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RESEARCH ARTICLE

HISTOPATHOLOGICAL AND DNA DAMAGE EVALUATION OF TRICLOSAN AND THE PROTECTOR ROLE OF VITAMIN E IN THE LIVER OF ALBINO MALE MICE

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ABSTRACT

Triclosan (TCS) is a widely used antimicrobial agent that is used worldwide since 1972. It can be found in various hygiene, medical and consumer products. Due to its widespread use, TCS has increasingly been a public health concern. The present study aimed to assess the histopathological potential and DNA damage induction of TCS treatment in liver tissues in male albino mice and to evaluate the protector role of vitamin E (Vit E) against TCS toxicity. TCS (15 mg/kg) was injected intraperitoneal for 2 consecutive days. Other group was orally administrated with Vit E (50 mg/kg) just before TCS injection. Mice were sacrificed after 24hr from the last treatment. Liver tissues were used for histopathological and molecular evaluations (Comet assay). In the present study, TCS treatment causes damage of liver tissues represented by congested central vein and hepatoportal vessels, mononuclear cells infiltration in portal area and slight hepatic degeneration with local area of leucocytic infiltration. Moreover, the results show a significant increase in tail length, % DNA in tail and tail moment for TCS group in comparison with the negative control group in comet assay. On the other hand, Vit E pretreatment was able to protect liver tissue from TCS toxic effect. In conclusion, the present study reports the toxic potential of TCS treatment and the ameliorative effect of Vit E pre-oral administration as a result of its antioxidant property to scavenge free radicals.

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INTRODUCTION

TCS has been used widely worldwide since 1972 in various hygiene, medical and consumer products (Wang *et al*., 2004; Calafat *et al*., 2008). The wide use of TCS leads to different hazards including allergy, irritation, endocrine disruption, cross resistance to certain antibiotics, ecosystem bioaccumulation, acute and chronic toxicity, carcinogenic and might be represented as a persistent environmental pollution (Halden, 2014). TCS effects might be due to its oxidative stress, reactive oxygen species (ROS) production that leads to cell damage and death. The common mechanism contributing to different tissue damage especially the liver is mainly the oxidative stress and ROS production. Therefore, it is highly recommended to use antioxidant compound to scavenge those ROS (Pradeep *et al*., 2007). Vitamin E, the most potent lipid peroxyl radical scavenger, has profound significance of being a potent antioxidant (Traber and Atkinson, 2007), a neuroprotective (Khanna *et al*., 2005; 2006), atherosclerosis, carcinogenesis and cardiovascular disease protector (Dutta and Dutta, 2003; Shekelle *et al*. 2004; Coulter *et al*., 2006). It also has the property of being a strong anti-inflammatory agent (Reiter *et al*., 2007).Vitamin E has been also reported to has anti-mutagenicpotential against the effects of various drugs, toxic chemicals and metal elements. The present study was aimed to evaluate the histopathological and DNA damage induction of TCS and the protector role of vitamin E in the liver of albino male mice.

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MATERIAL AND METHODS

Animals

Twenty-five albino male mice (*Mus musculus*; 8-10 weeks old; 26-30g body weight) were obtained from the animal house of the King Fahad Center for Medical Research, King Abdul-Aziz University in Jeddah. We have followed the European Community Directive (86/609/EEC) and National Rules on Animal Care.

Tested drugs

1- Triclosan (TCS) (Molekula) was dissolved in corn oil and injected intraperitoneally (i.p.) daily at 15 mg/kg for 2 consecutive days (Yueh *et al*., 2014).

2- Vitamin E (Vit E) (α-tocopherol acetate, Sigma) was dissolved in corn oil and orally was administrated daily for 2 consecutive days at dose level of 50 mg/kg (Haque and Gilani, 2005).

Treatment Schedule

Animals were divided into five groups (five mice per each) and were injected according to body weight for 2 consecutive.

Group 1: Negative control group (untreated mice);
Group 2: Vehicle group, mice were injected i.p. with 50µl corn oil;
Group 3: Vitamin E (Vit E) group, mice were administrated orally with 50µl Vit E in corn oil (50 mg/kg);
Group 4: Triclosan (TCS) group, mice were injected i.p. with 50µl TCS (15 mg/kg);
Group 5: Combined group (Vit E + TCS), mice were orally administrated with 50µl Vit E in corn oil followed by i.p. injection of 50µl TCS (15 mg/kg + 50 mg/kg).

