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Garlic Oil Nanoparticles: A Novel Approach to Enhancing Hepatoprotective Effects and Antioxidant Capacity in Food Science

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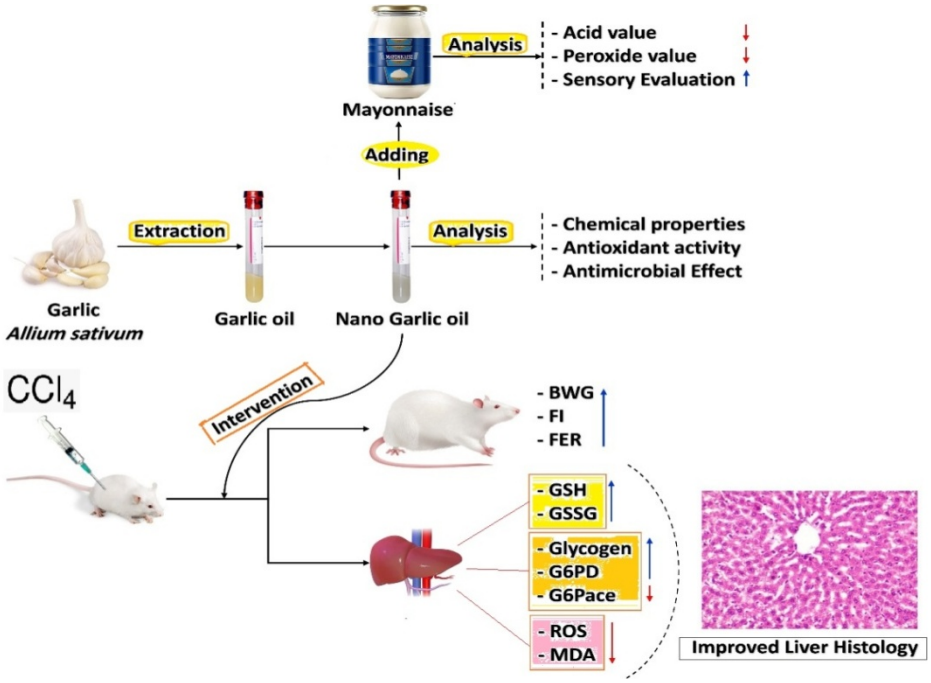
Abstract: This study investigated the potential of a garlic oil nanoemulsion to mitigate carbon tetrachloride-induced liver damage in rats and to act as a functional preservative in mayonnaise. An oil-in-water nanoemulsion of garlic oil was prepared, and mayonnaise samples were formulated with either normal garlic oil (GO) or nano garlic oil (NGO). In rats with liver damage, both GO and NGO reversed negative effects on body weight gain (+41.062% for GO vs. +48.953% for NGO), feed intake (+19.11% vs. +33.85%), and feed efficiency ratio (+16.949% vs. +23.728%), with the nano-formulation being more effective. NGO also showed superior therapeutic efficacy in restoring liver function, increasing hepatic glycogen by 73.19% and reducing G6Pase activity by 48.44%. It was also more effective in mitigating oxidative stress, reducing ROS by 55.45% and MDA by 43.75%. Histological analysis confirmed these protective effects, with NGO-treated rats showing more significant recovery of liver architecture. In mayonnaise, NGO exhibited superior antioxidant stability, with the lowest total change in acid value (56.08%) and peroxide value (1048.25%). Additionally, the nano-formulation improved sensory attributes, with a positive change in taste (+11.82%) and odor (+5.97%), overcoming the negative sensory impact of normal garlic oil. These results demonstrate that nanotechnology can enhance the therapeutic and functional properties of natural compounds.



Keywords: *Acid value, peroxide value, antioxidant activity, bacteriological inhibition, liver functions, ROS, GSH, mayonnaise, sensory attributes*

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1. Introduction

Nanotechnology has become a groundbreaking technology that has significantly transformed the food industry over the last several decades. This field operates on a minuscule scale, manipulating atoms, molecules, and macromolecules—typically sized between 1 and 100 nm—to create materials with unique, novel properties [1]. The nanomaterial's created through this process have at least one external dimension or an internal structure within the 1-100 nm range, which allows for the observation and manipulation of matter at the nanoscale. These materials exhibit distinct properties, such as color, solubility, strength, and diffusivity that are not seen in their larger-scale counterparts. This is primarily due to their high surface-to-volume ratio and other novel physicochemical characteristics, including toxicity, magnetic, optical, and thermodynamic properties [2,3]. The emergence of nanotechnology has spurred a new industrial revolution, with both developed and developing nations showing keen interest in increasing their investments in this area [4]. As a result, nanotechnology presents extensive opportunities for creating and applying new structures, materials, and systems in various fields, including agriculture, food, and medicine.

As reviewed by Singh et al. [5], the applications of nanotechnology in the food sector can be categorized into two main groups: food nanostructured ingredients and food nanosensing. Food nanostructured ingredients cover a broad spectrum from food processing to food packaging [6,7]. In food processing, these nanostructures can be used as food additives, carriers for smart delivery of nutrients, anticaking agents, or antimicrobial agents. When used in packaging, they can act as fillers to improve the mechanical strength and durability of the material. In contrast, food nanosensing is employed for more precise food quality and safety evaluation [1,8,9].

A particularly exciting application of nanotechnology is its combination with essential oils. Essential oils are volatile, aromatic compounds derived from plants, prized for their distinctive flavors, fragrances, and therapeutic properties. While they are celebrated for their antimicrobial and antioxidant qualities, their usefulness can be limited by their volatility and poor solubility in water [10]. Nanotechnology offers a solution to these issues by enclosing essential oils within nanocarriers like nanoemulsions, nanoliposomes, or solid lipid nanoparticles [11]. These specialized nanocarriers protect the essential oils from breaking down, regulate their release, and enhance their solubility and absorption. This allows for their more effective use as natural preservatives in food, boosting both safety and stability without the need for artificial additives [12].

The liver is the body's largest solid organ, performing a host of critical functions, including producing bile for digestion, metabolizing carbohydrates, fats, and proteins, storing vitamins and minerals, and detoxifying harmful substances and drugs [13,14,15]. Consequently, liver diseases represent a major global health concern, encompassing a variety of conditions that can impair its function. Common examples include hepatitis, caused by viral infections or autoimmune disorders, non-alcoholic fatty liver disease (NAFLD), linked to obesity and metabolic syndrome, and cirrhosis, a serious condition involving irreversible liver scarring [16]. The global prevalence of these diseases is increasing, largely due to modern lifestyle changes. NAFLD, in particular, is reaching epidemic proportions, affecting roughly 25% of the world's population. Furthermore, liver cancer remains a significant concern with consistently high incidence and mortality rates [17].

Traditional chemotherapy for liver diseases, particularly liver cancer, poses considerable difficulties. When these powerful drugs are administered systemically, they often cause severe side effects because they cannot distinguish between cancerous and healthy cells. This lack of specificity can damage healthy tissues, especially in the gastrointestinal tract and bone marrow, significantly lowering a patient's quality of life. Furthermore, the liver's intricate blood supply and the presence of drug-resistant cancer cells can limit the effectiveness of chemotherapy [18,19].

These limitations have created an opportunity for nanotechnology-based dietary treatments. Nanotechnology offers innovative solutions for both preventing and treating liver diseases. The liver's distinct vascular structure makes it an excellent target for nanomedicine. Nanoparticles can be designed to specifically target liver cells, known as hepatocytes, or the fibrotic tissue that develops during liver disease. This targeted delivery minimizes harm to healthy cells and enhances the effectiveness of therapeutic agents [20]. For example, nanoparticles can be loaded with anti-inflammatory drugs, antifibrotic agents, or genes to treat conditions like liver fibrosis, cirrhosis, and cancer. In the case of liver fibrosis, which is the excessive buildup of scar tissue, nanoparticles can deliver drugs directly to the activated stellate cells responsible for its production. This method is more effective than traditional treatments, which often have systemic side effects [21,22]. Additionally, nanoparticles can be used for imaging and diagnostics, enabling the early detection and monitoring of liver diseases. For instance, contrast-enhanced nanoparticles can be used in magnetic resonance imaging (MRI) to provide a clearer view of the liver's condition [23,24].

Given the potential of nanotechnology, this study aims to investigate the ability of garlic oil nanoparticles to reduce liver damage caused by carbon tetrachloride in rats. The research also explores the feasibility of incorporating garlic oil nanoparticles into an important food product: a functional mayonnaise-type dressing

2. Materials and Methods

2.1. Materials

2.1.1. Garlic Oil

Crude garlic oil (*Allium sativum*) was purchased from El-Masrayia Production for Natural Oil Extraction, located in Beheira, Damanhour, Egypt (License no. 532110310201629). All other mayonnaise ingredients, including oil, eggs, vinegar, salt, and sugar, were sourced

from a local market in Shebin El-Kom City, Menoufia Governorate, Egypt.

