

## Synthesis of Nano Sulfur Particles and their Antitumor Activity

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### Abstract

**Objectives:** The present study is designed to evaluate the antitumor activity of sulphur containing compounds in Nano formulations against Ehrlich Ascites Carcinoma (EAC) in female albino mice.

**Methods:** Preparation of sulfur nanoparticles (S-NPs) 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 Tetramethylammoniumbromide (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, and the determination of S-NPs antitumor activity against (breast carcinoma cell line (MCF7), liver carcinoma cell line (HEPG2), colon carcinoma cell line (HCT116), prostatic carcinoma cell line (PC3). Finally, determination biochemical assay of S-NPs by using female mice bearing EAC and of the assessment of liver functions and evaluation of kidney functions and determination of some heart functions in serum and hematology markers.

**Results:** This study revealed that S-NPs showed high strong activities in concentration (5 mg/kg) as it improvement of liver functions production compared to the positive control group, also showed the effect of S-NPs of the kidney functions, heart functions and hematology parameters in all studied groups and a significant increase in activities compared with positive control group.

**Conclusion:** S-NPs play an important role in improving liver functions, kidney functions and the heart functions of resulted in significantly increase activity against tumour and plays an important role in improving oxidative stress resulted in high antiproliferation activity against (MCF-7, HepG2, HCT116 and PC3) cell lines, and significantly increase with S-NPs reduced most of the hematological parameters towards the best compared to the positive control group.

**Keywords:** Sulfur Nanoparticles; Toxicity; Ehrlich ascites carcinoma; Viable cell count; Hematological

### Introduction

Cancer still represents one of the most serious human health related problem; despite the great progress in understanding its biology and pharmacology. The usual therapeutic methods for cancer treatment are individually useful in particular situations and when combined with other remedies, they offer a more efficient treatment for tumour. Nanotechnological improvements can be used for cancer patients; because nanotechnology can be used for better cancer diagnosis, more efficient drug delivery to tumour cells, and molecular targeted cancer therapy. First of all, nanotechnology can be used for better cancer diagnosis. Secondly, nanotechnology can be used for more efficient drug [1]. In this study evaluated the antitumor activity of sulphur containing compounds in Nano formulations against Ehrlich Ascites Carcinoma (EAC). It appears that Sulfur is an interesting element for tumour uptake because it plays an important role in cellular metabolism [2]. There are previous studies were on diallyl trisulfide (DATS) is a sulfane sulfur-containing compound, showed the highest biological activity in HepG2 cells. This compound increased the H<sub>2</sub>O<sub>2</sub> formation, lowered the thiol level and this is dependent on the presence of labile sulfane sulfur in their molecules [3]. Sulfur is an essential part of many enzymes and antioxidant molecules like glutathione and thioredoxin. Some sulfur-containing compounds can efficiently form a line of defence against reactive oxygen and nitrogen species [4]. Some sulfur-containing antioxidant systems showed decrease the levels of harmful ROS and help to reduce intracellular protein di-sulfide bonds, which are formed as a result of increased ROS levels. Therefore, sulfur containing antioxidants is essential in the maintenance of normal well-being of the cell and

health [5]. It appears that sulfane sulfur containing DATS can be bio-reduced in cancer cells and dependent on the presence of labile sulfane sulfur in their molecules thereby influencing the transmission of signals regulating cell proliferation [3].

### Material and Methods

#### Chemicals

The chemicals used for this study were taken from the following companies: Sodium thiosulfate (Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>·5H<sub>2</sub>O) (catalog 711906-551208) was obtained from the British drug houses LTD (B.D.H. laboratory Chemical group) and Tetramethylammonium bromide (TMAB) 98% (C<sub>4</sub>H<sub>12</sub>BrN) (catalog, 64-20-0) was obtained from Himedia laboratories Pvt.Ltd.(India)., RPMI-1640 medium, Trypan blue, Fetal Bovine Serum, Penicillin/ Streptomycin antibiotic and Trypsin- EDTA. From Sigma Aldrich Chemical. Kits [Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Total proteins (TP), Albumin (Alb), Bilirubin, Urea, Creatinine, Creatine kinase MB (CK-MB) and lactate dehydrogenase (LDH)] were from Biodiagnostic Company, USA.

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## Human tumor cell lines

Human tumour cell lines (breast carcinoma cell line (MCF-7), liver carcinoma cell line (HEPG2), colon carcinoma cell line (HCT116), prostatic carcinoma cell line (PC3). were used in this study obtained from the American Type Culture Collection (ATCC, Minisota, U.S.A.). The tumour cell lines were maintained at the National Cancer Institute, Cairo, Egypt, by serial sub-culturing.

## 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) 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 [6], 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  $2.5 \times 10^6$  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, sulfur nanoparticles (S-NPs) synthesized as follows: an appropriate amount of 50 ml (0.80 M) Sodium thiosulfate ( $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ ) dissolved in 50 ml of distilled water and using the Ultrasonic (WiseClean) device to speed the process of solubility then transferred into 250 ml conical flask 0.20 ml (0.02 M) of TMAB surfactant was dissolved in 20 ml of distilled water. A mixture of Sodium thiosulfate and TMAB was prepared by combining both solutions together and stirred mechanically at 120 r.p.m. and heated in constant  $40^\circ\text{C}$  respectively. Then 40 ml of (1.0 M) hydrochloric acid solution was added to the mixture to produce sized NPs under continuous stirring. After the reaction was stopped after 45 min, the produced yellow precipitates were collected, washed with distilled water and then dried according to the method of [7], [modified of Tetraoctylammonium bromide (TOAB) to Tetramethylammoniumbromide]. The nanoparticles were prepared in the laboratories of the Department of Chemistry, Faculty of Education for Pure Sciences, University of Diyala, Iraq.

