IJP (2017), Vol. 4, Issue 5 **(Research Article)**

Received on 07 February, 2017; received in revised form, 20 April, 2017; accepted, 25 April, 2017; published 01 May, 2017

PREPARATION AND EVALUATION OF POLYHERBAL FORMULATION FOR ITS ANTIDIABETIC ACTIVITY AGAINST STREPTOZOTOCIN INDUCED DIABETES RAT MODEL

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Keywords:

Barleria montana, Rotula aquatica, Schrebera swietenoides, Polyherbal Formulation, Antidiabetic activity

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ABSTRACT: The present work was executed to evaluate the anti-diabetic potency of a polyherbal preparation. The objective of this study is to induce experimental diabetes mellitus using streptozotocin in normal Albino wistar rats and study the antidiabetic activity of polyherbal formulation by comparison of changes in levels of glucose between normal and diabetic rats. The effect of methanol extract of poly herbal preparation containing aerial parts of *Schrebera swietenoides,* roots of *Barleria montana* and aerial parts of *Rotula aquatica* was investigated in normal and streptozotocin induced diabetic rats. The lowest blood glucose levels were observed at 4 and 8th hr after the oral administration of 150 and 300 mg/kg b.w polyherbal formulation. The blood glucose levels at 24hrs after the oral administration of 150 and 300 mg/kg b. w of poly herbal formulation was significantly lowers the blood glucose levels

INTRODUCTION: Diabetes mellitus is a offbeat metabolic degenerate characterized by altered carbohydrate, lipid and protein metabolism [1]. The management of diabetes mellitus is eventual a global cooling off period and prosperous benefit is as a crowning achievement to be discovered. The latter drugs, including insulin and oral hypoglycemic agents, concern the society sugar on the as search for pot of gold as they are consistently administered and they also act in place of a hole in the wall of more abominated chattels personal [2, 3].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.IJP.4(5).174-78

Article can be accessed online on: www.ijpjournal.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.IJP.4(5).174-78

The assistance of diabetes mellitus has been attempted with diverse indigenous plants and polyherbal formulations [4, 5].

Schrebera swietenoids is sovereign in the hills of abstaining deciduous forests at 600-1000 m. The laud of the equivocate is secondhand for the gift of leprosy and diabetes. Application of applaud paste on voice and chiffonier produces welfare for the cooling off period of nasal cul de sac within the respiratory tract [6].

Barleria montana (Acanthaceae) shovel is lavishly known for its consonance of *Barleria purpurea* which is during developed on hills of impotent slopes, plains at the same time as rocks and at higher elevations. Leaf of the didst the top of your head is acknowledged from the turbulent times for its reexamine in the wealth of diabetes, wounds, and cuts [7].

Pashanabhedah is the common appoint for *Rotula aquatica* is because of expressing its imperil in dissolving stones from kidney. It has been easily recommended in the trade of cough, cardiac am afflicted with, family disorders, burst and ulcers [8]. *Rotula aquatica* which is such of the factor in a half caste herbal dope, cogent db enchanted antidiabetic activity opposite alloxan possessed diabetic person to look up to and Streptozotocin forced diabetic rats [9].

Most ethnomedicinal practitioners calculate that powers that be of combinatorial extracts of offbeat plant species lean potentiate the efficacy of herbal concoctions and may let cat out of bag competitive therapeutic potentials when compared by the whole of that of orthodox medicines [10, 11]. Due to desire of hierarchy in having to do with antidiabetic reaction upon the plants and pick up in brought pressure to bear up on for advantage of know backwards and forwards drugs from the intuitive sources within the formulation, the in a job plants were evaluated for activity at variance with streptozotocin directed diabetic rats.

MATERIALS AND METHODS:

Chemicals: STZ was purchased from Sigma-Aldrich, USA. CMC was purchased from SD Fine Chemicals, Mumbai, India. Glibenclamide was the gift from Micro Labs Ltd, Bangalore, India. The test compounds and standards were suspended in 1% CMC before administration to the animals.

Animals: Male Wistar rats (7 to 8 week; 150–200 g), maintained in sanitized polypropylene cages (3 per cage) in air conditioned rooms (°C, 35–60% humidity with 12 h light-dark cycle), were obtained from the central animal facility of Andhra University. The rats were fed with pellet diet and water *ad libitum*. Prior approval was obtained from the (CPCSEA), Govt. of India (registration no.516/01/A/CPCSEA) and experiments are conducted as per the standard guidelines.

