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# DEVELOPMENT AND EVALUATION OF ANTIDIABETIC POTENTIAL OF POLYHERBAL FORMULATION IN STREPTOZOTOCIN INDUCED ANIMAL MODEL

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## ABSTRACT

*Vitex trifolia* L, *Vitex parviflora* and *Terminalia chebula* are well-known plants available throughout India and are commonly used for the treatment of various diseases including diabetes mellitus. The antidiabetic activity of the individual plant parts is well known, but the synergistic or combined effects are unclear. The concept of polyherbalism has been highlighted in *Sharangdhar Samhita*, an Ayurvedic literature dating back to 1300 AD. Polyherbal formulations enhance the therapeutic action and reduce the concentrations of single herbs, thereby reducing adverse events. The aim of the present study is to formulate a polyherbal formulation and evaluate its antidiabetic potential in animals. The polyherbal formulation was formulated using the hydroalcoholic extracts of the leaves of *V. trifolia*, *L. parviflora* and *T. chebula* in the ratio of 1:1:1. The quality of the finished product was evaluated as per the World Health Organization's guidelines for the quality control of herbal materials. The quality testing parameters of the polyherbal formulation were within the limits. The acute toxicity studies of the polyherbal formulation did not show any toxic symptoms in doses up to 2000 mg/kg over 14 days. The oral antidiabetic activity of the all extract and PHF (100 and 200mg/kg) was screened against streptozotocin (STZ) induced diabetes in rats and glibenclamide was used (500 mcg/kg body weight) as standard drug. The investigational drug was administered for 21 consecutive days, and the effect of the extract and polyherbal formulation on blood glucose levels was studied at regular intervals. At the end of the study, the blood samples were collected from all the animals for biochemical estimation. Polyherbal formulation showed significant antidiabetic activity at 100 and 200mg/kg, respectively and this effect was comparable with that of glibenclamide. The antidiabetic activity of polyherbal formulation is supported by biochemical analysis. The formulation has emerged as potential combination which can challenge the synthetic drug.

**Keywords:** Diabetes mellitus, *Vitex trifolia*, *Vitex parviflora, Terminalia chebula,* Polyherbal formulation, Glibenclamide.

## 1. INTRODUCTION

Diabetes mellitus is one of the most common disorders affecting almost 6% of the world population and the dynamics of the diabetes are changing rapidly in low- to middle-income countries [1]. According to International Diabetes Federation's (IDF) estimations, 80% of the world diabetic population will be from low- and middle-income countries in 2030. As per IDF 2011 report, China, India, and the United States of America have a diabetic population of 90.0, 61.3, and 23.7 million, respectively, which may be increased up to 129.7, 101.2, and 29.3 million, respectively in 2030 [2]. Globally, diabetes is one of the six major causes of death and also causing various systemic complications. Diabetes mellitus is treated by hormone therapy (insulin) or by administering glucose-lowering agents such as alpha-glucosidase inhibitors, sulfonylureas, biguanides and thiazolidinediones. Development of an adverse event is one of the complications in the treatment of any systemic disorder; hence, many of the research institutes and pharmaceutical companies are involved in drug development to find the molecules with good therapeutic potential and less adverse events [3]. In the USA, 10-25% of patients experience an adverse drug reaction and these adverse drug reactions are responsible for 3.4-7.0% of hospital admissions [4]. Plants are very useful to mankind. Many of them are used exclusively for medicinal purposes. According to the World Health Organization (WHO), a medicinal plant is a plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes, or which are precursors for chemo-pharmaceutical semi-synthesis.

Such plants are in great demand by pharmaceutical companies for their active ingredients [5, 6]. In traditional systems of medicine, many plants have been documented to be useful for the treatment of various systemic disorders. Many of the traditional/indigenous systems of medicine are effective than the modern system of medicine, but they suffer from lack of complete standardization which is one of the important challenges faced by the traditional system of medicine. The concept of polyhedral formulation is well documented in the ancient literature. Compared to the single herb, the polyherbal formulation has better and extended therapeutic potential. Hence, the present study was planned to formulate and standardize a polyherbal formulation using a plant having known antidiabetic activity and to evaluate its therapeutic effects in rodents.

