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Synthesis of Nano Sulfur particles and their Antitumor activity

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ABSTRACT

Objectives: Sulphur is among the most common minerals in the body mainly in proteins, but, also, commonly found in tissues joints and cells .At the same time there is a rapidly expanding literature on the effects, of sulphur in regulating biologicaly systems. **Methods:** Preparation of sulfur nanoparticles (S-NPs) was preparation and fabrication of nanosulfar were performed. 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 were size 50nm, 10nm and 5 nm , Then, Energy-dispersive X-ray spectroscopy (EDS) for the evaluation of their composition and purity, after that the X-Ray diffraction, and the determination of S-NPs antitumor activity against (MCF-7, HepG2, HCT116, and PC3) cell lines and were minimum inhibitory concentrations found to be 10.7 ng/ml, 3.7 ng/ml ,10.6ng/ml, and 3.34 ng/ml; respectively. Finaly, determination biochemical assay of S-NPs by using female mice bearing EAC and of the assessment of liver functions, kidney functions, heart functions 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.

1-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 tumors **(1)**. Nanotechnological improvements can be used for cancer patients; because nanotechnology can be used for better cancer diagnosis, more efficient drug delivery to tumor cells, and molecular targeted cancer therapy .First of all,

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nanotechnology can be used for better cancer diagnosis Secondly, nanotechnology can be used for more efficient drug **(2)**. It appears that Sulfur is an interesting element for tumor uptake because it plays an important role in cellular metabolism **(3)**.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 H2O2 formation, lowered the thiol level and this is dependent on the presence of labile sulfane sulfur in their molecules **(4)**. 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 defense against reactive oxygen and nitrogen species **(5)**. Some sulfur-containing antioxidant systems showed decrease the levels of harmful ROS and help to reduce intracellular protein disulfide 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 **(6)**. Also,There are previous studies was on It appears 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 **(4)**.

2-Material and methods:

2.1.1- Chemicals.

The chemicals used for this study were taken from the following companies: Sodium thiosulfate ($Na_2S_2O_3.5H_2O$) (catalog 711906-551208) was obtained from the British drug houses LTD (B.D.H. laboratory Chemical group)., and Tetramethylammonium bromide, (TMAB) 98% ($C_4H_{12}BrN$) (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 (AST, ALT, TP , Alb , Bilirubin ,Urea, Creatinine ,CK-MB and LDH) were from Biodiagnostic Company , USA.

2.1.2.Human tumor cell lines.

Human tumor cell lines MCF-7 (human breast cancer), HePG2(hepatocellular carcinoma) , HCT116 (human colon cancer), PC3 (human prostate cancer) were used in this study obtained from the American Type Culture Collection (ATCC, Minisota, U.S.A.). The tumor cell lines were maintained at the National Cancer Institute, Cairo, Egypt, by serial sub-culturing.

2.1.3. 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.*

2.1.4.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 **(7),** 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.

2.2-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 (0.80M) Sodium thiosulfate ($Na_2S_2O_3 .5H_2O$) 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 .20 ml (0.02M) of TMAB surfactant was dissolved in 20 ml of distilled water . Amixture 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 C^o 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 **(8),** [modified of Tetraoctylammonium bromide (TOAB) to Tetramethylammonium bromide]. The nanoparticles were prepared in the laboratories of the Department of Chemistry, Faculty of Education for Pure Sciences, University of Diyala , Iraq .

2.3. Samples characterization.

The (S-NPs) were characterized by FT-IR 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 SEM the shape and the morphology of the prepared S-NPs were characterized the images have 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, EDS Analysis, characterization 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.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 Rigaku Dmax 2500 diffractometer equipped with a graphite monochromatized CuKα radiation (k = 1.5407^{o}A) and particle size distribution in range 10 nm to 100 nm. **(9)**.

