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Anticancer Potential of Blue-Green Algae Extract (*Spirulina Plantesis)* and Curcumin Nanoparticles on Ehrlich Ascites Carcinoma–Bearing Mice

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Abstract

This research was accomplished to identify the possibility of preventing cancer by *Spirulina plantesis* (SP) and curcumin nanoparticles (Cur-NPs) being natural substances in the EAC model. 110 male mice were split up randomly into group (I): healthy control; group (II): mice received SP; group (III): mice received Cur-NPs; group IV: mice administrated with both SP and Cur-NPs; group V: EAC group; group VI: SP Protective (Pr) group; group VII: SP Therapeutic (Tr) group; group VIII: Cur-NPs Pr group; group IX: Cur-NPs Tr group; group X: SP & Cur-NPs Pr group; group In comparison to the control group, the biochemical assays throughout this study exhibited an extremely significant rise in serum and hepatic MDA, ALT, and AST levels and a greatly marked drop in levels of TAC in the EAC group. In contrast, administration of Cur-Nps alone or combined with SP results in a significant reduction and improvement in their levels. The histological and immunohistochemical findings confirm the biochemical observations since the liver's histology and immunohistochemistry were improved by SP and Cur-NPs.

Keywords: Spirulina plantesis (SP); Curcumin nanoparticles (Cur-Nps); Ehrlich ascites carcinoma (EAC)

1. Introduction

Even today, cancer remains a major public health concern with the millions of deaths it accounts for annually. **[1]** Experimental tumors are critical for modelling, and EAC is among the most frequent models. **[2]** Ehrlich tumors have the benefit of being transplantable tumor models in the ascites form, which makes it possible to study the anticancer effects of numerous synthetic and herbal drugs. **[3,4]** Various anti-cancer drugs are used, which, despite their great anti-tumor effect, cause undesirable side effects in humans **[5]** and significantly affect the host's normal cells. Therefore, natural, safe products to prevent and/or treat cancer have improved. **[6,7]**

In both human and animal systems, blue-green algae exhibit anti-tumor action against numerous tumors. A multicellular filamentous algal called *Spirulina platensis* (SP) can grow in both freshwater and saltwater environments. **[8]** It may significantly contribute to the inhibition of cancer owing to its modulation of the immune system's abilities and its antioxidant qualities. **[5]** It is a rich source of biological compounds including antioxidants, protein, carbohydrates, lipids, vitamins, minerals and phytopigments such as phycocyanin, chlorophyll, xanthophyll, beta-carotene, and zeaxanthin **[9]** and several kinds of necessary amino acids. **[10]** SP doesn't have cellulose cell walls; therefore, it does not need physical or chemical processing to be digestible. **[11]**

Turmeric is an herbal medication that is utilized to treat cancers of the mouth, breast, prostate, ovary, and skin. **[12]** Curcumin (CUR) is a highly active component of the turmeric root. **[13,14]** Traditional medicine views CUR as a helpful medicinal agent and believes it to have no significant side effects. **[15]** But CUR exhibits weak solubility in water, poor absorption in the free form in the gastrointestinal tract and fast biotransformation to inactive metabolites and therefore the systemic bioavailability is also very small. This is attributed to

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very poor absorption, faster metabolism, and systemic elimination after oral administration. **[16,17]** In addition, only organic solvents can dissolve CUR. So, biological uses are limited because of the reported toxicity of these solvents. **[18]** Consequently, its therapeutic activities are also significantly reduced, so, the nano-range formulations of CUR, popularly known as "Cur-Nps" have been developed to overcome this restriction. **[19]**

The field of biological study has shifted its focus towards nanoscience due to nanomaterials, which have emerged as one of the most important and useful sciences in various biological domains. **[20]** Cur-Nps is an oral CUR product industrialized in nanotechnology. **[21]** Because Cur-Nps can more easily cross biological systems' cell membranes, they interact with biological systems more quickly, **[22]** feature that makes Cur-Nps stand out in terms of its efficacy when compared to free CUR. **[23]** Cur-Nps supplementation has been shown to have beneficial effects on the risk of several chronic diseases in several epidemiological investigations. **[19]** The current research is done to estimate the antitumor activities of both SP and Cur-NPs against EAC in albino mice.

2. Materials & methods

2.1. Experimental design

110 male mice weighing 25–30 grams were bought from the National Cancer Institute's animal colony in Cairo, Egypt. Mice were housed under constant temperature conditions (24 ± 2°C) for two weeks before the experimental work. During the experiment, a standard rodent diet containing a high protein amount was supplied, and tap water was provided ad libitum. After 2 weeks of readjustment, the mice were allocated randomly into 11 groups of ten mice each. Group I (Healthy Control); Group II (Spirulina Group) mice received SP (500 mg/kg BW/day) for 30 days **[24]**; Group III (Cur-Nps Group) mice received Cur-NPs (15 mg/kg/day) for 30 days **[25]**; Group IV (SP and Cur-Nps Group) mice received both SP and Cur-Nps; Group V (EAC Group) mice were injected subcutaneously with 0.2 ml of ascetic fluid (2.5×10^6 EAC cells) in a right thigh to form a solid tumor without treatment. Group VI: (SP protective (Pr) group), mice processed orally with SP 15 consecutive days before and 15 days following tumor injection; Group VII: (SP therapeutic (Tr) group), mice orally treated with SP after tumor injection for 30 days; Group VIII: (Cur-Nps Pr group), mice processed orally with Cur-Nps for 15 consecutive days before and 15 days after tumor injection; Group IX: (Cur-Nps Tr group), mice processed orally with Cur-Nps following the tumor injection for 30 days. Group X: (SP & Cur-Nps Pr group), mice treated with both SP & Cur-Nps for 15 sequential days before & 15 days after injection of tumor; Group XI: (SP & Cur-Nps Tr group), mice treated with both SP & Cur-Nps after tumor inoculation for 30 days.

