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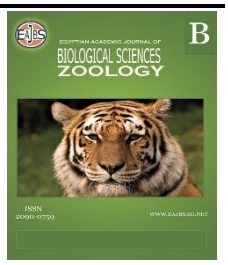

Effectiveness of l-carnitine Nanoparticles against Nephrotoxicity Induced by 5-Fluorouracil in *Ehrlich ascites* Carcinoma Bearing Mice

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ABSTRACT

This study aimed to investigate the potential modulatory effect of 5-Fluorouracil (5-FU) combined with L-carnitine nanoparticles (LCN) against 5-FU-induced nephrotoxicity in mice bearing, *Ehrlich ascites carcinoma* (EAC). Five sets of female albino mice were created. Group 1: CON, group 2: EAC induced by a single injection of 2.5 x 10^6 EAC/ml, group 3: EAC+5-FU received 25mg/kg of 5-FU i.p, group 4: EAC+LCN received orally 50 mg/kg, and group 5: EAC+5-FU+ LCN received the same dose and route as the preceding groups. The 5-FU+ LCN group saw a considerable reduction in mean tumor weight (MTW), an elevation in life span (ILS), and an improvement in mean survival time (MST). Furthermore, 5-FU+ LCN enhanced serum creatinine, urea, and albumin levels while decreasing MDA levels, which are related to increased levels of GSH, GPX, and SOD, as well as decreased levels of TNF-α and MCP-1 in kidney tissue. Moreover, 5-FU+ LCN reduced caspase-3 and P53 levels while increasing Bcl2 levels when compared to 5-FU alone. Microscopic examination showed lesions in the kidney tissue sections of mice exposed to 5-FU. The treatment of EAC mice with 5-FU+ LCN showed an enhancement in histological structure and an antitumor effect that was more protective than induced by 5-FU alone.

INTRODUCTION

Cancer has been identified as a significant contributor to morbidity and mortality on a global scale, and the diagnosis and treatment of cancer have been of extensive concern among scientists (Weber *et al*., 2020). Ehrlich ascites carcinoma (EAC) is an undifferentiated tumor that is commonly used to test various cancer therapy lines (Kumari *et al*., 2021). Chemo-therapeutic drugs are used to destroy tumor cells but also have side effects; some of them are severe due to their harm to normal cells (Fahad *et al*., 2021). 5-Fluorouracil (5-FU) is a long-used antimetabolite drug for several forms of malignancies. 5-FU generates damage to most major organs, especially the kidney. (Sethy and Kundu, 2021). 5-FU causes nephrotoxicity by producing reactive oxygen species and free radicals.

Depleting the antioxidant system and elevating oxidative stress induces apoptosis and damages DNA (Sengul *et al*., 2021).

Nanotechnology has gained greater importance in drug delivery systems. It can inhibit the incorporated variable drugs from degradation, increase the bioavailability of drugs, target drugs to specific sites, and control the release of drugs. It also almost removes side effects that are produced in normal tissues and organs (Marzi *et al*., 2022). Nanoparticles have many biological and physicochemical properties, such as selectivity, large surface area, chemical stability, the ability to increase half-life, specific structural features, and the ability to target tumors and prevent drugs from reaching normal cells, which induces lesser systemic toxicity (Klochkov *et al*., 2021).

Biological ingredients that have multiple effective actions on various biological molecular targets have some important anti-inflammatory and anti-cancer effects, such as l-carnitine. L-carnitine is a biologically active stereoisomer, having many properties such as adjustable release properties, hydrophobicity, biodegradable properties, and good biocompatibility (Farahzadi *et al*., 2023). L-carnitine nanoparticles (LCN) have recently gained great interest due to their powerful effects on nephrotoxicity and improving kidney functions. It has an antitumor effect and scavenges free radicals and ROS from the body (Hassan *et al*., 2022). So, the purpose of the current work is to explore the protective effect of LCN against 5-FU, which induces nephrotoxicity in female mice bearing Ehrlich cancer, and to determine whether l-carnitine nanoparticles affect the antitumor capabilities of 5-fluorouracil.

MATERIALS AND METHODS

Drugs and Chemicals:

5-Fluorouracil and l-carnitine nanoparticles were obtained from Sigma-Aldrich (St. Louis, USA). All additional reagents, solvents, and chemicals used in the study met the quality requirements set by international standards.

Animals:

The current experiment was done on female mice obtained from the National Cancer Institute at Cairo University since Ehrlich Ascites carcinoma cells demonstrate higher early progress in female mice than in male mice (Vincent and Nicholls, 1967).

247 female Swiss albino mice weighing 25–30 grams were obtained from the Theodore Bilharis Institute's animal home in Cairo. Mice were housed in plastic cages at room temperature with natural light. The animals were fed a usual pellet diet and given tap water. The animals were acclimated to their surroundings for 14 days before the trial. All animals were cared for according to animal testing methods permitted by the ethical committee at Ain Shams University's Faculty of Science in Cairo, Egypt.

Tumor Cell Line:

Ehrlich Ascites Carcinoma cells (EAC) were procured from Egypt's National Cancer Institute (NCI), Cairo University. EAC cells can proliferate in two forms: solids and ascites. Tumors in female mice are generated in ascites form by injecting $2.5x10^6$ tumor cells per animal into the peritoneal cavity and letting them develop according to the Egyptian National Institute of Cancer, Cairo University technique (Vincent and Nicholls, 1967).

EAC cells expand for 10 days before being retrieved by an i.p. puncture with a sterile syringe, diluted, and counted. Before injection, cells are counted using a Neubauer hemocytometer, and physiological saline is diluted. A solid tumor was created in female mice by injecting 0.2 ml of viable ($2.5x10^6$ cells per mouse) EAC cells subcutaneously in each mouse's left thigh and maintaining it for 10 days (Mahmoud *et al*., 2022).

Cell Viability:

Seven days following the initial EAC injection, ascites fluids were extracted and analyzed for cell viability using the trypan blue dye method (Strober *et al*., 2001). Under a microscope, a sample of viable (unstained) cells was counted, and the average of five squares was calculated. **Figure 1** depicts nonviable cells stained with trypan blue dye. $C = T \times D \times 10^4$ represents the viable cell concentration. Where C represents the concentration of viable cells per milliliter, T is the average number of viable cells per square, and D is the dilution factor.

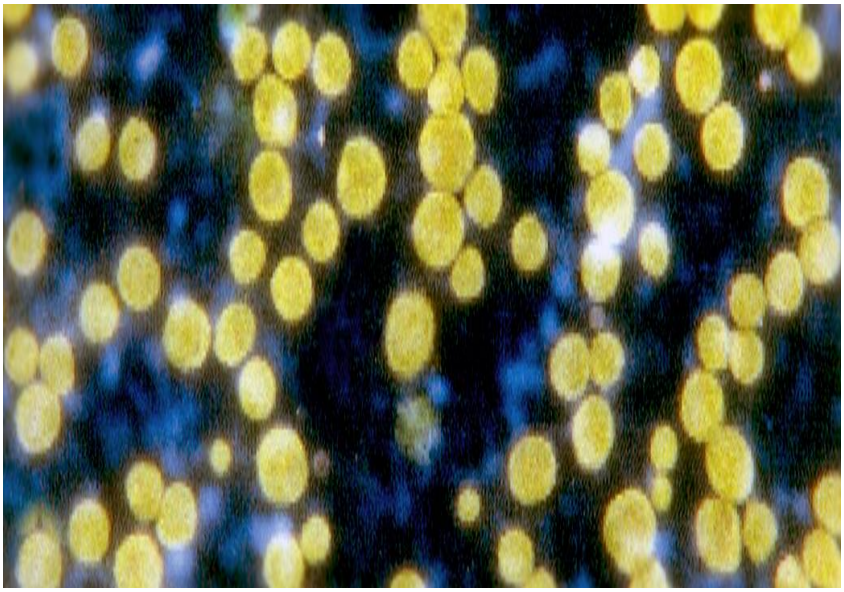

Fig.1. Showing the normal Ehrlich ascites carcinoma cells X 200

Induction of Ehrlich Solid Carcinoma:

Cells of Ehrlich Ascites Carcinoma (EAC) were isolated from the ascetic fluid of female mice with EAC and transplanted into 32 animals to produce Ehrlich Solid Carcinoma (ESC). On day zero, 2.5 x 106 viable EAC cells were injected intramuscularly into the left thigh of each mouse in 0.2 ml of diluted ascetic fluid (1:10) with saline (Mahmoud *et al*., 2022).

Experimental Design:

Antitumor Studies:

The anticancer properties of 5-FU and/or LCN were tested in 172 mice that were injected intramuscularly into each left thigh with 2.5×10^6 viable EAC cells to induce solid tumors, divided into two groups. The initial set of 32 mice for mortality rate. Following ten days of EAC cell injection, they were divided into four groups (n = 8), as follows: In the gr1 (EAC) group, in the gr2 the mice received 5-FU (25 mg/kg, i.p.) three times weekly for three weeks, in the gr3 the mice were given l-carnitine nanoparticles (50 mg/kg) three times weekly for three weeks, and in the gr4 the animals were administered with 5-FU and LCN at the same preceding period, dose, and method of administration (Wang *et al*., 1998; Shehatta *et al*., 2022). The second set of 140 mice was used to measure the mean tumor weight (MTW). Mice in this group were separated into four equal-sized groups (n = 35) and were treated as previously stated as follows: gr1: EAC, gr2: EAC+5-FU, gr3: EAC+LCN, and gr4: EAC+5-FU+LCN. The mice in this group were then left untreated until the study was completed. Every week, three mice were sacrificed to determine the control mean tumor weight (MTWc) and test mean tumor weight (MTWt), the control mean survival time (MSTc), the test mean survival time (MSTt), the percentage increase in life span (ILS%), and the tumor growth inhibition ratio (T/C%)(Fahim *et al*., 2003). The MST is defined as the period when only animals lived.

$$ILS\% = \frac{MSTt}{MSTc} \times 100$$

$$(T/C\ \%) = \frac{MSTt - MTWc}{MSTc} \times 100$$

Biochemical and Histopathological Studies:

75 mice were divided into five groups of 15 each. The group 1 mice were given an intraperitoneal injection of physiological saline and functioned as a normal control group (CON). In the group 2: EAC group. In group 3, the mice received 5-FU (25 mg/kg, i.p.) three times weekly for three weeks (EAC+5-FU). In group 4, the mice were given l-carnitine nanoparticles (50 mg/kg) three times weekly for three weeks (EAC+ LCN). In group 5, the mice received (25 mg/kg, i.p.) plus (50 mg/kg) three times a week for three weeks (EAC+5-FU+ LCN).

Blood Sampling:

At the completion of the 32-day trial, the mice were slain under ether anesthesia, and blood samples were collected in a dry, clean centrifuge tube for 15 minutes before being centrifuged at 3000 rpm for 15 minutes. Serum was obtained using Eppendorf tubes. Each mouse's solid tumors were separated, cleaned with distilled water, and weighed. The kidneys were separated, cleaned, and weighed individually. One kidney was quickly homogenized to produce a 10% (w/v) homogenate in an ice-cold phosphate buffer (pH 7.4) solution. The homogenate was centrifuged for 10 minutes at 4°C at 1800 g. For biochemical studies, the supernatant (10%) was separated and stored at 20 °C. For histological evaluation, the second kidney was fixed in 10% formalin-saline.

Assessment of Oxidative Stress:

Evaluation of oxidative stress by using the Biodiagnostic Company (Giza, Egypt) kit, Ohkawa *et al*. (1979) demonstrated that calorimetrically increased renal MDA levels. Renal superoxide dismutase (SOD) activity was calorimetrically evaluated using a kit from Biodiagnostic Company (Giza, Egypt), as described by Nishikimi et al. (1972). A spectrophotometer evaluated the amount of reduced glutathione (GSH) in kidney tissue using a kit from the Biodiagnostic Company (Giza, Egypt), as described by Beutler et al. (1963). Renal glutathione peroxidase (GPX) was tested colorimetrically using a kit from Bio-diagnostic in Egypt, following the manufacturer's instructions (Paglia and Valentine, 1967).

Determination of Inflammatory Markers:

TNF-α (tumor necrosis factor-alpha) was detected in the kidney using a TNF-α ELISA kit (Abbexa Ltd., Cambridge Science Park, Cambridge, CB4 0EY, UK), adhering to the manufacturer's instructions. The kidney's monocyte chemoattractant protein-1 (MCP-1) was quantified using a rat MCP-1 ELISA kit based on the manufacturer's instructions and guidelines (Cloud Clone Corp., USA).

Estimation of Apoptotic and Anti-Apoptotic Markers:

The quantitative estimation of renal caspase-3 was performed by using a rat CASP3 ELISA Kit in keeping with the supplier's instructions and guidelines. Tumor protein (P53) was measured using the P53 rat ELISA kit in accordance with the manufacturer's instructions and guidelines. To measure quantitative B-cell leukemia/lymphoma 2, a rat Bcl2 ELISA kit (Cloud-Clone Corp., USA) was employed.

Biochemical Determinations:

Renal creatinine was quantified using a rat ELISA kit of creatinine (Cloud-Clone Corp., USA), catalog number MBS749827. Urea was quantified using a rat ELISA kit of urea (Cloud-Clone Corp., USA), catalog number MBS9315229. A rat albumin ELISA kit (Cloud-Clone Corp., USA) with the catalog number CEB028Ra was used for quantitative albumin measurement.

Histopathological Investigation:

The kidney was autopsied and fixed in 10% neutral buffered formalin. Washing was carried out with tap water, and then dehydration occurred with serial dilutions of alcohol (methyl, ethyl, and absolute ethyl). Xylene is used in specimen clearance, and specimens are embedded in paraffin in a hot air oven at 56 degrees for twenty-four hours. Sectioning occurs by preparing Paralast wax tissue blocks at 4 microns in thickness with a rotatory microtome. Collect kidney tissue sections on glass slides, deparaffinized, and use hematoxylin and eosin for staining (Banchroft *et al*., 1996).

