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Research Article
Protective Effect of SO-SeNPs against DOX-induced Liver Toxicity and its Mechanism in Rats

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Abstract

Background and Objective: The administration of DOX induces hepatotoxicity by ROS and cytokines production that result in imbalanced redox potential that leads to oxidative stress, reduced antioxidant enzyme content. This study aimed to evaluate the protective activity of SO-SeNPs in rats against liver toxicity induced by DOX. **Materials and Methods:** In the current study, particle size and zeta potential SO-SeNPs was prepared and characterized. In addition, IC_{50} and LD_{50} SO-SeNPs were calculated. The liver-protective action of SO-SeNPs against DOX-induced liver toxicity of rats was assessed by 16 adult albino rats. **Results:** The SO-SeNPs were approximately 148.14±14.68 Nm tall with -29.4±0.84 negative zeta potential. IC_{50} for the Hep-G2 cell line and LD_{50} is equal to 161.17 μg mL^{-1} and 1650 mg kg^{-1} b.wt. A significant increase in plasma ALT, asT, asT and LDH as well as liver MDA, TNF-α, IL-6 and P53 has been observed through the 30 days of daily oral administration of SO-SeNPs at 33 and 82.5 mg kg^{-1} b.wt. to rats treated with DOX (20 mg kg^{-1} b.wt.). Oral SO-SeNP administration has increased SOD, GPx and GSH, on the other hand and in rats treated with DOX. The SO-SeNPs have also nearly normalized these DOX effects in the liver tissue. **Conclusion:** Current study's biochemical, histological and MRI results showed that SO-SeNPs have liver protective activity against DOX-induced liver toxicity in rats.

Key words: SO-SeNPs, doxorubicin, liver toxicity, IL-6, TNF-α, P53

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Doxorubicin (Dox) is a chemotherapeutic medication that is used to treat malignancies of the livers, stomach, ovary, breast, thyroid, sarcoma and children[1-3]. Long-term use of Dox, on the other hand, can have substantial negative effects on non-tumour tissues, limiting its therapeutic usefulness[4]. Hepatotoxicity, nephrotoxicity and cardiotoxicity are only a few of the adverse effects and Dox's harmful effects on the reproductive system and brain system are irreversible[5-8].

Adriamycin and hepatotoxic glycosides are also metabolites of Dox via hepatic microsomal enzymes and cytoplasmic reductase[9,10]. The liver as the body's largest metabolic organ, plays a key role in metabolism, which can be readily harmed by Dox. As a result, active lead compounds against Dox-induced liver damage must be investigated.

The creation of free radicals and the creation of Reactive Oxygen Species (ROS) can cause oxidative damage to organs, which can cause drug-induced hepatotoxicity[11-13]. Furthermore, Dox-induced cardiotoxicity has been linked to the stimulation of the inflammatory response, which can be decreased by inhibiting nuclear factor κB (NF-κB)[14-16]. Some investigations have shown that Dox-derived ROS can activate caspase-3, release cytochrome c and modify tumour suppressor P53 signalling to enhance apoptosis[17-19]. As a result, one effective treatment for Dox-induced hepatotoxicity is to simultaneously reduce oxidative stress, inflammation and apoptosis.

Natural antioxidants acquired from many alternative systems of medicine have been shown in numerous studies to have a wide spectrum of biological actions. To reduce Dox-induced oxidative stress in animal models, a variety of antioxidant-rich alternatives have been utilised[7,10,11]. Many plant extracts are useful in the treatment of organ toxicity[20-22].

The herb *Sonchus oleraceus* L. is found in the main areas of fields orchards, roadsides, gardens or cleared land and is native to Europe, Northern Africa and Asia (Compositae)[23]. This plant has been used in folk medicines for the treatment of diseases like diarrhoea, enteritis, pneumonia, hepatitis, appendicitis, bronchopneumonia, icterus, swelling in the throat, hematemesis and uraemia[24]. Flavonoids (primarily luteolin and apigenin), fatty acids, phenolic acids, volatile essentials and terpene oils have resulted in phytochemical studies[25-29].

Nanoparticles can benefit patients by directly treating the disease and removing the need for blood circulation[30,31]. Researchers have observed improved drug solubility, regulated release, increased organic bioavailability, increased stability and better long-term storage[32] when drugs are encapsulated into nanoparticles (as opposed to non-encapsulated drugs). These traits appear promising and may be required for disease prevention[33,34].

