Original Article

Nephro Protective Effects Of Beta Sitosterol In High Fat Diet-Induced Diabetic Nephropathy In Experimental Rats

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Abstract

Background: Diabetic nephropathy (DN) is one of the most serious complications of diabetes and is frequently associated with death in diabetic patients globally.β-sitosterol is a naturally occurring plant sterol found in rice, wheat bran, cereal grains, and a range of fruits and vegetables. Our previous studies have shown that β-sitosterol significantly improved glycaemic control in skeletal muscle and adipose tissue by facilitating insulin metabolic signalling and by altering pro-inflammatory signalling molecules but renal-protective effects of β-sitosterolhas not been identified.**Aim:** The present study was aimed to analyse whether β-sitosterol can control diabetic nephropathy. **Methods:**Adult male albino rats weredivided into four groups. Group-1: control; Group 2; high fat diet-induced type-2 diabetic rats; Group 3: Diabetic rats treated with β-sitosterol (20mg/kg b.wt, orally for 30 days) and Group-4: Type-2 diabetic rats treated with metformin (50mg/kg.b.wt, orally for 30 days).Insulin, liver and kidney

function makers were analysed in serum and antioxidant enzymes were measured using kidney tissue. **Results:**β-sitosteroltreatment to type-2 diabetic rats significantly reduced ($p<0.05$) liver (ALP, ALT and AST) and kidney function (urea, creatinine) and kidney injury molecule (KIM). It also declined hyperglycaemia, hyper insulinemia and dyslipidaemia compared to control with concomitant increase in tissue antioxidant enzymes**. Conclusion:**Our present findings for the first time show that β-sitosterol attenuates diabetic nephropathy by controlling biochemical profiles recorded in this study. Therefore, β-sitosterol could be considered as a therapeutic drug candidate for the treatment of diabetic nephropathy and associated complications.

KEY WORDS: Antioxidants;insulin resistance; kidney markers; phytosterols.

INTRODUCTION

Diabetic Nephropathy (DN) is characterised by albuminuria greater than 300 mg/ measured at least twice in 3-6 months. In addition to the significant prevalence of DN and the genetic component as one of the risks of DN emphasised by family-based studies, only 20 to 40% of diabetics will develop renal failure [1]. In humans, diabetic albuminuria develops together with the recognisable histological alterations such as glomerular basement membrane (GBM) thickness and mesangial enlargement. Glomerulosclerosis, arteriolar hyalinosis, and tubulointerstitial fibrosis develop when albuminuria increases and renal insufficiency worsens. T1 DM and T2 DM both have a persistent consequence called DN[2]. According to a research published in 1990, 50 percent of Pima Indians with T2 DM had nephropathy after 20 years, while 15 percent of them were in the latter stages of renal failure.The commercially available anti-diabetic medications have severe mechanism-based adverse effects, limited effectiveness tolerability, and limited efficacy, all of which can increase morbidity and mortality, necessitating the development of novel medicines for glycemic control.

Phytosterols are naturally occurring substances that are widely found in higher plants and resemble the structure of cholesterol found in human cells [3]. Numerous scientific studies have suggested that consuming these phytochemicals in large amounts can prevent atherosclerosis and lower serum levels of both total cholesterol and LDL cholesterol [4]. Campesterol (24-methylcholesterol), stigmasterol (22, 24-ethylcholesterol), and β-sitosterol (24-ethylcholesterol) are the three main phytosterols (90 percent). Studies on β -sitosterol's biological activity have risen in recent years among the numerous phytosterols[5]. It is a component of herbal medications used to treat a variety of illnesses, including benign prostatic hyperplasia, prostate cancer, breast cancer, hypercholesterolemia, and coronary artery disease[6]. Studies conducted in vitro and in vivo have shown that β-sitosterol has the ability to decrease cholesterol and mimic the effects of insulin. It has been demonstrated that β-sitosterol causes lipolysis in adipocytes and stimulates glucose uptake (0.1–100 M) in rat primary preadipocytes in a dose-dependent manner[7]. Our previous studies have shown that β-sitosterol significantly improved glycaemic control by regulating the expression of insulin signalling and by modulating the expression of pro-inflammatory signalling molecules in skeletal muscle, liver and adipose tissue in high fat diet and sucrose-induced type -2 diabetic rats. However, it is still unclear whether β-sitosterol can reduce the

risk the development of diabetic nephropathy. Therefore, the present study was to examine the antidiabetic potential of β-sitosterol in the kidney using high high-fat diet and sucrose-induced type-2 diabetic experimental rats.

MATERIALS AND METHODS

Chemicals

The entire chemicals and reagents used in this research were of the molecular and analytical grade acquired from Sigma Chemical Company (St. Louis, MO, USA); MP Biomedicals (Santa Ana, CA 92,707 USA) and Sisco Research Laboratories (Mumbai, India).

Animals

The present study was conducted at central animal house facility, MAHER, Kanchipuram-631552, Tamil Nadu, India as per the guidelines of institutional animal ethics committee (IAEC no: 006/2016dt 04.07.2016). Adult albino rats (180–200g) healthy adult male rats were maintanined in hygienic polypropylene cages in specific humidity (65%±5%) and temperature (21±2°C) with stable 12 h light and 12h dark.

