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Evaluation of Methyl-3,4 Dihydroxybenzoate for Renal Protection in Rat Model of Streptozotocin–Nicotinamide-Induced Diabetic Nephropathy

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ABSTRACT

Diabetic nephropathy (DN) is a serious microvascular impediment accompanying with type 2 diabetes that is characterized by progressive kidney damage resulting from increased oxidative stress and metabolic disruption. Many studies have described the antioxidant activity of phenols; as such, it can be speculated that these compounds may be used to treat some of the complications of diabetes. In this context, the current study investigated the nephroprotective efficacy of Methyl-3,4 Dihydroxybenzoate (MDHDB) against streptozotocin-nicotinamide-induced diabetic nephropathy (DN) in Wistar rats. DN was developed in Wistar rats using streptozotocin (40 mg/kg) given intraperitoneally 15 min after nicotinamide (100 mg/kg) administration. After confirming the presence of diabetes, the rats were divided into four groups: normal control, disease control, or two experimental groups receiving MDHDB at either 200 mg/kg or 400 mg/kg. Fasting blood glucose (FBG), body weight, food intake and water intake were assessed. In addition, The renal function test and the lipid profile were done. The status of oxidative stress markers were also carried out in the renal tissue. Finally, the the histopathology of the kidney was analyzed. MDHDB treatment lead to a reduction of fasting blood glucose (FBG) ,HbA1c and the symptoms of polydipsia and anorexia were enhanced in diabetic rats. Both of the doses of MDHDB exhibited a dose-dependent repair of the renal function indicators in diabetic rats, with the highest dose (400 mg/kg) exhibiting significant decreases in serum creatinine, blood urea nitrogen, and total proteins. Therefore, the results of this investigation indicate that MDHDB exhibit nephroprotective effects against DN through its multiple mechanisms including antihyperglycemic activity, improvement of oxidative stress indicators, improvement of lipid metabolism, and maintenance of the structure of the kidneys. .

Keywords: *diabetic nephropathy, Methyl-3,4 Dihydroxybenzoate, streptozotocin, nephroprotection, antioxidant enzymes*.

INTRODUCTION

Diabetes Mellitus is a global health disaster, estimated to affect 463 million people currently, and projected to increase by 50% till 2045 [1]. DM is a chronic metabolic disorder defined by persistent hyperglycemia resulting from an inability to produce enough insulin, poor sensitivity to insulin, or a combination of these factors. The pathology of diabetes can involve

many different organs and organ systems, and results in devastating microvascular and macrovascular complications, and therefore affects the morbidity and mortality of patients [2].

One of the major chronic complications of DM is Diabetic Nephropathy (DN), which is considered to be one of the most clinically relevant and costly complications of DM [3]. It is estimated that nearly 20-40% of diabetic patients have certain stages of DN, and it is estimated that DN accounts for almost 50% of the causes of End Stage Renal Disease (ESRD) worldwide and accounts for almost half of the individuals who require renal replacement therapy [4]. Patients with DN are expected to progress through stages of the disease until they reach ESRD, which usually occurs within a time frame of 5-10 years and results in higher healthcare costs and decreased life quality.

The pathogenesis of DN is a complex interaction of inflammatory, hemodynamic, and metabolic processes [5, 6, 2]. Multiple deleterious pathways are initiated by chronic hyperglycemia, this involves stimulation of the polyol pathway, leading to sorbitol accumulation and osmotic imbalance; enhanced formation of advanced glycation end products (AGEs), which modify the structure of proteins and trigger pro-inflammatory receptor signaling [7–8]; specific protein kinase C (PKC) isoforms activation that disrupt vascular permeability; and increased hexosamine biosynthetic pathway activity, resulting in altered gene expression [9].

Oxidative Stress, characterized by Reactive Oxygen Species (ROS) overproduction that overwhelms the human body ability to neutralize them, is central to the pathogenesis of DN [5,6]. Mitochondrial Superoxide Overproduction in response to hyperglycemia serves as the link between the multiple metabolic pathways that result in diabetic complications [10]. The oxidative damage produced by ROS causes injury to cellular macromolecules (lipids, proteins, DNA) eventually triggering cellular dysfunction and death [10].

In addition to oxidative stress, hemodynamic changes also contribute to the expansion of DN. Changes in glomerular filtration rate, increased intraglomerular pressure, and changes in renal autoregulatory responses contribute to the development of progressive glomerulosclerosis and tubulointerstitial fibrosis [4]. Clinically, these changes are manifested as proteinuria, hypertension and a decay in glomerular filtration rate (GFR) [4] .

The common of contemporary DN management is based on: i) controlling blood glucose levels; ii) managing hypertension with RAAS inhibiting drugs and iii) reducing lipids [11]. Although these approaches are able to delay DN disease progression, none are capable of arresting or reversing established DN [11]. The recent introduction of drugs such as GLP-1 receptor agonists and SGLT-2 inhibitors which show some promise [12], neither of these new classes of drug are able to target all of the pathophysiological processes involved in DN including oxidative stress [13]. Consequently, there is an urgent necessity to develop additional drugs which can target multiple aspects of DN disease simultaneously. A promising alternative to conventional drugs may be natural products with antioxidant properties since they exhibit multi-targeted actions and generally have favorable safety profiles [14].

Phenolic compounds are a various group of secondary metabolites defined by the presence of one or more hydroxyl substituents on an aromatic ring [15]. They have attracted significant attention in drug discovery and therapeutic research due to their antioxidant capacity, anti-inflammatory, and metabolic modulatory activities [15]. Phenolics exert antioxidant activities primarily through three mechanisms: ROS neutralization, pro-oxidant metal ions sequestration, and endogenous antioxidant defense systems upregulation [15]. Evidence from both clinical investigations and experimental models indicates that increased dietary consumption of phenolic compounds is linked to a reduced incidence of diabetes-related complications [16]. In particular, the specific phenolic compounds, resveratrol, quercitin and curcumin, have been reported to have protective effect against kidney damage in experimental models through mechanisms involving Nrf2 pathway activation, NF-κB inhibition and AMPK regulation [17]. MDHDB is a phenolic compound in which may be isolated form many plants as *kalimerisindic, Dioscorea alata L etc* [18, 19]. The neuroprotective effect of TBHP-induced oxidative damage in SG-SY5Y Cells has been well-studied [20]. In addition, It has previously been found to have significant antioxidant activity in DPPH radical scavenging assays. The position and number of the hydroxyl groups of phenolic compounds determine their antioxidant efficacy and biological activities. Since it is now extensively recognized that oxidative stress acts a essential role in the pathogenesis of DN [18] and since phenolic compounds have proven antioxidant properties, MDHDB appears to be a logical candidate for nephroprotection evaluation.

The streptozotocin (STZ)-nicotinamide (NA) induced DM model is a well-established method for investigating the consequences of Type 2 Diabetes Mellitus (TD2) and its complications [21]. STZ, a glucosamine-nitrosourea compound, induces pancreatic beta cells' selective destruction by alkylating DNA and generating ROS. Pre-treatment of rats with NA, a poly(ADP-ribose) polymerase inhibitor and antioxidant, diminishes the degree of STZ-induced beta cell necrosis and produces mild insulin deficiency similar to T2D [21]. This model provides a number of advantages including: (1) a stable level of moderate hyperglycaemia; (2) retention of some residual beta cell function; (3) development of microvascular complications including nephropathy; and (4) cost effectiveness and reproducibility [22]. Renal changes consistent with nephropathy typically occur in this model after 4-6 weeks, including: increased urinary albumin excretion; elevated serum creatinine and urea; glomerular hypertrophy; and mesangial expansion [22].

Although substantial progress has been achieved in elucidating the molecular and cellular processes involved in the diabetic nephropathy (DN) pathogenesis, therapeutic strategies capable of targeting multiple disease-driving pathways remain limited. Accordingly, this study aimed to evaluate the nephroprotective efficacy of MDHDB in diabetic nephropathy using the streptozotocin–nicotinamide (STZ–NA) model and to test the hypothesis that MDHDB will reduce the severity of DN via a combination of antihyperglycemic, antioxidant and renoprotective actions.

MATERIALS AND METHODS

Materials

All chemicals and reagents utilized in this research were of analytical quality. The STZ and NA used in this research were acquired from Sisco Research Labortaories Pvt.Ltd (SRL)-Mumbai-400 099, Maharastra, India. The MDHDB compound was procured from Avra Laboratories Pvt.Ltd-Hyderabad-500076-Telangana-India. The vehicle for drug administration was carboxymethyl cellulose (CMC). Commercially manufactured standard biochemical assay kits were also procured for measuring fasting blood glucose, glycosylated hemoglobin, serum creatinine, blood urea nitrogen, blood total protein, and lipid profile.

Experimental Animals

Male Wistar rats and Swiss Albino mice that weighed between 180-220g and 20-25g were 8-10 weeks old were used in the study. Those animals were obtained from Vyas Labs, Hyderabad, Telangana-501301, India. Those animals were then housed in an animal house at the Pharmacology division of College of Pharmaceutical Science, Andhra University, 530003 and kept in standard laboratory conditions, 12/12h light/dark, a temperature of 22 ± 2 °C, relative humidity of 55 ± 10%. They were fed with the standard food and had free access of water. The ethical approval was acquired from the Institutional Animal Ethics Committee (IAEC) (IAEC-15/AU-Pharma/2025-26), and they were carried out according to the CPCSEA guidelines.

