RESEARCH ARTICLE

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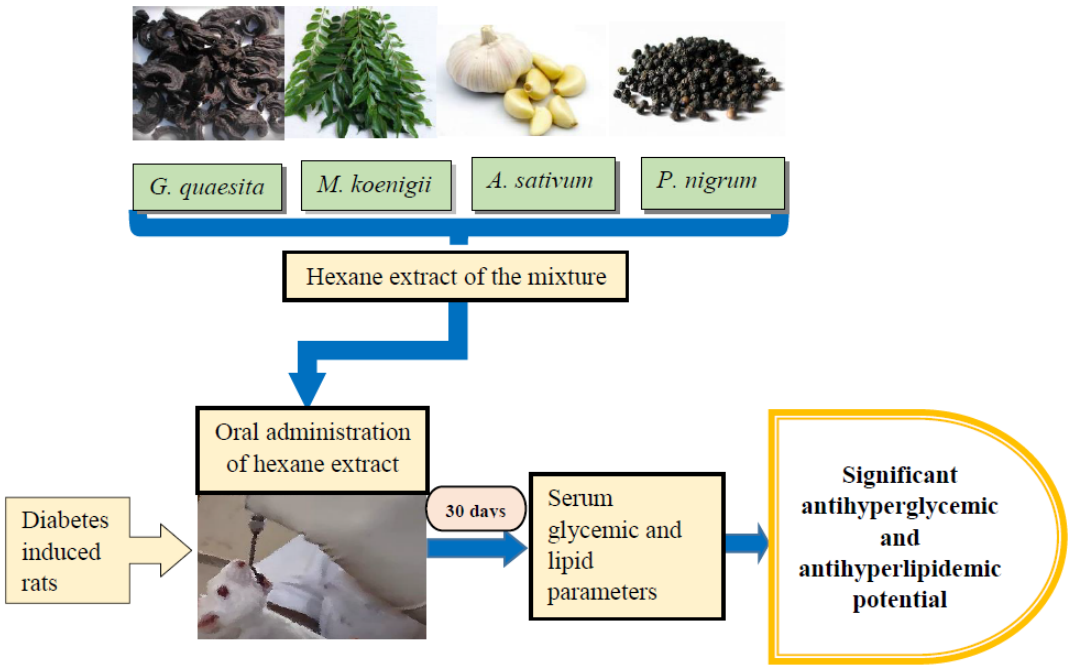

Highlights

- Hexane extract of the polyherbal mixture (*P. nigrum*, *M. koenigii*, *A sativum*, *G. quaesita*) showed significant antihyperglycemic potential.
- The hexane extract of the polyherbal mixture showed significant antihyperlipidemic effect on serum lipid parameters.
- Sub-acute treatment of the hexane extract of the polyherbal mixture reduced serum hyperglycemia and hyperlipidemia.

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RESEARCH ARTICLE

Sub-acute antihyperlipidemic and antihyperglycemic activity of the hexane extract of a polyherbal mixture in streptozotocin-induced diabetic rats

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Abstract: A folksy polyherbal remedy used in the traditional medicine system made of seeds of *Piper nigrum* L., leaves of *Murraya koenigii* L. Sprengel., cloves of *Allium sativum* L. and dried fruit rinds of *Garcinia quaesita* Pierre is considered as an efficient treatment for dyslipidaemia associated with diabetes mellitus by Ayurvedic medical practitioners in Sri Lanka. The effect of antihyperlipidemic and antihyperglycemic potential of the hexane extract (25 mg kg^{-1}) of the aforementioned polyherbal mixture during 30 days treatment in streptozotocin-induced diabetic rats was investigated. The oral administration of the hexane extract showed significant reduction of the glycated hemoglobin level (29.6%) and the fasting serum glucose concentration (34.3%) in addition to significant enhancement of the serum insulin (61.1%) in diabetic rats ($p < 0.05$). The hexane extract treated group showed significant antihyperlipidemic activity as evidenced by the reduction in serum total cholesterol (55.8%), low density lipoprotein cholesterol (75.0%), triglycerides (69.0%) and very low density lipoprotein cholesterol (55.8%) in diabetic rats ($p < 0.05$). The results of the present study demonstrated that the hexane extract of the polyherbal mixture possessed beneficial response on lipid and glycemic parameters, thus exhibiting its usefulness in developing a novel antihyperglycemic agent targeting the management of dyslipidemia associated the diabetes mellitus.

