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Ameliorative efficacy of polyherbal formulation in streptozotocin induced diabetic rats

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Abstract

The aim of the present study was to determine the anti-diabetic activity of a polyherbal formulation in streptozotocin induced diabetic rats. The methanolic extracts of Achyranthes aspera, Catharanthus roseus and Momordica charantia were used in 3:1:1 w/w/w ratio for polyherbal formulation (PHF) preparation. Fifty Sprague-Dawley rats were randomly divided into five groups (GI, GII, GIII, T1 and T2) based on body weight. The high fat diet treated rats of four groups (GII, GIII, T1 and T2) were injected with 45 mg/kg body weight of streptozotocin intra peritoneally to induce diabetes. Rats of group GI, GII were kept as normal and diabetic control respectively and GIII as standard control, were administrated with glibenclamide (2.5 mg/kg, orally for 60 days); and group T1 and T2 were administered with PHF daily at a dose of 375 mg/kg and 750 mg/kg body weight respectively for a period of 60 days. Administration of PHF showed dose dependent reduction in the blood glucose level on 30th and 60th days, which was comparable to that of standard drug glibenclamide. Significant decrease in body weight and increase in serum glucose level were observed in diabetic control, which was partially restored upon administration of PHF. Altered biochemical enzymes like AST, ALT and antioxidant enzymes (SOD) were normalized by administration of PHF in diabetic rats. Also, mean values of total cholesterol, triglyceride, HDL-cholesterol and LDL-cholesterol in rats treated with PHF were restored. The study indicated that PHF at higher dose showed significant decline ($p<0.001$) in blood glucose level.

Keywords: *Polyherbal formulation, Streptozotocin, type-2 diabetes.*

Diabetes mellitus is a group of chronic metabolic abnormalities, caused by the loss of glucose homeostasis due to improper insulin secretion or action or both resulting into defect in glucose metabolism and other energy-yielding fuels such as lipids and proteins (Moller, 2001). In diabetes, glucose in the blood fails to enter cells, thereby increasing the blood glucose level.

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Diabetes is a major healthcare problem, associated with nerves and blood vessels damage, leading to complications such as heart disease, stroke, kidney dysfunction, blindness, nerve problems, gum infections etc (Frances and Patrik, 2012). Chronic ailments occur mainly due to imbalance pro-oxidants and antioxidants in the organism, leading to oxidative stress (Tayyab and Lal, 2016) and is also an important cause of progression of β-cell dysfunction, insulin resistance, impaired glucose tolerance and type 2 diabetes mellitus (Wright *et al.,*2006).

At present, diabetes is mainly treated by the use of biguanides, thiazolidinediones, sulfonylurea, D-phenylalanine derivatives and α-glucosidase inhibitors besides insulin. However, because of side-effects the efficacies of these compounds are arguable and there is a demand for new compounds / molecules with good therapeutic potential and less adverse effects for the treatment of diabetes (U.K. prospective diabetes study16, 1995).

Medicinal plants are treasured supply of naturally occurring active phytochemicals, usually referred to as secondary plant metabolites, had been attributed to have different biological properties which provide protection against diverse diseases and give health benefits to humans and animals (Aathira *et al.*, 2021). The concept of polyherbal formulation (PHF) is well registered in the ancient literature, which has better and extended therapeutic potential as compared to the single herb. *Achyranthes aspera, Catharanthus roseus* and *Momordica charantia* have been individually studied and reported to have significant antihyperglycemic activities and are rich reservoir of pharmacologically established antidiabetic phytoconstituents (Akhtar and Iqbal, 1991; Chattopadhyay, 1994; Sarkar *et al*., 1996). Study with mixture containing all these three herbs have not been carried out in diabetic rat model. Thus, these three plants have been selected to prepare PHF to evaluate the effect on blood glucose level, lipid profile, anti-oxidant activity and other pathological changes in streptozotocin-induced diabetic rat model.

