The effect of formaldehyde on the renal cortex of adult male albino rats and possible protective role of vitamin C

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SUMMARY

Most of the people are exposed to formaldehyde. Vitamin C is a strong antioxidant that reduces reactive oxygen and nitrogen species. This study aimed to investigate the changes that occur in the renal cortex of adult male albino rats after formaldehyde administration and the possible protective effects of vitamin C. Forty-five adult male albino rats were divided randomly into three main groups. Group I (control groups) included 3 subgroups: (Ia), (Ib) and (Ic) which received no treatment, distilled water intraperitoneally and vitamin C (100 mg/kg /bwt) for 2 weeks respectively. Group II (formaldehyde group) contained 9 rats, which were injected intraperitoneally with formaldehyde (10 mg/kg /bwt) for 2 weeks. Group III (formaldehyde and vitamin C group) contained 9 rats that were received formaldehyde concomitantly vitamin C by same method mentioned before. At the end of the experiment, all animals were sacrificed. Kidney specimens were dissected out and processed for histopathological and immunohistochemistry examination. The results showed that formaldehyde exposure induced many histological changes in the renal cortex as hypertrophied, segmentation of glomeruli, degenerative changes of the convoluted tubules and dilatation of their lumens. On the other hand, vitamin C improved the state of oxidative stress as evidenced by inducible nitric oxide synthase immunohistochemistry and by Mallory trichrome staining. Statistical morphometric analysis showed that there was a significant difference in the area of tubules and in the height of epithelium of the tubules between control, formaldehyde treated group and formaldehyde and vitamin C treated group.

Key words: Formaldehyde – Kidney – Vitamin C – iNOS – Immunohistochemistry – Morphometry

INTRODUCTION

Formaldehyde (FA) is a monocarbon, colorless, flammable and highly water-soluble aldehyde with sharp odour (Kum et al., 2010). Formaldehyde, which is mostly taken into organisms in an exogenous manner, also exists in the natural structure of the organism. Its intake occurs through the skin, respiratory and the digestive system. Orally, it is digested in fresh water, vegetable, coffee and as protective additives in some drugs. It can be inhaled in cigarette smoke, in car exhaust and in the fumes of the paints of the furniture. The workers in anatomy, histology and pathology are exposed to FA as it is used for fixation (Yang et al., 2007). In the medical field FA is used in long period storage of organ and cadavers, dental coating materials, protective agent in some drugs and in hemodialysis solutions (Usanmaz et al., 2002). Even in infancy, children are exposed to formaldehyde by injection present in diphtheria, polio and tetanus vaccine preparations because of the manufacturing process. In addition, it is used as antiseptic in veterinary drugs and in fungicides (Metz et al., 2004).
and Thaysen-Andersen et al., 2007). Some cosmetics such as hair smoothing products contain FA. Several malignancy-treating drugs are formulated with formaldehyde, which is required for drug activation (Evion, 2007). Formaldehyde is metabolized into formic acid in the liver and erythrocytes then excreted by urine and feces (Cikmaz et al., 2010). Formaldehyde reacts with cellular components as proteins, lipids, and nucleic acids (Cheng et al., 2003). Formaldehyde, even at low concentrations is considered as a mutagenic and carcinogenic agent and it causes toxicity in a variety of organisms (Tang et al., 2009). Many studies reported that FA could induce severe tissue injury by forming reactive oxygen species (Wang et al., 2013).

Nitric oxide (NO) is a free radical molecule referred as reactive nitrogen species (RNS). It is one of the essential regulators of several biological processes as cell signaling, cell communication, cell mediated immunity and cell injury (Moncada et al., 1991). It is produced from arginine by the activity of three different nitric oxide synthase (NOS) isoenzyme: (1) endothelial NOS (eNOS), (2) neuronal NOS (nNOS) and (3) induced NOS (iNOS) (Kaplan, et al., 2012). Small quantities of NO are generated by (nNOS) and (eNOS), while large quantities of NO are formed by (iNOS). The (iNOS) is induced by cytokines and endotoxins. The (iNOS) has an important pathophysiological activity by forming large amount of NO gas in a short duration leading to cytotoxic effect (Mazroa et al., 2009).