***Keywords*:** Diabetes mellitus; antihyperlipidemic; antihyperglycemic; hexane extract; polyherbal mixture.

INTRODUCTION

Mortality and morbidity due to dyslipidemia associated diabetes mellitus has been a crucial public health poser in Sri Lanka over the last two decades. Recent data of International Diabetes Federation reported that there were 88 million cases of diabetes in South-east Asia including Sri Lanka out of a total of 463 million cases in the world (International Diabetes Federation, 2019). This disease is made up of a group of metabolic complications characterized by hyperglycemia, in which blood glucose levels are raised either due to the cells not responding correctly to the insulin metabolism or due to β-cells not being able to produce adequate insulin or both (American Diabetic Association, 2009). Hence, diabetes consists of a group of clinically heterogeneous complications with a common set of symptoms such as weight loss, excessive thirst and hunger, surplus urination, muscular weakness, elevation of the lipid parameters and staircase of the blood glucose levels (Marles and Farnsworth, 1995).

According to the current guidelines, diabetes mellitus is being treated with oral hypoglycemic agents such as metformin, acetohexamide, rosiglitazone, glibenclamide, etc. (Bodmer *et al*., 1995). However, most of the hypoglycemic drugs which have been used during a long period in the management of the diabetes mellitus have recorded various complications and adverse side effects (Halim, 2003). The reported side effects of the most common uses of antidiabetic agents are nausea, cramps, diarrhea, peripheral edema, bladder cancer, heart failure and macular edema (Vallon *et al*., 2009; Rhee *et al*., 2017). Additionally, recent findings showed that in spite of the rapid advancement in the treatment of diabetic mellitus with these monotherapy drugs, they would only affect selected targets. Therefore, one of the common problems that the world is facing today is to find new drug leads that can be used as a combined therapy to enhance the antidiabetic activity and while minimizing the side effects synergistically to surpass the barriers associated with such combined drug therapy.

Recently, as a primary solution to minimize these drawbacks, Ayurvedic polyherbal preparations and commercialized mixtures used against the management of diabetes mellitus have received increasing attention in Sri Lanka. A homemade Ayurvedic remedy made of the seeds of *Piper nigrum* L. (family: Piperaceae), leaves of *Murraya koenigii* L. Sprengel (family: Rutaceae), cloves of *Allium sativum* L. (family: Liliaceae) and the dried fruit rinds of *Garcinia quaesita* Pierre (family: Clusiaceae) has been practiced for the treatments of dyslipidemia and hyperglycemia by Ayurveda medical practitioners in Sri Lanka (Ponnamperuma, 2000). Taken individually, all of the above plants or their parts have been investigated for antihyperlipidemic activity or antihyperglycemic activity or both. For an example, *P. nigrum* has shown high antioxidant potential leading to the control of lipid parameters and serum glucose levels in obese rats (Meriga *et al*., 2017). Jain *et al*. (2012) reported numerous pharmacological activities including the antihyperlipidemic and antidiabetic activity of *M. koenigii*. The plant extract of the *A. sativum*

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showed significant reduction in serum glucose, triglyceride and total cholesterol levels in diabetic rats (Eidi *et al*., 2006). There are few reports of antihyperglycemic and antihyperlipidemic effects of the *G. quaesita* in addition to a study on the antihyperglycemic activity of three selected extracts of the plant by our research group (Liyanagamage *et al*., 2020a). In addition, subchronic and acute toxicity profile of the hot water and water: acetone extract of above polyherbal mixture was also assessed and found that it is safe and effective for *in vivo* use (Liyanagamage *et al*., 2020b). Our research group further identified that the hot water and water:acetone extracts of the polyherbal mixture has a strong antihyperglycemic and antihyperlipidemic effects with significant restoration of pancreatic β-cells (Liyanagamage *et al*., 2020c). However, in a parallel preliminary study, the hexane extract of this polyherbal mixture was recognized as the most active extract among the hexane, ethyl acetate and methanol extracts, based on the results of *in vitro* antioxidant and *in vivo* acute antihyperglycemic studies. (Sampath *et al*., 2020). Since hexane is not typically used to extract bioactive compounds, the above results while being interesting, were unexpected. Hence in this extensive current research, we aimed to investigate the antihyperlipidemic and antihyperglycemic potential of the hexane extract of the polyherbal mixture during 30 days of treatment in streptozotocin induced diabetic rats.

MATERIALS AND METHODS

Chemicals and equipment

Streptozotocin (STZ) and polyvinylpyrrolidone (PVP) were purchased from Sigma-Aldrich, USA. All the spectrophotometric enzyme assay kits used in the current study were purchased from Stanbio (EKF Diagnostics), USA and enzyme-linked immunosorbent assay (ELISA) kits are acquired from DRG International, USA. All other general chemicals, reagents and solvents used in present study were in analytical grade. A microplate reader (Thermoscientific, Singapore) was used for the measurement of bioassay parameters. A rotatory evaporator (Heidolph, Germany) was used in the preparation of the hexane extract.