2.4.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 **(10).**Cells were seeded in 96-well Microtiter plates at an initial concentration of 3 x10^{3} cell/well in a 150 µL fresh medium and left for 24 hours to attach to the plates. Different concentrations 0, 5, 12.5, 25, 50 µg/mL of drug were added. For each drug concentration, 3 wells were used. The plates were incubated for 48 hours.The cells were fixed with 50µL cold TCA 10% final concentration for 1 hour at 4 ^{0}C.The plates were washed with distilled water using (automatic washer Tecan, Germany) and stained with 50µL 0.4 % SRB dissolved in 1 % acetic acid for 30 minutes at room temperature. The plates were washed with 1 % acetic acid and air-dried. The dye was solubilized with 100µL/well of 10M Tris base (pH 10.5) and optical density (O.D.) of each well was measured spectrophoto metrically at 570 nm with an ELISA micro plate reader (Sunrise Tecan reader, Germany). The mean background absorbance was automatically subtracted and mean values of each drug concentration was calculated. The experiment was repeated 3 times, the percentage of cell survival was calculated as follows: (Surviving fraction = O.D. (treated cells)/ O.D.) control cells. The IC50 values (the concentrations of Resveratrol required to produce 50% inhibition of cell growth) were also calculated.

2.4. Determination median lethal dose (LD_{50}) of sulfur nanoparticles (S-NPs).

Approximate LD50 of sulphur nanoparticles (S-NPs) in mice were determined according to the method of **(11)**. 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 **(12)**. 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, where no mortality was observed for 2 months.

2.5.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 **(13)**. Studies carried out for determination of the most effective dose on tumor volume and count.

2.6. 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 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 (2.5×10^6 cells/ 0.3 ml/mouse) according to **(14)**.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 SNPs at 3, 5, 7, 9 days of EAC injection for 10 days.

2.7. 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 anesthesia. plasma was prepared by centrifuging EDTA-blood at 3000 r.p.m. for 10 minutes. 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 minutes. Serum was aliquot and stored at -20 °C until biochemical analysis. EDTA vacuum tube which run in a hematology analyser for estimating hematological parameters in experimental mice groups by using Automatic CBC analyzer (Sesmex Kx-21).

2.8. Biochemical Investigations.

The determination of serum alanine aminotransferase (ALT) activity were be according to the **(15)** ,determination of serum aspartate aminotransferase (AST) activity were being according to the **(16)** ,and serum total proteins were determined using Biuret method performed by**(17)**, determination of albumin in serum was carried according to **(18)**, serum bilirubin was determined according to **(19)** . Also, biochemical kidney functions tests, determination of urea assay in blood was measured according to the method described by **(20)**, and the creatinine concentration in the sample of determination of were being according to the **(21)**. Also, heart functions tests in serum. determination of serum creatine kinase MB (CK-MB) activity were measured according to **(22)**, determination of serum lactate dehydrogenase (LDH) activity were being accorded to **(21)**. Finally, the estimating hematological parameters by using an Automatic CBC analyzer (Sesmex Kx-21).

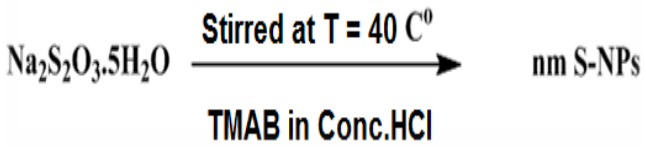
2.10.Statistical Analysis.

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 **(23)**.

3.Results.

-Sulfur nanoparticles preparation (Particle synthesis).

Quick precipitation method of sulfur nanoparticles 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.



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 S-NPs

The synthesized nanoparticles were characterized by FT-IR for the evaluation of

their composition and purity , **Fig. (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, Fig. (2,a,b,c) show the SEM images of all S-NPs samples which were prepared with TMAB surfactant.

-EDS characterization of sulfur nanoparticles.

Results in **Fig(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 **Fig. (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.

Chemical studies on sulfur nanoparticles in vitro .

