IJPSR (2019), Volume 10, Issue 5 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 19 August 2018; received in revised form, 16 November 2018; accepted, 06 December 2018; published 01 May 2019

EFFECTS OF GREEN TEA INFUSION AND EPICATECHIN ON DOXORUBICIN-INDUCED RENOCARDIOTOXICITY IN MALE ALBINO RATS

Osama M. Ahmed [*1], Manal M. Abdul-Hamid [2], Ahlam M. El-Bakry [3], Hanaa M. Mohammed [2] and Fatma El-Zahraa S. Abdel Rahman [4]

Physiology Division [1], Cell Biology, Histology and Genetics Division [2], Comparative Anatomy and Embryology Division [3], Department of Zoology, Faculty of Science, Beni-Suef University, Beni-Suef, Egypt.
Department of Basic Sciences [4], Faculty of Oral and Dental Medicine, Nahda University, Beni-Suef, Egypt.

Keywords:

Doxorubicin, Green tea, Epicatechin, Kidney, Heart, Oxidative stress

Correspondence to Author:
Osama M. Ahmed

Physiology Division,
Department of Zoology,
Faculty of Science, Beni-Suef University, Beni-Suef, Egypt.

E-mail: osamamoha@yahoo.com

ABSTRACT: This study aims to assess the preventive effects of green tea infusion and epicatechin on doxorubicin (Dox)-induced kidney and heart injuries and oxidative stress in male Wistar rats. The male Wistar rats administered Dox at an intraperitoneal dose of 4 mg/kg body weight (b.w.)/ week were orally treated with green tea infusion (200 mg/kg b.w.) and epicatechin (25 mg/kg b.w.) every other day for 6 weeks. The treatments of Dox-administered rats with green tea infusion and epicatechin resulted in a significant decrease in the elevated serum creatinine, urea and uric acid levels reflecting an improvement in kidney function. Similarly, the elevated serum CK-MB, LDH and AST activities were significantly ameliorated as a result of treatments of Dox-administered rats, thereby manifesting an amendment of heart function. The treatments also led to the prevention of the elevated lipid peroxidation and amelioration of the lowered GPx and GST activities as well as GSH content in kidney and heart. However, the SOD activity was not significantly altered in kidney and heart as a result of treatment of Dox-administered rats with green tea infusion and epicatechin. Also, the treatments remarkably improved Dox-induced deleterious histological alterations and inflammatory changes in kidney and heart in association with a significant decrease in serum TNF-α and an increase in serum IL-4 level. In conclusion, the green tea infusion and epicatechin may have chemopreventive potentials against Dox-induced nephrocardio-toxicity via suppression of oxidative stress, enhancement of antioxidant defense system and attenuation of inflammatory effects.

INTRODUCTION: Doxorubicin (Dox) is a traditional chemotherapeutic agent used for the treatment of a wide variety of cancers [1].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.10(5).2210-23

The article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.10(5).2210-23

Despite being highly effective, the use of Dox has many limitations due to the significant toxicity and side effects that occurred during and after hepatocellular carcinoma (HCC) treatment [2, 3].

These toxicities usually affect heart, brain, bone marrow and the consequences of these toxicities are often very apparent and will last for many years after treatment [2, 4]. Also, the kidney is also highly susceptible to toxicants for two reasons; a high volume of blood flowing through it and filtration of

large amounts of toxins which can concentrate in the kidney tubules. It can result in systemic toxicity causing decreased ability to excrete body wastes, inability to maintain body fluid and electrolyte balance and decreased the synthesis of essential hormones [5]. Several studies were conducted for antioxidants screening from natural medicine aiming to minimize oxidative injury by Dox [6]. Several natural anti-oxidants have been shown to alleviate the DOX-induced cell damage without compromising its anti-tumor efficacy in the animal studies [7].

Green tea and its constituting catechins are best known for their antioxidant properties, which have led to their evaluation in several diseases associated with reactive oxygen species (ROS), such as cardiovascular and neurodegenerative diseases and cancer [8, 9]. Several epidemiological studies, as well as studies in animal models, have shown that green tea can afford protection against various cancers such as those of the skin, breast, prostate and lung [10]. A role for epicatechin in the prevention of cancer was also reported; epicatechin promotes the maintenance of gap junctions between epithelial cells helping to prevent the progression of gastrointestinal lesions into malignant lesions [11]. Therefore, this study was designed to scrutiny the preventive effects of green tea infusion and epicatechin on Dox-induced kidney and heart injuries, oxidative stress, and inflammation in albino rats.

MATERIALS AND METHODS:

Experimental Animals: Male Wistar rats weighing about 140-180 g were used as experimental animals in the present investigation. They were obtained from the animal house of Research Institute of Ophthalmology, 2 El-Ahram Street, Giza, Egypt. They were kept under observation for about 15 days before the onset of the experiment to exclude any intercurrent infection. The chosen animals were housed in polypropylene cages with well-aerated covers at normal atmospheric temperature (25 ± 5 ºC) as well as 12 h daily normal light periods. Moreover, they were given access to water and supplied daily with standard pellet diet *ad libitum*.

All animal procedures are by the recommendations of the Canadian Committee for care and use of animals [12] and follow the guidelines and instructions for animal use and care of Experimental Animal Ethics Committee of Faculty of Science, Beni-Suef University, Egypt (Ethical Approval Number: BSU/FS/2014/8).

Chemicals and Drugs: Dox hydrochloride (adricin 10 mg vials), manufactured by Pharmacia Italia Nerviano Italy, was obtained through El Gomhuria Pharmacy, Cairo, Egypt. Green tea was purchased from Harraz Medicinal Plant Company, Cairo, Egypt (www.harrazegypt.com). Epicatechin was purchased from Sigma Chemical Company, St. Louis, MQ. Saline (0.9% sodium chloride) obtained from ADWIC Company and produced by El-Nasr Pharmaceutical Chemical Company, Cairo, Egypt. Carboxymethyl cellulose 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 Spectrum (LC/ESI-MS/MS): Liquid Chromatography (LC) coupled with Mass Spectrometry (MS) and a source of Electrospray Ionization Tandem Mass Spectrum (ESI-MS) was used for chemical analysis of green tea extract. The separation of the green tea sample was performed according to Yoshida *et al*., [13] method with some modifications on ZORBAX-C18 (4.6 × 100 mm id, 3.5 µm) analytical column using LC/ DAD/MS system Agilent 1100 quaternary pump (USA), coupled with a deuterium-arc-discharge (DAD) (Agilent 6120 quadruple spectrometer, CA, USA) wavelength setting of 280 nm.

The instrument conditions are as follow: pressure was 45.5 bar at the starting point and 55.9 bar at the stopping point; the flow rate of the mobile phase was 0.4 ml/min with a column compartment temperature of 40 ºC and an injection volume of 10 µl. The mobile phase was consisting 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 condition was: initial concentration of 10% B with programming to 15% B over 15 min. and to 27% B over 15 min. Then, the column was equilibrated under the initial condition for 10 min. The mass selective detector (MSD) instrument and ESI conditions were as

follows: vaporized temperature or gas temperature, 350 ºC; nebulizer gas, nitrogen, at a pressure 50 psi; drying gas, also nitrogen, at a flow rate 12 L/min and capillary voltage, -3500 V of negative mode and 3500 V of positive mode and scan range 100-1000 m/z. ESI of catechins in negative mode produces the $[M-H]^-$ molecular adduct ion while ESI of the caffeine in positive 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 one weekly dose (10:00-12:00 AM) for 6 weeks [14]. It was dissolved in sterile saline (0.9% NaCl) as a vehicle, and it was freshly prepared before injection.

Dose Preparation of Green Tea and Epicatechin: Green tea, *Camellia sinensis* (Theaceae) was authenticated by Dr. Walaa Azmy Hassan, Assistant Professor of Taxonomy and Flora, Botany Department, Faculty of Science, Beni-Suef University, Egypt). A voucher specimen was deposited in the herbarium of Botany Department, Faculty of Science, Beni-Suef University, Egypt. An electric grinder powdered the dried green tea leaves. The aqueous extract in the form of infusion was prepared according to the method of Swanston-Flatt *et al*., [15] and Ahmed [16]. The powder of green tea leaves was added to the already boiled distilled sterile water (2 g / 50 ml; 4% w/v) and infused for 15 min. The infusion was then filtered, and the filtrate was freshly used for oral administration to Dox-administered rats at a dose of 200 mg/kg b.w./day) according to Al-Hilfy [17]. Epicatechin was prepared by dissolving the required dose in 1% carboxymethyl cellulose (CMC) (25 mg/5 ml 1% CMC/ kg b.w./day).