## Samples characterization

The sulfur nanoparticles (S-NPs) were characterized by Fourier Transform Infrared Spectrometer (FT-IR) using device model Shimadzu Model FT/IR-8400. A material film (S-NPs) is prepared 1.5 mg of the solid sample is minced with 5 g of KBr or CsBr in an agate mortar, and a transparent disc (using a piston) is used free of abrasive and placed in the metering spectrometer. Employing a Scanning electron microscope (SEM) characterization. The shape and the morphology of the prepared S-NPs were characterized by Scanning electron microscope, the images have obtained using Inspect SEM (TESCAN veggie caechrepulic) type. A certain amount of S-NPs was taken with very little ethanol solvent (99%) and then placed on a special platform with the device with the coating plate gold and photographed to identify and determine their forms. Also, Energy dispersive X-ray spectroscopy (EDS) Analysis, characterization In this technique, the qualitative and quantitative analysis of the components and the linear survey of the components, as

well as the accuracy of the analysis of up to 0.1% and spatial accuracy of up to 20 mm, are used to analyse the different samples and give an idea of existing elements, using Inspect (Oxford X-Max 20). The synthesized nanoparticles were characterized by EDS for the evaluation of their composition and purity and the sample is prepared by dissolving the (S-NPs) with the ethanol (99%), then injecting it and observing the shape chart and the samples for X-Ray diffraction characterization using RigakuDmax 2500 diffractometer equipped with a graphite monochromatized  $\text{CuK}\alpha$  radiation ( $k=1.5407\text{\AA}$ ) and particle size distribution in range 10-100 nm. X-ray diffraction XRD technique was used to determine the structure and the size of the sulfur nanoparticles, x-ray structural analysis of the obtained samples shows that the samples are sulfur nanoparticles have an orthorhombic phase with S8 structure with traces of monoclinic by applying Scherrer equation on the XRD pattern, the particle size can be calculated by ( $D=K\lambda/b \cos\theta$ ). Where D is the mean size of crystallites (nm) K is a crystallite shape factor a good approximation is 0.9,  $\lambda$  is the X-ray wavelength, B is the full width at half the maximum (FWHM) in radians of the X-ray diffraction peak and  $\theta$  is the Bragg's angle (deg) [8].

## MTT assay in cultured human cancer cells

The effect of S-NPs on growth of cancer cells (MCF-7, HepG2, HCT116 and PC3), was assessed by MTT assay and cytotoxicity was carried out using Sulphorhodamine-B (SRB) assay following the method reported by Vichai and Kirtikara [9].

## Determination median lethal dose (LD 50) of sulfur nanoparticles (S-NPs)

Approximate LD 50 of sulphur nanoparticles (S-NPs) in mice was determined according to the method of Meier and Theakston [10]. Studies carried out for determination of the median lethal dose are important to help us to assess the limit dose recommended. To determine the median lethal dose of sulphur nanoparticles (S-NPs), a group of 10 mice were injected with doses 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, 8, 10 mg/Kg of sulphur nanoparticles (S-NPs); respectively, soluble in corn oil [11]. 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 sulphur nanoparticles (S-NPs).

## Determination dose response curve of sulphur nanoparticles (S-NPs)

The dose response curve of synthesized sulphur nanoparticles (S-NPs) in mice was determined according to the method of Crump et al. [12]. Studies carried out for determination of the most effective dose on tumour volume and count.

## Experimental design

The total number of 75 female Swiss albino mice weighing 20-25 g was divided into the three groups (25 mice in each group): Group (I): Negative Control: mice injected intraperitoneal (I.P.) with sterile saline for 10 days (day after day). Group (II): Positive Control (EAC bearing group): mice were injected with Ehrlich ascites carcinoma (EAC) by the concentration of ( $2.5 \times 10^6$  cells/0.3 ml/mouse) according to Amer [13], by I.P. injection once. Group (III) therapeutic group: mice were injected I.P. with S-NPs (5 mg/Kg) after EAC injection ( $2 \times 10^6$  cells/mouse), followed by I.P. injection of S-NPs at 3, 5, 7, 9 days of EAC injection for 10 days.

## Sampling and preparation

**Blood sampling:** At the end of the experiment, the blood samples

were collected from the retro-orbital venous plexus under light ether anaesthesia. Plasma was prepared by centrifuging EDTA- blood at 3000 r.p.m. for 10 min. Plasma samples were aliquot and stored at -20°C until biochemical analysis. Serum was prepared by centrifuging blood at 4000 r.p.m. for 10 min. Serum was aliquot and stored at -20°C until biochemical analysis. EDTA vacuum tube which runs in a hematology analyser for estimating hematological parameters in experimental mice groups by using Automatic CBC analyzer (Sesmex Kx-21).

