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Original Article

ANTI-HYPERGLYCEMIC AND ANTIOXIDANT ACTIVITIES OF THE AYURVEDIC DRUG - PREMAHAOUSHADHI CHOORNAM IN ALLOXAN INDUCED DIABETIC RATS

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Abstract

Diabetes, the most prevailing metabolic disorder, is attracting present research attention towards it. In the present study, we have designed to evaluate the antihyperglycemic and antioxidant potential of Premahaoushadhi choornam (POC), a poly herbal ayurvedic formulation, widely used in southern parts of India in the treatment of diabetes. The effect of POC on normoglycemic rats and on hyperglycemia induced with alloxan (120 mg/kg) on single and repeated administration of POC was evaluated. The antioxidant potential of the extract was estimated in the heart and pancreas after repeated administration of POC in alloxan induced diabetic rats. The results were satisfactory, as a significant decrease in the blood glucose levels after repeated administration was observed. The reversal of the decreased antioxidant enzyme levels in the heart and pancreatic tissues of diabetic rats suggest its efficacy against diabetes and oxidative damage.

Key words: Premahaoushadhi choornam, Diabetes, Alloxan, Glibenclamide, antioxidants.

INTRODUCTION

Diabetes is one of the major health problems the world is facing today. It is rising in an alarming rate necessitating alternative treatment methods. There are many formulations in Ayurveda, one of the oldest traditional medical systems of India, used in the treatment of diabetes. Some of the formulations are proved to be effective in various animal studies and many more are yet to be standardized for their intended use.

Premahaoushadhi Choornam (POC) is one of the Ayurvedic formulations widely used in the southern parts of India in the management and treatment of diabetes and is also claimed to be effective in the treatment of diabetes related disorders. Hence it was considered worthwhile to evaluate the antidiabetic and antioxidant potential of this polyherbal formulation. It was obtained from the Government Ayurveda College, Trivandrum, Kerala.

In the present study we had estimated the effect of POC on blood glucose level in normal and diabetic animals, on antioxidant defense agents and lipid peroxidation. Since, elevated extra and intra cellular glucose concentrations result in oxidative stress, which is due to an imbalance between pro oxidants and antioxidants. Several mechanisms have been reported in the genesis of oxidative stress in experimental diabetes in animals and in diabetic patients[1].

Premahaoushadhi Choornam consists of the following plants *Vermonia anthelmenthica* (whole plant), *Phyllanthus amarus* (whole plant), *Salacia ruticosa* (Leaves) and *Trigonella Foenum graecum* (seeds) in a definite ratio. It was suspended in warm water in the ratio of 1:4 for administration to the animals.

MATERIALS AND METHODS

Animals

Male wistar rats (150-200 gm) procured from the animal house of C. L. Baid Metha College of Pharmacy, Chennai were maintained in a controlled temperature of 26±2°C and humidity (30-40%), they were fed with pellet feed supplied by TANUVAS, Chennai and water *ad libitum*, necessary permission was obtained from the Institutional Animal Ethics Committee for the experiments on animals.

Effect of POC on glucose levels in normal fasting rats[2]

The animals were divided into 4 Groups each containing 6 rats. Group I animals served as vehicle control, Group II animals were treated with standard drug, Glibenclamide (1 mg/kg p. o.), Group III & IV animals, were treated with the test drug orally at a dose of 500 mg/kg & 1 gm/kg body weight respectively. All the animals were fasted for 18 hours before the administration of standard and test drugs. Blood glucose levels were estimated at 0, 4, 8 and 12 hours after administration of test and standard drugs by using GLUCOMEN GLYCO (Mfd. by A. Menarini Industries Farmacheutiche Reunite, Italy) - a one touch blood glucose monitoring system, blood collection was done by tail vein method.

Effect of POC on Alloxan - induced hyperglycemia

Animals were divided into 5 Groups (n = 6), Group I rats served as vehicle control to Group II, III, IV and V alloxan (120 mg/kg in normal saline) was administered intra peritoneally. Blood glucose levels were estimated before and 48 hours after the administration of alloxan[3]. The effect of test and standard drugs on the blood glucose levels were estimated after confirming the presence of diabetes. The blood samples were collected for estimation on 0, 1, 2, 4 and 6 hours. Fasting conditions of 18h were maintained before estimation of glucose levels[4]. Blood glucose levels were estimated on 1, 3, 7 and 10 days to estimate the effect of POC (500 mg/kg) and (1 g/kg) and Glibenclamide, after prolonged administration for 10 days[5]. On the 10th day all the animals were sacrificed by over dose of ether anesthesia.