Materials and methods

Plant materials and preparation of extracts

The aerial parts of *A. aspera* were procured from road side area of Ranchi Veterinary College, Ranchi. *Catharanthus roseus* collected from horticulture, BAU and *M. charantia* (MC) were grown from seeds and aerial part of plants were handpicked after flowering and fruiting. The plant materials were identified and authenticated by Central National Herbarium, Botanical survey of India, Howrah, West Bengal with reference number CNH/Tech.11/2021/27. All the plant materials (1 kg) were air dried and separately coarsely powdered in a mixer-grinder. A weight of 200 g of each powdered plant was placed in separate conical flasks and 500 ml of methanol was added and plugged with cotton and placed on magnetic stirrer for 72 h for extraction. After 72 h the supernatant was collected by filtration using Whatman paper No.1 and the solvent was evaporated at 50 ^{o}C in oven to make the crude extract. The residues obtained were stored in airtight bottles in a refrigerator for further use.

Chemicals and reagents

Streptozotocin was obtained from Himedia Private Ltd., Mumbai, India. Kits for biochemical parameters were purchased from ERBA Diagnostic Mannheim GmbH, Germany. All other chemicals and reagents were procured from Himedia Private Ltd.

Development of polyherbal formulation

The PHF was developed by combining the dried extracts of the plant materials based on the LD_{50} of individual plant extracts and oral glucose tolerance test in normal rats. The polyherbal formulation (PHF) were made by mixing *A. aspera*, *C. roseus*, and *M. charantia* extracts in the ratio of 3:1:1 w/w/w respectively in 0.5 per cent of gum acacia dissolved in distilled water as vehicle.

Effect of plant extracts on oral glucose tolerance test

Overnight fasted *Sprague-Dawley* normal rats of 6-8 weeks old of either sex

weighing 130-180 g were divided into five groups having 5 animals in each group. The rats were housed in polypropylene cages with free access to fresh water in departmental animal house at a temperature of 22 ± 2 ºC. Group I was kept as normal control; Group II received standard drug Glibenclamide at 2.5 mg/kg body weight; Group III, IV and V received 150 mg / kg body weight of *A. aspera* extract, *C. roseus* extract, and *M. charantia* extract respectively. The single dose of each extract dissolved in 0.5 per cent of gum acacia was administered orally in the rats. All animals received glucose (2g/kg) orally, 30 min after drug and extract treatment. Blood glucose levels were estimated by tail tipping method using Accuchek-Active Glucometer at 0, 30, 60, 90 and 120 min after treatment.

Preparation of high-fat diet.

The High-Fat Diet (HFD) was prepared by using normal pellet diet, raw cholesterol, and mixture of vanaspati ghee and coconut oil (2:1). Normal rat pellet diet was powdered by grinding and mixed with 2.5% cholesterol and mixture of vanaspati ghee and coconut oil (5%). The mixture was made into pellet form and put into freezer to solidify (Nekha *et al*., 2020).

Diabetic model

The HFD was fed orally to rats for 3 weeks to induce metabolic syndrome. The diabetes was induced in overnight fasted rats by a single intra peritoneal injection of a freshly prepared solution of streptozotocin (45 mg/ kg body weight) in 0.09 M cold citrate buffer having a pH of 4.8 (Tikkanen *et al.*, 1998). The rats were then kept for the next 24 h on 5 per cent glucose solution bottles in their cages to prevent hypoglycaemia and were monitored by periodic estimation of body weight and biochemical testing of fasting serum glucose. Only those rats with persistent blood glucose levels ≥200mg/dl for 7 days after streptozotocin administration were considered diabetic and included in the study.

Grouping and treatment schedule for hypoglycaemic activity

Based on the results of OGTT, we proceeded with 60 day hypoglycaemic activity study. The protocol of the experiment was approved by the Institutional Animal Ethics Committee, Ranchi University. Fifty Sprague-Dawley rats were divided into five groups, each containing ten animals, as follows:

Group I: Normal Control

Group II: Diabetic Control

Group III: Diabetic animals treated with glibenclamide @ dose of 2.5 mg/kg b.wt.

Treatment group 1 (T-1): Diabetic animals treated with PHF @ dose of 375 mg/kg b.wt.

Treatment group 2 (T-2): Diabetic animals treated with PHF @ dose of 750 mg/kg b.wt.

All the treatments were given once a day orally for 60 days on fixed time. On day 30th and 60thof the study, the blood samples were withdrawn from all the experimental animals through retro-orbital plexus puncture in plain and EDTA tubes for biochemical analysis. Finally, five animals from each group were sacrificed by using overdose of carbon dioxide and chloroform on day 30 and the remaining rats on day 60 of the experiment; liver tissues were excised and used for biochemical and oxidative stress analysis.