Statistical Analysis:

The current investigation's results were all assessed as mean±standard error of the mean. The significance of the two groups was compared using the Statistical Package for the Social Sciences, version 19.0. At $p < 0.05$, the difference was considered statistically significant. The percentage difference is the proportion of variance in the appropriate control group, as defined by the following rule:

$$\%Difference = \frac{treated\ value - control\ value}{control\ value} \times 100$$

RESULTS

The Anti-Tumor Studies:

In the current investigation, mice bearing EAC treated with only 5-FU and with 5-FU+LCN induced a marked effect on tumor growth retardation as showed in Table 1. This is supported by a remarkable rise in the survivor percentage. Cumulative mean survival time on day 40 and a marked decrease in the main tumor weight compared to the tumor-bearing mice group. As well, the present data show that the combined treatment caused a marked rise in survival time compared to the 5-fluorouracil treatment alone. It is worth noting that a noticeable effect was observed in the rise in the life span of mice (155.2%) in the 5-FU plus LCN-treated group and the inhibition ratio of tumors (55.2%) compared to tumor-bearing mice treated with 5-FU that recorded (118.4%) and (18.4%), respectively. This information is consistent with the earlier findings by (Kathigayan *et al*., 2007; Balamuruga, Reddy, and Menon 2010).

Effect of 5-fluorouracil and/or l-carnitine Nanoparticle therapy on renal oxidative stress marker MDA and antioxidant markers SOD, GSH, and GPX:

Table 2, shows that compared to the CON group, the EAC group had substantially higher ($P<0.05$) MDA levels and lower levels of SOD, GPX, and GSH. MDA levels increased considerably, although SOD, GPX, and GSH levels reduced ($P<0.05$) in the EAC+5-FU group compared to the EAC group. MDA levels dropped significantly, whereas SOD, GPX, and GSH levels rose in the EAC+5-FU+LCN group compared to the EAC treated with 5-FU alone.

Effect of treatment with 5-fluorouracil and/or l- carnitine Nanoparticle on renal pro-inflammatory markers(MCP-1) and (TNF-α).

Table 3, demonstrates that, compared to the CON group, the EAC group had a substantial rise in MCP-1 and TNF-α levels in the kidney compared to the CON group ($P<0.05$), While MCP-1 and TNF-α exhibited a modest substantial rise in the 5-FU treated group compared to the EAC group ($P<0.05$). In contrast to the 5-FU group, LCN and 5-FU+LCN significantly lowered MCP-1 and TNF-α levels in the kidney ($P<0.05$).

Table 1: The anticancer activity of 5-FU or/and LCN mice with Solid Ehrlich Carcinoma.

PTI (Days)	Gr. (EAC)		Gr. (EAC+5-FU)		Gr. (EAC+LCN)		Gr. (EAC+5-FU+LCN)	
	M	MTW±SE	M	MTW±SE	M	MTW±SE	M	MTW±SE
10	0/8	0.99±0.07	0/8	1.02±0.09	0/8	1.13±0.06	0/8	0.95±0.01
17	0/8	1.95±0.43	0/8	1.51±0.41	0/8	2.71±0.18	0/8	1.09±0.08
24	0/8	3.04±0.24	0/8	2.33±0.55	1/8	3.82±0.26	0/8	1.40±0.04
31	1/8	4.32±0.30	0/8	3.01±0.60	3/8	4.34±0.31	0/8	1.68±0.07
38	2/8	5.01±0.91	2/8	4.02±0.70	4/8	4.95±0.32	0/8	1.95±0.10
45	5/8	6.32±0.95	5/8	3.83±0.65	6/8	5.11±0.42	0/8	2.54±0.12
52	8/8	7.65±0.83	6/8	4.50±0.84	8/8	5.59±0.28	1/8	3.48±0.18
59			8/8	6.01±0.75			3/8	3.98±0.16
66							5/8	4.22±0.19
73							8/8	4.53±0.21
MTW	4.17		2.90		3.80		2.58	
MST (days)	38		45		34.5		59	
ILS%	-		118.4		90.7		155.2	
T/C%	-		18.4		-9.2		55.2	

PTI= Post tumor inoculation. M= Mortality. MTW= Mean tumor weight. MST (days) = Mean survival time. ILS= Increase of life span. T/C= Tumor inhibition ratio.

Table 2: Effect of treatment with 5-FU or/and 5-FU+LCN on MDA, SOD, GSH, and GPX of EAC-bearing mice.

Parameter / Group	MDA (nmol/g.tissue)	SOD (U/g.tissue)	GSH (mmol/g.tissue)	GPX (U/g.tissue)
CON	8.14±1.28	22.05±2.46	3.63±0.58	0.580±0.04
EAC	18.97±2.45[a] a (133.04%)	8.09±0.58[a] a(-63.31%)	0.33±0.03[a] a (-90.9%)	0.022±0.002[a] a(-96.2%)
EAC+5-FU	21.77±2.69[b] b (-12.86%)	5.80±0.42[b] b (-28.30%)	0.18±0.01[b] b(-45.45%)	0.066±0.004[b] b(200%)
EAC+ LCN	12.82±1.65[c] c (-41.11%)	13.26±1.73 [c] c (128.62%)	1.23±0.13[c] c (583.3%)	0.090±0.011[c] c(36.36%)
EAC+5-FU+ LCN	9.72±0.83[c] c(-55.35%)	20.09±2.04[c] c (246.37%)	2.52±0.21[c] c (1300%)	0.394±0.024[c] c(496.96%)

a: statistically significant at P<0.05 compared to CON group. b: statistically significant at P<0.05 compared to EAC group. c: statistically significant at P<0.05 compared to 5-FU group.

Table 3: The effects of 5-FU and 5-FU+LCN therapy on MCP-1 and TNF-α in EAC-bearing mice.

Parameter / Group	MCP-1(ng/ml)	TNF-α (pg/g tissue)
CON	4.59±0.50	202.67±16.32
EAC	22.78±3.54[a] a(396.29%)	750.19±20.22[a] a (270.15%)
EAC+5-FU	22.63±2.79[b] b(-0.658%)	848.87±23.51[b] b(13.15%)
EAC+LCN	18.90±2.31[c] c(-16.48%)	517.99±13.17[c] c(-38.97%)
EAC+5-FU+LCN	7.82±0.61[c] c(-65.44%)	258.95±14.04[c] c(-69.49%)

a: statistically significant at P<0.05 compared to (CON) group. b: statistically significant at P<0.05 compared to EAC group. c: statistically significant at P<0.05 compared to 5-FU group.

The Effect Of 5-Flourouracil or/and l-Carnitine Nanoparticles on Caspase-3, P53 and Bcl2 in Kidney Tissue In The Solid Ehrlich Carcinoma-Bearing Mice.

Table 4, illustrates the levels of pro-apoptotic markers Caspase-3, P53, and Bcl2 in the kidney. The EAC group had substantially higher renal caspase-3 and P53 levels than the CON group (P<0.05). Additionally, compared to the EAC group, renal Caspase-3 and P53 levels improved significantly, but renal Bcl2 levels fell markedly (P<0.05). Compared to the EAC+5-FU group, EAC+LCN and EAC+5-FU+LCN showed a substantial drop (P<0.05) in caspase-3 and P53 levels, as well as an increase in renal Bcl2.

Effect of treatment with 5-FU or/and 5-FU+LCN on serum creatinine, urea and albumin.

The data recorded in Table 5, illustrated a substantial elevation in ranks of creatinine and urea in the EAC set and a decline in albumin levels compared to the CON group (P<0.05). Moreover, creatinine, urea, and albumin produce a significant increase in the EAC+5-FU group compared with the EAC group (P<0.05). On the other hand, the EAC+ LCN and EAC+ 5-FU+LCN groups showed a significant decline (P<0.05) in levels of creatinine and urea and also showed an elevation in albumin levels compared to the EAC+ 5-FU group.

.

Table 4: Effect of treatment with 5-FU or/and 5-FU+LCN on caspase-3, P53, and Bcl2 of mice bearing EAC.

