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INNOVATIVE STRATEGIES IN THE USE OF VITAMIN E AND ROSEMARY AS ANTIMICROBIAL AGENTS FOR ENHANCING THE SAFETY AND PRESERVATION OF MINCED POULTRY MEAT DURING REFRIGERATION

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Abstract

This study evaluated the bactericidal efficacy of vitamin E and rosemary extracts in minced poultry meat stored at a refrigeration temperature of 4° C. Six distinct interventions were assessed: a control group (Group 1), three treatments involving thighs from broilers supplemented with vitamin E (300 mg/kg) (Group 2), and thighs from broilers fed rosemary leaf powder at two levels of 0.5% and 1% (Groups 3 and 4). Groups 5 and 6 utilized thighs from the control group that were sprayed with 0.5% of aqueous and alcoholic rosemary extracts, respectively. The study showed that incorporating rosemary and vitamin E extracts into minced chicken significantly reduced colony-forming units (CFU) of total bacteria, *E. coli*, and psychrophilic bacteria relative to the control group. The alcoholic rosemary extract exhibited the most significant reduction in bacterial counts, followed closely by the aqueous rosemary extract and rosemary leaf powder at

the 1% dosage. There were no significant differences in reductions in *E. coli* and psychrophilic bacteria between the vitamin E therapy and the control group. The findings indicate that rosemary extracts, especially in alcoholic form, function as potent antibacterial agents that preserve the quality of minced fowl during storage, potentially extending shelf life and reducing spoilage. Research suggests that utilizing natural preservatives like rosemary and vitamin E may effectively replace synthetic compounds in scenarios where maintaining consistent refrigeration is challenging. Natural preservatives can improve the safety and quality of meat products, and in meeting consumer demand for healthier and more sustainable food options.

Keywords: Vitamin E, Rosemary, Spoilage bacteria, Broilers, Dietary treatment.

ابتكار استراتيجيات حديثة باستخدام فيتامين E وإكليل الجبل كمضادات ميكروبية لتعزيز سلامة وحفظ اللحم المفروم للدواجن خلال الخزن بالتبريد

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الخلاصة

هدفت الدراسة إلى تقييم فعالية فيتامين E ومستخلصات إكليل الجبل في مقاومة البكتيريا في لحوم الدواجن المفرومة المخزنة في درجة حرارة 4 مئوية. قيمت ستة معاملات متداخلة تمثلت: مجموعة السيطرة (المجموعة 1)، وثلاث معاملات استخدم فيها أفخاذ فروج اللحم المغذاة على فيتامين E (300 ملغم/كغم) (المجموعة 2)، وأفخاذ فروج اللحم المغذّاة على مسحوق أوراق إكليل الجبل بمستويين مختلفين (0.5% و1%) (المجموعتان 3 و4). اما المجموعتان 5 و6 استخدم فيها أفخاذ فروج اللحم التي رشت بمستخلصات إكليل الجبل المائية والكحولية بنسبة 0.5% على التوالي. وقد أظهرت الدراسة أن دمج مستخلصات إكليل الجبل وفيتامين E في

الدجاج المفروم أدى إلى تقليل وحدات تكوين المستعمرات (CFU) من إجمالي البكتيريا، والإشريكية القولونية، والبكتيريا المحبة للبرودة بشكل كبير مقارنة بمجموعة التحكم. أظهر مستخلص إكليل الجبل الكحولي أكبر انخفاض في أعداد البكتيريا، يليه عن كثب مستخلص إكليل الجبل المائي ومسحوق أوراق إكليل الجبل بجرعة 1٪. أشارت النتائج إلى عدم وجود فروق كبيرة في تقليل البكتيريا القولونية والبكتيريا المحبة للبرودة بين علاج فيتامين E ومجموعة التحكم. تشير النتائج إلى أن مستخلصات إكليل الجبل، وخاصة في شكل كحولي، تعمل كعوامل مضادة للبكتيريا قوية تحافظ على جودة الدواجن المفرومة أثناء التخزين، مما قد يؤدي إلى إطالة العمر الافتراضي وتقليل التلف. تشير الأبحاث إلى أن استخدام المواد الحافظة الطبيعية مثل إكليل الجبل وفيتامين E قد يحل محل المركبات الاصطناعية بشكل فعال في السيناريوهات التي يكون فيها التبريد المستمر أمرًا صعبًا. يمكن للمواد الحافظة الطبيعية تحسين سلامة وجودة منتجات اللحوم، وتلبية طلب المستهلكين على خيارات غذائية أكثر صحة واستدامة.

