RESEARCH ARTICLE

Oral administration of hesperidin, a citrus flavonone, in rats counteracts the oxidative stress, the inflammatory cytokine production, and the hepatotoxicity induced by the ingestion of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)

Recep Bentli[1], Osman Ciftci[2], Asli Cetin[3], Merve Unlu[2], Nese Basak[2], Mahmut Çay[4]

[1] University of Inonu, Faculty of Medicine, Dept. of Internal Medicine, 44280, Malatya, Turkey
[2] University of Inonu, Faculty of Pharmacy, Dept. of Pharmaceutical Toxicology, 44280, Malatya, Turkey
[3] University of Inonu, Faculty Medicine, Dept. of Histology, 44280, Malatya, Turkey
[4] University of Inonu, Faculty of Medicine, Dept. of Anatomy, 44280, Malatya, Turkey

Correspondence: O Ciftci, Assoc. Prof., University of Inonu, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, 44 280, Malatya, Turkey
<osmciftci@gmail.com>
<ociftci@inonu.edu.tr>

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ABSTRACT. The objective of the current study was to investigate the protective effects of hesperidin against oxidative stress, altered cytokines levels and histological changes in rats induced by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Rats were divided randomly into four equal groups (Control, TCDD, hesperidin and TCDD+hesperidin). TCDD and hesperidin were given by gavage, dissolved in corn oil at doses of 2 μ/kg/week and 50 mg/kg/day respectively. The blood and tissue samples were taken from all rats on the 60th day, to be analyzed for the determination of oxidative stress, histological changes and cytokine levels. The results indicated that hesperidin prevented oxidative damage caused by TCDD via decrease lipid peroxidation and increased antioxidant defense systems. It also reversed the histological damage induced by TCDD. Although, TCDD led to a significant increase in TNF-α and IL-1β levels, hesperidin treatment was able to normalize these values in rats. In conclusion, it was shown that TCDD caused adverse effects as regards cytokine levels, histological alterations and oxidative stress in rats. However, hesperidin treatment mitigated these toxic effects. These results suggest that hesperidin could play a protective role against TCDD toxicity.

Key words: TCDD, hesperidin, cytokine, oxidative stress, histological alterations

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2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is a ubiquitous, environmental contaminant originating from certain chemical process (e.g. plastics manufacturing, paper processing, metal industries) [1, 2]. TCDD causes a variety of toxic effects including carcinogenesis, wasting syndrome, immunotoxicity, hepatotoxicity, reproductive toxicity, teratogenicity and endocrine changes in humans and in experimental animals [2-4]. The relative potency of TCDD is related to its binding affinity to the aryl hydrocarbon receptor (AhR) [5]. On the other hand, one of the important mechanisms of TCDD toxicity is oxidative stress [2, 6]. It has been shown that TCDD enhances lipid peroxidation, decreases glutathione (GSH) content and hepatic membrane fluidity, and disturbs the antioxidant enzyme balance in the liver [2, 5, 6]. In addition, our previous study [7] demonstrated that TCDD caused oxidative damage via an imbalance between oxidant and antioxidant status. Previous studies [8, 9] have shown that another prominent aspect of TCDD toxicity is immunotoxicity with altered cytokine levels. Ciftci and Ozdemir [4] showed that TCDD increased inflammatory and pro-inflammatory cytokine levels in rats. Also, Kim *et al*. [10] demonstrated that adipose cells were targets of persistent organic pollutants such as TCDD and suggested that inflammation (or inflammatory cytokine level disturbance) is one of the main toxicity pathways. The histological and immunological damage induced by TCDD may be associated with oxidative stress. Thus, antioxidant therapy can be beneficial against these adverse effects.

Hesperidin (HP), a bioflavonoid, plays a critical role in plant defense and is found abundantly in *Citrus* species such as lemon and orange. Previous studies [11, 12] have demonstrated that HP exhibits many pharmacological activities including antioxidant, anticarcinogenic, antihypertensive and anti-inflammatory activities. Pradeep *et al*. [13] indicated that oral administration of HP was found to offer protection against gamma irradiation-induced hepatocellular damage and oxidative stress in rats. It is therefore believed that HP is a powerful radical scavenger that promotes cellular antioxidant defense systems

[14, 15]. Thus, HP can prevent the oxidative stress and other adverse effects caused by toxic agents such as TCDD that lead to oxidative damage.

