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Research Article

Antidiabetic, Antihyperlipidemic and Antioxidant Activities of a Polyherbal Formulation in Type 2 Diabetic Rats

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ABSTRACT

Type 2 Diabetes Mellitus (T2DM) has developed into a significant cause of death and morbidity for millions of people worldwide. Antidiabetic drug toxicity concerns have shifted focus towards herbal remedies, which are both cost- was an effective and safe. Therefore, the objective of our study was to investigate the antidiabetic, antihyperlipidemic and antioxidant activities of a novel Polyherbal Formulation (PHF).

To prepare the PHF, aqueous extracts of various plant parts namely Commiphora mukul, *Azadirachta indica*, *Momordica*, *Curcuma longa L*, *Emblica officinalis Gaertn*, *Gymnema sylvestre Retz*, *Momordica charantia L*, *Garcinia cambogia Gaertn*, *cimum sanctum L and Trigonella foenum-graecum L* were used. The efficacy of the PHF was studied using various parts High Fat Diet (HFD) and low dose streptozotocin (STZ-35 mg/kg) induced in rats. An Oral Glucose Tolerance Test (OGTT) was also performed in diabetic rats for assessment of pancreatic β-cell function. On other hand, the group of diabetic rat were fed with the dose is 50, 100 and 200 mg/kg of PHF along with the normal, diabetic and positive control (glibenclamide-0.6 mg/kg) group for 28 days. In addition, the biochemical parameters such aspartate many AminoTransferase (AST), Alkaline Phosphatase (ALP), alanine aminotransferase, and blood glucose and lipid were profile were estimated by auto-analyzer. Insulin, leptin and tumor necrosis factor alpha (TNF-α) are also examined by ELISA kit.

The administration of 50, 100 and 200 mg/kg doses of PHF and glibenclamide (0.6 mg/kg), significantly ($p<0.05$) lower blood glucose level, Low-Density Lipoprotein Cholesterol (LDL-C), Very Low-Density Lipoprotein Cholesterol (VLDL-C), triglyceride, total cholesterol, glycated hemoglobin (HbA1c), TNF- α, leptin, ALT, AST, ALP, creatinine and urea as compared to diabetic control group. The levels of High-Density Lipoprotein Cholesterol (HDL-C), total protein and insulin were found to be significantly increase the compared with diabetic control group. Furthermore, the *in vivo* antioxidant enzymes such as CAT, SOD, GSH, GPx, and TBARS in liver and kidney tissues showed in significant ($p<0.05$) recovery in diabetic rats treated with the PHF.

Keywords: Polyherbal formulation; Type 2 diabetes mellitus; Oral glucose tolerance test; Antidiabetic activity; Antioxidant activity

INTRODUCTION

Diabetes Mellitus (DM) is a chronic disorder of carbohydrate, fat and protein metabolism characterized by hyperglycemia resulting from the defects in insulin secretion, insulin action and/or both. Chronic hyperglycemia and insulin resistance are major hallmark of DM having significant role in the pathogenesis of microvascular (neuropathy, retinopathy and nephropathy) and macrovascular (stroke, heart attack and peripheral vascular disease) complications [1,2]. Hyperglycemia can lead to the generation of free radicals that can cause oxidative stress through several mechanisms, such as auto-oxidation of glucose and

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activation of isoform protein kinase C (PKC) [3]. It is reported that, the existing diabetic population is 72.9 million in India and 425 million worldwide, which will be raised up to 134.3 million in India and 629 million worldwide at the end of 2045 [4]. With 90% to 95% of them existence T2DM [5]. The prevalence of diabetes is increasingly growing at an unprecedented pace all over the world. In 1995, the prevalence of diabetes in India and worldwide was about 4 percent [6]. Diabetes incidence increased more than 2 fold in two decades. In 2017, the prevalence of diabetes in India was around 10% and 9% worldwide, which by 2045 would rise to around 11% in India and around 10% worldwide. All proposed treatments for DM have problems of inadequate effectiveness, limited tolerability and/or side effects based on essential pathways [7,8]. Hence, an alternative approach for diabetes treatment pursues the use of medicinal plants in global milieu, as a potential source for developing new therapies and also as a dietary supplement for therapeutic purposes [9].

MATERIAL AND METHODS

Preparation of polyherbal formulation

The PHF was generously gifted by M/s. Lanson Biotech, Chennai. The PHF was prepared using a patented aqueous extraction procedure [10] which is briefly described [11]. The plants used for the preparation of the PHF and the percentages of individual plant in the PHF are provided in Table 1.

