

PAPER

Modulatory effects of catechin hydrate on benzo[a]pyrene-induced nephrotoxicity in adult male albino rats

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Abstract

Benzo [a] pyrene (B[a]P) is a potent mutagen and carcinogen, considered one of the commonest concomitants in the environment. The study aimed to evaluate the effect of catechin hydrate on benzo pyrene-induced kidney toxicity. Thirty-six adult male albino rats were divided into six groups: group I untreated control, group II received 10 mL/kg of corn oil (solvent of benzo [a] pyrene) twice a week, group III received 1 mL/kg 0.5% dimethyl sulfoxide (DMSO) (solvent of catechin) once per day, group IV received 50 mg/kg body weight of benzo[a]pyrene twice a week, group V received 20 mg/kg body weight of catechin in 1 mL/kg 0.5% DMSO once daily, and group VI received both catechin+benzo [a] pyrene with the same doses. All treatment was given by oral gavage for four weeks. At the end of the experiment, blood samples were collected for biochemical investigations, tissues were obtained for genotoxicity, RT-PCR, and histopathological studies. B[a]P exposure induced an increase in serum urea and creatinine levels along with severe renal histopathological changes. Our results showed a subsequent decrease in the antioxidant enzyme activities (catalase and superoxide dismutase), and conversely, (malondialdehyde) levels markedly elevated. Also, B[a]P induced DNA damage as well as activated an apoptotic pathway confirmed by upregulation of Bax, caspase-3, and downregulation of Bcl-2 expression. However, treatment with catechin-corrected kidney functions and antioxidant enzymes as well as regulated apoptosis. Histological results also supported the protective effects of catechin. These findings suggested that catechin hydrate is an effective natural product that attenuates benzo pyrene-induced kidney toxicity.

Key words: benzo [a] pyrene, superoxide dismutase, catechin hydrate, Bax, Bcl-2, kidney, nephrotoxicity, renoprotective

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental chemical carcinogens that lead to genetic damage with high bioaccumulation potentials [1]. B[a]P has been accepted as the indicator of PAH's presence in food due to its most intense carcinogenicity and to simplify the toxicological risk

measure assessment derived from different effects of these compounds [2].

The highest levels of B[a]P can be present during industrial food processing such as (heating, drying, and smoking), certain cooking practices (grilling, roasting, and frying) of meat, chicken, and fish [3], lower levels can be found in other foodstuffs as

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cereals (wheat), greens (kale, collard green), as well as smoke flavorings, vegetable fats, and oils and roasting or drying of coffee, cocoa beans, and tea leaves [4, 5].

The accepted levels of B[a]P in some foodstuffs as reported in European Commission (5 µg/kg for smoked meat and fish products; 2 µg/kg for fats and oil and 1 µg/kg for cereals) [6]. However, in a study conducted in Egypt on charcoal-grilled kebab and kofta; benzo [a] pyrene levels were 21–25 and 60–70 µg/kg, respectively [3].

B[a]P is absorbed simply through biological membranes due to its lipophilic nature [7]. It is bioactivated by binding to the aryl hydrocarbon receptor (AHR) in the liver and further metabolized to active carcinogen BaP-7-8-dihydrodiol 9, 10 epoxides (BAPDE) by cytochrome P450 enzymes. Since BAPDE (potent carcinogen) has an affinity for DNA, it forms DNA adduct (BAPDE-DNA) [8]. Alternatively, it can be metabolized into B[a]P quinines by dihydrodiol dehydrogenases that undergo redox cycling and induce oxidative stress via reactive oxygen species (ROS) and reactive nitrogen species (RNS) [9]. Thus, B[a]P exposure can lead to genetic mutations, chromosomal damage, single-strand breaks in DNA, apoptosis, oxidative, and inflammatory stress in various tissue.

Moreover, numerous toxicological studies proved the existence of aryl hydrocarbon receptor (AHR) in renal tissues, thus supporting the possible metabolic activation of benzo [a] pyrene to reactive intermediates in the kidneys similar to that occurring in the liver cells [10, 11].

Catechins are one of the potent antioxidant flavonoids. The flavan family is a mixture of chemicals such as galliccatechingallate, epicatechingallate, catechin hydrate, and epigallocatechingallate [12]. The beneficial effects of catechin on human health have been shown and proved by both epidemiological and *in vitro* studies. It has antioxidant, anti-inflammatory, and chemopreventive activities. Moreover, it plays an important role in declining the generation of malondialdehyde (MDA) level (MDA) and protecting against coronary heart disease in animal models [13, 14]. Thus, this study was designed to investigate the nephrotoxicity of benzo [a] pyrene and the beneficial properties of catechin hydrate on kidneys of adult male albino rats.

