Hypolipidemic and Cardioprotective Effects of Lion's Mane Mushroom (*Hericium erinaceus*) Ethanolic Extract in Male Albino Rats

Samah M. El-banna and Amira H. Darweesh

Department of Nutrition and Food Science, Faculty of Home Economics, Menoufia University, Shebin El-kom, Egypt.

Abstract:

Hyperlipidemia describes an irregularity in plasma lipid levels, which is the primary factor in the development of cardiovascular disease and atherosclerosis. *Hericium erinaceus* (HE) is an edible medicinal mushroom used as a highly valued food and medicine for its bioactive constituents. In this study ethanolic extracts of HE were assessed for their ability to reduce the levels of the lipid profile, the atherogenic index and cardiac risk factor values. Thirty adult male albino rats weighing an average of 120±5 g were used in this study .Rats were divided into five groups (six rats each).The first group was negative control group which was fed basal diet. The second group provided with hyperlipidemic diet as a positive control group, the third, fourth and fifth groups were fed on hyperlipidemic diet and received orally administered 100, 200, and 300 ml/kg alcoholic extract of HE for 28 days respectively. Biological experiments indicated that hyperlipidemic rats (model control) exhibited significantly ($p \leq 0.05$) increased in body weight, feed intake and feed efficiency ratio (FER), compared to the normal group. However, intervention with kg alcoholic extract of HE in feeding rats for 28 days led to a significant ($p \leq 0.05$) decrease in these parameters in the hyperlipidemic rats (model control). Also, biochemical parameters were substantially higher in the positive control group than in the normal group, but HDL-c concentration was significantly lower in the uninfected group. Treatment with the tested HE doses decreased TC, LDL-c, and TG levels, as well as the atherogenic index, leptin hormone, and cardiac risk factor values in hyperlipidemic rats, while increasing HDL-c content and insulin hormone. The biochemical results were supported by histopathological examination of heart tissue. As a result, the findings demonstrate that HE extract, is effective in improving serum lipid profile in hypolipidemic rats.

Key words: cardiac risk factor ,serum lipid profile, insulin hormone ,leptin hormone ,atherogenic index, Lion's Mane Mushroom.

Introduction :

High blood lipid levels are a hallmark of hyperlipidemia. It is defined by a rise in peripheral blood levels of triglycerides (TG), total cholesterol (TC), and low density lipoprotein cholesterol (LDL-C), along with a fall in HDL-C (high density lipoprotein cholesterol) levels **(Rauf *et al.*, 2022)**. Actually, a number of metabolic diseases, such as fatty liver, type 2 diabetes, hypertension and atherosclerosis, are linked to hyperlipidemia **(Mosa *et al.*, 2021)**. According to **Yao *et al.*, (2020)**, some endothelial dysfunctions are caused by prolonged, extended hyperlipidemia, which is the primary risk factor for atherosclerosis and cardiovascular problems. According to statistics, hyperlipidemia-related illnesses kill 30 million people worldwide each year, and this figure is steadily increasing. Elevated atherogenic indices, notable serum dyslipidemia (LDL, VLDL and TG), levels of ischemia-modified albumin, and histological examination of cardiac tissues can all be used to diagnose hypercholesterolemia **(Sreerekha *et al.*, 2021)**. Additionally, the heart's systolic function and cardiac electrophysiological response are directly impacted by hyperlipidemia, and these effects may be related to the body's general oxidative stress, pro-inflammatory state, and mitochondrial dysfunction brought on by the heart's ongoing buildup of cardiac lipids **(Hewage *et al*., 2018).**

According to **Kanakavalli *et al*., (2014)**, Primary and secondary HL can be generically classified into two categories. The most common kind, often known as familial HL since it results from a genetic abnormality, may be polygenic (many gene faults) or monogenic (a single gene flaw). The second type, acquired HL, is brought on by other conditions such as hypothyroidism, nephritic syndrome, persistent drinking, diabetes, the use of beta blockers, oral contraceptives, and corticosteroids. Pancreatitis can develop as a result of secondary hyperlipidemia and severe hypertriglyceridemia (**Onwe *et al.*, 2015)**.

The primary causes of hyperlipidemia include changed dietary habits that include more fat than 40% of total calories, more saturated fats than 10% of total calories, more cholesterol than 300 mg per day, or medical disorders that can be treated. Abnormal cholesterol levels can be caused by a high-fat diet, excessive alcohol consumption, being overweight, not exercising, and smoking, among other bad lifestyle choices. Additional contributory variables include pregnancy, diabetes, polycystic ovarian syndrome, renal disease and an underactive thyroid gland. It has been discovered that increased estrogen and other female hormones can change or elevate cholesterol levels. Age and gender play a significant role in the onset and progression of hyperlipidemia, according to earlier studies **(Yu *et al.*, 2019)**.

Mushrooms have long been utilized as a highly valued food and medication due to their bioactive compounds and biological activities **(Lee *et***

***al*., 2019; Gharib *et al*., 2022)**. Asian traditional medicine has long employed the edible medicinal mushroom *Hericium erinaceus*, which has recently received attention for its potential therapeutic and neuroprotective effects **(Yadav *et al*., 2020)**. The basidiomycete *Hericium erinaceus (HE)* is a member of the Russulales order, the Hericiaceae family, and the class Agaricomycetes. Broadleaf or coniferous woods and broadleaf mixed forests in the northern temperate zone—which encompasses Western Europe, North America, Japan, and Russia—are H. erinaceus's natural habitats. **(Mori *et al*., 2015)**. A wide range of biologically active chemicals derived from mushrooms have been shown to have pharmacological and therapeutic characteristics. Specifically, much study has been done on their bioactive polysaccharides for both current and potential uses in medications and functional meals **(Hiraki *et al*., 2017)**. Nonetheless, it is possible to identify the bioactive metabolites of mushrooms by keeping an eye on respiration, nutrient intake, and metabolite creation within the culture medium. Generally speaking, there are two categories of H. erinaceus bioactive metabolites: Low molecular weight substances like polyketides and terpenoids, as well as large molecular weight substances like polysaccharides **(Kuo *et al*., 2022)**. H. E fruiting bodies contain α-D-glucan, palmitic acid, corallocin, D-threitol, D-arabinitol, erinarols, 2,6-diethylpyrazine, 2-methyl-3-furanthiol, and antimicrobial, anticancer, antioxidant ,antidiabetic, antihyperglycemic, antiaging, antihyperlipidemic, gastroprotective, immunomodulating, and neuroprotective activity **(Hetland *et al*.,2020)**. When compared to untreated control groups, methanol and aqueous extracts of H. erinaceus have been shown to have significant anti-hyperlipidemic effects, decreasing plasma T.C, LDL-C, T.G, phospholipid, atherogenic index, and hepatic 3-hydroxy-3-methylglutaryl-coenzyme A (HMGCoA) reductase activity **(Liang *et al*., 2013)**. The mycelial ethanol extract of mushrooms effectively increased HDL-C by 31.1% while significantly lowering the serum LDL-C content in diabetic mice's blood by 45.5% **(Yang *et al*., 2003)**. **Gravina *et al*., (2023)** found that H.E has a high potential for treating metabolic disorders and preventing cardiovascular disease. Thus, the present trail was planned to prepare an ethanol extract of H.E and elucidate its hypolipidemic potential through the determination of the biochemical parameters of rats fed a high fat diet.