Cytotoxicity:-

The in vitro cytotoxic activities of S-NPs were showed in **Fig.(5)** found to be 10.7 ng/ml, 3.7 ng/ml ,10.6ng/ml, and 3.34 ng/ml against MCF-7, HepG2, HCT116, and PC3 cell lines; respectively,

- Toxicity study and dose response curve.

For determination of the median lethal dose (LD50) 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 .

-Viability count,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 **Fig. (6. a, b).**

-life span prolongation.

The life span showed a significant increase in theraputic group by (63.63%) compared to positive control group as shown in Table (1).

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 analyzed, 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 has 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 2).

-Biochemical Investigations.

Liver function tests

Data presented in Table (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 shown 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 increased 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 shown insignificant changes in these parameters. Data also shown significant decreased in A/G ratio in plasma levels ($p<0.001$), compared to EAC bearing tumor group, as in the Table (4) and these values are within normal range after administration of S-NPs.

Kidney function tests

The mean value of urea shown significant increased in EAC bearing tumor group by 89.5% ,($p<0.001$) compared to negative control group. While, their a significantly decreased by 20.7% in treated group ($p<0.01$) compared to EAC bearing tumor group and these values are within normal range as in Table (5). The mean value of creatinine shown significant increased in EAC bearing tumor 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 tumor group, Table (5).

Heart functions

The mean value of CK-MB shown significant increased in EAC bearing tumor group by 57.9%, ($p<0.001$), compared to negative control group. While, their a significant decreased by 15.8% in treated group ($p<0.01$) compared to EAC bearing tumor group, as shown in Table (6) . Also, the mean value of LDH shown increases in EAC bearing tumor group by 41.1%, ($p<0.001$), compared to negative control group. While, their a significantly decreased by 0.12%,in treated group ($p<0.01$) compared to EAC bearing tumor group, Table(6) , and these values are within normal range.

-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 , disturbances in hormonal and immune conditions can induce cancer **(24)**. 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 **(25)**. 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 **(26)**. In this study evaluated the antitumor activity of sulphur containing compounds in Nano formulations against Ehrlisch 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 **(27)**. Nanosize sulfur particles have many important applications like in pharmaceuticals **(28)**. It appears that sulfane sulfur containing DATS can be bioreduced in cancer cells to hydroperthiol that leads to H2O2 generation, thereby influencing the transmission of signals regulating cell proliferation and apoptosis **(4)**.

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

These sulfur nanoparticles were analyzed using the IR spectram to confirm the purity .They were characterized by FT-IR for the evaluation of their composition **Fig.(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 **(28)**. 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, (Fig. 2, a-b-c) to show the SEM images of all S-NPs samples more regular shape. The SEM micrographs showed of the size distribution is unanimous nanoscale which the scale range of sulfur nanoparticles is 5–50nm as show (Fig. 2, a-b-c), other studies revealed some results using (SEM) **(8)**, by Analysis of scanning electron microscope (SEM) Sulfur nanoparticles.
The synthesized nanoparticles were characterized by EDS for the evaluation of their composition and purity, Fig. (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 **(7)**. 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 Fig. (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 **(29)**. 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. 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 , 10.6ng/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 **(30)** , 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 , **(31)** .
The acute toxicity was estimated by intraperitoneal administration of the sulfur nanoparticles 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 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 causis a reduced in count and volume of EAC concentrations other. A similar study **(32)**, 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. That toxicity effects of toxicants and therapeutic agents are dose-dependent **(33)**.
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, the dose response curve for these S-NPs demonstrated that most effective dose was found to be "5 mg /kg" .Then, these sulfur nanoparticles were tested in mouse models (EAC model) to investigate the anti-tumor, anti-oxidant, and anti-apoptotic activities. While at non-cytotoxic doses sulphur nanoparticles is eco-friendly and clinical trials show no alarming toxic effects on eukaryotes **(34)**. 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 **(35)**.