Animal Grouping: Forty adult male Albino rats were randomly divided into four groups of 10 animals each as follows:

Group 1 (Normal Control Group): The rats of this group received the equivalent volume of saline intraperitoneally (i.p.) injected/week for 6 weeks. The rats of this group were given the same volume of the vehicle (CMC 1% solution) by oral gavage every other day for 6 weeks.

Group 2 (Dox-administrated Group): Dox (dissolved in saline) was injected i.p. as a total cumulative dose of 24 mg/kg b.w. divided into 6 equal doses, each of 4 mg/kg b.w. once weekly for 6 weeks [14]. The rats of this group were given the same volume of the vehicle (CMC 1% solution) by oral gavage every other day for 6 weeks.

Group 3 (Dox- administrated Rats Treated with Green Tea Infusion): This group was given Dox as group 2 plus green tea by oral gavage at a dose of 200 mg/kg b.w. every other day for 6 weeks [17]. The rats of this group were given the same volume of the vehicle (CMC 1% solution) as in groups 1, 2 and 4 by oral gavage every other day for 6 weeks.

Group 4 (Dox-administrated Rats Treated with Epicatechin): The rats of this group were administered Dox as group 2 plus epicatechin (dissolved in CMC 1% solution) by oral gavage at a dose of 25 mg/5 ml/kg b.w. every other day for 6 weeks [18].

Preparation of Blood and Tissue Homogenates: By the end of the experimental periods (6 weeks), rats were under diethyl ether anesthesia at fasting state. Blood samples were collected from jugular veins and allowed to coagulate at room temperature. The clear non-haemolysed supernatant sera were quickly aspirated and divided into three portions for each, and stored at -20 ºC for analysis of serum levels of kidney and heart function parameters, TNF-α and IL-4.

Kidney and heart were quickly excised, weighed and homogenized in a saline solution (0.9% NaCl) (10% w/v) using Teflon homogenizer (Glas-Col, Terre Haute, USA). The homogenates were centrifuged at 3000 r.p.m. for 15 min, and the supernatants were kept at -20 ºC for the assay of oxidative stress and antioxidant defense system markers.

Assay of Kidney Function Parameters: Serum creatinine level was measured according to Jaffe [19] by using kits purchased from Stanbio Laboratories (Texas, USA). Serum urea level was determined according to the method of Patton and Crouch 20 using reagent kits purchased from Diamond Diagnostic Chemical Company (Egypt). Serum uric acid level was determined according to the method of Fossati *et al*., [21] using reagent kits purchased from Spinreact Diagnostic Chemical Company (Spain).

Assay of Heart Function Parameters: CK-MB activity in serum was determined according to the method of Szasz [22] using reagent kits obtained from Stanbio Laboratories (Texas, U.S.A). The activity of LDH in serum was determined according to Henderson and Moss [23] using reagent kits purchased from Seppim S.A.S. Chemical Company (France). Serum AST activity was determined according to Murray [24] using reagent kits purchased from Spinreact, 7 E-17176 Sant Esteve De Bas (Gi), Spain.

Statistical Analysis: Statistical analysis was performed using SPSS version 20. Results were expressed as mean ± standard error (SE) and all statistical comparisons were made by Duncan's test post-hoc analysis. P-value at $P<0.05$ was considered significant while values of $P>0.05$ were considered non-significant. Percentage of changes were calculated by comparing Dox-administered group with the normal control and Dox-administered groups treated with green tea infusion and epicatechin with Dox-administered control group.

RESULTS: The chemical analysis of green tea extract by LC/ESI-MS/MS indicated the presence gallic acid, (-)-gallocatechin, (-)-gallocatechin, (-)-epigallocatechin, caffeine, (-)-epicatechin, (-)-epigallocatechin gallate, (-)-gallocatechin gallate, (-)-epicatechin gallate and (-)-catechin gallate **Table 1** and **Fig. 1**.

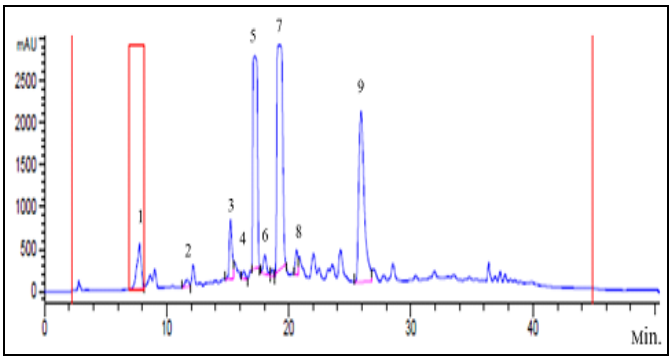

FIG. 1: LC/ESI-MS OF GREEN TEA EXTRACT. PEAKS IDENTIFICATION: 1: gallic acid; 2: (-)-gallocatechin; 3: (-)-gallocatechin; 4: (-)-epigallocatechin; 5: caffeine; 6: (-)-epicatechin; 7: (-)-epigallocatechin gallate; 8: (-)-gallocatechin gallate; 9: (-)-epicatechin gallate and (-)-catechin gallate.

TABLE 1: LC/ESI-MS DATA OF THE COMPOUNDS IDENTIFIED IN GREEN TEA AQUEOUS EXTRACT

Peak no.	t_R (min)	Compound Identity	$M-H]^-$ (m/z)	$M-H]^+$ (m/z)
1	7.83	Gallic acid	169	
2	11.76	(-)-gallocatechin	305	
3	15.33	(-)-epigallocatechin	305	
4	16.35	(+)-catechin	289	
5	17.27	Caffeine		195
6	19.16	(-)-epicatechin	289	
7	19.25	(-)-epigallocatechin gallate	457	
8	20.64	(-)-gallocatechin gallate	457	
9	26.00	(-)-epicatechin gallate and (-)-catechin gallate	441	

Concerning serum parameters of kidney function, the Dox-administered rats showed a significant increase ($P<0.05$) in serum levels of creatinine, urea, and uric acid recording percentage increases of 59.62, 54.09 and 126.23% respectively as compared to normal control group. The treatment of Dox-administered rats with green tea infusion induced a significant decrease of the elevated serum levels of creatinine, urea and uric acid ($P<0.05$) levels; the recorded percentage changes were -30.37, -40.41 and -59.96% respectively as compared to Dox-administered rats. On the other hand, the treatment of Dox-administered rats with epicatechin induced a significant decrease ($P<0.05$) in serum creatinine, urea and uric acid levels recording percentage changes of -36.04, -24.03 and -57.97% respectively as compared to Dox-administered rats. The treatment with epicatechin was more effective in improving the elevated serum creatinine level while the green tea infusion was more potent in decreasing the elevated serum urea level **Table 2**.

Regarding serum parameters related to heart function, the Dox-administered rats showed a significant increase ($P<0.05$) in serum level of CK-MB, LDH and AST activities recording percentage increases of 69.19, 76.47 and 31.35% respectively as compared to normal control group. The treatment of Dox-administered rats with green tea infusion induced a significant decrease of the elevated serum of CK-MB, LDH and AST

activities (P<0.05); the recorded percentage changes were -48.74, -64.57 and -35.78% respectively as compared to Dox-administered rats. Similarly, the treatment of Dox-administered rats with epicatechin induced a significant decrease (P<0.05) in serum CK-MB, LDH and AST activities recording percentage changes of -60.49, -31.94 and -24.51% respectively as compared to Dox-administered rats **Table 3**.