## 2. MATERIAL AND METHOD

### 2.1. Plant material

Leaves of *V. trifolia*, *L. parviflora* and *T. chebula* were collected from ruler area of Bhopal, Bhimbetka Bhojpur and Vindhya Herbals Bhopal respectively in the month of February. Plant material (leaves part) selected for the study were washed thoroughly under running tap water and then were rinsed in distilled water; they were allowed to dry for some time at room temperature. Then the plant material was shade dried without any contamination for about 3 to 4 weeks. Dried plant material was ground using electronic grinder. Powdered plant material was observed for their colour, odour, taste and texture. Dried plant material was packed in air tight container and stored for phytochemical and biological studies.

### 2.2. Chemical reagents

Glibenclamide (USV Pharma Ltd. India), Streptozotocin (Sigma-Aldrich), one touch glucometer (Johnson & Johnson, India) and all the chemicals used in this study were obtained from Hi Media Laboratories Pvt. Ltd. (Mumbai, India), Sigma-Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India). Lipid profile estimation kit was obtained from Transasia Bio Medical Limited, Mumbai, India. All the chemicals and solvent used in this study were of analytical grade.

### 2.3. Defatting of plant material

The shade dried leaves of *V. trifolia* (100gm), *L. parviflora* (75 gm) and *T. chebula* (124gm) were extracted with petroleum ether using maceration method. The extraction was continued till the defatting of the material had taken place.

### 2.4. Extraction with single solvent (Hydro-alcoholic) by maceration method

The air-dried and powdered defatted marc of leaves of *V. trifolia*, *L. parviflora* and *T. chebula* were subjected to extraction with ethanol: water in ratio of 80:20 v/v by maceration method. The resultant content was filtered with whatman filter paper no.1 and kept for evaporation of solvent to get the dry concentrated extract. The dried crude concentrated extract was weighed to calculate the extractive yield then transferred to glass vials (6×2 cm) and stored in a refrigerator (4°C), till used for analysis [7].

### 2.5. Preparation of polyherbal formulation

The polyherbal formulation (capsules) contained the extracts of *V. trifolia*, *L. parviflora* and *T. chebula* in the ratio of 1:1:1. The quality of the polyherbal formulation was tested as per the WHO guidelines for the quality control of herbal materials [8]. As per the guidelines, specific tests such as sampling, ash content, extractable matter, foaming index, loss on drying, tannin content, foreign matters and specific powder characteristic tests such as angle of repose and bulk density were undertaken and significant results were recorded.

### 2.6. Preparation of formulation by wet granulation method

The formulation preparation began with trials by adding a different ratio of binders and selecting the quantity of lubricants and preservatives, and finally the procedure was optimized. *V. trifolia*, *L. parviflora* and *T. chebula* extracts were finely powdered (sieve 40), and mixed in the ratio of 1:1:1 and taken for the preparation of capsules by wet granulation technique using lactose solution as a binder. The wet mass was passed through sieve number 22 to obtain granules. The granules were dried at 45°C in a tray dryer. The granules were lubricated with magnesium stearate. The optimized formulation showed very good flow properties. After this, the granules were filled in capsules colored yellow-red of size "0" in a capsule filling machine. The capsules were then deducted and transferred into poly bags, labeled and the samples were evaluated as per the testing requirements. Each 300 mg of herbal capsule contained the extracts of *V. trifolia* (100 mg), *L. parviflora* (100 mg), *T. chebula* (100mg), and lactose and excipients-quantity sufficient (q.s.).

**Table 1: Formulation of polyherbal formulation (Capsules)**

| Ingredients | Quantity (mg) |
|---|---|
| Extract of *Vitex trifolia* L. | 100 |
| Extract of *Vitex parviflora* | 100 |
| Extract of *Terminalia chebula* | 100 |
| Lactose | 30 |
| Magnesium stearate. | 10 |
| Talc | 10 |
| Total weight | 300 |

### 2.7. Preformulation studies

There are many formulations and process variables involved in mixing step and all these can affect characteristics of blend produced. Preformulation parameters such as bulk density, tap density, Carr's index, Hausner's ratio, and angle of repose were determined for the laboratory granules [9, 10].

