RESEARCH ARTICLE

Hypoglycemic and antihyperglycemic activity of polyherbal formulation in normoglycemic and Streptozotocin-induced diabetic rats

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Abstract

Aim: This study was carried out to investigate the hypoglycemic effects and antihyperglycemic activity of polyherbal (PH) formulation in normal and glucose-loaded and streptozotocin (STZ)-induced diabetic rats. **Materials and Methods**: PH formulation was tested for hypoglycemic activity for 4 h in normoglycemic rats, oral glucose tolerance test (OGTT) (2 h), and antihyperglycemic activity (28 days) in non-diabetic and STZ-induced diabetic rats.**Results and Discussion:** PH formulation at 200 and 400 mg/kg doses significantly ($P < 0.01$) reduced fasting glucose level in normal rats and similar to standard drug glibenclamide. The blood glucose reduction was greatest 32.56% for glibenclamide followed by 32.37% for PH (400 mg/kg) and 26.61% for PH (200 mg/kg), respectively. For OGTT, a significant ($P < 0.001$) plasma glucose lowering effects of PH (400 mg/kg) followed by PH (200 mg/kg) treated group was observed at all time intervals. Blood glucose lowering was more pronounced for diabetic rat given PH (400 mg/kg) followed by PH (200 mg/kg). Blood glucose levels were significantly lower ($P < 0.001$) in diabetic rat (STZ), treated with glibenclamide, and the two dose levels of PH up to 4 weeks. At 28 days, blood glucose reduction was greatest 67.48% for PH (400 mg/kg) followed by 62.27% for glibenclamide and 59.26% for PH (200 mg/kg). When compared to non-diabetics, liver glycogen levels in the untreated diabetic group were significantly lower ($P < 0.01$). Liver glycogen levels increased 2.3-fold in the glibenclamide-treated rats. Similarly, glycogen levels were increased significantly ($P < 0.01$) by 1.9- and 2.3-fold in PH (200 and 400 mg/kg)-treated groups, respectively. **Conclusion:** The treatment with PH (400 mg/kg) dose has shown a marked improvement in histological condition, as compared to diabetic control. These findings suggest that this PH formulation may be a potential source for the development of new antidiabetic drug.

Key words: Hypoglycemic, polyherbal, streptozotocin

INTRODUCTION

Diabetes mellitus (DM) is a persistent metabolic disorder of macronutrients such as fat, protein, and carbohydrate due to either inadequacy of insulin secretion or due to varying degree of insulin resistance.[1,2] Hyperglycemia and hypercholesterolemia are the characteristic features of DM.[3,4] Type 2 diabetes mellitus (T2DM) is a paramount money consuming and troublesome chronic diseases and is increasing in epidemic proportions all through the world.[5] Chronic hyperglycemia often leads to microvascular complications that include nephropathy, retinopathy, and neuropathy along with macrovascular complexities that lead to significant morbidity and mortality in T2DM patients.[6-10] Moreover, some present-day studies propose that free radicals, i.e. reactive oxygen species (ROS) are also involved in the initiation and development of vascular complications in DM.[11]

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A variety of antidiabetic drugs are available in the market, but they often produce undesirable side effects after prolonged consumption.[12,13] Consequently, there is a censorious need for new curative agents that prey the underlying pathogenic mechanisms and also can arrest or reverse the development of T2DM. Commonly practiced pharmacologic therapy includes antihyperglycemics, oral hypoglycemics, and insulin. There is a worldwide demand by patients for the products from natural origin and another dietary alleviator with antidiabetic activity. This tendency is due to unavailability of oral insulin, along with untoward effects of its repeated injections. Furthermore, oral hypoglycemics are not so fruitful in lowering the blood glucose in chronic T2DM patients.[14]

Plants are the richest source of emerging new drugs in market and play a cue role in complementary and alternative medicines.[15] Herbal drugs have been used quite effectively in treating T2DM all over the world and customarily considered as free from side effects and less toxic when compared to the available synthetic ones.[16,17] India the botanical garden of the world is the cosmic producer of therapeutic herbal agents.[18,19] In India, near about 80% of the countryside population utilize herbs, i.e., aboriginal systems of medicine.[20] Ethnopharmacological surveys showed more than 1200 herbs which are used for their so-called hypoglycemic activity as a traditional medicine.[21] The antidiabetic activity of the chemical constituents such as alkaloids, amino acids, alkyl disulfides, coumarins, glycopeptides, flavonoids, iridoids, inorganic ions, guanidines, lipids, phenolics, polysaccharides, peptides, steroids, triterpenoids, and xanthones has been reported.[22,23]

The current exploration was designed to assess the probable beneficial effects of these natural herbs in combination on blood glucose level of streptozotocin (STZ)-induced diabetic rats, which are commonly consumed along with the diet. All the herbs used in the study individually possess potent hypoglycemic or antihyperglycemic or antioxidant activity.