**Biochemical investigations:** The determination of serum alanine aminotransferase (ALT) activity were be according to the Hu et al. [14] determination of serum aspartate aminotransferase (AST) activity were being according to the Schumann et al. [15] and serum total proteins were determined using Biuret method performed by Burtis et al. [16], determination of albumin in serum was carried according to Cosgrove et al. [17], serum bilirubin was determined according to Jendrassik and Grof [18]. Also, biochemical kidney functions tests, determination of urea assay in blood was measured according to the method described by Tabacco et al. [19] and the creatinine concentration in the sample of determination of were being according to the Young and Friedman [20]. Also, heart functions tests in serum. Determination of serum creatine kinase MB (CK-MB) activity were measured according to Wu and Bowers [21] determination of serum lactate dehydrogenase (LDH) activity were being accorded to Young and Friedman [20]. Finally; the estimating hematological parameters by using an Automatic CBC analyzer (Sesmex Kx-21).

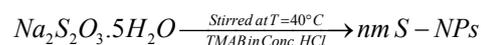
### Statistical analysis

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  $\pm$  SD. The levels of markers were analysed by ANOVA but the Mann Whitney U-test was used for comparisons between independent groups [22].

## Results

### Sulfur nanoparticles preparation (Particle synthesis)

Quick precipitation method of sulfur nanoparticles was by redox comproportionation of  $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$  in concentration HCL and using tetramethylammonium bromide (TMAB) as stabilizer according to equation following.



After particles prepared, the particles were centrifuged and washed extensively with water to remove any soluble impurities (such as unreacted sulfite) and then filtered. 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-Ray diffraction technique.

### Fourier transform infrared spectrometer (FT-IR) characterization of sulfur nanoparticles

The synthesized nanoparticles 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 and corresponds to sulfur element only.

### SEM characterization of sulfur nanoparticles

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

### EDS characterization of sulfur nanoparticles

Results in Figure 3 showed that the EDS analysis of the as prepared sulfur nanoparticles had broad peak(S).

- X-ray diffraction (XRD) characterization of sulfur Nanoparticles.

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

### Chemical studies on sulfur nanoparticles in vitro

**Cytotoxicity:** The *in vitro* cytotoxic activities of sulfur nanoparticles were shown in Figure 5. Minimum Inhibitory concentrations of synthesized sulfur nanoparticles were found to be 10.7 ng/ml, 3.7 ng/ml, 10.6 ng/ml, and 3.34 ng/ml against MCF-7, HepG2, HCT116, and PC3 cell lines; respectively.

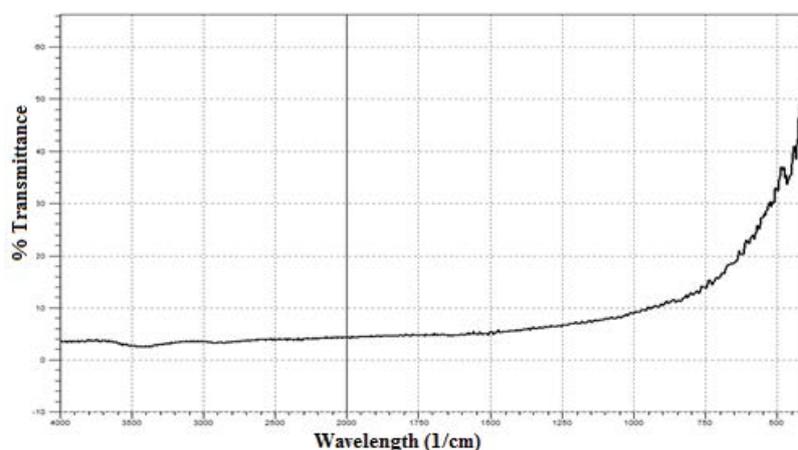


Figure 1: FT-IR spectra of sulfur nanoparticles.

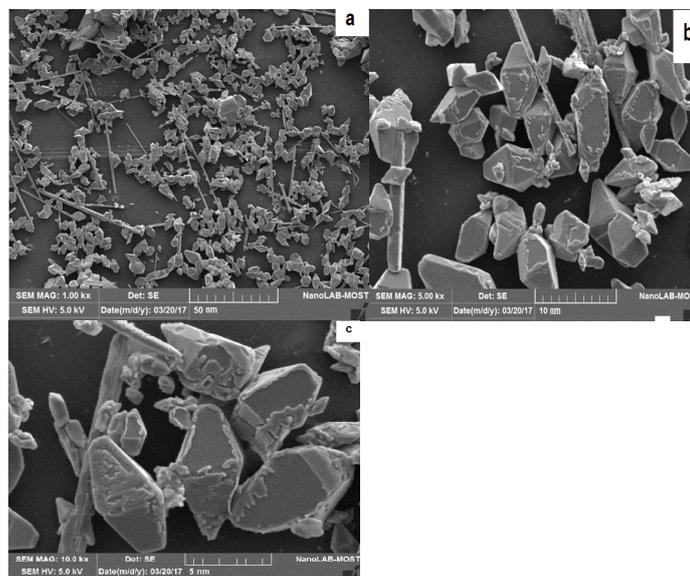


Figure 2: SEM images of the sulfur nanoparticles (a) size 50 nm, (b) size 10 nm (c) size 5 nm.