Plant materials

The seeds *P. nigrum*, leaves of *M. koenigii*, cloves of *A. sativum* and dried fruit rinds of *G. quaesita* were collected from Kandy, Sri Lanka, in April 2018 and the botanical specimens were identified by the National Herbarium, Royal Botanic Gardens, Peradeniya, Sri Lanka (Voucher No: 6/01/H/03).

Preparation of the plant extract

The hexane extract of the polyherbal mixture was prepared by grinding an equal amount (200 g) of each individual plant materials together and extracted into the hexane (three times a day) in a bottle shaker at room temperature (27 °C). The solvent was evaporated using a rotary evaporator. The sample was stored in a refrigerator at 0 °C until further used for the experiments.

Animals

Adult healthy albino Wistar rats (BW: 200 ± 20 g, 10 - 11 weeks of age) were purchased from the animal breeding unit of the Medical Research Institute (MRI), Sri Lanka. The Ethical Review Committee (Faculty of Medicine, University of Ruhuna, Galle, Sri Lanka.) granted ethical approval with the ref. no. 09.03.2016.3.8 for the use of animals in experiments. The rats were housed in an Animal Research Facility Centre of the Department of Biochemistry, Faculty of Medicine, University of Ruhuna, Sri Lanka. Animal handling was carried out according to the 3R principle of replacement, refinement and reduction involving accepted animal ethics. Free access was supplied to rats for standard pellet food and water *ad libitum* during their maintenance.

Development of diabetes mellitus

The adult male rats were kept fasting, overnight and then induced an intraperitoneal administration of a dose of 65 mg kg^{-1} of STZ drug, dissolved in freshly prepared buffer solution of citric acid/sodium citrate (0.1 M, pH 4.5) (Sharma and Gupta, 2017). After three days (72 h) of administration of STZ, the fasting blood/serum glucose concentration of experimental animals was evaluated following an enzyme assay protocol under the method of glucose-oxidase (Biorex diagnostics, UK). The rats with a fasted serum glucose concentration of > 11.1 mmol/L were considered as in a state of hyperglycemic condition and were used in the present experiments (Vasconcelos *et al*., 2011).

Experimental design

The experimental rats were allocated into four groups (n = 6/group). Group one and two were considered as healthy untreated and diabetic untreated rats, respectively. The rats with fasting serum glucose concentration between 4 - 5.6 mmol L^{-1} were considered as healthy rats and were used in the present experiment. Both groups of rats received standard animal food and water orally daily for 30 days. The plant extract at the optimum effective dose (25 mg kg^{-1}) dissolved in 50% PVP and glibenclamide (0.5 mg kg^{-1}), which served as the positive control, were administered orally to diabetic rats for 30 days in the group three and four, respectively. The selected dose of plant extracts was determined based on dose response (8, 25, 75 mg kg^{-1}) study of STZ-induced diabetic rats. During the experimental period, body weight of treatment and control groups rats were measured and oral glucose tolerance test (OGTT) was performed in all groups on the 1st, 7th, 14th, 21st and 28th day. Body weight increment of each group was calculated using the following formula. All overnight fasted experimental rats were euthanized using diethyl-ether in a desiccator on the 30th day of experiment and fasting blood was collected by cardiac puncture. These blood samples were used to determine serum lipid parameters and further analysis on serum glycemic parameters of the experimental rats.

$$\text{Increment in body weight} = \frac{(\text{Final body weight} - \text{Initial body weight}) \times 100}{\text{Initial body weight}}$$

Sub-acute antihyperglycemic activity of plant extract of the polyherbal mixture in diabetic rats

The effect of plant extract on oral glucose tolerance in diabetic rats

The oral glucose tolerance test was performed for overnight (12 h) fasted rats in each group on the 1st day and after 1st, 2nd, 3rd and 4th weeks after the commencement of the experiment. In the OGTT, a glucose solution (3.00 g kg^{-1}) was administered orally to rats in the four groups (group 1; healthy control, group 2; diabetic control, group 3; plant extract, group 4; glibenclamide) 30 min after the administration of plant extract/glibenclamide. Blood samples were collected from the tail tip of the tail vein at 0 (fasting, prior to glucose and drug administrations), 60, 120, 180 and 240 min after the administration of hexane extract and glibenclamide to STZ-induced diabetic rats. The blood glucose concentration was determined using a spectrophotometric assay kit (Biorex diagnostics, UK). The effect was analyzed through total area under the curve (TAUC) values. Area under the curve values were calculated with the formula in the trapezoidal method (Purves, 1992).

