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Nanoparticle-Enhanced The Protective Effects of MGN-3/Biobran Against Ehrlich Ascites Carcinoma-Induced Toxicity in the Liver, Kidneys, and Spleen of Mice

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Abstract: Background: Nanoparticle-enabled targeted drug delivery has proven effective in cancer treatment due to its ability to cross biological barriers, achieve therapeutic concentrations within tumors, and minimize adverse effects on surrounding healthy tissues. MGN-3/Biobran is a widely recognized natural compound known for its anti-inflammatory, antioxidant, and anti-carcinogenic properties. This study examined the effects of natural product MGN-3 nanoparticles on the toxicity to liver, kidney and spleen caused by Ehrlich ascites cancer (EAC) in an in vivo model.

Method: In addition to the negative control group, the EAC model was conducted by injecting EAC cells subcutaneously into the female albino mice's thighs. Free MGN-3 and MGN-3 nanoparticles were injected intraperitoneally every other day into mice with solid Ehrlich carcinoma (SEC) tumors. Body weight (BW) was monitored throughout the study and tumors were collected on day 22 post-inoculation and weighed. Liver, spleen and kidney organs were excised and weight. Lipid profile parameters and indicators for liver and kidney function were determined using serum.

Results: The results showed that EAC inoculation caused in addition to subcutaneous solid tumor formation, significant decrease in Body Weight, increase in organ weights, marked rise in serum concentrations of aspartate, alanine aminotransferases and gamma-glutamyl transferase (AST, ALT, GGT), urea, creatinine, uric acid, and Total cholesterol. Also, remarkable elevation in Low Density Lipoprotein / High Density Lipoprotein (LDL/HDL) ratio and triglycerides level was detected. Besides reducing tumor weight, the protective effects on BW and distant organs by MGN-3 were observed by maintaining normal BW, liver, spleen and kidney weights, ameliorating all biochemical parameters within normal values. Such results were more pronounced with Biobran nanoparticles.

Conclusion: In conclusion, Nanocapsulation of MGN-3/Biobran appears promising as a chemopreventive agent against tumor progression and EAC-induced toxicity in healthy liver, kidney and spleen organs.

keywords: MGN-3, Nanocapsulation, Ehrlich Ascites Carcinoma, liver, kidney and spleen organs.

1.Introduction

Cancer is a severe illness that affects people all around the world. Cancer is considered as the second disease that causes death globally as it can cause damage to most tissues and organs of the body (**1,2).** The rapid growth of abnormal cells and their spread within the body can lead the cells to attack other organs and tissues in the body causing great injury and organs dysfunction which is a major cause of death throughout the world (**3).**

Breast cancer is the fifth leading cause of cancer-related fatalities and one of the most common types of cancer. Approximately 99% of breast cancers occur in women and 0.5–1% of breast cancers affect men **(4).** Ehrlich ascites carcinoma (EAC) is one of the most common tumor models. Initially, EAC was categorized

as a spontaneous mammary adenocarcinoma in mice, which is similar to breast cancer. Transplanting EAC into immunocompetent mice is simple. Over the past forty years, the well-established mouse model of EAC has been used to study breast cancer. EAC is an undifferentiated carcinoma that was initially hyperdiploid and has special traits such as fast growth, high transplantability, short life expectancy, and 100% malignancy (**5,6)**. Furthermore, either solid or ascetic EAC cells have the ability to spread and infiltrate normal distant tissues including the kidney and liver, leading to severe toxicity and organ malfunction (**7-9).**

The potential of natural products to prevent cancer has been well investigated. The community's combined efforts have produced amazing progress, introducing natural products to clinical settings and opening up new therapeutic avenues **(10).** A significant advancement in cancer treatment would be the creation of novel anticancer drugs with little toxicity (**11).**

The well-known natural product MGN-3/Biobran is made by reacting the hemicellulose of rice bran with several enzymes that hydrolyze carbohydrates found in shiitake mushroom cells **(12)** It has anti-inflammatory, anti-carcinogenic, and antioxidant qualities. Research has demonstrated that MGN-3/Biobran has anti-tumor activity against solid Ehrlich carcinoma tumors **(13)** and neuroblastomas (**14**), can improve tumor regression brought on by radiation therapy (**15)**, protect rats from chemically induced glandular stomach carcinogenesis (**16)** and increase the apoptotic effect of a low dose of paclitaxel on tumor cells in mice **(17)**. Biobran's potential to function as a strong biological response modifier (BRM) that stimulates various immune system arms, such as dendritic cells (DCs) (**18)** and natural killer (NK) cells, has been linked to the processes via which it has anti-cancer effects (**13, 19-21).** Additionally, by regulating lipid peroxidation, strengthening the antioxidant defense system, and preventing oxidative stress, it can produce oncostatic activity **(22).**

