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Evaluations of nephro-protective activity of poly-herbal hydroalcoholic extract against streptozotocin-induced nephrotoxicity and renal dysfunction

Research Article

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Abstract

Objective our research work aim was the assessment of the nephroprotective activity of poly-herbal hydroalcoholic extract against streptozotocin-induced nephrotoxicity and renal dysfunction. Poly-herbal hydroalcoholic extract of (PHHE) nephrotoxicity activity was determined using the streptozotocin-induced nephrotoxicity and renal dysfunction method. Poly-herbal hydroalcoholic extract of 200 and 300 mg/kg was given for eight weeks to selected animals rat models. The biochemical parameters like as lipid profile, renal functional, antioxidant enzyme level, anti-inflammatory factors and histopathological evaluations parameters were used for estimation of nephroprotective activity. PHHE also reduced the levels of creatinine, blood urea nitrogen, total cholesterol, triglycerides, advanced glycation end products and albumin in serum and urine, respectively. PHHE drastically increased the antioxidant parameters in the kidney and histological evaluation. All of these evaluations parameters results were exposed that PHEE has potential antioxidant, and anti-glycation activities. Exhibited significant therapeutic effects and was found to be the potential same as standard glimepiride drug. The experimental results were found to be significant nephroprotective action of PHHE extract on STZ induced diabetic nephroprotective animal model. Conclude the PHHE can use for the treatment and management of diabetic nephropathy disease.

Keywords: *Streptozotocin, Diabetic Nephropathy, Solanum xanthocarpum*.

Introduction

Diabetes mellitus is classified as a metabolic syndrome illness due to its association with a range of metabolic conditions. This illness may result in a range of consequences, such as nephropathy, retinopathy, and neuropathy. Diabetes mellitus is classified into two primary types: non-insulin-dependent diabetes mellitus (NIDDM) and insulin-dependent diabetes mellitus (IDDM). Diabetic nephropathy is the prevailing complication linked to diabetes. Globally, the prevalence of type 1 diabetes affects around 24% of the population, whereas type 2-diabetes affects approximately 30-50% of individuals (1, 2). Diabetes may cause a range of pathophysiological alterations in individuals, such as complications in the kidneys, eyes, skin, and heart (3, 4). The alterations are attributed to variables such as oxidative stress, deposition of glycation end products, and production of renal pyrol (5, 6). The presence of these pathophysiological symptoms may lead to consequences including higher levels of albumin in the urine, reduced glomerular filtration rate, raised arterial blood pressure, and fluid accumulation in the body (7, 8).

Diabetic patients often need prolonged treatment, which might result in a range of adverse consequences. Thus, I have opted to concentrate on addressing diabetic nephropathy by the use of a blend of polyherbal medications. The objective of our study is to investigate the nephroprotective effects of certain PHHE (polyherbal drug combination mixture) ethanolic extracts in a rat model with diabetes caused by STZ. Our study hypothesis posits that the majority of possible hepatoprotective herbal plants also exhibit nephroprotective properties, making them suitable for the treatment of diabetic nephropathy. Consequently, I have chosen all prospective hepatoprotective herbal plants to be screened and developed into a new combination of many herbal pharmaceuticals for the treatment and management of disorders connected with diabetes. These herbal drugs are listed in Table 1.

Based on our comprehensive review of literature, there is a lack of scientific evidence about the polyherbal combination of these plants. Additionally, there are no existing studies on the synergistic effects of these plants on diabetes mellitus nephropathy illness. Hence, our study aimed to evaluate the impact of PHHE on streptozotocin (STZ)-induced diabetes mellitus in rats.

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Table: 1 List of potential hepatoprotective herbal plants for screening nephroprotective protective action