كلمات مفتاحية: فيتامين E، إكليل الجبل، البكتيريا المسببة للتلف، فروج اللحم، المعاملات الغذائية.

Introduction

Globally, meat is one of the most often used sources of protein (23), leading consumers to consistently seek high-quality and well-preserved meat products. During the handling of animals and processing into meat products, meat spoilage could impact its overall quality. This spoilage could rapidly deteriorate the meat if not properly preserved (2), leading to changes in color, texture, slime, or liquid production. Meat spoilage can be in various forms, including microbial growth, moisture, oxidation, enzymatic autolysis (18). Microbial spoilage is a significant factor that affects the quality of meat as it provides an ideal condition for the growth of microorganisms, including spoilage microorganisms (2). Meat products stored improperly and under unsafe temperatures risk spoilage and quality loss (16 and 19).

Maintaining poultry meat at a temperature of 4°C mitigates or inhibits the proliferation of numerous microorganisms. It helps extend the product's shelf life by diminishing the overall bacterial count, which protects the moisture content in the meat. However, there are various aspects related to the preservation of meat products. Unstable electrical supply is among them as it increases the risk of meat spoiling, making it vital to institute methods to mitigate this problem. Aromatic plants such as rosemary are already in use to preserve meat from bacterial spoilage. Rosemary is one of the most natural and basic preservatives and the most effective medicinal plant for reducing the rate of oxidative stress and bacterial growth in meat products, and extending their shelf life (6 and 22). The promising biological and functional properties of rosemary are due to active compounds such as phenolic, flavonoids, (9 and 25). These active substances are known for their antioxidant, antimicrobial, anti-inflammatory, and anti-cancer properties (13 and 17).

This study aimed to assess the impact of including vitamin E and rosemary into the diet of broilers for preserving broiler meat held at 4° C for 14 days, in comparison to the direct addition of an aqueous and alcoholic extract to minced thigh meat.

Materials and Methods

Experiment Design: The study was conducted at the Central Laboratory, College of Agriculture, University of Anbar. Six Ross 308 chickens, aged 42 days and weighing 2.5 kg, were slaughtered from each treatment, and randomized into treatments with two chickens per replication; thereafter, the complete thigh sections were excised and minced. This research conducted two experimental trials depending on the sort of meat used. The initial study utilized broiler meat from chickens subjected to identical treatments as those in the subsequent trial, which were then slaughtered and stored without supplementation, whereas the second trial employed broiler meat from chickens fed a commercial diet. The aqueous extract of rosemary leaves was produced using water, following the procedure outlined by (10), while their alcoholic extract was obtained utilizing the method described by (11). The preliminary trial had four treatments: a control group, vitamin E at 300 mg/kg, rosemary leaf powder at 0.5%, and rosemary leaf powder at 1%. The second experiment comprised two treatments: the first treatment (control) was identical to that in the first trial, while the second and third treatments involved aqueous rosemary leaf extract at 0.5% per kg and alcoholic dried rosemary leaf extract at 0.5% per kg, respectively. The meat samples were preserved in cork dishes, sealed in polyethylene bags, and refrigerated at 4° C for one week. The aging duration for samples was performed over three intervals of 1, 7, and 14 days.

Total Bacterial Count: The approach used for estimating psychrophilic bacterial populations is indicated by (5). Bacterial counts in the meat were conducted by inoculating 0.1 ml from the appropriate dilution into sterile Petri dishes and then overlaid with plate count agar (PCA); the plates were then incubated in a JSR type JSGI-50T incubator at 7° C for 24 hours. Following storage, the colony count was determined for each plate utilizing a colony counter. The total bacterial count per gram was determined by multiplying the mean number of colonies by the inverse of the dilution factor (24). This approach was also utilized by (4), wherein 0.1 ml of the suitable dilution was transferred into sterile Petri dishes. The PCA medium was distributed to entirely cover the plate. The plates were then incubated at 7-10° C, the colonies enumerated, and the quantity assessed per gram on day zero and throughout the storage intervals.

