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Evaluation on Safety and Efficacy of A Polyherbal Antidiabetic Nutraceutical Formulation

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ABSTRACT

In the present study, the polyherbal antidiabetic nutraceutical formulation was screened for its safety and efficacy using albino wistar rats (130-150gm). The formulation consists of *Gymnema sylvestre*, *Trigonella foenum-graecum*, *Allium cepa*, *Curcuma longa*, *Phyllanthus amarus*, *Tinospora cordifolia*, *Eugenia jambolana*, *Cinnamomum zeylanicum*. The safety of the formulation was studied by acute toxicity studies according to Organisation for Economic Co-operation and Development (OECD) guidelines and efficacy was studied by using streptozotocin induced diabetes model at 45 mg/kg b.w. The test drug did not show any signs of toxicity or mortality up to 5000 mg/kg which was fixed as the cut off point for the maximum tolerated dose. Antidiabetic activity was screened in streptozotocin induced diabetic rat model for 21 days using glibenclamide 1.5 mg/kg as standard and parameters like blood glucose level and weight variation were studied. After 21 days treatment the mean blood glucose level of the formulation, dose 1 (500mg/kg) treated group showed 142.50±1.11mg/dl, the formulation, dose 2 (250mg/kg) treated group showed 210.66±2.96 mg/dl, the standard group showed 137.66±2.10 mg/dl, Control group 433.33±9.89mg/dl, Normal group 94.83±0.30 mg/dl respectively and at a dose of 500mg/kg body weight. The formulation showed significant increase in the body weight. Therefore, the results indicated that the polyherbal neutraceuticals formulation is an efficacious and safer antidiabetic/ oral hypoglycemic formulation in Type II diabetes.

Keywords: Nutraceutical formulation, acute toxicity study, streptozotocin, antidiabetic activity.

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INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder affecting approximately 5% of the world's population. According to World Health Organisation Projections, the diabetic population is likely to increase to 300 million or more by the year 2025[1]. Drugs such as biguanides and sulfonylurea are currently available to reduce hyperglycemia in diabetes mellitus[2]. These drugs have side effects and complications which can lead to blindness, kidney failure, heart disease, nerve damage, limb amputations. Thus search for new drug/compound is essential to overcome the diabetic problems[3]. Management of diabetes without any side effect is still a challenge to medical community and there is continuous search for alternative drugs. Therefore it is prudent to look for options in herbal medicines for diabetes as well. Hence, in the present study the formulated Polyherbal antidiabetic nutraceutical formulation was evaluated for its safety & efficacy.

MATERIALS AND METHODS:

Plant material:

In the present study is framed orienting the aerial parts of *Gymnema sylvestre*, seeds of *Trigonella foenum-graecum*, bulb of *Allium cepa*, rhizomes of *Curcuma longa*, aerials parts of *Phyllanthus amarus*, stems of *Tinospora cordifolia*, seeds of *Eugenia jambolana*, barks of *Cinnamomum zeylanicum* were collected form JSS Ayurveda college, mysore and identified by Dr.J.Suresh, Professor authenticated by Dr. M.N. Naganandini Assistant professor ,dept. of phrmacognosy, JSSCP, Mysore.

Animals:

Wistar rats of either sex weighing 130-150 g were used. Animals used in the study were procured from JSS Medical College, animal facility Centre. The animal care and handling was carried out according to Committee for the Purpose of Supervision and Control on Experiments on Animals (CPCSEA) guidelines. The studies conducted were approved by the Institutional Animal Ethical Committee, JSS College of Pharmacy, Mysore, Karnataka (Approval No.: 098/2011).

Chemicals:

Streptozotocin procured from Sigma, Glimenclamide from Aventis, Pulsatom blood

Streptozotocin induced diabetes model[4]

Streptozotocin is a naturally occurring nitrosamide that has been used extensively to produce diabetes by destroying the insulin-producing beta cells of the pancreas in mammals. Wistar strain

albino rats of either sex weighing 130-150g were used for acute toxicity study and antidiabetic screening.

Acute toxicity study[5]

Acute toxicity study of the 2 potential doses of the formulation was done according to acute toxic classic method (Organisation for Economic Co- operation and Development guideline 425, 2001) using albino female rats to determine the safe dose. The animals were kept fasting for overnight providing only water. The suspension of formulation, prepared in 0.5% sodium carboxymethylcellulose, was administered orally for one animal at the limit dose of 2000 mg/kg and was observed for 14 days (with special attention for the first 4 hrs. of administration followed by next 20 hrs) for mortality. If the animal survived, another four animals were dosed sequentially so that total five animals were tested. If animals survived the same method as described above was adopted at a limit dose of 5000 mg/kg body weight.

Antidiabetic screening

The animals were weighed before to start the experiment and then they were divided randomly into different groups of six animals each.

