Effect of sage oil on tamoxifen-induced hepatotoxicity and nephrotoxicity in female rats

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Background and objective
Sage oil has anti-inflammatory and antioxidant activities. The current study was designed to evaluate the efficacy of sage oil at two different doses against hepatotoxicity and nephrotoxicity induced by tamoxifen in female rats.
Materials and methods
A total of 56 female Wistar albino rats were divided into control, sage control, tamoxifen-treated rats (45 mg/kg body weight), and rats treated with tamoxifen along with sage oil (0.2 ml/kg or 0.4 ml/kg). Each dose of sage oil was used as a protective (before tamoxifen) and as a therapeutic (after tamoxifen) treatment. At the end of the experiment, serum levels of tumor necrosis factor-alpha, as well as liver and kidney function biomarkers, were measured. Levels of lipid peroxidation (malondialdehyde), nitric oxide, and reduced glutathione and the activities of glutathione peroxidase and Na^+/K^+-ATPase were measured in liver and kidney homogenates. Sections of liver and kidney were examined for histopathological changes.
Results and conclusion
Tamoxifen-induced hepatic and renal impairment was evident from the significant elevation in alkaline phosphatase, aspartate aminotransferase, and alanine aminotransferase activities and bilirubin, urea, and creatinine levels. Furthermore, tamoxifen significantly increased serum tumor necrosis factor-alpha, as well as hepatic and renal malondialdehyde and nitric oxide levels. This was accompanied by a significant decrease in the levels of glutathione and the activities of glutathione peroxidase and Na^+/K^+-ATPase in liver and kidney. Treatment with sage oil at high dose (0.4 ml/kg) ameliorated almost all the biochemical and histopathological changes induced by tamoxifen in liver and kidney. In conclusion, sage oil has the ability to attenuate hepatotoxicity and nephrotoxicity induced by tamoxifen.

Keywords:
hepatotoxicity, nephrotoxicity, oxidative stress, sage oil, tamoxifen, tumor necrosis factor-alpha

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Introduction

Breast cancer, the most common disease among women in the world, is the second main cause of cancer death in this population [1]. There are several risk factors for developing breast cancer including obesity; use of oral contraceptives; unhealthy diets; sedentary lifestyles; hormone replacement therapy; menopause; and exposure to ionizing radiation, chemical substances, and environmental pollution [2]. Some of these risk factors are present in our population, putting our women at higher risks of having breast cancer.

Tamoxifen is an anti-estrogen nonsteroidal drug used in breast cancer therapy and also a chemopreventive drug in breast cancer-prone women [3]. Tamoxifen exerts its estrogen-antagonist effect by competing with estrogen for estrogen receptors (ER) in tumor tissues, thereby decreasing the risk of recurrence after the surgical removal of a primary ER-positive breast tumor [4]. Moreover, tamoxifen undergoes metabolic activation in the liver under the action of cytochrome *P450* enzymes, yielding 4-hydroxytamoxifen and endoxifen metabolites, which are responsible for the antitumor activity of tamoxifen [5]. Owing to the long-term treatment and widespread usage of tamoxifen, several adverse effects, especially hepatic and renal toxicity, were observed [5,6]. Tamoxifen toxicity was due to its reduction of pentose phosphate pathway, thereby enhancing oxidative stress and cellular injury [6]. Tamoxifen also induces overproduction of reactive oxygen species (ROS) during its metabolism [7]. The excessive production of ROS causes damage to cellular

and mitochondrial membranes, release of apoptotic factors into the cytoplasm, and activation of caspase, leading to apoptosis [8]. Therefore, there is a growing need to reduce the adverse effects of chemotherapeutic drugs. One of the strategies that may alleviate the adverse effects induced by tamoxifen is by reducing the free radical production. Natural antioxidants that inhibit ROS generation could be used to protect liver and kidneys against tamoxifen-induced injury.

The *Salvia* genus, the biggest member of the Lamiaceae or Mint family, contains more than 900 species worldwide [9]. *Salvia officinalis* (commonly known as sage), native to the Mediterranean, is used globally in traditional medicine and as flavoring spices [10]. Based on their observed pharmacological and curative efficacy, many studies have shown that sage species in many countries of Asia and the Middle East could be considered for drug development [10,11]. Sage essential oils have long been used in traditional medicines to treat a broad spectrum of diseases, such as digestive, cardiovascular, nervous, respiratory, metabolic, and endocrine diseases [11]. Sage oil possesses several pharmacological activities, such as anti-mutagenic, anti-inflammatory, antioxidant, hepatoprotective, and nephroprotective effects [12–14]. It has been reported that 1,8-cineole, camphor, α-thujone, β-thujone, borneol, and α-pinene are the main active constituents of sage oil [11]. These active ingredients have shown high antioxidant and free radicals scavenging activities [15].

Considering that sage oil has anti-inflammatory and antioxidant activities, the current study was designed to evaluate the protective and therapeutic effect of sage oil, at two different doses, against hepatotoxicity and nephrotoxicity induced by tamoxifen in female rats.

Materials and methods

Animals

A total of 56 female Wistar albino rats weighing 150–175 g obtained from the Animal House Colony of the National Research Centre, Giza, Egypt, were used in the present study. The animals were housed in stainless steel cages with *ad libitum* access to standard laboratory diet and tap water in a temperature-controlled (20–25°C) and artificially illuminated (12 h dark/light cycle) room free of any chemical contamination. Animal procedures were approved by the Ethics Committee of the National Research Centre (with ethical approval number of AR110506) and were performed in compliance with the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals (publication no. 85-23, revised 1985).

Chemical and drugs

Tamoxifen citrate (Nolvadex) was purchased from AstraZeneca UK Limited (Macclesfield, Cheshire, United Kingdom). It was dissolved in distilled water. Sage oil was purchased from Harraz Herbal Drugstore (Cairo, Egypt).