Total Coliform Count: The total coliform bacteria count was estimated as indicated by (12) using a petri dish and by the pouring plate method. This was done by placing 1 ml of appropriate dilution from the thigh meat sample onto a sterile Petri dish, followed by the addition of 15-20 ml of the culture medium MacConkey agar (Sigma-Aldrich Co. St. Louis, MO). The dish was stirred to mix the sample with the medium by turning it clockwise and vice versa. The dishes were left until the culture medium solidified and then incubated upside down in the incubator at a temperature of 37° C for 24 hours. After storage, the total number of coliform bacteria per gram was counted by multiplying the average number of colonies by the reciprocal of the dilution factor.

Statistical analysis: The statistical analysis of all traits was conducted in one direction (One Way Analysis). The trend included the effect of experimental

coefficients on the studied traits by following the General Linear Model and using the ready-made SAS statistical program version 9 (SAS Count) (7). The significant differences between the means were tested using Duncan's polynomial test (8) at the P=0.05 significance level.

Results and Discussion

Total Bacterial Count: Treatment of the minced chicken thigh meat with 0.5% alcoholic dried rosemary leaf extract resulted in a significant reduction ($P < 0.05$) in total bacterial count compared to the control and vitamin E treatment, with no significant differences seen among other treatments (Figure 1). No significant differences were observed between the control treatment and each of the treatments. The results indicated a marked reduction ($P < 0.05$) in the total bacterial count during the 7 and 14-day storage periods for all experimental treatments compared to the control.

The most substantial bacterial reduction was observed in minced chicken thigh meat treated with 0.5% alcoholic dried rosemary leaf extract, compared to the control and other treatments. The notable bacterial reduction ($P < 0.05$) occurred with rosemary leaf at 1% and aqueous rosemary leaf at 0.5% in comparison to the control treatment. No significant changes were identified in bacterial decrease among samples treated with vitamin E and rosemary leaf at a concentration of 0.5%. Furthermore, there was a significant decrease in the total bacterial count for the rosemary leaf treatment at 0.5% compared to the vitamin E treatment over a period of 14 days.

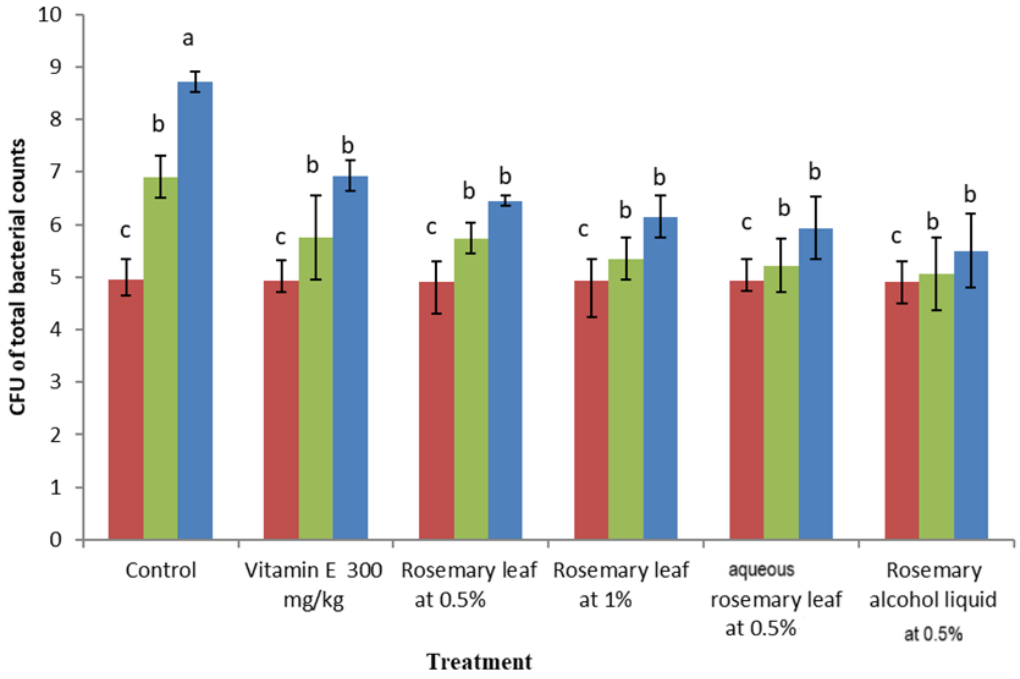

Figure 1: Colony-forming units (CFU) of total bacterial count in meat under different treatments and storage times. CFU of total bacterial count: red (after the first day of storage); green (after seven days storage); and blue (after fourteen days storage). Different letters on the axes are the significant differences between treatments in the CFU.