Acute Toxicity Study

Before evaluating the effectiveness of MDHDB, an acute toxicology assessment was performed using the OECD Guidelines 423 (Acute Toxicity Class Method) [18] to evaluate potential toxicity from oral administration of MDHDB. Mice (female Swiss albino) were utilized for toxicity evaluation, n=3 per group were evaluated per treatment group. A sequential dosing paradigm was employed, in which four different concentrations of MDHDB were administered orally: 5, 50, 300, and 2,000 mg/kg body weight. The initial concentration that would be used in the study was 2,000 mg/kg, based on the results of preliminary toxicity evaluations.

Observations were conducted immediately post-administration for 4 h regarding any symptoms of toxicity exhibited by the animal, such as alterations in behavior, motor function, hair and fur appearance, eye, mucous membrane, respiratory pattern, cardiovascular signs, autonomic responses, and any other central nervous system (CNS) manifestation. Animals were also evaluated at 24 h post-administration and daily for 14 days post-administration, noting any mortalities or morbidities, changes in body weight, and any gross abnormalities noted. Due to no mortalities or adverse reactions being reported during the toxicity testing at the highest concentration tested (2,000 mg/kg), the two lower concentrations were identified as follows: 1/5th of 2,000 mg/kg = 400 mg/kg, and 1/10th of 2,000 mg/kg = 200 mg/kg for use in subsequent efficacy studies [23].

Induction of Diabetes and Experimental Design

Diabetes Induction Protocol

The DN was developed by utilizing a widely accepted STZ-NA model [16]. Animals were given an overnight fast (approximately 16 h) with unrestricted access to water. After the fast, 100 mg/kg body weight of nicotinamide was administered intraperitoneally with the assistance of normal saline (0.9% NaCl). NA was directed 15 mins prior to the administration of freshly prepared STZ solution (40 mg/kg body weight), which had been dissolved in 0.1 M cold citrate buffer (pH 4.5). Citrate buffer used for STZ was made immediately before use and kept on ice at all times, as it is known that if this buffer is not refrigerated, the STZ will be unstable [24].

Control animals received equal amounts of citrate buffer and normal saline. In addition, following STZ-NA administration, animals were allowed to drink 10% glucose solutions for the first night post-administration to help reduce the risk of hypoglycemia due to the large amount of insulin released by damaged beta cells early in the course of diabetes [24].

Confirmation of Diabetes

Fasting Blood Glucose (FBG) was determined by glucometer on rats at the 7th day of induction after an 8-hour fasting. The rats were classified as diabetic if their FBG values were ≥ 250 mg/dL or excluded from further studies if their FBG values were less than 250 mg/dL [16].

Experimental Groups and Treatment Protocol

Successfully diabetic rats were randomly allocated into four experimental groups (6 animals per group)

The four experimental groups included:

• Group I (Normal Control): non-diabetic rats that received orally, 1% CMC solution.

• Group II (Disease Control): diabetic rats that received orally, 1% CMC solution.

• Group III (MDHDB Low Dose): diabetic rats that received orally, MDHDB 200 mg/kg dissolved in 1% CMC solution.

• Group IV (MDHDB High Dose): diabetic rats that received orally, MDHDB 400 mg/kg dissolved in 1% CMC solution.

DN was allowed to develop over the course of four weeks (28 days) after diabetes was induced in all the rats [6].

Treatment of DN with MDHDB was initiated on day 28 and lasted for an additional three weeks (21 days). The treatment was given once daily by gavage between 9:00 and 10:00 a.m.

Assessment Parameters

Body Weight, Food and Water Intake

Body weight, food and water intake were measured in each rat at baseline (on day 0), on day 7th (when diabetes was confirmed), on day 28th (the start of treatment), on day 35th (one week after treatment began), on day 42nd (two weeks after treatment began), and on day 50th (at the end of the study). Body weights were assessed for each rat individually every week during the course of this experiment on a calibrated electronic balance. Food and water consumption were measured weekly by determining the quantity of food or water that had been used and subtracting it from the amount of food or water that had been made accessible to the rats over a 24 h period. The results of these measurements were expressed as grams per week for food consumption and milliliters/week for water consumption.

Fasting Blood Glucose Monitoring

FBG levels were assessed in each rat at baseline (on day 0), on day 7th (when diabetes was confirmed), on day 28th (the start of treatment), on day 35th (one week after treatment began), on day 42nd (two weeks after treatment began), and on day 50th (at the end of the study), Blood glucose concentrations were determined with a hand held glucometer after the rat had been allowed to fast for eight hours. Two determinations were made, and then averaged to obtain one value.

Sample Collection

Blood Sample and Tissue Collection

At the termination of treatment duration, the blood sample were collected from the jugular vein under anesthesia for biochemical parameters assessment. [16].

Subsequent the collection of blood samples, each rat was euthanized via cervical dislocation and the kidneys were excised for oxidative stress markers and histopathology [25].

Biochemical Parameters Assessment

Glycosylated Hemoglobin (HbA1c)

HbA1c was evaluated in whole blood by means of commercial kits that utilized either ion exchange chromatography or immunoturbidimetric methods [26], and results are reported as (%) of total hemoglobin.

Renal Function Parameters

Serum Creatinine was assessed by the Jaffe Alkaline Picrate Method in which creatinine reacts with picric acid in an alkaline medium forming a chromogenic compound measured spectrophotometrically at 520 nm.

Assay for Blood Urea Nitrogen (BUN) via enzymatic assay employing the urease-glutamate dehydrogenase (UDH-GLDH) reaction in which urease catalyzes the hydrolysis of urea to ammonia, which is then measured utilizing the enzyme -coupled reaction involving glutamate dehydrogenase

Assay for total serum protein via the Biuret Reaction, in which proteins react with copper ions in an alkaline solution to produce a violet-colored complex measured at 540 nm.

Measurements of all biochemical parameters were performed according to the manufacturer's protocol utilizing a semi-automated analyzer [27, 28].

Lipid Profile Assessment

Serum lipid parameters were assayed using enzymatic colorimetric assays [27, 28]

Oxidative Stress Markers in Kidney Tissue

Catalase Activity Assay

The catalase activity was determined as described by Cohen et al. with some alterations. The capacity of catalase to break

down hydrogen peroxide (H_2O_2) was used to calculate its activity. thus, the quantity of H_2O_2 degraded is directly proportional to enzymatic activity. The assay principle involves monitoring the rate of H_2O_2 breakdown in the presence of catalase. Briefly, 5 mL of 30 mM H_2O_2 was added to test tubes containing 0.5 mL of tissue homogenate supernatant to start the reaction. One milliliter of 3 M sulfuric acid (H_2SO_4) was added to stop the reaction after three minutes. After adding 7 mL of potassium permanganate ($KMnO_4$), the absorbance was measured right away at 480 nm using a spectrophotometer [29].

Results were expressed as units per mL of tissue homogenate [29].

Superoxide Dismutase (SOD) Activity Assay

SOD activity was determined utilizing the pyrogallol autoxidation assay [30]. The assay uses the inhibition of pyrogallol autoxidation as an indicator of SOD. Pyrogallol solution (60 mM) was prepared in 1mM HCl at 37°C and combined with 0.05M Tris-HCl buffer (pH 8.4) containing 1 mM Na_2 EDTA to eliminate interference from metal ions. Buffer was used to adjust the final volume to 3000µL.

Control readings were generated by determining the absorbance of the buffer/pyrogallol mixture (without sample) at 325nm over 5 min with 30-second intervals. Absorbance readings for test samples were generated after the addition of pyrogallol solution to buffer containing the tissue homogenate; total volume was adjusted to 3000µL, and absorbance read at 325nm over 5 min @ 30 sec intervals.

The scavenging ability of superoxide anion ($\bullet O_2^-$) was calculated as absorbance increments using Eq.4:

$$\%\ Inhibition = \frac{[\Delta A\ \text{control/T} - \Delta A\ \text{sample/T}]}{\Delta A\ control/T} \times 100 \qquad (3)$$

Enzyme activity was expressed as units per milliliter of tissue homogenate.

Reduced Glutathione (GSH) Estimation

GSH levels were determined using Ellman's method [31]. A standard calibration curve was generated using known concentrations of reduced glutathione, and results were expressed as µM of GSH per gram of tissue.

Lipid Peroxidation Assessment

Malondialdehyde (MDA) was the main indicator used to measure thiobarbituric acid–reactive compounds (TBARS) in order to measure lipid peroxidation [32]. MDA and thiobarbituric acid (TBA) combine in acidic environments to produce a pink chromogen.

The resulting supernatant absorbance was measured at 532 nm. MDA levels were calculated using Eq. 5 :

$$Concentration\ \left(\mu\frac{\text{mol}}{\text{g}}\right) = \frac{Absorbance}{Molar\ extinction\ coefficient} \times 10^6 \qquad (4)$$

Histopathological Examination

Tissue Processing

Kidney tissues secured in 10% buffered formalin for at least 2 days were processed for histopathological examination. The tissues underwent dehydration through categorized series of alcohols (70%, 80%, 90%, 95%, and absolute alcohol), clean in xylene, and infiltrated with molten paraffin wax. Tissue blocks were prepared by embedding in paraffin and stained in hematoxylin and eosin (H and E) [33].

Microscopic Examination and Scoring

Stained sections were assessed under a light microscope at ×10. Histopathological changes were evaluated by an experienced pathologist blinded to treatment groups. Three histopathological parameters were evaluated: (i) coagulative necrosis of tubular epithelial cells, (ii) intratubular debris, and (iii) dilated hypocellular tubules. Each parameter was graded using a semi-quantitative scoring system [34] as follows,

Score 0, absence of detectable cellular damage; score 1, focal cellular injury involving <30% of the tissue; score 2, focal damage affecting 30–50% of the tissue; score 3, extensive yet localized lesions involving >50% of the tissue; and score 4, diffuse or global tubular necrosis.