This study aimed to provide an easy route for assessing the therapeutic potential of *Sonchus Oleraceus*-selenium Nanoparticles (SO-SeNPs) against doxorubicin-induced liver toxicity in determining the therapeutic value of medicinal plants.

MATERIALS AND METHODS

Study area: The current study was carried out at the Faculty of Applied Medical Sciences, October 6 University, Egypt during September, 2020.

Chemicals: Doxorubicin (DOX) was obtained from Sigma Chemical Co. (St. Louis MO, USA).

Plant material: Fresh aerial part (leaves, stem and flowers) of *Sonchus oleraceus* L. at maturity was collected from Horbite Village farms, Sharkia, Egypt. The plant was identified by Prof. Heba A. Elgizawy, Pharmacognosy Department, Faculty of Pharmacy, October 6 University.

Preparation of aqueous extract: Using a magnetic stirrer, an aqueous extract of air-dried aerial parts was prepared by dissolving a known amount of powdered air-dried aerial parts in distilled water. It was then filtered and evaporated under reduced pressure to dryness. To facilitate handling, an aqueous suspension, which is the form commonly used in folk medicine, was prepared.

Phytochemical screening: *Sonchus oleraceus* aerial parts were analyzed for alkaloids, cardiac glycosides, flavonoids, tannins, anthraquinones, saponins, volatile oil, coumarins and triterpenes[35].

Synthesis of *Sonchus Oleraceus*- Selenium Nanoparticles (SO-SeNPs): To begin, a 20 mM ascorbic acid (Vc) solution was freshly prepared by dissolving 35.2 mg powder in 10 mL water. SO-SeNPs was dissolved in deionized water and diluted in deionized water (90 mL) in a conical flask as follows: Dissolved selenious acid (H_2SeO_3, 0.013 gm., 0.01 mmol.) in 10 mL deionized water was added to the solution, with continuous stirring and heating at 60°C for 10 hrs, after which 200 mL of 40 mM ascorbic acid was added as a catalyst and the red SO-SeNPs were suspended and characterized by TEM.

SO-SeNPs characterization: The X-ray diffraction pattern of SO-SeNPs was determined at 25-28°C with nickel (Ni) (D8 Advance X-ray diffractometer) filtered using Cu (K = 1.54184 A°) radiation as the X-rayed source. The morphology and size of the SO-SeNPs were studied using a scanning electron microscope and a field transmission microscope at accelerating voltages of 15 and 200Kv, respectively.

Determination of SO-SeNPs cytotoxicity on cells: The 96 tissue plates were inoculated to form the entire monolayer sheet at 37°C with 1×10^5 cells mL^{-1} (100 uL/pitch) and incubated for 24 hrs. After a cell confluence sheet was formed, the cell monolayer was washed twice, the growth medium was decanted from 96 microplates. The tested sample was diluted double in a 2% serum RPMI medium (maintenance medium). The 0.1 mL of each dilution was tested in each well, with three wells used as checks and only maintaining medium. The platform was incubated and then examined at 37°C.

Physical signs of toxicity, such as partial or complete loss of the monolayer, rounding, shrinkage or cell granulation, were examined in the cells. MTT solution (5 mg mL^{-1} in PBS) was prepared (BIO BASIC CANADA INC).

Each well was supplemented with 20 uL MTT solution. Set the MTT thoroughly into the media on a table, 150-5 min. To allow MTT to be metabolized, incubate (37°C, 5% CO_2) for 1-5 hrs. Take the media away. (To remove residue dry plates, if necessary, on paper towels. Subsequently, resuspend formazan (MTT) in 200 uL DMSO. Formazan put in the solvent and thoroughly mixed on a shaking table for 5 min, 150 rpm. Optical density read at 560 nm and removes the bottom at 620 nm. Optical density should be correlated directly with the number of cells.

Animals: Male albino rats weighing approximately 150±10 g (84 rats, 60 for LD_{50} estimation and 24 rats for SO-SeNPs liver protective activity) were obtained from Cairo University's animal house in Giza, Egypt. The animals were kept in a light-controlled room at a temperature of 22°C and a humidity of 55-60%. The animals were kept for a week to acclimate before being fed a standard diet and given unlimited water.