Therefore, the present study focused on determination of the effects of TCDD on oxidative (TBARS, SOD, CAT, GSH), histological damage in liver tissue, and serum cytokine (TNF-α, IL-1β) level alterations in rats. The possible protective effects of HP against TCDD toxicity were also examined.

MATERIALS AND METHODS

Chemicals

2,3,7,8-TCDD (purity >99%) was obtained from Accustandart, Inc. (New Haven, CT, USA). All other chemicals, including HP were purchased from Sigma Chemical Co. (St. Louis, MO, USA) and were of analytical grade or of the highest grade available.

Animals and Treatment

A total of 28 healthy, adult male, Wistar Albino rats (aged 3-4 months), obtained from the Experimental Animal Institute Malatya-Turkey, were used for this experiment. Diet and drinking water were provided *ad libitum*. Experiments were performed on the basis of the animal ethics guidelines of the Institutional Animals Ethics Committee.

Rats were randomly divided into four equal groups (n = 7 in each group). Group 1 (Control), served as control and was given corn oil by gavage. In group 2 (TCDD), TCDD was diluted in corn oil and administered orally at a dose of 2 μg/kg/week, by gavage. Rats in group 3 (HP) were treated with HP suspended in corn oil, by gavage, at a dose of 50 mg/kg/day. In group 4, rats were treated with TCDD and HP (TCDD+HP) at the same time. The animals were sacrificed 60 days after the treatment, under ether anesthesia. For biochemical analysis, livers were immediately dissected and blood samples were collected from the left ventricle. Serum was obtained after whole blood centrifugation (3,000 g, 20 minutes, at 4°C). Tissue and serum samples were stored at -45°C in a deep freeze until analysis.

Cytokines

Cytokine production was determined by enzyme-linked immunosorbent assay (ELISA) using commercial kits according to the manufacturer's instructions. TNF-α levels were measured using anti-rat ELISA kits from Invitrogen Corporation (542 Flynn Road, Camarillo, CA 93012, USA, Cat No: KRC3011), and IL-1β levels were measured using anti-rat ELISA kits from eBioscience (San Diego, CA 92121, USA, Cat no: BMS630). The plates were read at 450 nm using the CA-2000 ELISA microplate reader (CIOM Medical Co., Ltd., China). Cytokine levels in the samples were calculated from standard curves of recombinant cytokines using a linear regression method.

Biochemical assay

The homogenization of tissue was described in our previous study [7]. The levels of thiobarbituric acid reactive substances (TBARS), total glutathione (GSH) levels, and catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities were determined by spectrophotometric methods; these methods have been described in our previous study [7].

Histological analysis

For light microscopic evaluation, liver samples were fixed in 10% formalin and embedded in paraffin blocks. Paraffin-embedded liver tissue was cut into 5 μm thick sections, mounted on slides and stained with Hematoxylin-Eosin. Tissue samples were examined using a Leica DFC280 light microscope and a Leica Q Win Image Analysis system (Leica Micros Imaging Solutions Ltd., Cambridge, UK).

We examined sections for eosinophilic-stained and pyknotic nuclei cells (necrotic cells), congestion, cell infiltration and intracytoplasmic vacuolization. Microscopic damage was identified as absent (0), slight (1), moderate (2) and severe (3) for each criterion.

Statistical analysis

Results were expressed as mean ± SEM. Cytokine, TBARS, SOD, CAT and GSH levels were compared between treatments using one-way analysis of variance (ANOVA) and the *post-hoc* Tukey's test. The histological results were compared by Kruskal-Wallis variance analysis. Where differences among the groups were detected, group means were compared using the Mann-Whitney U test. All analyses were carried out by SPSS statistical program ver. 18.0 (SPSS Corporation Inc., Chigago). Differences were considered as significant when p values were less than 0.05.

