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The Role of Lemon Grass Oil and Its Nano Emulsion on Shelf Life of Meat Balls and Studying Their Pathological Effects on Rats

Walaa M. Elsherif [1, 2], Dina N. Ali[1*], Abeer H. M. El Hendy[3] and Neven A. Elnisr[3]

[1]*Certified Food Hygiene Lab., Animal Health Research Institute (AHRI), Agriculture Research Center (ARC), Egypt.*
[2]*Dean of Faculty of Health Sciences Technology, New Assiut Technological University (NATU), New Assiut, Egypt.*
[3]*Pathology Department, Agriculture Research Center (ARC), Animal Health Research Institute (AHRI), Assiut Lab. Assiut, Egypt.*

Abstract

LEMONGRASS oil (LGO) is an aromatic herb; a rich source of citric acid, vitamin C, and phenolic compounds used as natural antioxidant and antibacterial agents in food processing but because of its distinctive citrus aroma it was transformed it into a nano emulsion. FTIR, PDI and TEM were used for nano emulsion characterization and we compared its antioxidant and antibacterial activity on sensory, chemical (pH, thiobarbituric acid reactive substance "TBARS and total volatile nitrogen "TVN") and microbiological (aerobic plate count "APC", total coliform count, total yeast and mold count and psychrotrophic count) quality of meat balls during cold storage at 4º C. The effects were studied. Sensory analysis indicated that 0.5% of lemon grass nano emulsion was the best concentration while chemical and microbiological analyses indicated significant advantages in using lemon grass and its nano emulsion at 1% concentration. In addition, we investigated the biochemical and histopathological effects of lemongrass oil extract and its nano emulsion post oral administration in vivo, and the results revealed that the lower concentration of 0.5% of lemongrass nano emulsion can be considered relatively safer than the higher concentration of 1% on glandular stomach, liver and kidney of tested rats.

Keywords: Lemongrass oil; nanoemulsions; meat balls, rats.

Introduction

Food safety is a major concern worldwide due to the challenges posed by microbial pathogens, toxins and the deterioration of food due to biochemical changes. Hence, materials with antibacterial and antioxidant properties have been widely studied for their application to ensure food safety [1].

Lemongrass oil (LGO) is a natural compound known for its rich composition of bioactive substances. It holds potential for various applications, including antibacterial, antifungal, anthelmintic uses, food preservation, agricultural practices, and animal health. Its ability to benefit normal cells while exhibiting anticancer properties suggests it could offer promising avenues for cancer treatment. However, further in-depth studies, both in vitro and in vivo, are necessary to confirm the antioxidant and anticancer mechanisms associated with lemongrass oil components [2].

Consumer awareness of the risks linked to synthetic preservatives, particularly their adverse health effects, has fueled interest in natural antioxidants and antimicrobials as alternatives to extend the shelf life of meat and meat products [3].

LGO exhibits strong antimicrobial activity against various microorganisms, but its stability and compatibility are often limited. Incorporating essential oils (EOs) into burger mixtures as nanoemulsions can help address these challenges. This approach ensures uniform distribution,

*Corresponding authors: Dina N. Ali, E-mail: Dr.dinaahri@ahri.org Tel.: +20 10 00716673
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enhances bioavailability, and improves stability and anti-aggregation properties. The antimicrobial activity of LGO is influenced by the size of the encapsulated emulsion droplets [4].

To address these limitations while preserving the beneficial attributes of LGO, several nano-technological strategies have been explored [5, 6]. Due to their small size, nanomaterials exhibit unique, innovative, and highly desirable properties [7].

Using raw lemongrass essential oil in food products is discouraged due to its adverse effects on sensory characteristics. However, incorporating lemongrass nano emulsions in burger formulations shows promise in inhibiting spoilage bacteria, extending shelf life, and enhancing cooking properties [8].

Although numerous studies have examined various applications of lemongrass essential oil, there is a lack of *in vivo* research to assess the biochemical and pathological effects of lemongrass nanoemulsions in experimental animals [9]. Thus, the present study aims to evaluate the impact of LGO and its nano emulsion on meatball quality while simultaneously investigating the biochemical and pathological effects of oral intake of LGO and its nano emulsions on experimental rats.

Material and Methods

Chemicals

We purchased lemon grass oil (LGO) from National Research Center, Giza, Egypt, Tween* 80 and Tween* 20 from El-Nasr pharmaceutical company" from El- Gomheria incorporation and deionized water was obtained from the Central Laboratory of Veterinary Medicine, Assiut University. Those chemicals were of analytical grade (AR) and classified as generally recognized as safe (GRAS).

Preparation of Nanoemulsion for Lemongrass Oil (LGO) [10]:

Nanoemulsion was prepared by dissolving 20% (v/v) Tween 80 in deionized water (5 ml of Tween 80 in 20 ml deionized water). The mixture was stirred using a magnetic stirrer at room temperature for 10 minutes to achieve homogeneity. Subsequently, 1 ml of LGO (3.8%) was slowly added dropwise at a rate of one drop every 10 seconds using a syringe. Acetic acid (20 µl) was introduced into the solution, and the mixture was stirred continuously for 1 hour using a hot plate stirrer (DAIHAN Scientific Co., Ltd, Korea). The resulting emulsion was then sonicated with an ultrasonic homogenizer (25 kHz, 650 W maximum power, USH650) for 20 minutes. The prepared nanoemulsion was stored at 4°C ± 0.2°C until use.

Characterization of the prepared Nano emulsion:

To analyze the nano emulsion, it was diluted 100-fold with distilled water at 25°C, and the mean droplet size and polydispersity index (PDI) were measured using a Zeta-Sizer (3000HS, Malvern Instruments, UK) at the Unit of Nanotechnology, Animal Health Research Institute, Egypt. The size of the particles was determined using a dynamic light scattering (DLS) instrument, and data were processed with Zeta-Sizer® software (version 7) following the methodology of Baboota et al. [11].

The morphology of the nanoemulsion was examined with a transmission electron microscope (TEM, JEOL-100CX II) at the Electronic Microscope Unit, Assiut University, Egypt. The sample was diluted with deionized water, and a small drop was placed on coated copper grids, negatively stained with uranyl acetate for three minutes, and dried with Whatman filter paper. This preparation method was adapted from Shakeel et al. [12].

Experimental Design:

The study aimed to evaluate the effect of LGO and its nano emulsion on meatball quality. Samples were divided into five groups:

- Control (C): No treatment.
- T1 & T2: Treated with 1% and 0.5% LGO, respectively.
- T3 & T4: Treated with 1% and 0.5% LGO nano emulsion, respectively.

Samples were stored at 4°C and monitored until signs of spoilage were detected.

Meatball Preparation:

Frozen beef (4.5 kg) was sourced from local butcher shops in Assiut Governorate, Egypt. The beef was minced immediately, packed in sterile polyethylene bags, and used for meatball preparation. The recipe consisted of 70% meat, 12% fat, 9% flour, 2.1% salt, 1.2% onion, 1% garlic powder, and 1.2% spice mix (black pepper, cumin, ginger powder, and turmeric powder). These ingredients were blended to form uniform meat dough, which was divided into the five experimental groups (C, T1, T2, T3, and T4) **[13]**. All samples were packed in polyethylene bags, stored at 4°C, and analyzed every two days for sensory, chemical, and microbiological properties. The experiment was performed in triplicate.

Sensory Analysis:

Negative control samples were assessed by a panel of 30 judges for sensory attributes, including taste, color, smell, texture, and overall acceptability, using a 9-point hedonic scale. A score of 9 indicated the highest acceptability, while 1 indicated the lowest. Before evaluation, the samples' color was examined in their uncooked

state. Meatballs were then cooked on a preheated grill for 5 minutes (2.5 minutes per side) until they reached an internal temperature of 70°C. After cooking, they were assessed for texture, odor, taste, and overall acceptability [14].

Chemical Quality Analysis:

- Thiobarbituric Acid-Reactive Substances (TBARs) were determined following the method of Ismail et al. [15].
- Total Volatile Base Nitrogen (TVBN): Measured according to Pearson [16].
- pH Determination: Conducted using the methodology described by Yalcin et al. [17].

Microbiological Evaluation:

Sample preparation for serial dilution was carried out under aseptic conditions according to ISO [18]. Ten grams of each sample were mixed with 90 ml of sterile 0.1% peptone water in sterile polyethylene bags and homogenized for 2 minutes to achieve a 1/10 dilution. Serial dilutions were prepared using test tubes containing 9 ml of sterile diluent.