Determination of LD_{50} of SO-SeNPs: Preliminary tests were performed on groups of four rats. SO-SeNPs were administered orally in various doses to determine the range of doses that cause 0-100% mortality in animals. The LD_{50} was determined in groups of ten animals by administering resveratrol nanoemulsion at different doses of 900, 1200, 1500, 1800, 2100 and 2400 mg kg^{-1}, orally. Animals were observed individually every hour for the first day and every day for the next 5 days following administration of the tested SO-SeNPs. Saganuwan's[36] method was used to calculate the LD_{50} using the following Eq:

$$LD_{50} = Dm - \left[\frac{\sum(Z\times d)}{n}\right]$$

where, Dm is the largest that kill all animals, $\sum$ is the sum of ($Z\times d$), Z is the Mean of dead animals between 2 successive groups, d is the constant factor between 2 successive doses and n is the Number of animals in each group

Experimental setup: This experiment was carried out to examine the protective effect of SO-SeNPs against DOX-induced liver toxicity. This experiment was conducted following guidelines established by the Animal Care and Use Committee of October 6th University. Adult albino rats were divided into four groups with six animals in each. The treatment groups are described in Table 1.

Blood samples were drawn from each animal's retro-orbital vein and collected in heparin-containing tubes after 30 days of treatment. The heparinized blood samples were centrifuged at 1000 xg for 20 min. Transaminases (L-alanine and L-aspartate)[38], alkaline phosphatase (ALP)[39] and lactate dehydrogenase (LDH)[40] activity were determined using separated plasma.

II-Preparation of liver samples: The liver was quickly removed after using cervical dislocation. A portion of each liver was weighed and homogenized with ice-cold saline in a glass homogenizer (Universal Lab. Aid MPW-309, mechanika precyzyjna, Poland) to make a 25% W/V homogenate. The homogenate was prepared in three aliquots. The first was deproteinized with ice-cold 12 percent trichloroacetic acid and the supernatant obtained after 1000 xg centrifugation was used to calculate GSH.

The supernatant from the second aliquot was used to calculate the levels of malondialdehyde (MDA), tumour necrosis factor- (TNF-α), interleukin 6 (IL-6) and tumour suppressor P53 (P53). The third aliquot of homogenate was used to prepare a cytosolic fraction of the liver by centrifuging it at 10500 xg for 15 min at 4°C in a cooling ultra-centrifuge (Sorvall comiplus T-880, Du Pont, USA) and the clear supernatant (cytosolic fraction) was used to determine the activities of SOD and GPx using rat ELISA kit, which is an *in vitro* enzyme- (ELISA). The test was carried out following the supplier's protocol (Rapid, Bio. Laboratories, Inc.).

Table 1: Decryption of treatment groups

Group	Group name	Treatment description
I	Normal control A	3 mL of distilled water orally for 30 days
II	DOX	Subcutaneous injection of 20 mg kg^{-1} b.wt. DOX[37] once a week for four weeks
III	SO-SeNPs+DOX	Oral suspension of 1/50 LD_{50} (33 mg kg^{-1} b.wt. SO-SeNPs) in water+subcutaneous injection of 20 mg kg^{-1} b.wt. DOX once a week for four weeks
IV	SO-SeNPs+DOX	Oral suspension of 1/20 LD_{50} (82.5 mg kg^{-1} b.wt. SO-SeNPs) in water+subcutaneous injection of 20 mg kg^{-1} b.wt. DOX once a week for four weeks

Table 2: Determination of LD_{50} of SO-SeNPs given orally in adult rats

Group number	Dose (mg kg^{-1})	No. of animals/group	No. of dead animals	(Z)	(d)	(Z×d)
1	900	10	0	0.5	300	150
2	1200	10	1	2.5	300	750
3	1500	10	4	5.0	300	1500
4	1800	10	6	7.5	300	2250
5	2100	10	9	9.5	300	2850
6	2400	10	10	0	00	7500

Z: Mean of dead animals between 2 successive groups and d: Constant factor between 2 successive doses

$$LD_{50} = Dm - \left[\frac{\sum(Z \times d)}{n}\right]$$

$$LD_{50} = 2400 - \left[\frac{7500}{10}\right] = 1650 \text{ mg kg}^{-1} \text{ b.wt.}$$

Table 3: Effect of SO-SeNPs on ALT, AST, ALP and LDH activity in rats treated with DOX

Groups	Treatment description	ALT (U L^{-1})	AST (U L^{-1})	ALP (U L^{-1})	LDH (U L^{-1})
I	Normal control A	45.76±4.67^{a}	80.75±6.50^{a}	115.40±4.37^{a}	95.40±8.44^{a}
II	DOX (20 mg kg^{-1} b.wt.)	103.67±8.60^{c}	155.90±10.45^{c}	238.08±8.73^{d}	225.74±14.37^{d}
III	SO-SeNPs (33 mg kg^{-1} b.wt.)+DOX (20 mg kg^{-1} b.wt.)	73.25±5.27^{b}	106.50±9.44^{b}	142.80±9.64^{c}	130.47±11.25^{c}
IV	SO-SeNPs (82.5 mg kg^{-1} b.wt.)+DOX (20 mg kg^{-1} b.wt.)	56.40±5.98^{a}	92.70±7.55^{a}	121.38±6.55^{b}	103.65±8.64^{b}

