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Antioxidant, Hypoglycemic, Antilipidemic, and Protective Effect of Polyherbal Emulsion (F6-SMONSECCE) on Alloxan-Induced Diabetic Rats

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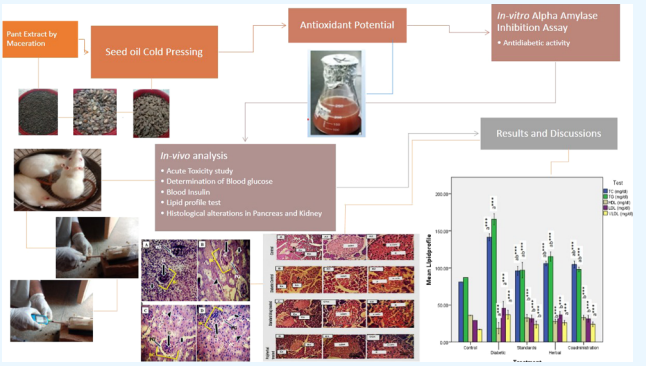
ABSTRACT: The current study focused on the antioxidant potential, α-amylase inhibitory activity, and hypoglycemic, hypolipidemic, and histoprotective (pancreas and kidney) effects of polyherbal emulsion on the alloxan-induced diabetic rats. Polyherbal formulations were prepared from extracts and oils of *Nigella sativa* (*N. sativa*), *Citrullus colocynthis* (*C. colocynthis*), and *Silybum marianum* (*S. marianum*). Out of nine stable formulations, one formulation named F6-SMONSECCE was found to be the best after its evaluation using antioxidant and in vitro α-amylase inhibition assay. The prepared herbal formulations showed significant ($p < 0.05$) antioxidant activity in terms of radical scavenging as 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric-reducing antioxidant power (FRAP) assays and also revealed the presence of a significant amount of total phenolic and flavonoid contents. "F6- SMONSECCE" (prepared with composition; *Silybum marianum* oil (SMO) + *Nigella sativa* extract (NSE) + *Citrullus colocynthis* extract CCE) was selected for an in vivo trial to ascertain its antidiabetic potential. The treatment dose was determined by using an acute toxicity trial on rats. Administration of alloxan (150 mg/kg b.w., i.p.) significantly ($P < 0.05$) augmented the blood glucose levels and lipid contents as total cholesterol (TC), triglycerides (TG), low-density lipoproteins (LDL-c), and very-low-density lipoproteins (VLDL-c). However, the levels of insulin and high-density lipoproteins (HDL-c) were found to be decreased, and the histopathological alterations were also found in the pancreas and kidney. The administration of the polyherbal formulation (F6-SMONSECCE) significantly attenuated the blood glucose levels (22.94%), TC (29.10%), TG (38.15%), LDL-c (27.58%), and VLDL-c (71.52%), whereas on the other side, the insulin (−149.15%) and HDL-c levels (−22.22%) were significantly increased. A significant histopathological normalization was observed in the pancreas and kidney tissues of the F6-SMONSECCE-treated rats. The current findings proposed that the prepared polyherbal formulation "F6-SMONSECCE" exhibited significant antioxidant, antilipidemic, and hypoglycemic potential and hence might be suggested as a remedy against diabetes or as a coadjuvant to synthetic medicines to maintain normal physiology.



INTRODUCTION

Diabetes mellitus (DM) is a condition linked with insulin abnormality in conjugation with defects in glucose and lipid metabolism. The condition develops when the pancreas is unable to produce sufficient insulin/when the insulin is ineffectively used.[1] Oxidative stress, obesity, lack of exercise, and inheritance are among the environmental and lifestyle factors that influence the onset of Type 1 and 2 diabetes.[2] The chronic outcome of diabetes disrupts glucose, protein, and lipid metabolism and thus results in persistent hyperglycemia and irregularities of the lipid profile.[3]

A variety of medications such as biguanides, sulfonylureas, thiazolidinediones, alpha-glucosidase inhibitors, etc., are available to manage diabetes and its associated abnormalities. However, the rate of mortality is still high due to the substantial side effects exhibited by such synthetic antidiabetic drugs.[4] In 2021, 537 million individuals worldwide (diagnosed or undiagnosed) were estimated to have diabetes, and by 2045, that number is expected to rise by 46% to 784 million.[5,6] It is also reported that "The International Diabetes Foundation" (IDF) estimates that 10.5% of adults worldwide have diabetes and that number is expected to rise to 12.2% by 2045.[7]

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Diabetes, if unchecked, can eventually cause serious problems that might injure the heart, blood vessels, eyes, teeth, kidneys, nerves, and other body parts, finally resulting in death. Cardiovascular disease, which increases mortality among diabetics, is one of the main consequences. Heart attacks and strokes are two to three times more likely in diabetic people. Diabetic neuropathy, or nerve damage brought on by high blood sugar, is a further consequence. Peripheral neuropathy, which primarily affects the feet, is the most prevalent kind. It increases the risk of foot ulcers and infections when coupled with decreased blood flow, ultimately resulting in limb amputations.[6] Due to the severe side effects of synthetic antidiabetic drugs, there has been a dire need to develop safe and effective antidiabetic medications with fewer physiological side effects. In recent years, researchers have been looking into medicinal flora to find out the most effective herbal formulations containing potent medicinal plants against diabetes.[8] The safety and efficacy of herbal medications for the treatment of diabetes have been proven in the past studies.[4,9] The antihyperglycemic properties of such medicinal plants might be linked to the presence of phytochemicals such as flavonoids, terpenoids, alkaloids, glycosides, and carotenoids, which are scientifically validated for producing antidiabetic effects either by enhancing insulin secretion from pancreas or lowering insulin resistance.[10]

N. sativa L., belonging to the Ranunculaceae family, is one of the most admired medical plants in history. *N. sativa* is reported in different studies for its potent pharmacological activities including antidiabetic, antioxidant, and hepatoprotective activities.[11] *S. marianum* is an herbal plant (*Asteraceae* family) and is native to the Mediterranean region. Earlier studies conducted by the researchers revealed that *S. marianum* possesses antidiabetic, antioxidant, and hepatoprotective activities.[12] *C. colocynthis* is a valued cucurbit plant, distributed in all deserts of the world. *C. colocynthis* fruit is generally familiar for a wide range of applications against various ailments, in addition to its pharmaceutical and nutraceutical potential. It has been used as a medicine against DM and is also recognized for its hypoglycemic effect.[13] Individually, the antidiabetic activities of these plants have already been cited in the literature. However, in the current study, our main concern is about the utilization of polyherbal formulations as an emulsion to treat diabetic patients with minimum side effects.