Multiple fields were examined for each section, and mean scores were calculated for each animal [34].

Statistical Analysis

All experimental results are expressed as mean ± standard error of the mean (SEM). Statistical analyses were performed using GraphPad Prism software (version 9.0). For parameters assessed at a single point, including fasting blood glucose levels, body weight, food, and water intake, biochemical parameters, lipid profiles, oxidative stress markers, and

histopathological scores that were determined at a single and multiple time points, we used a one-way and two-way ANOVA, along with the Tukey multiple-comparison post-hoc test to compare among multiple treatment groups. Also utilized the Kruskal-Wallis test (a nonparametric one-way ANOVA) to evaluate histopathological scores, with subsequent application of Dunn's multiple-comparison test.

Considered a p-value ≤ 0.05 to be statistically significant [11,12]. The specific comparisons made include:

• disease control group vs. normal control group (denoted by *)

• treatment groups vs. disease control group (denoted by @)

Established the threshold for statistical significance at $\alpha = 0.05$; therefore, considered p-values < 0.05 to be statistically significant (ns = not significant, $p > 0.05$).

Results and Discussion

Effect of MDHDB on Experimental Animals Body Weight

DN markedly affected body weight changes. Compared to the normal control group, the disease control group exhibited a progressive and statistically significant ($p < 0.05$) reduction in body weight over the course of the study. On day 0, there were no discernible variations in the four groups' mean body weight.

Following the administration of STZ-NA and the subsequent development of DN, however, the disease control rats demonstrated a significant weight loss.

By day 50th, animals in the disease control group exhibited a significant decrease in body weight compared to normal controls (Figure 1). Treatment with MDHDB with both doses produced a modest, non-significant (P>0.05) improvement compared to the disease control group.

Analysis of the weekly body weight changes demonstrated that the normal control animals demonstrated a consistent increase in body weight over the course of the study, whereas the disease control animals demonstrated a progressive decline in body weight beginning shortly after the onset of diabetes (Figure 2). Additionally, the MDHDB treated groups demonstrated a non significant stabilization compared to the 1st week.

Weight loss despite polyphagia is a distinctive feature of uncontrolled diabetes, representing a net negative energy balance as a result of impaired glucose use, gluconeogenesis, proteolysis, and lipolysis [29]. Consistent with these findings, the disease control animals in the current study demonstrated significant reductions in body weight, which correlated with their persistent hyperglycemia and catabolic states [35].

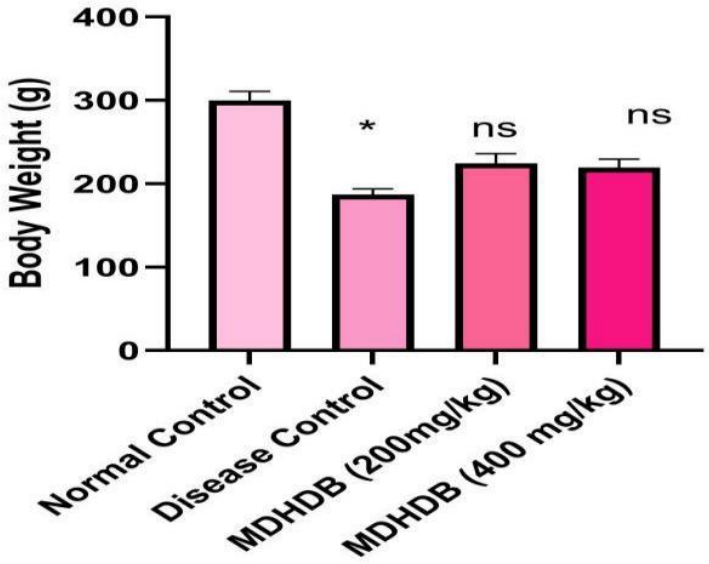

Figure (1): Effect of MDHDB on Body Weight.

Test compared to Disease control Group: P>0,05ns, P<0.05@ ; Compared to Normal Control Group: P>0,05ns, P<0.05*)

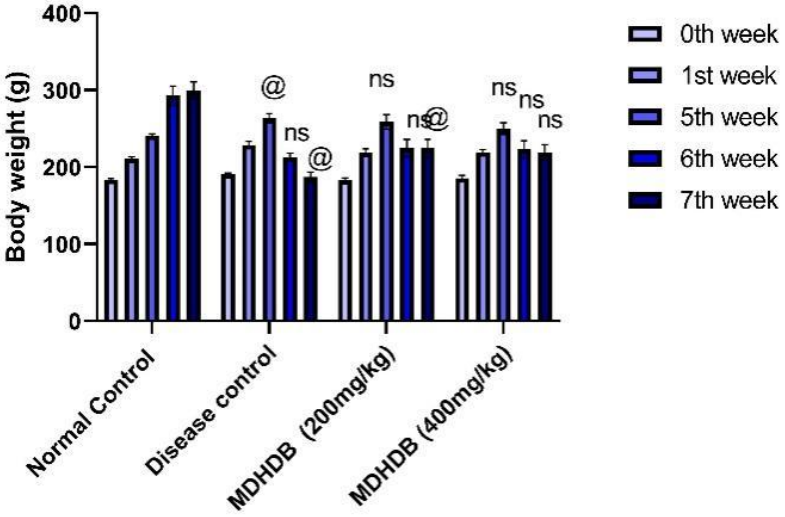

Figure (2): Effect of MDHDB on Body Weight.

(Test compared with 1st week: P<0.05@; P>0,05ns)

Effect of MDHDB on Food and Water Intake

Food Intake

Figure 3 illustrates the effect of MDHDB treatment on food intake measured at week 8 (50th day) of the study. Disease control animals demonstrated significantly reduced food intake (approximately 170 g/week) compared to normal control rats (approximately 230 g/week; p<0.05, denoted by *), representing a paradoxical hypophagia rather than the typical polyphagia often observed in uncontrolled diabetes [36]. This reduction in food intake in diabetic animals may reflect the severe metabolic disturbances, illness behavior, and nephropathy-associated anorexia that can occur in advanced diabetic complications [27].

MDHDB treatment produced dose-dependent restoration of normal feeding behavior . The low dose MDHDB (200 mg/kg) showed partial restoration of food intake to approximately 220 g/week (p<0.05 vs. disease control, denoted by @), approaching normal control levels. The high dose MDHDB (400 mg/kg) demonstrated superior efficacy, fully normalizing food intake to approximately 230 g/week (p<0.05 vs. disease control, denoted by @), achieving levels statistically comparable to normal controls (p>0.05).

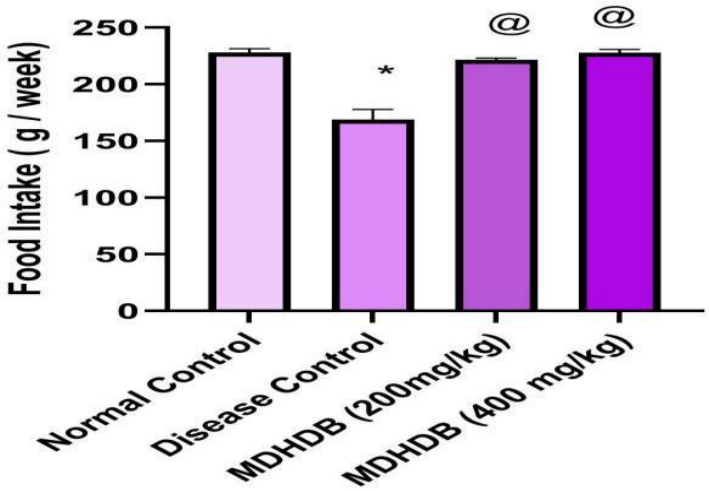

Figure (3): Effect of MDHDB on Food Intake (Test compared to Disease control Group: P>0,05ns, P<0.05@ ; Compared to Normal Control Group: P>0,05ns, P<0.05*)

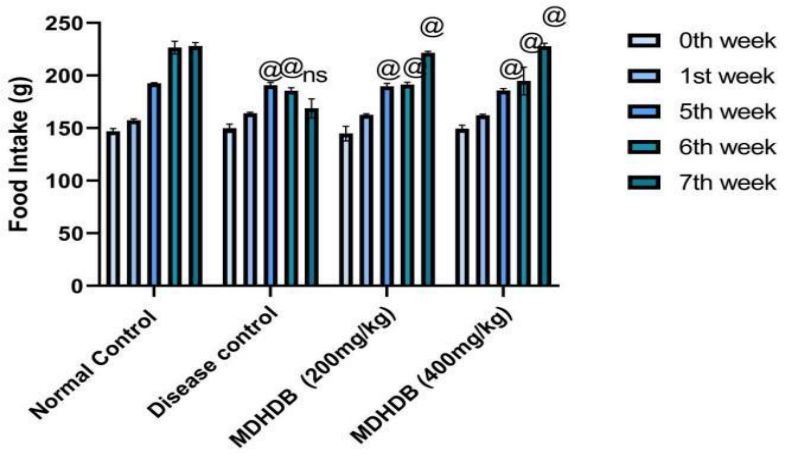

Figure (4): Effect of MDHDB on Food Intake.

