



INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES

Published by JK Welfare & Pharmascope Foundation

Journal Home Page: <https://ijrps.com>

Antioxidant and Antihyperlipidemic activity of Polyherbal formulation in Streptozotocin-induced diabetic rats

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Article History:

Received on: 18.04.2018

Revised on: 13.06.2018

Accepted on: 19.06.2018

Keywords:

Polyherbal Formulation (PH),
Streptozotocin (STZ),
Antihyperlipidemic,
Antioxidant,
Glibenclamide

ABSTRACT

In this current protocol, the antioxidant and antihyperlipidemic activity of Polyherbal formulation (PH) in streptozotocin (STZ)-induced diabetic rats were studied. STZ-induced diabetic rats had near about 50% higher serum cholesterol and 53.06 % higher serum triglyceride levels as compared to non-diabetic rats. Also, a significant increase in SGOT, SGPT and serum creatinine levels (65.02%, 69.11%, 53.05% respectively) were observed in STZ-induced diabetic rats. Quotidian therapy of diabetic group with standard & Polyherbal formulation (200 & 400 mg/kg) for four weeks significantly lowered ($P < 0.01$) serum cholesterol, triglyceride, hepatic enzymes and serum creatinine levels. Significant improvement in antioxidant activity with Glibenclamide & Polyherbal formulation (200 & 400 mg/kg) treated group were observed as a decrease in MDA level and increase in superoxide dismutase (SOD) and glutathione (GSH) levels. The treatment groups partially reversed the hindrance towards glucose utilizing system and oxidation status in diabetic rats. The study with PH (400 mg/kg) dose has shown marked improvement in histological condition, as compared to the diabetic control. The above results propose that this Polyherbal formulation may be a potential source for the development of the new antidiabetic drug.



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ISSN: 0975-7538

DOI: <https://doi.org/10.26452/ijrps.v9i4.1679>

Production and Hosted by

IJRPS | <https://ijrps.com>

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INTRODUCTION

Diabetes mellitus (DM) is a running course of altered metabolism, distinguished by an alteration in insulin secretion, action or both leading to mislay of glucose homeostasis. This results in the hindered metabolism of glucose and variant energy supplying fodder like lipids and protein (Scheen JA

1997). The WHO estimated that around the world nearby 346 million people agonise from diabetes and due to negligence, it may get doubled by 2030. Two main types of diabetes are Type-1, insulin deficiency state due to islet β -cell faulty functioning and Type-2, identified by resistance towards the action of insulin. Now, DM has to lead us to various severe micro and macrovascular impediments like neuropathy, nephropathy, retinopathy and cardiovascular disorders (Deshpande AD *et al.*, 2008). The hypoglycemic drugs used show untoward side effects like abnormally low blood sugar, lactic acidosis, oedema and abdominal malaise (Lorenzati B *et al.*, 2010) lead to the exploration of newer antidiabetic drugs in recent times. Thus, a growing inclination for herbal formulations has been observed due to drift towards healthy lifestyle employing natural sources.

A significant part of the world population and health care practitioners observed the entanglements of self-medication, untoward secondary effects and expensive therapy associated with the allopathic system thus are turning towards alternative therapies, (Chaudhary RR 1992) since, these systems are believed to be free from side effects and affordable. Experimental induction of diabetes in animals provide an understanding of the physiologic and biochemical derangement in hyperglycemic animals. This results in structural and oxidative changes along with vascular diseases (Baynes JW *et al.*, 1999). Diabetes also induces a significant alteration in lipid metabolism (Sochar M *et al.*, 1985), increased lipid peroxidation and hyperlipidemia (Morel DW *et al.*, 1989) which are often key determinants of the course and status of the disease. Liver and kidney are insulin dependent tissues that play a vital role during free fatty acids uptake, oxidation, and metabolism accompanied with the synthesis of variants like triglycerides, cholesterol, phospholipids etc. (Jia W *et al.*, 2004; Elder C 2004). Generally, a very compact biological knowledge of the plants has been found to contain chemical constituents like glycosides, flavonoids, terpenoids, phenols alkaloids etc. frequently showing glucose lowering activity as their mechanism of action (Loew D *et al.*, 2002). Traditional medicine system of India emphasised on a combination of plant formulation or extracts as the drug of choice relatively than an exclusive extract. All the herbs in the present protocol possess potent hypoglycemic, antihyperlipidemic or antioxidant activity. The present exploration was drafted to evaluate the potent and beneficial effects of some naturally occurring herbs in combination, which are commonly consumed along with the food, on the antioxidant and antihyperlipidemic status of streptozotocin-induced diabetic rats.

MATERIALS AND METHODS

Chemicals & Reagents

Streptozotocin was procured from HiMedia Laboratories, Mumbai. Glibenclamide was purchased from Sigma- Aldrich, (St. Louis, MO). A diagnostic kit for blood glucose estimation was purchased

from TransAsia Bio-Medicals Ltd., Himachal Pradesh (ERBA Diagnostics). Chemicals and reagents used in this protocol were of analytical grade.

Plant material

Collection of Medicinal plants from various regional places of India in August and September was done. Further, the plants are recognized and authenticated by National Herbarium of Cultivated Plants (NBPGR), Pusa Campus, New Delhi and Department of Botany and Chaudhary Charan Singh University, Meerut. The specimen voucher of the respective plant materials was lodged in the department.

