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Biological and Hypoglycemic Effects of a Polyherbal Extract on Alloxanized Diabetic Rats

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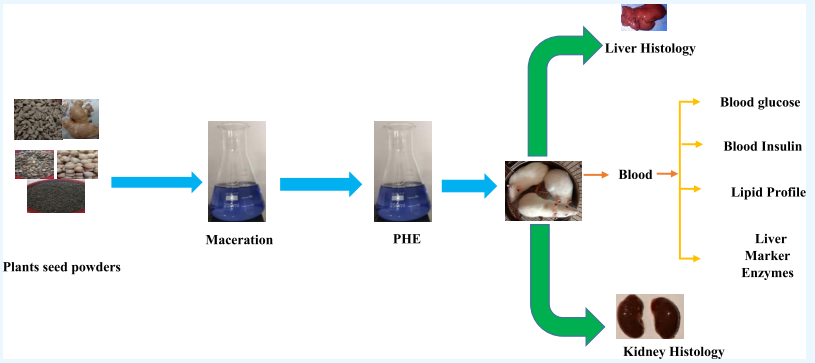

ABSTRACT: The current study investigates the antioxidant, antidiabetic, hepatoprotective, and nephroprotective potentials of a polyherbal mixture containing the methanolic extracts of seeds from *Nigella sativa*, *Cicer arietinum*, *Silybum marianum*, and *Citrullus colocynthis* and the rhizome of *Zingiber officinale*. The polyherbal extract (PHE) showed significant total phenolic contents (187.17 GAE/g), ferric reducing power (28%), and radical-scavenging activity (86.16%). The PHE also showed a substantial hypoglycemic effect in alloxan-induced diabetic rats by reducing the blood glucose level of the PHE-treated rats (−48.64%) and increasing the insulin level (107.5%) as compared with the diabetic control group. Likewise, an increase in high-density lipoprotein (HDL) contents (22.95%) with an associated decrease in low-density lipoprotein (LDL) levels (−43.93%) was also noted. A significant decrease in serum levels of liver marker enzymes, *e.g.*, SGPT (−36%), SGOT (−31%), and serum ALP (−12%), was also observed as compared with the standard drug-treated group. Based on the findings of the study, it may be suggested that PHE helps ameliorate the severity of diabetes as a herbal remedy and might be employed in nutra-pharmaceuticals, replacing synthetic antidiabetic compounds.

INTRODUCTION

The chronic metabolic syndrome, Diabetes mellitus, is characterized by hyperglycemia, glycosuria, and absolute deficiency of insulin. Previously conducted studies described oxidative stress as an important cause of diabetes and its complications. Oxidative stress induces the increased production of reactive oxygen species (ROS), which cause cellular damage, resulting in a decreased release of pancreatic $\beta(\beta)$ cells.[1] Many synthetic drugs, including biguanides, α-glucosidase inhibitors, and sulfonylureas, have been used to regulate elevated levels of blood glucose or treat hyperglycemic conditions. However, these drugs have adverse effects (hypoglycemia and hepatotoxicity) on diabetic patients due to their inefficiency in normalizing the lipid profile.[2] Hence, it is necessary to search for alternative products that may increase the insulin secretion from pancreatic β cells with less/no adverse effects. According to ethnobotanical data, about 800 plants have shown antidiabetic potential; hence, plants rich in antioxidants can be utilized to cure diabetes mellitus.[3] Various plants have shown moderate to good antidiabetic potential in different *in vivo* studies. Therefore, the current study explores five selected plants species (*Nigella sativa*, *Cicer arietinum* Linn., *Zingiber officinale*,*Citrullus colocynthis*, etc.) for the development of herbal products that may better regulate the blood glucose of diabetic patients with very few side effects.[4]

N. sativa, commonly known as black seed/kalonji, has been found to greatly affect the sugar digestion system in streptozotocin-induced diabetic rats by improving insulin

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secretion and C-peptide levels.[5] *C. arietinum* Linn. (Chickpea) leaves and seeds have exhibited good hypoglycemic effects by stimulating insulin secretion.[6] *Z. officinale* (ginger) is an underground rhizome that can help manage type 2 diabetes mellitus. It inhibited the activity of intestinal glucosidase and decreased amylase levels, resulting in a decrease in glucose absorption.[7] The extract of *C. colocynthis* (bitter apple) administered to diabetic rats showed a decrease in blood glucose levels and stimulated increased insulin secretion.[8] Likewise, the seeds of *S. marianum* (milk thistle) are also reported to have a positive effect in reducing the glucose level in patients with diabetes mellitus.[9]

Herbal formulations have plenty of bioactive compounds, including alkaloids, saponins, flavanoids, terpenoids, and glycosides, with various substantial bioactivities such as antidiabetic, antioxidant, and hypolipidemic activities.[10] Such herbal formulations work by inducing antihyperglycemic effects through the stimulation of β cell repair, rapid insulin production, and peripheral glucose utilization.[11] In the present study, the polyherbal extract (PHE) was prepared from methanolic extracts of seeds from *N. sativa*, *C. arietinum*, *S. marianum*, and *C. colocynthis* and the rhizome of *Z. officinale*. Various biological activities were tested to assess its effectiveness as an antioxidant, hepatoprotective, and nephroprotective agent.