TABLE 2: EFFECT OF GREEN TEA INFUSION AND EPICATECHIN ON SERUM CREATININE, UREA AND URIC ACID LEVELS IN DOX-ADMINISTERED RATS

Experimental conditions	Creatinine (mg/dl)	Urea (mg/dl)	Uric acid (mg/dl)
Control	0.52 ± 0.01	25.20 ± 1.56	2.44 ± 0.14
Dox	0.83 ± 0.06[a]	38.83 ± 1.74[a]	5.52 ± 0.26[a]
% Change[(A)]	(+60.19%)	(+54.09%)	(+126.23%)
Dox + GT	0.58 ± 0.06[b]	23.14 ± 1.20[b]	2.21 ± 0.26[b]
% Change[(B)]	(-30.37 %)	(-40.41%)	(-59.96 %)
Dox + Epi	0.53 ± 0.03[b]	29.50 ± 1.61[b]	2.32 ± 0.05[b]
% Change[(B)]	(-36.01%)	(-24.03%)	(-57.97%)

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 group respectively, at P<0.05. (A), (B): Percentage of changes about the normal control and Dox-administered group respectively. Dox: doxorubicin; GT: Green tea infusion; Epi: Epicatechin

TABLE 3: EFFECT OF GREEN TEA INFUSION AND EPICATECHIN ON SERUM CK-MB, LDH AND AST ACTIVITIES IN DOX-ADMINISTERED RATS

Experimental conditions	CK-MB (U/l)	LDH (U/dl)	AST (U/L)
Control	99.00 ± 2.76	1670.01 ± 47.38	154.80 ± 5.70
Dox	167.50 ± 2.73[a]	2947.01 ± 65.26[a]	203.33 ± 6.35[a]
% Change[(A)]	(+69.19 %)	(+76.47 %)	(+31.35%)
Dox + GT	85.86 ± 1.84[b]	1043.90 ± 37.21[b]	130.57 ± 2.50[b]
% Change[(B)]	(-48.74%)	(-64.58 %)	(-35.78%)
Dox + Epi	66.17 ± 0.98[b]	2005.72 ± 20.35[b]	153.50 ± 4.87[b]
% Change[(B)]	(-60.49 %)	(-31.94%)	(-24.51%)

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 group respectively, at α=0.05, P<0.05). (A), (B): Percentage of changes about the normal control, Dox-administered and Dox plus green tea group respectively. Dox: doxorubicin; GT: Green tea infusion; Epi: Epicatechin

Kidney SOD activity in Dox-administered rats was non-significantly decreased recording a percentage decrease of 0.26% as compared to normal control group. In contrast, kidney LPO was significantly increased (P<0.05) in Dox-administered rats recording a percentage increase of 69.41% as compared to normal control group. The treatment of Dox-administered rats with green tea infusion induced a non-significant increase of kidney SOD activity; the recorded percentage increase was 5.94%. In contrast, kidney LPO was significantly decreased (P<0.05) as a result of green tea infusion treatment recording a percentage decrease of 42.24% as compared to Dox-administered rats.

On the other hand, the treatment of Dox-administered rats with epicatechin induced a non-significant increase of kidney SOD activity; the recorded percentage increase was 3.15%. In contrast, kidney LPO was significantly decreased (P<0.05) as a result of epicatechin treatment recording a percentage decrease of 53.10% as compared to Dox-administered rats **Table 4**.

TABLE 4: EFFECT OF GREEN TEA INFUSION AND EPICATECHIN ON KIDNEY SOD ACTIVITY AND LIPID PEROXIDATION IN DOX-ADMINISTERED RATS

Experimental conditions	SOD activity (U/g tissue)	LPO (nmole/ 100 mg tissue/h)
Control	14.51 ± 0.55	8.86 ± 1.10
Dox	14.47 ± 1.42	15.01 ± 2.96[a]
% Change[(A)]	(-0.26 %)	(+69.41 %)
Dox + GT	15.33 ± 0.69	8.67 ± 0.96[b]
% change[(B)]	(+5.94 %)	(-42.24%)
Dox + Epi	14.92 ± 1.53	7.04 ± 1.52[b]
% Change[(C)]	(+3.15 %)	(-53.10 %)

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 about the normal control and Dox-administered groups respectively.

Kidney GSH, GPx and GST stores of Dox-administered exhibited a marked depletion

(P<0.05) as compared to the normal control group; the recorded percentage changes were -25.69, -14.92 and -28.22% respectively. The treatment of Dox-administered rats with green tea infusion significantly (P<0.05) prevented this depletion in GSH content and GPx activity recording percentage changes of 16.21 and 23.98% respectively. In contrast, GST activity was non-significantly affected as a result of green tea infusion treatment recording a percentage change of 28.56%. On the other hand, the treatment of Dox-administered rats with epicatechin induced a significant increase (P<0.05) of the serum GSH content and GPx activity recording percentage changes of 13.53 and 20.11% respectively. In contrast, GST activity was non-significantly affected as a result of epicatechin treatment recording a percentage change of 38.40% **Table 5**.

TABLE 5: EFFECTS OF GREEN TEA INFUSION AND EPICATECHIN ON KIDNEY GSH CONTENT AND GPX AND GST ACTIVITIES IN DOX-ADMINISTERED RATS

Experimental conditions	GSH (nmole/100mg tissue)	GPx (mU/100mg tissue)	GST (U/100mg tissue)
Control	125.15 ± 11.19	119.74 ± 4.02	77.38 ± 5.89
Dox	93.00 ± 6.51[a]	101.87 ± 10.77[a]	55.54 ± 6.99[a]
% change[(A)]	(-25.69%)	(-14.92%)	(-28.22%)
Dox+GT	108.08 ± 18.69[b]	126.30 ± 6.63[b]	71.40 ± 19.17
% change[(B)]	(+16.22%)	(+23.98%)	(+28.56%)
Dox+Epi	105.58 ± 6.07[b]	122.36 ± 2.57[b]	76.87 ± 16.09
% change[(C)]	(+13.53%)	(+20.11%)	(+38.40%)

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 group respectively, at P<0.05. (A), (B): Percentage of changes about the normal control and Dox-administered group respectively.

TABLE 6: EFFECTS OF GREEN TEA INFUSION AND EPICATECHIN ON HEART SOD ACTIVITY AND LIPID PEROXIDATION IN DOX-ADMINISTERED RATS

Experimental conditions	SOD activity (U/g tissue)	LPO (nmole/ 100 mg tissue/h)
Control	13.23 ± 0.23	7.23 ± 0.71
Dox	12.13 ± 0.375[a]	13.53 ± 0.92[a]
% change[(A)]	(-8.343%)	(+87.14%)
Dox+GT	12.52 ± 0.22	5.56 ± 0.79[b]
% change[(B)]	(+3.19%)	(-58.91%)
Dox+Epi	12.78 ± 0.263	8.43 ± 1.01[b]
% change[(C)]	(+5.42%)	(-37.69%)

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 group respectively, at P<0.05. (A), (B): Percentage of changes about the normal control and Dox-administered group respectively. Dox: doxorubicin; GT: Green tea infusion; Epi: Epicatechin.

The heart SOD activity was significantly (P<0.05) decreased in Dox-administered rats recording percentage change of -8.34% as compared to normal control group, In contrast, the heart LPO of Dox- administered rats exhibited a highly significant increase (P<0.05); the recorded percentage change was +87.14%. The treatment of Dox-administered rats with green tea infusion and epicatechin produced a non-significant change of SOD activity **Table 6**. The heart GSH, GPx and GST stores of Dox-administered rats showed a marked depletion (P<0.05) as compared to the normal control group; their recorded percentage changes were -31.75, -11.44 and -17.49% respectively. The treatment of Dox-administered rats with green tea infusion significantly (P<0.05) prevented this depletion in GSH content as well as GPx and GST activities recording percentage changes of 21.47, 10.85 and 21.19% respectively. Similarly, the treatment of Dox-administered rats with epicatechin induced a significant increase (P<0.05) of the serum GSH content, as well as GPx and GST stores as compared to the normal control group their recorded percentage changes, were 37.39, 13.04 and 19.39% respectively **Table 7**.

Serum TNF-α level was significantly (P<0.05) increased in Dox-administered rats recording percentage increase of 288.80% as compared with normal control. The treatment of Dox-administered rats with green tea infusion and epicatechin produced a significant decrease (P<0.05) of the elevated serum TNF-α level; the recorded percentage decreases were -63.29 and -51.06% respectively as compared to Dox-administered rats. Thus, green tea infusion was more effective in improving the elevated serum TNF-α level **Fig. 2**.