MATERIALS AND METHODS

Chemicals and Reagents

STZ was procured from HiMedia Laboratories, Mumbai, India. Glibenclamide was purchased from Sigma-Aldrich (St. Louis, MO). Diagnostic kit for blood glucose estimation was purchased from TRANSASIA Bio-Medicals Ltd., Himachal Pradesh (ERBA Diagnostics). All the chemicals and reagents used in the current study were of analytical grade.

Plant Material

All the medicinal plants were recognized and collected from various regional places of India in August and September. Further, they are authenticated by the National Herbarium of Cultivated Plants, Pusa Campus, New Delhi, and Department of Botany and Chaudhary Charan Singh University, Meerut. The voucher specimen of the respective plant material was deposited in the departments.

Preparation of Herbal Extract

The individual drugs were collected and dried in shade at room temperature of about 37°C. The crude drugs were then subjected to coarse powder by crushing or grinding. Further, in accordance with the quantity required, each drug was weighed on digital balance and subjected to continuous hot Soxhlet extraction using 95% ethanol (60–80°C) for 48 h (before extraction of fruits, seed, and stem, defatting was done with petroleum ether at 60–80°C). At the end using rotary flash evaporator under vacuum, the extract was concentrated to a semi-solid mass with the recovery of solvent. The traces of the solvents were separated by using lyophilizer. The resultant extract was stored at 4°C for future use.

Preparation of PH Formulation

The PH formulation was prepared by mixing all the extracts i.e., *Aloe vera, Camellia sinensis*, *Capparis deciduas*, *Musa sapientum, Phyllanthus amarus, Punica granatum* (flower and seed), and *Tinospora cordifolia* in equal concentration [Table 1].

EXPERIMENTAL ANIMAL MODEL

Adult albino rats (Wistar strain) weighing 150–200 g and albino mice (Swiss strain) weighing 20–30 g of either sex were procured from the animal house of M.I.E.T, Meerut, UP (India). Animals were housed under standard conditions of 24 ± 3°C temperature, 65 ± 10% RH, and 12 h light and dark cycle. They are fed with standard pellets and drinking water *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethics Committee of M.I.E.T, Meerut, and the experiments were conducted in compliance with

Table 1: Composition of PH Formulation

Plants	Parts	Extract Quantity (mg)
Aloe vera	Leaf pulp	100
Camellia sinensis	Leaves	100
Capparis decidua	Fruits	100
Musa sapientum	Flower	100
Phyllanthus amarus	Entire plant	100
Punica granatum	Flower	100
	Seed	100
Tinospora cordifolia	Stem	100

PH: Polyherbal

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 (711/02/a/CPCSEA/2013).

Study on Safety of the Formulation

According to the OECD guideline 423, acute toxicity study[24] was performed wherein randomly adult albino Swiss mice 20–30 g were allocated into four groups containing three animals in every group. Overnight fasted animals were administered orally with the PH formulation at various dose levels (5, 50, 300, and 2000 mg/kg body weight) dissolved in 1% carboxymethylcellulose (CMC). One group was maintained as control and administered the vehicle only. Then, the animals were observed individually at least once during first 30 min, periodically during first 24 h (special attention for first 4 h), and daily thereafter for a total period of 14 days. Furthermore, the behavior of animals and any other toxic symptoms were observed and recorded systematically (individual records being maintained for each animal).

Experimental Induction of Diabetes in Rats

Adult albino rats (Wistar strain) weighing 150–200 g were selected for the study. Blood sample were collected for the determination of baseline glucose levels. DM was induced in overnight fasted animals. For 3 consecutive days, each animal was injected intraperitoneally with 60 mg/kg/day of STZ freshly prepared in 0.01 M citrate buffer pH 4.5. Animals in control group received equivalent volume of the citrate buffer and are served as non-diabetic controls. After the elapse of 2-week period, animals in groups 2–5 were declared diabetic with hyperglycemia (blood glucose level >250–300 mg/dl).