Keeping in view the medicinal importance of these plants, different polyherbal emulsions were prepared from extracts and oils of *N. sativa*, *C. colocynthis*, and *S. marianum*. The aim of this study was to evaluate antioxidant, hypoglycemic, and antilipidemic potential of selected formulations. Moreover, histopathological alterations were also probed in the pancreas and kidney of the under-treatment rat model.

MATERIALS AND METHODS

The biochemical analysis was done in the Biochemistry Laboratory, Institute of Chemistry, University of Sargodha, Sargodha, Pakistan. The animal work was conducted in the animal house, Department of Pharmacy, University of Sargodha. All the reagents were of analytical grade and procured from Sigma-Aldrich.

Plant Material Collection and Sample Preparation. The seeds of *N. sativa (MP120)*, *S. marianum (MP290)*, and *C. colocynthis (MP246)* were purchased from National Agricultural Research Centre (NARC), Islamabad, and authenticated by a botanist, Department of Botany, University of Sargodha.

The individual extracts of *N. sativa*, *S. marianum*, and *C. colocynthis* seeds were obtained by the maceration process. Seed powders of selected plant seeds and vinegar solvent [(10% acetic acid (1:10)] were allowed to mix in an orbital shaker at 200 rpm for 24 h. After filtration, the excess solvent was removed by a rotary evaporator (Heidolph Laborota 4000 efficient HB Digital, Germany). The extract was stored at 4 °C until use for further studies.

Extraction of Oils through Cold Pressing. The seed oil extraction was performed by a cold press oil machine (Karaeier brand NF 500 model, Turkey). The cold press machine was preheated, and the temperature was set to 45−50 °C. The seeds of *N. sativa*, *S. marianum*, and *C. colocynthis* were pressed at a frequency of 15 Hz at 45 °C. The temperature of the seed oils obtained from the cold press machine was provided below 45 °C employing a recycling cooling system (4 °C).

Preparation of a Stable Herbal Formulation as an Emulsion. In the first step, the nonpolar phase of the emulsion was prepared by the dry gum method.[14] The fine powder of acacia gum *was triturated* with oil using a mortar and pestle. In the second step, the polar phase was made by thoroughly mixing honey and plant extract until the formation of a homogenous mixture. Honey was used due to its acidic pH as an antibacterial agent to increase the shelf life and viscosity of formulations. Both the nonpolar and polar components were mixed and again ground thoroughly to form a homogenous mixture of herbal formulations. The formulations prepared in the current study are as follows: F1 [(*Nigella sativa* oil (NSO) + *Silybum marianum* extract (SME)], F2 [(*Nigella sativa* oil (NSO) + *Citrullus Colocynthis* extract (CCE)], F3 [(*Nigella sativa* oil (NSO) + *Silybum marianum* extract (SME) + *Citrullus Colocynthis* extract (CCE)], F4 [*Silybum marianum* oil (SMO) + *Nigella sativa* extract (NSE)], F5 [(*Silybum marianum* oil (SMO) + *Citrullus Colocynthis* extract (CCE)], F6 [*Silybum marianum* oil (SMO) + *Nigella sativa* extract (NSE) + *Citrullus Colocynthis* extract (CCE)], F7 [*Citrullus Colocynthis* oil (CCO) + *Nigella sativa* extract (NSE)], F8 [*Citrullus Colocynthis* oil (CCO) + *Silybum marianum* extract (SME)], and F9 [*Citrullus Colocynthis* oil (CCO) + *Nigella sativa* extract (NSE) + *Silybum marianum* extract (SME)]. The O stands for oil, whereas E stands for the extract, of more than two extracts, half of each extract is used (Table 1). In each formulation, the extract was mixed with an appropriate amount of oil, whereas acacia gum and honey were used as biosurfactants/binders to join both polar and nonpolar components of the formulations.

Table 1. Composition of Herbal Formulations

oil (O)	extract (E)	acacia gum	honey
20 mL	35 mL	15 g	30 mL

Antioxidant Potential of Polyherbal Formulations. *Determination of Total Phenolic Contents (TPCs).* The TPCs of the prepared polyherbal formulations were determined using the Folin−Ciocalteu procedure.[15] The absorbance was taken at 765 nm using a UV−visible spectrophotometer (Edinburgh Instruments, 2 Bain Square Kirkton, UK).

Determination of Total Flavonoid Contents (TFCs). The TFCs of emulsions were estimated by using the method reported by.[16] The appearance of red color showed the presence of flavonoids. The absorbance was measured at 510 nm.

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DPPH Assay/Radical Scavenging Activity. The radical scavenging activity of prepared emulsions was measured using the method reported by Qadir et al.[17] The absorbance was measured at 517 nm using a UV−visible spectrophotometer. The solvent combination was used as a blank. DPPH and the solvent solution were used as a control. The radical scavenging activity was measured by calculating IC_{50} values using the following formula:

$$\text{percentage inhibition} = \frac{\text{absorbance of the control} - \text{absorbance of Sample}}{\text{absorbance of the control}} \times 100$$

Determination of the Reducing Power. The reducing potential of the developed emulsions was measured by ferric reducing power assay reported by Qadir et al.[17] 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and potassium ferricyanide were added to test tubes and incubated at 50 °C for 20 min. After incubation, 2.5 mL of 10% trichloroacetic acid was added and centrifuged at 3000 rpm for 10 min. A supernatant layer was taken and 0.5 mL of 1% ferric chloride solution was added. Absorbance was taken at a UV−visible spectrophotometer at 700 nm. Ascorbic acid was taken as a standard. The reducing power of the sample (emulsions) was calculated by comparing its values with the absorbance of ascorbic acid (standard).

