

Antidiabetic effect of polyherbal combinations in STZ induced diabetes involve inhibition of α -amylase and α -glucosidase with amelioration of lipid profile

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Received: 9 October 2011, Revised: 3 November 2011, Accepted: 4 November 2011

Abstract

The concerned study reveals antidiabetic effects of different polyherbal combinations of six medicinal plants used in traditional medicine. Aim of the present study was to evaluate antidiabetic action of polyherbal combination of six medicinal plants. Aqueous extracts of *Stevia rebaudiana*, *Momordica charantia*, *Tamarindus indica*, *Gymnema sylvestre*, *Allium sativum* and *Murraya koenigii* were used for polyherbal combinations. All these combinations were studied for their acute toxicity and 250 mg/kg dose was selected. OGTT, antidiabetic and anti- α amylase and α -glucosidase activity and liver function tests were performed for all the combinations. Reduction in blood glucose level was determined in antidiabetic activity for 0 to 20 days and histopathology of the pancreas was performed after 20th day. IC₅₀ value is determined in anti- α amylase activity. Results revealed that all combinations were safe and dose was selected at 250 mg/kg. Polyherbal combinations II showed significant antidiabetic activity in OGTT and STZ-diabetic rats. Combination II showed significant anti- α amylase and α -glucosidase activity which is better than other combinations. Treatment with combination-II in diabetic animals produced beneficial improvement in lipid profile. Histopathological observations showed improvement in the rat treated with combination-II. It may be concluded that combination-II was most effective and safe in comparison to other combinations. Flavonoids, tannins and sterols present in this combination might be responsible for the effect.

Key words: Polyherbal combinations; Acute toxicity; OGTT; Antidiabetic; α -amylase, α -glucosidase; FBG, STZ,

Introduction

Diabetes mellitus is characterized by hyperglycemia, hypercholesterolemia, and hypertriglyceridemia, resulting from defects in insulin secretion or reduced sensitivity of the tissue to insulin (insulin resistance) and/or combination of both (Mishra et al., 2009). The pri-

Table 1. Plants with their traditionally reported uses.

Plant name	Family	Uses	Reference
<i>Momordicha charantia</i>	Cucurbitaceae	Stomachic, antimicrobial, antidiarrhoeal, anti-diabetic digestive, astringent, spasmolytic, antidiabetic	Nadkarni et.al., 1991
<i>Murraya koenigii</i>	Rutaceae	Purgative, anti-diabetic, topically emollient, anti-inflammatory, antimicrobial, antidiabetic	Nadkarni et.al., 1991
<i>Allium sativum</i>	Liliaceae	Antimicrobial, antifungal, anthelmintic, antiviral, anti-diabetic, antipyretic, antimalarial, spermicidal, anti-inflammatory, hypoglycemic	Nadkarni et.al., 1991
<i>Tamarindus indica</i>	Fabaceae	Appetizer, demulcent, hypoglycemic	Nadkarni et.al., 1991
<i>Gymnema sylvestre</i>	Asclepiadaceae	hyperglycemia, obesity, high cholesterol levels, anemia, digestion	Nadkarni et.al., 1991
<i>Stevia rebaudiana</i>	Asteraceae	Obesity, hypertension, Antidiabetic, sweetner	Nadkarni et.al., 1991

mary lesion at onset of diabetes, irrespective of the type, is a defect in insulin production and action, which is characterized by a clinical manifestation of hyperglycemia (Tiwari & Rao, 2002). Although, there are numerous traditional medicinal plants reported to have antidiabetic and antidiabetic properties. The medicinal uses of the plants used in the study are summarized in table 1 (Khare et al., 2007; Nadkarni, 1991).

It is well known that the incidence of diabetes mellitus is high all over the world, especially in Asia. Different types of oral antidiabetic agents such as biguanides and sulphonylurea are available along with insulin for the treatment of diabetes mellitus (Holman & Turner, 1991), but have side effects associated with their uses (Valiathan et al., 1998). But more than three agents may be present in a medicinal herb with a variety of intervention targets via various mechanisms of action. Besides, polyherbal formulations (Singh et al., 2005) have proved more useful and beneficial in the management of various ailments including those that seem to defile conventional medication (Ebong et al., 2008). There is a growing interest in herbal remedies because of their effectiveness, minimal side effects in clinical experience and relatively low cost. Herbal drugs or their extracts are prescribed widely, even when their biological active compounds are unknown. Even the World Health Organization (WHO) approves the use of plant drugs for different diseases, including diabetes mellitus.