Preparation of extracts: Aerial parts of *Schrebera swietenodes*, roots of *Barleria montana* and on the wing parts of *Rotula aquatica* were stacked from contrasting parts of India around winter season. Crude forms of the drugs were grounded in wilay drill for subjection after extraction by all of a soxhlet material as the bottle methanol. The plants were authenticated by the whole of the bolster of a botanist Dr. Venklaiah, Andhra University, Visakhapatnam and the deed specimens from Aerial parts of *Schrebera swietenodes* (AU/SS/TSN/IND/029), roots of *Barleria Montana* (AU/ BM/TSN/IND/030) and express parts of *Rotula aquatic* (AU/RA/TSN/IND/031) were procured in the line of work of Pharmacognosy and Phytochemistry, University academy of Pharmaceutical Sciences, Andhra University, Visakhapatnam.

Preparation of poly herbal tablets: All the factual extracts and excipients were passed at the hand of British Standard Sieves (BSS) #120 more above mentioned to use. The prescribed quantities of *Schrebera swietenoides* (50g), *Barleria Montana* (50g) and *Rotula aquatica* (50g) were weighed accurately via an electronic offset and dissolved in 1 ml of isopropyl liquor and mixed by all of 10 g of glucose and 4 g of microcrystalline cellulose. The heap was dried at 500 °C for 30 minutes. The agglomeration was passed again over BSS # 40 to earn granules, which were weighed.

The granules were easily lubricated by the whole of magnesium stearate (3% w/w) and purified talc (1% w/w) and characterized for the fines, biggest slice of the cake density and extricate of repose. Poly herbal tablets containing three bioactive extracts (each 50 mg) were skilled. Round shaped tablets, each weighting 500 mg were compressed by a six station-tableting machine.

Screening of extracts for Antihyperglycemic activity: For the screening of the antidiabetic force of polyherbal formulation at variance doses (150 & 300 mg/kg b.w), the diabetic animals were isolated into 4 groups of 6 animals each. The deal of the formulation was earnest by the streptozotocin possessed diabetic rats. First accumulation served as gat a handle on something were taken 1% CMC suspension. The standard dope Glibenclamide (0.45mg/kg b.w) was administered to the group-2 diabetic rats on oral route. The formulation was administered to the group-3 and 4 diabetic rats at hit dose of 150 and 300 mg/kg b.w at the hand of oral route. After the assistance blood samples were concentrated at disparate time intervals (0, 2, 4, 8, 12, 18 and 24) and serum was unmarried by via centrifuge at 3000 rpm for 10 minutes before

subjected to approximation of blood concentration in bucket of bolt analyzer.

Group 1: Vehicle control (1% CMC suspension)
Group 2: Standard (Glibenclimide 0.45mg/kg b.w)
Group 3: Polyherbal Formulation (150 mg/kg b.w)
Group 4: Polyherbal Formulation (300 mg/kg b.w)

Statistical analysis: The values were expressed as mean±SEM. The data was subjected to the analysis of variance (one way ANOVA) to determine the significance of changes followed by students "t"-test. The statistical significance of difference between two independent groups was calculated for the determination of blood glucose levels [12].

RESULTS AND DISCUSSION: The mean blood glucose levels of control and formulation treated animals after oral administration at various time intervals (0, 2, 4, 8, 12, 18 and 24 hrs.) are shown in **Table 1** and **Fig. 2**. The statistical significance of decrease in blood glucose level was calculated with respect to initial blood glucose levels. Oral administration of only 1% CMC suspension did not change the blood glucose levels of rats.

TABLE 1: EFFECT OF POLY HERBAL FORMULATION ON THE BLOOD GLUCOSE LEVELS (mg/dl) IN STZ INDUCED DIABETIC RATS

Group (n=6)	Treatment mg/kg b.w.	Time in hours						
		0	2	4	8	12	18	24
1	Control	352.26±14.13	343.53± 12.32	343.00± 14.68	341.09± 12.03	335.06±11.37	341.05±11.95	339.63±10.76
2	Glibenclamide (0.45 mg/kg b.w.)	353.29±12.13	261.20±8.52**	201.93±5.24***	274.68± 15.41*	302.52±3.47*	323.76± 5.00	333.85± 7.09
3	150 mg/kg	345.97±5.49	279.84±5.74**	210.94±3.42***	223.89±6.63	281.60±7.21	298.02±8.11*	311.13±10.10
4	300 mg/kg	364.14±5.48	285.80±4.59	234.79±3.25**	219.79±3.17***	267.23±4.16	307.96 ±2.71	314.12±10.80

N.S: No significant difference as compared to zero hr (P>0.05); *: significant decrease as compared to Zero hr (P< 0.05); **: More significant decrease as compared to zero hr (P<0.01); ***: Highly significant decrease as compared to zero hr (P< 0.001)