α-Amylase Inhibition Assay of Polyherbal Formulations. The α-amylase inhibitory potential of polyherbal formulations was estimated spectrophotometrically using acarbose as the standard.[18] Initially, the polyherbal formulation was dissolved in phosphate buffer (0.02 mM) at 6.8 pH in different proportions. One milliliter of α-amylase solution was added to the sample solution of different concentrations ranging from 50 to 1000 mg/mL and incubated for 10−15 min at 37 °C. Afterward, 1 mL of starch solution (1% m/v) was added into test tubes and again incubated for 10−15 min at 37 °C. The solution mixture was then mixed with di-nitrosalicylic acid and heated up to 85 °C for 10 min and then diluted by adding 10 mL of distilled water, and absorbance was read at 540 nm. The control reaction was performed without the addition of extract. The same procedure was executed for the standard drug (acarbose) and inhibition was calculated by using the given formula;

$$\%\text{inhibition} = \frac{\text{absorbance of control} - \text{absorbance of test}}{\text{absorbance of control}} \times 100$$

In Vivo Antidiabetic Study. *Acute Toxicity Studies of Polyherbal Formulations.* Toxicity studies were employed on white male albino rats of 6−7 weeks and 130−180 g body weight, by using the acute oral toxicity method [OECD Guideline, 2001],[19] with prior permission of the ethical committee (SU/ORIC/359, dated: 11/ 04/ 2022). Different doses of herbal formulations were given orally to the rats. Animals were divided into three groups; each contained three animals and acclimatized for 5 days. Before dosing, animals were kept fasted overnight, and afterward, in the daytime, the formulation was ingested orally with different dose levels per body weight. Clinical signs of toxicity were observed in rats after two hours and in the next four hours, general behavior was noted, whereas at the end, after 24 h, mortality was observed in rats in each trial of 21 days.

Animal Research Study Protocol. Ninety male albino rats BALB/c (4 weeks old) weighing between 180 and 200 g were housed at the Institute of Pharmacy, University of Sargodha, Sargodha. The trial was conducted following the direction of the ethical committee. Rats were kept in the house for 15 days and acclimatized earlier to the beginning of the experiment. After the observation period, rats were divided into different numbers of groups (Table 2.).

Table 2. In Vivo Animal Study Trial Scheme

animal groups	treatments
Group I (control)	fed on the normal diet
Group II (diabetic control)	alloxanized rats
Group III (positive control/ standard drug-treated)	diabetic rats treated with glibenclamide: daily dose; 0.75 mL/kg.
Group IV (herbal formulation-treated)	diabetic rats treated with the polyherbal formulation: daily dose; 1.85 mL/kg.
Group V (coadministered)	diabetic rats treated with glibenclamide + herbal formulation: daily dose; 1.3 mL/kg.

Experimental Induction of the Diabetes and Administration of a Polyherbal Formulation. Single intraperitoneal injection of alloxan monohydrate in saline water induced diabetes in rats. After the induction of alloxan, the blood glucose levels (BGLs) were measured on day 0 (after the alloxan dose), day 3, and day 7 by using a commercially available glucometer OnCall*Ez II* (SN 303S0014E09). Rats having glucose levels of more than 300 mg/dL were used in the study, and doses of the polyherbal formulation were given orally to groups III, IV, and V up to the end of the study trial.[20]

Insulin Level Determination of F6-SMONSECCE-Treated Diabetic Rats. The determination of the insulin level in albino rat serum was performed using the protocol of enzyme-linked immunosorbent assay.[21]

Hypolipidemic Effect. The blood samples of all groups were analyzed for the lipid profile (triglyceride (TG), TG, LDL-c, VLDL-c, and HDL-c) using the standard assay kits (Analyticon Biotechnologies AG, Germany).

Histopathological Studies. After 21 days of trial, the animals were dissected, and body parts such as the pancreas and the kidney were separated according to the reported protocol.[22] These were fixed in Carnoy's fixative mixture made up of ethanol (85 mL), glacial acetic acid (5 mL), and formaldehyde (10 mL) for histological studies. The dehydration was done by submerging fixed tissues (pancreas and kidney) in different concentrations of alcohol for 3−5 h. After dehydration, the organs were kept in xylene for 5−6 h to get transparency. Blocks of paraffin wax with embedded organs were made by placing organs in molten wax at 56−57 °C. Sections of 5 μm thickness were made by a rotatory microtome. Albumenized histological glass slides were used for the stretching of sections. The slides of the separated organs were stained with eosin and hematoxylin dyes and mounted with Canada balsam. Histological alterations were further examined under a microscope at different magnifications, and photomicrographs were obtained. The number of the pancreatic cell (beta cells and alpha cells) and mean diameter of islets and beta and alpha cells were counted at 40X magnification. The same procedure was repeated for the measurements of the number of glomeruli per unit area (10 cm^2), average cross-sectional area (ACSA) of Bowman's capsule, ACSA of glomeruli, ACSA of proximal convoluted

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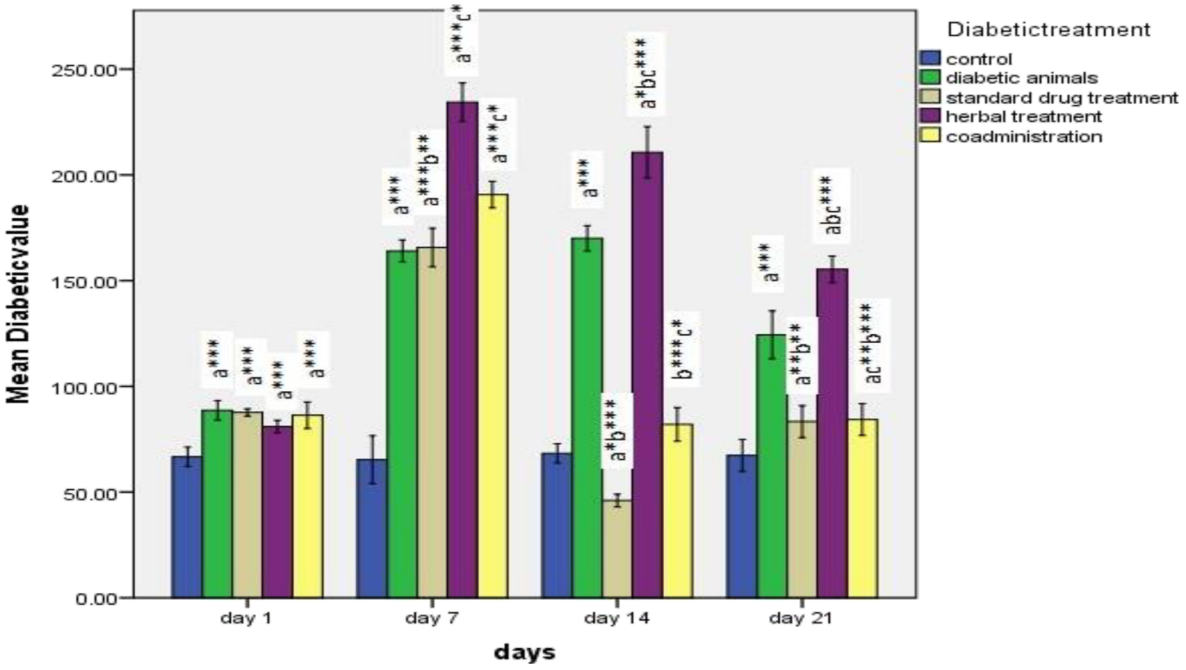

Figure 1. Comparison of the effect of polyherbal emulsion and standard drug treatment on weekly blood glucose levels in the diabetic rats. Values are given as Mean ± SEM and analyzed by One way ANOVA followed by Tukey's test. a = control vs other groups, b = diabetic group vs diabetic treated groups, and c = standard drug treated group vs polyherbal emulsion given group. ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$.