Serum IL-4 level was significantly (P<0.05) decreased in Dox-administered rats recording percentage decrease of -64.00% as compared with normal control. The treatment of Dox-administered

rats with green tea infusion and epicatechin produced a significant improvement of the lowered serum IL-4 level; the recorded percentage increases were 110.32 and 106.80% respectively as compared to Dox-administered rats. Thus, green tea infusion was more potent in improving the lowered serum IL-4 level **Fig. 3**.

TABLE 7: EFFECTS OF GREEN TEA AQUEOUS EXTRACT AND EPICATECHIN ON HEART GSH CONTENT AND GPx AND GST ACTIVITIES IN DOX-ADMINISTERED RATS

Experimental conditions	GSH (nmole/100mg tissue)	GPx (mU/100mg tissue)	GST (U/100mg tissue)
Control	90.57 ± 4.92	129.28 ± 3.57	51.12 ± 4.72
Dox	61.81 ± 7.46[a]	114.49 ± 7.01[a]	42.18 ± 1.22[a]
% change[(A)]	(-31.75%)	(-11.44%)	(-17.49%)
Dox+GT	75.08 ± 6.47[b]	126.91 ± 2.72[b]	51.12 ± 1.25[b]
% change[(B)]	(+21.47%)	(+10.85%)	(+21.19%)
Dox+Epi	84.92 ± 7.51[b]	129.42 ± 9.35[b]	50.36 ± 4.05[b]
% change[(C)]	(+37.39%)	(+13.04%)	(+19.39%)

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 group respectively, at P<0.05. (A), (B): Percentage of changes about the normal control and Dox-administered group respectively.

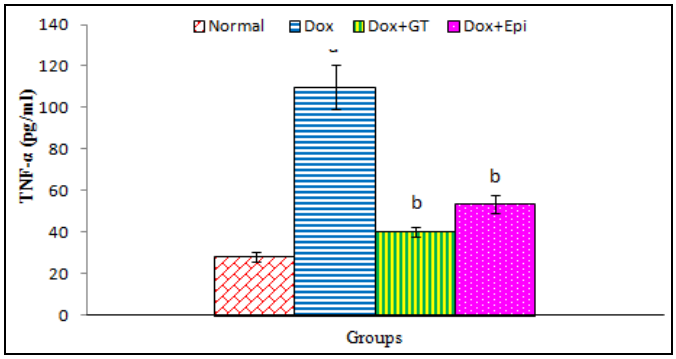

FIG. 2: EFFECT OF GREEN TEA INFUSION AND EPICATECHIN ON SERUM TNF-α LEVEL IN DOX-ADMINISTERED RATS. a, b: Significant difference in comparison with the corresponding normal control and Dox-administered group respectively, at P<0.05

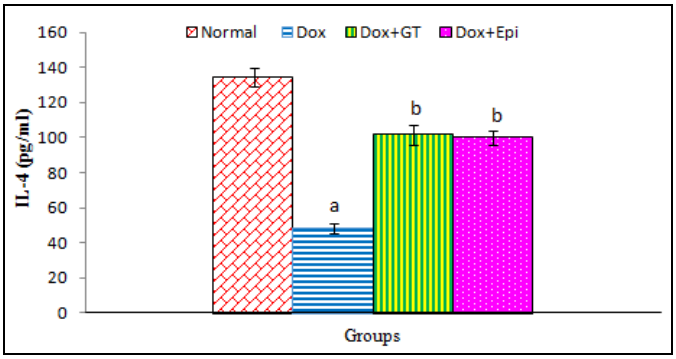

FIG. 3: EFFECT OF GREEN TEA INFUSION AND EPICATECHIN ON SERUM IL-4 LEVEL IN DOX-ADMINISTERED RATS. a, b: Significant difference in comparison with the corresponding normal control and Dox-administered group respectively, at P<0.05

The kidney sections of normal control rats depicted normal glomeruli with normal Bowman's capsule, proximal tubules and distal tubules (**Fig. 4**; Photomicrographs 4A and 4B).

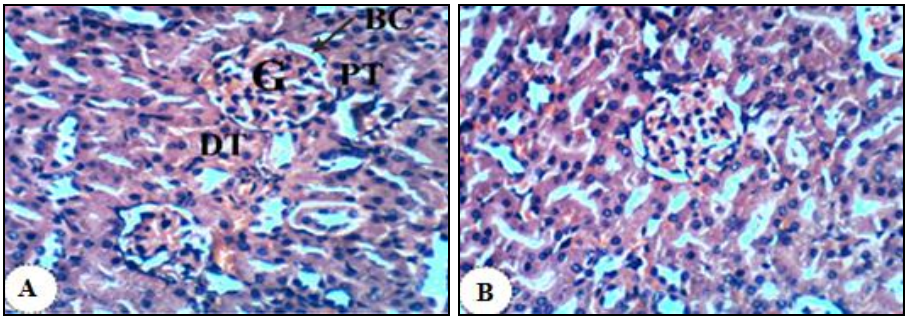

FIG. 4: PHOTOMICROGRAPHS OF KIDNEY SECTIONS OF NORMAL HISTOLOGICAL STRUCTURE OF RENAL PARENCHYMA (A & B) SHOWING NORMAL GLOMERULI (G) WITH NORMAL BOWMAN'S CAPSULE (BC), PROXIMAL TUBULES (PT) AND DISTAL TUBULES (DT). (H & E X400)

In contrast, the histopathological examination of kidney sections of Dox-administered rats showed disrupted histological architecture and integrity and several lesions represented by focal inflammatory cells infiltration between the tubules, perivascular edema and thickening of the parietal layer of Bowman's capsule, and atrophies of glomerular tufts (**Fig. 5**; Photomicrographs 5a, 5b and 5c).

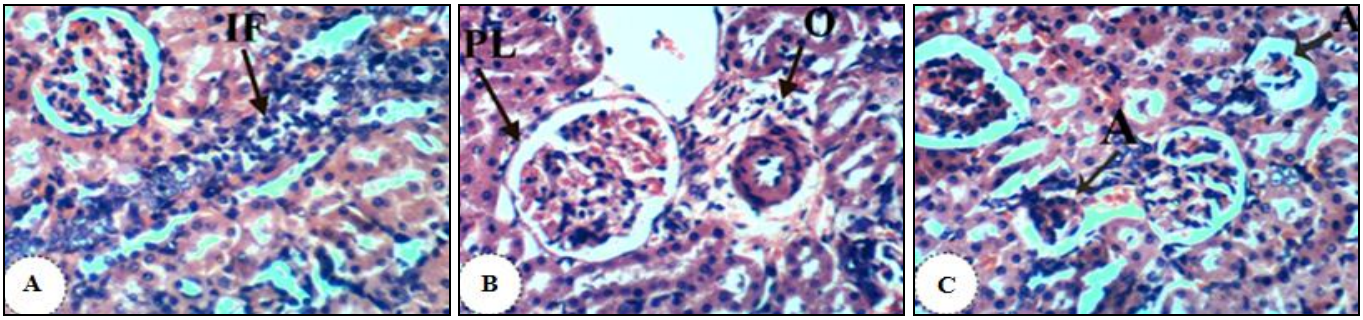

FIG. 5: PHOTOMICROGRAPHS OF KIDNEY SECTIONS OF DOX-ADMINISTERED RATS SHOWING: A) FOCAL INFLAMMATORY CELLS INFILTRATION (IF) IN BETWEEN THE TUBULES (H & E X 400). B) PERIVASCULAR OEDEMA (O) AND THICKENING OF THE PARIETAL LAYER (PL) OF BOWMAN'S CAPSULE (H & E X 400). C) ATROPHY (A) OF GLOMERULAR TUFTS. (H & E X 400)