2.1.2. Chemicals and Kits

Standard bioactive compounds such as gallic acid (GA), catechin (CA), α-tocopherol, and butylated hydroxytoluene (BHT), along with DPPH (2,2-diphenyl-1-picrylhydrazyl) and Tween 80, were acquired from Sigma Chemical Co., St. Louis, MO. Morgan Chemical Co., Cairo, Egypt, supplied the casein. Growth media, including nutrient broth, Mueller Hinton agar, Salmonella-Shigella (S.S.) agar, and Sabouraud dextrose agar, were all obtained from El-Ghomhorya Company for Trading Drug, Chemicals and Medical Instruments, Cairo, Egypt. Unless specified otherwise, all other analytical-grade chemicals, reagents, and solvents were also procured from El-Ghomhorya Company. Assay kits for glucose, glucose-6-phosphate dehydrogenase (G6Pase), glucose-6-phosphatase (G6PD), and malondialdehyde (MDA) were provided by BIODIAGNOSTIC, Dokki, Giza, Egypt. Additionally, El-Nasr Pharmaceutical Chemicals supplied kits for triglycerides (TGs), total cholesterol (TC), HDL-cholesterol, and LDL-cholesterol.

2.2. Methods

2.2.1. Garlic Oil Nanoemulsion Preparation

Garlic oil nanoemulsion was prepared at the National Research Centre's Nanotechnology Lab in Dokki, Cairo, Egypt, following the procedure by Vasiliki, [25]. The process used an oil-in-water system with a 1:4 volume ratio of garlic oil to deionized water (200 mL of oil combined with 800 mL of water). A 1% concentration of Tween 20 surfactant was mixed with the oil before the water was gradually added while the mixture was stirred at 2,000 rpm using a magnetic stirrer at room temperature. The nanoemulsion formation was confirmed by the appearance of a clear, transparent solution in the phase diagram, indicating complete solubilization. Specific amounts of oil and surfactant were combined to create a final 1,000 mL mixture, which was then homogenized for 20 minutes at 20,000 rpm using a high-speed homogenizer (Model: 400ELPC, PRO Scientific Inc., 01-02411ELPC HOMOGENIZER, USA), with an ice bath used for temperature control. Water was added drop by drop (50 µL every 30 seconds) to form a water-in-oil (w/o) nanoemulsion. The optimal homogenization temperature was set to 40°C, and care was taken to prevent air bubbles from forming. After preparation, all samples were stored at 4 ± 1°C. Finally, the mixture was subjected to 30 minutes of homogenization in an ultrasonic water bath (Ultrasonic Cleaner MTI Corporation, Model UD150SH3.8LQ, U.S.A) at 30°C.

2.2.2. Mayonnaise Preparation with Normal and Nano Garlic Oils

The mayonnaise formulation, based on the method by Guevara et al. [26] consisted of 74.7% oil, 14.5% egg yolk, 7.1% vinegar, 1.48% salt, and 1.92% sugar. For the control sample (MC), salt and sugar were first dissolved in vinegar, after which egg yolks were added and mixed at low speed for 10 minutes. Sunflower oil was then slowly incorporated while mixing continuously for another 10 minutes. Two additional mayonnaise samples were prepared: one with 500 ppm of normal garlic oil and another with 500 ppm of garlic oil nanoparticles. All samples were transferred to sterile lidded glass containers and stored at room temperature (25°C) until analysis. Over a 28-day storage period, chemical properties, microbiological content, and sensory evaluation of all samples were assessed on days 0, 10, 20, and 30.

2.2.3. Analysis of Normal and Nano Essential Oils

2.2.3.1. Acid Value

The acid value (AV) of the oil samples was determined using the AOCS Official Method Cd 3d-63 [27]. This method quantifies the amount of free fatty acids in a sample by measuring the milligrams of potassium hydroxide (KOH) needed to neutralize one gram of the oil.

2.2.3.2. Peroxide Value

The peroxide value (PV) was determined by following the AOCS Official Method Cd 8b-90 [28]. This protocol uses a solvent mixture of acetic acid and isooctane to measure all substances that can oxidize potassium iodide under specific conditions. The results are reported as milliequivalents of peroxide per 1,000 grams of the sample.

2.2.3.3. Antioxidant Activity

The antioxidant activity (AA) of the oils, as well as standard compounds like α-tocopherol and BHT, was assessed using a modified BCB assay based on the procedure from Marco [29]. In this test, 1 mL of a β-carotene solution (0.2 mg/mL in chloroform) was added to 50 mL round-bottom flasks, along with 0.02 mL of linoleic acid and 0.2 mL of Tween 20. The mixtures were then treated with 0.2 mL of 80% methanol (as a control) or the corresponding plant extract or standard. After evaporating the solvent under vacuum at room temperature, 50 mL of oxygenated distilled water was added and the solution was shaken to create a liposome suspension. The samples were then heated to 50°C to induce auto-oxidation for 2 hours. The absorbance at 470 nm was measured every 10 minutes using a Beckman DU-50 spectrophotometer. The rate of β-carotene bleaching was calculated through linear regression analysis over time. All samples were tested in triplicate, with BHT and α-tocopherol in 80% methanol serving as controls. Antioxidant activity was calculated using four different methods: 1) Antioxidant value (AOX): The absolute value of the slope from a plot of absorbance versus time [30], 2) Percent Inhibition: Calculated using the formula $AA=[(Rcontrol-Rsample)/Rcontrol]\times100$, where Rcontrol and Rsample are the rates of β-carotene bleaching without and with the plant extract, respectively, 3) Oxidation rate ratio (ORR): Determined by the ratio of the sample bleaching rate to the control bleaching rate, $ORR=Rsample/Rcontrol$, and 4) Antioxidant activity coefficient (AAC): Calculated using the formula $AAC=[(AbsS120-AbsC120)/(AbsC0-AbsC120)]\times100$, where AbsS120 is the absorbance of the antioxidant mixture at 120 minutes, AbsC120 is the absorbance of the control at

120 minutes, and AbsC0 is the absorbance of the control at time zero [31].

2.2.4. Antimicrobial Evaluation of Essential Oils

The antimicrobial activity of normal and nano garlic essential oils against four microorganisms (*E. coli*, *Salmonella*, and *Aspergillus flavus* and *niger*) was assessed using the agar well diffusion method. This technique involved culturing each bacterial species on Mueller Hinton Agar (MHA) agar, while fungi were cultured on Sabouraud dextrose agar. Essential oils were then placed into 6 mm diameter wells created in the inoculated agar media. The plates were then incubated overnight at 37°C for bacteria and for 72 hours for fungi. The effectiveness of the essential oils was determined by measuring the diameter (in millimeters) of the zone of inhibition that formed around each well [32].

2.2.5. Biological Experiments

2.2.5.1. Animals and Diet

Adult male albino rats, each weighing 150±6 g, were used for the study. The rats were sourced from the Research Institute of Ophthalmology, Medical Analysis Department, Giza, Egypt. The basal diet was prepared according to the formula by Reeves et al. [33], containing 10% protein, 10% corn oil, 1% vitamin mixture, 4% mineral mixture, 0.2% choline chloride, 0.3% methionine, 5% cellulose, and 69.5% corn starch. The specific compositions for the salt and vitamin mixtures were also based on the same reference.

2.2.5.2. Inducing Hepatotoxicity in Rats

Chronic liver damage was induced in 30 male albino rats by giving them intraperitoneal (IP) injections of carbon tetrachloride (CCl_4) in olive oil (50% V/V, 2 ml/kg body weight) twice a week for two weeks, following the method by Jayasekhar et al.[34]. Liver intoxication was confirmed by performing biochemical liver function tests on a random sample of four rats.

2.2.5.3. Experimental Design

All biological experiments were conducted in accordance with the regulations of the Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council, NRC, USA. A total of 24 rats were individually housed in wire cages under controlled conditions: 25±4°C, 55 ± 2% relative humidity, and a 12-hour light cycle. For two weeks before the experiment, the rats were acclimated to a basal diet (BD). Afterward, they were divided into two main groups. The first group (n=6) served as a negative control, receiving only the basal diet and olive oil injections (5 ml/kg body weight), which was the vehicle for the CCl_4 treatment. The second main group (n=18) was injected with CCl4 to induce liver damage and was subsequently divided into three subgroups of six rats each: a positive control (Group 2) fed the basal diet, and two treatment groups (Groups 3 and 4) fed the basal diet supplemented with 200 mg/kg body weight/day of nano-garlic oil (NGO) and normal garlic oil (GO), respectively. These concentrations were selected based on existing garlic oil toxicity data [35].