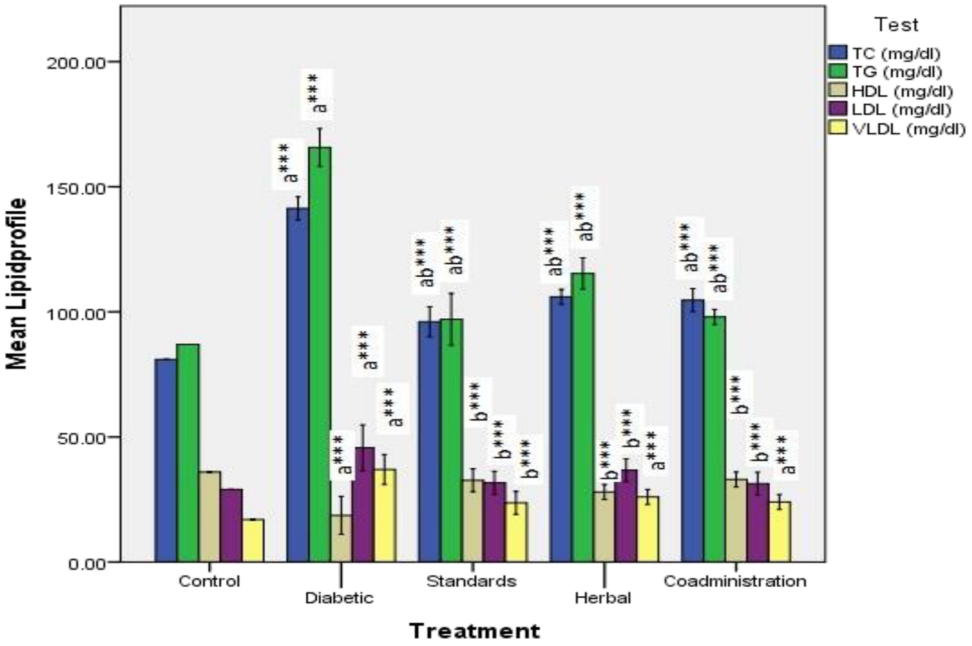

Figure 2. Effect of the polyherbal formulation on lipid profiles including TC, serum TG, high-density lipoproteins (HDL), low-density lipoproteins (LDL), and very low-density lipoproteins (VLDL) of diabetic rats. Values are given as Mean ± SD. The data were analyzed using one-way ANOVA and Tukey's post hoc test. a = control vs other groups, b = the diabetic group vs other diabetic treated groups, c = standard drug vs herbal and coadministered group, and d = herbal vs co-administered group. ***$P < 0.001$.

tubules, and ACSA of distal convoluted tubule from the kidney.

Statistical Analysis. The data collected from different experiments were analyzed through IBM SPSS Statistics for Windows, version 25.0 (IBM Corp., Armonk, N.Y., USA) by applying one-way analysis of variance (ANOVA) and Tukey's test.

RESULTS AND DISCUSSION

The findings of the current study revealed that the formulation F6 (SMONSECCE) showed the most effective antioxidant and alpha-amylase activities, whereas other formulations such as F1 (NSO + SME), F3 (NSO + SME + CCE), F4 (SMO + NSE), F5 (SMO + CCE), F7 (CCO + SME), and F8 (CCO + NSE), F9 (CCO + NSE + SME) offered moderate activities. Hence,

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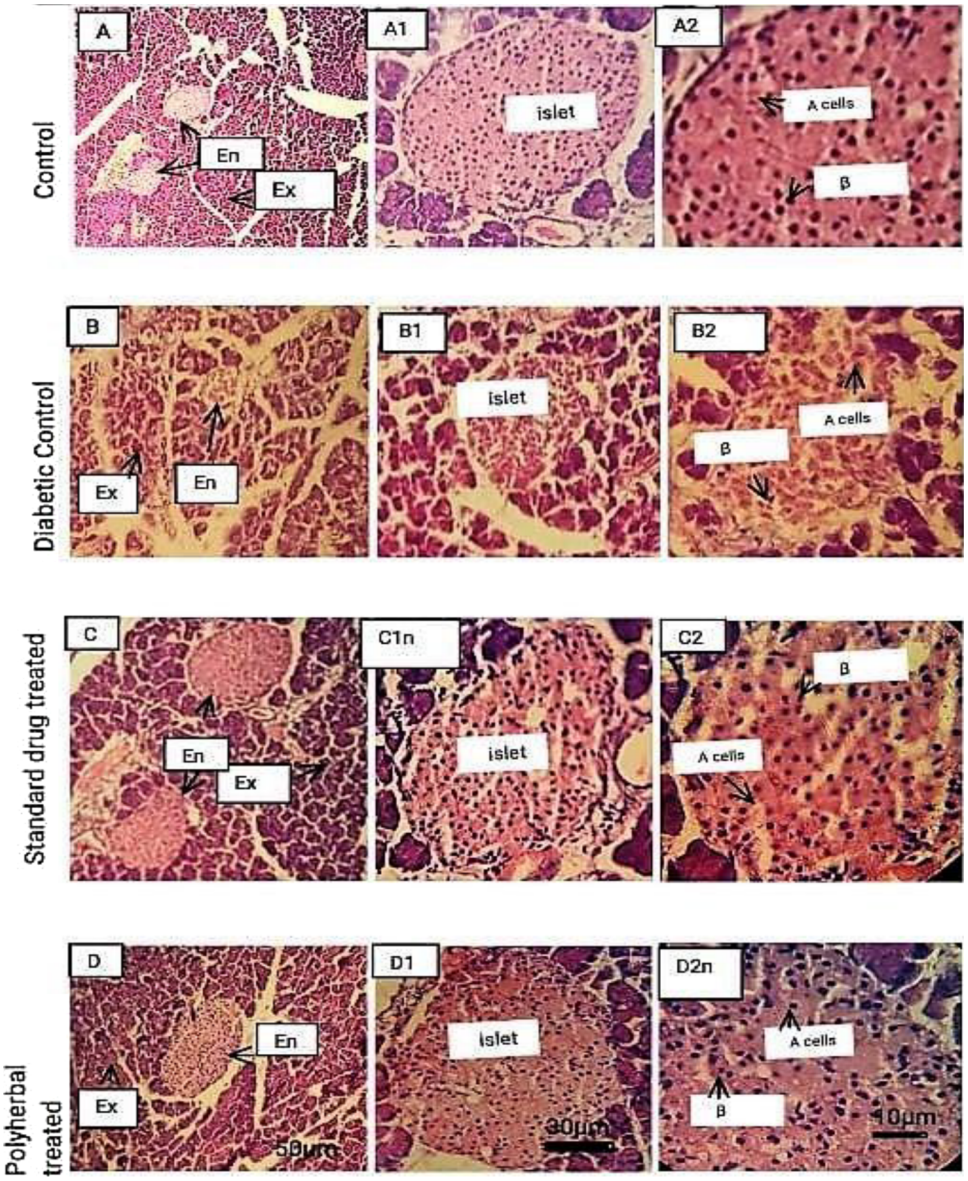

