

INFLUENCE OF *GANODERMA LUCIDUM* PREPARATIONS ON BLOOD GLUCOSE AND LIPIDS IN ALBINO RATS.

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Summary

Ganoderma lucidum, a mushroom, is traditionally used in Japan and China for various diseases. Commercially available *Ganoderma lucidum* preparations like fruit body product and mycelium product are widely used by diabetic patients. Consumption of these products by the patients concurrently with hypoglycemics may lead to serious hypoglycemia. Since there is paucity of information regarding their interaction with clinically used oral hypoglycemics, the present study was planned to investigate the same with glibenclamide, a commonly used oral hypoglycemic agent. Male adult Wistar rats were rendered hyperglycemic by administering 60mg/kg of alloxan (in 0.3 ml) through tail vein. Alloxan induced hyperglycemic animals were subdivided in to various groups (n=6 in each) to receive single dose of vehicle, glibenclamide (0.9mg/kg), fruitbody product (146 mg/kg) , mycelium product (243 mg/kg) and predetermined Sub Hypoglycemic Doses of fruitbody product (100 mg/kg), mycelium product (175 mg/kg), glibenclamide (0.45 mg/kg) together. Glucose was estimated in blood samples from tail vein collected at 0,1,3,5,7 and 9h after treatment. In chronic studies similar daily treatments were continued for 30 days in hyperglycemic rats and blood glucose and lipid levels were estimated after 24 h of the last dose.

In acute study fruitbody product, mycelium product & glibenclamide (0.9mg/kg) produced significant ($p<0.01$) hypoglycemia. In chronic studies glibenclamide (0.9 mg/kg), fruitbody product, mycelium product individually and Sub Hypoglycemic Doses of fruitbody product, mycelium product & glibenclamide together significantly lowered blood glucose. All the treatments significantly raised HDL and with an exception of glibenclamide, significantly lowered total cholesterol and LDL. Both triglycerides and VLDL were significantly lowered only in glibenclamide and mycelium product groups.

Both fruitbody product and mycelium product individually have hypoglycemic & hypolipidemic activity. Mycelium product appears to be more potent than fruitbody product. Synergistic hypoglycemic & hypolipidemic activity of fruitbody product and mycelium product with glibenclamide needs to be confirmed clinically.

Key words: Alloxan, Hyperglycemia, *Ganoderma-lucidum*, Glibenclamide

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Introduction

Diabetes mellitus, a metabolic disorder characterised by absolute or relative deficiency of insulin, affects carbohydrate, fat and protein metabolism, leading to hyperglycemia and dyslipidemia. Apart from insulins and several types of oral hypoglycemics, plants like *Aegle marmelos*¹, *Brassica oleracea*², *Allium cepa*³, *Eugenia jambolana*⁴, *Azadirachta indica*⁵ and *Trigonella foenum graecum*⁶ are shown to possess hypoglycemic activity in experimental animals.

Ganoderma lucidum, known as Reishi or Mannentake, in Japan and as Ling Zhi in China is revered as herbal medicine for more than thousand years in Japan and China. The Reishi which is usually grown on the hardwood trees, like oak and palm has been consumed as food as well as herbal remedy in Japan and China.

The herb is currently advocated for the treatment of various conditions like angina pectoris, hypertension, chronic bronchitis, hepatitis, leukopenia, autoimmune disorders etc⁷. *Ganoderma lucidum* also has been reported to restore steroid resistant glomerular dysfunction⁸ and to possess hypotensive⁹, to have antiproliferative action on human breast cancer tumoral cells¹⁰ as well as immunomodulator activity¹¹. Recent reports suggest that *Ganoderma lucidum* inhibits human hepatoma cells¹² as well as colorectal cancer cells¹³ and inhibits replication of hepatitis B virus¹⁴. The active principles of *Ganoderma lucidum* like ganoderan A and ganoderan B have been reported to exert hypoglycemic activity in experimental animals when given in single dose¹⁵.