Gas chromatography/mass spectrometry analysis

The analysis of the oil was carried out using gas chromatography/mass spectrometry (GC-MS) instrument (Model QP-2010 Ultra; Shimadzu Co., Kyoto 604-8511, Japan), equipped with head space AOC-5000 auto injector. The chromatographic separation was performed by using a RTX-5 column, 5% diphenyl, 95% dimethyl-polysiloxane, and 30 m×0.25 mm ID and 0.25-μm film thickness, fused-silica capillary column (Restek, Bellefonte, Pennsylvania, USA), with helium as a carrier gas at a flow rate of 0.99 ml/min. The gas chromatograph was coupled to a QP-2010 ultra MS detector. The sample was injected in 5-μl volume in splitless mode. The oven temperature was programmed to start at 40°C for 2 min, whereas the final temperature was 210°C for 5 min at a rate of 5°C/min.

Animals were divided randomly into eight groups (seven rats per group). The first group represents the control untreated group that received daily oral administration of distilled water. Rats in second and third groups (SAG 0.2 and SAG 0.4) received a daily oral administration of sage oil at 0.2 and 0.4 ml/kg, respectively, for 10 days by gavage [16]. Animals in the fourth group (TAM) received a daily oral administration of tamoxifen (45 mg/kg body weight) by gavage for 10 days [17]. In the fifth and sixth groups (SAG 0.2/Pr and SAG 0.4/Pr), rats were given sage oil at 0.2 and 0.4 ml/kg, respectively followed by tamoxifen with 1-h interval for 10 days. Rats in seventh and eighth groups (SAG 0.2/Tr and SAG 0.4/Tr) were given tamoxifen daily for 10 days, and then on the 11th day, animals were treated with sage oil at 0.2 and 0.4 ml/kg, respectively, for 10 days.

Preparation of samples

At the end of the experiment, all animals were killed by sudden decapitation after light anesthesia with ether, and blood samples were collected and centrifuged at 3000 rpm for 15 min at 4°C to separate sera. Serum was stored at −20°C till the measurement of tumor necrosis factor-alpha (TNF-α) and assessment of liver and

kidney functions. Kidney and liver of each rat were immediately dissected out, washed with saline, and a part of the liver tissue and the right kidney was fixed immediately in 10% formalin saline for histological study. Each of the other part of the liver tissue and the left kidney were weighed and homogenized in Tris-HCl buffer (pH 7.4). The homogenate was centrifuged at 3000 r/min for 10 min at 4°C, and the supernatant was stored at −20°C till the measurement of Na^+/K^+-ATPase and oxidative stress parameters.

Assessment of liver functions

Activities of alanine and aspartate aminotransferases (ALT and AST) and alkaline phosphatase (ALP) were measured using kits purchased from Biodiagnostic Company (Cairo, Egypt) based on methods of Reitman and Frankel [18] and Belfield and Goldberg [19], respectively. The serum levels of total and direct bilirubin were measured using kit supplied by Biodiagnostic Company according to the method of Walter and Gerade [20]. The indirect bilirubin levels were calculated as the difference between total and direct bilirubin levels.

Assessment of kidney functions

Urea and creatinine were estimated in serum spectrophotometrically using kits supplied from Biodiagnostic Company according to the methods described by Fawcett and Scott [21] and Schirmeister *et al.* [22], respectively.

Estimation of lipid peroxidation and nitric oxide

Lipid peroxidation in terms of malondialdehyde (MDA) formation was estimated according to the method of Ruiz-Larrea *et al.* [23].

Nitric oxide (NO) level in liver and kidney was indirectly assessed by measuring the nitrite levels by using a colorimetric method based on the Griess reaction [24].

Estimation of reduced glutathione and glutathione peroxidase

Reduced glutathione (GSH) was estimated according to the method of Beutler *et al.* [25] using Ellman's reagent.

The assay of glutathione peroxidase (GPx) was carried out using kit purchased from Biodiagnostic Company based on the spectrophotometric method described by Paglia and Valentine [26].

Estimation of Na^+/K^+-ATPase activity

Na^+/K^+-ATPase activity was measured spectrophotometrically in hepatic and renal tissue homogenates according to Bowler and Tirri [27] as described by Tsakiris *et al.* [28].

Estimation of serum tumor necrosis factor-alpha

TNF-α was measured in serum using rat TNF-α ELISA kit obtained from Glory Science Co. Ltd (Hangzhou, Zhejiang, China). The developed color was read at 450 nm.

Histopathological examination

The liver and kidneys of different groups were dissected and immediately fixed in 10% formalin saline. Sections of 4-μm thickness were cut by microtome from paraffin blocks. The sections were stained with hematoxylin and eosin and then investigated with light microscope for pathological investigation.

Statistical analysis

All results were expressed as means±SEM. Statistical difference between the groups was tested by one-way analysis of variance followed by post-hoc test using Duncan. Difference was considered significant at P value less than or equal to 0.05. Statistical Package for Social Sciences software (Chicago, Illinois 60606-6307, USA) (version 16) was used for all statistical calculations.

Results

Results of gas chromatography/mass spectrometry analysis

As shown in Table 1 and Fig. 1, GC/MS analysis revealed that the main constituents of sage oil were 1,8-cineole (59.75%), camphor (10.64%), α-pinene (6.60%), camphene (3.59%), α-terpineol (2.18%), (+)-aromadendrene (2.00%), 2β-pinene (1.60%), trans-caryophyllene (1.57%), cymene (1.35%), borneol L (1.30%), DL-limonene (1.10%), and α-terpinenyl acetate (1.01%). Moreover, other ingredients including linalyl propanoate (0.95%), veridiflorol (0.72%) β-myrcene (0.62%), 3-thujanone (0.56%), (−)-caryophyllene oxide (0.50%), methyl chavicol (0.45%), terpinen-4-ol (0.40%), α-fenchyl acetate (0.38%), β-thujone (0.37%), linalool (0.36%), methyl eugenol (0.35%), 3-pinanone (0.30%), α-humulene (0.27%), linalyl acetate (0.23%), ledene (0.22%), selina-3,7(11)-diene (0.22%) and 1 s,cis-calamenene (0.18%) were detected in low concentration.