Materials and Methods

Chemicals

Benzo [a] pyrene (C₂₀H₁₂) ≥96% HPLC, Cas. No. 50-32-8 in the form of pale yellow powder and catechin hydrate (C₁₅H₁₄O₆) ≥98% HPLCC as. No. 225937-10-0, yellow with tan cast dust were purchased from Sigma Aldrich Merck KGaA, Darmstadt, Germany). Corn oil was obtained in the form of an oily solution as a solvent agent for benzo [a] pyrene. DMSO is a colorless fluid, used as a solvent for catechin hydrate purchased from El Gomhoria pharmaceutical Co. Zagazig, Egypt.

Experimental protocol

Thirty-six adult male albino rats weighing 200–220 g were utilized. The rat species were obtained from the animal house of the Faculty of Medicine, Zagazig University. All rats were acclimatized for two weeks before the experiment. Rats were kept under standard environmental conditions (20–25 °C, 40–55% humidity, and a 12 h light/dark cycle) with free access to standard diet and water. All experimental procedures were conducted following the ZU-IACUC instruction and the guidelines for the care and use of laboratory rats of the National Institutes of Health Guide for Care and Use of Laboratory Animals [15].

The study design included six equal experimental groups, each containing six rats. Group I was used as control (received regular diet and tap water). Group II: each rat received 10 mL/kg of corn oil (solvent of benzo [a] pyrene) twice a week by oral gavage for four weeks, group III: each rat received 1 mL/kg 0.5% dimethyl sulfoxide (DMSO) (solvent of catechin) once per day by oral gavage for four weeks, group IV: each rat received 50 mg/kg (1/20 of LD₅₀ [16]) body weight of benzo [a] pyrene in 10 mL/kg of corn oil twice a week by oral gavage for four weeks [17], group V: each rat received 20 mg/kg body weight of catechin in 1 mL/kg 0.5% DMSO once daily by oral gavage for four weeks [18], and group VI: each rat received both catechin+benzo [a] pyrene with the same doses for four weeks.

Sample collection

At the end of the study, intraperitoneal injection of pentobarbital 150 mg/kg was used for anesthesia, then venous blood samples were collected from the retro-orbital plexus by capillary tubes with an orifice of 0.6 mm that was inserted into the orbit of the eye at an anterior angle, then rotated to drill through the conjunctiva in the direction of the site of the optic nerve and the blood spontaneously shoot into the capillary tube [19], the separation of the serum was done by blood centrifugation at 664 × g for 10 minutes, serum was used for the analysis of kidney functions (urea and creatinine) and oxidative stress markers (MDA, catalase (CAT), and superoxide dismutase (SOD)). Afterward, all rats were sacrificed by cervical dislocation, kidneys were immediately dissected out, and then one part of the tissue was transferred into 10% formal saline for histopathological study. The other parts were freshly frozen immediately at –20 °C, transported on dry ice, and stored at –80 °C to obtain homogenates for DNA fragmentation analysis and RT-PCR.

Biochemical Study

Kidney function assessment

Creatinine (mg/dl): The assay depends on creatinine reaction with sodium picrate described by Jaffé [20]. Creatinine reacts with alkaline picrate forming a red complex. The time interval chosen for measurements avoids interference from other serum constituents. The intensity of the formed color is proportional to the creatinine concentration in the sample.

Urea (mg/dl): urea in the sample is hydrolyzed enzymatically into ammonia (NH₃) and carbon dioxide (CO₂). Ammonia ions formed react with α-ketoglutarate in a reaction catalyzed by glutamate dehydrogenase (GLDH) with simultaneous oxidation of NADH to NAD⁺: decrease in the concentration of NADH is proportional to urea concentration in the sample [21].

Antioxidant enzymes assay

Serum catalase (ng/ml) was assayed using (CAT MyBiosource kit, Inc. Cat No. MBS2600683) based on peroxide removal [22].

Superoxide dismutase (U/ml) was assayed using (SOD Kit Cusabio Biotech Co., Ltd. Cat No. CSB-E08555r) based on inhibition of nitroblue tetrazolium reduction [23].

Lipid peroxidation assay

Serum MDA (nmol/ml) was assayed colorimetrically at a wavelength of 530–532 nm using MDA Biodiagnostic, Cat. No. MD 2529), where MDA can react with thiobarbituric acid (TBA) and give pink-colored trimethylene complex [24].

Table 1: The used primers targeting

Gene	Forward sequence	Reverse sequence	Lot
Bax	CGAATTGGCGATGAACTGGA	CAAACATGTCAGCTGCCACAC	NM_017059.2
Bcl-2	GACTGAGTACCTGAACCGGCATC	CTGAGCAGCGTCTTCAGAGACA	NM_016993.1
Caspase-3	GAGACAGACAGTGGAACTGACGATG	GGCGCAAAGTGAAGTGGATGA	NM_012922.2
Gapdh	GGCACAGTCAAGGCTGAGAATG	ATGGTGGTGAAGACGCCAGTA	NM_017008.4