S.No.	Botanical Name	English Name	Used part	% Used
1	Azadirachta indica A. Juss.	Neem	Seed kernel	10
2	Commiphora mukul	Indian Bdellium	Resin – guggul sterone	1
3	Curcuma longa L.	Turmeric	Rhizomes	10
4	Emblica officinalis Gaertn.	Indian Gooseberry	Fruit pulp	20
5	Garcinia cambogia Gaertn.	Gamboge	Fruit pulp	1
6	Gymnema sylvestre Retz.	Periploca of the woods	Leaves	20
7	Momordica charantia L.	Bitter gourd	Fruit pulp with seeds	20
8	Ocimum sanctum L.	Holy Basil	Leaves and seeds	8
9	Trigonella foenum-graecum L.	Fenugreek	Seeds	10

Table 1: Ingredient of the polyherbal formulation.

Experimental animals and housing

Healthy, male and female Sprague Dawley rats of age 8-10 weeks and weighing approximately 200-250 g was procured from Biogen, Bangalore. The animals were maintained in the animal facility of C.L. Baid Metha College of Pharmacy, Chennai. The animals were housed in polypropylene cage under standard laboratory conditions of relative humidity cage was procured from temperature, photoperiod (12 h light and 12 h dark cycle), normal pellet diet and water ad libitum. The experiment was performed as per the protocol approved by the Institutional Animal Ethics Committee (Approval No: IAEC/XLI/05/CLBMCP/2013).

Acute oral toxicity

The acute oral toxicity was performed to know any toxic effect in accordance with the guideline. The experiment was carried out in a step-wise procedure. Three female rats were used in each step. The test was initiated with the dose of 2000 mg/kg body weight since the PHF was expected to be safe as it contained only herbal ingredients. Since no mortality was observed in rats at 2000 mg/kg, a confirmatory test was carried out using another set of three rats. These rats were similarly treated with the same limit dose (2000 mg/kg) as in the case of Step I animals [12].

Development of HFD-Fed and STZ-Induced

Type 2 diabetic rats: The rats were distributed into two dietary regimens and fed with Normal Pellet Diet (NPD) and High Fat Diet (HFD) for a period of 2 weeks. The composition of one of kilogram HFD is normal pellet diet (365 g), casein (250 g), vitamin and mineral mix (60 g), cholesterol (10 g), dl-Methionine (3 g), sodium chloride (1 g), yeast powder (1 g) and lard (310 g) [13]. After 2 weeks of dietary manipulation the HFD animals were fasted overnight and injected intraperitoneally with Streptozotocin (STZ) at 35 mg/kg [13]. While the respective control rats were similarly injected with the vehicle, citrate buffer (pH 4.4), at a dose volume of 1ml/kg body weight. Animals were allowed free access to food and water after the STZ injection, and both STZ-injected and the non-injected animals were allowed to continue on their original diets (NPD or HFD) for the duration of the study. On day 7 (i.e. 6 days after the STZ injection), the animals were divided into subsets A and B. Subset animals were again divided into four groups for OGTT; each group had six rats. The rest of the rats (subset B) were subjected to fasting blood glucose and lipid profile tests prior to the repeated dose efficacy evaluation.

Subset A: Oral Glucose Tolerance Test (OGTT) in diabetic rats

The rats were selected randomly from the diabetic rats for the evaluation of OGTT. Subset A was divided into four groups as mentioned below. Six animals were used in each group

Group I: Normal rats administered double distilled water (10 ml/kg b. wt.)

Group II: Diabetic rats administered double distilled water (10 mg/kg b. wt.)

Group III: Diabetic rats administered PHF (200 mg/kg b. wt.)

Group IV: Diabetic rats administered GBL (0.6 mg/kg b. wt.)

The rats were fasted for 18 hours and the OGTT was performed by oral administration of 20 % glucose solution (2 g/kg b. wt.) through gavage [14] to diabetic and normal rats 30 min after dosing of the PHF or GBL. Blood sample was collected through tail region by using sterile needle at 0, 30, 60, 90 and 120 min of glucose administration [15] and blood glucose levels were estimated using a glucometer with glucose oxidase-peroxidase reactive strips. The results of the OGTT are expressed as integrated Area Under the Curve (AUC) over a period of 0-120 min. The glucose concentration versus time plot gives a curve showing rise and fall in the glucose level with time and expressed as integrated area under curve for glucose (AUCglucose). The value of AUC was calculated by using trapezoid rule [AUC=(C1+C2) ÷ 2× (t2-t1). The change glucose concentration over 120 minutes during OGTT were expressed as AUCglucose (mg/dl × 120 minutes) [16,17].