(Test compared with 1st week: P>0,05ns, P<0.05@)

Water Intake

Polydipsia (or an excessive need to drink fluids) is one of the most common signs of diabetes [36] in both animals and humans. The rats used in this study were found to have a higher amount of drinking fluid than normal controls ($p < 0.05$) at the time point of day 50 as shown in Figure 5; this increase in water consumption was likely due to osmotic diuresis (or loss of fluids through urine) resulting from the elevated blood sugar levels seen in the development of nephropathy [37].

Both doses of MDHDB presented a statistically substantial decrease in the excessive water consumption seen in diabetic rats in a dose dependent fashion, with the highest dose show the most marked effect ($p < 0.05$ vs. disease controls). Also, weekly water consumption measurements are shown in Figure 6 to indicate that the excessive need for water (polydipsia) occurred very quickly after the diabetes inducing procedure in the control animals with diabetes, but that it continued throughout the duration of the experiment. Treatment of the diabetic rats with MDHDB starting on day 28 caused a slow reduction of their water consumption over time, with the largest reductions occurring in week 7 (day 50) as seen especially in the rats treated with the 400mg/kg dose [36].

In addition to the improvement in water consumption, MDHDB also caused an improvement in the rats' food consumption (normalization). The development of polyphagia (an excessive need to eat) and polydipsia (an excessive need to drink) in diabetes occurs because of a lack of cellular energy (due to impaired glucose metabolism) and osmotic diuresis (loss of water through urine), respectively [38]. The fact that MDHDB treatment resulted in a reduction of these symptoms indicates that there was an improvement in the rats' cells to take up glucose and/or a reduction in the emission of glucose in the urine, which would be indicative of either an increase in the sensitivity of the rat to insulin, or an increase in some other process by which glucose can enter the cell independent of insulin [39].

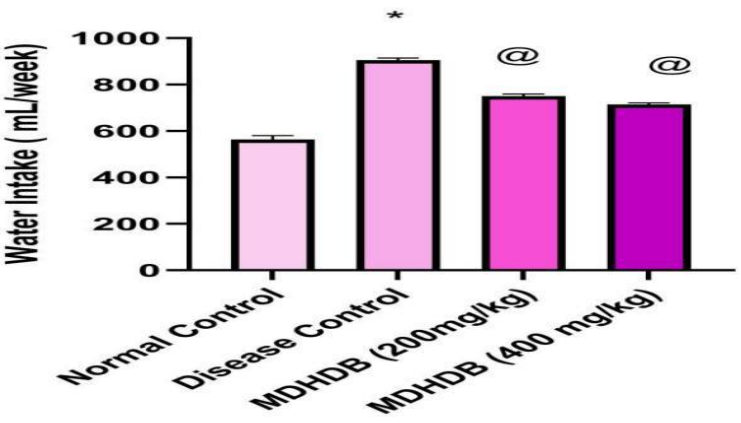

Figure (5): Effect of MDHDB on Water Intake. (Test compared to Disease control Group: P>0,05ns, P<0.05@; compared to Normal Control Group: P>0,05ns, P<0.05*)

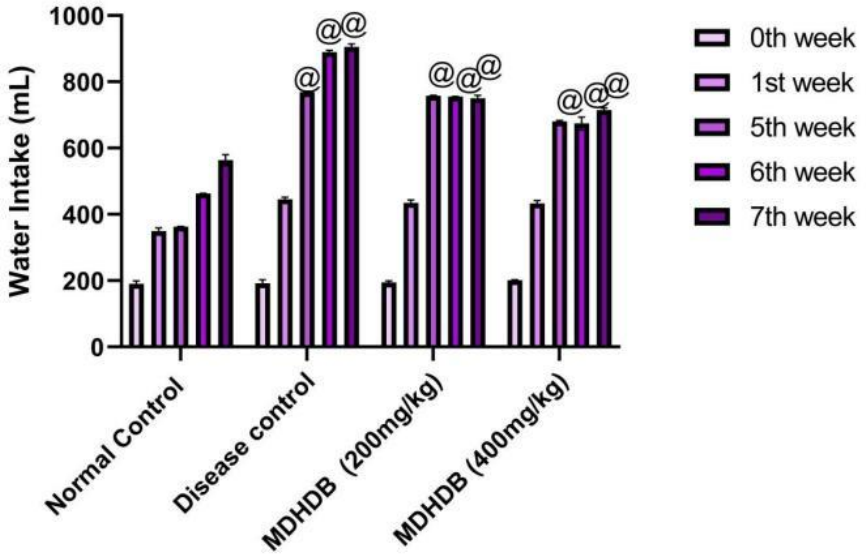

Figure (6): Effect of MDHDB on Water Intake.

(Test compared with 1st week: P>0,05ns, P<0.05@)

Effect of MDHDB on Fasting Blood Glucose

The level of FBG was the main indicator of glycemic control and the severity of the diabetic condition [11]. All the STZ-NA-treated rats had FBG ≥250 mg/dL on day 7 after diabetes induction, indicating a complete success of the diabetes induction. Disease control rats maintained a significantly elevated level of FBG during the duration of the experiment ($p<0.05$ compared to normal control) .

At the end of the study (day 50), fasting blood glucose (FBG) levels in the disease control group were approximately threefold higher than those in the normal control group (Figure 7). Administration of MDHDB resulted in significant, dose-dependent reductions in FBG levels. The low dose (200 mg/kg) produced a modest but significant decrease, whereas the high dose (400 mg/kg) elicited a more pronounced antihyperglycemic effect ($p < 0.05$ vs. disease control). However, the FBG level in the high dose treated rats remained higher than in the normal controls [40].

An analysis of FBG over time (Figure 8) indicated that the disease control rats maintained hyperglycemia for the entire 52-day experimental period . In contrast, the administration of MDHDB began to produce progressive glucose-lowering effects starting on day 35, with significant reductions in FBG evident by day 42 and even larger reductions by day 50 . The high dose of MDHDB appeared to be superior in terms of its ability to reduce FBG levels. Associated to the disease control group, the high dose treated rats reduced their FBG levels by about 35-40% at the end of the study.

MDHDB has been shown to have significant antihyperglycemic effects, and this includes a reduction of fasting blood glucose levels by about 35-40% in the high dose treated rats compared to disease controls. It is possible that these glucose lowering effects are the result of the multiple mechanisms typical of the actions of phenolic compounds [401,41], including: (1) enhanced peripheral glucose uptake through stimulation of GLUT4 translocation; (2) inhibition of hepatic gluconeogenesis through activation of AMPK [32,33]; (3) protection of the remaining pancreatic beta cells from apoptosis due to oxidative stress [29,31]; (4) inhibition of the enzymes involved in carbohydrate digestion (alpha-glucosidase and alpha-amylase), thereby decreasing the peak in glucose levels after eating [41]; and (5) improvement of insulin signaling through the reduction of the inflammatory mediators which can interfere with the function of the insulin receptor [38].

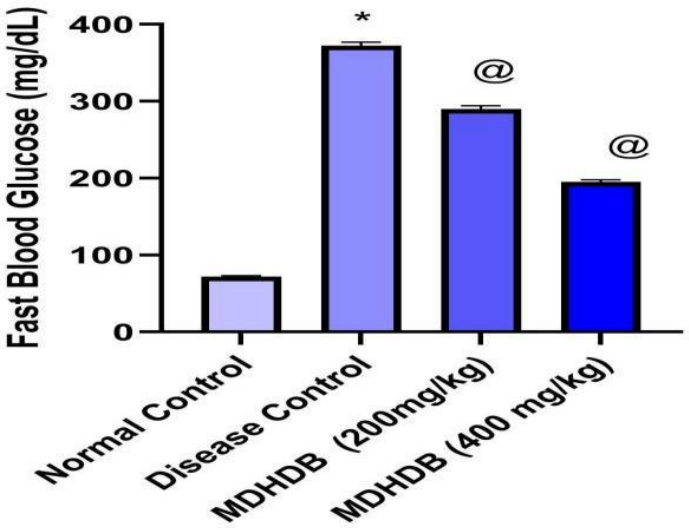

Figure (7): Effect of MDHDB on FBG.

(Test compared to Disease control Group: P>0,05^{ns}, P<0.05$^{@}$; Compared to Normal Control Group: P>0,05^{ns}, P<0.05*).

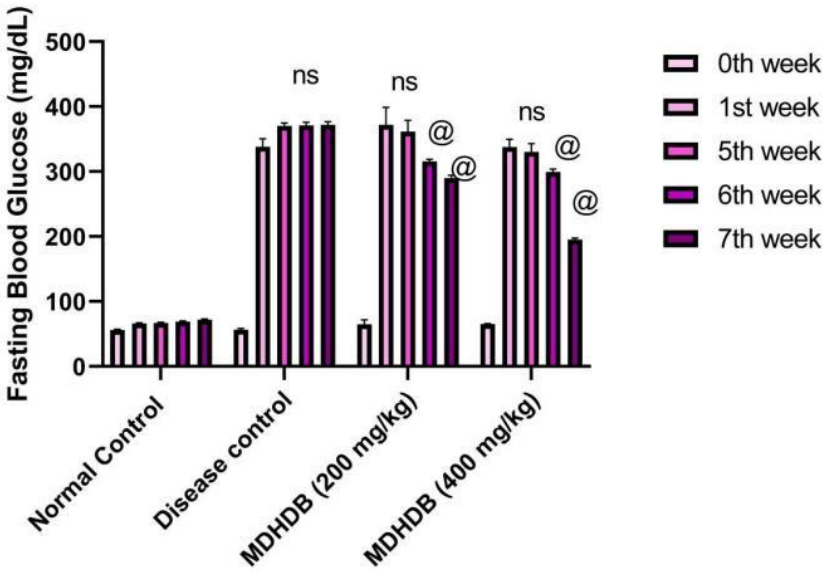

Figure (8): Effect of MDHDB on FBG.