2.2.5.4. Biological Evaluation

Daily food intake and weekly body weight measurements were recorded over the 28-day period. Body weight gain (BWG,%), food intake (FI), and food efficiency ratio (FER) were calculated using the methods of Chapman et al. (1959)[36] and the following formulas:

BWG (%) = (Final weight−Initial weight)/ Initial weight×100

FER=grams of feed consumed (g over 28 days)/grams of body weight gained (g over 28 days)

2.2.5.5. Blood and Organ Sampling

At the end of the four-week experiment, after a 12-hour fasting period, rats were anesthetized with ether before blood was collected from the abdominal aorta. The blood was placed in clean centrifuge tubes to clot at room temperature and then centrifuged at 3000 rpm for 10 minutes to obtain the serum [37]. The isolated serum was stored at -20°C until biochemical analysis. After euthanasia, liver samples were carefully dissected, rinsed with cold saline, weighed, and stored in a 10% formalin solution for histological examination.

2.2.5.6. Liver Homogenate Preparation

Liver homogenates were prepared using the method described by El-Khawaga et al. [38]. A portion of liver tissue was weighed and homogenized in ice-cold 0.9% saline. The resulting 5% (w/v) solution was centrifuged at 5000 rpm for 30 minutes at 4°C to remove cellular debris, and the supernatant was used for biochemical assays.

2.2.5.7. Biochemical Assays

Liver Functions

Liver glycogen content was measured as per. The activities of hepatic glucose-6-phosphate dehydrogenase (G6PD) and glucose-6-phosphatase (G6Pase) were determined following the methods of Chan et al. [39] and Rossetti et al. [40] respectively.

Glutathione Fractions

Reduced (GSH) and oxidized (GSSG) glutathione levels were measured colorimetrically in serum samples according to the procedure by Elman et al. [41].

ROS and MDA Content

Malondialdehyde (MDA) levels were assessed as described by Buege and Aust, [42]. A plasma sample was combined with thiobarbituric acid (TBA) reagent and heated, followed by centrifugation. Absorbance was read at 535 nm against a blank and compared to a standard curve. Reactive Oxygen Species (ROS) were detected by measuring the colorimetrically quantifiable blue formazan produced by ROS reducing Nitroblue Tetrazolium (NBT) [43].

2.2.6. Histopathological Examination

Liver specimens were immediately collected after the rats were sacrificed and preserved in 10% neutral buffered formalin. The fixed tissues were then dehydrated, cleared, and embedded in paraffin. Sections (4-6 μm thick) were

stained with hematoxylin and eosin before being examined under a microscope [44].

2.3. Statistical Analysis

All data were statistically analyzed using a one-way ANOVA via a computerized Costat program. The results are presented as the mean ± standard deviation (SD). Differences were considered statistically significant if $P \leq 0.05$ [45].

3. Results and Discussion

3.1. Chemical Properties, and Antioxidant and Antimicrobial Activities of Normal and Nano Garlic Oil

The provided data in Table 1 shows that nano garlic oil has superior initial chemical stability compared to normal garlic oil, which is supported by a significant decrease in both its acid value (AV) and peroxide value (PV). Specifically, the nano garlic oil's AV was 1.93±0.08 mg KOH/g oil and its PV was 2.61±0.13 meq peroxide/Kg oil, both significantly lower ($p \leq 0.05$) than the normal garlic oil's AV of 2.61±0.17 mg KOH/g oil and PV of 3.32±0.09 meq peroxide/Kg oil. This improved stability is consistent with scientific literature on nano-encapsulation, which explains that the nanoemulsion process encapsulates the oil's active components within a protective matrix, shielding them from environmental factors like oxygen and light that cause hydrolysis and oxidation [46]. This protective barrier prevents the chemical degradation of the oil from the outset, a finding supported by other studies, such as one by Hassanzadeh et al. [46] that demonstrated the superior ability of nano-formulated extracts to reduce peroxide value. Liao et al. [10] also confirmed that nano-encapsulation improves the oxidative stability of various essential oils, resulting in lower initial PVs and a slower rate of increase over time. The inherent stability of the nano-formulation makes it a more effective ingredient for food preservation, aligning with the observations of Francilia et al. [47], who noted that a higher acid value indicates lower storability and a greater risk of rancidity.

Table 1. Chemical properties of normal and nano garlic oil

Factor	Normal garlic oil	Nano garlic oil
Acid value (mg KOH/g oil)	2.61 ± 0.17 ^{a}	1.93 ± 0.08 ^{b}
Peroxide value (meq peroxide/Kg oil)	3.32 ± 0.09^{a}	2.61 ± 0.13 ^{b}

Values represent the mean ±SD (n=3). Means with various superscript letters in the same raw are different significantly at $p \leq 0.05$.

The provided data in Table 2, which includes a comprehensive analysis of the antioxidant activity of normal and nano garlic oil, demonstrates that the nano-formulation is significantly superior across all evaluated metrics. The nano garlic oil exhibited a lower Antioxidant Value (AOX) of 0.042, a higher Antioxidant Activity (AA) of 92.91%, a lower Oxidation Rate Ratio (ORR) of 0.071, and a higher Antioxidant Activity Coefficient (AAC) of 776.51 when compared to normal garlic oil. These results are well-supported by scientific literature, which attributes garlic oil's antioxidant properties to its organosulfur compounds, such as allicin, which scavenge free radicals to prevent lipid oxidation [46]. The enhanced performance of the nano-formulation is a direct result of nano-encapsulation, a process that improves the solubility and bioavailability of the oil's active compounds, allowing for more uniform distribution and greater interaction with oxidizing substrates [48]. Furthermore, the nano-matrix protects these volatile compounds from degradation by light, oxygen, and heat, ensuring they remain active for a longer duration and leading to a lower ORR [46]. This is in agreement with Huang et al. [49], who identified allyl sulfides in garlic essential oil as key contributors to its antioxidant effects through the scavenging of reactive oxygen species. The data also provides a valuable comparison with synthetic standards, showing that while high concentrations of BHT and α-tocopherol may be more potent, the nano garlic oil's performance is impressive. Its AA and AAC values are higher than BHT at a 50 mg/L concentration, demonstrating that nano-formulation can effectively bridge the gap between natural and synthetic antioxidants, offering a more effective, clean-label alternative for food preservation.

The provided data Table 3, in conjunction with supporting scientific literature, demonstrates that nano garlic oil is a significantly more potent antimicrobial agent than normal garlic oil. This is evident from the consistently larger inhibition zones produced by the nano-formulation against a range of microorganisms. Against bacterial strains, the nano garlic oil yielded a significantly larger inhibition zone for *E. coli* (25.12±0.54 mm) compared to the normal oil (16.28±0.65 mm), and a larger zone for *Salmonella spp.* (17.08±0.81 mm vs. 14.11±0.29 mm). A similar pattern was observed against fungal strains, with the nano oil producing a larger zone against both *Aspergillus niger* (19.70±0.67 mm vs. 12.20±0.87 mm) and *Aspergillus flavus* (26.18±1.04 mm vs. 20.89±0.92 mm). This enhanced efficacy is a direct result of the principles of nano-emulsification, which overcome the limitations of garlic oil's volatile, poorly soluble organosulfur compounds, such as allicin. The nano-formulation's superiority is due to three key factors: a higher surface area-to-volume ratio, which allows for more extensive contact with microbial cell walls [46]; enhanced permeability, enabling the tiny nano-droplets to more easily penetrate cell walls and membranes [50]; and improved stability, as nano-encapsulation protects the active compounds from degradation by light and oxygen [51]. The findings corroborate research by Batiha et al. [52], who showed that allicin's biocidal activity is effective against a wide variety of antibiotic-resistant, Gram-positive, and Gram-negative bacteria, and studies by Hassanzadeh et al. [46] and Ghorbani Gorji [50] that have highlighted the superior antibacterial and antifungal effects of nanoemulsions against foodborne pathogens. In essence, the data clearly illustrates that nano-formulation is a highly effective strategy for boosting the natural antimicrobial properties of garlic oil, making it a powerful tool for food preservation and safety.

