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Augmentation of the Metformin activity using *Pleurotus florida* Polysaccharide to control Hyperglycemia in Mice model: A Preliminary Study

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Abstract: The present study was aimed to establish the augmentative property of crude *Pleurotus florida* mushroom extract when taken with other established anti-diabetic drugs, i.e, metformin. Alloxan-induced diabetic mice were treated with metformin and metformin along with *Pleurotus florida* extract to compare the hypoglycemic profile of these two groups of mice. Results showed a good decline in blood glucose levels in both acute and sub-acute studies in group of mice having combined treatment. Malondialdehyde concentration was also assayed to find out any protective property of this mushroom against late diabetic complications through inhibition of ROS (Reactive Oxygen Species) formation in the liver.
Keywords: Alloxan, Diabetes, Malondialdehyde , Mushroom.

1. Introduction

Diabetes is a metabolic disorder of the endocrine system associated with high levels of blood sugar occurring in almost 6% of world population (1). It is often designated as a “silent killer” since over a long period of time it can actually lead to the various fatal consequences including damage to the retina (2); diabetic nephropathy (3), diabetic neuropathy (4) and even heart attack (5). As per WHO, 2011, fact sheet, 346 million people world wide have diabetes and more than 80% of diabetes death occur in low and middle income countries (1).

The pathogenesis of type II diabetes is complex involving progressive development of insulin resistance in liver and peripheral tissues accompanied by a defective insulin secretion from pancreatic beta cells leading to overt hyperglycemia (6). Generally current therapeutic strategies for type II diabetes are limited and involve some

classes of oral anti-diabetic agents that stimulate pancreatic insulin secretion (sulphonylureas and rapid-acting secretagogues/insulinotropics *e.g.*, glibenclamide, glipizide, rapaglinide), reduce hepatic glucose production (biguanides *e.g.*, metformin), or improve insulin action [thiazolidinediones (TZDs) *e.g.*, pioglitazone, rosiglitazone]. Each of above agents suffers from generally inadequate efficacy and number of serious adverse effects sometimes even leading to drug resistance (7). Thus, to overcome the above difficulties there is an urgent need to the discovery and development of novel drug. In recent years new natural therapeutic strategies are being examined i.e., the combination of the herbal remedies with oral hypoglycemic agent.

Consumption of mushroom is gaining interest in recent years, not only for their unique flavor, texture and gastronomic delicacy but also for their nutritional and medicinal properties (8-17). Parallelly, there are increasing evidences indicating that mushroom polysaccharides are potentially useful biologically active ingredients for pharmaceutical uses (18). Here, an attempt has been made to evaluate the addition of water soluble crude polysaccharide from *Pleurotus florida* (CPPf) as an adjunct to metformin for glycemic control in alloxan-induced diabetic mice.

2. Materials and Methods

Collection of White oyster Mushroom (*Pleurotus florida*)

Pleurotus florida was purchased from mushroom cultivation unit of Narendrapur Ramakrishna Mission Ashrama, Narendrapur, West Bengal, India.

Extraction of polysaccharide

Air dried and powdered fruit bodies of *Pleurotus florida* was extracted with ten volume of 99% ethanol at room temperature for two days to remove the alcohol soluble materials such as colored materials, phenolic compounds and lipid. After filtration, the residue was re-extracted with ethanol and filtered. The filtrate was air dried, then suspended and refluxed with distilled water at boiling condition for 8 hrs. The extract was filtered through gauze and the filtrate was concentrated to one-tenth of the volume with a rotary evaporator at 80ºC under vacuum. Polysaccharide was precipitated by addition of four volume of absolute alcohol and left at 4ºC overnight. After centrifugation, the pellets were washed with 70% (v/v) ethanol and then successively washed with ethyl acetate and acetone (19, 20). The washed pellets were dissolved in water and lyophilized to yield crude polysaccharide, CPPf.

Chemical properties of polysaccharide

Total sugar contained was measured by phenol sulfuric acid method at 490 nm using glucose as standard. The protein contained of protein-bound polysaccharide was determined by Bradford reagent. Presence of starchy polysaccharide was carried out by iodine reaction. Total phenolic compounds present in the crude polysaccharide were determined using Folin-Ciocalteau reagent where gallic acid was used as standard. All values were expressed as gm of standard equivalents/ 100 gm of crude dry polysaccharide (21).

Animals

Swiss albino mice (40-80g) of roughly identical age were used for the study. They were maintained at a temperature of 25 ± 3˚C and relative humidity of 45% to 55% under 12-h light: 12-h dark cycle. Water and feed were provided *ad libitum*. The animals were maintained in line with the guidelines recommended by the Animal Welfare Board and approved by our Institutional Animal Ethical Committee (IAEC) constituted following the guidelines of the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA), Ministry of Environment, Government of India, New Delhi. All the procedures complied with the Declaration of Helsinki, as revised in 1996. It has been established beforehand that incorporation of mushroom into the drinking water and diet has no effect on glucose homeostasis of mice (22).

