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RESEARCH ARTICLE

Cordyceps militaris (CM) Mushroom Powder Ameliorates Diet-Induced Hyperlipidemia in Albino Rats

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ABSTRACT

In the current study, we explored the ability of *Cordyceps militaris* (CM) mushroom powder to prevent hyperlipidemia in albino rats fed a high-cholesterol diet (HCD). Twenty-five male Albino Wister rats were divided into 5 groups, each consisting of five rats. PC (positive control group) was kept on a high-cholesterol diet, while rats in the negative control group (NC) were fed a standard basal diet and distilled water. The standard group (STD) (treatment group) received a dose of 10 mg/kg of body weight of Simvastatin. The low dose (LD) and high dose (HD) groups were administered CM powder, at 12g/kg b.wt. and 24 g/kg b.wt. /day, respectively, along with the basal diet, given orally daily for eight weeks. Our results clearly showed that CM significantly reduced the levels of TC, TG, and LDL levels by 55.65 %, 56.32 %, and 38.49 %, in LD respectively, and by 63.40 %, 64.08%, and 66.39 % respectively in HD. CM consumption significantly increased the value of HDL by 52.81 % and 57.45 % in LD and HD respectively. There was a significant decrease in serum MDA (Malondialdehyde) and serum TOS (Total Oxidative Stress) levels in the treatment groups of LD and HD, however, the level of serum catalase (CAT) and serum TAC (Total Antioxidant Capacity) followed an increasing trend in these groups. Moreover, CM at high doses triggered a significant drop in the amounts of aspartate transaminase (AST), and alanine transaminase (ALT) enzymes as compared to the NC by 27.2 %, and 34.82 % respectively. Furthermore, the normal histological structure was restored in the HD as compared to the PC group. Our results revealed that a high dose of CM could prevent HCD-induced hyperlipidemia and have the potential to be used as a functional food ingredient.

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INTRODUCTION

Compared to other ethnic groups, South Asian people have an elevated likelihood of developing atherosclerotic cardiovascular disease (CVD) (Raheem *et al.,* 2022). Recent research has revealed an increase in heart failure cases among South Asian populations, which can be linked to type 2 diabetes mellitus (T2DM), metabolic syndrome, hyperlipidemia, and premature coronary heart disease (Hussain *et al.,* 2023; Inderbir *et al*., 2024). For those who are at an elevated risk of CVD, it is essential to take preventative action to assist in avoiding the development of adverse events.

The most common health problem in the country today is hyperlipidemia, which is treated with a wide variety of drugs. Nevertheless, it has been shown that chemotherapeutics that reduce lipid levels can have major adverse effects, including constipation, heart toxicity, depression, anxiety, headaches, nausea, diarrhea, and vomiting (Gao *et al*., 2024). Therefore, the creation of a less toxic antihyperlipidemic drug with natural products and their bioconversion process has drawn increased attention in recent years. Cordyceps is among the thousands of mushrooms with countless bioactive components and health advantages (Adnan *et al*., 2017).

Therefore, it is reasonable to consider CM as a promising option for treating several illnesses. Numerous bioactive substances, including proteins, fats, carbohydrates, phenolic compounds, polysaccharides, cordycepin, cordycepic acid, adenosine, steroids, ergosterol, and

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terpenoids, etc. have been reported to be present in cordyceps. Cordycepin is the primary active ingredient that has undergone the greatest research regarding its medical and nutraceutical potential (Kuo *et al*., 2015).

Since cordyceps polysaccharides have so many uses in the cattle business, their use as a feed supplement has garnered significant attention in Asia. For instance, Cordyceps polysaccharides may boost the feed conversion ratio, encourage early chicken growth, and improve the daily growth increase of broilers (Han *et al*., 2015). According to Wang *et al.* (2015a), the CM waste medium reduced the feed-to-egg ratio (F/G) and raised the rate of egg production. Chickens' development performance and health index were enhanced by a hot-water extract made from the mycelia of *C. sinensis*, which also preserved the excellent microbiota in their intestines (Koh *et al.*, 2003).

The biological properties of CM for disease prevention and animal and human health protection are well documented (Sun *et al*., 2014; Doan *et al*., 2017). According to Han *et al*. (2015) and Cheng *et al*. (2016), CM greatly enhanced growth performance and immunity. Weaning pigs given fermented CM have also been shown to activate their cellular immune response (Cheng *et al*., 2016). The accumulation of 1.5 g/kg spent mushrooms reduced the cholesterol levels in the hypercholesterolemic profile compared to the pig control groups (Boontiam *et al.,* 2020). Research by Gao *et al*. (2011) found that the addition of 25 and 50 mg/kg cordycepin considerably reduced the levels of TC, TG, and LDL in rats and hamsters. Additionally, CM included 163 g kg−1 of dietary fiber, which may cause a hypercholesterolemic impact by increasing the synthesis of short-chain fatty acids. Short-chain fatty acids can then block the synthesis of cholesterol in the liver and gut and lower cholesterol levels (Jiao *et al*., 2020; Haghgoo *et al.,* 2023). According to a study, feeding fish with 10 g/kg of CM spent mushroom substrate (SMS) resulted in the largest increase in skin mucus lysozyme and peroxidase activities when given SMS through food (Doan *et al*., 2017). Recent studies have shown that cordycepin stimulates human and mouse immune systems by upregulating TNF-a, IL-10, IL1b, IL-6, and IL-8 (Koh *et al*., 2002; Doan *et al*., 2017).

