RESEARCH PAPER

Nephroprotective and Antidiabetic Effects of Polyherbal Formulation in Streptozotocin-Induced Diabetic Nephropathy Albino Wistar Rats

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ABSTRACT

Diabetic nephropathy (DN), one of the most important microvascular complications of diabetes mellitus, is characterized by the progressive deterioration of kidney function, oxidative stress, and inflammation. This research studied the nephroprotective and antidiabetic potential of a herbal formulation consisting of *Salvia hispanica* (chia), *Nigella sativa* (kalonji), and *Trigonella foenum-graecum* (fenugreek) against diabetic nephropathy induced by streptozotocin (STZ) in white male Wistar rats.

Twenty male albino rats were divided into five groups: normal control (NC), disease control (DC), which received STZ (55 mg per kg), standard treatment using metformin (100 mg per kg), and two groups that received a herbal formulation at doses of 250 and 500 mg per kg respectively. The following parameters were measured: blood glucose, urine volume, urine protein, blood urea, and levels of creatinine, uric acid and total protein in serum. The DC group showed considerable hyperglycemia, high levels of protein in urine, and increased levels of urea and creatinine indicating severe kidney damage. Members of the high-dose group (500 mg/kg) had nearly normal biochemical parameters and healthy kidney anatomy which were similar to those of the metformin group.

The authors attribute this effect to the combined effects of polyphenols, thymoquinone, and saponins, which eliminate oxidative damage, inflammation, and fibrosis. Therefore, the authors conclude that the polyherbal treatment is effective for diabetic nephropathy and has nephroprotective and anti-diabetic benefits specifically.

Keywords: Polyherbal formulation; *Salvia hispanica*; *Nigella sativa*; *Trigonella foenum-graecum*; Diabetic nephropathy; Streptozotocin; Nephroprotective; Antidiabetic; Oxidative stress; Wistar rats

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INTRODUCTION:

Diabetes mellitus, a long-term metabolic ailment, is distinguished by long-lasting hyperglycemia, caused by faults in either insulin metabolism, the action, or a combination of both [1],[2],[3]. This disease has become a global problem, which significantly affects mortality and morbidity and has serious social and economic consequences. The data provided by the International Diabetes Federation (IDF) indicate that the number of diabetes sufferers will grow all over the world from 463 million by 2019 to around 650 million; thus, showing its epidemic nature [4],[5]. Besides, the data announced by the World Health Organization (WHO) predict that in 2030 the mortality rate connected with diabetes will be twice as much as in 2005, and complications caused by diabetes will kill millions of people every year [6]. Moreover, diabetes is the important factor for many other diseases [7],[8],[9].

Diabetic nephropathy is one of the most serious consequences of diabetes mellitus among other microvascular complications that can lead to such a

severe condition as end-stage renal disease, which is common among one third of patients with diabetes mellitus [10].

This is indicated by the gradual progression of the functional & structural aspects of the kidney, which involves the thickening in glomerular basement membrane, expansion of mesangial matrix, tubular atrophy, interstitial fibrosis & finally glomerulosclerosis [11],[12],[13],[14]. Clinically, DN refers to the presence of albuminuria, decrease of GFR & increase of serum creatinine & BUN levels. In spite of the latest accomplishments in the treatment of diabetes & the advancement of renal therapy (i.e., ACE inhibitors & ARBs), the timely progress of DN cannot be managed properly, which shows the need for new treatment methods [15],[16],[17],[18].

Oxidative Stress and Pathogenesis of Diabetic Nephropathy:

The pathophysiology of DN is complicated as the development of oxidative stress is one of the leading factors. The chronic increase of blood sugar levels results in the formation of reactive oxygen species due to different processes, such as glucose autoxidation, formation of AGEs & activation of the polyolic cycle [19],[20],[21],[22],[23]. The presence of the ROS leads to the breakdown of the balance between the antioxidant and pro-oxidative systems of the organism resulting in the damage of the lipids, proteins & nucleic acids. In the kidney, oxidative stress causes the apoptosis of the cells of podocytes and epithelial cells, stimulating the production of extracellular matrix & activation of profibrotic pathways (i.e., TGF-beta, NF-κB) [24],[25],[26],[27].

