

Camellia sinensis and epicatechin abate doxorubicin-induced hepatotoxicity in male Wistar rats *via* their modulatory effects on oxidative stress, inflammation, and apoptosis

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ABSTRACT

This study aimed to assess the preventive effects of *Camellia sinensis* (green tea) leaf aqueous extract and epicatechin and to scrutinize their possible mechanisms of action in doxorubicin (Dox)-induced liver injury. Phytochemical screening of *C. sinensis* aqueous extract was performed by liquid chromatography electrospray ionization tandem mass spectrometry that revealed the presence of epicatechin and other polyphenols. Male Wistar rats were intraperitoneally injected with Dox (4 mg/kg/week) and were orally treated with *C. sinensis* aqueous extract (200 mg/kg) or epicatechin (25 mg/kg) every other day for 6 weeks. The treatments of Dox-injected rats with the extract and epicatechin resulted in a marked amelioration of the deteriorated effects on albumin, alpha-fetoprotein, and total bilirubin levels as well as alanine transaminase, aspartate transaminase, alkaline phosphatase, and gamma glutamyl transferase activities. The treatments also alleviated the altered serum tumor necrosis factor-alpha and interleukin-4, liver lipid peroxidation, and glutathione levels as well as liver superoxide dismutase, glutathione peroxidase, and glutathione-S-transferase activities. In association, the expression of liver nuclear factor-kappa B cells, cyclooxygenase-2, p53, and caspase-3 was remarkably decreased, the expression of Bcl-2 was significantly increased, and the liver histological architecture was remarkably amended by treatments. *Camellia sinensis* aqueous extract and epicatechin may have effective chemopreventive potentials against Dox-induced hepatotoxicity *via* reinforcement of antioxidant defense system and attenuation of the inflammatory and apoptotic effects.

INTRODUCTION

The liver, as vital organ, performs an array of complex functions coordinating metabolic, immunologic, and detoxification processes (Sendensky and Dufour, 2011). Despite the liver's substantiality and unique self-regeneration capability, viral infection, parasite infection, autoimmune diseases, fatty liver disorder, drug-induced hepatotoxicity, and alcohol abuse all contribute to the increasing prevalence of liver failure (Bai *et al.*, 2016).

Doxorubicin (Dox) is an anthracycline antibiotic that has been commonly used to treat multiple types of cancers (Xi *et al.*, 2012). Although Dox is an effective anti-cancer agent, its use is significantly limited owing to its side effects and toxicity (Ahmed *et al.*, 2019). This Dox toxicity often affects many organs such as liver, heart, brain, kidneys, and testes, thereby limiting its clinical application (Tacar *et al.*, 2013; Trivedi *et al.*, 2011). Mitochondria were suggested to be one of the main targets of Dox which produces its action through mitochondria-mediated apoptosis leading to modifications in mitochondrial membranes; this, in turn, results in alterations in oxidative phosphorylation and respiratory chain complexes in the mitochondria (Trivedi *et al.*, 2011). Moreover, Dox significantly deteriorates energy-signaling and -transducing systems like

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adenosine monophosphate-activated protein kinase and creatine kinase (Kuznetsova *et al.*, 2011).

Dox causes perturbation in the balance between the reactive oxygen species (ROS) generation on the one hand and the antioxidant defense system on the other hand leading to tissue injuries (Karaman *et al.*, 2006; Saad *et al.*, 2011). Several studies were conducted for the screening of the antioxidants derived from the medicinal plants and natural sources aiming to minimize oxidative damage by Dox (Kaiserova *et al.*, 2007). In this regard, many of these antioxidants were elucidated to amend the Dox-induced cellular degeneration without affecting its anti-cancer efficacies (Xin *et al.*, 2011).

Green tea, *Camellia sinensis* (*C. sinensis*) is one of the most commonly consumed beverages after water (Subhashini *et al.*, 2010). Its phytochemical screening by previous publications indicated the presence of phenolics, flavonoids, alkaloids, tannins, steroids, terpenoids, glycosides, and saponins (Geoffrey *et al.*, 2014; Rahman, 2016; Subhashini *et al.*, 2010). Polyphenols, including flavonoids, constitute up to 30% of the green tea leaves, but only 10% of black tea by dry weight (Subhashini *et al.*, 2010). Three basic flavonoids in tea leaves include catechins, theaflavins, and thearubigins (Cui *et al.*, 2008; Yanishlieva-Maslarowa and Heinonen, 2001).

Catechins have many biological activities and they are extensively used as potent protective and preventive agents (Rahmani *et al.*, 2015). They possess anticancer, anti-obesity, hypocholesterolemic, and hypoglycemic properties (Kanwar *et al.*, 2012). Green tea and its constituent catechins have widely been investigated for their therapeutic, protective, and preventive effects against cancer and other diseases (Cui *et al.*, 2008; Lecumberri *et al.*, 2013). Their benefits such as weight loss *via* stimulating the rate of metabolism, total cholesterol level reduction, high-density lipoprotein increase, and plague prevention have been previously demonstrated (Alappat *et al.*, 2015; Khan and Mukhtar, 2010). In addition, the green tea extracts and their catechin components have been elucidated to have potent antioxidant activities that play an important role in the prevention and therapy of multiple diseases (Rahman, 2016).

Therefore, this current study was conducted to assess the preventive efficacies of *C. sinensis* aqueous extract and its constituent, epicatechin, on Dox-induced liver injury and to suggest their probable mechanisms of action.

MATERIALS AND METHODS

Experimental animals

Male Wistar rats weighing from 140 to 180 g and aging 8–10 weeks were purchased from Research Institute of Ophthalmology, 2 El Ahram Street, Giza, Egypt. To exclude any interchange infection, the rats were overseen for about 2 weeks before the initiation of the experiment. The animals, chosen for study, were housed in polypropylene cages with good aerated stainless steel covers at normal atmospheric temperature (25°C ± 5°C) as well as 12-hour daily normal light periods in the Animal House in Faculty of Science, Beni-Suef University, Beni-Suef Governorate, Egypt. Moreover, the rats were daily supplied with excess water and standard pellet diet *ad libitum*. By the way, all animal experiments and procedures followed

the recommendations of the Canadian Council on Animal Care (CCAC) (2010) and are in concordance with the guidelines and instructions for animal use and care of Experimental Animal Ethics Committee of Faculty of Science, Beni-Suef University, Egypt (Ethical Consent Number: BSU/FS/2014/8).

Chemicals and drugs

Dox hydrochloride (adricin 10-mg vials), manufactured by Pharmacia Italia Nerviano Italy, was obtained from El Gomhuria Pharmacy, Cairo, Egypt. Green tea was purchased from Harraz Medicinal Plant Company, 1 Ahmed Maher Street, Bab Al Khalq, Cairo, Egypt (www.harrazegypt.com). Epicatechin of the chemical structure indicated in Figure 1 (Rozza *et al.*, 2012) was purchased from Sigma Chemical Company, St. Louis, MO. Saline (0.9% sodium chloride) was obtained from ADWIC Company and produced by El-Nasr Pharmaceutical Chemical Company, Cairo, Egypt. Carboxymethyl cellulose (CMC) was obtained from the Egyptian Center for Chemicals and Laboratory Supplies, Nasr City, Cairo, Egypt. All other used chemicals are ultrapure and are of analytical grade.

Analysis of green tea using the liquid chromatography electrospray ionization tandem mass spectrometry (LC/ESI-MS/MS)

Liquid chromatography (LC) coupled with mass spectrometry (MS) and a source of electrospray ionization tandem mass spectrum (ESI-MS) were used for chemical analysis of green tea extract.