MATERIALS AND METHODS

Plant Materials. Seeds of *N. sativa*, *C. arietinum*, *S. marianum*, *C. colocynthis*, and *Z. officinale* were purchased from the local market of Sargodha and authenticated by a taxonomist from the Department of Botany, University of Sargodha, Sargodha.

Preparation of PHE. Individual plant seeds were washed, dried, and ground to a fine powder using a mortar and pestle and stored in polythene bags. The following herbal combination was formulated to prepare the combined methanolic extract by maceration: *N. sativa*/*C. arietinum*/*S. marianum*/*C. colocynthis*/*Z. officinale* (D; 3:1:1:1:1). After thorough maceration with methanol (125 g/1 L), the filtrate was separated and concentrated using a rotary evaporator at 37 °C. The obtained extract (PHE) was stored at 4 °C for further analyses.

Infrared Spectroscopy. Preliminary characterization of the bioactive compounds was performed by identifying certain functional groups in PHE by infrared (IR) spectroscopy. The analysis was performed by placing the samples into KBr disks and desiccating under vacuum before scanning. FTIR spectra were recorded on an IR prestige-21 (200 V) Fourier-transform infrared (FTIR) spectrometer, SCHIMADZU (Japan), at High-Tech Laboratory, Department of Pharmacy, University of Sargodha, Sargodha, Pakistan.

Antioxidant Potential of PHE. *Total Phenolic Contents.* Total phenolic contents of PHE were determined using the Folin−Ciocalteu reagent following the cited method with some modifications.[12] The stock solution was prepared by adding the sample (100 mg) to methanol (1 mL). Different solutions (2, 6, 10, 14, and 18 mg/mL) of gallic acid (standard) were prepared from the stock solution (100 mg/mL). The reaction mixtures were incubated for 30 min at room temperature, and the absorbance was noted at 700 nm. The values were expressed in gallic acid equivalents (GAE) of dry mass.

Free-Radical-Scavenging Activity (DPPH Assay). The free-radical-scavenging activity of PHE was determined by a method reported earlier.[13] Stock solutions of PHE and the standard were prepared by dissolving the sample (100 mg) and the standard (100 mg) in methanol (1 mL). Ascorbic acid was used as the standard antioxidant, whereas the solvent (methanol) was used as the blank. The reaction mixtures were incubated for 30 min at room temperature. The absorbance was noted at 517 nm using a spectrophotometer. The radical-scavenging activity was determined using the following formula:

$$\text{inhibition (\%)} = [(\text{blank absorbance} - \text{sample absorbance})/\text{blank absorbance}] \times 100$$

Ferric Reducing Antioxidant Power Assay (FRAP Assay). The reducing power of PHE was determined by following a reported method with some modifications.[14] All test sample solutions (1 mL each) were mixed with phosphate buffer (1 mL) and potassium ferricyanide (1 mL). After incubation at 50 °C, trichloroacetic acid (2.5 mL) was added to the mixture and centrifuged (1000 rpm) for 5 min. Distilled water (1 mL) and ferric chloride solution (1 mL) were also added in aliquots. Ascorbic acid (100 mg/mL) served as the standard, and the absorbance was noted at 593 nm. Increased absorbance of the reaction mixture indicated an increase in its reducing power. The reducing power (%) was calculated using the following formula:

$$\text{reducing power (\%)} = \frac{\text{abs. of test} - \text{abs. of blank}}{\text{abs. of blank}} \times 100$$

***In Vivo* Antidiabetic Studies.** *Animals.* Albino rats (mean weight = 300 g) were purchased from the University of Veterinary and Animal Sciences, Lahore, Pakistan. The animals were kept in large spacious cages at the animal house of the Department of Pharmacy, University of Sargodha, and were maintained in line with the "Principals of Laboratory Animal Care" (NIH publication 85−23, revised in 1985). The rats were acclimatized for 15 days before the start of the experimental work and were provided a standard diet and water *ad libitum*.

Experimental Trial. The animals were divided into four groups with three rats per group: normal, diabetic, PHE-treated diabetic, and standard drug (glibenclamide)-treated diabetic groups. Following 14 h of fasting, diabetes was induced in the rats via intraperitonial injection of 10% alloxan (Sigma Chemical Co., St Louis, MO) at a dose of 150 mg/kg (dissolved in isotonic NaCl).[15] After 3 days of alloxan injection, diabetes was confirmed through increased levels of blood glucose (hyperglycemia). The PHE dose (200 mg/kg body weight) was administered orally to the rats daily for 14 consecutive days.[16]

Blood Sampling. During the study trial, all rats were fasted for 12 h before the collection of blood samples from the tail by puncturing with a needle, and glucose levels were determined using a glucometer (On Call EZ II, ACON Laboratories Inc.). The animals were sacrificed under ether anesthesia, and liver and kidney tissues were isolated and stored at −20 °C for histological studies comprising fixation, dehydration, clearing, embedding, sectioning, and staining with hematoxylin and eosin.

Insulin Level Determination of Polyherbal-Treated Diabetic Rats. The insulin level in albino rat serum was determined following the protocol of enzyme-linked immunosorbent assay (ELISA).

