Research Article

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Effects of Swimming Exercise and a Mixture of Garlic + Lemon Juice on body weight, Lipid profile, and Liver function in Obese hyperlipidemic Rats

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Abstract

Obesity is a chronic disease that leads to fatty liver, abnormal function tests, and hyperlipidemia. Exercise improved the lipid profile, liver histomorphology, and functions. Herbs were frequently included in obesity management. This study investigated the effects of swimming and a mixture of garlic and lemon juice on lipid profile, liver function, and body weight. Twenty adult male albino rats of about 240 ± 10-gram body weight and 12 weeks old were used and were randomly divided into four experimental groups. The first group received the standard pellet diet during the 28-day experiments. The second group was fed a high-formulated diet (HFD). The third group received the HFD and 28 ml of a garlic-lemon mixture orally. The final group, which was fed the HFD and engaged in 30 minutes of daily swimming, had their body weight, lipid profile, serum levels of AST, ALT, and ALP, and their liver histopathology evaluated. All these measurements showed a reduction in body weight, a significant decrease in liver function test parameters, a decrease in serum levels of cholesterol, triglycerides, and LDL, and an increase in HDL in both the exercise and treatment groups. Furthermore, the study concluded that both swimming and the administration of a mixture of

Allium sativum and Citrus limon significantly improved the lipid profile. Significant improvement in lipid profile decreases the cholesterol, TG, LDL, and liver function test parameters and ameliorates the liver histopathological lesions as well.

Keywords: Obesity, fatty liver, exercise, garlic.

Introduction

Obesity is a chronic, complex relapsing disease whereby excessive or abnormal fat accumulation (1) is an epidemic problem and has an impact on health and leads to complications such as increased fasting plasma triglycerides, LDL, and decreased HDL, resulting in fatty liver disease and increased levels of hepatic enzymes, including ALT and AST (2).

Obesity is a chronic, complex relapsing disease characterized by excessive or abnormal fat accumulation (1). It is an epidemic problem that impacts health and leads to complications such as increased fasting plasma triglycerides, LDL, and decreased HDL, resulting in fatty liver disease and increased levels of hepatic enzymes, including ALT and AST. Medicinal plants are frequently included in obesity management (8,6), and plant-derived nutraceutical formulas with anti-obesity action and no adverse effects on human health represent an innovative strategy for reducing obesity (9).

Various types of herbs have the potential to reduce serum lipid profile (10) through lipid regulation mechanisms that involve the entire process of lipid absorption, synthesis, transport, decomposition, and excretion (11). This is due to the presence of natural polyphenols in these plants (12). Similarly, plant compounds influence hepatic lipid metabolism (13) and plant-based antioxidants have both preventive and therapeutic effects on various liver diseases, including nonalcoholic fatty liver disease (14). A ubiquitous herb with a long history of medicinal use is garlic (15), which elicits anti-inflammatory, antioxidant, anticancer, antimicrobial, antidiabetic, cardio-protective, neuro-protective, immuno-modulatory, anti-inflammatory, and antihypertensive properties (16).

Citrus fruits represent one of the most consumable fruit genera globally Citrus fruits are a rich source of minerals and vitamins, as well as various dietary fibers that are exclusively crucial to human nutrition (17), these fibers possess numerous qualities, including antimicrobial, antifungal, anti-inflammatory, anti-cancer, depurative, and antiscorbutic (18), and they also have the ability to prevent kidney stones (19). Our current study explores the impact of swimming exercise combined with a mixture of garlic and lemon juice on lipid profiles, liver histology, and weight loss.

Material and Method

Preparation of a high- formulated diet (HFD): FD was prepared for the induction of obesity; it was done by mixing an ordinary diet with palm oil and cholic acid as a bile acid that promotes fat and

cholesterol absorption from the intestine (20).

Preparation of lemon and garlic mixture: The mixture was prepared by saps of three lemons weighted (95±5g) from the local market and each lemon firmly pressed to squeeze (13±2 ml) of its juice, combined with 100g crushed garlic bulbs (about 20gloves) poured in one liter of water (21).

Study design: Twenty adult male albino rats of about 240 g ± 10 body weight and 12 weeks old were randomly housed in four plastic cages bedded with wooden chips. Five animals were kept in each cage of control, model, and experimental groups in an animal house belonging to the Biology Department / College of Science and Education / Sulaimani University, under standard laboratory conditions, 12:12 light/dark at 18 ± 2ºC.