Figure 3. Photomicrographs from sections of pancreas of Control (A, A1, A2) rats exhibiting properly organized exocrine (Ex) and endocrine (En) tissue, islet of Langerhans, and α (A cells) and β cells. Diabetic control sections (B, B1, B2) show complete disruption of exocrine and endocrine tissue, regressed islets and reduced number of α and β cells are clearly seen. Standard drug-treated pancreas sections (C, C1, C2) and herbal-treated (D, D1, D2) show improvement in histological structure of exocrine and endocrine tissue, well-organized islets, and an increase in the number of α and β cells.

the prepared formulations especially F6 can be helpful in delaying the onset of oxidative pathologies such as diabetes.

Antioxidant Potential of Polyherbal Formulations. The total phenolic contents of different formulations ranged from 48.54 to 175 mg /GAE of dry weight. The lowest value was obtained for formulation F2, while the highest value was noted for F6 as shown in Figure 1. Bahadori et al. reported that phenolic compounds contribute to the overall antioxidant activities of plants.[23] Among phenolic acids, the presence of a 3-hydroxyl structure (2,3-hydroxybenzoic acid, gallic acid, caffeic acid, and caftaric acid) suppresses the production of reactive oxygen species and enhances the antioxidant effects.[24] The present results are in line with the findings of Abbas et al. who investigated that edible polyherbal formulations had the highest total phenolic content (346.88 ± 30.53 mg GAE/g).[25] The TPC values presented here are also similar to the results reported by (Iftikhar et al.) on a polyherbal formulation consisting of selected medicinal plant extracts.[26]

The amount of TFC in different formulations is given in Figure 2. The results of the present study revealed that the TFC values of prepared formulations ranged from 5.74 to 14.65 mg QE mg/g of dry weight, wherein the maximum value was observed for F2, while the minimum value for F1. Similar results were demonstrated by Patnala et al.[27] reported TFC values ranging from 22.24 to 82.57 RE/g for "Sitopaladichurna". Talebi et al. also determined TFC values in both hydroalcoholic and aqueous extracts as 36.27 and 17.79 mgQE/g of dry weight, respectively.[28]

The antioxidant potential of polyherbal formulations was measured in terms of DPPH scavenging activity as shown in Figure 3. The IC_{50} for the formulation ranged from 3.362 to

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Table 3. Antioxidant Evaluation (TPC, TFC, DPPH, and FRAP) of Polyherbal Emulsions

formulation	TPC	TFC	DPPH (IC_{50})	FRAP (EC_{50})
F1	53.45 ± 0.41b	5.48 ± 0.25a	4.30 ± 0.11a	3.23 ± 0.11a
F2	49.08 ± 0.53b	15.53 ± 0.50b	3.45 ± 0.15b	3.08 ± 0.07b
F3	69.65 ± 0.61c	7.43 ± 0.35c	5.64 ± 0.14c	3.08 ± 0.06b
F4	60.43 ± 0.93c	6.49 ± 0.21a	5.66 ± 0.13c	2.27 ± .05c
F5	59.78 ± 0.75c	8.53 ± 0.24c	3.73 ± 0.15b	1.53 ± .03d
F6	174.29 ± 0.79a	6.75 ± 0.15a	5.90 ± 0.16c	3.53 ± .40e
F7	75.52 ± 0.49a	14.80 ± 0.17b	4.13 ± 0.15a	1.04 ± .04f
F8	51.90 ± .027b	14.52 ± 0.49b	3.28 ± 0.16b	2.00 ± .02f
F9	63.85 ± 0.67c	10.70 ± 0.20d	1.84 ± .03c	1.84 ± .03 g

5.730 mg/m; the highest IC_{50}value was noted for F6 and lowest for F8.

The values of FRAP assay in terms of EC_{50} for different formulations ranged from 0.231 to 3.610 mg/mL, presenting the lowest value for F4 and the highest value for F6. The reduction potential for different formulations was in the order of F6 > F1 > F3 > F2 > F7 > F8 > F9 > F5 > F4 indicating the significant antioxidant potential of prepared formulations. The results of the present study are in agreement with the results of Babu and SJ, and Barku et al.[29,30]

The antioxidant potential of herbal preparations showed that almost all the formulations possessed the potential to cease the production of reactive oxygen species. The maximum phenolic contents were found in F6 herbal formulation. The maximum activity was shown by F6 might be linked to the presence of high contents of phenolic compounds (Table 3).

In Vitro Antidiabetic Activity of Polyherbal Formulations (α-Amylase Inhibition Assay). The α-amylase inhibitory effect in terms of IC_{50} for all prepared herbal formulations (F1-F9) was in the range of 152.12−257.25 μg/mL. The formulations F2, F3, F5, F6, F7, and F8 were noted to show maximum α-amylase inhibitory activity ranging from 50 to 1000 μg/mL at IC_{50} values such as 158.84, 179.11, 191.28, 221.20, 194.84, and 152.12 μg/mL, respectively. The formulations F2, F3, F5, F7, and F8 may attenuate the glucose levels below normal and thus lead to hypoglycemia; hence, to avoid such abnormal hypoglycemic circumstances, F6 (IC_{50}: 221.20 near standard IC_{50}) was employed for further in vivo studies (Table 4).

