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The Influence of *Allium sativum* and *Syzygium aromaticum* Powder Supplementation on Biochemical Markers, Oxidative Stress, and Histopathological Features of Albino Rats' Liver and Kidney Functions

Manal R. Bakeer[1]*, Esmat Seifelnasr[1], Marina M. Ibrahim[1], Eman I. Hassanen[2] and Aya Kh. Hendawy[1]

[1]*Physiology Department, Faculty of Veterinary Medicine, Cairo University, Giza 12211, Egypt.*

[2]*Department of pathology, Faculty of Veterinary Medicine, Cairo University, Egypt.*

Abstract

The garlic (*Allium sativum*) and clove (*Syzygium aromaticum*) are widely recognized for their medicinal and antioxidant properties. Thirty adult male Swiss albino rats (average body weight 150 - 170 gm.) divided into three groups and examined for two months. Group 1 (control group) was fed a conventional rat diet, Group 2 was given 5% garlic powder in their usual meal, and Group 3 was given 30 mg/kg BWT of clove powder every day. Biochemical investigation revealed significant decrease in AST levels in supplemented groups compared to the control. Kidney function indicators indicated a substantial decrease in uric acid, creatinine, and urea levels in the garlic-supplemented group, but no significant changes observed in the clove group. Oxidative stress study demonstrated a substantial increase in total antioxidant capacity in the garlic-treated group, as well as a decrease in malondialdehyde (MDA) levels in both the garlic and clove-treated groups. In addition, both treatment groups had considerably enhanced superoxide dismutase (SOD) activity. Histopathological analysis of liver and kidney tissues revealed no structural abnormalities in either group, suggesting no negative impact on organ morphology. These findings highlight the potential of garlic and clove supplementation in enhancing antioxidant defense mechanisms and improving certain biochemical markers of liver and kidney function.

Keywords: Garlic, Clove, Antioxidant, Oxidative Stress, Liver Function, Kidney Function, Superoxide Dismutase, Malondialdehyde, Total Antioxidant Capacity.

Introduction

Considering their high bioactive content and low side effects when compared to manufactured medications, medicinal plants have n extensively studied for their potential to promote health and manage diseases [1]. Garlic (Allium sativum) is a popular medicinal plant recognized for its therapeutic benefits, owing to its high quantity of sulfur-containing compounds such as allicin, diallyl sulfides, and ajoene [2]. These bioactive components exhibit strong antioxidant, anti-inflammatory, antimicrobial, and cardioprotective effects [3]. Garlic has been extensively studied for its potential to support overall health, including its role in protecting liver and kidney function [4]. Research suggests that garlic may help reduce oxidative stress, lower lipid peroxidation, and enhance detoxification processes, which are essential for maintaining the proper function of these vital organs [5] .

Clove (*Syzygium aromaticum*), another powerful medicinal herb, contains bioactive substances such as eugenol, flavonoids, and other phenolic components [6]. These chemicals contribute to clove's powerful antioxidant, anti-inflammatory, and hepatoprotective capabilities. Clove has long utilized to promote digestive health, immunological function, and metabolic balance [7], [8]. Its positive effects on liver and kidney function are due to its capacity to counteract oxidative stress, decrease inflammation, and control enzyme activity involved in detoxification and metabolic balance [9].

Given the importance of the liver and kidneys in general health, researching natural treatments like garlic and cloves is critical for avoiding or

*Corresponding authors: Manal R. Bakeer, E-mail: manalbaker36@yahoo.com, Tel.: 01095098696
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controlling organ disease. The aim of this study was to assess the effects of garlic and clove on liver and kidney function in male rats. providing valuable insights into their potential protective or therapeutic benefits.

Material and Methods

Animal and experimental design

The Medical Entomology Research Institute in Giza, Egypt, provided us with 30 mature male albino rats weighing between 150 and 170 grams. The animals were kept in standardized cages with natural ventilation and maintained at a regulated temperature of 23 ± 3°C, with a 12-hour light/dark cycle. To achieve adequate acclimation to the laboratory setting, the rats were given free access to food and water for two weeks. They were given a nutritionally balanced conventional rat meal that included carefully measured components such as L-cystine (3 g/kg), casein (200 g/kg), cellulose (50 g/kg), sucrose (500 mg/kg), corn starch (150 g/kg), corn oil (50 g/kg), vitamin mix V10001 (10 g/kg), and mineral mix S10001.

