The Possible Ameliorative Effect of Curcumin on Atrazine-Induced Oxidative Stress, DNA Damage, Mitochondrial Dysfunction, and Apoptosis in the Kidney of Adult Male Albino Rats

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Abstract

Introduction: Atrazine (ATR), a worldwide triazine herbicide, induces oxidative stress and DNA damage. It was found that oxidative stress is closely linked to apoptosis. Curcumin has strong antioxidant, antimicrobial, anticancer and anti-inflammatory actions.

Aim of the study: The aim of this study was to assess the possible ameliorative effect of curcumin on ATR- induced oxidative stress, oxidative DNA damage, mitochondrial dysfunction, and apoptosis in the kidney of adult male albino rats.

Material and methods: The current study was conducted on 40 adult male albino rats. Rats were divided into four equal groups. The first group was control. Group II was treated with curcumin (100mg/kg/day). Group III was treated with ATR (400mg/kg/day). Group IV was treated with both ATR (400mg/kg/day) and curcumin (100mg/kg/day). All were given orally for 21days. Body and kidney weights, blood urea and creatinine as well as 24 hours urinary protein levels were measured. Levels of malondialdehyde (MDA), reduced glutathione (GSH), and 8-Hydroxy-2′-deoxyguanosine (8-OHdG) as well as activities of catalase (CAT), heme oxygenase-1 (HO-1) and complex-1 were assessed. Histopathological and immunohistochemical examination of renal tissue were carried out.

Results: ATR treated group was significantly decreased in body and kidney weights, GSH level and HO-1 as well as complex 1 activities while there was significant increase in blood urea, creatinine, 24 hours urinary protein, MDA and 8-OHdG levels as well as CAT activity. Renal cloudy swelling was observed in H&E sections. Caspase 3 immunohistochemistry revealed high rate of apoptosis. Curcumin administration along with ATR significantly improved these observations.

Conclusion: It was concluded that administration of curcumin along with ATR ameliorated oxidative stress, DNA damage, mitochondrial dysfunction and apoptosis induced by ATR.

Recommendations: The current study recommend further studies to clarify issues related to human exposure to ATR and the ameliorative effect of curcumin in Egypt and elsewhere around the world.

Keywords   atrazine, oxidative stress, antioxidants, apoptosis, mitochondrial dysfunction, curcumin

Introduction

Atrazine (2 - chloro – 4 – ethylamino – 6 – isopropylamino – s – triazine) is a triazine herbicide used worldwide to control grasses and weeds in a variety of crop plantations, especially corn, maize and cereals, as well as different types of fruits (Lasserre, et al., 2009; Zhao, et al., 2014). Human exposure includes occupational and non occupational exposure. Occupational exposure occurs through both inhalation and dermal absorption during its manufacture, formulation as well as handling. Non occupational exposure may include consumption of contaminated fish as it bioaccumulates in fish (Ross, et al., 2009).

Many toxicological studies of atrazine (ATR) focus primarily on its effects on the endocrine and reproductive systems. Moreover, animal studies report that it causes developmental and immunological alterations in laboratory rodents (Pogrmic-Majkic, et al., 2010).

There is growing evidence suggesting that ATR can induce oxidative stress and DNA damage (Song, et al., 2009). Oxidative stress develops when there is an imbalance between pro-oxidants and antioxidants, leading to the generation of reactive oxygen species (ROS) at supra-normal levels. They attack all cellular components causing oxidation and fragmentation of nucleic acids, proteins, lipids, activation of cytoplasmic/ nuclear signal transduction pathways, modulation of gene and protein expression, alteration of activities of DNA and RNA polymerases and even cell death (Bhatti, et al., 2011).

Oxidative stress or generation of ROS has been closely linked to apoptosis (Xu, et al., 2012). Apoptosis is a form of programmed cell death; it is a genetically regulated active physiological process of cell suicide that causes cell deletion without inflammation, scarring, or release of cellular contents (Elmore, 2007). Mitochondria play a pivotal role in controlling life and death (Green and Reed, 1998).

Antioxidants are molecules capable of slowing or preventing the oxidation of other molecules. They are classified into enzymatic as catalase, glutathione peroxidase and non-enzymatic antioxidants as total thiol content and reduced glutathione which act as free radical scavengers (Blokhina, et al., 2002; Tian, et al., 2014).

Curcumin (diferuloylmethane or 1, 7-bis (4-hydroxy-3- methoxyphenol)-1, 6-heptadiene-3, 5-dione) is a yellow pigment extracted from rhizomes of the plant Curcuma longa (turmeric), it has strong antioxidant, antimicrobial, anticancer, and anti-inflammatory actions. It has been widely used as food additive and also as a herbal medicine throughout Asia (Al Moundhri, et al., 2013).

To the best of our knowledge no previous studies were conducted to show the ameliorative effect of curcumin on ATR nephrotoxicity. So, the aim of the present study was to investigate the possible ameliorative effect of curcumin on ATR- induced oxidative stress, oxidative DNA damage, mitochondrial dysfunction, and apoptosis in the kidney of adult male albino rats through biochemical and morphological methods.