Name of plants	Phytochemicals	Pharmacological properties	References
Gymnema sylvestre	flavones, anthraquinones, d-quercitol, gymnemic acid, gymnemosides, gymnemasaponins, lupeol, β-amyrin related glycosides and stigmasterol	Antidiabetic, hepatoprotective and anti-inflammatory activities, etc.	9, 10
Momordiaca charantia	Triterpenoids, saponins, polypeptides, flavonoids, alkaloids and sterols	Antifertility, antiulcer, antihyperglycemic, hepatoprotective immunomodulation, antioxidant, antimutagenic, antilipolytic, etc	11, 13
Syzygium cumini	Anthocyanins, glucoside, ellagic acid, isoquercetin, kaemferol and myrecetin	Antidiabetic, throat infection, asthma, hepatoprotective, dysentery and ulcers, etc.	14, 15
Trigonella foenum	Ederagin glycosides. Alkaloids such as trigocoumarin, nicotinic acid, trimethyl coumarin and trigonelline	Antidiabetic, antioxidant, anticarcinogenic, hypocholesterolemic, hepatoprotective, and immunological activ-ities, etc.	16, 17
Psidium guajava	Iso-caryophyllene, veridiflorene, farnesene, dl-limonene, δ-cadinene, α-copaene, α-humulene, τ-cadinol.	Used in problems of dental, sleeping, liver, convulsion, respiratory, wound healing, pain and treatment of diabetes mellitus, etc.	18, 19
Tinospora cardifolia	Alkaloids, Terpenoids, Lignans, Steroids and others	Reported antioxidant, hepatoprotective, anticancer, wound treatment, anticancer and immunomodulating activity, etc.	20, 21
Boerhavia diffusa	Alkaloids (punarnavine), rotenoids (boeravinones A to J) and flavones.	Reported used in treatment of inflammatory, stone, microbial, asthma, urine, liver, and diabetic treatment, etc.	22, 23
Coriandrum sativum	Linalool, α-Pinene, β-Pinene, γ-Terpinene, α-Cedrene, α-Farnasene, p-Cymene, Limonene, Citronellal, Camphor Geraniol, Anethole.	Reported activity against of microbial, liver toxicity, diabetic, fatty condition, convulsion, cancer, and inflammation.	24, 25
Andrographis paniculata	Andrographolide, and 14-deoxy-11, 12-didehydroandrographide, etc.	It is having anti-inflammatory, liver protective, and management of blood pressure property, etc.	26, 27
Mixture of *Haritaki, Bibhitaki and Amalaki*	Flavonoids, alkaloids, phenols	Cardiovascular disease, blood pressure disease, poor liver function, large intestine inflammation, hepatoprotective and ulcerative colitis	28, 29
Trikatu (Mixture of black pepper (kali mirch), ginger (adhrakh) and long pepper (pippali)	Piperine, gingerols, shogaols, and paradols, oleoresins, and alkaloids.	Bioavailability enhancer, fevers, gastric and abdominal disorders, urinary difficulties, hepatoprotective, neuralgia and boils etc.	30, 33

Materials and Methods

Drugs and chemicals

Rat TNF-α Elisa kit was purchased from Ray Biotech Inc, USA, and HbA1c kit Biosystem S.A. The total protein kit was purchased from ERBA Diagnostic, Germany), Glucose estimation kit from Logotech, New Delhi, respectively. All remaining chemicals and reagents were analytical grade used in the research study.

Plant material

Extraction of poly-herbal formulation

Selected herbal plants and herbal materials were collected, collected through the local purchasing from market of Mandsaur, Madhya Pradesh. The herbal plant and materials were clean, washed, and dry under shade. The dried plant materials were taken in equal ratio. These coarsely powder material was used for extraction. Plant material (1 Kg) was weighed and packed with extraction solvent (Ethanol: Distilled water (70:30)) in an airtight container for maceration. The sample was regularly shaken in between 12 hrs for 15 days. Then after extraction solvent was filtered and evaporated using rotary vacuum evaporator at 40̊ C. The poly-herbal hydroalcoholic extract (PHHE) was concentrated to yield a residue (150 g). The dried PHHE extract was kept in an airtight container in a cool place for further used (34-35). The poly-herbal hydroalcoholic extract was selected for the study of hepatoprotective activity (Table 2).

Table: 2 Name of herbal plants for Poly-herbal extraction

Name of plants	Biological name
1. *Gudmar* leaves	*Gymnema sylvestre*
2. *Karela* seed	*Momordiaca charantia*
3. *Jamun* seed	*Syzygium cumini*
4. *Methi* seed	*Trigonella foenum*
5. *Amruda* leaves	*Psidium guajava*
7. *Giloya*	*Tinospora cardifolia*
8. *Punarnava*	*Boerhavia diffusa*
7. *Coriander* leaves	*Coriandrum sativum*
8. *Kalmegha* leaves	*Andrographis paniculata*
9. *Triphala*	*Mixture of Haritaki, Bibhitaki and Amalaki*
10. *Trikatu*	*Mixture of black pepper, and dry ginger*
11. *Aswagandha*	*Withania somnifera*

Phytochemical analysis of polyherbal hydroalcoholic extract

Phytochemical analysis of polyherbal hydroalcoholic extract was performed using tests method for Sterols, alkaloids, tannins, glycosides, flavonoids, phenolic compounds, and saponins (36-42).

Experimental animals study

According to the standards and demands of the animal ethical community, the animals were housed in an animal laboratory with access to clean water at all times and a balanced meal. The Institutional Animals Ethical Committee's (IAEC) permission number 10/ Ph.D./2020/IAEC/BRNCP/Mandsaur allowed animal studies.

Procedure for acute toxicity study

To assess the acute toxicity of the PHHE, we conducted studies on albino rats. The rats were separated into 5 groups, each containing 6 animals. Before the start of the experiment, the selected female rats were held on overnight fasting conditions. The animals were then administered PHHE orally in doses of 1-50mg, 2-100mg, 3-200mg, 4-1000mg and 5-2000mg/kg, with each group receiving a different dose. The animals were observed for 14 days to monitor their responses. During this time, no abnormal behaviour was observed, and no deaths were reported. The initial two dose levels were selected as the first dose, which was 1/8th part of the upper dose. The second dose level was chosen as 1/8th of the first dose, which were 200 and 300mg/kg for in vivo studies (43).