The separation of the green tea sample was performed according to Yoshida and Majors (2006) method with some modifications on ZORBAX-C18 (4.6 × 100 mm id, 3.5 μm) analytical column using LC/deuterium-arc-discharge (DAD)/MS system Agilent 1100 quaternary pump, coupled with a DAD (Agilent 6120 quadrupole spectrometer, CA, USA) wavelength setting of 280 nm. The instrument conditions are as follows: pressure was 45.5 bar at starting point and 55.9 bar at stopping point; the flow rate of the mobile phase was 0.4 ml/minute with a column compartment temperature of 40°C and an injection volume of 10 μl. The mobile phase was composed of 0.2% formic acid in water (Solvent A) and 0.1% formic acid in acetonitrile (Solvent B) in a gradient elution mode. The gradient elution was conditioned as follows: initial concentration of 10%

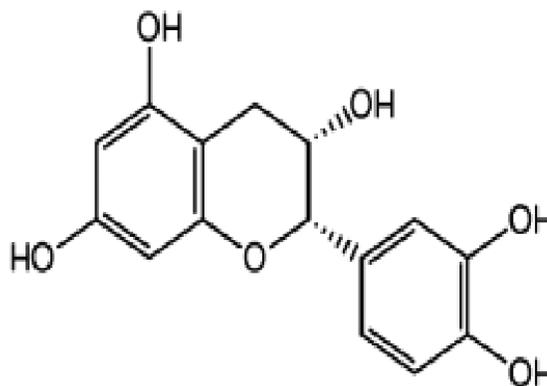


Figure 1. Chemical structure of epicatechin (Rozza *et al.*, 2012).

B with programming to 15% B for 15 minutes, and then to 27% B for another 15 minutes. The column was then equilibrated under the initial condition for 10 minutes.

The mass selective detector instrument and ESI conditions were as follows: vaporized temperature or gas temperature, 350°C; nebulizer gas, nitrogen, at a pressure 50 psi; and drying gas, also nitrogen, at a flow rate 12 l/minute and capillary voltage, -3,500 V of negative mode and 3,500 V of positive mode, and scan range 100–1,000 m/z. ESI of the catechins in -ve mode produces the $[M - H]^-$ molecular adduct ion while ESI of the caffeine in +ve mode produces the $[M + H]^+$ molecular adduct ion.

Dose preparation of Dox

Dox at dose 4 mg/kg body weight (b.w.)/week was prepared for intraperitoneal (i.p.) injection in 1 weekly dose for 6 weeks according to [Trivedi *et al.* \(2011\)](#).

Dose preparation of green tea aqueous extract and epicatechin

Green tea, *C. sinensis*, belonging to the Theaceae family, was authenticated by Dr. Walaa A. Hassan, Assistant professor of Taxonomy and Flora, Botany Department, Faculty of Science, Beni-Suef University, Salah Salem Street, Beni-Suef, Egypt. The herb was deposited in the Herbarium of Botany Department, Faculty of Science, Beni-Suef University, Egypt. The dried green tea leaves were grinded into powder by an electric grinder. The aqueous extract was prepared from the tea leaves powder based on the methods of [Ahmed \(2010\)](#) and [Swanston-Flatt *et al.* \(1990\)](#). The powder of green tea leaves was mixed with the already boiled distilled sterile water (2 g/50 ml; 4% w/v) and infused for 15 minutes. The aqueous extract was then filtered and the obtained filtrate was freshly used for oral administration to Dox-administered rats at a dose of 200 mg/kg b.w. according to [Al-Hilfy \(2012\)](#).

Epicatechin was prepared by dissolving the required dose in 1% CMC (25 mg/5 ml 1% CMC/kg b.w.).

Animal grouping

Forty Wistar rats were randomly divided into 4 groups of 10 animals each as follows:

Group 1 (Normal control group)

In this group, the rats received the equivalent volume of saline which was intraperitoneally (i.p.) injected/week for 6 weeks. The rats of this group were also given the equivalent volume of the vehicle (1% CMC solution) by oral gavage every other day for 6 weeks.

Group 2 (Dox-administered group)

Dox dissolved in saline was injected i.p. as a total cumulative dose of 24 mg/kg b.w. divided into six equal doses, each of 4 mg/kg b.w./week for 6 weeks [Trivedi *et al.* \(2011\)](#). The rats of this group were also given the same volume of the vehicle (1% CMC solution) by oral gavage every other day for 6 weeks.

Group 3 (Dox-administered rats treated with green tea aqueous extract)

This group was given Dox as group 2 plus green tea aqueous extract by oral gavage at a dose of 200 mg/kg b.w. every

other day for 6 weeks ([Al-Hilfy, 2012](#)). The rats of this group were given the same volume of the vehicle (1% CMC solution) as in groups 1, 2, and 4 by oral gavage every other day for 6 weeks.

Group 4 (Dox-administered rats treated with epicatechin)

In this group, the rats were administered Dox as group 2 plus epicatechin (dissolved in 1% CMC solution) by oral gavage at a dose of 25 mg/5 ml/kg b.w. every other day for 6 weeks ([Vasconcelos *et al.*, 2012](#)).

Preparation of blood and tissue homogenates

At the end of the 6 weeks of treatment periods, animals were sacrificed under diethyl ether anesthesia. Then, blood from each rat was collected from jugular veins into gel and clot activator tubes. The sera were quickly aspirated by Pasteur pipette and divided into three nearly equal portions for each individual rat, and kept in a deep freezer at -20°C for subsequent analysis. After decapitation by cervical dislocation and dissection, liver of each rat was quickly removed, weighed, and homogenized in a sterile saline (0.9% NaCl solution; 10% w/v) using Teflon homogenizer (Glas-Col; Terre Haute, IN). Then, the homogenates were centrifuged at 3,000 rpm. for 15 minutes and the supernatants were separated by Pasteur pipette and kept at -20°C pending determination of antioxidant defense system and oxidative stress biomarkers.

Assay of liver function parameters in serum

Serum alanine transaminase (ALT), aspartate transaminase (AST), gamma glutamyl transferase (GGT), and alkaline phosphatase (ALP) activities were assayed according to the methods of [Gendler \(1984\)](#), [Murray \(1984a; 1984b\)](#), and [Tietz *et al.* \(1983\)](#), respectively, using reagent kits purchased from Spinreact, 7 E-17176 Sant Esteve De Bas (Gi), Spain. Serum total bilirubin level was detected based on the procedure of [Jendrassik and Grof \(1938\)](#) using reagent kits purchased from Spectrum Diagnostics, Cairo, Egypt. Serum albumin level was estimated according to [Doumas *et al.* \(1971\)](#) using kits obtained from Diamond Diagnostics (Cairo, Egypt).

Assay of lipid peroxidation and antioxidant parameters

Glutathione (GSH) content in liver homogenates was detected based on the chemical method of [Beutler *et al.* \(1963\)](#). Lipid peroxidation (LPO) in homogenates was estimated based on the chemical method of [Preuss *et al.* \(1978\)](#). Glutathione peroxidase (GPx; EC 1.11.1.7), superoxide dismutase (SOD; EC 1.15.1.1), and glutathione-S-transferase (GST; EC 2.5.1.18) activities in liver homogenates were assayed based on the methods of [Kar and Mishra \(1976\)](#), [Mannervik and Guthenberg \(1981\)](#), and [Marklund and Marklin \(1974\)](#), respectively.

Detection of liver mRNA expression of various markers of apoptosis and inflammation

RNA purification from liver tissue was carried out by using GeneJET RNA Purification kits purchased from Thermo Fisher Scientific Inc., Rochester, New York, according to the method of [Chomzynski and Sacchi \(1987\)](#).

Reverse transcription and PCR amplification were carried out by Thermo Cycler PCR (Techne 32 Thermocycler)

using single step Verso 1-Step reverse transcriptase-polymerase chain reaction (RT-PCR) ReddyMix kit purchased from Thermo Fisher Scientific Inc. Rochester, New York, in the presence of specific primers (Table 1) of protein 53 (p53) (Asiri, 2010), B-cell lymphoma 2 (Bcl2) (Ashok and Sheeladevi, 2014), nuclear factor-kappa B cells (NF- κ B) (Ahmed *et al.*, 2016), and protein of housekeeping gene, β -actin (Ahmed *et al.*, 2017), supplied from Biosearch Technologies, South McDowell Blvd, Petaluma. After gel electrophoresis, the gel containing bands was transferred to Gel Documentation Unit and photos were taken. The photos of gene bands were analyzed with GelDocu Advanced Program from Raya for the Scientific Services, Giza, Egypt, and represented as numbers for statistical analysis. The mRNA expression of p53, Bcl2, and NF- κ B was related to β -actin.

ELISA assay

Serum tumor necrosis factor-alpha (TNF- α), interleukin-4 (IL-4), and alpha-fetoprotein (AFP) levels were detected by using specific enzyme-linked immunosorbent assay (ELISA) kits obtained from R&D Systems, Inc., Minneapolis, MN, according to manufacturer's instructions.