Ascorbic acid (vitamin C), a water soluble vitamin is as potent micronutrient that has two major functions as an antioxidant and as an enzyme cofactor (Combs, 2012). It acts as a biological antioxidant that scavenges free radicals and other reactive nitrogen and oxygen species by donating an electron to free radical species there by interrupting the radical chain reaction (Kesinger and Stevens 2009). Thus, even in small amounts; it can defend indispensable molecules in the body, such as proteins, lipids and nucleic acids against oxidative damage (Padayatty et al., 2010).

The aim of this study was to evaluate toxic effects of formaldehyde on renal cortex of albino rat and to investigate the possible protective effects of vitamin C supplementation during formaldehyde exposure by using histological, immunohistochemical and morphometrical study.

MATERIAL AND METHODS

Chemicals
- Formaldehyde (FA): in the form of liquid 37% concentration, was obtained from El Gomhouria Company for Chemical and Medical Trading, Zagazig, Egypt.
- Vitamin C: L-ascorbic acid) in the form of powder was obtained from El Gomhouria Company for Medical Trading and Chemical, Zagazig, Egypt. Fresh preparation of vitamin C by dissolving it in distilled water prior to administration at each time.
- Immunohistochemical kits were obtained from (DAKO life trade Egypt).

Animals
Forty-five adult albino rats, 60-day-old (weighing 270-300g) were obtained from the Laboratory Animals’ Unit at the Faculty of Medicine, Zagazig University. The animals were kept under standard hygienic laboratory conditions at 21±2 °C, fed with balanced diet and water ad-libitum and exposed to 12 h light / 12 h dark cycle for one week prior to the start of the experiments. All rats were handled in accordance to the standard guide for the care and use of laboratory animals (Institute of laboratory animal resources, 1996). These animals were equally divided into three groups

Group I: (Control): It was divided into three subgroups. Each subgroup included 9 rats:
- Subgroup (Ia) negative control: animals received no treatment.
- Subgroup (Ib) positive control: animals received daily dose (1 ml) of distilled water by intraperitoneal injection for 10 days.
- Subgroup (Ic) vitamin C positive control: received a daily dose of L-ascorbic acid (100 mg/kg BW) dissolved in distilled water by gavage for 2 weeks (Adeneye and Ologunju, 2009).

Group II: (FA treated group): It included 9 rats and they were administrated formaldehyde by intraperitoneal injection at dose, (10 mg/kg BW /day) for 2 weeks (Faghani, 2014).

Group III (FA and vitamin C treated group): This group included 9 rats. Animals were treated with formaldehyde (FA) (the same dose as mentioned before) and vitamin C (100mg/kg BW/day) at the same time by gavage for 10 days. Dose selection of FA and vitamin C was based on the published studies by Adeneye and Ologunju (2009) and Faghani (2014).

At the end of the experimental period, the rats were sacrificed and the kidney was rapidly removed and dissected out carefully, and was utilized for histopathological and immunohistochemistry examinations.

Histopathological study
The kidney specimens from each experimental group were fixed in 10% formalin for 48 hours then dehydrated through graded alcohols and embedded in paraffin. Transverse sections of 5 μm were obtained from all specimens, cut and stained with Hematoxylin and eosin and other sections stained with Mallory trichrome and PAS stains (Bancroft and Gamble, 2008). The specimens were examined in the Department of histology, Faculty of Medicine, Zagazig University.

Immunohistochemical study for iNOS
Sections of 5 μm in thickness were prepared from paraffin embedded tissues. After dewaxed in xylol and hydrated in descending grades of alcohol. They were immersed into preheated target retrieval solution to 95°C in water bath for forty minutes, then allowed to cool for twenty minutes. Sections were rinsed with phosphate buffered saline (PBS) for three times. Enough hydrogen peroxide H₂O₂ was applied for 5 minutes, then rinsed with PBS. Then the sections were incubated with polyclonal rabbit anti-iNOS (dilution 1:100) for 2 hours at room temperature. Biotinylated link was added for 10 minutes then sections were rinsed with (PBS). The sections were incubated with streptavidin HRP solution at room temperature for 10-15 min thin sections were rinsed with (PBS). The peroxidase activity was detected using 3,3’-diaminobenzidine (DAB) kit (Dako). DAB was used as a chromogen, which is converted into a brown precipitate. Slides were rinsed in distilled water, immersed in Hematoxylin for half minute then rinsed in tab water until blue. For negative controls slides, the sections were prepared by same method except that they were incubated with antibody diluents instead of the primary antibody. Brown-yellow granules in cytoplasm or nuclei were recognized as positive staining for iNOS (Purcell et al., 1997).