Experimental Design

Before the experiment, the selected animals were weighed and divided into five groups (n = 6). The complete duration of experiment was 28 days. Body weight and fasting blood glucose level were measured by GOD-POD method at every 7-day intervals and on the day of animal sacrifice in all the groups. At completion of experiment (on the 28th day), rats were fasted overnight, anesthetized, and blood was withdrawn through the retro-orbital plexus using a glass capillary and collected in EDTA-coated tubes. Food and water intake and body weight gain were measured for all the groups.

Group I - Control was fed orally with vehicle (1 ml/kg BW).
Group II - Diabetic was fed orally with vehicle (1 ml/kg BW).
Group III - Fed with glibenclamide orally (600 µg/kg BW).
Groups IV and V - Fed with PH orally (200 and 400 mg/kg BW, respectively).

Oral Glucose Tolerance Test (OGTT)

After overnight fasting, 0-min blood sample was taken from different groups of rats, namely, normal, diabetic control, diabetic + PH extract 200 and 400 mg/kg, and diabetic + glibenclamide 600 µg/kg by the retro-orbital sinus puncture,[25] and without any set back, a glucose solution (2 g/ml per kg) was dispensed by gavage. Four more blood samples were withdrawn at 30, 60, 90, and 120 min after glucose administration.[26] All the samples were collected for the estimation of glucose using commercially available kits.

Hypoglycemic Activity

Overnight fasted adult Wistar rats (water was allowed ad libitum) were segregated into four different groups containing 6 rats each. PH extract was administered orally in dose of 200 and 400 mg/kg body weight. Glibenclamide (600 µg/kg) was administered to one group, and 1 ml/kg body weight of 0.5% sodium CMC was administered to the control group. Commercially available kits were used to measure blood glucose level by glucose oxidase-peroxidase method at different time intervals, namely, 0, 30, 60, 120, and 240 min by retro-orbital plexus puncture.

Antihyperglycemic Activity

At the end of the 28th day, the rats were fasted for 16 h and the blood biochemical parameters were determined as follows: Blood was collected by retro-orbital plexus puncture and the serum was separated by centrifugation. Serum was further used to measure blood glucose level.

Determination of Blood Glucose Level: [27]

For determination of blood glucose level, 20 µL of serum was added to a mixture of distilled water (0.2 mL) and working glucose reagent (2 mL). The reaction mix was incubated at 37 ± 2°C for 10 min along with tubes containing the reagent blank and standard glucose 20 µl. Absorbance was measured at 505 nm against the reagent blank. The composition of the working glucose reagent contains (after reconstitution) a buffer (pH 7.0 ± 0.2 at 25°C), 0.52 mmol/L 4-aminoantipyrine, 10 mmol/L 4-hydroxybenzoic acid, 20,000 IU/L glucose oxidase, and 3250 IU/L peroxidase.

Liver Glycogen Estimation:[28]

First, liver samples (200 mg) were weighed and finely ground with 5% TCA (20 ml) in a homogenizer. The protein precipitate formed was filtered and utilized for liver glycogen analysis.

In a test tube, 2 ml of liver extract and 2 ml of 10N KOH were added and placed in boiling water for 1 h. After cooling, glacial

acetic acid (1 ml) was added to neutralize the excess of alkali and the mixture was made up to the mark (20 mL) with water. Further, 2 ml of above mixture was added slowly to 4 ml of anthrone reagent in a test tube, which was placed in cold water (prevents excessive heating). Mixing was done by lateral shaking, and then, the tube was placed in boiling water for exactly 10 min for color development and cooled with tap water. Within 2 h, the optical density was read at 650nm against a blank (prepared by subjecting 2 ml of 5% TCA to the same procedure).

Histopathological Study of Liver

The sample (liver) of treated rat and that of a normal healthy rat was dissected out and kept in 10% formalin and sent for histopathological study to report for organ toxicity.

Statistical Analysis

The results were expressed as mean±standard error of the mean. Statistical analysis was performed by student *t*-test (unpaired) and one-way analysis of variance (ANOVA) followed by Tukey's test. The values were tested for significance at a $P < 0.01$.

RESULTS

Toxicity Study

Acute toxicity study of the PH formulation was non-toxic in nature. Divergent doses of PH were not showing any toxic or lethal reaction at any of the dose selected until the end of the experimental period.

Preliminary Qualitative Phytochemical Screening

Preliminary phytochemical screening of the ethanolic extract of PH formulation showed the presence of various chemical constituents such as terpenoids, flavonoids, phenolic compound, tannins, saponins, and triterpenes [Table 2].