Parameter / Group	caspase-3 (ng/g. tissue)	P53 (pg/g, tissue)	Bcl2 (pg/g, tissue)
CON	11.53±1.52	86.20±7.08	61.02±4.24
EAC	41.17±4.65[a] a (257.06%)	201.33±12.62[a] a(133.56%)	15.52±2.22[a] a(-74.56%)
EAC+5-FU	58.61±5.32[b] b (41.26%)	245.33±15.22[b] b(21.85%)	16.68±1.96[b] b (7.47%)
EAC+LCN	33.93±4.66[c] c (-41.66%)	142.96±9.65[c] c (-41.72%)	37.35±4.11[c] c (123.92%)
EAC+5-FU+LCN	19.71±2.92[c] c (-66.11%)	113.01±7.19[c] c(-53.93%)	49.11±3.03[c] c (194.42%)

a: statistically significant at P<0.05 compared to CON group, b: statistically significant at P<0.05 compared to EAC group, c: statistically significant at P<0.05 compared to 5-FU group

Table 5: Effect of treatment with 5-FU or/and 5-FU+LCN on creatinine, urea and albumin of EAC-bearing mice.

Parameter / Group	Creatinine (mol/L. Tissue)	Urea (mmol/L.Tissue)	Albumin (g/dl.tissue)
CON	0.39±0.05	33.02±3.21	126.19±10.72
EAC	1.89±0.24[a] a (384.61%)	100.06± 7.98[a] a (203.03%)	34.76± 6.29[a] a (-78.56%)
EAC+5-FU	2.19 ±0.31[b] b (15.87%)	101.83± 9.25[b] b(1.76%)	41.85±4.61[b] b (20.39%)
EAC+LCN	0.88±0.041[c] c (-59.81%)	74.46±7.8[c] c(-26.87%)	107.37±7.32[c] c (156.55%)
EAC+5-FU+LCN	0.63±0.040[c] c(-71.23%)	57.34±6.66[c] c(-43.69%)	124.90±11.94[c] c(198.44%)

a: statistically significant at P<0.05 compared to CON group, b: statistically significant at P<0.05 compared to EAC group, c: statistically significant at P<0.05 compared to 5-FU group.

Histopathological Investigation:

Histopathological investigations of the kidney (Fig. 2A) revealed normal histology of the renal cortex and medulla. Furthermore, sections of the EAC group noticed a wide variety of inflammatory and necrotic changes. Some of the other sections showed hemorrhagic areas along with the detected interstitial nephritis. Mononuclear inflammatory cells are shown within the renal cortex (Fig. 2B). In the EAC +5-FU group, mild vacuolation in the lining epithelium of the renal tubules is seen within renal tissue (Fig. 2C). The EAC+ LCN group showed mononuclear inflammatory cell infiltration within the renal cortex (Fig. 2D). The EAC+5-FU+LCN group revealed an apparently normal medulla (Fig. 2E).

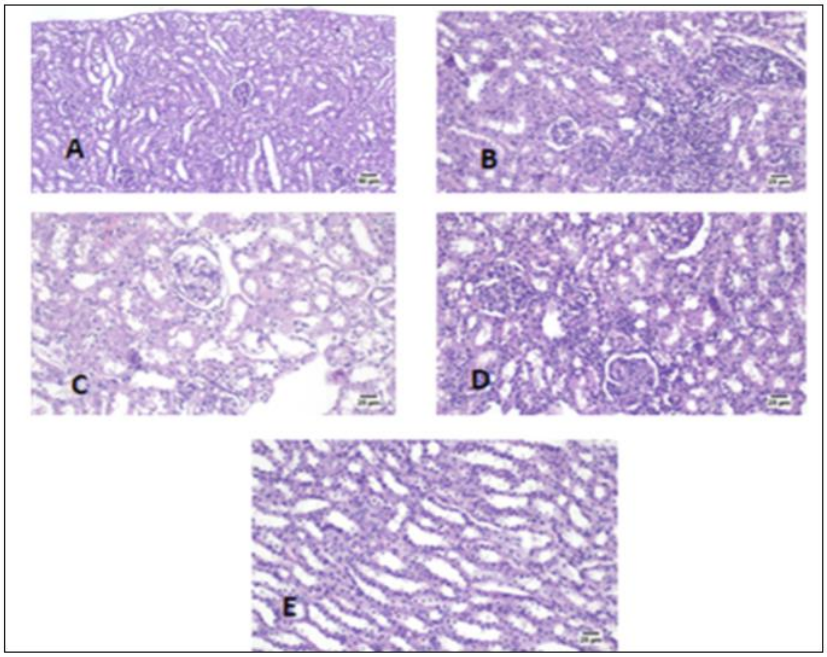

Fig 2: Photomicrographs of kidney slices stained with H and E from the CON, EAC, 5-FU, LCN, and 5-FU+ LCN groups. (**A**) Kidney photomicrograph, Normal group, demonstrating normal renal cortex (H&E). (**B**) A photomicrograph of the kidney, EAC group, at greater magnification, demonstrating mononuclear inflammatory cells within the renal cortex (H&E). (**C**) A photomicrograph of the kidney, EAC+ 5-FU group, demonstrating moderate vacuolation in the renal tubule lining epithelium (H&E). (**D**) A photomicrograph of the kidney from the EAC+ LCN group demonstrating mononuclear inflammatory cell infiltration within the renal cortex (H&E). (**E**) A photomicrograph of the kidney from the EAC+ 5-FU+ LCN group revealed an apparently normal medulla (H&E).

DISCUSSION

Conventional chemotherapies are used for the operation of cancer because of their ability to prohibit the proliferation of cancer cells and shrink primary tumors. This strategy led to killing both tumor and healthy cells, such as enzymes essential to DNA repair or synthesis. These features explain why healthy tissues, such as hair follicles and intestinal epithelium, are sensitive to the toxicity of chemotherapeutic drugs (Galluzzi *et al*., 2022).

5-Fluorouracil is considered a pyrimidine antimetabolite that has been used for 40 years in the treatment of a wide variety of cancers. 5-FU caused extensive side effects such as nephrotoxicity and weight loss in animals (Gelen *et al*., 2021). Several mechanisms have been suggested for 5-FU-induced toxicity, as 5-fluoro-2-deoxyuridine monophosphate (FdUMP), which is produced from the metabolization of 5-fluorouracil, inhibits the thynesis of thymidylate, an essential enzyme responsible for thymine synthesis, which suppresses the production of deoxythymidine monophosphate (dTMP), which is necessary for replication and repair of DNA, and its deficit leads to cellular toxicity (Rashid *et al*., 2014).

Nonselective endocytosis takes up nanoparticles, but chemical modification of the surface of these nanoparticles in such a way that these nanoparticles are recognized by tumor cell-specific cell-surface proteins would likely increase the capability of the endocytic process as well as tumor cell selectivity (Kou *et al*., 2017). The goal of this study was to combine nanotechnology with chemotherapeutic medications in the treatment of cancer in order to eliminate chemotherapy toxicity and protect organs from chemotherapy side effects. The current study investigated the burden deletion of solid tumors in mice carrying tumors treated with 5-FU+LCN. In addition, EAC-bearing mice's survival duration increased compared to the action with 5-FU alone. Likewise, the tumor inhibition ratio and animals' life span were elevated in the treatment of 5-FU plus LCN. The current finding showed that 5-FU plus LCN produces an antineoplastic effect through tumor proliferation inhibition, in agreement with Kou *et al*. (2017) who reported the ability of the l-carnitine nanoparticle to enter the tumor easily due to its nanometric size. In addition, it produces apoptosis in the tumor due to LCN acting as a more attainable carrier for anticancer drugs (Karthigayan *et al*., 2007).