Total aerobic plate count [19]:

From each dilution, 100 µl was plated onto dry plate count agar (HI-MEDIA, M091) and incubated at 30°C for 24-48 hours.

Coliform Count [20]: Violet Red Bile Glucose Agar (VRBG) was used. Plates were inoculated with 100 µl of the serial dilutions and incubated at 37°C for 24 hours. Colonies with pink to red or purple coloration, with or without precipitation halos, were counted.

Yeast and Mold Count [21]: Sabouraud's Dextrose Agar with 0.05 mg/ml chloramphenicol was used for inoculation. Plates were incubated at 25°C for 5 days. Yeast and mold counts were calculated per gram of the sample.

Psychrotroph Count [22]: From each dilution, 100 µl was plated on plate count agar and incubated at 7°C for 10 days.

Animal experimental design:

The experiments in this study were conducted according to the ethical guidelines for the care and the use of animals and approved by the institutional animal care and use committee (ARC-IACUC) Agricultural Research Center number ARC- AHRI 46 23

Female albino rats, weighing 120 ± 10 g aged 8 weeks, were obtained from laboratory animal house for experimental studies, faculty of veterinary medicine, Assiut University. Animals were kept in steel cages under laboratory conditions of a 25°C room temperature, 12:12 hours light: dark cycle and fed on rodent chow pellets with free access to water. Rats were left in their cages for 7 days for acclimatization to return to their normal conditions before the experiment. Then, rats were divided into 4 groups; a minimum of six rats was used in each group. The 1st group: was considered as control negative group without any treatment. 2nd group: each rat was gavaged orally with lemon grass oil 0.5% dissolved in corn oil 2ml/ day every other day continued for 30 days. 3rd group each rat was gavaged 2ml/ day every other day lemongrass nano-emulsion 0.5% for 30 days. 4th group: each rat received lemongrass nano-emulsion 1% 2ml/day by day for 30 days. On the fifteenth and thirtieth days of the experiment 3 rats from each group were selected, each animal was anaesthetized for blood samples collection then sacrificed by decapitation and tissues were harvested.

Biochemical blood analysis:

At the 15th and 30th days of the experiment, 2ml of blood sample was collected from retro-orbital plexus of each anesthetized rat into a clean centrifuge tube without anticoagulant then centrifuged for 15 min at 3000 r.p.m to separate the serum for biochemical assessment of the liver function enzymes [aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP)] were evaluated according to previous reports [23-25]. Measurement of serum renal function parameters [Urea (mg/dl) and Creatinine (mg/dl)] was done according to previous established methods [26, 27], and serum total antioxidant capacity (TAO) was measured according to Koracevic et al. [28]. The biochemical studies were conducted at the biochemistry department, faculty of Medicine, Assiut University.

Histopathological investigations:

For pathological examination, stomach, liver and kidneys were dissected from each euthanized rat and examined grossly to detect any abnormalities then fixed in neutral buffered formalin 10%. Fixed tissues were dehydrated with ethanol, cleared in clearing reagent and infiltrated in paraffin. Then thin tissues paraffin sections were obtained using a rotary microtome. The prepared tissue slides were stained with haematoxylin and eosin stain for histopathological investigation [29]. In addition, the degenerative changes, congestion and leucocytes infiltration in each section of rat glandular stomach, liver and kidneys were measured in five fields (15 fields for each group) by light microscopy (x10 objective lens) and were used to score the histopathological lesions of H&E stained sections of these tissues in terms of the degree of cell damage [30].

Statistical analysis

Analyses were performed with Graphpad prism version 9.5.1 software. Quantitative data are reported as mean ± standard error (SE). Two-way analysis of variance (ANOVA) followed by Tukey's post hoc multiple comparison test were used to determine the significant differences between groups. P-value less than 0.05 was considered statistically significant.

Results and Discussion

Lemongrass oil (LGO) has been recognized for its non-toxic mechanism of action and safety for human health and the environment. It is a food additive classified as GRAS and is registered under the REACH (Registration, Evaluation, Authorization, and Restriction of Chemicals) regulation by the European Chemical Agency (ECHA) [31]. However, the application of LGO in the food industry is limited due to its strong aroma and low efficiency. Nano emulsion technology presents an opportunity to overcome these limitations, enhancing the delivery of LGO using various carriers commonly employed in food systems. Nanotechnology in the food industry improves the effectiveness of ingredients by increasing bioavailability, absorption of bioactive compounds, stability, and sensory quality. Processed meat products, which are prone to bacterial damage, can benefit significantly from nanoemulsions with antimicrobial and antioxidant properties [32].

In this study, the nano emulsion of LGO was prepared and physically analyzed using the Zetasizer apparatus and Transmission Electron Microscopy (TEM). Table 1 reports the particle size and polydispersity index (PDI) of the nano emulsion. The average droplet size (nm) ± standard deviation was 79.2 ± 21.1, and the PDI was 0.331. A PDI value below 0.5 indicates superior stability and uniformity of the dispersion medium [33, 34]. The droplet size was within the typical nano emulsion range of 20 to 500 nm, as noted by Gupta [35]. Smaller particle sizes enhance antibacterial activity, while lower PDI values and zeta potential contribute to nano emulsion stability [36].

TEM analysis (Figure 1) revealed that the nano emulsion droplets were small, spherical, widely separated, and exhibited a dark appearance with an amorphous core. These physical characteristics contribute to the stability and functionality of the nano emulsion.

LGO demonstrates preservative benefits by protecting food sensory properties, inhibiting microbial activity, and extending shelf life. Its effectiveness is largely attributed to citral, the primary active component (≥45%) of LGO, which imparts a distinctive lime aroma [2]. Lipid oxidation and microbial growth are major factors leading to quality degradation and reduced shelf life of meat products during storage [37]. While synthetic preservatives are widely used to mitigate these effects, natural alternatives like LGO offer a safer and eco-friendly solution [38].

During meat storage, sensory changes in color, odor, taste, and texture occur due to bacterial growth and chemical changes such as oxidation and proteolysis. These undesirable changes diminish shelf life and consumer acceptability [39]. High concentrations of LGO may cause sensory issues, whereas lower concentrations have shown reliable effects on human health. In vitro studies suggest that LGO concentrations between 0.2–10 µL/mL are effective, though high concentrations may not be organoleptically acceptable [40].

Figure 2 evaluates sensory attributes—color, taste, odor, texture, and overall acceptability—to determine the optimal concentration of LGO and its nanoemulsion. Samples with 1% and 0.5% LGO exhibited poor sensory results, whereas those with 0.5% nanoemulsion achieved higher acceptability. Similarly, other studies indicate that while high concentrations of LGO have strong antibacterial and antioxidant effects, they are less acceptable organoleptically. A concentration of 0.05% LGO strikes a balance between antioxidant properties and sensory acceptability [41].

LGO contains terpenoid compounds such as citral, α-terpinene, geranial, linalool, neral, α-pinene, myrcene, and γ-terpinene. These terpenes exhibit cytotoxic effects on bacterial cell membranes and cytoplasm, causing cellular degradation, enzyme loss, membrane breakdown, and damage to genetic material. The antibacterial mechanism includes cellular lysis, structural changes, inhibition of septum formation, and production of deformed cells. Additionally, LGO disrupts membrane integrity, inhibits ATP production, alters pH, and increases membrane permeability [42].

(Figure 3) highlights the antibacterial effect of LGO and its nano emulsion on total aerobic plate counts. After 24 hours, bacterial counts increased, but the 1% nano emulsion showed superior effectiveness compared to the same concentration of LGO. These findings align with studies showing LGO disrupts bacterial biofilms and prevents growth [43, 44]. Hosny et al. [45] also reported that 0.5% LGO in beef kofta significantly reduced bacterial counts during 10 days of storage at 4°C. The antimicrobial activity of LGO is attributed to its high citral content and other bioactive compounds, which inhibit pathogens like *Salmonella typhi*, *Bacillus cereus*, and *Staphylococcus aureus* [46].

Figure 4 demonstrates the inhibitory effect of LGO and its nano emulsion on coliform counts within a week, with significant differences observed between treated and control samples. (Figure 5) indicates that 1% and 0.5% LGO and its nano emulsion effectively reduced yeast and mold counts from 9.2×10^6 in control samples to 10^5 CFU/g within a week. The antifungal activity of LGO has been reported against multiple fungi, with its volatiles like phenols and flavonoids demonstrating significant efficacy [47, 48].