Blood glucose management, hypolipidemia, anti-tumor, anti-microbial, anti-viral, anti-inflammatory, neuroprotective, antioxidant, and immunoprotective actions of this species have all been reported (Bi *et al.,* 2018; Jo *et al*., 2020; Guo *et al.,* 2020; Wu *et al.,* 2021; Wang et al., 2015b). As a result, CM may be regarded as a significant possibility for treating many diseases. Numerous bioactive substances, including proteins, fats, carbohydrates, exopolysaccharides, cordycepin, phenolic compounds, polysaccharides, cordycepic acid, adenosine, terpenoids, steroids, and ergosterol, etc. have been reported to be present in cordyceps. Cordycepin is the primary active ingredient that has undergone the greatest research regarding its medical and nutraceutical potential (Kuo *et al.,* 2015; Zhang *et al.,* 2021). It has a high price tag because of the challenges involved in harvesting. Despite its high price and scarcity, cordyceps has unmatched therapeutic uses that have elevated its status as an essential part of traditional Chinese and Tibetan medicine. In recent years, CM investigation has focused on assessing the active chemical ingredients, the pharmacological properties, and product development.

It's important to note that CM fruiting body water extract possesses anti-hyperlipidemic properties (Zhang *et al*., 2019; Huang *et al*., 2023). More prominently, not many studies have been done on the anti-hyperlipidemic effects of CM powder. In the current investigation, we delved into the efficacy of CM mushroom powder in mitigating hyperlipidemia among albino rats subjected to HCD. Our study not only explored the impact of CM powder on lipid levels but also delved into its effects on key blood parameters including LDL, HDL, TG, and Total Cholesterol. Furthermore, we meticulously examined its potential antioxidative properties by assessing its impact on serum CAT, MDA, TAC, and TOS. In tandem with evaluating its therapeutic potential, we meticulously assessed the safety profile of CM powder, scrutinizing its influence on liver functional enzymes such as AST and ALT activity, liver histopathology, and total blood count. This comprehensive approach sheds light on both the therapeutic benefits and safety considerations associated with CM powder consumption.

MATERIALS AND METHODS

Materials and Reagents: The Islamabad Mushroom Spawn Production Lab & Khan Mushroom Farm, Islamabad, Pakistan, served as the primary source for obtaining CM mushrooms. Chemicals, standards, and solutions were procured from authorized dealers, Faluka and Sigma Aldrich. After drying the cleaned CM fruiting bodies were finely ground into a powder using a grinder (MJ-176-NR-3899), as per the method described by Kujur *et al*. (2010). This powdered form of CM was then subjected to further dehydration in an air-drying oven (BOV-V7OF) at 70°C for 12 hours. Upon completion of the dehydration process, the CM powder was allowed to cool to room temperature within a desiccator and stored under refrigeration until further chemical analysis.

Quantification of active ingredients: Bioactive components in mushroom extract were analyzed by high-performance liquid chromatography (HPLC) through the method designated by Jamil *et al*. (2013).

Experimental animals and design: Twenty-five adult male albino rats weighing an average of 130±5 g were purchased from the University of Veterinary and Animal Sciences (UVAS), Lahore, Pakistan. They were housed in stainless steel cages in the environment-controlled animal house of the Department of Physiology, Faculty of Health Sciences, Government College University Faisalabad, Pakistan, which included a temperature of 25±2°C and a relative humidity of 60% with a 12-hour light-dark cycle. Rats were split up into five groups, each consisting of five rats. Albino rats were given a daily routine feed containing 1.5% and 0.5% of cholesterol (Cholesterol 90% E, Appli Chem, Darmstadt, Germany) and cholic acid (Sigma Aldrich) respectively to induce hyperlipidemia. All experimental groups were fed a high-cholesterol diet for the first fifteen days consisting of normal rat feed plus cholesterol. Rats in the NC were fed distilled water and a standard basal diet for a duration of the experiment (8 weeks). In contrast, the rats in the PC

were fed HCD (Iqbal *et al*., 2016). The third group was the STD; they were given a standard diet and a simvastatin tablet at a dose of 10 mg/kg body weight. The albino rats in the fourth (LD) and fifth (HD) groups were given a basal diet and a mixture of CM powder at daily doses of 12 g/kg body weight (b.wt.) and 24 g/kg/day b.wt. respectively for eight weeks, as indicated below.

NC: Standard feed + distilled water
PC: HCD and distilled water
STD: HCD and distilled water + Simvastatin 10 mg/kg b.wt.
LD: HCD and distilled water + 12 g/kg b.wt. CM powder
HD: HCD and distilled water + 24 g/kg b.wt. CM powder

Collection of Serum: The albino rats that had fasted overnight were put to death with urethane anesthesia following the eight weeks of the study. According to Uchida *et al*. (2001), the blood was drawn by cardiac puncture, and the serum was separated by centrifugation in the centrifuge machine (LABCENT 5000) at 3000 rpm for 15 minutes after that the blood was allowed to stand for at least 30 minutes at room temperature.

Serum Lipid Profile: The concentration of triglycerides in serum was measured using commercially available colorimetric assay kits made by Sigma-Aldrich, Germany (Catalog Number MAK266; mg/dl; Reactivity: 2 pmole-10 nmole; Range of measurement: 2-10,000 JM range). Commercially available colorimetric assay kits made by Sigma-Aldrich, Germany (Catalog Number C$0005; mg/dl; Range of measurement: 1–5 ug) were used to measure the total cholesterol levels in serum. The HDL-cholesterol phosphotungstic-precipitation method (bio research kit, catalog #CS009 1100) kit method was used to calculate HDL-cholesterol. The Friedrick equation, which is provided below, was used to determine the serum levels of LDL cholesterol.

Equation:
LDL-cholesterol = (Total-cholesterol) - (Triglyceride/5) - (HDL-cholesterol)

Oxidative stress parameters: CAT activity was determined by the following protocol outlined by Hadwan and Khabt (2018). The thiobarbituric acid (TBA) test was used to estimate the MDA concentration in blood serum. This test is based on the observation that the reaction between MDA and TBA forms a red color adduct, which is a secondary by-product that can be measured with a spectrophotometer. This assay's methodology was adapted from Ohkawa *et al*. (1979). Serum TOS was measured using a modified colorimetric technique, as reported by Erel, (2005). To estimate the TAC levels (mmol) in samples, an automated colorimetric method was employed that was also reported by Erel, (2004).