Preparation of Herbal extract: The individual drugs were collected and shade-dried at room temperature at about 37°C. The crude drugs were then subjected to a coarse powder by crushing or grinding, and each drug was then weighed as per the quantity required on a digital balance. The material was submitted to continuous hot soxhlet extraction using 95% ethanol (60-80°C) for about 48hrs (before extraction of fruits, seed and stem; defatting were done using petroleum ether at 60-80°C). Further, the extract was concentrated to semi-solid consistency employing rotary flash evaporator under vacuum with the recovery of solvent. The remnants of the solvent were removed by maintaining the extract in Lyophilizer. For future use, the extract was retained at 4°C.

Preparation of Polyherbal formulation

The polyherbal formulation was prepared by adding all the extracts, i.e. *Aloe vera*, *Camellia sinensis*, *Capparis decidua*, *Musa sapientum*, *Phyllanthus amarus*, *Punica granatum* (Flower & Seed) and *Tinospora cordifolia* in equal amount (1).

EXPERIMENTAL ANIMAL MODEL

Either sex adult albino Wistar rats (150-200g) and albino Swiss mice (20-30g) were procured from the animal house of M.I.E.T, Meerut, UP (India). They were encased at 24±3°C temperature, 65±10% RH and 12 hours of light and dark cycle. Animals were fed with standard pellets and drinking water *ad libitum*. The present protocol was ac-

Table 1: Composition of Polyherbal Formulation

Sr. no.	Plants	Parts	Extract dose (mg/kg)
1	<i>Aloe vera</i>	Leaf pulp	100
2	<i>Camellia sinensis</i>	Leaves	100
3	<i>Capparis decidua</i>	Fruits	100
4	<i>Musa sapientum</i>	Flower	100
5	<i>Phyllanthus amarus</i>	Entire plant	100
6	<i>Punica granatum</i>	Flower	100
		Seed	100
7	<i>Tinospora cordifolia</i>	Stem	100

cepted by the Institutional Animal Ethics Committee (IAEC), M.I.E.T, Meerut. The experiments were performed in compliance with guidelines as per "Guide for the care and use of laboratory animal" and with permission from Committee for Control and Supervision of Experiments on Animals (711/02/a/CPCSEA/2013).

Study on the safety of the formulation

An acute toxicity study was performed as per OECD guideline 423 wherein randomly adult albino Swiss mice 20-30g were allocated into four groups (each containing three animals). Polyherbal formulation at various dose levels (5, 50, 300 and 2000 mg/kg body weight) dissolved in 1% CMC was administered orally to overnight fasted animals; the control group was administered with the vehicle only. Further, an observation period of 14 days was set, i.e. individually once during the first 30 minutes, first 24 hours periodically (special attention for the first 4 hours) and after that daily. The behaviour and any other toxic symptoms of animals were also observed and recorded systematically (individual records being maintained for each animal).

Experimental induction of diabetes in rats

Adult albino Wistar rats (150-200g) were allocated into five groups (06 animals per group). Blood samples were collected for baseline glucose determination. Overnight fasted animals were used for induction of DM. For three consecutive days, each animal was injected intraperitoneally with streptozotocin (60 mg/kg/day) prepared freshly in 0.01 M citrate buffer (pH 4.5). Control group received an equivalent amount of the citrate buffer and served as non-diabetic controls. After the elapse of 2-week period groups, 2-5 were declared diabetic with hyperglycemia (blood glucose level >250-300 mg/dl).

EXPERIMENTAL DESIGN

All the selected animals were weight before the experiment and divided into 5 groups (n=6). Body weight of all the groups was measured in the 7-day interval and on the day of animal sacrifice. At the completion of the experiment (on 28th day), overnight fasted rats were anaesthetized and blood was withdrawn through heart puncture and collected in tubes. Blood samples were further used to measure antioxidant activity, lipid profiles (serum cholesterol, triglyceride, HDL and LDL), SGPT- SGOT, and serum creatinine employing an auto-analyzer.

Group I - control were fed orally with vehicle (1 ml/kg Body Weight)

Group II - diabetic were fed orally with vehicle (1 ml/kg Body Weight)

Group III - fed with glibenclamide orally (600 µg/kg Body Weight)

Group IV & V - fed with PH orally (200 and 400 mg/kg Body Weight respectively)

BIOCHEMICAL ANALYSIS

Estimation of serum lipid markers

The serum lipid markers, i.e. total cholesterol, triglyceride, HDL and VLDL levels were calculated using precipitation and enzymatic procedures from ERBA Diagnostics (Allain *et al.*, 1974, Fossati *et al.*, 1982). LDL-cholesterol level was calculated using the Friedwald equation.

Determination of aspartate transaminase activity (AST or SGOT)

Henry *et al.*, 1960 calculated the serum transaminase activity in rats by estimating the concentration of oxaloacetate hydrazone formed due to reaction with 2-4 dinitrophenylhydrazine. The resultant coloured solution was measured spectrophotometrically against a reagent blank at 546nm.

Determination of alanine transaminase (ALT or SGPT)

Henry *et al.*, 1960 estimated Alanine transaminase based on the reaction between α -ketoglutarate and L-alanine to produce L-glutamate and pyruvate. Enzyme activity was estimated by measuring the concentration of pyruvate hydrazone formed with 2,4-dinitrophenylhydrazine employing a UV spectrophotometer at 546 nm.