The antimicrobial properties of LGO are attributed to its high content of trans-citral isomers, which disrupt membrane integrity, ATP levels, pH, and membrane potential. LGO inhibits fungal growth and aflatoxin production, rendering fungal pathogens static and latent [49].

Figure 6 shows that psychrotrophic bacterial counts remained low in samples treated with LGO and its nano emulsion, while control samples exhibited significant bacterial growth. LGO's antibacterial effect likely arises from its ability to disrupt bacterial membrane integrity and function [50].

Figure 7 reports pH differences between treated and control samples during storage at 4°C. pH values decreased in treated samples, likely due to protein hydrolysis caused by essential oils' antimicrobial action [22]. These findings align with Salem et al. [50], who observed the highest pH values in samples treated with 1.5% and 1% LGO.

Reduction in pH:

The pH of meat can rise due to microbial activity producing alkaline compounds. LGO's antimicrobial action reduces microbial load, thereby limiting the production of these compounds and helping to maintain or slightly lower the meat's pH. Studies have observed that meat products treated with LGO maintain a more stable pH during storage, contributing to extended shelf life. Figure 8 reveals significantly lower thiobarbituric acid (TBA) values in samples treated with LGO and its nanoemulsion [51].

Reduction in TBA Values:

TBA values are indicative of lipid oxidation in meat, leading to rancidity. LGO's antioxidant compounds, such as citral, inhibit oxidative reactions, thereby decreasing TBA values. Studies have demonstrated that incorporating LGO into meat products results in lower TBA values during storage, indicating reduced lipid oxidation [52].

Figure 9 shows that total volatile basic nitrogen (TVBN) values were lower in samples supplemented with 0.5% and 1% LGO and its nanoemulsion compared to control samples, further supporting their preservative efficacy.

Reduction in TVBN Levels:

TVBN measures the concentration of volatile nitrogenous compounds, which increase due to microbial activity and protein degradation in meat. LGO's antimicrobial properties suppress the growth of spoilage microorganisms, leading to lower TVBN levels. Research has shown that meat products treated with LGO exhibit significantly lower TVBN values compared to untreated controls, highlighting its effectiveness in preserving meat quality [50].

In the present study, we investigated the biochemical and histopathological effects of lemongrass in its raw essential oil form and lemongrass nano-emulsion when administered orally to rats for 30 days.

Biochemical parameters:

Serum liver, kidney function enzymes and total antioxidants are summarized in Tables (2-3). As shown in Table (2) the aspartate aminotransferase was the highest significantly higher in value ($P<0.05$) at the 30th day post treatment with 1% lemongrass nano-emulsion group compared to control group and other two experimental groups. The alanine aminotransferase, ALP, Urea, Creatinine and TAO were not significantly affected ($P>0.05$) by oral administration of lemongrass oil or its Nano-emulsions during all experimental time.

As mentioned earlier, this study showed that increased serum level of AST enzyme after long time (30 days) of oral intake of higher concentration 1% nano emulsion. This elevation may be produced by the degenerative cumulative effect of higher concentration of nano emulsion particles on the liver cells. However, there was no significant effect on the other serum biochemical enzymes that were recorded in all treated groups. This was proportionally confirmed with previously published study about lemongrass EO oral treatment of mice for 21 days which recorded no signs of important changes in serum biochemical parameters only reduction in cholesterol level was recorded in animals treated with 100 mg/kg/day [53]. The same effect was described in rats [54].

Histopathological investigation:

The macro morphological examination of rats' organs in all groups revealed no apparent lesions were detected. There were only increase in stomach size associated with yellowish coloration that occurred in group two at the 15^{th} day of experiment then returned to its normal size after the 30^{th} day of experiment as illustrated in Fig.(10).

Microscopic examination of stomach, liver and kidneys tissues of rats orally administered lemongrass oil extract 0.5%, lemongrass nano-emulsions 0.5% and 1% were illustrated in Table

(4) and Figs (11-12-13). Regarding to Fig. (11A-B), the glandular stomach of control group at the 15th and 30th days of experiment revealed normal structure of rat gastric mucosa. In contrast, the gastric mucosa of group two revealed signs of gastritis in form of acute inflammatory cellular infiltration and congestion in mucosa after the 15th day of experiment (Fig. 11C). This pathology progressed to ulcer formation after the 30th day of LG oil extract orally administered (Fig. 11D). While, at the 15th day gastric mucosa of group three showed mild signs of gastritis (Fig. 11E) and returned to nearly normal structure after the 30th day of 0.05% nano-emulsion administration (Fig. 11F). Also, group four gastric mucosa demonstrated minimal hemorrhages post the 15th day (Fig. 11G). Then became congested associated with inflammatory cells infiltration in mucosa and sub mucosa at the 30th day of 1% nano emulsion oral uptake (Fig. 11H).

As shown in Fig. (12A-B) the liver tissue of control group at the 15th and the 30th days of experiment showed normal hepatic architecture of parenchyma and blood vessels. While, diffuse periportal vacuolar degeneration of hepatocytes and portal congestion were observed in group 2 rats at the 15th day (Fig. 12C). After the 30th day of LG oil extract administration the degenerative changes regressed to only minimal hepatocytes number containing one circumscribed vacuole scattered around portal area (Fig. 12D). In case of G3 at the 15th day of experiment hepatic tissue appeared nearly normal without any characteristic lesions (Fig. 12E). But, after the 30th day of 0.5% nano emulsion intake solitary hepatocytes necrosis scattered around portal area were observed (Fig. 12F). Rat's liver of G4 post the 15th day of orally 1% nano emulsion intake revealed mild hepatocytes degeneration associated with focal area of hepatocytes necrosis (Fig. 12G). Then, increased to marked periportal vacuolar degeneration of hepatocytes after the 30th day of experiment (Fig. 12H).

As illustrated in (Fig. 13A-B) the renal tissue of control group recorded normal structure of renal corpuscle and convoluted tubules of renal cortex at both the 15th and 30th days of experiment. Also, renal cortex of G2 rats appeared with nearly normal convoluted tubules except mild glomerular swelling of some glomeruli after the 15th day of oral 0.5% oil extract intake (Fig. 13C). But, at the 30th day the convoluted tubules revealed epithelial degenerative changes associated with minimal interstitial leukocytes infiltration and congested glomeruli (Fig. 13D). In case of G3 post the 15th day revealed congested glomeruli associated with thickening of Bowman's capsule of some corpuscles in addition to degeneration of convoluted tubular epithelium (Fig. 13E). But, at the 30th day of 0.5% nano emulsion intake the renal cortex restored their normal architecture with mild congested swollen some glomeruli (Fig. 13F). While, G4 after the 15th day of 1% nano emulsion intake the renal cortex exhibited mild glomerular disruption associated with some areas of interstitial hemorrhage (Fig. 13G). At the 30th day the convoluted tubular epithelium showed degenerative changes in addition to glomerular capillaries swelling (Fig. 13H).

Based on histopathology performed on the gastric, hepatic and renal tissues in this study, mild acute gastritis was observed after15 days of lemongrass EO exposure which progressed and formed small ulcers in gastric mucosa after longtime (30 days) of exposure. This may be attributed to the tested substance irritating action. While, hepatocytes vacuolar degeneration that was observed after 15 days of lemongrass OE administration started to decrease in size and number of affected hepatic cells after 30 days of exposure. This indicates that the injuries of the hepatic cells were reversible and the hepatocytes would return to its normal stable state within a certain limited-time. These degenerative changes of liver may be a consequence of lesions or disturbance occurred in the gastric mucosa of the stomach of the same group. These findings are consistent with previously published studies that did not demonstrate any remarkable histological effect of lemongrass oil extract on stomach, liver and kidney tissues [30, 55]. Interestingly, this study demonstrated increased hepatic vacuolar degeneration especially after long time of higher concentration 1% of nano emulsion intake as well as tubular and corpuscular degenerative alterations in kidneys that might be due to adverse effect of long-time exposure to nano molecules in hepatic and renal tissues. As reported previously an anxiolytic like effect of lemongrass essential oil has been documented which might be mediated by GABAergic system [55]. So the fact that has been speculated that herbal medicines secondary metabolites may act as alternatives to products of synthetic chemicals [57].