Material and methods:

Materials:

Mushroom HE utilized in this trail was purchased from Agricultural Seeds, Herbs, and Medicinal Plants Company, Cairo, Egypt.

Chemicals: The commercial kit and cholesterol were purchased from the Technogene Chemical Company in Dokki, Egypt, Giza. Dextrin, L-cysteine, casein, minerals mixtures, vitamins mixtures, starch and cellulose were purchased from the Cairo Corporation for Chemical Trade, in Cairo, Egypt.

Animals: Thirty male rats, approximately 120±5 g of fully grown male Sprague Dawley rats were acquired from the Medical Insects Research Institute located in Dokki, Cairo, Egypt.

Methods:

Preparation extract from *Hericium erinaceus*:

The powdered fruiting bodies of H E(1 kg) had been extracted with 80% ethanol (2 L) at room temperature (25 °C) and evaporated under low pressure using a rotator evaporator. The obtained crust was reconstituted in a solvent vehicle to be suitable for oral administration **(Shen *et al*., 2015).**

Induction of hyperlipidemia

70% maize flour, 10% casine, 10% corn oil, 5% bran, 4% mineral mixture, and 1% vitamin mixture made up the basic diet (**Reeves *et al*., 1993)**. The high-fat diet consisted of an 85% normal diet, 10% tallow, and 4% cholesterol. After one week of adaptation, rats fed a high-fat diet for three weeks developed hyperlipidemia; animals with hyperlipidemia of 200 mg/dL or above were employed as a model of hyperlipidemia in rats for investigation. The rats with hyperlipidemia were fed the experimental diet for four weeks **(Kamesh and Sumathi, 2012).**

The experimental design:

The basal diet was made using the recipe described by **Reeves *et al*., (1993).** This involved using vitamin mixture components according to the methods of **Campbell (1963)**. The salt mixture was produced according to the instructions supplied by **Hegsted *et al*., (1941)**.

Every biological experiment was conducted at Menoufia University's Home Economics Faculty's medical animal house. The rats were rehoused in clean, well-ventilated cages with controlled humidity, light/dark cycles of 12 hours and standard filtered feed. They were also given unlimited access to water. The Menoufia University Committee for the Care and Use of Laboratory Animals approved the study (Approval No: MUFHE /F/NFS/21/23), and all studies were conducted in accordance with the Guidelines on Ethical Standards for Animal Research. Each rat was given a basal diet for one week prior to the start of the experiment to allow for acclimatization. Subsequently, the rats were split into five groups of six, each of which was given a dietary regimen consisting of a basal diet serving as a negative control and a hyperlipidemic diet serving as a positive control. For a 28-days trial period, 100, 200, and 300 ml/kg Bw of *Hericium erinaceus* extract were administered to the hyperlepidemic third, fourth, and fifth groups. Rats were weighed after being deprived for the entire night and then exsanguinated.

Biological assessment

Daily and weekly records were made of the diet and body weight. Using the following formulae, the body weight gain (BWG), feed efficiency ratio

(FER), and certain organ weights were calculated using the data from **Chapman *et al.* (1959)**: Body weight gain = Final weight (g) – Initial weight (g)

Feed efficiency ratio (FER) = Body weight gain (g) / Feed intake (g).

Blood sampling

At the conclusion of the trial, the rats were weighed, and after a 12-hour fast, blood was taken from their hepatic portal veins. After being drawn and placed into sterile, dry centrifuge tubes, blood samples were allowed to coagulate for thirty minutes in a water bath at 37 degrees Celsius. The blood was centrifuged at 3000 rpm for 10 minutes in order to separate the serum. After that, the blood was aspirated into a clean cuvette tube and kept at -20°C for analysis **(Schermer, 1967)**.

Biochemical analysis

Using the techniques of **Fossati** and **Prencipe (1982), Allain *et al*. (1974), Burstein *et al*. (1980),** and **Friedewald *et al*. (1972)**, the levels of TG, TC, high-density lipoprotein cholesterol HDL-c, and LDL-c were measured in all serum samples. Using the formula proposed by **Lee and Nieman (1996)**, very low-density lipoprotein cholesterol (VLDL-c) was calculated as follows: VLDL-c = TG/5.

The atherogenic ratios were calculated by using the equations of **Bhardwaj *et al*., (2013)** as follows:

Atherogenic Index of serum (AI) = log TG/HDLc

Cardiac risk ratio (CRR), = TC/HDLc

Castelli's Risk Index (CRI) = LDLc/HDLc

Atherogenic Coefficient (AC) = (TC– HDLc)/HDLc

Atherogenic fraction (AF) was calculated according to **Aguilar *et al*., (2011)** by the difference between TC and HDL-C .

The techniques of **Considine *et al*., (1996)** and **Defronzo *et al*., (1979)** were used to measure the levels of the hormones insulin and leptin, respectively. The methods of **Tateishi *et al*., (1987)**, **Walker *et al*., (1990)**, and **Omole *et al*., (2018)** were applied to the analysis of LPO, CK-MB, and Troponin-1, respectively.

Histopathological examinations.

For fixation, the hearts of each rat were immersed in a 10% formalin solution. A segment of the myocardium was subsequently isolated, dehydrated in ethanol at concentrations of 40%, 70%, 80%, 95%, and 100%, and purified with xylene through the implementation of an automated processing system. Furthermore, the utilization of paraffin wax and automated tissue processing equipment was employed to eliminate xylene. Following this, the tissues were paraffin-waxed and obstructed in the coronal direction. Under light microscopy, the tissue was sectioned at a thickness of 4-5mm, stained with hematoxylin and eosin dye, and histological observations were conducted **(Drury and Wallington, 1980)**.

Statistical analyses.

Version 27 of SPSS was utilized for data entry and analysis. The results of the research were calculated and presented in the form of the mean ± standard deviation. A one-way analysis of variance (ANOVA) was conducted to ascertain the significance of the intergroup. Subsequently, a least significant difference (LSD) multiple range test was conducted, deeming a P-value of 0.05 to be indicative of statistical significance.