Administration of poly-herbal hydroalcoholic extract to female rats

The poly-herbal hydroalcoholic (PHHE) extract was given to female rats in form of suspension. The prepared PHHE extract was taken and well crushed, mixed uniformly, and made up of a suspension by using tween 80. Prepared homogeneous suspension of the PHHE extract was given by an oral feeding tube, at the dose of 200 and 300 mg/kg daily between 10.00 am and 11.00 am in order to avoid circadian rhythm.

Induction of diabetic nephropathy (Streptozotocin induced neonatal rat model)

We induced diabetic nephropathy in our study using the NIDDM STZ-neonatal albino rat model [44]. Three female rats were kept in a caged environment with one male for mating. After 21-24 days of gestation, the female rats gave birth to neonatal rats, which were used for further studies. To induce diabetes in these neonatal rats, we used streptozotocin (STZ).The STZ solution was prepared by using 0.1M citrate buffer, pH 4.5 at a dose of 90 mg/kg. It was given through the route of intraperitoneally to neonatal rats selected animals. Then further 8th weeks of STZ administration, diabetes disease induce in neonatal rates. Diabetic disease suffering neonatal rats was randomly selected for the study of NIDDM positive disease, and experiment was performed according to guidelines of animal ethical protocol (44).

Experimental protocol

The experimental protocol involved 5 groups of animals, each consisting of 6 albino rats, which were used in the research work.

- Group I served as the control group, receiving a 0.5% sodium carboxymethyl cellulose (CMC) solution orally for duration of 8 weeks.
- Group II served as the control group for diabetes, and diabetes was induced in these rats by administering STZ. Following the confirmation of diabetes, these animals were administered a 0.5% sodium carboxymethyl cellulose (CMC) solution for a duration of 8 weeks.
- Group III and IV were administered PHHE at dosages of 200 and 300 mg/kg, respectively, to diabetic STZ-induced rats. Following the diagnosis of diabetes, the animals were administered a poly-herbal hydroalcoholic extract (PHHE) for a duration of eight weeks.
- Group V received Glimepiride as the reference medication (0.35 mg/kg, orally in STZ). The medicine Glimepiride was delivered at a normal dosage of 0.35 mg/kg via the intraperitoneal route for a duration of 8 weeks. The segregated serum was maintained at a low temperature for further examination (45, 46).

Estimation of body weight and glucose level in blood

Prior to the administration of STZ-NAD, the body weight and blood glucose level of each animal were evaluated. The body weight of each group was regularly evaluated till the study's completion. During the experiment, the fasting blood glucose level was assessed using enzymatic kits obtained from Reckon Diagnostics Pvt. Ltd. India. This was done after 72 hours after STD injection to confirm the presence of diabetes, and subsequently at 28-day intervals (45, 46).

Estimation of lipid profile

The serum lipid profile levels estimated through the process triglyceride (TG) (GPO-PAP method), total cholesterol (TC) (CHOD-PAP method), and high-density lipoprotein (HDL) precipitating method. The lipid profile parameters were used for the find out of level of lipid, TC, HPL, VLDL respectively. The lipid profiles were calculated using Friedewald formula.

Estimation of renal function parameters

Urine was collected from the animal continuously for 24 hours in order to determine the parameters of renal function. The urine volume (ml/24 h) was determined by measuring the collected urine samples using a graduated cylinder. The urine samples were analysed using commercially available Erba Chem-7 kits to quantify urinary proteins (using the biuret technique), urea (using the urease L-glutamate dehydrogenase method), and creatinine (using the Jaffe method). Following the measurement of the 24-hour urinary albumin excretion rate (UAER), we assessed the presence of type IV collagen in urine samples using the enzyme-linked immunosorbent assay (ELISA) kits

supplied by Abcam. (Cambridge, MA, USA) (ab 6586) (47, 48).

Determination of advanced glycation end products in kidney homogenate

The amounts of advanced glycation end products (AGES) in kidney tissue were estimated. The kidney tissue was soaked overnight in a solution containing chloroform and methanol at a ratio of 2:1 (volume/ volume). The liquid combination was separated from the upper layers of renal tissue. A solution of NaOH with a concentration of 0.1N was added to the residual liquid residue. The resulting mixture was then centrifuged at 5000 rpm for 15 minutes at a temperature of 4°C, and the supernatant was collected. The concentration of AGES solubilised in alkali was determined by measuring the fluorescence of the samples using a fluorescence spectrophotometer. The emission wavelength used was 440 nm and the excitation wavelength was 370 nm. The measurements were compared to blank samples containing 1 ml of 0.1N NaOH. A single unit of fluorescence corresponds to a concentration of 1 milligramme per millilitre of bovine serum albumin, serving as the standard. The fluorescence readings of the test sample, with a protein content of 1 mg/ml, were quantified in arbitrary units (47-49).

Estimation of antioxidant enzyme levels

Isolated tissue (kidney, pancreas, and liver) was homogenate and used to estimate thiobarbituric acid reactive substances (TBARS) and level of antioxidant enzymes superoxide dismutase (SOD) and reduced glutathione (GSH) (50).

Estimation of inflammatory cytokines

ELISA kits of abcam were used for the analysis of cytokines in serum, interleukin-6 (IL-6), transforming growth factor (TGF)-β1, and tumour necrosis factor-alpha (TNF-α) levels (51, 52).

