Research Article

Formulation, characterization and biological evaluation of polyherbal tablet for antidiabetic potential in Streptozotocin induced diabetes in rats

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Abstract

Objective: Present work was aimed to evaluate polyherbal tablet containing methanol extracts of *Carissa carandus, Ocimum sanctum, Moringa oleifrea, Manilkara zapota.* **Material and methods:** The dried plant extracts of all proposed plants were mixed with different excipient using wet granulation method to prepare tablets using compressing machine. Prepared herbal formulation was tested for *in vivo* studies for antidiabetic activity by using Streptozotocin induced diabetes model Effect of formulation were assessed by measurement of biochemical parameters and histological observations. **Results**: Preformulation study of the granules showed that all the evaluated parameters were within the acceptable limit. Prepared polyherbal tablet was selected that showed acceptable data of all characterization parameters. After treatment with formulation, STZ induced diabetic animals showed significant reduction in body weight, blood glucose level, and lipid profile when compared to the control animals. The serum total cholesterol, and Triglyceride level were significantly ($P<0.01$) decreases and restore near to the normal level. Polyherbal formulation treated animals reversed the effect of STZ on the renal antioxidant level. This may be due to the antioxidant mechanism of the individual herbs present in the polyherbal formulation. **Conclusion** Observation of the present study was confirmed that prepared formulation was safe, and effective for antidiabetic activity. It was confirmed that prepared formulation was able to restore lipid profile, blood glucose level and antioxidants level to the nearly of normal. These positive effects may attribute to the presence of various flavonoid constituents present in methanol extracts of all plants. Additionally this formulation also showed antioxidant effect that may another possible mechanism for antidiabetic effect of formulation.

Keywords: *Carissa carandus, Ocimum sanctum, Moringa oleifrea, Manilkara zapota,* ployherbal tablets, antidiabetic, Streptozotocin

Introduction

Diabetes mellitus is a chronic metabolic disorder resulting from insulin deficiency, characterized by hyperglycaemia, altered metabolism of carbohydrates, protein and lipids, and an increased risk of vascular complication. The insulin deficiency may be absolute or relative and the metabolic abnormalities lead to the classic symptom of polyuria, polydipsia, polyphagia and fatigue. Long term complication of diabetes mellitus include gangrene, polyneuropathy and uraemia (Fowler and Williams, 1995).

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The toxic action of streptozotocin and chemically related alkylating compounds requires their uptake into the cells. Nitrosoureas are usually lipophilic and tissue uptake through the plasma membrane is rapid; however, as a result of the hexose substitution, streptozotocin is less lipophilic. Streptozotocin is selectively accumulated in pancreatic beta cells via the low-affinity GLUT2 glucose transporter in the plasma membrane. Thus, an insulin-producing cell that does not express this glucose transporter is resistant to streptozotocin. This diminishes cellular NAD+, and subsequently ATP, stores. The depletion of the cellular energy stores ultimately results in beta cell necrosis. Although streptozotocin also methylates proteins, DNA methylation is ultimately responsible for beta cell death, but it is likely that protein methylation contributes to the functional defects of the Beta cells after exposure to

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streptozotocin. Inhibitors of poly ADP-ribosylation suppress the process of DNA methylation (Bentley and Trimen, 2004).

Carissa carandas is a species of flowering shrub in the dogbane family, Apocynaceae. It produces berry-sized fruits that are commonly used as a condiment in Indian pickles and spices. It is a hardy, drought-tolerant plant that thrives well in a wide range of soils.

The chemical investigations of *C. carandas* had led to the isolation of several substances including β-sitosterol, lupeol, ursolic acid and a new cardioactive substance; glucosides of odoroside-H (Rastogi et al., 1967). Bark, leaves and fruit contain an unnamed alkaloid. The leaves are reported to have triterpene, tannins and carissic acid (Siddiqui et al., 2033). Fruits of this plant have been reported to contain a mixture of volatile principles like 2-phenyl ethanol, linalool, β-caryophyllene, isoamyl alcohol and benzyl acetate (Chandra, 1972) and a novel (Carissol) triterpenic alcohol. The karonda fruit is a rich source of iron and contains a fair amount of vitamin C. It is ant scorbutic and very useful for cure of anemia.

Ocimum sanctum, commonly known as holy basil, tulasi or tulsi, is an aromatic perennial plant in the family Lamiaceae. It is native to the Indian subcontinent and widespread as a cultivated plant throughout the Southeast Asian tropics.