The study effect of Sulfur nanoparticles (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 x 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, ($p<0.001$), Reduction of tumor volume and viable cell count of tumor-bearing mice, tumor cell growth inhibition determined the potency of an anticancer agent **(36)**. 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 **(37)**.

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 the positive control group as in Table (1).

It is apparent that sulfur nanoparticles 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 a 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 **(38)**. 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 **(39)**. progression of tumor was accompanied by hematological changes compared to normal gradual decrease in hemoglobin content, RBC count and gradual increase in leukocytes **(40)**, 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 raties that sulfur nanoparticles possess pronounced anticancer activity with a little or no host toxic effect.

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 shown 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 increased 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 shown insignificant change in these parameters. Data also shown significant decreased in A/G ratio in plasma levels ($p<0.001$) , compared to EAC bearing tumor 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 tumor 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 **(41)**.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 tumor-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 **(42)**. 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 **(43)**. 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 (**44)**. Serum albumins are important in regulating blood volume by maintaining the oncotic pressure (also known as colloid osmotic pressure) of the blood compartment **(45).**

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 increased in EAC bearing tumor group by 89.5% ,($p<0.001$) , compared to negative control group. While, their a significantly decreased by 20.7%, in treated group ($p<0.01$) compared to EAC bearing tumor group and these values are within normal range . The mean value of creatinine showed significant increased in EAC bearing tumor 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 tumor 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 buildup 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 **(46)**. Liver and kidney toxicity induced during tumor growth may be due to the excessive production of ROS that leads to oxidative damage **(47)**. 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 pro-mote the deterioration of cardiac and/ or renal dysfunctions **(48)**.

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 increased in EAC bearing tumor group by 57.9%, ($p<0.001$), compared to negative control group. While, their a significant decreased by 15.8% , in treated group ($p<0.01$) compared to EAC bearing tumor group . Also, The mean value of LDH shown increased in EAC bearing tumor group by 41.1%, ($p<0.001$) compared to negative control group. While, their a significantly decreased by 0.12%, in treated group ($p<0.01$) compared to EAC bearing tumor group and these values are within normal range. These results in were in agreement with **(49)** . 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 tumors with poor prognosis **(50)**. Have 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 **(51)**, 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.

These results are due to that the significant increase in LDH value resulted from heart tissue damage caused by tumor growth which were in agreement with **(52)**. Who reported that activity of LDH in tumor tissue along with mammographic characteristics could help in defining aggressive breast cancers as the elevation in LDH value was suggested for the same explaination . Free radical lead to oxidative stress and ROS generation which cause cardiac tissue damage releasing this enzyme and elevating its level in the blood stream **(53)**.

Conclusion: S-NPs play an important role in improving liver functions, kidney functions and the heart functions of resulted in significantly increase activity against tumor and plays an important role in improving oxidative stress resulted in high antiproliferation activity against (MCF7-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.

Aim of the work

The present study is designed to evaluate the antitumor activity of sulphur containing compounds in Nano formulations against Ehrlisch ascites carcinoma (EAC) in female albino mice.

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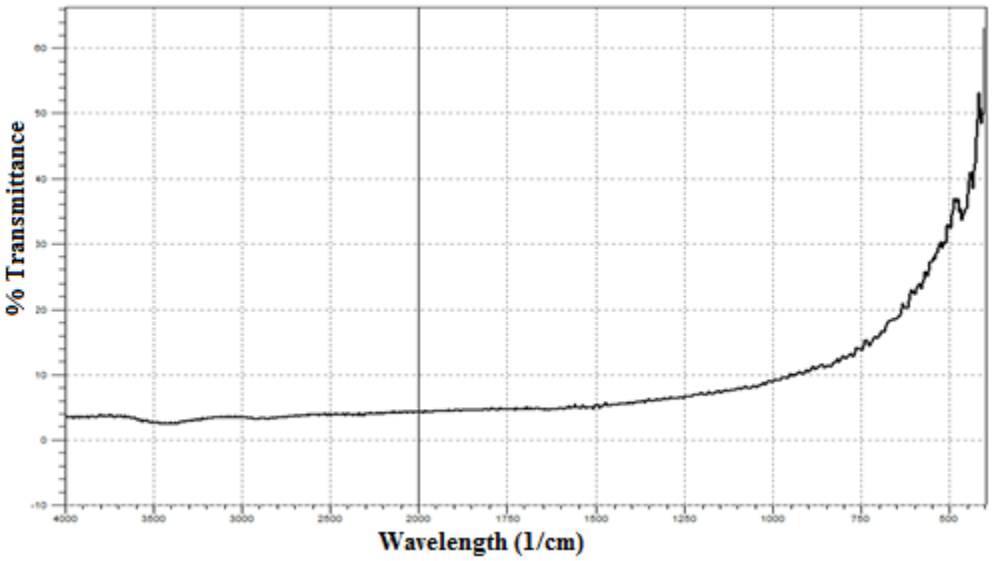