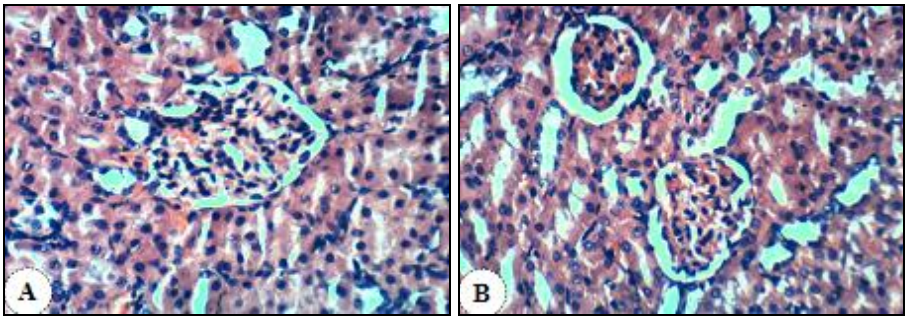

FIG. 6: PHOTOMICROGRAPHS OF KIDNEY SECTIONS OF DOX-ADMINISTERED RATS TREATED WITH GREEN TEA SHOWING NO HISTOPATHOLOGICAL CHANGES. (H & E X 400)

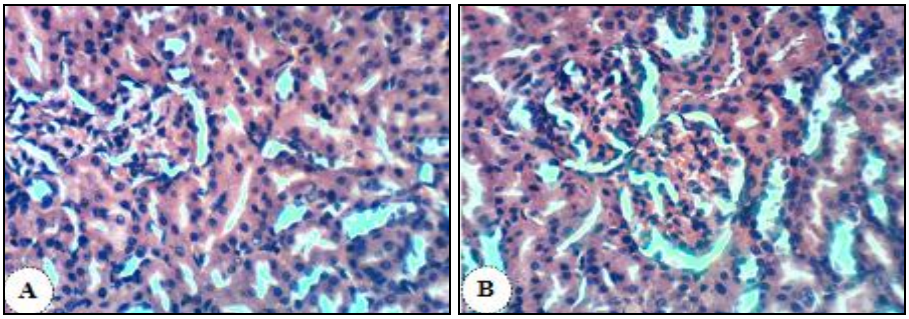

FIG. 7: PHOTOMICROGRAPH OF KIDNEY SECTIONS OF DOX-ADMINISTERED RATS TREATED WITH EPICATECHIN SHOWING NO HISTOPATHOLOGICAL CHANGES. (H & E X 400)

The treatment of Dox-administered rats with green tea infusion (**Fig. 6**; Photomicrographs 6a and 6b) and epicatechin (**Fig. 7**; Photomicrographs 7a and 7b) resulted in an amendment of Dox-induced kidney injuries and improvement of histological architecture and integrity.

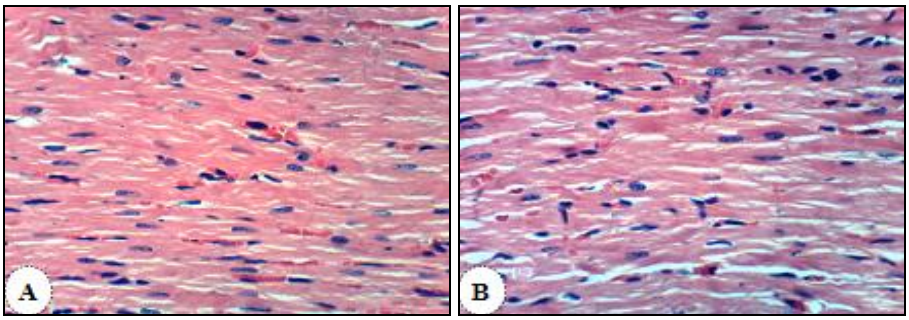

FIG. 8: PHOTOMICROGRAPHS OF HEART SECTIONS OF NORMAL HISTOLOGICAL STRUCTURE OF CARDIAC MYOCYTES. (H & E X 400)

The photomicrographs of the heart section of normal rats exhibited no histopathological alterations and normal histological structure of the cardiac myocytes (**Fig. 8**; Photomicrographs 8a and 8b).

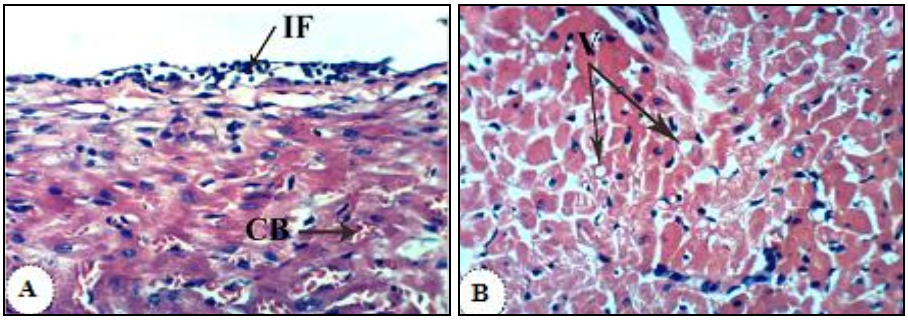

FIG. 9: A PHOTOMICROGRAPH OF DOX-ADMINISTERED RATS SHOWING: A) MONONUCLEAR CELLS INFILTRATION (IF) IN THE PERICARDIUM AND CONGESTION OF INTERMUSCULAR BLOOD CAPILLARIES (CB). (H & E X 400). B) VACUOLATION OF SARCOPLASM (V) OF CARDIAC MYOCYTES. (H & E X 400)

The photomicrographs of heart sections of Dox-administered rats exhibited disrupted architecture and integrity since many lesions represented by mononuclear cells infiltration in the pericardium, congestion of intermuscular blood capillaries, vacuolation of sarcoplasm of cardiac myocytes (**Fig. 9**; Photomicrographs 9a and 9b) were noticed. The treatment of Dox-administered rats with green tea infusion **Fig. 10** and epicatechin **Fig. 11** leads to a marked improvement of Dox-induced heart histological architecture and integrity. The deleterious histological changes observed in the heart of Dox-administered rats disappeared as a result of treatment with green tea infusion and epicatechin.

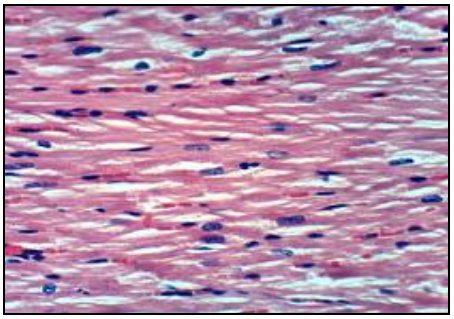

FIG. 10: A PHOTOMICROGRAPH OF HEART SECTION OF DOX-ADMINISTERED RATS TREATED WITH GREEN TEA SHOWING MARKED REGION OF NORMAL STRUCTURE. (H & E X 400)

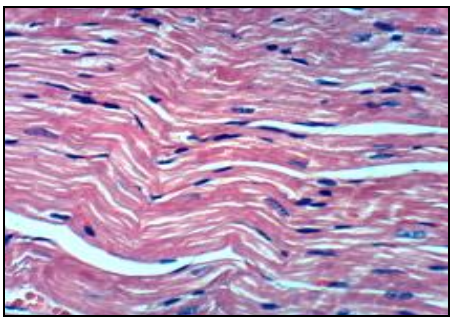

FIG. 11: A PHOTOMICROGRAPH OF HEART SECTION OF DOX-ADMINISTERED RATS TREATED WITH EPICATECHIN SHOWING MARKED REGION OF NORMAL STRUCTURE. (H & E X 400)

DISCUSSION: Kidneys are pivotal in the elimination of numerous xenobiotics, including drugs and environmental chemicals as well as endogenous metabolites. They have developed transport systems to prevent urinary loss of filtered nutrients, such as glucose, oligopeptides, and inorganic ions, as well as to facilitate the elimination of a variety of xenobiotics [25]. Markers commonly and routinely used to test kidney function include concentrations of creatinine and urea nitrogen [26]. Changes in these markers have been used to assess renal function for decades, but each marker has limitations [27].

Serum creatinine is an essential indicator of renal health since it is easily measured by the product of muscle metabolism since it produced from muscle at a constant rate. Creatinine is primarily filtered out of the blood by the kidneys (glomerular filtration and proximal tubular secretion), and it is sparsely or not reabsorbed by the renal tubules. If the filtering of the kidney is damaged, the serum creatinine level increases. Serum creatinine is probably the most widely used indirect measure of glomerular filtration rate (GFR) [28, 29].