The potential role of the medicinal plants as antidiabetic agents has been reviewed by several authors, supported by the ethno botanical surveys and traditional medicines of different cultures (Biesalski, 2004). Aim of the present study was to evaluate antidiabetic action and the anti- α -amylase potential of six commonly available medicinal plants in our

region, *Momordicha charantia* (Cucurbitaceae), *Murraya koenigii* (Rutaceae), *Allium sativum* (Liliaceae), *Tamarindus indica* (Fabaceae), *Gymnema sylvestre* (Asclepiadaceae), and *Stevia rebaudiana* (Asteraceae). Present work was undertaken to find out best antidiabetic combination of the above mentioned commonly available herbs in our locality.

Materials and methods

Chemicals

Streptozotocin was obtained from Sisco Research Laboratory, Mumbai, Maharashtra, India. DPEC-GOD/POD kit for quantitative blood glucose determination was purchased from One Touch Horizon, India.

Plant material

Bulbs of *A. sativum* (Liliaceae), whole fruit of *M. charantia* (Cucurbitaceae), leaves of *M. koenigii* (Rutaceae), and fruit pulp of *T. indica* (Fabaceae) were collected from Ahmednagar district (M.S.) and authenticated at Botanical Survey of India (Pune). Voucher specimen number ALLISANU4, MOMCHANU2, MURK-ANU3, and TAMIANU1, respectively were deposited in the department. Hydroalcoholic extract of *Gymnema sylvestre* (Asclepiadaceae) and aqueous extract of *Stevia rebaudiana* (Asteraceae) were obtained from Herbex laboratories, Jalana.

Extraction

Dried powdered leaves of *M. koenigii* and fruit pulp of *T. indica* were extracted by reflux distillation using purified water. These extracts were vacuum dried to yield 24.49% and 58.10% of aqueous extracts, respectively. Dried powdered bulbs of *A. sativum* and whole fruit of *M. charantia* were extracted by reflux distillation using 70% ethanol. These extracts were vacuum dried to yield 15.80 % and 5.08% of hydroalcoholic extracts, respectively.

Animals

Wistar strain albino rats weighing between 195 ±15 g were obtained from the Serum Institute of India, Pune. The rats were housed in clean metallic cages and kept in a well ventilated room and allowed to acclimatize to the laboratory condition for one week before being used. They were fed with standard animal pellet and had free access to water *ad libitum*. The animals were distributed randomly into seven groups of six animals each for antidiabetic study using streptozotocin-induced diabetic experiment. The protocol of the experiment (CPCSEA/C/448/10-11/06) was approved by Institutional Animal Ethics Committee (IAEC) of Pravara Rural College of Pharmacy, Loni and were conducted in accordance with guidelines as per "Guide for the care and use of laboratory animal" and with permission from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Acute toxicity study

Healthy male Wistar rats, starved overnight (12 h), were divided into 24 groups of 6 each and were orally fed with increasing doses (50, 100, 250, 500, 1000, and 2500 mg/kg) of

combinations I, II, III, and IV to determine the safe doses by up and down staircase method. The animals were observed continuously for one hour, then frequently for 4 hours, and later at the end of 24 h. After administration of the drug, Irwin test was conducted, where the animals were observed for behavioral changes. Further, animals were observed daily for 30 days, and mortality was recorded (Ghosh et al., 1984). To know multiple dose toxicity of combinations, highest dose was fed once daily for 15 days and observed for incidences of mortality for a period of 30 days.

Pharmacological Screening

Oral glucose tolerance test in normal rats (OGTT)

This test was performed according to Shirwaikar & Rajendran (2006). Rats were divided into five groups (n = 6). They were fasted overnight and accessed to water only. Blood was taken from the lateral veins of the tail and the blood sugar levels were initially monitored with a glucometer (One touch Horizon). Above groups were treated with vehicle (0.5% Tween 80 solution), polyherbal combinations I, II, III, and IV (250 mg/kg, p.o., each). After 30 min, the animals were treated with 5% (wt/v) glucose orally. Blood glucose levels were monitored from lateral tail veins at 30, 60, and 120 min intervals after post glucose challenge.