Safety assessment parameter: The kit method (ALT (GPT) SR, by Bio-active, catalog # 10498 99 93 183) was used to measure the serum ALT level. Serum samples were tested for aspartate aminotransferase (AST) using a commercially available liquiform method kit (Crescent® Diagnostic kit, Jeddah, catalog # 15204C).

Blood samples were taken in the EDTA-containing tube at the time of decapitation for a complete blood count, which was subsequently performed using a hematopathology analyzer (Model: Mindray Hematology Analyzer BC-10).

The histopathological evaluation was carried out using standard laboratory procedures. The tissues were fixed in paraffin blocks and then cut into 4 µm thick slices. Following that, the tissue slices were deparaffinized using xylene and rehydrated using different grades of ethanol (100%, 90%, and 70%). To stain the sections, hematoxylin, and eosin (H&E) were utilized. Every slide was inspected under a light microscope equipped with a digital camera and an X200 magnification (Fujisawa *et al*., 2012).

Statistical analysis: Using the Statistics 8.1 software program, a two-way ANOVA was used to statistically analyze the data about the current study's findings. The Tuckey HSD All Pair-Wise Comparison Test was used to determine the degree of significance between the sample mean values (Montgomery, 2008).

RESULTS

Bioactive Components Analysis by HPLC: Bioactive components include cordycepin, ergothioneine, cordycepic acid, chromotropic acid, quercetin, gallic acid, caffeic acid, chlorogenic acid, synergic acid, p-coumeric acid, m-coumeric acid, ferulate, vitamin E and ascorbic acid are detected in CM by HPLC, shown in Figure 1. Their concentration in the alcoholic extract of CM was 4.25, 610.67, 5.7, 15.98, 10.01, 127.81, 19.98, 30.01, 5.83, 5.21, 5.23, 24.21, 4.5, and 17.01 respectively as shown in figure 1. These values are expressed either in ppm or mg per kg dry weight.

Effect of CM powder on feed intake, water intake, and weight changes: The outcomes showed that, in comparison to normal rats, the hyperlipidemic rats' feed intake was decreased by an infusion of CM powder. Likewise, there was a significant increase in water intake in STD due to simvastatin medicine causing excessive thirst compared to the NC, followed by LD and HD as shown in Figure 2. Results showed a significant difference in weight measurements among all groups taken at intervals of 20 days. At the start of the trial, the weight of rats increased due to the HCD in PC, STD, LD, and HD in contrast to the group under negative control. Rats in the PC gained more weight than rats in the NC and treatment rats that received varying dosages of CM powder. The highest body weight gain percentage BWG % (19.38 %) was observed in PC. However, the administration of CM mushroom powder led to lower body weight in the LD and HD groups compared to PC and STD as shown in Figure 2. Additionally, the HD treatment group showed a greater reduction in weight with a percentage of 6.86% than the LD group (5.55%).

Effect of CM on Serum lipid profile: Results demonstrate that CM high significantly impacted the hyperlipidemic rats' total cholesterol levels. The data clearly showed that CM reduced the levels of cholesterol

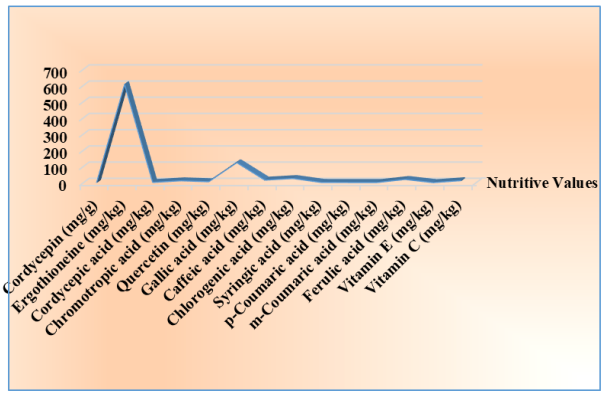

Fig. 1: A graphical representation of bioactive components present in the fruiting body of CM.

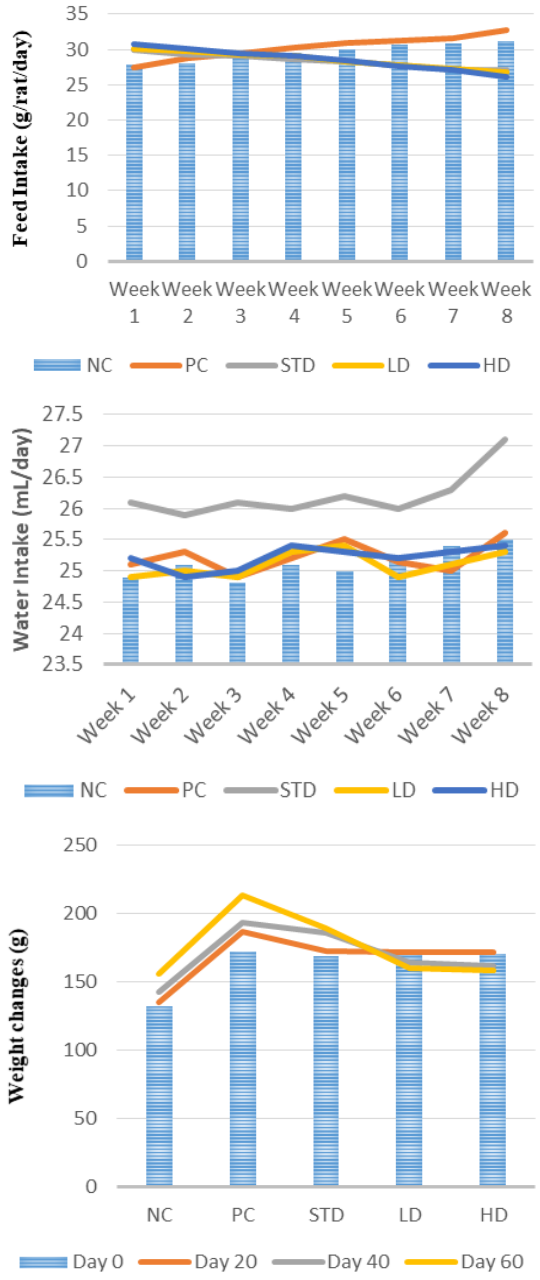

Fig. 2: (A) Feed consumption (g/rat/day) of rats in all groups during the study, B) Water intake (g/rat/day), and C) of rats in all groups during the study Weight changes (g) of rats in all groups during the study.

