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Der Pharmacia Lettre, 2018, 10 [5]: 7-26
[http://scholarsresearchlibrary.com/archive.html]

ISSN 0975-5071
USA CODEN: DPLEB4

Potential Role of Sulfur Nanoparticles as Antitumor and Antioxidant in Mice

Faten Zahran[1], Mustafa Hammadi[2], Muayad Al-dulaimi[1,2], Mahmoud Sebaiy[1*]

[1]*Faculty of Science, Department of Biochemistry, Zagazig University, Egypt*

[2]*Faculty of Education for pure Sciences, Department of Chemistry, Diyala, University, Baghdad, Iraq*

[3]*Faculty of Science, Department of Biochemistry, Zagazig University, Egypt*

[4]*Ministry of Education, Baghdad, Iraq*

***Corresponding author:** Sebaiy M, Faculty of Science, Department of Biochemistry, Zagazig University, Egypt, Tel: +20 55 2364612; E-mail: sebaiym@gmail.com*

ABSTRACT

***Objectives:** The role of elemental sulfur in human nutrition has not been studied extensively. Therefore, this study aimed to determine of some biochemical effects of sulfur nanoparticles (S-NPs) and emphasize the importance of this element as antioxidant and antitumor agent.*

***Methods:** Preparation of sulfur nanoparticles was done throughout. Firstly, the evaluation of their composition and purity by Fourier Transform Infrared Spectrometer (FT-IR) analysis. Secondly, The shape and size of S-NPs were investigated by scanning electron microscopy (SEM) techniques in all S-NPs samples which were prepared with Tetramethylammonium bromide (TMAB) surfactant, Then, Energy-dispersive X-ray spectroscopy (EDS) for the evaluation of their composition and purity, After that the X-Ray Diffraction patterns were measured for prepared S-NPs with TMAB surfactant. Final determination of sulfur nanoparticles antioxidant and antitumor activites in experiment female mice bearing EAC, determination of some molecular markers such as P53 and Cytochrome C. Also, histological examinations of liver and kidney tissues were done.*

Results: *This study revealed that sulfur nanoparticles showed high strong antioxidant activity in concentration (5 mg/kg) and antitumor activities as it increase SOD, GPx and TAC production compared to the positive control group. Also showed a significant increase in p53 and Cytochrome C compared with the positive control group.*

Conclusion: *Sulfur nanoparticles play an important role in improving oxidative stress resulted in significantly increase activity against EAC cells, and Also, sulfur nanoparticles reduced most of the pathological alterations induced by Ehrlich ascites carcinoma cells in mice which confirmed by histopathological examination, this may be supports the suitability of the use of sulfur nanoparticles as an antioxidant and anticancer agent. Further, more different types of Cancer cells have unique properties that can be exploited by nanoparticles to target this Cancer cell.*

Keywords: Sulfur nanoparticles, S-NPs toxicity, Anti-tumor activity, Anti-oxidant activity, Histopathology.

INTRODUCTION

All cancer registries constantly show striking differences in cancer incidence by age and among tissues. An analysis of these differences using basic concepts in cell biology indicates that cancer is the end-result of the accumulation of cell divisions in stem cells. Cell division can lead to a variety of cancer-promoting errors, such as mutations and epigenetic mistakes occurring during DNA replication, chromosome aberrations arising during mitosis, errors in the distribution of cell-fate determinants between the daughter cells, and failures to restore physical interactions with other tissue components [1]. Cancer cells may be more prone to the accumulation of reactive oxygen species (ROS) than normal cells; therefore increased oxidative stress can specifically kill cancer cells including cancer stem cells (CSCs). In order to generate oxidative stress in various cancer cell lines [2]. The accumulation of p53 protein has the potential to be one such marker in breast cancer [3]**.** Cancer remains a serious threat with higher risk of mortality. Nanotechnology, a well-known platform, has attracted notable interest in current times. The word ‘nano’ indicates one billionth of a meter. For pharmaceutical purposes, the phrase “nanotechnology” is now usually applied to mention the production of nanocarriers with dimensions in the range of 10 to 1000 nm for drug delivery purpose. The National Institute of Health (NIH) in the USA described Nanomedicine as utilization of nanotechnology in the diagnosis, therapy with simultaneous monitoring of biological systems [4]. Nanocarriers are competent enough to improve the pharmacokinetics and to enhance the biodistribution of existing therapeutic moieties to the target tissues, so that can improve efficiency [5]. The nanoparticles are finding important applications in the field of medicine for the fact that, biological processes also occur at the Nano scale and due to their amenability to biological fictionalization [6]. The applications of Sulfur Nanoparticles (S–NPs) were used as modification of metal and carbon nanotubes as anti-cancer agent [7]. Showed that the non-cytotoxic doses of sulphur nanoparticles are eco-friendly and clinical trials showed no alarming toxic effects on eukaryotes [8]. Biological sulfur-containing compounds, including cysteine, methionine, taurine, glutathione (GSH), N-acetylcysteine (NAC), and other sulfur compounds have been extensively studied for their antioxidant properties [9]**.** Antioxidants ameliorate oxidative damage caused by ROS, and research has focused on the role of antioxidants for the treatment and prevention of disease [10,11]. Numerous publications attribute “antioxidant” properties of sulfur compounds even when the compounds have no reducing or other radical-quenching groups and in most cases, there is no obvious antioxidant mechanism, [12]**.** Also, previous studies stated that Sulfur is an interesting element for tumor uptake because it plays an important role in cellular metabolism [13].

MATERIAL AND METHODS

Chemicals

The chemicals used for this study were taken from the following: Sodium thiosulfate (catalog 71166-508), was obtained from the British, drug houses LTD, (Chemical group) and Tetramethyl ammonium bromide (TMAB) (98%) (catalog, 66-22) was obtained from Himedia, laboratories (India), Kits, (SOD, GPx and TAC) were from Biodiagnostic, Company and (P53 and Cytochrome C) from were New test company - USA.

Animals

Adult female Swiss albino mice weigh (20-25) g was purchased [Abo Rawash - culture, Giza] used throughout this study. The animals were housed in steel mesh cages, (animal house, faculty of Science, Zagazig University, Egypt), and the animals were maintained in the controlled environment of temperature, humidity- light, and fed on a commercial, standard diet and tap water *ad- libitum*.

Ehrlich ascites carcinoma cells

Ehrlich ascites carcinoma (EAC) cells were initially supplied from the National Cancer Institute, Cairo, Egypt (only for the first transplantation), and maintained in female Swiss albino mice [14]. Through serial intraperitoneal (I.P.) inoculation of (0.2 ml) of freshly drawn ascites fluid (diluted in 1:5 saline solution), each inoculum contained approximately, (2.5×10^6 cells). This process was repeated every, (10 days) for keeping the strain available throughout the present study.