The study was carried out in Cairo University's Physiology Department and was authorized by the Faculty of Veterinary Medicine's Institutional Animal Care and Use Committee (IACUC) (Approval Number: (Vet Cu110520251111). The rats were randomly assigned to three experimental groups, each with ten individuals (n = 10):

• Group 1 (Control) received simply the regular rodent diet.

• Group 2 (Garlic Supplement Group) was fed a conventional diet plus 5% garlic powder.

• Group 3 (Clove Supplement Group) had a conventional diet that included 30 mg/kg body weight of clove flower powder each day.

The investigation lasted two months and was designed to investigate the effects of garlic and clove supplementation on kidney and liver function.

Preparation of Garlic and Clove powder

A local market (Harraz, Egypt) supplied the garlic powder and dried clove blossoms. For Group 2, 5% garlic powder was added to the usual rat diet (50 g/kg diet). Meanwhile, dried clove flowers were coarsely crushed using a grinding machine and given with ration to Group 3 at a dose of 30 mg/kg body weight every day.

Sampling

After anesthesia with ketamine (90 mg/kg body weight) and xylazine (10 mg/kg body weight), fasting blood samples were taken from each rat using the orbital sinus puncture procedure. Blood samples were extracted without anticoagulants, allowed to clot, and the resultant serum was kept at -20°C for biochemical analysis. Following the collection of blood samples, all rats were humanely euthanized under anesthesia.

Biochemical parameters

Liver Function Tests

Serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALp), albumin, and total bilirubin were quantitatively measured using commercial diagnostic kits obtained from the Biodiagnostic Company (Dokki, Giza, Egypt), following the manufacturer's protocols.

Renal Function Tests

Serum creatinine, urea, and uric acid concentrations were determined spectrophotometrically using standard commercial kits (Biodiagnostic Company, Dokki, Giza, Egypt). The analysis of creatinine, urea and uric acid were based on the colorimetric method described by Allain et al. (1974).

Oxidative Stress and Antioxidant Capacity

Serum levels of superoxide dismutase (SOD) Catalog No. SD 2521, total antioxidant capacity (TAC) Catalog No. TA 2513, and malondialdehyde (MDA) Catalog No. MD 2529. All measured parameters were assessed using commercial colorimetric kits obtained from the Biodiagnostic Company (Dokki, Giza, Egypt), according to the manufacturer's instructions. These biomarkers were used to evaluate the oxidative status and antioxidant defense in the serum of experimental animals.

Histological analysis

Samples from the liver and kidney were collected 60 days after the experiment began and stored in 10% neutral buffered formalin. Samples were treated by gradually increasing the concentration of ethanol solution and xylene before embedding them in paraffin wax. They were then cut into 4.5 µm thick slices with a conventional microtome. Hematoxylin and Eosin (H&E) staining was then used on each slice. The microscopic inspection was done using an Olympus light microscope, and photographs were taken using an Olympus digital camera connected to the CellSens Dimension program. [10]. The histological analysis was carried out at the Pathology Department, Faculty of Veterinary Medicine, Cairo university.

Statistical analysis

The data was analyzed using SPSS statistical software, version 11.0 (SPSS, 2001, Chicago, IL, USA). Results are reported as mean ± standard error of the mean. A one-way ANOVA was used to identify significant differences ($p \leq 0.05$) across groups, with statistical significance set at $p < 0.05$.

Results

Impact of Garlic and Clove Powder Supplementation on Biochemical Markers of Liver Functions.

Figures (1A), (1B), (1C), and (1D) show that garlic and clove supplementation did not significantly affect ALT, ALP, albumin, or total bilirubin levels. However, AST levels were much lower than compared of the control group.

Garlic and Clove Powder Supplementation and Biochemical Markers of Kidney Function.

Figures (2A), (2B), and (2C) reveal that the garlic-supplemented group had a substantial reduction in creatinine, uric acid, and urea levels, but the clove-supplemented group had no significant differences from the control group.

Impact of clove and garlic on oxidative stress and antioxidant parameters.