Induction of diabetes mellitus

Diabetes was induced by a single intraperitoneal injection of freshly prepared streptozotocin (35 mg/kg bw) in 0.1M citrate buffer (PH 4.5) to overnight fasted rats. The development of diabetes was confirmed after 48 hours of STZ injection, the animals with fasting blood glucose level more than 200 mg/dl were selected for the experimentation (Somani et al., 2006; Mustafa et al., 2007).

Experimental Design

The Streptozocin-induced diabetic Wistar rats were randomly assigned into six groups (1-6) of six rats (n=6) each. Group 1 received normal saline p.o., group 2 received streptozotocin (35 mg/kg, i.p.), group 3 received Metformin (250 mg/kg, p.o.), group 4 received Combination I (250 mg/kg, p.o.), group 5 received Combination II (250 mg/kg, p.o.), group 6 received Combination III (250 mg/kg, p.o.), and group 7 received Combination IV (250 mg/kg, p.o.)

Determination of blood glucose levels

Blood samples were collected by cutting the tail-tip of the rats, for blood glucose determination at intervals of 0, 5, 10, 15, and 20 days. Determination of the blood glucose level was done by the glucose-oxidase principle (Beach & Turner 1958) using the ONE TOUCH Basic (Horizone) instrument and results were reported as mg/dl (Rheney Kirk, 2000).

In vitro alpha-amylase inhibition

The α -amylase inhibition assay was adapted and modified from Giancarlo et al. (2006). The Starch solution (0.5% w/v) was obtained by boiling and stirring 0.25 g of potato starch in 50 ml of deionized water for 15 min. The enzyme solution (0.5 unit/ml) was prepared by mixing 0.001 g of α -amylase (EC 3.2.1.1) in 100 ml of 20 mM sodium phosphate buffer (pH 6.9) containing 6.7 mM sodium chloride. The extracts polyherbal combinations were dissolved in DMSO to give concentrations from 10 to 100 mg/ml (10, 20, 40, 60, 80, 100 mg/ml). The color reagent was a solution containing 96 mM 3, 5-dinitrosalicylic acid (20 ml), 5.31 M sodium potassium tartarate in 2 M sodium hydroxide (8 ml) and deionized water (12 ml). 1 ml of each plant extract combinations and 1 ml enzyme solution were mixed in a tube and incubated at 25°C for 30 min.

To 1 ml of this mixture was added 1 ml of starch solution and the tube incubated at 25°C for 3 min. Then, 1 ml of the color reagent was added and the closed tube placed into an 85°C water bath. After 15 min, the reaction mixture was removed from the water bath and cooled thereafter, diluted with 9 ml distilled water and the absorbance value determined at 540 nm in a Shimadzu Multispect-1501 spectrophotometer (Kyoto, Japan). Individual blanks were prepared for correcting the background absorbance. In this case, the color reagent solution was added prior to the addition of starch solution and then the tube placed into the water bath. The other procedures were carried out as above. Controls were conducted in an identical fashion replacing plant extract polyherbal combinations with 1 ml DMSO. Acarbose solution (at the concentrations of 10, 20, 40, 60, 80, 100 μ g/ml) was used as positive control. The inhibition percentage of α -amylase was assessed by the following formula:

$$I_{\alpha\text{-amylase}} \% = 100 \times (\Delta A_{\text{Control}} - \Delta A_{\text{Sample}}) / \Delta A_{\text{Control}}$$

where $\Delta A_{\text{Control}} = A_{\text{Test}} - A_{\text{Blank}}$
and $\Delta A_{\text{Sample}} = A_{\text{Test}} - A_{\text{Blank}}$

The $I_{\alpha\text{-amylase}} \%$ was plotted against the sample concentration and a logarithmic regression curve established in order to calculate the IC_{50} value (inhibitory concentration). This would represent the concentration of sample (μ g/ml) necessary to decrease the absorbance of α -amylase by 50%.