Morphometric study
Morphometric analysis was performed by computerized image analysis system "Image J 1.49v/Java 1.6.0_244 (64-bit)" (National Institutes of Health, USA). Spatial calibration to convert the image pixels into 1 micrometer units was done before each analysis. The following morphometric parameters were measured: area of proximal (PCT) and distal convoluted tubules (DCT) (μm²), glomerular tuft area (GA) (μm²) and the epithelial thickness of proximal and distal convoluted tubule (μm). All measurements were performed in PAS stained slides. 25 randomly selection of glomeruli, proximal and distal tubules in the cortex per animal were examined under high magnification (x400) (Mashhadi et al., 2014; Stojilkovica et al., 2016).

Statistical analysis
Data were statistically evaluated with SPSS, version 18.0 software. The values of parameters were presented as means ± standard deviations. Statistically significant difference was detected by one-way analysis of variance (ANOVA) followed by least significant difference (LSD) test for multiple comparison. P-values of ≤ 0.05 were considered statistically significant, while P-values of <0.01 indicate highly significant results.

RESULTS

I- Histopathological results

1- Hematoxylin and eosin

Examination of Hematoxylin and eosin stained sections of renal cortex of control adult male albino rats showed that no structural differences between the negative control groups and vitamin C treated positive control, thus in the following stains and groups the negative control slides represented the control groups. A normal histology of renal cortex was noted in the control group (Fig. 1a). The glomeruli are formed of a tuft of glomerular capillaries containing normal erythrocytes that surrounded by Bowman’s capsule. Bowman’s capsule is consisting of visceral and parietal layers of squamous epithelium with a lumen in between. The proximal convoluted tubules had brush border and a narrow star shaped lumen lined by pyramidal acidophilic cuboidal epithelium. The distal convoluted tubules were demonstrated with acidophilic cuboidal epi-
thelium that had a cleaner wider lumen and fewer nuclei. In addition, minimal interstitial tissue was present in between renal corpuscles and tubules.

In the FA treated group, there were significant histological changes to the renal cortex. Some glomeruli appeared hypertrophied with mesangial hypercellularity (Fig. 1b), whereas others appeared lobulated with marked congested glomerular capillaries (Fig. 1c). FA was found to cause damage to the architecture of the tubular epithelia and necrotic changes of cells. The convoluted tubules were observed in the form of flat cell with pyknotic nuclei. Some renal convoluted tubules were manifested by abnormal marked dilatation of their lumens that containing epithelial remnants (Fig. 1d). The interstitial tissue, in some perivascular areas, showed marked hemorrhage and infiltration by mononuclear inflammatory cells also interstitial tissue fibrosis was also observed. The capillary blood vessels appeared congested with blood with marked thickening of their wall (Fig. 1e).

In the FA and vitamin C treated group (Fig. 1f), there were marked improvement. Congestion of glomerular capillaries or inside the blood vessels was not seen. Most of the renal tubules were nearly similar to normal. The tubules had normal morphology and acidophilic cytoplasm and PCT had regular brush border. Also inflammatory cells and interstitial hemorrhage or fibrosis in the peritubular areas were not found.

2- The Mallory trichrome

Sections of kidney control group showed few collagen fibers among glomerular capillaries and around renal corpuscles and tubules (Fig. 2a). In formaldehyde-treated group, sections revealed the presence excess blue stained collagen fibers in the interstitium surrounding renal corpuscles and some renal tubules (Fig. 2b) and around blood vessels (Fig. 2c). Kidney sections of rats treated with FA and vitamin C showed few collagen fibers

Fig 2. 2a. Section from a control rat kidney showing: fine collagen fibers confined to Bowman's capsule, around the tubules and intraglomerular. 2b. Section from a FA treated rat kidney showing: excess collagen fibers in the interstitium around Bowman's capsule (arrow) and around tubules (arrowhead). 2c. Section from a FA treated rat kidney showing marked collagen fiber around blood vessels (arrow). 2d. Section from a FA and vitamin C treated rat kidney showing: few collagen fibers in the interstitium around Bowman's capsule, tubules and blood vessels. (Mallory trichrome, x400).