Determination of Serum creatinine level

To determine the concentration of serum creatinine, alkaline picrate method was employed using a diagnostic reagent kit (Henry *et al.*, 1974).

ANTIOXIDANT PARAMETERS

Estimation of GSH- Reduced glutathione

In an ice bath liver sample, 200 mg was homogenized using 0.02M of EDTA (8.0 mL) and maintained in the ice bath until further use. 5.0 mL sample of the homogenates were added to a mixture of distilled water (4.0 mL) and 50% trichloroacetic acid (1.0 mL). For 15 min the tubes were centrifuged at 3000rpm (4°C). 2.0 mL of supernatant was withdrawn and mixed with 0.4M Tris buffer pH 8.9 (4.0mL) and (0.1mL) [5,5-dithiobis-(2-nitrobenzoic acid)] (DTNB) and the sample was shaken. The absorbance was recorded at 412 nm within 5 min of the addition of DTNB against a reagent blank. Results for GSH were expressed as µg/mg protein (Ellman 1959).

Estimation of Superoxide Dismutase (SOD)

The reaction mix is composed of 0.1mM EDTA, 50 mM sodium carbonate and 96 mM of nitro blue tetrazolium (NBT). In a cuvette containing 0.05ml of the supernatant, 2 ml of mixture mentioned above and 0.05 ml of hydroxylamine was added. Due to auto-oxidation of hydroxylamine absorbance was taken at 560 nm for 2mins at 30s interval using the spectrophotometer and expressed in U/mg protein (Kono 1978).

Protein determination

In a test tube, 0.9mL of isotonic sodium chloride solution was added to 0.1 mL of plasma sample. Add 0.9 mL of triple distilled water and 10% trichloroacetic acid (1.5 mL) to 0.1 mL of the above diluted sample and kept cold for 4 hours. Using 0.1 N NaOH (1.0 mL) results in protein precipitation which was collected by centrifugation. In 0.1 mL of aliquot, 5 mL alkaline copper sulfate (mixture of 1.0% copper sulphate (1 mL) + 2% sodium potassium tartrate (1 mL) + 2.0% sodium carbonate (48 mL) in N NaOH) was added and incubated at 37°C for 30 minutes. At the same temperature, further Folin-Calteau reagent (0.5 mL) was added. Bovine serum albumin (standard protein solution) along with a blank was taken to study optical density (blue colour) at 625 nm by a spectrophotometer after 30 minutes (Lowry *et al.*, 1951).

Estimation of lipid peroxides (MDA)

Malondialdehyde (MDA) is a guide of free radical generation/lipid peroxidation determined. Add a mixture of 8.1% sodium lauryl sulphate (0.2 ml), 0.8% aqueous solution of thiobarbituric acid (1.5 ml) and 20% acetic acid (1.5 ml) of pH 3.5 to 0.2 ml of blood plasma. The concentration of the solution was made up to 4.0 ml with distilled water, heated to 95°C for 60 mins and then cooled under running

tap water. To this, 15:1 v/v of n-butanol: pyridine (5.0 ml) and distilled water (1.0 ml) was added. For 10 min the mixture was centrifuged at 3000 rpm which separates out the organic layer, absorbance was taken at 532 nm using double beam UV-Visible spectrophotometer against a blank. MDA values are expressed in nmol/mg protein (Ohkawa *et al.*, 1979).

Histopathological Study of Liver and Kidney

Liver and kidney of diabetic and non-diabetic rats were dissected and kept in 10% formalin. The sample was sent for histopathological studies to report any organ toxicity.

Statistical Analysis

The experimental results were expressed as Mean \pm S.E.M. Statistical analysis for all the groups was performed by student t-test (unpaired) and one-way analysis of variance (ANOVA) followed by Tukey test. The values were tested for significance at a P < 0.01 and 0.001.

RESULTS

Preliminary qualitative phytochemical screening

Preliminary phytochemical screening of the Polyherbal formulation showed the presence of various chemical constituents like flavonoids, phenols, terpenoids, triterpenes, tannins and Saponins (Table 2).

Serum Lipid Profile

To investigate the antihyperlipidemic activity of PH formulation on lipid profile following studies were performed. The concentration of total cholesterol, triglyceride, low-density cholesterol (LDL)

Table 2: Phytochemical Screening of Individual Extract

Constituent	Ethanollic Extracts							
	AV	CS	CD	MS	PA	PGS	PGF	TC
Alkaloids	-	+	+	-	+	+	-	+
Carbohydrates	+	+	+	-	+	+	-	+
Flavonoids	+	+	+	+	+	-	+	+
Glycosides	(a)+	(c)+	+	-	(c)+	(c)+	(c)-	+
Phenol	-	+	-	-	+	+	+	+
Proteins	-	-	+	-	-	-	-	+
Saponin	+	+	-	+	+	+	+	+
Steroids	-	+	+	-	+	+	-	+
Tannins	-	+	-	+	+	+	+	-
Fat & Fixed oil	-	-	-	-	-	-	-	-
Catechins	-	+	-	-	-	-	-	+
Terpenoids	-	-	-	+	+	+	-	+
Mucilage	-	-	-	+	-	-	-	-