Real-time quantitative RT-PCR assay

Total RNA was extracted from kidney tissue using Trizol. We used (Invitrogen; Thermo Fisher Scientific, Waltham, MA, USA) and HiSenScript™ RH (–) cDNA synthesis kit (iNtRON Biotechnology Co., South Korea) for cDNA synthesis according to the instruction of the manufacturer. We performed RT-PCR in Mx3005P real-time PCR system (Agilent Stratagene, USA) using TOPreal™ qPCR 2X PreMIX according to manufacturer's instructions [25]. The PCR-cycling condition includes an initial denaturation at 95 °C/12 min, followed by 40 cycles of denaturation for 20 s/95 °C, annealing for 30 s/60 °C, and extension at 72 °C/30 s. The oligonucleotide-specific primers were synthesized by Sangon Biotech (Beijing, China) (Table 1). Gapdh was used as an internal reference gene to normalize the expression of the apoptotic genes. The results were expressed as the ratio of reference gene to target gene by using the following formula: $\Delta\text{Ct} = \text{Ct}(\text{apoptotic genes}) - \text{Ct}(\text{Gapdh})$. The following formula was used: $\Delta\Delta\text{Ct} = \Delta\text{Ct}(\text{Treated}) - \Delta\text{Ct}(\text{control})$ to determine the relative expression levels. Thus, the expression levels were expressed as *n*-fold differences relative to the calibrator. The value was used to plot the expression of apoptotic genes using the expression of $2^{-\Delta\Delta\text{Ct}}$ [26].

Agarose gel electrophoresis of DNA

DNA was extracted from kidney samples with QIAGEN Total DNA Extraction Kit (QIAamp DNA Mini kit, Qiagen, Germany), solutions of CL (lysis buffer), 20 µL proteinase K, and 5 µL of RNase were added to samples and blended, then, the lysate was incubated at 56 °C for 30 minutes. Then, 200 µL of buffer BL (lysis/binding buffer), then incubated at 70 °C for 5 minutes. The sample tube was centrifuged at 13 000 rpm for 5 minutes, then 200 µL of absolute ethanol was added to the lysate, inverted to mix up for 5–6 times, then (wash buffer A) and (wash buffer B) were used then centrifuged, where 70 µL of buffer CE (elution buffer) was directly added to the membrane, incubated for 1 minute at room temperature, and then centrifuged for 1 minute at 13 000 rpm to elute the DNA. Electrophoresis was run in the TAE buffer. Electrophoresis was done at 100 mA and 70 volts for approximately 1 hour using the EC 360 Submarine Gel electrophoresis system (Maxicell, EC 360 M-E-C apparatus Cooperation St. Petersburg, Florida USA). The DNA was visualized using ethidium bromide and photographed [27], where the existence of the DNA ladder was assessed according to methods described earlier [28].

Histopathological study

The kidney was fixed in buffered formalin 10% then consistently processed, fixed in soft paraffin wax at 55 °C for 2 hours and in hard paraffin at 60 °C for 2 h, and divided to 5 µm-thickness sections that were stained by hematoxylin and eosin (H and E) [29], then examined by light microscope.

Statistical analysis

Results for studied groups were displayed as mean ± standard deviation ($X \pm SD$). SPSS program version 21 was used. Statistically, a significant difference was estimated by one-way analysis of variance (ANOVA), followed by the Least significant difference (LSD) test for multiple comparisons between different groups. The probability values (*P*) less than 0.05 were considered significant and highly significant when *P* values were less than 0.001 [30].

Results

Kidney function

There were no statistically significant differences ($P > 0.05$) in mean values of serum urea and creatinine in corn oil, DMSO, and catechin groups compared to the control group. However, there was a highly significant increase in serum urea (mg/dl) and creatinine (mg/dl) levels in the benzo [a] pyrene treated-group compared with the control group. Also, there was a significant decrease ($P < 0.05$) in catechin+benzo [a] pyrene group compared to benzo [a] pyrene treated group, as shown in Figs 1 and 2.

Oxidative stress parameters

Results revealed no significant differences ($P > 0.05$) in serum SOD, CAT and MDA levels of corn oil, DMSO and catechin groups compared to the control group. But a noteworthy difference ($P < 0.001$) was detected in the benzo [a] pyrene and catechin+benzo [a] pyrene group compared with the control group. A high significant decline in serum SOD and CAT and a highly significant increase in MDA levels were observed in the benzo [a] pyrene group compared to the control group. Whereas, there was a significant increase ($P < 0.05$) in SOD and a highly significant increase ($P < 0.001$) in CAT levels in catechin+benzo [a] pyrene group and a highly significant diminish in MDA level compared to B[a]P group, as shown in Fig. 3.

Qualitative assessment of DNA fragmentation (DNA laddering)

Gel electrophoresis of DNA isolated from kidneys of control groups showed normal DNA bands all over study periods (lanes 1, 2, and 3). While the administration of benzo [a] pyrene for four weeks resulted in advanced shearing of DNA (lane 4), regarding catechin+benzo [a] pyrene group, there was mild DNA fragmentation for kidney tissue (lane 6) (Fig. 4).

