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Comparative Studies on Serum Lipid Profile of *Ganoderma Lucidum* Extract And Atorvastatin In Normal and Diabetic Mice

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ABSTRACT

This work was carried out to investigate the lipid profile level in ethyl acetate extract of *Ganoderma lucidum* and atorvastatin in normal and alloxan induced diabetic mice. *Ganoderma lucidum* and atorvastatin have shown to reduce LDL level 10.4 and 14.59%, TC level 06.76 and 09.78%, TG level 07.40 and 09.82% alongside equipotently increased HDL level to 09.33 and 15.18% respectively in normal mice. However, in case of diabetic mice, these values were 11.61 and 14.46% for LDL, 10.56 and 13.00% for TC, 10.38 and 13.96% for TG, 14.94 and 20.02% for HDL as well. Therefore, these results suggested that, intra peritoneal administration of *Ganoderma lucidum* reduced bad cholesterol level and increased good one in blood plasma not more than that of atorvastatin which finally suggested that *Ganoderma lucidum* itself a good diet supplement provided health benefits by maintaining a normal lipid profile in serum.

Keywords: lipid profile; atorvastatin; cholesterol; diabetic.

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INTRODUCTION

Coronary artery disease is an important cause of morbidity and mortality worldwide. The plasma lipid profile characterized by low HDL cholesterol and increased total cholesterol, LDL cholesterol, and triacylglycerol levels plays an important role in the development of atherosclerosis[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 lifestyle 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]. Oxidative stress is an important mechanism underlying cardiovascular diseases[4]. The ability of macrophages to produce reactive oxygen species (ROS) and nitric oxide (NO) can increase the oxidative burden of low-density-lipoprotein (LDL) cholesterol and may accelerate the atherosclerotic process[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]. This mushroom shows bioactive compounds with pharmacological properties, mainly polysaccharides and triterpenes, which have hepatoprotective, hypoglycemic, hypolipidemic, antioxidant, and antitumor effects[7]. A previous study showed that G. lucidum metabolites modulate T_h-cell differentiation, resulting in the balance between Th1 and Th2 cells and the restriction of tumor growth[8].

The components of G. lucidum responsible for its hypolipidemic activity have not been established yet, but a large variety of biologically active polysaccharides, triterpenes, proteins, cerebrosides, and phenols may act upon specific signaling molecules and pathways, leading to its therapeutic effects[9, 10] demonstrated that lanosterol 14a-demethylase, which converts lanosterol into cholesterol, can be inhibited by oxygenosterols from G. lucidum. Further, indigestible fibers, such as b-glucans (1 → 3), present in G. lucidum reportedly reduce cholesterol absorption in the small intestine or bind to bile acids and consequently accelerate the enteric degradation of cholesterol[11]. The phytochemical properties of dried spawn of G. lucidum CG 144 showed the presence of cardiac glycosides and saponins. Saponins are known for their blood cholesterol-loweringactivity by blocking lumenal cholesterol absorption[12].

MATERIALS AND METHOD

Sample collection and processing

The dried mushrooms *G lucidum* was collected from the Mushroom Development and Extension Centre, Jessore. The cleaned mushroom was dried. Therefore the whole dried mushroom (i.e. pileus + stipe) was powdered to pass through a 40 mesh sieve and the powder was used for cold extraction.

Preparation of crude extracts

The coarse powders from this mushroom was 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 (What man filter paper no. 1). The Ethyl acetate solution was allowed to evaporate using rotary evaporator. Thus the highly concentrated Ethyl acetate extract obtained which was further dried completely under mild sun and by freeze-drying. The dried extract was then preserved in the refrigerator for the experimental use.

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 and HDL diagnostic kits; Crescent Diagnostics, Jeddah. The active drug, metformin hydrochloride and atorvastatin were the generous gift from Square Pharmaceuticals Ltd. Pabna in Bangladesh.

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 eight groups. Each group contains five 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, VII and IX were also kept normal to treat by extract, atorvastatin and metformin respectively.

Treatment of the animal

Group I and II served as non-diabetic and diabetic control group, 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 *Ganoderma lucidum* extract as a single intraperitoneal dose of 200 mg/kg body weight. Likewise group V (Diabetic) and VII (Non diabetic) treated with atorvastatin as a single intraperitoneal dose of 70 mg/kg body weight.

