



Ellagic acid ameliorates cisplatin toxicity in chemically induced colon carcinogenesis

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Abstract

Cis-diamminedichloroplatinum(II) (cisplatin) (CP) is an important chemotherapeutic agent used in the treatment of several cancers. However, it has several side effects including nephrotoxicity gonadotoxicity, hepatotoxicity, and ototoxicity. In *in vitro* experiments, antioxidants or reactive oxygen species scavengers have a cytoprotective effect on cells exposed to cisplatin (CP). Ellagic acid (EA) is one such bioactive polyphenol that is abundant in some fruits, nuts, and seeds. Various authors have reported that EA has strong antioxidant and antitumor potential. The present study was, therefore, carried out to explore the protective potential of EA on CP-induced gonadotoxicity and nephrotoxicity in colon tumor-bearing mice. Animals were divided into five groups: Group I: normal control, Group II: DMH treated. After 20 weeks of DMH treatment, the animals were divided into four subgroups, viz., Group III: no treatment, Group IV: EA, Group V: CP, and Group VI: CP + EA. Administration of EA significantly ameliorated the toxicity caused by CP as indicated by improved kidney function tests and reproductive function tests. EA treatment to CP-abused mice also led to a marked reduction in the extent of peroxidative damage to tissue as was evident from the improvement in the histopathological changes in kidney and testis. Blood counts were also improved on administration of EA to CP-treated mice. This article provides the evidence that antioxidant efficacy of EA has beneficial effects on CP-induced nephrotoxicity and gonadotoxicity and contributes to understanding the role of oxidative stress, and suggests several points as part of the mechanism of CP toxicity.

Keywords Cisplatin · Colon cancer · Nephrotoxicity · Testicular-toxicity · Ellagic acid

Introduction

Colorectal cancer is among the most commonly observed malignancies and is the third driving reason for disease-related mortality around the world [1]. Several genes have been directly implicated in the etiology of colorectal cancer [2]. Most colon cancers are adenocarcinomas (tumors developing from the glands lining the inner wall of colon). Inactivation of the adenomatous polyposis coli (APC) tumor-suppressor gene initiates colorectal neoplasia and also encodes a protein involved in cell adhesion and transcription [3, 4]. Rectal bleeding and anemia which are sometimes coupled with weight loss and changes in bowel habits are typical symptoms of colorectal cancer [5, 6]. Although surgical resection is the major curative

therapy in early stage, chemotherapy still remains an essential treatment modality for patients diagnosed with cancer. Chemotherapy drugs are powerful against cancer cells since they are intended to meddle with fast dividing cells [7]. Platinum-based drugs, for example, cisplatin (CP) is a generally utilized treatment in colorectal tumor [8]. Most of the tumor patients experience relapse and develop resistance in spite of initial reaction to CP. Increased ROS in cancer cells is crucial for its initiation and progression [9], in many cases, excessive ROS generation can cause danger and render cancer cells to be more prone to damage by increased oxidative stress caused by exogenous agents. Oxidative stress has been accounted to increase cell apoptosis by means of downstream pathways, for example, endoplasmic reticulum (ER) stress and mitochondrial cascade [10]. Along these lines, controlling ROS levels in cancerous cells is an approach to specifically execute growth, and has been included in the antitumor impacts of a few therapeutic drugs. Recognizing potential interactions between polyphenols and chemotherapeutics

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may provide information regarding the efficiency of polyphenol-containing foods in cancer therapy [11].

Numerous phytochemicals are well known for their anticancer properties, due to their ability to quench reactive oxygen species (ROS) and thereby protect critical cellular targets (i.e., DNA, proteins, lipids) from oxidative injury. Ellagic acid (EA) is a bioactive polyphenol present in some fruits, nuts, and seeds, for example, pomegranates, raspberries, strawberries, walnuts, and almonds [12]. It has been utilized for many years for the treatment of different ailments. Recent research has shown that EA has strong antioxidant and antitumor potential, inhibits malignant cell growth and instigates cancer cell apoptosis by prompting intracellular oxidative stress. Nowadays, combination chemotherapy has been observed to be an unrivaled treatment methodology. Hence, looking for a successful chemosensitizer that could expand the proficiency of anticancer medications, and, all the while, overcome multidrug resistance and side effects, is direly required.

Materials and methods

Animal model

Male laca mice (25–30 g each) procured from Central Animal House, Panjab University, Chandigarh (India) were housed in well-aerated conditions under a constant temperature and humidity in polypropylene cages bedded with sterilized rice husk. Mice in all the groups had free access to standard animal pellet diet (Ashirwad Industries Ltd., Ropar, Panjab, India) and tap water throughout the experiment. All the experimental protocols were approved by the Institutional Ethics Committee (Panjab University, Chandigarh, India) and conducted according to the Indian National Science Academy Guidelines for the use and care of experimental animals. Before starting the various treatments, the animals were allowed to acclimatize to experimental conditions for 1 week, and final day of their acclimatization period was considered as day 0 of the experiment.

Animals were divided into five groups: Group I: normal control, Group II: DMH treated (30 mg/kg body weight once in a week; sub-cutaneous from 0 to 20th week of treatment period), After 20 weeks of DMH treatment, the animals were divided into four subgroups viz. Group IIa: no treatment, Group IIb: EA (10 mg/kg b.wt p.o; orally from the 21st week to 26th week of treatment period), Group IIc: CP (5 mg/kg body weight once in a week; i.p in normal saline from the 23rd to 26th week of treatment period), Group IId: CP + EA (same as in Group IIb and IIc).