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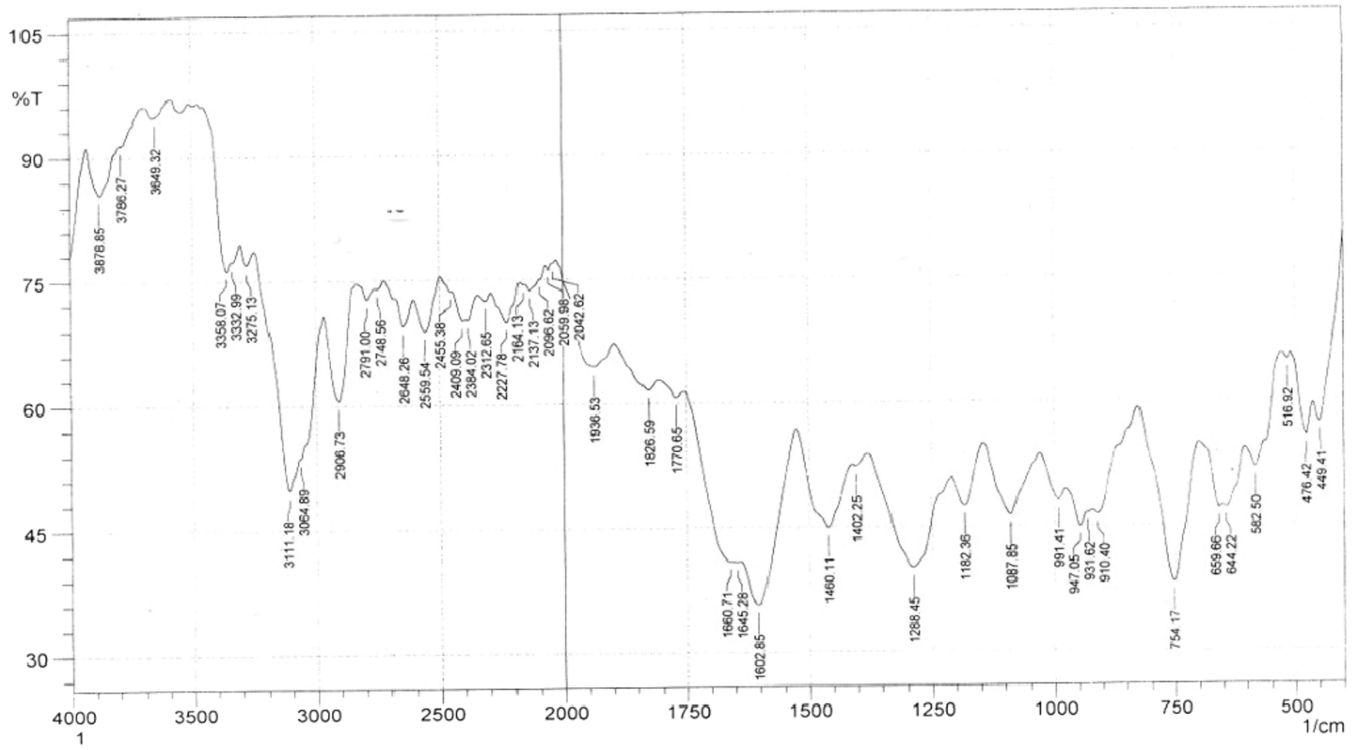

Figure 1. FTIR spectra of the methanolic polyherbal extract (PHE).

α-Amylase Inhibition Assay of PHE. *Sample Preparation.* The inhibitory potential of polyherbal formulations against α amylase was estimated by spectrophotometric analysis, and acarbose was used as the standard compound.[17] Initially, the polyherbal formulation was dissolved in 0.02 mM phosphate buffer at pH 6.8 in different proportions. The enzymatic solution was prepared by dissolving it (0.5 mg) in 1 mL of phosphate buffer. The same procedure was performed for the standard drug acarbose, and inhibition was calculated using the following formula:

$$\% \text{ inhibition} = \frac{\text{absorbance ofcontrol} - \text{absorbance oftest}}{\text{absorbance ofcontrol}} \times 100$$

Imaging and Micrometry. Digital images of 10× and 40× magnifications were used to obtain histological graphs to measure the average width and cross-sectional areas. Measurements of random areas were obtained from the digital graphs using a computer-assisted technique. Measurements of the glomeruli per unit area (10 cm^2), average cross-sectional area, Bowman's capsule, and proximal and distal convoluted tubules were recorded from the kidney and hepatocytes.

Statistical Analysis. Results are presented as mean ± standard deviation. Statistical analysis was carried out by the one-way ANOVA analysis and Tukey's post hoc test for multiple comparisons (differences among treatment means), using Statistical Package for Social Sciences (SPSS) software, version 21.0.

RESULTS AND DISCUSSION

FTIR Spectroscopy. The polyherbal extract was analyzed by FTIR spectroscopy for the identification of important functional groups of antioxidant compounds. The different peaks in the spectrum represent the presence of specific functional groups (Figure 1). Table 1 describes the IR ranges that are associated with the presence of specific functional groups in the PHE.