### 2.8. Characterization of prepared capsule formulation

The polyherbal capsules were evaluated for their description, uniformity of dosage units, weight variation, disintegration time and moisture content and compared with Indian Pharmacopoeial standards.

#### *2.8.1. Weight variation*

Twenty capsules were randomly selected and individually weighed, the average weight and standard deviation of 20 capsules was calculated. The individual weights of the each capsule should be within the limits of 90% and 110% of the average weight.

#### *2.8.2. Moisture content*

Moisture content was determined by using automatic Karl Fischer titration apparatus.

#### *2.8.3. Disintegration time*

Disintegration test was performed using the digital microprocessor based disintegration test apparatus (Electro lab, Mumbai, India). One capsule was introduced into each tube and a disk was added to each tube. The assembly was suspended in water (900ml) in a 1000 ml beaker. The volume of water at its highest point was at least 25 mm below the surface of the water and at its lowest point was at least 25 mm above the bottom of the beaker. The apparatus was operated and maintained at a temperature of 37±2°C.

### 2.9. Animals

Wistar rats (150-200 gm) were group housed (n= 6) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2°C, 55-65%). Rats received standard rodent chow and water *ad libitum*. Rats were acclimatized to laboratory conditions for 7 days before carrying out the experiments. All the experiments were carried in a noise-free room between 08.00 to 15.00 h. Separate group (n=6) of rats was used for each set of experiments. The animal studies were approved by the Institutional Animal Ethics Committee (IAEC), constituted for the purpose of control and supervision of experimental animals by Ministry of Environment and Forests, Government of India, New Delhi, India.

### 2.10. Toxicity study

Healthy adult male albino rats were fasted overnight prior to the experiment. Different doses (50-2000 mg/kg, P.O) of the hydroalcoholic extract leaves of *V. trifolia*, *L. parviflora* and *T. chebula* and polyherbal formulation were administered to each group of rats (Each group carries 6 rats) and they were observed continuously for 1 hour and then at half-hourly intervals for 4 hour, for any gross behavioural changes and further up to 72 hour, followed 14 days for any mortality as per the OECD (Organization for Economic Co-operation and Development) Guideline 425 [11, 12]. The hydroalcoholic extract leaves of *V. trifolia*, *L. parviflora* and *T. chebula* and polyherbal formulation was found to be non-toxic up to the maximum dose of 2000 mg/kg body weight. Dose selected for anti-diabetic evaluation was 100 and 200 mg/kg respectively.

### 2.11. Induction of experimental diabetes in rats

Rats were divided into different groups, each group consisted of six animals. After overnight fasting (deprived of food for 16 hours had been allowed free access to water), diabetes was induced in group II-XI by intraperitoneal injection of STZ dissolved in 0.1M sodium citrate buffer at pH 4.5, at a dose of 55mg/kg body weight. The control rats received the same amount of 0.1 M sodium citrate buffer. The animals were allowed to drink 5% glucose solution overnight to overcome the drug-induced hypoglycemia. Diabetes status was confirmed by estimating blood glucose levels after 72 hours of STZ injection. Animals showing fasting blood glucose levels above 250 mg/dl were selected for study. Body weight of rats was taken on pre and post treatment i.e. initial and final day of post treatment by

electronic balance. Fasting blood glucose level of rats were taken pre and post treatment i.e. 0, 8th and 21th day of post treatment.

Eleven groups of rats were employed in the present study and each group contains six animals, as follows

| | |
|---|---|
| Group I | Normal |
| Group II | Diabetic control received only STZ (negative control) |
| Group III | Received Glibenclamide orally at dose of 500 mcg/kg b.w. for 14 days |
| Group IV | Diabetic rats received hydroalcoholic extract leaves of *V. trifolia* (100 mg/kg/day p.o.) |
| Group V | Diabetic rats received hydroalcoholic extract leaves of *V. trifolia* (200 mg/kg/day p.o.) |
| Group VI | Diabetic rats received hydroalcoholic extract leaves of *L. Parviflora* (100 mg/kg/day p.o.) |
| Group VII | Diabetic rats received hydroalcoholic extract leaves of *L. Parviflora* (200 mg/kg/day p.o.) |
| Group VIII | Diabetic rats received hydroalcoholic extract leaves of *T. chebula* (100 mg/kg/day p.o.) |
| Group IX | Diabetic rats received hydroalcoholic extract leaves of *T. chebula* (200 mg/kg/day p.o.) |
| Group X | Diabetic rats received polyherbal formulation (100 mg/kg/day p.o.) |
| Group XI | Diabetic rats received polyherbal formulation (200 mg/kg/day p.o.) |