Figure 1 shows that including 0.5% alcoholic dried rosemary leaf extract into minced chicken thigh meat produced the largest reduction (P = 0.05) in total bacterial count during the 7 and 14-day storage periods, compared to the control and other treatments. Furthermore, adding both aqueous and alcoholic rosemary leaf extracts at 0.5% concentration to minced chicken thigh meat significantly decreased (P < 0.05) total bacterial numbers when compared to treatments involving the administration of 300 mg/kg of vitamin E and those supplemented with rosemary leaf powder at 0.5% and 1% kg forage, respectively. The treatments of minced meat for chickens supplemented with 300 mg/kg of vitamin E and 0.5% and 1% rosemary leaf powder considerably reduced (P<0.05) total bacterial count compared to the control.

Total Coliform Count (*E. coli*): Figure 2 shows the results of adding vitamin E and rosemary to broiler rations in comparison to adding aqueous and alcohol extract of rosemary directly to minced thigh meat. There were no significant differences in the number of *E. coli* on the first day of storage period between treatments. Thus, it is clear from the results that there was a significant increase in the *E. coli* counts in the control treatment compared with the experimental treatments.

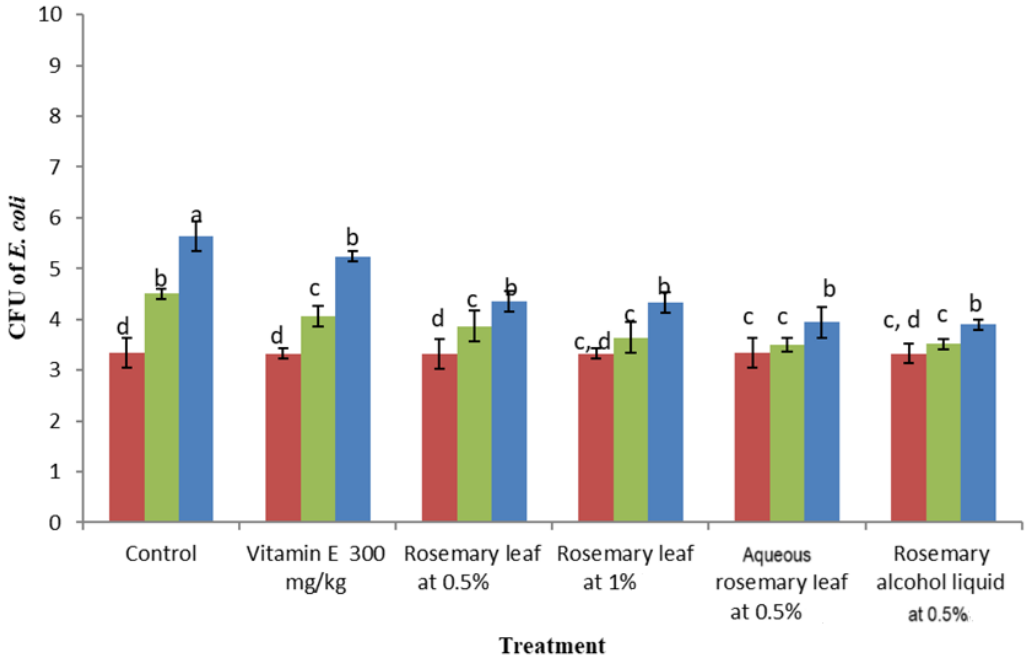

Figure 2: Colony-forming units (CFU) of E. coli in meat under different treatments and storage times. CFU of E. coli count: red (after the first day of storage); green (after seven days storage); and blue (after fourteen days storage). Different letters on the axes refers to the significant differences between treatments in the CFU.

