IJPSR (2020), Volume 11, Issue 11 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 06 February 2020; received in revised form, 12 October 2020; accepted, 17 October 2020; published 01 November 2020

EVALUATION OF ANTIHYPERGLYCEMIC ACTIVITY OF THE POLYHERBAL AND ALLOPOLYHERBAL FORMULATION ON NICOTINAMIDE-STREPTOZOTOCIN-INDUCED TYPE-2 DIABETIC RATS

S. N. Belhekar [1, * 2], P. D. Chaudhari [3] and R. L. Jadhav [2]

Centre for Research & Development [1], PRIST University, Vallam, Thanjavur - 613403, Tamil Nadu, India.
Gourishankar Institute of Pharmaceutical Education & Research [2], Limb, Satara - 415020 Maharashtra, India.
Modern College of Pharmacy [3], Yanuna Nagar, Nigdi, Pune - 411044, Maharashtra, India.

Keywords:

Supercritical CO_2 extracts, Antihyperglycemic, Polyherbal, Allopolyherbal, Hyperglycemia, Streptozotocin and Nicotinamide

Correspondence to Author:
Dr. Santosh Nivrutti Belhekar

Associate Professor,
Department of Pharmacology,
Gourishankar Institute of Pharmaceutical Education & Research, Survey No. 990, Near NH4 Highway, Limb, Satara - 415020, Maharashtra, India.

E-mail: santoshbelhekar@rediffmail.com

ABSTRACT: Amla, Arjuna, Gudmar, Guduchi, and Ginger have been usually used in the traditional system of medicine for the treatment of diabetes. The aim of the present study to evaluate antihyperglycemic activity of polyherbal and allopolyherbal formulation on Streptozotocin-Nicotinamide (STZ-NA) induced type-2 diabetes rats. All the extracts of herbs used for the present study were obtained by supercritical fluid extraction. The Oral glucose tolerance test (OGTT) and anti-hyperglycemic activities were performed on STZ-NA-induced diabetic rats. The effects of metformin, PHF, and allopolyherbal formulation (APHF) on plasma insulin, hemoglobin, liver glycogen, and glycated hemoglobin (HbA1c) were analyzed. Statistical analysis was done by using one-way ANOVA followed by Dunnett's multiple comparison tests. The OGTT of metformin, PHF, and APHF showed increased glucose tolerance. Single-dose anti-hyperglycemic activity of metformin, PHF, and APHF showed a significant ($P<0.01$) decrease in blood glucose level (BGL) in diabetic rats at 1st, 3rd, 5th, and 7th h. Multiple doses anti-hyperglycemic activity of metformin, PHF, and APHF showed significantly ($P<0.01$) reduction of BGL in diabetic rats after 14th, 21st, and 28th days of treatment. After treatment with metformin, PHF, and APHF for 28 days, there was a significant ($P<0.01$) decrease in HbA1c and a significant ($P<0.01$) increase in plasma insulin, hemoglobin, and liver glycogen levels in treated diabetic rats. Histopathology of the pancreas in treated groups showed the protection of β-cells of the pancreas. It is concluded that the simultaneous administration of polyherbal formulation and allopathic drugs increased the therapeutic effect, reduced dose and reduced side effects.

INTRODUCTION: Diabetes mellitus (DM) is a chronic endocrine metabolic disorder was characterized by chronic hyperglycemia, polyuria, polydipsia, polyphagia, emaciation, and weakness due to the disturbance in carbohydrate, fat and protein metabolism associated with an absolute or relative deficiency in insulin secretion and/or insulin action [1]. The disorder can be classified into type 1 DM and type 2 DM.

The latter accounts for more than 90% of all diabetes cases and is caused mainly by peripheral insulin resistance and impaired insulin secretion and largely the result of excess body weight and physical inactivity [2]. The total number of people with diabetes will be projected to rise from 171 million in 2000 to 366 million in 2030 [3]. WHO also projects that 9% of adults in the world suffer from

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.11(11).5812-22

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.11(11).5812-22

diabetes and this disease will be the 7th leading cause of death in 2030. According to recent data, 62.4 million Indians have been reported to have diabetes, and the figures are expected to reach 87 million by the year 2030 [4]. Treatment with sulfonylureas, biguanides, and insulin possesses undesirable side effects, so the management of diabetes without side effects is yet a challenge to the medical system. There is just an increasing demand to use natural products with anti-diabetic activity. Plants are useful sources for the development of antidiabetic drugs [5].

The concept of polyherbalism has been highlighted in Sharangdhar Samhita, Ayurvedic literature dating back to 1300 AD. When combining multiple herbs in a particular ratio, it will give a better therapeutic effect and reduce the toxicity [6]. The PHF enhances therapeutic action and reduces the concentrations of single herbs, thereby reducing adverse events. In the modern era, herbal formulations have gained greater importance than ever before, mainly due to their efficacy and easy availability as well as fewer side effects as compared to synthetic drugs [7].

WHO also estimated that 80% of the world's people still rely mainly on traditional medicines for their health care. The subcontinent of India is well-known to be one of the major biodiversity centers with about 45,000 plant species [8]. Plenty of herbs have been reported to possess antidiabetic effects. Many of these herbs have been demonstrated to have hypoglycemic effects in animal studies or clinical trials. Also, a number of them, such as Amla (*Emblica officinalis*) [9-11], Gudmar (*Gymnema sylvestre*) [12, 13], Arjuna (*Terminalia arjuna*) [14, 15], Guduchi (*Tinospora cordifolia*) [16, 17] and Ginger (*Zingiber officinale*) [18, 19] are well-known herbs available throughout India.

They are widely used for the treatment of various diseases, including diabetes and diabetic complications. These herbs have been proven scientifically for their anti-hyperglycemic, anti-hyperlipidemic, anti-oxidant, nephroprotective, cardio-protective activities and *etc.* They have different mechanisms of action, therefore, selected for the proposed study. All the extracts of herbs used for the present study were produced by supercritical fluid extraction (SFE) by a supercritical fluid such as CO_2 and co-solvent ethanol. The CO_2 extracts are pure, highly powerful, broad-spectrum, and extremely concentrated. The SFE is utilized on a large scale for the extraction of some food grade and essential oils and pharmaceutical products from plants [20]. The SFE allows the processing of plant material at low temperatures, hence limiting thermal degradation and prevent the use of toxic solvents [21].

The antidiabetic activity of these individual herbs is well known, but the synergistic or combined effects are unclear. The aim of the present study is to prepare a polyherbal formulation of CO_2 extract and evaluate its antihyperglycemic activity in the STZ-NA-induced diabetic rats, and to check PHF treatment could have a synergistic effect with metformin. The metformin is the most common drugs and routinely used in the treatment of types 2 DM, so selected for the present study.