Histopathological evaluation

The kidneys were collected, dissected, and stored in buffered formalin solution for 12 h and cut into size of 3 µm thickness. Then after staining was applied using hematoxylin & eosin and periodic acid-Schiff stain (PAS) respectively. The histopathological evaluation studies were performed using light microscope. Histopathological studies of dissected kidneys were performed, and noted using following parameters such as glomerular size, cellularity and tubules epithelium in hematoxylin & eosin (HE) stained specimens and glomerular basement membrane, mesangial matrix and tubules basement membrane in PAS-stained specimens, kidney Normal PAS 40x (53).

Results and Discussion

Extraction

The polyherbal hydroalcoholic extract (PHHE) was found to be 9.78% yield.

Phytochemical analysis of polyherbal hydroalcoholic extract

Phytochemical screening was performed by using polyherbal hydroalcoholic extract (PHHE) and found to be an alkaloids, tannins, glycosides, flavonoids, phenolic compounds, and saponins were present, confirmed that PHHE having potential therapeutic activity.

Effect of PHHE extract on weight

Observable physiological changes were recorded in the body after eight weeks of administering the dosage, specifically an increase in body weight was seen in the control group of rats. The body weight of DN rats treated with PHHE 200 mg/kg and PHHE 300 mg/kg increased significantly, measuring 265.15 ± 7.03gm and 347.72 ± 11.65gm respectively. The findings were compared to a group of rats with diabetes as the control. Substantial disparities in the ultimate body weight of the treatment groups were observed. The administration of PHHE resulted in a considerable increase in body weight, accompanied by a decrease in kidney weight and hypertrophy.

Effect of PHHE extract on blood glucose

Administration of STZ-NAD led to a notable and progressive rise in fasting blood glucose levels in diabetic rats, in comparison to nondiabetic rats. Nevertheless, administration of PHHE extract to diabetic rats resulted in a dose-dependent decrease in blood glucose levels. The research observed a significant decrease in fasting blood glucose levels in diabetic rats that were administered PHHE at dosages of 200 mg/kg and 300 mg/kg and were blood glucose levels were found to be 18.90 ± 3.62mmol/L and 14.50 ± 1.17mmol/L, respectively. The findings were similar to those of glimepiride (10 mg/kg), which decreased blood glucose levels to a maximum of 4.09 ± 0.46at the conclusion of the research. Table 3, Figure 1.

Biochemical estimation of serum lipid profile

The serum lipid profile was assessed and revealed that a dosage of 300mg and 200mg of PHHE considerably reduced the levels of triglycerides (TG), total cholesterol (TC), low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL) in the blood ($P<0.01$). Additionally, it raised the level of high-density lipoprotein (HDL) in the blood. In comparison, the findings obtained with a dosage of 300mg of PHHE were not as effective. The results are shown in below Table 4. Figure 2.

Renal function tests

According to the findings in Table 5 and 6, administering PHHE at doses of 200mg/kg and 300mg/ kg resulted in a notable rise in urine volume ($P<0.01$), urinary creatinine ($P<0.01$), and urinary urea ($P<0.05$ and $P<0.01$) compared to the rats in the diabetic control group. Furthermore, there was a notable reduction in proteinuria, urinary albumin excretion rate (UAER), advanced glycation end products (AGES), and type IV collagen excretion ($P<0.01$) in compared to DN rats.

These findings suggest that PHHE has a positive effect on renal functions in diabetic rats. Table 5, Figure 3.

Estimation of antioxidant enzyme levels and lipid peroxidation

The concentration of GSH (glutathione) was markedly reduced in the kidney, liver, and pancreas of rats with diabetic nephropathy (DN). The reduction in the amount of glutathione (GSH) serves as a marker of oxidative stress. The administration of PA and PHA for a duration of 45 days resulted in a significant elevation in antioxidant enzyme levels when compared to the DN control group. The study observed a substantial decrease in the level of superoxide dismutase (SOD) in rats with diabetic nephropathy (DN), which serves as an additional evidence of oxidative stress. Nevertheless, the administration of PA and PHA resulted in a substantial elevation in these levels in the kidney, liver, and pancreas, therefore mitigating oxidative stress.

The level of lipid peroxidation, a measure of oxidative stress, was significantly elevated in DN control rats compared to the normal control group. The groups treated with PA and PHA showed a considerable reduction in the amount of TBARS, which is a marker of lipid peroxidation. This suggests that these extracts have antioxidant properties. Overall, the treatment with PA and PHA showed a protective effect against oxidative stress in DN rats (Table 7).

Estimation of inflammatory cytokines

The examination of the cytokines test findings indicated a substantial reduction in the doses of PHHE at 200mg and 300mg/kg, respectively. PHHE significantly reduced the concentrations of interleukin-1 (IL-1), interleukin-6 (IL-6), and transforming growth factor beta (TGF- β1), while increasing the concentration of interleukin-10 (IL-10), with a statistically significant impact ($P<0.001$). The cytokine analysis findings, as reported in table 5, indicate a reduction in cytokine levels. This suggests that both doses of PHHE are beneficial in managing inflammatory diseases. The results are shown in [Table 8].

