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AN INVESTIGATION ON HYPOLIPIDEMIC AND HEPATOPROTECTIVE EFFECTS OF OYSTER AND REISHI MUSHROOM IN DIABETIC AND NORMAL MICE.

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ABSTRACT

Pleurotus osteratus (family: Pleurotaceae) and *Ganoderma lucidum* (family: ganodermataceae) are common edible mushrooms in Bangladesh. Ethyl acetate extract of *Pleurotus osteratus* and *Ganoderma lucidum* were used to find out their hypolipidemic and hepatoprotective activity in alloxan induced diabetic mice and normal mice. Lipid profile namely LDL, TG and TC level significantly reduced after 24 hours by the intraperitoneal administration of these extracts in normal and diabetic mice whereas equipotently increased HLD level in both cases. Further, diabetic condition increases alanine aminotransferase ALT/SGPT and aspartate aminotransferase AST/SGOT enzyme level in liver. Present treatment also showed that both the oyster and reishi has significantly inhibited the rise of both serum liver enzymes in the treated mice compared to controls.

KEY WORDS: hypolipidemic; hepatoprotective; lipid profile; diabetic mice.

1. INTRODUCTION

Hypercholesterolemia and low plasma concentration of high-density-lipoprotein (HDL) cholesterol play an important role in the initiation and progression of cardiovascular diseases.[1] Elevated serum triacylglycerol concentrations and hypersecretion of very low-density-lipoprotein (VLDL) cholesterol are also frequently associated with the development

of coronary atherosclerosis, mainly in patients with diabetes.[2] Although a life style change is often the first choice in such cases, hypolipidemic drugs generally help to control elevated levels of different forms of lipids in patients with hyperlipidemia. Treatment of dyslipidemia includes administration of drugs that inhibit lipoprotein production and increase lipid removal from plasma.[3] *Pleurotus ostreatus*, the oyster mushroom, is increasingly being recognized as an important food product with a significant role in human health and nutrition.[4] Oyster mushrooms are an ideal dietary substance for the prevention and treatment of hypercholesterolemia due to high content of dietary fiber, sterol, proteins, and microelements.[5] In recent years, there has been great interest in the lipid-lowering properties of medicinal mushrooms, including *Ganoderma lucidum*, popularly called ‘‘ling zhi,’’ ‘‘reishi,’’ or ‘mannentake’.[6] Fruiting bodies of *G. lucidum*, a basidiomycete belonging to the Ganodermataceae family, have long been used in folk medicine to treat hepatopathy, hypertension, diabetes, arthritis, asthma, cancer, and other diseases in Eastern countries.[7]

Reishi mushroom is a well-known traditional medicinal mushroom that has been shown to have obvious hepatoprotective effects. *G. lucidum* could represent a promising approach for the management of various chronic hepatopathies.[8] Similarly, oyster mushroom can suppress toxin induced increased levels of serum aminotransferase enzymes in animals when compared to those of mushroom untreated rats, indicating the hepatoprotective effect of mushroom.[9-10] The fact that lovastatin is present in high proportions in oyster mushroom, is an important food supplement for patients suffering from hypercholesterolemia.[11] Reishi mushroom shows bioactive compounds with pharmacological properties, mainly polysaccharides and triterpenes, which have hepatoprotective, hypoglycemic, hypolipidemic, antioxidant, and antitumor effects.[12] There is significant rise in serum GOT and GPT levels in diabetic mice, which could related to excessive accumulation of amino acids (glutamate and alanine) in the serum of diabetic animals as a result of amino acids mobilization from protein stores.[13] However, these mushrooms are an ideal dietary substance for the prevention and treatment of hypercholesterolemia due to high content of dietary fibre, sterol, proteins and micro elements.[14]

2. MATERIALS AND METHODS

2.1. Sample collection and processing

The dried mushrooms, *P steratus* and *G lucidum*, were collected from the National Mushroom Development and Extension Centre, Dhaka. The cleaned mushrooms were dried.

Therefore the whole dried mushrooms (i.e. pileus + stipe) were powdered to pass through a 40 mesh sieve and the powder was used for cold extraction.