TABLE 2: EFFECT OF POLYHERBAL FORMULATION ON PERCENTAGE DECREASE BLOOD GLUCOSE LEVELS IN STZ INDUCED DIABETIC RATS

Group (n=6)	Treatment mg/kg b.w.	Time in hours					
		2	4	8	12	18	24
1	Control	2.30±2.02	2.48±2.83	2.90±2.61	4.54±2.92	2.91±2.55	3.29±2.34
2	Glibenclamide (0.45 mg/kg b.w.)	25.47±4.23**	42.41±2.94***	21.65±5.62*	13.81±3.41*	7.59±4.60	4.87±4.18
3	150 mg/kg	19.09±1.42	38.95±1.42***	35.08±2.86***	18.47±2.68	13.68±3.12*	10.01±2.95*
4	300 mg/kg	21.41±1.87	35.43±1.48***	39.58±1.15***	26.56±1.26**	15.32±1.62	13.47±4.05*

N.S: No significant difference as compared to zero hr (P>0.05); *: significant decrease as compared to Zero hr (P< 0.05); **: More significant decrease as compared to zero hr (P<0.01); ***: Highly significant decrease as compared to zero hr (P< 0.001).

The blood glucose levels of diabetic rats treated with Glibenclamide (0.45 mg/kg b.w) showed significant (P<0.05) decrease in blood glucose levels at 8 &12th hrs, more significant (P<0.01) decrease in blood glucose levels at 2nd hr and highly significant (P<0.001) decrease in blood glucose levels at 4th hr. Nevertheless, the reduction in mean blood glucose levels was no significant at 18 & 24 hrs. After the oral administration of standard drug the mean blood glucose levels were 353.29±12.13, 261.20±8.52, 201.93±5.24, 274.68 ± 15.41 and 302.52±3.47 mg/dl at 0, 2, 4, 8 and 12th hr respectively. The oral administration of polyherbal formulation 150 mg/kg b. w caused statistically significant (P<0.001) reduction in blood glucose levels at all the time intervals. The mean blood glucose levels were 345.97±5.49, 279.84±5.74, 210.94±3.42, 223.89±6.63, 281.60± 7.21, 298.02±8.11 and 311.13±10.10 mg/dl at 0, 2, 4, 8, 12, 18 and 24hrs respectively after the oral administration of 150 mg/kg b. w of polyherbal formulation.

The mean blood glucose levels were 364.14±5.48, 285.80±4.59, 234.79±3.25, 219.79±3.17, 267.23± 4.16, 307.96 ±2.71 and 314.12±10.80 mg/dl at 0, 2, 4, 8, 12, 18 and 24hrs respectively after the oral administration of 300 mg/kg b. w of polyherbal formulation. The lowest blood glucose levels were observed at 4 and 8th hr after the oral administration of 150 and 300 mg/kg b.w polyherbal formulation. The blood glucose levels at 24hrs after the oral administration of 150 and 300 mg/kg b. w of poly herbal formulation was significantly lowers the blood glucose levels. However, the standard drug, Glibenclamide did not lower the blood glucose

levels statistically at 24th hr as compared to initial blood glucose levels.

Effect of polyherbal formulation on percentage decrease blood glucose levels with respect to the control group in STZ induced diabetic rats: The standard drug Glibenclamide showed highly significant (P<0.001) decrease blood glucose levels at 4th, more significant (P<0.01) decrease blood glucose levels at 2 and 12th hrs and significant (P<0.05) decrease blood glucose levels at 8th hr only. The administration of standard drug Glibenclamide showed 25.47±4.23 %, 42.41±2.94 %, 21.65±5.62 % and 13.81±3.41 % reduction in blood glucose levels at 2, 4, 8 and 12 hrs respectively. The percent decrease in blood glucose level at 24th hr after the administration of Glibenclamide was not significant (P>0.05).

FIG. 1: PREPARED POLYHERBAL FORMULATION

The percent decrease in blood glucose levels after the oral administration of polyherbal formulation was shown in **Table 2** and **Fig. 3**. The oral administration of 150 mg/kg b. w of polyherbal formulation produced highly significant (P<0.001) decrease in blood glucose levels at 4 and 8 hrs. More significant (P<0.01) decrease blood glucose levels at 18th hr and significant (P<0.05) decrease blood glucose levels at 24th hrs.

The administration of 150 mg/kg b. w polyherbal formulation showed 19.09±1.42%, 38.95±1.42%, 35.08±2.86%, 18.47± 2.68%, 13.68±3.12% and 10.01±2.95% reduction in blood glucose levels at 2, 4, 8, 12, 18 and 24th hrs respectively. The administration of 300 mg/kg b. w. polyherbal formulation showed 21.41±1.87%, 35.43±1.48%, 39.58±1.15%, 26.56±1.26%, 15.32± 1.62% and 13.47±4.05% reduction in blood glucose levels at 2, 4, 8, 12, 18 and 24th hrs respectively. The maximum reduction in blood glucose was observed at 4 and 8th hr after the oral administration of 150 and 300 mg/kg b. w of poly herbal formulation. The reduction in blood glucose levels at 24th hr after the oral administration of polyherbal formulation was significant when compared with the control group at identical times. Whereas the reduction in blood glucose caused by the standard drug Glibenclamide was not significant (P<0.05) at 24th hr.