Table 1. Identification of Important Functional Groups in PHE

sr. no.	IR range (cm^{-1})	peak value (cm^{-1})	functional group
1	3400−3200	3358	N−H stretching of amine
2	3400−3100	3100−3300	O−H stretching of phenol
3	2950−2800	2906	C−H stretching of alkyl group
4	1750−1600	1602	C═O stretching of carbonyl group
5	1400−1200	1288	C−N stretching of amine group
6	1400−1550	1460	C═C stretching of aromatic ring

Nivetha and Prasanna (2016) also identified bioactive constituents in the ethanol extract of *N. sativa* L. seeds by phytochemical screening through gas chromatography−mass spectrometry (GC−MS) and FTIR spectroscopy.[18] The FTIR analysis showed the presence of functional groups at different IR regions; phenols (3100−3300 cm^{-1}), alkaloids (358 cm^{-1}), alkanes (2906 cm^{-1}), esters (1602 cm^{-1}), and aromatic rings (1460 cm^{-1}).

Evaluation of Antioxidant Potential. After the indication of the presence of important antioxidant compounds in PHE through FTIR analysis, antioxidant activities were assessed by determining the total phenolic contents in it and also by performing DPPH and FRAP assays.

Total Phenolic Contents (TPCs). The calibration curve of gallic acid, used as the standard antioxidant, showed a linear increase in the antioxidant activity with an increase in the concentration of gallic acid (Figure 2).

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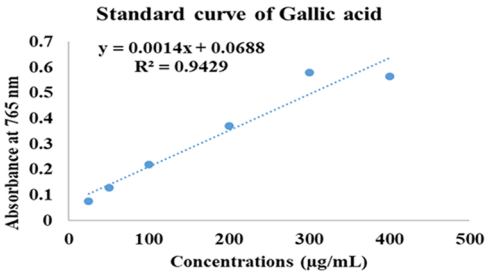

Figure 2. Standard curve of gallic acid.

The prepared polyherbal extract showed the presence of a significant amount of total phenolics (Table 2). According to the findings of Gupta et al. (2017), the increase in the radical-scavenging activity of PHE might be due to the presence of a significant amount of phenolics, which elevate the antioxidant potential.[19]

Table 2. Total Phenolic Contents of PHE[a]

sr. no.	concentration (mg/mL)	TPC value (GAE/g)
1	0.3	171.48 ± 1.62
2	0.6	175.18 ± 1.62
3	0.9	183.41 ± 1.71
4	1.2	189.03 ± 1.73
5	1.5	192.01 ± 2.01
6	1.8	197.05 ± 2.02
7	2.1	202.03 ± 2.07
	average TPC value (GAE/g)	187.17 ± 2.15

[a]Values are presented as the mean of triplicates; mean ± S.D.

Free-Radical-Scavenging Activity (DPPH Assay). The DPPH radical-scavenging activity of PHE at various concentrations ranging from 0.3 to 2.1 mg/mL was determined using ascorbic acid as the standard. The developed PHE showed a significant dose-dependent DPPH radical-scavenging activity compared with the standard (Table 3).

Table 3. Percentage Radical-Scavenging Activities of Different Concentrations of the Polyherbal Extract (PHE)[a]

sr. no.	concentration of dissolution (mg/mL)	scavenging activity of ascorbic acid (%)	scavenging activity of PHE (%)
1	0.3	80.99 ± 0.5	43.93 ± 0.55
2	0.6	82.24 ± 0.26	51.78 ± 0.66
3	0.9	84.19 ± 0.27	61.89 ± 0.35
4	1.2	85.88 ± 0.45	66.13 ± 0.32
5	1.5	87.20 ± 0.19	78.69 ± 0.55
6	1.8	88.14 ± 0.24	81.25 ± 0.25
7	2.1	90.79 ± 0.25	86.16 ± 0.33

[a]Values are presented as the mean of triplicates; mean ± S.D.

Nanthine et al. (2017)[20] also prepared a polyherbal formulation and reported that its free-radical-scavenging activity increased with its concentration. In another study, aqueous and alcoholic polyherbal formulations were reported to possess strong antioxidant properties due to the presence of phenolic and flavonoid content.[20]

Ferric Reducing Antioxidant Power Assay (FRAP Assay). The prepared polyherbal extract showed significant ferric reduction power as compared with ascorbic acid, used as the standard antioxidant (Table 4). This activity may be linked to the presence of a high content of phenolics in PHE, as Aldiab (2018)[21] associated the ferric-reducing property of the extracts with the presence of phenolic compounds that might quench the generated free radicals.

Table 4. Reduction Potential of Different Concentrations of Ascorbic Acid (Standard) and PHE (Sample)

sr. no.	concentrations (mg/mL)	absorbance of ascorbic acid (593 nm)	absorbance of PHE (593 nm)	reducing power (%)
1	0.3	0.395	0.084	21.27
2	0.6	0.427	0.089	20.85
3	0.9	0.567	0.102	17.99
4	1.2	0.684	0.104	15.21
5	1.5	0.786	0.129	16.42
6	1.8	0.812	0.175	22.00
7	2.1	0.875	0.245	28.00