Subset B: Efficacy evaluation of PHF in diabetic rats

The animals of the Subset B were used for the repeated dose efficacy evaluation. Before the commencement of dosing, the rats were fasted for overnight and their blood samples were collected through retro-orbital puncture under light ether anesthesia in anticoagulants containing tubes. The plasma was separated after centrifugation at 25°C for lipid profile estimations. Glucose levels were checked using glucose oxidase-peroxidase reactive strips and with a glucometer (AccuChek, Roche Diagnostics). The rats with fasting blood glucose in the range of 300 to 400 mg/dl were considered as diabetic and selected for the repeated dose efficacy study [18]. The lipid profiles of these rats were also considered for their selection. The animals were randomly divided into various groups as mentioned below.

Group I: Normal rats administered with double distilled water

Group II: Diabetic rats administered with double distilled water

Group III: Diabetic rats administered with PHF (50 mg/kg b. wt.)

Group IV: Diabetic rats administered with PHF (100 mg/kg b. wt.)

Group V : Diabetic rats administered with PHF (200 mg/kg b. wt.)

Group VI: Diabetic rats administered with GBL (0.6 mg/kg b. wt.)

The dose level of the positive control was selected based on available literature [19,20].

Orally treatment was given using an intragastric tube daily for 28 days continuously. The body weight was recorded weekly during 28 days dosing. Blood glucose was estimated at weekly interval using glucose strips and a glucometer. After 28 days dosing, the animals were anaesthetized using diethyl ether and sacrificed by decapitation after overnight fasting. The blood was collected with and without anticoagulant containing tube. Serum/Plasma was separated by centrifugation for 10 minutes at 2000 rpm for biochemical analysis.

Biochemical analysis

The blood glucose, total cholesterol, triglycerides, total protein, creatinine, urea, ALT, AST and ALP were measured using Erba kit (Erba Diagnostic Mannheim GmbH, Germany) auto-analyzer. Whereas the HDL-C was determined by using Autospan Liquid Gold Direct HDL-C (Autospan, India) kit by using auto-analyzer, LDL–C and VLDL-C were calculated using formula [21]. The HbA1c was carried out by using Agappe HbA1c Kit (Agappe Diagnostic kits, Ernakulam, Kerala, India). The insulin, leptin and TNF-α concentration were determined using Rat ELISA Kit (RayBiotech, Inc. Norcross GA 30092). Calculations of HOMA-B and HOMA-IR were done in accordance with the formula given below:

HOMA-B=20 × Insulin (μIU/l) ÷ Blood glucose (mmole/l)-3.5

HOMA-IR=Insulin (μIU/l) × Blood glucose (mmole/l) ÷ 22.5

Conversion factor:
Insulin (1μIU/l=7.174 pmol/l) and blood glucose (1 mmol/l=18 mg/dl).

Antioxidant activities

The liver and kidney of each animal were collected carefully. Blood from the tissues was removed by washing in cold saline. The tissues were then minced in Tris buffer (pH 7.4) using clean scissors. Homogenization of the tissues was done and the preparations were centrifuged (3000 g) at low temperature. The supernatant was collected from both liver and kidney separately. The protein estimation of the supernatant was carried out by Biuret method. The supernatant solutions were diluted to 1 mg/ml and used for various antioxidant assays. The antioxidant activities such as SOD, CAT, GPx, GSH, TBARS .

Statistical analysis

The obtained data were presented as mean ± SD which were analyzed by using one-way Analysis of Variance (ANOVA) with Tukey's HSD test (for data showing normality and homogeneity) or by Kruskal Wallis test (for data showing non-normal distribution or heterogeneity). Significance level was set at 5%

probability level. Statistical analysis was done using SPSS 21.0 version.

RESULTS AND DISSCUSSIONS

Acute oral toxicity

In acute oral toxicity, the administrated of PHF at dose level 2000 mg/kg b. wt. did not reveal any clinical signs of toxicity during 14 days observation period. The body weight and food consumption of treated animals was normal. The gross pathology results did not reveal any lesions. Therefore, 200 mg/kg, which is 10% of the dose which showed no behavioral or clinical alterations, was selected as the high dose for the efficacy study [29,30]. The other two doses selected were 100 mg/kg and 50 mg/kg b. wt.

Subset A: Effect of PHF on OGTT in diabetic rats

The blood glucose results of OGTT are presented in Figure 1. The administration of PHF (200 mg/kg b. wt.) and GBL resulted in reduction in the AUC glucose values which is mentioned in Figure 2.