Haematological parameters

Blood glucose levels were analysed using Accuchek-Active Glucometer (Roche Diagnostic GmbH Mannheim, Germany).

Estimation of lipid profile and biochemical parameters

Lipid profile parameters like total cholesterol (TC), HDL- cholesterol, LDL-cholesterol, triglyceride (TG) and biochemical parameters like alanine aminotransferase (ALT), aspartate amino transferase (AST) were estimated by using standard kits (Erba, Diagnostic Mannheim GmbH, Germany) with automatic biochemistry analyser (CHEM-5 Plus $_{v2}$,Transasia Bio-Medicals Ltd., Solan, HP, India).

Oxidative stress related parameters

Lipid peroxidation (LPO) in tissue

homogenate was estimated in terms of malondialdehyde (MDA) production by the modified method of Stock and Dormandy (1971) as described by Jain (1988).

Superoxide dismutase (SOD) was estimated as per the method described by Madesh and Balasubramanium (1998).

Statistical analysis Data were statistically evaluated by two- way ANOVA using the Graphpad Prism v 4.03 software program (San DIEGO, CA, USA) and the differences were considered statistically significant at *P<0.05 or lower (Snedecor and Cochran,1989).

Results and discussion

Effect of extracts on blood glucose in oral glucose tolerance test

The yield percentage of methanolic extracts of *A. aspera*, *C. roseus* and *M. charantia* were 12.13, 11.00 and 15.23 per cent, respectively.

The hypoglycaemic potential of each herbal extracts was tested by oral glucose tolerance test in normal rats. In OGTT model, it was observed that, at 30 min after starting the glucose tolerance test, the blood glucose level increased rapidly in the normal control groups but in treated groups the glucose induced hyperglycemia was prevented as compared to control group (Fig.1). The glibenclamide, *Catharanthus* and *Momordica* extract significantly reduced the elevation in blood glucose level at 30 min compared to the normal control group (P<0.001).The *Achyranthes* extract also reduced the elevated glucose level at 30 min but the reduction was not significant. Glibenclamide treated group also prevented the glucose induced hyperglycaemia at 30 min and 60 min as compared to normal control (P<0.001). There was not any significant decline of the blood glucose level in the normal control group during the observation period.

Symptoms and bodyweight

Rats of diabetic control group showed symptoms like dullness, sluggish movement, weight loss, polyuria, polydipsia and polyphagia whereas these symptoms were moderate in all other treatment groups except normal control group. The mean body weight in diabetic rats were reduced significantly (p<0.05) at the end of 30th and 60th day of experimental period (Table 1). The results obtained are in agreement with findings of Kumar *et al*. (2008) and Cheng *et al*. (2013) that increased appetite, weight loss and muscle weakness in diabetic rats might be due to insulin deficiency causing protein and fats catabolism due to negative energy balance. Polydipsia may be due to hyperglycaemia, causing intracellular water depletion and thus activating the thirst centres in the brain. PHF treatment groups (T1 & T2) and glibenclamide (G-III) treated group showed significant improvement in body weight, which indicates that polyherbal formulation and glibenclamide prevent the hyperglycemia induced muscle wastage. The results obtained are in agreement with findings of Vijayaraj and Kumaran (2018), Fernandes *et al*. (2007) and Hikmah *et al*. (2015) that the administration of *A. aspera*, *M. charantia* and *C. roseus* herbal extract respectively showed an improvement in mean body weight of diabetic rats. The increased body weight in PHF treated group could be attributed to the better utilization of nutrients, glucose, amino acids, fatty acids and other macro-molecular components due to improved insulin secretion by the beta-cells (Chander *et al*. 2015).

Serum glucose level

Diabetic control animals showed severe hyperglycemia compared to normal rats. It was observed that the standard drug, glibenclamide lowered the blood glucose level significantly, bringing it back to near normal level, whereas the PHF at 375 mg/kg and 750 mg/kg significantly (P < 0.001) decreased the fasting serum glucose level in the diabetic rats on 30th and 60th days, as compared to the diabetic control group (Table 2). The reduction in glucose levels may be due to increase in plasma insulin levels or enhanced transport of blood glucose in the peripheral tissue (Wilcox, 2005).The antihyperglycaemic activity of PHF may be due individual herb's active principles in the polyherbal formulation, stimulating remnant β-cells to release more insulin or improving