Experimental design

The mice were divided into four groups consisting of 3 animals in each group.

Group 1- Alloxan-induced diabetic mice.

Group 2- Diabetic mice treated with 50 mg/kg of CPPf.

Group 3- Diabetic mice treated with 70 mg/kg of aqueous metformin extract.

Group 4- Diabetic mice treated with both metformin (70 mg/kg) and CPPf (50 mg/kg).

Induction of Experimental type-2 diabetes mellitus (IDDM) Condition

Diabetes was induced by a solitary intraperitoneal injection of freshly prepared solution of alloxan monohydrate (SIGMA) at a concentration of 300 mg/kg body weight dissolved in sterile 0.85% saline (23). After 48 h, the animals showing levels of blood glucose greater than 200 mg/dl (diabetic) were chosen for further experiments. All the animals were permitted open access to drinking water and pellet diet.

Collection of blood and determination of glucose content

For blood glucose determination, the blood was obtained by snipping the tail by means of a sharp razor. Blood glucose level (BGL) was determined by means of a one touch horizon glucometer. Levels of glucose were expressed in mg/dl.

Preparation of liver homogenate

Animals were sacrificed on 3rd day, liver was dissected out and washed in saline and refrigerated until analysis. The liver was weighed and homogenized in ice cold 0.14 M KCl solution. The homogenate was centrifuged in a Micro-centrifuge (Denville 260D) at 10 000 × g for 15 min at 4°C.

Determination of MDA concentration.

Lipid peroxidation was assayed by measuring the malondialdehyde (MDA) concentration in the liver homogenate as thiobarbituric acid (TBA)-reactive material (24). MDA is formed as a secondary product when TBA and polyunsaturated fatty acid are heated in an acidic medium, its absorbance being measured at λ = 532 nm. The concentration of MDA was expressed as µM/g liver tissue.

3. RESULTS

Pleurotus florida crude polysaccharide (CPPf) appeared to be white powder which is highly soluble in water. The chemical composition of CPPf showed total carbohydrate and protein content of 68 % and 4 % respectively and very negligible amount of phenolics. Negative result from iodine reaction demonstrating that there is no starch in the polysaccharide. Acute toxicity studies revealed that the extract was harmless upto a dose of 5000 mg/Kg body wt.

Single administration of only crude polysaccharide (CPPf) or metformin or combination of metformin and crude polysaccharide reduced the levels of blood glucose in alloxan-induced diabetic mice which showed greatest diminution in the blood glucose at 4th hour following administration of the combined administration of CPPf with Metformin (Table 1).

Table 1. The combination effect of CPPf and Metformin on blood glucose level in alloxan-induced diabetic mice (Acute study). Values are the mean of three individual experiments.

Treatment of diabetic mice	Mean Glucose level (mg/dl)	
	0 hr	2 hr
Vehicle	437	430
CPPf (50 mg/Kg)	450	406
Metformin (70 mg/Kg)	410	278
CPPf (50 mg/Kg) + Metformin (70 mg/Kg)	433	82

Furthermore, sub-acute administration (once a day for 3 days) of same treatment sets caused decline in blood glucose levels as compared with the vehicle (only water) -treated set (Figure 1). Results showed that concomitant treatment of Metformin and CPPf resulted in a significant reduction in blood glucose level in comparison with other treated sets.

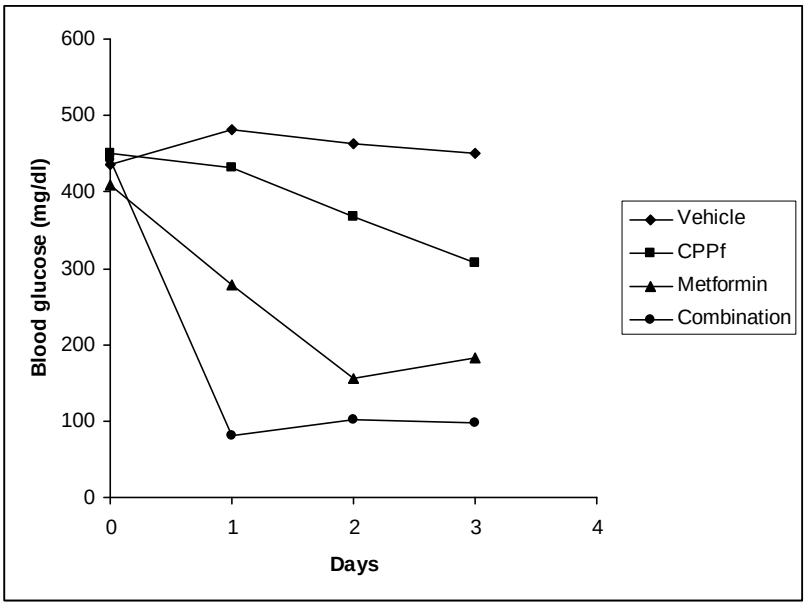

Figure 1. The combination effect of CPPf and Metformin on blood glucose level in alloxan-induced diabetic mice (Sub-acute study).Value are mean of three separate experiments.

