RESEARCH ARTICLE

Pre-clinical Study of Marketed Polyherbal Formulations on Blood Glucose Level and Associated Parameters in Streptozotocin-induced Diabetes

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ABSTRACT

The management of diabetes mellitus involves lowering blood glucose levels with anti-diabetic drugs. Globally and nationally, the demand for herbal goods is increasing correspondingly. There have been numerous polyherbal preparations with anti-diabetic activity discovered during the past few decades. For both their antidiabetes and potential to avert diabetic complications, two different commercial polyherbal preparations were tested. For the purpose of choosing a commercial polyherbal formulation, a survey was conducted. The formulations were chosen based on their similarity in terms of their constituents, their estimated efficacy, how frequently they were distributed, and their price range. STZ-induced diabetic rats were utilized as a model and metformin was the standard medication for evaluating the antidiabetic activity. Selected polyherbal formulations, including formulation A and formulation B, demonstrated effective anti-diabetic action. In comparison to diabetic control groups, polyherbal formulations meaningfully decreased glucose levels in diabetic rat's blood and other diabetic complications metrics such as serum Serum glutamic oxaloacetic transaminase (SGOT), Serum glutamate pyruvate transaminase (SGPT), triglyceride, and cholesterol, as a marker for liver problems, and organ weight variation.

Keywords: Herbal formulations, Diabetes, Hypoglycemic.

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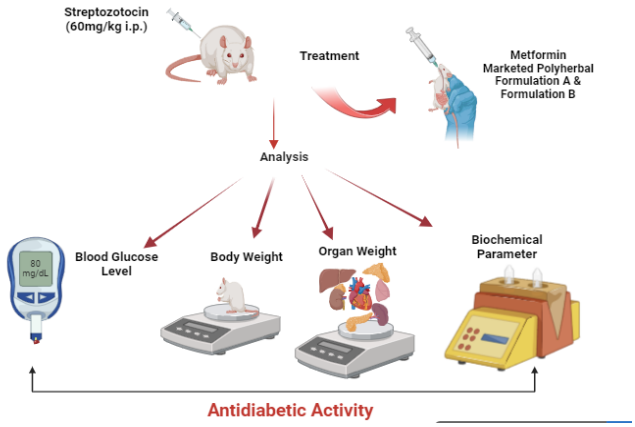

Graphical abstract

INTRODUCTION

Diabetes is a metabolic ailment that affects the breakdown of proteins, lipids, and carbohydrates. According to an international survey, diabetes affects around 10% of the world's population. Diabetes is predicted to be a serious danger to public health in the coming years. According to estimates, diabetes and the difficulties it brings would affect over 500 million people in emerging countries such as China and India.[1]

Furthermore, due to a lack of effective and economical therapies for both forms of diabetes, the disease's incidence will grow globally, having a significant impact on developing nations' populations. The beneficial effects on glycemic levels are well-documented in modern medicine, despite the fact that the preventive action of these medications against the progressive nature of diabetes and its consequences was limited and not always successful. Although insulin treatment efficiently manages blood sugar levels, it does have several downsides, such as being ineffective when given orally, having a limited shelf life, necessitating regular refrigeration, and providing a risk of fetal hypoglycemia in the event of an overdose. The usage of biguanide and sulfonylurea during treatment has its downsides as well. Plants are incredibly beneficial to mankind.

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A vast proportion of them are exclusively hired for medical reasons. The medicinal herb is that one "contains substances that can be used for therapeutic purposes, or that are precursors for chemo-pharmaceutical semi-synthesis, in one or more of its organs," according to the world health organisation (WHO). Pharmaceutical firms are highly interested in exploiting the active chemicals from these plants.[2]