RT-PCR

No significant differences ($P > 0.05$) in mean values of Bax, Caspase-3, and Bcl-2 expression could be detected in kidney tissue of corn oil, DMSO, and catechin groups compared to the

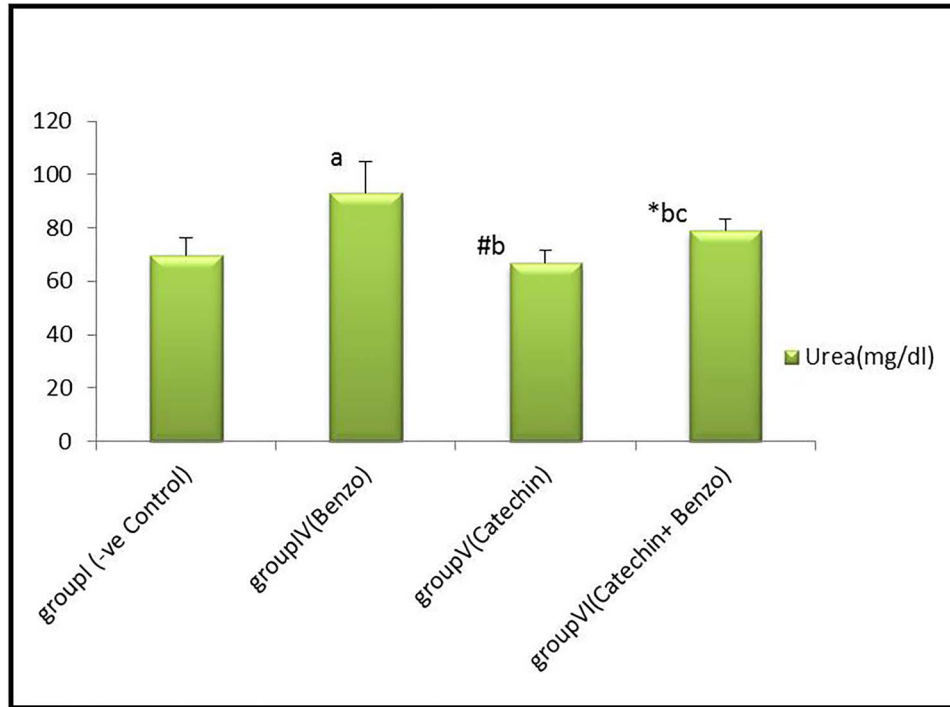


Figure 1: Bar chart showing comparative magnitude of mean values of serum Urea (mg/dl) in group I (-ve control), group IV (benzo[a]pyrene), group V (catechin) and group VI (catechin & benzo[a]pyrene) after four weeks of administration. # $P > 0.05$, * $P < 0.05$, ^a $P < 0.01$ compared to group I, ^b $P < 0.01$ compared to group IV, ^c $P < 0.01$ compared to group V.

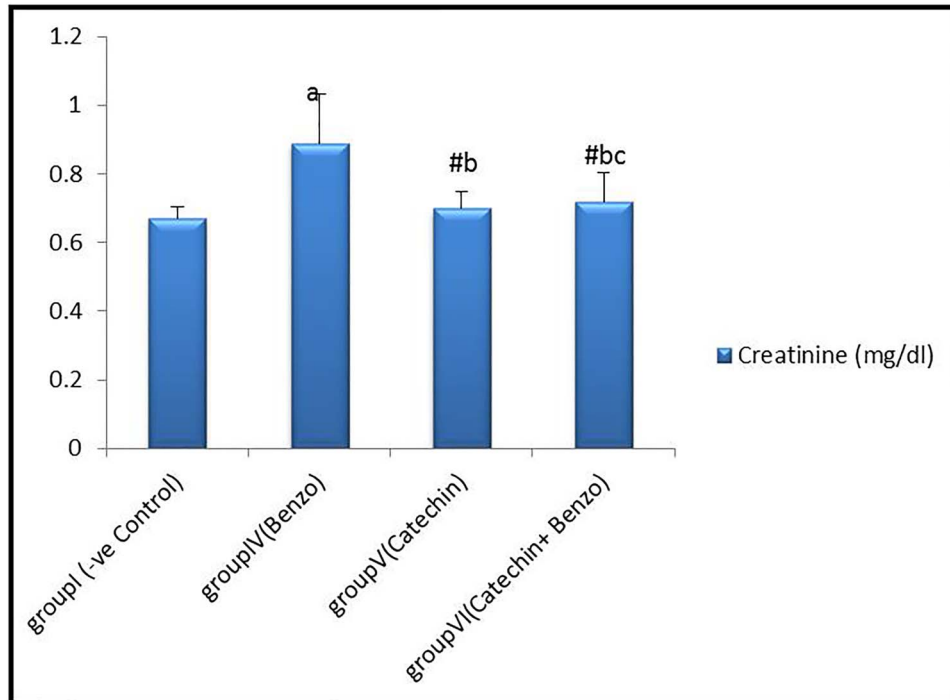


Figure 2: Bar chart showing comparative magnitude of mean values of serum Creatinine (mg/dl) in group I (-ve control), group IV (benzo[a]pyrene), group V (catechin), and group VI (catechin and benzo[a]pyrene) after four weeks of administration. # $P > 0.05$, ^a $P < 0.01$ compared to group I, ^b $P < 0.01$ compared to group IV, ^c $P > 0.05$ compared to group V.