2.2. Preparation of crude extracts

The coarse powders from these mushrooms were soaked in 95% Ethyl acetate solution for 7 days and were kept at room temperature with occasional shaking and stirring. When the solvent became concentrated, the liquid Ethyl acetate contents were filtered through cotton and then through filter paper (Whatman filter paper no. 1). The Ethyl acetate solution was allowed to evaporate using rotary evaporator. Thus the highly concentrated Ethyl acetate extracts were obtained which were further dried completely under mild sun and by freeze-drying. The dried extracts were then preserved in the refrigerator for the experimental use.

2.3. Drugs and chemicals

Compounds were purchased from commercial sources as follows: Alloxan monohydrate; Loba Chemiie, Mumbai, India. Total cholesterol (TC) and triglyceride (TG) kits; Boehringer Mannheim, GmbH, Germany. Serum LDL diagnostic kits; Crescent Diagnostics, Jeddah. Glycogen test diagnostic kit was o-toluidine reagent. The active drug, metformin hydrochloride was the generous gift from Square Pharmaceuticals Ltd. Pabna Bangladesh.

2.4. Induction of diabetes

Swiss albino female mice were purchased from Animal House of International Centre for Diarrheal Disease Research, Bangladesh (ICDDR, B). Prior to the commencement of experiment, the mice were acclimatized in a well-ventilated animal housed in animals cages under standard environmental conditions (22-25°C, humidity 60-70%, 12 hr light and dark cycle) for a period of one week. The mice were feed with standard pellet diet taken from the mice supplied lab. The animals used in this study were cared in accordance with the guidelines on animal experimentation of our institute. Mice were grouped into seven groups. Each group contains four mice. After overnight fasting, a freshly prepared solution of alloxan monohydrate (120 mg/kg body weight in normal saline) was administered intraperitoneally into group II-V. Group I kept as normal control group that did not receive the chemical. Group VI and VII were also kept normal to treat by these extract.

2.5. Treatment of the animal

Group I and II served as non-diabetic and diabetic control group, respectively. Group III stands for metformin control group in which metformin was administered as a single

intraperitoneal dose of 150mg/ kg body weight. Group IV (Diabetic) and VI (Non diabetic) received *Pleurotus osteratus* extracts. On the other hand group V (Diabetic) and VII (Non diabetic) received *Ganoderma lucidum* extract respectively as a single intraperitoneal dose of 200 mg/kg body weight.

2.6. Collection of blood serum

After completion of 24 hours experimental period mice were sacrificed and approximately 3-5 ml of blood samples were collected directly from heart by syringes. The collected blood samples were centrifuged at 4000 rpm for 15 minutes and the resulting supernatant was obtained as serum. Serum LDL, TC, TG, HDL, SGPT and SGOT concentrations were analyzed by UV spectrophotometric method (Shimadzu UV-1200, Tokyo, Japan) using wet reagent diagnostic kits according to the manufacturer's protocol.

2.6. Statistical analysis

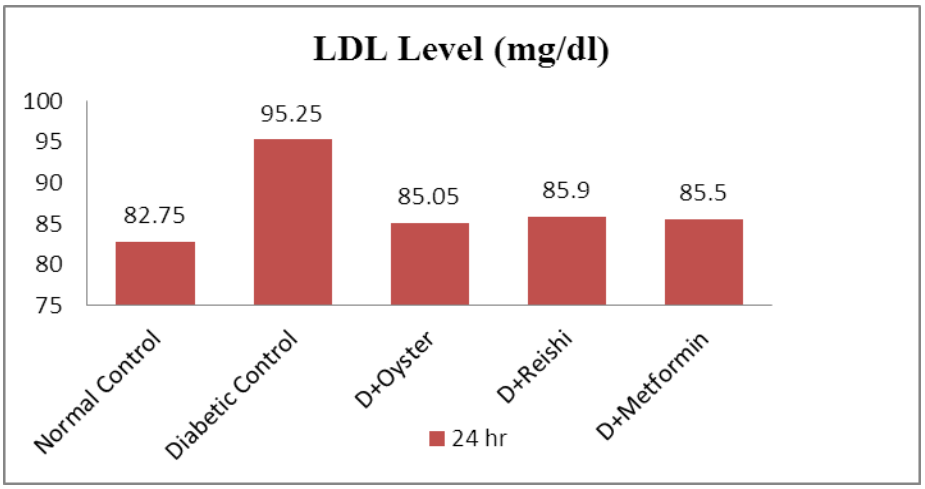
Data were expressed as mean ± standard error of mean (SEM). Statistical comparisons were performed by one-way analysis of variance (ANOVA) or students paired or unpaired *t*-test where appropriate. Results are considered to be significant when *p* values were less than 0.05 ($p<0.05$).

