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Research Article

ANTI DIABETIC ACTIVITY OF POLY HERBAL FORMULATIONS

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ABSTRACT

Objective: To evaluate the Anti diabetic activity of Azardirachta indica (Meliaceae), Mangifera indica (Anacardiaceae) and Musa sapientum (Musaceae) and to compare the activity between the plant extracts and a known anti diabetic drug Glibenclamide (4mg/kg body wt).

Methods: The plant extracts were investigated for Anti diabetic activity by Streptozotocin induced diabetes in rats. Dose selection was made on the basis of acute oral toxicity study a per OECD (423) guidelines. All the three plant extracts were formulated as poly herbal formulation in the ratio of 1:4:1.

Results: Oral administration of poly herbal formulation for 21 days resulted in significant reduction in blood glucose levels. Steptozotocin induced diabetic rat model was used for evaluation of anti diabetic activity. It also prevented body weight loss in diabetic rats.

Conclusion: All the plant extracts formulated as poly herbal formulation in the ratio of 1:4:1 exhibited significant Anti diabetic activity.

Key words: Anti diabetic activity, acute oral toxicity, poly herbal formulation, and streptozotocin.

INTRODUCTION

Diabetes is a complex disorder characterized by hyperglycemia resulting from defective insulin secretion, resistance to insulin action or both [1]. The increased oxidative stress is mainly involved in the pathogenesis and progression of diabetic tissue damage. Herbal medicines have been used for the treatment of diabetic patients since long and they are currently accepted as an alternative therapy for diabetic treatment. More than 1200 plants have been described in the scientific and popular literature as hypoglycemic agents [2].

MATERIALS AND METHODS

Plant material

The leaves of Azardirachta indica, Mangifera indica and flowers of Musa sapientum were collected from local area in Chennai. The plant materials was identified and authenticated by resident botanist, professor Dr.S.Jayaraman, Plant Anatomy Research Centre (PARC), Chennai. A voucher specimen was submitted at C.L.Baid Metha College of Pharmacy, Chennai. The dried leaves of Azardirachta indica, Mangifera indica and dried flowers of Musa sapientum were subjected to extraction by continuous hot percolation using chloroform as solvent and were subjected to standardization using pharmacognostical and phytochemical screening. The poly herbal formulation was prepared by 1:4:1 ratio using 1%CMC as a suspending agent.

Induction of diabetes mellitus in experimental animals [3,4]

Adult inbred wistar albino rats (32) of either sex were overnight fasted and received a freshly prepared solution of streptozotocin (STZ), [Sigma chemical Co, St Louis, MO, USA], (50mg/kg) in 0.1M citrate buffer, p^H 4.5, injected intraperitonially. After injection the animals had free access to food and water and were given 5% glucose in their drinking water for the first 24hrs to counter any initial hypoglycemia.

The development of diabetes was confirmed after 72 hrs of the STZ injection. The animals with fasting blood glucose level more than 200mg/dl were selected for the experimentation. out of 32 animals subjected for diabetes induction, 6 animals died before grouping and two animals were omitted from the study, because of sub-diabetic conditions (118mg/dl) and (122mg/dl). From the remaining 24 animals 4 groups of 6 animals were formed and used for the experimentation.

Procedure

Albino rats of either sex weighing between 200-250g were selected and acclimatized under standard laboratory conditions at 25±2ºc, 50±15% RH and normal photoperiod 12:12 light: dark cycle for 7 days. Then animals are divided into 5 groups each containing 6 rats. All the groups received single dose treatment in the following manner.

Group-I: Normal control animals received 1% SCMC.

Group-II: STZ (50mg/kg body wt) induced animals received 1%SCMC.

Group-III: STZ (50mg/kg body wt) induced animals received poly herbal formulation: 200mg/kg body wt/p.o.

Group-IV: STZ (50mg/kg body wt) induced animals received poly herbal formulation: 400mg/kg body wt/p.o.