Another important factor in the development of diabetic nephropathy (DN) is inflammation. The presence of high levels of glucose in the blood and the action of reactive oxygen species cause the release of inflammatory cytokines such as tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6), and monocyte chemoattractant protein 1 (MCP-1) which recruit a variety of immune cells into renal tissues [28],[29],[30],[31]. The inflammatory environment leads to increased damage of glomeruli and promotes the development of fibrosis. In addition, impaired endothelial function, reduced nitric oxide bioavailability and stimulation of the renin-angiotensin system (RAS) leads to additional damage of the kidney. All these factors form a vicious circle of oxidative stress, inflammation and fibrosis [32],[33],[34].

Limitations of Current Therapeutic Approaches:

Standard methods of treatment of DN include reduction of blood glucose and blood pressure levels and suppression of RAS system. However, these approaches only slow down the rate of disease progress but do not stop it completely. Pharmacological therapies including inhibitors of angiotensin converting enzyme (ACE) and angiotensin receptor blockers (ARBs) as well as sodium glucose transport protein 2 (SGLT2) inhibitors are known to provide partial protection against renal damage; however, they cause side effects and are not very effective in advanced cases [35],[36],[37],[38]. Dietary modifications are frequently recommended, such as limiting protein and sodium intake, but these changes can sometimes affect nutritional status and antioxidant defenses. Therefore, there is a need to develop alternative treatments for dealing with the mechanisms that cause DN, such as oxidative stress and inflammation [39],[40],[41],[42].

Role of Phytochemicals and Polyherbal Formulations:

The field of natural products, regarding plant-based phytochemicals, is now garnering great interest and has offered itself as an arsenal for defeating chronic ailments. The presence of bioactive phytochemicals such as polyphenols, flavonoids, alkaloids, and saponins in the plant material is responsible for a variety of biological effects, including anti-oxidative, anti-inflammatory, anti-diabetic, and nephroprotective abilities [43],[44],[45],[46]. Various studies made it possible to find phytochemicals (for example, quercetin, naringin, and chlorogenic acid) operating through the mechanism of scavenging ROS, modulation of certain pathways, and improving renal function in extensive models of diabetic nephropathy (DN). Notably, the phytochemicals are able to operate together, using all their individual capacities at once when mixed [47],[48]

Polyherbal products include a form of medicines that involve the use of various plant extracts. These products are the foundation of traditional medicinal systems like Ayurveda and Traditional Chinese Medicine, from which they acquire synergism and even additivity. An interesting development in pharmacology is the discovery of the possibility for using polyherbal formulations for treating metabolic disorders [49],[50].

***Salvia hispanica (Chia)*:**

Salvia Hispanica, better known as chia, has many nutrients as it is a great source of omega-3 fatty acids like α-linolenic acid, polyphenols, flavonoids, and dietary fiber. The antioxidant properties of chia seeds act on the levels of lipids and at the same time support endogenous antioxidant enzymes like SOD and catalase. Also, chia seeds are effective in improving glucose metabolism as they work on insulin sensitivity and reduce postprandial glycemia levels. In kidneys, chia polyphenols were observed to lower oxidative stress and inflammation supporting the possibility of nephroprotective benefits. Moreover, a high level of fiber is a good contributor to better lipid profile and lower CVD risk, what is very important in patients with diabetes [51],[52],[53],[54],[55],[56],[57],[58].

***Nigella sativa (Kalonji)*:**

Nigella sativa is often referred to as black seed or kalonji and has attracted significant attention in pharmacology. The principle bioactive ingredient of *Nigella sativa* is thymoquinone, a compound with powerful antioxidant, anti-inflammatory, and anti-apoptotic properties [59],[60],[61],[62],[63],[64],[65]. Thymoquinone has the ability to neutralize free radicals and inhibit lipid peroxidation while altering pathways involved in signaling such as NF-κB and Nrf2 [66],[67],[68]. In diabetes models, *Nigella sativa* enhances glycemic control, insulin secretion, and protects pancreatic β-cells from oxidative damage. The abundant evidence showing the potential of *Nigella sativa* to provide nephroprotection is derived from animal studies where it leads to decreased serum creatinine, BUN, and histopathological evidence of kidney damage. The extensive range of pharmacological actions exhibited by *Nigella sativa* indicates its suitability for use in combination with other medicines in polyherbal formulations targeting DN [69],[70],[71].

***Trigonella foenum-graecum (Fenugreek)*:**

Trigonella *foenum-graecum*, popularly known as fenugreek, is a herbal plant recognized for its anti-diabetic functionalities. Fenugreek seeds are rich sources of saponins, flavonoids, and alkaloids that have hypoglycaemic properties because they increase insulin secretion while improving glucose uptake and inhibiting intestinal glucose absorption. Fenugreek also has antioxidants and anti-inflammatory properties. Fenugreek has been found to lower oxidative stress markers while bringing down the levels of cytokines in diabetic models. Besides, fenugreek is said to protect the kidneys through oxidative stress and fibrosis pathways. Fenugreek's role in lipid metabolism also helps in preventing cardiovascular complications in diabetic patients [72],[73],[74].

