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Preparation and evaluation of polyherbal formulation for its antidiabetic activity against streptozotocin induced diabetes rat model

Dr. P. Solairaj[1], S. Jebaseelan[2]

[1] *Department of Pharmaceutical Chemistry, Sankaralingam bhuvaneswari college of pharmacy, Sivakasi.*
[2] *Department of Pharmaceutical Chemistry, Ultra college of pharmacy, Madurai-20*

Abstract

Objective: The present work was executed to evaluate the anti diabetic potency of a poly herbal preparation. The objective of this study is to induce experimental diabetes mellitus using streptozotocin in normal albino wistar rats and studied the antidiabetic activity of polyherbal formulation by comparison of changes in levels of glucose between diabetic and normal rats.

***Methods*:**A polyherbal formulation containing 3 antidiabetic herbs leaf parts of *Aegle Marmelos, Madhuca indica* , rhizome of *Costus speciosus* was investigated in normal and streptozotocin induced diabetic rats.

Results:The lowest blood glucose levels observed at Day 1,5,10 at the dose levels of 200mg/kg and 400 mg/kg b/w of polyherbal formulation. The blood glucose levels at 24 hrs after oral administration of 200mg/kg and 400 mg/kg b/w of polyherbal formulation was significantly lowers the blood glucose levels.

Conclusion: The display of synergy or anatagonism by the composite of herbal extracts in ameliorating hyperglycemia depended on the type and number of individual herbal extract used in constituting the experimental herbal formulations.

Keywords: Leaf parts of *Aegle Marmelos, Madhuca indica* , rhizome of *Costus speciosus* ,polyherbal formulation, antidiabetic activity.

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

A. marmelos is a native plant of India. *A. marmelos* belongs to Rutaceae family and commonly known as wood apple. In India, *A. marmelos* is grown as a temple garden plant and the leaves are used to pray Lord Shiva. *A. marmelos* is an important medicinal plant with several ethnomedicinal applications in traditional and folk medicinal systems. Traditionally, *A. marmelos* is used in the treatment of diarrhea and dysentery. Leaves of this plant used to cause infertility/abortion in women.[6]

Madhuca indica is a forest tree found in central and northern India and Malaysia. It is commonly observed in various parts of the Indian sub-continent, including Bangladesh. In the folk medicinal system of Bangladesh, various parts of the tree are used, namely whole young plants, leaves, stems, barks, roots, fruits, flowers, and seeds.[207]Extensive studies have been carried out on *M. indica*. A number of triterpenoids including α- amyrin acetate **(I)**, β-amyrin acetate **(II)**, 3β-monocaprylic ester of erythrodiol **(III)**, 3β-capryloxy oleanolic acid **(IV)** and 3β-capryloxy oleanolic acetate **(V)** from the mesocarp of *Madhuca indica* fruit. The other constituents isolated and characterized are n-hexacosanol **(VI)**, β-D-glucoside of β-sitosterol **(VI)** and free β-sitosterol **(VII)**. The nutshell extracts yielded β-D-glucoside of β-sitosterol, quercetin **(VIII)** and dihydroquercetin **(IX).**[7]

Costus speciosus Koen. (Keu, Crape ginger), an Indian ornamental plant, has long been medicinally used in traditional systems of medicine. This plant of Costaceae family is commonly known as *Keukand* (Hindi), Variegated Crepe Ginger (English). It is an erect, succulent, perennial herb, up to 2.7 m in height, arising from a horizontal rhizome, found in tropical region of India and also cultivated for ornament. The rhizomes and roots are ascribed to be bitter, astringent, acrid, cooling, aphrodisiac, purgative, anthelmintic, depurative, febrifuge, expectorant, tonic, improves digestion and stimulant herb that clears toxins. Juice of the rhizome is applied to head for cooling and relief from headache [8-13].

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.[14,15] 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.

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 and experiments are conducted as per the standard guidelines.

Preparation of Herbal Suspension

After extraction, evaporated residue extracts were suspended in water using tween-80 as a suspending agent. The residual extracts were mixed in the same concentration. The additives used are sodium CMC, Tween-80, Lemon oil, Sodium Saccharin, and Methyl paraben according to the quantity mentioned in table.