Preparation of sulfur nanoparticles with TMAB surfactant

In a typical reaction synthesis S-NPs synthesized as follows: an appropriate amount of **(**50 ml) 0.80 M, Sodium thiosulfate ($Na_2S_2O_3.5H_2O$) dissolved in 50 ml of distilled water, (20 ml) 0.02 M of TMAB surfactant was dissolved in 20 ml of distilled water, according to the method of [15] [modified of Tetraoctyl ammonium bromide to Tetramethyl ammonium bromide].

Samples characterization

The S-NPs were characterized by "Fourier Transform Infrared Spectrometer" (FT-IR), employing a Scanning electron microscope (SEM). Also, Energy dispersive X-ray spectroscopy, (EDS) Analysis, according to the method [15], the samples for, X-Ray diffraction characterization using Rigaku Dmax 2500 diffractometer equipped with a graphite monochromatized CuKα radiation (k = 1.5406^{o}A), and particle size distribution in range (5 nm to 100 nm) [16].

Determination median lethal dose (LD_{50}) of S-NPs

Approximate LD_{50}: of S-NPs in mice were determined according to the method [17]. To determine the median lethal dose of S-NPs, a group of 10 mice were injected with doses 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, 8 and 10 mg/Kg of S-NPs; respectively, soluble in

corn oil [18]. Another group of mice containing 5 mice was injected with doses 50, 100, 200, 500, 1000, 2000 mg/Kg of S-NPs ; respectively, to determine the safety of S-NPs.

Determination dose response curve of S-NPs

The dose response curve of synthesized S-NPs, in mice was determined according to the method [19]. Studies carried out for determination of the most effective- dose on tumor (volume and count).

Experimental design

The total number of 75- female Swiss albino mice weighing, 20-25 g were divided into, three groups 25 mice in each group ; Group (I): Negative Control, mice were injected I.P. with sterile saline for 10 days, (day after day). Group (II): Positive Control (EAC): mice were injected with Ehrlich ascites carcinoma by the concentration of (2.5×10^6 cells/0.3 ml/mouse), according to [20], by I.P, injection once; Group (III), therapeutic group: mice were injected I.P, with S-NPs (5 mg/Kg) after EAC injection, (2×10^6 cells/mouse), followed by I.P, injection of S-NPs [5 mg/kg] after EAC injection for 10 days.

Samples preparation

Blood sampling: At the end of the experimental period, Plasma was collected on anticoagulant (EDTA), by centrifuging blood at 3000 r.p.m. for 10 minutes Plasma samples were aliquot and stored at -20°C, and Serum was prepared by centrifuging blood at 4000 r.p.m. for 10 minutes. Serum was aliquot and stored at -20°C until biochemical analysis to carry out antioxidant assays (SOD, GPx and TAC), p53 and Cytochrome C.

Tissue sampling: Ehrlich ascites carcinoma cells were harvested from, the peritoneal cavity of each mouse in a centrifuge tube containing heparinized saline. Each sample was undergoing volum counting and viability- estimation and the evaluation of anti-apoptotic levels P53 and Cytochrome C in each studied groups. Part of the liver tissue was also collected and preserved in phosphate buffer Saline (pbs) (pH 7.4) until homogenate examination [21].

Viability of EAC cells: The viability of EAC cells was determined by the Trypan Blue Exclusion Method [22], where the total and viable cells (non-stained) were counted at magnification 40x; as the number of cells/ml was determined in the studied groups by using hemocytometer.

Life span prolongation: The life span calculation was carried out according to the method described by Mc Limans [23].

Biochemical investigations

The activity of Superoxide dismutase (SOD) was measured according to Nishikimi [24], Glutathione peroxidase activity (GPx) was determined by Paglia, and Valentine [25], and Total Antioxidant Capacity (TAC) was estimated according to Koracevic [26]. The level of p53 was determined according to the method [27], and Cytochrome C was determined according to the method [28] using Elisa technique.

Histo-pathological examination: The flattened sections of liver and kidney were placed on the surface of clean microscope slide accordingly [29]. Liver and kidney tissues from each group were collected and preserved in Formalin solution (10%) embedded in paraffin, sectioned and stained with Hematoxylin and Eosin (H&E) for histopathological examination.

Statistical analysis

All statistical analyses were done by a statistical for, social science package (SPSS-14.0), for Microsoft Windows [SPSS Inc. and considered statistically significant at a two, sided P<0.05]. Numerical data were expressed as mean ± SD. The levels of markers were analyzed by ANOVA but the Mann-Whitney, U-test was used for comparisons between independent groups [30].

RESULTS

Sulfur nanoparticles preparation'

Quick precipitation method of S-NPs was by redox comproportionation of ($Na_2S_2O_3.5H_2O$) in concentration HCl and using tetramethylammonium bromide (TMAB), as stabilizer according to equation following:

$$Na_2S_2O_3.5H_2O \xrightarrow[\text{TMAB in Conc. HCl}]{\text{Stirred at T= 40 °C}} \text{nm S-NPs}$$

After particles prepared the particles were centrifuged, and washed extensively, with water to remove any soluble impurities. The sulfur nanoparticles were collected in good yield. The purity of the product was formed to be (99%) and confirmed by FT-IR, EDS and X-RD technique.

Fourier Transform Infrared Spectrometer (FT-IR) characterization of S-NPs

The synthesized NPs were characterized by FT-IR for, the evaluation of their composition and purity (Figure 1) shows the spectrum of the FT-IR analysis. It is evident from the no peaks that the product is completely pure.