Urea is a commonly used a marker for the diagnosis of renal failure. Urea is a by-product of protein metabolism and is used as a marker in acute kidney injury for retention and elimination of uremic solutes [30]. Unlike creatinine, urea is not produced at a constant rate, and the rate can be influenced by extrarenal factors. Urea production can be increased by diet, critical illness, burns, trauma, gastrointestinal bleeding, and sepsis and can be influenced by drugs, such as corticosteroids [26]. Kidneys completely filter an excess of urea into the urine, but due to renal dysfunction, urea is released into the bloodstream as serum urea.

Therefore, higher serum urea level is directly proportional to the severity of renal damage [28]. Uric acid, that is another marker of kidney dysfunction, is the final oxidation product of purine metabolism and is really excreted. Therefore, elevated serum uric acid levels are seen in patients with reduced GFR[31].

The present study revealed that i.p. Injection of 4 mg Dox/kg b.w. for 6 weeks induced nephrotoxicity manifested biochemically by a significant increase of serum creatinine, urea and uric acid levels. These results are in accordance with Venkatesan [32], Chen *et al*., [33] and Mohana *et al*., [34]. In addition, it was reported that Dox-induced changes in the renal tissue of rats include increased glomerular capillary permeability and tubular atrophy [35].

Although, the exact mechanism of Dox-induced nephrotoxicity remains unknown, it is believed to be mediated through free radical formation, iron-dependent oxidative damage of biological macromolecules and membrane lipid peroxidation [36]. Concomitant with the altered serum biochemical changes related to liver dysfunction, the kidney sections of Dox-administered rats exhibited atrophy of glomerular tufts, thickening of the parietal layer of Bowman's capsule, focal inflammatory cells infiltration between the tubules, perivascular edema. The previous deleterious biochemical and kidney histological alterations, in the present study, were associated with a marked elevation of kidney LPO and a significant decrease of non-enzymatic antioxidant- GSH content and enzymatic antioxidants- SOD, GPx and GST activities. These results are in agreement with several authors [34, 37, 38]. Thus, it can be suggested that the deteriorated changes in oxidative stress and antioxidant defense system in Dox-injected rats may be involved in inducing kidney lesions.

In supporting this hypothesis, Deman *et al* suggested that the reduced glutathione content in the renal cortex of Dox-injected rats supports the idea of free radical involvement in nephrotoxicity of Dox [37]. In the same way, Kocahan *et al*., stated that kidney toxicity during doxorubicin is mediated by oxidative stress resulting from the elevation of oxidant radicals and the decline in antioxidants such as GPx, SOD, and catalase [39].

The treatment of Dox-injected rats with green tea infusion and epicatechin, in the present study, significantly improved the elevated serum creatinine, urea, and uric acid levels. These results are in concurrence with those of the previous finding of Chacko *et al*., who found that plasma creatinine and urea levels were reduced by the administration of green tea polyphenols in alloxan-induced diabetic rats [40]. In the same regard, Yokozawa *et al*., found that green tea significantly decreased serum creatinine and malondialdehyde levels, kidney excretion of glucose and proteins and oxidative stress in the kidney [41].

In consistency with the improvement in the serum variables related to kidney function in the current study, the kidney histological architecture and integrity of Dox-injected rats were remarkably ameliorated and normalized as a result of treatment with green tea infusion and epicatechin. The increased oxidative stress and suppressed antioxidant defense system in Dox-injected rats were also significantly ameliorated due to treatments with green tea and epicatechin; this was manifested by a decrease in kidney LPO and increase in kidney GSH content and GPx and GST activities.

Thus, it can be suggested that the decrease in oxidative stress and enhancement of the antioxidant defense system may contribute, at least in part, to improve the kidney function and histological integrity as a result of treatment of Dox-injected rats with green tea infusion and epicatechin. This suggestion goes parallel with other previous publications which depicted the roles antioxidant effects of green tea and its constituents in preventing and curing oxidative stress-induced diseases [40, 41, 42, 43]. Several serum or plasma biomarkers were established to detect heart function and cardiotoxicity. It has been reported that the enzymes AST, CK and LDH that leak from the tissue damage, are the best marker of cardiotoxicity due to their tissue specificity and serum catalytic activity [44].

The present study revealed that intraperitoneal injection of 4 mg Dox/ kg b.w. for 6 weeks induced cardiotoxicity that was manifested by elevation in serum CK-MP, LDH and AST activities. These results are in accordance with Pointon *et al*., [45] and

Tikoo *et al*., [46] who stated that the major mechanism of Dox cardiotoxicity is *via* damage or inhibition of electron transport chain (ETC) by involving alteration in the expression and translation of myocardial ETC genes, leading to ATP loss and caspase-3 activation which lead to elevation in the activities of serum CK-MP, LDH, and AST. Elevation of serum activities of CK-MP, LDH and AST enzymes are considered as important markers of early and late cardiac injury especially during clinical follow-up of Dox therapy [47]. Administration of Dox may lead to the damage of the myocardial cell membrane, or it becomes permeable, that resulted in the leakage of AST, CK-MB, and LDH in the blood [48, 49].

Dox-induced cardiotoxicity may also occur due to the formation of free reactive oxygen radicals, direct DNA damage, and interference with DNA repair and induction of immune reactions involving antigen-presenting cells in the heart [50, 51]. The enzymatic pathway of Dox-induced free radical generation involves the mitochondria and is an important mechanism of Dox cardiotoxicity. Dox has a high affinity for cardiolipin, a phospholipid that is enriched in the inner mitochondrial membrane. Due to this, Dox concentrates inside the myocytes [52]. Further, Dox-induced mitochondrial damage might lead to respiratory chain defects, resulting in continuous production of free radicals. Also, mitochondrial damage may result in the release of cytochrome C, leading to the induction of apoptosis. Dox is also believed to reduce the activity of respiratory complexes, adenine nucleotide translocator or the voltage-dependent anion channel, which are important in the generation and transport of ATP from the mitochondria to the cytosol. Increased oxidative stress is a common factor implicated across the numerous hypotheses of the cardiotoxicity of Dox [46, 48, 53, 54]. The implication of increased oxidative stress and production of free radicals in Dox-induced cardiotoxicity is also supported by the present study which revealed a significant increase in heart LPO and a significant decrease in kidney GSH content as well as suppression of GPx and GST activities.

Histological examination of the heart sections of Dox-administered rats, in the present study, supported the biochemical results related to heart dysfunction. The heart showed marked interstitial edema, necrosis of some myocardial fibers associated with blood congestion and extensive hemorrhage. These results are in concurrence with Ito *et al*., [55], Saad *et al*., [35] and Tikoo *et al*., [46] who noticed changes in the heart in the form of oedema, haemorrhage, necrosis and degenerative changes in Dox-treated rats and with Osman *et al*., [56] and Saraogi *et al*., [57] who showed swollen cardiac muscle fibers, interstitial edema and inflammatory infiltration.

The treatment of Dox-administered animals with green tea infusion and epicatechin significantly decreased the elevated heart LPO and improved the lowered heart GSH content and activities of the heart antioxidant enzymes including GPx and GST in association with the amendment of the Dox-induced deleterious histological changes. These results are in agreement with previously published reports of Khan *et al*., [49] who found that decreased activity of the antioxidant enzymes like GPx, GST, SOD, and catalase in Dox-injected rats was significantly improved as a result of pre-treatment with green tea extract. According to Khan *et al*., [49], green tea has been shown to neutralize reactive oxygen species, such as superoxide radical, singlet oxygen, hydroxyl radical, peroxyl radical, nitric oxide, nitrogen dioxide, and peroxynitrite, thereby reducing the damage to lipid membranes, proteins and nucleic acids in cell-free systems. In the same regard, Zheng *et al*., [58] found that green tea protects against Dox-induced cardiomyocytes injury and protects from Dox-induced LPO.