RESULTS

Immunological and biochemical results

Serum TNF-α and IL-1β levels are given in *figures 1* and *2*. Our results showed that TNF-α and IL-1β levels were significantly increased by TCDD compared to the control and other experimental groups. It was found that HP treatment reversed the increases in TNF-α and IL-1β levels when given together with TCDD, and decreased cytokine values compared with the group given TCDD only .

The liver SOD, CAT, GSH and TBARS levels are given in *table 1*. It was shown that TBARS levels were sig-

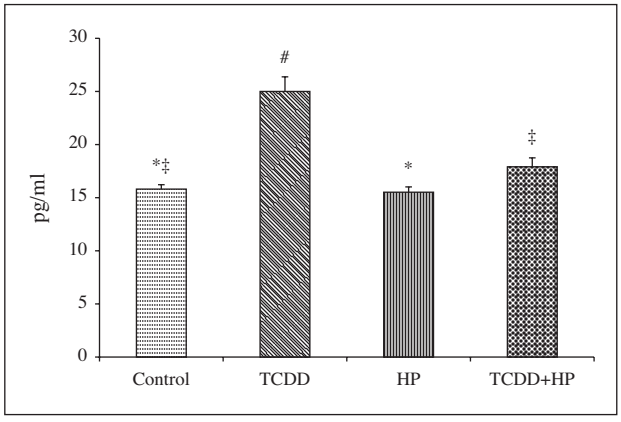

Figure 1
Serum TNF-α levels in rats (pg/ml ± SEM).
*, #, ‡ showed significant (P<0.05) differences between groups.

Table 1
The levels of SOD, CAT, GSH and TBARS in rat liver tissue.

	TBARS (nmol/g tissue)	Reduced GSH nmol/ml	CAT kU/mg protein	SOD (U/mg protein)
Control	7.16 ± 0.43^{ac}	207.5 ± 12.5^{a}	1.55 ± 0.089^{a}	26.59 ± 2.10^{a}
TCDD	13.24 ± 0.86^{b}	64.8 ± 2.23^{b}	0.56 ± 0.031^{b}	14.30 ± 0.73^{b}
HP	6.63 ± 0.40^{a}	206.7 ± 21.9^{a}	1.68 ± 0.152^{a}	32.36 ± 1.61^{c}
TCDD+HP	8.43 ± 0.44^{c}	122.7 ± 17.3^{c}	0.90 ± 0.052^{c}	24.97 ± 2.09^{a}

Means bearing different superscripts within same column were significantly different ($P<0.05$).

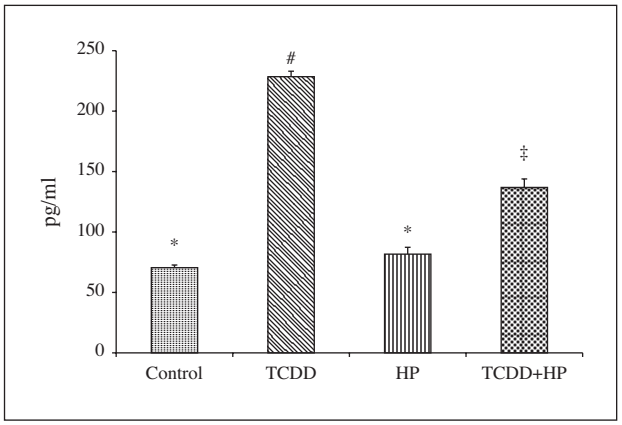

Figure 2
Serum IL-1β levels in rats (pg/ml ± SEM).
*, #, ‡ showed significant (P<0.05) differences between groups.

nificantly increased whereas GSH levels and SOD, CAT activity were significantly reduced in liver tissue treated with TCDD compared to other groups. Although there were no significant changes between the control and HP groups concerning TBARS, SOD and GSH levels, CAT activity was significantly induced by HP treatment. In the TCDD+HP group, the increased TBARS levels were decreased, and SOD and CAT activities, and GSH levels were increased compared with the TCDD group. These substances, when given together with TCDD, brought SOD, CAT, GSH and TBARS levels closer to the control level.