Eugenol, nerol, eugenol methyl ether, caryophyllene, terpinene-4-ol-decyladehyde, α-selinene, a and β-pinene, Camphor and carvacrol, Cineole, and linalool.

Different parts of Tulsi plant e.g. leaves, flowers, stem, root, seeds etc. are known to possess therapeutic potentials and have been used, by traditional medical practitioners, as expectorant, analgesic, anticancer, antiasthmatic, antiemetic, diaphoretic, antidiabetic, antifertility, hepatoprotective, hypotensive, hypolipidmic and antistress agents (Chopra and Nayer, 1956).

M. oleifera is a fast-growing, deciduous tree that can reach a height of 10–12 m (32–40 ft) and trunk diameter of 45 cm (1.5 ft). The bark has a whitish-grey colour and is surrounded by thick cork.

Chemical Constituents

Benzyl isothiocyanate, β- sitosteryl oleate, Stigmasterol, Oleic acid, Octadecene. The leaves are the most nutritious part of the plant, being a significant source of B vitamins, vitamin C, provitamin A as beta-carotene, vitamin K, manganese, and protein, among other essential nutrients (Chopra et al., 1956; Wealth of India, 1976).

Manilkara zapota, commonly known as the sapodilla is a long-lived, evergreen tree native to southern Mexico, Central America and the Caribbean.

The leaves of the sapodilla fruit can also be used as a medicine for inflammatory diseases. The leaves work as an oral anti-inflammatory agent. Take clean sapodilla fruit leaves and then boil them for about ten minutes. This boiled water which contains the extracts of Sapodilla can be used as medicine. It can be used for gargling as well (Wealth of India, 1976).

Sapodilla or chikoo is a natural sedative. It is used to relax the nerves and also to relieve stress. It is often suggested as a part of the diet of those struggling with insomnia and panic disorders.

Materials and methods

Collection and identification of plant materials

The proposed plant drug (*Carrisa carandus, Ocimum sanctum, Moringa olefera, Manilkara zapota)* were collected from nearest area of Bhopal (M.P.). The Leaves of all plant drugs were shade-dried, powdered into moderately coarse powder and stored in air tight container. Plant specimens were identified and authenticated in Department of Pharmacognosy, RKDF College of Pharmacy, Bhopal. The powder drug of all plants material was used for extraction.

Extraction and phytochemical studies

The powdered drug of *Carrisa carandus* plant (about 150 g) was defatted with petroleum ether and extracted with methanol (95%) in a soxhlet apparatus for 12 and 24 Hrs respectively. The solvent was removed under reduced pressure, with respect to dried plant material. The dried extract of was stored in a desiccator till further use.

Phytochemical investigation means to investigate the plant material in terms of its active constituents. It involves the identification of active constituents and to identify them qualitatively. Extract obtained from all plant drugs (*Carrisa carandus, Ocimum sanctum, Moringa olefera, Manilkara zapota)* were subjected to various qualitative tests for the identification of various plant constituents present in this species (Paech and Tracey, 1955; Sim, 1968; Kokate et al., 2002).

Preparation of Polyherbal tablets

The dried plant extracts of all proposed plants were mixed with different excipient using wet granulation method to prepare later solid pharmaceutical forms (Table 1). These prepared granules of each form were compressed into tablets using Compressing machine.

Characterization of Polyherbal tablets

Particle size measurement

Size affects the average weight of tablet. The method used for determination of particle size is Sieving.

Table 1. Composition of Polyherbal tablet formulation

Ingredients	Quantity per tablet (mg)			
	F1	F2	F3	F4
Carissa Carandus	100	100	100	100
Ocimum Sanctum	100	100	100	100
Moringa Oleifera	100	100	100	100
Manilkara Zapota	100	100	100	100
Ethyl Cellulose	50	40	40	30
Microcrystalline Cellulose	40	40	40	40
Dibasic calcium phosphate	30	40	30	50
PEG 400	20	10	20	20
Methyl paraben	10	20	20	10
Weight per tablet	550 mg	550 mg	550 mg	550 mg

Angle of repose

Angle of repose was determined by using funnel method. The accurately weighed blend was taken in a funnel. The drug excipient blend was allowed to flow through the funnel freely on to the surface (Lakade and Bhalekar, 2008). The height of the funnel was adjusted in such a way that the tip of the funnel just touches the apex of the heap or head of blend. The diameter of the powder cone was measured and angle of repose was calculated using the following equation:

$\tan \theta = h/r$

Where, h = height of powder cone formed

r = radius of the powder cone formed

Loose bulk density

Apparent bulk density was determined by pouring a weighed quantity of blend into graduated cylinder and measuring the volume and weight (Aulton, 2002).