However, as one of the primary concerns of oncological treatment, nanotechnology offers alternatives in the development of controlled release drug systems for the treatment of various diseases. These systems act at the target site, allowing the potentiation of therapeutic effects and the reduction of drug side effects **(23).** Nanoparticle-enabled targeted drug delivery has proven effective in cancer treatment due to its ability to penetrate biological barriers, achieve therapeutic concentrations within tumors, and minimize adverse effects on surrounding healthy tissues **(24).**

Researchers across various fields are extensively studying multifunctional nanoemulsions, primarily for cancer treatment. These studies demonstrate that nanoemulsions are efficiently absorbed by tumor cells, inhibit the spread of cancer to other organs, minimize toxicity to healthy cells, and suppress tumor progression (**25).** In the current investigation, we used lipid-based nanocapsulation to increase the therapeutic efficacy of the natural drug, MGN-3/Biobran, against tumor formation and the toxicity to distant organs caused by Ehrlich ascites carcinoma (EAC) in mice.

2. Materials and methods

2.1. Animals

Animal experiments were conducted in conformance with the "Guide for the Care and Use of Laboratory Animals" and with the consent of Mansoura University's Ethics Committee for Animal Experimentation **[MU-ACUC (SC.MS.23.07.28)**].

The study used two-month-old female Swiss albino mice weighing 22–25 g. In addition to receiving limitless water and regular cube pellets from Misr Oil & Soap Company in Cairo, Egypt, the animals were housed in cages with 12-hour cycles of light and dark and a constant temperature of 24°C ± 2°C. Dl-methionine (0.5%), lipids (1.0%), bran (3.3%), olive oil (2.3%), casein (12.5%), vitamins and salt mixture (0.2%), and water (0.2%) were the constituents of the pellets. Protein accounted for about 18% of total calories, carbs for 73%, and fat for 9%.

2.2. Tumor Cell Line and tumor inoculation

Ehrlich ascites carcinoma (EAC) is a non-differentiated, initially hyperdiploid carcinoma that is 100% malignant, has a high

transplanting capacity, and proliferates quickly. EAC cells proliferate quickly and lack differentiation. The National Cancer Institute at the University of Cairo in Egypt provided the initial murine EAC cells used in this investigation. The cells were kept in vivo via intraperitoneal passage of 2.5 $x10^6$ cells per week in female Swiss albino mice. In this experiment, 0.2 ml of EAC containing $2.5x10^6$ viable EAC cells was subcutaneously inoculated into each mouse's right thigh of the lower limb, resulting in solid tumors. Using the Trypan Blue dye exclude method, viability was assessed and determined to be greater than 95% **(17).**

2.3. MGN-3- rice bran extract

MGN-3/Biobran is a naturally occurring mixture of hemicelluloses made from rice bran that has been partially hydrolyzed using enzymes from shiitake mushrooms. MGN-3's primary chemical structure is that of an arabinoxylan, which has an arabinose polymer in its side chain and xylose in its main chain (**26**). According to Badr El-Din et al. (2016a), mice with solid Ehrlich ascites carcinoma tumors received intraperitoneal injections (i.p.) of MGN-3, which was prepared fresh by dissolving in 0.9% saline solution and given at a dose of 40 mg/kg body weight (BW)/day, three times per week. (**16**).

2.4. Lipidic MGN-3 nanoparticles preparation

Phase inversion was used in the formulation of lipid nano-capsules (LNs). Two distinct oils—avocado and cinnamon oils—were used to create LNCs. Oil-based LNCs were created by combining oils and surfactant in equal amounts, then adding 2.5 milliliters of distilled water while stirring constantly for 15 minutes. Finally, 1 milliliter of chilled distilled water was added.

2.5. Experimental design

On the first day, female Swiss albino mice received a subcutaneous injection of 0.2 mL of EAC cells (2.5×10^6 cells) into their right thigh. The study employed mice with a solid Ehrlich tumor mass of about 100 mm^3. Mice were divided into the following groups: -

Group 1: Normal control group (n = 6), mice without tumor.

Group 2: EAC group (n= 6), mice with solid tumors that are not treated.

Group 3: EAC+ MGN-3 (n= 6), From day 8 to day 22, when the study came to a conclusion, mice with solid tumors were given an intraperitoneal (i.p.) injection of 40 mg/kg BW of MGN-3 three times a week.