Treatment groups (n= 6):

1. Group 1: Normal
2. Group 2: Control= Streptozotocin
3. Group 3: Standard = Streptozotocin + Glibenclamide suspension (1.5 mg/kg bw)
4. Group 4: Test Dose 1= Streptozotocin+ Formulation suspension (500 mg/kg bw)
5. Group 5: Test Dose 2= Streptozotocin+ Formulation suspension (250 mg/kg bw)

The first group consisted of normal healthy rats while the other four groups of animals were given intraperitonially Streptozotocin in a dose of 45 mg/kg to induce diabetes. The animals were checked regularly and maintained properly and kept for 18 hrs fasting before experiment. These hyperglycemic rats were then divided into groups. The normal & control group of animals received orally 2 ml of normal saline, the third group were given glibenclamide, and the fourth and fifth group were given orally the formulation 500mg/kg, 250 mg/kg body weight respectively[6].

Blood glucose level[7]

The blood glucose levels of animals in all groups were estimated at 0, 7, 14 and 21 days after treatment by using glucometer and recorded and the efficacy of formulation was assessed in comparison with the reference standard ‘Glibenclamide’ and reported.

Body Weight Measurement[8]

Body weight has been measured totally four times during the course of study period (i.e., on before streptozotocin induction (initial values), 0th day, 7th day, 14th and 21st day of the treatment period), using a Digital weighing scale obtained from ORION Engineering weighs. After sacrificing the animals the whole pancreas from each animal was removed, collected in 10% formalin solution and immediately send for histopathological studies.

Statistical analysis:

The values were expressed as mean ± standard error of mean (SEM) of the indicated number of experiments/ animals. Statistical analysis was performed using Graph Pad Prism 5.04 by one-way ANOVA followed by post hoc Tukey's multiple comparison test. A value of $p < 0.05$ was considered significant. Graphs were prepared by Graph Pad Prism 5.04 software.

RESULTS AND DISCUSSION

Acute toxicity study:

Starting dose was selected to be 2000 mg/kg body weight up to 5000 mg/kg bw as specified for natural products. Animals were observed initially after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours. Additional observations like changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous system and somatomotor activity and behaviour pattern. Attention was also given to observation of tremors and convulsions.

No mortality and no signs of toxicity were found even after administration of a limit dose 5000 mg/kg of formulation, as per OECD guidelines the substance might be considered to have an LD_{50} value above 5000mg/kg body weight. So 1/10th and 1/20th of this dose was taken as experimental dose for subsequent antidiabetic studies.

Antidiabetic screening:

Blood glucose level (BGL):

Initially blood glucose level was estimated 72 hrs after streptozotocin administration and 7th, 14th and 21st day of the treatment with the granules of the formulated tablet for all animals. Blood glucose levels are expressed as mg/dl and were given in mean. The effect of formulation on BGL in streptozotocin induced diabetic rats has shown in Table 1. As expected, administration of streptozotocin led to elevation of fasting blood glucose levels, which was maintained over a period of study in diabetic control group and 21 days of daily treatment with formulation led to a fall in the BGL. There was a significant elevation in BGL in streptozotocin induced diabetic

control rats when compare with normal control. An oral treatment with formulation group was able to reduce blood glucose level significantly as compare to diabetic control. The formulation also significantly reduced the blood glucose level compared with diabetic control. The effect of formulation is more pronounced.

Table No. 1 Effect of formulation on blood glucose level of diabetic rats

Time period in days	Blood Glucose Level mg/dl				
	Normal	**Control**	**Standard**	**500mg/kg**	**250mg/kg**
Day 0	91.50±0.42	393.00±18.59	356.66±5.14	344.33±3.88	367.50 ±3.09
Day 7	91.66 ± 0.21	407.16±15.42	256.33±5.14	255.83±3.51	309.66 ±4.44
Day 14	92.83 ±0.30	419.16±12.59	182.50 ±3.81	188.33±2.10	265.00 ±4.47
Day 21	94.83±0.30	433.33[a]±9.89	137.66 [b]±2.10	142.50 [b]±1.11	210.66±2.96

All values are expressed as Mean ±SEM, n= 6, [a]*p <0.05* compared to normal, [b]*p <0.05* compared to control. All the data was analysed by one way ANOVA followed by post hoc Tukey's multiple comparison tests.

Body weight measurement:

The change in the body weight of animals in normal control, diabetic control and formulation during the study period is shown in Table. 2. Normal and standard group animals were found to be gained in their body weight but diabetic control rats showed significant reduction in the body weight, which is reversed by Formulation treated groups during 21 days of study. The treatment with formulation showed significant increase in the body weight compared with the Diabetic control in 0^{th}, 7^{th}, 14^{th} and 21^{st} day of study respectively.

Table 2 Effect of Formulation on body weight of diabetes rats

Time period in days	Blood Glucose Level mg/dl				
	Normal	**Control**	**Standard**	**500mg/kg**	**250mg/kg**
Day 0	180±5.77	177±4.78	181±3.07	175±4.16	130±0.25
Day 7	185 ±4.28	168±4.77	191±7.03	163±4.21	140±0.22
Day 14	195 ±3.41	160±3.65	200±7.30	163±4.21	142±0.55
Day 21	196 ±4.94	143±4.94[a]	206±6.14[b]	171±4.77[ab]	145±0.98

All values are expressed as Mean ±SEM, n= 6, [a]*p <0.05* compared to normal, [b]*p <0.05* compared to control. All the data was analysed by one way ANOVA followed by post hoc Tukey's multiple comparison tests.