control group. By the end of the fourth week, results revealed a highly significant increase in Bax and caspase-3 of kidney tissue and a significant decrease in Bcl-2 in group IV benzo [a] pyrene compared to the control group. A highly significant down

regulation ($P < 0.001$) in Bax and caspase-3 was observed in catechin+benzo [a] pyrene groups, while Bcl-2 showed a significant ($P < 0.05$) increased compared to benzo [a] pyrene group IV, as shown in Fig. 5.

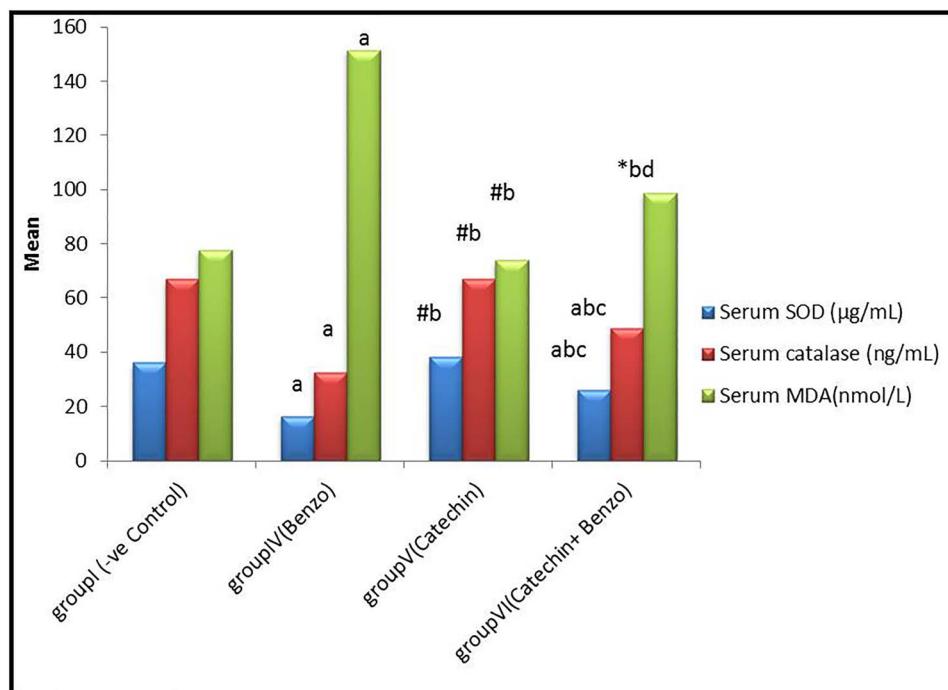


Figure 3: Bar chart showing comparative magnitude of mean values of serum SOD (U/ml), CAT(ng/ml), and MDA (nmol/ml) in group I (-ve control), group IV (B[a]p), group V (CH), and group VI (B[a]p + CH) after four weeks of administration. # $P > 0.05$, * $P < 0.05$, ^a $P < 0.01$ compared to group I, ^b $P < 0.01$ compared to group IV, ^c $P < 0.01$, ^d $P < 0.05$ compared to group V

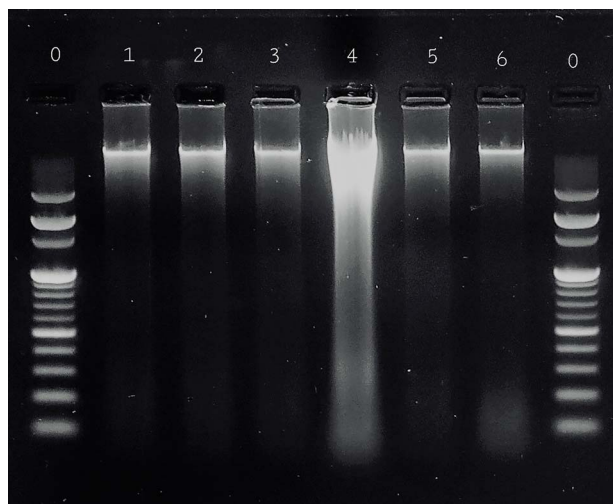


Figure 4: Agarose gel electrophoresis of DNA isolated from adult albino rat kidney showing lane 0: DNA ladder, lane 1: showing normal DNA band of control group I, lane 2: showing normal DNA band of group II (corn oil), lane 3: showing normal DNA band of group III (DMSO), lane 4: showing advanced shearing of DNA in benzo[a]pyrene-treated group IV, lane 5: showing normal DNA band (no DNA fragmentation) in catechin-treated group V, lane 6: showing very mild shearing of DNA in catechin & benzo[a]pyrene-treated group VI.