Data shown are mean±standard deviation of number of observations within each treatment. Data followed by the same letter are not significantly different at $p \leq 0.05$. ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, ALP: Alkaline phosphatase and LDH: Lactate dehydrogenase

Histological assessment: For histological examination, the liver was cut into pieces and fixed in a 10% buffered formaldehyde solution. The fixed tissues were processed with an automated tissue processing machine. Standard techniques were used to embed tissues in paraffin wax. The Bancroft and Steven method[41] was used to prepare 5 mL thick sections that were stained with hematoxylin and eosin for light microscopy analysis. The sections were then examined under a microscope for histopathological changes and photomicrographs were taken.

MRI protocol: Once placed on the handling platform, each mouse was fixed in a supine recumbence position and then introduced into the RF coil inside the MRI gantry. Many images and sequences are taken for 3 rats/group to evaluate and compare the results, including CORONAL T1, T2, SAGITAL T1, T2 and STAIR.

Statistical analysis: The results were expressed as mean±SD for each of the eight separate determinations. All the data were statistically analyzed using SPSS/18 software[42]. To test hypotheses, a one-way analysis of variance was used, followed by the least significant difference test ($p \leq 0.05$).

RESULTS

TEM analysis shows that SO-SeNPs had the size of around 148.14±14.68 nm with a negative zeta potential of −29.4±0.84 in Fig. 1.

Cultures were exposed to SO-SeNPs at doses of 31.2, 62.5, 125, 250, 500 and 1000 μg mL^{-1} to determine the effects of SO-SeNPs on liver cancer cells and normal cells. Dimethyl sulfoxide (DMSO, 0.1%) was used as the negative control. After 48 hrs, a dose-dependent reduction in cancer cell viability (IC_{50} value Hep-G2 cells = 161.7 μg mL^{-1} in Fig. 2 (a-b).

The results are reported in Table 2 showed that oral administration of SO-SeNPs in doses of 900, 1200, 1500, 1800, 2100 and 2400 mg kg^{-1} b.wt. resulted in mortalities of 0, 1, 4, 6, 9 and 10 rats, respectively. The dose of SO-SeNPs that killed half of the rats (LD_{50}) was 1650 mg kg^{-1} b.wt.

Plasma ALT asT, ALP and LDH activity are shown in Table 3. DOX (20 mg kg^{-1} b.wt.) i.p. administration resulted in

a significant increase in ALT asT, ALP and LDH activity when compared to the normal control group ($p>0.05$), indicating acute liver toxicity. When compared to the DOC-treated group, SO-SeNPs at 33 and 82.5 mg kg^{-1} b.wt. significantly reduced ALT asT, ALP and LDH activity ($p<0.05$).

Table 4 showed that DOX (20 mg kg^{-1} b.wt.) treated rats had significantly higher levels of MDA, TNF-α, IL-6 and P53 ($p>0.05$) when compared to the normal control group ($p = 0.05$). After 4 weeks, the administration of SO-SeNPs at 33 and 82.5 mg kg^{-1} b.wt. resulted in significantly lower MDA, TNF-α, IL-6 and P53 levels compared to the DOX-treated group of rats ($p<0.05$).

The activities of liver antioxidant parameters have significantly decreased ($p<0.05$) in Table 5 In comparison to the normal control group ($p<0.05$), SOD, GPx and GSH were observed in DOX-treated rats indicating acute liver toxicity. SO-SeNPs 33 and 82.5 mg kg^{-1}, respectively, significantly