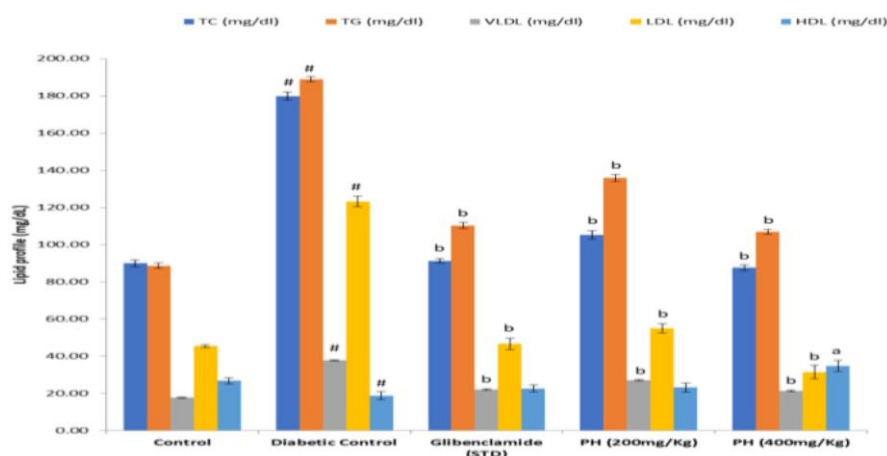
(a): Anthraquinone Glycosides (c): Cardiac Glycosides + Present - Absent

Aloe vera (AV), *Camellia sinensis* (CS), *Capparis decidua* (CD), *Musa sapientum* (MS), *Phyllanthus amarus* (PA), *Punica granatum* Seed (PGS), *Punica granatum* Flower (PGF) and *Tinospora cordifolia* (TC)

Table 3: Effect of Polyherbal Formulation on Lipid Profile of Diabetic Rats

Groups	Total Cholesterol (mg/dL)	Triglycerides (mg/dL)	VLDL (mg/dL)	LDL (mg/dL)	HDL (mg/dL)
Control	89.91± 1.83	88.68 ± 1.49	17.74 ± 0.30	45.40 ± 0.70	26.78 ± 1.73
Diabetic Control	179.83± 2.15 [#]	188.93±1.41 [#]	37.79±0.28 [#]	123.19±2.80 [#]	18.85±2.11 [#]
Glibenclamide (STD)	91.29± 1.22 ^b	110.34±1.71 ^b	22.07±0.34 ^b	46.55 ± 3.08 ^b	22.68 ± 1.96
PH (200mg/kg)	105.34± 2.27 ^b	135.85±2.00 ^b	27.17±0.40 ^b	54.95 ± 2.55 ^b	23.22 ± 2.37
PH (400mg/kg)	87.59± 1.57 ^b	106.83±1.41 ^b	21.38±0.28 ^b	31.51 ± 3.64 ^b	34.70±3.04 ^a

Values are expressed in Mean ± SEM (n = 6 in each group); [#] P < 0.01; when Diabetic control compared with normal control (Unpaired t-test); ^aP < 0.01; when other groups compared with Diabetic control (One Way ANOVA, followed by Tukey's test); ^bP < 0.001 when other groups compared with Diabetic control (One Way ANOVA, followed by Tukey's test).

**Figure 1: Effect of Polyherbal Formulation on Lipid Profile of Diabetic Rats**

Values are expressed in Mean ± SEM (n = 6 in each group); [#] P < 0.01; when Diabetic control compared with normal control (Unpaired t-test); ^aP < 0.01; when other groups compared with Diabetic control (One Way ANOVA, followed by Tukey's test); ^bP < 0.001 when other groups compared with Diabetic control (One Way ANOVA, followed by Tukey's test).

and very low-density cholesterol (VLDL) increased whereas the concentration of high-density cholesterol (HDL) decreased in the STZ (streptozotocin) induced diabetic group. Administration of PH (200 & 400 mg/kg) showed a significant reduction (P < 0.001) invariant lipid profile as compared to the diabetic control group whereas HDL level increased when compared to diabetic control in a significant manner (P < 0.01). PH at doses of 400 mg/kg was more efficacious than 200 mg/kg in decreasing the concentration of total cholesterol, triglyceride, LDL and VLDL & increasing the concentration of HDL cholesterol compared to the diabetic control group (Table 3 & Figure 1). Body weight of all the groups was also measured (Figure 7).

Hepatic Enzymes

A significant increase in SGOT ($\cong 3.25$ fold) and SGPT ($\cong 2.85$ fold) activity was found (p < 0.01) in diabetic group when compared to control group. Treatment with PH (200 & 400 mg/kg) resulted in the recovery of the levels of these two parameters

towards the control. When compared to the diabetic control a significant (p < 0.001) difference was noted in hepatic enzymes concentration between glibenclamide, PH (200 mg/kg) and PH (400 mg/kg) treated groups (Table 4 & Figure 2).

Serum Creatinine

The serum creatinine level ($\cong 2.15$ fold) in diabetic group was significantly increased (p < 0.01) as compared to non-diabetics group. Creatinine level decreased significantly (p < 0.001) in PH 400 mg/kg, followed by glibenclamide and PH 200 mg/kg treated groups (Table 4 & Figure 3).