Material and methods

Chemicals

Atrazine (6-chloro-N^2 - ethyl- N -isopropyl- 1, 3, 5-triazine- 2, 4- diamine) was available in the form of white powder (Syngenta Crop Protection AG- Basel-Switzerland).

Curcumin (also named diferuloylmthane) [(E, E)-1, 7- bis (4-hydroxy-3-methoxyphenyl)-1, 6-heptadiene-3, 5-Dione] was purchased from Sigma Chemical Co. (St. Louis, MO., USA).

All other chemicals were of analytical grade and were purchased from Sigma Chemical Co. (St. Louis, MO., USA).

Animals

This study was carried out on 40 adult male albino rats, their weight ranged from 150-200 g. They were obtained from the animal house of the Faculty of Medicine, Tanta University, Egypt. During the study, the animals were kept in wire mesh cages with ad-libitum access to water. The room temperature was about 22-24 ℃ and the animals were exposed to 12:12 hours light dark cycles.

Ethical considerations

Experimental procedures were performed according to the guide of care and use of laboratory animals approved by the Ethical Committee of Faculty of Medicine, Tanta University.

Fewer numbers of animals estimated to affair valid results were used.

Animal painless procedures conducted with appropriate sedation to avoid pain and stress

Experimental design

This current work was carried out in Forensic Medicine & Clinical Toxicology and Medical Biochemistry Departments. After one week of acclimatization, the animals were randomly divided into four main groups (ten rats each); all animals received their doses orally by syringe feeding method once daily for 21 days.

Group I: (control group): included 10 rats which received 0.2 ml corn oil (the diluting vehicle for atrazine and curcumin).

Group II: (Curcumin treated rats): included 10 rats that received curcumin in a dosage of 100 mg/kg/day (Ciftci, et al., 2011). Curcumin was prepared as 20mg/0.2ml solution in corn oil.

Group III: (ATR treated rats): included 10 rats that received 400mg/kg/day (about 13% of LD_{50}) (Juliani, et al., 2008). Atrazine was prepared as 80 mg/0.8ml solution in corn oil. According to Weed Science Society of America, (1994), oral lethal dose (LD_{50}) for ATR is 3090 mg/kg in rats.

Group IV: (ATR + curcumin treated rats): included 10 rats that received ATR in a dosage of 400 mg/kg/day concomitantly with curcumin in a dosage of 100mg/kg/day.

Body and kidney weights

Body weights of all studied groups were recorded at the beginning of the experiment, every day during the period of the experiment (for dose adjustment) and after 21 days of ATR and/or curcumin treatment before they were sacrificed. After 21 days, the kidneys were removed aseptically and weighed.

Sample collection

A-Blood sample

After 6 hours fasting, the animals were anaesthetized by light ether, and while the heart was still beating, blood was collected on heparin via cardiac puncture. Plasma was separated and stored at -80℃ till used for

the assay of the levels of malondialdehyde (MDA), 8-Hydroxy-2′-deoxyguanosine (8-OHdG), and reduced glutathione (GSH) as well as the activity of catalase (CAT). Plasma levels of creatinine and urea were estimated by commercial kits (Diamond Diagnostic, Egypt).

B- Urine sample

Twenty four hour urine samples were collected for assay of urinary protein level according to the method of Orsonneau, et al., (1989) by commercial kit (Diamond Diagnostic, Egypt).

Random urine samples were collected for the assay of 8-OHdG level by ELISA and creatinine level by commercial kits (Diamond Diagnostic, Egypt).

C- Tissue sample

After blood sampling, the animals were sacrificed by decapitation (after sedation with ether), the abdomen and the thorax were opened and both kidneys were removed, pieces of the kidney were taken for histopathological assessment and immunohistochemistry for Caspase 3. These pieces were immediately fixed in neutral buffered formaline (10%). The remaining portions were washed three times in ice cold saline and blotted individually on ash-free filter paper. These ice-washed portions of the kidney were separated into three pieces. Each piece was weighed and homogenized separately with a potter- Elvenhjem tissue homogenizer. The first pieces were homogenized in phosphate buffer saline (PBS) 10 mM pH 7.4 for estimation levels of MDA and GSH as well as the activity of CAT. The second pieces were homogenized in 10 mM Tris– HCl buffer pH 7.8 for mitochondrial separation followed by centrifugation at 700 xg for 10 min at 4 ˚C, then the supernatant was removed and centrifuged for another 20 min at 1000 xg to obtain mitochondria pellets. Mitochondria pellet was then re-suspended in 9 volumes of 10 mM Tris– HCl buffer pH 7.8 for the assay of complex 1 activity (Turpeenoja, et al., 1988).