Group 4: EAC+ LNPs (n= 6): mice bearing solid tumor, treated with plain lipid nanoparticles 3 times a week from day 8 until day 22, the end of the experiment.

Group 5: EAC+ MGN-3.LNPs (n= 8): mice bearing solid tumor and treated with MGN-3.lipid nanoparticles 3 times a week from day 8 until day 22, the end of the experiment

Adverse Effects of MGN-3 and MGN-3. lipid nanoparticles (Toxicity) For the duration of the treatment, the animals in each group were monitored every day for any negative side effects caused by MGN-3 or its nano-formulation, as measured by alterations in their regular feeding and drinking schedules and patterns of life activity.

2.6. Alternation in the Body weight

BW/g changes (initial, final, and net BWs at day 22) were assessed for each group. Final BW minus tumor weight is the net final BW. BW gain (g) and the percentage of change from the starting BW were computed.

2.7. Alternation in the tumor weight

Mice were put unconscious on day 22, and solid tumors were removed in order to determine TW.

2.8. Blood sample collections

The animals were sedated with diethyl ether and starved for 16 hours at the experimental endpoint (day 22). Blood was extracted from the abdominal aorta using vacuum tubes, and it was allowed to coagulate at room temperature. After centrifuging the serum for 20 minutes at 3000 r.p.m., it was stored until it was time for analysis. The parameters of the liver and kidney function tests were determined using serum.

In addition to lipid profile markers; Triglycerides, Total cholesterol, LDL, HDL levels.

2.9. Organs weight variations

Organs from G1–G5 animals were analyzed at Day 22. These comprised the kidney, spleen, and liver.

2.10. Analytical procedures

2.10.1. Liver function tests

Assessment of the activity of aspartate and alanine aminotransferases

Using a kinetic method described by the International Federation of Clinical Chemistry (IFCC) in accordance with the method of Breuer **(27)**, the levels of the enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in serum were measured using a diagnostic kit from ELITech Clinical System, France. The activity value of the transmittance was calculated using the following formula:

Activity (U/L) = ΔA/min×1750 (**27**)

Determination of gamma-glutamyl transferase activity

Gamma-glutamyl transferase (GGT) activity in serum was measured using a diagnostic kit from ELITech Clinical System, France. The estimation was carried out using Szasz **(28)** methodology. The activity value of the transmittance was calculated using the following formula:

Activity (U/L) = ΔA/min×2211 (**28**)

2.10.2. Kidney function tests

Determination of creatinine

The Colorimetric Method of Schirmeister et al. **(29)** was used to assess the quantity of creatinine activity in serum. The creatinine activity value was calculated using the formula below:

Creatinine in serum or plasma (mg/ dl) = (A_{Sample} / $A_{Standard}$) x 2 (**29**)

Determination of urea

Amount of urea activity in serum was estimated by Urease-Berthelot Method according to Fawcett and Scott **(30).** The urea activity value was calculated using the formula below:

Urea in serum (mg/ dl) = (A_{Sample} / $A_{Standard}$) x Standard Conc (**30**)

Determination of Uric acid

Amount of uric acid activity in serum was detected by the Enzymatic Colorimetric Method of Barham and Trinder **(31).** The following formula was used to determine the uric acid activity value:

Uric Acid in serum = (A_{Sample} / $A_{Standard}$) X Standard Conc (**31**)

2.10.3. Analysis of Lipid profile

Determination of Triglycerides

Fossati et al. **(32)** used the Enzymatic Colorimetric Method to assess the amount of triglyceride activity in serum. The following formula was used to determine the Triglycerides activity value:

Triglycerides Concentration = (A_{Sample} / $A_{Standard}$) X Standard Conc (**32**)

Determination of Total cholesterol

Using the Richmond Enzymatic Colorimetric Method, the amount of Total cholesterol activity in serum was evaluated (**33**). The Total cholesterol activity value was calculated using the following formula: Total cholesterol Concentration = (A_{Sample} / $A_{Standard}$) X Standard Conc.

Determination of HDL

HDL activity in serum was measured using the Enzymatic Colorimetric Method of Lopes-Virella et al. **(34)**. HDL activity value was calculated using the following formula:

HDL – Cholesterol in sample (mg / dl) = (A_{Sample} / $A_{Standard}$) X 55 (**34**)

Determination of LDL

Using the Enzymatic Colorimetric Method of Wieland and Seidel **(35),** the amount of LDL activity in serum was evaluated. The LDL activity value was calculated using the following formula:

Cholesterol content of the supernatant = (A_{Sample} / $A_{Standard}$) x standard x 11 (dil. Factor)

The LDL- cholesterol calculation:

LDL-chol. = Total chol – chol.in the supernatant (**35**)

Determination of LDL/HDL ratio

LDL/HDL ration was also calculated in the different groups by dividing LDL level by HDL level.