In traditional medical systems, several plants have been demonstrated to be beneficial in treating a variety of systemic disorders. Many traditional/indigenous medical systems are more successful than modern medical systems, but they are limited by a lack of total equality, which is one of the fundamental difficulties with traditional medical systems. There are several connections in ancient literature to the concept of polyhedral formulation. The therapeutic potential of a polyherbal formulation is higher and more diverse than that of a single plant. As a result, the current work sought to develop and standardize a polyherbal preparation based on plants known to have anti-diabetic action, as well as to estimate its therapeutic advantages in rats.[3]

MATERIALS AND METHODS

Chemicals and Reagents

Drug streptozotocin (STZ) was acquired from the American firm CAYMAN Chemical USV Pvt Ltd. supplied the metformin. Assay kits were obtained from ERBA Diagnostic Mannheim (TRANSASIA Bio-medicals Ltd. Solan), Arkray, Ultichem, and Pathozyme. Analytical-grade ingredients used.

Formulations Collection

Polyherbal formulations were collected from the local market of Nashik, Maharashtra.

Experimental Animal Model

Animals

Female and male albino Wistar rats weighing between 150 and 200 grams were purchased from Biotox, an animal facility in Nashik, India. The temperature, humidity, and lighting in the animal housing facilities were all maintained at ideal levels. Animals had free admittance to a standard pellet diet and water at all times. The IAEC at Biotox in Nashik gave their stamp of approval to the experiment (Biotox/IAEC/01/2023/RP-01). No animals were harmed in the course of these experiments, which were all carried out in accordance with CPCSEA regulations.

Acute toxicity study

Following OECD guideline 423 (2021), an acute toxicity investigation was done. Throughout the first 30 minutes, albino wistar rats were examined one-on-one at least once. Afterward, they were observed every day for the next 14 days, with special attention paid to them every four hours. Animal behavior was watched, and any additional harmful signs were methodically documented.

Experimental design

The chosen animals were weighed and then divided into nine groups of six individuals each, as depicted in. There were a total of 28 days of the experiment. Each group's weight gain or loss throughout the course of the week was measured.

There were a total of nine groups, each including six male wistar rats.

Group I- Vehicle (control).

Group II- Streptozotocin (Diabetic).

Group III- Streptozotocin-induced diabetic rat treated with metformin (standard).

Group IV- Streptozotocin-induced diabetic rat with Formulation A: High dose

Group V- Streptozotocin-induced diabetic rat with Formulation A: Medium dose

Group VI- Streptozotocin-induced diabetic rat with Formulation A: Low dose

Group VII- Streptozotocin-induced diabetic rat with Formulation B: High dose

Group VIII- Streptozotocin-induced diabetic rat with Formulation B: Medium dose

Group IX- Streptozotocin-induced diabetic rat with Formulation B: Low dose

Experimental induction of diabetes in rats

After an overnight fast, rats were administered 60 mg/kg of STZ dissolved in 0.1 M of ice-cold citrate buffer (pH 4.5) via intraperitoneal injection, at which point they developed experimental diabetes. Blood sugar readings taken on days 0 and 72 after STZ injection verified the presence of hyperglycemia.[4]

Estimation of blood glucose level

Blood sugar levels were checked using a One-Touch Horizon glucometer and gluco-strips.

Body weight and organ weight

On days 0, 7, 14, 21, and 28 of treatment time, the body weights of animals in every group were measured.

After surgery, blood samples were taken from the heart's aorta and kept in test tubes with an anticoagulant (EDTA). Cleansing was done on the liver, kidneys, heart, gonads, spleen, pancreas, and adrenal glands. Right away, organ weights (OW) were recorded, and a ratio of OW to body weight (O/B) was found. Organ parts were kept in 10% formalin and a refrigerator at 20°C so that chemistry tests could be done on them.[5]

Estimation of biochemical parameters

After the 28th day, all animals were anesthetized and blood samples were drawn to estimate biochemical parameters. Serum was parted by centrifugation and was used for assessment of glucose, triglycerides, sodium, serum glutamic-oxaloacetic transaminase (SGOT), urea, cholesterol, serum glutamic-pyruvic transaminase (SGPT), creatinine, bilirubin total (BIT), bilirubin direct (BID), albumin, potassium, total protein and calcium using spectrophotometer (Perkin Elmer, Germany).[6]

Statistical Analysis

The results were presented with means and standard errors. ANOVA was performed for comparisons, and Dunnett's

multiple comparison test was employed to determine statistical significance ($p < 0.05$). For the statistical analysis and graph creation, we utilized GraphPad Prism Software, version 5.0 (GraphPad Software, San Diego, CA, USA).