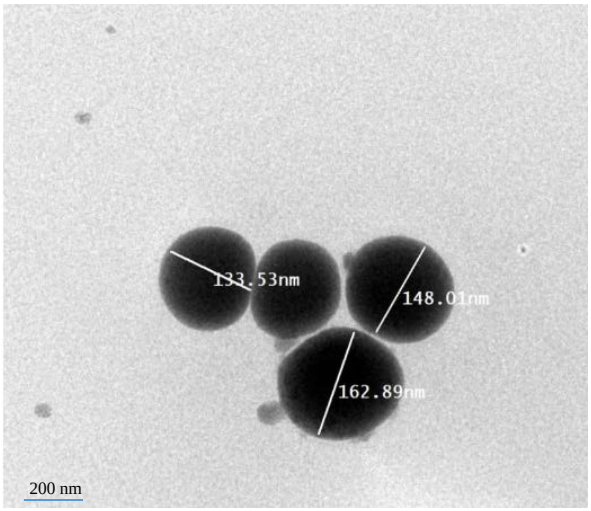

Fig. 1: TEM analysis of SO-SeNPs

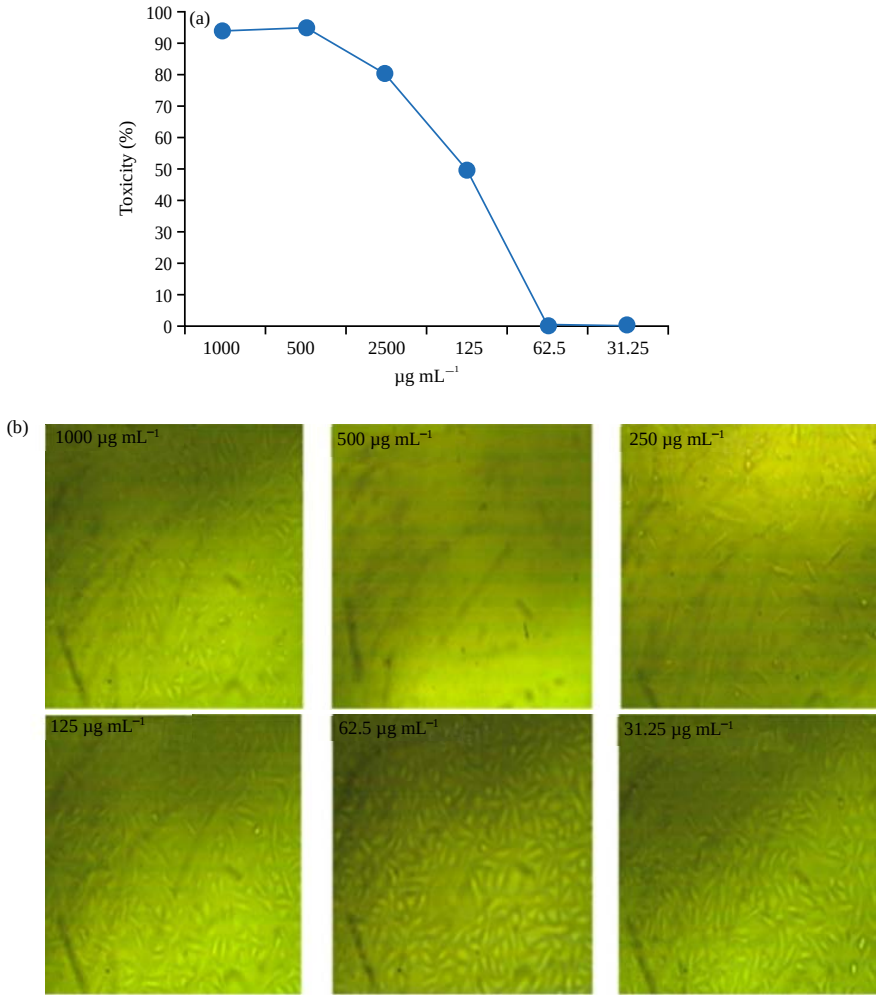

Fig. 2: Determination of SO-SeNPs cytotoxicity on cells (MTT protocol)

(a) Percentage of viability of liver cancer cells (Hep-G2) after treatment with of SO-SeNPs was assessed by the MTT assay after 48 hrs of treatment with DMSO

(b) Effect of SO-SeNPs on Hep-G2 liver carcinoma cell line at different concentrations