2.11. Statistical Analysis

Analysis of statistics for statistical analysis, GraphPad Prism® software version 7 was utilized. The significance of differences between mean values was assessed using the Newman–Keuls multiple comparison test in conjunction with one-way analysis of variance. Values are shown as mean ± standard error of the mean. At $P<0.05$, significance was established. *, **, ***, and **** denote statistical significances of $P < 0.05$, 0.01, 0.001, and 0.0001 for the figures, respectively.

3. Results

3.1. Adverse (toxicity) effects of MGN-3 and its nano-formulation

Mice were monitored daily to record any potential toxic side effects of MGN-3 or its nano-formulation. No adverse side effects were noticed throughout the experimental period.

3.2. Alternation in the Body and Tumor weights

Table 1 shows changes in the body weight of the different experimental groups. In comparison with the initial BW, the normal control group displayed 23.84% BW gain, Untreated EAC mice revealed loss of BW by 1.47%, $P<0.01$ of initial, and showed a highly significant difference($p<0.01$) versus all groups. On the contrary, EAC+MGN-3 treated mice recorded 23% BW gain, EAC+LNPs and EAC+MGN-3- LNPs revealed 23.1% and 20.82% BW gain, respectively of the corresponding initial BW and all treatments showed comparable BW change versus normal control group.

After 22 days of cell inoculation, the growth-dependent variance at tumor weight (TW/g) in various groups was used to assess the development and proliferation of EAC cells that were subcutaneously injected into mice. TW of untreated EAC mice showed high tumor growth rate by recording 2.18±0.36 g, meanwhile, treatment of EAC mice with MGN-3, plain LNPs and MGN-3-LNPs revealed markedly reducing tumor weight to record 0.31±0.08/g, 0.47±0.09/g and 0.08±0.01/g, respectively, representing a highly significant change ($p<0.01$) by -85.26%, -77.64% and -96.1%, respectively, when compared to the untreated EAC animals. Such reduction was much obvious with MGN-3-LNPs treatment.

Table (1): Effect of MGN-3, plain lipid nanoparticles and MGN-3. LNPs treatment on body weight (BW g) and tumor weight (TW g).

Groups parameter	Normal control	EAC	EAC+ MGN-3	EAC+ LNPs	EAC+MGN-3. LNPs
Initial BW (g)	22.93±0.45	22.62±1.3	22.27±0.7	22.2±0.6	22.43±0.7
# animals	6/6	6/6	6/6	6/6	8/8
Last BW (g)	28.4±1.6	24.4±0.9[a]	27.7±0.7	27.82±0.7	27.16±0.3
# animals	6/6	6/6	5/6	6/6	8/8
TW(g)	-	2.117±0.4	0.312±0.08 [B]	0.4733±0.09 [B]	0.08286±0.009 [B]
% change from EAC group	-	0	-85.26%	-77.64%	-96.1%
Net final BW	28.4	22.28[A]	27.4 [B]	27.34 [B]	27.1 [B]
BW gain (gm)	5.47	-0.33	5.12	5.14	4.64
% change from the initial BW	23.84%	-1.47%	23%	23.17%	20.73%

The mean ±SE of the appropriate number of animals/ groups is represented by each value. Net final BW= (Final BW-TW). BW gain+ (Net Final BW-Initial BW).

a. A: at the $p<0.05$ and $p<0.01$ levels, respectively, substantially different from the corresponding control group.

B: at the p<0.01 level, significantly different from the comparing EAC group.

3.3. Alternation in the liver, kidney and spleen weights.

Table (2) displays the weights of the mouse liver, spleen, and kidney for each experimental group. Weights of the liver, spleen, and kidney were found to rise significantly (p<0.01) after EAC injection without treatment. by 51.68%, 105.5% and 84.62%, respectively, as compared with the normal control group. In contrast, MGN-3 and MGN3-LNPs intake, reduced the increase of the different organ weights that caused by EAC inoculation, and maintained the weights within normal values. Such results were more marked with MGN-3 nanoparticles treatment.