Liver function biomarkers

Daily tamoxifen administration significantly increased ALP (+302.90%), ALT (+96.54%), and AST (+121.95%) activities. Moreover, tamoxifen administration induced significant elevation in total bilirubin (+695.45%), direct bilirubin (+445.45%), and indirect bilirubin (+654.55%) levels as compared with

Table 1 Gas chromatography/mass spectrometry analysis of sage oil

Peak	Retention time	Constituent name	% area
1	5.718	α-pinene	6.60
2	6.243	Camphene	3.59
3	7.285	2β-pinene	1.60
4	7.887	β-myrcene	0.62
5	9.316	Cymene	1.35
6	9.453	DL-limonene	1.10
7	9.601	1,8-cineole	59.75
8	12.949	Linalool	0.36
9	13.080	3-thujanone	0.56
10	13.591	β-thujone	0.37
11	14.856	Camphor	10.64
12	15.675	3-pinanone	0.30
13	16.037	Borneol L	1.30
14	16.116	Linalyl propanoate	0.95
15	16.523	Terpinen-4-ol	0.40
16	17.233	α-terpineol	2.18
17	17.573	Methyl chavicol	0.45
18	20.202	Linalyl acetate	0.23
19	21.537	α-fenchyl acetate	0.38
20	24.402	α-terpinenyl acetate	1.01
21	27.084	Methyl eugenol	0.35
22	27.215	trans-caryophyllene	1.57
23	27.851	Selina-3,7(11)-diene	0.22
24	28.041	(+)-Aromadendrene	2.00
25	28.669	α-humulene	0.27
26	30.440	Ledene	0.22
27	31.628	1 S,cis-calamenene	0.18
28	34.012	(–)-Caryophyllene oxide	0.50
29	34.413	Veridiflorol	0.72

control rats. Both protection and treatment with sage oil at 0.2 and 0.4 ml/kg succeeded in improving the increased activities of ALP, ALT, and AST induced by tamoxifen. Furthermore, only treatment with sage oil at 0.2 and 0.4 ml/kg succeeded in normalizing serum levels of total, direct, and indirect bilirubin (Table 2).

Kidney function biomarkers

Daily oral administration of tamoxifen induced a significant increase in serum levels of urea and creatinine, recording +139.23 and +644.44%, respectively, more than control values. Protection with sage oil at 0.4 ml/kg prevented the increase in urea level induced by tamoxifen. However, protection with sage oil at 0.2 ml/kg did not induce a similar effect. On the contrary, treatment with sage oil at 0.2 and 0.4 ml/kg restored the increased urea level to normal value. Both protection and treatment with either of the two doses of sage oil reduced the increase in creatinine induced by tamoxifen to a level that was still higher than the control level (Table 3).

Oxidative stress parameters

As shown in Table 4, the daily oral administration of tamoxifen for 10 days induced a significant elevation in hepatic and renal MDA (+133.86 and +94.31%, respectively) and NO (+116.67 and +313.04% respectively) levels above the control values. These findings were accompanied by a significant decrease in GSH (–58.26 and –52.96%) levels as well as the activity of GPx (–71.98 and –72.01%) in liver and kidney, respectively, as compared with control group. Both protection and treatment with sage oil at 0.2 and 0.4 ml/kg normalized the increased MDA and NO and improved the decreased GSH level and GPx activity induced by tamoxifen in hepatic tissues. In renal tissue, although sage oil (0.2 and 0.4 ml/kg) used before or after tamoxifen reduced the increased NO and the decreased GSH and GPx induced by tamoxifen, their levels were still higher than control values. However, only treatment with the two doses of sage oil restored the increase in renal MDA induced by tamoxifen.

Na^+/K^+-ATPase activity

The daily oral administration of tamoxifen for 10 days significantly decreased the hepatic and renal Na^+/K^+-ATPase, recording –46.15% and –53.57%, respectively, below control values. Both protection and treatment with sage oil at low and high dose normalized the significant decrease induced by tamoxifen in Na^+/K^+-ATPase, except for a significant decrease in hepatic Na^+/K^+-ATPase of rats protected with sage oil at low dose (Table 5).

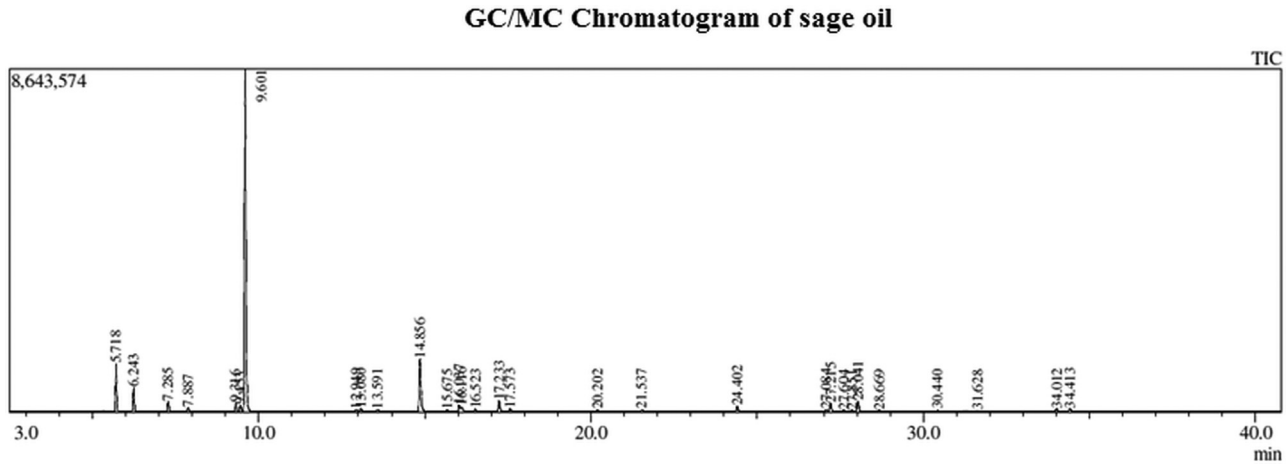
Tumor necrosis factor-alpha

A significant increase was observed in serum TNF-α level (+101.17) in tamoxifen-treated rats. Protection and treatment of animals with high dose (0.4 ml/kg) and treatment with low dose (0.2 ml/kg) of sage oil restored the increase in TNF-α level induced by tamoxifen. However, protection with low dose of sage oil failed to normalize TNF-α (Table 6).