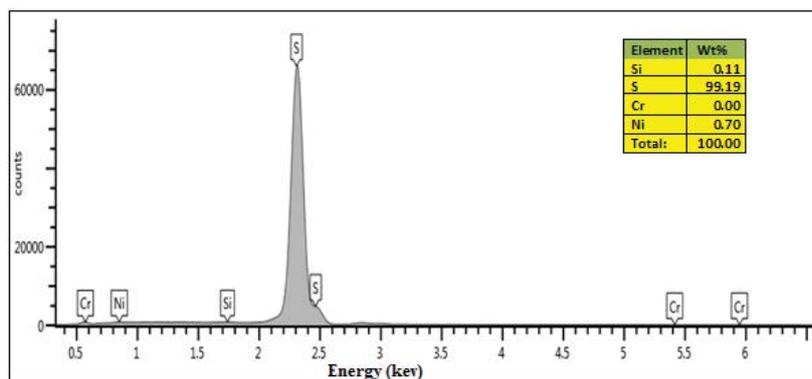


Figure 3: EDS spectrum of the sulfur nanoparticles.

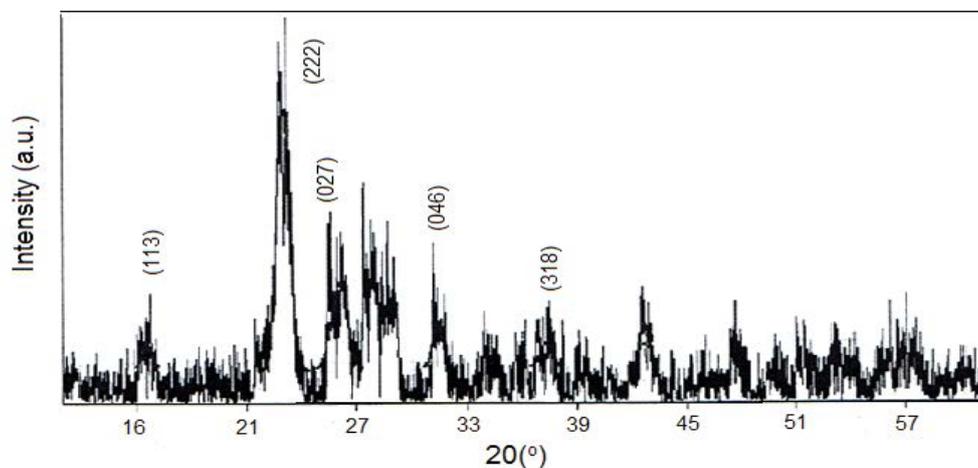


Figure 4: XRD pattern of the sulfur nanoparticles.

**Toxicity study and dose response curve:** For determination of the median lethal dose (LD 50) of sulfur nanoparticles, all doses up to 200 mg/ Kg mice were found to be nontoxic as no deaths were recorded which suggests that sulfur nanoparticles 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 6a and 6b.

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

**Life span prolongation:** The life span showed a significant increase in therapeutic group by (63.63%) compared to positive control group.

**Effect on hematological parameters:** In EAC-bearing mice, RBC count and packed cell volume were marked (P<0.05) declined, whereas total WBC count was enhanced as compared to the normal mice. Among the various white blood cells analysed, neutrophils were found to be elevated, while the lymphocytes were decremented in EAC-bearing mice when compared to normal mice. Sulfur nanoparticles to diseased animal have restored the above alterations to a significant extent.

There was a significant drastic fall in the hemoglobin content of the EAC control group as compared to a normal control group. Administration of sulfur nanoparticles significantly (P<0.001) reverted the above changes to normal (Table 1).

### Biochemical investigations

**Liver function tests:** Data presented in Tables 2 & 3 shown that S-NPs administration in mice lowered AST in therapeutic (p<0.01), ALT (p<0.001), activities and bilirubin levels, (p<0.001) compared to positive control group, but these values are with in normal range in mice so that they show insignificant change in these markers. Data shown also insignificant increase in T.P (p<0.05) and albumin levels (p<0.05), compared to positive control and these values are within normal range after administration of sulfur nanoparticles. Also administration of S-NPs shown significant increase in globulins by