General Formula for the Development of Antidiabetic Polyherbal Formulation

Ingredient	Quantity Taken
Aegle marmelos leaf ethanol extract	0.4 gm
Madhuca indica leaf methanol extract	0.4 gm
Costus speciosus rhizome methanol extract	0.4 gm
Tween 80	0.1%
Sodium CMC	2 gm
Lemon oil	0.15%w/v
Methyl Paraben	0.20%w/v
Sodium Saccharin	0.1%w/v
Purified Water q.s	100ml

Stability studies of polyherbal formulation

These studies were performed to determine the stability of formulation and to check the effect of environmental factor.

Physical tests for Polyherbal formulation:

The physical tests of polyherbal formulation was carried out for three month at room temperature (+25 ^{0}C) and 45 ^{0}C. The results were also same for 2nd and 3rd month.

Accelerated Stability Studies: The accelerated stability studies were carried out for polyherbal formulation for the period of three months. The different parameter such as sedimentation volume,redispersibility, rheology, flow rate ,viscosity, pH, particle size analysis, crystal growth were studied forthe formulation at 1st ,2nd and 3rd months.

1) **Sedimentation volume:** The sedimentation volume is ratio of the ultimate height (Vu) of the sediment to the initial height (Vo) of the total suspension as the suspension settles in a cylinder under standard conditions. It was determined by keeping a measured volume of suspension in a graduated cylinder in an undisturbed state for a certain period of time and note that the volume of the sediment which is expressed as ultimate height.

F = Vu / Vo = Ultimate Volume of the Sediment / Initial Volume of the Suspension

2) Redispersibility: The suspension was allowed to settle in a measuring cylinder. The mouth of the cylinder was closed and was inverted through 180^{0}and the number of inversions necessary to restore a homogenous suspension was determined. If the homogeneity of the suspension was attained in one inversion, then the suspension was considered 100% easily redispersible. Every additional inversion decreases the percentage of ease of redispersibility by 5%.

3) Rheology: The time required for each suspension sample to flow through a 10 ml pipette was determined the apparent viscosity by using the equation.

4) Viscosity: The viscosity of the sample was determined at room temperature using Brookfield viscometer at 50 rpm by using spindle no.3. All determinations were made in at least three times and the results obtain are expressed as the mean values.

5) pH: The pH of suspension was determined at intervals of one week for 3 months using pH meter.

6) Crystal Growth: Stability of suspension will also decrease because of crystal growth, which usually occurs from temperature fluctuation during storage and form broad particle size distribution. Crystal formation was determined at 4^{0}C, room temperature (RT) and 47^{0}C by taking test tube A, B, and C with sample for 24 hours, 48 hours and 72 hours.

Experimental induction of diabetes mellitus

The rats were injected streptozotocin dissolved in sterile normal saline at a dose of 150mg/kg body weight, intraperitonally. Since alloxan is capable of producing fatal hypoglycemia as a result of massive pancreatic insulin release, rats were treated with 10% glucose solution (8-10ml) intraperitonally after 6hrs.The rats were then kept for the next 24hrs on 5% glucose solution bottles in their cages to prevent hypoglycemia[16,17].

Treatment

Diabetic rats were randomly divided into four groups with 5rats in each group and all the drugs were given orally for 10days as follows. Group I was given aqueous suspension of 2% gum acacia as control, Group II was given aqueous suspension of polyherbal formulation containing 3 antidiabetic herbs 200mg/kg ,Group III was given aqueous suspension of polyherbal formulation containing 3 antidiabetic herbs 400mg/kg, Group IV was given Glibenclamide 10mg/kg The blood samples were collected before and after administration of drugs at 2, 4, 6 and 8hrs on 1st, 5th and 10th day of from retro orbital sinus puncture. The serum was separated from the blood samples by centrifugation and was analyzed for glucose, cholesterol and triglycerides levels by analytical method[18]. The concentration of glucose, cholesterol, and triglycerides levels in the blood at each time interval was calculated and expressed as mg/dL. Percentage reduction of glucose, cholesterol, and triglycerides levels in blood[19-21] from each group at different time [22]intervals was calculated. On 10th day after blood samples collection the rats were scarified by cervical dislocation and the pancreas were isolated and taken into container containing formalin and subjected to histopathological studies.