Animals were sacrificed after 24hr from last injection by cervical dislocation liver and tissues were used for further assays.

Histopathological evaluation

Liver sections were done and stained according to Carleton (1967) for the investigation of general histological changes under light microscope at magnification 400X.

Comet assay

The alkaline comet assay was performed as described in detail by Singh *et al*. (1988). The slides were stained by using 80 µl ethidium bromide (20 µg/mL) for 20 mins and viewed under an epifluorescence microscope (Zeiss epifluoresent) with an attached CCD camera. Fifty isolated comets were selected randomly and measured for comet tail length, %DNA in tail and tail moment using COMETSCORE software based on the definition by Olive and Banath (1993). Data were expressed as the mean±standard error (mean±SE). Statistical significances of differences between two groups were determined using Student's t-test, at the level of $p<0.05$ was considered as significance.

RESULTS AND DISCUSSION

Liver sections of negative control, vehicle and vitamin E (50 mg/kg) mice groups showed normal hepatic lobular architecture with hepatocytes (H) arranged in thin plates. The portal tracts were within normal limits and contained arteries, veins and bile ducts with no necrosis and inflammatory cells were observed. The hepatocytes contained rounded, regular nuclei with lymphocytes scattered between hepatocytes and the sinusoids (Figure 1A, 1B and 1C, respectively). Triclosan (15 mg/kg) treatment for 48hr leads to damage of liver tissue of mice TCS group. In which Figures 2A-C show different damaged shapes, congested central vein and hepatoportal vessels with slight mononuclear cells infiltrationin portal area. Moreover, liver tissue of TCS shows slight hepatic degeneration with local area of leucocytic infiltration. Pre-oral administration of vitamin E for TCS+Vit E group reduces the damage effect occurred as a result of TCS treatment. Results of Figure 3A showed normal hepatic lobular architecture with hepatocytes arranged in thin plates with central vein more or less as the liver tissue of negative control group. However, there is slight damage in other sections as congested central or portal vein (Figure 3B). This was also reported by Yueh *et al*. (2014), they confirmed that TCS administration causes chronic liver damage and apoptosis in mice. They believed that with the regenerative capacity of liver tissue, the surviving hepatocytes undergo compensatory proliferation and fibrogenesis. In addition, their tumorigenesis study demonstrated that TCS markedly increased animals' susceptibility to tumorigenesis, supporting the belief that fibrosis, when advanced, is a risk factor for developing hepatocellular carcinoma (HCC) (Bataller *et al*., 2005; Inokuchi *et al*., 2010). The most common and relevant mechanisms that are recognized in hepatic fibrogenesis are the production of ROS (Paik *et al*., 2014) and recruitment of inflammatory cells (Nieto *et al*., 2002). In our previous study (Hussien *et al*., 2016), we reported ROS induction and elevation of pro-inflammatory cytokines (TNF-α and IL-6) in the liver tissue of TCS-treated mice. Tsukamoto *et al*. (1995) demonstrated that ROS levels were positively associated with the release of TNF-α and IL-6 in Kupffer cells.

These results coincide with increased expression of TNF-α and IL-6 in the livers of TCS treated mice, suggesting that activation of inflammatory cells may represent a major source of oxidative stress-related molecules that subsequently mediate cytotoxic responses and profibrogenic effects. On the other hand, Vit E pre-oral administration reduces liver damage caused by TCS injection but not the same as negative control group. These results were in agreement with Hamadouche *et al*. (2013), they reported that administration of Vit E to lead acetate treated rats restored the testicular tissue damage. The main effect of Vit E in reducing liver tissue damage returned to its antioxidant property in scavenging free radicals in turn decrease ROS occurred as a result of TCS treatment.