by 55.65 %, and 63.40 % in LD, and HD correspondingly during an eight-week study period (Figure 3). In the STD group cholesterol levels decreased by 56.56%. The findings showed that, in comparison to the PC, the triglyceride levels of hyperlipidemic rats treated with CM powder in LD and HD correspondingly dropped by 56.32% and 64.08% during the eight-week study period. It can be assumed from the findings that the concentration level of CM was a significant factor in reducing triglycerides in rats with high levels of lipids (Figure 3).

Different levels of CM significantly altered the levels of low-density lipoprotein (LDL) in hyperlipidemic rats, according to the statistical results. The results showed that the highest value for LDL was spotted in PC (152.40 mg/dL), while LDL levels in STD, LD, and HD decreased by 63.44 %, 38.49 %, and 66.39 % respectively in comparison to the PC (Figure 3). Moreover, HDL levels increased in STD, LD, and HD. It is confirmed from the results that a maximum level of HDL was observed in hyperlipidemic rats that received HD with an increased percentage of 57.45 % as shown in Figure 3.

Oxidative stress parameters: There was a significant decrease in serum MDA level in the treatment groups of HD in comparison to the PC as shown in Table 1 (Figure 4A). However, the level of CAT in the serum showed an increase in the treatment groups of HD, LD, and STD and a decrease in the PC as shown in Table 1 (Figure 4A). An elevation in the catalase level protects against the peroxidation of cell wall lipids and lipoproteins. Additionally, our results implied that there was a significant decline in serum TOS level in the group treated with HD, LD, and STD while its level increased in the high fat-induced group PC. Conversely, the concentration of serum TAC was increased in the treatment groups of HD, LD, and STD when compared to the group under PC as shown in Table 1 (Figure 4A).

Safety assessment parameter

Liver functioning enzyme tests: Table 2 illustrates how CM affects the liver functional enzymes (ALT and AST) in rats that are normal and hyperlipidemic. As stated by the results, the peak levels of AST and ALT enzyme activities were documented in PC while the lowest levels were observed in HD. Treatment with HD triggered a significant drop in the amounts of AST, and ALT enzymes as compared to the NC by 27.2 %, and 34.82 % respectively at the end of the 8th week. On the other hand, the STD group also exposed a significant decline in AST and ALT enzymes when compared to PC as displayed in Table 1 (Figure 4B).

Liver Histopathology: An examination of the liver tissues in the NC that was kept in normal condition revealed a normal portal vein size and hepatocyte architecture. On the other hand, histological examination of the PC revealed loss of cellular contents, hepatocyte death, portal vein deformation, and fat accumulation in the liver as illustrated in (Figure 4C). Rats in the STD group had liver with average portal vein sizes and normal architectural distortion, as seen in (Figure 4C). Normal histological structure was restored in the T_2 (HD Group) as compared to

PC. While treated groups showed normal hepatic parenchyma and less fat accumulation (Figure 4C).

Complete Blood Count (CBC) tests: The effect of CM on the complete blood count in normal and hyperlipidemic rats has been shown in Table 2. According to the results, the highest values of Hb level were recorded in PC, while the lowest levels were observed in LD, which lie under the normal range for Hb blood level. Treatments with LD and HD in rats caused a non-significant decrease in the levels of blood Hb as compared to the NC. The levels of Total WBC and Platelets increase in PC, STD, LD, and HD as compared to NC respectively, which can be a risk factor for allergic reactions, stress, and hypertension as studied by Wang *et al.* (2018). Over all the results from this study indicate a non-significant effect of taking LD and HD of CM on CBC levels. The results are aligned with the reference values of a study presented by Delwatta *et al*. (2018).

DISCUSSION

Although CM extracts have a wide range of pharmacological activities, much of the research that has been done on them has been on their anti-tumor, anti-diabetic, and immune-regulating actions (Tran *et al*., 2019; Yang *et al*., 2021). To our knowledge, no study on CM powder's influence on lipid metabolism has been published. The outcomes showed that, in comparison to normal rats, the hyperlipidemic rats' feed intake was decreased by an infusion of CM powder. Likewise, there was a significant increase in water intake in STD due to simvastatin medicine causing excessive thirst compared to the NC, followed by LD and HD. However, the administration of CM mushroom powder led to lower body weight in the LD and HD groups compared to PC and STD. Because the CM powder may not have stimulated the rats' appetite, the rats with elevated lipid levels that got CM ingested less feed than the rats in the normal control group according to An *et al*. (2018). An earlier investigation also illustrated that administering CM enriched with cordycepin to mice with diet-induced obesity led to a notable reduction in the increase in body weight (Ahn *et al*., 2020).