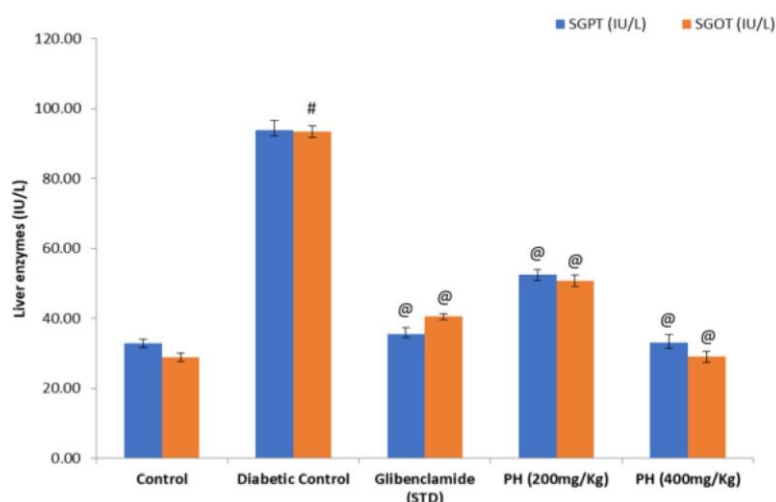
Antioxidant Enzymes - Lipid peroxidation, Reduced glutathione and Superoxide dismutase

The level of SOD and reduced glutathione significantly decreased (P < 0.01) in diabetic control whereas the level of MDA increased significantly (P < 0.01). Glibenclamide and PH (200 & 400 mg/kg) receiving groups have shown significant increase (p < 0.01) in SOD, reduced glutathione level and significant decrease (p < 0.001) in MDA level. The

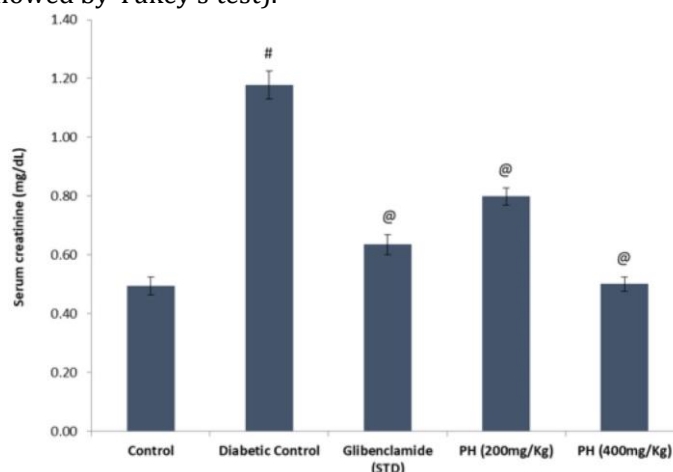
Table 4: Effect of Polyherbal Formulation on Hepatic Enzymes and Serum Creatinine of Diabetic Rats

Groups	SGPT (IU/L)	SGOT (IU/L)	Creatinine (mg/dL)
Control	32.85 ± 1.23	28.88 ± 1.24	0.49 ± 0.03
Diabetic Control	93.93 ± 2.61#	93.50 ± 1.64#	1.18 ± 0.04#
Glibenclamide (STD)	35.51 ± 1.95@	40.50 ± 0.97@	0.64 ± 0.03@
PH (200mg/kg)	52.59 ± 1.42@	50.69 ± 1.65@	0.80 ± 0.03@
PH (400mg/kg)	33.00 ± 2.47@	29.08 ± 1.56@	0.50 ± 0.02@

Values are expressed in Mean ± SEM (n = 6 in each group); # P < 0.01; when Diabetic control compared with normal control (Unpaired t-test); @ P < 0.001 when other groups compared with Diabetic control (One Way ANOVA, followed by Tukey's test).

**Figure 2: Effect of Polyherbal Formulation on Liver Enzymes of Diabetic Rats**

Values are expressed in Mean ± SEM (n = 6 in each group); # P < 0.01; when Diabetic control compared with normal control (Unpaired t-test); @ P < 0.001 when other groups compared with Diabetic control (One Way ANOVA, followed by Tukey's test).

**Figure 3: Effect of Polyherbal Formulation on Serum Creatinine of Diabetic Rats**

Values are expressed in Mean ± SEM (n = 6 in each group); # P < 0.01; when Diabetic control compared with normal control (Unpaired t-test); @ P < 0.001 when other groups compared with Diabetic control (One Way ANOVA, followed by Tukey's test).

net result suggests that significantly higher amount of SOD, reduced glutathione and lower amount of MDA in PH (400 mg/kg) is better than PH (200 mg/kg) dose in the diabetic group (Table 5 & Figure 4).

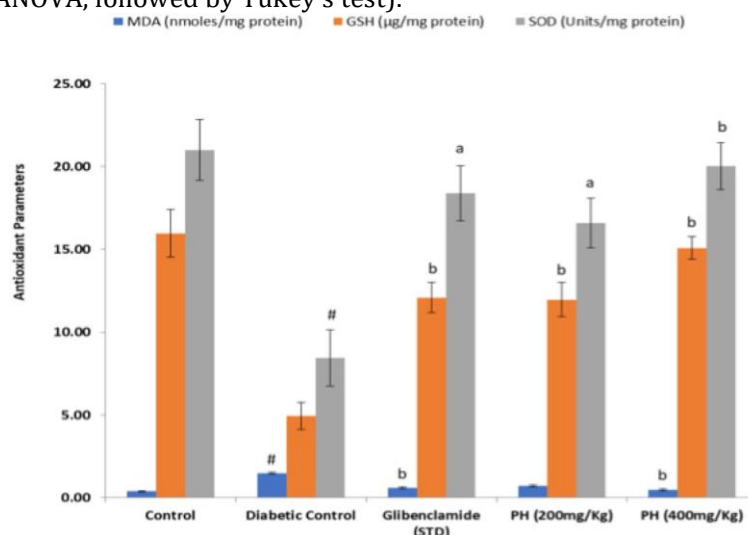
Liver histopathology

The histopathology of the liver in diabetic group displayed an increase in connective tissue component; blood vessels are remarkably dilated and showed degeneration, necrosis, and a macro droplet of fat captures congestion along with the build-up of fat as a substantial quarter of hepatocytes. Treatment with PH (200 & 400 mg/kg) has improved the histological condition. The results for