### 2.12. Blood sampling and glucose estimation

For blood glucose determination, blood was withdrawn by tail snipping technique. For various lipid profile and biochemical parameters estimation, blood was collected from cardiac puncture. Blood was collected in plain micro centrifuge tube at every second week throughout the study period from all the overnight fasted (16-20 hr.) animals, under anesthesia. Serum was separated from blood sample by centrifugation at 4000 r.p.m. for 10 minutes. Biochemical parameters were studied by using automated biochemistry analyzer Hitachi-902 [13, 14].

### 2.13. Estimation of oral glucose tolerance test

Glucose and the oxygen react in the presence of glucose oxidase producing gluconic acid and hydrogen peroxide subsequently. Hydrogen peroxide oxidizes the dyes in a reaction mediated by peroxidase resulting to a blue colour of the dyes. However instead of conventional and lengthy procedure of other diagnostic kits, the glucometer was found to be suitable diagnostically in term of test accuracy, where a drop of blood is sufficient to get the results and easy to access at ambient temperature >95°F and hence used.

### 2.14. Statistical analysis

All the data were expressed as mean±SEM. Statistical significance between the groups were tested using one-way analysis of variance (ANOVA) followed by Dunnett's *t*-test post-hoc test. P values less than 0.5 were considered significant.

## 3. RESULTS AND DISCUSSION

Preformulation parameters such as bulk density, tap density, Carr's index, Hausner's ratio, and angle of repose were obtained for the laboratory granules. The granules showed excellent flow property. The results are presented in table 2. The study on capsules revealed that they were uniform in content and weight. Further, the moisture content, pH and disintegration time were calculated and these values were found to be within normal limits (Table 3). Acute toxicity studies did not show any mortality up to 2000 mg/kg given as single oral administration. Hence, the study was carried out at the dose levels of 100 and 200mg/kg.

**Table 2: Preformulation studies and results of flow properties**

| Parameters | Results |
|---|---|
| Bulk density | 0.691 g/ml |
| Tapped density | 0.589 g/ml |
| Carr's index | 3.94% |
| Hausner's ratio | 1.11±0.05 |
| Angle of repose | 18.03° |

**Table 3: Results of evaluation of capsules**

| Parameters | Results* |
|---|---|
| Weight variation | 340±8 mg |
| Disintegration time | 12.3±0.2 min. |
| Moisture content | 0.85±0.05 mg |
| pH | 7.6 |

**Average of three determination (Mean±SD)*

Throughout the study, the diabetic animals showed significant reduction in body weight when compared to the control animals. However, the polyherbal formulation and glibenclamide inhibited the diabetes-induced body weight reduction (Table 4). Diabetic control animals showed severe hyperglycemia compared to normal animals. The mean serum glucose level in the

diabetic control group on day 0 was 290.40± 7.20mg/dl and on day 21 was 405.20±10.20mg/dl. It was observed that the standard drug glibenclamide lowered the serum glucose level significantly, bringing it back to near normal level, whereas the extract and polyherbal capsule at 100mg/kg and 200mg/kg significantly ($P < 0.001$) decreased the fasting blood serum glucose level in the diabetic rats on 7th, 14th, and 21st days, as compared to the diabetic control group. The results are presented in table 5. The diabetic rats showed significant ($P < 0.001$) increase in serum lipid profiles except HDL when compared to the control animals, whereas the levels in the treatment group remained within normal limits at the end of the study. Effects of extracts, herbal formulation and glibenclamide on the lipid profile of diabetic animals are presented in table 6. Diabetic animals showed significant reduction in total protein levels when compared to the control animals, whereas extract, herbal formulation and glibenclamide treated animals showed normal and total protein levels. Effects of extract, herbal formulation and glibenclamide on the liver and renal markers of diabetic animals are presented in table 7.