The current study discovered that a significant rise in the renal oxidative stress marker MDA was associated with a decline in renal antioxidant indicators such as SOD, GSH, and GPX in the EAC group as compared to the control group. This might be attributed to tumor development (Choudhury *et al*., 2008). These findings, on the other hand, align with those of Balamurugan, Reddy, and Menon (2010), who discovered that MDA levels rise as SOD and GSH levels decline, owing to the continuous creation of free radicals produced by increased tumor development, resulting in oxidative stress in patients.

The 5-FU-FUoup administered a rise in the level of renal MDA and reduced the levels of SOD, GPX, and GSH compared with the EAC group. This data was approved by Gelen *et al*. (2018) who reported that 5-FU is capable of producing oxidative stress, which has an accumulation of ROS that produces a reduction of antioxidants on oxygen disproportion, leading to attack and damage to DNA and then producing apoptosis in the cell.

This study demonstrated that the treatment of the EAC group with 5-FU and LCN caused a considerable decrease in MDA levels and a significant increase in renal enzymatic and non-enzymatic antioxidants in mice compared to the EAC+5-FU group. That is in agreement with Khan and Alhomida. (2011), reported that 5-FU-induced oxidative stress antagonization with 5-fluorouracil and l-carnitine nanoparticle treatment occurs due to the presence of antioxidant properties of l-carnitine, which were noticed by the rise of GSH, regulation of GPX, and SOD compared to the 5-FU group. These findings are consistent

with Aghebati-Maleki *et al*. (2020) who found that nanoparticles have the ability to improve retention and permeation of the vasculature of tumors and localize intrinsically into tumor vessels, producing high payloads and half-lives, continuing the production of therapeutic drugs, and decreasing their toxicity on normal cells. This study found that the increase in levels of antioxidants occurs due to the ability of LCN to scavenge free radicals and reduce lipid peroxidation.

In the contemporary study, in comparison to the CON group, the EAC group had a considerable rise in renal pro-inflammatory markers such as TNF-α and MCP-1. This is significant due to a pathological renal injury. These findings are consistent with Salem *et al*. (2021) who noticed a rise in the level of TNF-α in EAC-bearing mice compared to the normal control group. The EAC induces higher levels of pro-inflammatory markers, which occur due to the increasing production of ROS by macrophages that activate lipid peroxidation.

Administration of 5-FU to the EAC group produced TNF-α and MCP-1 levels in the kidneys that were higher in the 5-FU group (Sengul *et al*., 2021). This finding demonstrated the existence of inflammation in the kidneys of mice given 5-FU. In addition, the regulation of immune response by transcription factor NF-κB plays a major role in inflammation disease in many organs through genes that regulate and stimulate pro-inflammatory markers, leading to tissue damage pathogenesis and the formation of edema that causes tissue injury (Caglayan *et al*., 2018). This result is in agreement with Harishi *et al*. (2022) who stated that 5-FU caused overexpression of special proteins such as pro-inflammatory cytokines and COX-2 that induce the production of a large number of prostaglandins, matrix metalloproteinase activation result of this collagen fragmentation, basement membrane of epithelial, formation of edema that cause tissue injury, and pro-inflammatory TNF-α promoted to produce a large amount.

Contrariwise, the EAC-treated group with l-carnitine nanoparticles produced TNF-α, and MCP-1 levels in the kidney were lower in the EAC+5-FU group. These findings are comparable to those of Jiang *et al*. (2015) who recorded that l-carnitine plays an important role in scavenging reactive oxygen, reducing the production of superoxide anion, inhibiting the synthesis of fatty acids, and having the ability to decrease the level of inflammatory cytokines such as TNF-α and MCP-1 in chronic diseases such as cancer.

A recent study found that 5-FU plus LCN treatment significantly reduced renal TNF-α and MCP-1 levels compared to the EAC+5-FU group. These findings are consistent with those of Agarwal *et al*. (2019), who found that nanoparticles have specific benefits over traditional chemotherapy, such as enhanced tumor formation and less spread in normal organs. Nanoparticles can reduce inflammation by neutralizing TNF-α and MCP-1, which cause inflammation. The EAC+5-FU+LCN group had significantly lower levels of TNF-α and MCP-1 compared to the EAC+5-FU group.

Likewise, the present investigation discovered a rise in the renal levels of caspase-3 and P53, as well as a significant drop in Bcl2 levels in the EAC group as compared to the control group. These findings supported the findings of Abdallah *et al*. (2023), who said that if wounded cells did not undergo apoptosis, they might grow into cancer. This conclusion is congruent with that of Mansour *et al*. (2022), who found an increase in the expression of pro-apoptotic protein levels p53 and caspase-3, as well as a reduction in the expression of anti-apoptotic protein levels Bcl2 in EAC-bearing mice. Furthermore, because the Bcl2 family is associated with cancer, abnormal anti-apoptotic Bcl2 family members increase cancer cells in comparison to normal cells.

These findings stated that the EAC group treated with 5-FU resulted in a significant increase in P53 and caspase-3 levels, accompanied by a significant reduction in renal Bcl2 levels compared to the normal control group. These results follow De Angelis *et al*. (2006) who recorded that 5-FU treatment respondents induce excessive side effects such as nephrotoxicity in mice through inhibition of mitochondrial apoptosis of P53. So, it causes

an elevation in P53 and caspase-3 expression and reduces Bcl2 expression.

The present research has discovered that treating EAC-bearing mice with free LCN decreases pro-apoptotic markers such as P53 and caspase-3 while boosting the anti-apoptotic level of Bcl2. In view of our study (Lee *et al.*, 2022), demonstrated that the effect of l-carnitine on protection from autophagy-associated apoptosis also reduces ROS levels in the cytosol and mitochondria that act as antioxidants. On the other hand, Fathi *et al*. (2021) explained that treatment with l-carnitine produced an increase in the production of cytokines compared to the 5-FU treatment group. He mentioned that the occurrence of apoptosis changes due to the reduction in renal caspase-3 level as a pro-apoptotic protein that leads to apoptosis, as well as an increase in renal Bcl2 level as an anti-apoptotic protein, so l-carnitine has the capability to increase apoptosis in cancer cells of the kidney. L-carnitine has the ability to improve kidney function due to its capability to scavenge free radicals and its anti-apoptotic and antioxidant properties that lead to membrane permeability protection.

The current work revealed that treating EAC-bearing mice with free l-carnitine nanoparticles suppressed pro-apoptotic markers such as P53 and caspase-3 while enhancing the anti-apoptotic level of Bcl2. This study, in agreement with Lee *et al.* (2022), demonstrated that the effect of l-carnitine on protection from autophagy-associated apoptosis reduces ROS levels in the cytosol and mitochondria that act as antioxidants. On the other hand, Fathi *et al*. (2021) explained that treatment with l-carnitine produced an increase in the production of cytokines. In current research, the EAC-treated group with 5-fluorouracil plus l-carnitine nanoparticles resulted in a significant decrease in pro-apoptotic markers such as P53 and caspase-3, associated with an elevation in the level of the anti-apoptotic marker Bcl2 compared to the EAC+5-FU group. These results are in agreement with Hamida *et al*. (2020) who suggested that nanoparticles have a large surface area that allows particles to penetrate living cells easily and modify their surface by conjugation with target molecules to improve the delivery of drugs to cancer cells rather than normal cells. According to this study, the anti-apoptotic effect of EAC+5-FU+LCN is approved by an increase in Bcl2 level and a decline in caspase-3 level in nephrocytes, supporting the idea that the treatment of 5-fluorouracil plus l-carnitine nanoparticles can improve nephrotoxicity induced by 5-fluorouracil.