MATERIALS AND METHODS:

Drugs and Chemicals: Streptozotocin (STZ) was procured from Chemvenio, LIC, Gulbarga, Karnataka, Nicotinamide (NA) was procured from SDS Neutraceuticals, Karad and Metformin tablet was procured from USV Pharma, Mumbai. GOD-POD Kit was procured from Span Diagnostics, Surat, Glucometer was procured from Accu-Check, Roche Diagnostics India, Pvt Ltd. All other chemicals used in this study were of analytical grade.

Holistic Extracts: All the extracts were obtained by supercritical fluid extraction, and they are procured from Nisarga Biotech Pvt. Ltd, Satara, Maharashtra, as a gift sample.

Experimental Animals: Albino Wistar rats of either sex weighing 180-200 g ± 20 were procured from Shri Venkateshwara Enterprises, Banglore. All animals were maintained under standard laboratory conditions of temperature (22 ± 2 °C) and humidity 50 ± 15% with 12 h a day and 12 hours night cycle. Rats had free access to water and rodent pellet diet (Hindustan Lever Ltd, Bangalore, India). Animals were acclimatized to laboratory conditions one week prior to initiation of experiments. The experimental protocol has been approved by the Institutional Animal Ethics Committee of the Satara College of Pharmacy,

Satara (protocol approval No. is SCOP/IAEC/2011-12/38), and all the animal experiments were carried out according to CPCSEA guidelines [22].

Preparation of Polyherbal Formulation: The polyherbal formulation was prepared by mixing five holistic extracts by taking equal quantity and adding 1% CMC solution as a surfactant, continuously triturating till uniform suspension was formed. The quality of the finished product was evaluated as per the WHO guidelines for the quality control of herbal materials.

Supercritical Fluid Extraction: The supercritical fluid extraction was done by CO_2 and co-solvent ethanol; in supercritical CO_2 extraction, the critical temperature was 31.1 °C and pressure 73.8 bar. The holistic extracts were prepared by holistic blending technology; in this technology, high-quality organic CO_2 extracts, raw herb, and silica were mixed together in the right proportion to produce a blend of the herb, and this blend is known as a holistic extract. The obtained extracts were pure, potent, and finest quality blend in a powder form [20, 21].

Acute Oral Toxicity of Polyherbal Formulation: Acute oral toxicity of the polyherbal formulation (PHF) was carried out as per the revised OECD guidelines 423. The principle involves a stepwise procedure with the use of a minimum number of animals per step to obtain sufficient information on the acute toxicity of the test substance to enable its classification.

Healthy albino Wistar female rats (3 animals/dose) were used for the experiment. Overnight fasted rats were orally fed with a polyherbal formulation in increasing dose levels of 5, 50, 300, and 2000 mg/kg body weight, respectively. The animals were observed for their behavioral (alertness, restlessness, irritability, and fearfulness), neurological (spontaneous activity, reactivity, touch response, pain response, and gait), and autonomic (defecation and urination) profiles continuously for 24 h. After a period of 24 h, the animals were observed for 14 days for mortality [23].

Induction of Types 2 DM: Type 2 DM was induced in overnight fasted adult albino Wistar rats by a single intraperitoneal injection of 65 mg/kg streptozotocin (STZ) 15 min after the i.p. administration of 110 mg/kg of nicotinamide (NA). The STZ was dissolved in citrate buffer (pH 4.5), and NA was dissolved in normal saline. Hyperglycemia was confirmed by the elevated glucose levels in plasma, determined at 72 h and then on day 7 after injection. The rats found with permanent types 2 DM, and blood glucose concentration more than 250 mg/dl was used for the study [24-28].

Experimental Design for Hypoglycemic Study: In the experiment, a total of 36 healthy rats were used. The albino Wistar rats were divided into six groups (n=6). They are as follows.

Group I: Healthy rats treated with vehicle (1% CMC solution, 10 ml/kg, p.o.),

Group II: Healthy rats treated with Metformin (200 mg/kg/day, p.o.)

Group III: Healthy rats treated with polyherbal formulation (100 mg/kg/day, p.o.)

Group IV: Healthy rats treated with polyherbal formulation (200 mg/kg/day, p.o.)

Group V: Healthy rats treated with polyherbal formulation (400 mg/kg/day, p.o.)

Group VI: Healthy rats treated with Allopolyherbal formulation (Metformin 100 mg/kg + polyherbal formulation (100 mg/kg/day, p.o.)

Experimental Design for OGTT and Anti-hyperglycemic Study: In the experiment, a total of 42 rats (36 diabetic rats, 6 healthy rats) were used. The albino Wistar rats were divided into seven different groups of six animals, each as follows.

Group I: Healthy rats treated with vehicle (1% CMC solution, 10 ml/kg/day, p.o.)

Group II: Diabetic rats treated with vehicle (1% CMC solution, 10 ml/kg/day, p.o.)

Group III: Diabetic rats treated with Metformin (200 mg/kg/day, p.o.)

Group IV: Diabetic rats treated with polyherbal formulation (100 mg/kg/day, p.o.)

Group V: Diabetic rats treated with polyherbal formulation (200 mg/kg/day, p.o.)

Group VI: Diabetic rats treated with polyherbal formulation (400 mg/kg/day, p.o.)

Group VII: Diabetic rats treated with Allopolyherbal formulation (Metformin 100 mg/kg + polyherbal formulation (100 mg/kg/day, p.o.)

OGTT in Diabetic Fasted Rats: The OGTT was performed on STZ-NA-induced diabetic fasted rats. Diabetic rats were divided into seven groups (n=6). The animals fasted overnight before commencing the experiment. All rats were loaded with 2.5 g/kg, p.o., D-glucose solution (S.D. Fine-Chem. Ltd, Mumbai) after 30 min. of drug administration. The blood samples were obtained by the tail tip cutting method at just prior to drug administration and at 30, 60, 90, and 120 min after glucose loading. The BGL was measured by using the Accu-Check glucometer.

Acute and Sub-acute Anti-hyperglycemic Study: The oral anti-hyperglycemic activity of the PHF (100, 200 and 400 mg/kg), APHF (200 mg/kg), and metformin was screened against STZ (65mg/kg; i.p.) and NA (110mg/kg; i.p.) induced diabetes mellitus in rats. In an acute study, blood samples were obtained at 0, 1^{st}, 2^{nd}, 3^{rd}, 5^{th}, $7^{th,}$ and 24^{th} h. after treatment. Whereas in the sub-acute study, the PHF, APHF, and metformin were administered once a day for 28 consecutive days, and the effect of the PHF, APHF, and metformin on BGL were studied at 7^{th}, 14^{th}, $21^{st,}$ and 28^{th} day treatment.