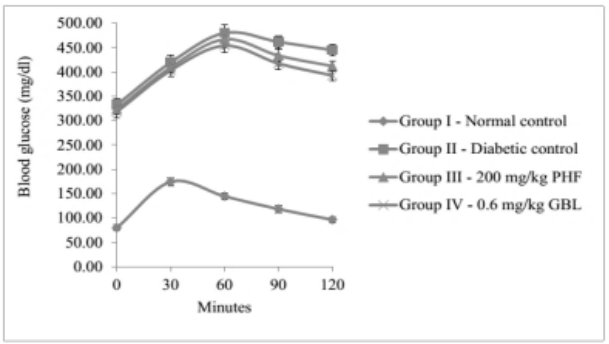

Figure 1: Effect of PHF on oral glucose tolerance test diabetic rats.

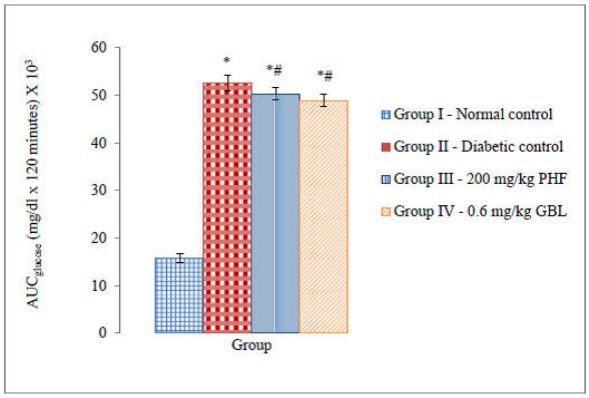

Figure 2: Effect of PHF on AUC glucose in diabetic rats.

Data are as the mean for six animals in each group; Vertical bars indicate standard deviation of the mean.

*Denotes significant difference in values with respect to group I at p<0.05

#Denotes significant difference in values with respect to group II at p<0.05

Note: PHF=Polyherbal Formulation; GBL=Glibenclamide; AUC glucose=Area Under Curve for glucose

Subset B: Efficacy evaluation of PHF in type 2 diabetic rats

The antidiabetic property of PHF was assessed using diabetic rats. The PHF was administrated to diabetic rats for 28 consecutive days. The blood glucose and lipid profile were estimated prior to the initiation of dosing. On day 29, biochemical analysis from blood and antioxidant activities from liver as well as kidney were sampled and evaluated.

Effect of PHF on body weight

The animals treated with PHF (Groups III, IV and V) and GBL (Group VI) revealed significantly high (p<0.05) body weight in comparison with Group II rats which are presented in Figure 3.

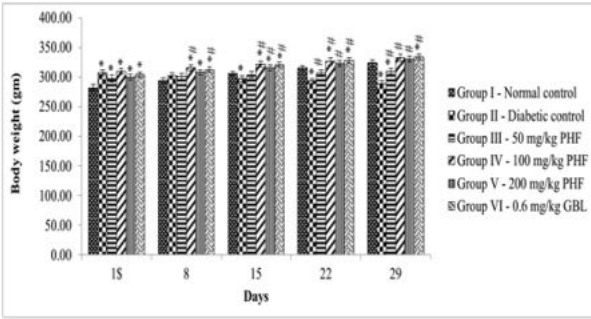

Figure 3: Effect of PHF on body weight in diabetic rats.

Vertical bars indicate standard deviation of the mean.

*Denotes significant difference in values with respect to Group I at p<0.05

#Denotes significant difference in values with respect to Group II at p<0.05

Note: PHF=Polyherbal Formulation; GBL=Glibenclamide; $=Before dosing

Effect of PHF on biochemical analysis

The blood glucose analysis performed on day 8, 15, 22 and 29 revealed significant decreases (p<0.05) in glucose values in animals treated with PHF (Groups III, IV and V) and GBL (Group VI) as compared to the diabetic control group (Group II) mentioned in Table 2. The administration of PHF (50, 100 and 200 mg/kg b. wt.) and GBL (0.6 mg/kg b. wt.) for 28 days was led to significant (p<0.05) recovery in triglyceride, total cholesterol, LDL-C, VLDL-C and HDL-C when compared with the diabetic control (Group II) which is mentioned in Table 3. On the other hand, the biochemical analyses such as HbA1c, insulin, leptin, TNF-α, total protein, AST, ALT, ALP creatinine, urea are significantly recovered in treated diabetic rats when compared with diabetic rats which is presented in. The results of