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 [23].

RESULTS AND DISCUSSION

Percentage reduction of glucose, cholesterol and triglycerides levels in blood with of **polyherbal formulation containing 3 antidiabetic herbs 200mg/kg in diabetic rats**.

The percentage reduction of glucose, cholesterol and triglycerides levels in blood with 200mg/kg of polyherbal formulation containing 3 antidiabetic herbs was found to be in (Day-1) 27.798, 21.388 and 20.46 (Day-5) 31.87, 27.42 and 21.98 (Day-10) 38.06, 30.402 and 24.818 after 4hrs of administration. Table 1,2,3

Table 1: ±SEM values of percentage reduction of glucose, cholesterol and triglycerides levels in blood with polyherbal formulation containing 3 antidiabetic herbs 200mg/kg of diabetic rats

GROUP	polyherbal formulation containing 3 antidiabetic herbs DAY1			
	2hr	4hr	6hr	8hr
% reduction of glucose	11.542 ±0.560*	27.798 ± 1.304**	17.79 ± 0.506**	11.5 ± 0.683*
% reduction of choloesterol	7.334 ±0.456*	21.388 ± 0.636**	15.51 ± 1.370*	9.24 ± 1.653*
% reduction of triglycerides	8.68 ± 0.897*	20.46 ± 0.654*	11.96 ± 0.745*	7.864 ± 1.224*

Table 2: ±SEM values of percentage reduction of glucose, cholesterol and triglycerides levels in blood with polyherbal formulation containing 3 antidiabetic herbs 200mg/kg of diabetic rats

GROUP	polyherbal formulation containing 3 antidiabetic herbs DAY 5			
	2hr	4hr	6hr	8hr
% reduction of glucose	14.24 ± 1.573*	31.87 ± 2.495**	17.43 ± 2.240**	10.494 ± 0.849*
% reduction of choloesterol	16.69 ± 1.905**	27.42 ± 0.4.65**	19.94 ± 0.494*	11.854 ± 0.5749*
% reduction of triglycerides	11.45 ± 0.572*	21.98 ± 0.760*	13.21 ± 0.687*	9.134 ± 0.982*

Table 3: ±SEM values of percentage reduction of glucose, cholesterol and triglycerides levels in blood with polyherbal formulation containing 3 antidiabetic herbs 200mg/kg of diabetic rats

GROUP	polyherbal formulation containing 3 antidiabetic herbs DAY 10			
	2hr	4hr	6hr	8hr
% reduction of glucose	23.31 ± 0.692**	38.06 ± 1.591***	24.12 ± 1.729**	14.66 ± 1.718*
% reduction of choloesterol	19.37 ± 0.833*	30.402 ± 0.5874**	22.41 ± 0.538**	13.16 ± 0.939*
% reduction of triglycerides	13.33 ± 0.441*	24.818 ± 0.881**	17.44 ± 0.629*	10.97 ± 0.555*

Percentage reduction of glucose, cholesterol and triglycerides levels in blood with polyherbal formulation containing 3 antidiabetic herbs 400mg/kg in diabetic rats.

The percentage reduction of glucose, cholesterol and triglycerides levels in blood with 400mg/kg of polyherbal formulation containing 3 antidiabetic herbs was found to be in (Day-1) 37.658, 27.358 and 23.274 (Day-5) 45.11, 34.95 and 27.06 (Day-10) 50.50, 40.90 and 32.74 after 4hours of administration. Table 4,5,6

Table 4: ±SEM values of percentage reduction of glucose, cholesterol and triglycerides levels in blood with polyherbal formulation containing 3 antidiabetic herbs 400mg/kg of diabetic rats

GROUP	polyherbal formulation containing 3 antidiabetic herbs DAY 1			
	2hr	4hr	6hr	8hr
% reduction of glucose	17.29 ± 1.659*	37.658 ± 1.075**	27.54 ± 1.477**	16.56 ± 0.440**
% reduction of choloesterol	13.81 ± 1.529*	27.358 ± 1.904**	19.02 ± 1.973**	16.50 ± 1.383*
% reduction of triglycerides	10.64 ± 1.381*	23.274 ± 0.825**	17.37 ± 1.856**	10.03 ± 0.490*