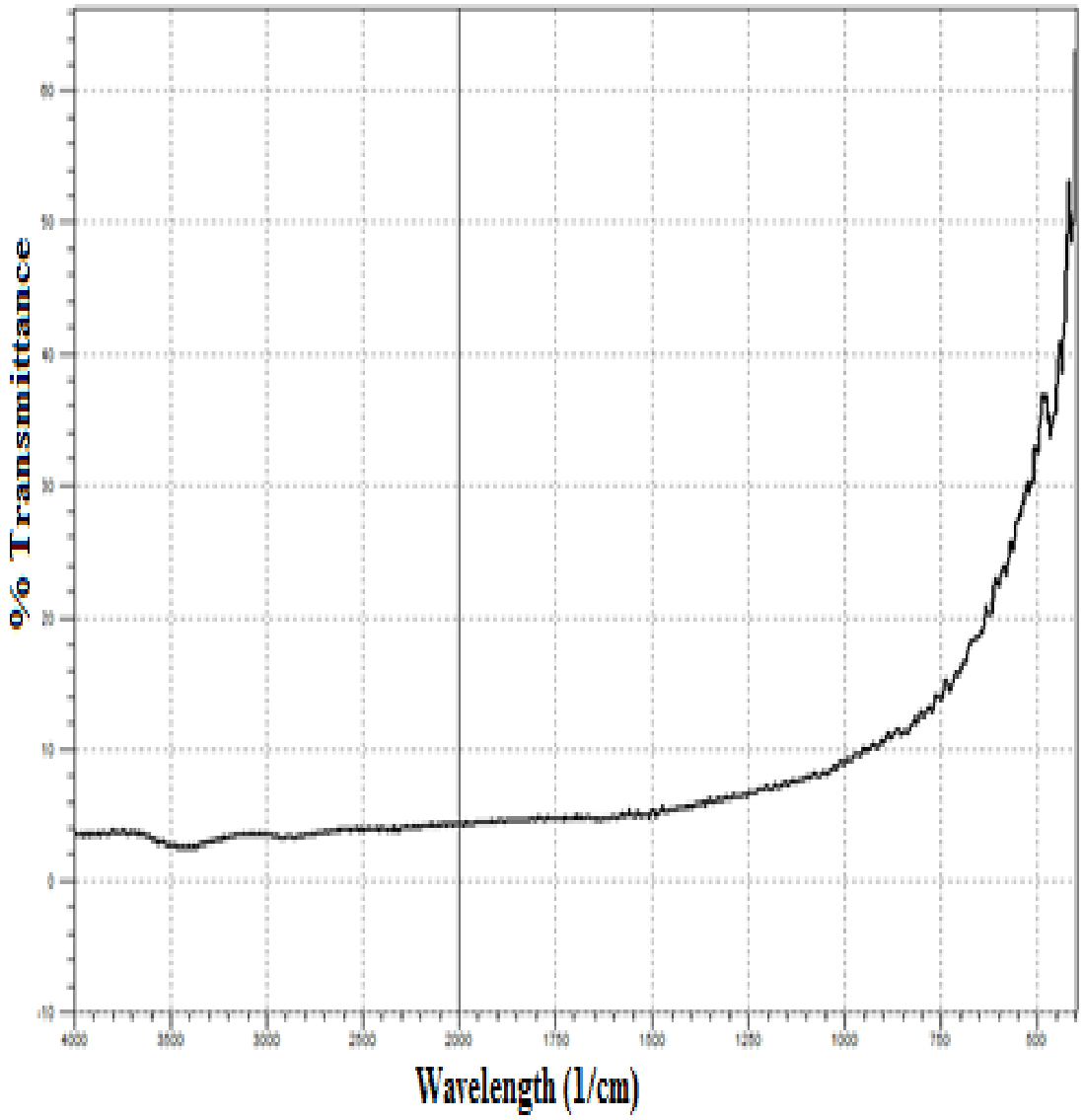

Figure 1: FT-IR spectra of S-NPs.

SEM characterization of sulfur nanoparticles

The shape and size of sulfur nanoparticles were investigated by SEM techniques, (Figures 2a, 2b and 2c) show the SEM images of all S-NPs, samples which were prepared with (TMAB) surfactant.

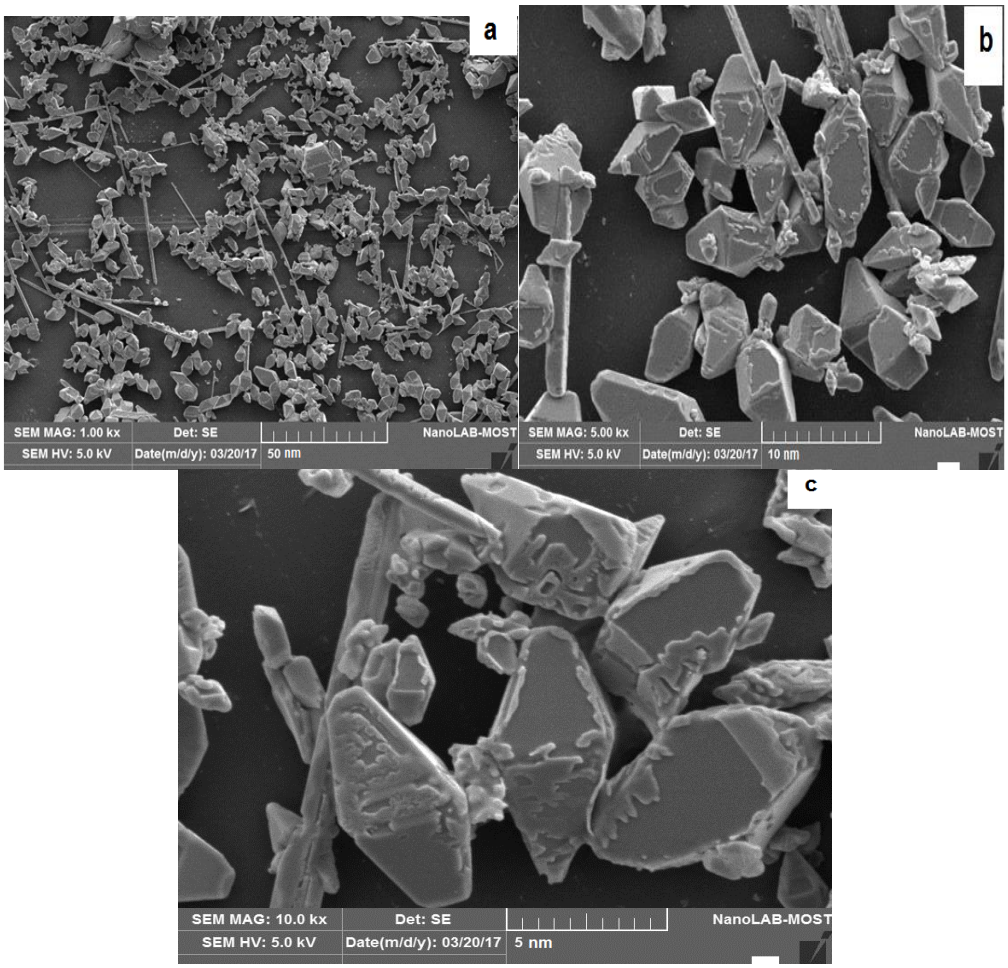

Figure 2: SEM images of the S-NPs, (**a**) size 50 nm, (**b**) size 10 nm and (**c**) size 5 nm

EDS characterization of S-NPs

Results in Figure 3, showed that the EDS analysis of the as prepared S-NPs had broad peak-S.