3. RESULTS AND DISCUSSION

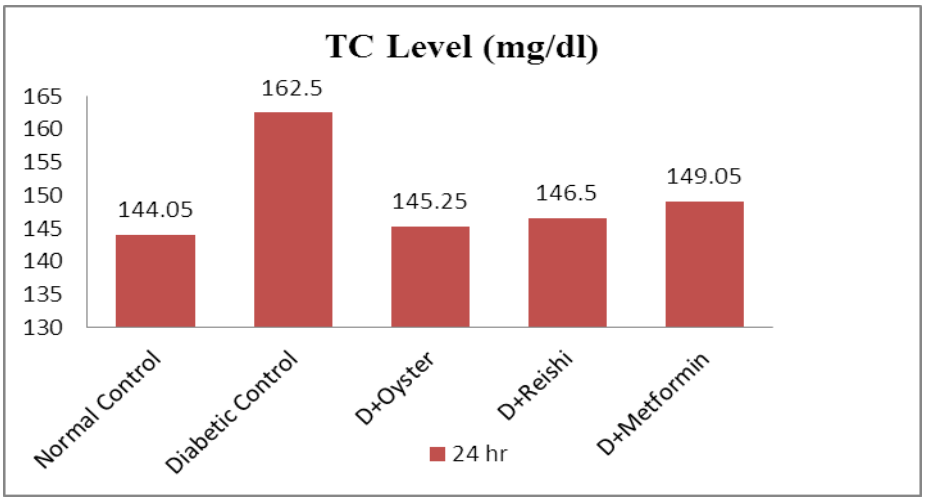
3.1. *Effect of Pleurotus osteratus and Ganoderma lucidum on low-density lipoprotein (LDL), total cholesterol (TC), triglyceride (TG) and high density lipoprotein (HDL) levels in alloxan induced diabetic mice*

After induction of diabetes, the LDL, TC and TG levels were increased alongside HLD level decreased significantly in alloxan induced diabetic mice. *P. osteratus, G.lucidum* and metformin HCl decreased the elevated serum LDL levels to 10.71, 09.82 and 07.09% respectively. The maximum reduction of 10.71% was observed by *P. osteratus* extract. The elevated serum TC levels were reduced to 10.62, 9.85 and 08.28%, by *P. osteratus, G.lucidum* and metformin HCl respectively. Likewise, serum TG level was reduced to 10.75, 09.89 and 08.24% when treated with *P. osteratus, G.lucidum* and metformin HCl. On the other hand *P. osteratus, G.lucidum* and metformin HCl increased serum HDL level 14.56, 12.66 and 06.46% respectively in alloxan induced diabetic mice.

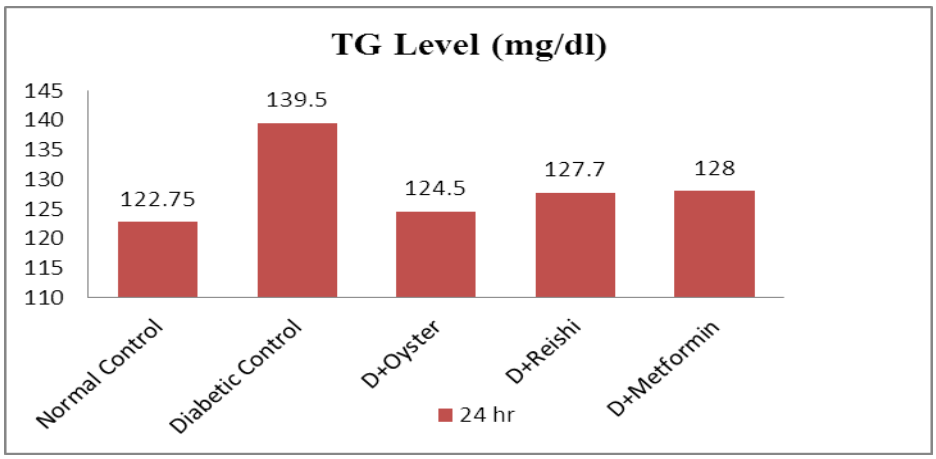
The summarized results are shown in the figure 1, 2, 3 and 4 for LDL, TC, TG and HDL levels, respectively.