RESULTS

Acute Oral Toxicity

Formulations A and B, when given orally at a dose of 2000 mg/kg, didn't cause any observed behavioral abnormalities or fatalities. When contrasted with the control group, there was no discernible variation in either body weight or food consumption. Formulations A and B can therefore be given without concern at doses up to 2000 mg/kg. In 100, 200, and 400 mg/kg were the doses chosen for additional research.

Estimation of Blood Glucose Level

Diabetic control animals had significant hyperglycemia compared to normal animals. Diabetic rats administered by formulation A and B at 100, 200, and 400 mg/kg had significantly ($p < 0.001$) reduced fasting blood serum glucose levels compared to the diabetic control group on days 7, 14, 21, and 28. Metformin, a common drug, was found to be quite effective at lowering blood glucose levels to near-normal ranges (Figure 1).

Body Weight

Diabetic rats showed a major body weight loss compared to normal rats. Weight loss persisted throughout the study's 28 day treatment period in diabetic control rats. The diabetes control group, however, experienced a much lower rate of body weight gain than the NC group after four weeks (Figure 2).

Organ Weight

Although the mean weight of all the animals decreased in group III standard, group IV formulation A low dose, group V formulation A intermediate dose, group VI formulation A high dose, group VII formulation B low dose, group VIII formulation B intermediate dose, and group IX formulation B high dose, a rise in weight of kidney (hypertrophy) was observed in STZ-treated animals compared with group I control animals in the current study.

Significantly less liver weight was seen in diabetic rats treated with formulation A, formulation B, and metformin than in untreated or control animals.

In group III standard, group IV formulation A low dosage, and group V formulation, pancreas weight was reduced. An intermediate dose, formulation of group VI, group VII, formulation B, high dosage compared to normal control group, treated group IX formulation B received a low dose, an intermediate dose for group VIII, formulation B, and a high dose.

Compared to a control group, the spleen weight of diabetic rats showed that splenic atrophy was a distinct characteristic.

As compared to group I (control group), group III (streptozotocin-treated group) displayed an exceedingly important rise in mean adrenal gland weight. In contrast to group I (the control group), which showed a modest rise in mean adrenal gland weight, group IX (the high dose of Formulation B) exhibited a minor drop in weight (Figure 3).

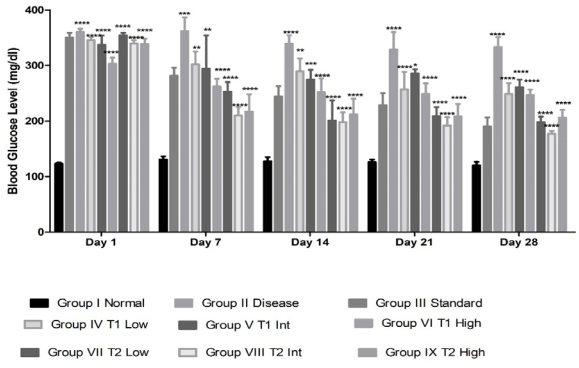
Biochemical Parameters

Results of the control and experimental groups for glucose, triglycerides, sodium, SGOT, urea, cholesterol, SGPT, creatinine, BIT, BID, albumin, potassium, total protein and calcium are shown in Figure 4.

The levels of glucose, triglycerides, sodium, SGOT urea, cholesterol, SGPT, creatinine, BIT, BID, albumin, potassium, total protein and calcium were significantly different in diabetic rats treated with metformin, formulation A, and B compared to diabetic rats not receiving treatment.