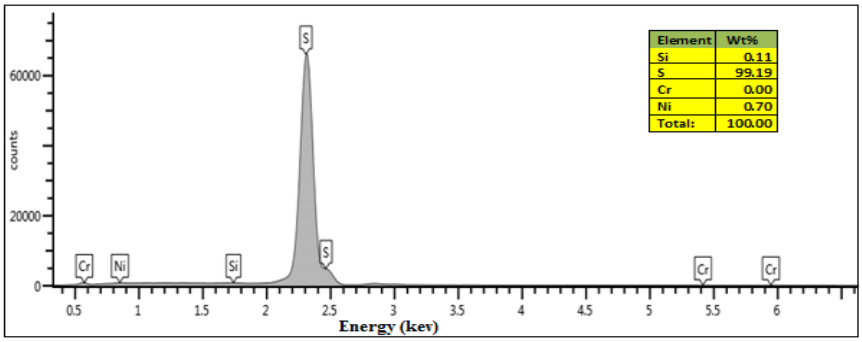

Figure 3: EDS spectrum of the S-NPs.

***X-ray diffraction (XRD), characterization of** S-NPs*

The XRD analysis of the as prepared, S-NPs had broad peaks were measured for preparing S-NPs with TMAB, surfactant as shown in Figure 4. The diffraction peaks were clearly, observed from the XRD of the S-NPs located near two, (16.8°, 23.0°, 25.9°, 31.7° and 37.7°) of 2θ positions, that are well-attributed to the (S-(113), S-(222), S-(027), S-(046) and S-(318); respectively.

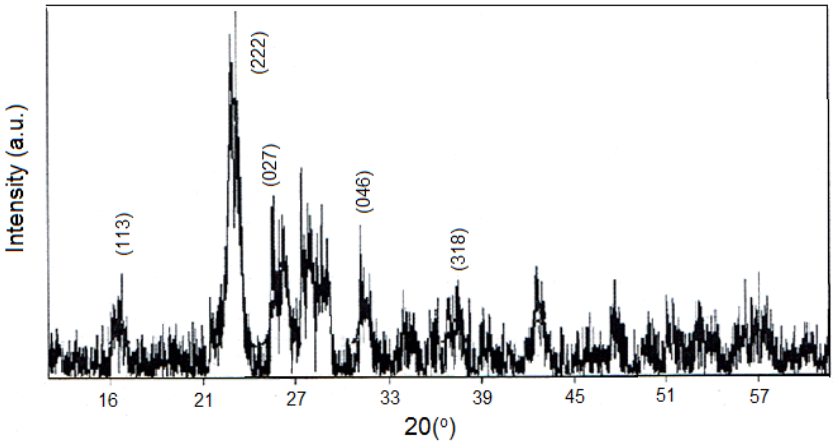

Figure 4: XRD pattern of the sulfur nanoparticles

Toxicity study and dose response curve

For determination, of the median lethal dose LD_{50} of S-NPs, all doses up to (200 mg/Kg), mice were found to be nontoxic as no deaths, were recorded which suggests that S-NPs may be a safe mixture. For dose- response curve it is clear that (5 mg S-NPs/Kg), mice was found to be the most effective, dose as it reduced the number of EAC cells, in treated mice group up to (78% of EAC cells), compared to the positive control mice group; as shown in Figure 5.

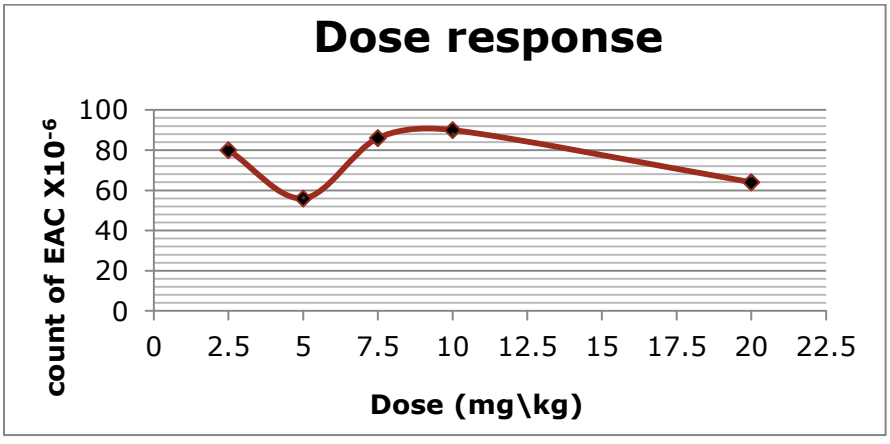

Figure 5: Dose response curve of S-NPs

Viability count and volume

From our results, it has been demonstrated that S-NPs have display anticancer activity, as they decreased EAC count and EAC volume by (82.5% to 73.3%); respectively in group bearing EAC (P<0.001) compared to positive control group as shown in (Figures 6a and 6b).

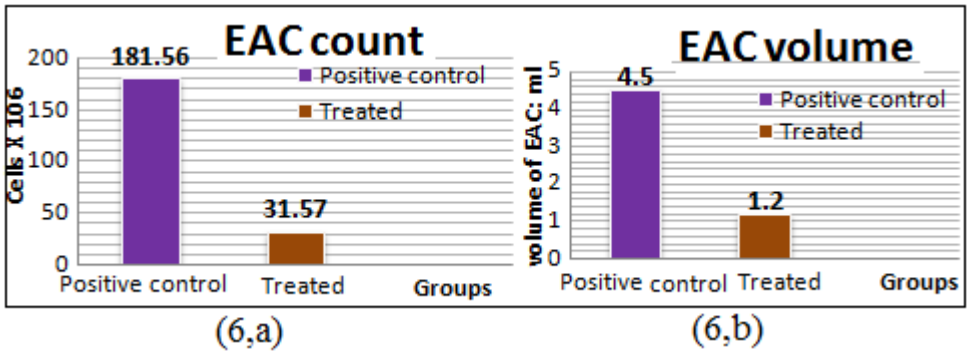

Figure 6: (a) EAC count, in studied groups after treatment with S-NPs. **(b)** EAC volume, in studied groups after treatment with S-NPs.

Life span prolongation

In Table 1, the life span showed a significant increase in therapeutic group, by (63.63%), compared to positive control group.