Reduced glutathione (GSH)

GSH was estimated according to the method described by Moron et al. [13]. This method is based on the reduction of 5,5-dithiobis-(2-nitrobenzoic acid) (DTNB) by SH group of GSH and formation of one mole of 2-nitro-5-mercaptobenzoic acid per mole of –SH. The 2-nitro-5-mercaptobenzoic acid formed has a deep yellow color, which can be used to measure –SH groups at 412 nm. A standard GSH plot was also performed to calculate the content of GSH. The assay was performed within 2–3 h after sacrificing the animal so as to avoid errors due to oxidation of GSH.

Lipid peroxidation

The assay for lipid peroxidation was performed according to the method of Trush [14]. Lipid peroxidation is the oxidative deterioration of lipids by free radicals generated due to various enzymatic and nonenzymatic reactions. Cycloperoxides formed in the deterioration process form Malondialdehyde (MDA) which is a degradation product. MDA forms a pink-colored complex with thiobarbituric acid (TBA) (MDA–TBA chromophore) which can be read at 532 nm.

Total leukocyte count

Total leukocyte counts in blood samples were carried out by method of Dacie and Lewis, 1968 [15]. Blood is diluted with Turk's solution (distilled water-98 ml, glacial acetic acid-2 ml) and a pinch of crystal violet stain. Turk's fluid was added to fresh blood to dilute the blood in the ratio 1:20 (v/v). A drop of diluted blood was immediately poured on Neubaur's chamber and covered with a glass coverslip. WBCs were counted in the four corner square of Neubaur's chamber.

Differential leukocyte count

The white blood cell differential count determines the number of each type of white blood cell, present in the blood. This was performed using the method described by Dacie and Lewis [16]. Acetone-free methyl alcohol is used as a fixative that preserves the cells in the same chemical and metabolic state (in order to prevent hemolysis). Giemsa stain stains the WBCs differently according to their nucleus (e.g., lymphocytes—non-lobed eccentric nucleus; monocytes—bean-shaped nucleus; and neutrophils—multilobed nucleus).

Hemoglobin

Hemoglobin content in the blood samples was assessed by the oxyhemoglobin method of Dacie and Lewis, 1991 [16]. Twenty microliters of fresh nonclotted blood was made up

to 4 ml by adding freshly prepared 0.04% ammonia solution. The OD was measured at 540 nm, and the hemoglobin content was determined from a prestandardized curve.

Reactive oxygen species (ROS) determination

It was determined using the method of Wang and Joseph [17]. In the presence of ROS, H₂-DCF is rapidly oxidized to become highly fluorescent dichlorofluorescein (DCF) which can be measured. Homogenate was added with DCFH-DA and buffer. This was then incubated at 37 °C for 30 min. After incubation DCF fluorescence intensity was detected at an excitation wavelength of 488 nm and emission wavelength at 525 nm.

Sperm motility

Spermatozoa from the vas deferens were teased out in a watch glass containing 1 ml normal saline (0.9% sodium chloride) at room temperature. Clumps were removed gently by mixing through a Pasteur pipette. 20 µl of this suspension was placed on a slide and was covered with a coverslip. The numbers of motile and nonmotile spermatozoa were counted in four random visual fields. Motility was expressed as percentage of motile sperm according to the following equation:

$$\% \text{ Motility} = \frac{\text{Number of motile sperms/field}}{\text{Total number of spermatozoa/field}} \times 100.$$

Sperm concentration

Epididymal spermatozoa were obtained by gentle squeezing and cutting of epididymis, suspended in 1 ml of normal saline. 20 µl of this suspension was placed on hemocytometer, and spermatozoa were counted in all the 64 cells of the hemocytometer and expressed as million per ml.

The sperm concentration was calculated using the following formulas:

$$\text{Sperm conc.} = N \times \frac{\text{Dilution factor}}{\text{Volume of sperm suspension}},$$

$$\text{Dilution factor} = \frac{\text{Final volume (1000 } \mu\text{l)}}{\text{Original volume taken (20 } \mu\text{l)}} = 50,$$

$$\text{Area of 4 WBC squares} = 4 \times 1 \text{ mm (length)} \times 1 \text{ mm (width)} = 4 \text{ mm}^2,$$

$$\text{Depth of the chamber} = 0.1 \text{ mm.}$$

Therefore, volume of sperm suspension in 4 WBC squares = 4 mm² × 0.1 mm = 0.4 mm³, *N* = total number of sperms in 4 WBC squares. Therefore, number of sperms in 1 mm³ of sperm suspension = $N \times \frac{\text{Dilution factor}}{0.4} = N \times 125 \times 10^3 / \text{ml}$.

Histology

Histopathological analysis of tissues was conducted using Hematoxylin and Eosin staining as described by Humanson [18]. Colons were removed and immediately transferred to neutral formalin and allowed to fix for 12 h. Next, the tissue was dehydrated gradually in ascending series of ethanol. For embedding, the dehydrated samples were placed in benzene, then sequentially in 1:1 benzene:paraffin wax and two changes in pure melted wax, before finally embedding in paraffin wax. Thin sections (5 µm) were obtained using a manual hand-driven microtome and transferred to the glass slides. These were dewaxed in xylene, rehydrated in descending series of ethanol, and stained with Hematoxylin and Eosin. Stained sections were mounted in DPX after dehydration and viewed under the light microscope.