Table 5: Effect of Polyherbal Formulation on Antioxidant Parameters of Diabetic Rats

Groups	MDA (nM/mg Protein)	GSH ($\mu\text{g}/\text{mg}$ Protein)	SOD (U/mg Protein)
Control	0.38 \pm 0.036	15.97 \pm 1.439	21.00 \pm 1.84
Diabetic Control	1.45 \pm 0.061 [#]	4.94 \pm 0.821 [#]	8.46 \pm 1.68 [#]
Glibenclamide (STD)	0.60 \pm 0.062 ^b	12.10 \pm 0.930 ^b	18.39 \pm 1.67 ^a
PH (200mg/kg)	0.71 \pm 0.061 ^b	11.97 \pm 1.043 ^b	16.60 \pm 1.49 ^a
PH (400mg/kg)	0.47 \pm 0.045 ^b	15.09 \pm 0.685 ^b	20.03 \pm 1.42 ^b

Values are expressed in Mean \pm SEM (n = 6 in each group); [#] P < 0.01; when Diabetic control compared with normal control (Unpaired t-test); ^aP < 0.01; when other groups compared with Diabetic control (One Way ANOVA, followed by Tukey's test); ^bP < 0.001 when other groups compared with Diabetic control (One Way ANOVA, followed by Tukey's test).

**Figure 4: Effect of Polyherbal Formulation on Antioxidant Parameters of Diabetic Rats**

Values are expressed in Mean \pm SEM (n = 6 in each group); [#] P < 0.01; when Diabetic control compared with normal control (Unpaired t-test); ^aP < 0.01; when other groups compared with Diabetic control (One Way ANOVA, followed by Tukey's test); ^bP < 0.001 when other groups compared with Diabetic control (One Way ANOVA, followed by Tukey's test).

PH (200 mg/kg) has been quite similar to that of glibenclamide. The 28-day treatment period with PH (400 mg/kg) dose has shown a marked decrease in the microdroplet build-up, as compared to the diabetic control group. Kidney histopathology of the streptozotocin-treated group revealed cellular infiltration, expanded bowman's space, atrophied Glomerulus, cloudy swelling of tubules and at last tubular necrosis. Amelioration of histological condition was observed when treated with glibenclamide and PH (200 & 400 mg/kg). The treatment with PH (400 mg/kg) dose has shown a marked decrease in anomalies followed by glibenclamide and PH (200 mg/kg) (Figure 5 & 6).

DISCUSSION

The present script considers the antioxidant, anti-hyperlipidemic effect along with serum creatinine level and hepatic enzyme markers of ethanolic extract of PH II (200 & 400 mg/kg) on normal and streptozotocin (STZ) induced rats. STZ is a cytotoxic compound (nitrosourea) obtained from *Streptomyces achromogenes*. This soil microbe produces reactive oxygen species (ROS). That enters the pancreatic β -cells of Langerhans via glucose

transporter (GLUT) and prompts breakdown of DNA strands, thereby decrease endogenous insulin release (Irudayaraj SS *et al.*, 2012). The polyherbal formulation consists of a huge variety of extracts that enumerate a crowd of phytochemical substances showing the efficacious result of the study.

Insulin poses a prime role in the metabolism of carbohydrates and lipids (a potent inhibitor of lipolysis). It also decreases liberation of free fatty acids and inhibits lipase activity in adipose tissue (Loci *et al.*, 1994). Diabetes increases fatty acids concentration and produces more of acetyl CoA which in turn commences β -oxidation of fatty acids resulting in lipid peroxidation (Oberley, 1988) and cholesterol production followed by generation of ROS. Streptozotocin-induced diabetic group of rats show the escalating amount of total cholesterol and triglyceride which are primary factors involved in the CHD and atherosclerosis, the secondary impediments occurring in diabetes (Ananthan R *et al.*, 2003). Experimental groups treated with standard drug and variant doses of extract brought the lipid profile back to normal level. This could be the result of increased level of insulin secretion, thereby inhibiting lipase activity, increase in the