Histopathology of pancreas:

Histopathological studies of pancreas of all treated and control group was done. Microscopic examined section of control rat pancreas showed that the islets are normal. The architecture is preserved. The acini are lined by round to oval cells with moderate cytoplasm and small round to oval nuclei. No fibrosis or any inflammation seen.

Microscopically, the pancreas section of diabetic control showed pancreas with engorged and congested blood vessels. The islets are damaged. The acini are lined by round to oval cells with moderate cytoplasm and small round to oval nuclei. The pancreas section of Standard Glibenclamide treated rats section showed pancreas with normal architecture and acini. The islet cells show moderate cytoplasm and round to oval nuclei. There is no evidence of inflammation. The section of formulation dose 1 (500 mg/kg) section shows pancreas, the islets are normal and no signs of inflammation, the islet cells show moderate cytoplasm and round to oval nuclei. The pancreas section of formulation dose 2 (250) mg/kg showed the islets are partly destroyed; the acini are lined by round to oval cells with moderate cytoplasm and small round to oval nuclei.

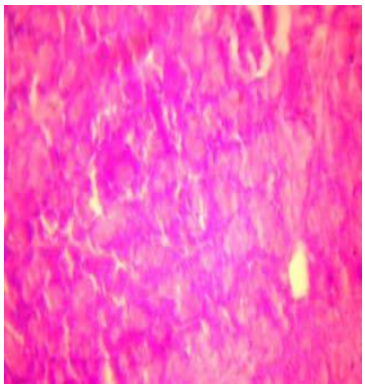

Figure 1: Pancreas of normal rats

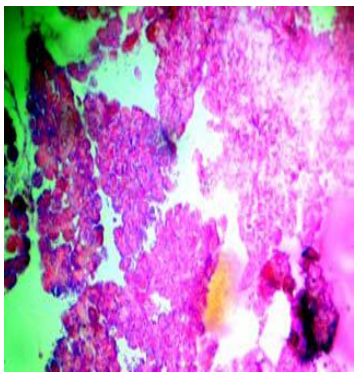

Figure 2: Pancreas of Control rats

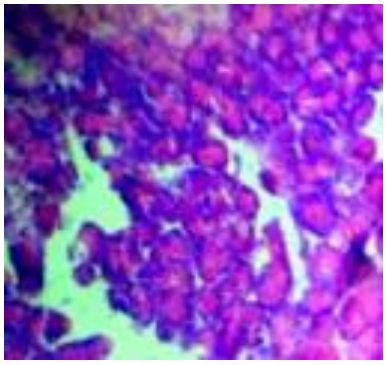

Figure 3: Pancreas of Glibenclamide treated rats

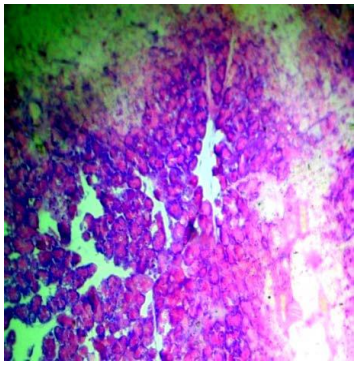

Figure 4: Pancreas of Formulation dose-1 (500 mg/kg) treated rats

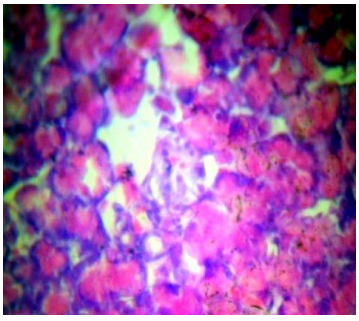

Figure 5: Pancreas of Formulation dose- 2 (250 mg/kg) treated rats

CONCLUSION

The polyherbal antidiabetic nutraceutical formulation consists of *Gymnema sylvestre*, *Trigonella foenum-graecum*, *Allium cepa*, *Curcuma longa*, *Phyllanthus amarus*, *Tinospora cordifolia*, *Eugenia jambolana*, *Cinnamomum zeylanicum*. In this study the formulation was screened for its safety and efficacy. Acute toxicity studies carried out conclude that formulation is safe up to a dose level of 5000mg/kg b.w. The formulation was screened for the antidiabetic activity using streptozotocin induced diabetes model and glibenclamide as standard. At a dose of 500mg/kg b.w. the formulation showed significant reduction in blood glucose level and increase in body weight. Microscopically, the pancreas section at the dose level of 500 mg/kg shows pancreas, the islets are normal and no signs of inflammation, the islet cells show moderate cytoplasm and round to oval nuclei. The results of the present investigation revealed that the polyherbal nutraceutical formulation is an efficacious and safer antidiabetic/ oral hypoglycaemic agent.

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