Histological investigation

After sacrifice and dissection at the end of the experiment, pieces of liver from each rat were immediately excised, fixed in 10% phosphate buffered formalin (pH 7.2), and transported to Pathology Department, Faculty of Veterinary Medicine, Beni-Suef University, Egypt, for further processing, sectioning, mounting of sections on slides, and staining with hematoxylin and eosin (H&E) (Bancroft *et al.*, 1996). The stained sections were examined for the detection of lesions.

Immunohistochemical investigation

Liver samples, fixed in 10% neutral phosphate buffered formalin, were dehydrated and embedded in paraffin, cut into 5- μ m sections, and mounted on positive slides. The COX-2 in liver tissue sections was detected by using anti-COX-2 and anti-caspase-3 specific IgG (H-62, sc-7951, Santa Cruz Biotechnology, Dallas, TX). The sections prepared from the liver were mounted on positive slides for detection of COX-2 (Therland *et al.*, 2004). Immunolocalization technique for caspase-3 was prepared on 5- μ m thickness liver sections according to Hussein and Ahmed (2010) and Pedrycz and Czerny (2008). The positive

reaction in cytoplasm of hepatocytes appeared brownish in color (Giorno, 1984).

Statistical analysis

Data were analyzed by SPSS v. 20. Results were expressed as mean \pm standard error mean (SEM) and all statistical comparisons were made by Duncan's test *post-hoc* analysis and least significance difference. *p* values at *p* > 0.05 were considered non-significant, while those at *p* < 0.05 were considered significant.

RESULTS

Phytochemical screening

The chemical analysis of green tea extract by LC/ESI-MS/MS indicated the presence gallic acid, (-)-gallocatechin, (-)-epigallocatechin, caffeine, (-)-epicatechin, (-)-epigallocatechin gallate, (-)-gallocatechin gallate, (-)-epicatechin gallate, and (-)-catechin gallate (Fig. 2).

Effect on liver function parameters in serum

Serum AFP and total bilirubin levels exhibited a significant increase (*p* < 0.05) in Dox-administered animals recording percentage increases of 418.18% and 137.50%, respectively, in comparison with the normal control group. On the contrary, serum albumin level was significantly (*p* < 0.05) decreased in Dox-administrated rats recording percentage change of -17.44%. The treatment of Dox-administered rats with green tea aqueous extract produced a significant decrease (*p* < 0.05) of the elevated serum AFP and total bilirubin levels; the recorded percentage decreases were -66.67% and -17.54%, respectively, in comparison with Dox-administered control. The decreased serum albumin level was non-significantly (*p* > 0.05) elevated as a result of treatment of Dox-administrated rats with green tea aqueous extract. The treatment of Dox-administered rats with epicatechin caused a significant decrease of the serum AFP (*p* < 0.05) levels recording percentage decrease -67.54% while it produced a non-significant decrease of serum total bilirubin level recording percentage decrease -5.26%. In contrast, the albumin level exhibited a non-significant increase (*p* > 0.05) in Dox-administered rats recording percentage increase of 1.10%. Green tea aqueous extract was more potent in improving the altered serum albumin and total bilirubin levels (Table 2).

Serum ALT, AST, ALP, and GGT activities showed a significant elevation (*p* < 0.05) in Dox-administered rats recording percentage increases of 65.23%, 31.35%, 56.60%, and 218.75%, respectively, when compared with the normal control group. The treatment of Dox-administered rats with green tea resulted in a significant decrease of the elevated serum ALT, AST, ALP, and GGT activities (*p* < 0.05); the recorded percentage changes were -46.02%, -35.78%, -52.45%, and -30.72%, respectively, in comparison with Dox-administered rats. The treatment of Dox-administered rats with epicatechin produced a significant decrease (*p* < 0.05) in serum ALT, AST, and ALP activities recording percentage changes of -42.57%, -24.51%, -57.43%, and -54.90%, respectively, in comparison with Dox-administered rats. While green tea aqueous extract was more potent in improving the elevated serum ALT and AST activities, epicatechin was more effective in alleviating the elevated serum ALP activity. The serum

Table 1. The sequences of forward and reverse primers of selected genes.

Detected gene	Primer sequence	Reference
P53	F: 5' d CAGCGTGATGATGGTAAGGA3'	Asiri (2010)
	R: 5' d GCGTTGCTCTGATGGTGA3'	
Bcl-2	F: 5' d GGGATGCCCTTTGTGGAACATA3'	Ashok and Sheeladevi (2014)
	R: 5' d CTCACCTTGTGGCCAGGTAT3'	
NF- κ B	F: 5' d TACCATGCTGTTTGGTTAC3'	Ahmed <i>et al.</i> (2016)
	R: 5' dTCAAGCTACCAATGACTTTC3'	
β -actin	F: 5TCACCCGTAAGTACCCCATGGAG3'	Ahmed <i>et al.</i> (2017)
	R: 5' d TTGGCCCTGGGGTTCAGGGGG3'	

GGT activity, on the other hand, was significantly ($p < 0.05$) decreased as a result of treatment of Dox-administered rats with green tea aqueous extract, while it was significantly ($p < 0.05$) increased due to epicatechin treatment (Table 3).

Effect on serum TNF- α and IL-4 levels

With regard to ELISA results, the Dox-administered rats showed a marked ($p < 0.05$) rise in serum TNF- α level and decrease in serum IL-4 level recording percentage changes of 288.81% and -64.10%, respectively, as compared with normal control group. As a result of treatment of Dox-administered rats with green tea aqueous extract, a significant ($p < 0.05$) decrease in serum TNF- α level and increase of IL-4 level were produced; the recorded percentage changes were -63.29% and 110.32%, respectively, as compared with Dox-administered rats. The treatment of Dox-administered rats with epicatechin caused a significant decrease in serum TNF- α level and increase of IL-4 serum level recording percentage changes of -51.06% and 106.80%, respectively, when compared to Dox-administered group. Thus, green tea aqueous extract seems to be more potent

in improving the proinflammatory cytokine, TNF- α , and anti-inflammatory cytokine, IL-4 (Table 4).

Effect on liver oxidative stress and anti-oxidant defense system

Liver SOD activity in Dox-administered rats exhibited a significant decrease ($p < 0.05$) recording percentage decrease of -20.92% when compared with the normal group. In contrast, LPO in the liver of Dox-administered rats was significantly increased ($p < 0.05$) recording percentage increase of 247.12% when compared with the normal group. The treatment of Dox-administered rats with green tea aqueous extract and epicatechin, respectively, resulted in a non-significant ($p > 0.05$) and a significant ($p < 0.05$) increases of liver SOD activity; the recorded percentage increases were 14.03% and 13.50%, respectively, as compared with Dox-administered rats. In contrast, hepatic LPO was significantly decreased ($p < 0.05$) in Dox-administered rats treated with green tea aqueous extract and epicatechin recording percentage decreases of -34.83% and -60.52%, respectively, when compared with Dox-administered rats, while the effect of green tea aqueous extract and epicatechin

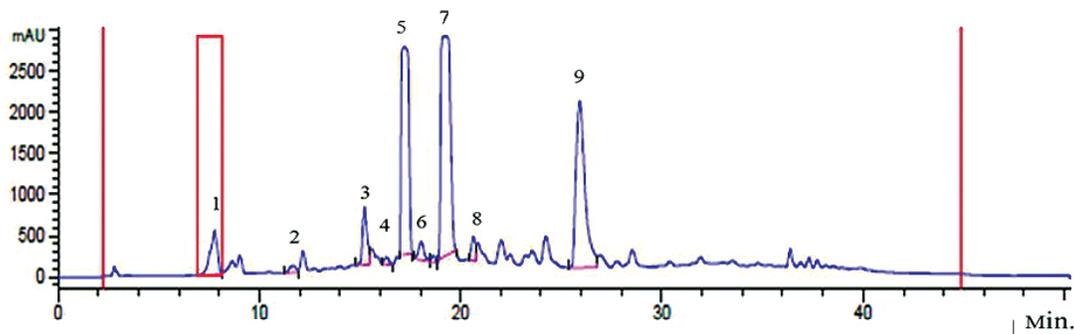


Figure 2. LC/ESI-MS of green tea extract. Peaks identification: 1: gallic acid; 2: (-)-gallocatechin; 3: (-)-gallocatechin; 4: (-)-epigallocatechin; 5: caffeine; 6: (-)-epicatechin; 7: (-)-epigallocatechin gallate; 8: (-)-gallocatechin gallate; and 9: (-)-epicatechin gallate and (-)-catechin gallate. These results are shared with our other publication (Ahmed *et al.*, 2019).