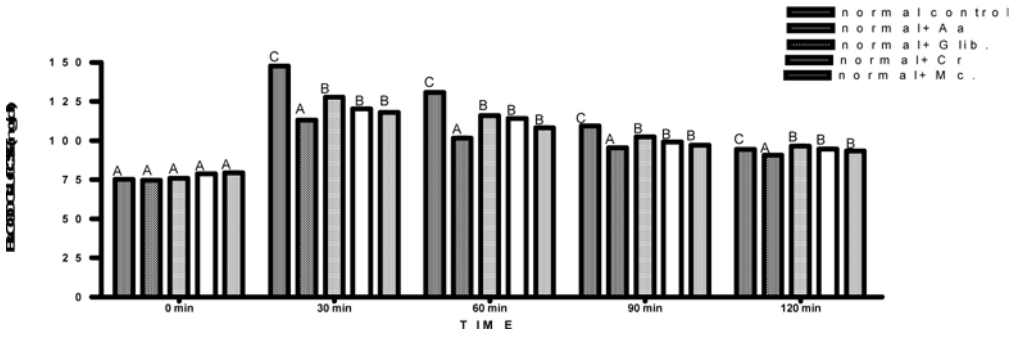

Fig.1. Histogram showing Glucose levels (mg/dl) in rats at different time intervals in different treatment groups under oral glucose tolerance test (OGTT).

insulin action at cellular level. The results were in agreement with Vijayaraj and Kumaran (2018) in *A. aspera* extract; Chattopadhyay, (1991) in *C. roseus*; Sathishsekar and Subramanian (2005) and Sarkar *et al*. (1996) in *M. charantia.* Chattopadhyay, (1994) reported a plant derived natural phytoconstituent named Vinculin, isolated from *C. roseus* having hypoglycaemicactivity. Ng *et al*. (1986) and Raman and Lau (1996), reported that *M. charantia* extract also contains phytoconstituents such as charantin, insulin-like peptides, lecithin and alkaloids which were responsible for hypoglycaemic property. Betaine, achyranthine and β-ecdysones (Akhtar and Iqbal, 1991) were reported to be isolated

Table1. Body weight (g) of experimental animals of different groups.

Body weight	Day 0 (Mean ± SE)	Day30 (Mean ± SE)	Day 60 (Mean ± SE)
Group-I	174.88 ± 3.83^{Aa}	199.47 ± 1.23^{Cb}	248.56 ± 5.7^{Ec}
Group-II	186.86 ± 1.43^{Bc}	147.08 ± 2.62^{Ab}	139.93 ± 5.21^{Aa}
Group-III	193.75 ± 0.56^{Ca}	210.99 ± 1.31^{Db}	221.13 ± 1.12^{Dc}
T-1	172.8 ± 2.51^{Aa}	183.40 ± 0.69^{Bb}	190.9 ± 0.55^{Bc}
T-2	171.12 ± 0.38^{Aa}	188.13 ± 0.38^{Bb}	197.18 ± 4.44^{Cc}

Values with different superscript in a column and row were significantly different (p<0.05).
(Capital superscript- Within column, Small superscript- Within row)

Table 2. Blood glucose (mg/dl) levels in different treatment groups.

Blood glucose	Day 0 (Mean ± SE)	Day30 (Mean ± SE)	Day 60 (Mean ± SE)
Group-I	95.28 ± 0.59^{Aa}	99.29 ± 1.88^{Aa}	98.42 ± 2.61^{Aa}
Group-II	212.83 ± 1.39^{Ca}	262.42 ± 1.35^{Eb}	291.08 ± 1.83^{Dc}
Group-III	204.55 ± 2.51^{Bc}	153.57 ± 3.64^{Bb}	130.26 ± 3.43^{Ca}
T-1	247.95 ± 1.31^{Dc}	190.96 ± 0.29^{Db}	134.45 ± 3.05^{Ca}
T-2	245.56 ± 2.86^{Dc}	169.09 ± 0.53^{Cb}	112.41 ± 3.11^{Ba}

Values with different superscript in a column and row were significantly different (p<0.05).
(Capital superscript- Within column, Small superscript- Within row)

Table 3. The mean ± SE haemoglobin (g/dl) values in different treatment groups.