LBD = Weight of the powder / volume of the packing

Tapped bulk density

It was determined by placing a graduated cylinder, containing a known mass of drug excipient blend. The cylinder was allowed to fall under its own weight on to a hard surface from the height of 10 cm at two second intervals (Banker and Anderson, 1987). The tapping was continued until no further change in volume was noted.

TBD = Weight of the powder/vol of the tapped packing

Compressibility index

The Compressibility index of the blends was determined by Carr's compressibility index (Mohsin et al., 2010).

Compressibility index (%) = (TBD-LBD) x 100/TBD

Hausner ratio

It is the measurement of frictional resistance of the drug. The ideal range should be 1.2-1.5 (Hamid et al., 2006). It is determined by using the following formula:

Hausner ratio= TBD / LBD

Color and appearance

Colour and taste can be made more appealing by adding coloring and flavouring agents. The externally added coloring and flavoring agents should match with the colour and taste of medicament. The compressed tablets were examined for their color and appearance (Lakade and Bhalekar, 2008). The result was shown in table

Weight variation test

With a tablet designed to contain a specific amount of drug in a specific amount of tablet formula, the weight of the tablet being made is routinely measured to ensure that a tablet contains the proper amount of drug. The average weight was determined by randomly selecting and weighing 20 tablets. Each tablet was also weighed individually. The deviation from the average weight in each case was Calculated and expressed as a percentage (Banker and Anderson, 1987).

All the values according to USP limit for the test (Lachman et al., 1987), not more than 2 tablets were outside the percentage limit and no tablet differs by more than 2 times the percentage limit (5%). The prepared tablets passed the limit.

Hardness and Friability test

Tablets require a certain amount of strength or hardness and resistance friability, to withstand mechanical shocks of handling in all processes. The hardness and friability were tested for the tablets by using calibrated hardness tester (Monsanto) and Roche friabilitor (4 minute at 25 rpm) tests respectively.

The friability of tablets for all formulations, were determined by Roche friabilator. The tablets was weighed and put into the plastic chamber and operated for 100 revolutions. Then, the tablets were dusted and reweighed. The percentage friability was calculated for each formulation and the values were given in table 4. The tablets passed the acceptable limit less than 1% (Lachman et al., 1987).

Disintegration test for tablets

The disintegration time of tablet for all formulations was determined by using the IP disintegration apparatus (Indian Pharmacopoeia, 1996). The 900 ml of 0.1N hydrochloric acid was the disintegration medium and the time to disintegrate completely was noted. A glass of plastic tube 80-100 mm long with an internal diameter of about 28 mm and external diameter 30-31 mm fitted at the lower end with a disc of rust proof wire gauge. Six tablets were placed in the

tube, raise and lower the tube in such a manner that the complete up and down movement is repeated 28 to 32 per minute. The tablets are disintegrated when no particles remains above the gauge, which readily pass through mesh (10 mesh screen).

Thickness and diameter

The thicknesses and Diameter of the tablets were evaluated by using Vernier calipers.

***In vitro* drug release**

The drug release testing of the formulated tablet was conducted by using USP paddle apparatus. It was carried out for 08 hrs with 900 ml 1.2 pH buffer acid solution maintained at 37±0.5◦C and agitated at 75 rpm. 5 ml of dissolution medium was withdrawn, filtered and diluted at regular intervals to determine the percentage drug release. The drug concentration was determined by calibration curve equation.

Pharmacological evaluation of Polyherbal tablets

Animals

Adult albino rats weighing about 150-200 g were used in the present investigation. All the rats were given a period of acclimatization for 15 days before starting the experiment. They were fed *ad libitum* everyday with standard chow diet and were given free access to water. Animals described as fasting were deprived of food for at least 16 h but were allowed free access to drinking water.