Histopathological results

The kidney section from control rats showed organized glomeruli as tufts of capillaries around mesangial cores of fibrous tissue and cells. Tubules are rounded or oval-shaped, lined with epithelial cells; the capillary basement membranes are thin. Epithelial cells are present over the surface of the capillaries but unremarkable in normal glomeruli. A flattened epithelium lines the

space around the glomerulus. There is a little more interstitium adjacent to the glomerulus, but it remains scanty. While kidney tissue from benzo [a] pyrene-treated group IV revealed disorganized glomeruli with congested blood capillaries, the tubules show loss of the brush border with an occluded lumen in most of them, and cast formation was present in some tubules. Kidney tissue from rats treated with catechin+benzo [a] pyrene (group VI) showed a near-normal histological appearance of glomeruli with wide Bowman's space; many tubules show preserved lumen with normal epithelial lining, others show still occluded lumens. While in group V (catechin-treated), glomeruli looked normal, and intervening round or oval tubules with apparently normal epithelial lining were seen (Fig. 6).

Discussion

Kidney tissue is vulnerable to injury by toxins triggering oxidative stress due to its high metabolic state, active enzymes, and massive oxygen demand [31, 32]. They also enclosed the highest physiological level of lipid peroxidation markers that are more sensitive to B[a]P toxic metabolites [33]. Moreover, B[a]P is mainly trapped in the kidneys. Thus, the kidney is considered a target organ for benzo[a]pyrene injurious effects [34, 11].

Our results showed that exposure to benzo[a]pyrene caused a highly significant increase in serum urea and creatinine. Similar to these findings, Ogbonna et al. and Deng et al. [35, 36] observed that B[a]P-treated rats showed significantly higher serum urea, BUN, and creatinine concentrations compared to other groups. Contrary to our results, Valentovic et al. [37] reported no change in blood urea nitrogen (BUN) following five weeks of B[a]P (10 mg/kg) administration in the normoglycemic animals, which may be due to different dosing and duration.

Reddy et al. [38] attributed the elevation in serum urea concentration to excessive breakdown of proteins and enzymes by

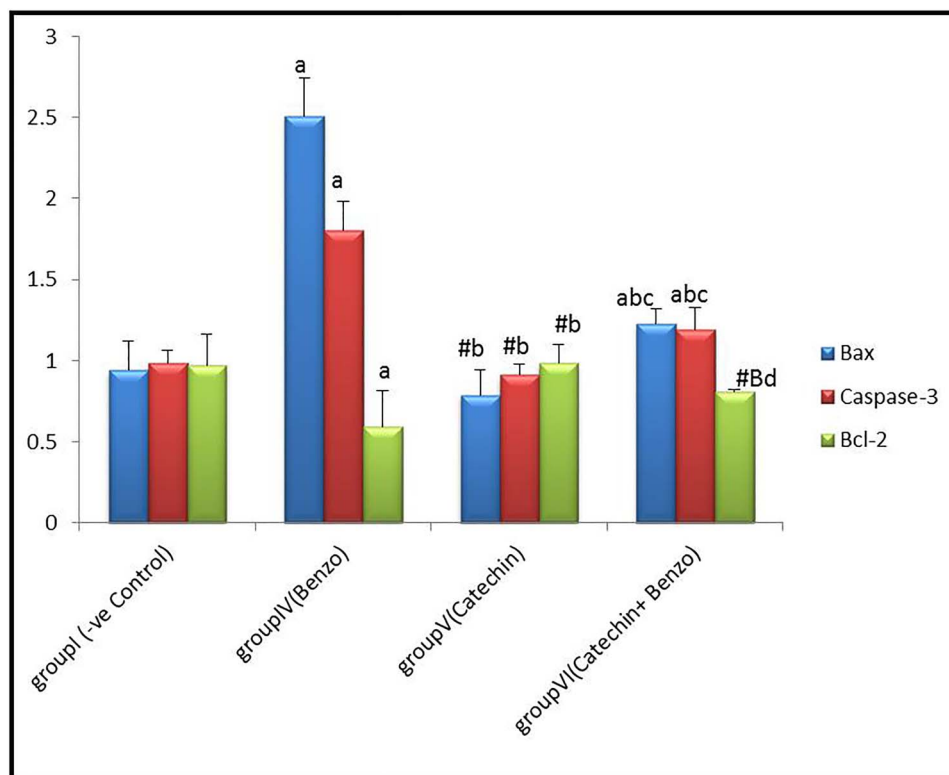


Figure 5: Bar chart showing comparative magnitude of the mean values of Bax, caspase-3 and Bcl-2 in group I (-ve control), group IV (benzo[a]pyrene), group V (catechin), and group VI (catechin and benzo[a]pyrene) in renal tissue after four weeks of administration. [#] $P > 0.05$, ^{*} $P < 0.05$, ^a $P < 0.01$ compared to group I, ^b $P < 0.01$, ^B $P < 0.05$ compared to group IV, ^c $P < 0.01$, ^d $P > 0.05$ compared to group V.

the presence of ROS. Secondly, reduced kidney activity resulted in urea holding back or accumulation. Serum urea accumulates when the rate of urea production exceeds the rate of clearance signals renal dysfunction.