Table 2. Effect of green tea aqueous extract and epicatechin on serum albumin, AFP, and total bilirubin levels in Dox-administered rats.

Experimental conditions	Albumin (g/dl)	AFP (ng/ml)	Total bilirubin ($\mu\text{mol/l}$)
Control	3.60 \pm 0.07	0.22 \pm 0.03	0.24 \pm 0.02
Dox	2.97 \pm 0.09 ^a	1.14 \pm 0.13 ^a	0.57 \pm 0.03 ^a
% change ^A	(-17.50%)	(+418.18%)	(+137.50%)
Dox + GT	3.13 \pm 0.10	0.38 \pm 0.15 ^b	0.47 \pm 0.02 ^b
% change ^B	(+5.39%)	(-66.67%)	(-17.54%)
Dox + Epi	3.00 \pm 0.06	0.37 \pm 0.08 ^b	0.54 \pm 0.02
% change ^B	(+1.10%)	(-67.54%)	(-5.26%)

Data are represented as a mean \pm SEM. The number of animals in each group is six.

^{a,b}Significant differences in comparison with the corresponding control and Dox-administered groups, respectively, at $p < 0.05$.

^{A,B}Percentage of changes in relation to the normal control and Dox-administered groups, respectively.

GT: green tea aqueous extract; Epi: epicatechin.

Table 3. Effect of green tea aqueous extract and epicatechin on serum ALT, AST, ALP, and GGT activities in Dox-administered rats.

Experimental conditions	ALT (U/l)	AST (U/l)	ALP (U/l)	GGT (U/l)
Control	34.60 ± 1.21	154.80 ± 5.70	193.80 ± 5.55	0.48 ± 0.09
Dox	57.17 ± 2.10 ^a	203.33 ± 6.35 ^a	303.51 ± 2.88 ^a	1.53 ± 0.09 ^a
% change ^A	(+65.23%)	(+31.35%)	(+56.60%)	(+218.75%)
Dox + GT	30.86 ± 1.45 ^b	130.57 ± 2.50 ^b	144.30 ± 8.32 ^b	1.06 ± 0.06 ^b
% change ^B	(-46.02%)	(-35.78%)	(-52.45%)	(-30.72%)
Dox + Epi	32.83 ± 1.45 ^b	153.50 ± 4.87 ^b	129.20 ± 4.49 ^b	2.37 ± 0.08 ^b
% change ^B	(-42.57%)	(-24.51%)	(-57.43%)	(+54.90%)

Data represented as a mean ± SEM. The number of animals in each group is six.

^{a,b}Significant difference in comparison with the corresponding control and Dox-administered groups, respectively, at $p < 0.05$.

^{A,B}Percentage of changes in relation to the normal control and Dox-administered groups, respectively.

GT: green tea aqueous extract; Epi: epicatechin.

Table 4. Effect of green tea aqueous extract and epicatechin on serum TNF- α and IL-4 levels in Dox-administered rats.

Experimental conditions	TNF- α (pg/ml)	IL-4 (pg/ml)
Control	28.25 ± 2.22	134.71 ± 5.33
Dox	109.84 ± 10.58 ^a	48.36 ± 2.90 ^a
% change ^A	(+288.81%)	(-64.10%)
Dox + GT	40.32 ± 2.54 ^b	101.71 ± 5.83 ^b
% change ^B	(-63.29%)	(+110.32%)
Dox + Epi	53.77 ± 4.40 ^b	100.01 ± 3.92 ^b
% change ^B	(-51.06%)	(+106.80%)

Data represented as a mean ± SEM. The number of animals in each group is six.

^{a,b}Significant differences in comparison with the corresponding normal control and Dox-administered groups, respectively, at $p < 0.05$.

^{A,B}Percentage of changes in relation to the normal control and Dox-administered groups, respectively.

GT: green tea aqueous extract; Epi: epicatechin.

on liver LPO activity was more or less similar, the effect of epicatechin was more potent on liver LPO (Table 5).

Dox-administration caused a marked depletion ($p < 0.05$) of hepatic GSH content as well as GPx and GST stores when compared with the normal group; the recorded percentage changes were -33.78%, -26.99%, and -33.45%, respectively. The treatment of Dox-administered rats with green tea aqueous extract significantly ($p < 0.05$) prevented this depletion in GSH content and GPx activity recording percentage changes of 21.25% and 39.40%, respectively. In contrast, GST activity was non-significantly affected as a result of green tea aqueous extract treatment recording a percentage change of 3.28%. The treatment of DOX-administered rats with epicatechin produced a significant increase ($p < 0.05$) of the serum GSH content as well as GPx stores; the recorded percentage changes were 20.29% and 34.22%, respectively. In contrast, GST activity was non-significantly affected due to epicatechin treatment recording a percentage change of -8.60%, while the effects of green tea aqueous extract and epicatechin were more or less similar on liver GSH content, the effect of green tea aqueous extract was potent on liver GPx activity (Table 6).

Effect on mRNA expression of liver p53, Bcl2, and NF- κ B

The mRNA expression of p53 relative to β -actin exhibited a significant ($p < 0.05$) elevation in Dox-administered rats recording percentage increases of 164.91% when compared

with the normal group. The treatment of Dox-administered rats with green tea aqueous extract and epicatechin produced a significant ($p < 0.05$) decrease of the elevated p53 mRNA expression recording percentage changes of -48.60% and -75.40%, respectively, when compared with Dox-administered control. Thus, the effect of epicatechin was more potent than green tea aqueous extract on decreasing the elevated mRNA expression of p53 relative to β -actin (Fig. 3).

The mRNA expression of Bcl2 relative to β -actin exhibited a significant decrease ($p < 0.05$) in Dox-administered rats recording percentage changes of -81.50% when compared with the normal control group. The treatment of Dox-administered rats with green tea aqueous extract and epicatechin produced a significant ($p < 0.05$) increase of the lowered Bcl2 mRNA expression recording percentage changes of 409.01% and 378.22%, respectively, when compared with Dox-administered control (Fig. 4).

The mRNA expression of liver NF- κ B relative to β -actin exhibited a significant ($p < 0.05$) increase in Dox-administered rats recording percentage increases of 48.51% as compared with the normal group. The treatment of Dox-administered rats with green tea aqueous extract and epicatechin produced a significant ($p < 0.05$) decrease of the elevated liver NF- κ B mRNA expression recording percentage changes of -31.20% and -49.10%, respectively, as compared with Dox-administered control. The epicatechin was more effective than green tea aqueous extract in

Table 5. Effect of green tea aqueous extract and epicatechin on liver SOD activity and lipid peroxidation in Dox-administered rats.

Experimental conditions	SOD activity (U/g tissue)	LPO (nmol/100 mg tissue/hour)
Control	16.49 ± 1.38	5.41 ± 0.57
Dox	13.04 ± 0.539 ^a	20.24 ± 1.65 ^a
% change ^A	(-20.92%)	(+274.12%)
Dox + GT	14.87 ± 1.722	13.19 ± 1.44 ^b
% change ^B	(+14.03%)	(-34.83%)
Dox + Epi	14.80 ± 0.56 ^b	7.99 ± 1.48 ^b
% change ^B	(+13.50%)	(-60.52%)

Data represented as a mean ± SEM. The number of animals in each group is six.

^{a,b}Significant difference in comparison with the corresponding normal control and Dox-administered groups, respectively, at $p < 0.05$.

^{A,B}Percentage of changes in relation to the normal control and Dox-administered groups, respectively.

GT: green tea aqueous extract; Epi: epicatechin.

Table 6. Effects of green tea aqueous extract and epicatechin on liver GSH content and GPx and GST activities in Dox-administered rats.

Experimental conditions	GSH (nmol/100 mg tissue)	GPx (mU/100 mg tissue)	GST (U/100 mg tissue)
Control	129.48 ± 10.21	122.08 ± 4.38	105.37 ± 3.24
Dox	85.74 ± 6.77 ^a	89.13 ± 4.02 ^a	70.12 ± 11.47 ^a
% change ^A	(-33.78%)	(-26.99%)	(-33.45%)
Dox + GT	103.96 ± 12.38 ^b	124.25 ± 5.37 ^b	72.42 ± 11.48
% change ^B	(+21.25%)	(+39.40%)	(+3.28%)
Dox + Epi	103.14 ± 14.12 ^b	119.63 ± 3.68 ^b	64.09 ± 18.75
% change ^B	(+20.29%)	(+34.22%)	(-8.60%)

Data represented as a mean ± SEM. The number of animals in each group is six.