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Heart[6] and pancreas[7] were dissected out quickly and rinsed with normal saline in ice cold condition. They were homogenized with 0.1 M Tris-HCL buffer and centrifuged. The supernatant fluid was used to estimate the antioxidant (SOD[8], CAT[9], GPX[10], GSH[11], LPO[12]) enzyme levels.

Statistical analysis

The data obtained in the studies were subjected to one way analysis of variance (ANOVA) for determining the significant difference. The intergroup significance was analyzed using Dunnett's t test. P-values<0.05 were considered to be significant. All the values were expressed as mean ± SEM.

RESULTS

Effect of POC on blood glucose levels in normal rats

The effect of POC on blood glucose levels of control, standard and test drug treated are illustrated in Table 1. It has been observed that the control group have almost similar blood glucose levels throughout the experiment, whereas Group II, III and IV animals showed a decrease in 4th & 8th hour, a slight increase was observed in the last phase.

Effect of blood glucose levels after single administration of POC in hyperglycemic rats

The blood glucose levels were observed after 48 hr of alloxan administration for the effect of single administration of POC and Glibenclamide. The results in Table 2 shows that the blood glucose levels in control group are almost normal and the diabetic control group showed a gradual increase. The group III, IV & V showed a decrease in blood glucose levels at 2, 4 and 6 hours after administration. Group III animals showed a marked decrease in blood glucose level at 2, 4 and 6 hours.

Effect of blood glucose levels after prolonged administration of POC in hyperglycemic rats

The blood glucose levels of control group did not show any significant change, whereas Group III and V, treated with Glibenclamide and POC (1 gm/kg) respectively showed a significant decrease when observed on 3, 7 and 10 days after treatment. The results were tabulated in Table 3.

Effect of POC on antioxidant levels in heart and pancreas

The change in the concentration and the activity of Reduced Glutathione (GSH) Glutathione peroxidase (GPx), Superoxide dismutase (SOD) and Catalase (CAT) in the heart and pancreas of normal and diabetic rats was evident. There was a significant reduction in GSH, GPx, SOD and CAT in diabetic control. A significant increase in the antioxidant enzyme levels was observed in Group IV and V rats, Glibenclamide treated groups attained almost normal levels. An increased LPO was observed in Group I, whereas Group V showed a significant decrease in lipid peroxidation levels in both heart and pancreas than that of Group IV.

TABLE 1: EFFECT OF POC ON NORMAL BLOOD GLUCOSE LEVELS IN RATS

Groups		Fasting Blood glucose levels (mg/dl)			
		0 hour	4th hour	8th hour	12th hour
I	Control	73.17 ± 4.28^{A}	75.24 ± 9.48^{B}	79.65 ± 17.2^{B}	78.14 ± 6.86^{B}
II	Glibenclamide (1 mg/kg)	85.63 ± 6.48^{A}	58.15 ± 4.33^{B}	48.13 ± 5.12^{B}	60.31 ± 5.86^{B}
III	POC (500 mg/kg)	78.23 ± 4.15^{A}	53.43 ± 6.18^{B}	49.82 ± 3.61^{B}	58.64 ± 6.17^{B}
IV	POC (1 gm/kg)	84.15± 7.18^{A}	58.22 ± 5.63^{B}	56.14 ± 8.32^{B}	73.48 ± 7.34^{B}

Values of B are compared with A
Values are mean ± SEM, n = 6
*p<0.05; **p<0.01; # p<0.001 ns - non significant