Table 2. Antioxidant activity of normal and nano garlic oil

Oil/Standard	Antioxidant value[a] AOX (A/h)	Antioxidant activity[b] AA (%)	Oxidation rate ratio[c] (ORR)	Antioxidant activity coefficient[d] (AAC)
Normal garlic oil	0.073 ± 0.009	87.15 ± 0.79 [c]	0.130 ± 0.007	678.38 ± 6.34
Nano garlic oil	0.042 ± 0.003	92.91 ± 0.54 [b]	0.071 ± 0.005	776.51 ± 5.98
Control	0.561 ± 0.011	0.00 ± 0.00	0.990 ± 0.00	0.00 ± 0.00
BHT, 50 mg/L	0.074 ± 0.003	86.94 ± 0.13 [c]	0.130 ± 0.09	683.73 ± 7.11
BHT, 100 mg/L	0.014 ± 0.001	97.83 ± 0.11[a]	0.021 ± 0.003	870.00 ± 8.79
α-tocopherol, 50 mg/L	0.012 ± 0.001	98.60 ± 0.09 [a]	0.019 ± 0.001	873.04 ± 4.56

[a] Antioxidant value (AOX, A/h) = The absolute value of slope (Abs was plotted against time).
[b] Antioxidant activity (AA, %) = (R control - R sample) / R control x 100 where: R control and R sample were the bleaching rates of beta-carotene in reactant mixture without antioxidant and with plant extract, respectively
[c] Oxidation rate ratio (ORR) = R sample / R control
[d] Antioxidant activity coefficient (AAC) = (Abs S120 - Abs C120) / Abs C 0 - Abs C 120) x 1000 where: .Abs S 120 was the absorbance of the antioxidant mixture at time 120 min, Abs C 120 was the absorbance of the control at time 120 min, Abs C 0 was the absorbance of the control at zero time.
[e] Each value represents mean ±SD. Values with different superscript letters in the same column are significantly different at p≤0.05.

Table 3. Antimicrobial effect of normal and nano garlic oil

Factor	Normal garlic oil	Nano garlic oil
Antibacterial effect (Agar well diffusion (mm)		
E. coli	16.28 ± 0.65 [b]	25.12 ± 0.54[a]
Salmonella spp.	14.11 ± 0.29 [b]	17.08 ± **0.81** [a]
Antifungal effect (Agar well diffusion (mm)		
Aspergillus niger	12.20 ± 0.87 [b]	19.70 ± 0.67 [a]
Aspergillus flavus	20.89 ± 0.92 [b]	26.18 ± 1.04 [a]

Values represent the mean ±SD (n=3). Means with various superscript letters in the same raw are different significantly at $p \leq 0.05$.

3.2. Biological Studies "Impact of Normal and Nanoparticle Garlic Oils on Rats with Liver Damage"

3.2.1. Body Weight Gain (BWG), Feed intake (FI) and Feed Efficiency Ratio (FER)

Effect of four weeks treatment with normal and nanoparticles garlic oils on BWG, FI and FER of hepatotoxic rats induced by CCl_4 was shown in Table 4. In rats with carbon tetrachloride (CCl4)-induced liver damage, the administration of nano garlic oil (NGO) and normal particle garlic oil (GO) at a dose of 200 mg/kg bw/day demonstrated significant protective and restorative effects on body weight gain (BWG), feed intake (FI), and feed efficiency ratio (FER). The data shows that CCl_4 induction in the model group (G2) caused a substantial decrease in BWG (−43.07%), FI (−33.27%), and FER (−28.048%) compared to the normal group (G1), which are classic signs of hepatotoxicity and impaired metabolic function. Both garlic oil treatments reversed these negative effects, with the NGO- treated group (G3) showing impressive increases in BWG (+48.953%), FI (+33.85%), and FER (+23.728%), and the GO-treated group (G4) showing significant increases as well, with BWG (+41.062%), FI (+19.11%), and FER (+16.949%). Interestingly, the nano garlic oil (NGO) appeared more effective than the normal-formulation (GO) for these specific physiological parameters, leading to greater percentage increases across all three metrics. This result indicates that nano oil may have a more effective therapeutic impact on these specific areas. It suggests the need for further research to explore factors such as dose-response relationships, the influence of encapsulation on the release of active compounds, and the potential presence of a wider range of synergistic compounds. This data is consistent with extensive literature on the hepatoprotective properties of garlic, which are attributed to its organosulfur compounds like allicin, known for their powerful antioxidant properties that scavenge free radicals and prevent lipid peroxidation, a primary mechanism of liver cell damage [53,54]. By mitigating liver injury, the treatments allow the liver to recover, thereby restoring metabolic functions, appetite, and the ability to efficiently utilize food. This restoration of BWG and FI is a well-documented consequence of liver toxicity [55,56,57,58,59] and similar bioactive compounds are also found in other parts of the garlic plant [15,60,61,62]. Our current findings indicate that for specific metrics such as BWG and FI, the nano oil offered a more substantial advantage. This is an important factor to consider for future research and development.

Table 4. Effect of four weeks treatment with normal and nanoparticles garlic oils on BWG, FI and FER of hepatotoxic rats induced by CCl_4*

Groups	Body weight gain (BWG, %)		Feed intake (FI, g/day/rat)		Feed efficiency ratio (FER)	
	Mean ±SD	% of change	Mean ±SD	% of change	Mean ±SD	% of change
G1: Normal	1.091± 0.009 [a]	0.00	12.62 ± 0.28 [a]	0.00	0.082 ± 0.006 [a]	0.00
G2: Model Hepatotoxic	0.621± 0.011 [c]	-43.07	8.89 ± 0.31 [d]	-33.27	0.059 ± 0.008 [c]	-28.048
G3: NGO (200 mg/kg bw/day)	0.925± 0.023 [a]	48.953	11.71 ± 0.27 [b]	33.85	0.073 ± 0.003 [b]	23.728
G4: GO (200 mg/kg bw/day)	0.876± 0.034 [b]	41.062	10.69 ± 0.60 [c]	19.11	0.069 ± 0.005 [b]	16.949

* Data presented as mean ± SD of six rats in each group. Means under the same column with different superscript letters indicates significant at $p \leq 0.05$. G1, normal group; G2, model group (hepatotoxic); G3 and G4, model group treated with nano- garlic oil (NGO) and normal garlic oil (GO) by 200 mg/kg bw/day. % of change (%), with comparisons made between the hepatotoxic (Model) group and the normal group, as well as between the groups treated with GO and the heptatoxic group. bw, body weight.

3.2.2. Liver Functions

The data in Table 5 clearly demonstrate that while both normal garlic oil (GO) and nano garlic oil (NGO) are effective in restoring liver function, NGO exhibits superior therapeutic efficacy in rats with CCl_4-induced liver damage. The hepatotoxic model group (G2) showed severe metabolic disruptions, evidenced by a sharp decrease in hepatic glycogen content (-60.66%) and

glucose-6-phosphate dehydrogenase (G6PD) activity (-50.79%), along with a dramatic increase in glucose-6-phosphatase (G6Pase) activity (+312.84%). These changes are classic signs of liver damage where the organ loses its ability to store glucose and regulate metabolism [63,64,65]. G6Pase is known to oppose glycogen synthesis, and the drop in G6PD reflects increased oxidative stress as the body's natural defense mechanism, which produces the crucial antioxidant NADPH, is compromised [64]. Both garlic oil formulations significantly improved these markers, but NGO (G3) provided a more substantial recovery. Specifically, NGO increased glycogen by 73.19% and G6PD by 54.57% while decreasing G6Pase by 48.44%, outperforming GO (G4), which showed increases of 37.93% and 35.3% and a decrease of 23.56%, respectively. This superior effect of NGO is consistent with modern nanomedicine research. Its enhanced bioavailability allows the active compounds to be more efficiently absorbed and delivered to the damaged liver cells, a key advantage of nanoparticles [23,66,67]. The nano-encapsulation process also protects the sensitive bioactive compounds of garlic from degradation and allows for a sustained therapeutic effect through controlled release [22,68]. The overall findings align with existing literature on garlic's hepatoprotective and metabolic effects. Garlic's sulfur compounds, such as allicin, are known to combat liver damage by reducing inflammation and oxidative stress, thereby helping to restore the liver's metabolic capacity and confirming its ability to regulate carbohydrate metabolism [53,54]. The data from this study align with the findings of numerous other researchers who have utilized various plant-based bioactive compounds, including those found in garlic [14,69,70,71,73,74].

