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Study the Impact of Garlic Essential Oils Loaded Lipid Nanoparticles (GLNS) on Some Physiological Parameters in Male Rats

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Abstract: The aims of work is detect the impact antioxidant garlic essential oils loaded lipid nanoparticles (GLNs) in male rats. Thirty adult male Wister rats weighing 148±10 g at 90 days of age were split into five groups.Group 1 were administered High-fat feed with 30% animal fat added was given for 60 days and Second group were control group , the Second group were administered garlic essential oil (GO) (10 mg\kg\day) for 28 days, fourth group administered with free LNPs (20mg \ Kg) and the fifth group administered with LNPs-GO (20mg\ kg \day). The result of X-Ray diffraction of LNPs-GO in major peak at 28.56° in the diffract to gram has moved to a lower 2θ value.The Zeta Potential (ZP) values of all formulations prepared were measured to be negative. The result indicates that the ZP ranges between 33.7 mV to -48.7 mV with the smallest size comprising the GLNs. The PDI value in the case of LNPs- GO were 0.265. regarding of Antioxidant Indicators the Blood Malondialdehyde Level (MDA) revealed that Glutathione Levels in Blood (GSH) significantly increased ($P<0.05$) in the G3, G4, and G5 groups relative to the G1 group, but they significantly decreased ($P<0.05$) in the G1 group.

The results also revealed that the G3 and G4 groups had significantly lower liver enzyme levels (AST, ALT, and ALP) than the G1 group, however the G5 groups did not significantly differ from the G1 group. Additionally, there was a noteworthy rise ($P<0.05$) in the G3 and G4.

Keywords: garlic essential oils, Nanoparticles, Physiological Parameters.

Introduction

Based on the unique organo-sulfur compounds, the garlic (Allium sativum L) plant is widely used as a seasoning of food and is believed to have a good effect on human health (1). The main components of garlic oil are diallyl disulfide, trisulfide, allyl propyl disulfide, disulfide, and most likely diallyl polysulfide (2). Particularly, it has been discovered that the essential oils extracted from garlic exhibit favorable antimicrobial, anti-diabetic, and anti-carcinogenic properties. This is noteworthy for various pharmacologic and medicinal applications (3). Moreover, they have the potential to lower blood pressure, cholesterol, and plasma aggregation (4).Nanoparticles (NP) are colloidal particles loaded with active chemicals that had been dissolved, entrapped, adsorbed, or adhered to the particle.(5). Some of the advantages associated with the use of nanotechnology in phytoceuticals are easy to manufacture as prolonged release systems, better permeability, better physicochemical stability, better tissue dispersion, and enhanced solubility and bioavailability. ROS and other intermediates caused by oxidative damage Death, fibrogenesis, and the extension of chronic inflammatory responses, especially hepatic chronic wound healing and liver fibrogenesis, are all affected by ROS and other oxidative stress-induced intermediate (6).Patients with HCV infection had lower levels of antioxidants and higher amounts of ROS/RNS (7). It has been

discovered that individuals who are overheated may benefit greatly from antioxidant therapy. Still, it appears that traditional antioxidants such as vitamins C and E are ineffective (8). The goal of this experiment was to determine the impact of antioxidant garlic -loaded lipid nanoparticles (GLNs)

Materials and methods

Animals and Housing

In this investigation, thirty adult male Wister rats weighing 150±10 g and 90 days old were utilized. Throughout the experiment, a 12-hour light/dark cycle was observed, with the light turning on at 6:00 a.m. and off at 6:00 p.m. The room temperature was kept at 23 ± 2 ºC.

Experimental design

The subsequent treatment was given to thirty male Wister rats for a total of continuous days when they were randomly equal groups of six animals each:

First group (G1).will be administered High-fat feed with 30% animal fat added was given for 60 days

Second group(G2) : will remain untreated as a negative control.

Third group(G3): Will be orally administered garlic essential oil (GO) (10 mg\kg\day) for 28 days

Forth group (G4) : taken orally with free lipid nanoparticles (LNPs) (20mg \ Kg) for 28 day .

Fifth group (G5): taken orally with LNPs-GO (20mg\ kg \day)for 28day.