❖ **Ethical statement**

The animal study was done after receiving approval from the Animal Ethics Committee of Fayoum University, Egypt, with approval number AEC2205; The experiments were sustained by the guidelines given by the faculty of science.

❖ **Sample collection**

After the experiment was ended, the animals fasted throughout the entire night. In the next morning, by using diethyl ether, animals were euthanized, then dissected, and from the retro-orbital venous plexus, blood samples were collected in two tubes; the first one contained EDTA to determine hematological measurements. In the second tube, blood was allowed to remain for 15 minutes at 37ºc, then centrifuged at 4000 rpm for 20 minutes for the separation of serum. Serum was removed and reserved in plastic vials at -20ºc until used for further biochemical analyses.

After blood collection, mice were sacrificed. The solid tumors were dissected out and weighed on a weighing balance. Liver tissue specimens were also taken from each animal. One part of the liver samples was conserved in a 10% formalin solution for histopathological and immunohistochemical examination. The tumor tissue and the other parts of the liver samples were weighed, then perfused with a phosphate buffered saline (PBS) solution, pH 7.4, and homogenized. The homogenates were reserved at -20°C until hepatic and tumor MDA levels were measured.

2.2. Preparation of spirulina dose

Spirulina platensis (SP), as a dark green powder, was purchased from the Algal Biotechnology Unit, National Research Centre (NRC), Dokki, Cairo, Egypt. In distilled water, SP was dissolved and administered orally via gavage to mice at a dose of 500 mg/kg BW/day for 30 days. **[24]**

2.3. Preparation of Cur-NPs dose

Curcumin nanoparticles (Size: 50 $\pm$ 5.5 nm) were purchased from Nanotech Company (Nanotech Egypt for Photo Electronics), Gate 3, Dreamland, 6th October, Cairo, Egypt. Cur-Nps were dissolved in distilled water and administered orally via gavage to mice at a dose of 15 mg/kg BW/day for 30 days. **[25]**

2.4. Ehrlich solid tumor induction in mice as a tumor model

The parent line of EAC cells was obtained from the National Cancer Institute (NCI), Cairo University, Egypt. For preserving EAC cells in vivo, 2.5 x 10^6 cells per mouse were intraperitoneally injected into Swiss albino female mice every 10 days, based on the method recommended by the Egyptian National Cancer Institute. At the time of the induction of solid tumors (day 0), peritoneal fluid was collected from a female mouse bearing an 8–10-day old ascetic tumor. The chosen concentration of tumor cells was obtained by diluting the ascetic fluid with normal saline (0.9% NaCl) (1:10). **[7, 26]** Solid Ehrlich carcinoma was induced by subcutaneous injection of 0.2 mL of diluted EAC, containing approximately 2.5×10^6 EAC cells, in the right thigh of the hind limb of each mouse. **[27]**

2.5. Laboratory analysis

2.5.1. The evaluation of tumor growth

2.5.1.1. Change in tumor volume

Tumor volume was calculated for each animal-bearing tumor in the thigh. By using an external caliper, the tumor's longest and smallest diameters were measured beginning on the ninth day following the transplantation of tumor cells and then recorded every three days throughout the duration of the 30-day experiment. The tumor volume of each animal was calculated based on **Jaganathan et al. [28]**, using the following formula:

Tumor volume (mm^3) = [length (mm) × width $(mm)^2$] /2

2.5.1.2. Tumor weight & volume

At the end of the experiment, the animals were euthanized, and solid tumors were dissected and weighed on a weighing balance. Tumor dimensions were also measured.

2.5.1.3. Tumor growth inhibition rate (TGIR)

TGIR is calculated from the next formula, in line with **Abd El-Dayem et al. [29]**: TGIR = [(average tumor volume of the EAC group − average tumor volume of the treated group) × 100] / average tumor volume of the EAC group.

2.5.2. Measurement of Body Weight Changes

The initial BW of mice was measured in grams (g) on day 0 at the time of EAC inoculation. **[30]** The initial BW on day 0 and the final BW on day 30 were used to assess BW changes in the animals.

2.5.3. Biochemical analysis

Oxidative stress assay: Lipid peroxidation was measured by determining the levels of MDA based on the procedure described by **Ohkawa et al. [31].** According to the procedure outlined by **Koracevic et al. [32]**, a quantitative assessment of TAC was estimated.