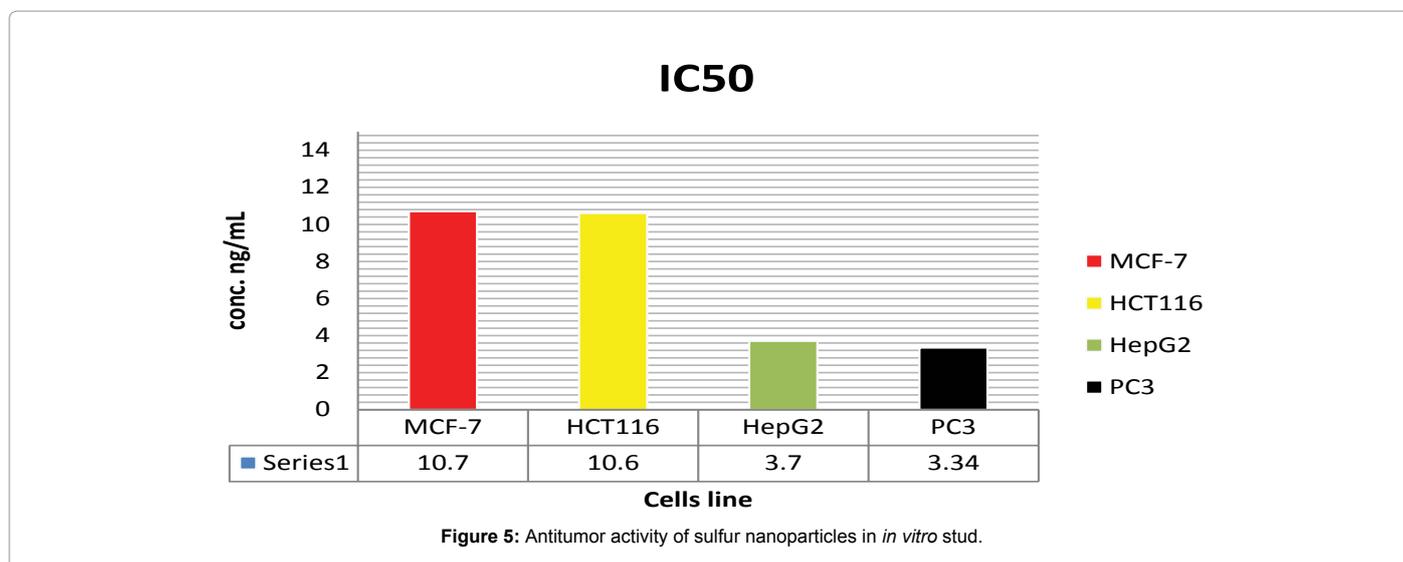
8.8%, (p<0.01), compared to positive control group but these values are within normal range in mice so that they show insignificant changes in these parameters. Data also shown significant decreased in A/G ratio in plasma levels (p<0.001), compared to EAC bearing tumour group, these values are within normal range after administration of S-NPs.

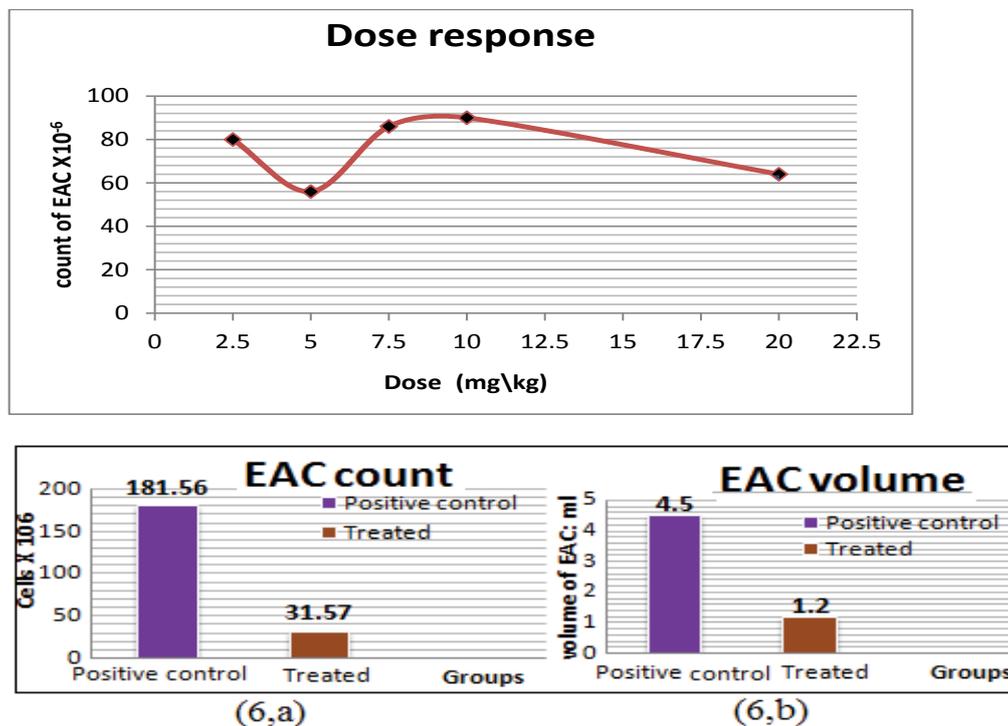
**Kidney function tests:** The mean value of urea shown significant increase in EAC bearing tumour group by 89.5%, (p<0.001) compared to negative control group. While, their significantly decreased by 20.7% in treated group (p<0.01) compared to EAC bearing tumour group and these values are within normal range as in Table 4. The mean value of creatinine shown significant increase in EAC bearing tumour group by 104% (p<0.001), compared to negative control group. While, their a significantly decreased by 0.68%, in treated group (p<0.01) compared to EAC bearing tumour group,

**Heart functions:** The mean value of CK-MB shown significant increase in EAC bearing tumour group by 57.9%, (p<0.001), compared to negative control group. While, their significant decreased by 15.8% in treated group (p<0.01) compared to EAC bearing tumour group, as shown in Table 5. Also, the mean value of LDH shown increases in EAC bearing tumour group by 41.1%, (p<0.001), compared to negative control group. While, their significantly decreased by 0.12% in treated group (p<0.01) compared to EAC bearing tumour group and these values are within normal range.

### Discussion

Cancer is a major health problem threatening the life in both developed and developing countries. It is a progressive uncontrolled degenerative disease predisposed by accumulation of toxins, disturbances in hormonal and immune conditions can induce cancer [23]. 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 [24], Ehrlich Ascities Carcinoma (EAC) is one of the commonest tumours. EAC is referred as an undifferentiated carcinoma and is originally hyper diploid, has the high Transplantable capability, no-regression, they are converted to the ascites form. EAC resembles human tumours, which are the most sensitive to chemotherapy due to the fact that they are undifferentiated and that they have a rapid growth





**Figure 6:** Dose response curve of S-N  
(a) EAC count in studied groups after treatment with sulfur nanoparticles.  
(b) EAC volume in studied groups after treatment with sulfur nanoparticles.