Fig(1): FT-IR spectra of sulfur nanoparticles.

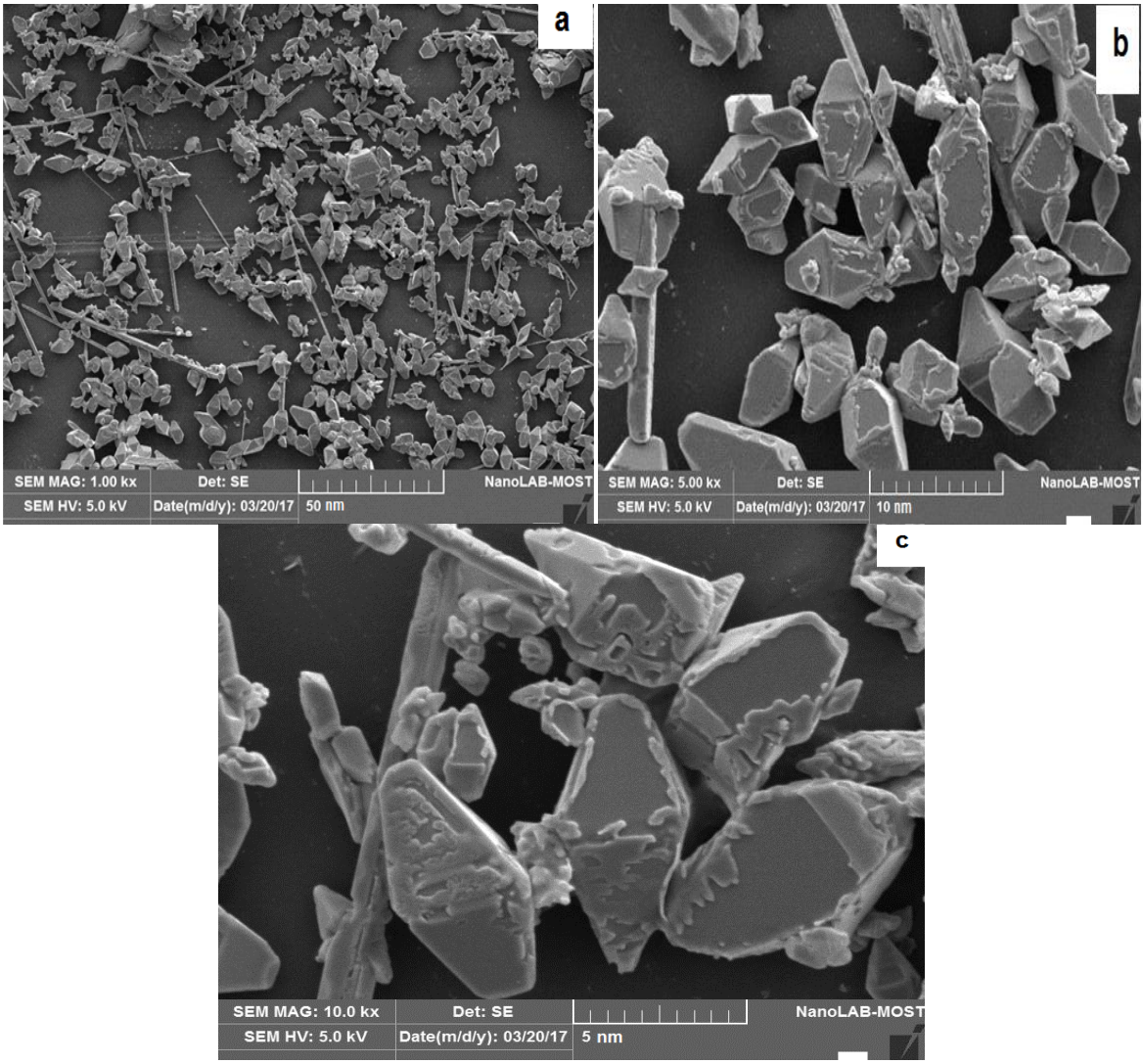


Fig (2) : SEM images of the sulfur nanoparticles (**a**)size 