Rationale for Polyherbal Formulation:

Combining the seeds of *Salvia hispanica*, *Nigella sativa*, and *Trigonella foenum-graecum* is possible because of their unique features, which enable effective treatment of diabetic nephropathy. *Salvia hispanica* is rich in omega-3 fatty acids and polyphenols that help combat oxidative stress and improve metabolism. *Nigella sativa* contributes thymoquinone, a powerful antioxidant and anti-inflammatory substance, to the formulation, while *Trigonella foenum-graecum* provides valuable saponins and flavonoids that improve blood sugar levels and protect the kidneys.

Thus, the new combination of these three seeds is potentially beneficial in a great number of diseases, including diabetic nephropathy because it can influence multiple molecular processes occurring in the human body. Additionally, it involves the consumption of seeds that are cheap and available for every person.

MATERIALS AND METHODS:

Protocol Approval:

The laboratory Wistar male rats were from a source that complied with institutional standards for animal health and welfare, and they were kept in a registered animal facility in accordance with institutional procedures. They weighed between 180 and 220 grams, were all adults, and were all albino, which suggests that the rats' skin tones were uniform. In order to provide an appropriate habitat, the rats were transferred to the laboratory under controlled circumstances with a 12-hour light/dark cycle, a temperature of 22 ± 2 °C, and a relative humidity of 50%–60%. While kept in the lab, the rats had unrestricted access to commercial pelleted food and water [75]. The CPCSEA of the Government of India's rules were followed in the laboratory's studies, which were authorized by the study's Institutional Animal Ethics Committee (IAEC/04/02/2025). Additionally, the studies followed all ethical guidelines pertaining to animal welfare in order to minimize animal suffering and instead promote maximization—replacing animals with non-animal methods, minimizing the number of animals used, and altering experimental procedures in any way that would reduce animal pain or discomfort.

Preparation & Formulation of extract:

Seeds of three types of plants, namely *Salvia hispanica*, *Nigella sativa*, and *Trigonella foenum-graecum*, were collected from certified sellers. The seeds were cleaned of dirt before being shade dried at room temperature until a constant weight was reached. The seed samples were ground in a grinder to obtain a coarse powdered form. Equal amount of each seed sample (500 g) was extracted with 95% ethanol through the Soxhlet extractor for a period of 72 hours. The extracts were cooled, filtered through filter paper (Whatman No. 1) and concentrated under reduced pressure through rotary evaporator to one-third of the original volume. The concentrated extracts were further dried in a water bath at 60 ± 2 °C and stored in desiccators before being used.

For preparing formulations, equal amounts of dried extracts of seeds of chia, kalonji, and fenugreek were combined to produce a homogeneous powder mixture. The combined powder was suspended in 0.5% of carboxymethyl cellulose solution to be given in the experiments.

The purpose of this polyherbal formulation was to use the synergistic pharmacological properties of the three seeds in terms of polyphenols from *Salvia hispanica*, thymoquinone from Nigella sativa, and saponins from Trigonella foenum-graecum. The study evaluated the nephroprotective and metabolic effects of the formulation in animal models of diabetic nephropathy based on the methodology proposed by Parmar et al. (2022).

Induction of diabetic nephropathy:

Streptozotocin (STZ), a synthetic analog of insulin that functions as an analog of insulin (i.e., when STZ is administered into an animal, it creates high levels

of glucose), was used to develop diabetic nephropathy in the diabetic rat model. A newly produced solution of STZ diluted in citrate buffer (pH 4.5) was injected intraperitoneally into each rat at a dose of 55 mg/kg of body weight, or one injection. Rats that had fasted for 72 hours without the use of anesthetics had their blood (serum) glucose levels measured so as to validate hyperglycaemia in an animal. Any blood glucose level in an animal that is more than 250 mg/dL is considered hyperglycaemia. Consistent with earlier research, the degree of hyperglycaemia and renal insufficiency was continually monitored for four weeks prior to assessing the advancement of nephropathy. It is well known that streptozotocin may specifically kill pancreatic β-cells and that it can replicate diabetes consequences in rodents, including nephropathy.