Mice Group	RBC (x10 <sup>6</sup> )	Hb g/dL	HCT g/L	MCV fL	MCH Pg	MCHC g/dL	WBC (x10 <sup>6</sup> )	Neutr
Negative control group	8.02±0.38	12.0±0.85	38.24±2.4	47.02±2.7	14.5±1.2	30.6±0.93	7.85±1.66	17.1±1
Positive control group	6.4±0.9	7.4±0.9	23.23±7.3	46.9±2.6	15.3±0.65	32.2±0.54	13.5±0.67	42.8±3
% Change	20.1%	38.3%	39%	0.25%	5.5%	5.22%	71.9%	150%
The group Change %	8.8±0.68	12.8±0.48	35.6±3.4	46.9±1.4	15.4±0.37	32.0±1.17	9.17±0.65	33.9±2
%	9.7%	6.6%	6.9%	0.25%	6.2%	4.5%	16.8%	98%

\* P<0.01

\*\*P<0.001

**Table 1:** Volume, Count and Hematological parameters in studied groups. Data were expressed as Mean±SD and percent %.

Variables	Negative control group		EAC bearing tumor		Treated (S-NPs)	
	Mean±SD.	% Change	Mean±SD.	% Change	Mean±SD.	% Change
AST (U/l)	197±12	-----	281±12.5***	42.6%	191±5.6**	32%
ALT (U/l)	43.4±3.5	-----	87.1±3.0**	100.6%	42.8±1.6***	50.8%
T.P (g/dl)	6.34±0.28	-----	4.05±0.34**	36.1 %	6.75±0.84***	66.6%
Alb (g/dl)	3.29±0.4	-----	2.11±0.4***	35.8%	3.42±0.2**	62%
Bilirubin (mg/dl)	0.56±0.09	-----	0.63±0.04**	12.5%	0.53±0.1***	15.8%

Significance at \*P<0.05, \*\*P<0.01, \*\*\*P<0.001

**Table 2:** Effect of sulfur nanoparticles on liver functions in plasma in all studied groups.

rate [25]. In this study evaluated the antitumor activity of sulphur containing compounds in Nano formulations against Ehrlich Ascites Carcinoma (EAC) in female albino mice. Sulfur is found in every cell in the human body and is involved in a wide range of biochemical functions. Sulfur's 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 [26]. Nanosize sulfur particles have many important applications like in pharmaceuticals. It appears that sulfane sulfur containing DATS can be bio reduced in cancer cells to hydroperthiol that leads to H<sub>2</sub>O<sub>2</sub> generation, thereby influencing the transmission of signals regulating cell proliferation and apoptosis [4].

Variable	Negative control group		EAC bearing tumor		Treated (S-NPs)	
	Mean±SD	% Change	Mean±SD	% Change	Mean±SD	% Change
Globulins (g/dl)	3.04±0.43	-----	1.94±0.65***	36.1%	3.31±0.91***	70.6%
A/G Ratio	1.11±0.28	-----	1.2±0.73***	8.1%	1.1±0.33***	8.3%

Significance at \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001

**Table 3:** Effect of sulfur nanoparticles on globulins and A/G ratio in plasma in all studied groups.

Variable	Negative control group		EAC bearing tumor		Treated (S-NPs)	
	Mean±SD	% Change	Mean±SD	% Change	Mean±SD	% Change
Urea (mg/dl)	28.96±1.6	-----	54.88±2.6**	89.5%	34.9±3.6***	36.4%
Creat (mg/dl)	0.346±0.03	-----	0.708±0.08***	104%	0.343±0.06**	51.5%

Significance at \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001

**Table 4:** Effect of sulfur nanoparticles on kidney functions in all studied groups.

Variable	Negative control group		EAC bearing tumor		Treated (S-NPs)	
	Mean±SD.	% Change	Mean±SD.	% Change	Mean±SD.	% Change
CK-MB (U/L)	22.2±0.5	-----	35.1±2.9***	57.9%	25.8±2.9***	26.4%
LDH (U/L)	1620±103	-----	2287±132***	41.1%	1622±77***	29%

Significance at \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001

**Table 5:** Effect of sulfur nanoparticles on heart functions in all studied groups.

Chemical characterization of sulfur nanoparticles was done by some methods to determine purity, composition and the structure of these particles.

These sulfur nanoparticles were analysed using the IR spectrum 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 Awwad et al. [27]. Who stated the FT-IR analysis was carried out to identify the possible biomolecules responsible for the capping and stabilization of sulfur nanoparticles, which were identical in terms of purity and stability.

Also, show the SEM images of sulfur particle 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 S-NPs samples more regular shape. The SEM micrographs showed of the size distribution is unanimous nano-scale which the scale range of sulfur nanoparticles is 5–50 nmas show Figures 2a-2c other studies revealed some results using (SEM) [7], by Analysis of scanning electron microscope (SEM) Sulfur nanoparticles.

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 sulfur nanoparticles had broad peak. Similar studies [6], Revealed by EDS analysis sulfur nanoparticles that the product is highly pure.