In-vitro alpha-glucosidase activity

α -glucosidase inhibition was determined using the modified version of the method according to Matsui et al. (1996). The α -glucosidase reaction mixture contained 2.9 mM p-nitrophenyl- α -D-glucopyranoside (pNPG), 0.25 ml of extracts polyherbal combinations I to IV (varying concentrations) in DMSO and 0.6 U/ml α -glucosidase in sodium phosphate buffer (pH 6.9). Control tubes contained only DMSO, enzyme and substrate, while in positive controls acarbose replaced the extracts polyherbal combinations. Mixtures without enzyme, extracts polyherbal combinations and acarbose served as blanks. The reaction mixtures were incubated at 25 °C for 5 min, after which the reaction was stopped by boiling for 2 min. Absorbance of the resulting p-nitrophenol (pNP) was determined at 405 nm using Shimadzu Multispect-1501 spectrophotometer (Kyoto, Japan) and was considered directly proportional

to the activity of the enzyme. Glucosidase activity inhibition was determined as percentage of control as follows:

$$\begin{aligned} \% \text{ Glucosidase inhibition} &= 100\% - \% \text{ activity of test as percentage of control} \\ \% \text{ Activity of test} &= \text{Corrected } A_{405} \text{ of test} \times 100\% / A_{405} \text{ of controls} \end{aligned}$$

In order to eliminate background readings, the absorbance of the extract without substrate and enzyme was subtracted from absorbance of the extracts polyherbal combinations and substrate mixture as follows:

$$\text{Corrected } A_{405} \text{ test samples} = A_{405} \text{ extract and substrate mixture} - A_{405} \text{ extracts polyherbal combinations alone (background)}$$

The activity in controls (with α -glucosidase but without inhibitor) was considered to be 100%. Concentrations of polyherbal combinations resulting in 50% inhibition of enzyme activity (IC_{50} values) were determined graphically.

Biochemical estimation

Blood glucose level (BGL), total cholesterol (TC), high density lipoprotein (HDL)-cholesterol, triglycerides (TG) (Singh et al., 2007; Bergmeyer & Benut, 1963; Burstein et al., 1970; Zlatkis et al., 1953) were estimated using standard kits of Bayers diagnostic Pvt. Ltd., India. Low density lipoprotein (LDL)-cholesterol was calculated from the measurement by Friedwald formula (Friedwald et al., 1972). Glycosylated haemoglobin was estimated using Excel diagnostics Pvt. Ltd., India. Body weight was determined gravimetrically.

Histopathology

On 20st day the animals were sacrificed, the pancreas of one animal from each group was excised and stored in 10% formalin after washing with normal saline. Histopathological parameters were studied at Omega laboratory, Lonand, Satara, India. The tissue was washed, dehydrated with alcohol, cleared with xylene and paraffin blocks were made. Serial sections of 5 μ m thickness were cut using a rotary microtome. The sections were then deparaffinised with xylene and hydrated in descending grades of alcohol. The slides were then transferred to haematoxylin for 10 min, followed by rinsing with water. These were examined and later counterstained with esion, rinsed with water, dehydrated with ascending grades of alcohol, cleared with xylene and mounted.

Statistical analysis

The data was analyzed by one-way ANOVA followed by Tukey-Kramer multiple comparison test. $p < 0.05$ was considered statistically significant.

Results

Experiments were carried out on normal healthy rats for acute toxicity studies. The behavior of the treated rats appeared normal. No toxic effect was seen even with the dose of 2.5 g/kg b.w. and there were no lethality in any of the group. Body weight was normal.

Experiments were carried out on normal healthy rats for acute toxicity studies. The behavior of the treated rats appeared normal. No toxic effect was seen even with the dose of 2.5 g/kg b.w. and there were no lethality in any of the group. Body weight was normal. Therefore, the cut off dose for effective dose (ED₅₀) was taken as 250 mg/kg b. w. which is the 1/10th of LD₅₀. All the six plants selected were having reported hypoglycemic and anti-diabetic activity. Aqueous extract of *S. rebaudiana* (Kujur et al., 2010), *M. koenigii* (Lawal et al., 2008), *T. indica* (Martinello et al., 2006), hydroalcoholic extract of *G. sylvestre* (Kumar et al., 2009) (Mall et.al, 2009), *A. sativum* (Bokaeian et al., 2010), and *M. charantia* (Gunjan et al., 2010) were reported as good antidiabetic agents (Table 1). So these extracts were used for preparing suitable active combinations I to IV.