Determination fasting serum glucose (FSG) in experimental rats

The FSG concentration of all experimental rats was estimated using the assay kit mentioned in OGTT according to the glucose-oxidase method followed by Trinder (1969) with slight modification for the blood samples collected on the 30th day. In this method, the 100 µL of serum sample was added to the 1.0 mL of glucose working reagent and mixed using a vortex. The mixture was incubated at room temperature for 10 minutes and the absorbance was measured at 500 nm using a UV-visible spectrophotometer. The same procedure was followed for the standard solution. The blood glucose concentration was calculated using the following formula.

$$FSG = [A_{Sample} / A_{Standard}] \times \text{concentration of the standard glucose (5.55 mmol } L^{-1})$$

Estimation of glycated hemoglobin (HbA_{1C}) in diabetic rats

Effect of hexane extract of the polyherbal mixture on HbA_{1C} percentage in diabetic rats was estimated using an enzymatic assay kit (Stanbio Laboratory, USA) for the blood samples collected on the 30th day. The ion exchange resin method was used to estimate the percentage of glycated hemoglobin as described by Abraham *et al*. (1978).

Estimation of serum C-peptide concentration

Serum C-peptide concentration in tested animals were estimated using a solid phase ELISA kit (DRG International, USA) based on the principle of competitive binding. In this method, a standard curve was constructed by plotting the mean absorbance against its concentration with absorbance value on the y-axis and concentration on the x-axis and the logistic model, $\{y = - 0.01 + [0.91 - (- 0.01)] / [1 + (x / 1.60)]^{0.66}, R^2 = 0.9873\}$ was developed to determine unknown C-peptide concentration in the serum sample.

Estimation of serum insulin concentration

A solid phase ELISA kit (DRG International, USA) based on the sandwich principle was followed to determine the insulin concentration in serum as described by the method according to Frier *et al.* (1981). In the protocol, a standard curve was constructed by plotting the mean absorbance obtained from each standard against its concentration (0, 6.25, 12.5, 25, 50 and 100 µI mL^{-1}) with absorbance value on the y-axis and concentration on the x axis. The insulin concentration of the serum sample was determined using the regression equation ($y = 0.0181, x - 0.1, R^2 = 0.9567$). Using the insulin concentration and FSG concentration of the serum samples obtained on the 30th day of study, pancreatic islet activity was evaluated under the homeostasis model assessment. The homeostasis model assessment for insulin resistance (HOMA-IR) and homeostasis model assessment for β-cell function (HOMA-β) were calculated according to following formulas described by Mattews *et al*. (1985).

$$\text{HOMA-IR} = [\text{fasting glucose (mmol } L^{-1}) \times \text{fasting insulin } (\mu U\ mL^{-1})] / 22.5$$

$$\text{HOMA-}\beta = [20 \times \text{fasting insulin } (\mu U\ mL^{-1})] / [\text{fasting glucose (mmol } L^{-1}) - 3.5]$$

Sub-acute antihyperlipdemic activity of the plant extract of the polyherbal mixture in diabetic rats

The serum lipid parameters, total cholesterol (TC), triglyceride (TG) and high density lipoprotein cholesterol (HDL-C) were estimated with spectrophotometric enzyme assay kits according to the previously described methods for the blood samples collected on the 30th day of experiment (Finley *et al*., 1978; Fredrickson *et al*., 1967). The serum very low density lipoprotein cholesterol (VLDL-C = 0.2 TC), low density lipoprotein cholesterol [LDL-C = TC – HDL-C – (0.2 TG)], atherogenic index of plasma [AIP = Log_{10} (TG/HDL-C)], coronary risk index (CRI = TC/ HDL-C) and cardio protective index (CPI = HDL-C / LDL-C) were calculated according to equations reported by Freidewald *et al*. (1972) and Jemila *et al*. (2017).

Statistical analysis of data

The data are represented as mean ± SEM (standard error value of the mean) and statistical analysis of the data was done by one-way ANOVA followed by Dunnett's post hoc test using the SPSS software (version 22). Significant difference was accepted when p value was less than 0.05 ($p < 0.05$).