Conclusion

Lemon grass oil and its nano emulsion were observed to possess high antimicrobial activity, so they can be used as a way of combating the growth of common causes of food poisoning and also this antimicrobial property makes them an effective option for bacterial and fungal infections. Therefore, the present microbiological, chemical, sensory, biochemical and pathological evaluation revealed that the lower concentration 0.5% of lemongrass nano emulsion can be considered relatively safer than the higher concentration 1%. In addition it is better than raw lemongrass oil form

because 0.5% nano emulsion did not provoke the degenerative effects on gastric and hepatic tissues.

Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical of approval

This study was conducted according to the ethical guidelines of the Animal Health Research Institute (AHRI).

Acknowledgments

Not applicable

Funding statement

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TABLE 1. Physical properties of formulated Nano-lemon grass.

Type of nano emulsion	Average ± SE dnm	PDI
Nano lemon grass	79.2±21.1	0.331

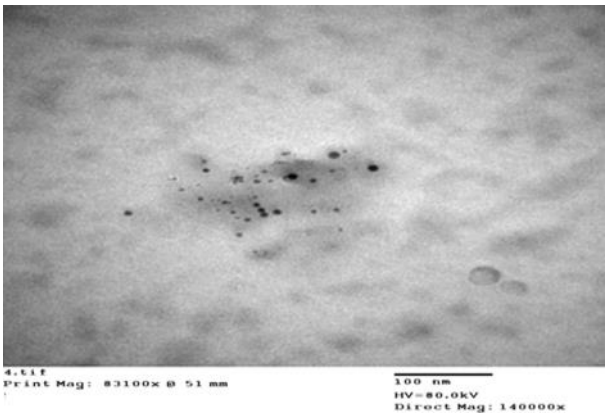

Fig. 1. TEM of lemon grass nano emulsion showed nano-sphere and without any aggregation with average size 48.73±5.54 nm.

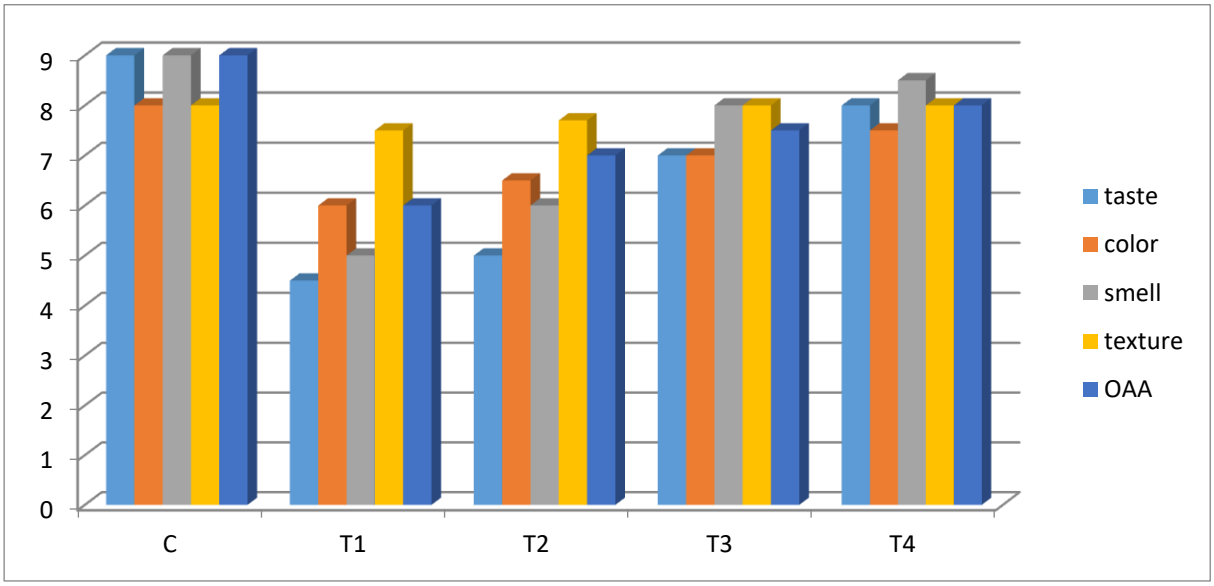

Fig. 2. Organoleptic of LG oil and its nano emulsion in laboratory manufactured meat balls.
OAA: Over All Acceptability

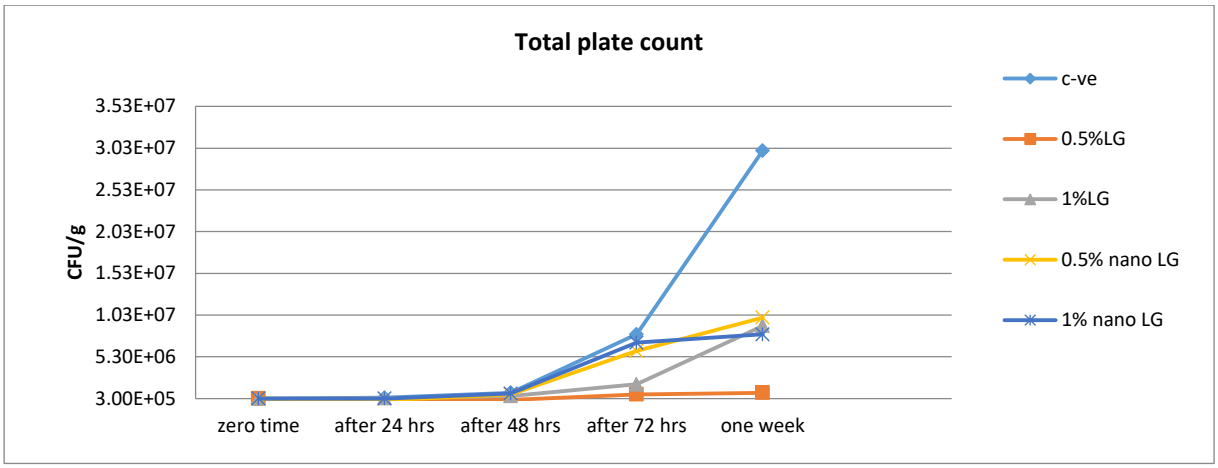

Fig. 3. Efficacy of LG oil and its nano emulsion on TBC of manufactured meat balls.

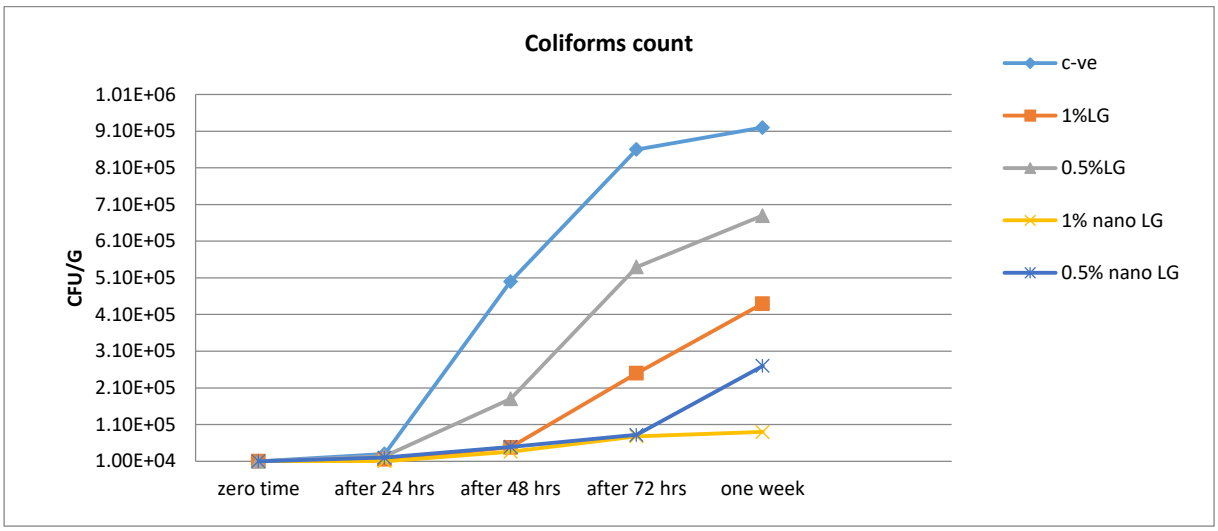

Fig. 4. Efficacy of LG oil and its nano emulsion on coliforms count of manufactured meat balls.