Biochemical Investigation: The treatments were given once daily to the respective group of animals for 28 days. At the end of the experiment, 12 h fasted rats were sacrificed by ether anesthesia. The blood samples were used to analyze blood glucose, insulin, hemoglobin, Glycated hemoglobin. All the assay procedures were carried out in accordance with the kit protocol.

Estimation of Insulin: The serum insulin level was assayed by enzyme-linked immunosorbent assay kit (Abcam, USA). Standards and samples were pipetted into the wells, and the insulin present in a sample is bound to the wells by the immobilized antibody. The wells were again washed, then a tetramethylbenzidine (TMB) substrate solution was added to the wells, and color is developed in proportion to the amount of insulin bound. The stop solution changes the color from blue to yellow; the intensity of the color is directly proportional to the amount of insulin present in a sample. The absorbance was measured at 450 nm.

Estimation of Hemoglobin: The serum hemoglobin level was assayed by using an enzyme-linked immunosorbent assay kit (BioAsaay System, U.S.A.). The hemoglobin assay kit is based on an improved Triton/NaOH method, in which the hemoglobin is converted into a uniform colored end product. The intensity of the color is directly proportional to hemoglobin concentration in the sample. The absorbance was measured at 450 nm.

Estimation Liver Glycogen: At the end of the experiment, 12 h fasted rats were sacrificed by ether anesthesia. Liver tissue (10 mg) was collected and rapidly homogenized with 100 µl ice-cold glycogen hydrolysis buffer for 10 min on ice. The homogenized tissue was centrifuged at 12000 rpm for 5 min, and then the supernatant was collected. After that, 1-50 µl samples were added into a 96 well plate. Subsequently, volume was adjusted to 50 µl per well with a glycogen hydrolysis buffer. The liver glycogen level was assayed by enzyme-linked immunosorbent assay kit (Abcam, USA).

In this assay, glycogen is hydrolyzed into glucose, which is oxidized to form an intermediate, which reduces a colorless probe to a colored product. The intensity of the color is directly proportional to the liver glycogen concentration in the tissue sample. The absorbance was measured at 450 nm. The amount of glycogen in the tissue sample was expressed in milligrams of glucose per gram tissue.

Estimation Glycated Hemoglobin: Glycated hemoglobin was estimated by the method of Sudhakar and Pattabiraman [29].

Histopathological Study: After completion of treatment, the rats were sacrificed by ether anesthesia, immediately blood samples of experimental rats were collected by cardiac puncture. The whole pancreas was quickly dissected out and washed immediately with saline. The collected pancreas was fixed in an individual container in a 10% formalin solution; after that, pancreatic tissue was dehydrated by various solvent and then embedded into the paraffin block. Sections of 5 µm thickness were cut and air-dried, then stained with hematoxylin and eosin (H and E) stain. Pancreatic tissues from all groups were subjected to histopathological examination [30].

Statistical Analysis: The results were expressed as mean ± S.E.M. Statistical difference was tested by using a one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests. A difference in the mean P-value <0.05 was considered as statistically significant.

RESULTS: Acute oral toxicity of PHF: The acute oral toxicity of PHF was carried out according to 423-OECD guidelines. According to these guidelines and by limit dose study, the lethal dose (LD50) of the present study was found more than 2000 mg/kg, so its therapeutic dose was 200 mg/kg. The therapeutic dose is $1/10^{th}$ of the lethal dose, the LD50 was 2000 mg/kg, and thus, the therapeutic dose of PHF was 200 mg/kg. The present study was carried out at three dose levels, a lower dose (100 mg/kg/day), middle dose (200 mg/kg/day), and a higher dose (400 mg/kg/day). Selected doses were used for all further studies.

Effect of Single Dose of Metformin, PHF and APHF on BGL in Normal Fasted Rats: Administration of a single dose of PHF-A (100 mg/kg), PHF-B (200 mg/kg), PHF-C 400 mg/kg) and (APHF 200 mg/kg) was not showing the significant hypoglycemic effect on normal fasted rats, when compared with a healthy control group. Whereas the administration of metformin showed a significant effect at 3 h (P<0.05), 5 h (P<0.01), and 7 h (P<0.01) and APHF only showed a significant (P<0.05) effect at 7 h. The % decrease in the BGL at 5h after treatment with metformin, PHF-A, PHF-B, PHF-C and APHF treated group when compared with 0 min BGL were found 6.02, 18.61, 7.02, 10.67, 11.53 and 12.90 respectively. However, the maximum reduction occurs in the metformin-treated group as compared to other treated groups **Fig. 1**.

Effect of Single Dose of Metformin, PHF and APHF on BGL in Normal Fasted Rats:

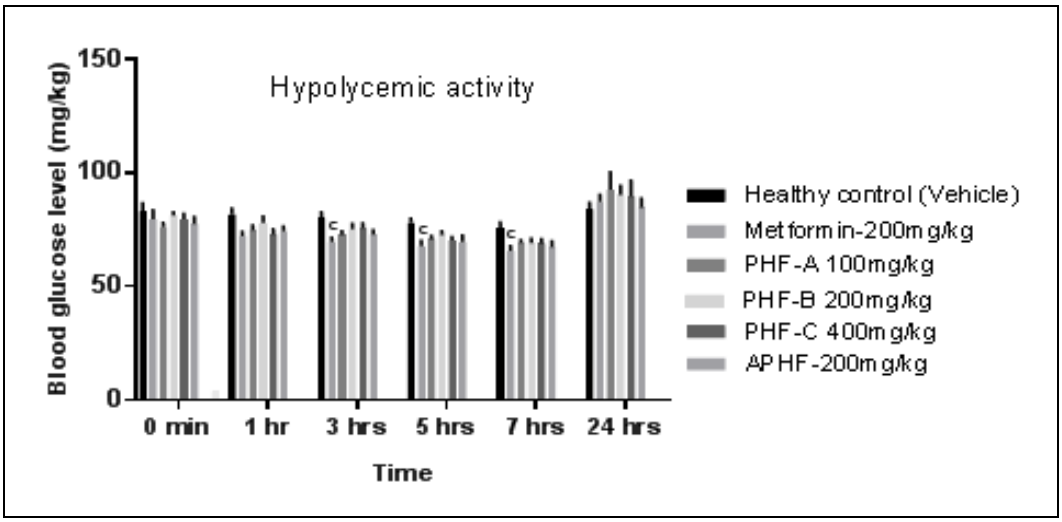


FIG. 1: EFFECT OF SINGLE DOSE OF METFORMIN, PHF AND APHF ON BGL IN NORMAL FASTED RATS: Values are expressed as Mean ± SEM (n = 6) in each group. Where, c = p<0.05, b = p<0.01, a = p<0.001, PHF = polyherbal formulation, APHF (allopolyherbal formulation) = PHF 100 mg/kg + metformin 100 mg/kg and BGl= blood glucose level

Effect of Metformin, PHF and APHF on OGTT in STZ-NA-induced Diabetic Fasted Rats: The BGL in the healthy control rats was elevated to a maximum value at 30 min after the glucose load and declined to near basal levels at 90 min, whereas, in STZ-NA-induced diabetic rats, the peak increase in blood glucose level was noticed even after 60 min and remained high over the next 60 min. Administration of metformin, PHF-A, PHF-B, PHF-C, and APHF to diabetic rats exhibited significantly ($p < 0.01$) decrease in blood glucose level at 30, 60, 90, and 120 min when compared with untreated diabetic rats. The % increase in the BGL at 60 min after glucose load in the diabetic control, metformin, PHF-A, PHF-B, PHF-C, and APHF treated group when compared with 0 min BGL were found 28.20, 19.66, 23.79, 20.53, 18.28 and 16.30 respectively.