Results

GSH

A significant decrease in GSH levels (Table 1) was observed in testis tissue of DMH alone group, DMH + EA, DMH + CP and DMH + CP + EA when compared with control ($p \leq 0.001$). CP and EA coadministration showed improvement from DMH alone, DMH + EA and DMH + CP + EA group at a significance level of $p \leq 0.01$. There were no significant alterations in GSH levels of kidney tissues when compared with control group (Table 1).

LPO

A significant increase in LPO (Table 2) level was observed in kidney tissue of DMH alone group when compared with control ($p \leq 0.01$). DMH + EA-treated group showed a marked increase in the kidney LPO level as compared to control ($p \leq 0.001$). A significant decrease was observed in the LPO level of CP and EA coadministered group when compared to DMH + CP group ($p \leq 0.05$). DMH + EA group

showed a remarkable increase in LPO levels of testis tissue when compared to control ($p \leq 0.001$) and decrease when compared with DMH alone group ($p \leq 0.05$). DMH + CP group showed an increase when compared to both DMH alone ($p \leq 0.05$) and DMH + EA ($p \leq 0.001$). CP + EA co

Table 1 Effect of cisplatin, EA, and cotreatment of cisplatin and EA on GSH levels of colon tumor-bearing mice

Groups/organ	Control	DMH	DMH+EA	DMH+CP	DMH+CP+EA
Kidney	2.81 ± 0.66	1.74 ± 0.44	2.05 ± 0.52	1.70 ± 0.62	2.50 ± 0.49
Testis	2.13 ± 0.23	1.49 ± 0.15 a ₃	1.47 ± 0.11 a ₃	1.19 ± 0.24 a ₃	1.56 ± 0.16 a ₃ , b ₂ , c ₂ , d ₂

Units μ moles of GSH/mg protein

Here 2, 1 denote $p < 0.01$ and $p < 0.05$, respectively. “a” represents the comparison between control and other groups; “b” represents comparison between DMH alone and other groups; “c” represents DMH+EA versus others; and “d” represents DMH+CP versus DMH+CP+EA

administration significantly decreased extent of lipid peroxidation as compared to DMH+CP group ($p \leq 0.01$) and increased when compared to control ($p \leq 0.05$).

ROS

CP administration to DMH-treated mice significantly increased the ROS levels in kidney tissue when compared with control (Table 3), DMH alone and DMH+EA mice ($p \leq 0.001$). Co-treatment group also showed elevated levels of ROS when compared with control, DMH alone and DMH+EA mice ($p \leq 0.001$). Similar trends were observed in testis tissue of DMH+CP group when compared with control, DMH alone and DMH+EA mice ($p \leq 0.001$). However, coadministered group showed a remarkable decrease in testis ROS levels compared with DMH+CP group ($p \leq 0.001$).

Hematological parameters (Table 4)

Hemoglobin

A marked decrease was observed in the Hb levels of DMH alone group, DMH+EA group and DMH+CP group than control ($p \leq 0.001$); ($p \leq 0.01$); ($p \leq 0.05$). DMH+CP group showed decrease in Hb levels than EA administered mice ($p \leq 0.05$). Co-treatment group showed improvement in Hb levels than DMH alone as well as DMH+CP group ($p \leq 0.05$); ($p \leq 0.01$).

Total leukocyte count

A marked decrease was observed in TLC of DMH+CP and DMH+CP+EA when compared to control as well as DMH alone group ($p \leq 0.05$); ($p \leq 0.01$).

Platelets

DMH+EA group showed decreased platelet count when compared with control and DMH ($p \leq 0.01$). DMH+CP animals as well as CP+EA coadministered animals also

showed a decreased platelet count as compared to control ($p \leq 0.01$) and DMH alone ($p \leq 0.05$).

Neutrophils

There were no significant alterations in neutrophil percentage of any group.

Lymphocytes

CP+EA coadministered animals showed increased lymphocyte percentage when compared to DMH+EA group ($p \leq 0.05$).

Kidney function tests

Creatinine A marked increase was observed in serum creatinine levels (Fig. 1) of DMH alone group and DMH+EA group when compared with control ($p \leq 0.05$). CP administration to tumor-bearing mice significantly increased the creatinine levels when compared to control ($p \leq 0.01$) and DMH alone group ($p \leq 0.01$). Co-treatment group also showed elevated levels of creatinine than control ($p \leq 0.05$).

Urea and blood urea nitrogen

DMH+EA group showed decreased levels of urea and BUN (Fig. 1) when compared with control ($p \leq 0.001$); ($p \leq 0.01$) and DMH alone group ($p \leq 0.001$); ($p \leq 0.01$). CP administration to tumor-bearing mice also showed elevated levels of both when compared with control ($p \leq 0.001$); ($p \leq 0.001$), DMH alone group and DMH+EA group ($p \leq 0.001$); ($p \leq 0.01$); ($p \leq 0.001$). Co-treatment group also showed increased levels of Urea and BUN when compared with control ($p \leq 0.01$); ($p \leq 0.01$) and DMH alone group ($p \leq 0.01$); ($p \leq 0.01$) but a slight decrease was observed from CP-treated animals.