Histological results

The microscopic damage score for each group was determined, and results are given in *table 2*. The livers of control (*figure 3A*) and HP (*figure 3B*) groups, showed the normal histological appearance. In the control group, microscopic examination showed liver parenchyma consisting of normal hepatocytes. Sinusoids and central vein were also clearly visible. The livers in the TCDD-treated group however, revealed severe histological alterations such as eosinophilicstained and pycnotic nuclei cells (*figure 4A*), congestion (*figures 4B-C*), intracytoplasmic vacuolization (*figures 4B-D*) and cell infiltration (*figure 4A*). Normal radial arrangements of hepatocytes from the central vein were disrupted. We also saw these effects in the TCDD+ HP group, but their severity was markedly decreased. We found eosinophilic-stained and pyknotic nuclei cells (*figure 5A*), but these cells were not as widespread as in the TCDD group. Histopathological findings were not as severe as in the TCDD group. We saw that cell infiltration (*figure 5A*), congestion and vacuolization (*figure 5B*) were decreased in the TCDD+ HP group.

DISCUSSION

The current study examined the potential toxic effects of TCDD on rat liver and the beneficial effects of HP against TCDD toxicity. To this end, we investigated liver oxidative stress status, involving the histological examination of liver, and measurement of serum TNF-α and IL-1β levels. Our results clearly indicate that TCDD induced oxidation and histological damage, and altered cytokine levels. Whereas HP treatment combined with TCDD mitigated these changes, and protected the rat liver against TCDD toxicity.

Oxidative stress is a condition where there is an imbalance between free radicals (such as TBARS) and the antioxidant defense system (activity of SOD, CAT and GSH levels), which leads to lipid peroxidation and inactivation of many enzymes. Some investigators [5, 16] have demonstrated that the liver is one of the major target organs for the toxic effects of TCDD. The present study clearly showed that exposure to TCDD induced significant oxidative damage by an increase the formation of TBARS and a decreases in GSH levels and antioxidant enzyme activity including SOD and CAT in liver tissue. Previous studies [17, 18] were confirmed by our findings and showed that TCDD treatment caused oxidative stress in certain tissues, such as the liver of laboratory animals. Our previous study [7] demonstrated that in similar doses, TCDD led to lipid peroxidation and destruction of antioxidant defense mechanisms in the liver. Similarly, Hassoun *et al*. [16] demonstrated that TCDD and its congeners significantly induce oxidative stress in hepatic and brain tissue at a dose range of 10-100 ng/kg/per day. In that investigation, the dose of TCDD used was lower than that used in our study; however, their results supported ours. In addition, previous studies [18, 19] have indicated that exposure to TCDD during development suppresses antioxidant enzymes in rat liver. On the other hand, HP treatment reduced increased TBARS levels and induced the antioxidant defense system (increased SOD, CAT enzyme activity and GSH levels), when given together with TCDD. Some studies [20, 21] have demonstrated that HP had strong antioxidant potency and caused a decrease in lipid peroxidation. Investigators [21, 22], including ourselves, have revealed that HP treatment protects liver tissue against many toxic agents, such as cadmium and dimethylbenzanthracene that induce oxidative stress. In this context, our study as well as those of others, [23, 24] suggested that the oxidative effect of TCDD may cause the generation of ROS using various mechanisms including binding AhR and a decrease in membrane fluidity. Alsharif *et al*. [24] showed that the decreased membrane fluidity and increased membrane damage may contribute to the toxic manifestations of TCDD as a con-

Table 2
Comparison of the effect of hesperidin on histological parameter changes caused by TCDD.

	Necrotic cells	Congestion	Cell infiltration	Vacuolization
Control	0.14 ± 0.42^{a}	0.20 ± 0.48^{a}	0.39 ± 0.49^{a}	0.43 ± 1.49^{a}
TCDD	2.80 ± 0.48^{b}	2.43 ± 0.60^{b}	2.09 ± 0.65^{b}	2.41 ± 0.83^{b}
HP	0.67 ± 0.78^{c}	0.31 ± 0.56^{a}	0.56 ± 0.63^{a}	0.99 ± 0.92^{c}
TCDD+HP	1.63 ± 0.68^{d}	1.43 ± 0.60^{c}	1.77 ± 0.73^{c}	1.81 ± 0.85^{d}

Means bearing different superscripts within same column were significantly different ($P<0.05$).