It is noticeable from the results that CM reduced the cholesterol levels by 55.65 %, and 63.40 % in LD, and HD respectively in an eight-week study period as compared to the STD where cholesterol levels decreased by 56.56%. These findings align with previous reports by (Yu et al., 2021), which demonstrated a significant reduction in TC, TG, and LDL levels after treatment with polysaccharides extracted from CM. The polysaccharide CM1, which is mostly composed of →4)-β-D-Glcp (1 → and →2)-α-D-Manp (1 → glycosyls), was extracted from the fruiting body of CM and purified for this investigation. Significantly, CM1 intervention reduced the plasma TC level by around 28% (Yu et al., 2021) In a different investigation, serum TC, TG, and LDL-c levels were considerably lower in hamsters fed the HFD when given cordycepin doses of 25 and 50 mg/kg than in hamsters fed the diet untreated (Peng et al., 2010).

The data demonstrate that, during the eight-week research period, the triglyceride levels of the hyperlipidemic rats (LD and HD) dropped by 56.32% and 64.08%, respectively, in comparison to the PC. It can be concluded from the results that the concentration level of CM was a substantial factor in reducing TG in hyperlipidemic rats. This study supports the findings of Yin *et al*.'s research from 2021, which showed that CM dramatically reduced plasma TG levels (∼37 % reduction, $p < 0.01$) (Yin *et al*., 2021). Furthermore, these results are also per the findings of previous reports by Yu *et al*. (2021), which demonstrated a significant reduction in TG levels after treatment with polysaccharides extracted from CM mushrooms.

Moreover, LDL levels in STD, LD, and HD decreased by 63.44 %, 38.49 %, and 66.39 % respectively as compared to the PC. The findings of current research agree with the study in which hamsters fed HFD with cordycepin dosages of 25 and 50 mg/kg significantly decreased the levels of LDL-C compared to untreated HFD-fed hamsters (Peng *et al*., 2010). It is also confirmed from the results that a maximum level of HDL was observed in hyperlipidemic rats that received HD of CM with a percentage of 57.45 %. Previous studies by Zhao *et al*. (2018) demonstrated an increase in HDL (High-Density Lipoprotein) levels after treatment with AE-PS (acidic-extractable polysaccharides) extracted from CM mushrooms (Zhao *et al*., 2018). In another study by Lin *et al*. (2021), it was mentioned that treatment with CM mushrooms decreased the risk of atherosclerosis (caused by hypercholesterolemia) by reducing the levels of LDL, TC, and TG, and increasing HDL levels.

The addition of 1.5 g/kg CM-SMS produced a cholesterol intensity in the hypercholesterolemic profile that was lower than that of the pig control groups (Boontiam *et al*., 2020). Previous research has demonstrated that cordycepin favorably induces a decrease in total cholesterol synthesis by inhibiting glycerol-3-phosphate acyltransferase and HMG-CoA reductase (Guo *et al*., 2010). Additionally, CM included 163 g kg−1 of dietary fiber, which may have a hypercholesterolemic impact by increasing the synthesis of short-chain fatty acids. This, in turn, may inhibit the synthesis of cholesterol in the liver and intestines and lower cholesterol levels (Jiao *et al*., 2020).

This contradicts earlier research, which found that pigs fed CM spent mushroom diet did not experience alterations in their cholesterol levels (Cheng *et al*., 2016). The pig's growth stage, the makeup of the feed, the health of the animal, or variations in the surroundings might have all contributed to the contentious result. Additionally, it was discovered that pigs given a diet enriched with CM spent mushrooms had higher HDL cholesterol. An expansion in HDL often indicates the ability to trigger the efflux of cholesterol into the hepatocytes for further metabolites. Weaning pigs' lipid metabolism can be managed with in-feed supplements thanks to these advantageous benefits (Boontiam *et al*., 2020).

The effect of CM on the liver functioning enzymes (ALT, and AST) in normal and hyperlipidemic rats was also determined as a safety parameter of CM. According to the results, the highest levels of AST and ALT enzyme