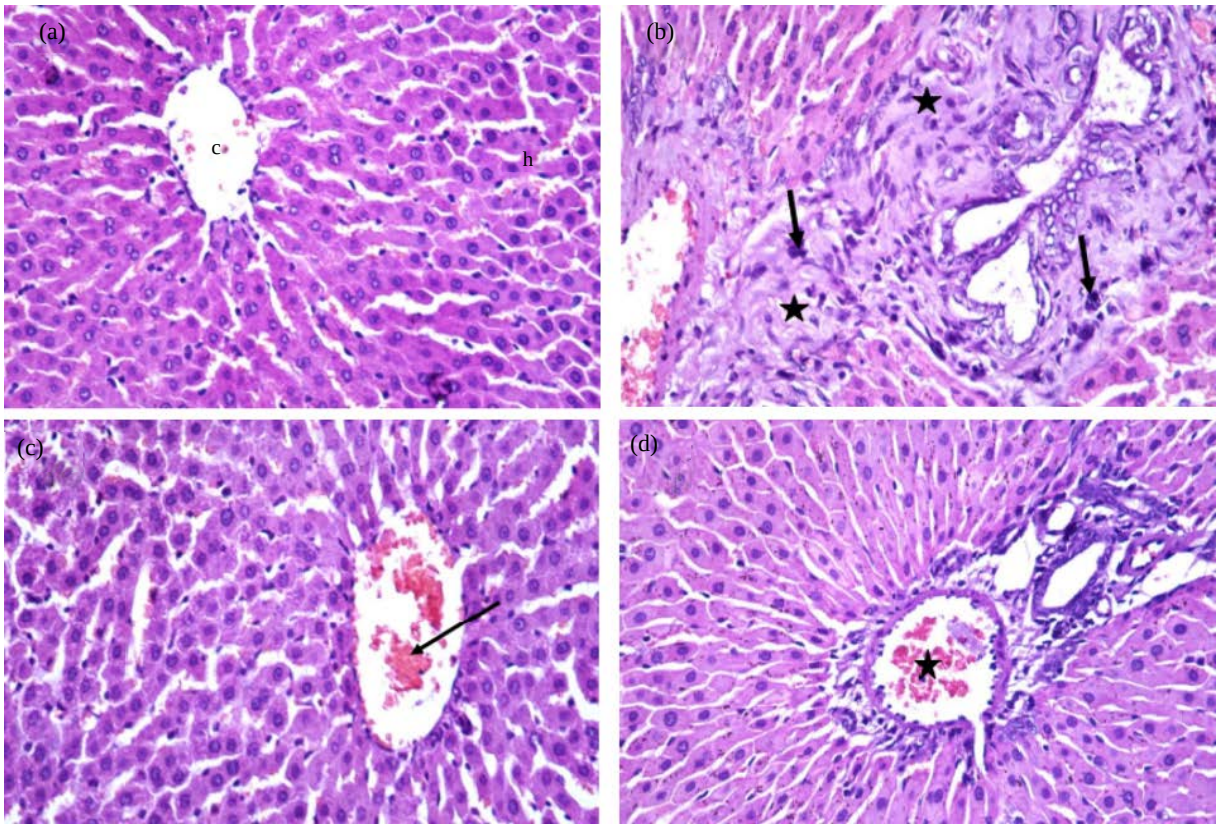

Fig. 3: Sections stained with hematoxylin and eosin (H and E, 400 X) histological examination of rats' liver of different groups compared to control group

(a) Group I normal control, (b) Group II DOX (20 mg kg-1 b.wt.), (c) Group III was administrate SO-SeNPs (33 mg kg-1 b.wt.)+DOX (20 mg kg-1 b.wt.), (d) Group IV was administrate SO-SeNPs (82.5 mg kg-1 b.wt.)+DOX (20 mg kg-1 b.wt.), Arrow: Congested hepato-portal blood vessel and Star: Leukocytic cells infiltration

Table 4: Effect of SO-SeNPs on levels of liver MDA, TNF-α, IL-6 and P53 in rats treated with DOX

Groups	Treatment description	MDA (nmol mg^{-1} tissue)	TNF-α (pg mg^{-1} tissue)	IL-6 (pg mg^{-1} tissue)	P53 (pg mg^{-1} tissue)
I	Normal control A	4.86±0.35^{a}	85.34±9.55^{a}	165.48±10.54^{a}	18.54±2.72^{a}
II	DOX (20 mg kg^{-1} b.wt.)	10.48±0.73^{c}	275.90±15.20^{c}	438.06±19.80^{c}	42.37±3.87^{c}
III	SO-SeNPs (33 mg kg^{-1} b.wt.) +DOX (20 mg kg^{-1} b.wt.)	7.99±0.85^{b}	125.48±11.70^{b}	210.70±22.65^{b}	27.60±3.00^{b}
IV	SO-SeNPs (82.5 mg kg^{-1} b.wt.) +DOX (20 mg kg^{-1} b.wt.)	4.86±0.65^{a}	92.10±7.42^{a}	183.66±16.83^{a}	20.84±2.65^{b}