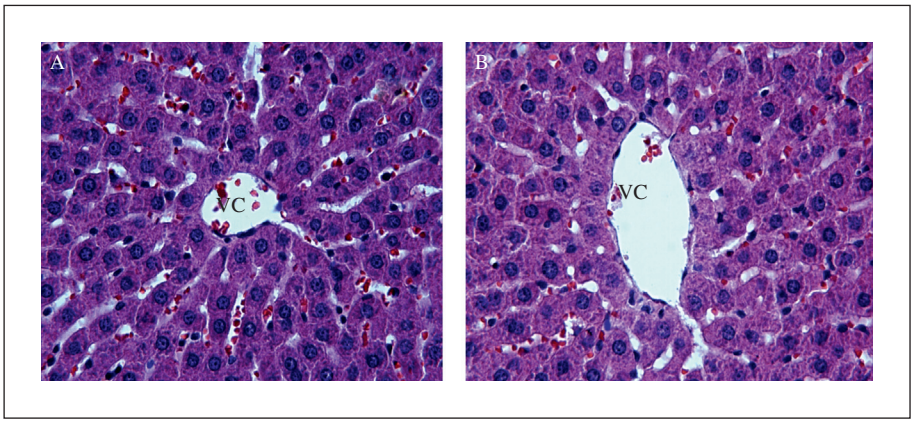

Figure 3
In control (A) and HP (B) groups, the liver showed normal histological appearence. VC: Vena centralis. H-X40.

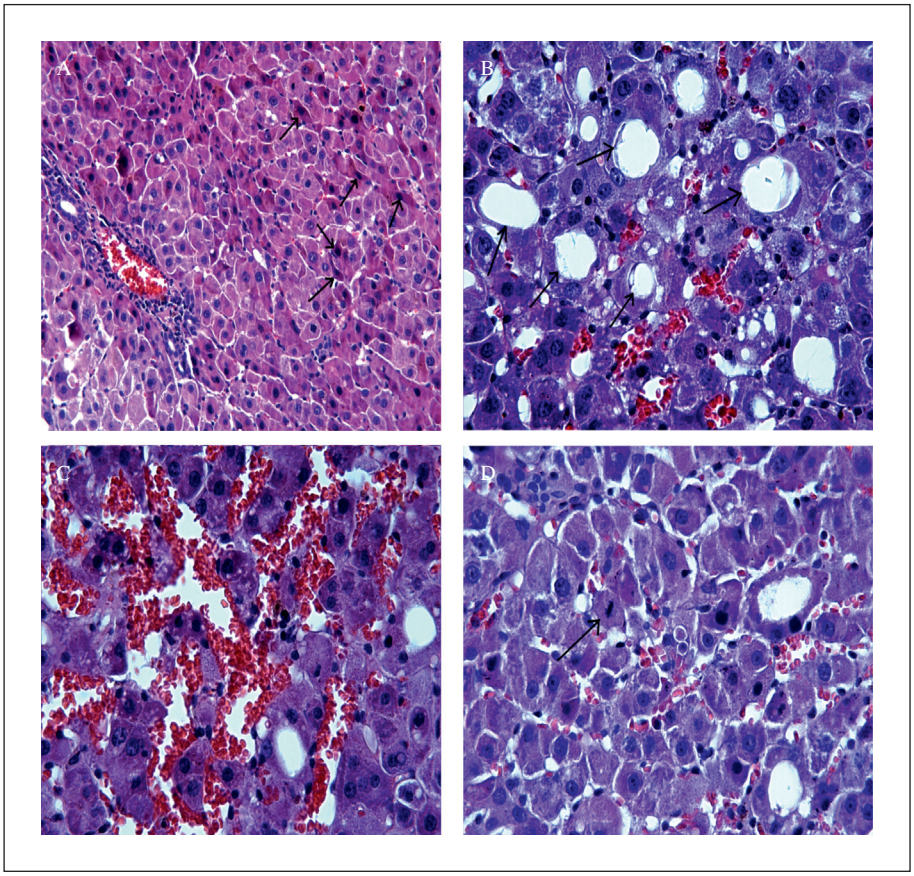

Figure 4
In TCDD group: (A) Eosinophilic stained and pyknotic nuclei cells, congestion and cell infiltration H-EX20; (B) Intracytoplasmic vacuolization (arrows) and congestion H-EX40; (C) Congestion and intracytoplasmic vacuolization H-EX40; (D) Intracytoplasmic vacuolization and mitosis in hepatocytes (arrow) H-EX40.