Body Weight and Food and Water Intake

The initial and final body weight of normal and diabetic rats is given in Table 3. A significant ($P < 0.01$) decrease in the body weight of diabetic control rats and diabetic + PH (200 mg/kg) was observed. Administration of glibenclamide and PH (400 mg/kg) did not influence any significant ($P < 0.01$) change in body weight in diabetic rats. The food and water intake in the experimental groups is presented in Table 3. Diabetic control rats showed significantly ($P < 0.001$) higher intake of food (35.13%) and water (61.83%) when compared with normal control group. The food intake was significantly ($P < 0.001$) decreased in diabetic rats when treated with glibenclamide and PH (400 mg/kg) (18.27% and 16.23%, respectively), as well the water intake (17.23% and 16.22%, respectively) when compared with diabetic control rats. Food (13.08%) and water (11.95%) intake decreased significantly ($P < 0.01$) in PH (200 mg/kg)-treated group when compared with diabetic control rats.

Blood Glucose Level

OGTT

The results were obtained for the rats administered with oral glucose load (3 g/kg) following various treatments. Compared

Table 2: Phytochemical screening of individual extract

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

(a): Anthraquinone Glycosides, (c): Cardiac Glycosides. +: Present, -: Absent. AV: *Aloe vera,* CS*: Camellia sinensis*, CD *:Capparis decidua,* MS: *Musa sapientum,* PA*: Phyllanthus amarus,* PGS*: Punica granatum* Seed, PGF: *Punica granatum* Flower, TC*: Tinospora cordifolia*

to non-diabetic control group, administration of oral glucose has increased plasma glucose levels significantly ($P < 0.01$) in the diabetic control over a period of 1.5 h. Further, when the diabetic control group was compared to any of the antidiabetic treatments, the plasma glucose level observed was significantly lower at all the time points for latter ($P < 0.001$). The plasma glucose lowering effects of the treatment group were pronounced in the 1st h following the glucose load. Comparing various treatments, decrease in blood glucose level was more marked for diabetic rat given PH (400 mg/kg) followed by PH (200 mg/kg) [Table 4 and Graph 1].

Hypoglycemic Effect

The study unveils hypoglycemic effect of PH in normoglycemic group where fasting blood glucose levels remain unchanged in control group over 4-h study period. Nevertheless, in groups treated with glibenclamide and two dose levels of PH (200 and 400 mg/kg), blood glucose levels were significantly lower. The blood glucose reduction was greatest 32.56% for glibenclamide ($P < 0.001$) followed by 32.37% for PH 400 mg/kg ($P < 0.001$) and 26.61% for PH 200 mg/kg W, respectively. Thus, PH (200 and 400 mg/kg) has significantly shown dose-dependent blood glucose lowering (hypoglycemic) effect similar to glibenclamide [Table 5 and Graph2a and 2b].

Antihyperglycemic Activity

The antihyperglycemic study revealed that fasting blood glucose level was significantly 3-fold more ($P < 0.01$)

Table 3: Effect of PH formulation on the body weight and food and water intake of STZ-induced diabetic rats

Groups	Body weight (g)		Food intake (g/day)	Water intake (mL/day)
	Initial	Final		
Control	162.5±4.54	163.3±3.24^{ns}	50.29±2.84	212.8±8.06
Diabetic control	163.7±5.68	146.2±5.10$^{@}$	67.96±2.28$^{\#}$	344.39±10.28$^{\#}$
Glibenclamide (STD)	164.0±5.16	162.83±6.32^{ns}	55.54±1.95^{b}	285.04±9.21^{b}
PH (200 mg/kg)	164.3±5.02	155.2±5.78$^{@}$	59.07±1.76^{a}	303.21±10.56^{a}
PH (400 mg/kg)	163.2±5.33	162.3±5.61^{ns}	56.93±2.05^{b}	288.50±9.25^{b}

Values are expressed in mean±SEM (n=6 in each group). $^{@}P$<0.01; when all groups compared with their respective initial body weight (paired t-test). $^{\#}P$<0.001; when diabetic control compared with normal control (unpaired t-test). ^{a}P<0.01; when other groups compared with diabetic control (one-way ANOVA, followed by Tukey's test). ^{b}P<0.001; when other groups compared with diabetic control (one-way ANOVA, followed by Tukey's test). PH: Polyherbal, STZ: Streptozotocin, SEM: Standard error of the mean