(Test compared with 1st week: P>0,05^{ns} P<0.05$^{@}$)

Effect of MDHDB on Glycosylated Hemoglobin (HbA1c)

HbA1c is a composite measure of the mean blood glucose concentration over the past two to 3 months and therefore represents a chronic period of exposure to hyperglycemia [28]. Rats used for disease control had HbA1c values significantly greater than those found in normal rats ($p < 0.05$) indicating suboptimal glycemic control throughout the study duration (Figure 9) [28].

Both doses of MDHDB resulted in significantly reduced HbA1c concentrations when related to disease control ($p < 0.05$). However, the high dose resulted in pointedly greater reductions in HbA1c levels. At the high dose of 400 mg/kg, HbA1c

levels were reduced to near normal ranges but remained significantly above normal rat control values; this suggests there was an improvement in glycemic control, but it was not normalized . This data correlates with the FBG data and demonstrates that MDHDB has ongoing antihyperglycemic properties.

The significant decrease in HbA1c levels resulting from MDHDB administration indicates that MDHDB produced ongoing improvements in glycemic control as opposed to short term glucose lowering [28]. HbA1c is an index of average glycemic exposure over the erythrocytes' life span (approximately 120 days in rats) and therefore provides an integrated measure of glycemic control over time [28,40]. The ~20-25% decrease in HbA1c values resulting from the high dose of MDHDB indicates a significant improvement in glycemic control that will reduce the risk of developing microvascular complications if administered chronically [28,37].

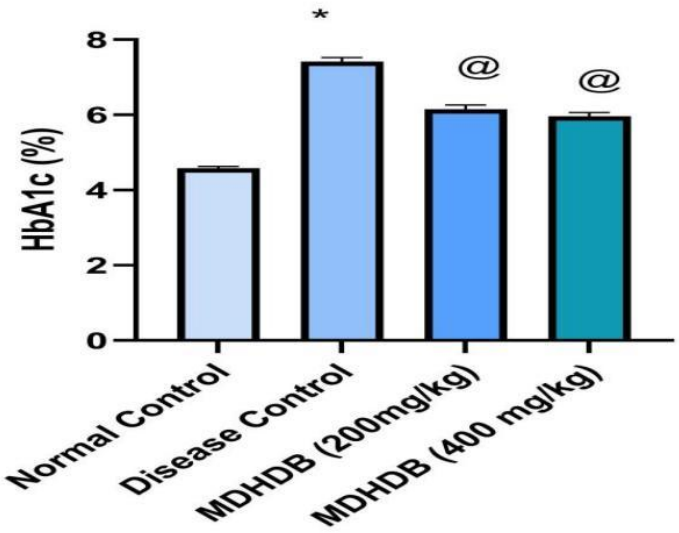

Figure (9): Effect of MDHDB on HbA1c. (Test compared to Disease control Group: P<0.05@; Compared to Normal Control Group: P>0,05ns, P<0.05*)

Effect of MDHDB on Renal Function Parameters

Serum Creatinine

Serum creatinine, a reliable indicator of glomerular filtration rate (GFR) and overall renal function, was significantly elevated in the disease control group (1.26 ± 0.03 mg/dL) compared to normal controls (0.78 ± 0.04 mg/dL; $p < 0.05$). The elevations in serum creatinine levels indicate that the kidneys of the disease control animals had impaired renal function and developed Nephropathy.

MDHDB treatment improved kidney function in a dose-dependent manner. Administration of the lower dose (200 mg/kg) reduced serum creatinine to 0.91 ± 0.15 mg/dL, representing a significant improvement compared to the disease control group ($p < 0.05$; Table 1). The higher dose of MDHDB resulted in a reduction in serum creatinine to 0.8 ±0.02 mg/dL ($p<0.05$ vs. disease control). The values of serum creatinine for the high-dose treated group were similar to those observed for the normal controls ($p >0.05$). Therefore, the high dose of MDHDB was able to restore kidney function to normal levels .

Blood Urea Nitrogen (BUN)

BUN is a pointer of kidney function and also of protein metabolism [43]. The BUN levels were significantly raised in the disease control group (39.15±0.57 mg/dL) when compared to the normal controls (13.81 ±0.24 mg/dL; $p < 0.05$) (Table 1). The increased levels of BUN in the disease control animals indicate severe impairment of the kidneys and changes in nitrogen metabolism [43].

Both the low and high-doses of MDHDB resulted in a significant decrease in the elevated levels of BUN ($p < 0.05$ vs. disease control). The low dose of MDHDB decreased the levels of BUN to 21.54 ±0.35 mg/dL. The high-dose of MDHDB resulted in an even greater reduction of BUN to 19.47 ±0.33 mg/dL, which represented about a 50% reduction in BUN levels from the disease control group. While there was improvement in the BUN levels of the treated groups, they still were slightly elevated relative to the normal controls, indicating some recovery of kidney function in the treated groups.

Total Serum Protein

Total serum protein levels were significantly elevated in the disease control animals (8.01 ±0.14 g/dL) when compared to the normal controls (6.58±0.12 g/dL; $p < 0.05$). It is likely that the increased levels of total serum proteins in the disease control animals reflected the combination of impaired renal protein clearance resulting from glomerular dysfunction and increased protein catabolism and abnormal protein synthesis by the liver in response to the diabetic state [42, 43].

MDHDB treatment normalized total protein levels in a dose-dependent fashion. The low-dose of MDHDB reduced total protein levels to 7.01±0.12 g/dL ($p < 0.05$ vs. disease control) (Table 1). The high-dose of MDHDB nearly completely normalized total protein levels to 6.76±0.07 g/dL ($p < 0.05$ vs. disease control), indicating improved renal protein processing and metabolic balance in the treated groups.

The significant reductions in serum creatinine and BUN levels after MDHDB treatment indicate preserved glomerular filtration and overall renal function [43,44]. The improvements of kidney function with MDHDB treatment are notable since the high-dose achieved almost complete normalization of creatinine levels and indicated a considerable preservation of nephron function despite diabetes and early nephropathy. Hemodynamic effects, anti-inflammatory action, inhibition of AGE formation and activity and anti-fibrotic effects are likely responsible for the protective effects on the kidneys [39].

Table (1): Effect of MDHDB on Renal Function Parameters

Groups	Creatinine level (mg/dL)	Blood Urea Nitrogen (mg/dL)	Total Protein (g/dL)
Normal Control	0,78±0,04	13,81±0,24	6,58±0,12
Disease Control	1,26±0,03*	39,15±0,57*	8,01±0,14*
MDHDB (200mg/kg)	0,91±0,15@	21,54±0,35@	7,01±0,12@
MDHDB (400 mg/kg)	0,8±0,02@	19,47±0,33@	6,76±0,07@

(Note: Data are reported as mean ±SEM. Test compared to Disease control Group: $P>0,05^{ns}$, $P<0.05^{@}$; Compared to Normal Control Group: $P>0,05^{ns}$, $P<0.05^{*}$)

Effect of MDHDB on Lipid Profile

DN often correlates with significant dyslipidemias which are directly linked to an increase in the formation of atherosclerotic plaques and subsequent cardiovascular complications [7]. Lipid profiles were examined in detail to assess the lipid status in disease control animals as well as how it was affected by MDHDB administration.

HDL Cholesterol

Compared to normal controls, disease control rats had significantly decreased HDL-cholesterol (33±0.57 mg/dl) [$p < 0.05$] (Table 2) and this decrease represented approximately a 21% decrease from the normal control values. Impaired reverse cholesterol transport and an increased risk of cardiovascular disease are directly correlated with decreased HDL-cholesterol levels [7].

Both the low and high doses of MDHDB produced dose dependent increases in HDL levels that were greater than disease control values ($p < 0.05$). Low-dose administration resulted in elevated HDL-cholesterol to 36.5±0.42 mg/dl while high-dose administration resulted in elevated HDL-cholesterol to 39.33±0.42 mg/dL. In addition, the HDL-cholesterol levels obtained with both the low and high doses of MDHDB were approximately 94% of the levels observed in the normal control group [44]. Elevated HDL-cholesterol levels indicate an improvement in lipid metabolism and an enhanced ability to protect against atherosclerosis.

LDL Cholesterol

LDL-cholesterol levels were found to be non-significantly elevated in disease control animals (91.5±4.12 mg/dL) as compared to normal controls (79.27±3.90 mg/dL) [$p > .05$] (Table 2). Administration of either the low or high dose of MDHDB resulted a non-significant improvement (82.33±1.64 and 79.83 ±2.56 mg/dL for low and high doses, respectively) [$p > 0.05$ vs disease control group]. These results suggest a selective enhancement in the protective lipid fraction [45]

VLDL and Triglycerides

VLDL-cholesterol levels were found to be pointedly raised in disease control rats (26.83 ± 0.70 mg/dL) as associated to normal controls (18.83±0.47 mg/dL) [$p < .05$] (Table 2). The increased levels of VLDL-cholesterol in disease control rats can result from increased production of VLDL by the liver due to insulin shortage and/or resistance, or from a decrease in

the clearance of VLDL from the circulation [7].

Administration of both the low and high doses of MDHDB resulted in decreases in VLDL-cholesterol levels [$p < 0.05$ vs disease control]. The high dose of MDHDB was found to produce the greatest level of reduction in VLDL-cholesterol levels (23.47 ± 0.79 mg/dL), resulting in a 50% reduction in the elevated levels of VLDL-cholesterol observed in disease control rats.