The third pieces were used for microsomal protein preparation; they were homogenized separately in cold sucrose solution (1 g of tissue in 5 ml of 0.25 M sucrose). Microsomal protein from the homogenized tissues was separated by differential ultracentrifugation. The final microsomal pellet was reconstituted in cold sucrose and stored at −80°C for the assay of heme oxygenase (HO-1) activity (Aboutabl, et al., 2009).

Protein concentration for each homogenate was determined by the method of (Lowry, et al., 1951).

Methods

Biochemical analysis

A- Spectrophotometric assay of MDA level

This method depends on the formation of MDA as an end product of lipid peroxidation which reacts with thiobarbituric acid producing thiobarbituric acid reactive substance (TBARS), a pink chromogen, which can be measured spectrophotometrically at 532 nm, by commercial kit (Biodiagnostic, Egypt), the results were expressed as nmol/ml or nmol/mg protein for plasma and tissue respectively (Ohkawa, et al., 1979).

B- Spectrophotometric assay of GSH level

The method is based on the reduction of 5,5 dithiobis (2-nitrobenzoic acid) (DTNB) with GSH to produce a yellow compound .The reduced chromogen is directly proportional to GSH concentration and its absorbance can be measured at 405 nm by using a commercial kit (Biodiagnostic, Egypt). The results were expressed as mg/dl or mg/g wet tissue for plasma and tissue respectively (Tietez, 1969).

C- Spectrophotometric assay of CAT activity

Catalase activity was assayed by the method of Sinha, (1972) which based on formation of chromic acetate from dichromate and glacial acetic acid in presence hydrogen peroxide, chromic acetate that produced was measured colorimetrically at 570 nm, the results were expressed as µm H O consumed/ min /L or µm H O consumed/ min / mg protein for plasma and tissue respectively.

D- Spectrophotometric assay of HO-1 activity

Heme oxygenase activity was determined in kidney microsomes. The microsomes were re-suspended in 1 ml of 0.1 mmol/l potassium phosphate buffer, pH 7.4, containing 2 mmol/L MgCl and analyzed for HO-1 activity by a spectrophotometric assay that measures the formation of bilirubin, the end product of heme degradation. The extracted bilirubin was measured by the difference in absorbance between 464 and 530 nm using a molar extinction coefficient of 40 $mM^{-1}cm^{-1}$, the results were expressed as pmol bilirubin/mg protein (Maines, 1996).

E-Determination of urinary and plasma level of 8-OHdG by ELISA technique

The supernatants were quantitatively assayed for oxidized DNA (Enzo Life Sciences, Catalog Number: ADI-EKS-350), the absorbance can be measured spectrophotometrically at 450nm. The results were averaged and expressed as ng/ml or ng/mg creatinine for plasma and urine respectively.

G- Spectrophotometric assay of mitochondrial complex-I activity (NADH: coenzyme Q oxido-reductase enzyme activity) in tissue mitochondria

It was measured according to the method of Birch-Machin, et al., (1994) by following the decrease in the absorbance due the oxidation of NADH at 340 nm. The results were expressed as nmol/min/mg tissue protein.

Histopathology

For light microscopic study, the fixed specimens of the kidney were dehydrated through ascending grades of ethyl alcohol, cleared in xylene and embedded in paraffin wax. Later, 4 µm thick sections were stained with haematoxylin and eosin (Bancroft and Gamble, 2008).

Immunohistochemistry

For caspase-3 immunohistochemical study, kidney sections were deparaffinized, hydrated and incubated with 10% hydrogen peroxide to block the endogenous activity of peroxidase. Sections were then incubated for two hours with primary rabbit anti-rat caspase-3 antibody directed against the cleaved activated caspase-3 (Neo Markers Fremont CA, Lab Vision). The sections were then rinsed three times in PBS and incubated with goat anti rabbit peroxidase-conjugated secondary antibody (Peroxidase-labelled streptavidin) for one hour and rinsed again three times in PBS. The immunoreactivity was visualized by applying streptavidin peroxidase as 3, 3 Diaminobenzidine (DAB) - hydrogen peroxide as a chromogen. Counterstaining was performed by haematoxylin. Positive caspase-3 cells have dark brown granules in their cytoplasm. For negative control, the primary antibody was replaced by PBS. Positive immunostaining was semiquantitated as follows: 0–1 (<30% of positive cells), 2 (30–50%) and 3 (>50%) (Meggiato et al., 2003). The result for each group was expressed as the mean score of the group.

Statistics

Data were analyzed using statistical package for social sciences (SPSS) version 16 for calculation of descriptive measures as mean and standard deviation. One way analysis of variance (ANOVA) test was used for comparison of mean among the studied four groups. Post-hoc test was used to compare mean between each two groups. Significance level was set at $p < 0.05$ (Field, 2005).

Results

Biochemical results

Table (1) shows comparative statistics of body and kidney weights as well as blood urea/creatinine and 24 hours urinary protein levels in all studied groups. Body and kidney weights were decreased significantly in ATR treated rats (group III) compared with the control and curcumin treated groups (group I and II), while treatment with curcumin along with ATR in group IV significantly improved this change. There was also a significant increase in blood urea/creatinine and 24 hours urinary protein levels in group III compared with group I and II, while treatment with curcumin concomitantly with ATR in group IV significantly reduced this increase.