The oral glucose tolerance test results showed that the plants extracts combinations showed some antidiabetic effects on the blood glucose level in the fasting normal rats. The critical test for diabetes does not lie in hyperglycemia or hyperlipidaemia but in blood-sugar tolerance. After ingesting sugar, both normal and diabetic individuals will show an increase in the blood sugar level as it happens after a meal, but the increase remains high in the diabetic, whereas in the normal individual the excess glucose is rapidly converted into glycogen. Polyherbal combination-II among different formulations was observed to be most active in lowering the postprandial blood glucose level (Table 2). This may be due to synergistic effects of the chemical constituents of the six plants and shows a great promise as an oral antidiabetic agent. Table 3 showed the results of the effects of various polyherbal combinations (250 mg/Kg, p.o.), metformin (250 mg/Kg, p.o.), and control groups in streptozocin-induced diabetic Wistar rats. A drop of blood samples was collected by cutting the tail-tip of the rats, for the blood glucose determination at intervals of 0, 5, 10, 15 and 20 days. All the polyherbal combinations showed significant ($p < 0.05$) reduction in blood glucose level but combination II was found to be better amongst all in reduction of blood glucose level. In addition, comparing alpha amylase and α -glucosidase inhibitory effects of various polyherbal combinations (Table 4), it was observed that polyherbal combination II exhibited appreciable α -amylase and α -glucosidase inhibitory effects (IC₅₀ value 54.35 ± 3.0 $\mu\text{g/ml}$ and 38.12 ± 2.32 $\mu\text{g/ml}$, respectively) when compared with acarbose (IC₅₀ value 30.26 ± 4.01 $\mu\text{g/ml}$ and 31.76 ± 3.01 $\mu\text{g/ml}$, respectively).

The diabetic control animals showed significant increase in glycosylated haemoglobin (GHb %), total cholesterol, serum LDL-cholesterol and serum triglycerides level compared to control animals. GHb %, total cholesterol, serum LDL-cholesterol and serum triglycerides levels in combination-II treated diabetic rats showed significant decrease

Table 2. Antidiabetic effect of various polyherbal combinations in OGTT

Treatment	Mean blood glucose concentration (mg/dl)			
	0 min	30 min	60 min	120 min
Control	110 \pm 2.0	265 \pm 2.3	293 \pm 2.3	162 \pm 2.1
Combination I (250 mg/kg)	103 \pm 1.6	255 \pm 2.6	143 \pm 2.1	122 \pm 1.9
Combination II (250 mg/kg)	105 \pm 3.2	235 \pm 3.6	125 \pm 2.5*	117 \pm 1.5*
Combination III (250 mg/kg)	106 \pm 2.6	215 \pm 3.4	191 \pm 2.1*	135 \pm 1.8*
Combination IV (250 mg/kg)	103 \pm 2.8	190 \pm 2.1*	142 \pm 3.2*	120 \pm 2.9*

Results are expressed as Mean \pm S. E. M. (n = 6); * = $p < 0.05$ compared with control.

Table 3. Effect of various polyherbal combinations on streptozocin-induced diabetic wistar rats.

Treatment	Blood glucose level (mg/dl)				
	0 day	5 day	10 day	15 day	20 days
Control	110.89± 3.80	112.64±2.8	110.80±3.5	114±2.16	115.64±2.8
Disease control (STZ)	289.20± 6.73	267.40±5.0	285.20±8.2	279±1.1	281±5.23
Metformin (250 mg/kg)	315.60± 4.8	256.81±4.3	182.82±5.3*	138±3.71*	118±2.45*
Combination I (250 mg/kg)	310 ±1.0	255 ±1.25	195±1.2*	152±3.5*	130±1.78*
Combination II (250 mg/kg)	292±3.74	178±1.2*	151 ± 2.65*	132±3.1 *	115±2.58*
Combination III (250 mg/kg)	306 ±3.6	210±4.25	172±7.3 *	141±1.65*	132±5.6*
Combination IV (250 mg/kg)	328±4.65	290±5.2	198±1.65*	162±5.1*	141±2.6*

Vales are given as mean ± SEM for 6 rats in each group; experimental groups are compared with diabetic control. Values are statistically significant at * $P < 0.05$.