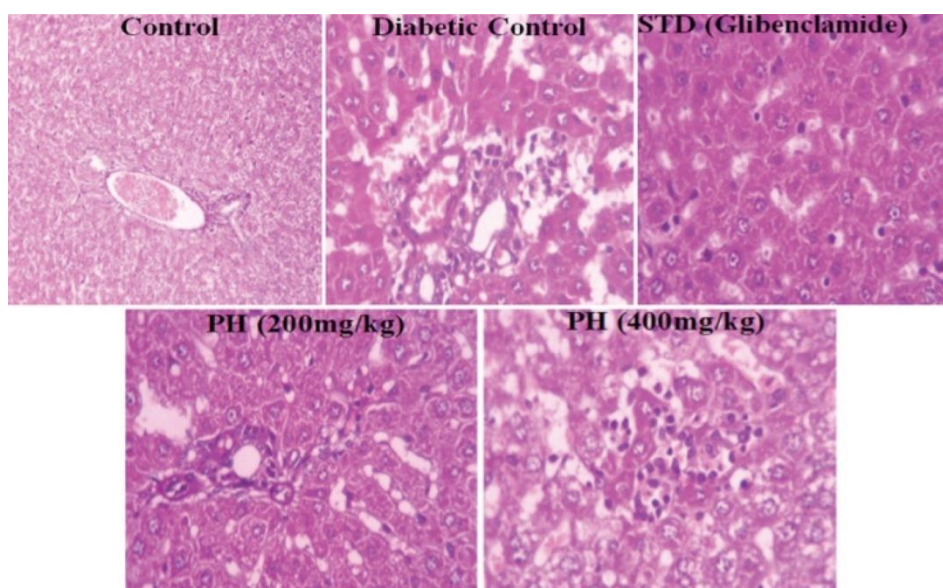


Figure 5: Histopathological studies of liver

(a) Control (b) Diabetic Control (c) Standard (STD-glibenclamide) (d) PH (400mg/kg) (e) PH (200 mg/kg)

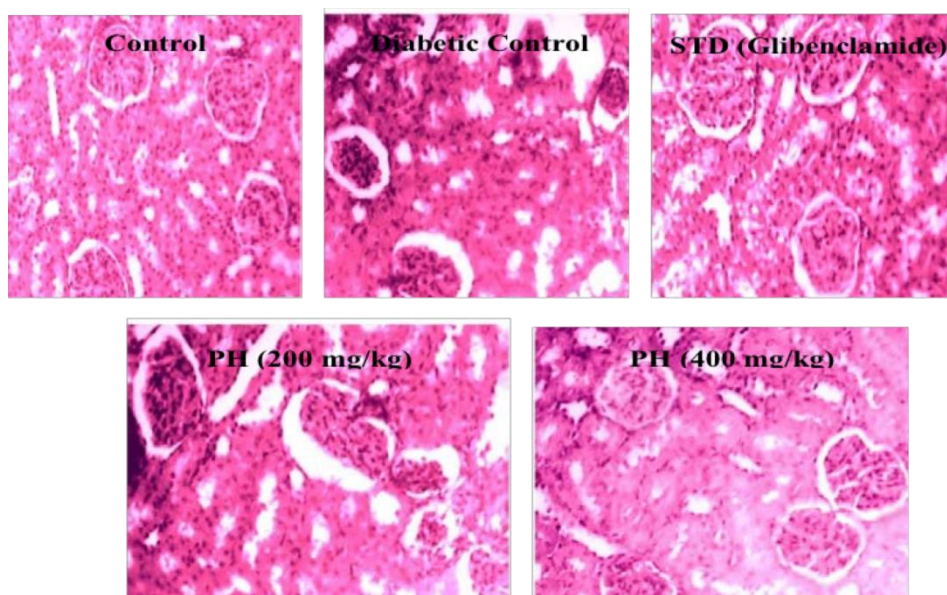


Figure 6: Histopathological studies of Kidney

(a) Control (b) Diabetic Control (c) Standard (STD-glibenclamide) (d) PH (200mg/kg) (e) PH (400 mg/kg)

utilization of glucose and decrease in free fatty acids mobilization from the fat depositions. STZ induced groups exhibited an alarming level of TC and TG along with LDL, VLDL and a decreased level of HDL. The treatment group showed a significant reduction in serum LDL, VLDL and increased the level of HDL. The outcome proposes that variant doses of the formulation significantly improved the lipid abnormalities.

Increased peroxidation of lipids impairs membrane fluidity and change in membrane linked enzyme activity. Further, the products formed like lipid radicals and peroxides, are detrimental to the cells causing atherosclerosis and brain damage

(Baynes 1995). Polyherbal formulation inhibits oxidative damage and reduces the lipid peroxidation markers of diabetic rats (due to the presence of anti-oxidative ingredients). Glutathione is a free radical scavenger and co-substrate for numerous intracellular enzymes playing a role in the degradation of hydrogen peroxide which undergoes oxidation utilising reduced form (GSH) to oxidized state (GSSG) (Meister A *et al.*, 1983). The GSH:GSSG ratio reveals the cellular redox balance system wherein decreasing GSH level is indicated in DM. In diabetes, a significant reduction in GSH level and GSH/GSSG ratio indicate debilitated glutathione defence system. In diabetic rats increasing