50nm, (**b**)size 10nm and (**c**)size 5nm.

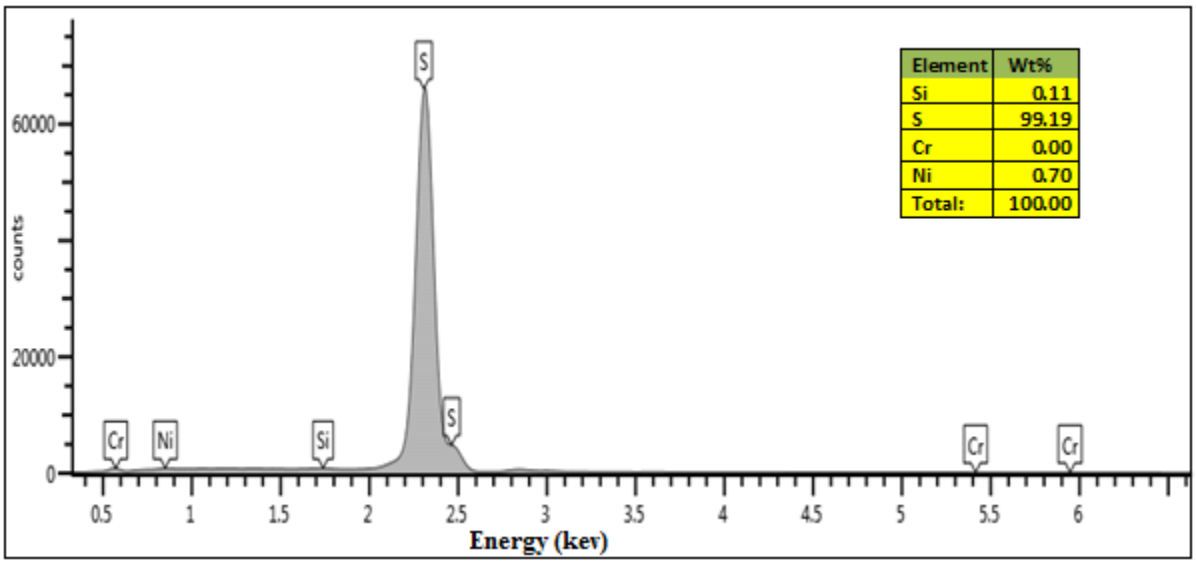


Fig (3) : EDS spectrum of the sulfur nanoparticles.