Levels of triglycerides were dramatically elevated in disease control animals (170.2 ± 15.71 mg/dL) as related to normal controls (97.5 ± 0.71 mg/dL) ($p < 0.05$). Although administration of MDHDB resulted in reductions in levels of triglycerides (158 ± 3.96 mg/dL and 149.7 ± 10.54 mg/dL for low and high doses, respectively), neither the low nor high dose of MDHDB produced statistically non-significant reductions in levels of triglycerides ($p > 0.05$ vs disease control), suggesting that MDHDB corrects the hypertriglyceridemia observed in disease control animals [46].

Total Cholesterol

Total cholesterol levels were found to be non-significantly elevated in disease control rats (151.3 ± 4.92 mg/dL) as compared to normal controls (139.9 ± 3.80 mg/dL) ($p > 0.05$) (Table 2). Total cholesterol levels in rats administered either the low or high dose of MDHDB were statistically non-significant (143.5 ± 2.70 and 142.6 ± 2.52 mg/dL for low and high doses, respectively).

The dyslipidemia observed in disease control rats, characterized by reduced HDL-cholesterol, elevated VLDL-cholesterol, and elevated levels of triglycerides, is a common metabolic abnormality that contributes to the development of accelerated atherosclerosis and cardiovascular disease in individuals with diabetic nephropathy [7]. Reduced insulin sensitivity and insulin deficiency contribute to several abnormalities in lipid metabolism, including: increased production of VLDL by the liver, reduced clearance of VLDL-triglycerides, and impaired maturation and secretion of HDL [7].

Administration of MDHDB resulted in significant improvements in the lipid profiles of treated animals, particularly through increases in HDL-cholesterol and decreases in VLDL-cholesterol. It is expected that some of the beneficial effects of MDHDB on lipid metabolism will result from its ability to activate PPAR, reduce oxidative stress and damage to lipoproteins, and activate AMPK [41].

Table (2): Effect of MDHDB on lipid profile

Animals	HDL (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)	Triglyceride (mg/dL)	Total Cholesterol (mg/dL)
Normal Control	41,83±0,30	79,27±3,90	18,83±0,47	97,5±0,71	139,9±3,80
Disease Control	33±0,57*	91,5±4,12ns	26,83±0,70*	170,2±15,71*	151,3±4,92^{ns}
MDHDB (200mg/kg)	36,5±0,42@	82,33±1,64^{ns}	24,67±1,22@	158±3,96^{ns}	143,5±2,70^{ns}
MDHDB (400mg/kg)	39,33±0,42@	79,83±2,56^{ns}	23,47±0,79@	149,7±10,54^{ns}	142,6±2,52^{ns}

(Note: Data are reported as mean ±SEM. Test compared to Disease control Group: P>0,05^{ns}, P<0.05@; Compared to Normal Control Group: P>0,05^{ns}, P<0.05*)

Effect of MDHDB on Oxidative Stress Markers in Kidney Tissue

Catalase Activity

Antioxidant enzyme catalase, shown to be a key player in the breakdown of hydrogen peroxide, showed statistically lower activity in kidney tissues from rats with kidney disease (26.5 ± 0.78 U/mL) as opposed to healthy rats (37.03 ± 0.88 U/mL; $p<0.05$) (Table 3). These findings indicate impaired antioxidant defenses [5 , 6].

MDHDB treatment resulted in a dose-dependent increase in catalase activity. At the lowest dose of MDHDB (200 mg/kg) there was a slight but statistically insignificant ($p>0.05$ vs. kidney disease) increase in catalase activity (27.48 ± 0.47 U/mL). However at the highest dose of MDHDB (400 mg/kg) there was a significant increase in catalase activity (29.52 ± 0.73 U/mL; $p<0.05$ vs. kidney disease), indicating an approximate 38% recovery from the diabetes-induced decrease in catalase activity.

Superoxide Dismutase (SOD) Activity

Activity of SOD, a crucial enzyme responsible for the neutralization of superoxide radicals, was also decreased in the kidney tissues of rats with kidney disease (1.88±0.04 U/mL) when compared to those of rats without kidney disease (3.39±0.28 U/mL; $p<0.05$) (Table 3). These results indicated a significant level of oxidative stress.

In addition to increasing catalase activity, both doses of MDHDB increased SOD activity ($p<0.05$ vs. kidney disease) to 2.04±0.05 U/mL, whereas the higher dose (400 mg/kg) increased SOD activity to 2.39±0.18 U/mL. Thus, MDHDB treatment resulted in an approximate 34% recovery in SOD activity. SOD activity was, however, still less than that observed in the kidney tissues of rats without kidney disease, indicating that the partial restoration of this important antioxidant enzyme had occurred [28].

Reduced Glutathione (GSH) Levels

The main non-enzymatic antioxidant, GSH, is a critical component in maintaining the redox balance within cells. GSH was significantly depleted in the kidney tissues of rats with kidney disease (1.77±0.04 µmol/g) when compared to those of rats without kidney disease (2.3±0.06 µmol/g; $p<0.05$) (Table 3) [6,22].

Treatments with MDHDB resulted in small increases in GSH levels that were dependent upon the dose administered (1.81±0.04 µmol/g and 1.85±0.07 µmol/g for low and high doses of MDHDB, respectively). However, these increases in GSH were not significant ($p>0.05$ vs. kidney disease). Thus, although MDHDB treatment may have supported the synthesis of GSH or prevented its depletion, the effect was not sufficient to restore GSH levels to normal under the experimental conditions used in this study [28].

Lipid Peroxidation (MDA Levels)

MDA (malondialdehyde), a marker of lipid peroxidation and oxidative membrane damage, was greatly elevated in the kidney tissues of rats with kidney disease (0.72±0.02 µmol/g) when compared to those of rats without kidney disease (0.38±0.05 µmol/g; $p<0.05$) (Table 3), indicating a severe level of oxidative injury [5,6].

MDHDB treatment produced dose-dependent decreases in MDA levels (0.58±0.06 and 0.56±0.02 µmol/g for low and high doses of MDHDB, respectively), but these decreases in MDA levels were not statistically significant ($p>0.05$ vs. kidney disease) [11,19]. Even though the decreases in MDA levels were not statistically significant, they represented a decrease of approximately 20-22%, which represents a relevant reduction in oxidative lipid damage. This reduction may contribute to the renoprotective effects of MDHDB [47].

Table (3): Effect of MDHDB on Oxidative Stress Markers in Kidney Tissue

Groups	CAT (Unit/mL)	SOD (Unit/mL)	GSH (umol/g of tissue)	MDA (umol/g of tissue)
Normal Control	37,03±0,88	3,39±0,28	2,3±0,06	0,38±0,05
Disease Control	26,5±0,78*	1,88±0,04*	1,77±0,04*	0,72±0,02*
MDHDB (200mg/kg)	27,48±0,47^{ns}	2,04±0,05$^{@}$	1,81±0,04^{ns}	0,58±0,06^{ns}
MDHDB (400 mg/kg)	29,52±0,73$^{@}$	2,39±0,18$^{@}$	1,85±0,07^{ns}	0,56±0,02^{ns}

(Note: Data are reported as mean ±SEM. (Test compared to Disease control Group: P>0,05^{ns}, P<0.05$^{@}$; Compared to Normal Control Group: P>0,05^{ns}, P<0.05*)

Histopathological Changes in Kidney Tissue

Histopathological examination of kidney tissue (Figure 12) (Table 4) provided qualitative and semi-quantitative assessment of structural damage and treatment effects [17].

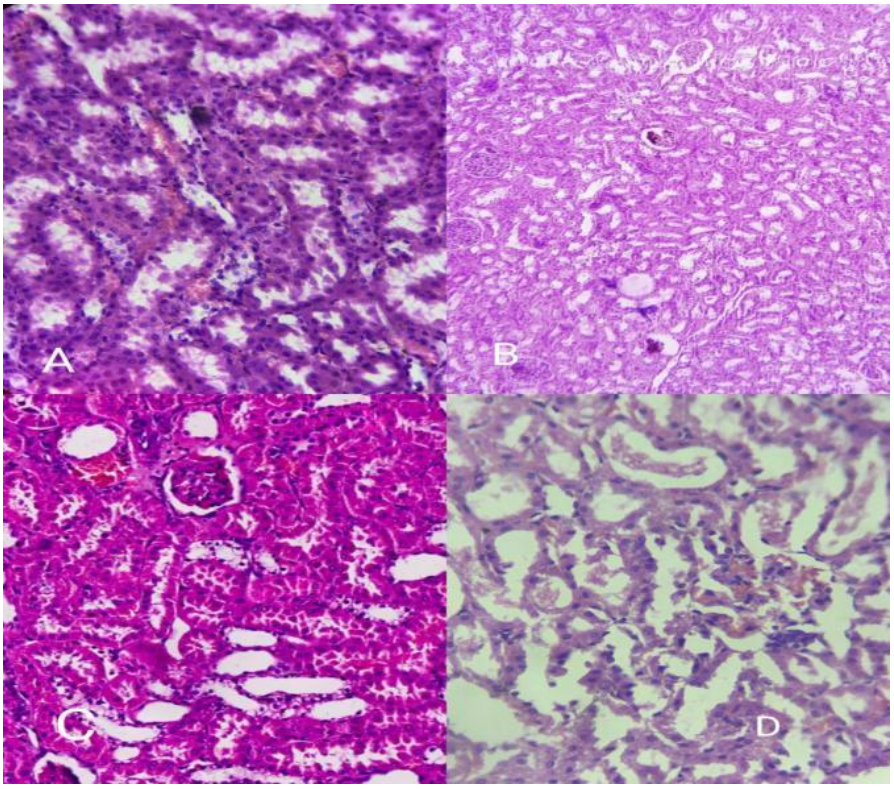

Figure 12: Histological change in Kidney of Wistar rats (x10): (A) normal control, (B) disease control, (C) MDHDB treatment at dose 200 mg /kg, (D) MDHDB treatment at doses 400 mg/kg.