HOMA-B and HOMA-IR are improved in treated diabetic rats which are mentioned in

Group	Days				
	1	2	4	22	21
Group I	80.17 ± 3.43	80.33 ± 3.14	80.83 ± 3.49	82.17 ± 3.43	85.83 ± 4.49
Group II	344.17 ± 7.86*	378.33 ± 8.41*	410.17 ± 7.19*	439.33 ± 5.35*	460.00 ± 6.99*
Group III	352.50 ± 6.63*	297.83 ± 6.43*#	249.17 ± 5.64*#	204.83 ± 5.12*#	166.17 ± 6.18*#
Group IV	345.17 ± 7.91*	289.17 ± 7.41*#	236.33 ± 6.86*#	189.83 ± 7.11*#	144.50 ± 5.68*#
Group V	338.67 ± 8.45*	280.83 ± 7.49*#	230.00 ± 6.03*#	180.50 ± 6.89*#	133.33 ± 5.16*#
Group VI	368.00 ± 7.62*	303.17 ± 7.08*#	241.17 ± 6.24*#	184.83 ± 6.01*#	130.50 ± 5.79*#

Table 2: Effect of PHF on blood glucose (mg/dl) in diabetic rats.

Group	HOMA B	HOMA-IR
Group I	284.49 ± 79.06	3.65 ± 0.25
Group II	8.60 ± 0.91*	10.75 ± 0.92*
Group III	17.21 ± 1.51*#	7.98 ± 0.77*#
Group IV	28.67 ± 2.49*#	7.13 ± 0.57*#
Group V	50.31 ± 4.69*#	5.87 ± 0.51*#
Group VI	49.32 ± 3.56*#	6.42 ± 0.52*#

Table 3: Effect of PHF on HOMA-B and HOMA–IR values in diabetic rats.

The values are mean ± standard deviation, N=6

*Denotes significant difference in values with respect to Group I at $p<0.05$

#Denotes significant difference in values with respect to Group II at $p<0.05$

Effect of PHF on *in vivo* antioxidant activities

Administration of PHF (50, 100 and 200 mg/kg b. wt.) and GBL (0.6 mg/kg b. wt.) are induced recovery in GPx, SOD, CAT, GSH and TBARS in liver and kidney which are presented in (Figures 4a-4d).

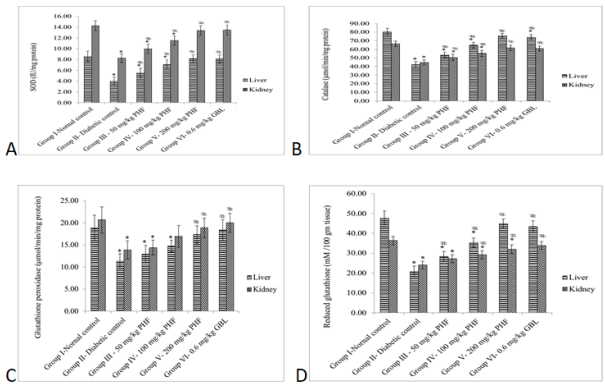

Figure 4: a) Effect of PHF on SOD in liver and kidney in diabetic rats; b) Effect of PHF on catalase in liver and kidney in diabetic rats; c) Effect of PHF on glutathione peroxidase in liver kidney in diabetic rats; d) Effect of PHF on reduced glutathione in liver and kidney in diabetic rats.

DISCUSSION

The OGTT is useful in forecasting β-cell function and insulin resistance . In our study, the 2 hour blood glucose in HFD fed and low dose STZ induced type 2 diabetic control rats (group II) was 3.3 fold higher than the normal control (group I) rats. The normal control rats showed highest blood glucose level at 30 minutes of observation time whereas type 2 diabetic rats (control as well as PHF and GBL treated rats) exhibited highest blood glucose level at 60 minutes of observation time. OGTT is used to evaluate the glucose tolerance which indirectly indicates the insulin sensitivity and pancreatic β- cell function. The OGTT data is also expressed as area under curve for glucose (AUCg). The AUCg is higher in diabetic control rat when compared to normal. The higher AUCg indicated pancreatic β–cell dysfunction and/or insulin resistance in diabetic patient. The results suggested that the PHF may have potential to improve beta-cell function and insulin sensitivity.

The antidiabetic property of PHF was assessed using diabetic rats. The PHF was administrated to diabetic rats for 28 consecutive days. The blood glucose and lipid profile were estimated prior to the initiation of dosing.