Table (2) shows comparative statistics of plasma and kidney tissue MDA level, GSH level, CAT activity and HO-1 activity in all studied groups. Plasma and kidney tissue MDA level showed significant increase in group III compared with group I and II, while treatment with curcumin along with ATR significantly reduced this increase in group IV. As regards changes in the antioxidants, there was significant decrease in plasma and kidney tissue GSH content in rats treated with ATR (group III) compared with the control and curcumin treated rats (group I and

II), while the treatment with curcumin significantly improved these changes in group IV. Similarly, kidney HO-1 activity was significantly decreased in group III with significant improvement on administration of curcumin concomitantly with ATR in group IV. On the other hand, there was significant increase in plasma and kidney tissue CAT activity in rats treated with ATR (group III) compared with the control and curcumin treated rats (group I and II), while the treatment with curcumin significantly reduced this increase in group IV.

Table (3) shows comparative statistics of plasma and urinary levels of 8-OHdG and kidney tissue complex-I activity in all studied groups. There was significant increase in plasma and urinary 8-OHdG level in group III compared with group I and II, while treatment with curcumin concomitantly with ATR significantly reduced this increase in group IV. Complex-1 activity showed significant decrease in kidney tissue in ATR treated rats (group III) compared with the control and curcumin treated groups (group I and II), while the treatment with curcumin significantly improved this change in group IV.

Histopathological results

Examination of H & E stained renal sections from the control and curcumin treated rats (group I and II) by light microscope showed normal appearance of the renal tissue (photomicrograph 1). Sections form ATR treated rats (group III) revealed extensive cloudy swelling of the cells of the renal tubules (photomicrograph 2), while sections from ATR & curcumin treated rats (group IV) showed mild cloudy swelling of the cells of renal tubules (photomicrograph 3).

Immunohistochemical result

Examination of caspase-3 stained renal sections from the control and curcumin treated rats (group I and II) by light microscope showed negative staining for caspase-3 in renal tubules (photomicrograph 4). Sections in ATR treated rats (group III) showed cloudy swelling of the renal tubules with marked positivity for caspase-3 (photomicrograph 5). Caspase positivity was cytoplasmic in the kidney tissue. Sections from ATR & curcumin treated animals (group IV) showed mild caspase positivity in the renal tubules (photomicrograph 6).

Table (4) shows comparative statistics of caspase 3 positivity in the kidney tissue in all studied groups. The highest mean positivity was detected in ATR treated rats (group III) compared with the control and curcumin treated rats (group I and II). There was significant improvement on administration of curcumin in group IV. There was a statistically significant difference between the four groups.

Table (1): One way analysis of variance (ANOVA) with post-hoc test of all studied groups (each 10 rats) as regards body and kidney weights, blood urea/creatinine and 24 hours urinary protein levels.

Parameter/Group	Group I [a] (n=10)	Group II [b] (n=10)	Group III [c] (n=10)	Group IV [d] (n=10)	ANOVA F	P
Body weight (g)	188.6±3.52 [cd]	195.6±13.2 [cd]	141.2±5.3 [abd]	163.5±8.6 [abc]	6.03	<0.05*
Kidney weight (g)	4.20±0.18 [cd]	4.5±0.3 [cd]	2.84±0.20 [abd]	3.24±0.27 [abc]	8.19	<0.05*
Blood creatinine level (mg/dl)	0.51±0.09 [cd]	0.52±0.07 [cd]	0.81±0.03 [abd]	0.75±0.09 [abc]	7.52	<0.05*
Blood Urea level (mg/dl)	51.1±0.12 [cd]	51.63±0.75 [cd]	67.41±1.95 [abd]	59.41±1.63 [abc]	16.33	<0.05*
24 hour urinary protein level (mg/24h)	8.55±1.01 [cd]	9.36±1.68 [cd]	23.64±1.03 [abd]	16.74±1.33 [abc]	5.99	<0.05*

a-d: significant difference between groups at p<0.05*; n= number. Data are mean± standard deviation.