Streptozotocin induced diabetic model

Diabetes was induced in albino wistar rats (150–200 g) by a single intraperitoneal (i.p.) injection of 50mg/kg of streptozotocin (STZ), reconstituted in freshly prepared normal saline (0.9% W/V) after overnight fasting. After 72 h of STZ administration, glucose levels were measured in blood samples collected from retro orbital sinus of rats. Rats with fasting serum glucose levels more than 200mg/dL were considered diabetic and selected for further Study. Animal grouping was made are given below:

Group I- Control rats received vehicle solution normal saline

Group II- Diabetic control rats received vehicle solution normal saline

Group III – Reference drug (Metformin; 250 mg/kg) treated group

Group IV – Diabetic rats treated with polyherbal tablet 550mg/kg

Effects of polyherbal tablets on Biochemical Parameters in Streptozotocin Induced Diabetes

The effects of herbal formulation in normal and diabetic rats were assessed by measuring blood glucose level, serum lipid profile and changes in body weight. Finally the one animal from each group was select to sacrifice by diethyl ether anesthesia, and liver and kidney tissues were separate for biochemical and histopathological observation.

Enzymatic and non-enzymatic antioxidant assay

In both models, one part of liver and kidney tissues were used for antioxidant assay. Catalase was estimated following the breakdown of hydrogen peroxide according to the method of Beers and Sizer (1952). Superoxide dismutase (SOD) was assayed according to Misra and Fridovich (1972) based on the inhibition of epinephrine autoxidation by the enzyme. Reduced glutathione (GSH) content was determined in granuloma tissue by the method of Moron *et al*, 1979.

Histopathological study

In case of STZ induced diabetic rats, animals were anaesthetized before taking liver and kidney tissue sample using diethyl ether. Tissue specimen from control, diabetic control, formulation treated and reference group were collected and store in 10% formalin after that usual processing 6 µm thick sections were cut and stained with haematoxylin and eosin (McManus and Mowry, 1965).

Statistical analysis

The results of the study were subjected to analysis of variance followed by Dunnett's t-test for multiple comparisons. Values with $P<0.01$ were considered to be significant.

Results and discussion

Extraction of different plant materials

The moderately coarse powder of the leaves of *Carissa Carandus* (150g) was subjected to extraction with petroleum ether and methanol by increasing order of polarity from non-polar to polar. The yields were found to be 2.8g (1.86% w/w of crude drug) of petroleum ether extract with semisolid mass of brown colour, 12.2g (8.13% w/w of crude drug) of methanolic extract with blackish brown colour semisolid mass, was observed. The moderately coarse powder of the leaves of *Ocimum Sanctum* (150g) was subjected to extraction with petroleum ether and methanol by increasing order of polarity from non-polar to polar.

Phytochemical investigation was performed for petroleum ether and methanolic extracts of the all proposed plants *(Carissa carandus, Ocimum sanctum, Moringa oleifrea, Manilkara zapota).* Petroleum ether extract of *Carissa carandus* showed the presence of Sterols while methanolic extract of *Carissa carandus* showed the presence of

flavonoids and Tannins. Petroleum ether extract of *Ocimum sanctum* showed the presence of Proteins and amino acids while methanolic extract of *Ocimum sanctum* showed the presence of Alkaloids, flavonoids and saponins. Petroleum ether extract of *Moringa oleifrea* showed the presence of Sterols, Acidic compounds and Resins while methanolic extract of *Moringa oleifrea* showed the presence of Alkaloids flavonoids and Tannins. Petroleum ether extract of *Manilkara zapota* showed the presence of Sterols and amino acids while methanolic extract of *Manilkara zapota* showed the presence of Flavonoids, Phenolic compounds, Carbohydrates and Tannins.

Acute toxicity Study

Animals were observed at regular time intervals. In all the cases no death was observed within first 24 hours. Additional observations like behavioral changes in skin, fur, eyes, mucous membranes, respiratory, autonomous and central nervous systems and somatic motor activity and behavioral pattern. Attention was also given to observation of tremors and convulsions. The toxicity studies were carried out as per the OECD guidelines. The polyherbal formulation did not show any mortality or adverse event up to 2000 mg/kg. Hence, the study was carried out at the dose levels of 550 mg/kg.

Preparation and Characterization of polyherbal tablets

Polyherbal tablet formulations were prepared using the four herbal drug extracts. The various combinations of dried granules of powdered extracts of *Carissa carandus, Ocimum sanctum, Moringa oleifrea, Manilkara zapota* were prepared and characterized on the basis of pre-formulation studies including parameters like angle of repose, loose bulk density, tapped bulk density, compressibility index and hausner ratio etc. Preformulation study of the granules showed that all the evaluated parameters were within the acceptable limit.

The results of bulk density, angle of repose, Compressibility Index and Hausner's ratio were indicated that the polyherbal powder mixture possess good flow properties and good packing ability (Table 2 and 3). After a formulation by a direct compression method using automated punching machine, developed polyherbal tablets were subjected to measuring of post compression parameters like uniformity of weight, uniformity of content, hardness, friability, thickness, and disintegration time of the tablets. All the parameters of the test products are complied with the Pharmacopeial requirements.