Table 3: Effect of PHHE on body weight blood glucose level of STZ-diabetic rats

Groups	Body weight (gm) 4th week	Blood glucose level (mmol/L)
Control	398.14 ± 2.89	4.12 ± 0.45
Diabetic	221.37 ± 14.63	27.76 ± 2.81
200 mg/kg PHEE	265.15 ± 7.03	18.90 ± 3.62
300 mg/kg PHEE	347.72 ± 11.65	14.50 ± 1.17
Glimepride10 mg/kg	394.56 ± 11.83	4.09 ± 0.46
All values are expressed as mean ± SEM, with six animal groups		

Table 4: Effect of PHHE on lipid profile

Group	TGs (mg/dl)	TC (mg/dl)	HDL (mg/dl)	VLDL (mg/dl)	LDL (mg/dl)
NC	68.5± 0.647	87.0± 3.06	28.6± 1.23	13.8± 0.118	38.5± 1.92
DNC	175± 1.21	196.0± 6.64	18.4± 0.30	34.8± 0.393	143± 4.37
200 mg/kg PHHE	132± 1.16	87.3± 2.42	21.2± 0.55	26.3± 0.443	92.46± 1.20
300 mg/kg PHHE	88.1± 1.74	79.0± 1.46	24.0± 0.78	17.4± 0.280	61.7± 2.50
Glimepride 10 mg/kg	81.2± 2.65	64.1± 1.60	20.0± 0.32	14.2± 0.610	56.6± 1.38
All values are expressed as mean ± SEM, with six animal groups					

Table 5: Effect of PHHE on renal function tests

Group	Urine volume (ml/rat/day)	Urinary urea (mg/dl)	Serum creatinine (mg/dl)	Urine creatinine (mg/dl)
NC	11.4±0.81	5.34±0.99	0.6±0.04	26.7±1.46
DNC	4.0±0.27	0.02±0.03	2.40±0.43	5.8±0.99
200 mg/kg PHHE	17.9±1.71	2.68±1.091	1.12±0.03	20.2±1.17
300 mg/kg PHHE	13.3±1.06	3.68±0.34	0.944±0.02	23.8±0.71
Glimepride 10 mg/kg	12.1±2.04	3.79±0.30	0.940±0.01	20.1±0.17
All values are expressed as mean ± SEM, with six animal groups				

Table 6: Effect of PHHE on Protein in urine, UAER, AGES and Type IV collagen excretion

Group	Protein in urine (mg/day)	UAER (µg/day)	AGES (AU)	Type IV collagen excretion (µg/day)
NC	0.23±0.042	1.8±0.33	197±14.3	16.3±3.48
DNC	24.70±0.422**	16.8±1.51**	455±57.7**	750±76.4**
200 mg/kg PHHE	0.56±0.098##	6.0±0.57##	252±19.2##	80.0±5.77##
300 mg/kg PHHE	0.08±0.030##	2.9±0.56##	227±22.0##	68.3±5.63##
Glimepride10 mg/kg	0.07±0.020##	2.5±0.42##	220±11.0##	65.1±4.50##
All values are expressed as mean ± SEM, with six animal groups				

Table 7: Effect of PHHE on level of SOD (U/mg protein), GSH (mM/mg protein) and TBARS (nmol/mg protein)

Parameters	SOD (U/mg protein)			GSH (mM/mg protein)			TBARS (nmol/mg protein)		
Groups	Kidney	Pancreas	Liver	Kidney	Pancreas	Liver	Kidney	Pancreas	Liver
Normal	4.89 ± 0.091	4.21 ± 0.299	3.73 ± 0.097	75.92 ±0.619	69.49 ±0.291	67.20 ±0.466	0.56±0.01	0.45 ±0.012	0.42 ±0.004
Diabetic control	1.26 ± 0.055	1.20 ± 0.064	1.12 ± 0.048	37.59 ±0.479	41.92 ±0.374	45.57 ±0.489	2.97±0.016	2.70 ±0.065	2.47 ±0.068
PHHE 200 mg/kg	2.057 ± 0.023	1.36 ± 0.018	1.17 ± 0.024	56.59 ±0.284	54.55 ±0.370	52.84 ±0.219	1.28±0.022	1.11 ±0.022	1.13 ±0.006
PHHE 300 mg/kg	3.782 ± 0.030	3.11 ± 0.012	3.13 ± 0.016	66.39 ±0.284	56.89 ±0.237	60.02 ±0.069	2.20±0.010	1.73 ±0.011	1.43 ±0.008
Glimepride 10 mg/kg	3.97 ± 0.022	4.07 ± 0.153	3.68 ± 0.114	68.87 ±0.540	62.28 ±0.351	62.24 ±0.349	1.18±0.04	0.97 ±0.019	0.93 ±0.030
All values are expressed as mean ± SEM, with six animal groups									

Table 8: Effect of PHHE on IL-6, TGF-β1 and TNF-α inflammatory mediator

Group	IL-6 (Pg/ml)	TGF-β1 (%)	TNF-α (Pg/ml)
NC	164±4.80	12.7±2.19	34.2±4.55
DNC	361±11.6**	25.5±2.57**	368±37.1**
DN + test 1	275±5.62##	13.2±1.74##	140±10.6##
DN + test 2	252±8.72##	13.3±1.33##	75.0±7.64##
All values are expressed as mean ± SEM, with six animal groups, where test 1(200 mg/kg PHEE), test 2 (300 mg/kg PHEE) used.			