Table 4: Effect of PH formulation in OGTT

Groups	0 min	30 min	60 min	90 min	120 min
Control	80.52±1.50	165.18±1.92	144.11±1.82	115.92±1.29	81.05±1.05
Diabetic control	231.16±2.12$^{\#}$	304.88±3.81$^{\#}$	355.89±3.84$^{\#}$	322.93±2.20$^{\#}$	291.99±1.50$^{\#}$
Glibenclamide (STD)	89.38±2.25	176.38±3.94^{a}	154.72±4.19^{a}	120.86±3.73^{a}	98.99±2.52^{ns}
PH (200 mg/kg)	86.68±3.44	175.05±2.31^{a}	153.50±3.88^{a}	120.86±1.48^{a}	109.98±2.56^{a}
PH (400 mg/kg)	87.31±3.08	166.19±2.12^{a}	145.12±2.56^{a}	108.86±3.74^{a}	90.76±3.00^{ns}

Values are expressed in mean±SEM (n=6 in each group). $^{\#}P$<0.01; when diabetic control compared with normal control (unpaired t-test). ^{a}P<0.001; when groups compared with their respective "0" min (one-way ANOVA, followed by Tukey's test). SEM: Standard error of the mean. PH: Polyherbal, OGTT: Oral glucose tolerance test

Table 5: Effect of PH formulation on blood glucose level (mg/dL) in normal rats

Groups	0 min	30 min	60 min	120 min	240 min
Control	82.80±4.35	81.26±4.35$^{\#}$	80.84±4.0$^{\#}$	81.21±1.56$^{\#}$	81.32±3.09$^{\#}$
Glibenclamide (STD)	85.08±4.00	69.27±4.00^{a}	54.56±2.63^{b}	50.11±2.62^{b}	57.38±0.80 ^{b}
PH (200 mg/kg)	80.57±3.20	66.51±3.20^{a}	53.40±1.71^{a}	54.51±1.49^{a}	59.13±1.11^{a}
PH (400 mg/kg)	81.16±2.32	61.09±2.32^{b}	45.65±1.09^{b}	50.21±1.57^{b}	54.88±1.90^{b}

Values are expressed in mean±SEM (n = 6 in each group). $^{\#}P$>0.01 (not significant); when groups compared with their respective "0" min (one-way ANOVA, followed by Tukey's test). ^{a}P<0.01; when groups compared with their respective "0" min (one-way ANOVA, followed by Tukey's test). ^{b}P<0.001; when groups compared with their respective "0" min (one-way ANOVA, followed by Tukey's test). PH: Polyherbal, SEM: Standard error of the mean

in STZ-induced group to that of the non-diabetic control (hyperglycemia continued in diabetic control over 4-week period). The blood glucose level observed in treatment groups (glibenclamide and PH 200 and 400 mg/kg) was significantly lower. At termination (week 4), blood glucose reduction was greatest 67.48% for PH (400 mg/kg) followed by 62.27% for glibenclamide and 59.26% for 200 mg/kg PH. PH (200 and 400 mg/kg) significantly ($P < 0.01$) showed dose-dependent blood glucose lowering effect at termination [Table 6 and Graph 3a and 3b].

Liver Glycogen

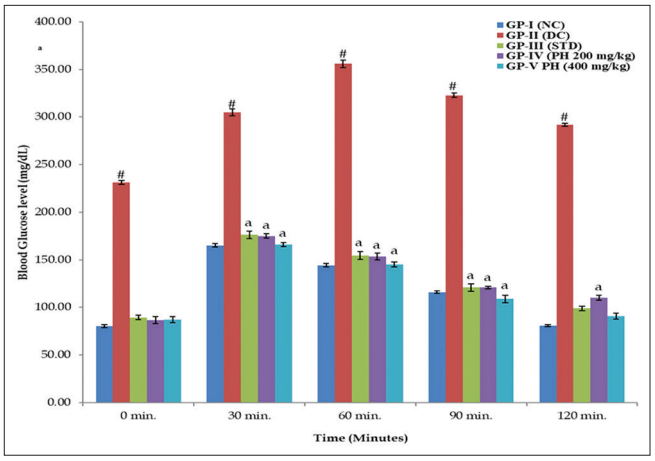
The liver glycogen load significantly raised 1.8–2.5 fold in treated diabetic groups, i.e., 2.3-fold for glibenclamide ($P < 0.001$), 1.9-fold for PH200 mg/kg ($P < 0.01$), and 2.3-fold for PH400 mg/kg ($P < 0.001$)-treated groups, respectively, as compared to their diabetic control, whereas the liver glycogen level was significantly lower ($p < 0.01$) in diabetic control as compared to normal control [Table 7 and Graph 4].