Zeta potential and particle size

The particle size, polydispersity index, and zeta potential of the LNs formulations were ascertained using the dynamic light scattering method (ZetaSizer Nano-ZS) (9).

Calculating the Efficiency of Encapsulation and Loading

EE be referenced as the perentage of total quantity of the PL received through the formulation after doing the procedure. Loading capacity (LC) of the stimulation mass of entrapped GO to the lipid's (stearic acid) total mass. EE and LC were calculated as was explained above. The 10 mg LNPs-PL formulations were well weighed by dissolving them with 10 ml methanol. This was followed by centrifuging of the samples at 9,000 rpm within 30 minutes. Using a UV/VIS spectrophotometer (PG Instruments Ltd., T80+ UV/VIS Spectrophotometer)

Stands for differential scanning calorimetry (DSC).

GO and LNPs-GO DSC scans were performed using a Mettler DSC 821e (Mettler Toledo, Germany). Five milligrams of the samples were put into pans made of aluminum oxide, sealed, and then examined. An empty metal pan served as a reference. The melting point of SLN dispersions was compared to that of the bulk lipid (10), and DSC was performed at a temperature range of 25 to 250 °C at a rate of 5 °C/min under N2 flow.

Liver function test

2µl of serum was utilized for serum Alkaline phosphatase (ALP), Alanine aminotransferase (ALT), and Aspartate aminotransferase (AST) assays using the COBAS C 111 auto-analyzing protocol.

Antioxidant Indicators

the kit assays the Antioxidant Indicators auto-analyzing technique using the enzyme-linked immune sorbent test (ELISA), which is based on the Biotin double antibody sandwich technology.

Statistical Analysis

The Chi-square test and SPSS Preogram (version 18) software (2010) were used to analyze the data. Statistical significance was defined as a P value of $p \leq 0.05$ (11).

Result and Discussion

Characterization of LNPs-GO

X-Ray diffraction of LNPs-GO

X-Ray diffraction (XRD) The x-ray patterns of LNPs have been captured distinctive peaks that are crisp and strong are readily identifiable in both LNPs and LNPs- GO . On the other hand, compared to pure PL, the LNPs major peak at 28.56° in the diffract to gram has moved to a lower 2θ value. The high overlap occurring in the peaks indicates that the two oxides are superimposed together, and the width of the band indicates its small crystalline size. he Debye-Scherrer equation was used to determine the average crystallite size of the synthesized LNPs-GO. LNPs nanoparticles had an average crystal size of 88 nm (12).

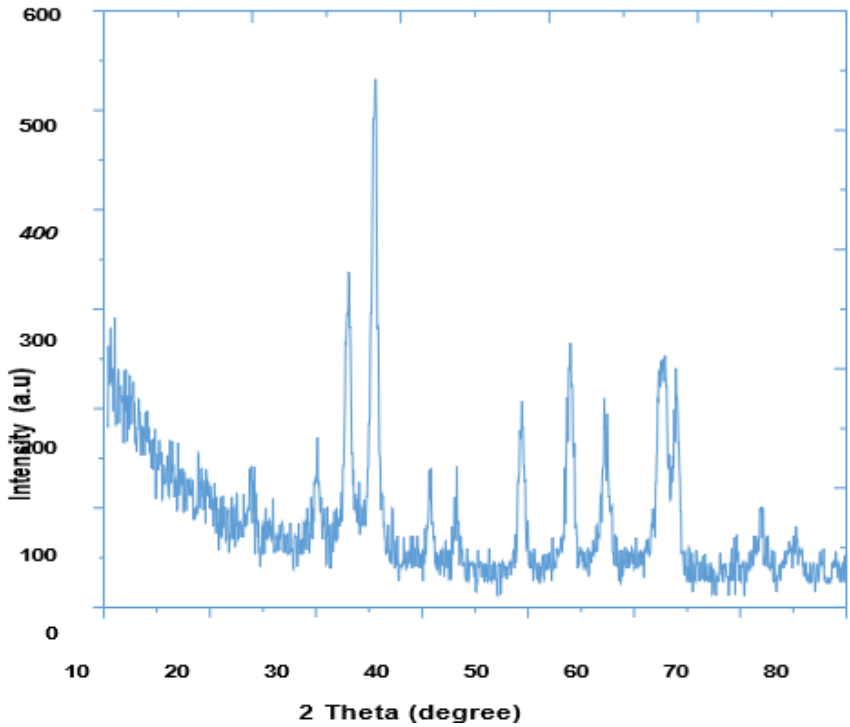

Figure.1: XRD of **LNPs- GO**

Scanning electron microscopy using field emission (FE-SEM)