Results and discussion

Biological results

The effects of various doses of HE extract on the body weight gain, feed intake, feed efficiency ratio, and relative heart weight in hyperlipidemic rats.

The impact of various dosages of ethalonic extract of HE on body weight gain, feed intake, feed efficiency ratio, and relative heart weight in rats with hyperlipidemia is illustrated in Table 1. Significantly superior biological outcomes were observed in the positive control group compared to the negative control group. Significant decreases in body weight gain, feed intake, and efficiency ratio were observed in hyperlipidemic rats administered different doses of HE in comparison to the positive control group ($P \leq 0.05$). In addition, the 300ml HE/kg dose caused a significantly greater reduction than the other doses. The rate of increase in BWG and FI was observed to be dose-dependent in hyperlipidemic rats.

In the current investigation, an HFD successfully improved hyperlipidemia in rats, and the FER value was altered to reflect weight gain and feed consumption. The therapy for hyperlipidemia patients with HE works by inhibiting HMG-CoA reductase functionality. Our findings therefore confirmed that they have cholesterol-lowering properties. Despite their global prevalence, it is beneficial to broaden the range of treatment alternatives for hyperlipidemia by utilizing an ethanolic extract of HE to enhance the exo-biopolymer concentration. Furthermore, **Han *et al*. (2013)** and **Vigna *et al*. (2019)** discovered that HE contains polysaccharides, oligosaccharides, fatty acid, sterol, erinacine and hericenone which have piqued medical researchers' interest over the last two decades due to their diverse clinical and biological properties, as well as their antioxidant activity. HE has anti-obesity effects in ovariectomized mice because of its ability to reduce fat absorption caused by dietary fiber and four lipase-inhibiting compounds (hericenone C, hericenone D, hericenone F, and hericenone G) **(Hiraki *et al*., 2017)**.

Table (1): The effects of various doses of HE extract on the body weight gain, feed intake, feed efficiency ratio, and relative heart weight in hyperlipidemic rats.

Parameters / Groups	BWG (g)	FI (g/day)	FER (g/rat/day)	RHW(g)
Negative control	35.51^{d}± 1.65	13.94^{a}±0.23	0.091^{d}±0.011	0.44^{e}±0.01
Positive control	53.34^{a}± 0.67	16.14^{c}±0.01	0.118^{a}±0.026	0.57^{a}±0.02
HEE(100 ml/kg, orally)	48.22^{b}±1.52	15.79^{d}±0.36	0.109^{b}±0.001	0.54^{b}±0.01
HEE(200 ml/kg, orally)	43.48^{c}± 0.83	15.37^{c}±0.08	0.101^{c}±0.017	0.50^{c}±0.02
HEE(300 ml/kg, orally)	37.35^{d}±1.95	14.34^{b}±0.33	0.093^{d}± 0.002	0.47^{d}±0.01
LSD	3.58	0.46	0.017	0.02

Means in the column followed by the same letter are not significantly different ($p \leq 0.05$). HEE: *Hericium erinaceus* extract. BWG: body weight gain. FI: feed intake. FER:feed efficiency ratio. RHW: Relative Heart Weight.

Biochemical results:

The effects of various doses of HE extract on serum lipid profiles, CRI and CRR on hyperlipidemic rats.

Consequently, the levels of TC, LDL-c, and TG were significantly greater in the positive control group compared to the negative group (refer to Table 2). The HDL-c concentration in the positive control group was significantly reduced in comparison to the negative control group; however, the HFD altered these results. HE supplementation increased HDL-c levels while decreasing TC, TG LDL-c, and VLDL-c levels significantly (p 0.05). Significant modifications took place with the escalation of HE dosage, suggesting a substantial decrease in cholesterol levels in comparison to the positive control group. Cardiovascular risk was predicted utilizing the CRI, which is the ratio of LDL to HDL. The CRI was lower in all groups compared to the hyperlipidemia group following an HFD (Table 2). The CRR of the negative control group, representing the TC to HDL ratio, was 1.78. In contrast, the CRI of the positive control group was 3.38, and the CRR for that group was 5.59. Compared to the positive control group, those treated with HE doses were able to decrease both of them. This effect was significantly amplified by increasing the HE doses, specifically by 300ml/kg, whereas the negative control group exhibited no significant changes in any parameter with the exception of TG. **Han *et al*., (2013)** reported that *Hericium erinaceus* significantly inhibited lipid peroxidation and enhanced the activity of antioxidant enzymes in mice used in their studies. It demonstrates that HE treatment can aid in lipid profile restoration by regulating blood glucose and insulin levels with HE chemicals, given that the mechanism of action of polypeptides is HMG-CoA reductase inhibition **(Wang *et al*., 2022)**.

Hetland *et al*.,(2020) believed that HE could scavenge oxygen-free radicals and prevent lipid peroxidation by regulating the glucose/insulin system. They discovered that HE has direct or indirect anti-diabetic effects via

lowering oxidative stress, which could explain why it improves glycometabolism. Thus, the current study discovered that HE possesses substantial antioxidant activity, which could be responsible for its hypolipidemic characteristics. **Gravina *et al.*, (2023)** demonstrated that H. erinaceus flourished in circumstances containing A. scoparia. Scoparone, a possible chemical, is apparently overproduced in HE. Scoparone is a primary coumarin that possesses a hypolipidaemic effect by retarding pathomorphological alterations in hypercholesterolaemic diabetic rabbits. As a result, scoparone in HE or exo-polymers in HE explain the hypolipidaemic activity of HE.

Table (2): The effects of various doses of HE extract on serum lipid profiles, CRI and CRR on hyperlipidemic rats.