Reproductive potential

Sperm motility CP administration to tumor-bearing mice significantly decreased the sperm motility (Fig. 2a) when

Table 2 Effects of cisplatin, EA, and cotreatment of cisplatin and EA on LPO levels of colon tumor-bearing mice

Groups/organ	Control	DMH	DMH+EA	DMH+CP	DMH+CP+EA
Kidney	2.89±0.64	3.94±1.05 a ₂	3.27±1.10	5.18±0.83 a ₃	4.42±0.74 a ₃ , d ₁
Testis	11.8±2.54	17.3±3.34	14.3±1.79 a ₃ , b ₁	19.1±3.26 b ₁ , c ₃	15.4±1.57 a ₁ , d ₂

Units n moles of MDA–TBA/mg protein/min

Here 3, 2, 1 denote $p < 0.001$, $p < 0.01$ and $p < 0.05$, respectively. “a” represents the comparison between control and other groups; “b” represents comparison between DMH alone and other groups “c” represents DMH+EA versus others; and “d” represents DMH+CP versus DMH+CP+EA

Table 3 Effects of cisplatin, EA, and cotreatment of cisplatin and EA on ROS levels of colon tumor-bearing mice

Groups/organ	Control	DMH	DMH+EA	DMH+CP	DMH+CP+EA
Kidney	74.9±7.91	92.6±5.21 a ₁	86.2±6.15	135±16.6 a ₃ , b ₃ , c ₃	126±16.1 a ₃ , b ₃ , c ₃
Testis	230±37.7	282±32.9	257±18.7	517±88.6 a ₃ , b ₃ , c ₃	462±49.1 a ₃ , b ₃ , c ₃ , d ₃

Units Relative fluorescence intensity of DCF

Here 3, 1 denote $p < 0.001$ and $p < 0.05$, respectively. “a” represents the comparison between control and other groups “b” represents comparison between DMH alone and other groups; “c” represents DMH+EA versus others; and “d” represents DMH+CP versus DMH+CP+EA

Table 4 Effects of cisplatin, EA, and cotreatment of cisplatin and EA on blood parameters of colon tumor-bearing mice

Groups/estimation	Control	DMH	DMH+EA	DMH+CP	DMH+CP+EA
Hemoglobin (g/dl)	12.0±1.32	8.17±0.76 a ₃	8.83±1.04 a ₂	6.87±0.78 a ₃ , c ₁	10.4±1.29 b ₁ , d ₂
Total leukocyte count	8257±731	9083±1395	7203±205	5333±581 a ₁ , b ₂	5500±723 a ₁ , b ₂
Platelets	61.1±3.10	64.3±9.07	51.0±2.00 a ₂ , b ₂	45.4±7.51 a ₂ , b ₁	48.0±4.58 a ₂ , b ₁
Neutrophils %	31.0±2.64	26.3±2.52	33.3±6.11	27.3±4.05	25.33±3.51
Lymphocytes %	69.0±2.62	73.7±2.52	66.7±6.11 b ₁	69.3±2.51	74.7±3.51 c ₁

All values are mean±SD ($n=6$). Data are analyzed using one-way ANOVA followed by post-hoc test. Here 3, 2, and 1 denote $p < 0.001$, $p < 0.01$, and $p < 0.05$, respectively. “a” represents the comparison between control and other groups; “b” represents comparison between DMH alone and other groups; “c” represents DMH+EA versus others

compared to control, DMH alone, DMH+EA group ($p \leq 0.001$). Co-treatment group also showed a remarkably decreased sperm motility when compared to control, DMH alone, DMH+EA group ($p \leq 0.001$) but showed significant improvement when compared to DMH+CP group ($p \leq 0.01$).

Sperm concentration DMH alone group showed significant decrease in sperm number (Fig. 2b) when compared to control ($p \leq 0.001$). DMH+EA group showed improvement in sperm count when compared to DMH alone group ($p \leq 0.001$) but a decrease was observed with respect to control ($p \leq 0.01$). A marked decrease in sperm count was observed in CP administered group ($p \leq 0.001$). Co-treatment group showed improvement in sperm count when compared with DMH+CP group ($p \leq 0.001$) but a decrease was observed as compared to other groups.

Histology Colon from normal animals showed normal histoarchitecture where as DMH administration for 20 weeks

showed timorous growth as well as irregular colon epithelium (Fig. 3). Kidneys from control animals show normal morphology with well-preserved glomerulus and no loss of tubular epithelial cells. DMH-treated and DMH+EA groups showed mild tubulointerstitial injury. Cisplatin-treated kidneys show extensive loss of tubular epithelial cells, tubular dilation, intratubular debris, tubular cell atrophy, inflammatory cell infiltration, and increased interstitial areas (Fig. 4). Testis from control animals showed normal spermatogenesis, DMH-treated animals showed marginally low mature sperms than normal, EA-treated animals also showed low mature sperms, DMH+CP-treated group showed atrophy, shrunken tubules as well as low population of germ cells, and sperms all over and reduced leydig cells. CP+EA cotreated animals showed mild atrophy and distorted germinal epithelium but the testicular structure remained intact despite the loss of germ cells, there was no loss of connective tissue when compared to CP+DMH group (Fig. 5).