Liver function assays: According to **Reitman & Frankel [33]**, AST& ALT activities were measured using a Hitachi 7180 biochemistry spectrophotometer and a commercial kit from Bio-diagnostic in Egypt.

2.5.4. Hematological Analysis

Blood samples were collected from mice for the evaluation of HB content, total RBC, total WBC count, and measurement of HCT. For the estimation of HB content, blood was diluted with Drabkin's reagent and measured in a spectrophotometer. Blood cells were suspended in RBC and WBC-diluting fluid and counted in a hemocytometer under a light microscope.

2.5.5. Histopathological investigation

Animals were sacrificed, and the liver was taken out for histological preparations. They were immediately fragmented into tiny pieces and preserved (24 hours) in a 10% neutral formalin solution. The samples were fixed and then dehydrated in ethyl alcohol in ascending order (Merck, Germany): 70, 80, 90, and 96% for 20 minutes each, following that for 30 minutes each in 2 changes using absolute ethanol. Tissues were cleared (20 minutes) in xylol (two changes), then impregnated in wax paraplast (three changes) at 60°C for three hours and embedded in wax paraplast, forming paraffin tissue blocks. Sections 4 to 5 µm thickness were made and inserted on coated slides. Sections were cleaned of paraffin by dipping in xylol two times, ten minutes each, and hydrated in descending concentrations of ethyl alcohol (100-100-95-90-80-70), followed by distilled water, and stained with hematoxylin HX & eosin (Ehrlich Hematoxylin). **[34,35]** The sections were then photographed using a light microscope (Leica, with a digital camera, Wetzlar, Germany).

2.5.6. Immunohistochemical analysis of p53

Immunohistochemical staining of p53 was performed according to **Tousson et al. [36]**. Liver sections of 4 µm thick were prepared, and paraffin was removed by dipping in xylene. After using the appropriate primary antibody, all sections were incubated for an overnight period at 4°C. Sections were incubated with an anti-rabbit p53 monoclonal antibody. All sections were viewed under a light microscope, and computer software for image analysis (Leica QWin, Microsystems, Germany) was utilized. Positive nuclei for p53 accumulation were stained brown. The tumor was considered p53-positive if more than 10% of cells showed positive staining. **[37]**

2.5.7. Statistical analysis

The analysis was done with Microsoft Excel (version 10) and the Statistical Package for the Social Science (SPSS software version 16) on a personal computer. The following analyses were performed in

line with the technique described by **[38, 39]**. All values are provided as mean ± standard deviation (SD) **[40]**. When p values are ≤ 0.05, the difference in means is significant.

3. Results

3.1. Results of tumor growth

Due to the high growth rate in Ehrlich tumor model, change in tumor volume was monitored over 30-day period of experiment for EAC group and all other groups. Mice injected with EAC cells developed a palpable solid tumor by day 10 following inoculation in EAC group, while it appeared in all other groups of the SP and Cur-Nps by day 12 post-inoculation.

Compared to the untreated EAC group, supplementation of mice with SP & Cur-Nps 15 days prior to tumor cell inoculation and throughout the experimental period resulted in highly significant inhibition of tumor weight & volume in all protection groups (P< 0.001). Treatment of tumor-bearing mice with SP only (SP Tr group) resulted in neither a significant reduction in tumor volume nor tumor weight. In contrast, administration of Cur-Nps alone or in combination with SP (Cur-NPs Tr group / SP& Cur-NPs Tr group) resulted in a significant reduction in tumor weight & tumor volume (p< 0.05). **(Figure 1(a)& (b))**.

Also, significant inhibition of tumor growth was observed in varying degrees in these groups. The maximal TGIR (72.1%) was detected in group X, whereas the lowest TGIR (8.5%) was observed in group VII. **(Figure 1(c))**.