Type-2 diabetes induction in animals

Type-2 diabetes in the experimental was induced based on report of Sampath et al[8]. After the treatment period, rats showed the fasting blood glucose above 120 mg/dL, were considered as type-2 diabetic rats.

Experimental Design

Animals were grouped into five and each group consisted of 6 animals. Normal rats (Group 1); Type-2 diabetic rats (Group 2); Type-2 diabetic rat treated with β-sitosterol (20 mg/kg) was served as group 3; Type-2 diabetic rats treated with metformin (50 mg/kg) was served as group 4. After the completion of treatment period, the animals were anaesthetized, serum was collected. Kidneys from both control and treated animals were removed and used for the assessment of various assays.

Fasting blood glucose (FBG)

On-Call Plus blood glucose test strips (ACON Laboratories Inc., USA) method was used to measure fasting blood glucose levels and concentration of same was displayed in terms of mg/dl.

Oral Glucose Tolerance Test (OGTT)

Oral glucose tolerance in control and treated animals was quantified based on the report of Sampath et al. (2014) by OGTT[8].

Fasting serum insulin

In this study we used rat insulin ultrasensitive ELISA kit (Crystal Chem Inc., USA) in order to measure fasting serum insulin and concentration of insulin in was expressed in terms of ng/ml.

Serum lipid biomarkers

Lipid biomarkers such free fatty acid (FFA), cholesterol, LDL-c, TG and HDL-c were analysed using Spin React biochemical assays kits procured commercially and displayed as mg/dL.

Liver and renal function markers

ALP, ALT, and AST (the liver function markers), creatinine, urea and blood urea nitrogen (renal function markers) in the serum of healthy and treated animals were measured using biochemical kits purchased from Spin React commercially.

Serum electrolytes (Na^+ and K^+)

Serum electrolytes such as sodium and potassium were measured by biochemical methods and the results were expressed as mmol/L.

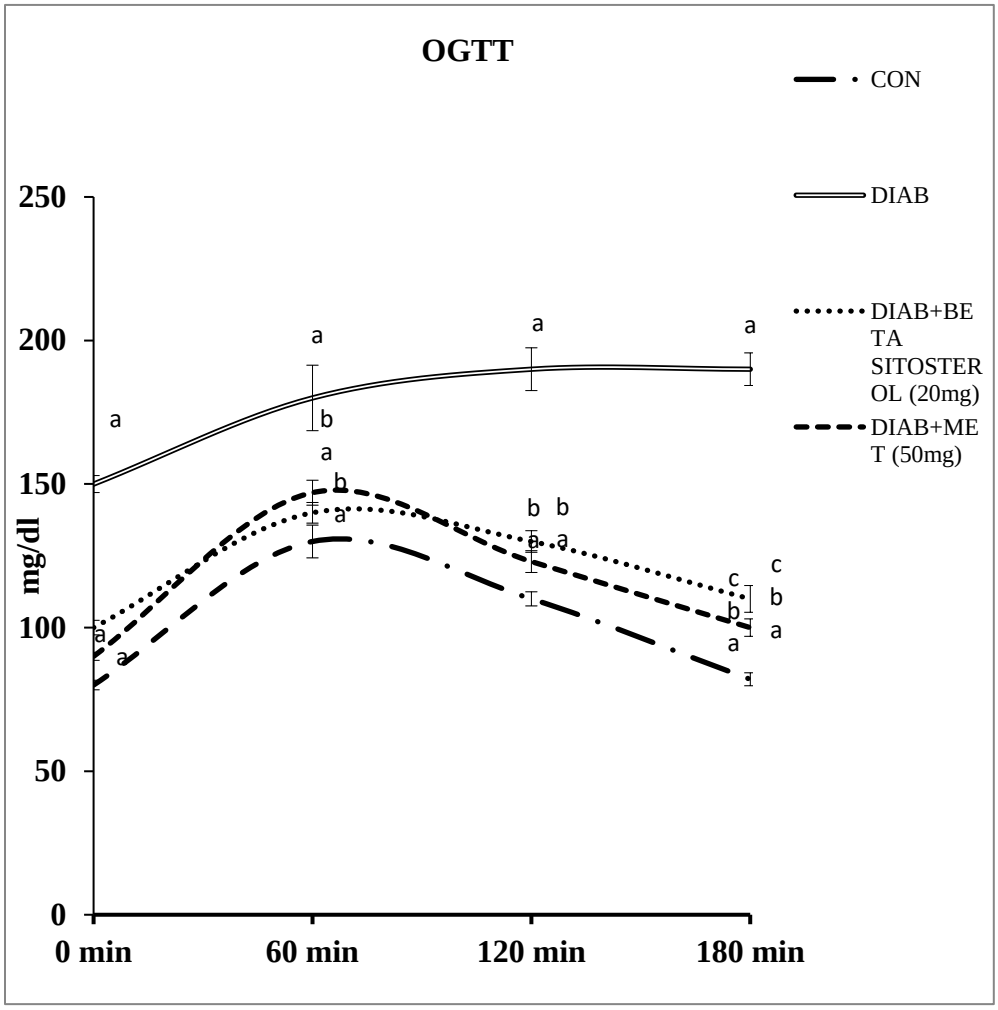
Antioxidant enzymes

Tissue antioxidant enzymes such as reduced glutathione (GSH), Catalase (CAT), super oxide dismutase (SOD) and glutathione peroxidase (GPx) in the kidney tissue were measured using rat ELISA kits procured from Abbkine (Bldg C17, Optics Valley International Biomedicine Park, Wuhan, China, 430223) and results were displayed as ng/L, ng/L, pg/L and Pg/L respectively.