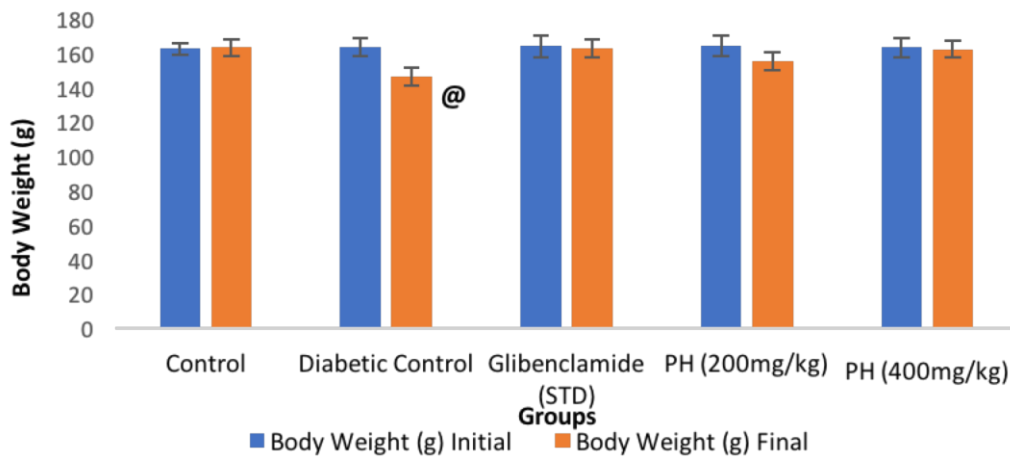


Figure 7: Effect of Polyherbal Formulation on the Body weight of Streptozotocin-Induced Diabetic Rats

Values are expressed in Mean \pm SEM (n = 6 in each group); @P < 0.01; when all groups compared with their respective initial body weight (Paired t-test).

level of MDA might also contribute to impaired glutathione concentration (Maritim AC *et al.*, 2003). Our results implicated that, the polyherbal formulation has increased GSH; suggesting its beneficial effects.

In the mitochondria, the surplus level of glucose alternates the electron transport chain; thus, forming a glut of superoxide anions (Wiernsperger NF *et al.*, 2003). Elevated blood glucose may increase the production of superoxide radical in diabetic control rats leading to increased SOD activity. As SOD converts superoxide anions to hydrogen peroxide (H_2O_2), increased SOD activity increases H_2O_2 turnover (Kono Y *et al.*, 1982). The different dose of polyherbal formulation significantly improved endogenous antioxidant SOD level and prevents membrane damage by decreasing lipid peroxidation when compared to diabetic control.

Many herbs in the formulation have already been reported as hepatoprotective. The raised levels of liver enzymes were brought down significantly by the polyherbal formulation. Hence, improvement in liver function, increase in glucose uptake and its utilization subsequently may be the viable mechanism for this consideration (Vetrichelvan T *et al.*, 2002). Generally, kidney damage is correlated with an increase in plasma serum creatinine level (Bartels A *et al.*, 1971) observed in diabetes-induced animals which decreased after administration of the Polyherbal formulation. This could be due to its antihyperglycemic activity by increasing the uptake and utilization of glucose by the tissues.

The liver histopathological study disclosed that STZ-induced group have acutely deteriorated liver with cytotoxic injury showing necrosis and fibrotic changes. Comparatively, glibenclamide and polyherbal formulation cure seeming abnormalities as

antidotes with minimal sign of hepatotoxicity. Histopathology study of kidney revealed that normal control group and standard treated group have normal glomeruli and tubules as compared to streptozotocin treated group (showing necrosis in kidney). Polyherbal low dose and high dose treated group showed almost return of normal appearance of glomeruli and tubules.

According to microscopic examinations, pathological alterations elicited by streptozotocin were notably reduced by dispensing glibenclamide and polyherbal formulation. Though glibenclamide showed good efficacy for different biochemical parameters results for all the parameters concluded that polyherbal formulation was most effective. No toxicity was observed in 28 days study period, regarding hepato and nephrotoxicity. So, the polyherbal formulation may be a flawless alternative for the prevailing hypoglycemic drugs with an auxiliary advantage of hypolipidemic effect thereby diminishing cardiovascular risk factors analogous to DM. Thus, herbal formulation requires future allowance for a clinical trial to be fruitful in treating diabetes mellitus and making its existence in the antidiabetic drug business.

CONCLUSION

Diabetic Mellitus treatment is life-long therefore curative agents devoid of side effects are treasured and one such approach is the use of a complementary and alternative system of medication comprising herbal products. The study provides evidence for the effectiveness of several plant extracts in controlling blood glucose level as a combined formulation in comparison with individual plant extract. Since the extracts produced hypolipidemic

and antioxidant action, the formulation may be expected to be better in controlling manifestations of chronic diabetes.

Our findings explain that polyherbal formulation in variant doses have antihyperlipidemic and antioxidant activity, which is manifested by decreasing total cholesterol, triglyceride, LDL, VLDL, SOD, reduced glutathione and increasing HDL and MDA level. The data propose that the combined extract showed a consistent and significant antioxidant, hypolipidemic activity along with ameliorated hepatic and renal function, in the diabetic group. Polyherbal formulation (PH-II) 400mg/kg; followed by glibenclamide and PH- II (200 mg/kg) has manifested the most effective improvements the diabetic complications.

The formulation has shown a dose-dependent reversal of high lipid levels observed in the diabetic rat; accompanied by decreased oxidative stress in both diabetic and normal rat. A need for future studies in human subjects is required to determine if the above results can be appropriately extrapolated to human beings. It was a determined attempt to use herbal therapy along with allopathic drugs to minimize the risk factors accompanying diabetes mellitus and the side effects of the allopathic system in long-term use. It provides us with a new ray of hope for new generation oral hypoglycemic drugs and may have beneficial effects in type II diabetes mellitus.

Acknowledgements

Authors are thankful to the management of M.I.E.T, Meerut and I.T.S College of Pharmacy, Muradnagar, Ghaziabad for providing all the necessary facilities and support for successful completion of the research protocol.

Conflict of interest statement

The authors report no conflict of interest.

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