TABLE 2: EFFECT OF POC ON BLOOD GLUCOSE LEVELS AFTER SINGLE ADMINISTRATION

Groups		Blood glucose levels (mg/dl) on single administration				
		0 hour	1st hour	2nd hour	4th hour	6th hour
I	Control	85.63 ± 6. 75^{A}	84.86 ± 12.34^{Bns}	86.58 ± 7.36^{Bns}	87.47 ± 9.54^{Bns}	86.21 ± 10.66^{Bns}
II	Diabetic control	260.18 ±10.25^{A}	258.38 ± 18. 17^{Bns}	262.92 ± 16.63^{Bns}	265.85 ± l3.16^{Bns}	264.25 ± 16.49^{Bns}
III	Glibenclamide (1 mg/kg)	274.3 1 ± 16.46^{A}	236.78 ± 31.27^{Bns}	183.98± 24.84^{B*}	175.42 ± 13.40^{B*}	164.69 ± 11.28$^{B\#}$
IV	POC (500 mg/kg)	256.47 ± 23.05^{A}	243.56 ± 24.59^{Bns}	210.00 ± 23.61^{B*}	195.57 ± 8.46^{B*}	187.63 ± 21.89^{B*}
V	POC (1 gm/kg)	248.58 ± 34.71^{A}	235.36 ± 22.84^{Bns}	196.62 ± 38. 82^{B*}	189.38 ± 17.20^{B*}	181.64 ± 15.28^{B*}

Values of B are compared with A
Values are mean ± SEM, n = 6
*P<0.05, **P<0.01, # p<0.001, ns - non significant.

TABLE 3: EFFECT OF POC ON BLOOD GLUCOSE LEVELS AFTER DAILY ADMINISTRATION FOR 10 DAYS

Groups		Blood glucose levels (mg/dl)			
		Day 1	Day 3	Day 7	Day 10
I	Control	85.63 ± 6.75^{A}	89.34 ± 11.63^{Bns}	92.14 ± 16.85^{Bns}	88.73 ± 9.47^{Bns}
II	Diabetic control	260.18 ± l0.25^{A}	273.42 ± 39.36^{Bns}	287.59 ± 23.33^{Bns}	305.76 ± 18.93^{Bns}
III	Glibenclamide (1 mg/kg)	274.31 ± 16.46^{A}	238.31 ± 19.57^{Bns}	206.75 ± 8.43 $^{B\#}$	1 94.37 ± 6.43$^{B\#}$
IV	P.C (500 mg/kg)	256.47 ± 23.05^{A}	236.55 ± 11.84^{Bns}	212.3 ± 8.57^{B*}	193.28 ± 6.42^{B**}
V	P.C (1 gm/kg)	248.58 ± 34.71^{A}	214.23 ± 14.16^{Bns}	195.4 ± 10.14$^{B\#}$	1 87.63 ± 5.33$^{B\#}$

Values of B are compared with Values of A
Values are mean ± SEM, n = 6
*P<0.05, **P<0.01, # p<0.001, ns - non significant.

TABLE 4: EFFECT OF POC ON ANTIOXIDANT LEVELS IN RAT PANCREAS

Groups		Effect of POC on antioxidant levels in rat Pancreas				
		SOD	CAT	GSH	GPX	LPO
I	Control	4.56 ± 0.32$^{A\#}$	13.72 ± 3.16$^{A\#}$	18.27 ± 11.35$^{A\#}$	28.43 ± 12.66$^{A\#}$	38.21 ± 14.38$^{A\#}$
II	Diabetic control	2.11 ± 10.14	5.56 ± 10.72	9.65 ± 10.73	15.64 ± 11.84	56.36 ± 16.15
III	Glibenclamide (1 mg/kg)	3.88 ± 10.18^{B*}	12.13 ± ll.03^{B*}	17.18 ± 14.01^{B*}	24.87 ± 13.08^{B**}	40.89 ± 14.29$^{B\#}$
IV	POC (500 mg/kg)	3.08 ± 0.21^{B*}	9.38 ± 11.82^{B*}	14.8 ± 13.42^{B**}	19.17 ± 12.04^{B*}	47.5 ± 15.26^{B*}
V	POC (1 gm/kg)	3.82 ± 10.15^{B*}	11.85 ± 2.43^{B*}	15.22 ± 12.83^{B**}	23.45 ± 12.14^{B**}	42.0 ± 14.79^{B**}

