 4: GSH content in liver

Groups	GSH content (mM /100g tissue)
Group I	1635±153[a]
Group II	538±47[b]
Group III	932±84[c]
Group IV	1115±109[d]
Group V	1390±121[e]

Results are expressed as mean ±SD. Values with the same superscript do not differ significantly. Significance accepted at $p<0.05$

Effect of CI on lipid peroxidation status in liver

The metabolic dysregulation in diabetes leads to increased ROS and promotes the accumulation of lipid peroxidation products in the liver[50]. In the present study, the level of major lipid peroxidation products, such as TBARS, HP and CD were significantly increased in diabetic rats as compared to normal (Table 5). Interestingly, CI and Glibenclamide treated rats showed significantly decreased levels of TBARS, HP and CD when compared to the untreated diabetic rats. A significant effect was shown by CI at a dose of 400 mg when compared to glibenclamide.

Table 5: Lipid peroxidation product concentration in liver

Groups	TBARS (mM /100g tissue)	HP (mM /100g tissue)	CD (mM /100g tissue)
Group I	0.59±0.05[a]	103.13±9.93[a]	31.56±2.74[a]
Group II	1.13±0.09[b]	194.13±18.14[b]	63.52±5.94[b]
Group III	0.97±0.08[c]	163.53±15.87[c]	54.53±4.77[c]
Group IV	0.86±0.07[d]	152.92±14.77[d]	49.37±4.13[d]
Group V	0.75±0.6[e]	131.52±12.98[e]	39.93±3.26[e]

Results are expressed as mean ±SD. Values with the same superscript do not differ significantly. Significance accepted at $p<0.05$

IV. Discussion

Studies have shown that the association of diabetes mellitus with atherosclerosis can have a strong correlation with diabetic dyslipidemia and oxidative stress[51,52]. Since long back herbal medicines have been a highly esteemed source of phytochemicals with remarkable potential to prevent and cure diverse diabetic complications. The present study evaluates the effect of CI extract on diabetic dyslipidemia and oxidative metabolism in the liver of experimental diabetic rats. The study demonstrates the potential pharmacological application of CI in modulating diabetes and associated metabolic dysfunction.

Dyslipidemia is a common feature of diabetes characterized by alterations in lipid metabolism and lipid profile, mainly due to factors like insulin deficiency or resistance, adipocytokines, and hyperglycemia[53]. Insulin deficiency or resistance activates intracellular hormone-sensitive lipase that promotes the release of non-esterified fatty acids (NEFA) from triglycerides stored in adipose tissue which in turn increases hepatic triglyceride production [54]. Due to the loss of the normal inhibitory effect of insulin on hepatic apoB production and triglyceride secretion in VLDL, more triglyceride-rich VLDL will be produced[55,56,57]. Furthermore, the diminished catabolism of VLDL exacerbates hypertriglyceridemia and lipoprotein lipase that mediates the removal of triglycerides from the circulation is down regulated in states of insulin resistance or deficiency. There exists a positive correlation of triglycerides with cholesterol, obesity, glucose intolerance, cigarette smoking, and hyperuricemia, whereas it can be negatively correlated with HDL cholesterol[58]. The risk of CVD is greater at any given level of serum cholesterol in patients with diabetes and its association with hypertriglyceridemia is stronger than in the general population[59]. In agreement with all these reports, we observed an altered lipid profile in diabetic conditions that was significantly attenuated by CI. In connection with the altered lipid profile in DM, we have evaluated the activities of major lipid metabolizing enzymes in the liver. This modulatory effect of CI over the altered lipid profile in diabetes mellitus was supported by the decreased activities of major lipogenic enzymes, viz; HMG CoA reductase, ICD and ME.

The derangement of metabolic processes in DM, especially hyperglycaemia and dyslipidemia, leads to increased oxidative stress that further triggers the inflammatory cascade[60]. The liver is among the primary organs susceptible to these stress signaling pathways, which may lead to serious liver tissue injury. The excessive ROS in the liver causes irreversible oxidative modification of lipids, proteins and carbohydrates along with the release of inflammatory cytokines and the induction of apoptosis in hepatocytes[61]. In addition, evidence suggests that a decrease in antioxidant activities during hyperglycemic state leads to oxidation-induced liver damage[62,63]. The lipid peroxidation mediated by the interaction of polyunsaturated fatty acids with ROS leads to the accumulation of lipid peroxides and aldehydes that in turn cause tissue damage[64]. Notably, studies show that there is a prominent increase in the level of lipid peroxidation products in the liver during DM[65]. In agreement with these reports, we observed a significant decrease in the activities of antioxidant enzymes along with increased lipid peroxidation products in the liver of diabetic rats. Interestingly, CI supplementation could significantly reverse these oxidative changes and thus protected the liver from irreversible tissue damage.

V. Conclusion

CI supplementation significantly ameliorated the complications of diabetic dyslipidaemia, normalized the activities of lipogenic enzymes and protected the liver from oxidative damage. Hence, considering the therapeutic applications of CI as an adjunct in the treatment of diabetes can be a useful pharmacologic overture for the management of diabetes.

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