Parameters / Groups	TC (mg/dl)	TG (mg/dl)	HDL-c (mg/dl)	LDL-c (mg/dl)	VLDL-c (mg/dl)	CRI	CRR
Negative control	107.56^{d} ±4.92	97.56 ^{e} ±3.76	60.43 ^{a} ±1.87	27.62 ^{d} ±1.72	19.51 ^{d} ±1.43	0.46 ^{d} ±0.08	1.78 ^{d} ±0.43
Positive control	205.54 ^{a} ±6.34	220.45 ^{a} ±7.04	36.76 ^{d} ±8.93	124.15^{a} ±10.77	44.09 ^{a} ±3.45	3.38 ^{a} ±0.87	5.59^{a} ±0.58
HEE(100ml/kg, orally)	175.89 ^{b} ±6.87	191.44 ^{b} ±8.32	43.45 ^{c} ±2.56	94.15 ^{b} ±9.51	38.29 ^{b} ±2.04	2.17 ^{b} ±0.93	4.05^{b} ±0.69
HEE(200ml/kg, orally)	142.33 ^{c} ±8.04	157.45 ^{c} ±4.81	49.87 ^{b} ±5.77	60.97 ^{c} ±2.99	31.49 ^{c} ±3.58	1.22 ^{c} ±0.47	2.85^{c} ±0.11
HEE(300ml/kg, orally)	107.33 ^{d} ±3.97	127.66 ^{d} ±3.88	55.67 ^{a} ±3.06	31.13 ^{d} ±6.01	20.53 ^{d} ±1.92	0.56 ^{d} ±0.25	1.93^{d} ±0.67
LSD	10.87	12.67	5.23	14.71	2.04	0.33	0.42

Means in the column followed by the same letter are not significantly different ($p \leq 0.05$). HEE: *Hericium erinaceus* extract. TC: Total cholesterol; TG: Triglycerides; LDL-C:Low-density lipoprotein cholesterol; (VLDL-C):Very low-density lipoprotein cholesterol; HDL-C:High-density lipoprotein cholesterol ; CRI: Coronary Risk Index (TC/HDL-C); CRR: Cardiac Risk Ratio (LDL-C/HDL-C).

The effects of various doses of HE extract on serum atrial fibrillation (AF), atherogenic coefficient (AC) and atherogenic index (AI) on hyperlipidemic rats.

The serum AF, AC, and AI of hyperlepidemic rats supplemented with different dosages of HE are shown in Table 3. In comparison to the negative control rats, the levels of cardiac parameters were significantly elevated in untreated hyperlipidemic rats ($P \leq 0.05$). The administration of HE at concentrations of 100, 200, and 300 ml/kg b.w. resulted in significantly reduced levels of AF, AC, and AI on day 28 compared to the positive control group ($P \leq 0.05$). Consequently, the high treated dose exhibited a more pronounced and statistically significant decrease in these parameters in comparison to the other doses, while the negative control group did not experience any significant changes in AF and AC ($P \approx 0.05$). LDL-C/HDL-C, also known as the AI, has demonstrated potential as an effective prognostic

indicator for atherosclerosis and coronary heart disease. The cholesterol concentration of all atherogenic lipoproteins is denoted by AC. It is determined by the difference between high-density cholesterol and TC, AF is the most common type of treatable heart arrhythmia, which occurs when the heart beats too slowly, too rapidly, or irregularly (**Friedman,2015**). So, the study of **Shrivastava and Jain(2023)** was matched with the obtained results which revealed that *Hericium erinaceus* has antidiabetic , cardioprotective ,antibiotic, anticarcinogenic, antihyperlipodemic, antihypertensive, antifatigue, antisenescence, nephroprotective, hepatoprotective and neuroprotective properties and improves anxiety, cognitive function, and depression properties due to their bioactive phytochemicals such as hericenones, erinacines, hericerins, erinarols, 2,6-diethylpyrazine, 2-methyl-3-furanthiol and corallocin.

Furthermore, *Hericium erinaceus* contains dietary fiber that has the potential to bind with cholesterol, which is a significant aspect in determining its ability to absorb lipophilic components. By chelating and absorbing organic molecules, the surface functional groups of DF reduce plasma and liver cholesterol levels. The primary environments utilized to simulate cholesterol adsorption capacity were the stomach (pH 2.0) and the intestine (pH 7.0). At pH 7.0, the adsorption capacity was considerably greater than at pH 2.0. The gut was found to be the primary site of DF's cholesterol adsorption capacity, according to a study **(Du *et al*., 2021)**. The reduction in cholesterol levels, specifically VLDL and LDL cholesterol, and the elevation in HDL-c led to changes in the cardic index, which are expressed as AI, AF, and AC, respectively **(Gravina *et al*., 2023).**

Table (3): The effects of various doses of HE extract on serum Atrial fibrillation(AF), Atherogenic Coefficient (AC) and Atherogenic Index (AI) on hyperlipidemic rats.

Parameters / Groups	AI	AF	AC
Negative control	$0.21^{e}\pm0.03$	$47.13^{d}\pm1.87$	$0.78^{d}\pm0.13$
Positive control	$0.78^{a}\pm0.21$	$168.78^{a}\pm9.03$	$4.59^{a}\pm0.84$
HEE(100 ml/kg, orally)	$0.64^{b}\pm0.09$	$132.44^{b}\pm7.56$	$3.05^{b}\pm0.65$
HEE(200 ml/kg ,orally)	$0.49^{c}\pm0.11$	$92.46^{c}\pm6.99$	$1.85^{c}\pm0.06$
HEE(300 ml/kg, orally)	$0.36^{d}\pm0.04$	$51.66^{d}\pm6.82$	$0.93^{d}\pm0.12$
LSD	0.09	13.73	0.35

Means in the column followed by the same letter are not significantly different ($p \leq 0.05$). HEE: *Hericium erinaceus* extract. AF: Atrial fibrillation;AC: Atherogenic Coefficient ; AI: Atherogenic Index (log TG/HDL-c).

The effects of various doses of HE extract on serum LPO, Troponin-1 and CK-MB on hyperlipidemic rats.

Table (4) shows the serum LPO, troponin-1, and CK-MB levels in the three experimental groups at the end of the experiment (28 days). The study found that serum levels of LPO, troponin-1, and CK-MB were considerably higher in the positive control group ($P \leq 0.05$) compared to the negative control group. Treatment with HE for 28 days resulted in significant reductions in LPO, troponin-1, and CK-MB levels compared to the untreated hyperlipidemic group ($P \leq 0.05$). After 28 days of administering HE (300 ml/kg b.w.), serum LPO, troponin-1, and CK-MB levels decreased, with significant changes except for CK-MB levels ($P \leq 0.05$). Trophonin in contrast to CK-MB, I is a more effective cardiac marker for myocardial infarction due to its reduced sensitivity and specificity towards myocardial damage. A myocardial infarction or the presence of additional myocardial complications may be suggested by elevated CK-MB levels. Myocarditis constitutes one of these conditions. This is a heart muscle infection with inflammation. Increased LDL levels are especially atherogenic since they can be oxidized and trigger atheroma formation. As a result, an elevated LDL level is considered a significant risk factor in the occurrence of a myocardial infarction. Those who tested positive for troponin had an average LDL level that was higher **(Jaffe *et al*., 2011)**. Treatment with HE resulted in decreased levels of LDL cholesterol and greater levels of HDL, which contained dietary fiber and an active molecule with antihyperlipidemic, cardioprotective, hepatoprotective, nephroprotective, and neuroprotective properties **(Gravina *et al*.,2023)**.