Table 5: ±SEM values of percentage reduction of glucose, cholesterol and triglycerides levels in blood with polyherbal formulation containing 3 antidiabetic herbs 400mg/kg of diabetic rats

GROUP	polyherbal formulation containing 3 antidiabetic herbs DAY 5			
	2hr	4hr	6hr	8hr
% reduction of glucose	25.62 ± 0.822**	45.11 ± 2.155***	31.07 ± 2.902**	21.406 ± 1.755**
% reduction of choloesterol	18.45 ± 0.482*	34.95 ± 3.073**	25.92 ± 1.863**	17.50 ± 1.337*
% reduction of triglycerides	15.52 ± 1.428*	27.06 ± 1.517**	18.42 ± 2.415**	12.704 ± 1.812*

Table 6: ±SEM values of percentage reduction of glucose, cholesterol and triglycerides levels in blood with polyherbal formulation containing 3 antidiabetic herbs 400mg/kg of diabetic rats

GROUP	polyherbal formulation containing 3 antidiabetic herbs DAY 10			
	2hr	4hr	6hr	8hr
% reduction of glucose	29.69 ± 0.699**	50.50 ± 1.906***	33.14 ± 1.849**	22.95 ± 1.042**
% reduction of choloesterol	21.79 ± 0.530*	40.904 ± 1.949**	28.02 ± 1.476**	18.32 ± 0.9316*
% reduction of triglycerides	18.11 ± 0.775*	32.742 ± 1.418**	21.15 ± 2.886*	14.41 ± 1.723*

Percentage reduction of glucose, cholesterol and triglycerides levels in blood with Glibenclamide 10mg/kg in diabetic rats

The percentage reduction of glucose, cholesterol and triglycerides levels in blood with 10mg/kg of Glibenclamide was found to be in (Day-1) 39.18, 21.20 and 18.65 (Day-5) 49.11, 23.81 and 20.87 (Day-10) 57.91, 29.35 and 24.06 after 4hrs of administration. Table 7,8,9.

Table 7: ±SEM values of percentage reduction of glucose, cholesterol and triglycerides levels in blood with with Glibenclamide 10mg/kg of diabetic rats

GROUP	Glibenclamide 10mg/kg DAY 1			
	2hr	4hr	6hr	8hr
% reduction of glucose	19.09 ± 1.319*	39.18 ± 3.47**	23.78 ± 2.823**	18.39 ± 0.883**
% reduction of choloesterol	10.52 ± 0.347*	21.20 ± 0.739**	19.40 ± 2.317**	11.33 ± 1.215*
% reduction of triglycerides	9.482 ± 1.180*	18.65 ± 0.986**	11.75 ± 0.786**	10.21 ± 0.785*

Table 8: ±SEM values of percentage reduction of glucose, cholesterol and triglycerides levels in blood with Glibenclamide 10mg/kg of diabetic rats

GROUP	Glibenclamide 10mg/kg DAY 5			
	2hr	4hr	6hr	8hr
% reduction of glucose	23.08 ± 1.088**	49.11 ± 2.977***	34.33 ± 1.396**	22.156 ± 0.974**
% reduction of choloesterol	10.65 ± 0.526*	23.81 ± 0.875**	15.08 ± 0.626**	10.682 ± 0.518*
% reduction of triglycerides	10.356 ± 0.706*	20.87 ± 0.372**	13.21 ± 1.053**	9.656 ± 0.407*

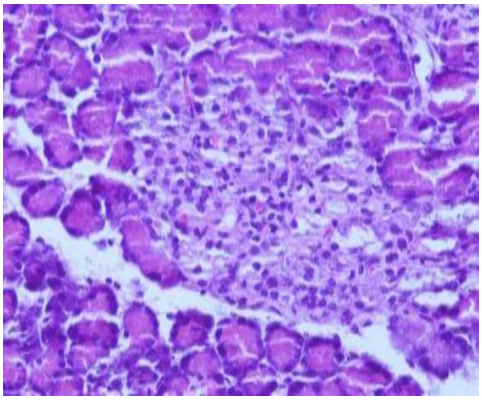
Table 9: ±SEM values of percentage reduction of glucose, cholesterol and triglycerides levels in blood with Glibenclamide 10mg/kg of diabetic rats