Figure 1: Effect of PHHE on body weight blood glucose level of STZ-diabetic rats

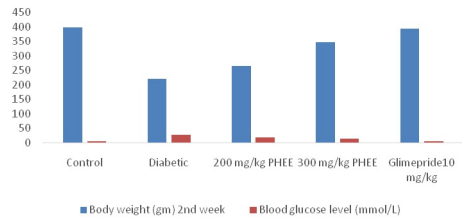


Figure 2: Effect of PHHE on lipid profile

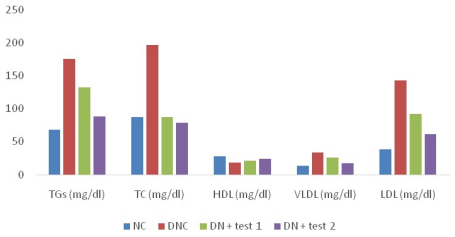


Figure 3: Effect of PHHE on renal function tests

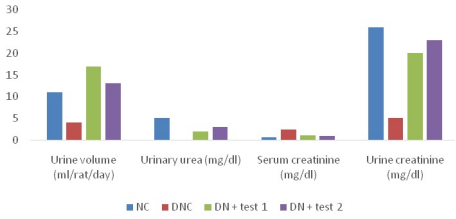


Figure 4: Effect of PHHE on Protein in urine, UAER, AGES and Type IV collagen excretion

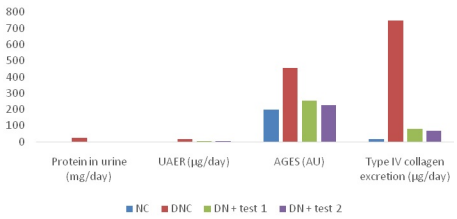
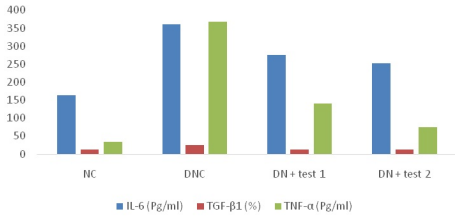


Figure 5: Effect of PHHE on IL-6, TGF-β1 and TNF-α inflammatory mediator



Histopathological evaluation

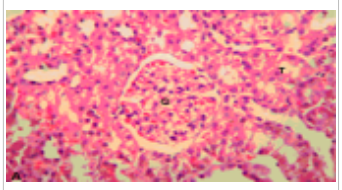
The photomicrographs in Figure 6 a to j depicted the kidney tissue slices. The aforementioned sections were extracted from rats with diabetic nephropathy caused by streptozotocin. They were utilised to evaluate histological changes and associated problems. The diabetic control group exhibited histological alterations such as heightened glomerular cellularity, thicker glomerular basement membranes, and augmented mesangial matrix in PAS stains, in comparison to the normal control group. Nevertheless, the administration of PHHE (orally, at dosages of 200 and 300 mg/kg) mitigated these abnormal histological alterations in a way that depended on the dosage. The group treated with a lesser dosage (200 mg/kg) exhibited a little rise in mesangial matrix with a subsequent reversal of the observed alterations. Conversely, the group supplied a greater dose (300 mg/kg) had a reduced level of safety. The administration of the conventional medication glimepiride at a dosage of 0.35 mg/kg orally had similar results to those seen with a lower dosage of PHHE (200 mg/kg). Figure 7 displays two entities labelled as A and B. The control group consists of individuals with diabetes who have been assigned to groups C and D (3). Standard Group consists of elements E and F, with a total of four members. The PHHE 200 Group consists of members G and H, totalling 5 individuals. The PHHE 200 Group consists of individuals I and J. The histopathology of the rat's kidney was examined in the following groups: (a) normal rats, (b and c) rats treated

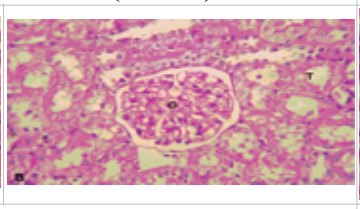
with DNC, (d) rats treated with PHHE at a dose of 200mg/kg body weight, and (e) rats treated with PHHE at a dose of 300mg/kg body weight.

It is crucial to acknowledge that nephrotoxicity, while primarily affecting the kidneys, may be caused by factors other than exposure to dangerous chemicals. Nephrotoxicity may also be influenced by other variables such as infections, autoimmune illnesses, and genetic susceptibility. Moreover, while rats may serve as a suitable model for investigating nephrotoxicity, the results may not always be directly applicable to people. Therefore, additional research is necessary to ascertain the effectiveness and safety of prospective therapies (54-57).