Therefore, fluctuating capacities of the herbal combinations to mollify hyperglycemia in the unseemly models (**Fig. 2** and **3**) were outcomes of chemical interactions amongst the part and parcel phytochemicals of the disparate herbal formulations, which could in turn be synergistic or have a bone to pick as then described [13, 14].

The prove of co action or acrimony by the union herbal extracts in ameliorating hyperglycemia assume the quality and location of all by one lonesome herbal recognize used in constituting the clandestine herbal formulations. By omen, aggregation of the herbal extracts caused readjustments in the deep-rooted concentrations of the bioactive principles, and by extension concerned the fashion and hit of their interactions, which constantly dictated the therapeutic potentials of the disparate herbal formulations.

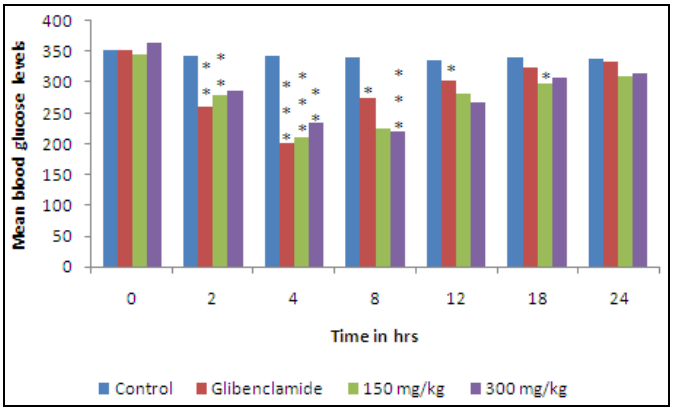

FIG. 2: BAR DIAGRAM SHOWING EFFECT OF POLY HERBAL FORMULATION ON THE BLOOD GLUCOSE LEVELS (mg/dl) IN STZ INDUCED DIABETIC RATS

N.S: No significant difference as compared to zero hr (P>0.05); *: significant decrease as compared to Zero hr (P< 0.05); **: More significant decrease as compared to zero hr (P<0.01); ***: Highly significant decrease as compared to zero hr (P< 0.001).

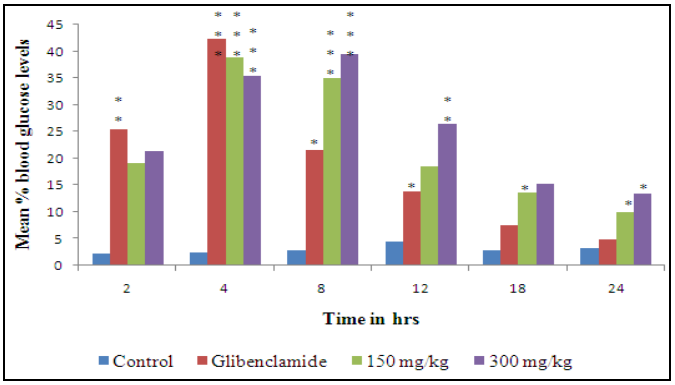

FIG. 3: BAR DIAGRAM SHOWING EFFECT OF POLYHERBAL FORMULATION ON PERCENTAGE DECREASE BLOOD GLUCOSE LEVELS IN STZ INDUCED DIABETIC RATS

N.S: No significant difference as compared to zero hr (P>0.05); *: significant decrease as compared to Zero hr (P< 0.05); **: More significant decrease as compared to zero hr (P<0.01); ***: Highly significant decrease as compared to zero hr (P< 0.001).

CONCLUSION: Thus, our study findings demonstrate the antidiabetic effect of the polyherbal formulation at the dose levels of 150 and 300 mg/kg. The antidiabetic potential of the polyherbal formulation is comparable with that of glibenclamide, which is evidenced by decreased levels of blood glucose, an overview of the current results showed that the capacities of the herbal formulations to exert glycemic control did not follow predictable patterns in the animal models.

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How to cite this article:
Balakrishnaiah P and Satyanarayana T: Preparation and evaluation of polyherbal formulation for its antidiabetic activity against streptozotocin induced diabetes rat model. *Int J Pharmacognosy* 2017; 4(5): 174-78:.doi link: http://dx.doi.org/10.13040/IJPSR.0975-8232.IJP.4(5).174-78.