After short term treatment with individual and combination therapy the MDA concentration was measured in liver tissue homogenate. The results are presented in Figure 2. The concentration of MDA measured in the liver of normal mice was 13.14 μM/g of tissue. The changes in the concentration was measured in the liver of alloxan-induced diabetic mice (Group 1), (16.10 μM/g of tissue), diabetic mice treated with CPPf (Group 2), (12.90μM/g of tissue), diabetic mice treated with metformin (Group 3), (12.38 μM/g of tissue), and diabetic mice with combination treatment (Group 4), (12.64 μM/g of tissue). Result signifies all the treatments protect liver from the oxidative stress.

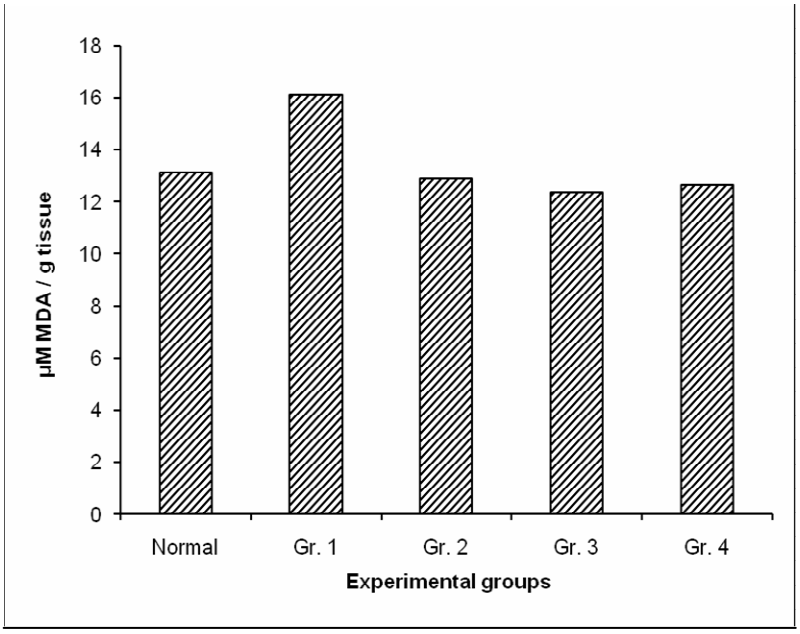

Figure 2. MDA concentration in the liver. Results are mean of three separate experiments.

4. Discussion

Overall results suggest that a beneficial effect can be additive blood glucose lowering effect when metformin is combined with crude polysaccharide of *P. florida*. So, when the standard drug metformin is treated in combination with CPPf, it might alter the other pharmacokinetic profiles like absorption, distribution, metabolism, and/or excretion. Our results coincide with the observations on the reduction of hyperglycemia upon combined application of glibenclamide and plant extract, as has been reported previously for *Zingiber officinale* (25), *Cassia auriculata* (26), *Aloe vera* (27), *Trigonella foenumgraceum* (28) and *Momordica charantia* (29).

Badole *et al* (2008) showed potent and synergistic blood glucose lowering effect of a mushroom *Pleurotus pulmonarius* agarous extract in combination with glibenclamide in alloxan induced diabetic mice (30).

In the pathophysiology of diabetes, the cells are exposed to oxidative stress due to increased production of reactive oxygen species produced by glucose auto-oxidation and protein glycation (31) leading to lipid peroxidation which ultimately invites late diabetes complications (32). Mushrooms are known to have strong free radical scavenging activity. Different extract/fractions/polysaccharides showed potential *in vitro* free radical scavenging activity and inhibitor of lipid peroxidation (33-37). In the present study, the higher rate of lipid peroxide formation including MDA in alloxan-induced diabetic mice was inhibited by both the CPPf and Metformin, strongly suggests its protective activity towards secondary complication.

In conclusion, the present study demonstrated that, the hypoglycemic effect of metformin was further augmented by the additional dose of crude *Pleurotus florida* polysaccharide. Additionally a strong antioxidative action probably via a reduction in hypoglycemia, thus leading to a delay in development of late diabetic complication associated with hyperglycemia. Further studies are needed to understand the mechanism behind this augmentation.

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