RESULTS

β-sitosterolsitosterol on FBG and OGT

Oral glucose tolerance was significantly improved in diabetic rats treated with the drug β-sitosterol compared to control (Fig.1). Similarly, FBG levels was also significantly reduced by β-sitosterol as efficiently as standard drug metformin (Fig.2).


OGTT
CON
DIAB
DIAB+BETA SITOSTEROL (20mg)
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250
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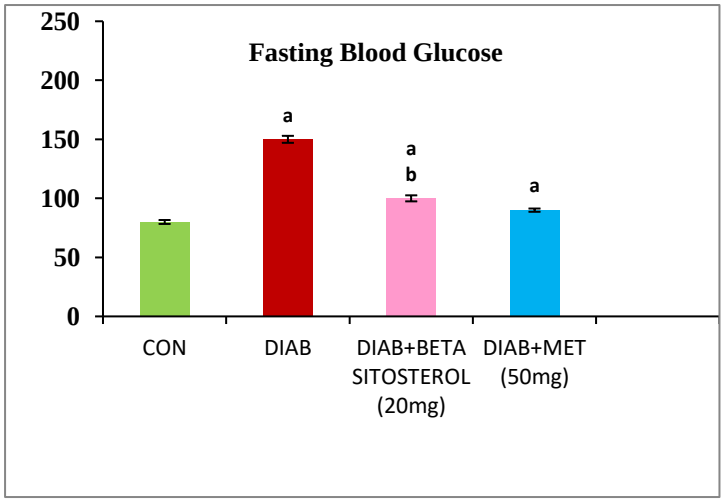
Fasting Blood Glucose
250
200
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100
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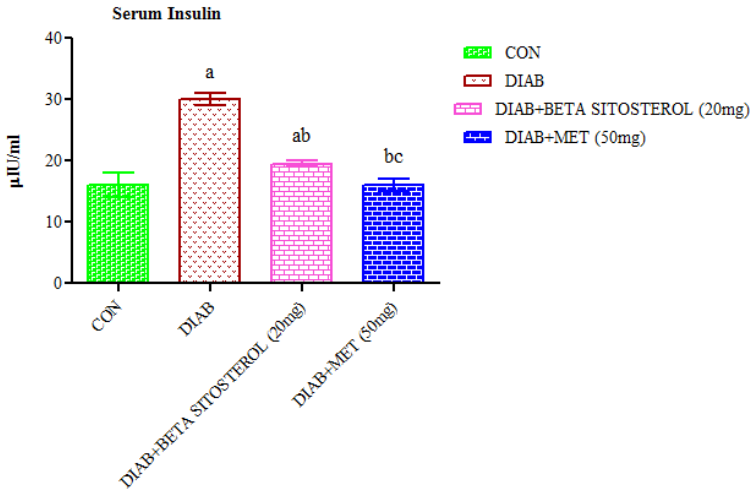
0
CON
DIAB
DIAB+BETA SITOSTEROL (20mg)
DIAB+MET (50mg)

"Fig. 1& 2: Role of β-sitosterolon FBG and OGT. Each column indicates mean ±SEM of sixrats. p<0.05 was considered as significant change, a- comparison to control; b-comparison to diabetes induced; c-comparison with diabetes and β-sitosterol".

β-sitosterolon fasting serum insulin

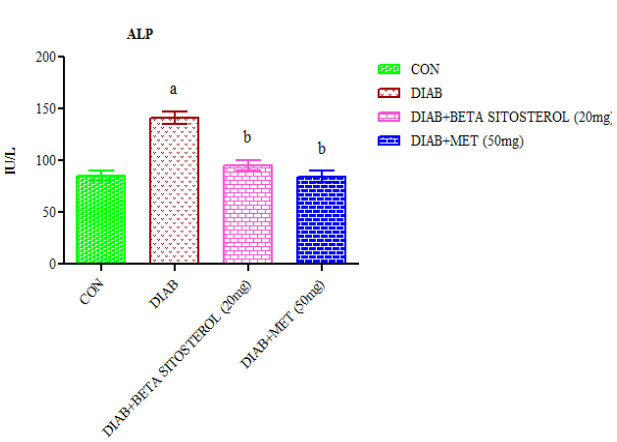
As expected serum insulin levels was significantly reduced in diabetic animals administered with β-sitosterol comparison with control effectively indicating that the compound reduces hyperinsulinemia (Fig.3).