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 and HDL concentrations were analyzed by UV spectrophotometric method (Shimadzu UV-1200, Tokyo, Japan) using wet reagent diagnostic kits according to the manufacturer's protocol.

Statistical analysis

Data were expressed as mean ± standard error of mean (SEM). Statistical comparisons were performed by one-way analysis of variance (ANOVA) following Dunnet's test through the SPSS software (version 20; IBM Corporation, New York, USA). ($P < 0.05$*vs.*control) was considered statistically significant.

RESULTS AND DISCUSSION

Effect of Ganoderma lucidum and atorvastatin 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. *G.lucidum,* atorvastatin and metformin HCl decreased the elevated serum LDL levels to 10.4, 14.59 and 06.69 % respectively in normal mice. The maximum reduction of 14.59% was observed by atorvastatin compare to the normal mice. The elevated serum TC levels were reduced to 06.76, 09.78 and 04.16 %, by *G.lucidum,* and atorvastatin and metformin HCl respectively. Impairment of the plasma triacylglycerol level is related to the presence of cholesterol mainly in lipoproteins of increased size[13]. In this study serum TG level was reduced to 07.40, 09.82 and 04.52% when treated with ethyl acetate extract of *G.lucidum*, atorvastatin and metformin HCl. *G.lucidum,* atorvastatin and metformin HCl increased serum HDL level to 09.33, 15.18 and 04.49% respectively in normal mice also.

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

Table 1: Serum level of different lipids after 24hours in normal mice

Group	LDL	HDL	TC	TG
Normal control	82.25±0.64	49.05±0.75	144.25±0.15	121.75±0.39
Normal Extract	73.70±0.75	52.75±0.45	134.50±0.75	112.75±0.28
Normal atorvastatin	70.25±0.62	56.50±0.64	130.15±0.40	109.80±0.65
Normal metformin	76.75±0.25	51.25±0.27	138.25±0.25	116.25±0.05

Values are expressed as Mean ±SEM (n=5).

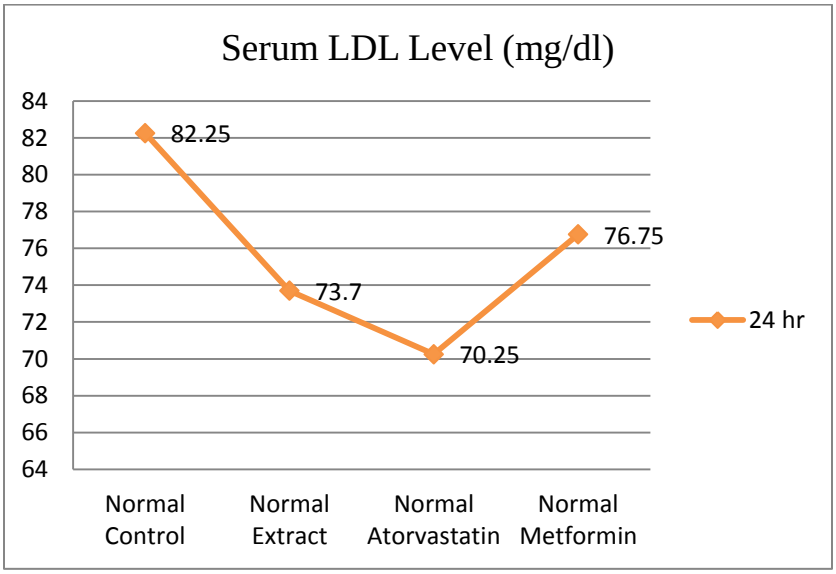

Figure 1: Effect of *Ganoderma lucidum*, atorvastatin and metformin on LDL levels in normal mice.