Table (4): The effects of various doses of HE extract Serum LPO, Troponin-1 and CK-MB on hyperlipidemic rats.

Parameters / Groups	LPO (nmol-ml)	Troponin-1 (ng/l)	CK-MB (IU/L)
Negative control	$2.23^{e} \pm 0.41$	$0.04^{e} \pm 0.003$	$3.45^{d} \pm 0.99$
Positive control	$12.65^{a} \pm 2.87$	$4.98^{a} \pm 0.59$	$9.06^{a} \pm 0.84$
HEE(100 ml/kg, orally)	$10.01^{b} \pm 1.34$	$3.32^{b} \pm 0.45$	$8.03^{b} \pm 1.33$
HEE(200 ml/kg, orally)	$8.34^{c} \pm 1.22$	$2.65^{c} \pm 0.34$	$6.10^{c} \pm 1.04$
HEE(300 ml/kg, orally)	$5.87^{d} \pm 0.76$	$0.42^{d} \pm 0.08$	$3.99^{d} \pm 0.55$
LSD	1.11	0.26	0.98

Means in the column followed by the same letter are not significantly different ($p \leq 0.05$). HEE: *Hericium erinaceus* extract. LPO: Lipid peroxidation. CK-MB: Creatine Kinase MB.

The effects of various doses of HE extract on insulin and leptin levels on hyperlipidemic rats.

Data on fasting serum insulin and leptin levels are presented in Table 5. The insulin levels of the hyperlipidemic group (positive control) decreased significantly after 28 days. Variable concentrations of HE led to significantly

elevated levels of insulin in comparison to the positive control ($P \leq 0.05$) and a significant decrease in insulin levels ($P \leq 0.05$) in comparison to the negative control group. Regarding leptin, it was observed that the positive control group exhibited a significantly elevated level of leptin compared to the normal group. However, administration of different doses of HE resulted in a reduction in leptin hormone levels, with the magnitude of this decrease being significantly amplified as the extract dose increased. Additionally, no statistically significant differences were observed in leptin levels when comparing the fifth group to the negative control group ($P \leq 0.05$).

HE is a mushroom that offers medicinal and edible properties. It has been utilized historically to treat diabetes. It contains the active component Hericene A (HA), which stimulates insulin production by inhibiting ATP-dependent potassium channels in pancreatic cells and inhibits glucosidase when blood glucose levels rise. An additional investigation revealed that the probable pathway through which HE induces an antihyperglycemic response in diabetic rats involves either the pancreatic synthesis of insulin from pre-existing -cells or its liberation from its bound state. This mechanism enhances glycemic regulation and inhibits weight loss. Furthermore, its vitamin B, polysaccharide, and high concentration of essential amino acids resulted in improved blood sugar and overall health **(Liang *et al*., 2013 and Wang *et al*., 2022)**.

Table (5): The effects of various doses of HE extract on insulin and leptin levels on hyperlipidemic rats.

Parameter / Groups	Insulin(U/ml)	Leptin(ng/ml)
Negative control	18.75 [a] ±2.42	8.98 [d] ±1.34
Positive control	6.96 [e]±1.17	30.96 [a] ±2.76
HEE(100 ml/kg Bw, orally)	9.75 [d]±1.04	24.67 [b]±3.56
HEE(200 ml/kg Bw, orally)	13.07 [c]±2.01	18.93 [c]±1.87
HEE(300 ml/kg Bw, orally)	16.64 [b]±3.12	11.98 [d]±0.89
LSD	2.03	4.87

Means in the column followed by the same letter are not significantly different (p ≤0.05). HEE: *Hericium erinaceus* extract.

Histopathological examination of heart:

When examining the heart of a rat from group 1, a normal histological architecture of cardiac myocytes was observed under the microscope (Photos 1 and 2). On the contrary, the myocytes of the sarcoplasm were vacuolated in the hearts of rats in group 2 (Photos 3 and 4), which was accompanied by inflammatory cell infiltration (Photo 4) and intermyocardial edema (Photo 5). Meanwhile, the hearts of rats in groups 3 and 4 exhibited no histological alterations, with the exception of cardiac blood channel congestion in a few investigated sections (Photos 6, 7, 8, and 9). Furthermore, there were no

histopathological changes detected in the cardiac tissue of the rats in group 6. (Photos 10 & 11).

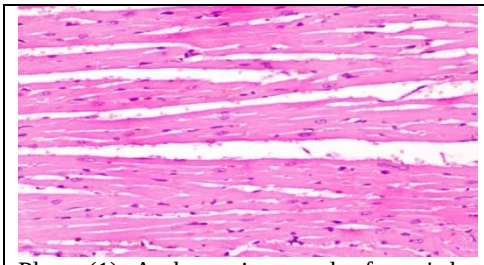

Photo (1): A photomicrograph of a rat's heart from group 1 (negative control) with normal histological architecture of cardiac myocytes (H&E, X 400).

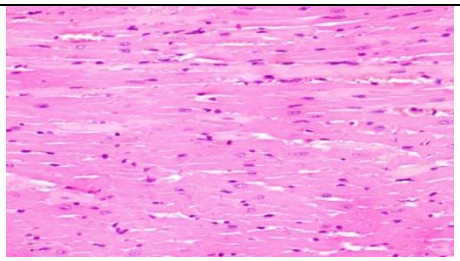

Photo (2): A photomicrograph of the heart of a rat from group 1 (negative control) demonstrating the normal histological architecture of cardiac myocytes (H & E, X 400).

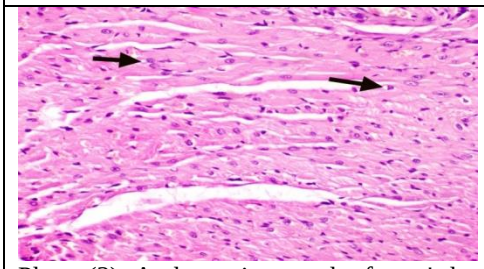

Photo (3): A photomicrograph of a rat's heart from group 2 (Positive control) shows vacuolation of the sarcoplasm of cardiac myocytes (H&E, X 400).

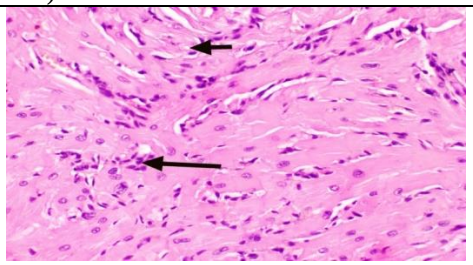

Photo (4): A photomicrograph of the heart of a rat from group 2 (Positive control) shows vacuolation of the sarcoplasm of cardiac myocytes, together with inflammatory cell infiltration (H & E, X 400).