**Table 4: Effect of plant hydroalcoholic extracts of leaves of *V. trifolia, L. parviflora and T. chebula* and PHF on body weight in rats**

| Group | Drug and Dose | Body Weight(gm) | |
|---|---|---|---|
| | | Initial weight (gm) | Final weight (gm) |
| I | Normal Control (Saline) | 190.00±9.80 | 206.00±10.17 |
| II | Diabetic Control (STZ) | 200.00±10.0 | 168.00±8.300 |
| III | STZ+ Glibenclamide | 194.10±9.70 | 213.00±10.60* |
| IV | STZ+ *V. trifolia* 100mg/kg | 174.00±9.60 | 210.00±10.10* |
| V | STZ+ *V. trifolia* 200 mg/kg | 172.00±9.50 | 207.00±10.00* |
| VI | STZ+ *L. Parviflora* 100 mg/kg | 200.00±9.90 | 215.00±11.00* |
| VII | STZ+ *L. Parviflora* 200 mg/kg | 199.40±9.60 | 214.00±10.50* |
| VIII | STZ+ *T. chebula* 100mg/kg | 200.60±9.90 | 215.20±10.00* |
| IX | STZ+ *T. chebula* 200 mg/kg | 199.70±9.60 | 214.10±10.50* |
| X | Diabetic + PHF (100 mg/kg) | 167.00±5.00 | 191.00±6.00* |
| XI | Diabetic + PHF (200 mg/kg) | 170.00±5.10 | 208.00±7.10* |

**Table 5: Effect of plant hydroalcoholic extracts of leaves of *V. trifolia, L. parviflora and T. chebula* and PHF on serum glucose level in rats**

| Group | Drug and Dose | Serum glucose levels (mg/dl) | | |
|---|---|---|---|---|
| | | 0 Day | 8th Day | 21th Day |
| I | Normal Control (Saline) | 70.20 ±3.65 | 85.400±4.20 | 106.10±6.32 |
| II | Diabetic Control (STZ) | 290.40±7.20 | 387.00±9.65# | 405.20±10.20# |
| III | STZ+ Glibenclamide | 259.00±6.50 | 129.10±6.40* | 110.00±5.80* |
| IV | STZ+ *V. trifolia* 100 mg/kg | 264.00±6.25 | 130.00±7.90 | 119.00±6.90* |
| V | STZ+ *V. trifolia* 200 mg/kg | 261.00±5.00 | 129.20±7.40* | 116.00±6.00* |
| VI | STZ+ *L. Parviflora* 100 mg/kg | 270.50±6.77 | 140.30±7.80 | 134.20±7.00* |
| VII | STZ+ *L. Parviflora* 200 mg/kg | 264.10±6.32 | 137.10±7.20* | 122.00±5.90* |
| VIII | STZ+ *T.chebula*100 mg/kg | 270.50±6.77 | 140.00±7.80 | 134.10±7.00* |
| IX | STZ+ *T. chebula* 200 mg/kg | 264.10±6.32 | 137.00±7.20* | 122.10±5.90* |
| X | Diabetic + PHF (100 mg/kg) | 265.00 ±6.00 | 132.00±4.20* | 118.00±3.40* |
| XI | Diabetic + PHF (200 mg/kg) | 262.00±5.00 | 130.00±3.10** | 115.00±3.00*** |

*Values are expressed as mean ± S.E.M. (n = 6). Values are statistically significant at p<0.05 (One-way ANOVA followed by Dunnett's test)*

The polyherbal formulation was formulated using the hydroalcoholic extracts of the leaves of *V. Trifolia*, *L. Parviflora* and *T. Chebula* in the ratio of 1:1:1. The antidiabetic activity of the individual plants has been proven. The leaves of all three plants showed significant antidiabetic potential in rodents. In the modern era, herbal formulations have gained greater importance than ever before, mainly due to their efficacy and easy availability [15], as well as less side effects as compared to the synthetic drugs [16]. These advantages have led

the people move toward herbal preparations, for disease treatment and prevention, as they are claimed to display synergistic, potentiative, and agonistic/antagonistic actions and the mixture of species in them shows better therapeutic effect than either species on its own [17]. The concept of polyherbalism has been highlighted in *Sharangdhar Samhita*, an Ayurvedic literature dating back to 1300 AD [18]. Polyherbal formulations enhance the therapeutic action and reduce the concentrations of single herbs, thereby reducing the adverse events.