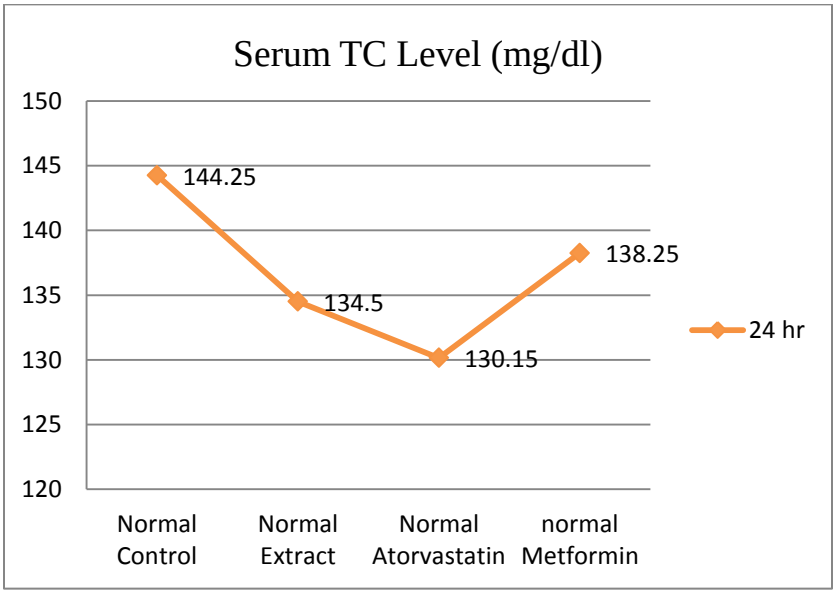

Figure 2: Effect of *Ganoderma lucidum*, atorvastatin and metformin on TC levels in normal mice.

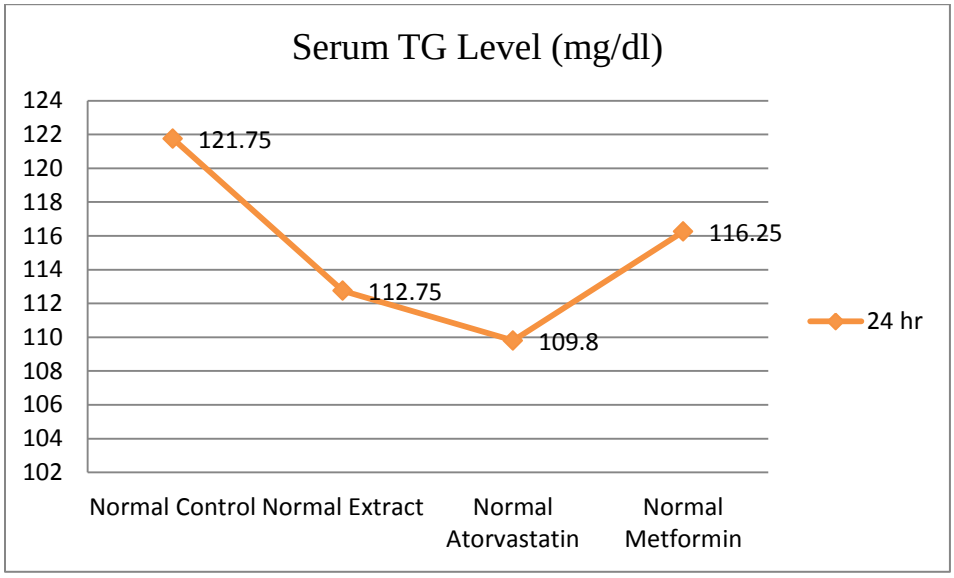

Figure 3: Effect of *Ganoderma lucidum,* atorvastatin and metformin on TG levels in normal mice.