During 4 weeks of the experimental period, 1st group was respected as the control group, only fed the standard pellet diet, 2nd group was respected as the model group fed with HFD for induction of obesity, 3rd the group was fed HFD daily and received 1ml of the mixture of garlic and lemon, that administered orally for each rat via a technique of oral gavage, involves the passage of a gavage needle into the esophagus, and the last 4th group rats fed with the HFD and doing swimming 30 minute/day. The exercise was performed by swimming in a plastic bucket (high 100 cm, diameter 40 cm), containing tap water at a depth of 50cm maintained at 32-34 ºC. The duration of the first swimming experience was limited to 10 min and increased by 10 min daily until it reached one 30 minutes (22).

Body weight: On the last day of the experiment, before taking blood samples and euthanasia, the body weight for each rat in different groups was got in grams by putting rats directly on balance (JADAVER-SKY 600).

Collection and preparation of blood samples: After 28 days of experiments, anesthesia for rats was done by overnight fasting and intraperitoneal injection of a combination of ketamine hydrochloride (50 mg/kg/bw) and xylazine (5 mg/kg/bw) (20). Blood samples were aspirated by a 5ml disposable syringe through a cardiac puncture and injected into the gel tubes to be centrifuged at 3000 rpm for 15 min. Finally, the serum obtained was analyzed for biochemical parameters such as serum cholesterol (Ch), triglyceride (TG), High-density lipoprotein (HDL), and low-density lipoprotein (LDL), and parameters of liver function test ALT, AST, and ALP were determined by using an automatic biochemistry analyzer (Cobas E 411, Roche Germany).

Histopathology: After euthanasia, a piece of hepatic tissue from each rat of different groups was fixed with 10% formalin in the proper container to prevent autolysis and purification. The biopsies were processed and embedded in molds with liquid paraffin to make tissue blocks. By using a manual microtome, 3µm tissue slices were obtained. After floatation in a water bath, ribbons of tissues were immobilized on glass slides to be stained with hematoxylin and eosin

(H&E) before adhering coverslips over them (20).

The histopathological checking of tissue slides was done using a binocular light microscope (Olympus), and micrographs of the pathological changes in liver tissues were taken by an eyepiece camera guided by (S-EYE-2) computer software.

Statistical analysis: The IBM SPSS Statistics 23 application is used for the interpretation and analysis of the data of the research. The one-way ANOVA was used for the comparison of means between groups. The significance level is calculated at 0.01 level.

Results

There was a significant (P<0.01) between groups of administered mixture and exercise compared with the Obese model group. There were significantly decreased blood cholesterol, triglycerides, and LDL inthe rats' blood cholesterol, triglycerides and LDL in groups of administered mixture and exercise compared with the Obese model group (P value ≤0.01). The means were (61.25 ± 1.23), (53.50 ± 1.29) compared to (71.00 ± 1.29), (62.60 ± 2.88), (71.00 ± 2.91) compared to (105.20 ± 2.58) and (25.50 ± 1.29), (22.25 ± 1.70) compared to (27.50 ± 1.29). There was a significantly increase in blood HDL of the rats in groups of administered mixture and exercise compared with the Obese model group (P value ≤0.01). The means were (21.50 ± 1.29) (25.25 ± 1.70) compared to (14.50 ± 1.29). There were significant decreases in body weight and liver function test parameters ALT, AST, and ALP of the rats in groups of administered mixture and exercise compared with the obese model group (P value ≤0.01). The means were body weight (363.00 ± 0.81), (343.75 ± 11.08) compared to (377.50 ± 11.90), LFT (30.00 ± 1.8), (32.75 ± 1.70) compared to (44.25 ± 1.70), (103.50 ± 1.29) (103.75 ± 3.59) compared to (114.75 ± 5.50) and (133.00 ± 4.16) (207.00 ± 13.42) compared to (417.66 ± 31.21) respectively, and preserving histology of liver as well compared to fatty liver in model group. The liver's tissue in the control group was preserved. However, in the model group, there were histopathological changes, including disappearing issue of liver in control group was preserved. However, the model group, there were histopathological changes including disappearing sinusoids due to hepatocytes swelling by fatty change accompanied by hyperplasia of Kupffer cells. In the exercise group, there ere foci of hemosiderosis and scattered inflammatory cells throughout the hepatic tissue. In the mixture group, there were no histopathological changes in the liver tissue figure (1).