^{a,b}Significant difference in comparison with the corresponding normal control and Dox-administered groups, respectively, at $p < 0.05$.

^{A,B}Percentage of changes in relation to the normal control and Dox-administered groups, respectively.

GT: green tea aqueous extract; Epi: epicatechin.

decreasing the elevated mRNA expression of NF- κ B relative to β -actin (Fig. 5).

Histopathological and immunohistochemical findings

The normal liver histological structure is shown in Figure 6. The hepatocytes are arranged in hepatic trabeculae that radiate from the central vein (CV) forming sinusoids in between. The sinusoids are lined by Kupffer cells (Kc) and endothelial cells. The photomicrograph of liver section showed organized architecture and integrity.

The liver of Dox-administered rats showed congested CVs, Kupffer cell proliferation as well as hyperemic sinusoids. There were mild strands of fibroblasts around the hepatocytes and cytoplasmic vacuolization of hepatocytes (Fig. 7; photomicrograph 7a). Fibrosis of hepatic capsule and vacuolization of hepatocytes were also noticed (Fig. 6; photomicrograph 7b) in addition to fibroplasia of the bile duct and vacuolization of hepatocytes (Fig. 7; photomicrograph 7c).

The treatment with green tea aqueous extract produced marked amelioration of Dox-induced liver histological deleterious changes. Mild vascular changes and few Kupffer cells activation and binucleated hepatocytes were noticed in the liver of Dox-administered rats.

The treatment of Dox-administered rats with epicatechin induced a remarkable amelioration of liver histological changes. Slight thickening of hepatic capsules and apparent normal hepatocytes were noticed in liver sections of Dox-administered animals treated with epicatechin (Fig. 9; photomicrographs 9a and 9b).

The immunohistochemical sections of COX-2 expression in normal rats depicted a weak immunohistochemical reaction for COX-2 in hepatocytes (Fig. 10; photomicrograph 10a). On the other hand, the liver of Dox-administered rats exhibited an evoked strong reaction in the form of brownish color in the cytoplasm of hepatocytes (Fig. 10; photomicrograph 10b). The liver of Dox-administered rats treated with green tea aqueous extract (Fig. 10; photomicrograph 10c) and epicatechin (Fig. 10; photomicrograph 10d) showed a weak expression of COX-2.

Immunohistochemical staining of caspase-3 in liver of normal rat exhibited a weak expression of caspase-3 (Fig. 11; photomicrograph 11a). In contrast, the liver of Dox-administered rats showed a strong activated expression of caspase-3 represented by dense cytoplasmic brownish color (Fig. 11; photomicrograph 11b). On the other hand, Dox-administered rats treated with green tea aqueous extract (Fig. 11; photomicrograph 11c) and epicatechin (Fig. 11; photomicrograph 11d) exhibited a weak expression of caspase-3.

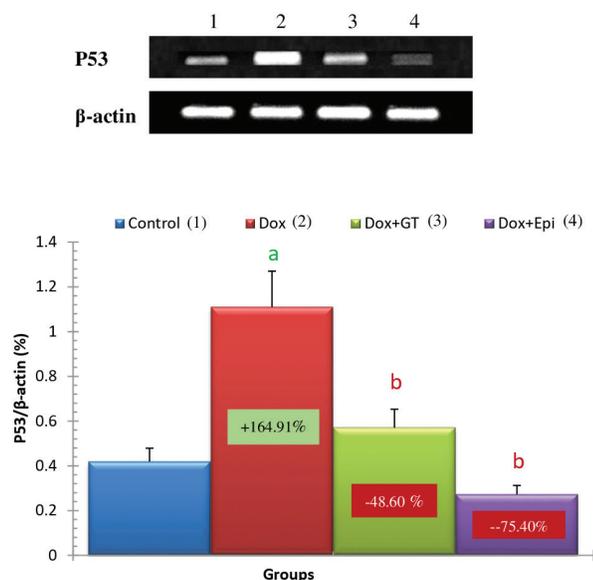


Figure 3. Effects of green tea aqueous extract and epicatechin on liver p53 mRNA expression relative to β -actin in Dox-administered rats. The number of detected samples in each group is three. ^{a,b}Significant difference in comparison with the corresponding control and Dox-administered groups, respectively, at $p < 0.05$. Percentage changes were calculated in relation to the normal control and Dox-administered groups, respectively. GT: green tea aqueous extract; Epi: Epicatechin.

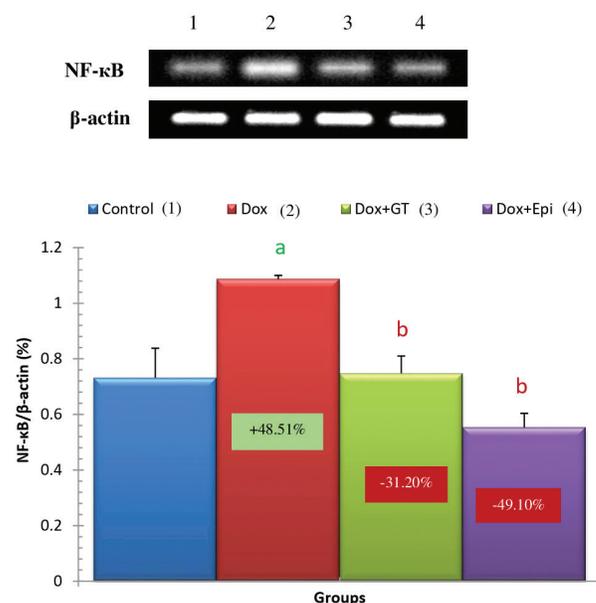


Figure 5. Effect of green tea aqueous extract and epicatechin on liver NF- κ B mRNA expression relative to β -actin in Dox-administered rats. ^{a,b}Significant difference in comparison with the corresponding control and Dox-administered groups, respectively, at $p < 0.05$. Percentage changes were calculated in relation to the normal control and Dox-administered groups, respectively.

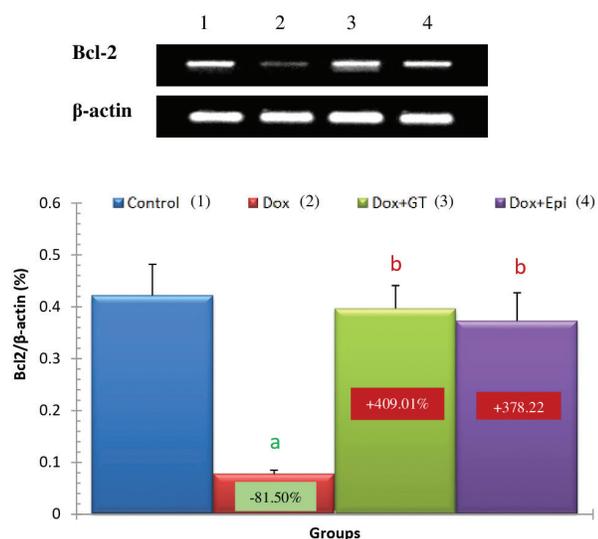


Figure 4. Effect of green tea aqueous extract and epicatechin on liver Bcl2 mRNA expression relative to β -actin in Dox-administered rats. The number of detected samples in each group is three. ^{a,b}Significant difference in comparison with the corresponding control and Dox-administered groups, respectively, at $p < 0.05$. Percentage changes were calculated in relation to the normal control and Dox-administered groups, respectively.

DISCUSSION

Dox is considered as one of the most potent anticancer agents. Its therapeutic application is limited due to its deleterious effects on normal tissues, such as the liver and heart (Ibrahim *et al.*, 2010; Wang *et al.*, 2012). However, the mechanisms of

Dox-mediated cytotoxicity in normal tissues and cancer cells are different (Wang *et al.*, 2012). In its mechanisms of action, Dox may act through DNA intercalation, membrane function alteration, and ROS formation (King and Perry, 2001). Dox is considerably metabolized in the liver (King and Perry, 2001); thereby, the liver is one of the organs that are mostly affected by Dox toxicity.