RESULTS AND DISCUSSION

The present study evaluates the antihyperlipidemic and antihyperglycemic potential of the plant extract of the polyherbal mixture composed of seeds of *P. nigrum* L, leaves of *M. koenigii* L. Sprengel., cloves of *A. sativum* L. and dried fruit rinds of *G. quaesita* Pierre in STZ-induced diabetic rats. The application of STZ to develop diabetic model is widely recommended and the diabetic rat model often resembles human diabetes conditions as reported

by weight loss, hyperglycemia, glycosuria and polydipsia (Kumar *et al*., 2010). According to Jacobson *et al*. (2010), the STZ administered diabetes is correlated with weight loss in rodent models due to proteins being utilized in a condition of carbohydrate inaccessibility during energy production. The effects of the plant extract on the body weight in the STZ - induced diabetic rats were analyzed under present investigation and results are graphically shown in Figure 1. All groups at the first day of experiment showed no apparent difference in body weight compared to the normal control group. However, there was a 34%, 9%, 23% increment of body weight observed in healthy untreated, plant extract and glibenclamide treated groups, respectively while reduced the body weight of diabetic control rats by 13% on 28th day when compared to the initial body weight. In contrast, the body weight of the diabetic untreated group was significantly reduced at the 28th day compared to the healthy untreated group ($p < 0.05$). When diabetic rats were treated with the plant extract for 30 days, glycemic control was significantly enhanced and this might lead to improvement of the body weight of diabetic rats as in the case of healthy untreated rats.

Sub-acute antihyperglycemic activity of the plant extract of the polyherbal mixture in diabetic rats

In the present research work, antihyperglycemic activity of

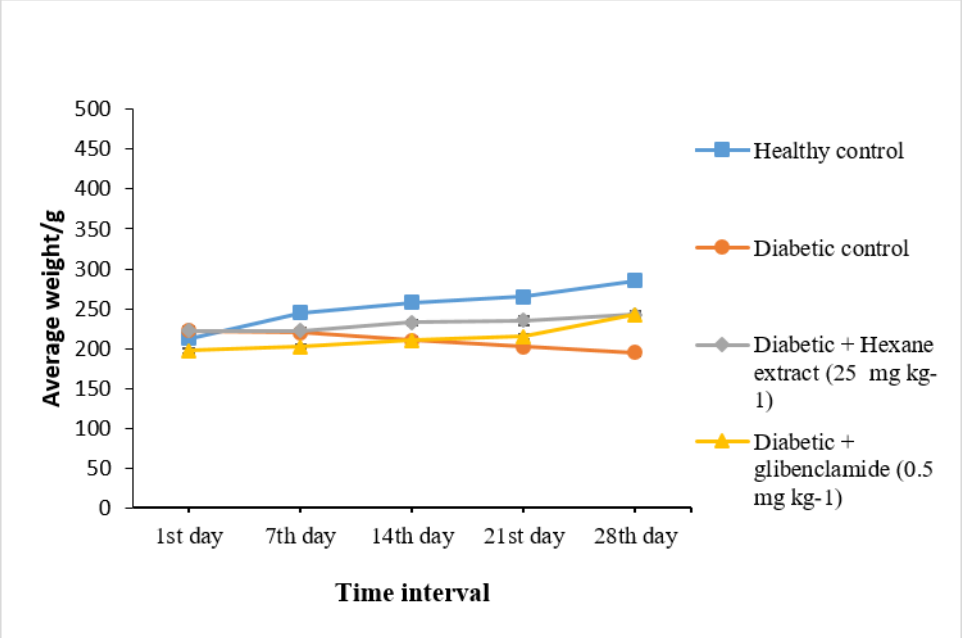

Figure 1: Effect of plant extract on body weight of STZ-induced diabetic rats. The values are expressed as mean ± SEM (n = 6/group). *Significant difference with healthy control group ($p < 0.05$).