A = Group I Vs Group II, B = Group III, IV and V Vs Group II
Values are mean ± SEM, n = 6
*P<0.05, **P<0.01, # p<0.001, ns - non significant.
Units: SOD (Units/mg protein)
CAT (µmoles of H_2O_2 consumed/min/mg protein)
GPx (µg of GSH utilized/min/mg protein)
GSH (µg/mg protein)
LPO (nmoles of MDA liberated/min/mg protein)

TABLE 5: EFFECT OF POC ON ANTIOXIDANT LEVELS IN RAT HEART

Groups		Effect of POC on antioxidant levels in rat heart				
		LPO	GPX	CAT	GSH	SOD
I	Control	0.62 ± 0.02$^{A\#}$	1.05 ± 0.08$^{A\#}$	5.78 ± 0.15$^{A\#}$	12.59 ± 0.27$^{A\#}$	13.18 ± 1.02$^{A\#}$
II	Diabetic control	0.81 ± 0.01	0.68 ± 0.04	3.86 ± 1.14	5.81 ± 0.46	6.34 ± 0.26
III	Glibenclamide (1 mg/kg)	0.66 ± 0.02^{B*}	0.98 ± 0.06^{B*}	4.81 ± 0.16^{B**}	10.37 ± 0.16^{B**}	12.54 ± 027*
IV	POC (500 mg/kg)	0.7 ± 0.02^{B*}	0.89 ± 0.05^{B*}	4.06 ± 0.31^{B*}	8.35 ± 0.22^{B*}	9.78 ± 0.85^{Bns}
V	POC (1 gm/kg)	0.65 ± 0.03^{B*}	0.94 ± 0.04^{B**}	4.39 ± 0.18^{B*}	9.72 ± 0.81$^{B\#}$	11.72 ± 0.11^{B*}

A = Group I Vs Group II, B = Group III, IV and V Vs Group II
Values are mean ± SEM, n = 6
*P<0.05, **P<0.01, # p<0.001, ns - non significant.
Units: SOD (Units/mg protein)
CAT (µmoles of H_2O_2 consumed/min/mg protein)
GPx (µg of GSH utilized/min/mg protein)
GSH (µg/mg protein)
LPO (nmoles of MDA liberated/min/mg protein)

DISCUSSION

It was observed that, treatment of diabetic rats with POC had not only shown a good hypoglycemic activity but also had an effective antioxidant activity which is evident from the observations.

Diabetes inducing agents like alloxan and streptozotocin are reported to induce diabetes with the generation of free radicals, a significant reduction in antioxidant enzyme levels is indicated as the potential reason for the susceptibility of organs to atrophy in diabetic states[13]. POC had shown an increased antioxidant enzyme levels in both heart and pancreas, thus protecting the tissues from oxidative damage by GSH, which is regenerated from its oxidized form (GSSH)[14]. The oxidative free radicals of alloxan might have contributed to the inactivation of the enzyme in lipid peroxides of alloxan treated animals, thus leading to the damage of pancreatic tissue resulting diabetes in animals, which showed an increase in LPO.

POC treated groups at both the doses have shown reversal of the decreased enzyme levels thus protecting further damage of the tissue from oxidative free radicals and hydro peroxides. Many recent experimental findings suggest that overproduction of reactive oxygen and nitrogen species, lowered the antioxidant defense and alterations of enzymatic pathways in humans with poorly controlled diabetes mellitus, can contribute to endothelial, vascular and neurovascular dysfunction.

Increased lipid peroxidation is due to the enzyme fatty acyl co enzyme-A oxidase which initiates oxidation of fatty acids[15]. CAT, GPx and GSH are important in protecting the tissue from oxidative free radicals, altered levels of both enzymatic and non enzymatic anti oxidants poses a serious threat to diabetic animals resulting in impairment of tissues, organs, enzymes and cell membranes. A decreased GSH is an indication of increased oxidative stress, its increase in POC and Glibenclamide treated groups showed the efficacy of the Ayurvedic preparation and standard drug counteracting the oxidative stress. Increased SOD in POC treated rats will be responsible for the break down of superoxide anion to oxygen and hydrogen peroxide.

All these observations show that POC is effective in lowering the blood glucose level in alloxan induced Type-I diabetes and protect from the development of diabetic complications, atherosclerosis and associated cardiovascular diseases. This can be attributed to the combined effect of various chemical constituents of the plant ingredients used in POC.

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