3.4. Liver function results

The detrimental impact of EAC induction and development on liver function tests, as well as the protective function of MGN-3 with and without nano-formulation on hepatic toxicity, are depicted in Figures (1, A, B, and C). By the end of the treatments, the levels of serum ALT, AST, and GGT were estimated. Serum levels of transaminases (AST & ALT) and GGT were considerably elevated by untreated EAC tumor by 93.59%, 234.48%, and 45.90%, respectively, in comparison to the equivalent control values (p<0.0001). MGN-3 and MGN-3-LNPs decreased the serum activity of AST, ALT, and GGT in EAC-bearing mice to levels comparable to normal.

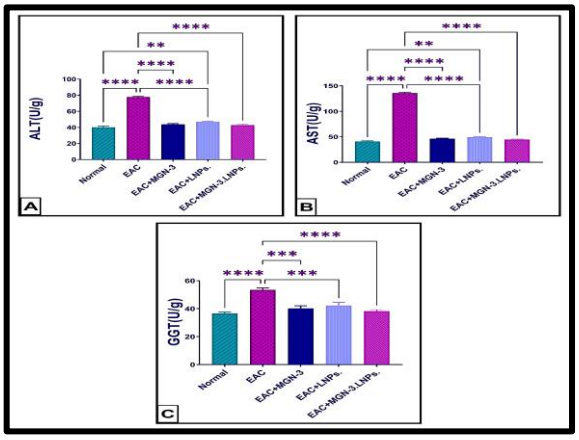

Figure 1: The impact of the various treatments on the levels of (A) ALT, (B) AST, and (C) GGT. The symbols ∗, ∗∗, ∗∗∗, and ∗∗∗∗ denote statistical significance between groups at P < 0.05, P < 0.01, P < 0.001, and P < 0.0001 levels, respectively. Each value represents the mean ± SEM for five animals/group

Table (2): Effect of MGN-3, plain lipid nanoparticles and MGN-3. LNPs treatment on organs weight.

Groups parameters	Normal	EAC	EAC+MGN3	EAC+LNPs	EAC+MGN3-LNPs
Liver weight	1.48±0.05	2.25±0.1^{A}	1.77±0.09^{B}	1.8±0.08^{ab}	1.6±0.08^{B}
% changes from normal control group	-	+51.68%	+19.1%	+23.6%	+10.1%
% changes from EAC-group	-34.07%	-	-21.48%	-18.52%	-27.41%
Spleen weight	0.36±0.02	0.74±0.09^{A}	0.46±0.05^{b}	0.5±0.03^{b}	0.38±0.04^{B}
% changes from normal control group	-	+105.5%	+27.78%	+38.9%	+5.6%
% changes from EAC-group	-51.35%	-	-37.84%	-32.4%	-48.65%
Kidney weight	0.26±0.02	0.48+0.02^{A}	0.32±0.04^{B}	0.36±0.02^{b}	0.3±0.03^{B}
% changes from normal control group	-	+84.62%	+23.1%	+38.46%	+15.4%
% changes from EAC-group	-45.8%	-	-33.3%	-25%	-37.5%

According to the number of animals/ groups, each statistic is the mean ± SE. Number of mice/groups are: Control (6), EAC (6), EAC+MGN-3 (6), EAC+ LNPs (6) and

EAC+MGN-3- LNPs (8). a. A: at the $p<0.05$ and $p<0.01$ levels, respectively, substantially different from the corresponding control group. b. B: at the $p<0.05$ and $p<0.01$ levels, respectively, substantially different from the corresponding EAC group.

3.5. kidney function findings

Figure 2 (A, B, and C) shows the serum creatinine, urea, and uric acid levels for each group. Creatinine, urea, and uric acid levels in untreated EAC mice were significantly higher than those in the normal control group. On the other hand, kidney function markers were significantly lower in the Biobran, plain LNPs, and MGN-3-LNPs groups compared with the normal control group.