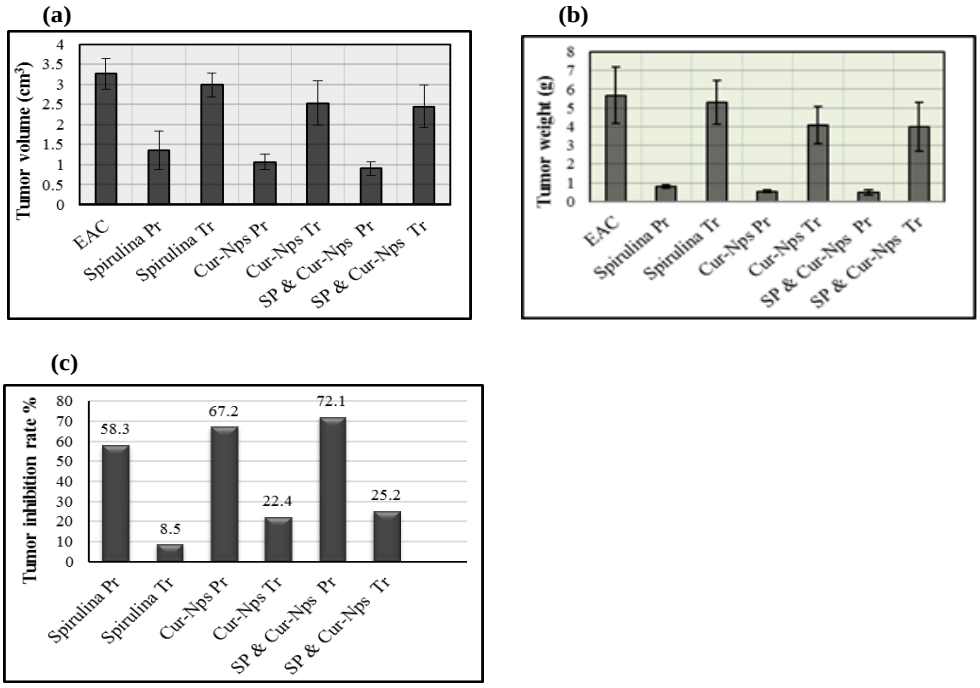

Figure (1): (a): Impact of various treatments on the volume of a solid tumor. **(b):** Impact of various treatments on weight of a solid tumor. **(c):** Tumor growth inhibition rate in the studied groups.

3.2. Changes in the body weight

At the end of the experiment all studied groups showed some increases in their body weights if compared to the beginning of the experiment. In comparison to the control group, there was a significant

increase in the BWs of the EAC group. Also, a highly significant decrease in body weights was observed in groups from Group VI to Group XI when compared with Group V as shown in **Table (1).**

3.3. Biochemical analysis

Our results showed a highly significant rise in serum& hepatic MDA level and a highly group (Group V) in comparison to the control group with p value <0.001. In all protective & therapeutic groups from Group VI to Group XI, treatment of EAC-bearing mice with SP alone or combined with Cur-Nps induced a highly significant reduction in serum & hepatic MDA (P<0.001) and a highly significant increase of serum TAC (P<0.001) when compared with the EAC group. as shown in **table (2).**

In comparison with healthy control group, EAC group indicated an extremely significant rise in serum ALT & AST activity (P<0.001).

Table (1): Mean ± SD of body weight (gm) at the start and end of the experiment in different groups

	Mean ± SD At the start	Mean ± SD At sacrifice	P^a value	P^b value	% of change
Group I (Control)	30 ± 1.02	33.9 ± 1.2			13%
Group II (Spirulina)	30.1 ± 1.4	33.01 ± 0.9	0.085		9.7%
Group III (Cur-Nps)	30± 1.2	32.75 ± 1.5	0.1		9.2%
Group IV (Spirulina & Cur-Nps)	30.2 ± 1.1	32.94 ± 1.1	0.08		9.1%
Group V (EAC)	30.1 ±1.3	35.3 ± 0.63	**< 0.05**		17.28%
Group VI (Spirulina Pr)	30 ± 0.9	32.86± 0.99	0.06	**< 0.001**	9.53%
Group VII (Spirulina Tr)	30 ± 0.7	33 ± 0.81	0.07	**< 0.001**	10%
Group VIII (Cur-Nps Pr)	30 ± 1.1	33.01±1.06	0.1	**< 0.001**	10.03%
Group IX (Cur-Nps Tr)	30 ± 1.01	33.05 ± 0.9	0.09	**< 0.001**	10.17%
Group X (SP & Cur-Nps Pr)	30 ± 1.4	33.38 ± 1.06	0.287	**< 0.001**	11.27%
Group XI (SP & Cur-Nps Tr)	30 ± 1	33.24 ± 0.9	0.175	**< 0.001**	10.8%

Significant at p-value ≤ 0.05 $p > 0.05$ is considered non-significant (NS)
[a] Significant p-value versus the control group (group I). [b] Significant p-value versus EAC group (group V).

On the other hand, protective and therapeutic groups of SP, Cur-Nps and SP plus Cur-Nps resulted in a highly significant decline (P<0.001) in ALT& AST activity comparing with EAC group as shown in **table (2)**.

Table 2. Mean ± S.D. of serum, liver, and tissue MDA, TAC, ALT, and AST activities in the different groups

	S-MDA (nmol/ml)	L-MDA (nmol/g tissue)	T-MDA (nmol/g tissue)	TAC	ALT	AST
Group I (Control)	11.34 ± 0.0288	26.92±0.644		0.995±0.056	37.8±1.81	29.6±2.75
Group II (Spirulina)	11.556 ± 0.44	27. 4±1.07		1.02±0.025	39.3±1.7	30.4±4.59
Group III (Cur-Nps)	11 ± 0.5	27.26±1.69		1.004±0.025	38.42±1.895	30.9±3.51
Group IV (SP & Cur-Nps)	11.36 ± 0.76	26.2±1.398		1.03±0.028	39.6±2.22	30.5±3.63
Group V (EAC)	20.46 ± 0.35[a]	83.84±0.92[a]	11.835±0.228	0.64±0.0519[a]	79.8±1.751[a]	55.3±5.67
Group VI (Spirulina Pr)	14.02 ± 0.64[ab]	42.28±2.5[ab]	2.8±0.467[b]	0.823±0.018[ab]	46.57±1.679[ab]	40.6±3.06[ab]
Group VII (Spirulina Tr)	15.24 ± 0.46[ab]	47.73±3.16[ab]	5.25±0.19[b]	0.796±0.023[ab]	45.3±3.529[ab]	39.2±4.54[ab]
Group VIII (Cur-Nps Pr)	13.2 ± 0.51[ab]	40.4±1.65[ab]	2.05±0.194[b]	0.797±0.017[ab]	43.3±3.02[ab]	35.1±5.6[ab]
Group IX (Cur-Nps Tr)	14.38 ± 0.37[ab]	43.62±1.44[ab]	3.24±0.312[b]	0.774±0.023[ab]	42.45±2.287[ab]	34.9±3.75[ab]
Group X (SP& Cur-Nps Pr)	15.06 ± 0.13[ab]	40.19±1.04[ab]	2.593±0.34[b]	0.881±0.038[ab]	44.12±3.9[ab]	35.6±5.68[ab]
Group XI (SP& Cur-Nps Tr)	15.66 ± 0.33[ab]	43.24±1.54[ab]	3.27±0.18[b]	0.838±0.022[ab]	41.6±2.22[ab]	34.5±3.54[ab]