In the weight variation test the percentage weight variation in all the tablet formulations was within the pharmacopoeial limit. The variation was in range of 1.85±0.21 to 2.65±0.31% indicating the maximum percentage variation in F4 and minimum in F1. Thus all the formulations pass the test (Table 4 and 5).

The hardness of formulation was measured in kg/cm^2 with the help of Monsanto tester. Amongst all the formulations prepared, F1 has been found to be the most acceptable one in terms of weight variation and *in vitro* disintegration time. This formulation showed appreciable hardness characteristics, which facilitated its fast disintegration. The friability of formulation indicated that the tablets were

Table 2. Angle of repose, loose bulk density and Tapped bulk density of formulated tablets

S.N.	Batches	Angle of repose (θ)	Loose bulk density (g/ml)	Tapped bulk density (g/ml)
01	F1	22.1±1.04	0.48±0.07	0.45±0.04
02	F2	29.3±1.85	0.54±0.02	0.53±0.07
03	F3	24.5±1.38	0.56±0.04	0.51±0.10
04	F4	27.6±1.94	0.61±0.06	0.49±0.12

Table 3. Color, Hausner Ratio and Compressibility index (%) of prepared polyherbal tablet

S.N.	Batches	Color	Hausner Ratio	Compressibility index (%)
01	F1	Grey-Brown	1.32±0.14	15.35±0.85
02	F2	Grey-Brown	1.68±0.20	19.85±1.0
03	F3	Grey-Brown	1.54±0.16	17.50±0.64
04	F4	Grey-Brown	1.95±0.31	16.45±0.82

Table 4. Weight variation, Hardness (Kg/cm^2) and Friability (%) test of prepared polyherbal tablet

S.N.	Batch	%Weight variation (±5%)	Hardness (Kg/cm^2)	Friability (%)
01	F1	1.85± 0.21	3.1 ± 0.02	0.59 ± 0.01
02	F2	2.30±0.18	3.8 ± 0.05	0.89 ± 0.01
03	F3	2.35±0.24	4.1 ± 0.03	0.76 ± 0.03
04	F4	2.65±0.31	3.6 ± 0.04	0.92 ± 0.01

Table 5. Thickness, diameter and disintegration time of formulated tablets

Batch	Thickness (mm)	Diameter (mm)	Disintegration time (min)
F1	4.3 ± 0.01	10.1± 0.02	18.40±1.27
F2	4.7 ± 0.03	10.8± 0.05	24.15±1.83
F3	4.6 ± 0.01	10.5± 0.03	20. 20±1.75
F4	3.9 ± 0.02	10.3± 0.01	21.30±1.61

mechanically stable. As the average weight of tablets was 550 mg, the acceptable weight variation range is ±5%. Hence the entire formulated tablet passed the weight variation test. The disintegration time of formulation was not more than 25 Minutes.

The tablets require a certain amount of strength or hardness and resistance friability to withstand mechanical shocks of handling in all processes. The hardness of tablets was determined by Monsento hardness tester. The hardness of tablets was in range of 3.1 ±0.02 to 4.1± 0.03 indicating maximum in F3 and minimum in F1. The hardness of tablets was within the Pharmacopoeial limit. Thus all the tablets were pass the hardness test.

The friability of tablets was determined by Roche friabilator. The percentage friability was in range of 0.59 ± 0.01 to 0.92 ± 0.01 indicating maximum with F4 and minimum with F1. The range of percentage friability was within the pharmacopoeial limit. Thus all the formulations passed the friability test.

The disintegration time of tablets of all the formulations was determined using IP disintegration test apparatus. The time required to disintegrate the tablets was in range of 18.40±1.27 to 24.15±1.83 min. indicating maximum with F2 and minimum with F1. The range of disintegration time was within the pharmacopoeial limit. Thus all the formulation passed the disintegration test. The formulations F1-F4 were evaluated for in vitro drug release. The formulation F1 was found suitable which gave 100% drug release in 12hrs.

Antidiabetic activity

The present study aims to evaluate the antidiabetic effect of prepared herbal tablets *Carissa carandus, Ocimum sanctum, Moringa oleifera, Manilkara zapota* plants extract in different proportion. These plants are already reported to consist of some glucose lowering potential in the available literature. On the basis of available literature and previous studies on these plants, here we prepared herbal tablets of these plants extract and investigated antidibetic activity by using two different in vivo animal models i.e. Streptozotocin induced diabetes in rats.