Table (2): One way analysis of variance (ANOVA) with post-hoc test of all studied groups (each 10 rats) as regards malondialdehyde level, reduced glutathione level, catalase activity and heme oxygenase-1 activity

Parameter/ group		Group I [a] (n=10)	Group II [b] (n=10)	Group III [c] (n=10)	Group IV [d] (n=10)	ANOVA F	P
MDA level	Plasma (nmol/ml)	2.86±0.33 [cd]	3.22±0.74 [cd]	4.88±0.35 [abd]	4.71±0.16 [abc]	7.62	<0.05*
	Kidney tissue (nmol/mg protein)	1.20±0.30 [cd]	1.12±0.37 [cd]	3.86±0.28 [abd]	3.56±0.41 [abc]	4.22	<0.05*
GSH level	Plasma (mg/dl)	3.11±0.52 [cd]	3.19±0.24 [cd]	2.20±0.58 [abd]	2.42±0.36 [abc]	10.33	<0.05*
	Kidney tissue (mg/g wet tissue)	4.62±0.25 [cd]	4.74±0.34 [cd]	1.86±0.38 [abd]	2.41±0.19 [abc]	5.635	<0.05*
CAT activity	Plasma (μmol/min /L)	53.36+4.5 [cd]	59.6±11.3 [cd]	274.3±35.6 [abd]	250.2±+14.5 [abc]	15.36	<0.05*
	Kidney tissue (μmol/min /mg protein)	112.2±16 [cd]	113.5±7.5 [cd]	341.3±19.6 [abd]	328.5+22.5 [abc]	23.52	<0.05*
HO-1 Activity	Kidney tissue (pmol bilirubin/mg protein)	13.91±0.2 [cd]	13.11±0.1 [cd]	7.53±0.06 [abd]	10.1±0.02 [abc]	9.66	<0.05*

a-d: significant difference between groups at p<0.05*; n=number; MDA= malondialdehyde; GSH= reduced glutathione; CAT= catalase; HO-1= heme oxygenase-1 activity. Data are mean± standard deviation

Table (3): One way analysis of variance (ANOVA) with post-hoc test of all studied groups (each 10 rats) as regards of complex-1 activity and 8-Hydroxy-2′-deoxyguanosine level
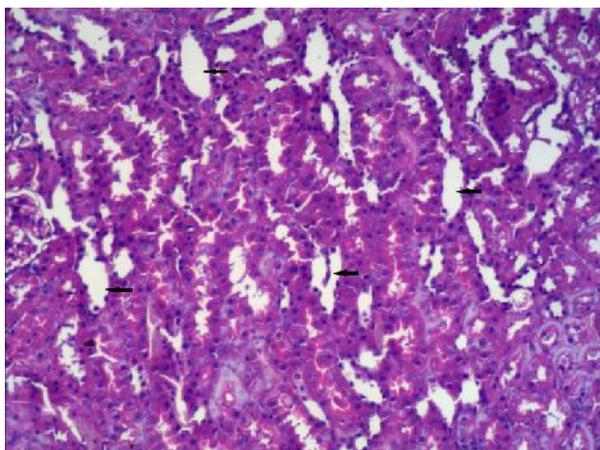
Parametr/group		Group I [a] (n=10)	Group II [b] (n=10)	Group III [c] (n=10)	Group IV [d] (n=10)	ANOVA F	P
Complex I activity	Kidney tissue (nmol/min/mg tissue protein)	18.41±0.18 [cd]	18.41±0.96 [cd]	15.1±0.33 [abd]	17.5±0.08 [abc]	22.36	<0.05*
8-OHdG level	Urine (ng/mg creatinine)	27.63±1.96 [cd]	28.99±0.3 [cd]	42.85±0.36 [abd]	35.11±0.13 [abc]	5.46	<0.05*
	Plasma (ng/ml)	15.42±0.21 [cd]	16.1±0.01 [cd]	21.41±0.88 [abd]	18.74±0.99 [abc]	10.35	<0.05*

a-d: significant difference between groups at p<0.05*; n= number; 8-OHdG = 8-Hydroxy-2'-deoxyguanosine. Data are mean± standard deviation.
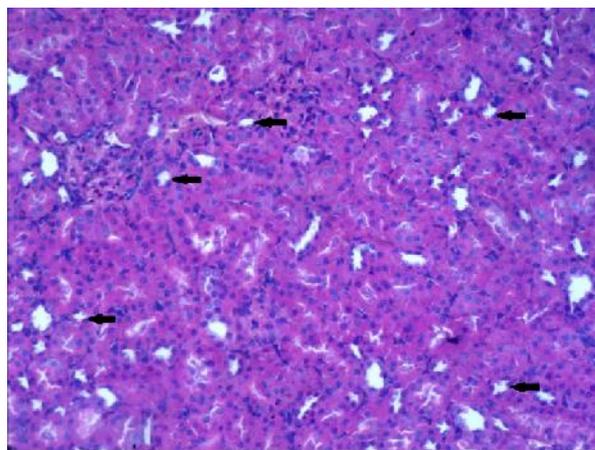
Table (4): One way analysis of variance (ANOVA) with post-hoc test of all studied groups (each 10 rats) as regards of caspase- 3 positivity in renal tissue among all studied groups