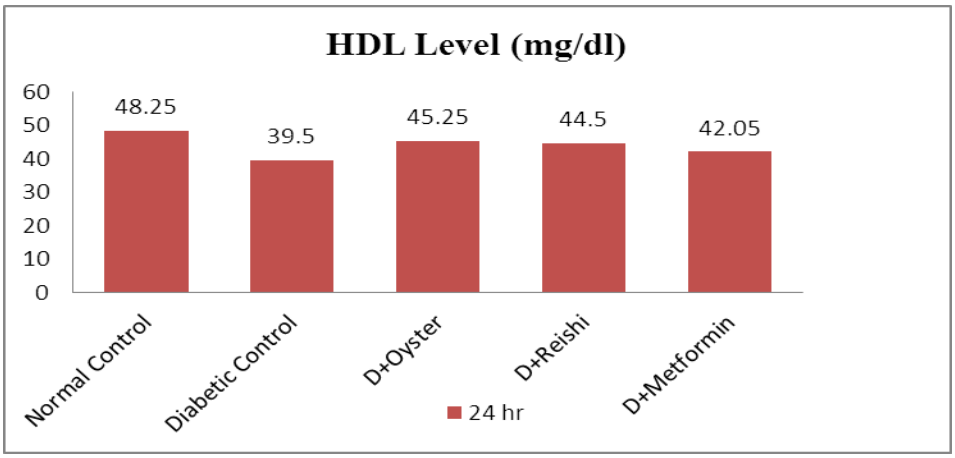
"Fig.1" Effect of *Pleurotus osteratus* and *Ganoderma lucidum* on LDL levels in alloxan induced diabetic mice.



"Fig.2" Effect of *Pleurotus osteratus* and *Ganoderma lucidum* on TC levels in alloxan induced diabetic mice.



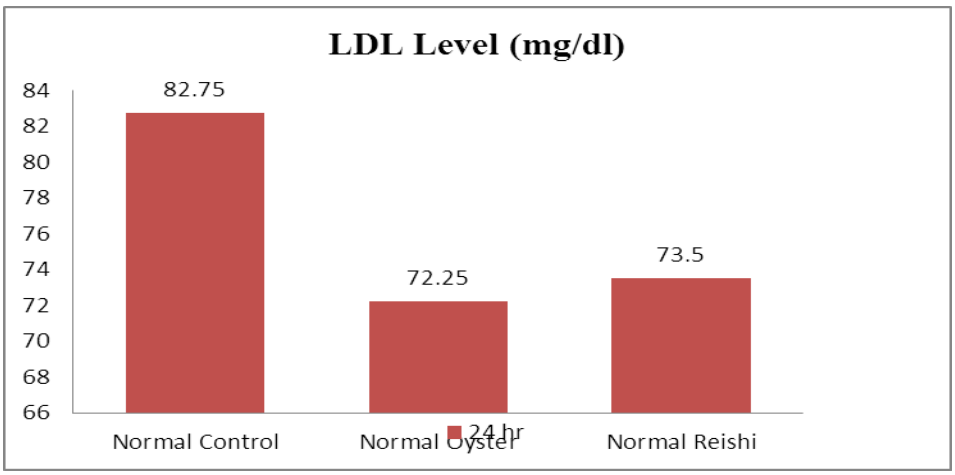
"Fig.3" Effect of *Pleurotus osteratus* and *Ganoderma lucidum* on TG levels in alloxan induced diabetic mice.