Significant at p-value ≤ 0.05. $p > 0.05$ is considered non-significant (NS).
[a] Significant p-value versus the control group (group I). [b] Significant p-value versus EAC group (group V).
S-MDA: Serum malondialdehyde; L-MDA: Liver malondialdehyde; T-MDA: Tumor malondialdehyde.

3.4. Result of hematological analysis

Our results of hematological analysis showed a highly significant decrease in HB content, RBCs count, and HCT% and a highly significant increase WBCs count in EAC group (Group V) and all protective & therapeutic groups of spirulina and/or Cur-Nps when compared with the control one (P<0.001). Instead, there was a marked rise in both HB conc. & RBCs count in Group VI (spirulina Pr group), Group VIII (Cur-Nps Pr group) and Group IX (Cur-Nps Tr group) (*P<0.05*) when compared with the EAC group. Also, Group X and Group XI induced a highly significant increase in HB conc. & RBCs count (*P<0.001*) when compared with the EAC group. While Group VII showed improvement in HB conc. & RBCs count but this difference does not reach statistical significance. In comparison with EAC group, all protective and therapeutic groups from Group VI to Group XI, exhibited a highly significant decrease in WBCs count and a highly significant increase in Hematocrit% (P<0.001) as shown in **table (3).**

3.5. Histopathological examinations

Figure 2(A) is a photomicrograph of a control group's liver segment, demonstrating the standard architecture of a hepatic lobule and the central vein (CV) enclosed by hepatocytes (H), the hepatic sinusoids (S) are shown contain Kupffer cells (K) and normal nuclei (N). While sections of liver of mice in EAC group **(Figure 2(B))**; Showing the liver sections with disturbance of the hepatic lobules architecture, vacuoles (V) in the hepatocytes, pyknotic Kupffer (K) cells, massive lymphocyte infiltration (arrow) in the portal and periportal spaces with dilated and congested (PV) veins.

Table 3. Mean ± S.D. of HB, RBCs, WBCs, and HCT% in the different groups**.**

	HB (g/dl)	RBCs $\times 10^6$ /mm^3	Total WBCs $\times 10^3$ / mm^3	HCT %
Group I (Control)	12.66±1.26	6.44±0.7	3.76±0.41	41.66±2.93
Group II (Spirulina)	12.07±0.36	5.77±0.18	4.02±0.336	40.545±0.57
Group III (Cur-Nps)	12.034±0.48	5.89±0.117	3.496±0.49	40.885±0.429
Group IV (SP & Cur-Nps)	12.1±0.47	6.165±0.29	3.86±0.217	41.175±1.487
Group V (EAC)	9.498±1.06[a]	3.72±0.51[a]	9.12±0.487[a]	32.43±2.31[a]
Group VI (Spirulina Pr)	10.775±1.02[ab]	4.437±0.82[ab]	6.38±0.439[ab]	36.615±3.528[ab]
Group VII (Spirulina Tr)	10.275±0.76[a]	4.09±0.41[a]	7.974±0.776[ab]	34.925±2.6[ab]
Group VIII (Cur-Nps Pr)	10.4±0.21[ab]	4.295±0.42[ab]	6.58±0.597[ab]	35.385±0.758[ab]
Group IX (Cur-Nps Tr)	10.097±0.865[ab]	4.12±0.23[ab]	7.3±0.41[ab]	35.03±2.12[ab]
Group X (SP& Cur-Nps Pr)	11.2±0.29[ab]	5.075±0.139[ab]	5.85±0.616[ab]	38.1±0.966[ab]
Group XI (SP& Cur-Nps Tr)	10.95±0.316[ab]	4.9175±0.13[ab]	6.852±0.497[ab]	37.175±1.067[ab]

Significant at p-value ≤ 0.05. $p > 0.05$ is considered non-significant (NS).
[a] Significant p-value versus the control group (group I). [b] Significant p-value versus EAC group (group V).