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

position and the relative intensity of the diffraction peaks match well with the standard monoclinic phase sulfur diffraction pattern [28]. 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 Sulfur nanoparticles [8].

### The goal of the *in vitro* study was to explore the mechanism underlying

Sulfur nanoparticles-induced cell death in human MCF-7, HepG2, HCT116 and PC3 cell lines , here, we report that treatment with sulfur nanoparticles inhibited cell proliferation and viability. To investigate the effect of sulfur nanoparticles on cells viability of MCF-7, HepG2, HCT116 and PC3 cells, cells were treated with or without different concentrations of sulfur nanoparticles for 48 h. Our data showed that sulfur nanoparticles significantly decreased cells viability of treated MCF-7, HepG2, HCT116, and PC3 cells as confirmed by MTT assay as in Figure 5. The proliferation of MCF-7, HepG2, HCT116 and PC3 cells was significantly inhibited by sulfur nanoparticles at (10.7 ng/ml, 3.7 ng/ml ,10.6 ng/ml, and 3.34 ng/ml); respectively. Indicating anti-proliferation activity of sulfur nanoparticles on these cell lines and raising the possibility that sulfur nanoparticles might be a potential chemo-preventive or therapeutic agent.

Those effective doses showed no cytotoxic effects on different cell lines [29], other studies revealed some results using a sulfane sulfur-containing compound, showed the highest biological activity in HepG2 cells. This compound produced the strongest inhibition of cell proliferation [30].

The acute toxicity was estimated by intraperitoneal administration of the sulfur nanoparticles to determine the median lethal dose (LD 50). Our results revealed that, doses up to 200 mg/kg in mice were may be considered safe for sulfur nanoparticles 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 [31], was conducted using for sulfur nanoparticles has

been given orally to the rabbits at a concentration of 2000 mg/kg body weight (3 animals/group) was considered safe for sulfur nanoparticles. Those toxicity effects of toxicants and therapeutic agents are dose-dependent [32].

A dose-response curve is done to know which is the most effective dose of sulfur nanoparticles on the reduction of the EAC cell count by (78%) as in Figure 6, 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-tumour, 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 [33]. 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 [34].

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  $4.5 \pm 0.5$  ml and  $(181.3 \pm 11.3) \times 10^6$  cells/ml in EAC bearing tumour 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 tumour group, ( $p < 0.001$ ). Reduction of tumour volume and viable cell count of tumour-bearing mice, tumour cell growth inhibition determined the potency of an anticancer agent [35]. 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 [36].

As to life span prolongation (T/C%) in treating a group with S-NPs the life of treated animals was prolonged by 163.63% compared to the positive control group.

It is apparent that S-NPs increased the RBC cells count and hemoglobin content and decreased the WBC cells count to the normal level in therapeutic group and also note changes in the components of the other which illustrate significant increase in RBC cells count by 9.7%, Hb content by 6.6%, HCT content by 6.9%, MCV content by 0.25%, MCH content by 6.2%, MCHC content by 4.5% and significant decrease in WBC cells count by 16.8%, Neutrophil cells count by 98%, Lymphocyte cells count by 40.5% and platelet count by 10.9% in therapeutic group compared to positive control group ( $*P < 0.05$ ), ( $**P < 0.001$ ). This result supports the suitability of the sulfur nanoparticles as an anticancer agent which indicates that sulfur nanoparticles has a protective action on the homeopathic system. Results in the present study were resembles as recorded by Pandya et al. [37]. One of the major problems in cancer chemotherapy is myelosuppression, followed by anemia due to the reduction of RBC and hemoglobin content. This is probably owing to the deficiency of iron in a hemolytic or myelopathic condition [38]. Progression of tumor was accompanied by hematological changes compared to normal gradual decrease in hemoglobin content, RBC count and gradual increase in leukocytes [39], which was also observed in control mice. The RBC count was almost reversed back to normal range on the treatment of sulfur nanoparticles in EAC bearing mice. It also improves the WBC level efficiency. The hemoglobin level was in the near normal range in the therapeutic group. Recovery of the hematological parameters like hemoglobin content, RBC and WBC cells counts in the experimental mice indicates the protective action of sulfur nanoparticles on the hemopoietic system and TIS certainly ratios

that sulfur nanoparticles possess pronounced anticancer activity with a little or no host toxic effect [40].