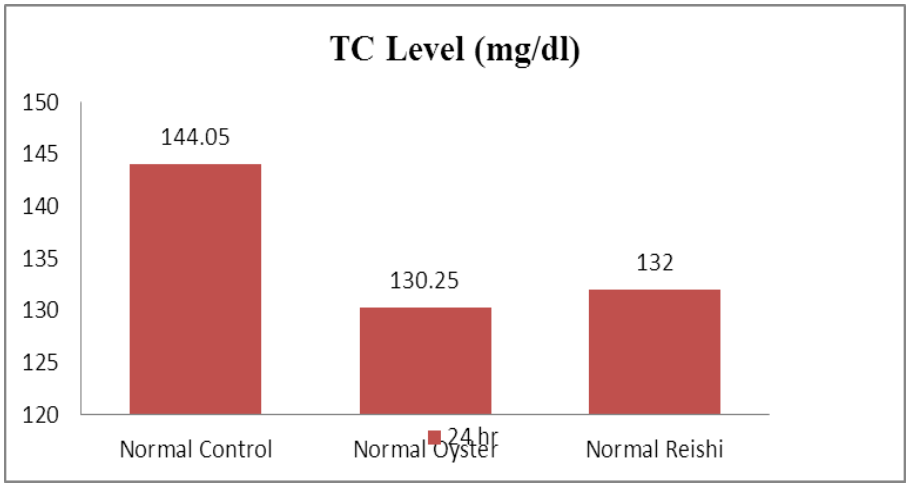
"Fig.4" Effect of *Pleurotus osteratus* and *Ganoderma lucidum* on HDL levels in alloxan induced diabetic mice.

3.2. *Effect of Pleurotus osteratus and Ganoderma lucidum on low-density lipoprotein (LDL), total cholesterol (TC), triglyceride (TG) and high density lipoprotein (HDL) levels in normal mice*

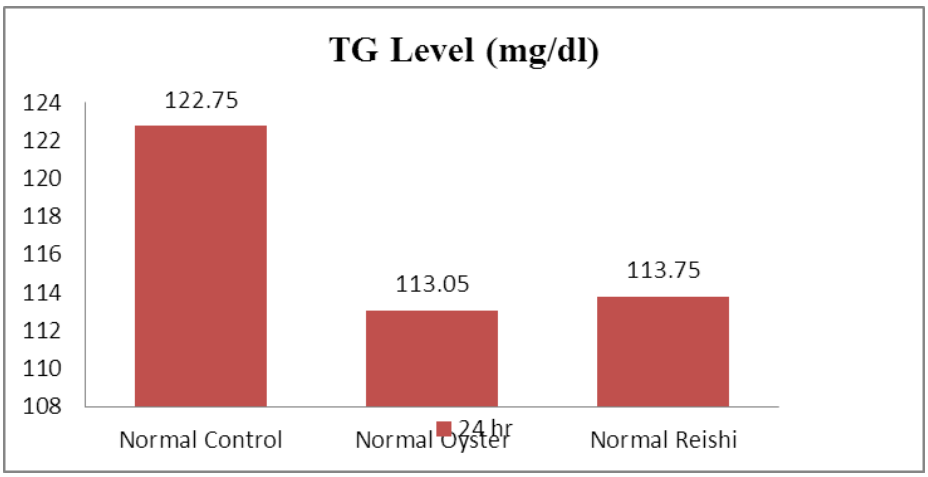
HDL is known as good cholesterol which helps to maintain or decrease LDL level. *P. osteratus* and *G.lucidum* decreased the elevated serum LDL levels to 11.50 and 09.33% respectively in normal mice. The maximum reduction of 11.50% was observed by *P. osteratus* extract compare to the normal mice. The elevated serum TC levels were reduced to 09.58 and 08.37%, by *P. osteratus* and *G.lucidum* respectively. Similarly, serum TG level was reduced to 07.90 and 07.33% when treated with ethyl acetate extract of *P. osteratus* and *G.lucidum. P. osteratus* and *G.lucidum* increased serum HDL level 11.50 and 09.33% respectively in normal mice also. The summarized results are shown in the figure 5, 6, 7 and 8 for LDL, TC, TG and HDL levels, respectively.