According to the current study, mice with Ehrlich ascites carcinoma have altered renal function, resulting in elevated levels of creatinine and urea and a reduction in albumin levels. These findings are in agreement with Chen *et al*. (2019), who observed that the increase in creatinine and urea causes Ehrlich ascites carcinoma damage to the renal tubules and cortex, atrophy of the glomerulus, and harm to the renal tissue. Moreover, Ehrlich ascites carcinoma-induced ROS is a significant factor in oxidative stress-related injury to renal tissue compared to the control group. In like manner, Hex *et al*. (2017) concluded that an essential serum protein, albumin, levels alter to reveal the presence of malnourishment, chronic inflammation, and the development of kidney cancer. Correspondingly, Adikwn, Biradee, and Ogungbaike, (2019) revealed that, in comparison to the normal control group, 5-FU administration decreased albumin levels and increased the blood levels of urea and creatinine. Additionally, the study's biochemical results concur with those published by Badwoud *et al*. (2017) who reported that when treatment with 5-FU causes kidney impairment, nephrotoxicity is produced, leading to tubule and glomerule degeneration, an increase in creatinine and urea, and a decrease in albumin. In keeping with our findings, 5-FU-treated individuals had higher levels of creatinine and urea and lower albumin levels than the EAC group.

According to Zheng *et al*. (2021), the current study validates the efficacy of l-carnitine in treating reductions in urine protein excretion and improvements in renal function. L-carnitine therapy results in lower levels of urea and creatinine as well as less tubular necrosis and cast formation, in agreement with McCann *et al*. (2021), who recorded

that nanoformulations of l-carnitine improve bioavailability and pharmacokinetic characteristics for passive or active delivery of hydrophobic or hydrophilic compounds, as well as the improvement of its biological activity while decreasing the side effects of 5-FU. According to these findings, compared to treating with 5-FU or LCN alone, the 5-FU+LCN group had higher levels of albumin and lower levels of serum creatinine and urea (Aydogdu *et al*., 2006).

Furthermore, several studies have indicated that l-carnitine nanoparticles have anticancer and anti-inflammatory features that stimulate apoptosis and prevent metastasis. Additionally, l-carnitine nanoparticles could improve drug cytotoxicity induced by oxidative stress damage; they work as a delivery system for various drugs through the production of free radicals and ROS instead of using the drug-free.

The normal control group's kidney histopathology demonstrated normal histology in both the renal cortex and medulla. The glomeruli and both types of renal tubules were found in the renal cortex, whereas the renal medulla included both types of renal tubules and collecting ducts. Differently, the kidney tissue of the EAC group revealed a wide variety of inflammatory and necrotic changes. Interstitial nephritis was noticed in the renal cortex, which was manifested by the presence of a large number of mononuclear inflammatory cells that had infiltrated the renal cortex. Specific parts of the tested sections revealed hemorrhagic regions in addition to the identified interstitial nephritis. The renal tubules suffered degeneration and necrosis. Some of the degenerating renal tubules contained eosinophilic protein. In view of our findings, Mutar *et al*. (2020) reviewed several pathological alterations seen in the glomeruli and renal tubules, including glomerular shrinkage, severe injury and degeneration of the renal tissues, and Malpighian corpuscles that lost their distinctive composition.

Regarding the histopathological alterations in the EAC+ 5-FU group, showing mild vacuolation of the renal tubules was quite a common finding. Some other sections showed focal mononuclear inflammatory cell aggregations within the renal cortex. Some renal tubules exhibited degenerative changes and even necrosis. Based on these results, Elbanan *et al*. (2023) recorded in the kidney the appearance of disarray with irregular tubules and necrosis, focal areas of intense tubular degeneration, congestion, and interstitial edema, and an irregularly shaped atrophy glomerulus with enlarging capsular spaces. The EAC group that was treated with LCN only appeared apparently normal. Few individuals showed mild focal mononuclear inflammatory cell aggregations. Some other sections exhibited mild inflammatory reactions in the renal medulla with degenerative changes in the renal tubules. Furthermore, 5-FU+LCN in the EAC-treated group were apparently normal histologically. A few sections showed vacuolation in the epithelial lining of the renal tubules. Some severely affected sections showed extensive perivascular mononuclear inflammatory cell infiltration. In line with our finding (Hassan *et al*., 2022), which showed that l-carnitine nanoparticles have powerful effects on nephrotoxicity due to the ability of LCN to target tumors and prevent drugs from reaching normal cells, which induces lesser systemic toxicity and improves kidney functions, It has an antitumor effect and deletes free radicals and ROS from the body.

CONCLUSION

Although there are wide uses for 5-FU as a chemotherapeutic agent, it has limited clinical applications due to its toxicity in kidney tissue. So, drug delivery systems containing l-carnitine nanoparticles are used to enhance the effect of 5-FU. All parameters of kidney tissue showed improvement in the result of using 5-FU+LCN compared to 5-FU only and EAC-bearing mice. Furthermore, the goal of this work is to show that l-carnitine nanoparticles containing 5-fluorouracil are more protective than free 5-FU.

Declarations:

Ethical Approval:Ethical Approval is not applicable.

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REFERENCES

Abdallah, R.; Al-Saleem, M.; Abdel-Mageed, W.; Al-Attar, A.; Shehata, Y.; Abdel-Fattah, D.;and Atta, R. (2023). LCMS/MS Phytochemical Profiling, Molecular, Pathological, and Immune-Histochemical Studies on the Anticancer Properties of Annona muricata. *Molecules,* 28(15), 5744. https://dio.org/1420-3049/28/ 15/ 5744.

Adikwu, E.; Ebinyo, N.; and Amgbare, B. (2019). Protective activity of selenium against 5-fluorouracil-induced nephrotoxicity in rats. *Cancer Translational Medicine*, *5*(3), 50-55. https://search.proquest.com/openview/ bc2367a04e676b57 cd53ab85becc 0eb3/1?pq-origsite=gscholar&cbl=2042886.

Agarwal, H.; Nakara, A.; and Shanmugam, V. (2019). Anti-inflammatory mechanism of various metal and metal oxide nanoparticles synthesized using plant extracts: A review. *Biomedicine & Pharmacotherapy,* 109, 2561-2572. https://dio. org/ S0753332218371956.

Aghebati-Maleki, A.; Dolati, S.; Ahmadi, M.; Baghbanzhadeh, A.; Asadi, M.; Fotouhi, A.; and Aghebati-Maleki, L. (2020). Nanoparticles and cancer therapy: Perspectives for application of nanoparticles in the treatment of cancers. *Journal of cellular physiology,* 235(3), 1962-1972. https://dio.org/10.1002/jcp.29126.