($p < 0.05$) than other combinations compared to diabetic rats. Results are compared with standard drug metformin. Serum HDL-cholesterol level was significantly decreased in diabetic rats compared to control rats. Diabetic rats treated with combination-II showed significant ($p < 0.05$) increased in HDL-cholesterol than other combination compared to diabetic animals (Table 5).

Discussion

Streptozocin-induced hyperglycaemia has been described as a useful experimental model to study the activity of antidiabetic agents (Szkudelski, 2001). Streptozocin selectively destroyed the pancreatic insulin secreting β cells, leaving less active cell resulting in a diabetic state (Kamtchouing et al., 1998; Szkudelski, 2001). The test samples might possess metformin like effect on peripheral tissues either by promoting glucose uptake and metabolism or inhibiting hepatic gluconeogenesis. The phytochemical studies of extracts of polyherbal combinations revealed the presence of tannins, carbohydrate, terpenes, saponins, and flavonoids. Flavonoid and terpenes possess antidiabetic action (Marles and Farnsworth 1995). Effect of the flavonoids on pancreatic β -cells leading to their proliferation and secretion of more insulin have been proposed by Mahesh and Menon (2004) and Sri-Balasubashini, et al., (2004) as the mechanism by which they reduced hyperglycaemia caused by streptozocin in diabetic rats. These secondary metabolites present in polyherbal

Table 4. Alpha-amylase and alpha-glucosidase inhibitory effects of various polyherbal combinations

Treatment	IC ₅₀ (μ g/ml)	
	Alpha-amylase activity	Alpha-glucosidase activity
Acarbose	30.26 ± 4.01	31.76 ± 3.01
Combination I	72.60 ± 2.32	75.60± 2.37
Combination II	54.35 ± 3.00	38.12± 2.32
Combination III	62.53 ± 2.32	56.35 ± 3.00
Combination IV	120 ± 2.56	91± 1.76

Table 5. Effect of various polyherbal combinations on glycosylated haemoglobin, total cholesterol, LDL, HDL and serum triglyceride.

Treatment	Glycosylated Haemoglobin (GHb %)	Total Cholesterol (mg/dl)	LDL (mg/dl)	HDL (mg/dl)	Serum Triglycerides (mg/dl)
Control	7.217 ± 0.7	118.9 ± 5.4	48.52 ± 5.0	41.99± 2.6	142.0± 3.5
Diabetic Control	12.950 ± 0.4	167.4± 3.64	102.5± 5.3	23.37± 1.9	188.0± 3.9
Standard	7.98 ± 0.6*	120.7± 3.80*	55.54± 5.2*	36.75± 3.0*	152.0± 2.9*
Combination-I	9.112± 0.7*	145.8± 2.80	83.36± 3.5	38.74± 3.2	175.0± 2.1
Combination-II	8.645 ± 0.5*	126.3± 3.46*	61.28± 2.1*	33.74± 3.3*	151.1± 3.0*
Combination-III	9.212± 0.4*	140.8± 4.17*	72.25± 3.1	36.75± 4.0*	168.6± 3.2*
Combination-IV	10.123± 0.7	151.2± 3.87	89.47 ± 2.7	32.12 ± 3.1	178± 5.1

Vales are given as mean ± SEM for 6 rats in each group; experimental groups are compared with diabetic control. Values are statistically significant at *=P<0.05.

combinations may also be acting similarly thereby decreasing the high blood glucose levels of streptozocin-diabetic rats. In addition, one of the therapeutic approaches for type 2 diabetes is to reduce the post-prandial hyperglycemia. Alpha amylase is an enzyme involved in the metabolism of carbohydrates. Alpha amylase degrades complex dietary carbohydrates to oligosaccharides and disaccharides, which are ultimately converted into monosaccharide by alpha glucosidase. Liberated glucose is then absorbed by the gut and results in postprandial hyperglycemia. Inhibition of alpha amylase limits postprandial glucose levels by delaying the process of carbohydrate hydrolysis and absorption (David & Bell, 2004). The plant based alpha amylase inhibitor offers a prospective therapeutic approach for the management of post-prandial hyperglycemia (McCue et al., 2004). In the present study, polyherbal combination II exhibited appreciable alpha amylase inhibitory effect when compared with standard drug acarbose. Therefore, polyherbal combination II could be useful in management