Histopathological results

In control rats, the histological examination of liver tissue showed normal architecture of hepatic cords separated by blood sinusoids with normal central vein. Likewise, liver tissues of normal rats treated with sage oil (0.2 and 0.4 ml/kg) showed the same normal hepatic architecture. On the contrary, liver sections of rats in tamoxifen group showed massive steatosis with mild infiltration of inflammatory cells, a condition termed as steatohepatitis. However, both protection and treatment with sage oil at 0.2 and

Figure 1



Chromatogram of sage oil fractionation by GC/MS. GC/MS, gas chromatography/mass spectrometry.

Table 2 Effect of sage oil protection and therapy on liver function tests in serum of rats treated with tamoxifen

	ALP (U/l)	ALT (U/l)	AST (U/l)	Total bilirubin (mg/dl)	Direct bilirubin (mg/dl)	Indirect bilirubin (mg/dl)
Control	121.3^{a}±21.6	17.92^{a}±0.34	40.59^{a}±0.61	0.22^{a}±0.01	0.11^{a}±0.01	0.11^{a}±0.01
SAG 0.2	123.2^{a}±10.3	18.10^{a}±0.69	40.70^{a}±0.69	0.16^{a}±0.02	0.07^{a}±0.02	0.10^{a}±0.01
SAG 0.4	120.6^{a}±12.3	17.36^{a}±0.42	40.45^{a}±0.37	0.16^{a}±0.02	0.09^{a}±0.01	0.10^{a}±0.01
TAM	488.72^{b}±20.55	35.22^{b}±0.64	90.09^{b}±1.48	1.75^{b}±0.08	0.60^{b}±0.12	0.83^{b}±0.09
SAG 0.2/Pr	312.69^{c}±7.22	22.83^{c}±0.67	69.30^{c}±1.09	0.51^{c}±0.03	0.30^{a}±0.05	0.23^{a}±0.03
SAG 0.4/Pr	269.70^{d}±13.68	21.42^{c}±0.64	68.91^{c}±1.12	0.85^{d}±0.01	0.31^{a}±0.04	0.48^{c}±0.02
SAG 0.2/Tr	221.32^{e}±7.87	19.74^{d}±0.36	63.87^{d}±1.38	0.37^{a}±0.05	0.27^{a}±0.01	0.13^{a}±0.06
SAG 0.4/Tr	172.30^{f}±8.66	18.75^{ad}±0.50	52.25^{e}±1.91	0.34^{a}±0.03	0.23^{a}±0.04	0.12^{a}±0.06

Values represent mean±SE.ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase.Different letters indicate significantly different means at *P* value less than 0.05.Same letters indicate nonsignificant changes.

Table 3 Effect of sage oil protection and therapy on kidney function tests in serum of rats treated with tamoxifen

	Urea (mg/dl)	Creatinine (mg/dl)
Control	4.18^{a}±0.20	0.18^{a}±0.01
SAG 0.2	3.79^{a}±0.31	0.22^{a}±0.04
SAG 0.4	3.39^{a}±0.25	0.15^{a}±0.02
TAM	10.00^{b}±1.03	1.34^{b}±0.08
SAG 0.2/Pr	7.55^{c}±0.13	0.93^{c}±0.02
SAG 0.4/Pr	6.14^{a}±0.27	0.80^{c}±0.04
SAG 0.2/Tr	6.38^{a}±0.09	0.47^{d}±0.02
SAG 0.4/Tr	5.15^{a}±0.07	0.39^{d}±0.01

Values represent mean±SE.Different letters indicate significantly different means at *P* value less than 0.05.Same letters indicate nonsignificant changes.

0.4 ml/kg showed mild degree of steatosis together with presence of normal hepatocytes (Fig. 2).

In control group, the histological examination of kidney tissue showed normal histological structure of glomeruli and tubules. Moreover, renal tissue from normal rats treated with sage 0.2 and 0.4 ml/kg showed the same normal architecture of renal glomeruli and tubules. However, renal sections of rats treated with tamoxifen showed mini foci of degenerated tubular epithelium. Both protection and treatment with sage oil at 0.2 and 0.4 ml/kg showed normal histological appearance of renal tissue (Fig. 3).

Discussion

Tamoxifen significantly increased serum ALT, AST, and ALP activities and serum bilirubin levels in the current study. ALT and AST are released into circulation when hepatocytes are exposed to damage, resulting in a loss of cell membrane integrity and cellular leakage [29]. Custódio *et al.* [30,31] reported that tamoxifen and its active metabolite (4-hydroxytamoxifen) interact adversely with lipid bilayers of cell membranes, an effect which could precipitate in the cytostatic effects of tamoxifen. ALP, an indicator for hepatobiliary system integrity and bile release, discharges in response to cholestasis and increased biliary pressure [32]. Consequently, the present increase in ALP activity indicates obstructive event or cholestatic effect following tamoxifen administration. In addition, the present increase in serum bilirubin (total and direct) levels reveals posthepatic toxicity, probably caused by disruption of