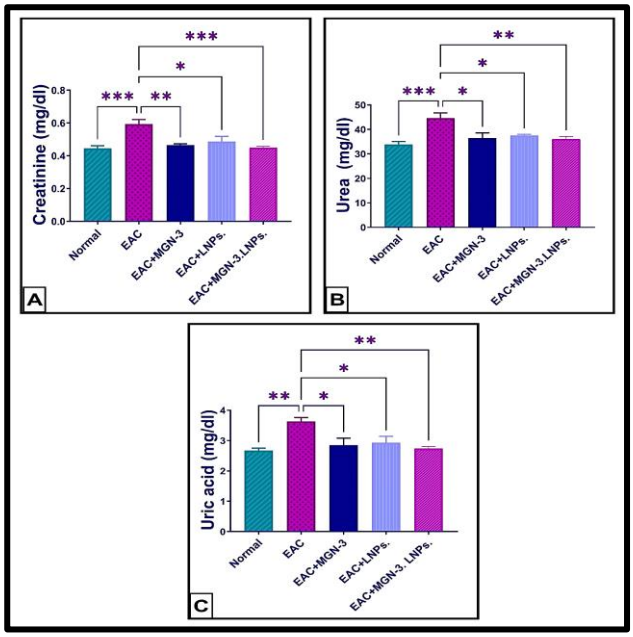

Figure2: Effect of the different treatments on (A)Creatinine, (B)Urea and (C) Uric acids levels. The symbols ∗, ∗∗, and ∗∗∗ denote statistical significance between groups at $P < 0.05$, $P < 0.01$, and $P < 0.001$ levels, respectively. Each value represents the mean ± SE for five animals/group.

3.6. Lipid profile

The serum levels of triglycerides and total cholesterol in the untreated EAC mice showed marked significant rise ($p<0.001$) versus normal control group by 107.3% and 104.7%, respectively. In contrast, EAC+ MGN-3, EAC+LNPs and EAC+ MGN-3-LNPs groups, revealed that the rise in these parameters was slowed down and the levels of triglycerides and cholesterol markedly decreased to yield insignificant values versus the normal control group. Such decrease was more prominent in MGN-3-LNPs group, and a marked significant decrease versus untreated EAC group was recorded (Figure 3 A and B).

Moreover, the good cholesterol lipoprotein (HDL) in mice with different treatment was comparable with the normal control mice and significantly increased by 28.34%, 31.82% and 71.6 % in EAC mice treated by MGN-3, Plain LNPs and MGN-3-LNPs, respectively, versus the EAC mice group which showed marked decline by 46.42% of normal control group. However, the untreated EAC animals' levels of lipid lipoprotein (LDL) were noticeably higher by 280.56% of normal controls. Serum LDL levels in EAC-bearing mice significantly decreased after receiving various treatments in particular when treated with MGN-3–LNPs followed by MGN-3 then plain LNPs (Figure 4 A& B).

Additionally, Chl/HDL and LDL/HDL ratios showed a remarkable enhancement by MGN-3–LNPs treatment followed by MGN-3 then the plain LNPs treatments in comparison with the group of untreated EAC mice. As treatments to EAC bearing groups reduced the Chl/HDL ratio by 45.72%, 44.27% and 55.32% and decreased LDL/HDL ratio by 61.5%, 46.8% and 88.58% for MGN-3, Plain LNPs and MGN-3-LNPs treatments, respectively, versus untreated EAC group as shown in (Figure 4 C & D).

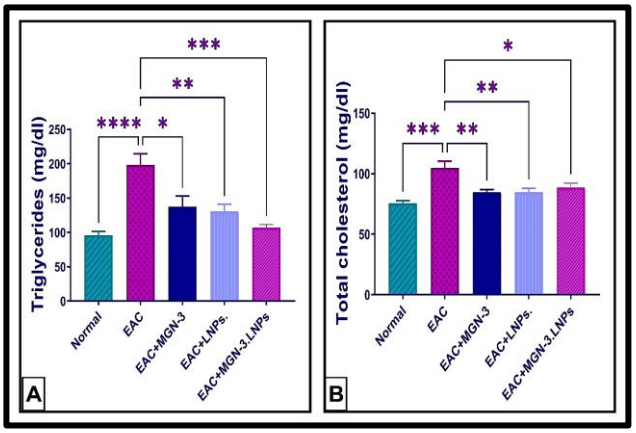

Figure 3: Impact of the various treatments on the levels of (A) total cholesterol and (B) triglycerides. The symbols ∗, ∗∗, ∗∗∗, and ∗∗∗ signify statistical significance between groups at $P < 0.05$, $P < 0.01$, $P < 0.001$, and $P < 0.0001$, respectively. Each value represents the mean ± SEM for five animals per group.