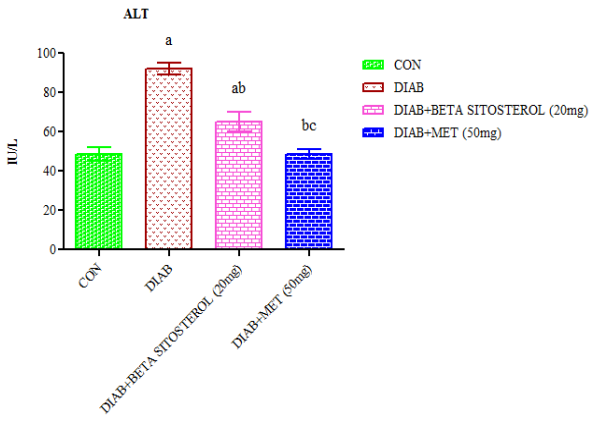


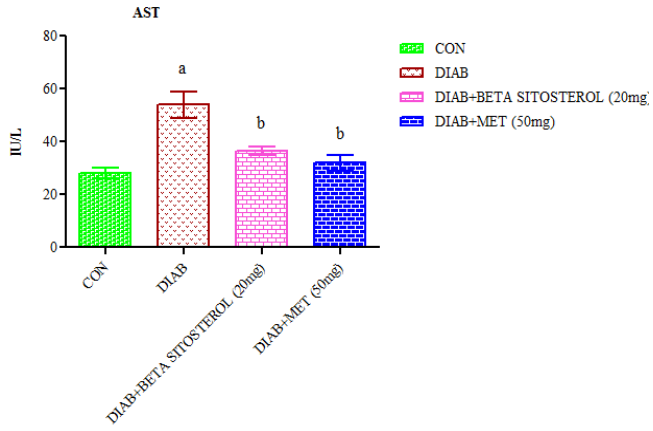
"Fig. 3: Role of β-sitosterol on serum insulin. Each column indicates mean ±SEM of six rats. p<0.05 was considered as significant change, a- comparison to control; b-comparison to diabetes induced; c-comparison with diabetes and β-sitosterol".

β-sitosterolon liver and kidney function markers

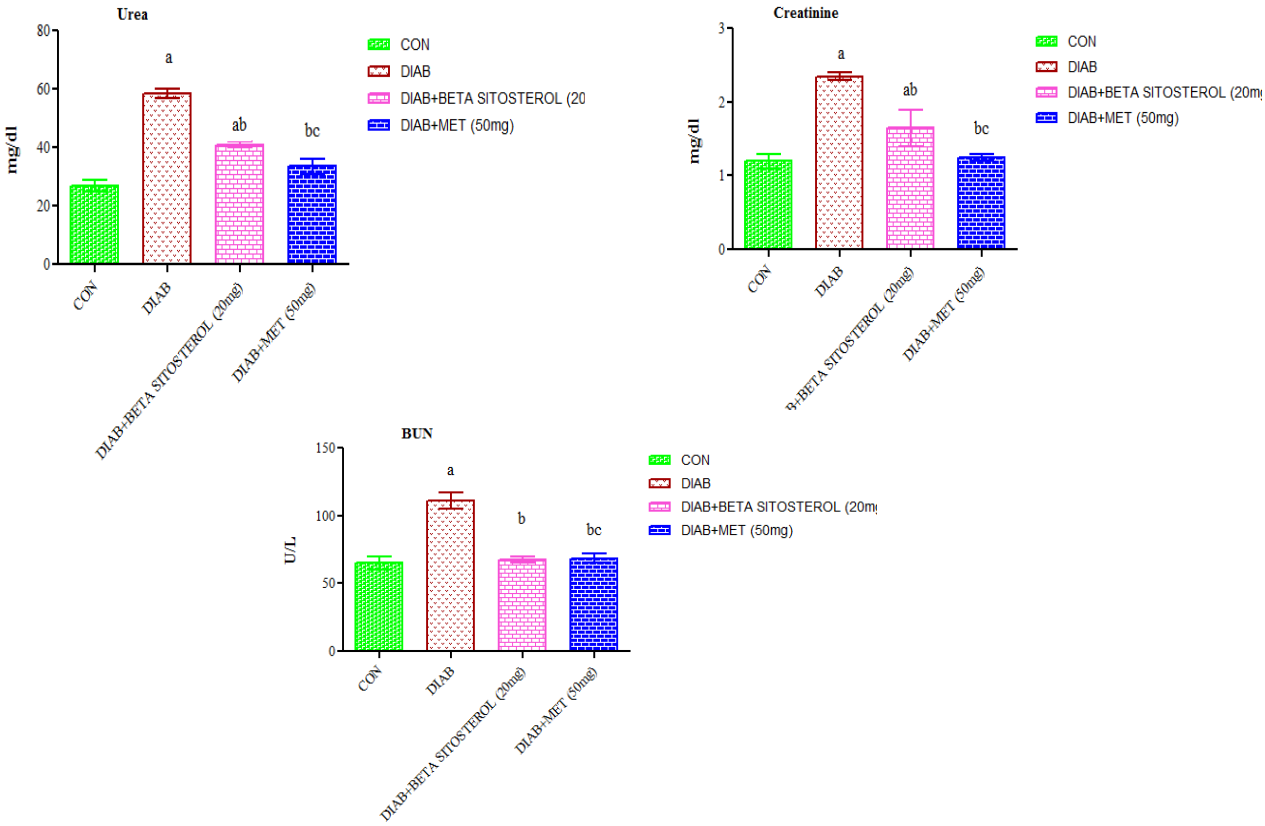
β-sitosterol exhibited a significant reduction in the AST, ALP and ALT in the serum (Fig.4 a-c). Renal function markers (creatinine, urea and blood urea nitrogen) were also effectively reduced by the drug β-sitosterol whose effects were comparable with standard drug metformin (Fig.5 a-c).