In Spirulina therapeutic (Tr) group **(Figure 2(C))**; the hepatic lobule appeared less like normal as dilated and congested central vein (CV), surrounded by some lymphocyte infiltration (arrow), but, In Cur-NPs therapeutic (Tr) group **(Figure 2(D))**; the hepatic lobule appeared with little improvement except the dilated and little congested CV, and little infiltration of lymphocytes (arrow). Also, in Spirulina & Cur-NPs Tr group **(Figure 2(E))**, the hepatic lobule appeared less like normal as dilated and little congestion CV, with usual nuclei (N) and Kupffer cells that are enlarged (K).

In protective groups of Spirulina, Cur-NPs, and Spirulina& Cur-NPs **(Figure 2 (F, G& H))** respectively: all of them showing the hepatic lobule similar to normal control groups.

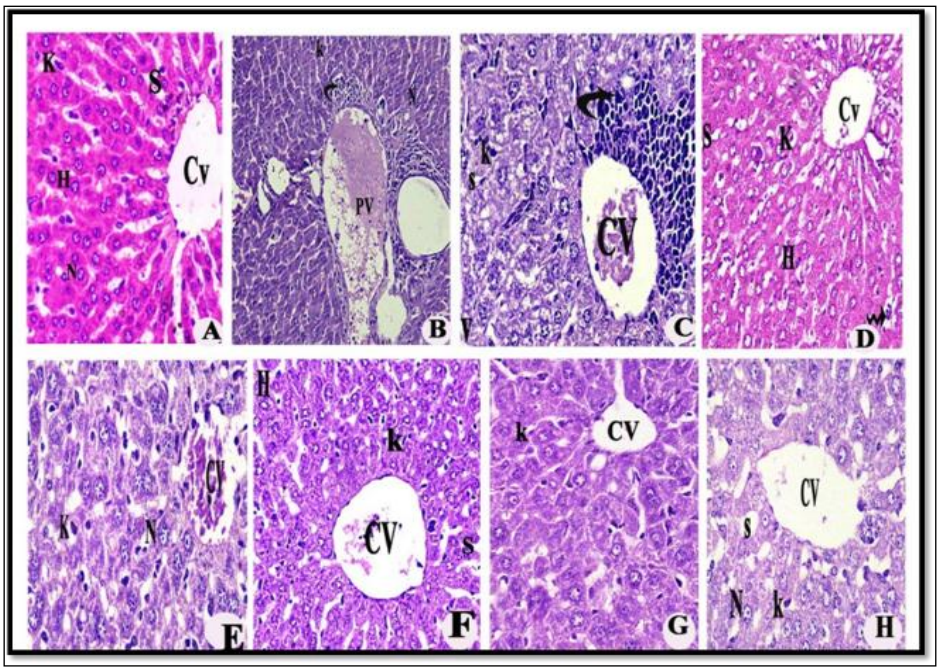

Figure 2: A photomicrograph of a liver section of control groups (A): showing normal architecture of a hepatic lobule, the central vein (CV) surrounded by the hepatocytes (H), the hepatic sinusoids (S) are shown contain Kupffer cells (K) and normal nuclei (N); EAS group (B): Showing the liver sections with disturbance of the hepatic lobules architecture, vacuoles (V) in the hepatocytes, pyknotic Kupffer (K) cells, massive lymphocyte infiltration (arrow) in the portal and periportal spaces with dilated and congested (PV) veins; Therapeutic (Tr) groups (C, D& E): in (C) Spirulina Tr group: the hepatic lobule appeared less like normal as dilated and congested central vein (CV), surrounded by some lymphocyte infiltration (arrow), In (D) Cur-NPs Tr group: the hepatic lobule appeared with little improvement except the dilated and little congested central vein (CV), and little lymphocyte infiltration (arrow), In (E) Spirulina & Cur-NPs Tr group: showing the hepatic lobule that appear less like normal as dilated and little congestion central vein (CV), with normal nuclei (N) and hypertrophied Kupffer cells (K); Protective groups of Spirulina, Cur-NPs, and Spirulina + Cur-NPs (F, G& H) respectively : all of them showing the hepatic lobule similar to normal control groups. (H & E Stain-Scale Bar: 20 mm).

3.6. Immunohistochemical examination

A photomicrograph of liver of control groups **(Figure 3(A))**; showing negative nuclear P53 immuno-expression (arrow), while that of EAC group **(Figure 3(B))**; Showing strong P35 nuclear immune-expression (arrowhead). Therapeutic groups of Spirulina, Cur-NPs, and Spirulina + Cur-NPs **(Figure 3(C, D& E))**, respectively; showing decrease of P35 nuclear immune-expression when compared with EAC groups. While protective groups of Spirulina, Cur-NPs, and Spirulina + Cur-NPs **(Figure 3(F, G& H))**, respectively; Showing very weak P35 nuclear immune-expression (arrowhead indicates nuclear and cytoplasmic expression).