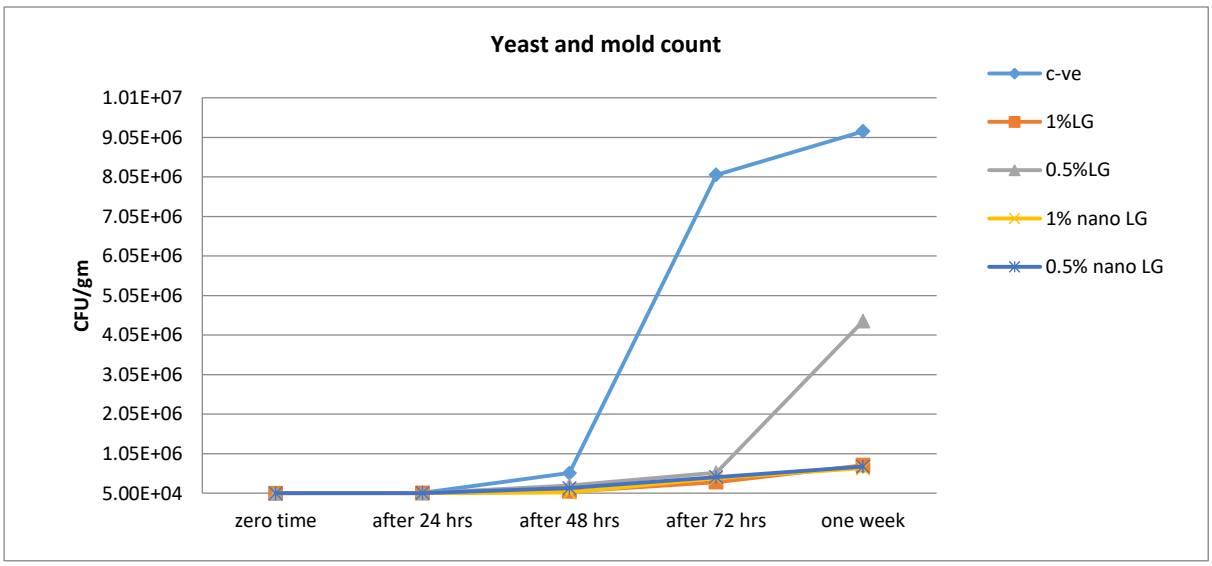

Fig. 5. Efficacy of LG oil and its nano emulsion on yeast and mold count of manufactured meat balls.

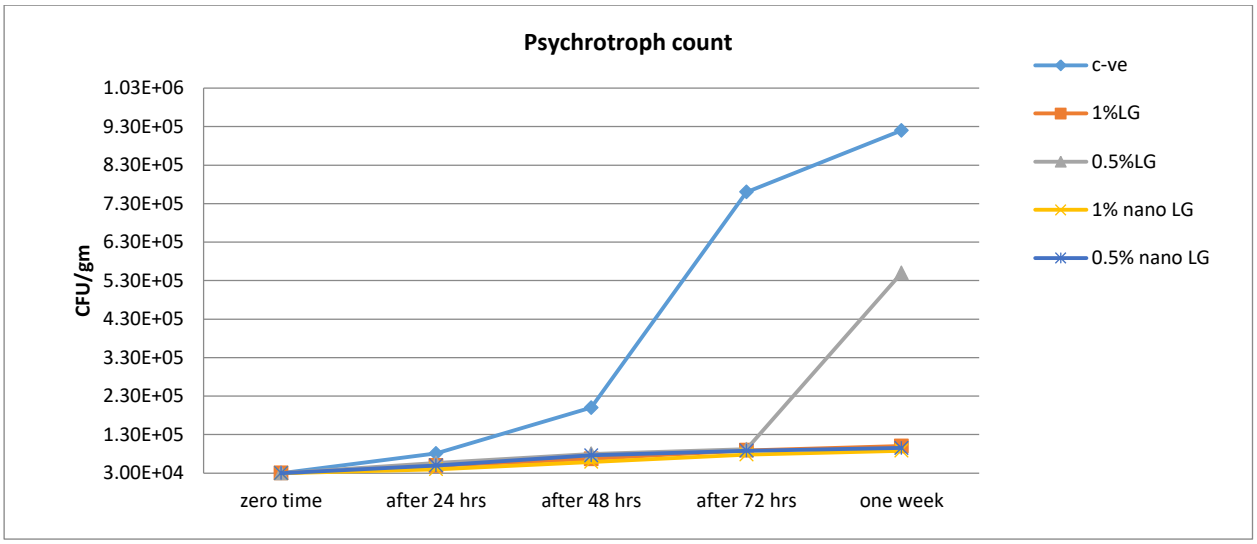

Fig. 6. Efficacy of LG oil and its nano emulsion on psychrotroph count of manufactured meat balls.

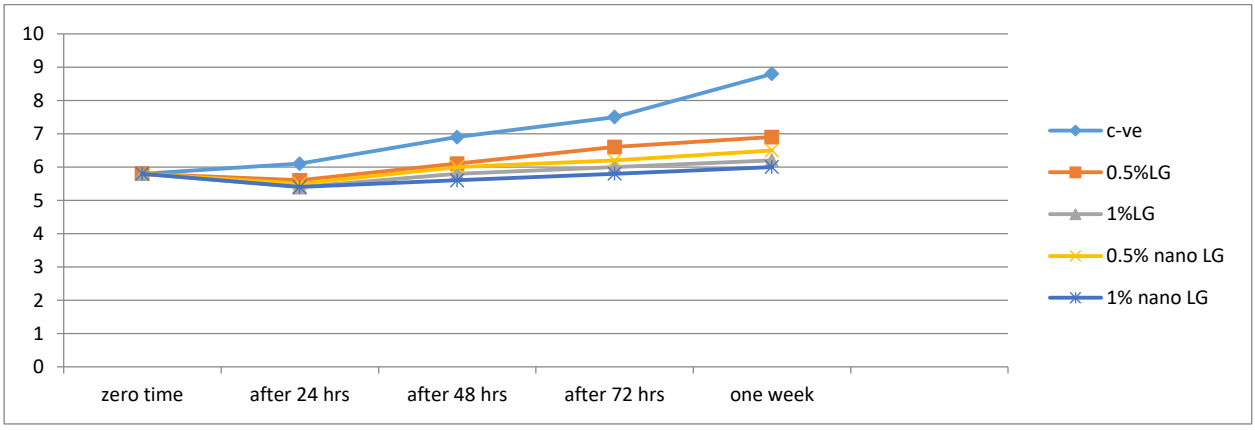

Fig. 7. Efficacy of LG oil and its nano emulsion on pH of manufactured meat balls.

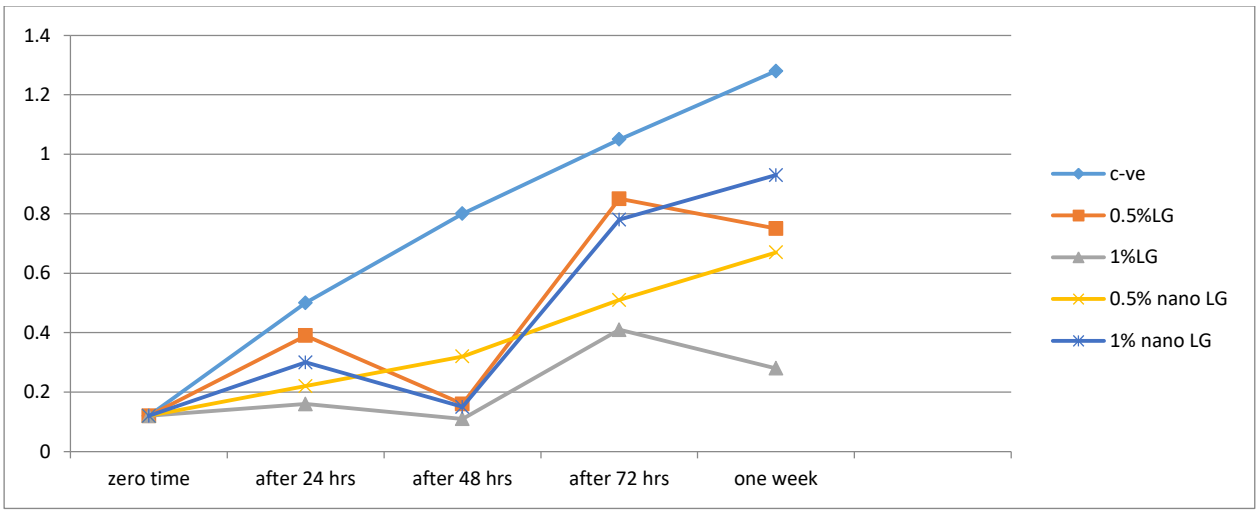

Fig. 8. Efficacy of LG oil and its nano emulsion on TBA of manufactured meat balls.