Groups (Hb%)	Day 0 (Mean ± SE)	Day30(Mean ± SE)	Day 60 (Mean ± SE)
Group-I	11.60 ± 0.02^{Ca}	13.33 ± 0.03^{Cb}	13.9 ± 0.01^{Db}
Group-II	9.75 ± 0.12^{Ab}	9.01 ± 0.12^{Ab}	7.76 ± 0.07^{Aa}
Group-III	10.36 ± 0.1^{Ba}	10.88 ± 0.03^{Ba}	11.76 ± 0.12^{Bb}
T-1	9.84 ± 0.15^{Aa}	11.17 ± 0.09^{Bb}	12.54 ± 0.18^{Cb}
T-2	9.69 ± 0.3^{Aa}	11.79 ± 0.05^{Bb}	14.67 ± 0.14^{Dc}

Values with different superscript in a column and row were significantly different (p<0.05).
(Capital superscript- Within column, Small superscript- Within row)

Table 4. The mean ± SE ALT (IU/L) values in different treatment groups.

Groups (ALT)	Day30 (Mean ± SE)	Day 60 (Mean ± SE)
Group-I	22.80 ± 0.75^{Aa}	23.07 ± 0.42^{Aa}
Group-II	45.97 ± 0.47^{Ea}	55.35 ± 0.34^{Db}
Group-III	34.1 ± 0.37^{Db}	31.93 ± 0.34^{Ca}
T-1	31.72 ± 0.2^{Cb}	28.13 ± 0.1^{Ba}
T-2	29.62 ± 0.41^{Bb}	24.04 ± 0.51^{Aa}

Values with different superscript in a column and row were significantly different (p<0.05).

(Capital superscript- Within column, Small superscript- Within row)

Table 5. The mean ± SE AST (IU/L) values in different treatment groups.

Groups (AST)	Day30 (Mean ± SE)	Day 60 (Mean ± SE)
Group-I	87.35 ± 1.38^{Aa}	87.87 ± 0.92^{Aa}
Group-II	147.66 ± 0.76^{Ea}	170.22 ± 2.27^{Eb}
Group-III	128.50 ± 0.33^{Da}	134.05 ± 0.88^{Db}
T-1	108.91 ± 0.33^{Cb}	101.97 ± 0.49^{Ca}
T-2	102.48 ± 0.54^{Bb}	98.06 ± 0.46^{Ba}

Values with different superscript in a column and row were significantly different (p<0.05).

Table 6. The mean ± SE serum cholesterol (mg/dl) values in different treatment groups.

Groups (TC)	Day30 (Mean ± SE)	Day 60 (Mean ± SE)
Group-I	101.90 ± 1.59^{Aa}	111.32 ± 1.19^{Ab}
Group-II	178.68 ± 0.42^{Ca}	232.84 ± 0.9^{Eb}
Group-III	140.59 ± 0.70^{Bb}	134.21 ± 0.60^{Ca}
T-1	142.38 ± 1.38^{Bb}	138.09 ± 0.47^{Da}
T-2	140.16 ± 0.73^{Bb}	127.45 ± 0.53^{Ba}

Values with different superscript in a column and row were significantly different (p<0.05).
(Capital superscript- Within column, Small superscript- Within row)

from *Achyranthes* which helps in regulating carbohydrate digestion and absorption and thus responsible for hypoglycaemic activity.

Haemoglobin (Hb %)

The mean Hb% in diabetic control rats were observed to decline drastically from 30th to 60th day of experiment (Table 3). Decreased Hb content in diabetic rats might be due to increased formation of glycosylated Hb. In agreement with the present results, Emmanuel *et al*., (2010)also reported that in *diabetes* ailment the excess of glucose present in the blood reacts with haemoglobin to form glycated hemoglobin (HbA_{1C}) thus exhibiting low level of Hb in diabetic animals. The improvement in Hb values in PHF (T-1,T-2) treated groups might be due to reduction in blood glucose levels thereby decreasing the excess glucose reacting with haemoglobin forming glycosylated haemoglobin or might be due to improvement in insulin secretion by β-cells; thus reducing the severity of hyperglycaemia; were reported by Vijayaraj and Kumaran (2018), Jayanthi *et al*. (2010) and Ali *et al*. (1993) in *A. aspera, C. roseus* and *M. charantia* extracts treated diabetic animals, respectively.