Data shown are mean±standard deviation of the number of observations within each treatment. Data followed by the same letter are not significantly different at $p \leq 0.05$. MDA: Malondialdehyde, TNF-α: Tumour necrosis factor-α, IL-6: Interleukin 6 and P53: Tumour suppressor P53

Table 5: Effect of SO-SeNPs on levels of liver SOD, GPx and GSH in rats treated with DOX

Groups	Treatment description	SOD	GPx	GSH (mg (%))
I	Normal control A	18.66±1.65^{c}	25.40±2.16^{c}	17.64±1.54^{c}
II	DOX (20 mg kg^{-1} b.wt.)	7.22±0.43^{a}	9.70±1.00^{a}	9.88±0.66^{a}
III	SO-SeNPs (33 mg kg^{-1} b.wt.)+DOX (20 mg kg^{-1} b.wt.)	12.87±0.58^{b}	16.38±1.06^{b}	13.70±0.93^{b}
IV	SO-SeNPs (82.5 mg kg^{-1} b.wt.)+DOX (20 mg kg^{-1} b.wt.)	17.90±1.06^{c}	22.75±2.11^{bc}	15.44±0.98^{bc}

Values are given as mean±SD for groups of six animals each. Values Data followed by the same letter are not significantly different at $p \leq 0.05$. SOD: Superoxide dismutase, GPx: Glutathione peroxidase, GSH: Reduced glutathione, SOD: One unit of activity was taken as the enzyme reaction, which gave 50% inhibition of NBT reduction in 1min/mg protein and GPx: μg of GSH consumed/min mg protein

improves treatment ($p<0.05$) as opposed to the DOX-treated group, of the liver, enzymes SOD, GPx and GSH activities.

As shown in Fig. 3a, histopathological examination of liver sections of the normal group (I) showing normal hepatic parenchyma, note the normal polyhedral hepatocytes (h), blood sinusoids and central vein (c), (H and E X400).

On the other hand, in the liver of DOX-treated control group (II), showing changes in the portal area, note the

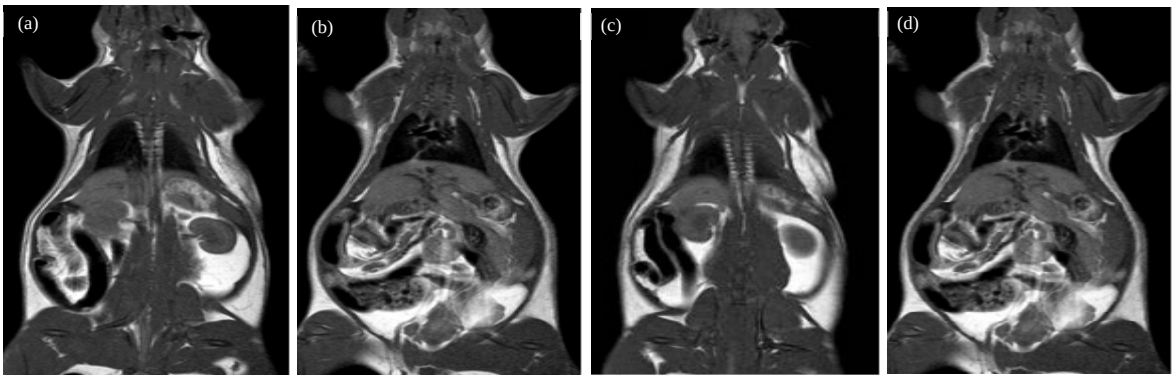

Fig. 4: Magnetic resonance imaging (MRI) examination of rats' liver of different groups compared to control group
(a) Group I normal control, (b) Group II DOX (20 mg kg-1 b.wt.), (c) Group III was administrate SO-SeNPs (33 mg kg-1 b.wt.)+DOX (20 mg kg-1 b.wt.) and (d) Group IV was administrate SO-SeNPs (82.5 mg kg-1 b.wt.)+DOX (20 mg kg-1 b.wt.)

congested hepato-portal blood vessel (arrowhead), hyperplastic bile duct (arrow) and leucocytic cells infiltration (*), (H and E X200) (H and E X200) in Fig. 3b.

The history study also showed mild blood vessel congestions of the hepatoportal (*) without infiltration of fibrosis or leukocytic cells in SO-SeNPs 33 mg kg^{-1} b.wt. treated rats. Groups III and healthy and organized hepatic cords in the central vein with slight congestion (arrow) showed almost the same data as group IV in comparison with the group DOX-treated with SO-SeNPs82.5 mg kg^{-1} b.wt. in Fig. 3c and d.