Table 1: Effect of sulfur nanoparticles on life span prolongation

Groups	**Positive Control Group**	**Compound (S-NPs) Treated**
Parameters	**Life span prolongation**	**Life span prolongation**
Days	**11**	**18**
% Change	---------	**63.63**
T/C ratio (%)	---------	**163.63**

Biochemical Investigations

Antioxidant assay

The effects of sulfur nanoparticles on antioxidants in Ehrlich ascites carcinoma bearing mice were examined, data in Table 2 showed that administration of sulfur nanoparticles revealed a significant increase in Superoxide Dismutase (SOD), Total Antioxidant Capacity (TAC) and Glutathione peroxidase activity (GPx), (p<0.001) in therapeutic group compared to positive control group.

Also, the effects of sulfur nanoparticles on antioxidants in liver tissue were examined, data in Table 3 showed that administration of sulfur nanoparticles revealed a significant increase in Superoxide Dismutase (SOD), Total Antioxidant Capacity (TAC) and Glutathione peroxidase activity (GPx) ($p<0.001$) in therapeutic group compared to positive control group.

Table 2: Effect of sulfur nanoparticles on SOD, GPx and TAC in EAC cells in all studied groups.

Variables	EAC bearing tumor		Treated (S-NPs)	
	Mean ± SD.	Change %	Mean ± SD.	Change %
SOD (U/mL)	175.3 ± 21.2	------	267 ± 19.4***	52.30%
GPx (mU/ml)	84.8 ± 12.3	------	332 ± 33.1**	291.50%
TAC (mM/L)	0.40 ± 0.14	------	2.40 ± 0.19***	500%
Note: Significance at *P<0.05, **P<0.01, ***P<0.001				

Table 3: Effect of sulfur nanoparticles on SOD, GPx and TAC in liver tissue in all studied groups

Variable	Negative control group		EAC bearing tumor		Treated (S-NPs)	
	Mean ± SD.	% Change	Mean ± SD.	%Change	Mean ± SD.	%Change
SOD (U/mL)	808 ± 50.2	-------	564 ± 30.8***	30.1%	861.5 ± 51.2***	52.6%
GPx (mU/ml)	202.5 ± 3.3	-------	102 ± 2.8**	49.6%	375.9 ± 51.2**	268%
TAC (mM/L)	0.338 ± 0.02	-------	0.247 ± 0.02***	26.9%	0.395 ± 0.03***	59.9%
Note: Significance at *P<0.05, **P<0.01, ***P<0.001						

Apoptotic effects

Data in Table 4 revealed that administration of the sulfur nanoparticles showed a significant increase in p53 and Cytochrome C levels in therapeutic group ($p<0.001$) compared to positive control group. Also, data in Table 5 revealed that administration of the sulfur nanoparticles showed significant increase in p53 and Cytochrome C levels in therapeutic group ($p<0.001$) compared to positive control group.

Table 4: Effect of sulfur nanoparticles on P53 and cytochrom c in EAC cells in all studied groups

Variables	EAC bearing tumor		Treated(S-NPs)	
	Mean ± SD	% Change	Mean ± SD	% Change
P53 ng/ml	0.465 ± 0.13	----------	3.747 ± 0.81***	705%
Cyto-c ng/ml	0.88 ± 0.3	----------	3.64 ± 0.5***	313%
Note: Significance at *P<0.05, **P<0.01, ***P<0.001				

Table 5: Effect of sulfur nanoparticles on P53 and cytochrom c in liver tissue in all studied groups

Variables	Negative control group		EAC bearing tumor		Treated(S-NPs)	
	Mean ± SD.	% Change	Mean ± SD.	% Change	Mean ± SD.	% Change
P53 ng/ml	3.5 ± 0.39	-------	0.87 ± 0.12***	150%	3.71 ± 0.28***	57.60%
Cyto-c ng/ml	13.35 ± 3.0	-------	7.75 ± 0.5***	41.90%	13.5 ± 1.1***	74%
Note: Significance at *P<0.05, **P<0.01, ***P<0.001						

Histopathological examination

The two vital detoxification organs, (liver and kidney) were removed from all test groups at the end of the study and were carefully observed microscopically, therapeutic group revealed no observable gross lesions when compared with Negative control group, as shown in Figures 7-12.

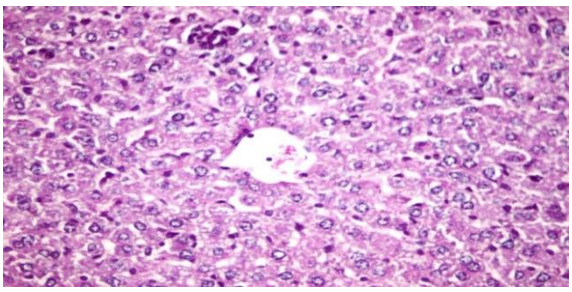

Figure 7: A photomicrograph of the liver from negative control group showing normal hepatic parenchyma; hepatocytes, blood sinusoids, and Portal tract (H&E 400x).

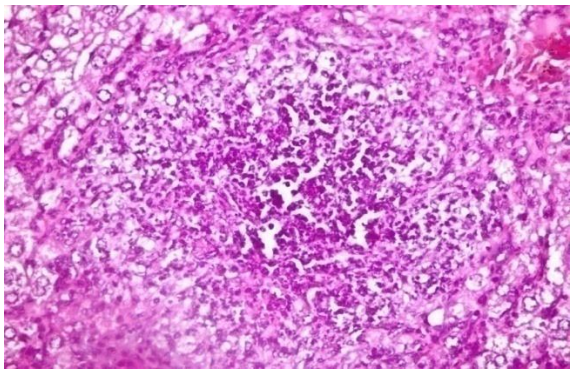

Figure 8: A photomicrograph of EAC group liver showing solid carcinoma of aggregated malignant cells infiltrated with inflammatory cells (arrow), (H&E 400x).

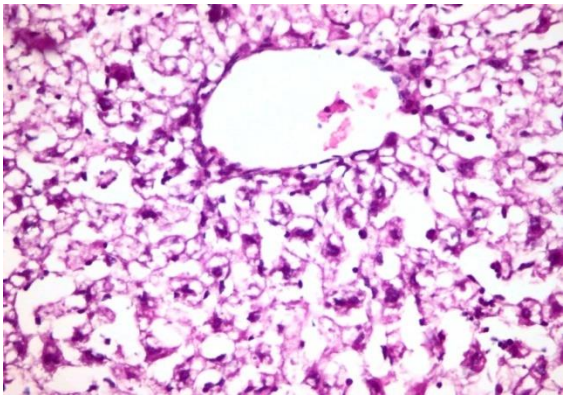

Figure 9: A photomicrograph of the Liver from therapeutic group showing the absence of the solid carcinoma with regeneration of normal hepatocytes (arrow), (H&E 400x).