Assessment of liver toxicity enzyme test

Serum ALT and AST mean values in diabetic rats were significantly (P<0.001) higher as compared to normal control animals throughout the study period (Table 4, 5), similar findings were also reported by many workers (Shibib *et al*., 1993; Chaudhari *et al*., 2009). Streptozotocin induced hyperglycaemia causes elevation of plasma levels of ALT and AST, which are significant markers of liver (hepatic) abnormality. Supporting our findings, Ohaeri (2001) has reported that the structure of liver was modified in diabetic rats. Therefore, elevated activities of ALT and AST in serum

Table 7. The mean±SE serum triglycerides (mg/dl) values in different treatment groups.

Groups (TG)	Day30 (Mean ± SE)	Day 60 (Mean ± SE)
Group-I	84.29 ± 0.45^{Aa}	85.17 ± 0.60^{Aa}
Group-II	196.02 ± 2.02^{Ea}	220.52 ± 1.55^{Eb}
Group-III	140.04 ± 1.94^{Db}	128.66 ± 0.35^{Da}
T-1	132.13 ± 0.94^{Cb}	123.97 ± 0.66^{Ca}
T-2	121.03 ± 1.08^{Bb}	108.46 ± 1.24^{Ba}

Values with different superscript in a column and row were significantly different (p<0.05).

Table 8. The mean ± SE serum LDL-cholesterol (mg/dl) values in different treatment groups.

Groups (LDL-c)	Day30 (Mean ± SE)	Day 60 (Mean ± SE)
Group-I	52.68 ± 0.32^{Aa}	54.89 ± 0.59^{Aa}
Group-II	151.78 ± 0.28^{Ea}	171.12 ± 0.45^{Eb}
Group-III	85.03 ± 0.27^{Db}	78.19 ± 0.26^{Da}
T-1	72.88 ± 0.33^{Cb}	67.33 ± 0.23^{Ca}
T-2	68.74 ± 0.23^{Bb}	62.24 ± 0.32^{Ba}

Values with different superscript in a column and row were significantly different (p<0.05).
(Capital superscript- Within column, Small superscript- Within row)

Table 9. The mean±SE serum HDL-cholesterol (mg/dl) values in different treatment groups.

Groups (HDL-c)	Day30 (Mean ± SE)	Day 60 (Mean ± SE)
Group-I	30.32 ± 0.36^{Ca}	34.54 ± 0.27^{Cb}
Group-II	17.88 ± 0.55^{Ab}	15.41 ± 0.32^{Aa}
Group-III	25.73 ± 0.35^{Ba}	29.4 ± 0.23^{Bb}
T-1	37.69 ± 0.20^{Da}	40.83 ± 0.32^{Db}
T-2	38.87 ± 0.25^{Ea}	42.02 ± 0.05^{Eb}

Values with different superscript in a column and row were significantly different (p<0.05).

Table 10. The mean ± SE SOD activity(U/mg protein) in liver tissue collected from rats under different treatment groups.

Groups (SOD)	Day30 (Mean ± SE)	Day 60 (Mean ± SE)
Group-I	25.43 ± 3.78^{Da}	26.72 ± 4.0^{Ca}
Group-II	19.49 ± 2.66^{Ab}	16.45 ± 2.12^{Aa}
Group-III	21.53 ± 3.03^{Ba}	21.85 ± 6.95^{Ba}
T-1	23.46 ± 3.4^{Ca}	26.98 ± 4.02^{Cb}
T-2	25.7 ± 3.79^{Da}	29.78 ± 4.54^{Db}

Values with different superscript in a column and row were significantly different (p<0.05).
(Capital superscript- Within column, Small superscript- Within row)

might be due to the drop out of these enzymes into the blood stream from the liver cytosol due to increased hepatocytes membrane permeability or membrane damage (Concepcion *et al*., 1993); or due to increased activity of amino acid, responsible for elevated gluconeogenesis and ketogenesis (Nikkila and Kekki, 1973). In the present study, PHF and glibenclamide treated groups showed significant (P<0.001) reduction in ALT and AST levels as compared to diabetic control animals. This result may be due to the ameliorative effect of the individual herbs present in the polyherbal formulation (Shah *et al*., 2010). Findings are in agreement with previous reports by Bafna and Mishra (2004), Al-Shaqha*et al*. (2015) and Dandagi *et al*. (2008) related to hepatoprotective activity of *A. aspera, C. roseus* and *M. charantia* extracts, respectively in restoring the elevated levels of ALT and AST in diabetic rats. Chaudhari *et al*. (2009) also attributed the hepatoprotective effect of *M. charantia* due to presence of flavanoids, ascorbic acid and other components such as saponins, tannins, triterpenes and alkaloids in the extract etc.