	Group I [a] (n=10)	Group II [b] (n=10)	Group III [c] (n=10)	Group IV [d] (n=10)	ANOVA F	P
Renal caspase- 3 positivity	0.30±0.2 [cd]	0.30±0.15 [cd]	2.80±0.4 [abd]	1.30±0.48 [abc]	15.6	0.001*

a-d: significant difference between groups at p<0.05*; n= number. Data are mean± standard deviation.
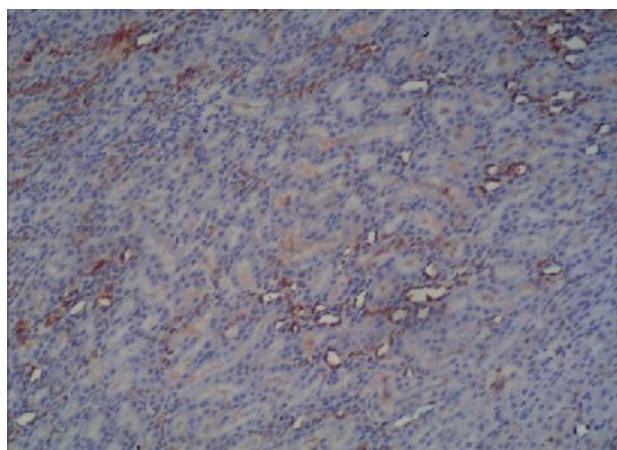
Photomicrograph (1): showing normal renal tubules with wide lumen (arrows) from control group (G I) [H& E x100]
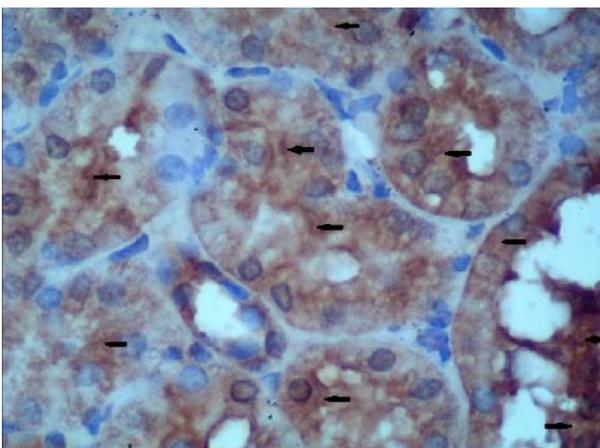

Photomicrograph (2): showing extensive cloudy swelling in renal tubules with swollen eosinophilic granular cytoplasm and narrow lumens (arrows) from atrazine treated group (G III) [H& E x100].
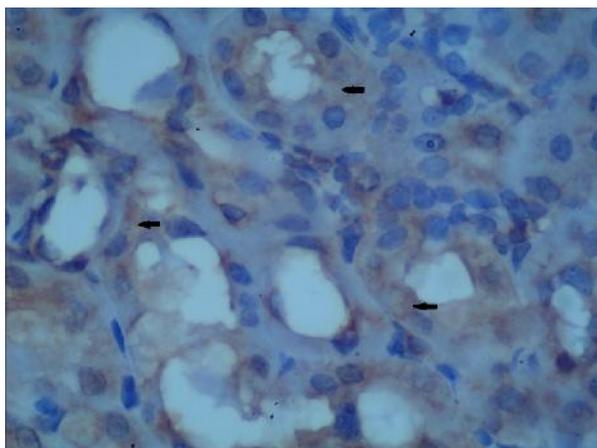

Photomicrograph (3): showing mild cloudy swelling (mild cytoplasmic swelling) from atrazine & curcumin treated group (G IV) [H& Ex100].

Photomicrograph (4): showing negative staining for caspase-3 in the renal tubules from control group (G I) [immunoperoxidase x100]

Photomicrograph (5): showing marked positivity for caspase- 3 in the renal tubules suffering from extensive cloudy swelling (atrazine treated group, GIII) [immunoperoxidase x400].

Photomicrograph (6): showing mild caspase- 3 positivity from atrazine & curcumin treated group (G IV) [immunoperoxidase x400].

Discussion

Atrazine has various health effects for the exposed persons including congestion of the heart, lungs and kidneys, low blood pressure, muscle spasms, weight loss, damage to adrenal glands, cardiovascular damage, retinal degeneration, muscle degeneration, cancer and disruption of the endocrine system function, lipid metabolism and reproduction (Chu, et al., 2003).

The present study was carried out to investigate the possible ameliorative effect of curcumin on ATR induced oxidative stress, oxidative DNA damage, mitochondrial dysfunction, and apoptosis in the kidney of adult male albino rats through biochemical and morphological methods.

The current study showed statistically significant decrease in body and kidney weights in ATR treated rats (group II). This result was in agreement with that obtained by Gluzczak, et al., (2007) who reported that ATR inhibits the activity of the key enzymes of glyconeogenesis such as hexokinase, glycogen synthase, and glucokinase, so decrease glycogen content in the liver with reduction of body weight. These results may be also due to interfering effect of ATR with food absorption mechanism (Wilhelms, et al., 2005).