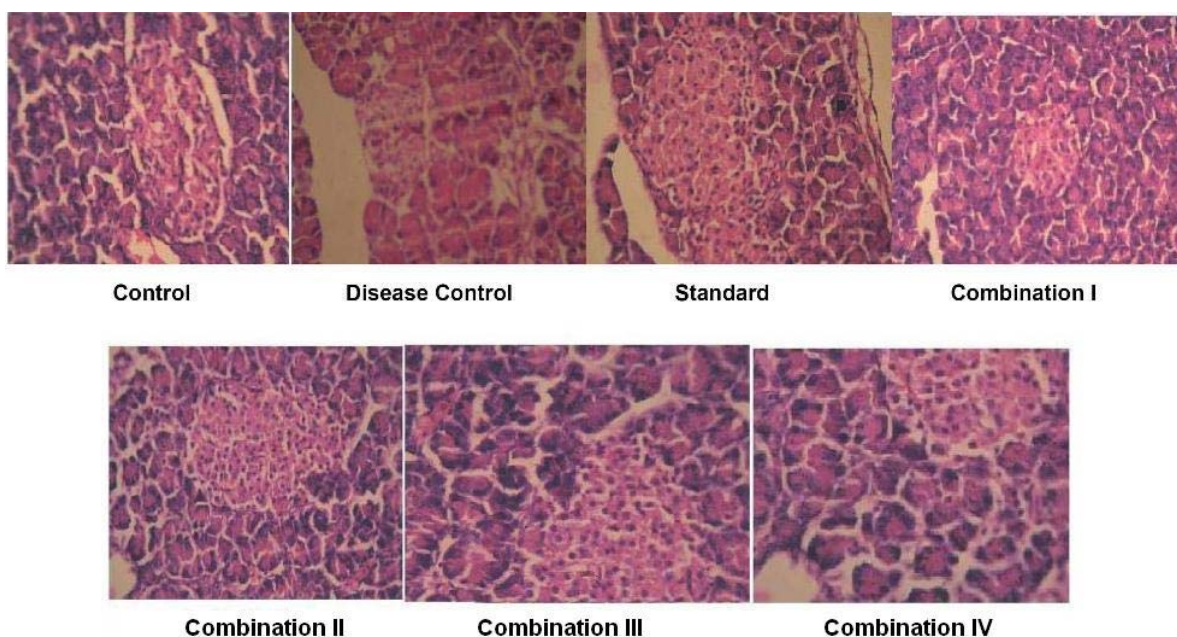


Figure 1. Effects of various polyherbal combinations on histopathology of the pancreas.

of post-prandial hyperglycemia due to inhibition of alpha amylase enzyme. Increased non-enzymatic and auto-oxidative glycosylation is one of the mechanism linking hyperglycemic and vascular combinations. In present study, diabetic rats showed higher GHb% indicating their poor glycemic control. Treatment with combination-II decreased GHb% level. Lipids play important role in pathogenesis of diabetes mellitus. Level of serum lipids is usually raised in diabetic conditions and it is a risk factor for cardiovascular diseases like coronary heart disease and atherosclerosis. In present study elevated serum total cholesterol, triglycerides, LDL-cholesterol, reduced-HDL-cholesterol was observed. Treatment with combination-II in diabetic animals produced beneficial improvement in lipid profile. In the diabetic Control, decrease of pancreatic islet numbers and their size, atrophy and vacuolation and invasion of connective tissues in parenchyma of pancreatic islets were detected but these abnormal histological signs were dramatically decreased in combination-II dosing groups compared to that of control. Similar histopathological changes of the pancreas were observed in metformin dosing group (Figure 1).

It may be concluded that combination-II was most effective in comparison to other combinations and metformin and there were no toxic effects during the 7 hr of study. Combination-II has shown remarking effect on blood glucose level and marked improvement on hyperlipidemia due to diabetic. Its specific effect on HDL has additional advantage in checking coronary risks.

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