Normal Control Group

There was no evidence of tubular dilation, debris in the tubules, or necrosis in the tubules. The interstitial space around the tubules was normal as well with no evidence of inflammation or fibrosis. The mean injury score for all of the measured factors was low (dilated tubules = 0.4±0.24, debris in the tubules = 0±0, necrosis = 0.2±0.2) [11].

Disease Control Group

Kidney tissues from diabetic animals that served as disease controls showed marked nephropathic changes typical of diabetic nephropathy [46]. These changes were prominent and included: Severe tubular dilatation with a significant decrease in the height and density of the normal epithelial cells, which affected over half of the total area examined (score: 2.6 ±0.24; $p<0.05$ vs normal control) ; Significant accumulation of proteinaceous casts and cellular debris in the lumen of the tubules, observed throughout the examined sections (score: 3 ±0; $p<0.05$ vs normal control) ; Significant necrosis of the tubular epithelial cells as evidenced by an outline of the cell bodies, but lack of definition of the nucleus and cytoplasmic eosinophilia, which affected greater than half of the tubular structures (score: 2.8±0.2; $p<0.05$ compared to normal control)

MDHDB Low Dose Group (200 mg/kg)

Administration of MDHDB 200mg/kg resulted in partial amelioration of nephropathic changes . Tubular dilatation remained apparent but was significantly less severe (score: 1.8 ±0.37; $p>0.05$ compared to disease control), intratubular debris was reduced (score: 1.4±0.24; $p>0.05$ compared to disease control), and tubular epithelial necrosis showed improvement (score: 1.6±0.24; $p>0.05$ compared to disease control]. Although some degree of improvement was noted, these did not reach statistical significance indicating that protective effects may be evident at this dose, but are likely limited.

MDHDB High Dose Group (400 mg/kg)

High-dose MDHDB administration provided significant renoprotection. Tubular architecture was largely preserved with only minor dilatation (score: 1 ±0; $p<0.05$ compared to disease control), a significant reduction in intratubular debris (score: 0.8±0.2; $p<0.05$ compared to disease control), and a significant reduction in tubular epithelial necrosis (score: 0.8 ±0.2; $p<0.05$ compared to disease control). The kidneys of the high-dose group appeared to have a normal architecture with the majority of the tubules being structurally normal and there being little or no glomerular damage and significantly reduced

inflammation.

These histopathologic findings provide direct visual evidence of the renoprotective effects of MDHDB at the tissue level [16]. In addition to the presence of severely damaged tubules in disease-control animals characterized by dilated hypocellular tubules, proteinaceous cast and cellular debris accumulation in the tubular lumens, and widespread tubular epithelial necrosis, represent characteristic diabetic nephropathy pathologies resulting from prolonged exposure to hyperglycemia -induced oxidative stress, ischemia and inflammatory injury [48].

The significant renoprotection afforded by the high-dose MDHDB, with histological scores near those of normal control animals, indicate that basic kidney architecture is preserved [49,50]. The reduction in tubular necrosis indicates that MDHDB protects against various pathways of cell death such as necrosis reduction, apoptosis prevention, and autophagy modification.

Table (4): Histopathological changes in kidney

Groups	dilated hypocellular tubules	intratubular debris	Coagulate necrosis of tubular epithelial cell
Normal Control	0,4±0,24	0±0	0,2±0,2
Disease Control	2,6±0,24*	3±0*	2,8±0,2*
MDHDB (200 mg/kg)	$1,8\pm0,37^{ns}$	$1,4\pm0,24^{ns}$	$1,6\pm0,24^{ns}$
MDHDB (400 mg/kg)	$1\pm0^{@}$	$0,8\pm0,2^{@}$	$0,8\pm0,2^{@}$

(Note: Data are reported as mean ±SEM. Test compared to Disease group: $P>0,05^{ns}$, $P<0.05@$; compared to normal control group test: $P>0,05^{ns}$, $P<0.05*$).

CONCLUSION

Overall, the study provided a wealth of information about the efficacy of MDHDB in treating diabetic nephropathy according to its significant effetcs on hyperglycemia, renal function parameters, oxidative stress (*in vivo*). Therefore, MDHDB seems to be a very capable new potential therapeutic drug for the dealing of DN, a major complication of diabetes ..

REFERENCES

[1] Saeedi P, Petersohn I, Salpea P, et al. (2019). "Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9th edition". Diabetes Research and Clinical Practice. 157: 107843. doi:10.1016/j.diabres.2019.107843

[2] Kaur N, Kishore L, Singh R (2018). "Dillenia indica L. attenuates diabetic nephropathy via inhibition of advanced glycation end products accumulation in STZ-nicotinamide induced diabetic rats". *Journal of Traditional and Complementary Medicine*. 8 (1): 226–238. doi:10.1016/j.jtcme.2017.06.007

[3] Alicic RZ, Rooney MT, Tuttle KR (2017). "Diabetic kidney disease: challenges, progress, and possibilities". *Clinical Journal of the American Society of Nephrology*. 12 (12): 2032–2045. doi:10.2215/CJN.11491116

[4] Tuttle KR, Bakris GL, Bilous RW, et al. (2014). "Diabetic kidney disease: a report from an ADA Consensus Conference". *Diabetes Care*. 37 (10): 2864–2883. doi:10.2337/dc14-1296

[5] Forbes JM, Coughlan MT, Cooper ME (2008). "Oxidative stress as a major culprit in kidney disease in diabetes". *Diabetes*. 57 (6): 1446–1454. doi:10.2337/db08-0057

[6] Jha JC, Banal C, Chow BS, Cooper ME, Jandeleit-Dahm K (2016). "Diabetes and kidney disease: role of oxidative stress". *Antioxidants & Redox Signaling*. 25 (12): 657–684. doi:10.1089/ars.2016.6664

[7] Singh R, Devi S, Gollen R (2015). "Role of free radical in atherosclerosis, diabetes and dyslipidemia: larger-than-life". *Diabetes/Metabolism Research and Reviews*. 31 (2): 113–126. doi:10.1002/dmrr.2558

[8] Ramasamy R, Vannucci SJ, Yan SS, Herold K, Yan SF, Schmidt AM (2005). "Advanced glycation end products and RAGE: a common thread in aging, diabetes, neurodegeneration, and inflammation". *Glycobiology*. 15 (7): 16R–28R. doi:10.1093/glycob/cwi053

[9] Brownlee M (2001). "Biochemistry and molecular cell biology of diabetic complications". *Nature*. 414 (6865): 813–820. doi:10.1038/414813a

[10] Han H, Cao A, Wang L, et al. (2017). "Huangqi decoction ameliorates streptozotocin-induced rat diabetic nephropathy through antioxidant and regulation of the TGF-β/MAPK/PPAR-γ signaling". *Cellular Physiology and Biochemistry*. 42 (5): 1934–1944. doi:10.1159/000479834

[11] Gross JL, De Azevedo MJ, Silveiro SP, Canani LH, Caramori ML, Zelmanovitz T. Diabetic nephropathy: diagnosis, prevention, and treatment. Diabetes care. 2005 Jan 1;28(1):164-76.https://***doi***.org/10.2337/diacare.28.1.164

[12] Bae JH.SGLT2 Inhibitors and GLP-1 Receptors Agonist in Diabetic Kidney Disease: Evolving Evidence and Clinical Application. Diabetes Metab J.2025 May;49(3):386-402.doi:10.4093/dmj.2025.0220.

[13] Valle-Velázquez E, Zambrano-Vásquez OR, Cortés-Camacho F, Sánchez-Lozada LG, Guevara-Balcázar G, Osorio-Alonso H. Naringenin — A potential nephroprotective agent for diabetic kidney disease: A comprehensive review of scientific evidence. Biomolecules and Biomedicine. 2024 Dec 1;24(6):1441.doi:**10.17305/bb.2024.10511**.

[14] Kumar S, Sharma UK, Sharma AK, Pandey AK. Protective efficacy of Solanum xanthocarpum root extracts against free radical damage: phytochemical analysis and antioxidant effect. Cellular and Molecular Biology. 2012 Dec 22;58(1):174-81.

[15] Ullah N, Azam Khan M, Khan T, Ahmad W. Protective potential of Tamarindus indica against gentamicin-induced nephrotoxicity. Pharmaceutical Biology. 2014 Apr 1;52(4):428-34.doi: **10.3109/13880209.2013.840318**.

[16] Singla K, Singh R (2020). "Nephroprotective effect of *Curculigo orchioides* in streptozotocin-nicotinamide induced diabetic nephropathy in Wistar rats". *Journal of Ayurveda and Integrative Medicine*. 11 (4): 399–404. doi:10.1016/j.jaim.2020.05.006

[17] Kishore L, Kaur N, Singh R (2017). "Nephroprotective effect of *Paeonia emodi* via inhibition of advanced glycation end products and oxidative stress in streptozotocin-nicotinamide induced diabetic nephropathy". *Journal of Food and Drug Analysis*. 25 (3): 576–588. doi:10.1016/j.jfda.2016.08.009

[18] Zhang Z, Zhou X, Zhou X, Xu X, Liao M, Yan L, Lv R, Luo H. Methyl 3, 4-dihydroxybenzoate promotes neurite outgrowth of cortical neurons cultured in vitro ★. Neural regeneration research. 2012 May 5;7(13):971-7 .doi:10.3969/j.issn.1673-5374.2012.13.002.