**Table 6: Effect of plant hydroalcoholic extracts of leaves of *V. trifolia, L. parviflora and T. chebula* and PHF on serum lipid profiles i.e. total cholesterol, triglyceride, HDL, LDL level in rats**

| Group | Drug and Dose | Total Cholesterol (mg/dl) | Triglyceride (mg/dl) | HDL (mg/dl) | LDL (mg/dl) |
|---|---|---|---|---|---|
| I | Normal Control | 142. 90±5.7 | 112.10±4.50 | 49.50±2.47 | 75.60±5.79 |
| II | Diabetic Control | 247.66±9.4 | 214.30±4.60 | 29.10±1.50 | 178.10±5.98 |
| III | STZ+ Glibenclamide | 187.10±5.51*** | 132.50±5.20*** | 46.90±1.57*** | 88.00±5.90*** |
| IV | STZ+ *V. trifolia* 100 mg/kg | 199.71±8.00** | 152.65±4.39* | 41.50±1.65** | 115.20±5.79** |
| V | STZ+ *V. trifolia* 200 mg/kg | 194.08±9.2*** | 150.10±4.58** | 42.37±1.58*** | 106.44±5.24*** |
| VI | STZ+ *L. Parviflora* 100 mg/kg | 205.10±8.00* | 168.60±4.65* | 39.40±1.50** | 121.30±5.90** |
| VII | STZ+ *L. Parviflora* 200 mg/kg | 200.50±9.6*** | 162.30±4.58** | 41.55±1.68*** | 117.50±5.84*** |
| VIII | STZ+ *T. chebula* 100 mg/kg | 205.70±7.00* | 165.65±4.39* | 40.40±1.50** | 124.49±5.79** |
| IX | STZ+ *T. chebula* 200 mg/kg | 201.50±9.6*** | 161.00±4.44** | 41.55±1.68*** | 115.44±5.24*** |
| X | Diabetic + PHF 100 mg/kg | 197.73±7.00** | 149.70±4.55* | 42.50±1.50** | 112.30±5.00** |
| XI | Diabetic + PHF 200 mg/kg | 193.10±9.4*** | 140.30±4.60** | 43.55±1.58*** | 100.50±5.84*** |

*Values are expressed as mean ± S.E.M. (n = 6). Values are statistically significant at p<0.05 (One-way ANOVA followed by Dunnett's test)*

**Table 7: Effect of plant hydroalcoholic extracts of leaves of *V. trifolia, L. parviflora and T. chebula* and PHF on total protein, SGPT, SGOT (U/L), SALP (U/L) in rats**

| Group | Drug and Dose | Total Protein (g/dl) | SGPT(U/L) | SGOT(U/L) | SALP(U/L) |
|---|---|---|---|---|---|
| I | Normal Control | 5.7±6.10 | 56.00±3.50 | 41.83±3.88 | 140.24±3.66 |
| II | Diabetic Control | 16.4±4.5 | 135.10±4.30 | 133.17±3.00 | 298.90±4.00 |
| III | STZ+ Glibenclamide | 6.0±3.00*** | 77.15±4.20*** | 57.57±3.00*** | 165.20±5.00*** |
| IV | STZ+ *V. trifolia* 100 mg/kg | 7.5±3.60** | 99.02±4.72** | 84.98±2.87* | 198.99±4.50 |
| V | STZ+ *V. trifolia* 200 mg/kg | 6.30±7.00*** | 84.19±3.19** | 70.10±2.70** | 181.62±5.00** |
| VI | STZ+ *L. Parviflora* 100 mg/kg | 7.9±3.50** | 104.10±4.70** | 86.98±2.87* | 201.79±5.50 |
| VII | STZ+ *L. Parviflora* 200 mg/kg | 6.40±2.90*** | 89.50±4.10** | 75.00±2.97** | 184.72±5.20** |
| VIII | STZ+ *T. chebula* 100 mg/kg | 7.10±3.50** | 105.30±4.70** | 87.88±2.87* | 202.99±4.50 |
| IX | STZ+ *T. chebula* 200 mg/kg | 6.38±7.10*** | 88.60±4.10** | 76.10±2.70** | 187.80±5.00** |
| X | Diabetic + PHF 100 mg/kg | 7.4±3.50** | 97.02±4.75** | 78.98±2.97* | 190.79±5.50* |
| XI | Diabetic + PHF 200 mg/kg | 6.10 ± 2.90*** | 81.20±3.19** | 68.00±3.00** | 177.10±5.20** |