Table 4 Effect of sage oil protection and therapy on the levels of malondialdehyde (nmol/g), nitric oxide (μmol/g), and glutathione (mmol/g) and activity of glutathione peroxidase (U/g) in the liver and the kidney of rats treated with tamoxifen

	Liver				Kidney			
	MDA	NO	GSH	GPx	MDA	NO	GSH	GPx
Control	17.19^{a}±0.32	0.12^{a}±0.04	25.80^{a}±0.79	282.70^{a}±3.88	15.12^{a}±1.04	0.23^{a}±0.02	27.49^{a}±0.82	575.77^{a}±22.68
SAG 0.2	15.72^{a}±0.92	0.08^{a}±0.02	25.47^{a}±0.81	278.38^{a}±7.03	17.01^{a}±0.37	0.30^{a}±0.02	25.67^{a}±0.35	608.48^{a}±31.83
SAG 0.4	13.36^{a}±1.63	0.06^{a}±0.01	27.42^{a}±1.03	315.17^{a}±28.46	15.99^{a}±2.71	0.20^{a}±0.05	26.72^{a}±0.65	640.73^{a}±26.39
TAM	40.20^{b}±2.41	0.26^{b}±0.03	10.77^{b}±0.69	79.21^{b}±3.53	29.38^{b}±1.12	0.95^{b}±0.15	12.93^{b}±1.25	161.18^{b}±16.72
SAG 0.2/Pr	23.01^{a}±1.65	0.14^{a}±0.02	13.70^{c}±0.43	144.23^{c}±16.88	22.96^{c}±0.64	0.86^{b}±0.11	15.35^{c}±0.38	272.65^{c}±8.62
SAG 0.4/Pr	21.88^{a}±0.55	0.14^{a}±0.03	15.19^{c}±0.28	205.93^{d}±16.67	21.14^{c}±0.74	0.76^{b}±0.08	16.32^{c}±0.33	283.76^{c}±7.30
SAG 0.2/Tr	17.37^{a}±1.16	0.20^{ab}±0.03	18.87^{d}±0.53	222.03^{e}±18.24	18.14^{a}±1.52	0.63^{c}±0.15	18.58^{d}±0.48	413.75^{d}±18.57
SAG 0.4/Tr	13.03^{a}±1.24	0.12^{a}±0.03	20.95^{e}±0.69	281.99^{a}±4.44	18.07^{a}±0.45	0.39^{ac}±0.04	20.77^{d}±0.33	435.10^{d}±15.85

Values represent mean±SE. GPx, glutathione peroxidase; GSH, glutathione; MDA, malondialdehyde; NO, nitric oxide. Different letters indicate significantly different means at *P* value less than 0.05. Same letters indicate nonsignificant changes.

Table 5 Effect of sage oil protection and therapy on the activity of and Na^+/K^+-ATPase (μmol Pi/min/g) in the liver and the kidney of rats treated with tamoxifen

	Liver Na^+/K^+-ATPase (μmol Pi/min/g)	Kidney Na^+/K^+-ATPase (μmol Pi/min/g)
Control	0.13^{a}±0.01	0.28^{a}±0.03
SAG 0.2	0.14^{a}±0.01	0.26^{a}±0.02
SAG 0.4	0.14^{a}±0.01	0.33^{a}±0.02
TAM	0.07^{b}±0.003	0.13^{b}±0.01
SAG 0.2/Pr	0.09^{c}±0.001	0.26^{a}±0.01
SAG 0.4/Pr	0.11^{a}±0.002	0.27^{a}±0.02
SAG 0.2/Tr	0.11^{a}±0.01	0.25^{a}±0.02
SAG 0.4/Tr	0.11^{a}±0.01	0.29^{a}±0.04

Values represent mean±SE. Different letters indicate significantly different means at *P* value less than 0.05. Same letters indicate nonsignificant changes.

Table 6 Effect of sage oil protection and therapy on the levels of tumor necrosis factor-alpha (pg/g) in the serum of rats treated with tamoxifen

	TNF-α (pg/ml)
Control	5.14^{a}±0.49
SAG 0.2	4.23^{a}±0.34
SAG 0.4	6.73^{a}±1.54
TAM	10.34^{b}±1.84
SAG 0.2/Pr	10.13^{b}±0.95
SAG 0.4/Pr	5.30^{a}±0.33
SAG 0.2/Tr	5.40^{a}±0.37
SAG 0.4/Tr	4.47^{a}±0.32