The present study revealed a significant increase in 24 hours urinary protein, plasma urea and creatinine levels in this study. These results were in a harmony with those of Santa María, et al., (1986) who reported an altered kidney function and an increase in protein content of urine in rats treated with ATR. There was also statistical improvement upon curcumin administration along with ATR in group III. It was observed that the results of the current study went hand in hand with the results of Tapia, et al., (2013) who reported that curcumin may play a key role in the attenuation of proteinuria and progression of renal damage in rats with partial nephrectomy.

The present study revealed that ATR administration caused increased plasma and renal tissue MDA level. These results were similar to those obtained by Jin, et al., (2010) who reported significant increase in MDA level in ATR treated rats compared to control. These results could be explained on the basis that ATR induced oxidant damage is triggered by activation of certain signaling pathways which are involved in development of inflammation and oxidative stress (Seo, et al., 2010).

Findings of this study showed that treatment of rats with ATR caused significant decrease in plasma as well as tissue level of GSH content. Depletion of cellular GSH content below the critical level prevents the conjugation of xenobiotics like ATR to GSH and thus enables them to freely combine covalently with cell proteins causing their damage (Singh, et al., 2010). Reduction in GSH level is an indication that detoxification is going on (Yamano and Morita, 1995).

The increase in CAT activities observed in the present study may be in response to H_2O_2 production as CAT is responsible for the detoxification of H_2O_2 to water and oxygen as reported by Saravanan, et al., (2008).

The current study revealed statistically significant decrease in HO-1 activity in ATR treated group compared to the control group. Decreased activity of HO-1 could be attributed to an insufficient induction of HO-1 in conditions of severe prolonged oxidative stress, where levels of HO-1 are unable to provide adequate cellular antioxidant protection (Peterson, et al., 2009).

In the present study, there was an improvement in oxidant/antioxidant status upon curcumin administration along with ATR in group III. These results were in agreement with Scapagnini, et al., (2002) who defined curcumin as a potent inducer of HO-1 in the protection of tissues against inflammatory and neurodegenerative conditions. Curcumin treatment inhibited the lipid peroxidation process, which results in decreased MDA formation (Belviranlı, et al., 2013; Correa, et al., 2013 and Tokaç, et al., 2013).

This current study revealed that rats treated with ATR had significantly higher plasma and urine 8-OHdG levels, the major product of DNA oxidation, compared to control group. This finding was in accordance with that of Cavas, (2011) who revealed significant increase in the frequencies of micronuclei and DNA strand breaks in erythrocytes of C. auratus, following exposure to commercial formulation of ATR, and thus demonstrated the genotoxic potential of ATR on fish. This could be attributed to the ability of ATR to attack hydroxyl radicals on DNA which results in single or double strand breaks (Dizdaroglu, 1992). Curcumin supplementation in the current study caused significant decrease in plasma and urine 8-OHdG levels, the results were in harmony with Manju, et al., (2013) who reported the genoprotective effect of curcumin.

The main source of ROS in most cells is the mitochondria, where they are formed upon incomplete reduction of oxygen at several sites on the electron transfer chain (ETC). The results of this current study revealed that administration of ATR caused decreased renal tissue complex-I activity increasing its role in mitochondrial dysfunction with more ROS generation with consequently increased apoptosis. This could be explained on the basis that ATR might bind to mitochondrial complexes I and II, resulting in the suppression of mitochondrial oxidative phosphorylation (Cheney, et al., 1997).

The present study revealed that curcumin administration improved renal tissue complex-I activity. This was in agreement with Sood, et al., (2011) who reported that curcumin supplementation was able to normalize significantly the reduced activities of complex I, II and IV, as well as GSH content in rat brain.

Biochemical results were confirmed by morphological changes seen in H& E and caspase-3 stained sections. H& E stained sections of the kidney showed extensive cloudy swelling of the renal tubules in ATR treated animals indicating cell injury. Interference with the antioxidant defense mechanism and ROS generation may induce stress in renal cells to undergo cell injury (Abdelhalim and Jarrar, 2011). These results were in accordance with Xing, et al., (2012) who demonstrated different degrees of cloudy swelling of epithelial cells of renal tubules in the histopathology of the kidney of common carp exposed to ATR. In the current study, there was improvement on administration of curcumin concomitantly with ATR in group III as section of the kidney showed only mild cloudy swelling.

Apoptosis is mediated by a family of cysteine proteases called caspases. Among the group of 11 caspases identified, caspase-3 has been recognized as a central layer in the execution of apoptosis and may provide a target for regulating cell death (Kumar, et al., 2005).

In the current study, immunostaining of kidney sections treated with ATR revealed high caspase-3 expression indicating apoptosis of renal cells. These results were in line with that of Zhang, et al., (2011) who reported a higher expression of active caspase-3 in the spleen of mice after ATR exposure. Additionally, Abarikwu, et al., (2011) reported that ATR mediated nuclear changes associated with apoptosis and increased caspase-3 activity.

The present study revealed mild caspase-3 positivity in group III indicating improvement upon administration of curcumin concomitantly with ATR. Duan, et al., (2013) reported the protective effect of curcumin on contrast-induced nephropathy through reducing renal cell apoptosis via up-regulating HO-1 expression and increasing HO-1 activity in rat.