Figure 3. Garlic treatment resulted in a considerable increase in overall antioxidant capacity when compared to other experimental groups. In terms of malondialdehyde concentration, groups treated with cloves and garlic had considerably lower MDA concentrations than the control group. In terms of superoxide dismutase activity, the groups treated with clove and garlic had a substantial increase compared to the control group.

The Effects of Garlic and Clove Powder Supplementation on Histopathological analysis.

No changes were detected in liver and kidney sections across groups. Liver showed normal histological architecture of hepatic cords, central veins, and portal triads, whereas kidneys displayed typical histological architecture of renal tubules, glomeruli, and interstitial tissue Figure (4).

Discussion

The current study discovered that garlic supplementation significantly reduced AST levels while having no significant influence on ALT or ALP levels. These findings are similar to those of [11], who investigated the effects of garlic supplementation on liver enzymes. ALT and AST values are often regarded as reliable measures of liver function. Research Yu et al [12] found that garlic can prevent liver cell breakdown and lower ALT and AST levels in people with non-alcoholic fatty liver disease**.** Supporting this, Shojaei-Zarghani et al [13] reported that garlic supplementation effectively reduced serum liver enzyme levels. Furthermore, a study by [14] discovered that a 12-week supplementation with 1600 mg/day of garlic powder improved hepatic steatosis and decreased liver enzyme levels. Another study found that garlic extract therapy retained liver structural integrity while improving histopathological alterations, as shown by substantial decreases in AST, ALT, and ALP levels. Garlic's preventive effect is due to its capacity to reduce oxidative damage induced by free radicals in the liver [15]. Conversely, Noori [16] discovered that giving garlic extract at a dosage of 500 mg/kg body weight for 13 days resulted in a substantial rise in ALT levels. This rise is thought to occur because large dosages of garlic can influence ALT, a liver-specific enzyme, disrupting the plasma membrane of liver cells and therefore increasing its blood levels.

In the current study, clove powder supplementation had no significant effect on ALT or ALP levels, however it did reduce AST levels significantly. These findings are consistent with those reported by Oladokun et al [17], who found that clove essential oil dramatically reduced AST levels in broilers. Similarly, [18] discovered that clove supplementation did not affect AST or ALT levels in broilers. In contrast, research by [19] found that clove powder supplementation at 5% and 6% significantly elevated AST levels, suggesting that large dosages of clove may have a deleterious influence on liver function. Furthermore, Naser et al. [20] found that clove powder supplementation in broilers had no significant effect on liver enzymes, except for ALP, which rose considerably when compared to the control group. In general, elevated AST and ALT levels indicate hepatocellular deterioration. As a result, the drop in AST or ALT levels may have been interpreted as proof of garlic powder and clove powder supplementation's hepatoprotective properties.

The current findings showed that the inclusion of garlic powder at a dose of 5% of the diet did not significantly alter total bilirubin or albumin levels. Research done by [21] indicated that administering garlic extract at a dosage of 300 mg/kg did not affect total bilirubin or albumin levels. However, at dosages of 600 and 1,200 mg/kg, albumin concentration increased significantly. Another research found that ingesting 500 mg/kg body weight caused a substantial increase in bilirubin levels [16]. Bilirubin is a key hemoglobin breakdown product, and elevated bilirubin levels can be caused by poor liver excretion. Several investigations have demonstrated that modest dosages of garlic boost antioxidant effects; however, excessive doses negate these benefits and produce morphological alterations in liver function [16].

The study found that adding garlic powder to rats' food significantly reduced their creatinine, uric acid, and urea levels ($p < 0.05$). These findings are consistent with those of [22], who found that garlic extract enhanced kidney function in diabetic rats. They discovered that garlic significantly reduced creatinine, uric acid, and urea levels. Similarly, Yahya et al [23] observed that garlic protects the kidneys, lowering urea, creatinine, and uric acid levels. Another study discovered that giving garlic

extract to male rats reduced creatinine and urea levels while creating a non-significant rise in uric acid. The current study found that supplementation with clove powder had no significant effect on creatinine, urea, or uric acid levels when compared to the control group. In contrast, Abbas et al [25] discovered that giving clove extract (300 mg/kg) for 4 weeks resulted in a decrease in creatinine, urea, and uric acid levels. This decrease might be attributable to the secondary metabolites in clove syrup, which have antioxidant properties [25]. These variations in clove's effects might be attributed to variances in preparation or intake length.