3.2.3. Glutathione Fractions

The provided data from Table 6 demonstrates that both normal garlic oil (GO) and nanoparticle garlic oil (NGO) effectively mitigate the severe depletion of hepatic glutathione fractions caused by CCl4-induced hepatotoxicity, a condition characterized by significant oxidative stress and a compromised antioxidant defense system. The hepatotoxic model group (G2) showed a marked decrease in both reduced glutathione (GSH) (−50.37%) and oxidized glutathione (GSSG) (−35.21%) compared to the normal group (G1), a clear indicator of the liver's rapid consumption of GSH to detoxify free radicals. Both garlic oil treatments restored these levels, with the NGO group (G3) showing a substantial increase in GSH (+70.97%) and GSSG (+41.30%) and the GO group (G4) showing significant increases of +29.77% and +17.39%, respectively. Consistent with previous findings, NGO proved more effective than GO in restoring these antioxidant markers, suggesting a more robust therapeutic response. This is consistent with extensive research highlighting garlic's hepatoprotective effects, which are primarily attributed to its organosulfur compounds like allicin that act as potent antioxidants, scavenging free radicals and enhancing the activity of antioxidant enzymes [53,54]. The observed restoration of the glutathione system is a key mechanism behind this protective effect. The consistent and compelling finding of nano garlic oil's (NGO) superior effectiveness compared to its regular form is intriguing. Several factors could explain this. It might be due to a more efficient absorption and metabolism of active compounds from the nano oil, a wider array of synergistic compounds in the NGO that work more effectively in combination, or the possibility that the nanoparticle formulation process itself activates these compounds. The data from the current study align with findings by Mihailovic et al.[66] who demonstrated the superior antioxidant effects of nanoparticles. Additionally, our results are consistent with the work of many other researchers who have used a variety of plant-based bioactive compounds, including those from garlic [14,53,68,70,73,74].

3.2.4. Reactive Oxygen Species and Malondialdehyde Content

The data presented in Table (7), showing the effects of garlic oil (GO) and nano garlic oil (NGO) on oxidative stress in rats, indicates that both formulations are effective in mitigating liver damage caused by CCl_4, but with distinct differences in efficacy. The hepatotoxic model group (G2) exhibited a dramatic increase in both Reactive Oxygen Species (ROS) and Malondialdehyde (MDA), with levels rising by 151.19% and 140.0%, respectively, confirming the successful induction of severe oxidative stress. However, when treated with the garlic oil formulations, these markers were significantly reduced. While normal GO (G4) was effective, decreasing ROS by 46.44% and MDA by 25.00%, the NGO (G3) demonstrated a superior therapeutic effect, leading to a remarkable reduction of 55.45% in ROS and 43.75% in MDA. This finding is consistent with and supported by a broad range of studies that highlight the antioxidant and hepatoprotective properties of garlic and its nanoparticles. Research by Bedawy, [53] and Shang et al., [54] shows that garlic's organosulfur compounds like allicin are potent antioxidants that combat oxidative stress by neutralizing free radicals and preventing lipid peroxidation. The superior efficacy of NGO is a recurring and intriguing finding in related research, often attributed to the enhanced properties of nanoparticles. This includes improved bioavailability, where their small size allows them to be more efficiently absorbed and reach target liver cells, as discussed by Linh et al. [76] and Bartneck et al. [67]. Nanoparticles can also provide controlled release of the active compounds over time, ensuring a sustained therapeutic effect [22], and the formulation process itself might even activate the bioactive compounds, making them more potent [23]. These results, which are in agreement with numerous other studies on plant-based compounds for liver protection [14,68,69,73,77], collectively suggest that nanotechnology offers a promising approach to improving the therapeutic potential of natural products for treating liver diseases.

3.2.5. Liver Histology

Figure 1 shows the effect of four weeks of treatment with normal and nanoparticle garlic oils on the liver histology of hepatotoxic rats induced by CCl_4. Histopathological examination of liver sections from Group 1 (control group) revealed the normal histoarchitecture of the hepatic parenchyma (Photos A and

B). In contrast, liver sections from Group 2 (hepatotoxic model control) exhibited marked histopathological lesions, including multifocal hepatocellular necrosis with inflammatory cell infiltration (Photo C), Kupffer cell proliferation (Photo D), inflammatory cell infiltration in the portal triads (Photos D, E, and F), sporadic hepatocyte necrosis (black arrow), and fibroplasia in the portal triad (Photo G). Meanwhile, liver sections from Group 3 (nanoparticle garlic oil-treated rats) showed Kupffer cell proliferation, slight vacuolization of some hepatocytes (Photo H), and sparse hepatocellular necrosis (Photo I). On the other hand, liver sections from Group 4 (normal garlic oil-treated rats) exhibited vacuolar degeneration of sporadic hepatocytes (Photos J, K, and L), focal hepatocellular necrosis associated with inflammatory cell infiltration (Photo L), and mild leucocytic exocytosis (Photo M).

Table 5. Effect of four weeks treatment with normal and nanoparticles garlic oils on liver functions of hepatotoxic rats induced by CCl_4*

Groups	Glycogen content (mg/g wet tissue)		Glucose-6-phosphate dehydrogenase activity (G6PD, U/g wet tissue)		Glucose-6-phosphatase activity (G6Pase, µmole/min/g wet tissue)	
	Mean ±SD	% of change	Mean ±SD	% of change	Mean ±SD	% of change
G1: Normal	12.33±1.89[a]	0.00	14.45±1.73[a]	0.00	2.57±0.25[d]	0.00
G2: Model (Hepatotoxic)	4.85±1.02[d]	-60.66	7.11±1.04[c]	-50.79	10.61±1.02[a]	312.84
G3: NGO (200 mg/kg bw/day)	8.40±0.55[b]	73.19	10.99±0.06[b]	54.57	5.47±0.56[c]	-48.44
G4: GO (200 mg/kg bw/day)	6.69±1.26[c]	37.93	9.62±0.64[b]	35.3	8.11±0.92[b]	-23.56

* Data presented as mean ± SD of six rats in each group. Means under the same column with different superscript letters indicates significant at $p \leq 0.05$. G1, normal group; G2, model group (hepatotoxic); G3 and G4, model group treated with nano- garlic oil (GO) and normal garlic oil (NGO) by 200 mg/kg bw/day. % of change (%), with comparisons made between the hepatotoxic (Model) group and the normal group, as well as between the groups treated with GO and the heptatoxic group. bw, body weight.

Table 6. Effect of four weeks treatment with normal and nanoparticles garlic oils on hepatic glutathione fractions of hepatotoxic rats induced by CCl_4*

Groups	GSH (mmole /g wet tissue)		GSSG (mmole /g wet tissue)	
	Mean ±SD	% of change	Mean ±SD	% of change
G1: Normal	10.76±1.06[a]	0.00	0.71±0.03[a]	0.00
G2: Model (Hepatotoxic)	5.34±0.62[b]	-50.37	0.46±0.05[b]	-35.21
G3: NGO (200 mg/kg bw/day)	9.13±1.03[a]	70.97	0.65±0.05[a]	41.30
G4: GO (200 mg/kg bw/day)	6.93±1.08[b]	29.77	0.54±0.04[ab]	17.39

* Data presented as mean ± SD of six rats in each group. Means under the same column with different superscript letters indicates significant at $p \leq 0.05$. G1, normal group; G2, model group (hepatotoxic); G3 and G4, model group treated with nano- garlic oil (NGO) and normal garlic oil (GO) by 200 mg/kg bw/day. % of change (%), with comparisons made between the hepatotoxic (Model) group and the normal group, as well as between the groups treated with GO and the heptatoxic group. bw, body weight; GSH, reduced glutathione; GSSG, oxidized glutathione.

Table 7. Effect of four weeks treatment with normal and nanoparticles garlic oils on hepatic reactive oxygen species and malondialdehyde content of hepatotoxic rats induced by CCl_4*

Groups	Reactive oxygen species (ROS, U/ml tissue)		Malondialdehyde content (MDA, nmole/g wet tissue)	
	Mean ±SD	% of change	Mean ±SD	% of change
G1: Normal	0.84 ± 0.09[c]	0.00	611.56 ± 9.44[d]	0.00
G2: Model (Hepatotoxic)	2.11 ± 0.21[a]	151.19	894.34 ± 11.45[a]	140.0
G3: NGO (200 mg/kg bw/day)	0.94 ± 0.05[bc]	-55.45	687.52 ± 7.18[c]	-43.75
G4: GO (200 mg/kg bw/day)	1.13 ± 0.12[b]	-46.44	751.69 ± 10.06[b]	-25.00

* Data presented as mean ± SD of six rats in each group. Means under the same column with different superscript letters indicates significant at $p \leq 0.05$. G1, normal group; G2, model group (hepatotoxic); G3 and G4, model group treated with nano- garlic oil (NGO) and normal garlic oil (GO) by 200 mg/kg bw/day. % of change (%), with comparisons made between the hepatotoxic (Model) group and the normal group, as well as between the groups treated with GO and the heptatoxic group. bw, body weight.