Streptozotocin (STZ) is widely used to induce diabetes mellitus in animals. It can be administered through either intravenous (IV), intraperitoneal (IP) or subcutaneous (SC) ways. The mechanisms of action STZ is quite understandable. STZ is selective cytotoxic agents and consequently destroy the pancreatic beta cells selectively. In short, STZ is transported to pancreatic beta cells by GULT2 glucose transporter since both are glucose analogues. The STZ splits into glucose and methylnitrosourea. Methylnitrosourea possesses alkylating properties. The alkylation of DNA by methylnitrosourea leads to the destruction of the beta cells (Lenzen, 2008).

The streptozotocin induction causes suppression of insulin secretion by the destruction of pancreatic beta cells. Streptozotocin is a nitrosourea analogue which has a hexose moiety linked to N-methyl-Nnitrosourea moiety. Since the nitrosourea is lipophilic, the cellular uptake of STZ into plasma membrane is fast. Additionally, the STZ is selectively accumulated and transported via GLUT2 glucose transporter (Karunanayake et al., 1976; Tjälve, 1976). Consequently, the insulin producing cells could be STZ resistant if the cell does not express any GULT2 transporter (Ledoux and Wilson, 1984; Schnedl et al., 1994; Elsner et al., 2000). The underlying mechanism of toxic effects of STZ to the pancreatic beta cell is assumed to be taken place by the alkylation of DNA from the interaction of methylnitrosourea moiety of STZ. The consequence of the alkylation initiates a bunch of events which lead to fragmentation of the DNA (Pieper et al., 1999). Another hypothesis for STZ mechanism of action claims that the intracellular nitric oxide (NO) donor is responsible for the diabetogenic effects of STZ (Turk et al., 1993). Chemically, STZ could liberate NO as it possesses nitroso group. Moreover, effect of NO is attributed to the elevated action of guanyl cyclase and the formation of cGMP by STZ. Therefore, the most toxic alkylating agent known as methyl methanesulphonate is not a NO donor which may suggest that the NO donating could not be considered as the underlying reason for toxic effects of STZ (Delaney et al., 1995).

Table 6. Effect of Poly herbal tablet on body weight of STZ induced diabetic rats

Groups (n = 6)	Body weight (g) on different days			
	Day 0	Day 07	Day 14	Day 21
Control	194.7±7.5	195.0 ± 18.5	195.9 ± 15.9	196.8 ± 16.1
Diabetic Control	195.0 ± 21.7	245.0 ± 20.3	252.0 ± 22.6	253.5 ± 19.0
Diabetic treated with standard	197.6 ± 19.3	202.5 ± 17.9	198.6 ± 19.6	198.9 ± 22.4
Treated with Polyherbal tablet	198.8 ± 17.7	199.4 ± 26.5	198.4 ± 24.6	200.1 ± 20.8

Data shown as mean±standard deviation (SD). *p<0.01 compared to control group

Table 7. Effect of Poly herbal tablet on blood glucose of STZ induced diabetic rats

Groups (n = 6)	Blood glucose (mg/dl)	
	Day 0	Day 21
Control	128.2±4.6	137.2±3.56
Diabetic Control	218±10.5	315.4±2.59
Diabetic treated with standard	221±5.8	130.1±3.5*
Treated with Polyherbal tablet	223±5.2	138±2.5*

Values are mean ± SD; *p > 0.01

Table 8. Effect of Poly herbal tablet on Lipid profile of STZ induced diabetic rats

Groups (n = 6)	Day 21	
	Total Cholesterol (TC) (mg/dL)	Triglyceride (TG) (mg/dL)
Control	121.3±2.8	82.6±2.3
Diabetic Control	226.2±2.6	203.2±2.4
Diabetic treated with standard	128.0±1.8*	88.0±2.2*
Treated with Polyherbal tablet	123.1±2.0*	90.1±2.1*

Values are mean ± SD; *P > 0.01

Throughout the study, the diabetic animals showed significant reduction in body weight when compared to the control animals (Table 6). However, the polyherbal formulation and metformin inhibited the diabetes-induced body weight reduction. The rats treated with herbal tablets showed an unchanged in body weight at different time duration. After days 21, body weight of group treated with herbal tablets (200.1 ± 20.8) and reference drug (198.9 ± 22.4) were not changes. The results showed significant increase in animal's body weight were found after induction of diabetes. But in case of herbal tablets and reference antidiabetic drug treatment it was not increased.