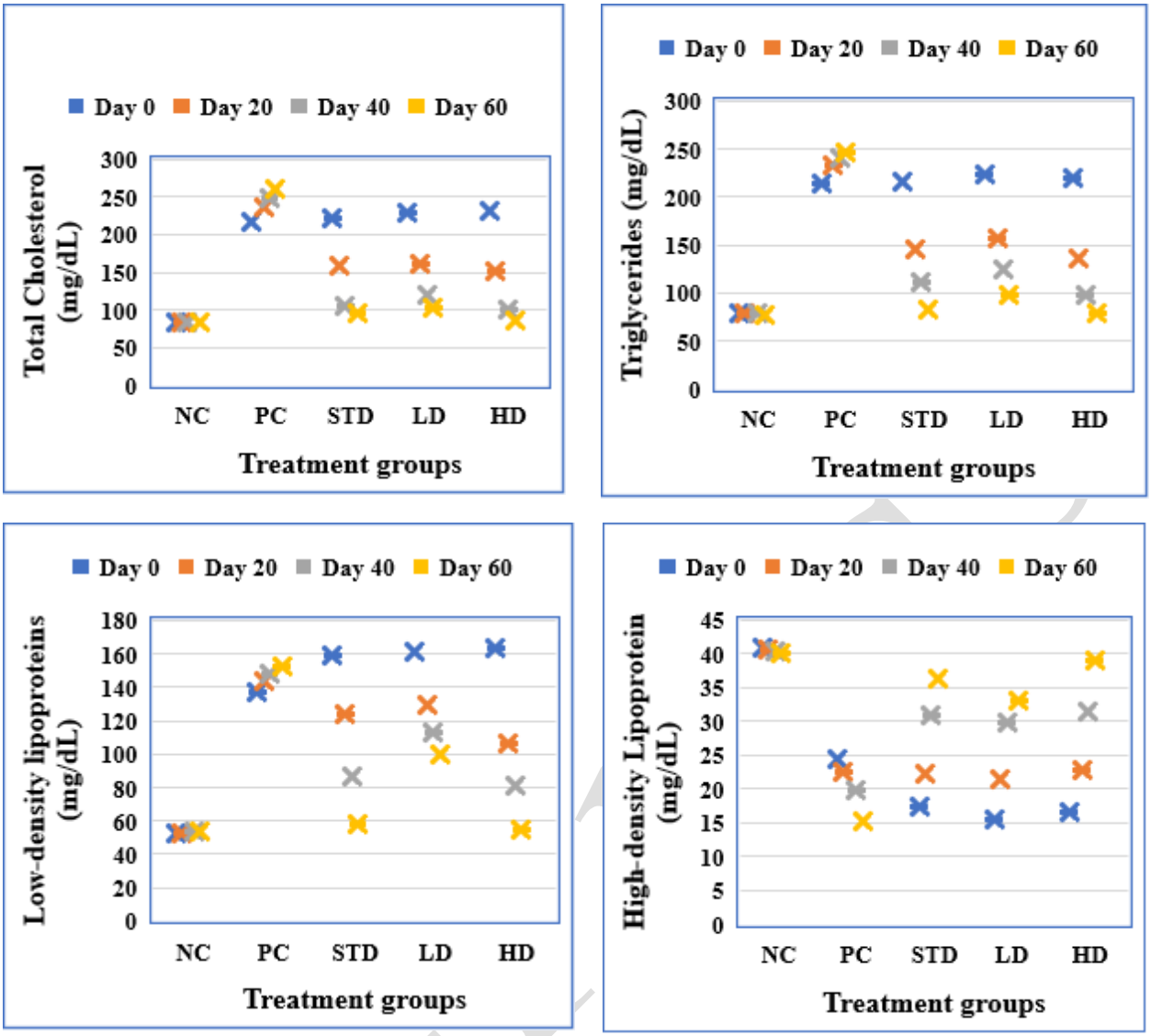

Fig. 3: Graphical presentation of levels of TC, TG, LDL, and HDL of different rat groups during the study period.

activities were recorded in the PC while the lowest levels were observed in HD. Treatment with HD in rats triggered a significant reduction in the levels of AST, and ALT enzymes as compared to the PC by 27.2 %, and 34.82 % respectively at the end of the 8th week. These results are supported by Cha *et al*. (2013), indicating that serum AST and ALT levels decreased after treatment with cordycepin-enriched CM in Sprague-Dawley rats. In another study, Serum ALT, and AST actions were significantly lowered using CM in comparison with the control group (Choi *et al*., 2014).

There was a significant decrease in serum MDA and TOS levels in the treatment groups of CM treatment, high and low doses. However, the level of catalase in the serum CAT showed an increase in the treatment groups of high doses of CM. Similarly, the concentration of serum TAC was increased in the treatment groups of high doses of CM. The cordyceps polysaccharide that was separated from *C. militaries* was able to inhibit the immune response that was induced by cyclophosphamide, which improved the activities of macrophages and lymphocytes. It also raised the levels of catalase, superoxide dismutase, and glutathione peroxidase and decreased the amount of malondialdehyde in the mice's blood (Wang *et al*., 2015b). On the other hand, nothing is known about how CM polysaccharide (CMP) supplementation affects laying hens. Researchers noted that feeding *C. militaries* waste medium to laying hens resulted in a considerable decrease in feed conversion ratio and an increase in egg production. Additionally, they demonstrated that the reducing capacity of ascorbic acid and *C. militaries* waster medium is about equal (Wang *et al*., 2015a). As a result, it was anticipated that the hens in a trial receiving more CMP would have a lower feed-to-egg ratio and produce more eggs (Xiaochen *et al*., 2020). Important factors for measuring egg quality were eggshell thickness, eggshell strength, Haugh unit, and egg shape index. These characteristics were associated with the freshness and shelling rate of eggs. According to research, there was no discernible change in the three groups' egg quality (Xiaochen *et al*., 2020). Similarly, researchers found that adding *C. militaries* waster medium to a meal did not increase the strength and thickness of the eggshell (Wang *et al*., 2015a).

An examination of the liver tissues in the NC that was kept in normal condition revealed a normal portal vein size and hepatocyte architecture. On the other hand,