All of the histopathological data offer critical insights into the hepatoprotective potential of these formulations. In the control group (Group 1), liver sections revealed normal histoarchitecture with well-preserved hepatocytes and intact portal triads, consistent with typical hepatic tissue. In contrast, the hepatotoxic model group (Group 2) displayed pronounced histopathological alterations including multifocal hepatocellular necrosis, Kupffer cell proliferation, inflammatory infiltration in portal triads, and fibroplasia-pathological features widely attributed to oxidative stress induced by CCl_4 exposure, as supported by several authors [72,75,77,78,79,80,81]. Remarkably, the group treated with nanoparticle garlic oil (Group 3) exhibited moderate improvements such as slight vacuolization and sparse necrosis, indicating that the nano-formulation may improve garlic oil's bioavailability and therapeutic efficacy, as previously suggested by Zhang and Wang [82].

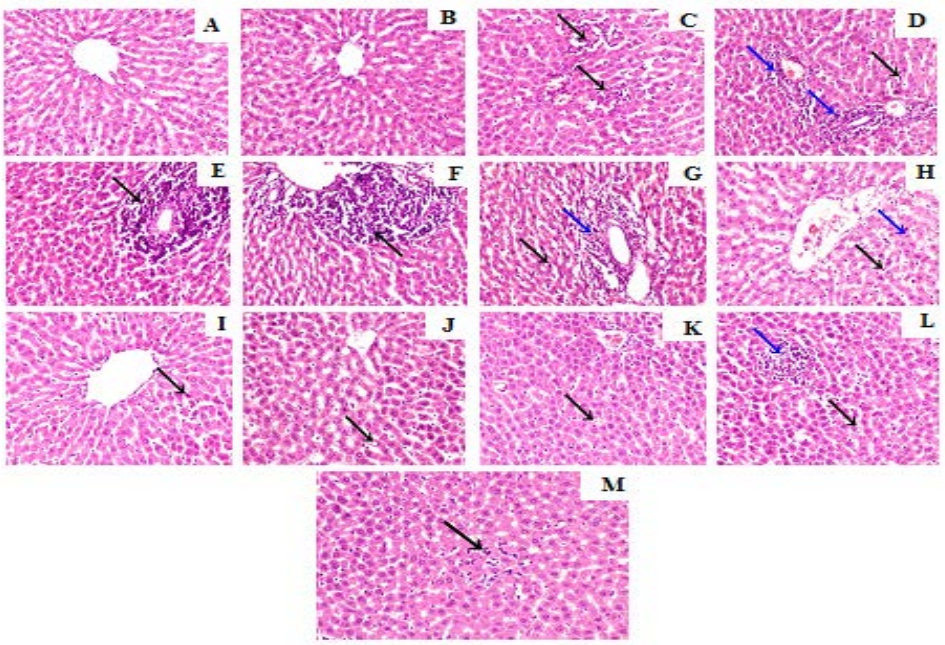

Photo A, liver of rat from group 1 showing the normal histoarchitecture of hepatic parenchyma; Photo B, liver of rat from group 1 showing the normal histoarchitecture of hepatic parenchyma; Photo C. liver of rat from group 2 showing multifocal hepatocellular necrosis with inflammatory cells (black arrow); Photo D, liver of rat from group 2 showing Kupffer cells proliferation (black arrow) and inflammatory cells infiltration in the portal triads (blue arrow); Photo E, liver of rat from group 2 showing inflammatory cells infiltration in the portal triads (black arrow); Photo F, liver of rat from group 2 showing inflammatory cells infiltration in the portal triads (black arrow); Photo G, liver of rat from group 2 showing necrosis of sporadic hepatocytes (black arrow) and fibroplasia in the portal triad (blue arrow); Photo H, liver of rat from group 3 showing Kupffer cells proliferation (black arrow) and slight vacuolization of some hepatocytes (blue arrow); Photo I, liver of rat from group 3 showing sparsely hepatocellular necrosis (black arrow); Photo J liver of rat from group 4 showing vacuolar degeneration of sporadic hepatocytes (black arrow); Photo K, liver of rat from group 4 showing vacuolar degeneration of sporadic hepatocytes (black arrow); Photo L, liver of rat from group 4 showing vacuolar degeneration of sporadic hepatocytes (black arrow) and focal hepatocellular necrosis associated with inflammatory cells infiltration (blue arrow); Photo M, liver of rat from group 4 showing mild leucocytic exocytosis (black arrow) (H & E X 200).

Figure 1. Effect of four weeks treatment with normal and nanoparticles garlic oils on liver histology of hepatotoxic rats induced by CCl_4 (H & E X 200)

Meanwhile, the group treated with normal garlic oil (Group 4) showed partial histological recovery with vacuolar degeneration and focal necrosis, reflecting a degree of hepatoprotection though less pronounced than the nanoparticle formulation, and suggesting that the effect is influenced by formulation type and possibly dosage, in line with observations by Zhang and Zhang [83].

The histopathological improvements observed in the nanoparticle garlic oil group underscore the significance of nanotechnology in enhancing the therapeutic efficacy of natural compounds. Nanoparticles can improve the solubility, stability, and bioavailability of bioactive compounds, leading to more pronounced pharmacological effects [82]. In contrast, the normal garlic oil group, while showing beneficial effects, exhibited residual liver damage, highlighting the need for optimized formulations to achieve maximal therapeutic benefits. These findings align with existing literature that reports the hepatoprotective effects of garlic oil against various hepatotoxic agents, including CCl_4 and NDEA (*N*-Nitrosodiethylamine) [78]. The observed histopathological changes, such as inflammatory cell infiltration and hepatocellular necrosis, are consistent with the known mechanisms of liver injury induced by these agents. The partial restoration of liver architecture in the garlic oil-treated groups further supports the potential of garlic oil as a therapeutic agent for liver protection.

3.3. Technology application "Mayonnaise"

3.3.1. Evaluation of Rancidity Progression in Mayonnaise Supplemented with Normal And Nano- garlic Oil Over the Storage Period

3.3.1.1. Acid Value

The provided data (Table 8 and Figure 2 & Figure 3) illustrates the protective effect of normal and nano garlic oil on the acid value (AV) of mayonnaise stored at room temperature over a 30-day period. A higher AV signifies increased lipid oxidation and a decrease in product quality. All three mayonnaise samples showed a progressive increase in AV over time, but the rate of increase differed significantly. The control mayonnaise, without any garlic oil, exhibited the most substantial rise in AV, with a total change of 122.61%, indicating a high susceptibility to oxidative rancidity. In contrast, adding normal garlic oil effectively slowed this process, resulting in a total AV change of 70.66%. The most effective treatment was the nano-formulation of garlic oil, which yielded the lowest final AV and a total change of only 56.08%, demonstrating superior antioxidant stability. This clear dose-response relationship, where nano-sized garlic oil particles provide the greatest protective effect, is consistent with scientific research. The primary reason for these differences is the antioxidant capacity of garlic oil's

active organosulfur compounds, which scavenge free radicals and inhibit lipid oxidation [48]. The superior performance of the nano garlic oil (Nano GO) is attributed to nano-encapsulation, a concept explored in various food science studies, which significantly improves the antioxidant activity, solubility, and stability of natural compounds. The smaller particle size of the nanoemulsion increases the surface area for the active compounds to interact with the lipids, thereby enhancing their efficiency in preventing oxidation [48]. A study by Hassanzadeh et al. [46] similarly found that garlic extract-loaded nanoemulsions had a positive impact on primary oxidation products in mayonnaise, which directly correlates with the AV changes observed in this data [46]. Furthermore, similar research confirms the effectiveness of other natural antioxidants like rosemary and green tea essential oils in extending the shelf life of high-fat foods by reducing lipid oxidation indicators, reinforcing the conclusion that natural antioxidants, particularly in nano-emulsified forms, can effectively mitigate food deterioration [50].

Table 8. Impact of incorporating normal and nano garlic oil on the acid value (AV) of mayonnaise stored at room temperature over a 30-day period*

Product	Storage period (days)							
	0		10		20		30	
	mg KOH /g oil	% of change	mg KOH /g oil	% of change	mg KOH /g oil	% of change	mg KOH /g oil	% of change
Mayonnaise (MY)	0.261± 0.030 ^{a}	0.00	0.363 ± 0.041 ^{a}	39.08	0.498 ± 0.037 ^{a}	90.80	0.581 ± 0.045 ^{a}	122.61
MY + 500 mg normal GO	0.259 ± 0.022 ^{a}	0.00	0.349 ± 0.031 ^{ab}	34.75	0.395 ± 0.034 ^{b}	52.51	0.442 ± 0.029 ^{b}	70.66
My + 500 mg Nano GO	0.255 ± 0.021 ^{a}	0.00	0.318 ± 0.025 ^{b}	24.71	0.361 ± 0.041 ^{b}	41.57	0.398 ± 0.025 ^{c}	56.08

* Each value represents mean of three replicates ±SD. Means with various superscript letters in the same column are different significantly at $p \leq 0.05$.GO, garlic oil. The percentage of change (%), with comparisons made between the storage period (days) and the zero time values.