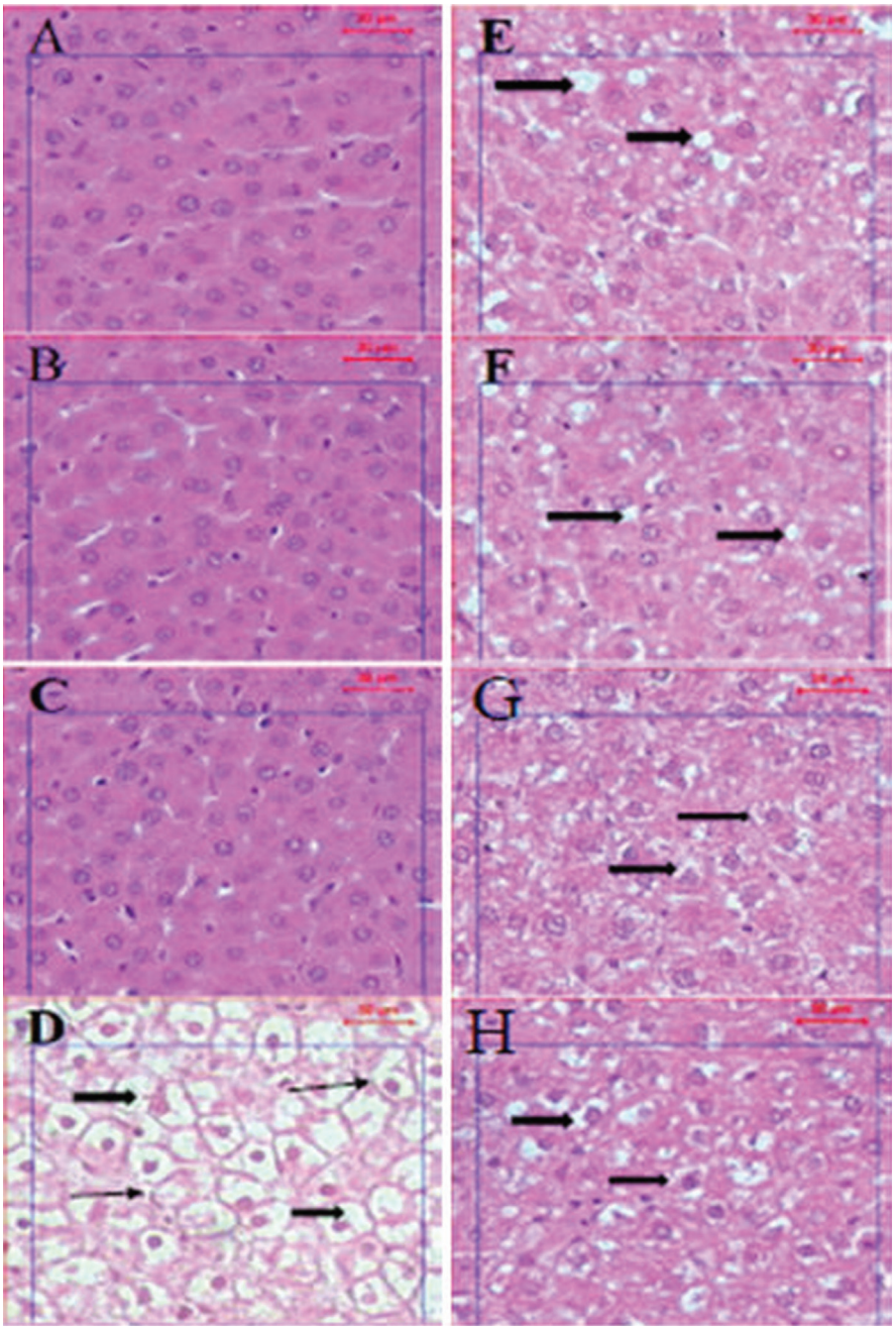
Values represent mean±SE. TNF-α, tumor necrosis factor-alpha. Different letters indicate significantly different means at *P* value less than 0.05. Same letters indicate nonsignificant changes.

bile drainage in the biliary system following tamoxifen exposure.

High concentrations of tamoxifen and its metabolites were found in lungs, liver, and kidneys [33]. Tamoxifen-induced renal impairment was evident from significant elevation in serum urea and creatinine levels, reflecting a reduction in glomerular filtration rate and nephrotoxicity [34]. The nephrotoxic mechanism of tamoxifen could be attributed to the ability of tamoxifen to activate mitochondrial calcium influx, causing overproduction of reactive oxygen and nitrogen species and subsequently induction of oxidative stress and mitochondrial apoptosis [35]. These, in turn, result in renal tubular dysfunction and accumulation of urea and creatinine in blood. Moreover, it has been reported that elevated bilirubin levels induce severe alterations in renal function and renal epithelium injury [36]. Consequently, the current increase of serum urea and creatinine could be attributed to hyperbilirubinemia and renal oxidative stress.

The significant elevation in liver and kidney function biomarkers induced by tamoxifen was correlated with oxidative stress, which was indicated from the significant increase in lipid peroxidation (MDA) and NO combined, with the significant decline in GSH and GPx in liver and kidney tissues. These findings are consistent with the previous studies that reported the contribution of oxidative stress and lipid peroxidation in tamoxifen-induced liver and kidney injuries [5,6]. Tamoxifen is a potent inhibitor of the mitochondrial electron transport chain, leading to massive production of ROS and apoptosis [37]. ROS can attack cell membrane phospholipids, causing the present increased lipid peroxidation induced by tamoxifen in