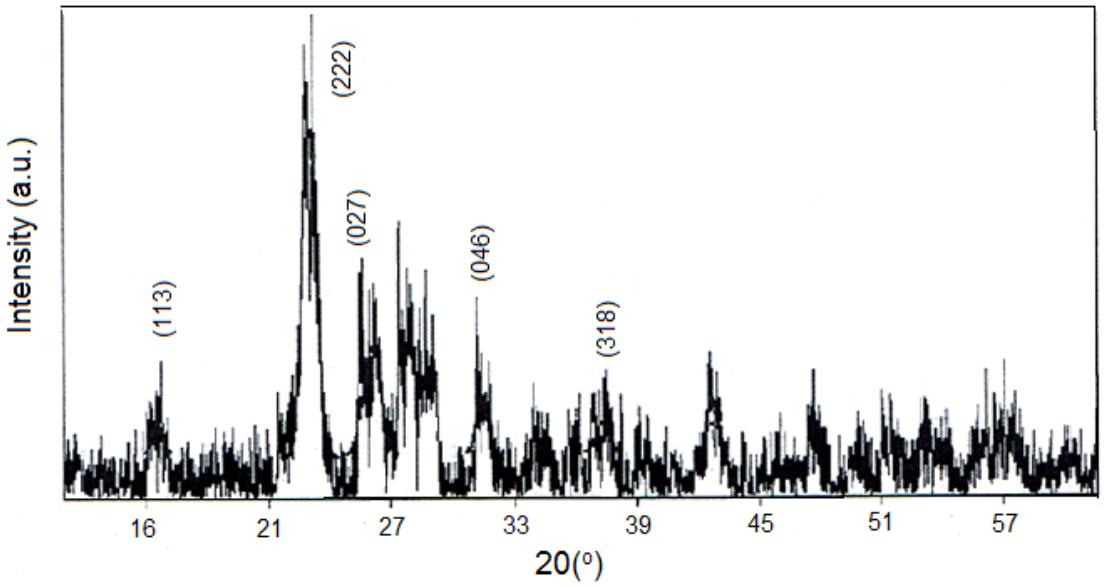


Fig (4): XRD pattern of the sulfur nanoparticles.

Table (1): Effect of sulfur nanoparticles (S-NPs) on life span prolongation .

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

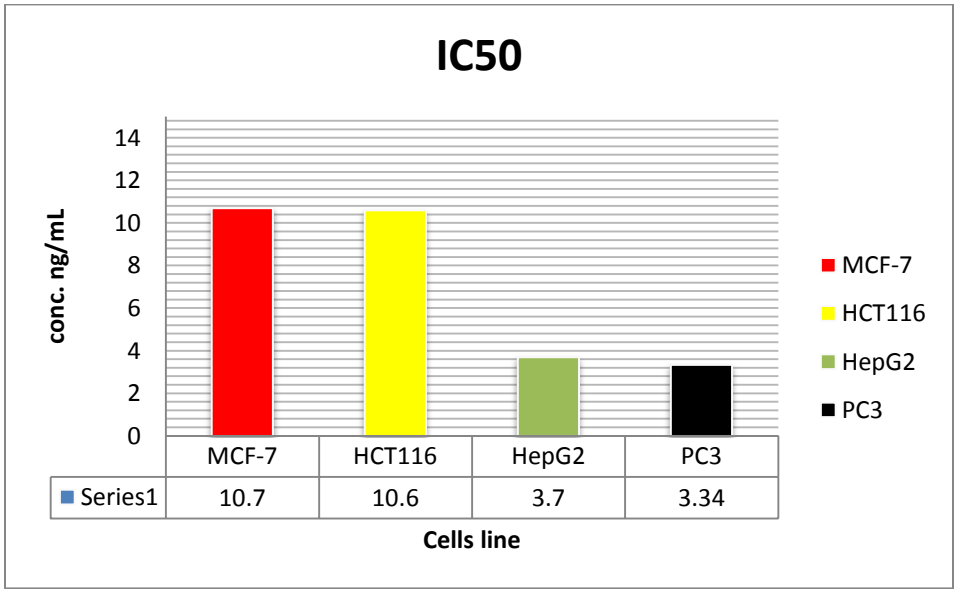


Fig (5): antitumor activity of sulfur nanoparticles in invitro study.