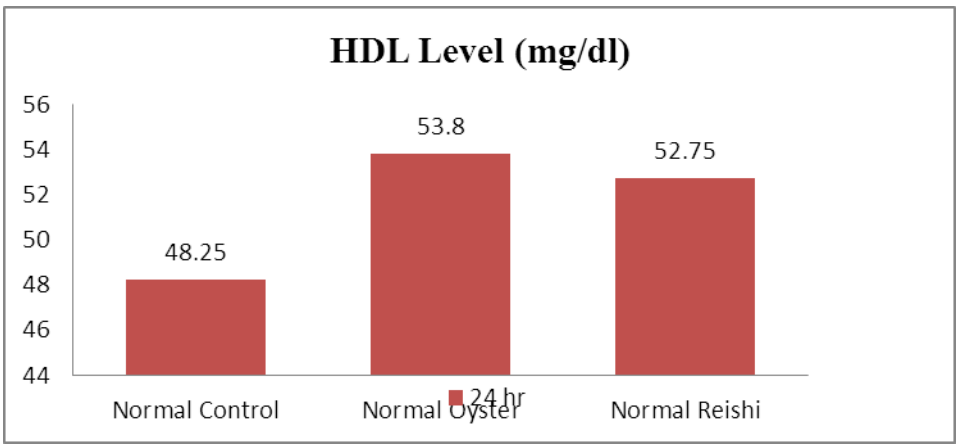
"Fig.5" Effect of *Pleurotus osteratus* and *Ganoderma lucidum* on LDL levels in normal mice.



"Fig.6" Effect of *Pleurotus osteratus* and *Ganoderma lucidum* on TC levels in normal mice.



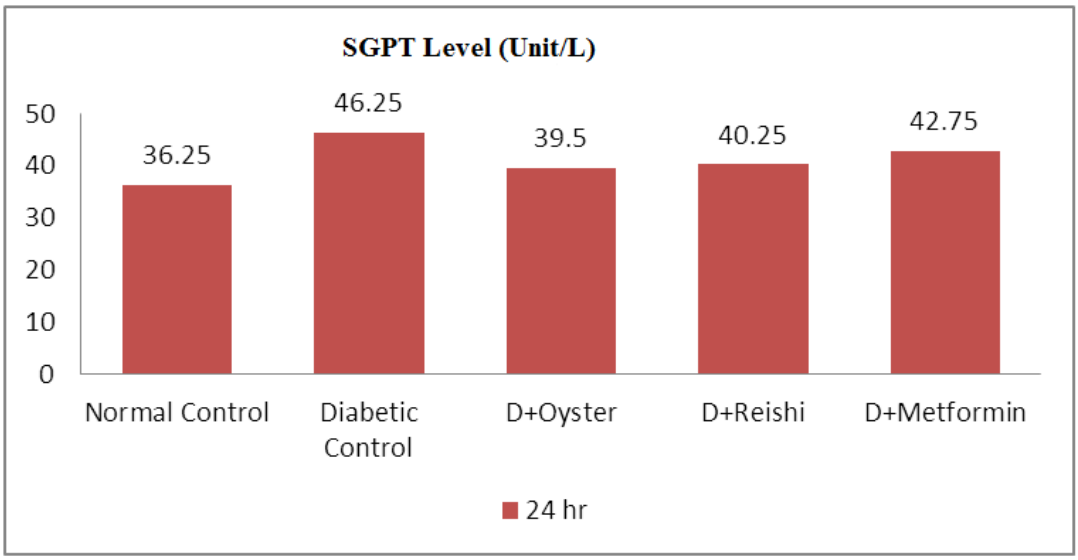
"Fig.7" Effect of *Pleurotus osteratus* and *Ganoderma lucidum* on TG levels in normal mice.