Group-V: STZ (50mg/kg body wt) induced animals received glibenclamide 4mg/kg body

Wt/p.o.

The body weight gain and fasting blood glucose levels of all rats were recorded at regular intervals during the experimental period.

Glucose tolerance test

After 21 days of treatment, a fasting blood sample was collected from all groups. Blood samples were also collected at the time intervals of 30 min, 1hr, 2hr, and 3hr after administration of glucose at a concentration of 2g/kg of body wt [5].

Biochemical assay

After 21 days of treatment, the fasted rats of various groups were sacrificed by cervical decapitation. Fasting blood glucose was estimated by using a commercial glucometer and test-strips (Accuchek sensor test meter). At the end of the study, all the animals were sacrificed under light ether anesthesia. The rats were sacrificed by decapitation and blood was collected by bleeding of retro-orbital plexus using micro capillary technique from all the groups of overnight fasted rats and serum was seperated to study the biochemical parameters. The level of glycosylated hemoglobin was estimated by using glycosylated haemogloobin test kit. Total protein, Total cholesterol, HDL, Triglycerides, LDL, VLDL, Creatinine were estimated by using kit.

Table 1: Effect of PHF on Blood Glucose Level in Normal Glucose Loaded Rats (GTT) and in STZ Induced Diabetic Groups

Test sample mg/kg	Blood glucose levels mg/dl						Blood glucose levels mg/dl				
	0 min	30 min	60 min	90 min	120 min		0 min	30 min	60 min	120 min	240min
Control	88.6 ± 2.531	92.5 ± 2.34	110.2 ± 3.01	105.8 ± 3.01	97.5 ± 3.42	Control	82.1 ± 1.6	90.5 ± 2.29	91.0 ± 2.53	94.3 ± 2.26	96.0 ± 2.54
						Diabetic control-STZ	252.6 ± 6.9	266.4 ± 3.90**	306.6 ± 5.07**	332.7 ± 3.2**	356.5 ± 4.28**
PHF-200	90.1 ± 2.603	89.7 ± 1.96	102.5 ± 2.63ns	94.4 ± 2.21*	89.1 ± 2.6*	PHF-200+ STZ	244.5 ± 5.4	266.2 ± 3.5ns	299.3 ± 4.51	305.3 ± 4.0**	326.3 ± 3.28**
PHF-400	92.4 ± 2.812	88.2 ± 3.408	99.6 ± 2.540*	85.6 ± 2.55**	79.3 ± 3.15**	PHF-400 +STZ	240.2 ± 3.5	264 ± 4.94ns	271.8 ± 4.57**	288.7 ± 3.0**	299.7 ± 4.29**
Glibenclamide	93.2 ± 2.706	85.7 ± 1.856	81.1 ± 2.617**	74.3 ± 3.12**	68.4 ± 3.62**	Glibenclamide +STZ	253.3 ± 4.5	251.7 ± 2.9*	260.3 ± 3.78**	273.5 ± 2.9**	292 ± 4.0

Table 2: Effect of Sub Acute Treatment of PHF on Body Weight Changes and Blood Glucose Level on STZ Induced Diabetic Rats