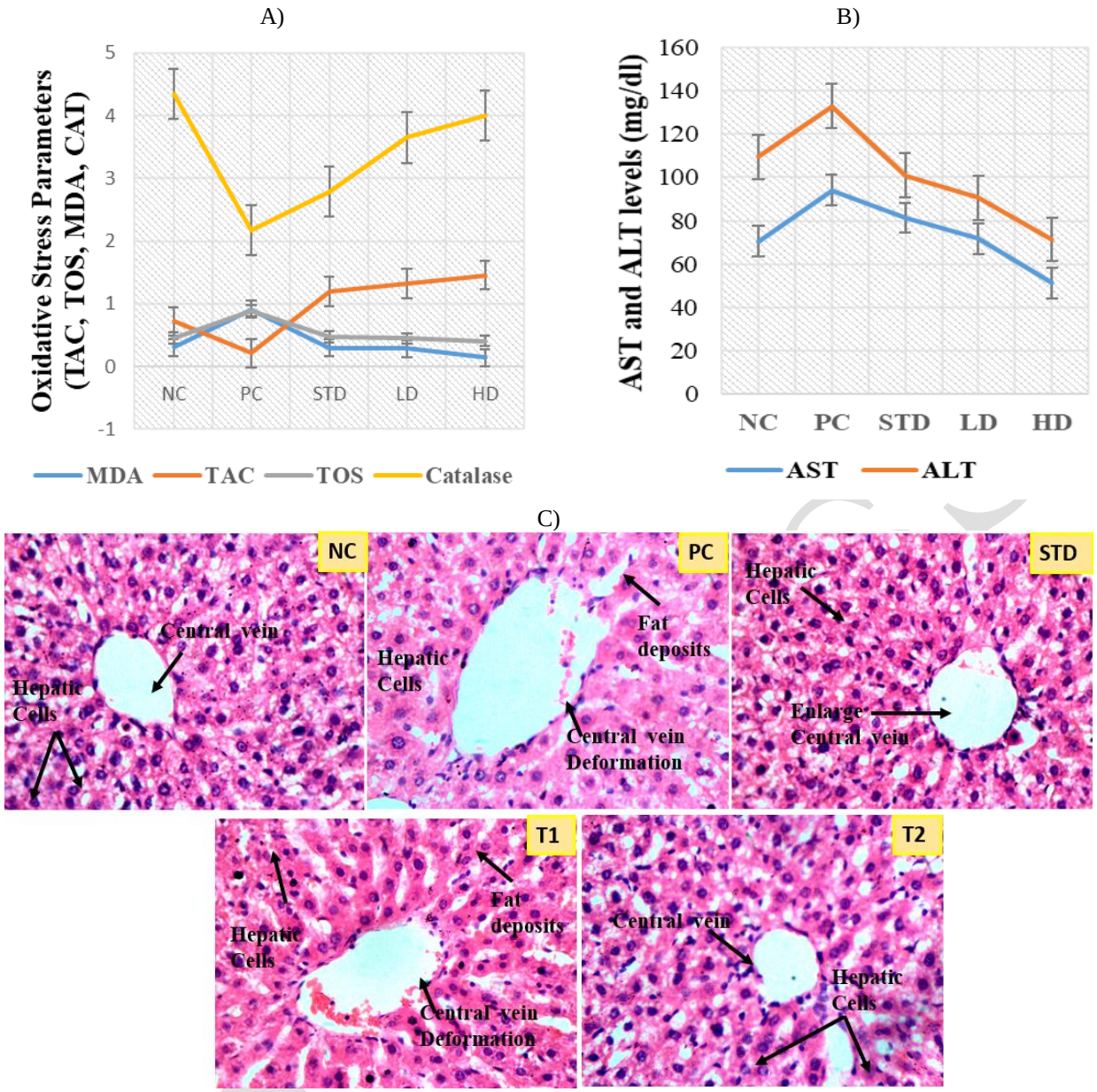

Fig. 4: A) Graphical presentation for oxidative stress parameters (TAC, TOS, MDA, CAT), the effect of CM on different rat groups after 8 weeks: B) For liver functioning enzymes (ALT, and AST), the effect of CM on different rat groups after 8 weeks: C) effects of CM on lipid accumulation in rats fed HCD; NC treated with normal conditions showed a normal portal vein size and hepatocyte architecture; PC were hyperlipidemic rats treated with HCD that revealed loss of cellular contents, hepatocyte death, portal vein deformation, and fat accumulation; STD hyperlipidemic rats treated with simvastatin drug (10mg/kg b.wt.) had liver with average portal vein sizes and normal architectural distortion. T1) The low-dose treated group (12 g/kg b.wt. of CM) showed less deposition of fat and portal vein deformation. T2) The high-dose treated group (24 g/kg b.wt. of CM) restored normal histological structure as compared to PC.

Table 1: Mean±Standard deviation values for oxidative stress parameters (TAC, TOS, MDA, CAT), and liver function enzymes (AST and ALT), the effect of CM on normal and hyperlipidemic rats after 8 weeks.

Treatments	TAC	TOS	MDA	CAT	ALT (mg/dl)	AST (mg/dl)
NC	0.72 ± 0.014^{D}	0.45 ± 0.069^{B}	0.30 ± 0.11^{AB}	4.34 ± 1.21^{A}	109.62 ± 58.74^{AB}	70.47 ± 8.82^{B}
PC	0.21 ± 0.047^{E}	0.88 ± 0.020^{A}	0.91 ± 0.50^{A}	2.16 ± 0.59^{B}	132.83 ± 11.87^{A}	94.18 ± 5.51^{A}
STD	1.19 ± 0.016^{C}	0.47 ± 0.024^{B}	0.29 ± 0.01^{AB}	2.78 ± 0.78^{AB}	100.98 ± 7.49^{AB}	81.39 ± 5.21^{AB}
LD	1.32 ± 0.037^{B}	0.45 ± 0.013^{B}	0.28 ± 0.25^{AB}	3.65 ± 0.31^{AB}	90.56 ± 5.03^{AB}	71.74 ± 9.27^{B}
HD	1.45 ± 0.048^{A}	0.4098 ± 0.032^{B}	0.13 ± 0.049^{B}	4.00 ± 0.260^{AB}	71.45 ± 7.29^{B}	51.30 ± 5.14^{C}

Values are Mean ± STD (n=5); Along the column, values denoted by distinct superscript letters show a significant difference ($P < 0.05$).

histological examination of the PC revealed loss of cellular contents, hepatocyte death, portal vein deformation, and fat accumulation in the liver. Rats in the STD group had livers with average portal vein sizes and normal architectural distortion. The Normal histological structure was restored in the HD group as compared to the PC.

Since cordyceps polysaccharides have so many uses in the cattle business, their use as a feed supplement has garnered significant attention in Asia. For instance, Cordyceps polysaccharides may boost the feed conversion ratio, encourage early chicken growth, and improve the daily growth gain of broilers (Han *et al.*, 2015).

Table 2: Mean±Standard deviation values for complete blood count (Hb, Total WBC, Total RBC, Platelets, HCT, MCV, MCH, RDW-CV, Neutrophils, Lymphocytes, Monocytes, Eosinophils), the effect of CM on normal and hyperlipidemic rats after 8 weeks.