[19] N. S. Aminah(2017). "Methyl-3,4-dihydroxybenzoate and 9-10-dihydrophenanthrene-2,4,7-triol two phenolic compounds from *Dioscorea alata* L. and their antioxidant activity". *AIP Conf. Proc.* 21 1888 (1): 020050. doi.org/10.1063/1.5004327

[20] Cai L, Wang LF, Pan JP, Mi XN, Zhang Z, Geng HJ, Wang JH, Hu SH, Zhang W, Gao Q, Wu WT. Neuroprotective effects of methyl 3, 4-dihydroxybenzoate against TBHP-induced oxidative damage in SH-SY5Y cells. Molecules. 2016 Aug 22;21(8):1071.doi:10.3390/molecules21081071

[21] Szkudelski T (2012). "Streptozotocin-nicotinamide-induced diabetes in the rat: characteristics of the experimental model". *Experimental Biology and Medicine*. 237 (5): 481–490. doi:10.1258/ebm.2012.011372

[22] Masiello P, Broca C, Gross R, et al. (1998). "Experimental NIDDM: development of a new model in adult rats administered streptozotocin and nicotinamide". *Diabetes*. 47 (2): 224–229. doi:10.2337/diabetes.47.2.224

[23] OECD (2002). "Test No. 423: Acute Oral Toxicity – Acute Toxic Class Method". *OECD Guidelines for the Testing of Chemicals, Section 4*. Paris: OECD Publishing. doi:10.1787/9789264071001

[24] Szkudelski T.Streptozotocin-nicotinamide-induced diabtes in the rats. Characteristics of the experimental model. Experimental biologiy and medicine 237,no.5(2012):481-490.

[25] Kpemissi M, Eklu-Gadegbeku K, Veerapur VP, Negru M, Taulescu M, Chandramohan V, Hiriyan J, Banakar SM, Nv T, Suhas DS, Puneeth TA. Nephroprotective activity of Combretum micranthum G. Don in cisplatin induced nephrotoxicity in rats: In-vitro, in-vivo and in-silico experiments. Biomedicine & Pharmacotherapy. 2019 Aug 1;116:108961.https://doi.org/10.1016/j.biopha.2019.108961

[26] Palsamy P, Subramanian S (2011). "Resveratrol protects diabetic kidney by attenuating hyperglycemia-mediated oxidative stress and renal inflammatory cytokines via Nrf2-Keap1 signaling". *Biochimica et Biophysica Acta*. 1812 (7): 719–731. doi:10.1016/j.bbadis.2011.03.008

[27] Kishore L, Kaur N, Singh R (2017). "Distinct biomarkers for early diagnosis of diabetic nephropathy". *Current Diabetes Reviews*. 13 (6): 598–605. doi:10.2174/1573399812666161207123007

[28] Beutler E (1984). *Red Cell Metabolism: A Manual of Biochemical Methods*. 3rd ed. Orlando: Grune & Stratton.doi :10.7326/0003-4819-75-6-985_1

[29] Darkazally AZ, Alnour A, Homsi S. Troxerutin effect on gastric ulcers induced by ketorolac in rats: Relation with oxidative stress. Heliyon. 2024 Oct 15;10(19.DOI: 10.1016/j.heliyon.2024.e38893

[30] Li X. Improved pyrogallol autoxidation method: a reliable and cheap superoxide-scavenging assay suitable for all antioxidants. Journal of agricultural and food chemistry. 2012 Jun 27;60(25):6418-24.doi: 10.1021/jf204970r

[31] Annouf YA, Al-Laham SH, Al-Shatti EY. Effect of Amlodipine on enteropathy induced by Indomethacin in rats. International Journal of Current Pharmaceutical Research. 2020;12(3):144-50.*doi*:10.22159/ijcpr.

[32] Patil NR, Rasal VP, Malabade RH. Screening of mandarin oil on indomethcin induced inflammatory bowel disease in wistar rats. Indian J. Pharm. Educ. Res. 2014 Oct 1;48:1-6.*DOI*: 10.5530/ijper.48.4s.1.

[33] Bhave AL, Bhatt JD, Hemavathi KG. Antiulcer effect of amlodipine and its interaction with H2 blocker and proton pump inhibitor in pylorus ligated rats. Indian journal of Pharmacology. 2006 Nov 1;38(6):403-7.doi :**10.4103/0253-7613.28206**.

[34] Mehenni C, Atmani-Kilani D, Dumarçcay S, PerinD, Gérardin P, Atmani D. Hepatoprotective and antidiabetic effect of Postacia lentiscus leaf and fruit extratc.J.Food Drug Anal. 2016;24(3):653-669.doi:10.1016/j.jfda.2016.03.002

[35] Ruch RJ, Cheng SJ, Klaunig JE (1989). "Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea". *Carcinogenesis*. 10 (6): 1003–1008. doi:10.1093/carcin/10.6.1003

[36] Iorember FM. Malnutrition in chronic kidney disease. Frontiers in pediatrics. 2018 Jun 20;6:161.Strominger JL, Brobeck JR. A mechanism of regulation of food intake. The Yale Journal of Biology and Medicine. 1953 Apr;25(5):383.

[37] Umanath K, Lewis JB (2018). "Update on diabetic nephropathy: core curriculum 2018". *American Journal of Kidney Diseases*. 71 (6): 884–895. doi:10.1053/j.ajkd.2017.10.026

[38] Donate-Correa J, Martín-Núñez E, Muros-de-Fuentes M, Mora-Fernández C, Navarro-González JF (2015). "Inflammatory cytokines in diabetic nephropathy". *Journal of Diabetes Research*. 2015: 948417. doi:10.1155/2015/948417

[39] American Diabetes Association (2021). "Standards of medical care in diabetes—2021". *Diabetes Care*. 44 (Suppl 1): S1–S232. doi:10.2337/dc21-Sint

[40] Ceriello A, Esposito K, Piconi L (2008). "Oscillating glucose is more deleterious to endothelial function and oxidative stress than mean glucose in normal and type 2 diabetic patients". *Diabetes*. 57 (5): 1349–1354. doi:10.2337/db08-0063

[41] Rani V, Deep G, Singh RK, Palle K, Yadav UC (2016). "Oxidative stress and metabolic disorders: pathogenesis and therapeutic strategies". *Life Sciences*. 148: 183–193. doi:10.1016/j.lfs.2016.02.002

[42] Kpemissi M, Potârniche AV, Lawson-Evi P, Metowogo K, Melila M, Dramane P, Taulescu M, Chandramohan V, Suhas DS, Puneeth TA, Vlase L. Nephroprotective effect of Combretum micranthum G. Don in nicotinamide-streptozotocin induced diabetic nephropathy in rats: in-vivo and in-silico experiments. Journal of Ethnopharmacology. 2020 Oct 28;261:113133.doi :**10.1016/j.jep.2020.113133**

[43] Kamel FO, Shagroud O, Ahmad MA, Abd El-Aziz GS, Burzangi AS, Bakhshwin D, Jamal M, Karim S. Agmatine ameliorates diabetes type 2-induced nephropathy in rats. Asian Pacific Journal of Tropical Biomedicine. 2024 Jan 1;14(1):8-16.doi **:10.1097/APT.0000000000000455**.

[44] Mima A (2022). "Mitochondria-targeted drugs for diabetic kidney disease". *Antioxidants*. 11 (12): 2334. doi:10.3390/antiox11122334

[45] Pedersen LR, Olsen RH, Anholm C, Astrup A, Eugen-Olsen J, Fenger M, Simonsen L, Walzem RL, Haugaard SB, Prescott E. Effects of 1 year of exercise training versus combined exercise training and weight loss on body composition, low-grade inflammation and lipids in overweight patients with coronary artery disease: a randomized trial. Cardiovascular diabetology. 2019 Oct 1;18(1):127.DOI: 10.1186/s12933-019-0934-x

[46] Kashihara N, Haruna Y, Kondeti VK, Kanwar YS (2010). "Oxidative stress in diabetic nephropathy". *Current Medicinal Chemistry*. 17 (34): 4256–4269. doi:10.2174/092986710793348581

[47] Giacco F, Brownlee M (2010). "Oxidative stress and diabetic complications". *Circulation Research*. 107 (9): 1058–1070. doi:10.1161/CIRCRESAHA.110.223545

[48] Bahadoran Z, Mirmiran P, Azizi F (2013). "Dietary polyphenols as potential nutraceuticals in management of diabetes: a review". *Journal of Diabetes and Metabolic Disorders*. 12 (1): 43. doi:10.1186/2251-6581-12-43

[49] Kanwar YS, Sun L, Xie P, Liu FY, Chen S (2011). "A glimpse of various pathogenetic mechanisms of diabetic nephropathy". *Annual Review of Pathology*. 6: 395–423. doi:10.1146/annurev.pathol.4.110807.092150

[50] Mogensen CE, Christensen CK, Vittinghus E (1983). "The stages in diabetic renal disease: with emphasis on the stage of incipient diabetic nephropathy". *Diabetes*. 32 (Suppl 2): 64–78. doi:10.2337/diab.32.2.s64.