Table (1) Mean and standard deviation of biochemical parameters in control, model, and treatment rats. Values are means ± S.E.M., *p<0.001 difference between treated groups and models.

Groups	Control	Model	Exercise	Garlic and Lemon
	Mean ± Sd	Mean ± Sd	Mean ± Sd	Mean ± Sd
Cholesterol	60.44 ± 0. 93	71.00 ± 1.29	53.50 ± 1.29*	61.25 ± 1.23*
Triglyceride	58.60 ± 6.80	105.20 ± 2.58	62.60 ± 2.88*	71.00 ± 2.91*
HDL	16.50 ± 1.29	25.25 ± 1.70	14.50 ± 1.29*	15.50 ± 1.29*
LDL	16.50 ± 1.29	14.50 ± 1.29	25.25 ± 1.70*	21.50 ± 1.29*
ALT	36.25 ± 0.95	44.25 ± 1.70	32.75 ± 1.70*	30.00 ± 1.82*
AST	87.75 ± 1.70	114.75 ± 5.50	103.75 ± 3.59*	103.50 ± 1.29*
ALP	123.00 ± 1.00	417.66 ± 31.21	207.00 ± 13.42*	133.00 ± 4.16*
Body Weight	210.00 ± 8.16	377.50 ± 11.90	343.75 ± 11.08*	363.00 ± 0.81*