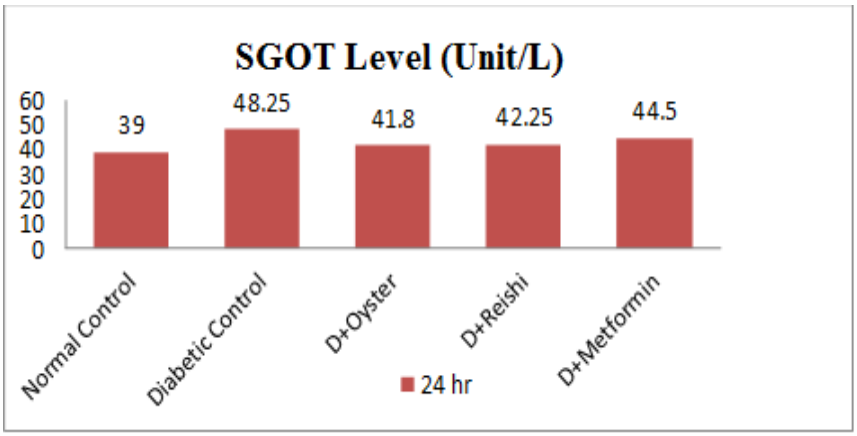
"Fig.8" Effect of *Pleurotus osteratus* and *Ganoderma lucidum* on HDL levels in normal mice.

3.3. *Effect of Pleurotus osteratus and Ganoderma lucidum on liver enzyme SGPT and SGOT level in alloxan induced diabetic mice.*

Besides metabolic functions liver performs bile formation and secretion, synthesis of some proteins including clotting factors, detoxification of drugs and endogenous compounds, storage of vitamins and minerals.[15] Both liver enzymes alanine aminotransferase and aspartate aminotransferase were increased in diabetic condition. *Pleurotus osteratus*, *Ganoderma lucidum* and Metformin HCl reduced SGPT level to 14.59, 12.97 and 07.57% respectively. 13.37, 12.44 and 07.77% serum SGOT level reduced by *Pleurotus osteratus*, *Ganoderma lucidum* and Metformin HCl respectively in diabetic mice. The summarized results are shown in the figure 9 and 10 for SGPT and SGOT levels, respectively.



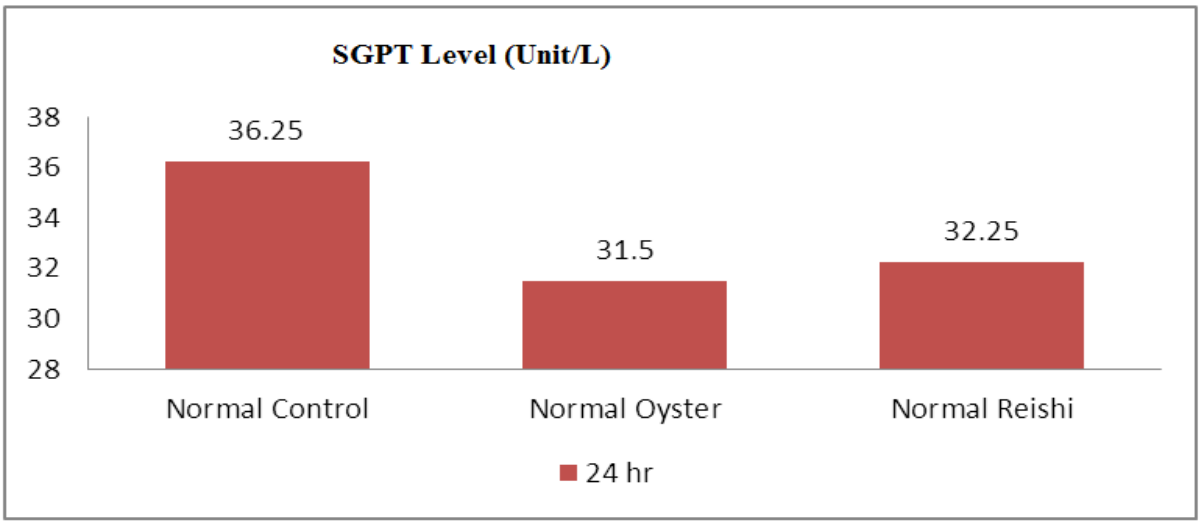
"Fig.9" Effect of *Pleurotus osteratus* and *Ganoderma lucidum* on SGPT levels in alloxan induced diabetic mice.