Biochemical Potential of PHE. *Hypoglycemic Effect of PHE.* The results showed that the administration of alloxan and the polyherbal extract to the rats significantly ($p < 0.001$) altered their blood glucose levels. A significant increase in the blood glucose level of the diabetic group (201.17%) was noted after the injection of alloxan to normal rats (Figure 3). However, the administration of PHE and glibenclamide to rats for up to 14 days effectively lowered the blood glucose levels of animals as compared with the diabetic control group (−48.64 and −59.44%, respectively). Earlier, Majeed et al. (2018) also reported a decrease in blood glucose levels of alloxanized rats after treatment with the polyherbal formulation.[22] Similarly, Shah et al. (2019) reported a significant decrease in blood glucose levels of streptozotocin-induced diabetic rats after the polyherbal treatment.[23] In a recent study, Murthy et al. (2020) reported that the administration of the polyherbal formulation considerably normalized the blood glucose levels of alloxanized diabetic rats by regenerating pancreatic β cells.[24]

Effect on the Lipid Profile and Liver Markers. The lipid profile of treatment groups was also analyzed, and the beneficial effect of the polyherbal extract was noted in diabetic rats, which normalized the lipid status significantly by increasing the levels of good fats, HDL-cholesterol (+22.95%), and decreasing bad fat, LDL (−43.93%), as compared with the standard drug-treated group. Previous data reports also revealed that the hypolipidemic effect of polyherbal formulations was due to the synergistic action of its constituents such as phenolic, flavonoids, alkaloids, and saponins.[25]

The liver damage in diabetic rats was assessed by evaluating liver marker enzymes, including alkaline phosphatase, aspartate transaminase, and alanine transaminase. Significant decreases in serum SGPT (−36%), serum SGOT (−31%), and serum ALP (−12%) levels were observed in PHE-treated diabetic rats as compared with glibenclamide-treated rats (Table 5). Diabetes affects many organs including the liver, which plays a key role in the regulation of carbohydrate, lipid, and protein metabolism. When glucose levels are increased in the blood, levels of liver enzymes also increase significantly, affecting normal metabolism.[26] Earlier, Ahmad et al. (2020) also reported the effectiveness of a polyherbal formulation containing thymoquinone, which significantly decreased liver marker enzymes in carbon tetrachloride-induced hepatorenal injury in rats[27]

Effect of the Polyherbal Extract on Insulin Levels in Diabetic Rats. The results of insulin determination in the

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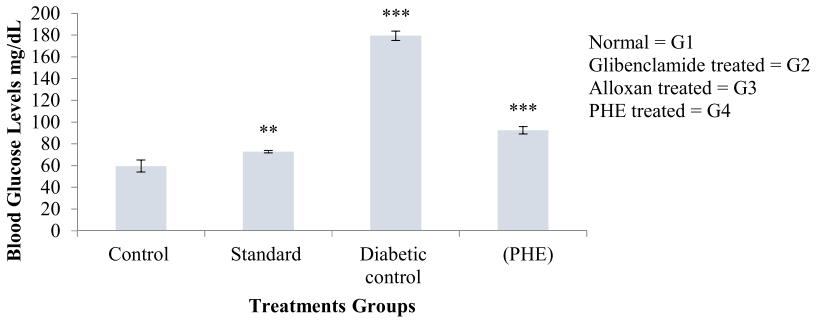

Figure 3. Hypoglycemic effect of the methanolic polyherbal extract (PHE): G3 was compared with G1 (normal), while G2 and G4 were compared with G3 (diabetic control) at $P < 0.05$. The data were subjected to one-way ANOVA analysis and Tukey's post hoc test with $n = 3$.

Table 5. Hypolipidemic and Hepatoprotective Effects of PHE in Diabetic Rats

groups	total protein (g/dL)	HDL (mg/dL)	LDL (U/L)	creatinine (mg/dL)	Tc (mg/dL)	TG (mg/dL)	SGPT (U/L)	SGOT (U/L)	ALP (U/L)
glibenclamide (10 mg/kg)	5.53 ± 0.40	66.53 ± 1.48	1094.9 ± 1.57	0.96 ± 0.15	32.82 ± 1.42	79.76 ± 1.0	38.6 ± 1.2	41.5 ± 1.4	198.6 ± 1.8
PHE (200 mg/kg)	7.4 ± 0.50	81.8 ± 0.53	613.83 ± 1.5	1.06 ± 0.20	53.8 ± 1.19	109.6 ± 2.23	24.7 ± 2.4	28.63 ± 0.80	174.7 ± 1.6

blood showed a significant ($p < 0.05$) decrease in insulin levels of alloxan-treated rats as compared with the normal control group. After the administration of the formulation and glibenclamide to diabetic rats, significant ($p < 0.05$) elevation in the level of insulin was observed as compared with the diabetic control group (Table 6)

Table 6. Effect of the Polyherbal Formulation on the Insulin Level in Alloxan-Induced Diabetic Rats[a]

groups	insulin level (U/L)
normal control	17.5 ± 1.1[a]
diabetic control	6.6 ± 0.97[e]
diabetic treated with F6-SMONSECCE	13.7 ± 1.00[a] (−107.5%)
diabetic treated with glibenclamide	16.3 ± 1.00[ab] (−146.9%)

[a]Values expressed as means ± SD. Means ± SD with different superscript letters (a−d) within the column indicate significant differences ($p < 0.05$).