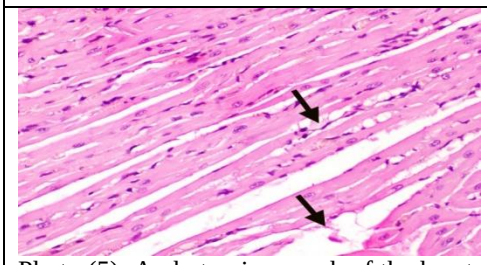

Photo (5): A photomicrograph of the heart of a rat from group 2 (Positive control) reveals intermyocardial edema (H & E, X 400).

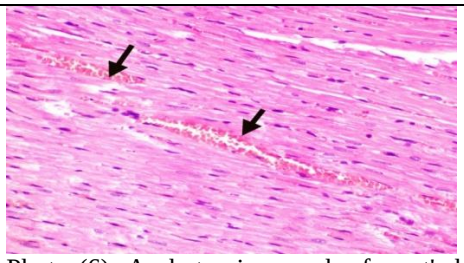

Photo (6): A photomicrograph of a rat's heart from group 3 (treated with 100ml/kg Bw of HE extract) shows myocardial blood vessel congestion (H & E, X 400).

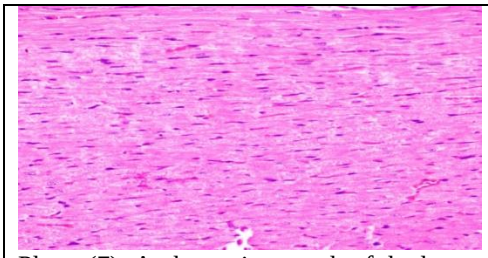

Photo (7): A photomicrograph of the heart of a rat from group 3 (treated with 100ml/kg Bw of HE extract) revealed no histopathological changes (H & E, X 400).

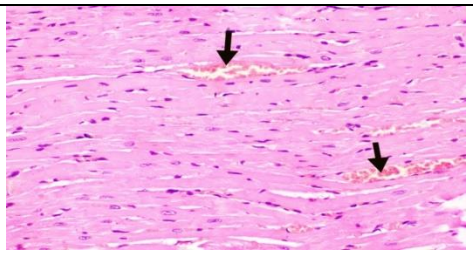

Photo (8): A photomicrograph of a rat's heart from group 4 (treated with 200ml/kg Bw of HE extract) shows myocardial blood vessel congestion (H&E, X400).

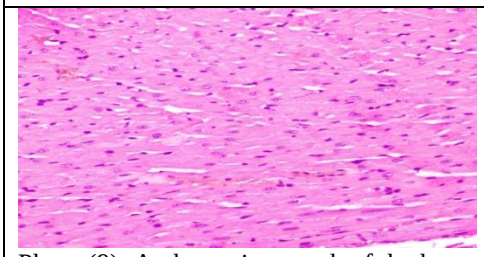

Photo (9): A photomicrograph of the heart of a rat from group 4 (treated with 200ml/kg Bw of HE extract) revealed no histopathological changes (H & E, X 400).

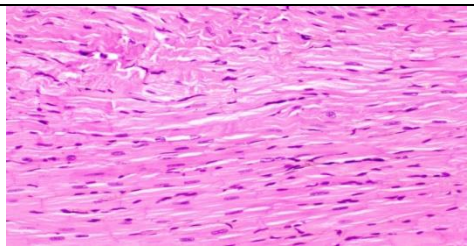

Photo (10): A photomicrograph of the heart of a rat from group 5 (treated with 300ml/kg Bw of HE extract) reveals no histopathological changes (H&E, X 400).

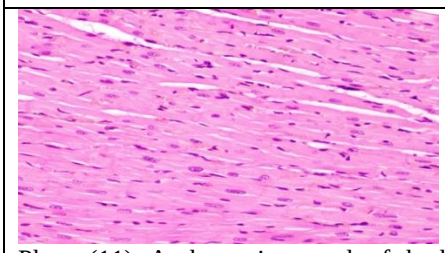

Photo (11): A photomicrograph of the heart of a rat from group 5 (treated with 300ml/kg Bw of HE extract) revealed no histopathological changes (H & E, X 400).

Conclusion:

The study showed that administration with lion's mane mushroom led to a decrease in the levels of TC, LDL, TG, the rate of atherosclerosis, the hormone leptin, and risk factors for heart disease in mice with high blood fats. There was also an increase in the levels of HDL and the hormone insulin. The results showed that mushroom extract can treat hyperlipidemia in rats. The best results were for the group treated with mushrooms at a dose of 300 ml/kg. Therefore, it may be an effective natural medicine for treating hyperlipidemia in humans.

References :

Aguilar, E. C.; Queiroz, M. D. G. M. N.; Oliveira, D. A. D. and Oliveira, N. J. F. D. (2011): Serum lipid profile and hepatic evaluation in mice fed diet containing pequi nut or pulp (Caryocar brasiliense Camb.). Food Science and Technology, 31 (4): 879-883.

Allain, C. C.; Poon, L. S.; Chan, C. S.; Richmond, W. F. P. C.; and Fu, P. C. (1974): Enzymatic determination of total serum cholesterol. Clinical chemistry, 20(4), 470-475.

Bhardwaj, S.; Bhattacharjee, J.; Bhatnagar, M. K.; Tyagi, S. and Delhi, N. (2013): Atherogenic index of plasma, castelli risk index and atherogenic coefficient-new parameters in assessing cardiovascular risk. Int. J. Pharm. Biol. Sci., 3(3), 359-64.

Burstein, M.;Scholnick,H.R. and Morfin,R. (1980): Rapid method for the isolation of lipoproteins from human serum by precipitation with polyanions. J. Clin. Lab. Invest., 40: 583-595.

Campbell, J. A. (1963): Methodology of Protein Evaluation. RAG Nutr. Document R. 101 Led.37 June Meeting, New York.

Chapman, D.G.; Castillo, R. and Campbell, J.A. (1959):

Evaluation of protein in foods:1. A method for the determination of protein efficiency ratio. Canadian Journal of Biochemistry and Physiology, 37(5): 679-686.

Considine, R.V.; Sinha, M.K.; Heiman, M.L. and Kriunas, A. (1996):Serum immunoreactive- leptin concentration in normal weight and obese humans. N. Engl. j. Med., 334(5):292-295.

Defronzo, R.A.; Tobin, J.D. and Andres, R. (1979): Glucose clamp technique: Amethod for quantifying insulin secretion and resistance. Am. J. Physiol., 237: 214-223.