Grouping and Treatment Protocol:

Monitoring and Sample Collection:

During the experimentation period, measurements were taken of body weight, food and liquid consumption, and blood glucose amounts for the purposes of testing. After the experimentation, the animals were put on an overnight fast before undergoing anesthesia with ketamine and xylazine. Blood samples were taken from the animal bodies by puncturing the covering with a needle. The blood serum was centrifuged and preserved for later testing.

Biochemical Parameters:

The diagnosis was based on the measurement of the concentrations of glucose, urea, creatinine, uric acid, and total protein in the blood serum, using diagnostic instruments available in the markets following the guidelines provided by the manufacturers. These parameters can be used to determine the level of blood glucose control and state of kidney functionality in the animals.

Histopathological Examination:

The kidneys were removed, washed with cold saline, and dried before weighing. One kidney from each animal was preserved with 10% neutral buffered formalin for 24 hours, passed through a series of ethanol concentrations for dehydration, then paraffinized after being treated with xylene. Thin sections of the tissue (5 µm) were made, stained with H&E, and examined under a light microscope (at a magnification of ×400). The degree of nephropathy was assessed according to histological characteristics such as glomerular hypertrophy, tubular epithelial damage, and interstitial fibrosis.

Statistical Analysis:

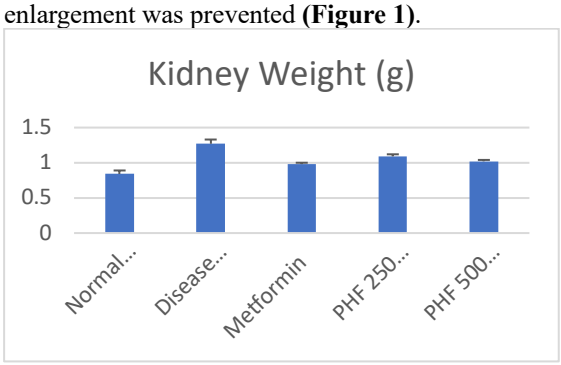
All the data are presented as Mean ± SEM (n = 6). The groups were compared statistically using one-way analysis of variance (ANOVA), and Tukey's post-hoc test was performed using GraphPad Prism 10.0 software. The value of $p < 0.05$ indicates statistical significance. The experiment was designed adequately in order to achieve enough power (β = 0.8) to detect the treatment effect on renal biomarkers and histological scores.

RESULTS

Blood Glucose Control and Kidney Weight:

The administration of streptozotocin (STZ) led to permanent hyperglycemia in the disease control group throughout the experiment, thus providing evidence for the successful induction of the diabetic nephropathy condition. The blood glucose of the disease control group increased progressively from 301.5 mg/dL at the beginning of the experiment to 337.2 mg/dL at the end of the experiment. In contrast, using the polyherbal formulation reduced the blood glucose level in a dose-dependent manner. The doses of 250 mg/kg of the polyherbal formulation resulted in a moderate decrease in the blood glucose concentration at the same time when 500 mg/kg of the formulation resulted in a significant decrease. By the end of treatment, the final blood glucose level in the high-dose group (172.4 mg/dL) was similar to that in the metformin group (158.5 mg/dL).

There has been an observable increase in weight for kidneys in the diseased control group (that weighed 1.27g) as compared to that of the normal control group (0.84g), indicating renal hypertrophy due to diabetic nephropathy. With the administration of metformin and polyherbal formulations in test animals, the observed increase in kidney weight was countered. The group that was treated with the formulation that contained 250 mg of the active formulation per kg showed some effect that reduced kidney weight to 1.09g while the group treated with the formulation of 500 mg/kg showed a further decline in kidney weight down to 1.02g. In this instance, the decline in kidney weight registered in the higher doses is very much equal to the kidney weight recorded in the metformin control group (0.98g) proving that diabetes-induced kidney enlargement was prevented **(Figure 1)**.



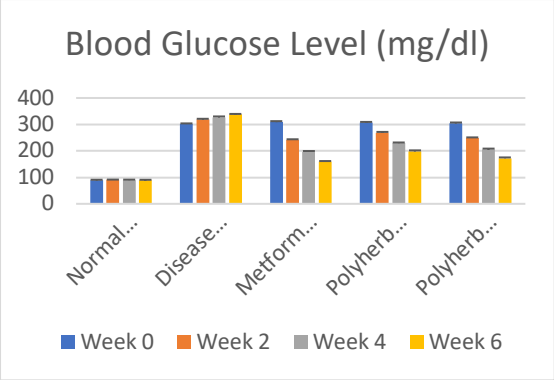


Figure 1. Effect of polyherbal formulation on blood glucose levels and kidney weight in STZ-induced diabetic nephropathy rats.