Marketed preparations like Reishi gano (Fruitbody Product-FP) and Ganocelium (Mycelium Product-MP) though widely consumed by both diabetic and non-diabetic individuals in India, they are not evaluated for their hypoglycemic and hypolipidemic activity. If these preparations have significant hypoglycemic activity as reported, could produce severe hypoglycemia in diabetic patients on hypoglycemic agents. Paucity of information regarding interactions of *Ganoderma lucidum* products with commonly used oral hypoglycemic like glibenclamide (GLB) prompted the present study.

In the present study single and multiple doses of both the preparations of ganoderma, FP and MP individually, and together in their sub hypoglycemic dose (SHD)s with that of GLB were explored for their hypoglycemic and hypolipidemic activity and for their possible interaction with glibenclamide in alloxan induced hyperglycemic Wistar rats.

Materials and methods

Animals:

The complete course of experiments were carried out using healthy male rats of Wistar strain, weighing between 200-250 g. The animals were acclimatized to normal laboratory conditions with 12:12-h light-dark cycle and were maintained on standard laboratory diet (Amrut feeds) with free access to water.

Hyperglycemia was induced by injecting alloxan monohydrate (Sigma Chemical Co., USA) in single dose of 60mg/kg (freshly dissolved in 0.3ml of saline) through the tail vein. After 48 h of alloxan treatment, glucose was estimated in tail vein blood with the help of glucometer (Pulsatum), and animals with blood glucose ≥ 200 mg/dl were included in the study.

Drugs used:

As per the manufacturer's brochure, the chemical constituents of fruitbody product (reishi gano) are polysaccharide, organic germanium, adenosine, ganoderic essence, triterpenoids, protein and fibre, whereas mycelium product (ganocelium) contains polysaccharide, organic germanium, vitamins and minerals. FP (reishi gano) and MP (ganocelium) of *Ganoderma lucidum* were procured from (DXN-Products, Marketed by Daeshan Trading India Pvt.Ltd) local market and used orally in the dose of 146 mg/kg and 243 mg/kg respectively. These rat equivalent doses calculated on the basis of advocated human dose were administered orally in 1ml of 2% gumacacia suspension. To assess subhypoglycemic dose (SHD), a series of experiments using different fractions of calculated hypoglycemic dose for FP, MP & GLB were individually administered to different groups of animals. The maximum fraction showing insignificant change in hyperglycemic animals were selected as SHDs and were found to be 100 mg/kg for FP, 175 mg/kg for MP and 0.45 mg/kg for GLB.

Glibenclamide (Courtesy Hoechst Marrion – Roussel, Mumbai) was used orally in the dose of 0.9 mg/kg and 0.45 mg/kg (SHD) in 1 ml of 2% gumacacia suspension. For interaction studies SHDs of GLB, FP and MP were administered in total volume of 2 ml p.o together.

Acute Studies:

Overnight fasted hyperglycemic rats were divided in five groups (n=6 in each) to receive: (i) Vehicle (2% gum acacia), (ii) GLB (0.9 mg/kg), (iii) FP (146 mg/kg), (iv) MP (243 mg/kg), (v) SHDs of FP (100 mg/kg), MP (175 mg/kg) and GLB (0.45 mg/kg) together. Drugs were administered in a single dose at 8:00 am and food was withheld until the completion of studies. A drop of blood from tail vein was taken on glucose strip at 0,1,3,5,7, & 9 hours, after treatment to estimate the glucose with the help of glucometer (Pulsatum).