To evaluate the protective effect of sulfur nanoparticles on liver functions in all studied groups in AST, ALT, Bilirubin, T.P, Albumin, Globulins and A/G ratio concentration level were estimated, showed AST in therapeutic ( $p < 0.01$ ), ALT ( $p < 0.001$ ), activities and bilirubin level, ( $p < 0.001$ ) compared to positive control group, but these values are within normal range in mice so that they show insignificant changes in these markers. Data shown also insignificant increase in T.P ( $p < 0.05$ ) and albumin level ( $p < 0.05$ ), compared to positive control and these values are within normal range after administration of sulfur nanoparticles. Also administration of S-NPs shown significant increase in globulins by 8.8%, ( $p < 0.01$ ), compared to positive control group, but these values are within normal range in mice so that they show insignificant change in these parameters. Data also shown significant decreased in A/G ratio in plasma levels ( $p < 0.001$ ), compared to EAC bearing tumour group, and these values are within normal range after administration of S-NPs. These results are due to free radical released from oxidative stress caused by tumour growth which lead to DNA and tissue damage which result in changes in membrane permeability and metabolism disturbances causing elevation of the enzyme after released from mitochondria which are in agreement with Abd El-Aziz et al. [40]; who reported that Liver damage induced by tumor cells generally reflects disturbances in liver cell metabolism, which lead to characteristic changes in serum enzyme activities. The increased levels of AST in serum may be interpreted as a result of liver damage or as changes in membrane permeability indicating the severity of hepatocellular damage by EAC. As the serum of AST is a clinical indicator of tumour-induced toxicity. AST or ALT activities are a valuable aid primarily in the diagnosis of liver disease. However, when body tissue or an organ such as the liver or heart is diseased or damaged, additional AST and ALT are released into the bloodstream, causing activities of the enzyme to rise. Therefore, the amount of AST and ALT in the blood is directly related to the extent of the tissue damage [41]. Liver is the most important key organ in the metabolism, detoxification and secretory functions in the body and it is highly affected primarily by toxic agents that why we studied the following parameters which are found to be of great importance in the assessment of liver damage. Aspartate aminotransferase (AST) enzyme, Alanine aminotransferase (ALT) enzyme, Total protein (T.P), Albumin (Alb) and Bilirubin is found mainly in the liver, but also found in red blood cells, heart cells, muscle tissue and other organs, such as the pancreas and kidneys [42]. Bilirubin, another substance commonly measured in the blood to detect liver disease, is produced from the breakdown of red blood cells. A normal bilirubin level is maintained as the liver continually removes bilirubin from the bloodstream for further processing. If the liver is impaired, however, bilirubin is not removed, and the level in the bloodstream will rise [43]. Serum albumins are important in regulating blood volume by maintaining the oncotic pressure (also known as colloid osmotic pressure) of the blood compartment [44].

To evaluate the protective effect of sulfur nanoparticles on kidney functions in all studied groups in urea and creatinine concentration levels was estimated and the mean value of urea showed significant increase in EAC bearing tumor group by 89.5%, ( $p < 0.001$ ), compared to negative control group. While, their significantly decreased by 20.7%, in treated group ( $p < 0.01$ ) compared to EAC bearing tumour group and these values are within normal range. The mean value of creatinine showed significant increase in EAC bearing tumour group by 104%, ( $p < 0.001$ ), compared to negative control group. While, their a significantly decreased by 0.68%, in treated group ( $p < 0.01$ ), compared

to EAC bearing tumour group. Also, the kidneys are important because they keep the composition, or make up, of the blood stable, which lets the body functions like: prevent the build-up of wastes and extra fluid in the body, keep levels of electrolytes stable, such as sodium, potassium, and phosphate, make hormones that help, regulate blood pressure, make red blood cells and bones stay strong [45]. Liver and kidney toxicity induced during tumour growth may be due to the excessive production of ROS that leads to oxidative damage [46]. It has been established that ROS play an important role in inflammatory conditions by interacting with pro-inflammatory cytokines. The over-produced pro-inflammatory cytokines may lead to inflammation, enhance systemic inflammatory stress and also promote the deterioration of cardiac and/or renal dysfunctions [47].

To evaluate the protective effect of sulfur nanoparticles on heart functions in all studied groups in CK-MB and LDH concentration levels was estimated and the mean value of CK-MB showed significant increase in EAC bearing tumour group by 57.9%, ( $p < 0.001$ ), compared to negative control group. While, theirs significant decreased by 15.8%, in treated group ( $p < 0.01$ ) compared to EAC bearing tumour group. Also, the mean value of LDH shown increased in EAC bearing tumour group by 41.1%, ( $p < 0.001$ ) compared to negative control group. While, their significantly decreased by 0.12%, in treated group ( $p < 0.01$ ) compared to EAC bearing tumour group and these values are within normal range. These results in were in agreement with Maghamiour and Safaie [48]. Who reported that elevated CK-MB fraction is seen in prostatic carcinoma and other underlying malignancy, such as breast cancer. Injury or stress to muscle tissue, the heart, or the brain can be associated with increased total CPK levels due to CPK leakage into the circulation. Increase in any particular type of CPK would define the type of damaged tissue. Increased serum levels of CK isoenzymes variously signal heart, brain, or skeletal muscle damage. They may also be markers for advanced tumours with poor prognosis [49]. Which shown that CK-MB level measured by mass assay, is elevated significantly in serum on day I after myocardial infarction in rats, induced by coronary artery ligation. A significant elevation in the level of CK-MB has been observed in the heart effluent during myocardial ischemia and reperfusion in isolated rat hearts [50], during recent years, CK-MB activity assays have been replaced by CK-MB mass assays which measure the protein concentration of CK-MB, rather than its catalytic activity. Enzyme immunoassays have become the choice for measuring CK-MB in the laboratory because analytical interferences which lead to false positive test results are less frequent.

## Conclusion

These results are due to that the significant increase in LDH value resulted from heart tissue damage caused by tumour growth which were in agreement with Radenkovic et al. [51]. Who reported that activity of LDH in tumour tissue along with mammographic characteristics could help in defining aggressive breast cancers as the elevation in LDH value was suggested for the same explanation. Free radical leads to oxidative stress and ROS generation which cause cardiac tissue damage releasing this enzyme and elevating its level in the blood stream [52].

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