Table 11. The mean ± SE MDA level (nmol/mg protein) in liver tissue collected from rats under different treatment groups.

Groups (MDA)	Day30 (Mean ± SE)	Day 60 (Mean ± SE)
Group-I	29.19 ± 0.36^{Ca}	29.65 ± 0.41^{Ca}
Group-II	43.10 ± 0.63^{Ea}	50.998 ± 0.38^{Eb}
Group-III	33.96 ± 0.67^{Da}	31.46 ± 0.41^{Da}
T-1	21.80 ± 0.41^{Bb}	17.81 ± 0.14^{Ba}
T-2	19.00 ± 0.24^{Ab}	15.04 ± 0.26^{Aa}

Values with different superscript in a column and row were significantly different (p<0.05).
(Capital superscript- Within column, Small superscript- Within row)

Lipid profile

In *diabetes mellitus*, hyperglycemia is accompanied with dyslipidemia that is characterized by increase in total cholesterol, triglyceride, LDL cholesterol and decrease in HDL cholesterol (Table 6, 7, 8 and 9). The abnormally high concentration of serum lipids in diabetics is mainly due to increased congregation of free fatty acids from the peripheral fat depots (Gupta *et al*., 2009). In the present study, the mean values of HDL-cholesterol were significantly (P<0.001) increased whereas TC, TG and LDL-cholesterol were significantly (P<0.001) decreased in both PHF and glibenclamide treated groups as compared to diabetic control on day 30 and 60. Supporting to our findings, Jayanthi *et al*. (2010), reported a significant lowering of serum total lipid levels treated with *C. roseus* in streptozotocin induced diabetic rats. Significant improvement in hyperlipidemia were in agreement with the findings of Khanna *et al*. (1992), who showed alcoholic extract of *A. aspera* had lowered serum cholesterol in triton induced hyperlipidemic rats. The findings were also in agreement with Krishnakumari and Priya (2006), who reported hypolipidemic efficacy of *A*. *aspera* in sesame oil fed rats. In agreement with present results, Nerurkar (2005) also reported that treatment with methanolic extract of *M. charantia* significantly decreased the total serum cholesterol, triglycerides, low density lipoproteins and increased the high-density lipoproteins in obese rats. This might be due improved insulin release, enhanced uptake of glucose and better utilization of nutrients like glucose, amino acids, fatty acids and other macromolecular components (Singh *et al.,* 1989).

Antioxidant defense system

Superoxide dismutase (SOD) is an antioxidant enzyme that catalyses the dismutation of superoxide to produce hydrogen peroxide and oxygen. Increased production of superoxide ion leads to elevation in SOD activity to alleviate the auto-oxidation and oxidative stress and thus provides cellular protection against the damage caused by free radicals and ROS (Kant *et al*., 2011). Increased levels of lipid peroxides and reactive oxygen species by streptozotocin cause alkylation, breakage of DNA strands or tissue damage through peroxidation of unsaturated fatty acids finally leading to death of beta cells (Alireza *et al*., 2009). The PHF treated animals showed improved oxidative stress (Table 10 and 11), which may be due to the free radical scavenging properties of the individual herbs present in it or due to prevention of glucose auto-oxidation, protein glycation and the polyol pathway that generates free radicals (Atalay and Laaksonen, 2002). Results related to improved oxidative stress markers in diabetic animals treated with PHF (T-1, T-2) in the present study are in agreement with previous findings reported by Vijayaraj and Kumaran (2018), Salah *et al*. (1995), and Sathishsekar and Subramanian (2005) related to antioxidant activity of *A. aspera, C. roseus* and *M. charantia,* respectively.

Conclusion

Polyherbal formulation (3:1:1w/w/w/) with extracts of aerial plant parts of *A. aspera, C. roseus* and *M. charantia* respectively at 375 and 750 mg/kg doses, showed ameliorative effect against streptozotocin

induced alterations in diabetic rats, due to active principles. These findings suggested the hypoglycemic, hypolipidemic, hepatoprotective and antioxidant potentials of the PHF and thus help in preventing future complications of diabetes.

Conflict of interest

We declare that we have no conflict of interest.

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