Fig. 1 Effect of cisplatin, EA, and cotreatment of cisplatin and EA on kidney function of colon tumor-bearing mice

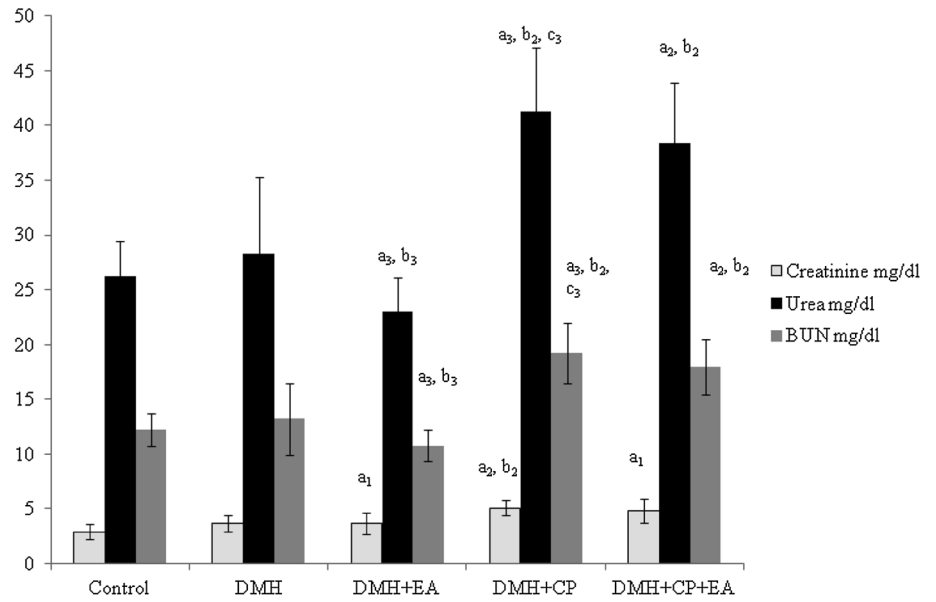
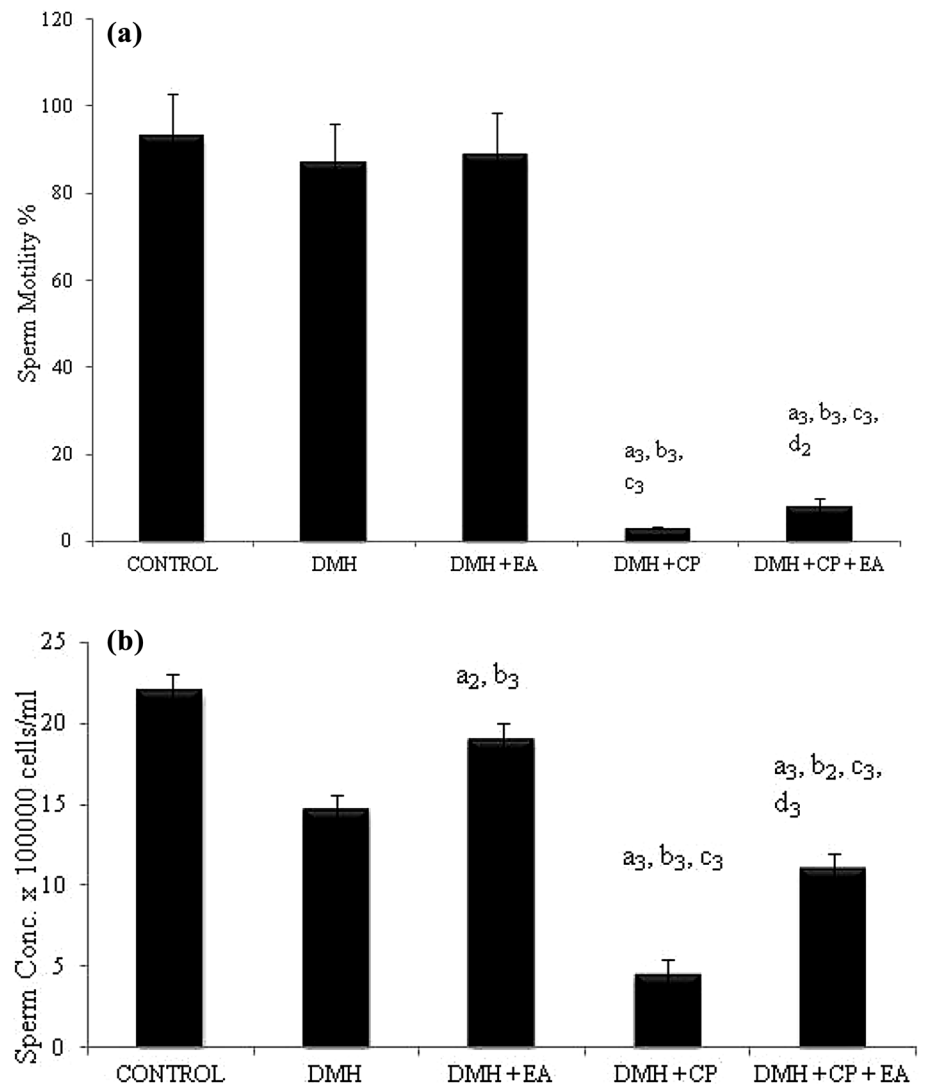


Fig. 2 Effect of cisplatin, EA, and cotreatment of cisplatin and EA on reproductive potential of colon tumor-bearing mice (sperm motility & sperm concentration)



Statistics All values are mean \pm SD ($n=6$). Data are analyzed using one-way ANOVA followed by post-hoc test. Here 3, 2, and 1 denote $p < 0.001$, $p < 0.01$, and $p < 0.05$, respectively. “a” represents the comparison between control and other groups; “b” represents comparison between DMH alone and other groups; “c” represents DMH+EA versus others.