Liver Histopathology

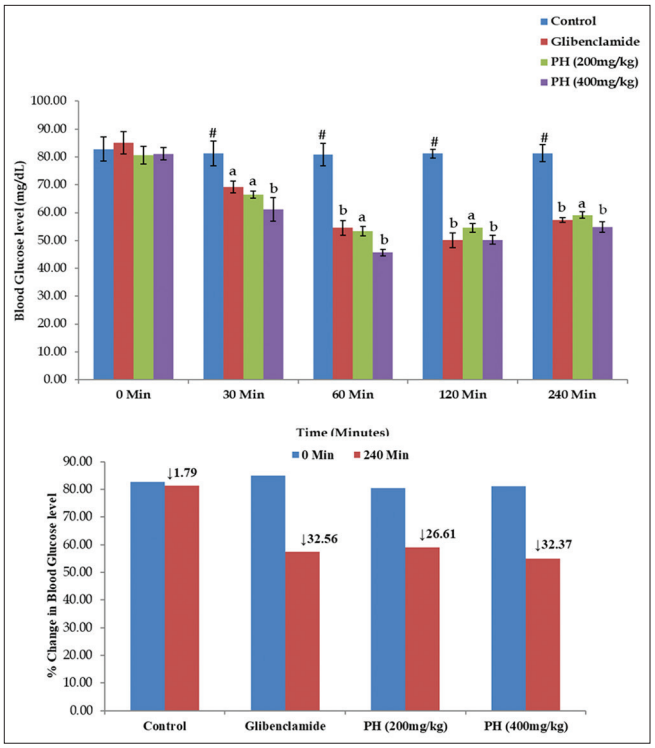
The histopathology of the liver in diabetic group displayed an increase in connective tissue component; blood vessels are markedly dilated and showed degeneration, necrosis, and congestion along with build-up of fat as substantial quarter of hepatocytes is captured by macro droplet of fat. Treatment with PH (200 and 400 mg/kg) has improved the histological condition. The results for PH (200 mg/kg) are quite similar to that of glibenclamide. The treatment with PH (400 mg/kg) dose has shown marked decrease in the microdroplet build-up, as compared to diabetic control [Figure 1].

DISCUSSION

The present manuscript discusses about the hypoglycemic and antidiabetic activity of ethanolic extract of PH (200 and 400 mg/kg) on normal and STZ-induced diabetic rats. STZ has been used to produce animal model of insulin-dependent DM for ages. STZ is a cytotoxic compound (nitrosourea compound) obtained from *Streptomyces achromogenes* (soil microbe). STZ enters the β-cells of Langerhans through glucose transporter and produce ROS to induce DNA strand breakdown which leads to decrease in endogenous insulin release.[29] The breakage of DNA is due to nitrourea moiety. Certain changes start after 2 h of STZ administration, further developing hyperglycemia and concomitant thrust in insulin concentration.[30] Finally, severe hyperglycemia develops with a decrease in insulin level.[31] The toxicity studies revealed that there were no fatality or noxious reactions found in the groups receiving different doses of the extract till the end of experimental period. Thus, the PH formulation was found to be safe as it consists of huge variety of herbs that enumerate a list of phytochemical substances showing efficacious result for the current protocol.

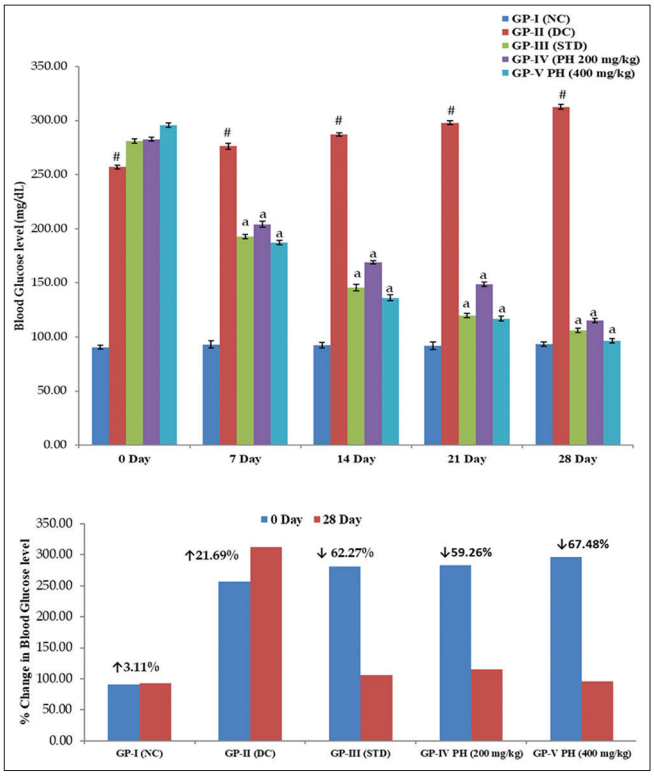


Graph 1: Effect of polyherbal formulation in oral glucose tolerance test Values are expressed in mean±standard error of the mean (n = 6 in each group). #P < 0.01; when diabetic control compared with normal control (unpaired t-test). aP < 0.001; when groups compared with their respective "0" min (one-way ANOVA, followed by Tukey's test).