Conclusion

Based on evidence presented in this study, it can be concluded that exposure to ATR resulted in altered oxidant/ antioxidant status, oxidative DNA damage, mitochondrial dysfunction, and apoptosis of the rat kidney. Administration of curcumin along with ATR resulted in improvement of the toxic effects of ATR thus highlighting the ameliorative action of curcumin, by attenuating oxidative stress, DNA damage, mitochondrial dysfunction and apoptosis.

Recommendation

To the best of our knowledge no previous studies were conducted to show the ameliorative effect of curcumin on ATR nephrotoxicity so further studies are definitely needed to clarify issues related to human exposure to ATR and the ameliorative effect of curcumin in Egypt and elsewhere around the world.

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الملخص العربي

التأثير الملطف المحتمل للكركمين على الإجهاد التأكسدي و تلف الحمض النووي (د ن أ) و اختلال وظائف الميتوكوندريا و موت الخلايا المبرمج في كلى ذكور الجرذان البيضاء البالغة الناجم عن الأترازين

منى محمد أبو النور و أميرة أمين وهدان[1] و محمد مصطفى شريف[2] و نعمة سليمان و ولاء عرفة كشك[3]

المقدمة: يؤدي الأترازين (مبيد الأعشاب التريازيني المنتشر حول العالم) إلى الإجهاد التأكسدي وتلف الحمض النووي (د ن أ) .وقد وجد أن الإجهاد التأكسدي وثيق الارتباط بموت الخلايا المبرمج. للكركمين تأثير قوي كمضاد للأكسدة و مضاد للميكروبات و مضاد للسرطان و مضاد للالتهابات.

الهدف من الدراسة: كان الهدف من هذه الدراسة تقييم التأثير الملطف المحتمل للكركمين على الإجهاد التأكسدي و تلف الحمض النووي (د ن أ) و اختلال وظائف الميتوكوندريا وموت الخلايا المبرمج في كلى ذكور الجرذان البيضاء البالغة الناجم عن استخدام الأترازين.

مواد و طرق البحث: أجريت الدراسة الحالية على ٤٠ من ذكور الجرذان البيضاء البالغة .تم تقسيم الجرذان إلى أربع مجموعات متساوية . المجموعة الاولى استعملت كمجموعة ضابطة. المجموعة الثانية تم إعطاؤها ١٠٠ ميلليجرام/ كجم من الكركمين يوميا. المجموعة الثالثة تم إعطاؤها ٤٠٠ ميلليجرام/ كجم من الأترازين يوميا. المجموعة الرابعة تم إعطاؤها ٤٠٠ ميلليجرام/ كجم من الأترازين مع ١٠٠ ميلليجرام من الكركمين يوميا. وقد تم إعطاء جميع الجرعات عن طريق الفم لمدة ٢١ يوما. ثم تم قياس أوزان الجسم و الكلى و مستويات اليوريا والكرياتينين في الدم وكذلك مستوى البروتين في بول الأربع والعشرين ساعة. كما تم تقييم مستويات المالون داي الديهيد والجلوتاثيون المختزل و ٨ هيدروكسي-2 – ديوكسي جوانوزين وكذلك أنشطة الكاتالاز و الهيم أوكسيجيناز– ١ و الكومبليكس– ١. كما تم عمل فحص هيستوباثولوجي و مناعي لأنسجة الكلى.

النتائج: أظهرت نتائج هذه الدراسة نقصا ذا دلالة إحصائية في أوزان الجسم و الكلى و مستويات الجلوتاثيون المختزل وكذلك أنشطة الهيم أوكسيجيناز– ١ و الكومبليكس– ١ في الجرذان التي تم معالجتها بالأترازين مع زيادة ذات دلالة احصائية في مستويات اليوريا والكرياتينين في الدم و مستوى البروتين في بول الأربع والعشرين ساعة و المالون داي الديهيد و ٨ هيدروكسي-2 – ديوكسي جوانوزين و كذلك نشاط الكاتالاز. مع ملاحظة تضخم غائم في أنسجة الكلى المصبوغة بالهيماتوكسلين والإيوزين. كما كشفت الصبغة المناعية كاسباس–٣ عن ارتفاع معدل موت الخلايا المبرمج. و بإعطاء الكركمين مع الأترازين حدث تحسن ملحوظا في النتائج السابقة

الاستنتاج: نخلص من هذه الدراسة إلى أن استخدام الكركمين مع الأترازين يحسن الإجهاد التأكسدي و تلف الحمض النووي (د ن أ) و اختلال وظائف الميتوكوندريا وموت الخلايا المبرمج في الكلى.

التوصيات: توصي الدراسة الحالية بالمزيد من الدراسات لتوضيح القضايا المتعلقة بالتعرض البشري للأترازين و التأثير الملطف للكركمين في مصر وأماكن أخرى حول العالم.

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