Discussion

The present study was designed to explore the protective potential of EA against toxicity caused by CP in murine colon cancer model (DMH induced) which limits its use as a drug of choice over other available drugs.

DMH is a metabolic precursor of methylazoxymethanol (MAM) which is reported to induce tumors in rodents. Repetitive treatment with this methylating agent was found to induce colon tumors in mice [19]. In the present study, DMH administration for 20 weeks led to development to colon tumors as evidenced by H/E staining.

GSH concentrations were reduced significantly in CP-treated mice. The introduction of an exogenous antioxidant is thought to help the endogenous antioxidant system in scavenging the ROS produced during an imbalance in redox status induced by CP. In the current study, treatment with EA to CP-abused mice seems to maintain the GSH concentrations at a level approaching to those in the control mice. It was reported that when 10 mg/kg EA is combined with CP treatment, the level of GSH is improved [20]. Extent of lipid peroxidation and ROS generation is also a very reliable marker of oxidative damage, in the present study, CP administration to tumor-bearing mice increased lipid peroxidation in both kidney and testis tissue much more than DMH alone group as well as control did. This increase could be attributed to the cytotoxic pathway followed by CP which

is known to elevate ROS and LPO in rapidly dividing cells (apart from cancerous cells) [21] and in kidneys due to accumulation. At a dose of 10 mg/kg, EA treatment improved most of the biochemical parameters and histology. The consistency of EA at this dose in improving the kidney and testis status may be due to either the ability to scavenge the ROS as antioxidant [22] and protect the kidney from oxidative stress or EA may affect CP uptake by the kidney cells. It is known that the kidneys accumulate CP more than other organs [23], resulting in necrosis in the terminal portion of the proximal renal tubules and apoptosis in the distal nephron. Biochemical markers such as Cr and BUN elevate significantly following administration of nephrotoxic drugs [24]. However, these markers, although reliable for detection of renal injury, lack the sensitivity and specificity for early kidney damage detection. This increase may be either due to tubular injury, which affects water reabsorption leading to dehydration, or to CP cytotoxic effects on the gastrointestinal tract, which affects the eating behavior, alters gastrointestinal rhythm, and delays gastric emptying, which subsequently causes altered metabolism [25]. Damage is caused by an increase in the glomerular volume and cellular degenerative changes, including cytoplasmic vacuolization of the proximal tubular cells, and tubular dilation [26]. Histopathological examination revealed acute tubular necrosis in most of the examined sections of kidney in mice treated with CP [27]. Testis histology also revealed an altered histoarchitecture in the animals undergoing CP treatment. Many researchers have reported a reduction in germ cell number and deceleration in spermatogenesis [28]. In the present study, decreases in sperm motility and concentration were also observed. Seminiferous tubules contain arrested spermatogenic cells at various stages of division. A reduced mean seminiferous tubule diameter and degenerative changes in germinal cell layer thickness support decreases in sperm motility and concentration [29]. Some improvements were also observed

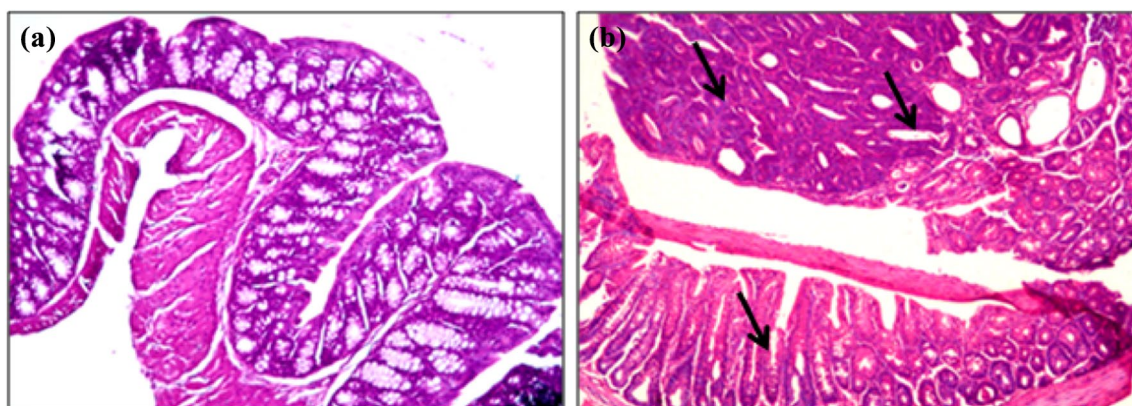


Fig. 3 Histopathological assessment of colon in different groups ($\times 100$): **a** control; **b** DMH alone (showing cancerous cells, irregular lining of colon epithelium, atypical colonocytes, and vacuolization)