Graph 2: (a) Effect of polyherbal formulation on normoglycemic rats (hypoglycemic effect). Values are expressed in mean±standard error of the mean ($n = 6$ in each group). #$P > 0.01$ (not significant); when groups compared with their respective "0" min (one-way ANOVA, followed by Tukey's test). a$P < 0.01$; when groups compared with their respective "0" min (one-way ANOVA, followed by Tukey's test). b$P < 0.001$; when groups compared with their respective "0" min (one-way ANOVA, followed by Tukey's test). (b) Effect of polyherbal formulation on percentage change in blood glucose level in normoglycemic rats (hypoglycemic effect)

OGTT is used to identify the altered carbohydrate metabolism throughout post glucose administration. PH formulation was found to be efficacious in controlling fasting blood glucose, decreases hyperglycemia caused by OGTT, and suggests that rats served with non-identical doses of PH extract have better glucose utilization ability.[32] The results elaborate that improved level of glucose tolerance is due to insulin release from β -cells and increased glucose transportation and consumption in different doses of extract-treated groups.[33]

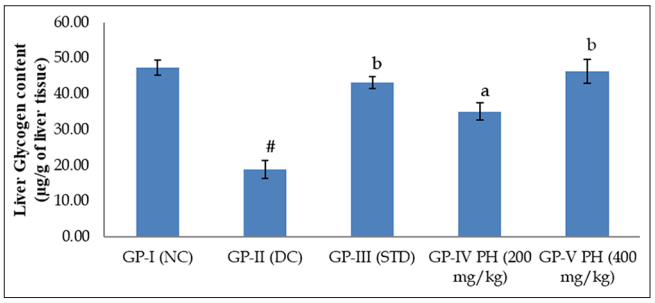


Graph 3: (a) Effect of polyherbal formulation on diabetic rats (antihyperglycemic effect). Values are expressed in mean±standard error of the mean (n = 6 in each group). #P < 0.01; when diabetic control compared with normal control (unpaired t-test). aP < 0.01; when other groups compared with respective "0" day (one-way ANOVA, followed by Tukey's test). (b) Effect of PH formulation on percentage change in blood glucose level (antihyperglycemic effect)

One of the utmost important allopathic treatments for DM is insulin; a lot of research has been carried to find its substitute, secretagogues, or sensitizers from synthetic or plant source for the effective treatment.[19,34,35] For 28 days, oral administration of PH formulation resulted in significant hypoglycemic activity as compared to glibenclamide (standard drug); the results suggested that the mode of action of PH formulation and glibenclamide may be similar.

Glycogen displays a salient role in the storage of glucose in the guise of intracellular energy source by stimulating glycogen synthesis and inhibiting glycogen phosphorylase. The stores of liver glycogen were markedly depleted in diabetic rats, which directly affect the insulin and cause insulin deficiency.[36] STZ-induced group treated with PH formulation (200 and 400 mg/kg) brings back the liver glycogen near to normal rat, which shows increase in the level of insulin secretion. Hence, improvement in increase in the uptake of glucose and its utilization may be a possible mechanism for this observation.[37]

The liver histopathological study revealed that STZ-induced diabetic group has severely deteriorated liver with cytotoxic injury showing necrosis and fibrotic changes. Comparatively, glibenclamide and PH formulation cure as antidotes to seeming abnormalities with minimal sign of hepatotoxicity. According



Graph 4: Effect of polyherbal formulation on liver glycogen content. Values are expressed in mean±standard error of the mean (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)

Table 6: Effect of PH formulation on blood glucose level (mg/dL) in diabetic rats