Garlic powder consumption has been linked to elevated TAC and SOD activities. For example, one study found that broiler chicks fed diets supplemented with 0.75 g/kg of garlic powder had significantly higher levels of TAC and SOD than the control group [26].

MDA is a biomarker for lipid peroxidation and oxidative stress. Studies have revealed that garlic powder treatment dramatically reduces MDA levels. The control group had the highest MDA concentrations in the study, but groups given garlic powder supplementation had reduced MDA levels, demonstrating garlic's ability to inhibit lipid peroxidation [27].

These findings highlight the effect of garlic powder in improving antioxidant defenses (elevating TAC and SOD) and lowering oxidative stress indicators like MDA. Incorporating garlic powder into your diet may provide antioxidant protection. In compared to the control, the clove-treated group increased only SOD and decreased MDA. Clove (*Syzygium aromaticum*) is known for its powerful antioxidant capabilities, particularly the capacity to increase superoxide dismutase (SOD) activity. SOD is an important enzyme that protects cells from oxidative stress by facilitating the dismutation of superoxide radicals into oxygen and hydrogen peroxide [28]. A study conducted by [29] found that administration of clove (*Syzygium aromaticum*) has been recognized for its potent antioxidant properties, particularly in reducing malondialdehyde (MDA) levels, a biomarker of lipid peroxidation and oxidative stress.

The absence of pathological lesions in the liver and kidneys suggests that neither garlic powder nor clove powder exhibited cytotoxic effects at the tested concentrations. Garlic, which is abundant in organosulfur compounds such as allicin, has been shown to support liver function and reduce oxidative stress-related damage [30]. Likewise, clove, with eugenol as its primary bioactive component, has demonstrated protective properties by minimizing lipid peroxidation and modulating inflammatory pathways [31], [32]. Research by Lestari and M. Rifai [33] discovered that providing a single-bulb garlic oil extract to mice for 28 days caused no adverse histological alterations in their livers or kidneys, suggesting that it is safe for long-term usage. Similarly, Fowotade et al [34] found that moderate dosages of garlic extract (250-350 mg/kg body weight/day) had no adverse effects on the liver and kidneys of Wistar rats, but larger doses (>400 mg/kg body weight/day) caused structural changes in these organs. These data suggest that prolonged low-dose garlic consumption may give cytoprotective effects against oxidative stress [35], but excessive consumption might cause considerable cellular damage. Similarly, Ali et al [36] found that the eugenol-rich fraction (ERF) of clove increased liver cell growth and reduced oxidative stress in liver cirrhosis patients. Furthermore, Elgharib et al [37] discovered that when taken at 200 mg/kg body weight, clove protected the liver and kidneys by lowering damage caused by cadmium-induced hepatorenal toxicity.

Conclusion

The findings of this investigation indicate that garlic and clove powder supplementation can positively influence biochemical markers and antioxidant parameters without causing histopathological alterations in liver and kidney tissues. Garlic supplementation significantly improved kidney function markers by reducing creatinine, uric acid, and urea levels. Garlic and clove supplementation successfully reduced oxidative stress, as seen by lower MDA levels and enhanced SOD activity. The findings indicate the potential health benefits of these natural supplements in mitigating oxidative stress-related damage while maintaining normal liver and kidney structure and function. Further research is needed to better understand the underlying molecular processes and possible therapeutic implications of garlic and clove supplements in illness prevention and management.

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Author Contributions.

Manal R. Bakeer: Led investigation, visualization, supervision, review, and editing, as well as methodology development and conducted the final revision. Aya KH Hendawy: Contributing to methodology and data curation. E.Seifelnasr: Led investigation, visualization, and supervision,

Marina M. Ibrahim: Contributed to drafting the original manuscript and developing the methodology

Eman I Hassaneen: Contributing to methodology and histopathology.

Ethical of approval

The study was carried out in Cairo University's Physiology Department and was authorized by the Faculty of Veterinary Medicine's Institutiona Animal Care and Use Committee (IACUC) (Approval Number: (Vet Cu110520251111).