Chronic Studies:

As mentioned in acute studies various groups (n=6 in each) of hyperglycemic animals received the similar treatments once daily orally at 8:00 am for 30 days. After 24 hrs of last dose, under ether anesthesia 2-3 ml of cardiac blood was collected to estimate glucose by glucose oxidase/ peroxidase (GOD/POD) method using a standard kit (Beacon Diagnostics, India). Blood lipid profile was assessed by estimating cholesterol and HDL cholesterol with the help of cholesterol oxidase/ peroxidase kit (Beacon Diagnostics, India). The blood level of triglycerides were estimated by using a separate kit (Beacon Diagnostics, India) and VLDL, LDL levels were calculated using the formula: $VLDL = \text{Triglycerides}/5$ and $LDL = \text{Total cholesterol} - HDL - VLDL$.

The body weights of each animal in different groups were noted to compare the same with that of (0 day) pretreatment, before sacrificing the animals. All the procedures were performed in accordance with the guidelines of Institutional animal ethics committee constituted as per the recommendations of CPCSEA, under Ministry of Animal Welfare Division, Government of India.

Statistical Analysis:

For both acute and chronic studies, data were expressed as Mean \pm S.E.M. and were analysed by using one way ANOVA followed by Dunnet's test. $P \leq 0.05$ was considered to be significant.

Results

In acute studies, when compared to controls, significant ($p < 0.05$) hypoglycemia was observed at 3 h in FP treated group and at 5 and 7 h in GLB treated group, while in MP treated animals at 3 h ($p < 0.05$), 5 h ($p < 0.01$) and 7 h ($p < 0.05$). However SHDs of FP, MP & GLB together failed to produce significant hypoglycemia (Table I).

In chronic studies 24 hour after the last dose GLB (0.9 mg), FP, MP individually and SHDs of FP, MP & GLB together produced significant ($p < 0.01$) hypoglycemia (Fig I). All the treatments significantly ($p < 0.05$, $p < 0.01$) raised the HDL. Except GLB other treatments significantly ($p < 0.01$) lowered total cholesterol and LDL while MP and GLB significantly ($p < 0.05$, $p < 0.01$) lowered triglycerides and VLDL (Table II).

There was no significant change in food intake of different treated groups when compared to that of controls. Twenty four hours after the last dose of treatment significant ($p < 0.01$) weight gain was observed only in FP (+ 9.4 \pm 0.96 g), MP (+ 11.83 \pm 0.61 g) and SHDs of FP, MP & GLB together (+ 8.69 \pm 0.46g) treated groups as compared to their respective pretreatment weights (0 day). In GLB treated group animals had lost the weight (-6.61 \pm 0.22 g) though it was statistically insignificant ($p > 0.05$) whereas in vehicle treated control there was significant ($p < 0.01$) reduction in the bodyweight (-20.50 \pm 0.77 g).

Table I. Effect of single dose on blood glucose of hyperglycemic rats.

Treatment (mg/kg) n=6	Blood glucose (mg%) Mean± SEM					
	0 hours	1 hours	3 hours	5 hours	7 hours	9 hours
Control (vehicle)	308.0 ±32.42	309.2 ±10.69	317.7 ±7.03	320.5 ±26.41	347.5 ±6.93	361.2 ±4.35
GLB(0.9)	278.2 ±7.83	272.0 ±6.68	250.7 ±12.68	143.5 * ±11.38	209.2 * ±5.67	271.3 ±7.06
FP(146)	295.8 ±42.75	191.8 ±33.10	174.0 * ±27.97	260.3 ±59.47	268.3 ±56.42	262.0 ±56.66
MP(243)	236.7 ±18.66	189.0 ±22.75	159.0 * ±23.96	114.5 ** ±10.20	189.5 * ±35.08	253.0 ±24.12
FP(100) +MP(175) +GLB(0.45)	408.2 ±57.70	394.7 ±51.04	343.0 ±63.22	305.5 ±97.37	288.8 ±75.13	261.3 ±51.95

ANOVA followed by Dunnet's test. *p<0.05, **p<0.01 as compared to control.
GLB - Glibenclamide, FP – Fruitbody product, MP- Mycelium product.