The results demonstrate a significant decrease in *E. coli* count over the seven-day storage period, favoring the 0.5% aqueous rosemary leaf extract and the 0.5% alcoholic dried rosemary leaf extract compared to the control and other treatments. This was followed by the treatments of rosemary leaf powder at 1%, rosemary leaf powder at 0.5%, and vitamin E at 300 mg/kg. The aqueous and alcoholic extract treatments showed significant decrease in *E. coli* compared to the other treatments, with no significant difference between them. Following that, two treatments of minced thigh meat were administered with powdered rosemary leaves at

concentrations of 0.5% and 1%, compared to treatment with the vitamin E 300 mg/kg feed. This similarly showed significant reductions in *E. coli* count relative to the control treatment. The results revealed no significant variations in the efficacy of the aqueous and alcoholic extracts against *E. coli*, since there were no significant differences between them.

Adding 0.5% alcoholic and aqueous rosemary leaf extract to minced chicken thigh meat had the most significant decrease in the CFU of *E. coli* during the of 7 and 14-day storage periods compared with the control and other treatments. Additionally, the minced meat treatment of chickens fed on rosemary leaf powder at 0.5 and 1% feed, respectively, significantly reduced the CFU of *E. coli* compared with the control and treatment with vitamin E 300 mg/kg feed. This also significantly decreased the CFU of *E. coli* bacteria compared with the control treatment.

Psychrophilic Bacteria: Figure 3 shows the effects on psychrophilic bacteria growth of including vitamin E and rosemary to the broiler diets and adding aqueous and alcoholic rosemary leaf extracts directly to minced thigh meat refrigerated for 14 days. No significant differences were observed in the bacteria counts on the first day of storage period between treatments. However, there was a significant increase in the CFU of the bacteria in the control compared to the experimental treatments.

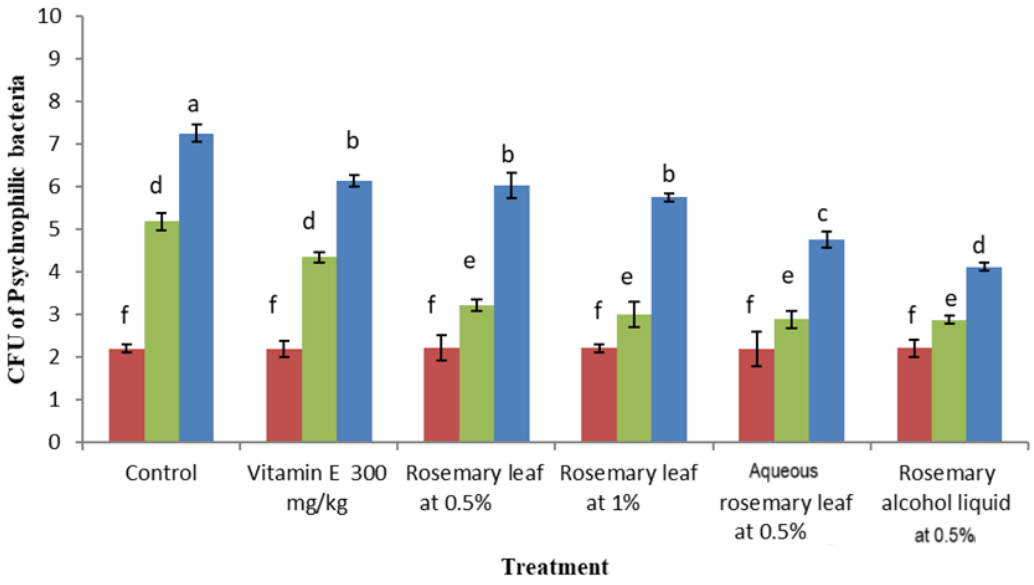

Figure 3: Colony-forming unit (CFU) of psychrophilic bacteria in meat under different treatments and storage times. CFU of psychrophilic bacterial count: red (after the first day of storage); green (after seven days storage); and blue (after fourteen days storage). Different letters on the axes refers to the significant differences between treatments in the CFU.

The results indicate a significant decrease in the CFU of psychrophilic bacteria during the seven-day storage period, favoring the treatments of aqueous rosemary leaf at 0.5% and rosemary alcohol liquid when compared to the control and other treatments. The results from treatments with rosemary leaf at 1% and 0.5%, and vitamin E at 300 mg/kg show no significant differences between the aqueous and alcoholic extracts in countering psychrophilic bacteria. Furthermore, there was a notable increase in the levels of psychrophilic bacteria in the control when compared

to the experimental treatments. The treatment with the alcoholic extract demonstrated a significant decrease in psychrophilic bacteria count, followed by the aqueous extract treatment, and subsequently the minced thigh meat from chickens fed rosemary leaf powder at 0.5% and 1%, respectively. This was in comparison to the vitamin E treatment at 300 mg/kg feed, which also significantly reduced psychrophilic bacteria CFU when compared to the control treatment.