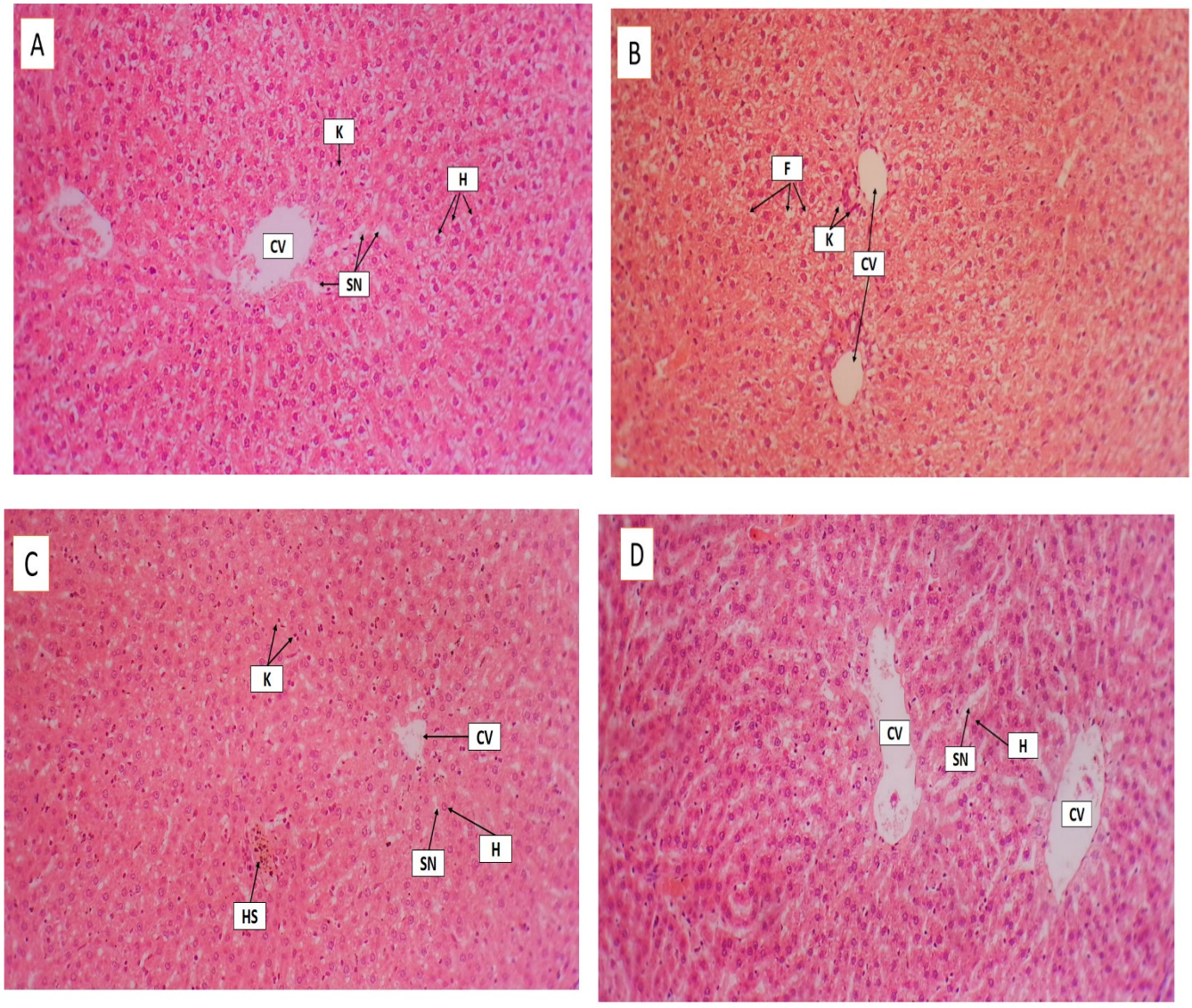

Figure (1) Panel A control group revealed liver histology, central vein (CV), sinusoids (SN), Hepatocytes, and Kupffer cells could be seen (K), B model group revealed central vein (CV), sinusoids disappeared due to hepatocytes swelling by fatty change (F), and there is hyperplasia of Kupffer cells (K), C exercise group revealed histological feature of liver including central vein (CV), sinusoids (SN) surrounded by hepatocytes (H) without any pathological changes, there is foci of hemosiderosis and scattered inflammatory cells though out the section Kupffer cells (K), D lemon group throughout the section normal histological features of liver were preserved revealed including central vein (CV), sinusoids (SN) surrounded by hepatocytes (H) without any pathological changes. H & E staining 10x.

Discussion

Results indicated a significant decrease in cholesterol, Triglycerides, LDL, body weight, and serum parameters of liver function in treated and exercise groups compared to model rats (P value ≤0.01). At the same time, there as an increase in HDL and preserved live histology.

These results are in line with published literature, such as the work of (21), that administration of garlic plus lemon juice resulted in an improvement in lipid levels of patients with hyperlipidemia, and other studies that garlic juice can offers hypolipidemic effects in male Wistar rats including a decrease in the total cholesterol, triglycerides and LDL concentrations and increase in the HDL (23).

These confirmed results might be because citrus juice is source of dietary fiber (24). This soluble fiber assists in lowering general blood cholesterol levels (25), and garlic components to can bind to fatty acids and bile and prevent the absorption of fats (26).

Allium sativum (Garlic) is an important source of biologically active Phyto molecules that have antithrombotic, antimicrobial, and antiproliferative effects. They reduce the effect of cholesterol and play a role in preventing oxidative damage caused by reactive oxygen species (27). *Citrus limon* (lemon) is a potential source of antioxidant agents, scavenging free radicals and preventing their degenerative effects because of polyphenols, flavonoids, limonoids, carotenoids, and vitamin C. (28).

Flavonoids are among the major constituents of polyphenols found in different parts of citrus fruits (skin, peels, seed, pulp membrane, and juice) (29). Naringin, naringenin, nobiletin, narirutin, and hesperidin are the most important flavonoids thus far isolated from citrus fruits (30). Hesperidin may improve hypercholesterolemia and fatty liver by inhibiting both the synthesis and absorption of cholesterol and regulating the expression of mRNA for cutaneous fatty acid binding protein (31) while naringin and naringenin, including regulation of lipid digestion, reverse cholesterol transport, and LDL receptor expression, also activate adenosine monophosphate-activate protein kinase (AMPK) pathways to increase lipid oxidation and inhibit Hyrroxymethylglutaryl CoA reductase (32) that lowers the expression of cholesterol synthesis in humans as well as in most animals (33).