Patwekar et al. demonstrated that the inhibitory activity of PHF of hot water extract might be due to the presence of various phytoconstituents including phytol, sterols, and phenols.[31] In another study conducted by Keshala et al. it was reported that the hot infusion of Malewana Madhumeha Choorna (MMC) polyherbal formulation showed hypoglycemic activity by the inhibition of carbohydrate digesting enzymes, especially α-amylase. The enzyme inhibitory action may be due to the presence of flavonoids and phenolic compounds in MMC.[32]

In Vivo Antidiabetic Activity of Polyherbal Formulations. *Acute Toxicity Study of F6-SMONSECCE.* Before executing the in vivo studies, acute toxicity of the developed polyherbal formulation F6 was observed and no change in behavior was noted up to the dose level of 2000 mg/kg body weight as well as no sign of mortality was observed during the observation period (21 days) (Table 5).

Determination of BGLs for Antihyperglycemic Therapeutic Effects of Polyherbal Formulation; F6-SMONSECCE. The diabetic rat model was used for evaluating the antihyperglycemic effects of the prepared formulation (F6-SMONSECCE). Animals were induced diabetes by the alloxan and the results showed that the diabetogenic agent such as alloxan significantly ($0 < 0.05$) increased the blood glucose levels after the 7th day of the study period. The administration of polyherbal formulation (F6- SMONSECCE) and glibenclamide (G) and coadministration of (G + H) to the alloxan-treated rats significantly lowered the blood glucose levels after the 7th day of treatment. Moreover, significant fluctuations in the standard drug (G) treated group were noted as compared to the herbal-treated (F6- SMONSECCE) group. F6 formulation exhibited consistency in attenuating the BGLs which clearly indicated the steady control of the formulation on metabolism. However, when the herbal drug was administered along with the standard drug, more prominent and steady effects were observed in the regulation of BGLs (Figure 1).

Majeed et al. described that alloxan injected into rats significantly raised the glucose and glycosylated hemoglobin levels in different animals, while oral administration of the polyherbal formulation and glibenclamide drug considerably reduced glucose, glycosylated hemoglobin, and liver glycogen concentration due to increased insulin and leptin levels. Moreover, they found that the administration of the polyherbal formulation also enhanced the activity of pancreatic beta cells by upregulating the expression of PDX-1 (pancreatic and duodenal homeobox 1), INS-1 (insulin-1 gene precursor), and INS-2 (insulin-2 precursor) genes which are part of the insulin signaling cascade. Past studies support the results reported in our current work and demonstrate the effectiveness of the herbal formulations.[33] Similarly, Han et al. also explained the beneficial effect of Chinese herbal formulation (Yitangkang) on type-2 diabetic rats.[34] Administration of streptozotocin (STZ) to rats significantly ($P < 0.01$) increased the BGLs, total cholesterol (TC), TG, and LDL-c but decreased the HDL-c levels in the blood. It was also noted that the administration of STZ changed the activity of antioxidant enzymes (SOD, MDA, and other related enzymes) and endocrine and cyclic nucleotide system in comparison to the control group. The treatment with Chinese herbal formulation (Yitangkang), and metformin revealed that both of them effectively regulated the blood parameters, regulatory enzymes, energy metabolism, and hormonal level of the diabetic rats.[34]

Currently, several researchers are investigating the efficacy of the polyherbal formulations of medicinal plants in diabetic rats. In a recent study conducted by Gbekley et al., the hypoglycemic effect of herbal recipe Diabeto-Dolvo (DD) on streptozotocin-treated rats was also observed. According to the results, the administration of DD into diabetic rats might have repaired the oxidative damage, hyperglycemia, and hyper-

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Table 4. Alpha-Amylase Inhibition of Polyherbal Formulations[a]

Sr.No	concentrations (μg/mL)	F1	F2	F3	F4	F5	F6	F7	F8	F9	standard
1	50	42.05 ± 1.00$^{a}_{D}$	26.33 ± 0.96$^{a}_{B}$	30.54 ± 1.02$^{a}_{C}$	41.56 ± 1.11$^{a}_{D}$	32.30 ± 0.80$^{a}_{C}$	39.52 ± 0.66^{a}	31.51 ± 1.00$^{a}_{C}$	20.27 ± 1.01$^{a}_{A}$	41.60 ± 0.70$^{a}_{D}$	31.55 ± 1.03$^{a}_{C}$
2	100	45.01 ± 0.96$^{b}_{G}$	29.58 ± 1.03$^{b}_{B}$	32.46 ± 1.02$^{b}_{C}$	43.36 ± 0.74$^{b}_{F}$	34.60 ± 1.0$^{b}_{C}$	41.11 ± 0.91$^{b}_{E}$	39.02 ± 0.99^{b}	24.15 ± 0.88$^{b}_{A}$	46.31 ± 1.93^{b}	36.00 ± 0.96$^{b}_{D}$
3	250	46.24 ± 0.82$^{b}_{E}$	30.60 ± 0.98$^{b}_{A}$	33.32 ± 1.00$^{b}_{B}$	45.64 ± 0.97^{c}	39.64 ± 1.10$^{c}_{C}$	43.05 ± 1.0$^{c}_{D}$	42.63 ± 1.00$^{c}_{D}$	30.39 ± 0.96$^{c}_{A}$	53.51 ± 1.52$^{c}_{F}$	44.44 ± 0.41$^{c}_{D}$
4	500	48.06 ± 0.98$^{c}_{C}$	35.02 ± 0.98$^{c}_{A}$	37.65 ± 1.07$^{c}_{A}$	49.58 ± 0.99$^{d}_{C}$	41.54 ± 1.02$^{d}_{B}$	46.08 ± 1.10$^{d}_{C}$	41.62 ± 1.10$^{c}_{B}$	36.83 ± 0.98$^{d}_{A}$	56.63 ± 1.15$^{d}_{D}$	47.01 ± 0.94$^{d}_{C}$
5	1000	51.04 ± 0.81$^{d}_{E}$	37.31 ± 1.00$^{d}_{A}$	45.13 ± 1.04$^{d}_{D}$	50.26 ± 1.40^{d}	43.20 ± 1.00$^{e}_{C}$	51.44 ± 1.30^{e}	40.16 ± 1.60$^{b}_{B}$	40.48 ± 0.87$^{e}_{B}$	59.20 ± 1.47$^{e}_{F}$	51.00 ± 0.64$^{e}_{E}$
	IC50	232.41$^{e}_{F}$	158.84$^{*e}_{A}$	179.11$^{*e}_{B}$	230.45$^{e}_{F}$	191.28$^{*f}_{C}$	221.23$^{f}_{E}$	194.94$^{*d}_{G}$	152.12$^{*f}_{A}$	257.25$^{f}_{H}$	210$^{f}_{D}$

[a]* Showing the minimum IC_{50} value, $n = 3$.