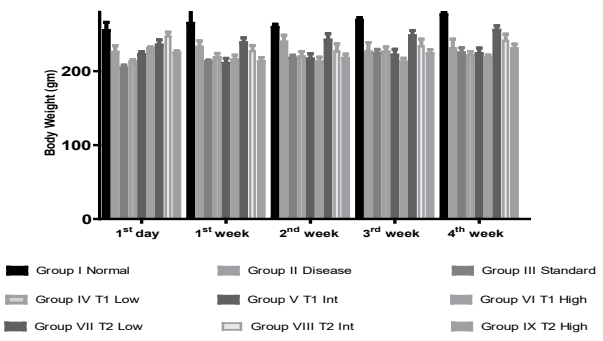
SGPT and SGOT concentrations were determined for assessing the hepatic and renal functions, respectively, while creatinine and urea levels were investigated to assess the influence of formulation A and Bon the control group of biochemical parameters in diabetic rats. Compared to the control group, STZ caused substantial increases in SGPT, SGOT, creatinine, and serum urea levels.

Significant increases in serum urea and creatinine levels in contrast to the control group demonstrate that STZ significantly reduced renal function. Treatment with formulation A and B at low, intermediate, and high doses decreased urea, creatinine, BIT, BID, triglyceride, cholesterol, total protein, calcium,



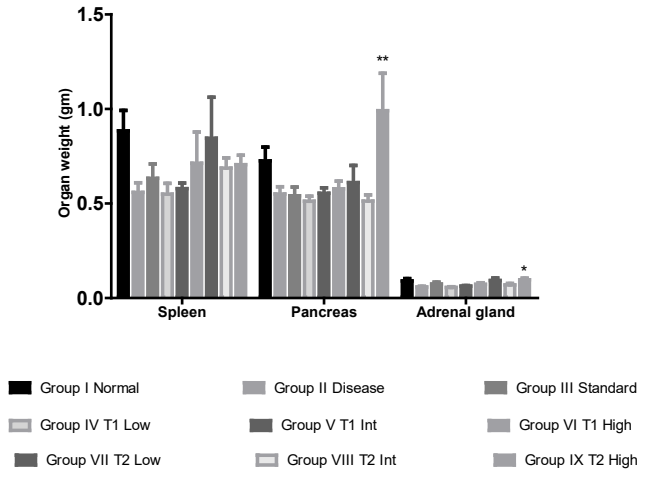
Outcomesstated as mean ± SEM (n = 6).* $p < 0.001$ as associated to diseasecontrol.

Figure 1: Effect of formulation A and B on glucose level of STZ-induced diabetic rat.



Results expressed as mean ± SEM (n = 6).* $p < 0.001$ as compared to disease control.

Figure 2: Effect of formulations A and B on body weight of STZ-induced diabetic rat.



Results expressed as mean ± SEM (n=6).* $p < 0.001$ as compared to disease control.

Figure 3: Effect of formulation A and B on organ weight of STZ-induced diabetic rat

potassium, sodium, SGPT, SGOT, and albumin in comparison to a disease control group.