It is worth mentioning from many previous publications that Dox is considered as the most toxic anthracyclines and causes weight loss (Danz *et al.*, 2009; Panjraht *et al.*, 2007; Sayed-Ahmed *et al.*, 2010). For this reason, overdosage and long-term administration of this drug causes toxicity and death.

The main toxic and deleterious effects on hepatocytes include disruption of electron transport and oxidative stress and arrest cell cycle of hepatocytes (Kassner *et al.*, 2008; Zhao *et al.*, 2012). To reduce the toxic and side effects of Dox, various strategies were reported (EL-Hak *et al.*, 2018; Jabłońska-Trypuć *et al.*, 2018). The newly developed chemotherapeutic drugs often cause many side effects that restrict their use which are mostly oxidative stress-dependent (Ahmed and Abdella, 2010). Therefore, recent studies hypothesized that the combination of the chemotherapeutic drug such as Dox together with a potent natural antioxidant may be the appropriate approach to mitigate the toxic side effects of this kind of drugs (Ahmed and Abdella, 2010; Injac *et al.*, 2008).

The present study revealed that Dox injected weekly at dose 4 mg Dox/kg b.w. through intraperitoneal route for 6 weeks induced hepatotoxicity which was biochemically manifested by a significant elevation of serum ALT, AST, ALP, and GGT activities and bilirubin level in addition to a significant lowering in serum level of albumin. These changes run parallel with Ahmed *et al.* (2013) and Injac *et al.* (2008) who attributed the elevation in the

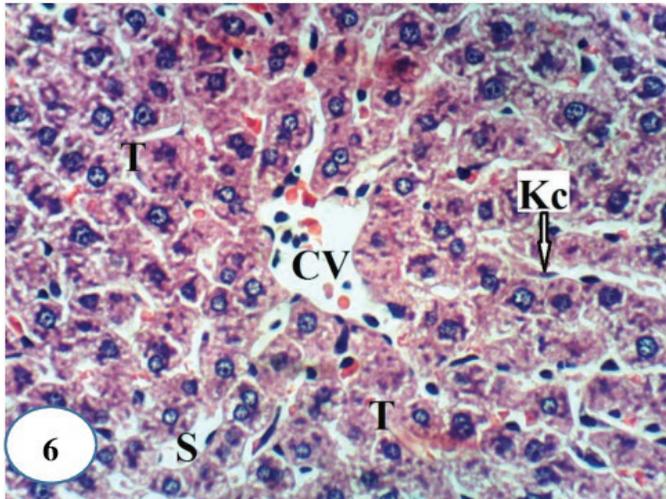


Figure 6. A photomicrograph of liver section of normal rats showing a normal histological structure of hepatic lobule and depicting a CV, hepatic strands or trabeculae (T) with sinusoids (S) and Kupffer cells (Kc) in-between (H&E $\times 400$).

serum enzyme activities to their excess leakage from degenerated hepatocytes and damage in bile ductular cells membranes as a result of toxicity. The significant decrease in serum albumin level in Dox-administered rats is in accordance with [EL-Maraghy *et al.* \(2009\)](#) who attributed this change to alterations in protein and free amino acid metabolism and their synthesis in the injured hepatocytes and/or increased protein degradation. On the other hand, the elevation in the total bilirubin level in serum of Dox-administered rats may be due to blockage of bile canaliculi as a result of inflammatory cells infiltration, fibroblast proliferation and fibrosis in the portal areas, and/or may be owing to regurgitation of direct (conjugated) bilirubin from the necrotic liver cells to sinusoids ([Ahmed, 2001](#); [Deepa and Varalakshmi, 2003](#); [Saad *et al.*, 2001](#)).

The previous deleterious biochemical alterations of liver biomarkers in serum of Dox-administered rats in the current study were accompanied with a remarkable increase in liver LPO and a significant suppression in the liver levels of non-enzymatic antioxidant (GSH) as well as enzymatic antioxidants (SOD, GPx, and GST). These results are in concurrence with those obtained by several authors ([Abd El-Aziz *et al.*, 2001](#); [Ahmed *et al.*, 2013](#); [Balachandar *et al.*, 2003](#); [Kalender *et al.*, 2005](#); [Patel *et al.*, 2010](#); [Yagmurca *et al.*, 2007](#)) who stated that one of the most convincing hypotheses of hepatic injury due to Dox injection is the ability of the drug to produce excess free radicals and lipid peroxides and to suppress free radical scavenging capacity and antioxidant defensive mechanism.

Histopathological investigation of liver sections of Dox-administered rats in the present study supported the previous biochemical results. The liver of Dox-administered rats showed congested CVs, hyperemic sinusoids, Kupffer cell multiplication, strands of fibroblasts around the hepatocytes, cytoplasmic vacuolization of hepatocytes, fibrosis of hepatic capsule, and periductal fibroblastic proliferation around the bile ductules. These results are in concordance with [Yagmurca *et al.* \(2007\)](#) who reported

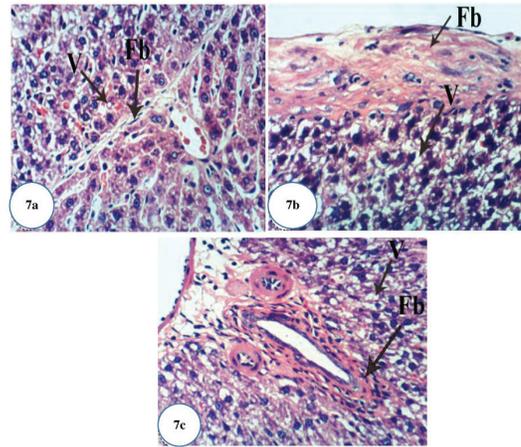


Figure 7. Photomicrographs of liver section of Dox-administered rats showing: (a) strands of fibroblasts (Fb) around the hepatocytes and vacuolization (V) of hepatocytes (H&E $\times 400$); (b) fibrosis of hepatic capsule and vacuolization (V) of hepatocytes (H&E $\times 400$); and (c) fibroplasia of bile duct and vacuolization (V) of hepatocytes (H&E $\times 400$).

destructive damage of hepatocytes, necrotic foci, blood congestion, and proliferation of bile canaliculi in Dox-supplemented rats.

Antioxidants obtained from natural sources and plants represent a logical treatment strategy for therapy of liver diseases. In this regard, there are many plant-derived chemicals with powerful antioxidant properties that may serve as primary compounds for developing novel hepatoprotective drugs ([Girish and Pradhan, 2012](#); [Pradhan and Girish, 2006](#)). Green tea and its constituting catechins are best known for their free radical scavenging capabilities, which have led to their evaluation in a number of diseases associated with exacerbated production of ROS, such as diabetes mellitus, cancer, neurodegenerative diseases, and cardiovascular disorders. Several epidemiological and meta-analysis studies as well as studies in animal models have depicted that green tea can afford protection against various types of cancers including breast, skin, lung, and prostate cancers ([Mukhtar and Ahmad, 2000](#); [Yang *et al.*, 2002](#)). The epicatechin as one of the major constituent of green tea is considered as one of the most potent antioxidant component of catechin ([Ishige *et al.*, 2001](#)) due to its higher free radical scavenging activity ([Kostyuk *et al.*, 2004](#)) and its greater bioavailability over other catechin components ([Baba *et al.*, 2001](#)).