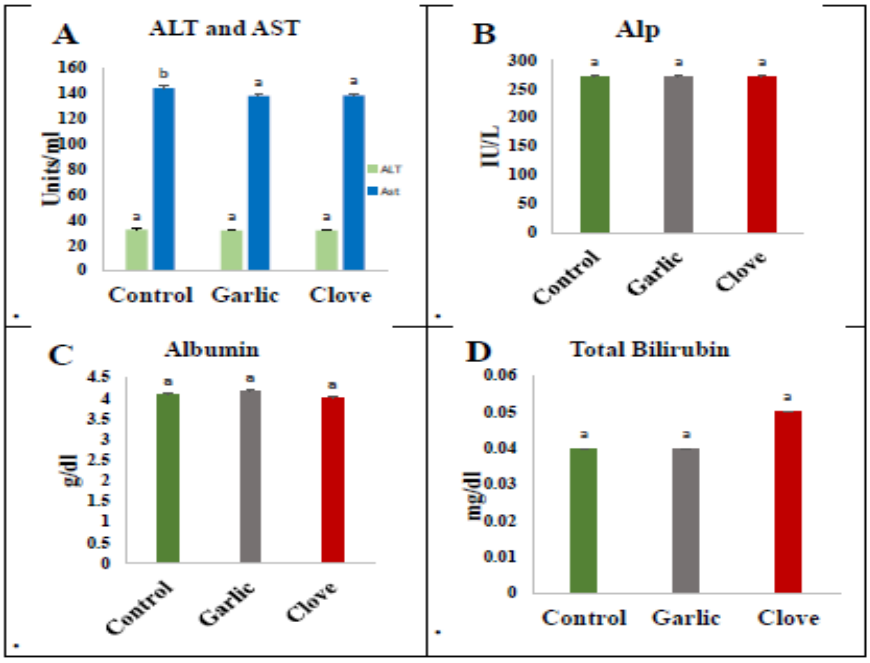

Fig. 1. Shows how garlic and clove supplementation affected ALT, AST, ALp, Albumin, and Total Bilirubin levels in adult male rats. Data is shown as mean ± SE (n = 5 rats per group). Means with different letters differ substantially (p≤0.05).

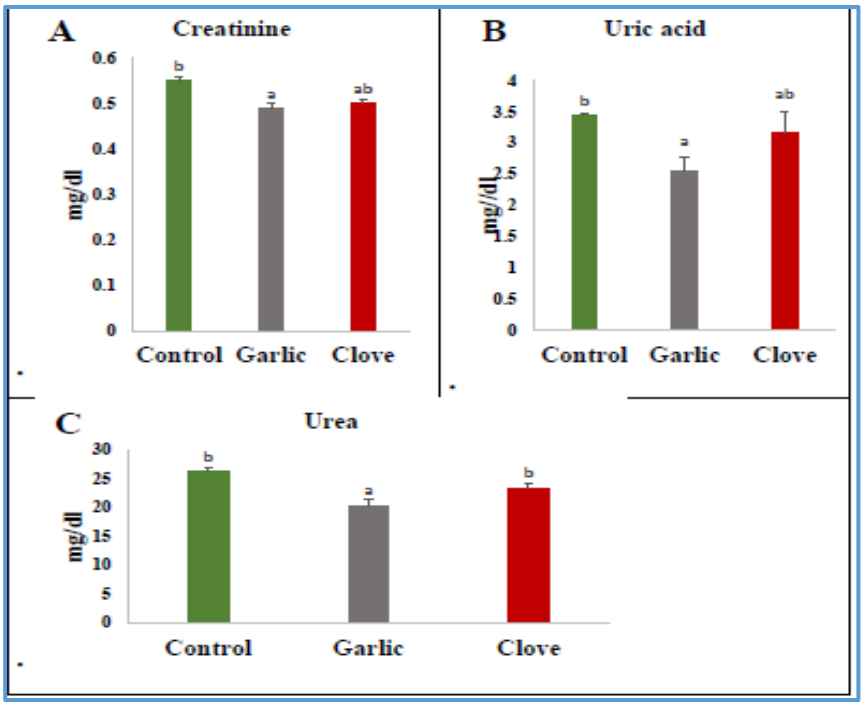

Fig. 2. Depicts the effects of garlic and clove supplementation on Creatinine, Uric acid, and Urea levels in adult male rats. Data is shown as mean ± SE (n = 5 rats per group). Means with different letters differ substantially p≤0.05).