Garlic contains rutin (34) and polyphenol with hypolipidemic effects (35). The mechanisms of action are likely attributed to the ability of rutin to reduce diet cholesterol absorption (36) and the presence of quercetin, which improves dyslipidemia and reduces lipid accumulation in the liver (37) by inhibiting Lenovo fatty acid synthesis through the Acetyl-CoA Carboxylase 1/adenosine monophosphate-activated protein kinase (AMPK) protein phosphatase 2 (PP2) axis (38). These results are consistent with other studies that suggest garlic can be beneficial for the suppression of dyslipidemia, hepatosteatosis, and oxidative stress in rats.

In the presented study, swimming for 30 minutes/day has been shown to reduce body weight and improve hyperlipemia and liver function parameters in blood. Likewise, studies by (40) established that swimming exercise is effective in weight loss and reducing blood cholesterol levels in hypercholesterolemic rats. And there is a study that swimming training is effective in the serum levels of ALT, AST, ALP in young females (41) and prevent liver damage in rats (42).

Swimming can burn many calories and improve the musculoskeletal system (43); however, in exercised muscle, there is increasing sensitivity to insulin that decreases blood glucose levels and enhances the ability of the muscles to burn fat to a greater extent, which is mediated by activation of several enzymes that are necessary for lipid metabolism which affects positively in decreasing TG, C, and LDL and increasing the levels of HDL (44,45).

Conclusion

This study concluded that obesity can induce fatty liver and increase its parameters in blood AST, ALP, accompanied by hyperlipidemia. Swimming and administering a mixture of Allium sativum and Citrus limon significantly decrease the cholesterol, TG, LDL, and liver function test parameters while increasing HDL and ameliorating the liver histopathological lesions. Also, it was demonstrated that swimming exercise had more promising health benefits than administering a mixture.

Conflicts of interest

The authors declare that there is no conflict of interest**.**

Ethical Clearance

This work is approved by The Research Ethical Committee.

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تأثيرات تمرين السباحة ومزيج الثوم وعصير الليمون على وزن الجسم ونسبة الدهون في الدم ووظائف الكبد في الفئران المصابة بالسمنة وارتفاع الدهون في الدم

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الخلاصة

السمنة هي مرض مزمن يؤدي إلى تشحم الكبد واختلال وظائفه وارتفاع الدهون في الدم. تحسن التمارين الرياضية من نسبة الدهون في الدم وبنية الكبد النسيجية ووظائفه. غالبًا ما يتم استعمال الأعشاب في علاج السمنة. تهدف هذه الدراسة إلى التحقيق في آثار السباحة ومزيج من الثوم وعصير الليمون على نسبة الدهون في الدم ووظائف الكبد ووزن الجسم. تم استخدام عشرين فأرًا أبيض ذكرًا بالغًا بوزن جسم حوالي 240 ± 10 جرام وعمر 12 أسبوعًا، وتم تقسيمهم عشوائيًا إلى أربع مجموعات تجريبية. خلال 28 يومًا من التجارب، تم تغذية المجموعة الأولى بالنظام الغذائي القياسي، والمجموعة الثانية بنظام غذائي عالي الدهون(HFD) ، والمجموعة الثالثة بنظام HFD مع إعطاء 28 مل من مزيج الثوم والليمون عن طريق الفم، والمجموعة الأخيرة بنظام HFD مع ممارسة السباحة لمدة 30 دقيقة يوميًا. تم قياس وزن الجسم ونسبة الدهون في الدم ومستويات AST و ALTو ALPفي الدم، بالإضافة إلى تقييم التغيرات النسيجية المرضية في الكبد. أشارت جميع النتائج إلى فقدان وزن الجسم وانخفاض كبير في معايير اختبار وظائف الكبد ومستويات الكوليسترول والدهون الثلاثية و LDLفي الدم وزيادة HDL في مجموعات التمارين والعلاج، إلى جانب الحفاظ على بنية الكبد النسيجية مقارنة بالكبد الدهني في المجموعة الثانية. خلصت الدراسة إلى أن السمنة يمكن أن تسبب تشحم الكبد وتدهور وظائفه، مصحوبة بارتفاع نسبة الدهون في الدم. من ناحية أخرى، أدت السباحة وإعطاء مزيج من الثوم والليمون إلى تحسن كبير في نسبة الدهون في الدم وانخفاض الكوليسترول والدهون الثلاثية و LDLومعايير اختبار وظائف الكبد وتحسين الآفات النسيجية المرضية في الكبد.

الكلمات المفتاحية: السمنة, الكبد الشحمي, التمارين, الثوم.