Diabetic control animals showed severe hyperglycemia compared to normal animals. The mean blood glucose level in the diabetic control group on day 0 was 218±10.5 mg/dl and on day 21 was 315.4±2.59 mg/dl. It was observed that the standard drug metformin lowered the blood glucose level significantly, bringing it back to near normal level, whereas the polyherbal tablets at 550 mg/kg significantly ($P < 0.01$) decreased the blood glucose level in the diabetic rats on 21st days, as compared to the diabetic control group. There was a significant decrease in blood glucose level were found upon treatment with herbal tablets (138±2.5) and it was also found comparable to reference drug treated group (130.1±3.5). The results of blood glucose level of different groups were shown in table 7.

The lipid profile was also observed after days 21 of treatments. The serum total cholesterol, and Triglyceride level were significantly ($P<0.01$) decreases and restore near to the normal level (Table 8). The observed serum total cholesterol levels of herbal tablet and reference group were found as 123.1±2.0and 128.0±1.8, respectively. The Triglyceride level was observed as 88.0±2.2 and 90.1±2.1 for reference treated and herbal tablet treatment group, respectively. The diabetic rats showed significant ($P < 0.01$) increase in serum lipid profiles when compared to the control animals, whereas the levels in the treatment group remained within normal limits at the end of the study.

The polyherbal formulation treated animals reversed the effect of STZ on the renal antioxidant level. This may be due to the antioxidant mechanism of the individual herbs present in the polyherbal formulation. STZ diabetic rat has decreased levels of SOD, CAT and GSH which cause hyperglycemia. Incessant generation of free radicals can lead to tissue damage through peroxidation of unsaturated fatty acids (Munday, 1988). Upon treatment with polyherbal formulation and standard metformin, there was a significant improvement was observed in antioxidant markers level (Table 9). The polyherbal formulation treated animals

Table 9. Effect of tablet formulations and reference drug on enzymes and non enzymatic level of tissues in Streptozotocin induced diabetic model in rats

Groups	Enzymatic and non-enzymatic assay		
	SOD(µg/50 mg tissue)	CAT(µmol/50 mg tissue)	GSH(µmol/50 mg tissue)
Control	41.64±5.24	32.17±2.85	37.20±2.79
Diabetic Control	24.73±3.42	17.52±2.17	20.38±1.78
Diabetic treated with standard	38.24±2.81	28.94±2.61	35.48±2.04
Treated with Polyherbal tablet	36.08±3.64	27.30±2.62	35.21±2.67

n = 6 albino rats per group, value represents Mean S.D. *P<0.01, when compared each treated group with control group

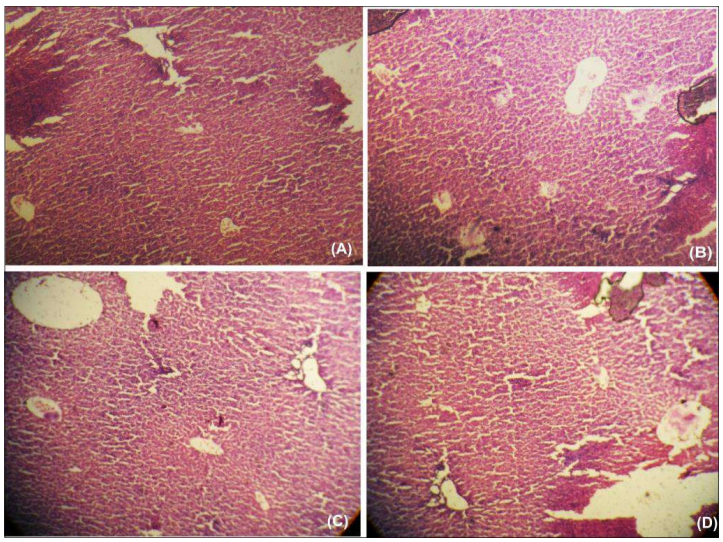

Figure 1. Photomicrograph of liver tissues collected from different groups of STZ induced diabetes in rats: (A) Normal Control; (B) Diabetic Control; (C) Diabetic treated with standard (Metformin); (D) Treated with Polyherbal tablet