However, the maximum reduction of BGL occurs in APHF treated rats as compared to other rats at different time interval **Fig. 2**.

Effect of Metformin, PHF, and APHF on OGTT in STZ-NA-induced Diabetic Fasted Rats:

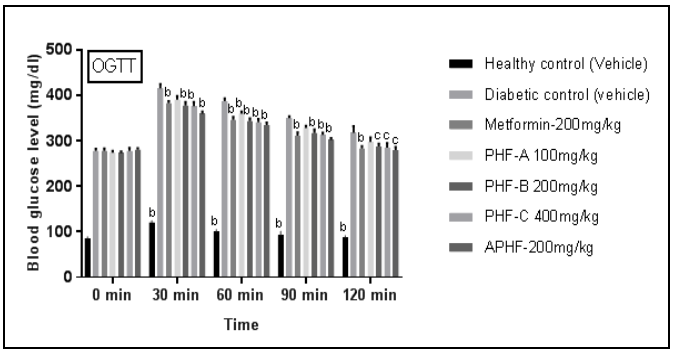


FIG. 2: EFFECT OF METFORMIN, PHF AND APHF ON OGTT IN STZ-NA-INDUCED DIABETIC FASTED RATS: Values are expressed as Mean ± SEM (n = 6) in each group. Where, c = P<0.05, b = P<0.01, a = P<0.001, PHF = Polyherbal formulation, APHF (Allopolyherbal formulation) = PHF 100 mg/kg + Metformin 100 mg/kg and BGL= blood glucose level.

Effect of a single dose of metformin, PHF, and APHF on BGL in STZ-NA-induced Diabetic Fasted Rat: The STZ-NA-induced diabetic rats showed a markedly higher BGL than that of healthy control rats, whereas a single dose treatment of Metformin, PHF-A, PHF-B, PHF-C, and APHF group were exhibiting a significant anti-hyperglycemic effect (P<0.01) at 1 h, 3 h, 5 h and 7 h.

The % decrease in the BGL at 5 h after treatment with metformin, PHF-A, PHF-B, PHF-C, and APHF treated group when compared with 0 min BGL were found 36.75, 30.52, 32.88, 35.50, and 40.64 respectively. However, the maximum reduction occurs in APHF treated group at 1 h, 3 h, 5 h, and 7 h. as compared to other treated groups **Fig. 3**.

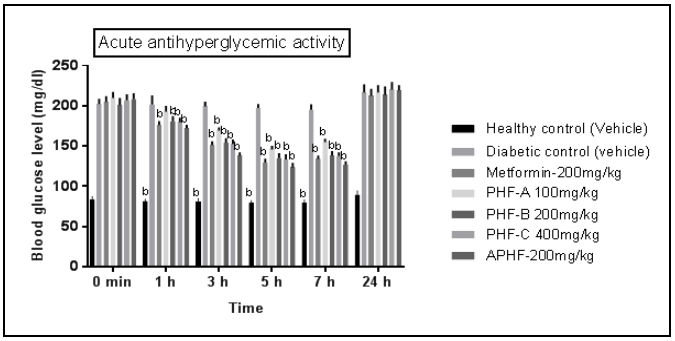


FIG. 3: EFFECT OF SINGLE DOSE OF METFORMIN, PHF AND APHF ON BGL IN STZ-NA-INDUCED DIABETIC FASTED RAT: Values are expressed as Mean ± SEM (n = 6) in each group. Where, c = P<0.05, b = P<0.01, a = P<0.001, PHF = Polyherbal formulation and APHF (Allopolyherbal formulation) = PHF 100 mg/kg + Metformin 100 mg/kg and BGL= blood glucose level

Effect of Multiple Doses of Metformin, PHF, and APHF on BGL in STZ-NA-induced Diabetic Fasted Rat: After daily administration of the metformin, PHF-A, PHF-B, PHF-C, and APHF for 28 days significantly decreased BGL in diabetic rats compared to untreated diabetic rats. Treatment with metformin, PHF-A, PHF-B, PHF-C, and APHF significantly (P<0.01) reduced BGL by 61.64%, 52.30%, 58.05%, 62.39%, 65.13%, respectively when compared with the diabetic control group. BGL was measured randomly on 0, 7^{th}, 14^{th}, $21^{st,}$ and 28^{th} days of study. BGL in diabetic rats was raised nearly to 2.5–3.0-fold as compared to healthy control rats. The raised levels of BGL declined sharply after oral administration of PHF-A, PHF-B, PHF-C, and APHF. When comparisons were made between '0' day and 28^{th} day of treated groups, there was a highly statistically significant (P < 0.01) reduction in blood glucose levels. As far as the relative efficacy is concerned, polyherbal formulation (400 mg/kg b.wt.) and allopolyherbal formulation (200 mg/kg b.wt.) produced more significant anti-hyperglycemic activity than metformin **Fig. 4**.

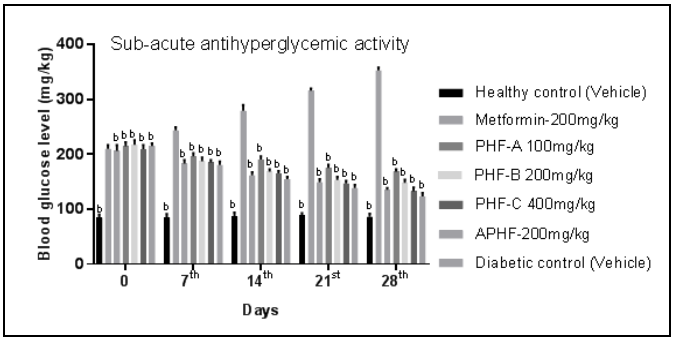


FIG. 4: EFFECT OF MULTIPLE DOSES OF METFORMIN, PHF AND APHF ON BGL IN STZ-NA-INDUCED DIABETIC FASTED RAT: Values are expressed as Mean ± SEM (n = 6) in each group. Where, c = P<0.05, b=P<0.01, a = P<0.001, PHF = Polyherbal formulation, APHF (Allopolyherbal formulation) = PHF 100 mg/kg + Metformin 100 mg/kg and BGL= blood glucose level

Effect of Multiple Doses of Metformin, PHF and APHF on Glycated Hemoglobin, Insulin, Hemoglobin, and HbA1c in STZ-NA-induced Diabetic Fasted Rat: After daily administration of the metformin, PHF-A, PHF-B, PHF-C, and APHF for 28 days significantly (P<0.01) decrease in the glycated hemoglobin level, whereas plasma insulin and hemoglobin levels, were significantly (P<0.01) increased in diabetic rats when compared with diabetic control rats.