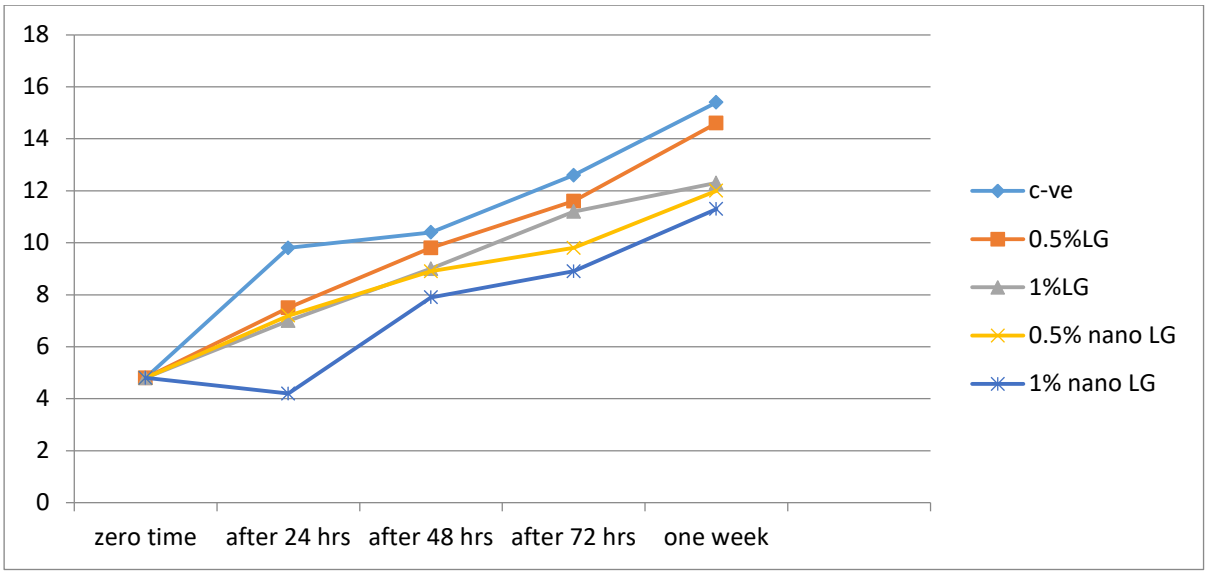

Fig. 9. Efficacy of LG oil and its nano emulsion on TVBN of manufactured meat balls.

TABLE 2. Biochemical parameters of rat serum liver enzymes in all experimental groups.

Treatments	AST U/L		ALT U/L		ALP IU/L	
	15th day	**30th day**	**15th day**	**30th day**	**15th day**	**30th day**
Control	26.8±3.5	11.3±3.9[a]	19.03±6.2	16.73±7.8	561.3±156.8	272.3±123.7
G2 oil 0.5%	29.6±4.3	14.43±5.9 [a]	24.8±5.1	18.37±3.0	799±101.5	238.5±96.8
G3 nano 0.5%	28.73±0.37	14.63±3.63 [a]	37.43±3.6	29.83±8.5	500.9±152.7	394.7±28.8
G4 nano 1%	30.17±5.11	45.8±7.59[b]	22.6±9.2	37.23±11.6	336.23±112.8	658.9±108.2

Notes: Data are presented as means ± standard error (SE). Values followed by the different letter within the same column are significantly different (P<0.05) according to ANOVA two way analysis followed by tukey's post hoc multiple comparison test.

TABLE 3. Biochemical parameters of rat serum renal function enzymes and total antioxidants.

Treatments	Urea mg/dL		Createnin mg/dL		TAO mM/L	
	15th day	30th day	15th day	30th day	15th day	30th day
Control	58.5±5.4	63.9±2.7	1.6±0.3	0.61±0.17	0.76±0.02	0.6±0.03
G2 oil 0.5%	67.3±2.3	111.03±12.4	2.4±0.3	0.48±0.2	0.77±0.03	0.5±0.07
G3 nano 0.5%	99.4±5.4	109.37±15.9	2.5±1.1	1±0.25	0.66±0.01	0.5±0.06
G4 nano 1%	63.9±5.4	69.3±3.3	0.65±0.2	1.2±0.4	0.66±0.05	0.5±0.09

Notes: Data are expressed as mean ± SE (P<0.05).

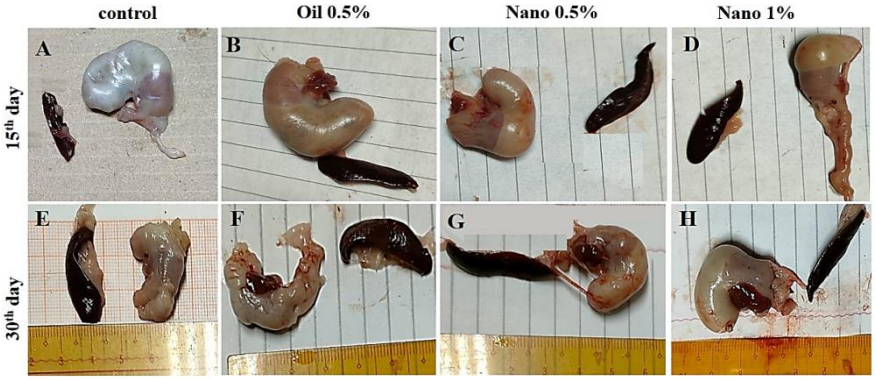

Fig.10. Macro morphological photos of rat stomach and spleen at 15th & 30th day of experiment showing (A) normal stomach size and color of control group. (B) Increase size and yellow coloration of stomach tissue of group 2. (C) Nearly normal stomach size with yellowish color of group 3. (D) Normal stomach size with yellow color of group 4. And at 30th day showing (E) control normal stomach. (F-G-H) nearly normal size and color compared to control group with other groups 2-3-4.