In conduction with the previous studies, the treatment of Dox-administered rats with green tea aqueous extract and epicatechin potentially alleviated the raised serum ALT, AST, and ALP enzyme activities and bilirubin level. The lowered serum albumin level was detectably increased in Dox-administered rats treated with green tea aqueous extract and epicatechin. These improvements in serum biomarkers of liver function were associated with potential amendment of liver integrity and architecture and amelioration of Dox-induced deleterious hispathological changes. These alterations are in concordance with the previously published report of [Mandziuk *et al.* \(2015\)](#) who depicted that Dox-induced inflammation, eosinophilic degeneration, and interstitial edematous changes were markedly reduced by green tea. The ameliorative effects of green tea

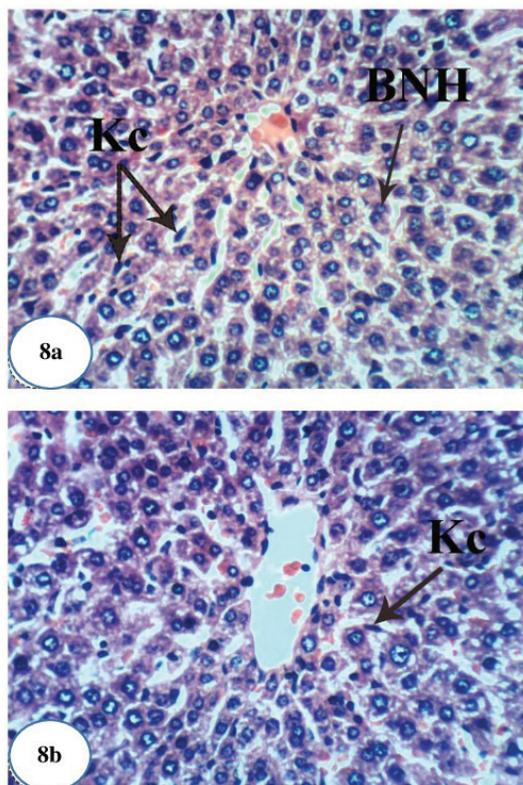


Figure 8. Photomicrographs of liver sections of Dox-administered rats treated with green tea. (a and b) Marked recovery of normal structure except the presence of few Kupffer cells (Kc) activation and binucleation of hepatocytes (BNH) (H&E $\times 400$).

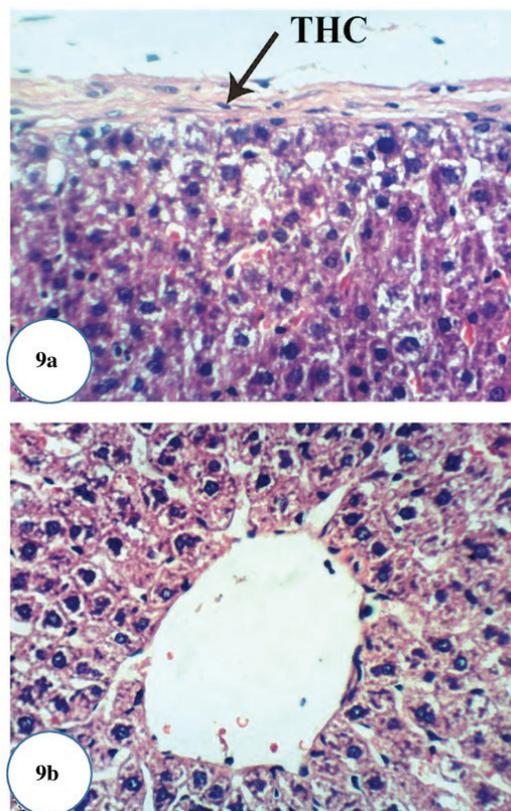


Figure 9. Photomicrographs of liver sections of Dox-administered rats treated with epicatechin. (a) Slight thickening of hepatic capsule (THC) (H&E $\times 400$) and (b) apparent normal hepatocytes (H&E $\times 400$).

aqueous extract and epicatechin may be attributed to the potentiation of the antioxidant defense system and suppression of ROS generation. It is important here to mention that the ALP activity was more decreased in the Dox-administered rats treated with green tea aqueous extract and epicatechin than the normal control. The decrease lower than the normal due to treatment with green tea may be attributed to the presence of epigallocatechin gallate and gallic acid gallate, which have inhibitory effects on phosphatases (Okamoto *et al.*, 2003) due to the presence of galloyl moiety in the structure. The epicatechin may also have inhibitory effects on ALP activity. The serum activity of GGT, responsible for extracellular GSH, was significantly increased as a result of treatment of Dox-administered rats with epicatechin. This up-regulation of GGT activity may be attributed in the light of suggestion of Chinta *et al.* (2006) who hypothesized that increased GGT activity may be an adaptive response to the loss of glutathione to conserve intracellular GSH content and results in a compensatory effect on mitochondrial complex I activity rather than in its inhibition and decrease following improvement of hepatobiliary function.

In the present study, the treatment of Dox-injected rats with green tea aqueous extract and epicatechin reduced liver LPO and increased the liver GSH level and GPx, GST, and SOD enzyme activities. These antioxidative features of green tea and its catechin have been reported in previous *in vivo* studies, which demonstrated that dietary intake of green tea catechins can improve total

antioxidant capacity and can decrease the level malondialdehyde (MDA), as a biomarker of LPO, in the rat's liver, blood, and brain (Skrzydłowska *et al.*, 2000). In another study, Quine and Raghu (2005) showed that epicatechin supplementation at a dose of 15 and 30 mg/kg b.w. in diabetic rats produced a significant decrease in MDA levels and increase in GSH concentration and SOD, GPx, and catalase (CAT) activities in the liver. In the same regard, Rizvi *et al.* (2005) had revealed that epicatechin treatment produced an elevation in GSH content in red blood cells of both normal and type 2 diabetic subjects. Rizvi and Zaid (2001) have also stated that tea catechins (epicatechin is being one of the components) protect type 2 diabetic red blood cells from tert-butyl hydroperoxide-induced oxidative stress. Moreover, Cuevas *et al.* (2009) found that epicatechin produced a significant decrease in LPO and reactive oxygen species in amyloid- β -treated rats. Mohamed *et al.* (2011) reported that SOD, CAT, and GPx in the brain tissues in Dox-administered rats were normalized and the elevated level of MDA was decreased upon using epicatechin supplementation. The strong free radical scavenging activity of epicatechin might be due to its antioxidant property as a result of the presence of adjacent hydroxyl (OH) groups on the same ring (Cuevas *et al.*, 2009; Haque *et al.*, 2006; Mohamed *et al.*, 2011; Rahman, 2016). In the present study, the chemical analysis of green tea extract indicated the presence of many free radical scavenging constituents including gallic acid, (-)-gallic acid, (-)-epigallocatechin, (-)-epicatechin,

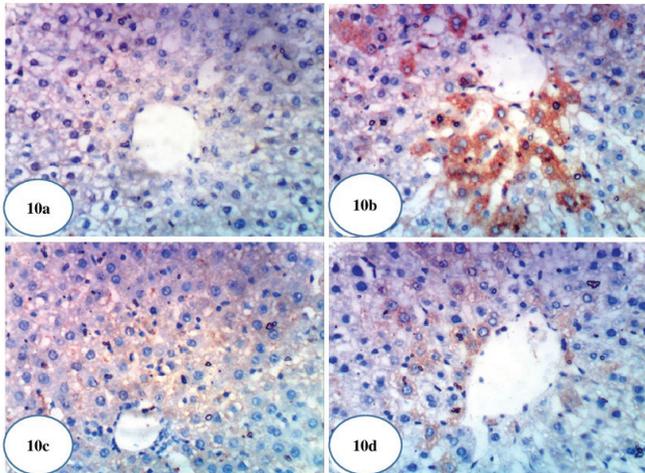


Figure 10. Photomicrographs of immunohistochemical stained liver sections for COX-2 expression detection. (a) A photomicrograph of immunohistochemical staining of COX-2 in liver of normal control rat showing a weak expression of COX-2 ($\times 400$). (b) A photomicrograph of immunohistochemical staining of COX-2 in liver of Dox-administered rats showing a strong stimulated expression of COX-2 (immunopositivity indicated by brownish color) ($\times 400$). (c) A photomicrograph of immunohistochemical staining of COX-2 in liver of Dox-administered rats treated with green tea showing a weak expression of COX-2 (immunopositivity indicated by brownish color) ($\times 400$). (d) A photomicrograph of immunohistochemical staining of COX-2 in liver of Dox-administered rats treated with epicatechin showing a weak expression of COX-2 (immunopositivity indicated by brown color) ($\times 400$).

(-)-epigallocatechin, (-)-epigallocatechin gallate, (-)-gallocatechin gallate, (-)-epicatechin gallate, (-)-catechin gallate, and caffeine.

Tumor marker AFP, a specific glycoprotein secreted from fetal liver and yolk sac, that rapidly falls few weeks after birth (Patil *et al.*, 2013), is the most reliable serum marker for the diagnosis of hepatocellular carcinoma (HCC) (Wen-Jun *et al.*, 2013). There was a marked elevation in serum AFP level in rats injected with Dox alone. This finding is consistent with the previous report which revealed that the injection of Dox to rats resulted in the increase of serum AFP level reflecting increased probability of and susceptibility to HCC (Kim *et al.*, 2010). On the other hand, the Dox-injected groups treated with green tea and epicatechin showed an improvement in the serum level of AFP reflecting their potent anti-cancer and hepatoprotective effects. In agreement with the previous publication of Abozalim *et al.* (2016), Nakagami (2004), Rani *et al.* (2014), Shanmugam *et al.* (2017), and Tadesse *et al.* (2015) who reported the anticancer and antioxidant effects of green tea and epicatechin.