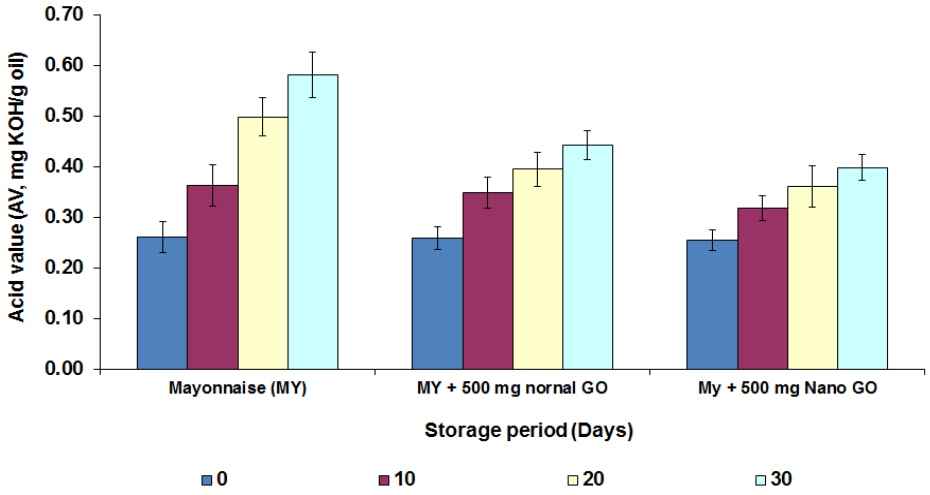

Figure 2. Impact of incorporating normal and nano garlic oil on the acid value (AV, mg KOH /g oil) of mayonnaise stored at room temperature over a 30-day period. Each value represents mean of three replicates ±SD. Means with various superscript letters in the same column are different significantly at p≤0.05.GO, garlic oil. The percentage of change (%), with comparisons made between the storage period (days) and the zero time values.

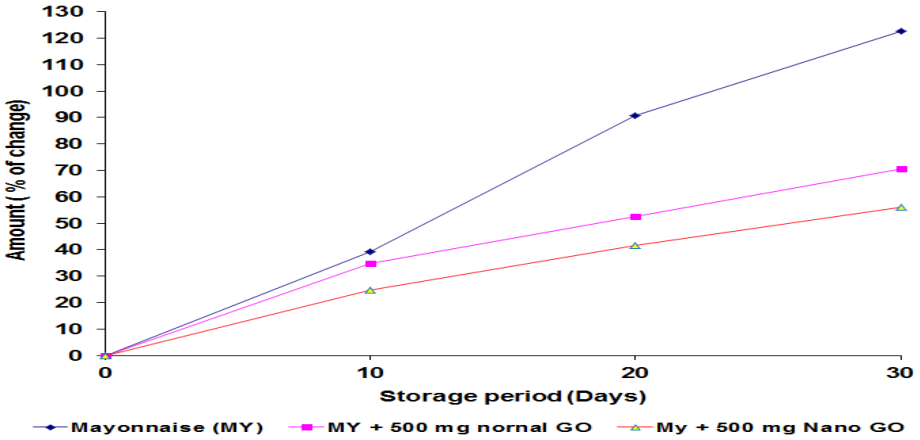

Figure 3. Impact of incorporating normal and nano garlic oil on the acid value (AV, as a percent of change) of mayonnaise stored at room temperature over a 30-day period. Each value represents mean of three replicates ±SD. Means with various superscript letters in the same column are different significantly at p≤0.05.GO, garlic oil. The percentage of change (%), with comparisons made between the storage period (days) and the zero time values.

Table 9. Impact of incorporating normal and nano garlic oil on the peroxide value (PV) of mayonnaise stored at room temperature over a 30-day period*

Product	Storage period (days)							
	0		10		20		30	
	meq peroxide /Kg oil	% of change	meq peroxide/ Kg oil	% of change	meq peroxide /Kg oil	% of change	meq peroxide /Kg oil	% of change
Mayonnaise (MY)	1.19 ± 0.096 [a]	0.00	11.27 ± 0.566 [a]	847.06	14.91 ± 0.959 [a]	1152.94	15.89 ± 0.996 [a]	1235.29
MY + 500 mg normal GO	1.17 ± 0.085 [a]	0.00	6.93 ± 0.456 [b]	492.31	13.01 ± 0.843 [a]	1011.97	14.33 ± 0.74 [a]	1124.79
My + 500 mg Nano GO	1.14 ± 0.076 [a]	0.00	5.34 ± 0.240 [c]	368.42	10.81 ± 0.742 [b]	848.25	13.09 ± 0.640 [b]	1048.25

* Each value represents mean of three replicates ±SD. Means with various superscript letters in the same column are different significantly at p≤0.05.GO, garlic oil. The percentage of change (%), with comparisons made between the storage period (days) and the zero time values.

3.3.1.2. Peroxide Value

The provided data, including Table 9 and Figure 4 and Figure 5, demonstrates the protective effect of normal and nano garlic oil on the peroxide value (PV) of mayonnaise during 30 days of room-temperature storage. PV is a key indicator of lipid oxidation, with a lower value signifying greater product stability. The results show a dramatic increase in PV for all three mayonnaise samples, but the rate of increase varies significantly. The control sample, without any garlic oil, experienced a colossal PV increase from an initial 1.19±0.096 to a final 15.89±0.996 meq peroxide/Kg oil after 30 days, representing a 1235.29% change and indicating severe oxidative rancidity. The addition of normal garlic oil significantly curbed this increase, resulting in a final PV of 14.33±0.746 meq peroxide/Kg oil (a total change of 1124.79%). However, the nano-formulation of garlic oil proved to be the most effective, limiting the PV increase to a final value of 13.09±0.640 meq peroxide/Kg oil, the lowest among all samples, with a total change of 1048.25%. This superior antioxidant stability is attributed to the enhanced performance of the nano garlic oil (Nano GO), which aligns with the scientific consensus on the use of nanotechnology in food preservation. Nano-encapsulation of natural antioxidants, such as garlic essential oil, improves their activity and stability by providing a larger surface area for active compounds to interact with lipids, thereby more effectively preventing oxidation [48]. This principle is supported by studies on garlic nanoemulsions in edible oil, which showed they are more effective than bulk extracts in reducing oxidation markers like PV [46]. Similar research on other plant-based nanoemulsions, such as those from rosemary and green tea, also highlights the importance of these natural additives and the role of nanotechnology in maximizing their efficacy in high-fat foods [50]. While the data presented here indicates significant oxidation under room-temperature conditions, another study by Hady et al., [84] found that mayonnaise stored under refrigerated conditions had much lower final PV values (less than 10 meq/kg oil), suggesting that while garlic oil is an effective antioxidant, storage temperature is a critical factor in preventing rancidity. Numerous researchers have reported similar findings for other natural antioxidants used in food products, which is consistent with these results [59,80,85,86,87,88,89,90].

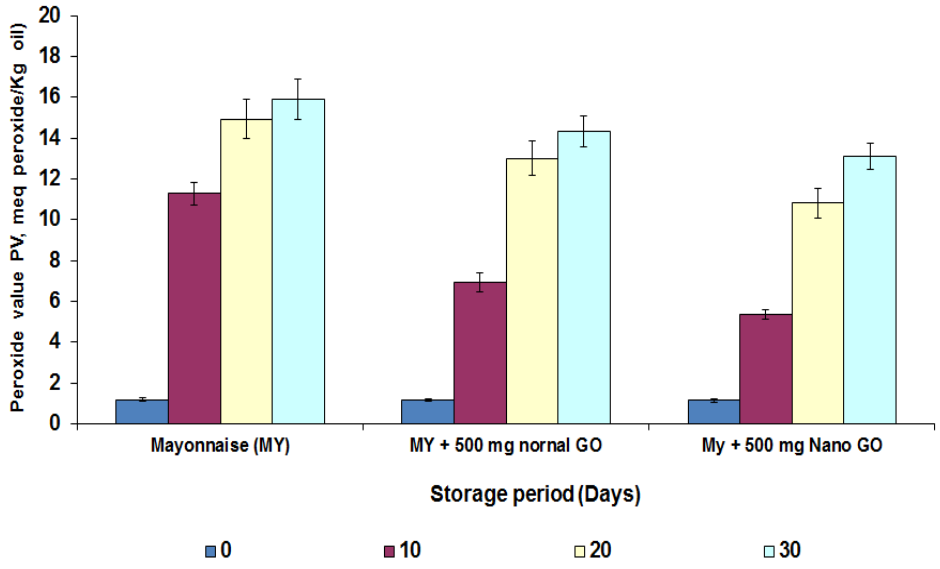

Figure 4. Impact of incorporating normal and nano garlic oil on the peroxide value (PV, meq peroxide/ Kg oil) of mayonnaise stored at room temperature over a 30-day period. Each value represents mean of three replicates ±SD. Means with various superscript letters in the same column are different significantly at p≤0.05.GO, garlic oil. The percentage of change (%), with comparisons made between the storage period (days) and the zero time values.