Fig 3. 3a. Section from a control rat kidney showing positive PAS reaction in basal lamina of tubules (arrowhead) and in the brush border of PCT (arrows). 3b. Section from a FA treated rat kidney showing variable PAS reactions, strong PAS positive reaction in the basement membrane of the glomeruli and tubules (arrows) and weak reaction (curved arrows) or absence (arrowhead) in the brush borders of most of PCT. 3c. Section from a FA and vitamin C treated rat kidney showing positive PAS reaction in basal lamina of tubules and in the brush border of PCT (arrowhead). (PAS, x400).
among glomerular capillaries and around renal corpuscles and tubules (Fig. 2d).

3- Periodic acid Shiff (PAS)
Examination of PAS stained sections of renal cortex of control adult male albino rats demonstrated PAS reaction in brush border of PCT and in basement membrane of renal corpuscles and tubules (Fig. 3a). In formaldehyde-treated group, sections stained with PAS showed a strong PAS reaction in basement membrane of the renal corpuscles and tubules and decrease (broken) or even absence (loss) in the reaction of brush border of some tubules (Fig. 3b). Kidney sections of rats treated with FA and vitamin C showed a strong PAS reaction in glomerular and tubular basement membranes and in the renal tubular brush borders (Fig. 3c).

4- Immunohistochemical reaction of iNOS enzyme
The control-group stained sections revealed a negative reaction for iNOS in renal corpuscles and tubules (Fig. 4a). In FA treated (group 2), a positive immune reaction for iNOS was found in renal corpuscles and tubules (Fig. 4b). Meanwhile, stained sections of the FA & vitamin C treated group revealed mild immune reaction for iNOS in renal corpuscles and tubules (Fig. 4c).

II- Morphometric results
Administration of FA at a dose of 10 mg/kg BW for 2 weeks in rats resulted in a significant increase in the mean of PCT area and DCT area when compared with that of control (P<0.05, P <0.001 respectively ) and with FA and vitamin C treated group (P < 0.05). No significant statistical difference (P > 0.05) between control and FA and vitamin C treated group. Regarding GA, the study showed a non-significant statistical difference among the different groups (P > 0.05) (Figs. 5, 6, 7) (Tables 1, 2).

As regard the height of tubules, there was a significant decrease in the height of both the PCT and DCT in FA treated group when compared with that of control (P < 0.001, P < 0.05 respectively ) and with FA and vitamin C treated group (P < 0.05). Meanwhile administration of both FA and vitamin C was not statistically significant when compared
Humans are exposed to FA every day due to its presence in different kinds of medicine and industrial products such as building materials, cosmetics, cigarette smoke, and photochemical smog as well as even various fruits vegetables and seafood that may be illegally preserved with formalin (Tang et al., 2009).

Indeed exposure to FA seemed to be associated with toxicity in many species, including humans, following inhalation, ingestion or injection FA has been documented to be potentially carcinogenic (Metz et al., 2004; Abdulqader and Mustafa, 2014). The present study investigated the effect of FA on the kidney and the possible protective effect of vitamin C.

The results of this study showed that the administration of FA by intraperitoneal injection of (10mg/kg BW) for 2 weeks to adult albino rats led to marked degenerative changes on the renal cortex (when compared to normal control rats) including different forms of glomerular degeneration (hypertrophy, shrinkage or lobulation with marked congested glomerular capillaries). The results of this work are similar to a previous study mentioned by Treesh et al. (2014), who had demonstrated specific pathological changes in renal tissue that was exposed to formaldehyde in the form of hypertrophy, dilation in Bowman’s capsule and congestion in the glomeruli. Cikmaz et al. (2010) also suggested that there is a close relationship between the severity of tissue changes and the amount and
duration of exposure to formaldehyde.

This Study showed that FA induced extensive degree of degeneration of the renal convoluted tubules and architecture of the tubular epithelia such as abnormal marked dilatation of their lumens, flat cell with pyknotic nucli, vacuolar degeneration of their cytoplasm. All these changes indicated cellular necrosis. These finding are in accordace with those of Abdulqader and Mustafa (2014) and Treesh et al. (2014), who gave FA by daily gavage of 1 ml FA and 30 mg/kg BW of FA intraperitoneal injection for 5 days respectively.

In the present work, it was observed that FA produced extensive infiltration of mononuclear inflammatory cells and interstitial hemorrhage in some peritubular and perivascular areas. These findings were compatible with those of Abdulqader and Mustafa (2014), who observed that FA exposure by gavage for 7 days caused high inflammatory degree and interstitial nephritis in rat kidney. However Agarwal and Behari (2007) and Golalipour et al. (2009) argued that there were no evidences of fibrotic changes or inflammatory cell infiltration of interstitial tissue in FA inhalation in 1.5 ppm to rat kidney. This may be due to different route of administration.