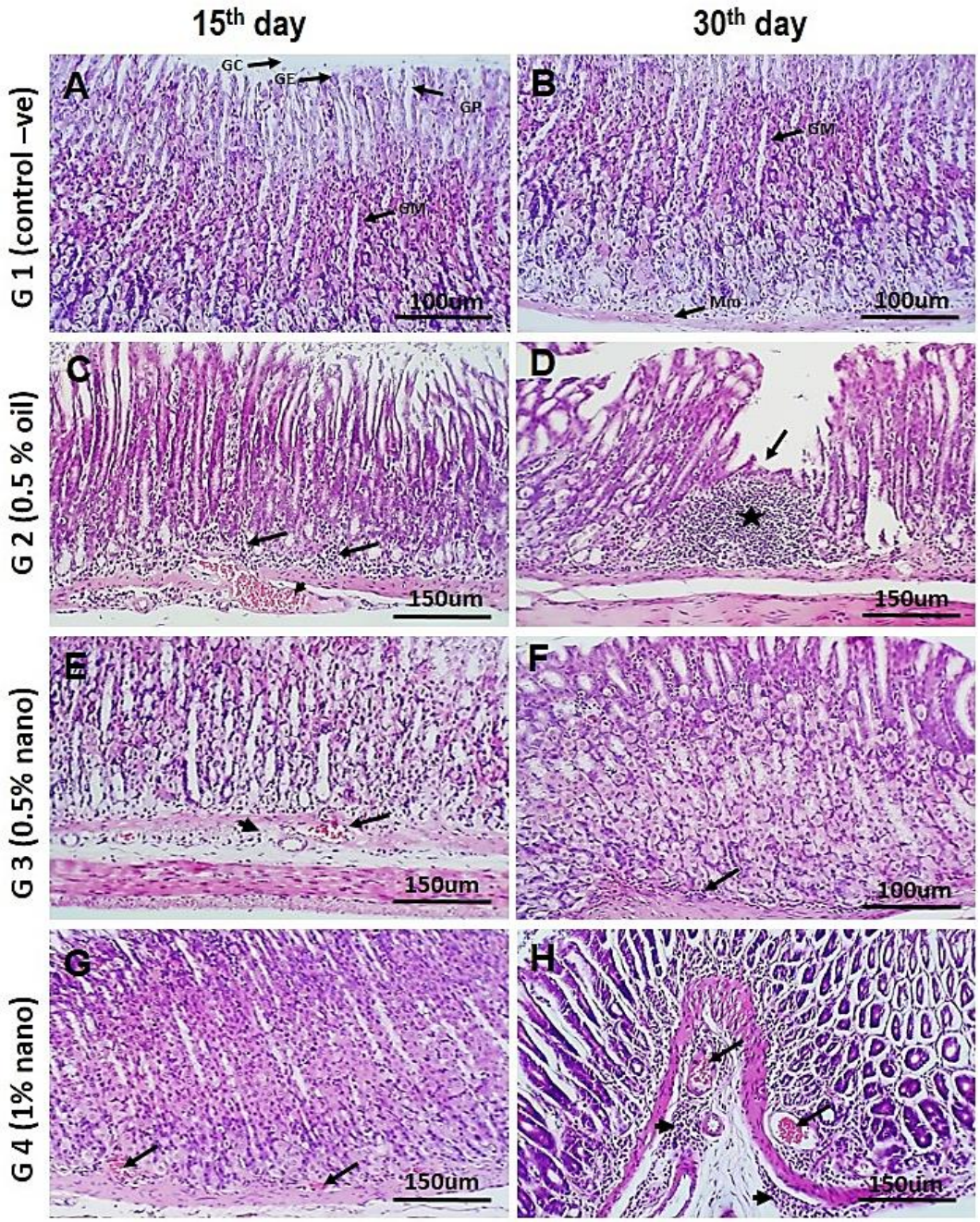

Fig.11. Rat glandular stomach photomicrographs stained with H&E stain showing (A-B) control group post 15th & 30th day normal histological structure of gastric mucosa [GC] gastric cavity, [GE] gastric epithelium, [GP] gastric pits, [GM] gastric mucosa, [Mm] muscolaris mucosa. (C) Group 2 post 15th day gastritis characterized by inflammatory mononuclear lymphocytes and eosinophil granulocytes infiltration in mucosa (arrows) dilated congested blood vessel (arrow head). (D) G2 post 30th day mucosal ulcer (arrow) associated with chronic inflammatory cells aggregation (star). (E) G3 post 15th day mild gastritis congested blood vessel (arrow) and edema in sub mucosa (arrow head). (F) G3 post 30th day nearly normal gastric mucosa except minimal inflammatory cell infiltration (arrow). (G) G4 post 15th day minimal hemorrhages in mucosa (arrows). (H) G4 post 30th day congested blood vessels of mucosa and sub mucosa associated with inflammatory cells infiltration (arrow head) and edema. Scale bars= 100um, 150 um

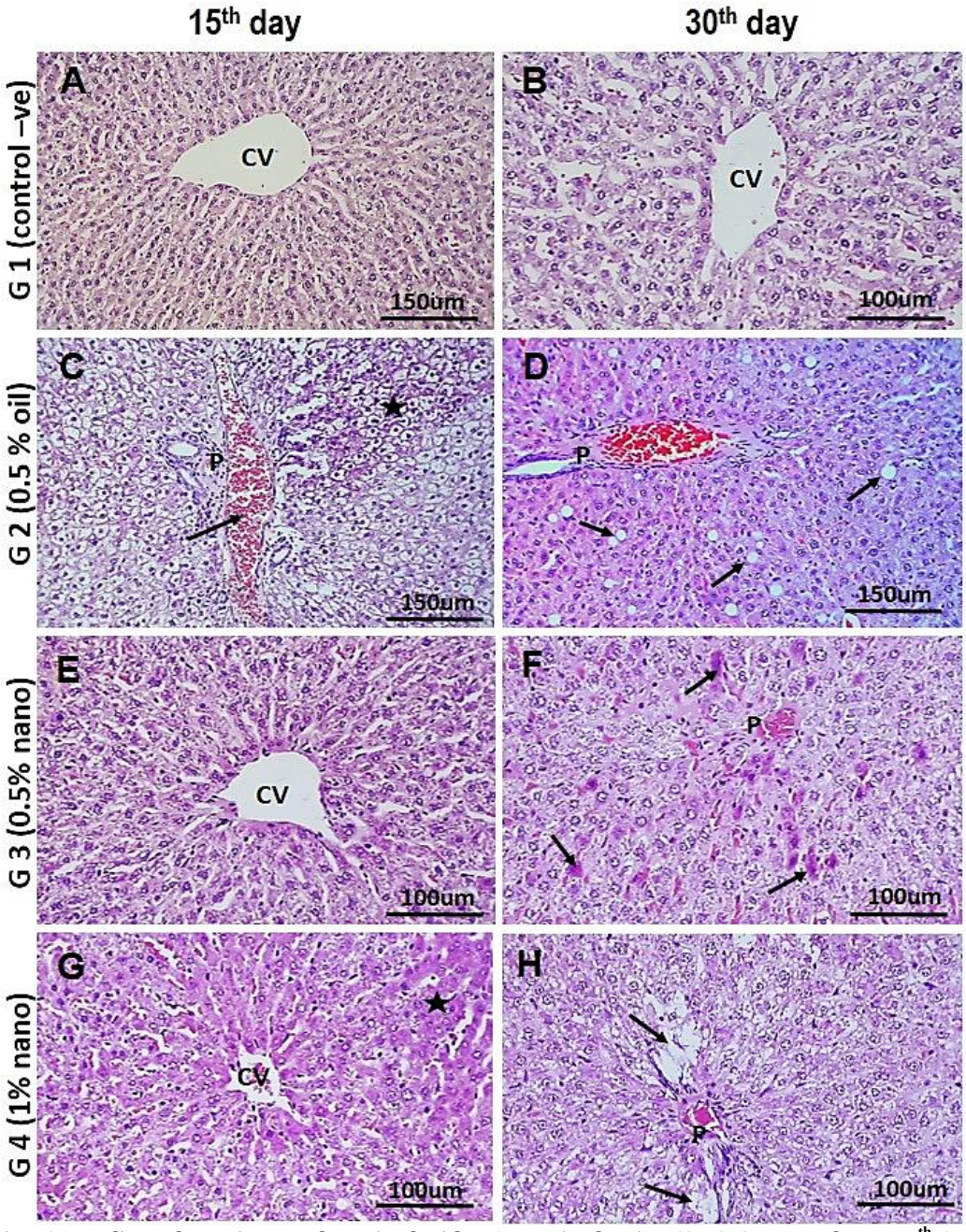

Fig. 12. Rat liver photomicrographs stained with H&E stain showing (A-B) G1 control post 15th & 30th day normal hepatic parenchymal cells and [CV] central vein. (C) G2 post 15 day diffuse periportal hepatocytes vacuolar degeneration and necrosis (star) congested portal blood vessel (arrow). (D) G2 post 30th day reduction in vacuolar hepatic degeneration to only separate hepatocytes with prominent circumscribed vacuole surrounding portal tract (arrows) and congested portal blood vessel. (E) G3 post 15th day nearly normal hepatic parenchyma except one layer of perivenuler [CV] hepatocytes degeneration. (F) G3 post 30th day discrete periportal hepatocytes necrosis (arrows). (G) G4 post 15th day focal area of hepatic degeneration (star) and perivenuler [CV] hepatocytes degeneration. (H) G4 post 30th day marked vacuolar hepatic degeneration in periportal area (arrows). Scale bars= 100um &150um.