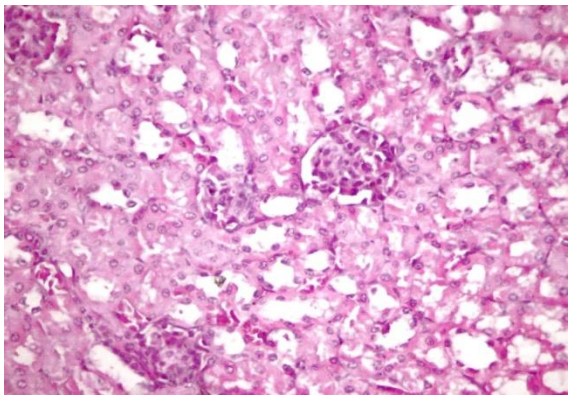

Figure 10: A photomicrograph of the kidney from negative control group showing normal renal parenchyma; renal glomeruli and renal tubules (H&E 400x).

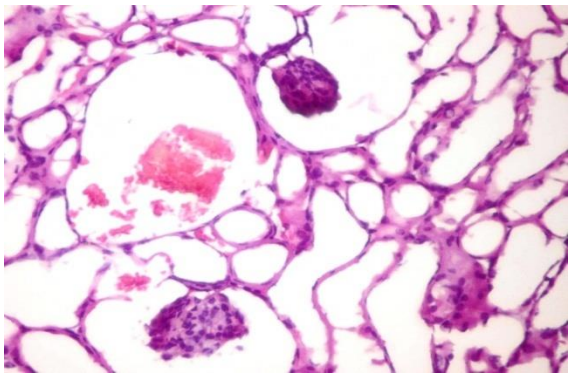

Figure 11: A photomicrograph of the kidney from positive control group showing severe glomerular tuft necrosis (arrow) with poor vascularization (arrow head) (H&E 400x).

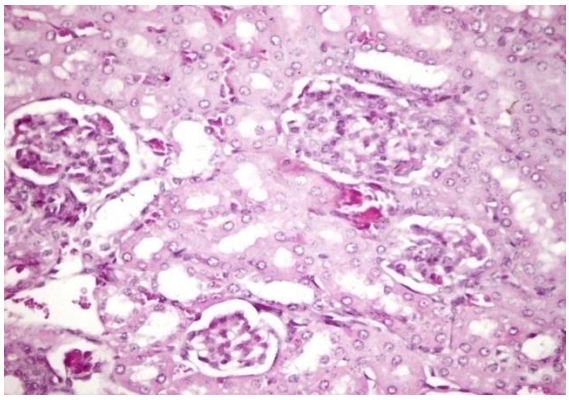

Figure 12: A Photomicrograph of the kidney from therapeutic group showing improvement of the glomerular tuft and normal renal tubules (arrow) (H&E 400x).

DISCUSSION

Cancer is a major health problem threating the life in both developed and developing countries. It is a progressive uncontrolled degenerative disease predisposed by accumulation of toxins through carcinogenic food, smoking, alcohol drinking, toxic medicines environmental pollution and radiation and exposure to infectious organisms that considered being external factors. Also, internal factors as mutations, disturbances in hormonal and immune conditions can induce cancer [31]. Cancer is the most harm disease in human and also health problem worldwide. Despite its high occurrence, the exact molecular mechanisms of the development and progression are not fully understood [32]. Ehrlich Ascities Carcinoma (EAC) is one of the commonest tumors. EAC is referred as an undifferentiated carcinoma and is originally hyper diploid, has the high Transplantable capability, no-regression, they are converted to the ascities' form. EAC resembles human tumors, which are the most sensitive to chemotherapy due to the fact that they are undifferentiated and that they have a rapid growth rate [33]. Sulfur is found in every cell in the human body and is involved in a wide range of biochemical functions. Sulfur involvement in the human body ranges from Cellular energy production/metabolism. Maintaining blood glucose levels and Antioxidant protection - scavenges or neutralizes free

radicals and recycles oxidized antioxidants and Blood flow – produces both blood clotting factors as well as anticoagulants and Proper immune [34]. Nanosize sulfur particles have many important applications like in pharmaceuticals [35]. Suppress cell growth and induce apoptosis in multiple cancer cell lines [36]. It appears that sulfane sulfur containing DATS can be bioreduced in cancer cells to hydroperthiol that leads to H_2O_2 generation [37]. This study aimed to evaluate the antitumor activity of sulphur nanoparticles against Ehrlisch ascites carcinoma (EAC) in female albino mice.

Chemical characterization of S-NPs was done by some methods to determine purity composition, and the structure of these particles.

These S-NPs were analyzed using the IR spectram, to confirm the purity. They were characterized by (FT-IR), for the evaluation of their composition, (Figure 1) shows the spectrum of the FT-IR, analysis. It is evident from the no peaks that the product is completely pure and corresponds to sulfur element only. These results are in agreement with [35]. Who stated the FT-IR analysis, was carried out to identify the possible biomolecules responsible for the capping and stabilization of S-NPs, Which were identical in terms, of purity and stability.

Also, show the SEM images of S-NPs, synthesize by (1M), of HCl catalyzed in the presence of TMAB surfactants. The shape and size of S-NPs were investigated by SEM techniques, (Figures 2a -2c), to show the SEM images of all sulfur nanoparticles samples more regular shape. The SEM micrographs showed of the size distribution is unanimous nanoscale which the scale range of S-NPs is 5–50nm as show, (Figures 2a-2c), other studies revealed some results using, (SEM) [15], by Analysis of SEM S-NPs.

The synthesized nanoparticles were characterized by EDS for the evaluation of their composition and purity. Figure 3 shows the spectrum of the EDS analysis, it is evident from the peaks, that the product is highly pure and corresponds to sulfur element only. Also, showed that the EDS analysis of the as prepared S-NPs had broad peak. Similar studies [38]. Revealed by EDS analysis S-NPs, that the product is highly pure.

The XRD analysis of the as prepared S-NPs had broad peaks were measured for preparing S-NPs, with TMAB surfactant: as shown in Figure 4. The diffraction peaks were clearly observed from the XRD, of the S-NPs located near two, (16.8°, 23.0°, 25.9°, 31.7° and 37.7°) of 2θ positions, that are well-attributed to the (S-(113), S-(222), S-(027), S-(046) and S-(318);respectively. Synthesized S-NPs are well-crystalline, the position and the relative intensity of the diffraction peaks match well with the standard monoclinic phase sulfur diffraction pattern [39]. There is no other phase found, which means that phase pure monoclinic sulfur was prepared under these experimental conditions, and a similar study was revealed by XRD analysis S-NPs [15].