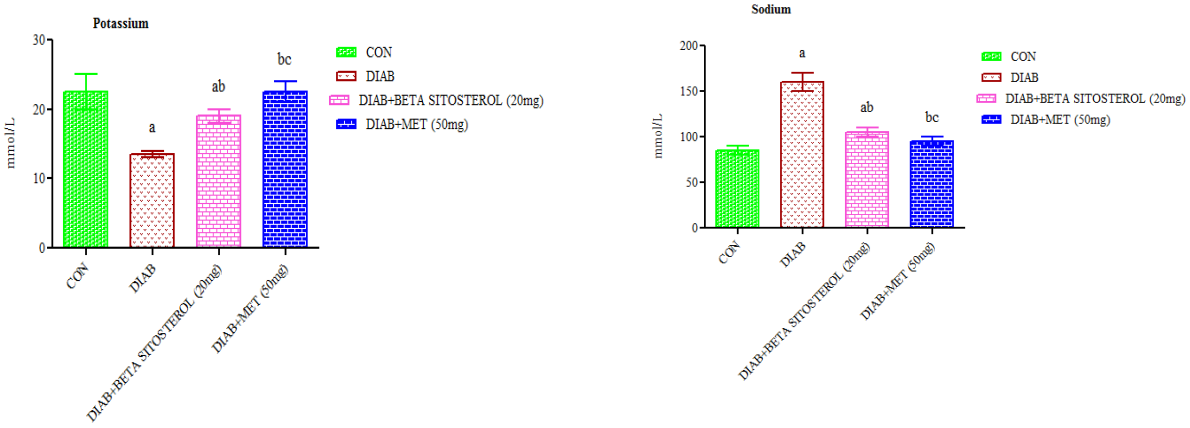
"Fig.4a-c: Role of β-sitosterol on ALT, ALP and AST. Each column indicates mean ±SEM of six rats. $p<0.05$ was considered as significant change, a- comparison to control; b-comparison to diabetes induced; c-comparison with diabetes and β-sitosterol".



"Fig.5a-c: Role of β-sitosterol on creatinine, urea and BUN. Each column indicates mean ±SEM of six rats. $p<0.05$ was considered as significant change, a- comparison to control; b-comparison to diabetes induced; c-comparison with diabetes and β-sitosterol".

β-sitosterolon serum electrolytes

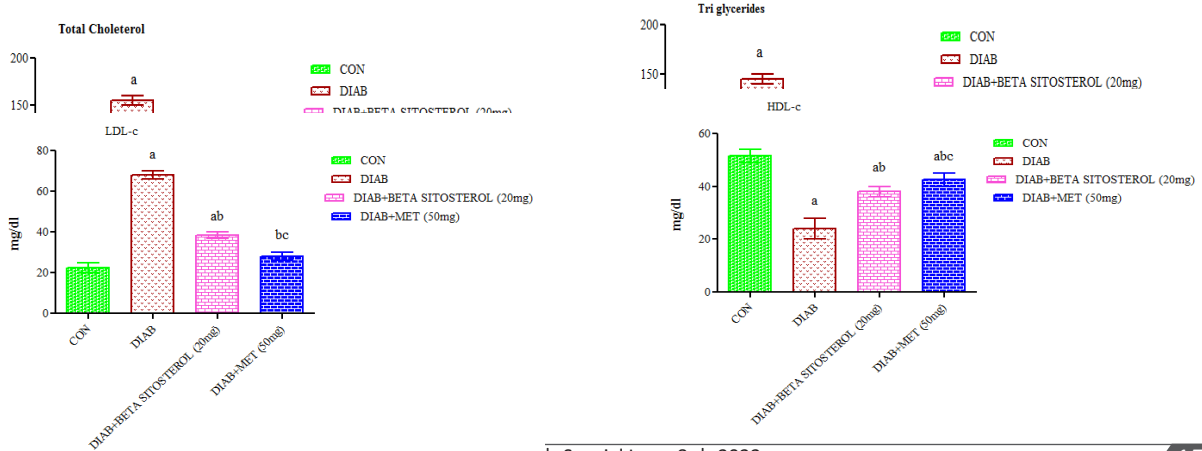
Serum potassium concentration was significantly improved and sodium concentration was reduced in diabetic rats treated with 20mg dose of β-sitosterol. These results clearly show that β-sitosterol significantly regulates serum electrolytes effectively (Fig.6 a and b).



"Fig.6a & b: Role of β-sitosterol on serum electrolytes. Each column indicates mean ±SEM of six rats. $p<0.05$ was considered as significant change, a- comparison to control; b-comparison to diabetes induced; c-comparison with diabetes and β-sitosterol".