Also, increased serum creatinine indicates the retention of creatinine in the blood resulted from a gradual degeneration of the kidney due to exposure to reactive metabolites of benzo [a] pyrene, which readily elicits free radicals [39, 35].

The present study revealed a significantly decreased serum urea and creatinine in (B[a]p + CH) co-treated group. So, if serum urea and creatinine elevation are an index of nephrotoxicity, their significant reduction by catechin indicates its relieving effects [40]. Similarly, Mostafa [41] reported that administration of high catechin levels plus green tea significantly decreased all investigated kidney functions compared to control groups.

According to our results, there was a highly significant decrease in serum SOD and CAT levels and a highly significant increase in MDA level in the benzo [a] pyrene group. These findings matched with previous research of Kamaraj et al., Shahid et al., and Almatroodi et al. [42–44].

The antioxidant enzymes represent the defense response system to oxidative stress and normalize the adverse effects caused by oxidative stress. Whereas, the superoxide-dismutase enzyme catalyzes the dismutation of two superoxide anions to hydrogen peroxide and oxygen, then the catalase enzyme reduces two hydrogen peroxide molecules to water and oxygen [45, 46].

On the other hand, the combined (B[a]p + CH) group showed a significant increase in SOD, a highly significant increase in CAT levels, and a highly significant decrease in MDA level compared to B[a]P group.

Shahid et al. [43] proved that SOD and CAT levels decreased after B[a]p administration, while MDA increased, and co-administration of catechin hydrate ameliorated these effects, In line with the outcome of our study, indicating that catechin restored the intensity of antioxidants along with its anti-lipid peroxidative action.

In the current study, the administration of benzo[a]pyrene for four weeks resulted in advanced shearing of DNA in kidneys. Furthermore, the combined administration of catechin with benzo[a]pyrene restored DNA integrity. This reduction in endogenous damage can indicate increased DNA protection by catechin against the free radical attack and accelerated DNA repair. As reported by Shahid et al. and Alshatwi et al., catechin has succeeded in reducing cellular injuries and DNA fragmentation, showing that catechin may also protect against B[a]P-induced renal genotoxicity [43, 47].

Sinha and Dash [48] stated that a single oral dose of B[a]P (125 mg/g b.wt) induced renal damage with significant DNA fragmentation and alteration in DNA integrity in the kidney compared to the control group. In the same context, Delgado and his team [49] suggested that B[a]P metabolites can cause oxidative DNA damage by creating adducts with DNA with subsequent dose-dependent breaks of DNA strands.

The production of ROS by B[a]P and the ensuing oxidative stress play a pivotal role in apoptosis. A cascade of events leads to activation of various downstream pro-apoptotic proteins and blockage of antiapoptotic proteins, thereby leading to apoptosis [50].

Our results revealed a highly significant increase in Bax and caspase-3 of kidney tissues and a highly notable diminish in Bcl-2 in group B[a]p. However, the treatment with catechin led

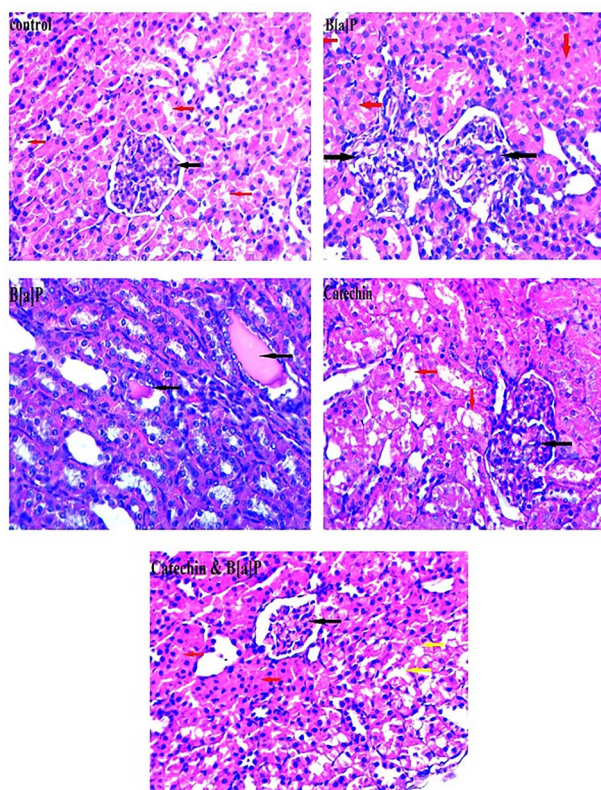


Figure 6: Photomicrograph of the kidney in different experimental groups. Control group showing normally looking glomeruli (black arrow) with intervening round or oval tubules with epithelial lining (red arrow). Benzo[a]pyrene-treated group showing disorganized glomeruli with congested blood capillaries (black arrow). The tubules show loss of the brush border with occluded lumen in most of them (red arrow), other showing cast formation in some tubules (black arrow). Catechin-treated group showing normally looking glomeruli (black arrow) with intervening round or oval tubules with epithelial lining (red arrow). Catechin & benzo(a)pyrene-treated group showing the glomeruli with near normal histologic appearance with wide Bowman's space (black arrow), many of the tubules show retained lumen with normal epithelial lining (yellow arrow), other tubules show still occluded lumens (red arrow) (H&E $\times 400$).