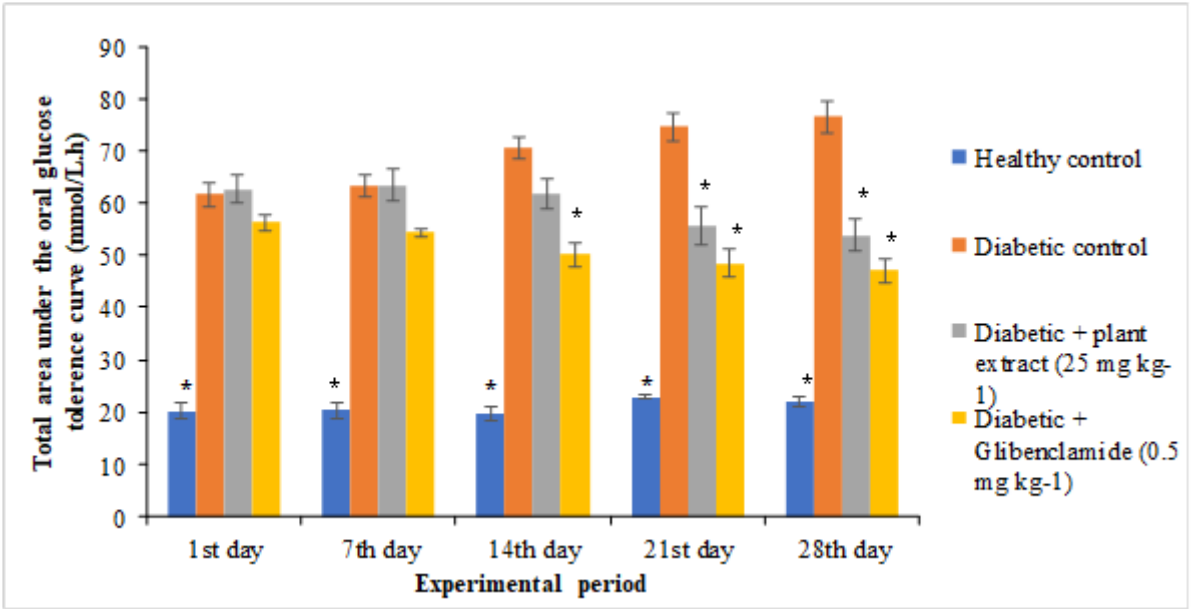

Figure 2: Effect of the plant extract on the total area under the oral glucose tolerance curve values in streptozotocin induced diabetic rats at weekly intervals. Each data point is expressed as the mean ± SEM (n = 6/group). *Significant difference with diabetic control ($p < 0.05$).

the plant extract of the polyherbal mixture was assessed in the oral glucose tolerance, FSG concentration, serum HbA_{1C} percentage, serum insulin concentration, C-peptide concentration through the homeostasis model in diabetic rats. The TAUC values of the treated groups with the plant extract (25 mg kg^{-1}) and glibenclamide (0.5 mg kg^{-1}) and the untreated groups of rats in the healthy control and the diabetic control at weekly intervals are shown in Figure 2. The low value in TAUC reflects enhancement or high efficacy in oral glucose tolerance in the tested group and *vice versa*. There was a reduction in TAUC values in healthy rats when compared to the diabetic rats at weekly intervals. The extract of the polyherbal mixture at the dose of 25 mg kg^{-1} showed a statistically significant enhancement by 25% of the oral glucose tolerance starting on the 21st day of experiment when compared to diabetic control group ($p < 0.05$). Moreover, the oral administration of the plant extract and glibenclamide, lowered the TAUC values by 30% and 38%, respectively and these values were statistically significant with the TAUC value of the diabetic untreated group ($p < 0.05$) on the 28th day of experiment. These results therefore indicate that the plant extract has significant oral glucose tolerance potential in diabetic rats during the 30 days treatment period. A feasible mechanism for the antihyperglycemic activity might be due to increased streaming of glucose to the outlying/peripheral tissue or through enhancement of insulin secretion from pancreatic beta cells.

The effect of plant extract on the serum glycemic parameters which were analyzed in current study, namely serum insulin, serum C-peptide, percentage of HbA_{1C} and FSG concentrations in streptozotocin induced diabetic rats are shown in Table 1. The insulin hormone is the major constituent that which affects regulation of blood glucose concentration directly in the body. According to Horwitz *et al.* (1975), the C-peptide concentration is a more accurate indicator of insulin secretion because insulin level changes rapidly. The present results show that the administration of glibenclamide and the plant extract of the polyherbal mixture increased the serum fasting insulin concentration and the C-peptide concentration in diabetic rats than in the untreated diabetic control rats. The plant extract therefore would be effective in increasing the secretion of insulin through the β-cell islets and is further corroborated by the serum C-peptide concentration in the plant extract treated diabetic rats. Furthermore, the insulin activity was confirmed *via* the homeostasis model for insulin resistance (HOMA-IR) and for β-cell function (HOMA-β) (Mattews *et al*., 1985). The determinations of HOMA-β and HOMA-IR in experimental rats are shown in Table 2. A statistically significant enhancement in the β-cell function was found for the glibenclamide (79.5%) and the hexane extract (75.9%) treated groups compared to the diabetic control group ($p < 0.05$). In contrast, the HOMA-IR index of the glibenclamide and the plant extract was lowered by 25.4% and 23.4%, respectively, when compared to the diabetic control group.