Administration of metformin, PHF-A, PHF-B, PHF-C, and APHF tends to bring these parameters towards near normal. The liver glycogen levels of diabetic rats were significantly lower than those in healthy rats, and they were increased significantly (P<0.01) after the treatment in **Fig. 5** and **6**.

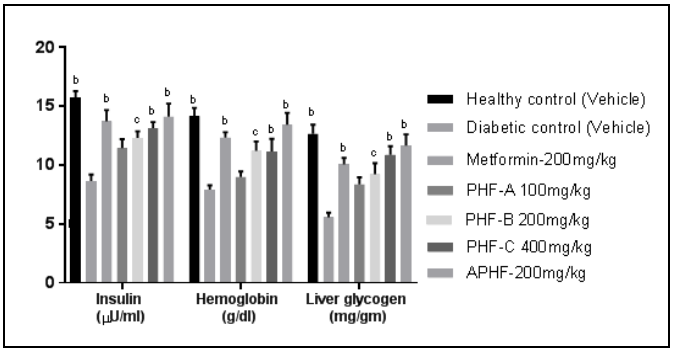


FIG. 5: EFFECT OF MULTIPLE DOSES OF METFORMIN, PHF AND APHF ON INSULIN, HEMOGLOBIN AND LIVER GLYCOGEN IN STZ-NA-INDUCED DIABETIC FASTED RAT: Values are expressed as Mean ± SEM (n=6) in each group. Where, c = P<0.05, b = P<0.01, a = P<0.001, PHF = Polyherbal formulation and APHF (Allopolyherbal formulation) = PHF 100 mg/kg + Metformin 100 mg/kg

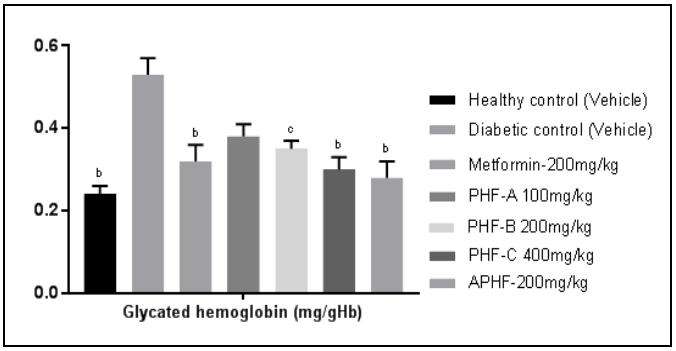
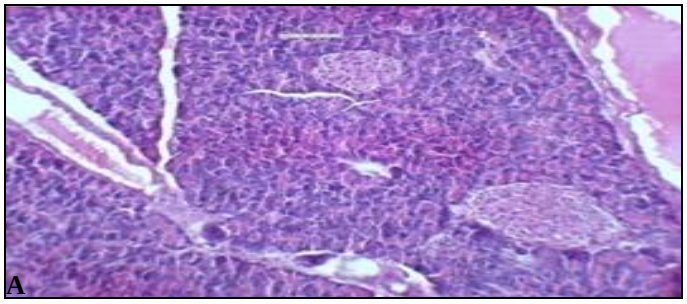
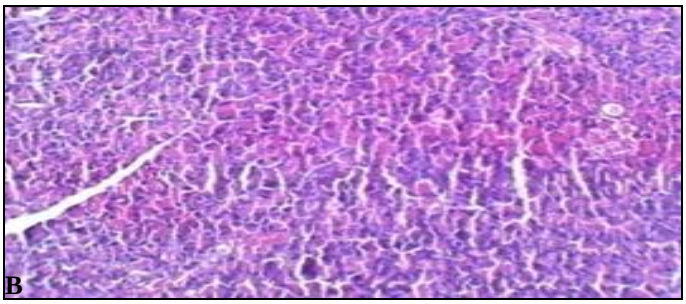


FIG. 6: EFFECT OF MULTIPLE DOSES OF METFORMIN, PHF AND APHF ON GLYCATED HEMOGLOBIN IN STZ-NA-INDUCED DIABETIC FASTED RAT. Values are expressed as Mean ± SEM (n = 6) in each group. Where, c = P<0.05, b = P<0.01, a = P<0.001. PHF = Polyherbal formulation and APHF (Allopolyherbal formulation) = PHF 100 mg/kg + Metformin 100 mg/kg

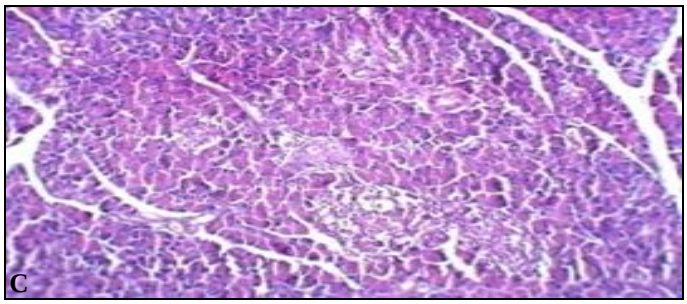
Histopathological Examination of Pancreas:



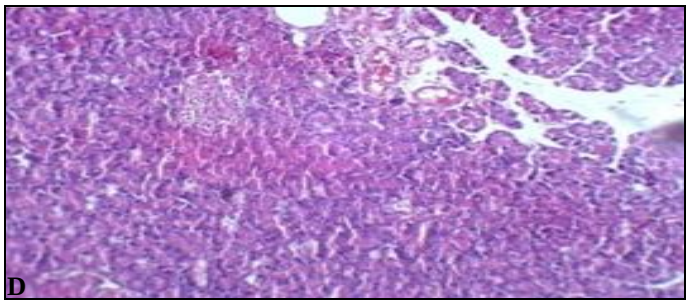
HEALTHY CONTROL: (0.5 ML SALINE) H&E IMAGE (100X)