Renal Function Biomarkers:

Infusion of streptozotocin led to significant kidney function deterioration in the control group as indicated by the rise of serum urea, creatinine, uric acid, and notable amount of proteinuria. The biochemical changes were accompanied by the growth of urine volume as a sign of osmotic diuresis and malfunctioning tubular reabsorption. The control group rats had increased glucose levels constantly, proving the advancement of diabetic nephropathy.

Administering the multi-phytotherapy preparation resulted in an increase of kidney function in a dose-dependent manner, which became visible at the dose of 250 mg/kg as a decrease in serum urea and creatinine levels, as well as partial recovery of urine protein. In the high-dose group (500 mg/kg), almost total normalization of the kidney parameters was achieved, with values being close to those of the metformin group. In addition, there was a significant drop in urine protein output which shows that the glomerular membrane is sound and the leakage of the basil membrane is reduced.

High levels of urine volume in the disease control group were normalized in the treated rats thus indicating recovery of tubular reabsorption function. The reduction in blood urea levels advanced the progress in the excretion of nitrogenous wastes whereas uric acid levels showed the decrease in oxidative stress and complications in purine metabolism. Overall, the data demonstrated the nephroprotective effect of the preparation on kidney functioning despite hyperglycaemia.

The overall biochemical recovery seen in the treated groups highlights the synergistic effect of the phytochemicals that were present. The antioxidants originating from Salvia hispanica help alleviate oxidative stress, while Nigella sativa alleviates inflammation through thymoquinone, and, finally, saponins from Trigonella foenum-graecum promote the effectiveness of insulin and facilitate renal metabolism. The reestablishment of the normal range in urine output, protein excretion, and serum biomarkers proves the importance of the polyherbal formulation in alleviating symptoms of diabetic nephropathy through its antioxidative, anti-inflammatory, and metabolic effects **(Table 1)**.

Table 1: Effect of Polyherbal Formulation on Urine and Serum Biomarkers in STZ-Induced Diabetic Nephropathy Rats.

Group	Treatment	Urine Volume (mL/24h, Mean ± SEM)	Urine Protein (mg/dL, Mean ± SEM)	Blood Urea (mg/dL, Mean ± SEM)	Serum Creatinine (mg/dL, Mean ± SEM)	Serum Uric Acid (mg/dL, Mean ± SEM)	Serum Total Protein (g/dL, Mean ± SEM)
I	Normal Control (Saline)	12.5 ± 0.8	8.2 ± 0.6	28.4 ± 1.2	0.72 ± 0.05	2.1 ± 0.2	6.8 ± 0.3
II	Disease Control (STZ 55 mg/kg)	32.6 ± 1.4 *	42.5 ± 1.8 *	68.7 ± 2.5 *	1.85 ± 0.12 *	5.6 ± 0.4 *	3.2 ± 0.2 *
III	Standard (Metformin 100 mg/kg)	15.8 ± 0.9	12.4 ± 0.7	32.1 ± 1.3	0.84 ± 0.06	2.4 ± 0.3	6.5 ± 0.4
IV	Polyherbal Formulation (250 mg/kg)	20.6 ± 1.1	18.7 ± 0.9	45.2 ± 1.7	1.12 ± 0.08	3.5 ± 0.3	5.4 ± 0.3
V	Polyherbal Formulation (500	16.9 ± 0.8	13.6 ± 0.7	34.8 ± 1.4	0.91 ± 0.07	2.6 ± 0.2	6.2 ± 0.3

	mg/ kg)						

Following the protein leakage from the glomerular basement membrane damage, the serum protein levels diminished significantly in the disease control group, while these were restored in both the treatment groups. The efficacy of the high-dose formulation was stronger than the efficacy of the low-dose formulation, which can indicate the improvement in renal absorption and reduced proteinuria grade. The data reported indicate that the polyherbal formulation is capable of improving renal function by normalizing biochemical parameters and maintaining nephron integrity.

The results obtained in the high-dose treatment group can be explained by the synergistic action of its active components—*Salvia hispanica* is rich in polyphenols and omega-3 fatty acids that counteract oxidative stress, *Nigella sativa* is a good source of thymoquinone known for its anti-inflammatory and antioxidant properties, while *Trigonella foenum-graecum* is a remedy high in saponins and flavonoids that mediate insulin sensitivity and renal metabolism.