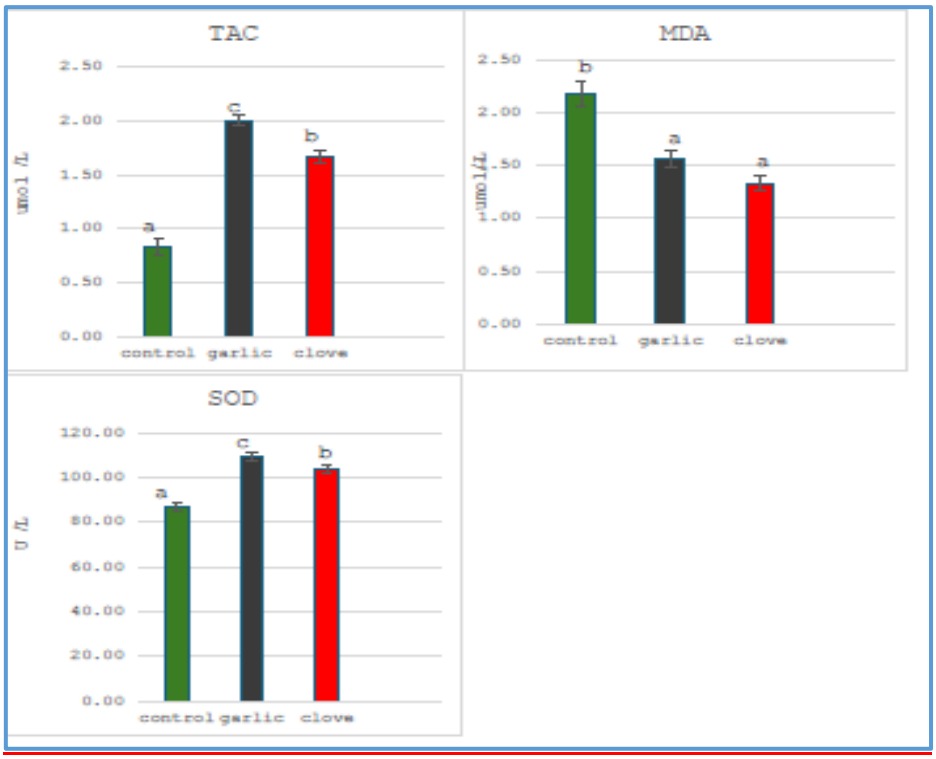

Fig. 3. The impact of clove and garlic on certain antioxidant parameters, (A): Total antioxidant capacity (TAC), (B): MDA levels, and superoxide dismutase activity (SOD). Each value is reported as mean ± SE. (n = 5 rats per group). Bars with different letters in the same parameter show substantial differences.