The findings showed that the polyherbal extract normalized the level of insulin in the blood of rats, which might be linked to the regeneration of pancreatic cells. The results are in line with the findings of Gopalakrishnan et al. (2020), who reported that the administration of polyherbal drugs decreases the maximum combination of glucose with hemoglobin and thus results in decreased blood glucose levels due to increased insulin levels.[28]

***In Vitro* Antidiabetic Activity of PHE (α-Amylase Inhibition Assay).** The α-amylase inhibitory effect of PHE from 50 to 1000 μg/mL was observed, and the IC_{50} value was found to be 194.84 μg/mL (Table 7). PHE significantly decreased glucose levels. Acarbose was used as a standard and showed 52.32 ± 0.64% inhibition of α-amylase activity at a concentration of 1000 μg/mL, and the IC_{50} value was found to be 210 μg/mL.

Hyperglycemia is a vital factor that induces diabetes, and blood glucose control is critical in the treatment of diabetes as well as the prevention of microvascular and macrovascular consequences. Reducing postprandial hyperglycemia by inhibiting starch hydrolysis, such as with PPA inhibitors, is useful in curing DM.[29]

Table 7. α-Amylase Inhibition of Polyherbal Formulations[a]

sr. no.	concentrations (μg/mL)	PHE	standard
1	50	33.17 ± 1.00	34.51 ± 1.03
2	100	35.42 ± 0.99	36.66 ± 0.96
3	250	40.17 ± 1.05	41.01 ± 0.41
4	500	42.92 ± 1.11	45.50 ± 0.94
5	1000	43.16 ± 1.63	52.32 ± 0.64
	IC_{50}	194.84*	210

[a]*minimum IC50 value; $n = 3$.

PHE showed an α-amylase inhibitory activity (194.84 μg/mL) even lower than that of the standard, acarbose (210 μg/mL), suggesting that the polyherbal formulation could have promising antidiabetic potential due to the presence of various phytochemicals or proteins that act as possible α-amylase inhibitors. The results revealed that the polyherbal formulation possibly inhibited maltose production and controlled the glucose level. Gopinath, et al. also demonstrated that the inhibitory activity of PHE in hot extract might be possibly due to the presence of various phytoconsituents, including phytol, sterols, phenols, and many other components.[30]

Histological Observation. *Kidney Histology.* Histological sections of the kidney are shown in the photomicrographs (Figure 4), which show the changes in the glomerular diameter of kidney tissues extracted from rats of the standard group and the PHE-treated group after 14 days of oral administration. Light microscopic observation of kidneys of rats treated with PHE (B) showed a significant ($p < 0.05$) decrease in the glomerular diameter (GD). The highest mean of the number of glomeruli was recorded in the PHE-treated group followed by the glibenclamide-treated group. However, in the case of C and D, glibenclamide and PHE significantly reduced the vacuolization close to that in the control group. Alloxan also changed the diameter of glomeruli and caused vacuolization in the kidney of diabetic rats. When the diabetic rats were treated with PHE and the standard drug, significant changes were observed in the PHE-treated diabetic rat group as compared with the standard drug-treated group (Figure 4). PHE reduced oxidative stress, which caused a change in the diameter of glomeruli by necrosis. PHE more effectively ceased the

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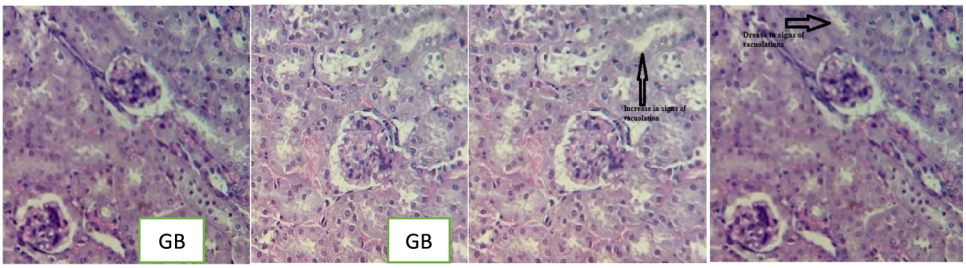

Figure 4. Micrographs showing kidney transverse sections of (A) control, (B) diabetic control, (C) PHE-treated, and (D) glibenclamide-treated rats. Transverse sections of the kidney tissues showing a reduction in the glomerular diameter (GR) and signs of vacuolization in the PHE- and glibenclamide-treated rats in (C, D) (H and E, 400×).