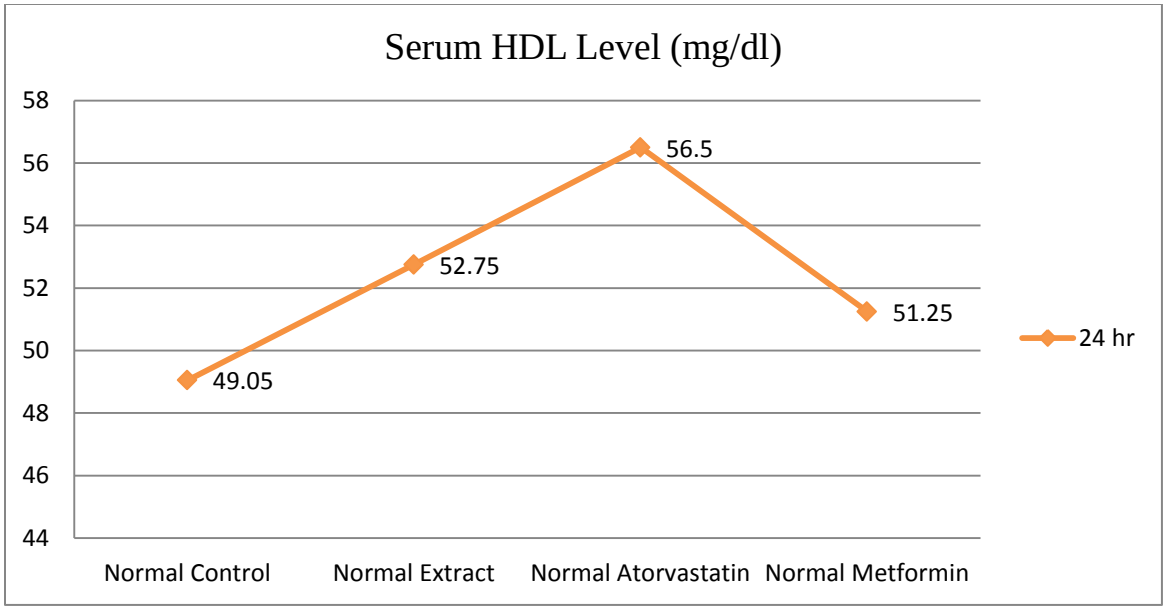

Figure 4: Effect of *Ganoderma lucidum,* atorvastatin and metformin on HDL levels in normal mice.

Effect of Ganoderma lucidum extract and atorvastatin 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 and HLD level decreased in alloxan induced diabetic mice. *G.lucidum,* atorvastatin and metformin HCl decreased the elevated serum LDL levels to 11.61, 14.46 and 05.54 % respectively. The maximum reduction of 14.46 % was observed by atorvastatin drug. The microsomal enzyme 3-hydroxy-3-

methylglutarylcoenzymeA (HMG-CoA) reductase is the major rate-limiting enzyme in cholesterol biosynthesis, which converts HMG-CoA to mevalonate. Therefore, inhibiting HMG CoA reductase decreases intracellular cholesterol biosynthesis[14]. The elevated serum TC levels were reduced to 10.56, 13.00 and 06.57 %, by *G.lucidum*, atorvastatin and metformin HCl respectively. Likewise, serum TG level was reduced to 10.38, 13.96 and 04.11% when treated with *G.lucidum*, atorvastatin and metformin HCl. On the other hand *G.lucidum*, atorvastatin and metformin HCl increased serum HDL level to 14.94, 20.02 and 04.49% respectively in alloxan induced diabetic mice.

The summarized results are shown in the table 2 and figure 5, 6, 7 and 8 for LDL, TC, TG and HDL levels, respectively.

Table 2: Serum level of different lipids after 24hours in diabetic mice.

Group	LDL	HDL	TC	TG
Diabetic control	94.75±0.25	38.5± 0.64	163.50±0.47	139.75±0.53
Diabetic extract	83.70±0.87	44.25±0.75	146.25±0.21	124.25±0.33
Diabetic atorvastatin	81.05±0.47	46.25±0.40	142.25±0.29	120.25 ±0.62
Diabetic metformin	89.50±0.85	40.05±0.47	152.75±0.65	134.05±0.95

Values are expressed as Mean ±SEM (n=5).

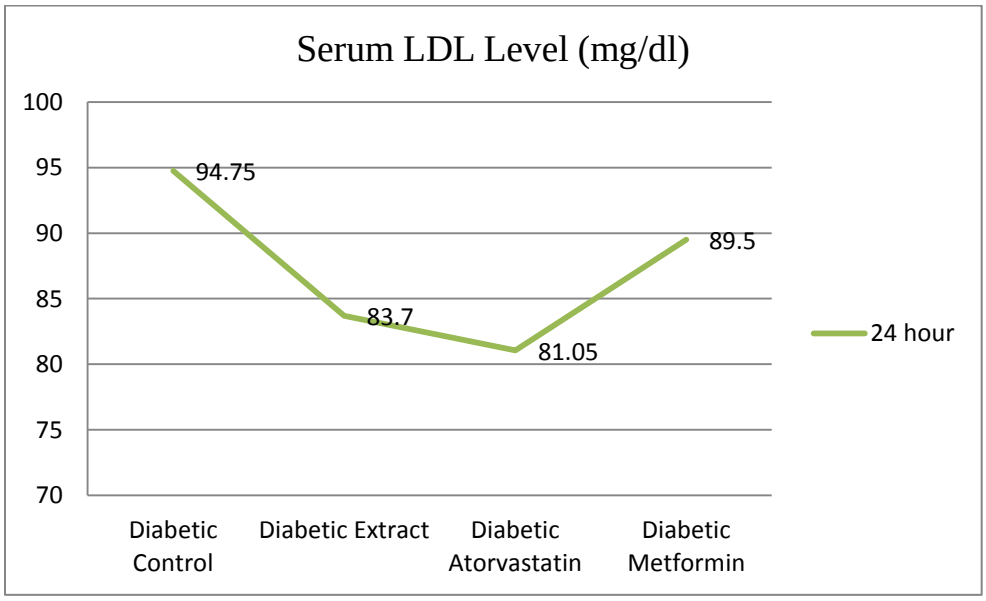

Figure 5: Effect of *Ganoderma lucidum*, atorvastatin and metformin on LDL levels in diabetic mice.