Table 5. Acute Toxicity Study of Formulation

animal groups with formulation doses (F6-SMONSECCE)	glucose level of rats at day 1	glucose level of rats at day 21	no. of dead animals
1 (100 mg/kg b.wt.)	65	59	nil
	71	65	
	68	73	
2 (250 mg/kg b.wt)	61	64	nil
	89	56	
	75	65	
3 (500 mg/kg b.wt)	77	62	nil
	83	55	
	81	52	

lipidemia in the same way as the treatment with glibenclamide.[35]

Determination of Lipid Status for Therapeutic Effects of Polyherbal Formulation (F6- SMONSECCE). The results showed that the alloxan significantly ($P < 0.005$) increased the levels of TC, TG, LDL-c, and VLDL-c), whereas a significant reduction was noted for HDL-c as compared to the normal control group animals (Figure 2). The standard drug (glibenclamide) as well as co-administered (G + H) group showed a more prominent effect by lowering the levels of TC, TG, and LDL-c, while notable elevation was observed in the levels of HDL-c. The administration of polyherbal formulation (SMONSECCE) significantly attenuated the BGLs (22.94%), TC (29.10%), TG (38.15%), LDL-c (27.58%), and VLDL-c (71.52%), whereas on the other side, the insulin (−149.15%) and HDL-c levels (−22.22%) were significantly increased. The coadministered rats showed a prominent reduction in TC (26.64%), TG (16.86%), LDL-c (13.79%), and VLDL-c (55.88%) while elevation in HDL-c (−11.11%) was also observed. It may be inferred from the results that herbal formulation significantly reduced the levels of bad contents of lipids, and increased the healthy lipid content with less or no side effects.

The diabetogenic agent such as alloxan, alter the serum levels of TC, TG, LDL-c, VLDL-c, and HDL-c referring that it also happens in diabetes. The administration of polyherbal formulation in the diabetic rats can restore normal levels of serum lipids.[36] The lipid-lowering effect of the aqueous extract of polyherbal formulation might be owed to the presence of flavonoids which have been reported to lower TC and TG levels.[37] In another study, Chaudhuri and Sharma, (2016) described that STZ-induced diabetes caused the elevation of TC, TG, and LDL while a reduction in HDL levels. However, the treatment with polyherbal formulation declined the elevated levels of TC, TG, and LDL-c, whereas it improved the concentration of HDL-c.[38]

Effect of F6-SMONSECCE on the Insulin Level of Diabetic Rats. The result revealed a significant ($P < 0.05$) decline in the insulin levels of alloxan-treated rats as compared to the normal control group. After the ingestion of formulation (F6-SMONSECCE) and glibenclamide in diabetic rats, the insulin level was substantially improved as compared to the diabetic control group (Table 6).

In the glucose-loaded model, F6-SMONSECCE showed a significant hypoglycemic effect. Excessive blood glucose stimulates the release of insulin. This insulin might increase peripheral glucose uptake and regulate glucose metabolism via a variety of methods.[39] Nevertheless, it was clear from the experiment that insulin requires a minimum time to control

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

groups	insulin (μ/L)
normal control	16.4 ± 1.14[a]
diabetic control	5.9 ± 0.97[e]
diabetic treated with F6-SMONSECCE	14.7 ± 1.00[a] (−149.15%)
diabetic treated with glibenclamide	15.3 ± 1.00[ab] (−159.32%)

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

the glucose level up to the normal. The glucose levels in the F6-SMONSECCE and glibenclamide-treated animals did not rise above those in the negative control group, indicating a robust activity of the F6-SMONSECCE and glibenclamide in glucose usage. According to the literature cited in previous studies, glibenclamide improves the pancreatic cells ability to release insulin, which has a positive effect on glucose tolerance.[26,36] Therefore, the mechanism behind the F6-SMONSECCE antidiabetic activity includes an insulin-like effect, perhaps via increased glucose consumption / improved cell sensitivity by enhancing insulin secretion.[40] Numerous plants with similar hypoglycemic potential have also been reported.[31]

Effect of F6-SMONSECCE on Histological Alterations in Pancreas and Kidney of Diabetic Rats. *Histology of Pancreas.* Histological sections of pancreatic tissue of the normal control group showed normal anatomy with proper arrangement of α and β cells in islets. Compact and round nuclei were observed in cells (Figure 3). Pancreatic sections of the diabetic group showed that severe necrotic changes were caused by alloxan especially in the center of islets. Other changes including karyolysis, the disappearance of the nucleus, and in some places residues of destructed cells were also noted. A visible reduction in the size and the number of islets around the vessel and a severe reduction in the number of β cells were observed. The administration of polyherbal formulation and glibenclamide significantly ($P < 0.001$) normalized the architecture of a pancreas.

Effect of Polyherbal Formulation (F6- SMONSECCE) on Diabetic Pancreatic Cells and Their Diameters. The statistical analysis showed that the mean number of α and β cells and mean diameter of islets, α and β cells significantly decreased ($P < 0.001$) in all the diabetic groups as compared to the normal control. The ingestion of polyherbal formulation and glibenclamide significantly ($P < 0.001$) elevated the attributes of the pancreas as compared to the diabetic group (Table 7).

Histology of Kidneys. Histology of the kidney section in the normal control group exhibited all normal structures in which Bowman's capsules were well organized, glomeruli rounded, confined in proper peri-glomerular space, luminal spaces were appropriate in the proximal and distal tubules and epithelial nuclei of tubular epithelium were aligned properly (Figure 4A). The histological analysis of the kidney section of the diabetic control group (Figure 4B) showed ruptured Bowman's capsule, wide peri-glomerular spaces, deformed and swollen glomeruli, and dilation of proximal and distal tubules with large luminal space.

The kidney section of standard drug-treated diabetic rats (Figure 4C) showed recovery signs having the normal shaped Bowman's capsules, organized glomeruli, regular luminal spaces and less dilated proximal and distal tubules. The kidney section of the herbal drug-treated diabetic group (Figure 4D) presented a resemblance to the normal control group.

Micrometric Analysis of Kidneys. The average cross-sectional area of Bowman's capsule, the glomeruli, proximal tubule, and distal tubule in the diabetic control group were significantly ($P < 0.05$) increased as compared to the normal control group, whereas on the other side, in drug and formulation-treated groups, no notable difference was seen in the ACSA of Bowman's capsule as compared to that of control group. Significant reduction ($P < 0.001$) in the ACSA of Bowman's capsule, glomeruli, proximal tubule, and distal tubule in treated diabetic (standard drug/ herbal emulsion) rats was found as compared to diabetic control (Table 8).