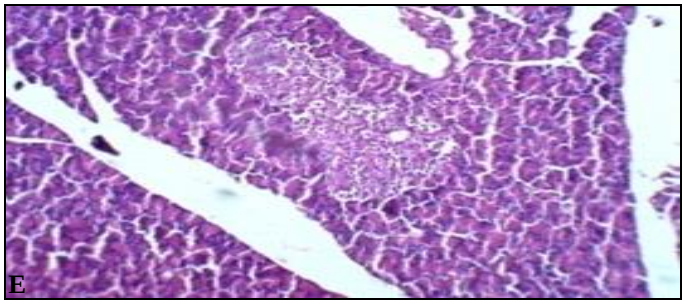
DIABETIC CONTROL: (0.5 ML SALINE) H&E IMAGE (100X)



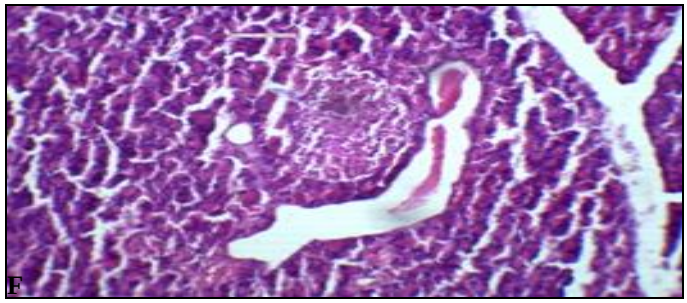
METFORMIN: (200 mg/kg) H&E IMAGE (100X)



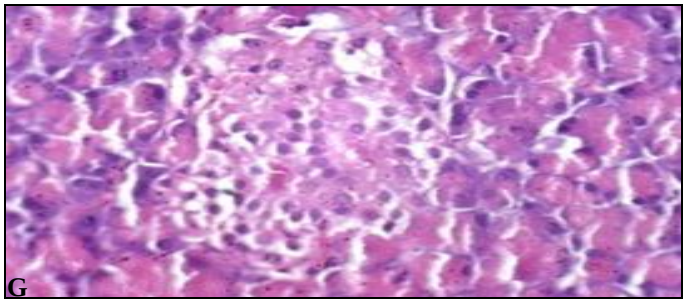
PHF-A: (100 mg/kg) H&E IMAGE (100X)



PHF-B: (200 mg/kg) H&E IMAGE (100X)



PHF-C: (400 mg/kg) H&E IMAGE (100X)



APHF: (METFORMIN-100 mg/kg PLUS PHF-100 mg/kg) H&E IMAGE (400X)

Histopathological Study: Healthy control rat (A) showed a normal acinar pattern, shape, and the average size of islets and normal pancreatic structure of islets of Langerhans. Diabetic control rat (B) showed a damaged and destroyed islet of Langerhans. The islets of Langerhans were irregular in shape and size, and most of them were smaller than healthy control rats. Diabetic rats (C) treated with metformin showed normal acinar cells. The islet present in adequate proportion and having slightly reduced cellularity of β-cells than normal. Diabetic rats (D) treated with PHF-A (100 mg/kg) showed the presence of a few islets of β-cells. Some of the islets of β-cells were found to be increased as compared to the diabetic control rat.

Diabetic rat (E) treated with PHF-B (200 mg/kg) showed a gradual restoration of pancreatic endocrine cells; degeneration of some of the pancreatic acini was still observed. The numbers of islets of β-cells were found to be increased. Diabetic rats (F) treated with PHF-C (400 mg/kg) showed viable cellularity with distinct β-cell mass, β-cell was evenly distributed, and an increased number of β-cells was also observed. The islets were present with a large proportion as compared to the diabetic control rat. Diabetic rats (G) treated with APHF (metformin 100 mg/kg + PHF 100 mg/kg) showed more number of islets and viable cellularity with distinct β-cell mass.

DISCUSSION: The STZ and NA injection caused type 2 diabetes mellitus, which may be due to the destruction of β-cells of the islet of Langerhans of the pancreas [24]. The STZ an antibiotic, has a selective toxic effect on pancreatic β-cells and induces diabetes [25]. Further, STZ causing alkylation or breakage of DNA strand and a consequent increase in the activity of poly-ADP-ribose synthetase, an enzyme depleting nicotinamide adenine dinucleotide (NAD) in β-cells, finally leading to energy deprivation and death of β-cells are reported [26]. Administrations of NA before STZ injection have been shown to either prevent or lessen the severity of the induction of diabetes [27]. NA partially protects the β-cells against the STZ-mediated cytotoxic damages.

The NA was founded to preserve the intracellular pool of NAD either by acting as a precursor of NAD or by inhibiting the activity of poly (ADP-ribose) synthetase, which is a NAD-consuming enzyme activated by STZ [26]. As NA is an antioxidant, which exerts a protective effect on the cytotoxic action of STZ by scavenging free radicals and causes only minor damage to pancreatic β-cell mass-producing type 2 diabetes [28].

All the extracts were obtained by supercritical fluid extraction; SFE utilizes supercritical fluids, which above their critical point, exhibit liquid-like as well as gas-like properties. SFE is the process of separating one component from another using supercritical fluid as the extracting solvent [21]. Carbon dioxide (CO_2) is a commonly used supercritical fluid, sometimes modified by the co-solvents such as ethanol or methanol. SFE occurs at high pressure to extract the higher value products from natural materials.

Unlike conventional extraction, the SFE didn't leave any solvent residue behind. Moreover, the CO_2 is non-toxic, non-flammable, odorless, tasteless, inert, and inexpensive [30]. It is a relatively rapid process because of the low viscosity and high diffusivity associated with supercritical fluids. Supercritical CO_2 extracts are pure, highly powerful, and extremely concentrated [20]. SFE allows the processing of plant material at low temperatures, hence limiting, thermal degradation and also avoids the use of toxic solvents [21]. Supercritical CO_2 behaves like a lipophilic solvent,

whereas co-solvent ethanol acts as a hydrophilic solvent, as a result non-polar as well as polar phytochemicals are extracted from raw herbs. The polyherbal formulation was prepared by taking an equal quantity of all holistic extract and mixed all the five extracts, by adding 1% CMC solution as a surfactant and continuously triturated till uniform suspension is formed. The antidiabetic activity of the individual herb has been proven [9, 13, 14, 16, 31]. In the present study, treatment with polyherbal formulation (PHF-A, PHF-B, PHF-C) and allopolyherbal formulation (APHF) produced a significant decrease in blood glucose level in STZ-NA-induced diabetic rats.

The hypoglycemic effect may be due to increased secretion of insulin from the β-cells of the pancreas, *i.e.,* pancreatotrophic action 12 and significantly increased in glucose utilization in the peripheral tissues, increased insulin sensitivity, as well as the anti-oxidant property of formulation [32]. The result of OGTT also substantiates that PHF and APHF showed improvement of glucose metabolism in the diabetic rat.