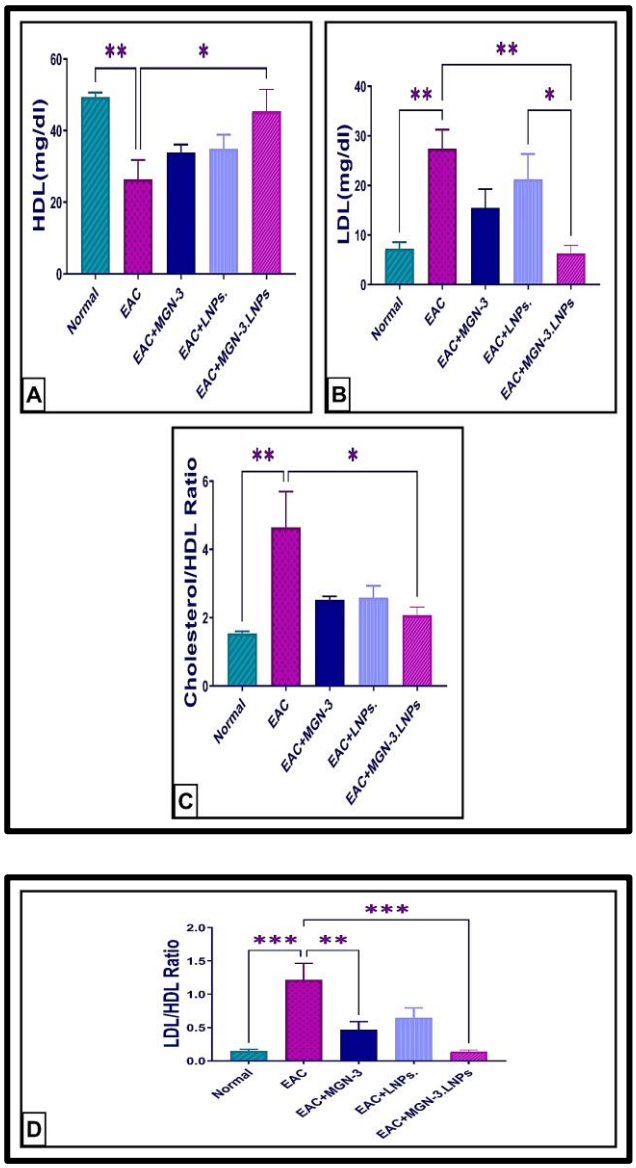

Figure 4: Effect of the different treatments on (A) HDL-C, level (B) LDL-C level and (C) Cholesterol/HDL ratio, (D) LDL/LDL ratio. The symbols ∗, ∗∗, ∗∗∗, and ∗∗∗ signify statistical significance between groups at $P < 0.05$, $P < 0.01$, $P < 0.001$, and $P < 0.0001$, respectively. Each value represents the mean ± SEM for five animals per group.

4. Discussion

Pharmaceutical formulations made of particles of a nanometer range are known as nano-emulsions. They are a stable and secure way to distribute medications because they are also made of safe gradient excipients. (**25).** Recently, loading chemotherapeutic medicines into nanoemulsion systems has become more popular in medicine because of their capacity to increase a drug's effectiveness and lessen its side effects. In this context, using natural ingredients in nanoformulation could improve the anticancer medicines' stability and bioavailability (**36)**. Furthermore, because lipid-based carriers are effective and safe, they have emerged as desirable options for the creation of medicines, vaccines, diagnostics, and nutraceuticals (**37).**

A well-researched natural product made from rice bran is MGN-3/Biobran **(12)** and possesses anti-inflammatory, antioxidant and anti-carcinogenic properties **(13, 22)**. A recent study examined the efficacy of Biobran encapsulated in nanofiber as a bioactive material that may enhance wound healing (**38**). According to the study, Biobran was delivered from the nanofiber scaffolds in a controlled and sustained manner as the release rate of Biobran dropped as the concentration of Biobran increased. According to these results, Biobran-loaded core/shell nanofiber scaffolds may be used as the perfect multipurpose wound dressing in wound healing. Through nano-formulation, the current study sought to increase MGN-3's anticancer activity against EAC-induced Toxicity in distant organs in mice. Researches conducted by our team and others has indicated that EAC tumor formation in an animal can influence several critical organ systems. (**39, 40**).

The current statistical analysis showed that MGN-3-LNPs remarkably reduced tumor growth in terms of tumor weight compared with tumor implanted mice who received free MGN-3 and void NPs. Our Earlier studies have shown that free MGN-3/Biobran exhibited anti-tumor activity against a solid tumor of Ehrlich carcinoma in mice **(13, 15, 17, 22)** and against other types of cancer in different experimental models (**16, 41).** The mechanisms included immune system regulation, apoptosis induction, tumor cell growth suppression, and free radical counteraction.