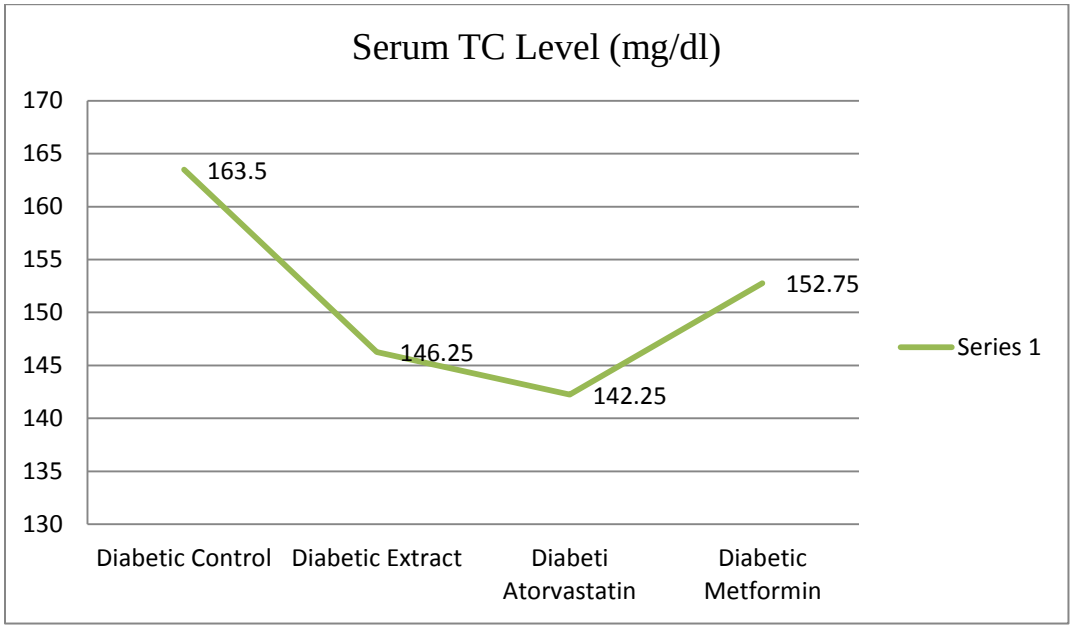

Figure 6: Effect of *Ganoderma lucidum*, atorvastatin and metformin on TC levels in diabetic mice.