Aydogdu, N.; Atmaca, G.; Yalcin, O.; Taskiran, R.; Tastekin, E.; and Kaymak, K. (2006). Protective effects of l-carnitine on myoglobinuric acute renal failure in rats. *Clinical and experimental pharmacology and physiology*, *33*(1-2), 119-124. https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1440-1681.2006.04336.x

Badawoud, M.H.; Elshal, E.B.; Zaki, A.I.; and Amin, H.A. (2017). The possible protective effect of L-arginine against 5-fluorouracil-induced nephrotoxicity in male albino rats. *Folia Morphologica*, *76*(4), 608-619. https://journals.viamedica. pl/ folia_ morphologica/article/view/50730

Balamurugan, E.; Reddy, B.V.; and Menon, V.P. (2010). Antitumor and antioxidant role of Chrysaora quinquecirrha (sea nettle) nematocyst venom peptide against ehrlich ascites carcinoma in Swiss Albino mice. *Molecular and cellular biochemistry*, 338, 69-76. https://dio.org/10.1007/s11010-009-0339-3.

Banchroft, J.; Stevens, A.; and Turner, D. (1996). Theory and practice of histological techniques. 4th Ed. New York, London, San Francisco, Tokyo: Churchil Livingstone. https://cir.nii.ac.jp/crid/1370848662559314694

Beutler, E.; Duron, O.; and Kelly, B. M. (1963). Improved method for the determination of blood glutathione. *The Journal of laboratory and clinical medicine*, 61, 882-888., https://dio.org/13967893/.

Caglayan, C.; Temel, Y.; Kandemir, F.; Yildirim, S.; and Kucukler, S. (2018). Naringin protects against cyclophosphamide-induced hepatotoxicity and nephrotoxicity through modulation of oxidative stress, inflammation, apoptosis, autophagy, and DNA damage. *Environmental Science and Pollution Research*, 25, 20968-20984, https://dio.org/10.1007/s11356-018-2242-5.

Chen, K.; Demarco, B.; Heilig, R.; Shkarina, K.; Boettcher, A.; Farady, C.; and Broz, P. (2019). Extrinsic and intrinsic apoptosis activate pannexin-1 to drive NLRP 3 inflammasome assembly. *The EMBO journal*, *38*(10), e101638. https://www.

embopress.org/doi/abs/10.15252/embj.2019101638

Choudhury, S.; Roy, G.; Gupta, M.; and Majumder, U. (2008). The central nervous system depressant activities of Mycotoxin MT81 and its acetylated and benzoylated analogues). *Al Ameen Journal of Medical* Sciences, 1: 104–114, http://dio. org/AJMS.2.P-104-114-C.

De Angelis, P.; Svendsrud, D.; Kravik, K.; and Stokke, T. (2006). Cellular response to 5-fluorouracil (5-FU) in 5-FU-resistant colon cancer cell lines during treatment and recovery. *Molecular cancer*, 5(1), 1-25, https://dio.org/10.1186/1476-4598-5-20.

Elbanan, M.E.; Amer, M.E.; El-Missiry, M.A.; Othman, A.I.; and Shabana, S.M. (2023). Melatonin protected against kidney impairment induced by 5-fluorouracil in mice. *Journal of Experimental Zoology Part A: Ecological and Integrative Physiology*, *339*(8), 777-787. https://onlinelibrary.wiley.com/doi/abs/10.1002/jez. 2728

Fahad, O.; Abdulkareem, F.; Mohammed, A.; and Kareem, O. (2021). Produce of Selenium Nanoparticles by Pulsed Laser Ablation for Anticancer Treatments. *International Journal of Nanoscience*, 20(06), 2150055. https://dio. org/ 10. 1142/S0219581 X21500551.

Fahim, A.; Esmat, E.; Mady, E.; and Ibrahim, K. (2003): Antitumor activities of Iodo acetate and Di methyl sulphoxide against solid Ehrlich carcinoma growth in mice. Biological Research, 36:253-62. https://dio.org /S0716-97602003000200015.

Farahzadi, R.; Sanaat, Z.; Movassaghpour-Akbari, A.; Fathi, E.; and Montazersaheb, S. (2023). Investigation of L-carnitine effects on CD44+ cancer stem cells from MDA-MB-231 breast cancer cell line as anti-cancer therapy. *Regenerative Therapy*, 24, 219-226, https://dio.org/S2352320423000627 .

Fathi, F.; Sanei, B.; Ganjalikhani Hakemi, M.; Saidi, R.; and Rezaei, A. (2021). Liver resection promotes (regulates) proinflammatory cytokines in patients with hepatocellular carcinoma. *Canadian Journal of Gastroenterology and Hepatology*, 2021, https://dio.org/2021/5593655/.

Galluzzi, L.; Humeau, J.; Buqué, A.; Zitvogel, L.; and Kroemer, G. (2020). Immunostimulation with chemotherapy in the era of immune checkpoint inhibitors. *Nature reviews Clinical oncology*, 17(12), 725-741, https://dio. org/s41571-020-0413.

Gelen, V.; Şengül, E.; Yıldırım, S.; and Atila, G. (2018). The protective effects of naringin against 5-fluorouracil-induced hepatotoxicity and nephrotoxicity in rats. *Iranian journal of basic medical sciences*, 21(4), 404. https://dio.org/PMC5960758/.

Gelen, V.; Şengül, E.; Yıldırım, S.; Senturk, E.; Tekin, S.; and Kükürt, A. (2021). The protective effects of hesperidin and curcumin on 5-fluorouracil–induced nephrotoxicity in mice. *Environmental Science and Pollution Research*, 28, 47046-47055, https://dio.org/10.1007/s11356-021-13969.

Hamida, R.; Ali, M.; Goda, D.; Khalil, M.; and Al-Zaban, M. (2020). Novel biogenic silver nanoparticle-induced reactive oxygen species inhibit the biofilm formation and virulence activities of methicillin-resistant Staphylococcus aureus (MRSA) strain. *Frontiers in bioengineering and biotechnology*, *8*, 433, https://dio. org/ 10.3389/fbioe.2020.00433/.

Harish, B.; Shivakumar, P.; Kumar, B.; Ramya, B.; Begum, N.; Rani, M.; and Kumar, B.; (2022). Amelioration of experimental nephrotoxicity due to 5-Flourouracil by Resveratrol in comparison to Vitamin-E, https://dio.org/Vol11_Issue4_03.

Hassan, S.; Saeed, A.; Rahim, O.; and Mahmood, S.; (2022), Alleviation of cisplatin-induced hepatotoxicity and nephrotoxicity by L-carnitine. *Iranian Journal of Basic Medical Sciences*, 25(7), 897, https://dio.org/PMC9392569/.

He, L.; Zhu, H.; Zhou, S.; Wu, T.; Wu, H.; Yang, H.; and Guo, Z. (2018). Wnt pathway is

involved in 5-FU drug resistance of colorectal cancer cells. *Experimental & molecular medicine*, *50*(8), 1-12. https://www.nature.com/articles/s12276-018-0128-8

Jiang, P.; Chan, C.; Chan, K.; Cheng, S.; Wong, J.; Wong, V.; and Lo, Y. (2015). Lengthening and shortening of plasma DNA in hepatocellular carcinoma patients. *Proceedings of the National Academy of Sciences*, 112(11), E1317-E1325, https://dio.org/10.1073/pnas.1500076112.

Karthigayan, S.; Balasubashini, M.; Balasubramanian, T.; and Somasundaram, S. (2007). PGE from Octopus aegina induces apoptosis in Ehrlich's ascites carcinoma of mice. *Toxicology Mechanisms and Methods*, 17(8), 451-458, https://dio. com/ 10.1080/15376510601177688.