Table II. Lipid profile (mean \pm S.E.M) of hyperglycemic rats after a month long treatments with various agents.

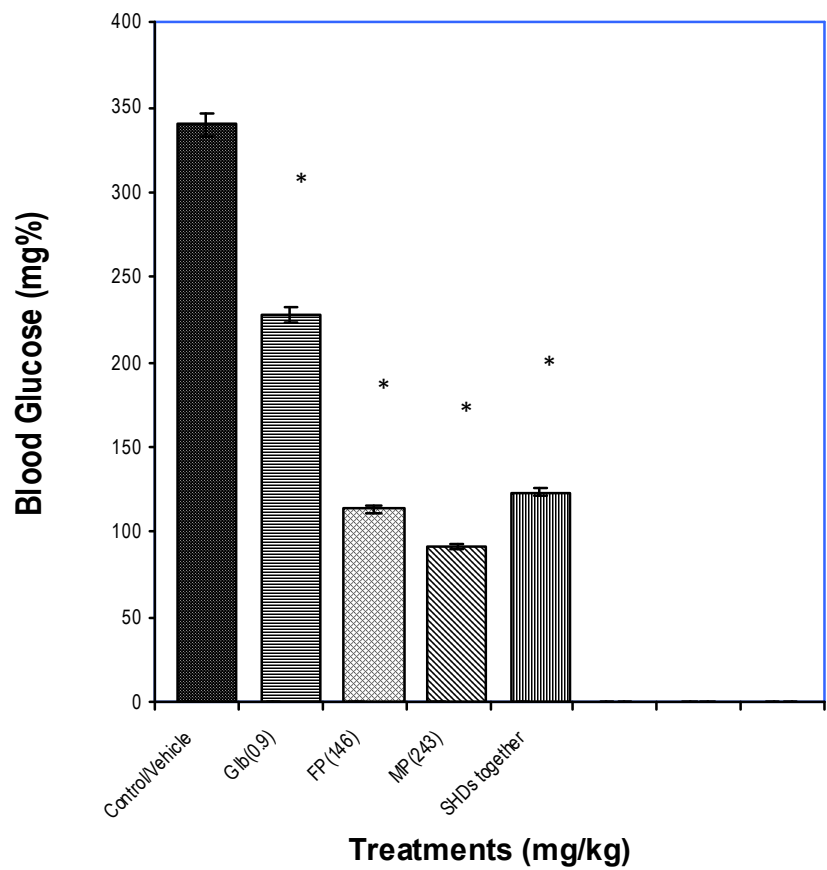
Treatment (mg/kg) n=6	Lipids (mg%) Mean \pm SEM				
	Total Cholesterol	Triglycerides	HDL	LDL	VLDL
Control (vehicile)	162.20 \pm 4.02	98.83 \pm 1.94	13.83 \pm 1.62	128.60 \pm 5.25	19.77 \pm 0.39
GLB(0.9)	154.80 \pm 3.37	69.50* \pm 8.47	30.00* \pm 2.27	110.90 \pm 3.52	13.90* \pm 1.69
FP(146)	57.13** \pm 3.43	79.17 \pm 11.21	40.53** \pm 6.85	9.09** \pm 3.84	15.83 \pm 2.24
MP(243)	49.83** \pm 8.55	58.17** \pm 4.55	35.82** \pm 3.63	2.38** \pm 7.83	11.63** \pm 0.91
FP(100) +MP(175) +GLB(0.45)	93.17** \pm 5.24	83.83 \pm 4.10	29.83** \pm 2.17	54.57** \pm 7.99	16.80 \pm 0.82

ANOVA followed by Dunnet's test. * p <0.05, ** p <0.01 as compared to control.

GLB - Glibenclamide, FP – Fruitbody product, MP- Mycelium product.

Fig. I: Effect of chronic (30 days) treatment on blood glucose.