The SEM pix of LNPs- GO is displayed in Figure 2. The SEM scan revealed that the prepared LNPs had a spheriical shape. Particle size disitribution was similar to DLS. After loding, the nanocapsules had a spherical form, high dispersion, and a limited size distribution (13).

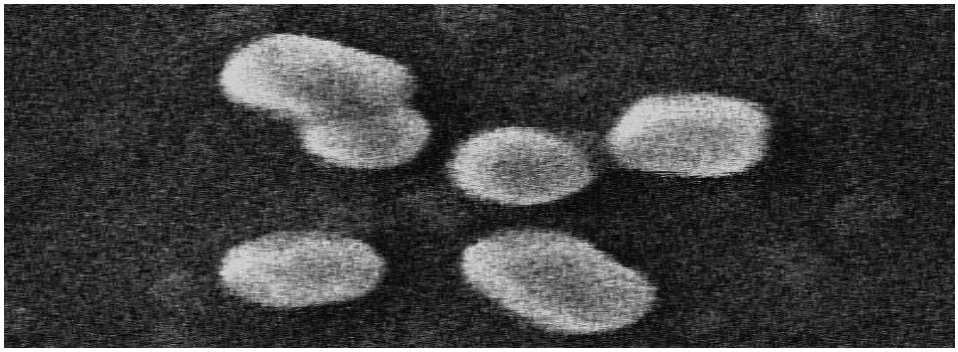

Figure 2: Appearance of LNPs-GO under scanning electron microscopy (SEM image of LNPs-PL at 50,000× magnification. Scale bar: 200 nm)

Polydispersity Index (PDI)

The PDI values provide critical insights into the size distribution and homogeneity of the nanoparticle populations. A PDI of 0.420 for the free LNPs . suggests a moderately broad size distribution, indicating some variability in particle size. Conversely, a PDI of 0.270 for the LNPs-GO (Figure .1). indicates a more homogeneous and uniform size distribution compared to the free LNPs. This suggests that the incorporation of garlic essential oil has contributed to a more uniform population of nanoparticles. Generally, PDI values below 0.3 are considered acceptable for pharmaceutical applications, indicating a relatively homogeneous population. The figure elicits a very clear size increase after loading of garlic essential oil. The LNPs-GO peak is shifted to the right and is bigger than that of the free LNPs. The decreased width of the peak in LNPs- GO corresponds to lower PDI value indicating more homogenous size distribution. Noticeably, the LNPs- GO sample has the small peak at the larger size range, which implies that a small portion of the nanoparticles becomes aggregated. overall, the data indicate that the addition of the garlic essential oil to the lipid nanoparticles has caused to increase in size and uniformity of the size distribution of the particles. The comparison of the PDI of LNPs- GO in the lower value underlines the idea of a better homogeneity which is beneficial in the pharmaceutical context. The intensity graph, along with size distribution data substantiates these results. because SLNs are spherical, they can be useful in the controlled release and preservation of encapsulated essential oil. This is explained by the fact that the spherical form of nanoparticles has the shortest contact area with the aqueous phase of the dispersed phase and the longest channel of access for essential oil filled nanoparticles when compared to other forms of nanoparticles (14).