Glucose level in blood was significantly increased in type 2 diabetic control rats when compared to normal control. The administration of the PHF at 50, 100 and 200 mg/kg b. wt. resulted in significant decrease in blood glucose levels. Though the blood glucose values were not recovered to the normal level, the recovery was substantial in the high dose group (200 mg/kg b. wt.) wherein 71% decrease was observed in the glucose levels in comparison to the diabetic control. The decrease observed in the blood glucose values of the animals treated with 200 mg/kg dose of the PHF was comparable with the positive control (GBL at 0.6 mg/kg b. wt.) group. May be an increase in the duration of treatment would have improved the blood glucose values to the normal level. The hypoglycemic action might be due to modulation of insulin secretion and/or insulin action as reported . Hydroxycitric acid, an active compound of Garcinia cambogia Gaertn which is reported to cause delayed intestinal

glucose absorption in rats might have also contributed to the hypoglycemic effect. Azardirachta indica, one of the plants used in the PHF, is reported to block the action of epinephrine, the reduction in peripheral utilization of glucose and glycogenolytic effect in diabetic animals. In addition, the acetone extract of fruit of Momordica charantia, another plant used in the PHF, is conveyed to regenerate beta cells in islets of Langerhans of pancreas in diabetic rats . Therefore, the PHF may help in the regeneration of damaged β-cells, due to presence of bioactive compounds flavonoids, terpenoids and steroids in the PHF.

The body weight of the type 2 diabetic rats showed gradual decrease over the 28 days study period as compared to normal control group. STZ-induced diabetes is reported to cause loss of body weight due to increased muscle destruction or degradation of structural proteins in diabetic rats. Furthermore, insulin resistance induces muscle wasting *via* suppression of structural Phosphatidylinositol 3 Kinase (PI3K)/Akt signaling important to activation of caspase-3 and proteolytic pathway which may involve degradation of muscle protein . The structure proteins are well known for their contribution to body weight . When type 2 diabetic rats were administrated with PHF at dose level of 50, 100 and 200 mg/kg b. wt. for 28 days, they revealed an enhancement in body weight as compared to diabetic control. The results indicate the protective effect of PHF in controlling muscle wasting or gluconeogenesis. In addition to that the ability to defend body weight loss seems like to be the result of PHF capability to decrease hyperglycaemia as reported.

DM is reported to be associated with hyperlipidemia. The mobilization of free fatty acid from adipose tissue occur when reduction in insulin takes place which may lead to increased production of LDL-C and dyslipidemia. In our study, type 2 diabetic rats exhibited significant elevation in plasma total cholesterol, triglyceride, LDL-C and VLDL-C levels whereas HDL-C level was significantly declined as compared to normal control. The noticeable hyperlipidaemia that is characterized in diabetic state may consequently be regarded as a importance of the abandoned actions of lipolytic hormones for the fat deposition. Treatment with the PHF at 200 mg/kg b. wt. showed significant improvement in lipid profile of the rats. The level of harmful lipids such as total cholesterol, triglyceride, LDL-C, VLDL-C were significantly reduced by 36%, 35%, 51% and 35%, respectively, with respect to the diabetic control (Group II) whereas the HDL-C level was significantly increased by 121% at highest dose level 200 mg/kg b. wt. On the other hand, the diabetic rats treated with GBL at 0.6 mg/kg b. wt. revealed reduction in total cholesterol, triglyceride, LDL-C and VLDL-C by 40%, 38%, 56% and 38% respectively as compared to diabetic control group whereas the HDL-C level exhibited significant increased by 133% as compared to diabetic control. Extract of C. mukul gum resin, one of the plants used in the PHF, was reported to have antihyperlipidemic activity in diabetic rats, which may inhibit lipogenic enzymes and HMG-CoA reductase in the liver, increase uptake of cholesterol by the liver through stimulation of LDL receptor binding, directly activate the thyroid gland and/or increase biliary and faecal excretion of cholesterol.

During DM, as the blood glucose level is increased in the blood, the blood glucose reacts with haemoglobin to form HbA1c. The Fasting Blood Glucose (FBG) is directly proportional to amount of HbA1c. In diabetic rats, the HbA1c is increased due to hyperglycemia and results in glycation of haemoglobin. The concentration of HbA1c is linked with diabetic retinopathy, nephropathy and neuropathy and it is considered as a diagnosis and prognosis tool for diabetes associated complications. The increased level of HbA1c up to 12% was reported in diabetic patients . In diabetes, relative deficiency of insulin leads to reduction in protein synthesis in all tissues and consequently the synthesis of haemoglobin is also reduced . In our study, the HbA1c level was significantly increased by 146% in type 2 diabetic rats as compared to normal control. The administration of the PHF at 50, 100 and 200 mg/kg b. wt. for 28 days reduced the HbA1c levels by 14, 32 and 48 % respectively. The capability of the PHF to reduce HbA1c levels in diabetic rats revealed its potentiality to preclude the diabetic associated complications.