Data expressed as Mean \pm S.E.M. (n=6 in each group) ANOVA followed by Dunnet's test. * P<0.01 as compared to control. GLB - Glibenclamide, FP – Fruitbody product, MP- Mycelium product. SHDs together- Subhypoglycemic doses of GLB,FP and MP together.



Discussion

Findings of the present studies clearly indicate that single dose administration of FP,MP and GLB in their therapeutic equivalent doses produced significant ($p<0.05$) hypoglycemia in alloxan induced hyperglycemic rats. The hypoglycemic activity was maximum and more sustained in MP treated animals as compared to the other groups. However combination treatment with subhypoglycemic doses of FP,MP and GLB failed to produce significant hypoglycemia.

In hyperglycemic animals, on chronic administration (for 30 days) of FP,MP and GLB in their therapeutic equivalent dose showed significant ($p<0.01$) hypoglycemia even after 24 hours of the last dose. Similarly, significant ($p<0.01$) hypoglycemia was observed in animals treated with subhypoglycemic doses of FP,MP and GLB together for 30 days. Significant hypoglycemic activity observed only on chronic treatment with SHD combination of FP,MP and GLB indicates cumulative action of FP and MP.

To the best of our knowledge no study has reported hypoglycemic activity of commercial preparations of *Ganoderma lucidum*, though the active principles like ganoderan A, ganoderan B and water extract of fruitbody of *Ganoderma lucidum* have been shown to produce significant hypoglycemia on single dose administration in hyperglycemic animals¹⁵. Hypoglycemic activity of *Ganoderma lucidum* products is obviously attributed to the glycans (ganoderan A and ganoderan B) present in them. The mechanism by which these glycans lower the blood glucose is not clearly elucidated. Ganoderan B has been shown to increase plasma insulin both in euglycemic and hyperglycemic animals¹⁶. Hypoglycemic activity of ganoderan B attributed to its ability to increase glucose utilization and its metabolism in the liver¹⁶. This mechanism appears to be more plausible than the previous one since water extract of *Ganoderma lucidum* has been reported not to increase plasma insulin¹⁷. Hypoglycemic activity of *Ganoderma lucidum* has also been attributed to blockade of adrenergic β receptors and stimulation of α receptors leading to enhanced glucose utilization¹⁷. In the present study significant hypoglycemia in fasting hyperglycemic rats caused by FP and MP and significant hypoglycemia persisting even after 24 hours of the last dose in chronic study rule out the possibility of their interference with intestinal glucose absorption. Considering above mentioned earlier reports, synergistic interaction between GLB and FP/MP appears to be pharmacodynamic rather than pharmacokinetic.

In the present study FP, MP individually and SHD combination of FP, MP and GLB produced favourable effects on lipid profile, in hyperglycemic animals. MP appears to be better hypoglycemic and hypolipidemic as compared to FP. These findings of the present study agree with earlier report wherein, fruitbody extract has been shown to significantly reduce total cholesterol, triglycerides and phospholipids⁹. The hypolipidemic activity of *Ganoderma lucidum* products has been attributed to triterpenes like ganoderic acid B and ganoderic acid C present in them¹⁸. The chemical derivatives of functional groups of these triterpenes have been reported to inhibit cholesterol synthesis by interfering with cytochrome P450 enzyme¹⁸.

As advised by the manufacturer, both FP and MP are consumed together for a variety of conditions including diabetes mellitus. The findings of the present study if extrapolated to humans, indicate that MP alone is sufficient to control hyperglycemia and dyslipidemia in diabetes mellitus and also that concomitant use of FP and MP with glibenclamide in diabetic patients could precipitate severe hypoglycemia. The commercial preparations like Reishigano and Ganocelium are increasingly used with the presumption that they are safe and free from toxicity. Therefore these *Ganoderma lucidum* products merit clinical evaluation with respect to their potential interactions not only with hypoglycemic agents but other drugs that are likely to be coadministered.

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