GROUP	Glibenclamide 10mg/kg DAY 10			
	2hr	4hr	6hr	8hr
% reduction of glucose	34.79 ± 1.020**	57.91 ± 0.904***	41.98 ± 1.295**	27.00 ± 0.764**
% reduction of choloesterol	17.64 ± 0.611*	29.352 ± 1.117**	21.61 ± 0.973**	15.69 ± 0.947*
% reduction of triglycerides	14.99 ± 1.127*	24.068 ± 1.068**	16.17 ± 0.757**	10.74 ± 0.932*

Histopathological studies

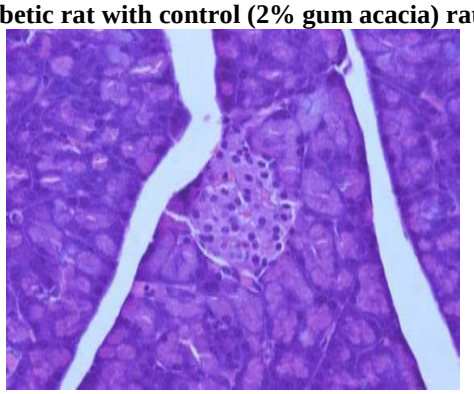
1). Control Rat without diabetes: Shows normal pancreatic acini and ducts. Islets are also normal with round nuclei and fine abundant cytoplasm. (Fig 1)

Fig. 1: Microscopic features of control rat without diabetes



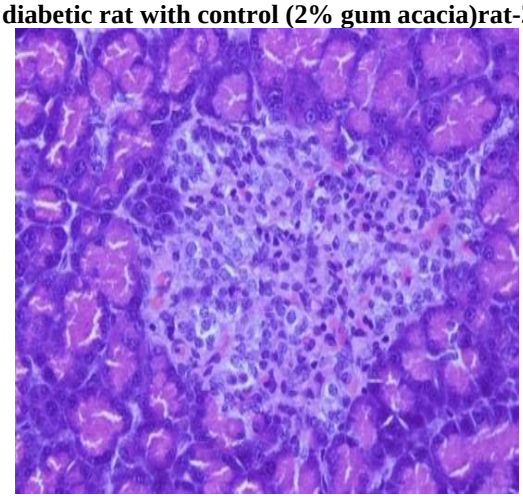
2).Streptozotocin induced diabetic rat with control Rat-1 (2%gumacacia): Acini are normal. However the islets are decreased in number. There is increased vascular congestion seen with infiltration by lymphocytes. Many cells have pyknotic nuclei with vacuolated cytoplasm. Few cells have elongated nuclei. (Fig 2)

Fig. 2: Microscopic features of streptozotocin induced diabetic rat with control (2% gum acacia) rat-1



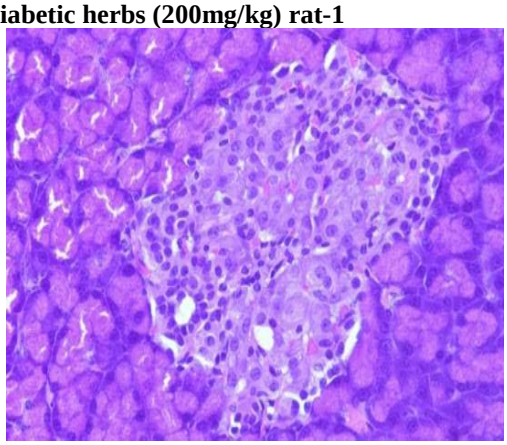
3). Streptozotocin induced diabetic rat with control Rat-2(2% gum acacia): Acini are normal. Number of islets cells is decreased in number. These cells have vacuolated cytoplasm and round to oval nuclei. The stroma shows congestion and mild lymphocytic infiltrate. (Fig 3)