In the current study, tumor growth was correlated with alteration in body and organs weights. Significant decrease in BW accompanied by remarkable increase in liver, kidney and spleen weights of untreated EAC bearing mice were recorded. In addition, the developed solid Ehrlich carcinoma produced vital organs dysfunction in mice. It was found that EAC cells when inoculated in mice, The expanding tumor increases the likelihood that it

will spread to other organs, including as the kidney, liver, and lung, by causing localized inflammation and increased vascular permeability (**42, 43**).

One important organ that plays a significant role in many metabolic and detoxifying processes is the liver. Therefore, assessing serum levels of the liver enzymes ALT and AST is a helpful technique, especially for liver condition monitoring and follow-up **(44, 45**) Our findings revealed a significant increase in the levels of AST, ALT, and GGT in the serum of untreated EAC bearing animals. Since these enzymes are typically found in the cytoplasm and released into the bloodstream following cellular damage, the significant rise in serum hepatic enzymes has been linked to hepatic structural damage (**46).** Our results are in line with our earlier study (**39**) and consistent with **(47)** who found that implantation of EAC in mice harmed the liver and induced hepatic damage in mice, also They can result in liver damage and oxidative stress (**48).** Our findings are also agreed with the findings of other studies **(49, 50).**

Further, the current study's use of the EAC in a mice model was found to cause kidney dysfunction as approved by the higher-than-normal control group levels of serum creatinine, urea, and uric acid. Raised creatinine concentration is an index of kidney dysfunction **(51)**. Similar findings were observed by **(52)** who recorded high elevation in the concentration of creatinine and urea in the serum of mice transplanted by EAC cells. They attributed the damage to the kidney of mouse by such cancer cells that metastasized to invade mouse organs. Our current study showed that the rise in liver and kidney biomarkers was slowed down and the levels were non-significant comparing to the normal outcomes in the groups that received free MGN-3 and MGN-3-LNPs, however the effect of lipid-based encapsulated MGN-3 was more effective when compared with the free MGN-3 in ameliorating hepato-renal functions toward the normal levels.

Furthermore, lipid profile in the current study showed marked increase in serum total cholesterol, triglycerides, LDL concentrations in untreated EAC. In contrast, marked decrease in EAC+ MGN-3, EAC+LNPs and EAC+ MGN-3-LNPs groups was obtained to yield insignificant values versus the normal control group. Such improvement was more prominent in MGN-3-LNPs group. Moreover, the good cholesterol HDL level in mice with different treatments was comparable with the normal control mice but significantly elevated versus the untreated EAC-bearing mice group which showed marked decline. Additionally, Chl/HDL and LDL/HDL ratios showed a remarkable reduction by MGN-3–LNPs treatment followed by MGN-3 then the plain LNPs treatments in comparison with the group of untreated EAC mice. Actually, when MGN-3/Biobran was transformed into nanoparticles, its effectiveness was significantly increased. Our results demonstrated that lipid-based MGN-3-LNPs might be able to improve the drug's cytotoxic effects and extend its release rate against solid Ehrlich carcinoma tumor and to prevent the distant organs damage caused by EAC cells migration. In a similar manner, many researchers in various fields are investigating multifunctional nanoemulsions, mostly for the treatment of various cancers. These investigations all show that effectively absorbed by tumor cells, nanoemulsions prevent cancer cells from spreading to other organs, reduce toxicity to healthy cells, and restrict tumor growth **(25).**

Also, it was evident that nanoparticles exhibit distinct physical and chemical properties compared to their bulk counterparts in addition to their excellent bioavailability and biodegradability qualities, because of their tiny size and wide surface area (**53)**. Furthermore, targeted drug delivery made possible by nanoparticles has been shown to be effective in the treatment of cancer because it can overcome some biological barriers, reach therapeutic concentrations in tumors with lower drug dosages, and avoid harmful effects on nearby normal tissues (**54).** The results confirmed that the stability, bioavailability, and tissue distribution of MGN-3 to the liver and kidney were all improved by lipid-based nano-formulation, which increased MGN-3-LNPs' anticancer potential. against EAC cells-induced liver and kidney damage in comparison with the native MGN-3 treatment.

5. Conclusion

The study demonstrates that MGN-3-loaded lipid nanoparticles (MGN-3-LNPs) offer significant advantages due to their nanosize. These nanoparticles enhance tissue distribution and absorption, making MGN-3 more effective in targeting cancer cells. Additionally, MGN-3-LNPs inhibit cancer progression and provide protection to distant organs from the damage caused by Ehrlich Ascites Carcinoma (EAC) cells. This innovative formulation strategy holds promise for improving cancer treatment outcomes and minimizing adverse effects on healthy tissues.

4. References

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