In the MRI examination of normal group (I) liver, parenchyma was shown to be free of all weights with regular boundaries, homogeneous and with normal intensity. Normal sagittal, T1, T2 and STAIR coronary showed in Fig. 4a.

In addition, the liver of the DOX-treated control group (II), MRI, showed an irregular, heterogeneous, alternative-intensity liver mass in Fig. 4b.

The MRI was shown to have a ground texture and the defective focal lesion has regressed with SO-SeNPs 33 mg kg^{-1} b.wt. compared to DOX treated rats (group III) at various levels of DOX treated rats in Fig. 4c. Furthermore, the MRI of the liver of DOX-treated rats which administrated orally SO-SeNPs 82.5 mg kg^{-1} b.wt. showed marked improvement in the group (IV) without an injury and there were no assessments of inflammatory cells in Fig. 4d.

DISCUSSION

In the present study, SO-SeNPs was prepared to evaluate their cytotoxicity against Hep-G2 cell line and showed that the IC_{50} of SO-SeNPs against Hep-G2liver carcinoma cell line = 161.17 μg mL^{-1}. Also, Yin *et al.*[43] reported that hot aqueous extract of *Sonchus oleraceus* had inhibitory effects on the liver cancer cell line HepG-2 and K562 cells by decreasing cell sustainability and prompting apoptosis which could block the tumour cell cycle. Khan[20] investigated the cytotoxicity of *Sonchus oleraceus* showed moderate inhibition of Hep-G2 and HCT-116 cells, while *Sonchus oleraceus* showed low inhibition against all cells (Hep-G, HCT-116 and MCF-7). The cytotoxic effect of both cold and hot aqueous extracts on AMN-3 and Hep-2 cell lines was higher than that of the ethanolic extract[44].

The DOX (20 mg kg^{-1}) has been given once a week for four weeks in the current study. The results have demonstrated that the increased plasma effects of ALT asT, ALP and LDH have been shown to have toxic to the same dose and have confirmed the toxicity of DOX without death[45,46]. In addition, DOX toxicity[47] is demonstrated by the elevation of cytokines and the oxidative stress indicator (MDA).

Also oral administration of SO-SeNPs at doses of 33 and 82.5 mg kg^{-1} b.wt. provided significant protection against DOX-induced liver toxicity. These effects could be attributed to the presence of terpenes, sterols, flavonoids and coumarins, which have been linked to a significant potential for human health benefits[20].

It was found that, an increase in TNF-α, IL-6, P53 and oxidative stress marker (MDA) in DOX-treated rats. Current findings corroborate those of Michela *et al.*[48] and Rong *et al.*[49] who discovered an increase in inflammatory mediators in DOX-treated rats.

The current findings show that SO-SeNPs could normalize liver levels of TNF-α, IL-6, P53 and MDA in the DOX-treated group. Wu *et al.*[49] and Li *et al.*[50] reported anti-inflammatory effects of *Sonchus oleraceus* extract.

Free radicals, oxidative stress and lipid peroxidation have all been linked to organ damage, according to evidence[51].

Increased liver TNF-, IL-6 and P53 concentrations as well as decreased activity of SOD, GPx and GSH has been shown to induce mitochondrial toxicity and free radical generation in chronic liver toxicity[52].

SO-SeNPs are a powerful Reactive Oxygen Species (ROS) scavenger[53] and normalized the oxidative stress biomarkers SOD, GPx, GSH and MDA, resulting in decreased oxidative stress, which contributes to DOX's suppression of liver inflammation.

SO-SeNPs have a liver-protective effect, according to histological and MRI studies. Because liver proliferation is an early event in toxicity-related changes, the attenuation of liver injury and fibrosis in rats by SO-SeNPs could be associated with a reduction in the inflammatory response. To the best of our knowledge, the prophylactic effect of SO-SeNPs against DOX-induced liver toxicity has never been reported and this study may be the first of its kind.

CONCLUSION

The current study found that SO-SeNPs have potent liver protective activity against DOX-induced liver toxicity by normalizing the levels of oxidative stress biomarkers and inflammatory mediators.

SIGNIFICANCE STATEMENTS

This study discovers the protective activity of SO-SeNPs that can be beneficial for the treatment of DOX-induced liver toxicity. This study will help the researcher to uncover the critical areas that focus on evaluate of SO-SeNPs as a promising new agent in the treatment of a certain type of liver inflammation that many researchers were not able to explore. Thus, a new theory to explain the correlation between the protective activity of SO-SeNPs and the degree of cytokines suppression in liver tissue may be arrived at.

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