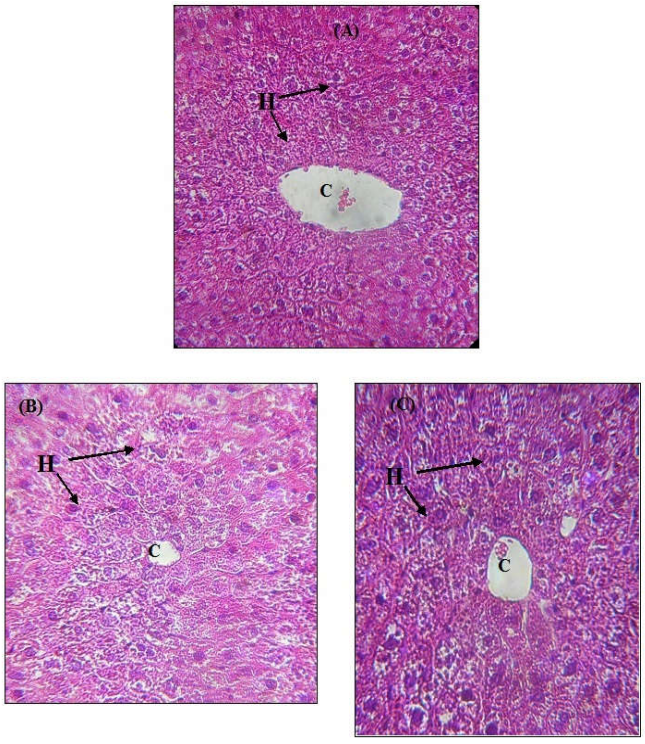

Figure 1. Photomicrographs of mice liver section of negative control group (A), vehicle group (B) and vitamin E group (Vit E). In which: C, central vein; H, hepatocytes. H&E(X400)

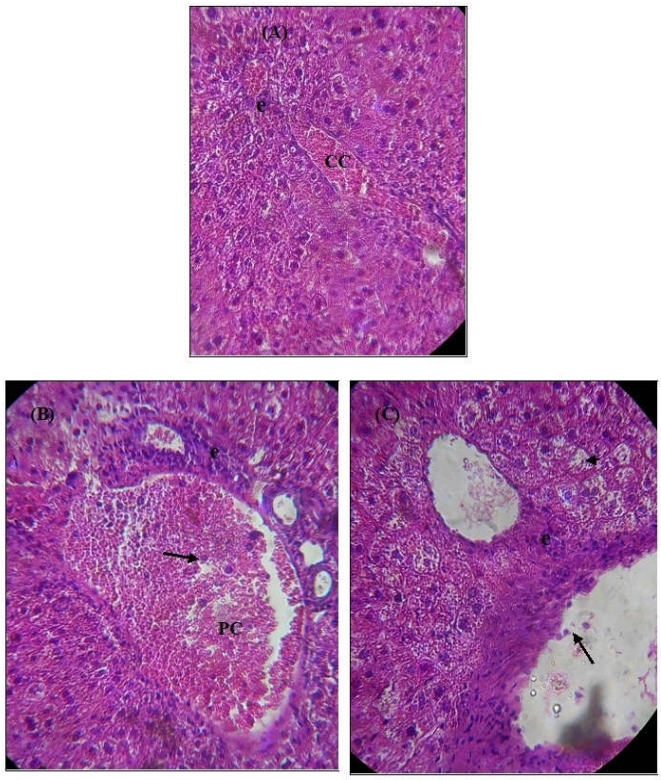

Figure 2. Photomicrographs of mice liver section of triclosan group (TCS) (A-C) showing central vein (CC) and portal vessel (PC) congestion, local aggregation of eosinophils (e), slight eosinophilic infiltration in the portal area (arrow) and slight hepatocytes degradation (arrow head). H&E(X400)

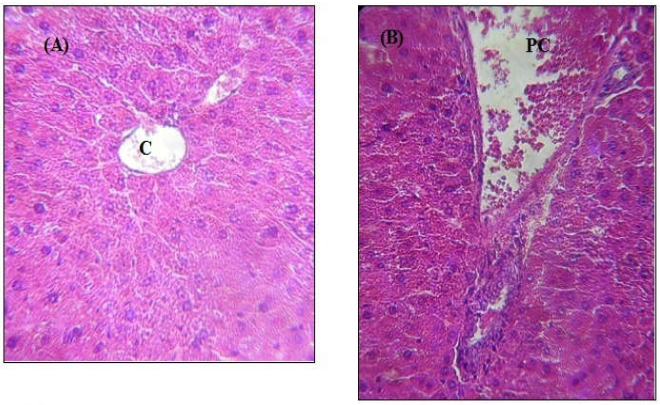

Figure 3. Photomicrographs of mice liver section of vitamin E+triclosan group (Vit E+TCS) (A&B). In which: C, central vein; PC, portal vessel congestion. H&E (X400)