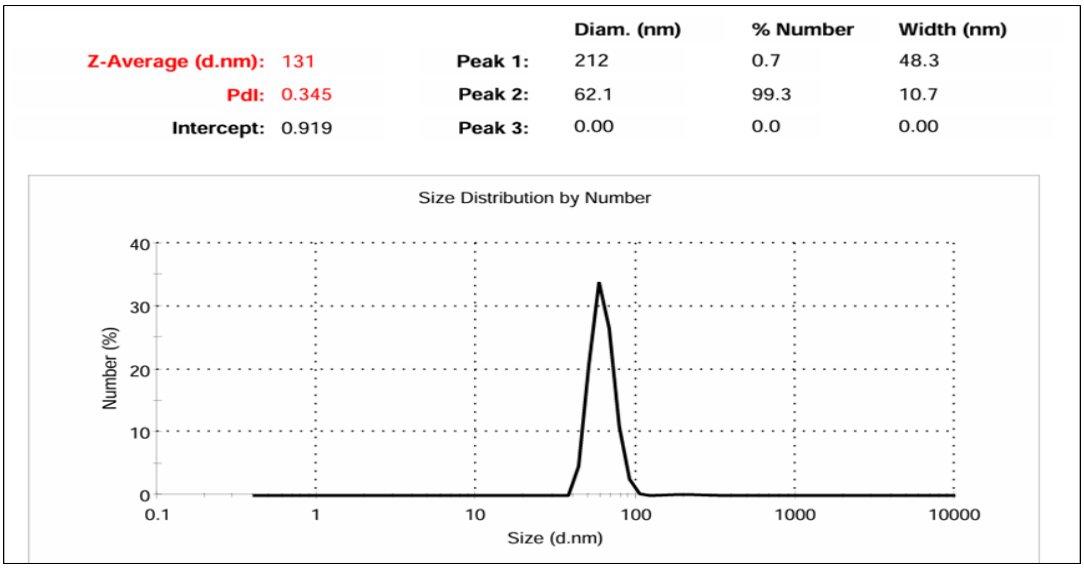

Figure 1: The size particle (z-average diiameter) and polydispersity index (PDI) of the LNs-GO formiulations.

Zeta Potential

The particle size and its distribution are among the more significant factors related to quality, affecting other macroscopic characteristics. The formulation component, manufacturing techniques, and environmental factors . the size of liquid nanoparticles is affected by a number of factors, including duration, temperature, pressure, number of cycles, and equipment (35). The experiment's particle size was 110.5±3 nm, with a zeta potential of -36.6±0.5 mV and a PDI of 0.320±0.04 based on the Z-average of LNs-GO (figure.2).