Furthermore, Babu *et al*. found that green tea extract lowers the lipid peroxidation in heart and aorta of diabetic rats [59]. In our opinion, the preventive effect of green tea infusion and epicatechin on the decrease of non-enzymatic and enzymatic antioxidants and increase in LPO could be attributed to the antioxidant and ROS scavenging properties of green tea infusion constituents and epicatechin. In the present study the green tea infusion was reported to contain many antioxidant constituents including gallic acid, (-)-gallocatechin, (-)-gallocatechin, (-)-epigallocatechin, caffeine, (-)-epicatechin, (-)-epigallocatechin gallate, (-)-gallocatechin gallate, (-)-epicatechin gallate, (-)-catechin gallate, caffeine.

In the present study, the level of the pro-inflammatory cytokine, TNF-α, in serum was significantly elevated in Dox-administered rats while the level of the anti-inflammatory cytokine, IL-4, was significantly decreased reflecting the preponderance of Th1 and the presence of elevated Th1: Th2 cell ratio. These alterations were associated with presence of inflammatory cells' infiltrations in the kidney and heart. By these changes, Shankar *et al*. found that injection of Dox augments a peripheral increase in the cytokine TNF-α, which stimulates several inflammatory pathways [60].

The treatment of Dox-injected rats with green tea infusion and epicatechin significantly improved the elevated serum TNF-α level and the lowered serum IL-4 level and the inflammatory leucocytic infiltration in renal and cardiac tissues. Thus, both green tea infusion and epicatechin have potent anti-inflammatory effects. The anti-inflammatory, as well as the antioxidant effects of green tea infusion, may be attributed to its constituting epicatechin, other catechins, gallic acid and caffeine [61, 62, 63, 64].

CONCLUSION: Both green tea infusion and epicatechin successfully prevented Dox-induced kidney and heart injuries manifested by improvement in the function and histological integrity of kidney and heart. These preventive effects may be mediated *via* suppression of oxidative stress and inflammation as well as enhancement of the antioxidant defense system.

ACKNOWLEDGEMENT: The authors deeply thank Prof. Dr. Kawkab A. Ahmed, Professor of Histopathology, Pathology Department, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt for help in examining and identifying lesions in the histological sections.

CONFLICT OF INTEREST: The authors declare that there no conflict of interest.

REFERENCES:

1. Xi L, Zhu S, Das A, Chen Q, Durrant D, Hobbs DC, Lesnefsky EJ and Kukreja, RC: Dietary inorganic nitrate alleviates doxorubicin cardiotoxicity: Mechanisms and implications. Nitric Oxide 2012; 26(4): 274-284.
2. Tacar O, Sriamornsak P and Dass CR: Doxorubicin: an update on anticancer molecular action, toxicity and novel drug delivery systems. J Pharm Pharmacol 2013; 65: 157-70.
3. American Association for Cancer Research [AACR], Abstract #2168, CTI Meeting Technology, USA (2016).
4. Patel N, Joseph C, Corcoran GB and Ray SD: Silymarin modulates doxorubicin-induced oxidative stress, Bcl-xL and p53 expression while preventing apoptotic and necrotic cell death in the liver. Toxicology and Applied Pharmacology 2010; 245(2): 143-52.
5. Oduola T, Bello I, Adeosun G, Ademosun AW, Raheem G and Avwioro G: Hepatotoxicity and nephrotoxicity evaluation in Wistar albino rats exposed to *Morinda lucida* leaf extract. N Am J Med Sci 2010; 2: 230-33.
6. Kaiserova H, Šimůnek T, van der Vijgh WJ, Bast A and Kvasničková E: Flavonoids as protectors against doxorubicin cardiotoxicity: role of iron chelation, anti-oxidant activity and inhibition of carbonyl reductase. Biochim Biophys Acta 2007; 1772: 1065-74.
7. Xin Y, Wan LL, Peng JL and Guo C: Alleviation of the acute Doxorubicin-induced cardiotoxicity by *L. barbarum* polysaccharides through the suppression of oxidative stress. Food & Chemical Toxicology 2011; 49(1): 259-64.
8. Cui Y, Morgenstern H, Greenland S, Tashkin DP, Mao JT, Cai L, Cozen W, Mack TM, Lu Q and Zhang ZF: Dietary flavonoid intake and lung cancer- A population-based case-control study. Cancer 2008; 112(10): 2241-48.
9. Lecumberri E, Dupertuis YM, Miralbell R and Pichard C: Green tea polyphenol epigallocatechin-3-gallate (EGCG) as an adjuvant in cancer therapy. Clin Nutr 2013; 32(6): 894-03.
10. Zaveri NT: Green tea and its polyphenolic catechins: medicinal uses in cancer and non-cancer applications. Life Sci 2006; 78(18): 2073-80.
11. Kang KS, Kang BC and Lee BJ: Preventive effect of epicatechin and ginsenoside Rb2 on the inhibition of gap junctional intercellular communication by TPA and H_2O_2. Cancer Letters 2000; 152(1): 97-06.
12. Canadian Council on Animal Care (CCAC). Guide to the care and use of Experimental Animals, CCAC, Ottawa, Ontario, Canada 1993; 1: 298.
13. Yoshida T and Majors RE: High-speed analyses using rapid resolution liquid chromatography on 1.8-µ porous particles. J Sep Sci 2006; 29: 2421-32.
14. Trivedi PP, Kushwaha S, Tripathi DN and Jena GB: cardioprotective effects of hesperetin against doxorubicin-induced oxidative stress and DNA damage in rat. Food and Chemical Toxicology 2011; 11(3): 215-25.
15. Swanston-Flatt SK, Day C, Flatt PR and Bailey CJ: Evaluation of antihyperglycemic properties of traditional plant treatment for diabetes in streptozotocin diabetic and db/db mice. In: Frontiers in Diabetes Research. The lesson for Animal Diabetes. London: Smith-Gordon 1990; 3: 286-93.
16. Ahmed OM: Antihyperglycemic effects of water extract of *Ulva lactuca* and its polysaccharides in nicotinamide-streptozotocin-induced diabetic rats. Egypt J Zool 2010; 54: 273-97.
17. Al-Hilfy JHY: Effect of green tea aqueous extract on body weight, glucose level, and kidney functions in diabetic male Albino rats. Journal of Al-Nahrain University 2012; 15(3): 161-66.
18. Vasconcelos PC, Seito LN, Di Stasi LC, Akiko Hiruma-Lima C, and Pellizzon CH: Epicatechin used in the treatment of intestinal inflammatory disease: an analysis by experimental models. Evid Based Complement Alternat Med 2012; 2012: 508902.
19. Jaffe M: Ueber den niederschlag, welchen picrinsare in normalem harn erzeugt und uber eine reaction des kreatinine. Z Physiol Chem 1886; 10: 391-00.