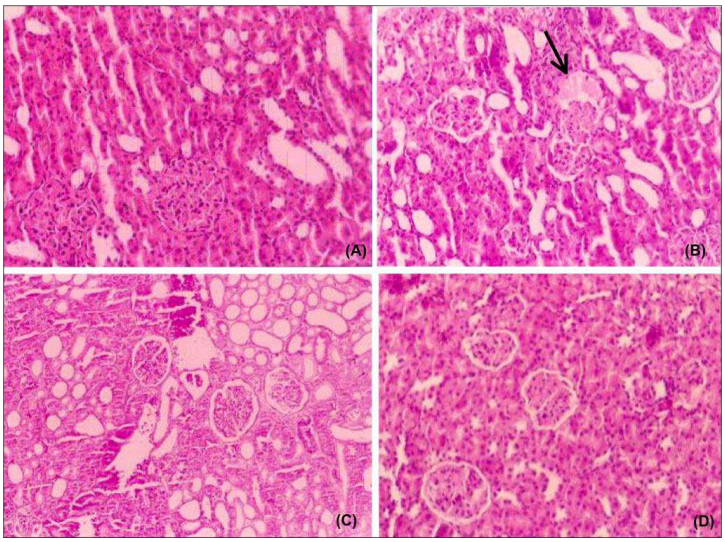

Figure 2. Photomicrograph of kidney tissues collected from different groups of STZ induced diabetes in rats: (A) Normal Control; (B) Diabetic Control; (C) Diabetic treated with standard (Metformin); (D) Treated with Polyherbal tablet

inhibited the hyperglycemia induced by STZ, which may be due to the free radical scavenging properties of the individual herbs present in it.

It has been reported that STZ-induced diabetic rats showed serious degeneration in the liver, mainly embodied with focal necrosis, inflammatory cell infiltration, mussy hepatic cords, and congestion in central vein (Turk et al., 1993). In contrast, normal rats had an organized hepatic architecture with normal hepatocyte morphology. As shown in figure, the liver of diabetic rats treated with metformin almost recovered to normal with focal necrosis. The polyherbal tablets treatments significantly relieved the mussy hepatic cords of liver (Figure 1), and exhibited a marked improvement in the liver histopathology evidenced by a diminution of inflammatory cell infiltration and attenuation of lipid droplet accumulation against STZ induced histological alteration. Of note, no clearly histopathological abnormalities were found in liver tissue in the metformin treated rats and herbal tablets treated groups, indicating the remarkable effect ameliorating liver defects.

The histology of the kidney (Figure 2) tissue revealed that non diabetic control group shows the normal arrangement of the nephron cells with normal glomeruli. The diabetic group of animal's kidney showed the improper arrangement of the nephron cell with the presence of high endocytic vacuoles which was absent in the other group animals. The metformin and herbal tablet formulation treated group of animals showed recovery of vaguely arranged nephron cells into properly arranged cells with glomeruli.

Nevertheless, architecture of the kidney in STZ-induced diabetes rats exhibited various pathological damages, such

as glomeruli partly sclerosis, mesangial expansion, glomerular hypertrophy, interstitial lymphocyte infiltration, vacuolation in the renal epithelia and pyknotic nuclei. The kidney histology of metformin treated rats was recovered to normal as compared with normal control rats.

It was worth noting that the kidney of diabetic rats treated with herbal tablets appeared nearly normal. These results showed that the herbal extracts containing tablet formulation treatments notably alleviated STZ-induced histology damages in different degrees. Herein, polyherbal tablets had the most demonstrable effect on the protection of liver and kidney against injuries in diabetes rats.

Flavonoids are considered as one of the most abundant secondary metabolites of the plant. Approximately, more than 8000 unique flavonoids from different plants have so far been isolated and characterized through various isolation and spectroscopic techniques (Cook and Samman, 1996; Hollman and Katan, 1999). In addition to that, the flavonoids are mostly found in fruits, vegetables, nuts, seeds, stem, flowers, etc. The flavonoids are low molecular weight compounds and have been reported to exert various important pharmacological effects including the cell synthesis (Heim et al., 2002). Moreover, the scientific claims for flavonoids as a cure of diabetes are noteworthy owing to its wide range of mechanisms of action.

Conclusion

Observation of the present study was confirmed that prepared formulation was safe, and effective for antidiabetic activity. It was confirmed that prepared formulation was able to restore lipid profile, blood glucose level and antioxidants level to the nearly of normal. These positive effects may attribute to the presence of various flavonoid constituents present in methanol extracts of all plants. Additionally this formulation also showed antioxidant effect that may another possible mechanism for antidiabetic effect of formulation.

Conflicts of interest: Authors do not have any conflicts in present study.

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