Test sample mg/kg	Biochemical estimation											
	Hb	TP	TC	HDL – C	TG	LDC	VLDL	Cre	LPO	SOD	CAT	GP_X
Control	5.34±0.3	6.16±0.	85.83±5.	34.67±2.	85.83±2.	34.0±1.5	17.16±1.	0.691±	13.86±0.	6.79±0.	3.69±0.	18.14±0.
	3	30	45	24	31	9	48	0.08	37	23	18	02
Diabetic	11.83±0.	3.66±0.	176.5±2.	23.33±1.	171.7±3.	118.9±1.	34.34±1.	1.85±0.	33.80±0.	3.26±0.	1.96±0.	8.21±0.0
control-STZ	47**	33**	04**	45**	33**	06**	75**	1**	26**	21**	14**	1**
PHF-	10.3±0.4	4.83±0.	123.7±2.	31.33±2.	127.5±2.	66.17±1.	25.5±1.0	1.56±0.	23.60±0.	4.86±0.	2.74±0.	16.13±0.
200+STZ	2*	36*	36**	02*	81**	7**	6**	05*	30**	36**	13*	10*
PHF-	7.16±0.3	5.16±0.	105.5±3.	39.0±1.3	108.3±3.	44.9±2.2	21.6±2.0	1.25±0.	19.26±0.	4.92±0.	3.23±0.	13.29±0.
400+STZ	0**	32**	46**	3**	80**	**	**	03**	32**	26**	21**	14**
Glibenclamid	5.83±0.3	5.66±0.	97.33±4.	47.67±2.	90.0±1.8	31.6±1.5	18.0±1.2	1.12±0.	16.56±0.	4.30±0.	3.36±0.	17.13±0.
e+STZ	0**	53**	16**	09**	2**	**	1**	03**	24**	24**	15**	03**

Table 3: Effect of PHF on Biochemical parameters on STZ Induced Diabetic Rats

Test sample mg/kg	Body weight gms				Body Glucose level mg/dl			
	1st day	7th day	14th day	21st day	1st day	7th day	14th day	21st day
Control	225.28 ± 3.58	226.5 ± 3.55	231.8±3.31	234.1±3.96	82.1±1.6	85.4±2.6	91.0±4.3	94.6±3.5
Diabetic control-STZ	210.6 ± 3.74	108.7±5.13**	163.4±5.59**	156.16±3.03**	252.6±3.6	286.3±5.2**	301.4±6.8**	315.4±10.5**
PHF-200+STZ	215.14 ± 3.98	182.40±2.24ns	181.6±3.39*	196.7±3.98**	244.5±5.4	182.3±4ns	152.2±6.2*	125.0±5.1*
PHF-400+STZ	206.14 ± 3.09	199.58±3.59ns	195.33±3.03**	201.45±3.20**	240.2±3.5	175.2±9.8*	131.5±8.2**	106.1±5.3**
Glibenclamide+STZ	210.17 ± 3.8	198.3±3.31*	202.85±3.98**	206.14±3.20**	235.3±4.5	165.1±3.9ns	125.2±4.1**	105.4±6.8**

Values are mean ±SEM, n=6, ns- non significant, **P<0.01

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Liver homogenate preparation

Liver was removed, homogenized with buffer containing 0.25M sucrose and 0.1M Tris-Hcl bufferant p^H 7.4. 10% homogenate was prepared by using Teflon pestle and glass homogenizer and centrifuged at 600rpm for 10min to obtain post mitochondrial supernatant (PMT). This was used to analyze the protein concentration by biuret's method. The post mitochondrial supernatant was again centrifuged at 8000rpm for 15min. This supernatant was used to analyse the antioxidant enzyme level (lipid peroxidation, superoxide dismutase, catalase, glutathione peroxidase) in the liver.

Histopathology & Statistical analysis

The organs like liver and pancreas were dissected out and washed with ice-cold saline. The pancreatic tissues were preserved in 10% formalin solution for histopathologiacal study. The data were expressed as mean ± SEM (standard error mean). The significance of differences among the groups was assessed using one-way analysis of variance (ANOVA) followed by Dunnet's test. P values of <0.05 were considered as significance.

RESULTS

Glucose tolerance test

The effect of poly herbal formulation on glucose tolerance was illustrated at different time points. Statistical analysis by one way ANOVA showed that the rats which are loaded with glucose after 60 min of poly herbal formulation administration of 200mg/kg body wt/p.o and 400mg/kg body wt /p.o reduced blood glucose level compared to glibenclamide (4mg/kg body wt/p.o). Poly herbal formulation 200mg/kg body wt/p.o reduced blood glucose level less significantly (p<0.05) and 400 mg/kg body wt/p.o more significantly (p<0.01) when compared to contol.

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