Effect of β-sitosterol on serum lipid profile in type-2 diabetic rats

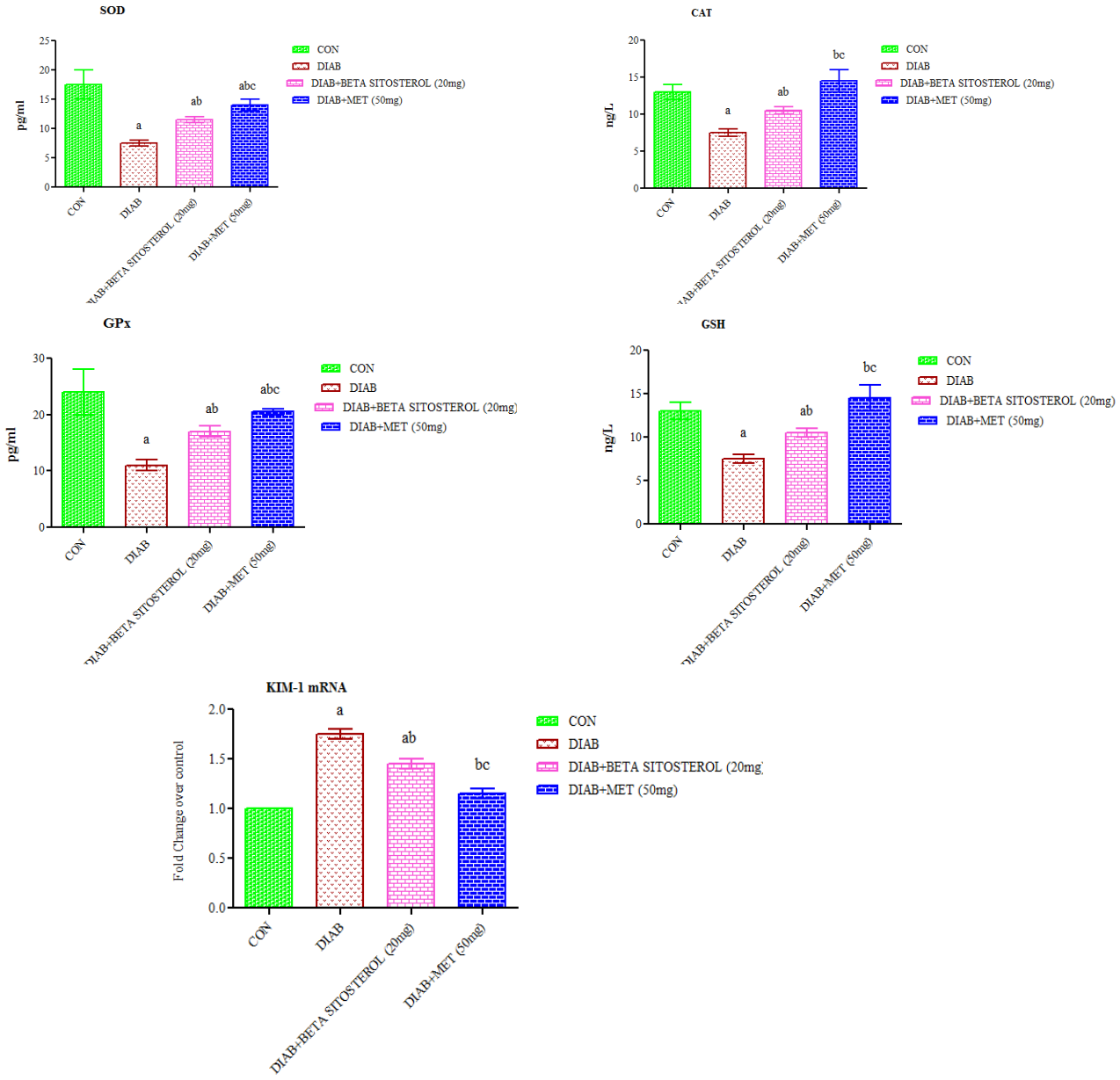
β-sitosterol alleviated dyslipidemia in diabetic rats as effectively as metformin by restoring these lipid profile values to normal level and results of the same are illustrated infigure 7a-d.



"Fig.7 a-d: Role of β-sitosterol on serum lipid markers. Each column indicates mean ±SEM of six rats. $p<0.05$ was considered as significant change, a- comparison to control; b-comparison to diabetes induced; c-comparison with diabetes and β-sitosterol".

β-sitosterol elicits the levels of Antioxidant Enzymes in the kidney of type-2 diabetic rats

In order to check whether β-sitosterol can enhance the levels of antioxidant enzymes in the kidney we measured the levels of GSH, CAT, SOD and GPx by ELISA method. In the diabetic animals treated with β-sitosterolfascinatingly improved these antioxidant enzymes in diabetic animals as effectively as metformin (Fig. 8a-d).



“Fig.8a-d: Role of β-sitosterol on KIM-1 protein levels in the kidney. Each column indicates mean ±SEM of six rats. $p<0.05$ was considered as significant change, a- comparison to control; b-comparison to diabetes induced; c-comparison with diabetes and β-sitosterol”.