The acute toxicity was estimated by intraperitoneal, administration of the S-NPs to determine the median lethal dose (LD50). Our results revealed that, doses up to (200 mg/kg) in mice were may be considered safe for S-NPs where no mortality was observed and mice were healthy and active during the observation period. Also, it was found that, (5 mg/kg) was considered to be the most effective dose causes a reduced in count and volume of EAC concentrations other. A similar study [40], was conducted using for S-NPs has been given orally to the rabbits at a concentration of (2000 mg/kg) body weight (3 animals/group), was considered safe for S-NPs. Those toxicity effects of toxicants and therapeutic agents are dose-dependent [41].

A dose-response, curve is done to know which is the most effective dose of S-NPs on the reduction of EAC cell count by 78%, as in Figure 5, the dose response curve for these S-NPs demonstrated that most effective dose was found to be (5 mg/kg). Then, these S-NPs were tested in mouse models, (EAC model) to investigate the anti-tumor, anti-oxidant, and anti-apoptotic activities. While at non-cytotoxic doses S-NPs, is eco-friendly and clinical trials show no alarming toxic effects on eukaryotes [6]. Dose-response curve is a simple x-y graph relating the magnitude of a stressor such as, concentration of a pollutant, the amount of a drug, temperature, intensity of radiation) to the response of the receptor (organism under study, the response may be a physiological or biochemical response, or even death [42].

The study effect of S-NPs on volume and viable EAC cell count in studied groups. The mean values of EAC volume and count were found to be, (4.5 ± 0.5 ml) and (181.3 ± 11.3 × 10^6 cells/ml), in EAC bearing tumor group, while treated group were demonstrated a significant decrease in EAC volume by 73.3% and significant reduction in EAC cells, count by 82.5%, compared to EAC bearing tumor group, ($p<0.001$), (Figure 6). Reduction of tumor volume and viable cell count of tumor-bearing mice, tumor cell growth inhibition determined the potency of an anticancer agent [43]. When anticancer agents *in vivo* are used for treatment in cancer cell population large changes may occur in the cell and in result of that many cells are killed by the treatment induction of apoptosis in cancer cells is one of the goals of anticancer potential of any drug [44]. As to life span prolongation, (T/C %) in treating a group with sulfur nanoparticles the life of treated animals was prolonged by, (163.63%) compared to positive control group: as in Table 1.

The anti-oxidant effect of S-NPs was evaluated in the present study, through the estimation of SOD, GPx activities and TAC level in EAC cells. Our results found that, the mean value of SOD activity in EAC cells in positive control group was found to be (175.3 ± 21.2 U/ml), ($p<0.001$). Meanwhile, in treating group; it was significantly increased to (267 ± 19.4 U/ml), by 52.3% ($p<0.01$) compared to EAC group, as in Table 2. Also, the mean value of GPx concentration in positive control group was found to be (84.8 ± 12.3 mU/ml), ($p<0.001$). Meanwhile, in treating group; it was significantly increased to (332 ± 33.1 mU/ml), by 291.5%, ($p<0.01$) compared to EAC group, as in Table 2.

But, TAC level in positive control group was found to be (0.40 ± 0.14 mM/L), ($p<0.001$). Meanwhile, in treating group ; it was significantly increased to (2.40 ± 0.19 mM/L), by 500%, ($p<0.01$) compared to EAC group, as in Table 2. The anti-oxidant effect of sulfur nanoparticles were evaluated in the present study, through the estimation of SOD, Gpx activities and TAC level in liver tissue. The mean value of SOD activity in liver tissue in positive control group was found to be (564 ± 30.8 U/ml), ($p<0.001$). Meanwhile, in treating group; it was significantly increased to (861.5 ± 51.2 U/ml), by 52.6% ($p<0.01$) compared to EAC group, as in Table 3.

Also, Gpx concentration in liver tissue in positive control group was found to be (102 ± 2.8 mU/ml), ($p<0.001$). Meanwhile, in treating group; it was significantly increased to (375.9 ± 51.2 mU/ml), by 268% ($p<0.01$) compared to EAC group, as in Table 3. On the other hand, the mean value of TAC level in liver tissue in positive control group was found to be (0.247 ± 0.02 mM/L), ($p<0.001$). Meanwhile, in treating group; it was significantly increased to (0.395 ± 0.03 mM/L) by 59.9% ($p<0.01$) compared to EAC group, as in Table 3. Antioxidants, including polyphenols, sulfur- and selenium containing compounds, enzymatic antioxidants such as superoxide dismutase (SOD) and glutathione peroxidase (GPx) [45]. Glutathione peroxidase (GPx) is a type of an enzyme that serves as a cellular antioxidant. It reduces the peroxide group to a relatively un-reactive alcohol group, using glutathione as the reducing agent, and thus protects the cell from oxidative damage [46]. H_2O_2 is the product of SOD, the

increased production of H_2O_2 in the cancer cells treated with garlic-derived sulfur compounds, may be attributable to the increased activity of this enzyme as a result of addition of sulfane sulfur to the enzyme [37]. The apoptotic effect of sulfur nanoparticles was evaluated by measurement p53 level and Cytochrome C concentration in the EAC cells in all studied groups. The mean value of p53 level in positive control was found to be (0.465 ± 0.13 ng/mL). Furthermore, the treatment with sulfur nanoparticles (5 mg/kg, I.P.) showed a significant increase in p53 level (3.747 ± 0.81 ng/ml) by 705%, ($p<0.001$), compared to positive control group as shown in Table 4 (Figures 13 and 14).

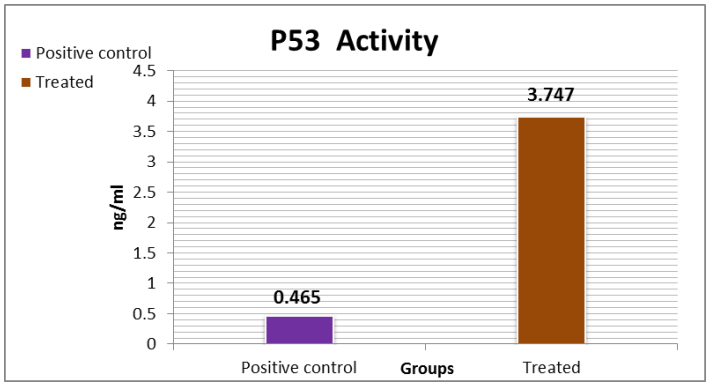

Figure 13: P53 activity in EAC cells in all studied groups.