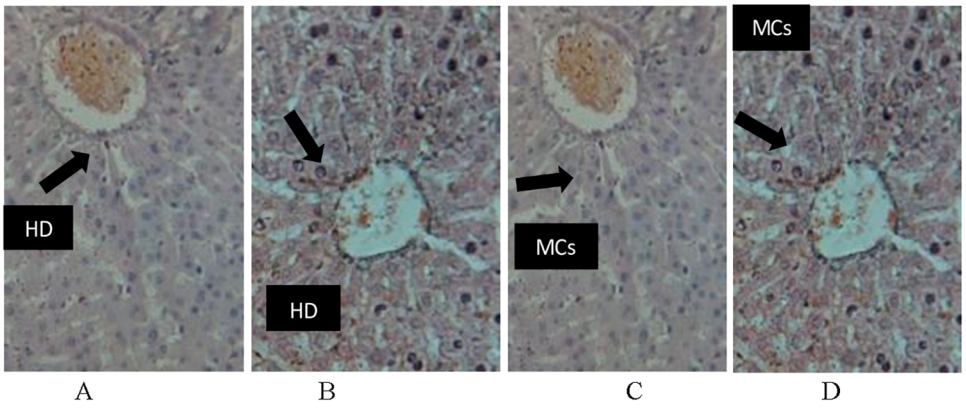

Figure 5. Sections of the liver of rats from the standard group and rats treated with the methanolic polyherbal mixture (PHE): (A) normal, (B) diabetic, (C) standard drug-treated, and (D) PHE-treated groups, respectively; the highest mean hepatocytes diameter (HD) and multinucleated cells (MCs) were found in rats in the standard group, followed by the PHE-treated group (H and E, 400×).

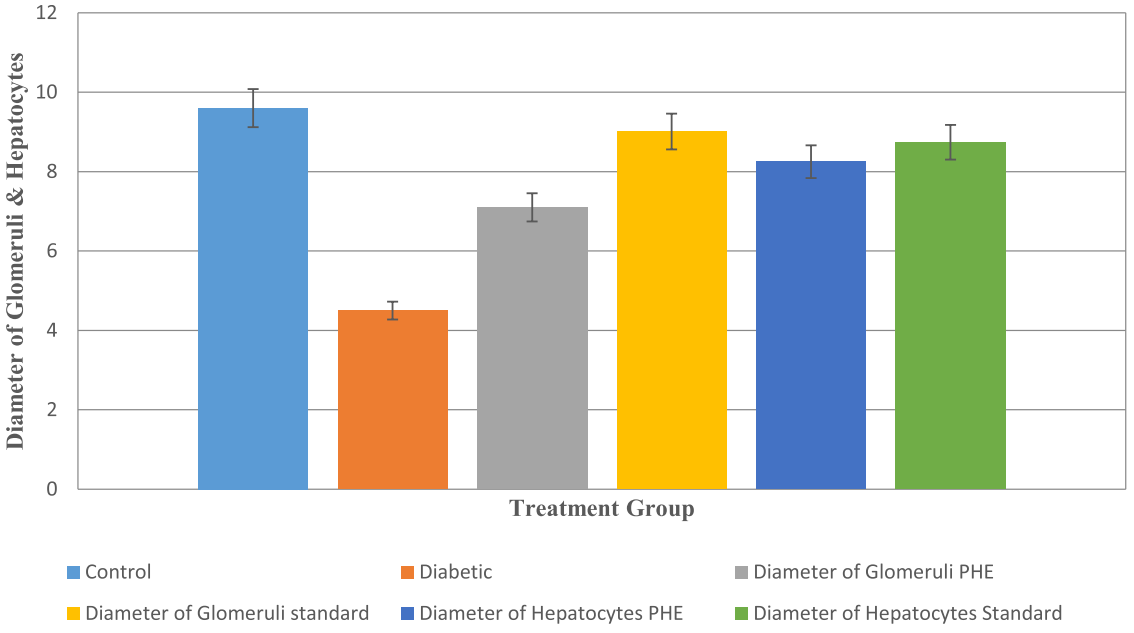

Figure 6. Effects of PHE and glibenclamide on the ACSA of glomeruli (kidney) and hepatocytes (liver) of diabetic rats; the mean diameter of glomeruli was found to be reduced in the PHE-treated group (values expressed as means ± SD).

alteration of cytoplasmic components of renal tubules than that in the standard drug-treated diabetic rats (Figure 4). Our results are comparable to the findings of Abuzinadah and Aftab (2020), who explained the nephron-protective effect of polyherbal formulations in cisplatin-induced hepatorenal injury by normalizing the renal architecture and restoring cellular organization/function.[27] Another study also reported the nephron-protective effect of polyherbal formulations in carbon tetrachloride-induced hepatorenal injury in rats. Polyherbal formulations caused tubular contraction, hydropic regeneration

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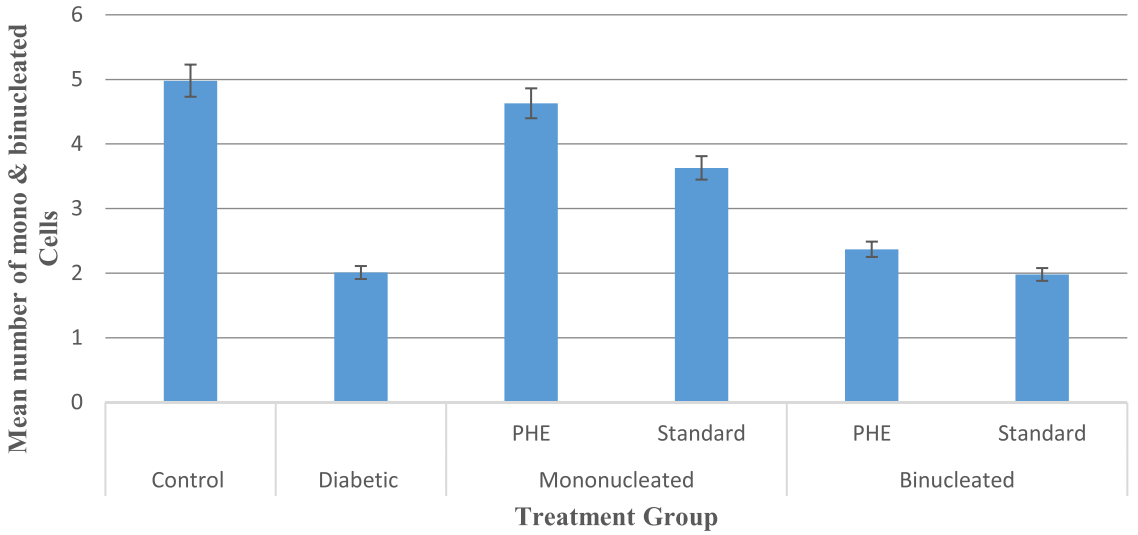