20. Patton CJ and Crouch R: Colorimetric of determination of blood urea. Anal Chem 1977; 49: 464-69.
21. Fossati P, Prencipe L and Bertil G: Use of 3,5-dichloro-2-hydroxybenzenesulfonic acid/4-aminophenazone chromogenic system in direct enzymic assay of uric acid in serum and urine. Clin Chem 1980; 26 (2): 227-31.
22. Szasz G: Proceedings of the second international symposium on clinical enzymology. Chicago 1975.
23. Henderson AR and Moss DW: Enzymes. Tietz Fundamentals of Clinical Chemistry. W.B. Saunders eds. Philadelphia USA, Edition 5th, 2001: 352.
24. Murray R: Aspartate aminotransferase. Methods in Clinical Chemistry. The C.V. Mosby Co. St Louis. Toronto. Princeton 1984: 1112-16.
25. Cheng X and Klaassen CD: Tissue distribution, ontogeny, and hormonal regulation of xenobiotic transporters in mouse kidneys. Drug Metab Disposit 2009; 37: 2178-85.
26. Bagshaw SM and Gibney RT: Conventional markers of kidney function. Crit Care Med 2008; 36: 152-58.
27. Ronco C and Bagshaw SM: Kidney function tests and urinalysis in acute renal failure. In: Critical Care Nephrology. Philadelphia, PA: Saunders Elsevier, Edition 2nd, 2009: 251-59.
28. Dickey DT, Muldoon LL, Doolittle ND, Peterson DR, Kraemer DF and Neuwelt EA: Effect of N-acetylcysteine route of administration on chemoprotection against cisplatin-induced toxicity in rat models. Cancer Chemother Pharmacol 2008; 62: 235-41.
29. Ferguson MA and Waikar SS: Established and Emerging Markers of Kidney Function. Clin Chem 2012; 58(4): 680-89.
30. Stevens LA and Levey AS: Measurement of kidney function. Med Clin North Am 2005; 89: 457-73.
31. Giordano C, Karasik O, King-Morris K and Asmar A. Uric acid as a marker of kidney disease: Review of the current literature. Disease Markers 2015; 1-6.
32. Venkatesan N. Curcumin attenuation of acute adriamycin myocardial toxicity in rats. Pharma Br J 1998 124: 425-27.
33. Chen A, Sheu LF, Ho YS, Lin YF, Chou WY, Chou TC and Lee WH: Experimental focal segmental glomerulosclerosis in mice. Nephron 1998; 78: 440-52.
34. Mohana M, Sarika K, Prakash G and Sanjay K: Protective effect of *Solanum torvum* on doxorubicin-induced nephrotoxicity in rats. Food and Chemical Toxicology 2010; 48(1): 436-40.
35. Saad SY, Najjar TA and Al-Rikabi AC: The preventive role of deferoxamine against acute doxorubicin-induced cardiac, renal and hepatic toxicity in rats. Pharma Col Res 2001; 43: 211-18.
36. Pritsos CA and Basal JM: Drug-induced antioxidant enzyme activities correlate with age-dependent doxorubicin oxidative toxicity. Chem Biol Interact 2000; 127: 1-11.
37. Deman A, Ceyssens B and Pauwels M: Altered antioxidant defence in a mouse adriamycin model of glomerulosclerosis. Nephro Dialy Transplan 2001; 16(1): 147-50.
38. Yagmurca M, Erdogan H, Iraz, M, Songur A, Ucar M and Fadillioglu E: Caffeic acid phenethyl ester as a protective agent against doxorubicin nephrotoxicity in rats. Clinica Chimica Acta 2004; 348(1-2): 27-34.
39. Kocahan S, Dogan Z, Erdemli E and Taskin E: Protective effect of quercetin against oxidative stress induced toxicity associated with doxorubicin and cyclophosphamide in rat kidney and liver tissue. Iranian Journal of Kidney Diseases 2017; 11(2): 124-31.
40. Chacko SM, Smitha K and Kuttan R: Anti-diabetic activity of green tea polyphenols and their role in reducing oxidative stress in experimental diabetes. J Ethnopharmacol 2002; 83: 109-16.
41. Yokozawa T, Nakagawa T, Lee KI, Cho EJ, Terasawa K and Takeuchi S: Effects of green tea tannin on cisplatin induced nephropathy in LLC-PK1 cells and rats. J Pharm Pharmacol 1999; 51: 1325 -31.
42. Yokozawa T, Nakagawa T and Kitani K: Antioxidative activity of green tea polyphenol in cholesterol-fed rats. J Agric Food Chem 2002; 50: 3549-52.
43. Mao X, Gu C, Chen D, Yu B and He J: Oxidative stress-induced diseases and tea polyphenols. Oncotarget 2017; 8(46): 81649-61.
44. Singal PK, Deally CM and Weinberg LE: Subcellular effects of adriamycin in the heart: a concise review. Mol Cell Cardiol 1987; 19(8): 817-23.
45. Pointon AV, Walker TM, Phillips KM, Luo J, Riley J, Zhang S, Parry JD, Lyon JJ, Marczylo EL and Gant TW: Doxorubicin *in-vivo* rapidly alters expression and translation of myocardial electron transport chain genes, leads to ATP loss and caspase-3 activation. Plos One 2010; 5: e12733.
46. Tikoo K, Mukta SS and Chanchal G: Tannic acid ameliorates doxorubicin-induced cardiotoxicity and potentiates its anti-cancer activity: Potential role of tannins in cancer chemotherapy. Toxicology and Applied Pharmacology 2011; 251(3): 191-00.
47. Fadillioglu E, Erdogan H, Sogut S and Kuku I: Protective effects of erdosteine against Doxorubicin-induced cardiomyopathy in rats. Appl J Toxicol 2003; 23: 71-74.
48. Deepa PR and Varalakshmi P: Protective effect of low molecular weight heparin on oxidative injury and cellular abnormalities in adriamycin-induced cardiac and hepatic toxicity. Chemico-Biological Interac 2003; 146: 201-10.
49. Khan G, Haque SE, Anwer T, Ahsan MN, Safhi MM and Alam MF: Cardioprotective effect of green tea extract on doxorubicin-induced cardiotoxicity in rats. Acta Pol Pharm 2014; 71(5): 861-8.
50. Chlebowski RT: Adriamycin (doxorubicin) cardiotoxicity: a review. West J Med 1979; 131: 364-68.
51. Di Cosimo S and Baselga SJ: Targeted therapies in breast cancer: where are we now? Eur J Cancer 2008; 44: 2781-90.
52. Goormaghtigh E, Huart P, Praet M, Brasseur R and Ruysschaert JM: Structure of the adriamycin-cardiolipin complex. Role in mitochondrial toxicity. Biophys Chem 1990; 35: 247-57.
53. Heide RSV and L'Ecuyer TJ: Molecular basis of anthracycline-induced cardiotoxicity Heart Metabol 2007; 35: 1-4.
54. Othman AI, El-Missiry MA, Amer MA and Arafa M: Melatonin controls oxidative stress and modulates iron, ferritin, and transferrin levels in adriamycin-treated rats. Life Sci 2008; 83(15-16): 563-8.
55. Ito H, Miller SC, Billingham ME, Akimoto H, Torti SV and Wade R: Doxorubicin selectively inhibits muscle gene expression in cardiac muscle cells *in-vivo* and *in-vitro*. Proc Natl Acad Sci USA 1990; 87: 4275-4279.
56. Osman AM, Nemnem MM, Abou-Bakr AA, Nassier OA and Khayyal MT: Effect of methimazole treatment on doxorubicin-induced cardiotoxicity in mice. Food and Chemical Toxicology 2009; 47(10): 2425-30.
57. Saraogi P, Krishna KP, Bhulan KS and Kiran D: Rosiglitazone and pioglitazone aggravate Doxorubicin-induced cardiomyopathy in Wistar rats. Biomedicine & Aging Pathology 2011; 1(1): 65-71.
58. Zheng J, Lee HC, Bin Sattar MM, Huang Y and Bian JS: Cardioprotective effects of epigallocatechin-3-gallate

against Doxorubicin-induced cardiomyocyte injury. Eur J Pharmacol 2011; 652: 82-8.
59. Babu P, Sabitha K and Shyamaladevi C: Therapeutic effect of green tea extract on oxidative stress in aorta and heart of streptozotocin-diabetic rats. Chem Biol Interact 2006; 162: 114-20.
60. Shankar S, Suthakar G and Srivastava RK: Epigallo-catechin-3-gallate inhibits cell cycle and induces apoptosis in pancreatic cancer. Frontiers in bioscience: a Journal and Virtual Library 2007; 12: 5039-51.
61. Sutherland BA, Sutherland BA, Rahman RMA and Appleton I: Mechanisms of action of green tea catechins, with a focus on ischemia-induced neurodegeneration. Journal of Nutritional Biochemistry 2006; 17: 291-06.
62. Chacko SM, Thambi PT, Kuttan R and Nishigaki I: Beneficial effects of green tea: A literature review. Chinese Medicine 2010; 5 (13): 1-9.
63. Ohishi T, Goto S, Monira P, Isemura M and Nakamura Y: Anti-inflammatory action of green tea. Anti-Inflammatory & Anti-Allergy Agents in Med Chem 2016; 15 (2): 74-90.
64. Reygaert WC: An update on the health benefits of green tea. Beverages 2017; 3(6): 1-14.

How to cite this article:
Ahmed OM, Abdul-Hamid MM, El-Bakry AM, Mohammed HM and Rahman FEZSA: Effects of green tea infusion and epicatechin on doxorubicin-induced renocardiotoxicity in male Albino rats. Int J Pharm Sci & Res 2019; 10(5): 2210-23. doi: 10.13040/IJPSR.0975-8232.10(5).2210-23.