Figure 2

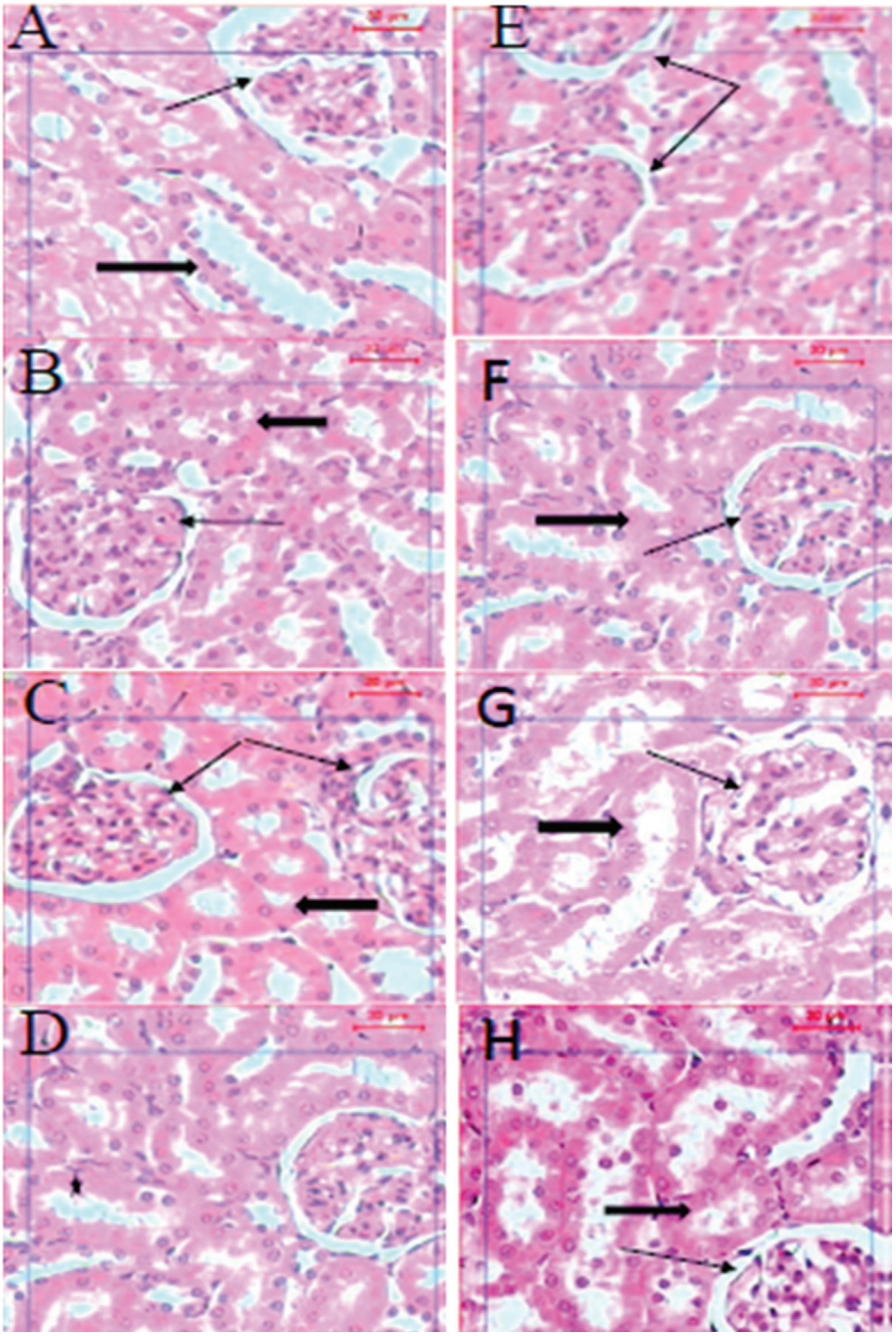


The effect of sage oil protection and therapy on the histopathological changes induced by *tamoxifen* in the liver of rats (hematoxylin and eosin, ×400). (a) Section of the liver of control rat showing the normal structure of liver tissue with normal architecture of hepatic cords and normal central vein. Section of liver tissue from rat receiving sage oil at (b) 0.2 ml/kg and (c) 0.4 ml/kg showing the same normal picture of liver. (d) Hepatic tissue section of tamoxifen-treated rat showing steatosis (thick arrow) with mild infiltration of inflammatory cells (thin arrow). Section of liver tissue from rat receiving tamoxifen and sage oil at (e) 0.2 ml/kg and (f) 0.4 ml/kg showing mild degree of steatosis (thick arrow). Section of liver of tamoxifen-treated rats treated with sage oil at (g) 0.2 ml/kg and sage oil at (h) 0.4 ml/kg showing mild degree of steatosis (thick arrow).

the liver and kidney. On the contrary, the current decline in the antioxidants GSH and GPx activity in hepatic and renal tissues could be explained by their exhaustion in combating the ROS produced by tamoxifen. Another possible mechanism for decreased GSH and increased MDA in tamoxifen-treated group may be owing to impaired pentose phosphate pathway [6], thereby reducing the availability of NADPH for glutathione reductase to recycle oxidized glutathione to GSH [38,39]. Moreover, the present decrease in GSH could result in an inhibition of GPx enzyme, making hepatic and renal tissues more susceptible to oxidative stress.

It has been reported that tamoxifen activates mitochondrial NO synthase enzyme by increasing

Figure 3



The effect of sage oil protection and therapy on the histopathological changes induced by *tamoxifen* in the kidney of rats (hematoxylin and eosin, ×400). (a) Section of the kidney of control rat showing the normal structure of kidney tissue with normal renal tissue architecture of glomeruli (thick arrow) and tubules (thin arrow). Section of kidney tissue from rat receiving sage oil at (b) 0.2 ml/kg and (c) 0.4 ml/kg showing the same normal picture of kidney. (d) Section of the kidney of tamoxifen-treated rat showing degenerated tubules (*). Section of kidney tissue from rat receiving tamoxifen and sage oil at (e) 0.2 ml/kg and (f) 0.4 ml/kg showing more or less normal glomerular and tubular architecture. Section of kidney of tamoxifen-treated rats treated with sage oil at (g) 0.2 ml/kg and sage oil at (h) 0.4 ml/kg showing more or less normal glomerular and tubular architecture.

mitochondrial Ca^{2+} levels [35]. Therefore, the current increase in NO levels in liver and kidney may be owing to the stimulation of mitochondrial NO synthase enzyme by tamoxifen. The generated NO can react with ROS, particularly superoxide anion, to form peroxynitrite that induces oxidative stress and apoptosis [40]. This may be one of the mechanisms behind tamoxifen-induced hepatic and renal damage.

Na^+/K^+-ATPase is a membrane-bound enzyme that transports sodium and potassium ions across cell membrane against electrochemical gradient [41]. As mentioned above, tamoxifen is a potent inhibitor of the

mitochondrial electron transport chain, thereby reducing the ATP-producing capacity of cells [37]. Cellular depletion of ATP, which is the key substrate for Na^+/K^+-ATPase, may underlie the current decline of Na^+/K^+-ATPase activity in liver and kidney. It has been shown that Na^+/K^+-ATPase requires phospholipids for its activity, which is inhibited by ROS [42,43]. This may also have a role in the present inhibition in hepatic and renal Na^+/K^+-ATPase activity induced by tamoxifen. Na^+/K^+-ATPase is important in kidney for filtering waste products from blood, glucose and amino acids reabsorption, electrolytes regulation, and pH maintenance [44]. Moreover, the liver requires Na^+/K^+-ATPase for bile secretion [45]. As Na^+/K^+-ATPase is necessary for the maintenance of different cellular functions, its inhibition may mediate the present impairment in liver and kidney functions.