DISCUSSION

Diabetes affects 463 million people worldwide and is currently responsible for millions of fatalities. By 2030, there will be 578 million cases of diabetes worldwide, and by 2045, there will be 700 million cases [9]. With an estimated 77 million people in India suffering from diabetes, the biggest number of any country in the world, and with the condition expected to quadruple in prevalence over the next 20 years, diabetes is quickly gaining the status of a possible epidemic. India now has a significant diabetes problem, making it the undisputed diabetes capital of the world. When compared to its population, China is said to have 116.4 million diabetics, but India is the undisputed diabetes capital of the globe. According to reports, lifestyle changes made against the backdrop of nutrition, genetic predisposition, high-fat food (Western diet) intake, and decreased physical activity have resulted in an unusual rise in the prevalence of diabetes in India [9]. Additionally, there are significant regional differences in the prevalence of diabetes and how it is treated.

Numerous scientific studies contend that changes in biology and metabolism serve as fundamental regulators of the prevalence and pathogenesis of obesity and type-2 diabetes. They argue that even a moderate (5 percent) weight loss achieved by low-calorie dieting greatly improves metabolic capacities in adipose tissue, liver, kidney, and muscle insulin sensitivity, as well as pancreatic beta-cell function [10]. In order to find new and anticipated possible treatments for the prevention and treatment of metabolic issues, it is crucial to understand the biology and pathology of adipose tissue. Particularly, a large body of scientific information demonstrating that adipose tissue has recently been discovered thermogenic and endocrine properties strongly suggests that focusing on adipose tissue as a treatment approach is both conceivable and doable[11].

Growing and producing herbal medicines could be beneficial to both economic development and community health in the current situation because of the significant rise in the use of herbal medicines and the emphasis in published studies from developed countries that a significant portion of the medicines they supply have a herbal origin. According to the WHO, the majority of people on Earth (approximately 80%) rely on herbal medicine for their main healthcare needs, which uses plant materials or their bioactive components[12].

Due to their long-term efficacy and safety, natural remedies consistently receive increasing attention in diabetic medications. One such perfect and secure anti-diabetic medication with a significant ability to manage hyperglycemia is SIT. This natural micronutrient has a chemical structure similar to cholesterol, as does the main phytosterol found in nuts, oils, and vegetables. SIT is a useful phytomedicine for treating obesity, diabetes, atherosclerosis, and cancer because of its anti-oxidant, immunomodulatory, antidiabetic, and hepatoprotective effects [13]. More importantly, SIT is thought to be a safe medication for pharmaceutical applications because it has no cytotoxic or genotoxic effects on experimental mice. In the adipose tissues of obesity-induced type-2 diabetic rats, our prior research has demonstrated

that SIT regulates hyperglycemia and insulin resistance by increasing insulin signalling through activation of insulin receptor and glucose transporter 4 (GLUT 4) proteins [14].

The main cause of diabetes complications is abnormalities in the metabolism of protein, lipids, and carbohydrates. A chronic metabolic condition called diabetes is characterised by either insufficient insulin production, insulin action resistance, or both. It is linked to anomalies in the metabolism of proteins, carbohydrates, and lipids, which result in hyperglycemia, hyperlipidemia, hyperinsulinemia, and hypertension [15]. In high fat diet and sucrose produced diabetic rats, the oral effective dose of β -sitosterol (20 mg/kg body weight) was administered once daily until the completion of the trial (30 days post induction of diabetes). At the conclusion of the trial, antioxidant enzymes (SOD, CAT, GR, GPx, GSH, and GST) were evaluated after biochemical tests such as fasting blood glucose (FBG), oral glucose (OGT) and tolerances (IT), Homeostasis Model Assessment for Insulin Resistance (HOMA-IR), and Quantitative Insulin Sensitivity Check Index (QUICKI), serum lipid profile (LDL, HDL, VLDL, TC, and FFA.

The high fat diet introduction in the current study's rats resulted in a considerable increase in body weight. On the other hand, therapy with β-sitosterol resulted in a considerable decrease in body weight because of a possible hypocholesterolemic impact. Rats fed a high-fat diet displayed greater FBG and fasting blood insulin levels as a result of hyperglycemia brought on by insulin resistance. However, the increased insulin sensitivity and insulin receptor-mediated increase in glucose uptake and oxidation by β -sitosterol may be the cause of the lower levels of FBG and insulin seen in the current study.

Elevated levels of serum liver and kidney function indicators, a sign of the organs' functional impairment, were induced by a high-fat diet. The liver is essential for the metabolism of protein, fat, and carbohydrates. The metabolic products of those pathways and the enzymes involved in those metabolisms that are more susceptible to the occurrence of abnormalities may be viewed as biomarkers for liver dysfunction [16]. When a person consumes a high fat diet, their liver often experiences turbulence in their metabolism, which causes distinct variations in their serum biomarker enzyme activity. As they are present in the cytoplasm and released into the blood circulation after cellular damage, the most prevalent indicators like AST, ALT, and ALP are used in the diagnosis of liver injury [17]. The levels of AST, ALT, and ALP were dramatically lowered after treatment with beta-sitosterol. investigates how scavenging the free radicals caused by a high-fat diet protects the structural integrity and membrane stability against oxidative damage[18]. Subsequent Increased levels of urea and creatinine, which are possible biomarkers of renal impairment, were seen in diabetic rats. According to Duan et al. (2018), a high-fat diet-induced inflammation and hyperglycemia can result in higher levels of cytokines (TNF- and IL-6) that are toxic to glomerular epithelial and mesangial cells and cause kidney injury[19]. Sharmila et al. (2016) have clarified the protective effect of -sitosterol on DEN and Fe-NTA treated nephrotoxicity induced male albino Wistarrats[20], which is consistent with the current study's findings on the ameliorative effect of -sitosterol on kidney function. Due to the presence of active functional groups like a four-ring steroid nucleus with five and six carbon double bonds and three hydroxyl groups, β -sitosterol treatment has been proposed to protect renal cell membranes from damage against nephrotoxicants by improving antioxidant status

through scavenging elevated ROS generation and play a role in the stabilisation of phospholipid bilayers in cell membranes[20].