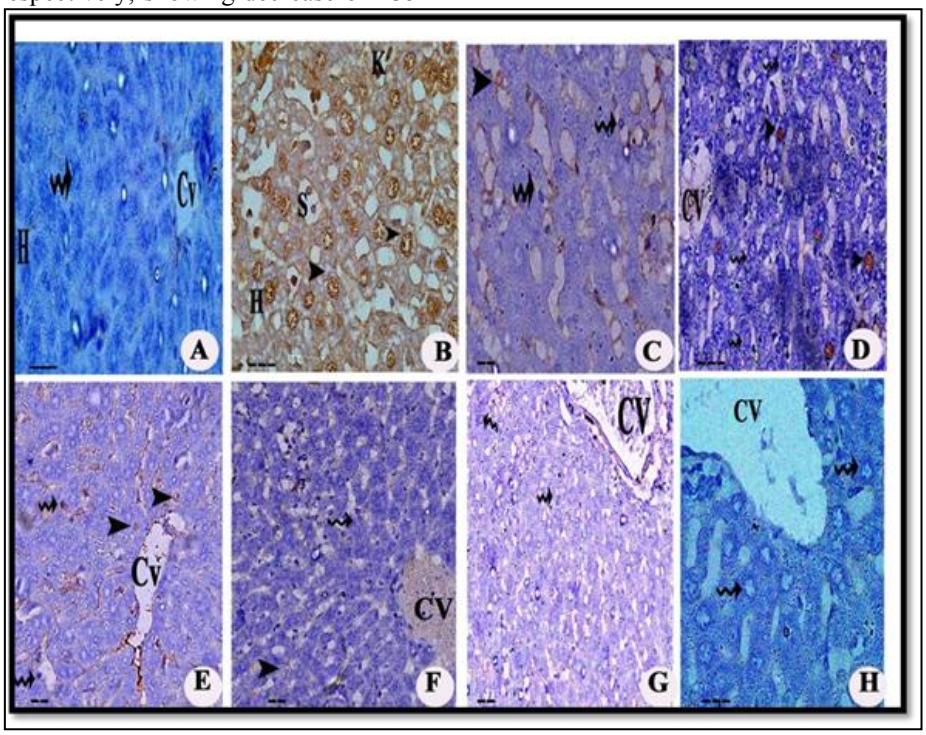

Figure 3: photomicrograph of liver of control groups (A): showing negative nuclear P53 immuno-expression (arrow); EAC group (B): Showing strong P35 nuclear immune-expression (arrowhead); Therapeutic groups of Spirulina, Cur-NPs, and Spirulina + Cur-NPs (C, D& E) respectively: showing decrease of P35 nuclear immune-expression in compared with EAC groups; protective groups of Spirulina, Cur-NPs, and Spirulina + Cur-NPs (F, G& H) respectively: Showing very weak P35 nuclear immune-expression (arrowhead indicates nuclear and cytoplasmic expression). (P35 antibody, IHC ×200).

4. Discussion

The most widely utilized form of cancer treatment is chemotherapy. **[41]** Using plants as medicine to inhibit carcinogenesis and treat cancer is an important and rapidly growing field of cancer research due to less toxicity of natural products in comparison to modern chemotherapy **[42]** Cur-NPs have been synthesized, to enhance hetmedicinal value of curcumin for cancer prevention. [**43]**

A specific kind of blue-green algae called Spirulina has long been served as a supplement to the diet. **[44,45]** According to research by **Bhat & Madyastha, [46]** and **Subhashini, et al, [47]**, Spirulina offers numerous medicinal advantages, such as anti-cancer, anti-inflammatory, and hepatoprotective properties. A Current investigation was completed to recognize the defensive action of SP & Cur Nps against EST experimentally induced in mice. Mice injected with EAC cells developed a palpable solid tumor by day 10 following inoculation in group V, while it appeared in all other groups of the SP and Cur-Nps by day 12 post-inoculation. This is in keeping with other previous studies that used the same model. **[27,48]** For groups VI, VII, and X, the tumor volumes were greatly decreased, this result was arranged with **El-Atrsh et al, [49]** who informed that spirulina exhibited antitumor activity against EST by a reduction in a mice tumor's volume.

The present investigation declared that using Cur-Nps causes a considerable decrease in tumor volume. Using spirulina and Cur-Nps together produced a further reduction in volume in comparison with their corresponding single treatment. This finding is along with many reports such **as El-Azab et al, [50]** who described that a single treatment with curcumin produced a significant reduction in tumor weight as compared to the EAC

animals. This is also convenient with the study done by **Karmakar et al, [51[& Pandey et al, [52]**. Body weight variations are generally an important factor in toxicological studies **[53]**. Except for group V, there was no change between the body weight of the control and other groups in the present study where both of them were increased.

MDA, an end result of lipid peroxidation, represents a marker for oxidative stress. **[54]** In contrast to control group in the current study, the EAC group's MDA levels significantly increased. The observed raise in MDA could be because EAC induced the free radical's development and also through exhaustion of antioxidants leading to oxidative stress **[55]**. These outcomes harmonized with **Kabel, [56]** findings who found that, regarding the control group, tissue MDA levels significantly increased. This is also convenient with the study done by **Nisari et al, [57]**; who estimate that the MDA levels in the kidney and liver were increased by the tumor development in the EAC-cell injected mice.