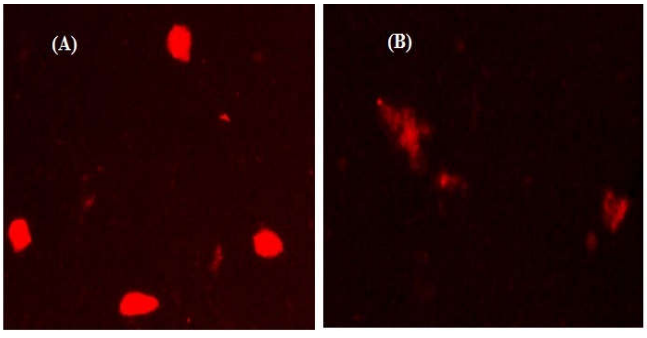

Figure 4. Representative photomicrograph showing (A): typical nuclei of undamaged cells of negative control group and (B): DNA damage observed as comets that were seen in triclosan (TCS) group

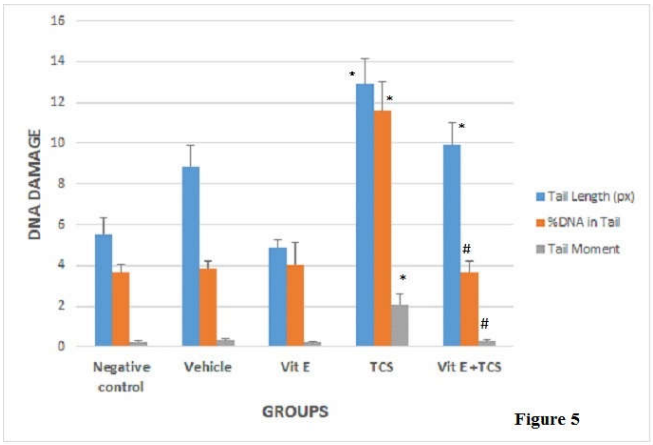

Figure 5. Effect of triclosan (TCS) on the DNA (DNA damage were represented by Comet assay) in mice liver cells. Significant difference (P < 0.05) using Student's t-test, in which: *Statistically compared with negative control group; # Statistically compared Vit E+TCS group with TCS group

The genotoxic potential of triclosan was evaluated by using comet assay, in which Figure 4(A) shows typical nuclei of undamaged cells for negative control group; while Figure 4(B) is a representative photomicrograph for TCS group showing DNA damage observed as comets. The results show a significant increase in tail length, % DNA in tail and tail moment for TCS group in comparison with the negative control group as shown in Figure 5. However, Vit E pre-oral administration to TCS group shows remarkable decrease in tail length but still significant in comparison to negative control group. In addition, Vit E+TCS group shows a significant reduction in %DNA in tail and tail moment in comparison to TCS group. These results were in agreement with Lin *et al*. (2010); Sethuraman and Ramesh Kumar (2014), in which they reported the genotoxic effect of TCS using comet assay on earthworms (different concentrations) coelomocytes and zebra fish (sub-lethal concentrations) blood erythrocyte, respectively. Their results report that TCS exposure significantly increased tail DNA damage at different sampling time. Moreover, Ciniglia *et al*. (2005) reported a significant genotoxic effects of TCS on *C. ehrenbergii* at 0.25 mg L^{-1}, and that higher concentrations irreversibly altered the DNA strands. In addition, Jirasripongpun *et al*. (2008) assessed the genotoxicity of TCS using comet and apoptosis assays on KB and Vero cell lines using the 50% inhibition concentration (IC50, 0.034 and 0.036 mM respectively) and the maximum concentration of TCS in personal care products (0.023 mM). They reported that, the number of comet cells increased as the concentration and exposure time to TCS increased in both cell lines. Moreover, genetic damage was accrued from the exposure to TCS at concentrations in the IC20-30range, following a 5 days exposure period. This DNA damage exposed to TCS might have been due to oxidative stress and the consequent production of reactive oxygen species (ROS) leading to cell damage and death. However, the results show the protective effect of Vit E pre-oral administration to TCS group by decreasing DNA damage in comparison to TCS group as a result of its antioxidant property to scavenge free radicals induces by TCS treatment.

Conflict of Interest

The authors have declared no conflict of interest.

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