*Values are expressed as mean ± S.E.M. (n = 6). Values are statistically significant at p<0.05 (One-way ANOVA followed by Dunnett's test)*

The toxicity studies were carried out as per the OECD guidelines. The polyherbal formulation did not show any mortality or adverse event up to 2000 mg/kg. Hence, the study was carried out at the dose levels of 100 and 200mg/kg. STZ is toxic glycoside obtained from *Streptomyces achromogenes*, a gram-positive bacterium. It accumulates in pancreatic β cells via the glucose transporter 2 (GLUT2) and reduces their expression. The alkylating properties of the STZ modify the biological macromolecules, fragment DNA and destroy the β cells, causing insulin-dependent diabetes [19]. In the diabetic control group, severe body weight loss was observed, which may be due to increased muscle wasting and loss of tissue proteins [20]. In the present study, the treatment groups showed significant improvement in body weight, which indicates that extract; polyherbal formulation and glibenclamide prevent the hyperglycemia-induced muscle wastage. The reduction in glucose levels may be due to increase in plasma insulin levels or enhanced transport of blood glucose in the peripheral tissue [21]. Our study gives evidence that the polyherbal formulation increases the plasma insulin levels and has promising antidiabetic activity. The diabetic hyperglycemia induced by STZ causes elevation of plasma levels of SGPT, SGOT which are considered as significant markers of liver dysfunction. The polyherbal formulation treated animals reversed the effect of STZ on the liver markers. This may be due to the hepatoprotective mechanism of the individual herbs present in the polyherbal

formulation [22]. STZ diabetic rat has increased levels of lipid peroxides and reactive oxygen species, which cause hyperglycemia. Incessant generation of free radicals can lead to tissue damage through peroxidation of unsaturated fatty acids [23]. The polyherbal formulation treated animals inhibited the hyperglycemia induced by STZ, which may be due to the free radical scavenging properties of the individual herbs present in it.

## 4. CONCLUSION

Diabetes mellitus is one of the most common disorders affecting almost 6% of the world population and the dynamics of the diabetes are changing rapidly in low- to middle-income countries. In traditional systems of medicine, many plants have been documented to be useful for the treatment of various systemic disorders. Many of the traditional/indigenous systems of medicine are effective than the modern system of medicine, but they suffer from lack of complete standardization which is one of the important challenges faced by the traditional system of medicine. The concept of polyhedral formulation is well documented in the ancient literature. Compared to the single herb, the polyherbal formulation has better and extended therapeutic potential. Hence, the present study was planned to formulate and standardize a polyherbal formulation using a plant having known antidiabetic activity and evaluate its therapeutic effects in rodents.

Thus, our study findings demonstrate the antidiabetic effect of the hydroalcoholic extracts of leaves of *Vitex trifolia* L, *Lagerstroemia parviflora*, *Terminalia chebula* and polyherbal formulation at the dose levels of 100 and 200 mg/kg. The antidiabetic potential of the polyherbal formulation is comparable with that of glibenclamide and individual extract of *Vitex trifolia L, Lagerstroemia Parviflora* and *Terminalia chebula*, which is evidenced by decreased levels of blood glucose, total cholesterol, triglyceride, low density lipoprotein (LDL)-cholesterol, SGOT, and SGPT, and increase HDL-cholesterol.

### *Conflict of interest*

None declared

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