Histopathological:

Histopathological analysis of the kidney showed that there were differences in the structure of the kidney tissue among the groups observed in this study. In the control group detailed in Figure I, the kidneys were healthy in all aspects and there were no visible traces of disease. However, the kidneys of the disease control group presented with multiple pathological changes. Observations from Figure II have shown that the lumina of most of the tubules contained deposits, the staining of cells in the tubules had become more severe, and there was significant distortion of glomeruli. Specifically, Grade 5 kidney damage had occurred since 70–80% of the kidney was affected. The kidneys of the metformin-treated normal control animals showed most signs of recovery according to what is presented in Figure III. Though there were still some deposits in the lumen of the tubules, the kidney structure remained rather intact and mostly normal.

The polyherbal formulation was effective in a dose-dependent manner. At a dosage of 250 mg/kg (Figure IV) mild tubular deposits and epithelial changes were observed in the kidneys, indicating only moderate protection as compared to the disease control. At 500 mg/kg (Figure V) the histological results were much more pronounced with very little deposits and nearly normal epithelial structure. This group had the least amount of damage when compared to the other test groups, which illustrates the high nephroprotective potential exhibited by the polyherbal formulation at high doses. Thus, the findings with respect to the histopathology conform with the biochemical observations indicating that the polyherbal formulation protects the kidneys in diabetic nephropathy **(Figure 2)**.

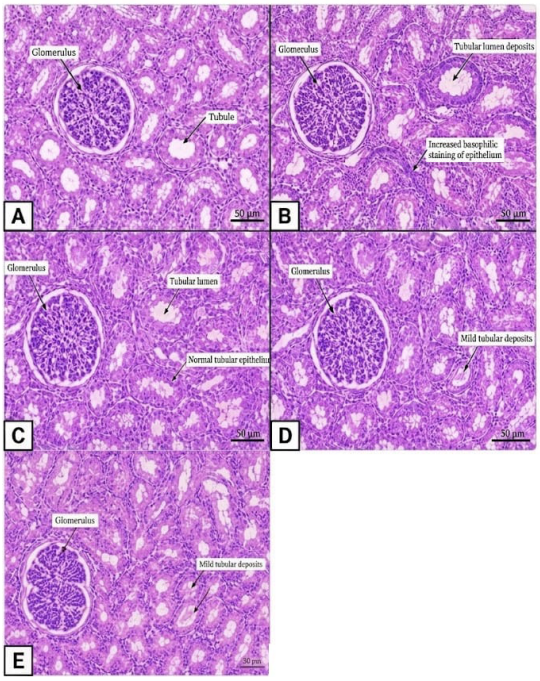


Figure 2: Histology examination of wistar rat's kidney.

CONCLUSION:

The present experiment showed that a herbal mixture which included *Salvia hispanica*, Nigella sativa, and Trigonella foenum-graecum had effective nephroprotective and anti-diabetic properties in Wistar rats suffering from diabetic nephropathy. Biochemical study reported an increase in the effectiveness of markers of renal function such as creatinine, urea, uric acid, and serum proteins depending on the dosage used while histopathological investigations showed that the glomerular and tubular structure had remained unchanged.

It's important to note that in the group that received 500 mg/kg of the mixture, the drug worked better than metformin, thus showing that polyphenols, thymoquinone, and saponins acted synergistically on oxidative stress, inflammation, and fibrosis.

ABBREVIATIONS:

ACE – Angiotensin-converting enzyme
AGEs – Advanced glycation end-products
ANOVA – Analysis of variance
ARB – Angiotensin receptor blocker
BUN – Blood urea nitrogen
CMC – Carboxymethyl cellulose
CPCSEA – Committee for the Purpose of Control and Supervision of Experiments on Animals
CVD – Cardiovascular disease
DN – Diabetic nephropathy
GFR – Glomerular filtration rate
H&E – Hematoxylin and eosin
IAEC – Institutional Animal Ethics Committee
IDF – International Diabetes Federation
NF-κB – Nuclear factor kappa-light-chain-enhancer of activated B cells
Nrf2 – Nuclear factor erythroid 2-related factor 2
ROS – Reactive oxygen species
RAS – Renin-angiotensin system
SEM – Standard error of mean
SGLT2 – Sodium-glucose cotransporter-2
SOD – Superoxide dismutase
STZ – Streptozotocin
TGF-β – Transforming growth factor beta
WHO – World Health Organization

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