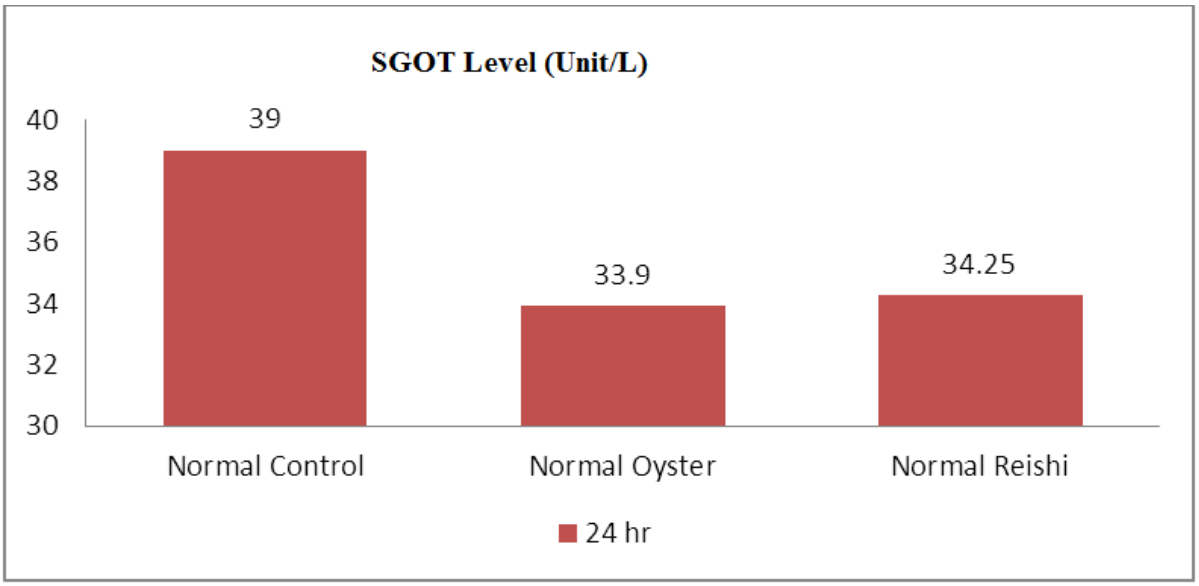
"Fig.10" Effect of *Pleurotus osteratus* and *Ganoderma lucidum* on SGOT levels in alloxan induced diabetic mice.

3.4. *Effect of Pleurotus osteratus and Ganoderma lucidum on liver enzyme SGPT and SGOT level in normal mice.*

In these experiments it has been shown that 13.10 and 11.03% of serum SGPT level reduced by *Pleurotus osteratus* and *Ganoderma lucidum* respectively when compare with normal mice. Unlike SGPT, *Pleurotus osteratus* and *Ganoderma lucidum* reduced SOPT level 13.08 and 12.18% after 24 hour of drug treatment in normal mice. The summarized results are shown in the figure 11 and 12 for SGPT and SGOT levels, respectively.



"Fig.11" Effect of *Pleurotus osteratus* and *Ganoderma lucidum* on SGPT levels in normal mice.



"**Fig.12" Effect of *Pleurotus osteratus* and *Ganoderma lucidum* on SGOT levels in normal mice.**

D+Oyster=Diabetic+Oyster, D+Reishi=Diabetic+Reishi, D+Metformin=Diabetic+Metformin.

4. CONCLUSION

High level of TC, TG and LDL increase the risk of heart diseases, hypertension and diabetes. The present design study showed that the bad cholesterols and liver enzymes levels were higher alongside good cholesterol level was lower in alloxan induced diabetic mice; however, extract of oyster and reishi mushrooms have shown significant hypolipidemic and hepatoprotective effects in diabetic and normal mice. Further study is necessary for the screening of chemical compounds and structure elucidation of the respective effects leads as well as their exact mechanism.

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