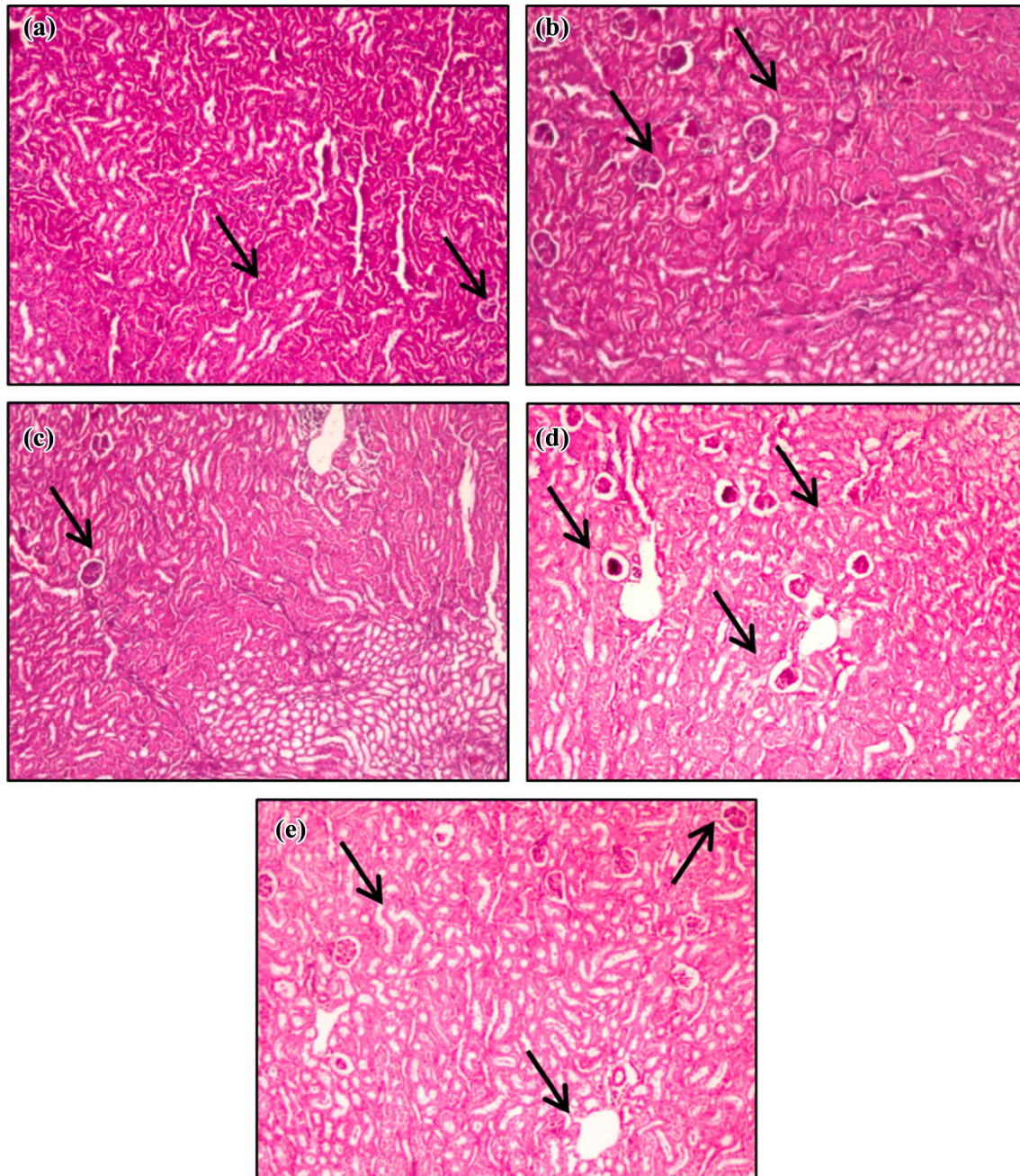


Fig. 4 Histopathological assessment of kidney (cortex region) in different groups ($\times 100$): **a** control; **b** DMH alone; **c** DMH+EA; **d** DMH+CP; **e** DMH+CP+EA

in mice treated with CP combined with EA. Histopathological examination of the kidney is considered to be the golden standard method to detect renal injury. It has been reported that CP can induce epithelial cell atrophy, loss of brush border, tubular dilation, necrosis, and apoptosis [30], leading to severe renal injury through increased expression of intercellular adhesion molecule 1 (ICAM-1) in renal proximal tubules, macrophage infiltration, nuclear factor- κ B (NF- κ B) activation, and ROS production [31]. However,

Atessahin et al. reported that EA, at a dose of 10 mg/kg, ameliorated the histopathological changes induced by CP in the kidney. They reported that EA at 10 mg/kg protected the kidney from tubular necrosis, degeneration, desquamation, and tubular dilatation. Upon application of chemotherapy, not only the rapidly dividing cells get affected but also some of the normal cells get affected, which are rapidly dividing such as hair, the lining of mouth, the cells lining the intestinal tract, and the blood cells (WBC, RBC, and platelets)