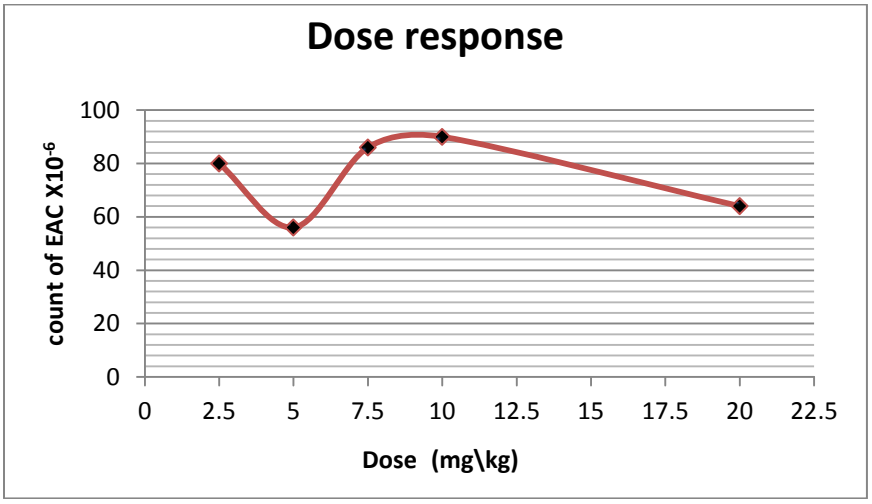
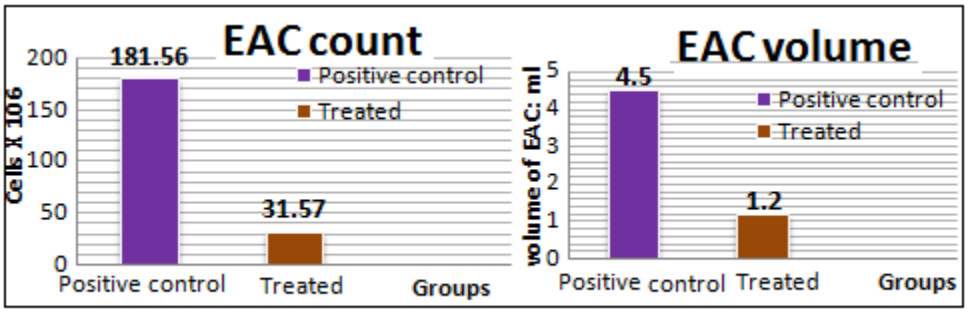


Fig (5): Dose response curve of S-NPs.



(6,a) (6,b)

Fig. (6) : 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 ($x10^6$)	Hb g/dL	HCT g/L	MCV fL	MCH Pg	MCHC g/dL	WBC ($x10^6$)	Neutrophil%	Lymphocyte %	PLT ($x10^3$)
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±1.9	52.4±4.1	455±58.5
Positive control group% Change	6.4±0.9 20.1%	7.4±0.9 38.3%	23.23±7.3 39%	46.9±2.6 0.25%	15.3±0.65 5.5%	32.2 ±0.54 5.22%	13.5± 0.67 71.9%	42.8±3.5 150%	69.5±4.2 32.6%	563 ±30.3 23.7%

Table (2): volume, count and hematological parameters in studied groups.

Thr. group Change%	**8.8± 0.68 9.7%**	**12.8± 0.48 6.6%**	**35.6± 3.4 6.9%**	**46.9± 1.4 0.25%**	**15.4± 0.37 6.2%**	**32.0± 1.17 4.5%**	**9.17± 0.65 16.8%**	**33.9± 2.3 98%**	**41.3± 4.1 40.5%**	**505± 35 10.9%**

Data were expressed as Mean± SD and percent %
-* P value < 0.01
-**P < 0.001

Table (3): Effect of sulfur nanoparticles on liver functions in plasma in all studied groups.

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(4):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 %
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 (5): 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 %
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 (6): Effect of sulfur nanoparticles on Heart 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