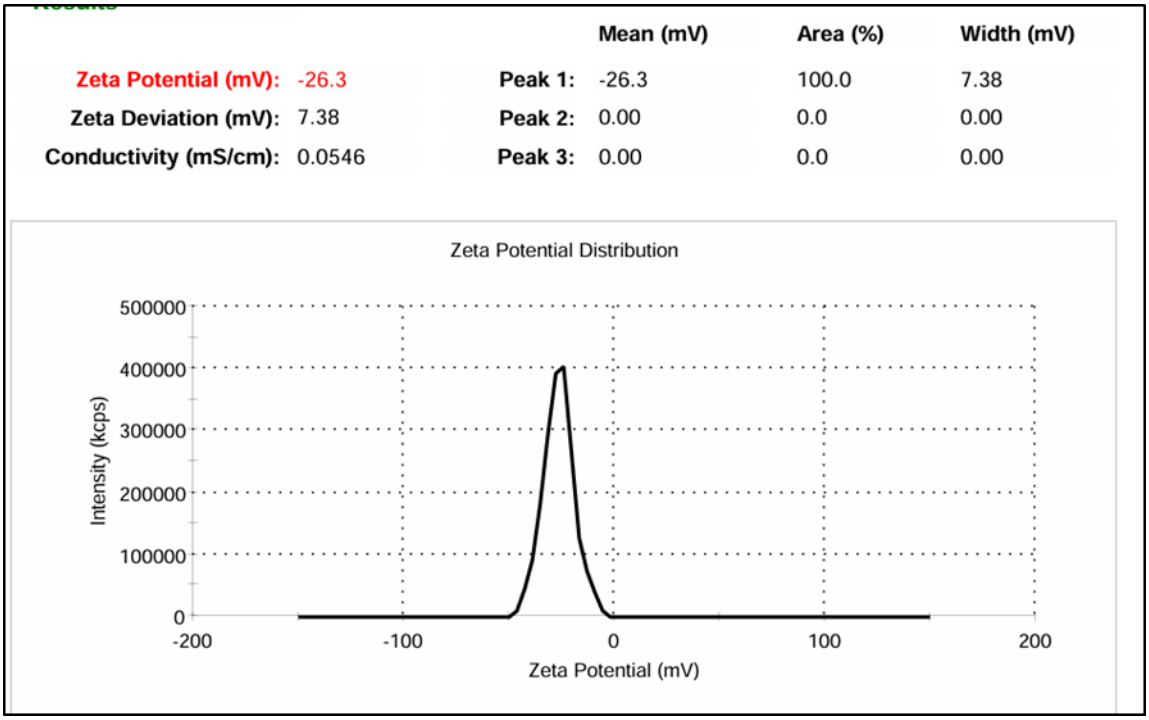

Figure 2: The zeta potential (zp) of the LNs- GO-formulations.

Antioxidant Indicators

Blood Malondialdehyde Level (MDA)

MDA levels were significantly higher (P<0.05) in the G1 group that received the high-fat diet than in the control group (G2), according to the statistical analysis results in Table (1). The results also revealed significant differences between the G3, G4, and G5 groups and the control group (G2), as well as a significant drop (P<0.05) between the G3 and G5 groups and the G1 group. Additionally, notable distinctions between the G3, G4, and G5 were noted.

Glutathione Levels in Blood (GSH)

GSH levels were significantly lower (P<0.05) in the G1 treated with the high-fat diet than in the control group, according to the results in Table (1). Additionally, compared to the G1 group, the G3, G4, and G5 groups exhibited a substantial increase (P<0.05) in the previously described table. In comparison to the control group (G1), a notable decline was noted in the G3, G5, and G5 groups. Additionally, notable distinctions between the G3, G6, and G5 were noted.

Groups	(µmol/mg) MDA	(µmol/mg) GSH
G1	1.598±0.015 e	2.859± 0.061 a
G2	3.221± 0.009a	1.600± 0.018 e
G3	1.923±0.006 d	2.587± 0.006 b
G4	2.753± 0.011 b	2.098± 0.007 d
G5	2.311± 0.006 c	2.241± 0.025 c
LSD	0.212	0.232

Table.1. the levels of MDA and GSH in serum of in male Rats

G1: will be administered High-fat feed with 30% animal fat added was given for 60 days . **G2**: will be kept without treatment as negative control. **G3:** Will be orally administered garlic essential oil (GO) (10 mg\kg\day) for 28 days **. G4:** Will be orally administered with free lipid nanoparticles (LNPs) (20mg \ Kg) for 28 day .

G5: will be orally administered with LNPs- GO (20mg\ kg \day)for 28day.

the results of the current study showed a significant decrease in glutathione (GSH) levels, accompanied by a significant increase in malondialdehyde (MDA) levels, in the group of animals treated with a high-fat diet (1T) when compared to the control group. These results are consistent with the findings of other studies (15). These results are also consistent with the study by Noeman(16), which indicated a decrease in GSH levels with an increase in MDA levels in the liver, kidney, and heart of rats treated with a high-fat diet. This may be due to damage Oxidative stress caused by a high-fat diet in these organs. Chen et al.'s study (17) also indicated a decrease in the levels of GSH, CAT, and SOD, along with an increase in lipid levels in the serum and livers of rats treated with a high-fat diet for thirty days. This was attributed to an imbalance between oxidants and antioxidants, and the resulting oxidative stress. Oxidative stress has led to the generation of large quantities of reactive oxygen species (ROS), which increases the rate of glutathione consumption, which is the most important non-enzymatic antioxidant in removing free radicals and their products, and is then converted to its inactive oxidized form, GSSG (18). Previous studies have confirmed the existence of a relationship between obesity and increased oxidative stress, which is observed in the increase in MDA levels with a decrease in the level of antioxidants (GSH, SOD, CAT), which increases lipid peroxidation, which is one of the most important reasons that make obesity a risk factor in the development of heart disease .In addition, the current results showed that giving the animals sage garlic essential oil and LNPs- GO with a high-fat diet led to a reduction in MDA levels and an increase in GSH levels compared to the hyperlipidemia group (G2). This may be due to the short duration of treatment, which was insufficient, or to the low dose of the drug used in the treatment. This may be due to the flavonoids found in garlic essential oil, which are known for their diverse biological properties. These antioxidants work to scavenge free radicals by activating antioxidant enzymes in the body and preventing the oxidation of proteins and lipids in cell membranes (19). The most important of these is LNPs- GO which plays an important role in stimulating the immune system and improving DNA gene expression within cells by stimulating the enzyme glutathione, due to its antioxidant role, which contributes to protecting the body from damage caused by toxic substances. (20) confirmed the role of LNPs- GO in reducing GSH consumption, as it is one of the most important natural antioxidants that reduces oxidative stress by scavenging free radicals. The reason may also be due to phenols. The active ingredients in garlic essential oil play an effective role in reducing oxidative stress by activating cellular antioxidant pathways, which enhances their role in scavenging free radicals. Among the most important of these is rosmarinic acid, which is a powerful antioxidant that helps protect phospholipids in cell membranes from oxidative stress (21).