In the present evaluation, the antihyperglycemic effect of the plant extract of the polyherbal mixture was also evaluated using the percentage of HbA_{1C}. The HbA_{1c} is considered as the gold standard assay, which is a benchmark or diagnostic test (Sehrawat *et al*., 2018). After the induction of diabetes mellitus in diabetic rats, blood glucose level was uplifted due to insufficient insulin synthesis and/or malfunction of pancreatic β-cells (Noor *et al*., 2017). Hence, the increase of the HbA_{1c} within the red blood cells correlates to the average level of glucose to which the cell has been exposed during its lifespan. The percentage of HbA_{1c} was important to determine the efficiency of oral administration of the plant extract of the polyherbal mixture by monitoring subacute serum glycemic regulation in the current study. The diabetic rats who were treated with the plant extract of the polyherbal mixture and glibenclamide, exhibited a statistically significant reduction in percentage of HbA_{1C} on the 30th day ($p = 0.01$). Glycated hemoglobin was found to increase in uncontrolled diabetes and the increase is directly proportional to the fasting serum glucose level. The treatment of the plant extract and glibenclamide significantly lowered the fasting serum glucose concentration in streptozotocin induced diabetic rats by 34.3% and 40.7%, respectively on the 30th day ($p < 0.05$). The plant extract of the selected polyherbal mixture, therefore, has the potential to minimize the binding ability of glucose molecules to hemoglobin in erythrocytic cells by decreasing the glucose concentration in the blood. These serum glycemic parameters, namely serum insulin, serum C-peptide, percentage of HbA_{1C} and FSG concentrations, therefore, they described well the antihyperglycemic

Table 1: Effect of the plant extract on glycemic parameters in diabetic rats.

Glycemic parameter/ Index	Healthy control rats	Diabetic control rats	Diabetic + plant extract (25 mg/ kg)	Diabetic + Glibenclamide (0.5 mg/kg)
HbA_{1C}%	5.09 ± 0.37	9.22 ± 0.64	6.49 ± 0.40[a]	5.97 ± 0.27[a]
FSG (mmol/L)	5.30 ± 0.31	14.04 ± 0.92	9.23 ± 0.99[a]	8.32 ± 0.49[a]
Serum Insulin (µIU/mL)	15.32 ± 0.69	8.39 ± 0.15	13.52 ± 0.73[a]	13.94 ± 0.76[a]
Serum C-peptide (ng/mL)	4.80 ± 1.31	1.60 ± 0.20	3.06 ± 0.65	3.29 ± 0.85
HOMA-IR	3.40 ± 0.24	6.46 ± 0.57	4.95 ± 0.79	4.82 ± 0.25
HOMA-β	167.18 ± 0.80	16.34 ± 0.77	51.46 ± 1.31[a]	60.33 ± 1.17[a]

The values are expressed as mean ± SEM (n = 6/group). Data were analyzed by ANOVA followed by Dunnett's post hoc test. a- significant difference of treated groups with diabetic control at $p < 0.05$.

activity of the selected polyherbal mixture. STZ causes hyperglycemia by selective reduction of β-cell amount, which directly leads to a depletion of insulin release (Husain *et al*., 2009). Reduction of insulin synthesis could compensate in disordered control of glucose by minimizing the efficiency of glucose utility in insulin-sensitive tissues and lessening suppression of hepatic glucose synthesis. Minimized insulin also could result reduction of adipocyte metabolism, causing in increased fatty acid level and elevated lipolysis (Kahn *et al*., 2006). It is well known that standard drug of glibenclamide normalize hyperglycemia condition by elevating the synthesis and secretion of insulin from the surviving pancreatic β-cells (Proks *et al*., 2002). The antidiabetic effect of plant extract is usually dependent upon the capacity of β-cell demolition. Treatment in chemically induced diabetic rats with some medicinal plant extracts affected on the stimulation of β-cells function (Atta-ur-Rahman and Zaman, 1989; Grover *et al*., 2000). In view of present investigation, antidiabetic effect of the plant mixture of selected polyherbal mixture may be due to potentiation of insulin synthesis and secretion from existing β cells in Langerhans. In addition, the antihyperglycemic effect of plant extract would be due to the reported availability of a higher number of antidiabetic compounds such as mahanimbine, isomahanimbine, koenimbidine, murrayacine and koenimbine, myricetin, diallyl sulfide, diallyl disulfide, diallyl trisulfide, piperine and garcinol present in the selected plants and the synergism of the active phytoconstituents (Dineshkumar *et al*., 2010; Handral *et al*., 2012; Padiya and Banerjee, 2013; Park *et al*., 2016; Jain *et al*., 2017; Radi´c *et al*., 2019; Liyanagamage *et al*., 2020a).

Sub-acute antihyperlipdemic activity of the plant extract of the polyherbal mixture in diabetic rats