Blood CBC	NC	PC	STD	LD	HD
Hb (g/dl)	14.16 ± 0.87^{AB}	15.06 ± 0.58^{A}	13.93 ± 0.25^{B}	13.43 ± 1.30^{B}	13.96 ± 0.20^{B}
Total WBC (cumm)	6933.3 ± 1761.62^{A}	12167 ± 4652.23^{A}	10200 ± 3274.14^{A}	12267 ± 1887.67^{A}	13333 ± 2055.07^{A}
Total RBC (Mill/Cu.mm.)	7.6 ± 0.54^{A}	7.64 ± 0.36^{A}	7.33±0.10A	7.06 ± 0.68^{A}	7.21 ± 0.24^{A}
Platelets (cumm)	554000 ± 179518.8^{A}	694333 ± 43924.18^{A}	611333 ± 77777.46^{A}	836667 ± 161001^{A}	680000 ± 129780.6^{A}
HCT (%)	47.80 ± 3.81^{AB}	48.23 ± 1.12^{A}	43.56 ± 1.38^{AB}	41.03 ± 3.59^{B}	43.03 ± 1.66^{AB}
MCV (fi)	62.76 ± 2.32^{A}	63.20 ± 2.74^{A}	59.46 ± 1.49^{A}	58.16 ± 0.65^{A}	59.70 ± 1.70^{A}
MCH (pg)	18.63 ± 0.25^{A}	19.73 ± 1.70^{A}	17.63 ± 0.15^{A}	17.56 ± 0.60^{A}	18.03 ± 0.40^{A}
MCHC (g/dl)	29.66 ± 1.20^{A}	31.23 ± 1.70^{A}	29.70±0.51A	30.20 ± 0.88^{A}	30.23 ± 0.87^{A}
RDW-CV (%)	16.20 ± 0.4^{A}	15.36 ± 0.49^{AB}	15.26 ± 0.25^{AB}	14.90 ± 0.34^{B}	15.10 ± 0.36^{B}
Neutrophils (%)	33.0 ± 2.64^{A}	33.33 ± 0.57^{A}	33.33 ± 0.57^{A}	33.00 ± 1.73^{A}	33.3 ± 2.08^{A}
Lymphocytes (%)	92.33 ± 2.88^{A}	90.66 ± 2.08^{A}	92.66 ± 2.08^{A}	90.66 ± 3.21^{A}	92.0 ± 2.64^{A}
Monocytes (%)	3.33 ± 0.57^{A}	4.00 ± 1^{A}	3.33 ± 1.15^{A}	3.66 ± 1.15^{A}	3.33 ± 0.57^{A}
Eosinophils (%)	1.66 ± 0.57^{A}	2.00 ± 1^{A}	1.33 ± 0.57^{A}	2.66 ± 0.57^{A}	1.66 ± 1.15^{A}

Along the column, values denoted by distinct superscript letters show a significant difference ($P < 0.05$) for $n = 5$. Hb: hemoglobin, WBC: white blood cell, RBC: red blood cell, HCT: hematocrit, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin content, RDW-CV: red cell distribution-coefficient of variation.

According to Wang *et al*. (2015a), the CM waste medium reduced the feed-to-egg ratio (F/G) and raised the rate of egg production. Chickens' development performance and health index were enhanced by a hot-water extract made from the mycelia of C. sinensis, which also preserved the better microbiota in their intestines (Koh *et al*., 2003).

According to a study, feeding fish with 10 g/kg of CM-SMS resulted in the largest increase in skin mucus lysozyme and peroxidase activities when given SMS through food (Doan *et al*., 2017). According to the current study's findings on serum immune measures, feeding Nile tilapia with SMS dramatically raised their non-specific immunological parameters (Doan *et al*., 2017). Research on humans, dairy cows, elk, and mice has shown that CM-SMS contains immunostimulants, antioxidants, and antibacterials (Zhu *et al*., 2011; Park *et al*., 2012; Zeng *et al*., 2015).

One possible mechanism of the effect of antihyperlipidemic effect of CM can be attributed to the presence of AMPK activity (phospho–AMP-activated protein kinase) and PA-CoA carboxylase activity in the liver and adipose tissues, both of which play a role in regulating lipid metabolism. These activities inhibit glycerol-3-phosphate acyltransferase, an enzyme that regulates triglycerides, as well as HMG CoA reductase, an enzyme that regulates total cholesterol (Thomson and Winder, 2009). Further investigation however is necessary to determine the impact of CM on lipid metabolism as well as how other possible effects support CM-mediated lipid homeostasis.

Conclusion: Conclusively the results demonstrated a significant reduction in lipid levels, including total cholesterol, triglycerides, and low-density lipoprotein cholesterol, accompanied by an elevation in high-density lipoprotein cholesterol levels. These alterations suggest a potential lipid-lowering effect of CM mushroom powder, indicating its promise as a natural intervention against hyperlipidemia.

In light of the research findings, CM may be utilized as a medication or functional food to prevent hyperlipidemia and oxidation in a rat model of high cholesterol-induced hyperlipidemia. This is likely due to cordycepin and other polyphenolic compounds that have anti-oxidative and anti-hyperlipidemic properties.

The findings of current research will stimulate continued inquiry, fostering a deeper understanding of the bioactive components of CM mushroom powder and their intricate interactions within the complex milieu of lipid metabolism. Ultimately, the exploration of CM as a hypolipidemic agent holds promise for enhancing human health and well-being, paving the way for future advancements in preventive and therapeutic nutrition.

Ethics statement: This study was approved by The Ethical Committee GCUF. This study did not raise any ethical issues.

Competing interests: The authors declare no competing interests

Authors' contributions: Muhammad Awais Saleem conducted the experimental research, collected and processed the data, and drafted the manuscript. Aftab Ahmed oversaw the overall research process and ensured compliance with ethical standards. Nazir Ahmad contributed to the conception and design of the study, performed the data analysis, and revised the manuscript critically for important intellectual content. Abid Rashid provided substantial input on the study's methodology, assisted with data interpretation, and contributed to the revision of the manuscript. Muhammad Afzaal supported the statistical analysis and interpretation of results and contributed to the drafting and finalization of the manuscript.

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