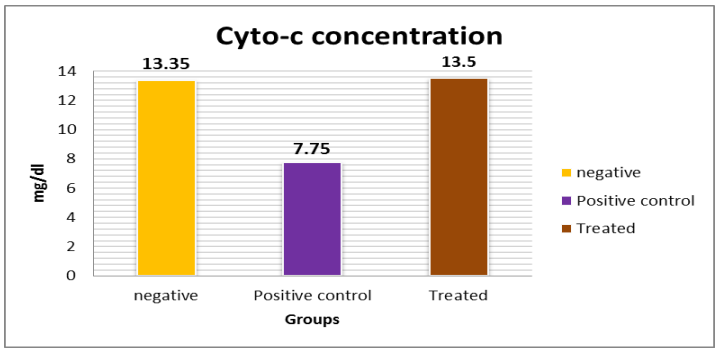

Figure 14: Cyto-c concentration in liver tissue in all studied groups.

Also, The mean value of Cytochrome C concentration in positive control group was found to be (0.88 ± 0.3 ng/mL). Furthermore, the treatment with sulfur nanoparticles (5 mg/kg, I.P.) showed a significant increase in Cytochrome C concentration (3.64 ± 0.5 ng/ml) by 313%, ($p<0.001$), compared to positive control group as shown in Table 4 and Figure 15.

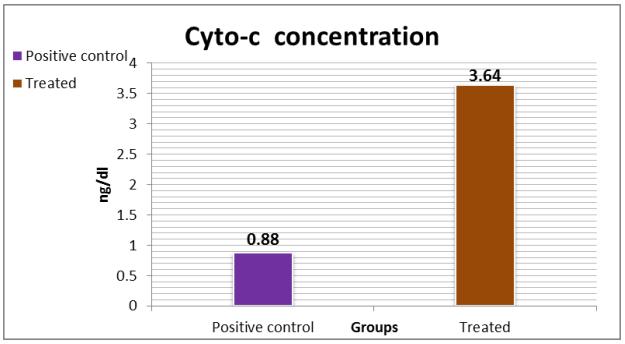

Figure 15: Cyto-c concentration in EAC cells in all studied groups.

The apoptotic effect of sulfur nanoparticles were evaluated by measurement p53 level and Cytochrome C concentration in the liver tissue in all studied groups. The mean value of p53 level in positive control was found to be (0.87 ± 0.12 ng/ml). Furthermore, the treatment with sulfur nanoparticles (5 mg/kg, I.P.) showed a significant decreased in p53 level (3.71 ± 0.28 ng/ml) by 57.6%, ($p<0.01$) compared to EAC group, as shown in Table 5 and Figure 16.

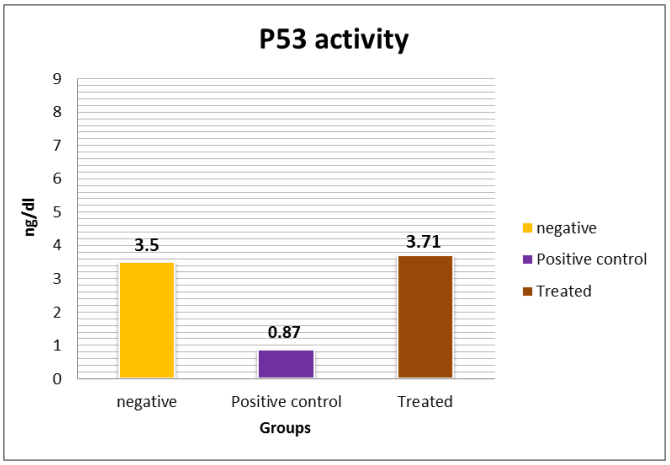

Figure 16: P53 activity in liver tissue in all studied groups.

Also, the mean value of Cytochrome C concentration in positive control group was found to be (7.75 ± 0.5 ng/ml). Furthermore, the treatment with sulfur nanoparticles (5 mg/kg, I.P.) showed a significant increase in Cytochrome C concentration (13.5 ± 1.1ng/ml) by 74% ($p<0.01$), compared to the EAC group as in Table 5 and Figure 16.

CONCLUSION

Apoptosis is an ordered and orchestrated cellular process that occurs in physiological and pathological conditions. Apoptosis plays an important role in the treatment of cancer as it is a popular target of many treatment strategies [47]. Therefore, we studied p53 level in positive and therapeutic groups to know the effect of sulfur nanoparticles in the recoveryof tumor edmice. Cytochrome C is also involved in the initiation of apoptosis. Upon release of Cytochrome C to the cytoplasm, the protein binds

apoptotic protease activating factor [48]. There are previous studies stated that sulfane sulfur containing DATS can be bioreduced in cancer cells and dependent on the presence of labile sulfane sulfur in their molecules thereby influencing the transmission of signals regulating cell proliferation and apoptosis [37].

The histological examinations of liver and kidney tissues with Hematoxylin and Eosin stain in the different studied groups confirm the biochemical study in all different groups, as shown in Figures 7–12. The histological examination of liver control mice (Negative control Group) showing normal central vein, hepatocytes, and blood sinusoids, (H & E 400x), (Figure 7). however, EAC group showing solid carcinoma of aggregated malignant cells infiltrated with inflammatory cells, as shown in (Figure 8). Nevertheless, Treated group liver showing dilated hepatoportal blood vessels which permeated with leucocytic cells as illustrated in (Figure 9). The histological examination of kidney in control mice Negative control Group showing normal renal parenchyma; Renal Glomeruli and renal tubules (H & E 400x), (Figure 10). However, EAC group kidney showing severe renal tubular degeneration as shown in (Figure 11). Nevertheless, treated group showed alliterations in the kidney tissues Treated group kidney showing improvement of the glomerular tuft and normal renal tubulesand mononuclear cells infiltration and interstitial blood vessel dilatation and congestion as illustrated in Figure 12. Similar studies revealed of sulfur species occurring in prostate cancer tissue has been observed significant changes by tissue affected and improve most of their parts [49]. The gold standard for diagnosis and staging of many diseases is histopathology. Histopathology refers to the microscopic examination of tissue in order to study the manifestations of disease, specifically, in clinical medicine [50]. The cells or tissues are more healthy and vigorous in the presence of the sulfur compounds [12].

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