In the present study, the apoptotic mediators, p53, and caspase 3 expressions in liver were markedly elevated in Dox-administered rats while expression of anti-apoptotic marker Bcl-2 was significantly lowered. The treatment of Dox-administered rats with green tea aqueous extract and epicatechin prevented these alterations. The augmented apoptosis in Dox-administered rats may be attributed to the activated oxidative stress. This attribution was supported by Patel *et al.* (2010) who stated that oxidative stress has been implicated as a contributor factor to various forms of cell death, involving a specific inducer of apoptosis. In turn, the reduced apoptosis in the liver as a result of the treatment of Dox-injected rats with green tea aqueous

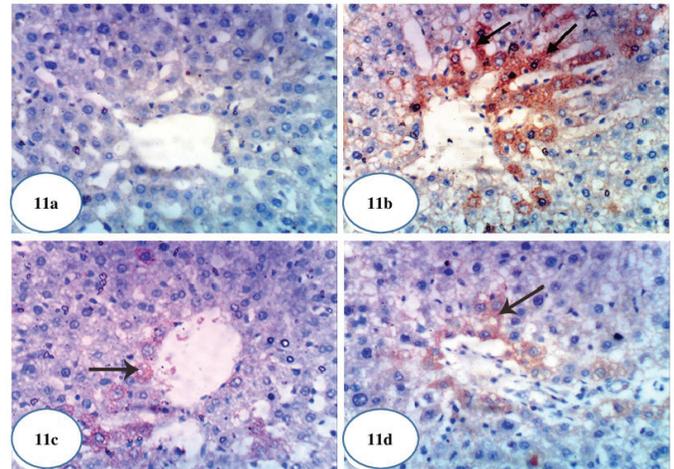


Figure 11. Photomicrographs of immunohistochemical stained liver sections for caspase-3 expression detection. (a) A photomicrograph of immunohistochemical staining of caspase-3 in liver of normal control rat showing a weak expression of caspase-3 ($\times 400$). (b) A photomicrograph of immunohistochemical staining of caspase-3 in liver of Dox-administered rats showing a strong augmented expression of caspase-3 (immunopositivity indicated by brownish color) ($\times 400$). (c) A photomicrograph of immunohistochemical staining of caspase-3 in liver of Dox-administered rats treated with green tea showing a weak expression of caspase-3 (immunopositivity indicated by brownish color) ($\times 400$). (d) A photomicrograph of immunohistochemical staining of caspase-3 in liver of Dox-administered rats treated with epicatechin showing a weak expression of caspase-3 (immunopositivity indicated by brownish color) ($\times 400$).

extract and epicatechin may result secondary to the suppressed oxidative stress and enhanced antioxidant defense system. This suggestion runs parallel with Spencer *et al.* (2001) who evidenced that 3'-O-methyl epicatechin inhibits H_2O_2 -induced cell death and that the mechanism involves attenuation of caspase-3 activity as an apoptotic marker.

To assess the effect on the inflammatory status, the levels of serum TNF- α and IL-4 were determined by ELISA, liver NF- κ B mRNA expression was assayed by RT-PCR, and COX-2 was assayed by immunohistochemical technique.

In the current study, the level of pro-inflammatory cytokine TNF- α in serum was significantly elevated in Dox-administered rats while the level of anti-inflammatory cytokine IL-4 was significantly decreased reflecting the preponderance of T helper 1 (Th1) and the presence of elevated Th1: T helper 2 (Th2) cell ratio. These data are in concurrence with Shankar *et al.* (2007) who found that injection of Dox augments a peripheral increase in the cytokine TNF- α , which stimulates several inflammatory pathways. As indicated by Tangpong *et al.* (2006), TNF- α induces mitochondrial malfunction by its downstream consequences, leading to further increase in cytochrome C release, oxidative stress, caspase 3 activity, and TUNEL-positive cell death, all of which are implicated as inducers of apoptosis following Dox injection. On the other hand, IL-4 was reported to inhibit multiple functions of activated macrophages, including macrophage production of TNF- α and interleukin-1 β (IL-1 β), and the macrophage secretion of reactive oxygen intermediates and up-regulate expression of interleukin-1 (IL-1) receptor antagonist (Abramson and Gallin, 1990; Hart *et al.*, 1989; Vannier *et al.*, 1992). It also activates macrophage 15 lipoxygenase activity, which

may suppress the synthesis of pro-inflammatory leukotriene B4 (LTB4) (Katoh *et al.*, 1994). The treatments of Dox-administered rats with green tea aqueous extract and epicatechin, in the present study, improved the deteriorations in both TNF- α and IL-4 levels reflecting dominance of Th2 cells. Thus, both green tea aqueous extract and epicatechin may have potent anti-inflammatory effects by activating the production Th2 cytokines and suppressing the activity of Th1 cells.

It was also evidenced in the present study that the liver NF- κ B and COX-2 expressions were remarkably increased in Dox-administered rats and were recovered toward normal levels as a result of treatments of Dox-administered rats with green tea aqueous extract and epicatechin. These results are in concurrence with many previous reports. Lagha and Grenier (2015) found that black and green tea polyphenols suppress the inflammatory response of monocytes/macrophages mediated by *Fusobacterium nucleatum* (*F. nucleatum*). They also first stated that the black and green tea extracts, theaflavins, (-)-epigallocatechin-3-gallate (EGCG) reduce the NF- κ B activation induced by *F. nucleatum* in monocytes. It is also relevant here to mention that NF- κ B is a key regulator of genes coding for inflammatory mediators including of interleukin-1 β (IL-1 β), TNF- α , IL-6, and C-X-C Motif Chemokine Ligand 8 (CXCL8) by macrophages (Lagha and Grenier, 2015). Many other publications revealed the inhibitory effects of EGCG, which is a major component of green tea on NF- κ B activation as well as TNF- α and COX-2 expression (Aggarwal and Shishodia, 2006; Gupta *et al.*, 2004; Shankar *et al.*, 2008; Shimizu *et al.*, 2004). In the same way, Mohamed *et al.* (2011) reported that the treatment with epicatechin prior to Dox in rats significantly prevented the increase in TNF- α , iNOS, and NF- κ B expressions.

In conclusion, the *C. sinensis* aqueous extract and epicatechin have potent preventive effects against Dox-induced hepatotoxicity. The suppression of oxidative stress, the enhancement of antioxidant defense system, the modulatory effects of inflammatory signaling pathways, and anti-apoptotic actions all are implicated to prevent the Dox-induced hepatotoxicity and to improve the liver architecture and integrity. Thus, *Camellia sinensis* aqueous extract and epicatechin may be useful substances for patients treated with Dox.

ABBREVIATIONS

AFP: alpha-fetoprotein; ALP: alkaline phosphatase; ALT: alanine transaminase; AST: aspartate transaminase; b.w.: body weight; kg: kilogram; Bcl2: B-cell lymphoma 2; BNH: binucleation of hepatocytes; CAT: catalase; CCAC: Canadian Council on Animal Care; CMC: carboxymethyl cellulose; COX-2: cyclooxygenase-2; CV: central vein; Dox: Doxorubicin; Fb: fibroblasts; GGT: gamma glutamyl transferase; GPx: glutathione peroxidase; GSH: glutathione; SEM: standard error mean; GST: glutathione-S-transferase; H&E: Haematoxylin and Eosin; i.p.: intraperitoneally; IL-1 β : interleukin-1 β ; IL-4: interleukin-4; Kc: Kupffer cells; LPO: lipid peroxidation; LTB4: leukotriene B4; MDA: malondialdehyde; NF- κ B: nuclear factor-kappa B cells; p53: protein 53; ROS: reactive oxygen species; RT-PCR: reverse transcriptase-polymerase chain reaction; S: sinusoids; SOD: superoxide dismutase; T: trabeculae; Th1: T helper 1; Th2: T helper 2; THC: thickening of hepatic capsule; TNF- α : tumor necrosis factor-alpha; V: vacuolization.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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