For the 7 and 14-day storage, the minced meat treatments of chickens fed rosemary leaf powder at 0.5% and 1% feed, respectively resulted in significantly lower numbers of psychrophilic bacteria. The control treatment and treatment with vitamin E 300 mg feed also showed significant declines in the bacteria numbers. It can be concluded that adding 0.5% alcoholic and aqueous rosemary leaf extract to minced chicken thigh meat produced the most significant decrease in the number of psychrophilic bacteria.

Rather than having a significant increase in bacterial CFU through prolonged cryopreservation durations, the inhibition of colonic bacteria, which are categorized as meat spoilage bacteria, was achieved by using plant extracts abundant in bacterial-inhibiting bioactive compounds. This was especially evident with the use of rosemary extracts rich in phenolic compounds. Non-volatile compounds such as carnosine, carnosol, rosmanol, rosmariquinone, rosmaridiphenol, rosmadial, 12-methoxyeamosic, epi, and iso which were tested in rosemary extract for meat applications, demonstrated good antioxidant activity in mitigating lipid oxidation and antibacterial effects (20).

The study by (21) examined the impact of incorporating rosemary extract in varying concentrations (18.6 mg/kg, 480 mg/kg) to assess physical and chemical parameters, including color, TBA index, sensory evaluation, and bacterial count at intervals of 0, 30, 60, 120 days for frozen chicken burgers. The results indicated that rosemary extract did not influence the color or the physical and chemical parameters of the chicken burgers. After 120 days at -18° C, the rosemary extract sample at 480 mg/kg concentration exhibited comparable indicators to BHA at 20 mg. However, sensory evaluation revealed no significant differences between treatments throughout the storage duration, as the 480 mg/kg rosemary extract effectively substituted the industrial antioxidant BHA at 20 mg/kg in chicken burgers. Consequently, the preservation of four-month frozen storage is assured without altering sensory evaluation and bacterial development, as demonstrated by (1, 3 and 15) on the aqueous and alcoholic extracts of rosemary, basil, and mint. Adding 3% of these extracts at a temperature of 4° C for 14 days enhanced the quality characteristics of pomegranate chicken. Moderate ethanolic rosemary extract (82.09 mg/ml), in comparison to BHT (95.86 mg/ml), demonstrates the potential of rosemary extracts as a natural source of antioxidants and preservatives to prolong the shelf life of berries. This effectively inhibits the growth of pathogenic microorganisms, particularly when the ethanolic extract is stored under cold conditions, thereby offering consumers healthier chicken meat.

Another study indicated that dried rosemary extract in chicken broiler exhibited significant antioxidant capacity, inhibiting 48.29% of fat oxidation over 21 days of storage at 4° C, compared to the control, which showed a reduction in

malondialdehyde levels, as confirmed by the researcher regarding the rosemary extract. Ethanolic rosemary leaf extract exhibits significant antioxidant and antibacterial properties in chicken burgers, likely attributable to the robust antioxidant and bacteriostatic activities of phenolic compounds, including gallic acid, catechin, coumaric acid, ferulic acid, cinnamic acid, quercetin, and kaempferol. The efficacy of these compounds is enhanced under refrigeration, suggesting that rosemary leaf extract can be effectively utilized in chicken products.

Conclusions

Rosemary and vitamin E can be employed as antibacterial agents in meat without apprehensions over customer approval. This study found that utilizing antimicrobial agents produced variable antibacterial efficacy against the investigated microorganisms, hence maintaining the quality of minced poultry.

Supplementary Materials:

No Supplementary Materials.

Author Contributions:

Study conception and design: Sofyan M. Farhan and Zahra H. Mohammad; Data collection: Saad I. Yousif, Salwan M Abdulateef, and Nadia Jebril; Analysis and interpretation of results: Osama A. Saeed, and Thafer T. Mohammed; Draft manuscript preparation: Amar A. Al-Azzami. All authors reviewed the results and approved the final version of the manuscript.

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No Data Availability Statement.

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The authors declare no conflict of interest.

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