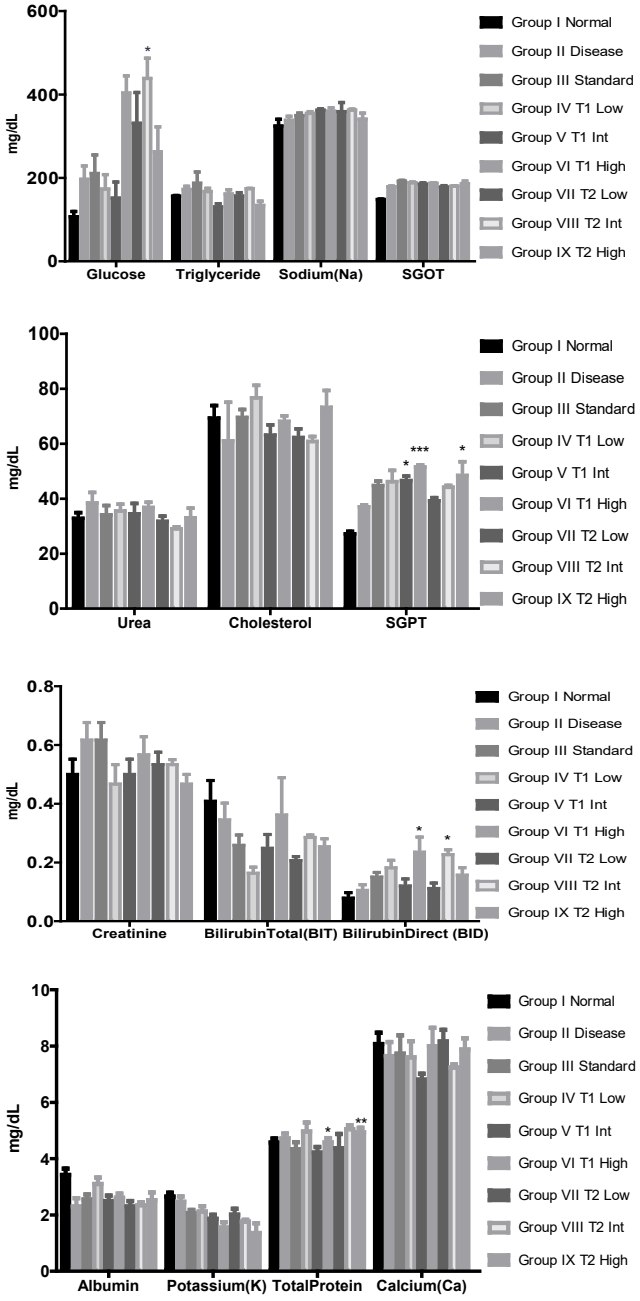
DISCUSSION

Traditional DM treatment has a long list of adverse effects, many of which lead to treatment failure. It is expected that herbal therapies will have fewer side effects while yet being as effective as conventional medications. These days, more than 1200 plant species are used to treat symptoms of diabetes mellitus; of these, more than half have had their hypoglycemic qualities confirmed by experimentation.

The purpose of this investigation was to compare the efficacy of several marketed polyherbal treatments for diabetes. To determine which polyherbal anti-diabetic formulations were the best available, a survey of the Nashik local market was conducted. A number of essential variables were taken into consideration when conducting the survey, such as related ingredients, formulation belief, formulation frequency of sales, and formulation price range. Two of the four commercialized polyherbal formulations formulation A and B were selected due to their ability to prevent diabetes.

In the current study, rats were given 60 mg/kg of streptozotocin to induce diabetes while clinical symptoms, blood glucose levels, body weight, and organ weight were observed.

Elevated blood glucose levels should always be treated seriously since they can create problems with the eyes and kidneys, among other organs, and interfere with their capacity to perform vital functions. Lowering blood glucose levels and reducing the problems associated with diabetes are therefore critical. In the current work, PFA dramatically decreased blood glucose in STZ-induced diabetic rats.[7] Both formulations A and B lower blood sugar levels and prevent STZ-induced diabetes from starting. For the first four weeks, however, there was no discernible alteration in glucose levels among a diabetic group



Results stated as mean ± SEM (n=6).* $p < 0.001$ as associated to disease control.

Figure 4: Effect of formulation A and B on biochemical parameters of STZ-induced diabetic rat.

and groups treated with formulations A and B. One possible explanation for this could be the extract's lack of a strong anti-diabetic action. It is likely that the doses' concentration of active components will not be sufficient to manage hyperglycemia during the first several weeks. Active components in the extract may take some time to concentrate to a point where they are useful. Certain circumstances, such as reducing sugars or other carbs, may prevent formulations A and B's hypoglycemic effects from starting to perform as planned. As per many reports,

the existence of reducing sugars may lead to the digestion-induced release of free glucose, hence potentially elevating blood sugar levels against the hypoglycemic effects of active hypoglycemic medicines. Its potential to lower blood sugar would be diminished as a result of this.