The combination of all five holistic extracts provides the best anti-hyperglycemic effect in diabetic rats. That might be possible by the positive herb-herb interaction produce synergism, which could be pharmacokinetics synergism or pharmacodynamics synergism. Polyherbal formulations enhance the therapeutic action and reduce the concentrations of single herbs, thereby reducing adverse events [7]. They have a wide therapeutic range, eco-friendly, cheaper, and readily available, and they also effective at a low dose and safe at a high dose. Also, PHF treatment could have a synergistic effect with standard oral hypoglycemic agents like metformin. The combination of PHF and metformin showed a more glucose-lowering potential than either drug alone 33. PHF-A, PHF-B, PHF-C, and APHF have significantly reversed the hyperglycemia in STZ-NA-induced diabetes rat; however, a four-week treatment with APHF was better than metformin.

Histopathology of rats treated with STZ-NA showing the disorganized structure of the endocrine and exocrine cells. There was a reduction of islets of β-cells, and the ratio of acinar to islet cell also was not maintained [34].

The pancreas of diabetic control rats showing ruptured and destructed Islets of Langerhans with damage in β-cells, whereas pancreas of rats treated with by PHF (100 mg/kg 200 mg/kg, and 400 mg/kg) showing protection of Islets of Langerhans against STZ administration. At the same time pancreas of rat treated with APHF showing more protection of β-cells and stopped the further destruction of the remaining β-cells in the islet.

These preliminary data clearly show that APHF is more effective than a well-known oral hypoglycemic agent like metformin. The allopathic drug metformin was also reduced the BGL below normal level in normal and STZ-NA-induced diabetic rats [35]. The antihyperglycemic activity of a single dose of PHF-A, PHF-B, PHF-C, and APHF was evaluated in STZ-NA-induced diabetic rats. PHF and APHF showed significantly ($P<0.01$) decrease in BGL at 1, 3, 5, and 7 h. and maximum reduction of BGL occurred at 5 h. Multiple-dose treatment of the PHF-A, PHF-B, PHF-C, and APHF for a period of 28th days showed a significant decrease ($P<0.01$) in the BGL at 21st and 28th day and from this duration, the maximum reduction occurred at 28th days in diabetic rats. APHF showed the maximum reduction (65.13%) in blood glucose levels.

In uncontrolled or poorly controlled diabetes, there is increased glycosylation of a number of proteins, including hemoglobin and β-crystalline of the lens. The non-enzymatic, irreversible covalent bonding of excessive glucose with Hb in circulation results in the formation of Glycated hemoglobin (HbA1C), which is a critical parameter for assessing long-term glycemic control [36]. The HbA1c was significantly increased in diabetic control animals, and this increase was found directly proportional to the fasting blood glucose level. During uncontrolled or poorly controlled diabetes, the excess glucose present in the blood reacts with hemoglobin [37]. Therefore, the total hemoglobin level was decreased in diabetic rats.

After administration of PHF, APHF and metformin for 4 weeks prevent a significant elevation in HbA1c, thereby increasing the level of plasma insulin and total hemoglobin in diabetic rats. This could be due to the result of PHF, APHF, and metformin treatment.

Glycogen is the primary intracellular storable form of glucose, and its level in various tissues, especially in the liver, indicates the direct reflection of insulin activity [38]. The decrease in hepatic glycogen observed may be due to insufficient insulin and inactivation of glycogen synthetase system in the diabetic state [39]. However, after the treatment with the PHF, APHF, and metformin, there was a significant increase in the liver glycogen levels in the diabetic rats.

CONCLUSION: On the basis of the aforementioned results and discussion, it may be concluded that the Polyherbal formulation and Allopolyherbal formulations can be considered as an adjuvant in the treatment of type 2 diabetes mellitus. Simultaneous administration of poly-herbal formulation and allopathic drugs increased the effect, reduced dose, and reduced side effects. However, the molecule(s) responsible for such an effect requires further investigation.

ACKNOWLEDGEMENT: The authors are sincerely thanks to Dr. Kailas Khadtare, Disha Diagnostic Center, Satara, for doing blood biochemical investigation and pathophysiological study. The authors also would like to thank Mr. Girish Soman, Managing Director, Nisarga Biotech Pvt., Ltd., Satara, for providing gift samples of holistic extract.

CONFLICTS OF INTEREST: Authors do not have any conflict of interest.

REFERENCES:

1. Deb L and Dutta A: Diabetes mellitus: It's possible pharmacological evaluation techniques and naturopathy. International Journal of Green Pharmacy 2006; 1: 7-28.
2. Prabhakar PK and Doble M: Effect of natural products on commercial oral antidiabetic drugs in enhancing 2-deoxyglucose uptake by 3T3-L1 adipocytes. Ther Adv Endo Metab 2011; 2: 103-14.
3. Wild S, Roglic G, Green A, Sicree R and King H: Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care 2004; 27: 1047-3.
4. Baruah MP, Pathak A, Kalra S, Das AK, Zargar AH and Bajaj S: A revisit to prevailing care and challenges of managing diabetes in India: Focus on regional disparities. Indian J Endocrinol Metab 2014; 18: 254-3.
5. Jung HW, Jung JK, Ramalingam M, Yoon CH, Bae HS and Park HS: Anti-diabetic effect of Wen-pi-tang-Hab-Wu-ling-san extract in streptozotocin-induced diabetic rats. Indian J Pharmacol 2012; 44: 97-102.
6. Parasuraman S, Thing GS and Dhanaraj SA: Polyherbal formulation: concept of ayurveda. Pharmacogn Rev 2014; 8: 73-80.
7. Petchi RR, Chockalingam V and Parasuraman S: Antidiabetic activity of polyherbal formulation in streptozotocin-nicotinamide induced diabetic wistar rats. J Tradit Complement Med 2014; 4: 108-17.
8. Grover JK, Yadav S and Vats V: Medicinal plants of India with anti-diabetic potential. J Ethno-pharmacol 2002; 81: 81-100.
9. Mai AE and Elham AA: Antidiabetic efficacy of aqueous fruit extracts of Amla (*Emblica officinalis*), Gaertn in streptozotocin-induced diabetes mellitus in male rats. Trop J Pharm Res 2015; 14: 801-06.
10. Faizal P, Suresh S, Satheesh KR and Augusti KT: A study on the hypoglycemic and hypolipidemic effects of an Ayurvedic drug Rajanyamalakadi in diabetic patients. Indian J Clin Biochem 2009; 24: 82-7.
11. Ansaria A, Md Shahriarb SZ, Md Mehedi H, Das SR, Rokeyad B and Md Haqueb A: *Emblica officinalis* improves glycemic status and oxidative stress in STZ induced type 2 diabetic model rats. Asian Pac J Trop Biomed 2014; 7: 21-5.
12. Shanmugasundaram ER, Gopinath KL, Shanmugasundaram KR and Rajendran VM: Possible regeneration of the Islets of Langerhans in streptozotocin-diabetic rats given *Gymnema sylvestre* leaf extracts. J Ethnopharmacology 1990; 30: 265-79.
13. Okabayashi Y, Tani S, Fujisawa T, Koide M, Hasegawa H and Nakamura T: Effect of *Gymnema sylvestre*, R.Br. on glucose homeostasis in rats. Diabetes Res Clin Pract 1990; 9: 143-8.
14. Ragavan B and Krishna KS: Anti-diabetic effect of *T. arjuna* bark extract in alloxan induced diabetic rats. Indian J Clin Biochem 2006; 21: 123-8.
15. Vaidya AB: *Terminalia arjuna* in cardiovascular therapy. J Assoc Physicians India 1994; 42: 281-2.
16. Sivakumar V and Dhana RMS: Antioxidant effect of *Tinospora cordifolia* extract in alloxan-induced diabetic rats. Indian J Pharm Sci 2010; 72: 795-8.
17. Tamboli SB, Sontakke SP and Parsode RB: Study of hypoglycemic activity of *Tinospora cordifolia* in alloxan induced diabetic rabbits. Int J Basic Clin Pharmacol 2013; 2: 559-61.
18. Al-Amin ZM, Thomson M, Al-Qattan KK, Peltonen-SR and Ali M: Anti-diabetic and hypolipidaemic properties of ginger (*Zingiber officinale*) in streptozotocin-induced diabetic rats. Br J Nutr 2006; 96: 660-6.
19. Stoilovaa I, Krastanova A, Stoyanovab A, Denevc P and Gargovaa S: Antioxidant activity of a ginger extracts (*Zingiber officinale*). Food Chem 2007; 102: 764-70.
20. Abbas KA, Mohamed A, Abdulamir AS and Abas HA: A review on supercritical fluid extraction as new analytical method. American Journal of Biochemistry and Biotechnology 2008; 4: 345-3.
21. Andrea C, Massimo EM and Andrea O: Supercritical fluid extraction of plant flavors and fragrances: A review. Molecules 2013; 18: 7194-38.
22. Goverdhan P: Hypoglycemic and antidiabetic activity of *Glochidion velutinum* on streptozotocin-nicotinamide induced type 2 diabetic rats. European Journal of Biological Sciences 2011; 3: 126-30.
23. OECD 423: OECD guidelines for the testing of chemicals: Acute oral toxicity-Acute toxic class method. Organization for Economic Cooperation and Development, 17th Dec, 2001; 1-14.
24. Shirwaikar A, Rajendran K and Barik R: Effect of aqueous bark extract of *Garuga pinnata* Roxb. in streptozotocin-nicotinamide induced type-II diabetes mellitus. J Ethnopharmacol 2006; 107: 285-90.

25. Atsuo T, Akiko MY, Ryosuke N, Yuka S and Masayuki S: Hypoglycaemic effects of antidiabetic drugs in streptozotocin-nicotinamide-induced mildly diabetic and streptozotocin-induced severely diabetic rats. Journal compilation 2008; 103: 560-8.
26. Syed IR, Devi K and Khanam S: Protective role of glibenclamide against nicotinamide-streptozotocin-induced nuclear damage in diabetic Wistar rats. J Pharmacol Pharmacother 2010; 1: 18-3.
27. Srinivasan K and Ramarao P: Animal models in type 2 diabetes research: An overview. Indian J Med Res 2007; 125: 451-72.
28. Shirwaikar A, Rajendran K and Punitha ISR: Antidiabetic activity of alcoholic stem extract of *Coscinium fenestratum* in streptozotocin-nico-tinamide induced type 2 diabetic rats. J Ethno-pharmacol 2005; 97: 369-4.
29. Sudhakar NS and Pattabiraman TN: A new colorimetric method for the estimation of glycosylated heamoglobin. Clin Chim Acta 1981; 109: 267-4.
30. Sapkale GN, Patil SM, Surwase US and Bhatbhage PK: Supercritical fluid extraction: a review. Int Journal of Chemical Sciences 2010; 8: 729-43.
31. Yiming L, Tran VH, Duke CC and Roufogalis BD: Preventive and protective properties of *Zingiber officinale* (Ginger) in diabetes mellitus, diabetic complications, and associated lipid and other metabolic disorders: A brief review. Evid Based Complement Alternat Med 2012; Article ID 516870: 10.
32. Patel SS, Goyal RK, Shah RS, Tirgar PR and Jadhav PD: Experimental study on effect of hydroalcoholic extract of *Emblica officinalis* fruits on glucose homeostasis and metabolic parameters. An International Quarterly Journal of Research in Ayurveda 2013; 34: 440-4.
33. Shah S, Bodhankar S, Bhonde R and Mohan V: Combinative therapeutic approach for better blood sugar level control in alloxan diabetic mice. International Journal of Diabetes and Metabolism 2006; 14: 104-5
34. Mandlik RV, Desai SK and Naik SR: Anti-diabetic activity of polyherbal formulation (DRF/AY/5001). Indian J Exp Biol 2008; 46: 599-6.
35. Singh AB, Chaturvedi JP, Narender T and Srivastava AK: Preliminary studies on the hypoglycemic effect of *Peganum harmala*. L. seeds ethanol extract on normal and streptozotocin-induced diabetic rats. Indian J Clin Biochem 2008; 23(4): 391-93.
36. Jayanthy G and Subramanian S: Rosmarinic acid, a polyphenol, ameliorates hyperglycemia by regulating the key enzymes of carbohydrate metabolism in high fat diet-STZ induced experimental diabetes mellitus. Biomedicine & Preventive Nutrition 2014; 4: 431-7.
37. Pari L and Satheesh MA: Effect of pterostilbene on hepatic key enzymes of glucose metabolism in Streptozotocin-nicotinamide induced diabetic rats. Life sci 2006; 79: 641-5.
38. Sharma B, Balomajumder C and Roy P: Hypo-glycemic and hypolipidemic effects of flavonoid rich extract from *Eugenia jambolana* seeds on streptozotocin-induced diabetic rats. Food Chem Toxicol 2008; 46: 2376-3.
39. Kondeti VK, Badri KR, Maddirala DR, Thur SKM, Shaik SF and Kasetti RB: Effect of *Pterocarpus santalinus* bark, on blood glucose, serum lipids, plasma insulin and hepatic carbohydrate metabolic enzymes in streptozotocin-induced diabetic rats. Food Chem Toxicol 2010; 48: 1281-7.

How to cite this article:
Belhekar SN, Chaudhari PD and Jadhav RL: Evaluation of antihyperglycemic activity of the polyherbal and allopolyherbal formulation on nicotinamide-streptozotocin-induced type-2 diabetic rats. Int J Pharm Sci & Res 2020; 11(11): 5812-22. doi: 10.13040/IJPSR.0975-8232. 11(11).5812-22.