Fig. 3: Microscopic features of streptozotocin induced diabetic rat with control (2% gum acacia)rat-2



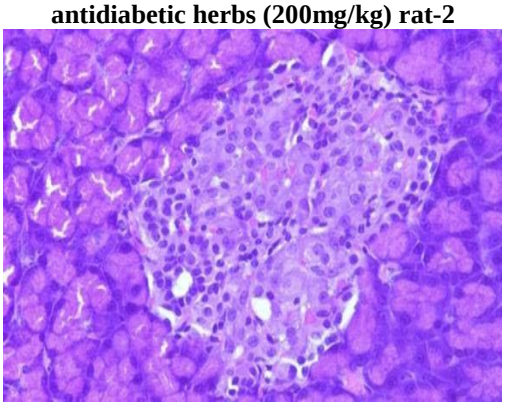
4). Streptozotocin induced diabetic rat with polyherbal formulation containing 3 antidiabetic herbs (200mg/kg) in Rat-1: Islet cells are still decreased in number. The cells have round to oval nuclei with fine chromatin while few have dark nuclei. Cytoplasm of many cells is vacuolated. There is mild lymphocytic infiltrate seen. (Fig 4)

Fig. 4: Microscopic features of Streptozotocin induced diabetic rat with polyherbal formulation containing 3 antidiabetic herbs (200mg/kg) rat-1



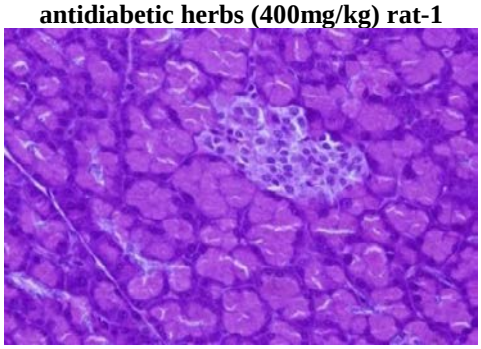
5). Streptozotocin induced diabetic rat with polyherbal formulation containing 3 antidiabetic herbs (200mg/kg) in Rat-2: Acini are normal. Islets are decreased in number. Their nuclei vary from round to oval with fine to dark chromatin. The cytoplasm of many cells is still vacuolated. The stroma shows few congested blood vessels or lymphocytic infiltrate. The findings are almost similar to position or Streptozotocin induced control rats. (Fig 5)

Fig. 5: Microscopic features of Streptozotocin induced diabetic rat with polyherbal formulation containing 3 antidiabetic herbs (200mg/kg) rat-2



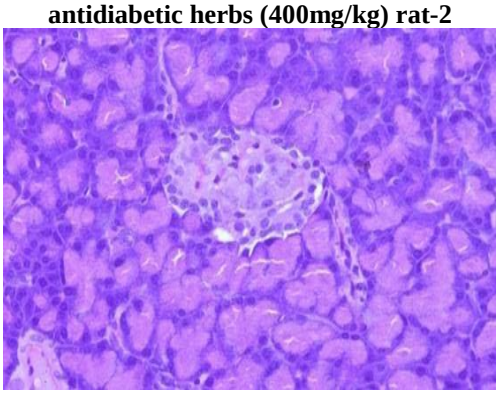
6). Streptozotocin induced diabetic rat with polyherbal formulation containing 3 antidiabetic herbs (400mg/kg) in Rat-1: Acini are normal. The islets are in process of regeneration with mild increase in numbers. Vacuolated cytoplasm is persisting in some cells. The remaining cells have moderate eosinophil cytoplasm. Number of inflammatory cells has decreased. Regeneration is seen and the changes are less severe when compared to positive control and standard. (Fig 6)

Fig. 6: Microscopic features of Streptozotocin induced diabetic rat with polyherbal formulation containing 3 antidiabetic herbs (400mg/kg) rat-1