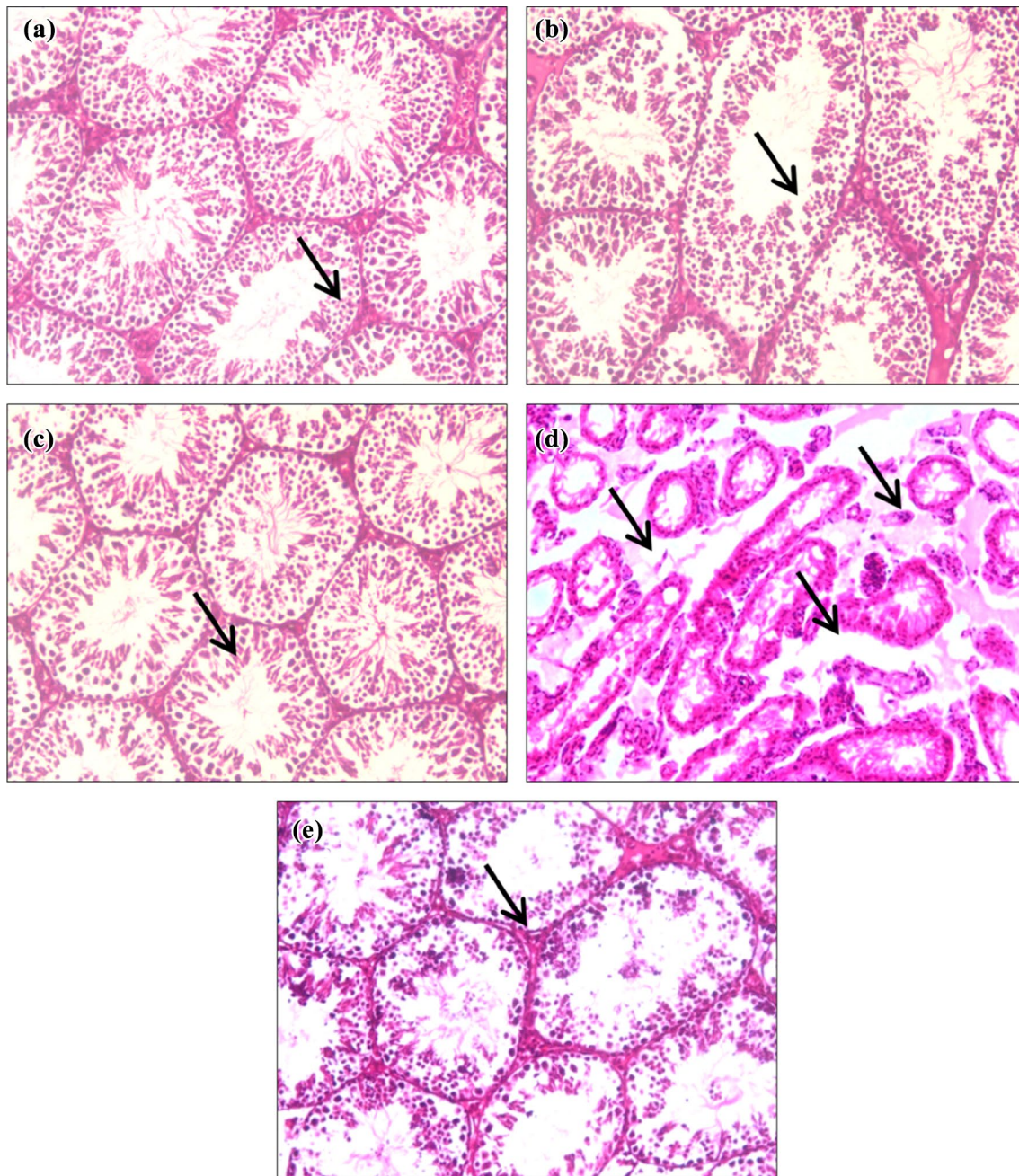


Fig. 5 Histopathological assessment of testis in different groups ($\times 200$): **a** control; **b** DMH alone; **c** DMH+EA; **d** DMH+CP (shrinkage of tubules, increased vacuolization, disruption of germinal epithelium, and loss of connective tissue); **e** DMH+CP+EA

[32, 33]. A marked decrease is observed in the blood cells due to chemotherapy which is also known as the “Nadir effect.” Nadir means ‘low point.’ The period of time beginning 7–12 days after each chemotherapy dose—and possibly lasting up to 1 week. This effect renders the patient more prone to infections and fatigue, which further becomes the reason for the plight of patients undergoing chemotherapy [34, 35]. A similar trend was observed in the present study (in the groups administered with CP), which suggests that

there must be a supplement given along with the chemotherapeutic drug, which enhances the bone marrow activity so that nadir effect is reduced. Presently, administration of EA reduced the nadir effect significantly in the group undergoing chemotherapy. Many earlier reports have also suggested an increase in blood count on administration of phytochemicals in various diseases. Polyphenols may enhance the total antioxidant-scavenging capacities of blood by binding to the RBCs [36], thereby lowering the reduction in blood counts.

Mice treated with CP showed significant reduction in water intake and urine output post CP treatment. However, it has been reported that CP administration damages the renal tubule and distorts its ability to reabsorb water and cause polyuria [37], which can also lead to polydipsia [38]. Recent studies reported that CP administration leads to increase urine output. Administration of EA at a dose of prior to and post CP treatment increased water intake and urine output (data not shown), which suggests improved kidney function. Additional conventional biochemical markers used in the present study to detect kidney injury were plasma urea, creatinine (Cr), and BUN. A decreased glomerular filtration rate is evidenced by increased plasma creatinine levels, following CP administration [39]. In the present study, plasma urea, Cr, and BUN increased, following either CP treatment or CP combined with EA. However, urea, Cr, and BUN concentrations decreased to some extent when EA was administered prior to and post CP treatment. EA also has antihepatotoxic property that improves the hepatic architecture and functions against toxic and pathological conditions. The molecular mechanisms of EA activates the scavenging of free radicals, regulation of phase I and II enzymes, modulation of proinflammatory and profibrotic cytokines synthesis, the regulation of biochemical pathways involved in synthesis, and degradation of lipids as well as the maintenance of essential trace element levels [40].

It has been reported that EA has been accounted to induce apoptosis particularly in colon adenocarcinoma cells when contrasted with normal colon cells [41]. In outline, our examination showed that EA is an effective agent to be used in combination with CP to reduce the underlying side effects such as nephrotoxicity and gonadotoxicity induced by chemotherapy and may be a potential candidate for CP-based combination treatment in clinical application.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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