Drury, R. A. B. and Wallington, E. A. (1980): Carleton's histological technique 5th ed. New York: Churchill Livingstone.

Du, X.; Wang, L.; Huang, X.; Jing, H.; Ye, X.; Gao, W., ... and Wang, H. (2021): Effects of different extraction methods on structure and properties of soluble dietary fiber from defatted coconut flour. Lwt, 143, 111031.

Fossati, P. and L. Prencipe, (1982). Serum triglycerides determined calorimetrically with an enzyme that produces hydrogen peroxide. Clin. Chem., 28(10): 2077-80.

Friedewald, W.T.; Levy, R. and Fredrickson, D.S. (1972).Estimation of the concentration of low density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. Clin. Chem., 18, 499–502.

Friedman, M. (2015):Chemistry, Nutrition, and Health-Promoting Properties of *Hericium erinaceus* (Lion's Mane) Mushroom Fruiting Bodies and Mycelia and Their Bioactive Compounds. J. Agric. Food Chem., 63 (32): 7108- 7123.

Gharib, M. A. A.; Elhassaneen, Y. A. E. E., and Radwan, H. (2022): Nutrients and nutraceuticals content and in vitro biological activities of reishi mushroom (ganoderma lucidum) fruiting bodies. Alexandria Science Exchange Journal, 43(2), 301-316.

Gravina, A.G.; Pellegrino, R.; Auletta, S. and Palladino, G. (2023): *Hericium erinaceus*, a medicinal fungus with a centuries-old history: Evidence in gastrointestinal diseases. World J. Gastroenterol., 29(20): 3048–3065.

Han, Z.H.; Ye, J.M. and Wang, G.F. (2013):Evaluation of in vivo antioxidant activity of *Hericium erinaceus* polysaccharides. Int. J. Biol. Macromol.,52:66–71.

Hegsted, D.; Mills, R. and Perkins, E. (1941): Salt mixture. J. Biol. Chem., 138: 459.

Hetland, G.; Tangen, J.M.; Mahmood, F.; Mirlashari, M.R.; Nissen-Meyer, L.S.H.; Nentwich, I.; Therkelsen, S.P.; Tjonnfjord, G.E.and Johnson, E. (2020): Antitumor, Anti-Inflammatory and Antiallergic Effects of Agaricusblazei Mushroom Extract and the Related Medicinal Basidiomycetes Mushrooms, Hericium erinaceus and Grifola frondosa: A Review of Preclinical and Clinical Studies. Nutrients, 12:1339.

Hewage, S. M.; Sid, V.; Prashar, S.; Shang, Y.; Karmin, O.; and Siow, Y. L. (2018):Impact of lingonberry supplementation on high-fat dietinduced metabolic syndrome and hyperlipidemia. Atheroscler, 32: 65.

Hiraki, E.; Furuta, S.; Rika Kuwahara, R.; Takemoto, N.; Nagata, T. and Akasaka, T. (2017): Anti-obesity activity of Yamabushitake (Hericiumerinaceus) powder in ovariectomized mice, and its potentially active compounds. Journal of Natural Medicines; volume 71:482–491.

Jaffe, A.S.; Lindahl, B.and Katus, H.A.(2011): Sensitive tropinin I assay in patients with suspected acute coronary syndrome. J. Am. Med. Assoc.,306(5):488–489.

Kamesh, V.; and Sumathi, T. (2012): Antihypercholesterolemic effect of Bacopa monniera linn. on high cholesterol diet induced hypercholesterolemia in rats. Asian Pacific Journal of Tropical Medicine, 5(12), 949-955.

Kanakavalli, K.; Thillaivanan, S.; Parthiban, P.; Vijayalakshmi, G.; Sudha, M.; and Sutha, J. (2014): Antihyperlipidemic herbs in siddha system of medicine. Int. J. Pharma. Sci., 4 :541- 545.

Kuo, Y.; Lin, T.;Lin, J.; Chen, Y.; Li, T. and Chen, C. (2022):Identification of Common Liver Metabolites of the Natural Bioactive Compound Erinacine A, Purified from *Hericium erinaceus* Mycelium. J. Appl. Sci., 12(3): 1201.

Lee, L.Y.; Li, I.C.; Chen, W.P.; Tsai, Y.T.; Chen, C.C.; Tung, K.C. (2019): Thirteen-Week Oral Toxicity Evaluation of Erinacine A Enriched Lion's Mane Medicinal Mushroom, Hericiumerinaceus (Agaricomycetes), Mycelia in Sprague-Dawley Rats. Int. J. Med. Mushrooms, 21, 401–411.

Lee, R.; and Nieman, D. (1996): National Assessment. 2nd Ed., Mosby, Missouri, USA.

Liang, B.; Guo, Z.; Xie, F.; and Zhao, A. (2013): Antihyperglycemic and antihyperlipidemic activities of aqueous extract of *Hericium erinaceus* in experimental diabetic rats. BMC complementary and alternative medicine, 13(1), 1-7.

Mori, K.; Ouchi, K. and Hirasawa, N. (2015): The Anti-Inflammatory Effects of Lion's Mane Culinary-Medicinal Mushroom, Hericiumerinaceus (Higher Basidiomycetes) in a Coculture System of 3T3-L1 Adipocytes and RAW264 Macrophages. Int. J. Med. Mushrooms, 17, 609–618.

Mosa, A.U. ; Naser, I.H and AbdAlkareem, Z. (2021): Hyperlipidemia: pathophysiology, causes, complications and treatment. Kerbala journal of pharmacy and pharmaceutical science,118.

Omole, J.G.; Ayoka, O.A. and Alabi, Q.K. (2018): Protective effect of kolaviron on cyclophosphamide-induced cardiac toxicity in rats. J Evid Based Integr Med.,23(1):1–11.

Onwe, P. E.; Folawiyo, M. A.;AnyigorOgah, C. S.; Umahi, G.; Okorocha, A. E.; and Afoke, A. (2015): Hyperlipidemia: etiology and possible control. IOSR J. Dent. Med. Sci., 14: 93-100.

Rauf, A.; Akram, M.; Anwar, H.; Daniyal, M.; Munir, N.; Bawazeer, S.; ... and Khan, H. (2022): Therapeutic potential of herbal medicine for the management of hyperlipidemia: latest updates. Environmental Science and Pollution Research, 29(27), 40281-40301.

Reeves,P. G.;Nielsen, F. H. and Fahey, G. C. Jr.(1993):AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. J. Nutr.,123(11): 1939– 1951.

Schermer, S. (1967): The Blood Morphology of Laboratory Animal. Longmans, Printed in Great Britain, Green and Co. Ltd., pp.350.