Liver enzymes

the liver enzyme levels (AST, ALT, and ALP) in the serum of male rats fed the high-fat diet (G1) were significantly higher ($P<0.05$) than those of the control group (G2), according to the results in Table 2. While there was no discernible difference between the G5 and G1 groups, the data also revealed a considerable decline in the G3 and G4 groups relative to the G1 group. The G3 and G4 also showed a substantial rise ($P<0.05$).

Table.2. the levels Liver enzymes in serum of in male Rats

Groups	(U\L) ALP	((U\L) ALT	((U\L) AST
G1	81.85± 0.202 d	10.661 ±0.128 d	15.377 ±0.176 d
G2	96.11± 0.019 a	22.43 ± 0.026 a	26.563± 0.024 a
G3	84.55± 0.015 c	13.543 ± 0.07 c	18.518± 0.129 c
G4	93.822±0.010 a	20.864 ±0.02 a	24.869± 0.03 a
G5	94.927± 0.294 b	16.687± 0.05 b	20.672± 0.27 b
LSD	2.302	1.652	1.857

G1: will be administered High-fat feed with 30% animal fat added was given for 60 days . G2: control group . G3: Will be orally taken garlic essential oil (GO) (10 mg\kg\day) for 28 days . G4: Will be orally administered with free lipid nanoparticles (LNPs) (20mg \ Kg) for 28 day .

G5: orally taken with LNPs- GO (20mg\ kg \day)for 28day.

he statistical analysis revealed that the animals in Group G1 (fed a high-fat diet) had significantly higher levels of the liver enzymes AST, ALT, and ALP ($P<0.05$) than the control group. These outcomes aligned with the conclusions of (22).A high-fat diet damages the body, especially the liver tissue, which results in elevated liver enzyme levels (AST, ALT, and ALP). Our histological analysis supported this by showing necrosis, inflammatory infiltration in the liver, and the release of these enzymes into the circulation (23). A high-fat diet is positively associated with liver steatosis and elevated saturated fatty acids in the liver, which occur concurrently with acute liver tissue necrosis and cause an increase in the release of liver enzymes (AST, ALT, and ALP) into the circulation, as Zhang and his group (24) also confirmed.

Following a 28-day treatment course of administered garlic essential oil (GO, the serum levels of AST, ALT, and ALP reduced. The antioxidant properties of garlic have an added function in regulating calcium levels. It has been suggested that garlic should be recognized as a powerful antioxidant. This supports the findings of a research conducted by (25), who assessed the effects of vitamin D on renal and liver damage. The administration of free lipid nanoparticles (LNPs) and LNPs- GO led to a significant decrease in liver enzymes AST, ALT, and ALP. Besides, the nanoparticles serve as potent antioxidants, and their therapeutic efficacy enhances with reduced diameter and decreased dosages. The improved effectiveness finally results in the observed liver protection. Nanoparticles (NPs) have a crucial role in improving the structure and performance of the liver (26). This conclusion is in line with a prior study done by (27) that involved the oral administration of LNPs- GO Nevertheless, it presented conflicting results with the research conducted by (28).

Conclusion

Essential oils of garlic have a low water solubility and bioavailability. Using lipid systems in lipophilic substances increases the solubility and bioavailability of poorly soluble drugs. Rat liver enzyme antioxidant levels are positively impacted by garlic essential oils Loaded Lipid Nanoparticles (GLNs), which also boost anti-oxidative enzymes.

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