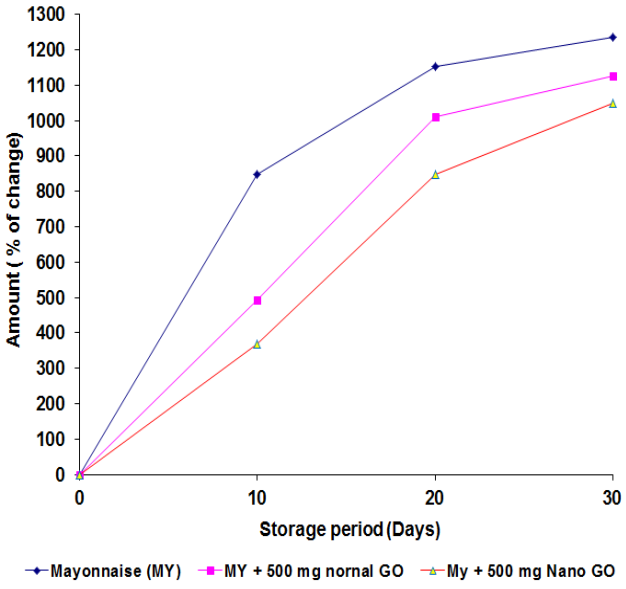

Figure 5. Impact of incorporating normal and nano garlic oil on the peroxide value (PV, as a percent of change) of mayonnaise stored at room temperature over a 30-day period. Each value represents mean of three replicates ±SD. Means with various superscript letters in the same column are different significantly at p≤0.05.GO, garlic oil. The percentage of change (%), with comparisons made between the storage period (days) and the zero time values.

3.3.2. Sensory Evaluation

The provided data, from Table (10), its accompanying analysis demonstrate that the nano-formulation of garlic oil (Nano GO) offers superior sensory properties in mayonnaise compared to both a control sample and one with normal garlic oil (normal GO). While the control serves as a baseline for initial quality, the normal GO sample showed significant negative impacts on the mayonnaise's sensory profile, with a −23.58% change in odor and a −17.42% change in taste, leading to an overall acceptability decline of −5.18%. This is likely because the pungent organosulfur compounds in garlic essential oil, like allicin, overpower the mayonnaise's delicate flavor, a common issue with strong, volatile plant extracts [46]. Conversely, the Nano GO sample was highly effective at improving or maintaining sensory attributes, with taste and overall acceptability improving by 11.82% and 12.61%, respectively, and odor by 5.97%. These findings align with scientific literature on nanotechnology in food science, which explains that nano-encapsulation can effectively mask the harsh flavor and aroma of these compounds, leading to a milder, more acceptable taste. This process also allows for a slower, more controlled release of the active ingredients, which not only enhances the flavor profile but also prolongs the antioxidant effect. Research by Ghorbani Gorji et al. [50] supports this, noting that nano-emulsion systems provide a solution for balancing the functional benefits of natural extracts with their potential negative sensory impacts [50]. The data, therefore, confirms that nano-emulsification is a crucial tool for enhancing the sensory quality of food by mitigating the undesirable characteristics of raw extracts while still delivering their functional benefits [46].

Table 10. Impact of incorporating normal and nano garlic oil on the sensory evaluation of mayonnaise stored at room temperature over a 30-day period*

Parameter	Mayonnaise (MY)		MY + 500 mg normal GO		My + 500 mg Nano GO	
	Degree	% of change	Degree	% of change	Degree	% of change
Texture	8.24 ± 0.23 [a]	0.00	7.98 ± 0.14 [a]	-3.16	8.19± 0.25 [a]	-0.61
Color	7.90 ± 0.19 [a]	0.00	6.87 ± 0.31 [b]	-13.04	7.97± 0.16 [a]	0.89
Odor	6.70 ± 0.12 [a]	0.00	5.12 ± 0.15 [b]	-23.58	7.10± 0.19 [a]	5.97
Taste	6.43 ± 0.23 [a]	0.00	5.31 ± 0.12 [b]	-17.42	7.19± 0.14 [a]	11.82
Over all acceptability	7.14 ± 0.20 [b]	0.00	6.77 ± 0.11 [b]	-5.18	8.04± 0.13 [a]	12.61

* Each value represents mean of three replicates ±SD. Means with various superscript letters in the same raw are different significantly at $p \leq 0.05$.GO, garlic oil. The percentage of change (%), with comparisons made between the treated samples with GO and the normal Mayonnaise (MY).

Conclusion

This study successfully demonstrated the enhanced therapeutic and functional properties of garlic oil when formulated as a nanoemulsion. The nano-formulation exhibited superior antioxidant and antimicrobial activities compared to its normal counterpart, which directly translated to improved performance in both biological and food-related applications. The findings from the biological experiments provide strong evidence of the hepatoprotective potential of nano garlic oil. Its ability to more effectively restore physiological and biochemical markers, mitigate oxidative stress, and improve liver histology in rats with induced liver damage highlights its promising role as a natural remedy for liver diseases. The enhanced bioavailability and stability conferred by nano-encapsulation were critical to these therapeutic effects, suggesting that this delivery system can significantly improve the efficacy of essential oils. Furthermore, the results from the food application section confirm the commercial feasibility of incorporating nano garlic oil into products like mayonnaise. The nano-formulation not only offered superior protection against lipid oxidation but also improved the product's sensory attributes, effectively masking the pungent flavor and aroma of garlic while retaining its beneficial properties. This dual benefit of preservation and sensory enhancement addresses key challenges in the food industry, paving the way for the development of healthier, more stable food products. Based on these findings, it is recommended to conduct further research into the long-term toxicity and metabolic fate of nano garlic oil in animal models to ensure its safety for human consumption. Additionally, future studies should explore the application of this nano-formulation in a wider range of food products and investigate the specific mechanisms by which it enhances drug delivery and therapeutic outcomes.

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Ethical Approval

All biological procedures for this study were approved by the Scientific Research Ethics Committee (Approval # 29-SREC-12-2023), Faculty of Home Economics, Menoufia University, Shebin El-Kom, Egypt.

Conflicting Interests

The authors have intentionally excluded some information from this article to aid in its publication.

Author Contributions

Yousif Elhassaneen: Spearheaded the study's design and execution, including the development of protocols and supervision of experiments. He also validated all data and statistical analyses, and was a key contributor to the drafting and revision of the manuscript. Dina Muhammad: Executed the primary experimental work, including data collection, organization, and analysis. She also gathered foundational information and wrote the initial draft of the manuscript. Mai Gharib: Assisted with the study's protocol preparation and oversight of practical experiments. She also contributed to the collection of conceptual data, validation of results, and manuscript drafting. Hesham Saad: Aided in the preparation of the study protocol, gathering conceptual insights and confirming data accuracy.

Abbreviations

AA, antioxidant activity, Abs, absorbance, AV, acid value, BCB, β-carotene bleaching (BHT, butylated hydroxytoluene, BWG, body weight gain, CCl_4, carbon tetrachloride, DPPH, 2,2-diphenyl-1-picrylhydrazyl, FI, feed intake, dimethyl sulfoxide, G6PD, glucose-6-phosphatase, FER, feed efficiency ratio, GO, normal garlic oil, G6Pase, glucose-6-phosphate dehydrogenase, GSH, reduced glutathione, GSSG, oxidized glutathione, HDL-c, High density lipoprotein-cholesterol, $IC_{50,}$ LDL-c, low density lipoprotein-cholesterol, MDA, malondialdehyde, NGO, nano garlic oil, ROS, reactive oxygen species, PV, peroxide value, SD, standard deviation, TBA, thiobarbituric acid, TGs, triglycerides.

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