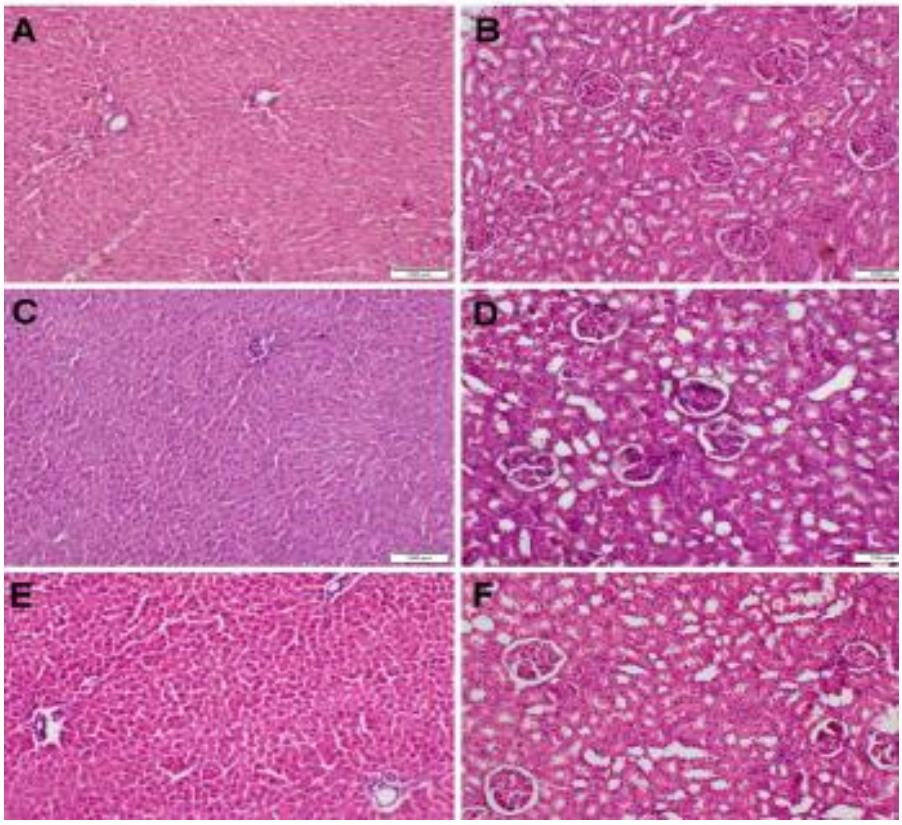

Fig. 4. Garlic and clove powder supplementation's effects on liver and kidney histopathology. Microscopic images of liver and kidney slices stained with H&E from various experimental groups. (A-B) control group. (C-D) Group that received garlic. (E-F) The clove receiving group. All of the groups had a normal histological structure. (Scale bar 100 um corresponding to magnification power x 10)

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تأثير مكملات الثوم ومسحوق القرنفل العطري على المؤشرات الكيميائية الحيوية، والإجهاد التأكسدي والسمات النسيجية المرضية لوظائف الكبد والكلى لدى الجرذان البيضاء

منال رمضان بكير[1] ، عصمت سيف النصر[١] ، مارينا منير إبراهيم[1] ، ايمان حسينين[2] وآية خليل هنداوي[1]

[1] قسم الفسيولوجيا، كلية الطب البيطري، جامعة القاهرة، الجيزة ١٢٢١١، مصر
[٢] قسم الباثولوجيا، كلية الطب البيطري، جامعة القاهرة، الجيزة ١٢٢١١، مصر

الملخص

يُعرف الثوم والقرنفل على نطاق واسع بخصائصهما الطبية ومضادات الأكسدة. قُسّم ثلاثون جرذًا سويسريًا أبيض بالغًا (بمتوسط وزن ١٥٠-١٧٠ جم) إلى ثلاث مجموعات، وفُحصت لمدة شهرين. تم تغذية المجموعة ١ (مجموعة الضبط) على نظام غذائي تقليدي للفئران، وأعطيت المجموعة ٢ مسحوق الثوم بنسبة ٥٪ في وجبتها المعتادة، وأعطيت المجموعة ٣ ٣٠ ملغ / كغ من وزن الجسم من مسحوق القرنفل يوميًا. أظهر الفحص الكيميائي الحيوي انخفاضًا كبيرًا في مستويات AST في المجموعات المكملة مقارنةً بالمجموعة الضابطة. أشارت مؤشرات وظائف الكلى إلى انخفاض كبير في مستويات حمض البوليك والكرياتينين واليوريا في المجموعة التي تناولت مكملات الثوم، ولكن لم تُلاحظ أي تغييرات كبيرة في مجموعة القرنفل. أظهرت دراسة الإجهاد التأكسدي زيادة كبيرة في إجمالي قدرة مضادات الأكسدة في المجموعة المعالجة بالثوم، بالإضافة إلى انخفاض في مستويات مالونديالدهيد (MDA) في كل من مجموعتي الثوم والقرنفل المعالجة. بالإضافة إلى ذلك، أظهرت كلتا المجموعتين المعالجتين نشاطًا معززًا بشكل كبير لإنزيم أكسيد الفائق .(SOD) لم يكشف التحليل النسيجي لأنسجة الكبد والكلى عن أي تشوهات هيكلية في أي من المجموعتين، مما يشير إلى عدم وجود تأثير سلبي على مورفولوجيا الأعضاء. تُسلّط هذه النتائج الضوء على إمكانات مكملات الثوم والقرنفل في تعزيز آليات الدفاع المضادة للأكسدة وتحسين بعض المؤشرات الكيميائية الحيوية لوظائف الكبد والكلى.

الكلمات الدالة: الثوم، القرنفل، مضاد للأكسدة، الإجهاد التأكسدي، وظائف الكبد، وظائف الكلى، إنزيم أكسيد الفائق ديسميوتاز، مالونديالدهيد، السعة الكلية لمضادات الأكسدة.