Dyslipidemia is associated with elevated TC, TG, VLDL-C and LDL-C and lowered HDL-C levels than expected normal levels. These changes can be attributed to an improvement of cardiovascular diseases in diabetic patients. According to Liu *et al*. (2015), HDL-C negatively and LDL-C positively could be associated with the complications of coronary heart diseases. In the present evaluation, the effect of the plant extract of the polyherbal mixture on serum lipid parameters after treatment during 30 days in streptozotocin-induced diabetic rats are shown in Table 2. The serum TC, LDL-C, TG, and VLDL-C concentrations were significantly increased by 60.5%, 81.9%, 71.3 % and 60.5%, respectively in diabetic rats after the induction of diabetes mellitus ($p < 0.05$). The administration of the plant extract and glibenclamide showed efficient reduction of serum concentration of TC (55.8%, 49.1%), LDL-C (75.0%, 63.7%), TG (69.0%, 66.0%), and VLDL-C (55.8%, 49.1%) in diabetic rats. The HDL-C concentrations were elevated in the plant extract treated group by 28.9% when compared with the diabetic control group. Furthermore, the plant extract treated groups showed a rise of CPI (91%) and a depreciation of AIP (82.8%) and CRI (69.9%) in diabetic rats. Lower AIP and CRI values reflect a lower cardiovascular risk and *vice versa* (Adaramoye. and Akanni, 2014). The CPI index represents protection against coronary risk factors and the higher the value of CPI, the greater is the protecting effect against cardiovascular diseases (Attanayake *et al*., 2018). These indices therefore indicate that the plant extract of the polyherbal mixture could have a potent cardio protective activity in diabetic conditions. The oral administration of the plant extract therefore restored the serum lipid parameters significantly at the end of the intervention by lowering the serum TC, LDL-C, TG, VLDL-C levels while by increasing HDL-C level in diabetic rats and this leads to control cardiovascular outbreaks, which are associated with diabetes mellitus. Chemically-induced diabetes mellitus

Table 2: Effect of the plant extract of the polyherbal mixture on lipid parameters in diabetic rats.

Lipid parameter/Index	Healthy control rats	Diabetic control rats	Diabetic + plant extract (25 mg/ kg)	Diabetic + Glibenclamide (0.5 mg/kg)
TC (mg/dl)	78.91 ± 0.97	199.64 ± 0.30	88.28 ± 0.42[a]	101.57 ± 0.81[a]
TG (mg/dl)	59.74 ± 0.85	208.11 ± 0.85	64.41 ± 0.45[a]	70.80 ± 0.72[a]
HDL-C (mg/dl)	43.98 ± 0.69	31.03 ± 0.87	43.65 ± 0.52	35.51 ± 0.11
VLDL-C (mg/dl)	15.78 ± 0.59	39.93 ± 0.26	17.66 ± 0.08[a]	20.31 ± 0.16[a]
LDL-C (mg/dl)	22.99 ± 0.58	126.98 ± 0.13	31.75 ± 0.86[a]	46.16 ± 0.33[a]
AIP	0.15 ± 0.07	0.83 ± 0.07	0.14 ± 0.05a	0.21 ± 0.10a
CRI	1.85 ± 0.14	6.64 ± 0.75	2.00 ± 0.29a	2.24 ± 0.19a
CPI	1.92 ± 0.25	0.25 ± 0.03	2.75 ± 0.42	1.31 ± 0.31

Each data point is expressed as the mean ± SEM ($n = 6$/group). Same letter in the same raw indicate significant difference at $p < 0.05$ for lipid parameter of treated groups vs diabetic control group. TC : total cholesterol; TG : triglycerides; HDL-C : high density lipoprotein cholesterol; VLDL-C: very low density lipoprotein cholesterol; LDL-C : low density lipoprotein cholesterol; AIP :atherogenic index of plasma; CRI : coronory risk index; CIP : cardio protective index.

is connected with singed imbalance in lipid metabolism (Gadi and Samaha, 2007). Present hypolipidemic effect of the selected polyherbal mixture in STZ-induced diabetic rats is consonant with previous investigations conducted by our research group (Liyanagamage *et al*., 2020c).

CONCLUSION

Administration of the hexane extract of the polyherbal mixture made of seeds of *P. nigrum* L., leaves of *M. koenigii* L. Sprengel., cloves of *A. sativum* L. and dried fruit rinds of *G. quaesita* Pierre for 30 days showed a significant antihyperglycemic activity with a decrease in serum glucose concentration and percentage of glycated hemoglobin while elevating serum insulin and C-peptide concentration in diabetic rats. Furthermore, the plant extract of the selected polyherbal mixture showed beneficial effects in parameters such as body weight, oral glucose tolerance and lipid profiles. The hexane extract, unlike water or water : acetone extracts, would seem not feasible to emerge as a homemade Ayurvedic remedy. However the results obtained in this study is novel and warrants disclosure to the scientific community perticulary as a validation and scientific scrutinization of the use of the above plant mixture in the treatment of dyslipidemia associated diabetes mellitus. Additionally, these data suggested that plant extract of the above polyherbal mixture might be useful in developing novel therapeutic agents for diabetes mellitus and dyslipidemia, and could also be used as a potential source to isolate natural antidiabetic and antihyperlipidemic agents.

DECLARATION OF CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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