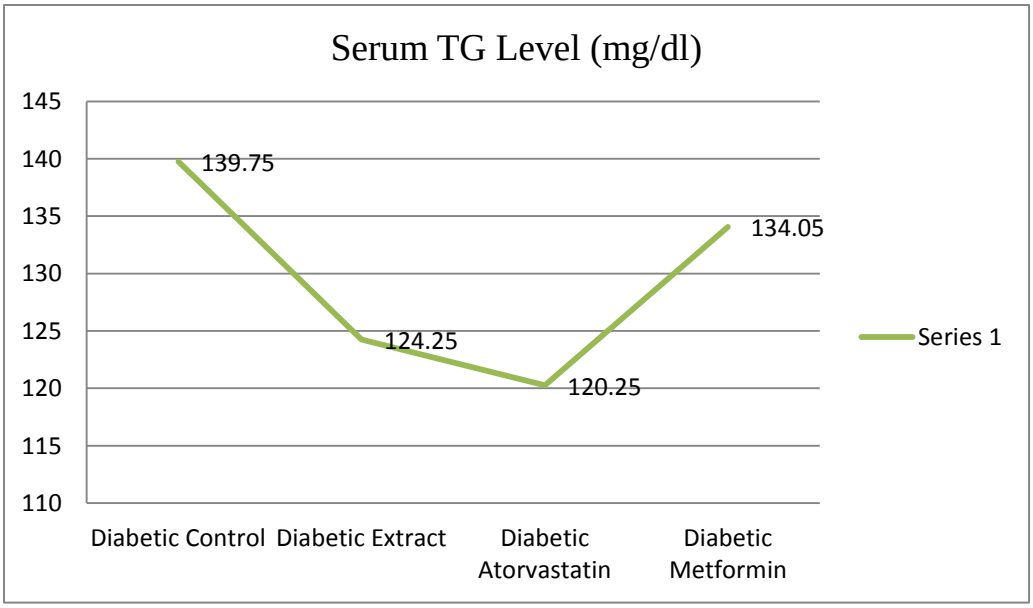

Figure 7: Effect of *Ganoderma lucidum*, atorvastatin and metformin on TG levels in diabetic mice.

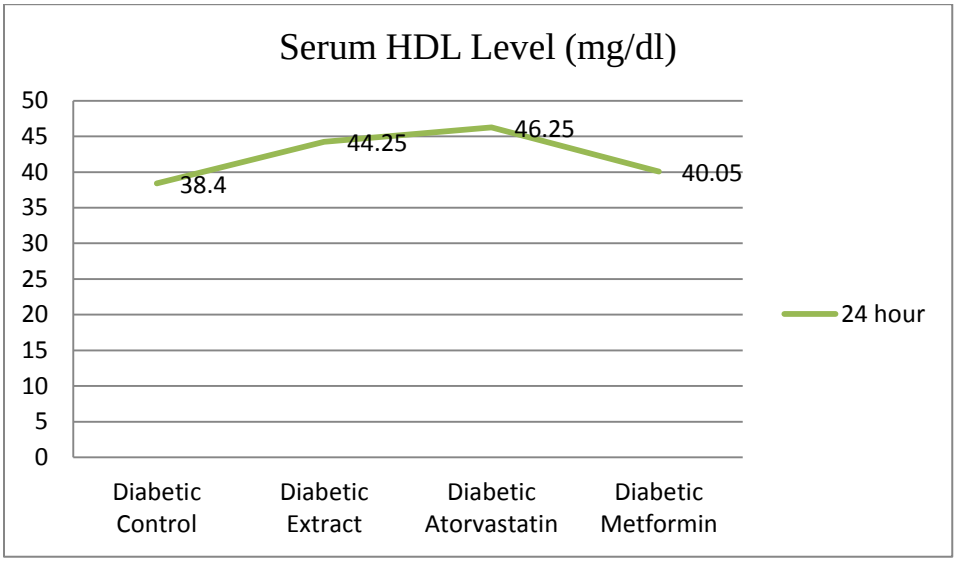

Figure 8: Effect of *Ganoderma lucidum,* atorvastatin and metformin on HDL levels in diabetic mice.

CONCLUSION

High level of TC, TG and LDL increase the risk of heart diseases, hypertension and diabetes. Hypercholesterolemia and low plasma concentration of high-density-lipoprotein (HDL) cholesterol play an important role in the initiation and progression of cardiovascular diseases[15] and cardiovascular disease severe as a chronic complication is 2-4 times higher in diabetic patients than the healthy population[16]. Consumption of fat-rich diet directly affects the serum lipid profile and the fatty acids composition, which is an important factor in the modulation of lipid metabolism. The high saturated fatty acids intake increases the low-density lipoprotein cholesterol (LDL-c) and reduces the high-density lipoprotein cholesterol (HDL-c) in the bloodstream. This is a known condition for the development of coronary artery disease because the HDL-c is inversely related to risk of atherosclerosis, while LDL-c is an important risk factor to cardiovascular events since, when present in elevated levels in the blood, it migrates to the arterial intimal layer triggering the development of atherosclerosis[17, 18,]. Therefore, a balance of unsaturated fatty acids is important when selecting food sources and those rich in monounsaturated and polyunsaturated fatty acids; particularly, the long-chain n-3 polyunsatured fatty acids should be used preferentially since they reduce the risk for atherosclerosis[19, 20].

The present design study showed that the bad cholesterols levels were higher alongside good cholesterol level was lower in alloxan induced diabetic mice; however, extract of *G.lucidum* have shown hypolipidemic effects in normal and diabetic mice in comparison with atorvastatin .

However, further study is necessary for the screening of exact chemical compounds and structure elucidation of the respective effects leads as well as their mechanism.

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