Shen, T.; Morlock, G.; and Zorn, H. (2015): Production of cyathane type secondary metabolites by submerged cultures of *Hericium erinaceus* and evaluation of their antibacterial activity by direct bioautography. Fungal biology and biotechnology, 2, 1-7.

Shrivastava, A and Jain, S. (2023): Hericiumerinaceus (Bull.) and *Hericium coralloides*. BookEdible and Medicinal Mushrooms of the Himalayas. ImprintCRC Press. Pages17. eBook ISBN9781003259763

Sreerekha, P.R.; Dara, P.K. and Vijayan, D.K. (2021): Dietary supplementation of encapsulated anthocyanin loaded-chitosan nanoparticles attenuates hyperlipidemic aberrations in male Wistar rats. Carbohydrate Polymer Technologies and Applications, 2: 100051.

Tateishi,T.; Yoshimine, N. and F Kuzuya, F (1987):Serum lipid peroxide assayed by a new colorimetric method. Exp. Gerontol., 22(2):103-111.

Walker, H. K.; Dallas, M.W.; Hall, M.D. and Hurst, J.W. (1990): The History, Physical, and Laboratory Examinations.Clinical Methods, 3rd edition. Boston: Butterworths; ISBN-10: 0-409-90077-X

Vigna, L.; Morelli, F.; Agnelli, G. M.; Napolitano, F.; Ratto, D.; Occhinegro, A.; ... and Rossi, P. (2019): *Hericium erinaceus* improves mood and sleep disorders in patients affected by overweight or obesity: could circulating pro-BDNF and BDNF be potential biomarkers?. Evidence-Based Complementary and Alternative Medicine, 2019.

Wang ,P.N.; Tong,Z.; Wang, D.; Zhang, Y. and Liu, T. (2022): Effects of Hericiumerinaceus polypeptide on lowering blood lipids of mice with hyperlipidemia induced by a high-fat diet. Journal of Future Foods, 2 (4):346-357.

Yadav, S.K.; Ir, R.; Jeewon, R.; Doble, M.; Hyde, K.D.; Kaliappan, I.; Jeyaraman, R.; Reddi, R.N.; Krishnan, J. and Li, M. (2020): A Mechanistic Review on Medicinal Mushrooms-Derived Bioactive Compounds: Potential Mycotherapy Candidates for Alleviating Neurological Disorders. Planta. Med., 86, 1161–1175.

Yang, B.K.; Park, J.B. and Song, C.H. (2003): Hypolipidemic effect of an exo-polymer produced from a submerged mycelial culture of *Hericium erinaceus*. Biosci. Biotechnol. Biochem.,67:1292–1298.

Yao, Y. S.; Li, T. D.; and Zeng, Z. H. (2020): Mechanisms underlying direct actions of hyperlipidemia on myocardium: An updated review. Lipids Health Dis., 19 :1-6.

Yu, X. H.; Zhang, D. W.; Zheng, X. L.and Tang, C. K. (2019):Cholesterol transport system: an integrated cholesterol transport model involved in atherosclerosis. Prog. Lipid Res., 73: 65- 91.

تأثير المستخلص الإيثانولي لفطر عرف الأسد علي خفض دهون الدم والوقاية من أمراض القلب في ذكور الفئران الألبينو

د / سماح البنا ، د / أميرة حمدى
قسم التغذية وعلوم الأطعمة – كلية الاقتصاد المنزلى – جامعة المنوفية

ملخص البحث :

يقصد بارتفاع دهون الدم عدم انتظام مستويات الدهون في الدم، وهو العامل الرئيسي في تطور أمراض القلب والأوعية الدموية وتصلب الشرايين. فطر عرف الأسد هو فطر طبي صالح للأكل ويستخدم كغذاء ودواء لاحتوائه علي بعض المركبات النشطة بيولوجيا. وهدفت الدراسة إلي التعرف علي تأثير المستخلص الإيثانولي للفطر علي مستوي دهون الدم ومعدل تصلب الشرايين وعوامل الخطورة لأمراض القلب. تم استخدام ثلاثون فأرا ذكر بالغ من نوع الألبينو متوسط وزنهم 120 ± 5 جرام في هذه الدراسة. تم تقسيم الفئران إلى خمس مجموعات (ستة فئران في كل مجموعة). تم تغذية المجموعة الأولي علي الوجبة الأساسية كمجموعة ضابطة سالبة، وتغذت المجموعة الثانية علي وجبات مرتفعة الدهون كمجموعة ضابطة موجبة، بينما تغذت المجموعات الثالثة والرابعة والخامسة علي وجبات مرتفعة الدهن مع المستخلص الكحولي لفطر عرف الأسد بجرعات 100 و 200 و 300 مل / كجم من وزن الجسم عن طريق الفم لمدة 28 يوما . أظهرت الدراسة أن الفئران التي تغذت على مجموعات عالية في الدهون (المجموعة الضابطة الموجبة) زيادة كبيرة بشكل ملحوظ ($p\leq0.05$) في وزن الجسم، وكمية المأخوذ الغذائي ومعدل كفاءه الغذاء مقارنة بالمجموعة الطبيعية. ومع ذلك، أدى التدخل بالمستخلص الكحولي من فطر عرف الأسد في تغذية الفئران لمدة 28 يومًا إلى انخفاض كبير بشكل ملحوظ ($p\leq0.05$) في هذه المعايير لدى الفئران التي تغذت على غذاء عالي الدهون (المجموعة الضابطة الموجبة). كانت المعاملات البيوكيميائية أعلى بشكل كبير في المجموعة الضابطة الموجبة مقارنة بالمجموعة الطبيعية، ولكن كانت تركيزات HDL–c أقل بشكل ملحوظ في المجموعة غير المصابة. أدى العلاج بالجرعات المختبرة من HE إلى تقليل مستويات TC و LDL–c و TG، بالإضافة إلى معامل الإصابة بتصلب الشرايين وهرمون اللبتين وقيم عامل الخطر القلبي في الفئران التي تغذت على نظام غذائي عالي الدهون، في حين زيادة محتوى HDL–c وهرمون الأنسولين تم دعم النتائج البيوكيميائية بالفحص النسيجي لأنسجة القلب. ونتيجة لذلك، تظهر النتائج أن المستخلص الايثانولى لفطر عرف الأسد فعّال في تحسين صورة دهون الدم في الفئران التي تتغذى على نظام غذائي عالي الدهون.

الكلمات الرئيسية: عامل الخطر القلبي، مستوى الدهون في الدم، هرمون الأنسولين، هرمون اللبتين، مؤشر تصلب الشرايين, فطر عرف الأسد.