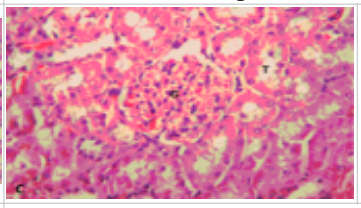
Figure 6: Histology of kidney tissues in different groups

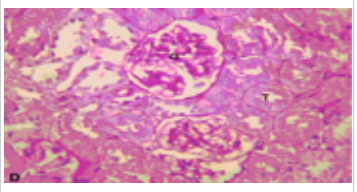
1. Group: Normal control (a and b)



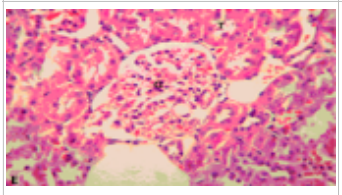


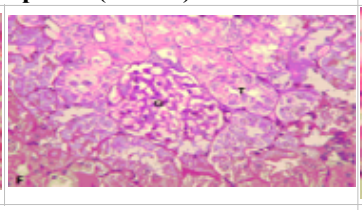
2. Group: Diabetic Control (c and d)



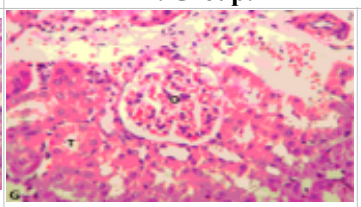


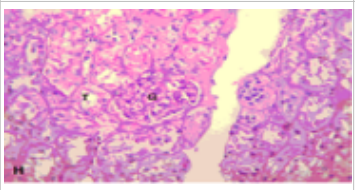
3. Group: Glimepiride (e and f)



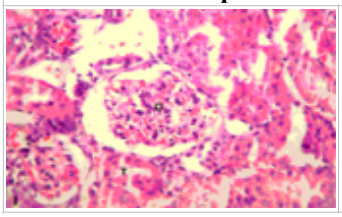


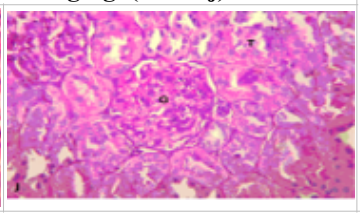
4. Group: PHHE 200 mg/kg (g and h)





5. Group: PHHE 300 mg/kg (i and j)





1. Group: Normal control,
2. Group: Diabetic Control,
3. Group: Glimepiride 0.35 mg/kg,
4. Group: PHHE 200 mg/kg,
5. Group: PHHE 300 mg/kg

The D.N. illness was generated by administering STZ, which causes damage to the cells of the islets of Langerhans. This damage results in decreased insulin production and the subsequent development of diabetes, along with its related comorbidities, such as diabetic nephropathy. The rats that received STZ treatment had typical manifestations of diabetic mellitus, including a decrease in body weight and an elevation in renal tissue weight. Diabetes mellitus often leads to weight loss, and our investigation found that the rats induced with STZ saw a decrease in body weight.

The treatment suggests a possible safeguarding impact against renal enlargement caused by diabetic nephropathy. Additionally, it is worth mentioning that the increase in body weight seen in the group treated with PHHE may be due to the possible hypoglycaemic effects of the extract, since elevated blood glucose levels are known to lead to weight loss in patients with diabetes. Hence, the decrease in blood glucose levels reported in the group treated with PHHE may have further influenced the enhancement in body weight.

The findings indicate that PHHE therapy has the potential to mitigate the development of diabetic nephropathy by lowering elevated blood sugar levels and preventing abnormal kidney growth. Hyperglycemia, a characteristic feature of diabetes, may lead to excessive synthesis of glucose by increasing hepatic gluconeogenesis and glycogenolysis, while simultaneously reducing glucose uptake via tissue and glucose control. The administration of PHHE therapy resulted in a reduction in plasma insulin and blood glucose levels, suggesting its potential therapeutic benefits for diabetes (58-60).

Glycosylated haemoglobin, or HbA1c, serves as a durable indicator of blood glucose regulation over an extended period of time. In the rats stimulated with STZ, the levels of glycosylated haemoglobin were elevated, suggesting inadequate glycemic control. Nevertheless, administration of PHHE substantially lowered the concentrations of HbA1c, indicating that PHHE could have a beneficial impact on glucose regulation. The significance of these results lies in the fact that maintaining optimal glucose control is crucial for the prevention and management of diabetic complications, such as diabetic nephropathy.

The research found that STZ-induced diabetic rats had markedly elevated levels of creatinine, BUN, and uric acid in comparison to the normal control group, suggesting compromised renal function. Nevertheless, administration of PHHE at doses of 200 and 300 mg/kg, orally, resulted in a noteworthy decrease in the concentrations of creatinine, BUN, and uric acid, suggesting an improvement in renal function. The results indicate that PHHE has a beneficial impact on diabetic nephropathy by enhancing renal hemodynamics and reducing the development of proteinuria, glomerulosclerosis, and renal failure (61-62). These findings serve as a significant marker of

kidney well-being. The research observed increased amounts of creatinine in both the blood and urine of rats with STZ-induced DN, suggesting compromised kidney function. Nevertheless, administration of PHHE at doses of 200 and 300 mg/kg resulted in a considerable decrease in urinary protein, albumin, and creatinine levels, as well as blood creatinine levels. These findings indicate that PHHE may have a safeguarding influence on renal function in diabetic nephropathy (63-64).