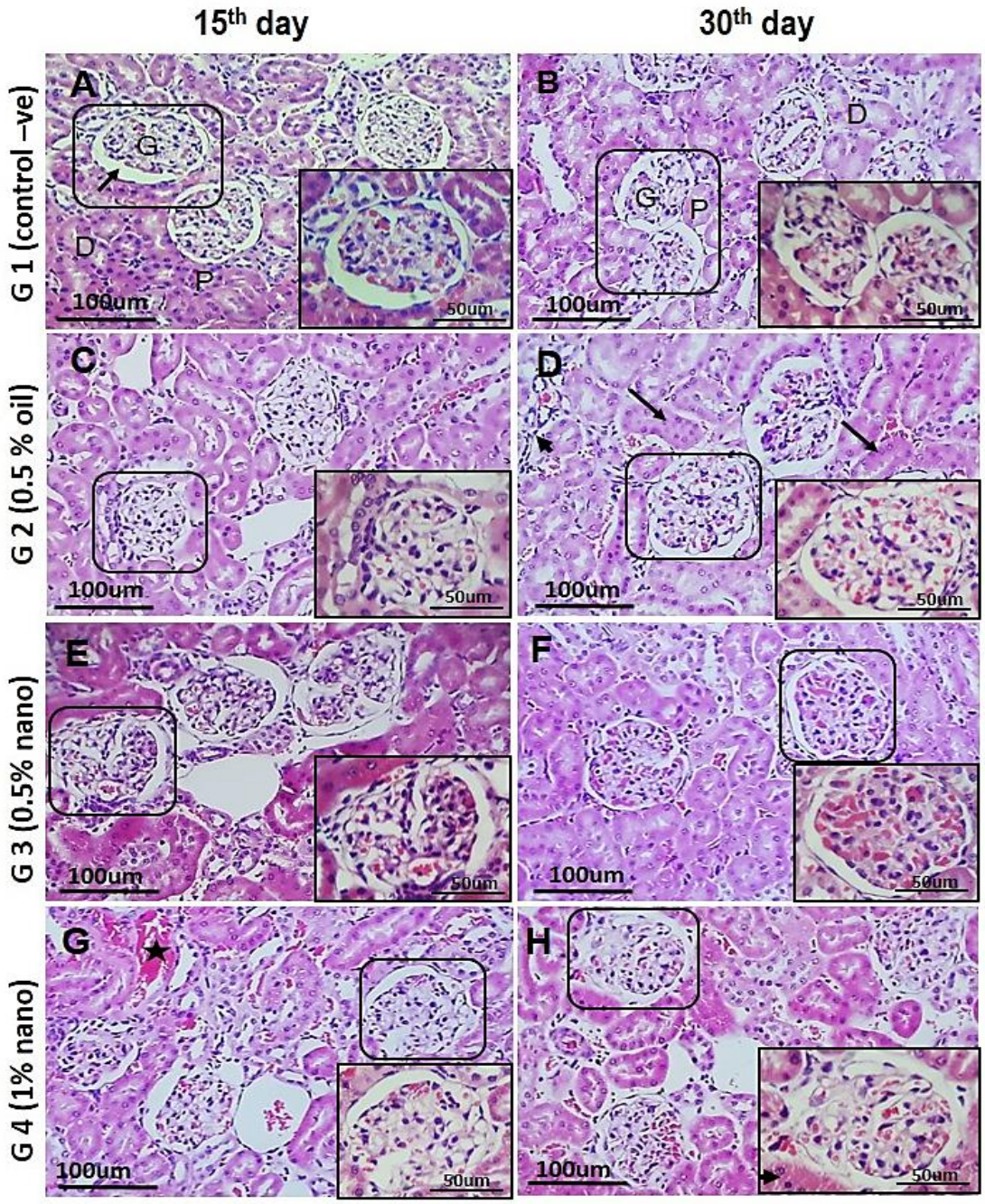

Fig.13. Rat renal cortex photomicrographs stained with H&E stain showing (A-B) G1 control post 15th & 30th day normal structure of renal cortex [G] a glomerular tuft of renal corpuscles with Bowman's space (arrow), [P] proximal convoluted tubules and [D] distal convoluted tubules. (C) G2 post 15th day nearly normal convoluted tubules and mild swollen glomerular tuft with narrow Bowman's space in the box. (D) G2 post 30th day epithelial degeneration of convoluted tubules with pyknotic cells (arrows), minimal interstitial inflammatory cells infiltration (arrow head) congestion of glomerular capillaries in the box. (E) G3 post 15th day degeneration of cortical convoluted tubular epithelium, congested glomerular tuft with thickening Bowman's capsule in the box. (F) G3 post 30th day nearly normal cortical tubules and mild congested swollen glomerular tuft in the box. (G) G4 post 15th day area of interstitial hemorrhage (star) and mild glomerular disruption in the box. (H) G4 post 30th day epithelial degenerative changes of convoluted tubules (arrow head) and congested swollen glomerular capillaries in the box. Scale bars= 50um & 100um.

TABLE 4. Histopathological changes in rat gastric tissue, liver and kidney stained with H&E stain based on scoring lesion severity.

Organs histopathology	Control		G2 oil 0.5%		G3 nano 0.5%		G4 nano 1%	
	15th day	30th day	15th day	30th day	15th day	30th day	15th day	30th day
Glandular stomach								
Epithelial degeneration	0	0	1	2	0	0	0	1
Congestion	0	0	2	1	1	0	1	2
Leukocytic infiltration	0	0	2	2	1	1	1	2
Liver								
Vacuolar degeneration	0	0	2	1	0	0	1	2
Hepatocytes necrosis	0	0	1	0	0	1	1	1
Congestion	0	0	2	1	0	1	1	1
Inflammatory cells infiltration	0	0	1	0	0	0	1	1
Kidneys								
Glomerular swelling & congestion	0	0	1	1	1	1	2	1
Thickening of Bowman's capsule	0	0	0	0	1	0	1	1
Degeneration of tubular epithelium	0	0	0	1	1	1	1	1
Inflammatory cells infiltration	0	0	0	1	1	0	1	1

The pathological injury in rat gastric tissue, liver and kidney are scored in terms of degree of cell damage as following: 0 = no change; 1 = cell damage < 25%; 2 = cell damage is 26-50%; 3 = cell damage is 51-75; and 4 = cell damage is 76-100%.

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دور زيت عشبة الليمون ومستحلبه النانوي على مدة صلاحيات كرات اللحم ودراسة تاثيراتهما الباثولوجية على الفئران

ولاء محمود علي الشريف [1، 2] ، دينا نورالدين علي مصطفي [1] ، عبير هاشم مصطفي[3] و نيفين عبدالغني النسر[3]

1 معمل صحة الاغذية المرجعي، معهد بحوث الصحة الحيوانية باسيوط، مركز البحوث الزراعية، مصر.

2 كلية تكنولوجيا العلوم الصحية، جامعة اسيوط الجديدة التكنولوجية، اسيوط الجديدة، مصر.

3 قسم الباثولوجيا، معهد بحوث الصحة الحيوانية باسيوط، مركز البحوث الزراعية، مصر.

الملخص

زيت الليمون هو عشب عطري؛ مصدر غني بحمض الستريك، وفيتامين ج، والمركبات الفينولية التي تُستخدم كعوامل مضادة للأكسدة ومضادة للبكتيريا في معالجة الطعام، ولكن بسبب رائحته الحمضية المميزة تم تحويله إلى مستحلب نانوي. تم استخدام FTIR و PDIو TEMلتوصيف المستحلب النانوي وقارنّا نشاطه المضاد للأكسدة والمضاد للبكتيريا على الجودة الحسية والكيميائية ، مادة التفاعل مع حمض الثيوباربيتوريك والنيتروجين المتطاير الكلي و الحالة الميكروبيولوجية عن طريق العدد الكلي للبكتريا، عدد البكتريا القولونية، عدد الخمائر والعفن الكلي وعدد البكتيريا المحبة للبرودة في كرات اللحم أثناء التخزين البارد عند 4 ºمئوية. أشارت التحليلات الحسية إلى أن 0.5% من مستحلب الليمون العشبي النانوي كان أفضل تركيز ، بينما أشارت التحليلات الكيميائية والميكروبيولوجية إلى مزايا كبيرة في استخدام الليمون العشبي ومستحلبه النانوي بتركيز 1%. بالإضافة إلى ذلك، قمنا بدراسة التأثيرات الكيميائية الحيوية والهيستوباثولوجية لاستخراج زيت الليمون العشبي ونانوهيمولسيون الخاص به بعد تجريعه للفئران، وكشفت النتائج أن التركيز المنخفض بنسبة 0.5% من نانوهيمولسيون الليمون العشبي يمكن اعتباره أكثر أمانًا نسبيًا من التركيز الأعلى بنسبة 1% على المعدة والكبد والكلى للفئران المختبرة.

الكلمات المفتاحية: زيت الليمون العشبي ، كرات اللحم ، الفئران ، الحالة الميكروبيولوجية.