Numerous workers have reported that HFD-STZ induced type 2 diabetes mimicked the T2DM in humans .The HFD-STZ animals become hyperglycaemic due to the decline of pancreatic β-cell function accompanied by loss of insulin sensitivity. The alkylation nature of STZ causes pancreatic β-cells DNA strand breaks that induce the activation of poly ADP-ribose polymerase followed by reduction lethal Nicotinamide Adenine Dinucleotide (NAD). This function also, generation of potential free radicals such as Nitric Oxide (NO) by intracellular metabolism of STZ aggravates the situation and triggers the further pancreatic β-cells DNA strands breaks. In our study, the plasma insulin values in type 2 diabetic rat were significantly decreased by 45% when compared to the normal control rats. Similar decrease in insulin was reported in diabetic rat model. The administration of the PHF at dose level of 50, 100 and 200 mg/kg b. wt. for 28 days helped to increase the insulin levels by 16, 36 and 52% respectively. The increase in plasma insulin level indicates that the PHF might stimulate insulin secretion from the remaining β-cells or regenerated β-cells.

Leptin is a hormone produced by adipose tissue and it regulates various physiological processes and behaviours, including appetite, body weight, neuroendocrine functions and glycaemia. It is reported that insulin resistance is related with raised up leptin levels in plasma independent of body fat mass . In T2DM, hyperglycemia results from insulin resistance of peripheral tissue and relative insulin deficiency. The plasma leptin concentration is increased in type 2 diabetic patients and it stimulates monocyte chemotactic peptide-1 synthesis via the mitogen-activated protein kinase/extracellular signal-regulated kinase pathway. In our study, type 2 diabetic rats revealed 81% increase in plasma leptin value as compared to normal control. The administration of the PHF at dose level of 50, 100 and 200 mg/kg b. wt. for 28 days reduced the leptin levels by 13, 25 and 35% respectively. The results suggest that the PHF could improve insulin sensitivity by reducing the leptin levels.

TNF-α is an inflammatory marker which is secreted by several types of cells such as macrophages and monocytes. TNF-α is considered as a contributing factor in obesity, insulin resistance and pathogenesis of T2DM reported that the TNF-α level in

plasma is elevated in type 2 diabetes. In our study, the TNF-α level in type 2 diabetic rats was increased by 110% as compared to the normal control (Group I). Treatment with the PHF at dose level of 50, 100 and 200 mg/kg b. wt. for 28 days resulted in decrease in TNF-α level by 16, 29 and 46% respectively. Oleic acid, one of the major bio constituent of the PHF, may have played a role in reducing the elevated TNF-α level as reported.

In our study, plasma total protein levels were significantly decreased about 45% in type 2 diabetic rats as compared to the normal control (Group I). Similar reduction in plasma total protein level was reported earlier in diabetic rats. The decline of total protein in plasma could be due to increased protein catabolism, enhanced proteolysis and lowered protein synthesis. The reduction of total protein in plasma could also be due to progressive proteinuria followed by a gradual decline in renal function as reported. The administration of the PHF at dose level of 50, 100 and 200 mg/kg b. wt. for 28 days revealed increase in plasma protein by 38, 49 and 70% respectively. The administration of Emblica officinalis, one of the plant present in the PHF, is conveyed to increase the level of plasma total protein in rats.

The ALT, AST and ALP levels are consistent markers of liver function. In our study, the values of AST, ALT and ALP were increased by 130, 136 and 146% in diabetic rats at dose level 200 mg/kg b. wt., respectively when compared to normal control. The activities of ALT, AST and ALP were increased in plasma, which may be mostly due to the leakage of these enzymes from the liver cytosol, indicating the hepatotoxic effect of STZ as reported. Jaeschke et al reported similar elevation in ALT, AST and ALP in type 2 diabetic control rats. Ghosh et al reported that the gluconeogenesis and ketogenesis is increased in diabetic complications, which may be due to increased transaminase activity. The administration of the PHF revealed decreased about the AST, ALT and ALP values by 42%, 40% and 46% as compared to the diabetic control group respectively at highest dose level. The treatment with methanolic extract of leaves of Emblica officials Gaertn to diabetic rats is reported to have reduced the activities of ALT, AST and ALP. Similarly, AST and ALT activities were reduced in diabetic rats administered with Commiphora mukul gum resin. Furthermore, treatment with alcoholic extract of Momordica charantia also showed decrease in the AST, ALP and ALT activity in diabetic rats.