Zararsiz et al. (2007) has searched for a relationship between toxic effects of FA on many organs and the production of reactive oxygen species. It was reported that reactive oxygen species (ROS) and lipid peroxidation resulting from formaldehyde exposure disturb or alter antioxidant cellular defenses and induce oxidative damage to cellular components such as DNA, lipids and proteins (Tang et al., 2003; Im et al., 2006; Duong et al., 2011). Oxidant injury is now recognized as playing a key role in the induction of experimental renal diseases (Ailani, 2009).

The other objective of this Study is to evaluate the possible protective effect of vitamin C. Up to date, several studies have examined the protective effects of vitamin C on some organs including the kidney. There is evidence that administration of vitamin C orally produces major protection against kidney damage induced by oxonate injection in rats (Kensara, 2013). Also Derakhshanfar et al. (2013) detected a significant protection of vitamin C in gentamicin produced nephrotoxicity in rat. To our knowledge, no studies are performed on protective effect of vitamin C against FA induced nephrotoxicity.

The present study revealed that administration of vitamin C was effective in production of marked improvement of the kidney of rat. There were disappearance of the glomerular congestion and the reduction of Bowman’s capsule space to almost normal width. Architecture of the tubules was also restored and the cytoplasmic vacuolations were disappeared. There was an improvement in the
brush border of the proximal convoluted tubules. This observation is in agreement with Adeneye and Ologunju (2009), who found that tubulonephritis, vacuolar degeneration and lymphocytic infiltration were reduced after being treated with vitamin C and they suggested that vitamin C can diminish kidney injury induced by acetaminophen.

The studies of Idogun and Ajala (2005) and Odigie et al. (2007) in animals and in human considered that vitamin C as effective antioxidant agent that stabilize the intracellular defense systems and can scavenge strongly free radicals as reactive oxygen species (ROS). The present study revealed that administration of vitamin C was effective in decreasing or even disappearing the toxic effect of FA most likely via free radical scavenging activity.

In the current work, Mallory’s trichrome stained sections of FA-treated group revealed a marked increase in collagen fibers in the glomerular capillaries and also around renal corpuscle and tubules. These results are in concordance with those of Treesh et al. (2014). PAS stained sections of FA-treated group showed decrease or even loss in the PAS reaction in the brush border of the proximal tubular epithelium. (Maeda et al., 2003) suggested that a decrease of PAS is due to a change of carbohydrate composition of the glycocalyx that reported to occur in damaged tubules in many pathological conditions.

Ucmakil et al. (2013) postulated that, no exactly clear information about mechanism of the effect of FA on the expression of iNOS. Considering that this effect occurs by one of the two ways, the first is that FA or its metabolites act like a stimulator that induces iNOS protein synthesis directly. This is supported by Speit (2006), who stated that as FA is a water-soluble molecule, it can easily diffuses into membranes and directly cross reacts with DNA-protein chains. However, Ferrer et al. (2010) pointed out that ROS mediates the induction of iNOS gene expression. The second way may be through cytokines and this theory is supported by Persoz et al. (2010), who reported that FA has a stimulating impact on cytokines, which affects iNOS metabolism. Several studies showed that cytokines such as TNF-α and IL-1β are effective on iNOS mRNA synthesis and iNOS activity or expression (Horie et al., 2009).

The morphometric results of this study showed significant differences of PCT area and DCT area between FA treated and control groups. Also height of both the PCT and DCT revealed a significant decrease in FA treated group. With regard to these findings, morphometric results are adapted with histopathologic findings. Administration of both FA and vitamin C showed a significant improvement in the height and area of the tubules also this findings are in harmony with histopathologic results.

This study proved histologically toxic effect of FA to kidney. Even short duration exposure to FA can cause that damaging effect. As well as this work confirmed that the vitamin C has a protective role in FA toxicity, as it can reverse the tissue damage that occurred in kidney.

Much more attention should be paid for limiting the occupational and environmental exposure to formaldehyde. Special precautions must be taken to limit the level of the environmental, water and food contamination by formaldehyde and many alternatives should be developed to improve the safety profile of formaldehyde. Moreover, highly exposed individuals advised to take vitamin C supplementation to limit the toxic effects of formaldehyde on the kidney.

REFERENCES


EIVISON (2007) Pixantrone can be activated by formaldehyde to generate a potent DNA adduct forming