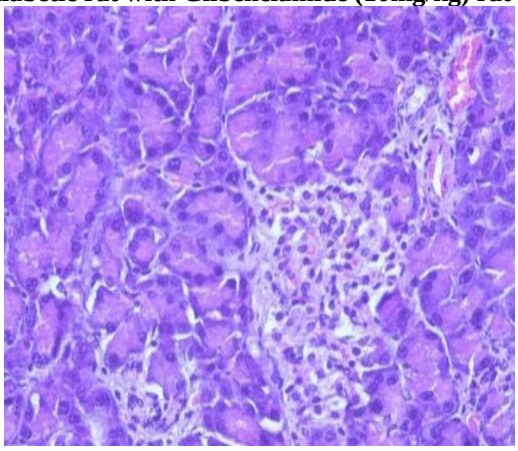
7). Streptozotocin induced diabetic rat with polyherbal formulation containing 3 antidiabetic herbs (400mg/kg) in Rat-2: Acini are normal. Regeneration is seen in islets and the histopathological changes are less in severity than the standard and positive control. (Fig 7)

Fig. 7: Microscopic features of Streptozotocin induced diabetic rat with polyherbal formulation containing 3 antidiabetic herbs (400mg/kg) rat-2



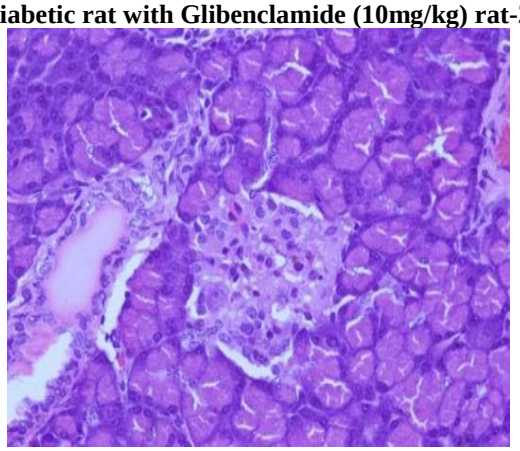
8). Streptozotocin induced diabetic rat with Glibenclamide (10mg/kg) in Rat-1: Acini are normal. Islets are however slightly decreased in number with most show vacuolated cytoplasm. The nuclei are vesicular. The number of lymphocytes is also less. (Fig 8)

Fig. 8: Microscopic features of Streptozotocin induced diabetic rat with Glibenclamide (10mg/kg) rat-1



9). Streptozotocin induced diabetic rat with Glibenclamide (10mg/kg) in group-2: Acini are normal. Islets are however slightly less in number with many having vacuolated cytoplasm. The nuclei are vesicular**.** (Fig 9)

Fig. 9: Microscopic features of Streptozotocin induced diabetic rat with Glibenclamide (10mg/kg) rat-2



As polyherbal formulation containing 3 antidiabetic herb also showed reduction in the glucose levels at 4hours same as Glibenclamide. It indicates that may be polyherbal formulation containing 3 antidiabetic herb also as same mechanism of action as Glibenclamide. It is well established that sulphonylureas produce hypoglycemia by increasing the secretion of insulin from pancreas [281] and these compounds are active in mild streptozotocin induced diabetes where as they are inactive in intense streptozotocin induced diabetes nearly all beta cells have been destroyed. Since results showed that Glibenclamide reduced blood glucose levels hyperglycemic animals. The state of diabetes is not severe. Streptozotocin treated animals receiving the polyherbal formulation containing 3 antidiabetic herb showed rapid normalization of blood glucose levels in comparison to control and this be due to the possibility that some beta cells remaining still surviving to act upon by polyherbal formulation containing 3 antidiabetic herb to exert its insulin releasing effect. More over this suggests that the mode of action active ingredients of polyherbal formulation containing 3 antidiabetic herb is probably mediated by enhanced secretion of insulin, like sulphonylureas. The anti-hyperglycemic and hypoglycemic effects of polyherbal formulation containing 3 antidiabetic herb may be due to multiple effects of chemical constituents of different plants in polyherbal formulation containing 3 antidiabetic herb Histopathological results showed the regeneration of pancreatic islets in polyherbal formulation containing 3 antidiabetic herb 400mg/kg when compared to standard Glibenclamide 10mg/kg.

CONCLUSIONS

Thus, our study findings demonstrate the antidiabetic effect of the polyherbal formulation at the dose levels of 200 and 400 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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