It is clear from the present data that tamoxifen induced a marked increase in serum TNF-α, indicating the development of severe inflammation. In agreement with our results, El-Dessouki *et al.* [17] reported that tamoxifen induced a significant increase in TNF-α level by increasing the gene expression of nuclear transcription factor kappa B. In addition, the massive production of ROS induced by tamoxifen can increase the production of TNF-α [46]. These findings emphasize the cytotoxic effects of tamoxifen on liver and kidney. Therefore, inhibition of either TNF-α production or its activity could recover tamoxifen-induced liver and kidney injuries.

The histopathological examination of liver sections of tamoxifen-treated rats showed massive steatosis with mild infiltration of inflammatory cells (steatohepatitis). Tamoxifen is among the medications that can induce steatohepatitis [5,47]. This effect could be ascribed to tamoxifen-induced disturbance in the mitochondrial respiratory chain, which causes not only abnormal fatty acids oxidation and steatosis but also increased production of ROS. The massive production of ROS may be a crucial element in the pathogenesis of tamoxifen-induced steatohepatitis by activating lipid peroxidation and stimulating the production of pro-inflammatory cytokines like TNF-α by Kupffer cells and other inflammatory cells [47]. In tamoxifen-treated rats, the histopathological examination of renal sections revealed mini foci of degenerated tubular epithelium. Accordingly, the current histopathological changes observed in liver and kidney of tamoxifen-treated rats are induced by oxidative damage caused by the massive production of reactive oxygen and nitrogen species and inflammatory reactions triggered by increased TNF-α.

In the current study, GC/MS analysis revealed that sage oil contains many active constituents like 1,8-cineole, camphor, α-pinene, camphene, 2β-pinene, trans-caryophyllene, cymene, borneol L, DL-limonene, α-terpinenyl acetate, β-thujone, α-humulene, and linalyl acetate. These active ingredients exhibited powerful antioxidant, free radical scavenging, and lipid peroxidation inhibitory activities [15,48–50].

The present data clearly revealed that sage oil possesses hepatoprotective and nephroprotective effects against tamoxifen-induced toxicity. These effects are indicated from the ability of sage oil either as a protection or treatment to improve the changes induced by tamoxifen in ALT, AST, and ALP activities and total and direct bilirubin levels (liver functions) and in urea and creatinine levels (kidney functions). In agreement with our results, Fahmy *et al.* [51] reported that treatment with sage oil attenuated the hepatotoxicity and nephrotoxicity induced by carbon tetrachloride. This improvement could be attributed to the ability of sage oil to prevent the oxidative stress induced by tamoxifen by reducing lipid peroxidation and NO production and increasing GSH and GPx activity in both liver and kidney.In agreement with the present findings is the study by Kozioł *et al.* [52], who attributed the hepatoprotective effect of sage oil to its major constituent, 1,8-cineole, which has various pharmacological effects, including anti-inflammatory, antioxidant, and antinociceptive activities. In addition, 1,8-cineole was found to suppress the elevated serum transaminase activity and stopped the necrosis and hemorrhage associated with the septic shock to an extent greater than dexamethasone [53]. The mono-terpenoid, 1,8-cineole also revealed a hepatoprotective effect mediated by the activation of the antioxidant defense mechanism, ameliorating the oxidative damage caused by the environmental pollutant 2,3,7,8-tetrachlorodibenzo-p-dioxin [54]. In addition, β-pinene, a mono-terpene found in a relatively high amount in the currently investigated sage oil, was also reported to exhibit a potent antioxidant capacity [55]. This implies that the major constituents of the essential oils may contribute to their apparent biological activities. Moreover, it has been shown that sage oil enhances GPx activity, which protects hepatocytes against dimethoxy naphthoquinone and hydrogen peroxide-induced DNA damage. It has been reported that drinking water rich in sage extract improved oxidative stress in rat hepatocytes [56].

Furthermore, it has been reported that other active ingredients of sage oil like α-humulene, caryophyllene, and caryophyllene oxide inhibited the activity of

cytochrome P450A [57], thereby reducing the production of ROS and oxidative stress. Rehman *et al.* [48] reported that DL-limonene significantly suppressed the expression of inducible NO synthase enzyme, thereby reducing NO production.

The present data clearly demonstrated that sage oil was effective in normalizing the reduction of hepatic and renal Na^+/K^+-ATPase activity induced by tamoxifen. The efficacy of sage oil in restoring Na^+/K^+-ATPase activity could be attributed to its antioxidant and lipid peroxidation inhibitory activities. In addition, the efficacy of sage oil in restoring the activity of Na^+/K^+-ATPase may help to improve the decrease in liver and kidney function biomarkers induced by tamoxifen.

Several studies reported that active constituents of sage oil like 1,8-cineole (eucalyptol), camphor, veridiflorol, and DL-limonene possess anti-inflammatory activity through their inhibitory effects on gene expression of TNF-α and inactivation of nuclear transcription factor kappa B [48,58–60], thereby decreasing TNF-α levels and preventing the inflammatory effect induced by tamoxifen.

The current study clearly revealed that sage oil ameliorated the histopathological changes induced by tamoxifen in liver and kidney. However, a mild degree of steatosis in hepatic tissues was observed. The antioxidant and anti-inflammatory activities of sage oil could mediate the improvement of histopathological changes induced by tamoxifen in liver and kidney.

Conclusion

In the light of the data obtained from the present study, it could be observed that the therapeutic effect of sage oil was more prominent than its protective effect against the adverse effects induced by tamoxifen. Moreover, therapy with high dose (0.4 ml/kg) was more effective than low dose of sage oil. This could be attributed to that the high dose contains more active ingredients than the low dose. Further studies are needed to verify the beneficial role of sage oil in ameliorating the hepatotoxicity and nephrotoxicity induced by tamoxifen.

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Conflicts of interest

There are no conflicts of interest.

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