to a highly significant decrease in Bax and caspase-3 and an increase in Bcl-2 compared to B[a]p group. These results matched those of Shahid et al. [43], where the B[a]p-apoptotic effect was regulated with catechin treatment in a dose-dependent manner. These results further supported apoptosis involvement in organ damage and oxidative stress in B[a]p-induced toxicity.

According to Silva Santos et al. [51], catechin can act directly within the mitochondria to inhibit ROS generation, mitochondrial membrane potential loss, and apoptosis by restoring the activity of mitochondrial complex I and ATP biosynthesis. As well as Shahid et al. and Gheysarzadeh and Yazdanparast [43, 52] demonstrated that catechin could also inhibit apoptosis by enhancing the expression of the antiapoptotic protein B-cell lymphoma-2 (Bcl-2) and suppressing the expression of apoptotic protein Bcl-2-associated X (Bax).

Oxidative stress contributes to the pathogenesis of cancer. The abnormal histological tissue may further progress into a precancerous lesion, even cancer in organisms. This supports the histopathological findings of the B[a]p-treated group in the current study, whereas kidney tissue showed disorganized glomeruli with congested blood capillaries, the tubules show loss of the brush border, most of them with an occluded lumen and cast formation in some others [36].

Near our results, Ogbonna et al. [35] observed that exposure to benzo [a] pyrene caused distortions and damage in the kidney tissues. They discussed that damaged renal tissue could not perform in the clearance of reactive substances from the body leading to more tissue injury. The distortions in the glomeruli architecture could cause an increase in serum creatinine and urea concentration of rats exposed to B[a]P. Also, Adedara et al. [53] documented that kidneys of rats exposed to B[a]P alone at a dose of 10 mg/kg for 15 days showed numerous protein casts tubular lumen indicating nephrotic glomerular dysfunction.

In contrast, in (B[a]p + CH) group, the glomeruli showed near-normal structure with wide Bowman's space, many tubules have lumen with normal epithelial lining, and other tubules show still occluded lumens.

In line with the outcome of our study, Wongmekiat et al. [54] demonstrated that catechin could protect against oxidant-induced renal injury in different pathologic settings. The marked reduction in renal oxidative stress coupled with significant improvement in renal function and renal morphology by catechin indicates that its protective effect is based on the free radical scavenging activity. Catechin directly scavenges oxy-free radical species leading to the protection of lipid membranes, proteins, and nucleic acids [55, 56].

Catechin behaves like other flavonoids, may also function indirectly as antioxidants through inhibiting the redox-sensitive transcription factors, nuclear factor-kappa B, and activator protein. It suppresses prooxidant enzymes such as inducible nitric oxide synthase, lipoygenases, cyclooxygenases, and xanthine oxidase. Furthermore, catechin induces phase II antioxidant enzymes, such as glutathione S-transferases and superoxide dismutases [57].

Anjaneyulu et al. [58] attributed the catechin renoprotective-effects inhibition of angiotensin formation and direct vasorelaxation effect along with antioxidant properties. Catechin has been speculated to have angiotensin-converting enzyme (ACE) inhibition properties and hypotensive activity.

Flavonoids from natural products constitute the largest category of AhR ligands, demonstrating that they can suppress AhR transformation [59, 60]. On the other hand, Hui et al. [61] reported that aryl hydrocarbon receptor activation mediates kidney disease and renal cell carcinoma. This can explain the salvaging effect of flavonoids on the B[a]P-induced toxicity.

Limitation: Our findings give the only emphasis on the protective effect of catechin on benzo [a] pyrene-induced renal toxicity, however further studies focusing on the exact mechanism of action of catechin, are needed to define the precise mechanism of action of catechin.

Conclusions

Our results showed that B[a]p exposure induced toxic kidney effects in albino rats, evidenced by oxidative, genotoxic, apoptotic, and histopathological changes, while catechin hydrate has shown a renoprotective effect on benzo [a] pyrene-induced toxicity mediated through its antioxidant and possible direct nephroprotective actions. This gives rise to the prospect of developing a renal preventive strategy for individuals who are at risk of B[a]p contamination utilizing catechin supplementation. Limiting environmental exposure to such chemicals has a great impact on controlling their toxic profile. Further researches are needed to establish and validate well-designed protocols for toxicity examination and protective measures. Since bioflavonoids have emerged as compounds of clinical potential, various

standardization procedures, and clinical trials may make catechin a useful clinical moiety.

Conflict of interest statement

There is no conflict to declare.

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