Groups	0 Day	7 Day	14 Day	21 Day	28 Day
Control	90.45±1.92	92.94±3.63	92.36±3.51	91.83±3.04	93.26±3.63
Diabetic control	256.95±3.03#	276.38±3.01#	287.10±2.14#	297.98±2.74#	312.69±2.44#
Glibenclamide	280.94±1.80	192.89±3.26a	145.54±2.32a	119.75±3.71a	106.00±1.96a
PH (200 mg/kg)	282.70±1.88	204.03±2.78a	168.90±1.53a	148.67±1.99a	115.18±2.07a
PH (400 mg/kg)	295.91±1.97	187.15±2.17a	136.09±2.88a	116.83±2.23a	96.23±2.00a

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 respective "0" day (one-way ANOVA, followed by Tukey's test). PH: Polyherbal, SEM: Standard error of the mean

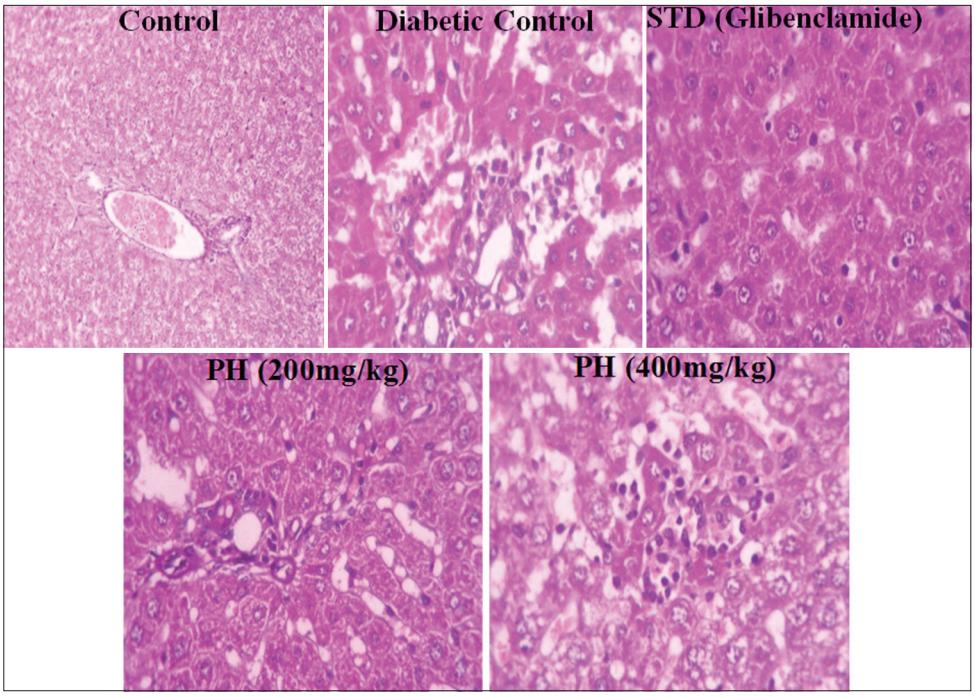


Figure 1: Histopathological studies of liver. (a) Control (b) Diabetic Control (c) STD (glibenclamide), (d) PH (400mg/kg) (e) PH (200 mg/kg)

Table 7: Effect of PH formulation on liver glycogen content in diabetic rats

Groups	Liver glycogen (µg/g of wet tissue)
Control	47.36±2.13
Diabetic control	18.89±2.56[#]
Glibenclamide	43.17±1.69[b]
PH (200 mg/kg)	35.16±2.48[a]
PH (400 mg/kg)	46.42±3.37[b]

Values are expressed in mean±standard error of the mean (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: Polyherbal

to microscopic examinations, pathological alterations elicited by STZ were notably reduced by dispensing glibenclamide and PH formulation. Although glibenclamide showed good efficacy for divergent biochemical parameters, results for all the parameters concluded that PH formulation was most effective.

CONCLUSION

Diabetic treatment is lifelong wherein medicines devoid of any side effects would be treasured and one of such viewpoints is the use of alternative system of medicine comprising herbal products. Our findings reveal that different doses of PH formulation 400 mg/kg, followed by PH (200 mg/kg), respectively, have most effective antidiabetic activity. OGTT has shown that PH formulation is having better glucose utilization capacity. The data also propose that the PH formulation exhibits significant and consistent hypoglycemic activity in diabetic rats. The PH formulation has delivered a dose-dependent reversal of high plasma glucose level observed in diabetic rat. The study provides evidence for the effectiveness of several plant extracts in controlling blood sugar as a PH mixture in comparison with individual plant extract. Further studies are necessitated in human subjects to determine if these results can be appropriately extrapolated to human beings. It was a determined attempt to use herbal therapy along with allopathic drugs so as to decrease the risk factors analogous with DM and the drawbacks of allopathic system in long-term use.

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