The serum urea and creatinine level increased in diabetic rats, which are considered as important markers for renal dysfunction. In our study, the serum urea and creatinine levels were increased by 157% and 281% respectively in type 2 diabetic rats as compared to the normal control. Diabetic rats treated with the PHF at dose level of 50, 100 and 200 mg/kg b. wt. for 28 days revealed decrease in serum urea levels by 14, 29 and 48% respectively. The creatinine level also showed significant decrease (19, 40 and 62%) in animals treated with the PHF in comparison with the diabetic control (Group II) in dose dependent manner. On the other hand, the administration of alcoholic extract of Momordica charantia in diabetic rats is reported to reduce the serum urea and creatinine levels. The administration of Trigonella foenum graecum seed extract to diabetic rats is reported to protect morphology and kidney function in diabetic rats, which may be due to antioxidant activity has reported protective effects Garcinia cambogia, one of the components of the PHF, against obesity-induced nephropathy. Furthermore, the administration of Curcumin, the active principle of Curcuma longa, another component of the PHF is reported to ameliorate nephropathy in diabetic rats.

The insulin sensitivity and insulin secretion is evaluated by homeostasis model assessment (HOMA) for possibility of DM. HOMA-IR and HOMA-B are assessed for insulin resistance and β-cell function respectively with high risk of DM. In our study, type 2 diabetic rats exhibited significant decrease in HOMA-B value whereas the HOMA-IR value exhibited a significant increase as compared to the normal control group. The administration of the PHF at dose level of 50, 100 and 200 mg/kg b. wt. for 28 days resulted in recovery in the levels of HOMA-B and HOMA-IR. The results of HOMA-B and HOMA-IR suggest that the PHF is capable of recovering the β-cell function as well as insulin resistance.

Oxidative stress, arbitrated mostly by hyperglycaemia-induced generation of free radicals is developing gradually DM and diabetic complication. Under normal conditions, $\bullet O_2^-$ is eliminated by antioxidant defence mechanisms. $\bullet O_2^-$ is dismutated to H_2O_2 by SOD. The H_2O_2 is converted into H_2O and O_2 by either GSH-Px or CAT. Johansen et al repotrted that the H_2O_2 and protects the tissues against hydroxyl radicals. GSH acts as a direct scavenger as well as a co-substrate for glutathione peroxidase. GPx is minimizing oxidative damage which works along with GSH for decomposition of H_2O_2 to other non-toxic products. Reduced activities of GPx may result from radical-induced inactivation and glycation of the enzyme. The SOD and CAT are well known to be inhibited in DM as a result of non-enzymatic glycosylation and oxidation. In our study, the activities of GPx, SOD, CAT and GSH in liver and kidneys were significantly decreased in type 2 diabetic rats.

The administration of the PHF at dose level of 50, 100 and 200 mg/kg b. wt. for 28 days resulted in significant rise in the activity of CAT, GPx, SOD and GSH in liver and kidney. Commiphora mukul gum resin, one of the components of the PHF, is known to increase the activities of CAT, GPx, SOD and GSH in the liver of diabetic rats. The administration of Azadirachta indica A. Juss is also reported to increase the antioxidant markers such as SOD, CAT, GPx and GSH in rat liver . The activities of SOD, CAT, GPx were recovered to the near normal level by the PHF indicating the efficacy of the PHF in attenuating the oxidative stress in diabetic liver. The TBARS in plasma/tissue is a marker for lipid peroxidation and it is to assess the amount of tissue damage. Numerous studies have reported that TBARS and hydroperoxides are increased in liver, kidney and plasma in diabetic rat. Our study also revealed increase in TBARS values in the liver and kidney of type 2 diabetic rats. The administration of the PHF for 28 days helped to reduce the TBARS values of liver and kidney. Aqueous extract of fruit of Emblica officinalis is reported to have radical scavenging activity and inhibit the lipid and protein oxidation in alcohol induced toxicity in rat. On the other hand, Bukan. reported that sulfonylurea glyburide is capable of employing

direct insulin-like effect on heart CAT, GPx and SOD activities of diabetic rats.

CONCLUSION

The efficacy study of the PHF revealed antidiabetic properties in the HFD-STZ induced type 2 diabetic rats. The PHF at 200 mg/kg b. wt. brought about significant decrease in blood glucose level and recovery in the HOMA-B and HOMA-IR levels in diabetic rats. The biochemical analyses such as HbA1c, insulin, total protein, ALT, AST, ALP, creatinine and urea are improved in diabetic rats. Anti-inflammatory markers like leptin and TNF-α and lipid profile of the diabetic rats also revealed marked recovery following 28 days treatment with the PHF. The antidiabetic activity of the PHF was found to be on a par with the positive control (GBL) at 200 mg/kg dose level. The study also revealed significant improvement in GSH, GPx, SOD and, CAT activities as well as TBARS value in liver and kidney of diabetic rats treated with the PHF indicating in vivo antioxidant activity of the PHF.

AUTHOR CONTRIBUTIONS

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CONFLICT OF INTEREST

No conflicts of interest.

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