In contrast to non-diabetics, the current study looked at formulations A and B's impact on liver weight reduction and their capacity to stop hyperglycemia brought on by STZ in rats.[8]

When rats are given STZ, their kidneys get bigger compared to their body weight. This is called renal enlargement. One theory suggests that variations in growth factor production are responsible for renal enlargement. Proximal convoluted tubule cells and glomerular mesangial cells are overexpressing transforming growth factor-beta 1, which causes this. Protein synthesis may potentially accelerate or the breakdown of renal extracellular components may slow down, leading to renal hypertrophy. The kidney/body weight ratio was lower in the MPGL-treated STZ-diabetic rats, suggesting a potential reversal of kidney hypertrophy.[9]

Rats with hyperglycemia in this study showed abnormal cardiac tissue and a smaller heart weight.[10]

As expected, streptozotocin lowered body mass and shrank the spleen while raising blood glucose levels.[11]

Reduction in pancreatic weight could perhaps be attributed to the elimination of insulin-producing cells and the subsequent destruction and disappearance of pancreatic islets.[12]

The functions of the adrenal glands in biology are diverse and include functions in immunity, inflammation, metabolism, osmoregulatory regulation, and development. Adrenal hormone-induced modifications to the body's glucose, protein, and fat metabolism directly affect all major organs. The development of DM symptoms is also influenced by the adrenal gland.[13,14]

In our research, at the fifth week, the diabetic group receiving formulation A and B treatment had considerably lower SGOT and SGPT values than the diabetic group receiving no treatment. It is an indication that the hepatoprotective nature has improved and that the damage to the liver has been corrected. According to the current study, total protein, albumin, and globulin levels are lowered until the end of the fifth week when diabetes is caused by streptozotocin. The proximal tubule damage that results in microalbuminuria in the early stages of streptozotocin-induced diabetes in rats may be linked to the harmful effect of STZ on hepatocytes by inducing oxidative damage to the liver that includes hepatic dysfunction, leakage of hepatic enzymes responsible for protein synthesis, and loss of albumin due to effects.[15] Thus, in the proximal tubule of diabetic rats, we found that advanced diabetic nephropathy increased albumin filtration at the glomeruli and thus enhanced tubular reabsorption of albumin. This is because there was a reduction in megalin expression together with a decrease in albumin endocytosis. Our findings regarding agreement and serum total protein and albumin levels were significantly impacted negatively by the induction of streptozotocin.[16] This illustrates how the considerable loss of blood proteins (albumin and globulin) caused the STZ diabetic rats to have decreased kidney functioning (nephropathy).[17] In the current investigation, we found that patients with type 2 diabetes had significantly reduced serum levels of TBIL. Based on current experimental findings, bilirubin has the ability to disrupt the expression levels of TNF-, IL-2, TNF-, and cell adhesion molecules. Additionally, it has been shown to downregulate the appearance of major histocompatibility complex class II in macrophages. T-cell differentiation can also be suppressed by it. In certain diseases, there has also been evidence of a negative relationship between bilirubin and inflammatory markers such as C-reactive protein (CRP), NLR, and others.[18]

According to the present study's findings, rats given a dose of 60 mg/kg body weight of streptozotocin develop diabetes, as well as reduced body and organ weight and elevated glucose levels. Both formulation A and B have a good anti-diabetic effect.

CONCLUSION

As a consequence, our study's findings demonstrate that formulations A and B exhibit anti-diabetic effects when taken at doses of 100, 200, and 400 mg/kg. Lower blood sugar, total cholesterol, triglycerides, cholesterol, urea, creatinine, and SGOT and SGPT levels demonstrate the polyherbal formulation's comparable antidiabetic potential to metformin.

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