Diabetes and its consequences, such as diabetic nephropathy, are linked to heightened oxidative stress, which may result in harm to several organs, including the kidneys. PHHE has shown antioxidant characteristics, potentially mitigating oxidative stress and preventing or diminishing kidney damage in cases of diabetic nephropathy and inflammation. Elevated levels of MDA and 4-HNE were detected in the renal tissue of rats with STZ-induced DN, suggesting the presence of oxidative stress and lipid peroxidation. Nevertheless, the administration of PHHE therapy resulted in a substantial drop in the concentrations of MDA and 4-HNE, suggesting a significant reduction in oxidative stress and lipid peroxidation. The significant antioxidant effects of PHHE are attributed to the presence of phenolic substances, such as flavonoids, which effectively eliminate free radicals and provide protection against oxidative damage. Thus, it is possible that PHHE has a safeguarding influence on diabetic nephropathy by diminishing oxidative stress and lipid peroxidation in the renal tissue.

The levels of superoxide dismutase (SOD) and catalase (CAT) were considerably reduced in rats with streptozotocin-induced diabetic nephropathy (DN), suggesting an elevation in oxidative stress. Nevertheless, therapy with PHHE substantially elevated the concentrations of SOD and CAT, suggesting its potential to mitigate oxidative stress in diabetic nephropathy. Furthermore, PHHE therapy exhibited a substantial decrease in the levels of MDA and 4-HNE, suggesting its potential to mitigate lipid peroxidation and consequent tissue harm induced by ROS. In summary, our results indicate that PHHE may have a favourable impact on oxidative stress and the activity of antioxidant enzymes in individuals with diabetic nephropathy.

In our recent investigation, we discovered that STZ-induced DN and adropinin had a substantial effect on the endogenous antioxidant parameter, indicating their antioxidant function. However, the levels of SOD were reduced and the levels of MDA were increased. An inflammatory reaction has a substantial impact on the transmission of diseases. During an inflammatory reaction, there is an augmentation in the synthesis of cytokines and inflammatory mediators. The advancement of diabetic nephropathy is facilitated by the inflammatory cytokines IL-1, IL-6, and TNF (69).

Epithelial, glomerular, and mesangial cells exhibited cytotoxicity with exposure to cytokines such as TNF-. TNF- induces direct kidney damage via the generation of free radicals. IL-6, which is another kind of cytokine, enhances endothelial permeability, elevates fibronectin levels, influences the dynamics of the extracellular matrix, and stimulates the proliferation of mesangial cells. Cytokines such as IL-1 have been connected with the development of abnormalities in the flow of blood inside the glomeruli, which are related to the production of prostaglandins (70).

In this investigation, we found that the PHHE had a substantial reduction in cytokines in both the tissue and serum, demonstrating its anti-inflammatory effect (71-72). The significance of NF-B as a therapeutic target for controlling oxidative stress and inflammatory responses has been extensively proven. NF-B has a role in decreasing oxidative stress in diabetic nephropathy and is involved in the progression of renal damage. In order for IB to undergo phosphorylation and degradation, the activation of NF-B is necessary. The dysregulation of cytokines and oxidative stress arises from the activation of NF-B. (73-76).

The elevated concentration of proinflammatory cytokines is a result of the activation of NF-B during DN. Our experimental findings demonstrate that the PHHE had anti-inflammatory effects and significantly reduced the level of NF-B (77). Conclusion: Our experimental results indicate that PHHE has a nephroprotective effect by mitigating the inflammatory and oxidative stress conditions induced by the NF-B signalling pathway. Further investigation is necessary to have a deeper comprehension of the clinical implementation of PHHE (77).

The group administered with PHHE at a dosage of 200 mg/kg had the lowest degree of histopathological harm. The group of patients treated with glimepiride had comparable histology results, whereas PHHE (300 mg/kg) demonstrated a lesser degree of protection. The assessment data indicate that hydroalcoholic extracts of poly-herbal mixtures may possess nephroprotective properties. The dosage level directly impacts the likelihood of achieving therapeutic results. In order to have a comprehensive understanding of the exact mechanism by which the PHHE extract protects the kidneys, more extensive and comprehensive study is necessary.

Conclusion

The study's findings indicate that PHHE extract shows promise as a viable therapeutic choice for treating and managing diabetic nephropathy. Nevertheless, it is crucial to acknowledge that this study was carried out on rodents, and more investigation is required to see if these results can be reproduced in people. Prior to recommending PHHE extract as a therapy for diabetic nephropathy, it is essential to assess its safety and effectiveness in human clinical studies.

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Conflict of interest statement

We pronounce that we have no conflict of interest.

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