In this study, the diabetogenic agent alloxan altered the internal structure of the kidney cells by increasing the cross-sectional area of Bowman's capsule, glomeruli, distal tubule, and the proximal tubules. The treatment with a standard drug (glibenclamide), and polyherbal formulation significantly ($P < 0.05$) normalized the internal structures of the kidney as compared to the diabetic group.

Our results are inconsistent with the findings of Madic et al., who described that the polyherbal formulation effectively normalized the glomerular size, proximal tubular cells and renal collagen that might be linked to its antioxidant activity.[41] In another experiment, Iroanya et al. analyzed the protective effects of polyherbal formulation (GOV), prepared from leaves of *Gongronema latifolia*, *Ocimum gratissimum* and *Vernonia amygdalin*, in acetaminophen-treated rats. He demonstrated that the diabetic rats treated with polyherbal formulation (GOV) significantly normalized the tubular degeneration, edema, and arrested necrosis.[42]

CONCLUSIONS

The current study findings exhibited that the prepared herbal-formulation F6-SMONSECCE exhibited potent antioxidant and antidiabetic activities. The in vitro activities showed that few herbal-formulations possessed a high level of activity as compared to the standard drug (glibenclamide). The in vivo

Table 7. Effect of Polyherbal Formulation on the Diabetic Pancreatic Cells and Their Diameters[a]

groups	no. of Cells		mean diameter (μm)		
	beta cells /10cm^2	alpha cells /10 cm^2	islet	beta cells	alpha cells
normal control	23.75 ± 0.59	9.37 ± 0.32	9.38 ± 0.32	0.39 ± 0.01	0.23 ± 0.01
diabetic control	5.63 ± 0.38[a]***	5.00 ± 0.27[a]***	5.00 ± 0.27[a]***	0.12 ± 0.01[a]***	0.06 ± 0.01[a]***
drug-treated	17.37 ± 0.32[ab]***	7.63 ± 0.18[ab]***	7.63 ± 0.18[ab]***	0.29 ± 0.01[ab]***	0.16 ± 0.04[ab]***
polyherbal-treated	13.5 ± 0.33[abc]***	5.75 ± 0.25[ac]***	5.75 ± 0.25[ac]***	0.25 ± 0.01[ab]***c*	0.12 ± 0.04[abc]***

[a]Values are presented as Mean ± SEM, a = The normal control vs all diabetic groups, b = The diabetic control vs drug/ emulsion treated groups, c = standard drug-treated vs herbal emulsion-treated. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

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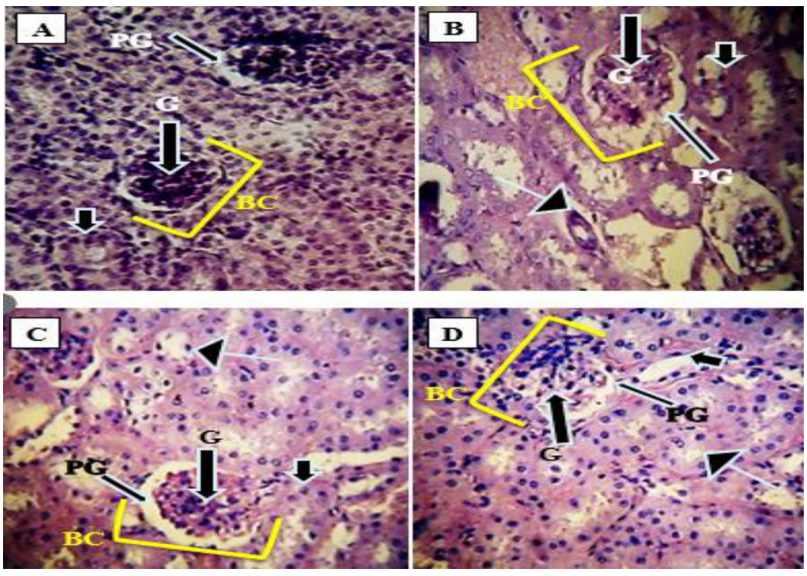

Figure 4. Photomicrographs from sections of the kidney of the normal untreated rat (A) presenting well organized Bowman's Capsule (B) with narrow periglomerular space (PG), a dense bunch of capillaries in the glomerulus (G), the normal proximal and distal tubules (arrows), the diabetic control (B) kidney sections show highly disorganized Bowman's capsule, wide peri-glomerular space, and few capillaries in the glomerulus and the tubules with wide lumen. Sections from glibenclamide (G) treated diabetic rat kidney (C) and polyherbal emulsion-treated diabetic group (D) presents recovery signs in the arrangement of Bowman's capsule, the glomeruli, and tubules.

Table 8. Effect of the Herbal-Formulation on Different Kidney Attributes of Rats[a]

	average cross sectional area (μm)			
groups	Bowman's capsule	glomeruli	distal tubule	proximal tubule
normal control	17.25 ± 0.52	14.37 ± 0.26	12.37 ± 0.18	10.50 ± 0.18
diabetic control	24.12 ± 1.00^{a}*** (39.82%)	21.12 ± 0.22^{a}*** (46.97%)	15.87 ± 0.22^{a}*** (28.29%)	13.37 ± 0.18^{a}*** (27.33%)
glibenclamide treated diabetic	16.65 ± 0.26^{b}*** (−3.47%)	14.75 ± 0.25^{b}*** (3.50%)	12.50 ± 0.26^{b}*** (1.05%)	10.37 ± 0.18^{b}*** (−1.23%)
herbal-emulsions treated diabetic	16.25 ± 0.41^{b}*** (−5.79%)	15.37 ± 0.32^{b}*** (6.95%)	12.62 ± 0.26^{b}*** (2.02%)	10.50 ± 0.18^{b}*** (0%)

[a]Values are presented as Mean ± SEM, a = The normal control vs all diabetic groups, b = diabetic control vs drug/ emulsion-treated groups, c = standard drug-treated vs herbal- emulsion treated. ***$P < 0.001$, **$P < 0.01$.

trial also showed the potential antidiabetic activities of F6-SMONSECCE in the diabetic rats along with the normalization of the lipid profiles and alteration in the levels of biochemical attributes. The selected herbal-formulation, F6-SMONSSECCE, also conserved the architecture of the kidney as nephron-protective activities in the diabetic rats. The F6-SMONSECCE herbal-formulation may be suggested as a herbal remedy due to its hypoglycemic and antioxidant potential in the nutra-pharmaceutical sector.

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Notes

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