Figure 7. Mean numbers of mononucleated and binucleated cells per unit area (10 cm^2): the highest mean value was found in the PHE-treated group (values expressed as means ± SD).

in the tubular epithelium, enhancement of the brush border on tubular epithelium cells, inhibition of tubular necrosis, and progression in the size of Bowman's capsule.[31]

Liver Histology. The hepatocyte diameter (HD) and mononucleated cells (MC) of the standard and PHE-treated rats were determined through liver transverse sections. The highest mean of the hepatocyte diameter (HD) was recorded for the rats in the standard group followed by the PHE group, while in the case of MCs, the highest means were observed in the rats in the PHE-treated group followed by those in the standard group (Figure A−D). Nephrotoxicity signifies a condition of renal dysfunction that arises due to exposure to environmental chemicals or drugs. Oxidative stress and inflammation are the main pathological pathways involved in its etiology.

For liver cells, histological studies showed that alloxan severely affected hepatocytes by reducing their diameter and the mean numbers of mononucleated and binucleated cells. However, after treatment with PHE and glibenclamide, the diameter of hepatocytes and the numbers of mononucleated and binucleated cells increased significantly. A more significant increase in the diameter was observed in glibenclamide-treated diabetic rats (8.74 ± 0.75) than the PHE-treated diabetic rats (8.25 ± 0.28) (Figure 5), while the numbers of mononucleated and binucleated cells were significantly increased (4.63 ± 0.22 and 2.37 ± 0.11) in the liver of PHE-treated diabetic rats compared with that in the standard drug-treated rats. Gulati et al. (2019) also reported the beneficial effect of polyherbal formulations of D-galactosamine, which ameliorated liver damage by reconstructing hepatocytes to their original structures.[28] Similarly, they also reported that polyherbal formulations containing thymoquinone led to the regeneration of damaged hepatocytes by inhibiting oxidative stress, which is a cause of hepatic congestion, hemorrhage, and appearance of necrotic cells.[32]

Micrometry Studies. *Micrometry Studies of the Average Cross-Sectional Area of Glomeruli and Hepatocytes.* The average cross-sectional areas (ACSAs) of glomeruli and hepatocytes in the kidneys and liver of rats in the treatment groups were determined, respectively. Alloxan significantly decreased the ACSAs of glomeruli and hepatocytes. After the administration of PHE and the standard drug (glibenclamide), the kidneys of rats treated with the standard drug showed the highest ACSA (9.01 ± 0.23), whereas those of PHE-treated rats showed a reduced ACSA (7.10 ± 0.20); however, an increased ACSA value was noted in rats in the glibenclamide-treated group (8.74 ± 0.75) as compared with those in the PHE-treated group (8.25 ± 0.28) [Figure 6].

Micrometry Studies of Liver and Kidney Sections. The administration of alloxan to rats significantly reduced the number of mononucleated and binucleated cells per unit area (10/cm^2). After treatment with PHE and the standard drug (glibenclamide), the highest mean number of mononucleated cells per unit area (10/cm^2) was recorded in rats in the PHE-treated group (4.63 ± 0.22) as compared with those in the glibenclamide-treated group (3.63 ± 0.15), while the mean number of binucleated cells per unit area (10/cm^2) was found to be increased (2.37 ± 0.11) in the hepatocytes obtained from the PHE-treated group as compared with those from the glibenclamide-treated group (1.07 ± 0.06) (Figure 7).

However, in the case of the number of glomeruli, a significant ($p < 0.001$) increase was observed in the kidneys of rats treated with PHE (1.73 ± 0.06) as compared with those treated with glibenclamide (1.62 ± 0.06).

CONCLUSIONS

A preliminary phytochemical screening and FTIR study were conducted to characterize and develop a polyherbal combination from *N. sativa*, *C. arietinum*, *S. marianum*, *C. colocynthis*, and *Z. officinale*. The developed polyherbal combination showed significant antioxidant, antidiabetic, and hepatorenal protective activities in diabetic rats. As the administration of alloxan to rats damages pancreatic β cells, amelioration of blood glucose levels upon administration of PHE to diabetic animals suggests its action through an increase in insulin secretion from the repaired pancreatic cells. The developed polyherbal combination can be explored further for the management of drug-induced diabetes, hepatotoxicity, and nephrotoxicity as a potential natural remedy.

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Notes

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