The present investigation demonstrates that mice administrated with Cur-Nps recorded a reduction in serum & tissue MDA levels. These data are supported by the results of **Barakat, [58]** who showed that Cur-Nps diminishes harmful effects incited by EAC in mice through the decrease of inflammatory and biochemical (MDA & NO) parameters. In the current study, EAC-bearing mice displayed a significant decrease in serum TAC. Previous research showed that tumor expansion impairs the antioxidant mechanism and raises lipid peroxidation (LPx) in the crucial organs that are hosting the tumor **[59,60].** Cur-NPs have been related to scavenge oxygen free radicals, to inhibit lipid peroxidation, and has anticarcinogenic activities in experimental models. **[61]** The results of our investigation revealed that supplementing the diet with Cur-Nps helped to reduce the MDA levels and increase the antioxidant defense system. This is also supported by **Abd El-Monem et al, [62]**; who revealed that Cur-Nps as a strong cytochrome p450 inhibitor can stabilize antioxidant enzymes and non-enzymatic antioxidants. Cur-Nps also improves antioxidant enzyme levels.

The hepatic enzymes, ALT & AST are the best biochemical parameters for the detection of liver diseases. **[63]** Hepatocytes are severely damaged in mice having EAC cells. **[64]** As a result, mice with EAC may have higher levels of the enzymes ALT & AST in their serum. **[65]** Reduced level of these hepatic enzymes in serum is a marker of the antitumor potential. **[66]**

Dolai et al, [67]; indicated that increased liver enzyme activity, including ALT and AST, were seen in the EAC group. The current study's findings displayed that the activities of AST&ALT were increased inEAC bearing mice as compared with that of normal mice. This finding was similar to those of **Tousson et al, [68];** who informed that AST & ALT were increased in the EAC group. Relative to the EAC group, ALT & AST were considerably lower in mice treated with spirulina & Cur-Nps, and this goes in consistent with **Alheeti et al., [69]**; who conclude that the liver capacities of mice can be improved by using spirulina for treating EAC. **Abd El-Monem et al, [62]**; revealed that Cur-Nps significantly reduced liver enzyme levels and lipid peroxidation.

These conclusions were supported by the outcomes of the histopathological evaluation of liver tissues, since the liver section of EAC-bearing mice showed various histopathological alternations including an increased number of necrotic hepatocytes with deeply pyknotic nuclei, congestion associated with brown pigment deposition and thickening of the central vein's wall by increased collagen content. **[70]** These data indicate that numerous important organ processes including liver function can be affected by the growth of tumors in animal bodies. The results reported here are close to those of **Tousson et al, [68] and Mutar et al, [71]**; who described that; EAC initiated tissue damage in the liver.

The achievement of an anticancer treatment was evaluated by tumor volume and viable cell count reduction in tumor-bearing mice, tumor cell growth suppression, besides hematological profile. **[72; 73]** Our results demonstrated a very significant drop in HB content, RBCs count, and HCT% in EAC group in comparison to the control one. Reduction of RBC or hemoglobin occurs because of myelopathy condition or iron deficiency leads to anemia in tumor icebearing m. **[74]** Reduced RBC or HB levels are the cause of anemia in tumor-bearing mice, which can also be brought on by myelopathy or iron shortage **[75]**

In the present study; tumor sections in EAC group exhibited high positive reactions for apoptotic P53. Our results decide with **Aldubayan et al. [76]** who find that Ehrlich tumors were shown to significantly enhance P53 immunoreactivity. Additionally, **Abd Eldaim et al, [77]**, reported that; EAC induced apoptosis and DNA impairment in tissues. The findings of **Abd El-Monem et al, [62];** who stated that; The liver building's histological appearance significantly improved after Cur-Nps treatment. supported these conclusions. Curcumin works to protect cells by controlling lipid peroxidation, biochemical marker enzymes, and the antioxidant defense system. **[78]**

5. Conclusion:

In conclusion, SP & Cur-NPs administration enhanced the histological and immunohistochemical changes in the liver tissue as well as the biochemical

changes in mice with EAC. According to our research, SP and Cur-NPs may have potent anti-inflammatory, antioxidant, and anti-apoptotic properties that protect the livers of mice against the carcinogenicity of EAC.

6. Abbreviation

Curcumin Nanoparticles (Cur-NPs); Ehrlich Ascites Carcinoma (EAC); Spirulina (SP); Total Antioxidant Capacity (TAC); Malondialdehyde (MDA); Alanine aminotransferase (ALT); Aspartate aminotransferase (AST); Protective (Pr)**;** Therapeutic (Tr); Curcumin (CUR); Ethylene Diamine Tetra- Acetic Acid (EDTA); Tumor Growth Inhibition Rate (TGIR); Phosphate Buffered Saline (PBS); National Cancer Institute (NCI)**;** Body weight (BW); Hemoglobin (HB); Red Blood Cells (RBCs); White blood cells (WBCs); Hematocrit (HCT); Statistical Package of the Social Science (SPSS); Standard Deviation (S.D); Nitric Oxide (NO); Lipid peroxidation (LPx); Reactive Oxygen Species (ROS); Ehrlich Solid Tumor (EST).

7. Conflicts of interest

There are no conflicts of interest

8. References

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