Dyslipidemia, which is a significant factor in the development and consequences of diabetes, is a further significant issue. According to the current study, rats fed a high-fat diet and sucrose had significantly higher blood levels of cholesterol, triglycerides, and free fatty acids. This could be because the liver is producing more lipids at a higher rate and exchanging them with plasma lipids, according to Padmanabhan et al. (2014)[21]. The dietary fat contributes to raised blood lipid levels by providing precursor molecules for endogenous lipid production. In type-2 diabetic rats, an elevated level of LDL was found, which may be related to reduced LDL receptor activation, which is responsible for the removal of LDL from plasma. The present study found a significant increase in cholesterol levels, which may have been caused by the suppression of the genes for LDL receptor, which results in an accumulation of LDL in plasma[22]. According to Klop et al. (2013), the exchange of triglycerides for cholesterol esters between triglyceride-rich lipoproteins (VLDL) and cholesterol esters-rich lipoproteins (LDL, HDL) is mediated by cholesteryl ester transfer protein[23]. In the adipose tissue of high fat and sucrose fed diabetic rats, treatment with -sitosterol significantly reduced the levels of FFA, cholesterol, triglyceride, and LDL while increasing the levels of HDL, which may be the result of inhibiting cholesterol absorption and influencing/regulating the cholesterol metabolism.

The biological system is shielded from oxidative damage by antioxidant defence mechanisms. Enzymatic and non-enzymatic routes are among the two categories. Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase are the three main enzymatic antioxidants (GSH-Px). Cu/Zn SOD (SOD1), Mn-SOD (SOD2), and SOD3 are the three SOD isoforms that are found in the cytosol, mitochondria, and extracellular matrix, respectively. It changes superoxide into H2 O2, which CAT, a hememetalloenzyme found in mitochondria, peroxisomes, cytoplasm, and the nucleus, then breaks down into oxygen and water. Additionally, GSH-Px (selenoprotein), which is expressed in both intracellular and extracellular environments, contributes to the breakdown of lipid peroxides. In the presence of reduced glutathione (GSH), which is oxidised into glutathione disulfide (GSSG), and then reduced to GSH by the action of glutathione reductase, it transforms hydroxyl radicals and peroxides into harmless forms[24].

The control of cellular redox status and the prevention of the generation of lipid peroxidation are both significantly aided by the detoxification of hydroxyl radicals. The reduction in the GSH:GSSG ratio, tocopherols, ascorbic acid, cysteine, and methionine amino acid levels mimics the cellular redox status. Flavanoids, polyphenols, sterols, and anthocynins are examples of dietary herbal antioxidants (Exogenous antioxidants) that serve as ROS scavengers [25]. In high fat-fed type-2 diabetic rats, levels of enzymatic antioxidants (SOD, CAT, GPX, GR, and GST) and non-enzymatic antioxidants (GSH) were significantly decreased. This may be due to an increase in the use of antioxidants in scavenging free radicals to protect cells from ROS-mediated damage and lipid peroxidation, whereas β-sitosterol treatment reversed the altered antioxidant levels close to normal levels in diabet According to the current finding, Radika et al. (2013) revealed that administering β -sitosterol to high-fat diet and sucrose-fed rats decreases oxidative and nitrosative stress by increasing NO levels and decreasing lipid formation[26].

The renal tissues in high fat diet-induced nephrotoxic rats showed dilated congested vascular space and aggregation of inflammatory cells between the renal tubules. Evident histological alterations in β-sitosterol treated rats showed mild condensed glomerulus and mild aggregates of inflammatory cells. This study clearly showed the nephroprotective potential of β-sitosterol. In diabetes-induced nephrotoxic rats, mRNA level of KIM-1 was significantly increased. Oral effective dose of β-sitosterol significantly restored the altered levels of the mRNA to that of the normal range.

CONCLUSION

Our study concludes that β-sitosterol significantly reduced diabetic nephropathy by modulating high fat diet and sucrose-induced detrimental changes in the levels of renal biomarkers, kidney injury molecules-1, liver function makers, serum electrolytes and antioxidant enzymes as a result of hyperglcaemia, dyslipidaemia, in the renal tissues. Hence, for the first time we report that β-sitosterol has the potency to reduce diabetic nephropathy and hence it may be considered as a therapeutic drug candidate for the treatment of diabetic kidney disease and associated complications. Further studies on the effect of β-sitosterol on the expression of insulin and inflammatory signalling molecules in the kidney tissue needs to be done in order to potentiate the mechanisms of action.

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DECLARATION OF CONFLICTS OF INTEREST

The authors report no conflicts of interest.

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