Khan, H.; and Alhomida, A. (2011). A review of the logistic role of l-carnitine in the management of radiation toxicity and radiotherapy side effects. *Journal of Applied Toxicology*, 31(8), 707-713. https://dio.org/10.1002/jat.1716.

Klochkov, S.; Neganova, M.; Nikolenko, V.; Chen, K.; Somasundaram, S.; Kirkland, C.; and Aliev, G. (2021). Implications of nanotechnology for the treatment of cancer: Recent advances. In Seminars in cancer biology (Vol. 69, pp. 190-199). *Academic Press*. https://dio.org/S1044579X19301877.

Kou, L.; Yao, Q.; Sivaprakasam, S.; Luo, Q.; Sun, Y.; Fu, Q.; and Ganapathy, V. (2017). Dual targeting of l-carnitine-conjugated nanoparticles to OCTN2 and ATB0,+ to deliver chemotherapeutic agents for colon cancer therapy. *Drug Delivery*, 24(1), 1338-1349, https://dio.org/10.1080/10717544.2017.1377316.

Kumari, R.; Saini, A.; Chhillar, A.; Saini, V.; and Saini, R. (2021). Antitumor effect of bio-fabricated silver nanoparticles towards ehrlich ascites carcinoma. *Biointerface Research in Applied Chemistry*, 11, 12958-12972, https://dio. org/ 2021/01/ 20695837115.1295812972.

Lee, Y.; Chou, H.; Chen, Y.; Tung, S.; Ko, T.; Buyandelger, B.; and Juan, S. (2022). l-Carnitine reduces reactive oxygen species/endoplasmic reticulum stress and maintains mitochondrial function during autophagy-mediated cell apoptosis in perfluorooctanesulfonate-treated renal tubular cells. *Scientific Reports*, 12(1), 4673, https://dio.org/s41598-022-08771-3.

Mahmoud, M.; Abdollah, M.; Elsesy, M.; Abou El Ella, D.; Zada, S.; and Tolba, M. (2022). The natural isoflavone Biochanin-A synergizes 5-fluorouracil anticancer activity in vitro and in vivo in Ehrlich solid-phase carcinoma model. *Phytotherapy Research*, *36*(3), 1310-1325, https://dio.org/10.1002/ptr. 7388.

Mansour, S.; Hashem, A.; Abd-El-Maksoud, M.;El-Hussieny, M.; El-Makawy, A.; Abdel-Aziem, S.; and Soliman, F. (2022). The synthesis and antineoplastic activities of thiaziridine, sulfidometylphosphonium, and dithiaphosphitane-sulfide against the Ehrlich ascites carcinoma. *Fundamental & Clinical Pharmacology*, 36(3), 536-552, https://dio.org/10.1111/fcp.12751.

Marzi, M.; Osanloo, M.; Vakil, M.K.; Mansoori, Y.; Ghasemian, A.; Dehghan, A.; and Zarenezhad, E. (2022). Applications of metallic nanoparticles in the skin cancer treatment. *BioMed Research International*, 2022. https://dio.org/2022/2346941/.

McCann, R.; George De la Rosa, V.; Rosania, R.; and Stringer, A. (2021). L-carnitine and acylcarnitines: mitochondrial biomarkers for precision medicine. *Metabolites*, *11*(1), 51. https://www.mdpi.com/2218-1989/11/1/51

Mutar, F.; Tousson, E.; Hafez, E.; Abo Gazia, M.; and Salem, B. (2020). Ameliorative effects of vitamin B17 on the kidney against Ehrlich ascites carcinoma induced renal toxicity in mice. *Environmental Toxicology*, *35*(4), 528-537. https:// onlinelibrary.wiley.com/doi/abs/10.1002/tox.22888

Nishikimi, M.; Roa, N.; and Yogi. K. (1972). Colorimetric determination of superoxide

dismutase. Biochemical Biophysical research Communication, 46:849-854, https://dio.org/S0006291X72802183.

Ohkawa, H.; Ohishi, W.; and Yagi, K. (1979). Assay for lipid peroxidase in animal tissue by thiobarbituric acid reaction. https://dio.org/0003269779907383.

Paglia, D.; and Valentine, W. (1967). Studies on the Quantitative and Qualitative Characterization of Erythrocyte Glutathione Peroxidase. *Journal of Laboratory and Clinical Medicine*, 70:158-169,https://dio.org/0022-2143(67)90076-5/.

Rashid, S.; Ali, N.; Nafees, S.; Hasan, S.; and Sultana, S. (2014). Mitigation of 5-Fluorouracil induced renal toxicity by chrysin via targeting oxidative stress and apoptosis in wistar rats. *Food and Chemical Toxicology*, 66, 185-193. https://dio.org/S0278691514000404.

Salem, A.; Medhat, D.; Fathy, S.; Mohamed, M.; El-Khayat, Z.; and El-Daly, S.; (2021). Indole glucosinolates exhibit anti-inflammatory effects on Ehrlich ascites carcinoma cells through modulation of inflammatory markers and miRNAs. *Molecular Biology Reports*, 48(10), 6845-6855, https://dio.org/10.1007/s11033-021-06683-5

Sengul, E.; Gelen, V.; Yildirim, S.; Senturk, E.; Dag, Y.; Eser, G.; and Gok, M. (2021). Investigation of effects of Silymarin in 5-fluorouracil hepatotoxicity and nephrotoxicity in Mice, https://dio.org /rs-448267/latest.

Sethy, C.; and Kundu, C.; (2021). 5-Fluorouracil (5-FU) resistance and the new strategy to enhance the sensitivity against cancer: Implication of DNA repair inhibition. *Biomedicine & Pharmacotherapy*, 137, 111285., https://dio.org/ S0753332221000706.

Shehatta, N.; Okda, T.; Omran, G.; and Abd-Alhaseeb, M. (2022). Baicalin; a promising chemopreventive agent, enhances the antitumor effect of 5-FU against breast cancer and inhibits tumor growth and angiogenesis in Ehrlich solid tumor. *Biomedicine & Pharmacotherapy*, 146, 112599, https://dio.org/ S075333222101386X.

Vincent, P.; and Nicholls, A. (1967). Comparison of the growth of the Ehrlich ascites tumor in male and female mice. *Cancer Research*, 27(6_Part_1),1058-1065, https:// dio.org/27/6_Part_1/1058/476692.

Wang, J.; Guo, X.; Barron, L.; and Szoka, F. (1998). Synthesis and characterization of long chain alkyl acyl carnitine esters. Potentially biodegradable cationic lipids for use in gene delivery. *Journal of medicinal chemistry*, 41(13), 2207-2215, https://dio.org/10.1021/jm950802i.

Weber, D.D.; Aminzadeh-Gohari, S.; Tulipan, J.; Catalano, L.; Feichtinger, R.G.; and Kofler, B. (2020). Ketogenic diet in the treatment of cancer–where do we stand?. *Molecular metabolism*, *33*, 102-121. https://www.sciencedirect.com/ science/article/pii/S2212877819304272

Zhang, D.; Ma, X.L.; Gu, Y.; Huang, H.; and Zhang, G.W. (2020). Green synthesis of metallic nanoparticles and their potential applications to treat cancer. *Frontiers in Chemistry*, *8*, 799. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7658653/