sequence of oxidative stress. However, HP prevents the oxidative effects of TCDD and these protective effects are mainly due to its antioxidant properties such as being a scavenger of free radicals or an inhibitor of bio-molecule oxidation. Also, Yang *et al.* [25] have claimed that HP suppressed the AhR receptors, thus the beneficial effects of HP against TCDD toxicity may be partially accounted for by the suppression of these AhR receptors.

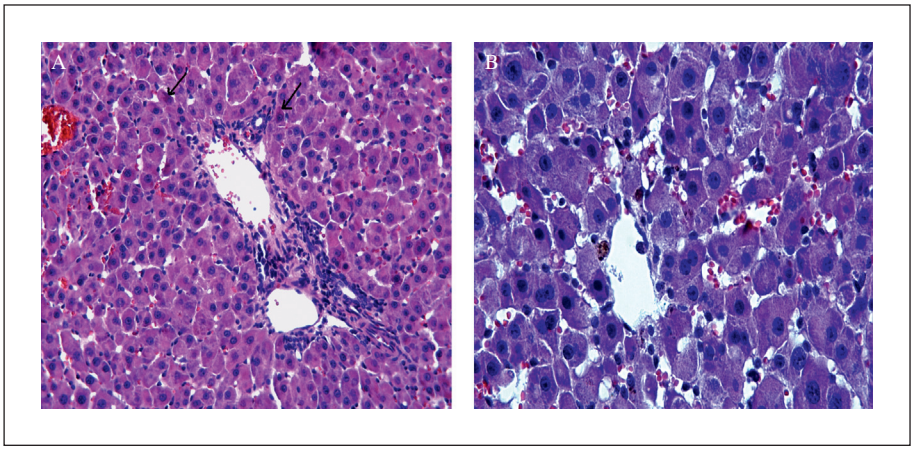

Figure 5
In TCDD+ HP group: (A) Eosinophilic stained and pyknotic nuclei cells (arrows) and cell infiltration H-EX20; Intracytoplasmic vacuolization and congestion H-EX40.

The present study demonstrated that TCDD treatment caused severe histological damage (eosinophilic-stained and pyknotic nuclei cells, congestion, intracytoplasmic vacuolization and cell infiltration) in liver tissues of rats. These results are in agreement with some previous studies [26, 27]. On the other hand, HP treatment together with TCDD prevented these adverse effects of TCDD and protected liver tissue against TCDD toxicity. As far as we are aware there have been no studies involving the effects of HP on the liver, but it is known that similar antioxidant agents such as curcumin, myrcen and cineol, protect liver tissue against many toxins [4]. These results confirmed our findings. It was thought that the liver damage was as a result of oxidative stress and there was a correlation between oxidative and histological damage results. Therefore, the beneficial effects of HP on the liver as regards oxidative stress might be the result of its antioxidant properties and its binding to AhR receptors.

TNF-α, also known as cachectin, is a cytokine involved in systemic inflammation and is a member of a group of cytokines that stimulate the acute phase reaction. The primary role of TNF is in the regulation of immune cells [28]. IL-1β, also known as catabolin, is produced by activated macrophages as a proprotein. This cytokine is an important mediator of the inflammatory response, and is involved in a variety of cellular activities, including cell proliferation, differentiation, and apoptosis [28]. In the present study, it was determined that TCDD treatment significantly increased serum TNF-α and IL-1β levels, and led to negative effects on the immune system and cytokine levels, as found in our previous studies [3, 4]. However, HP treatment, together with TCDD, significantly reduced the adverse immunological effects of TCDD via decreased serum cytokine levels. Similarly, Fan *et al*. [29] showed that different doses (1, 3, 10, 30 and 90 μg/kg) of TCDD caused transient increases in levels of TNF-α and IL-1β mRNA in rat, and confirmed our results. In addition, previous studies, which confirm our study, showed that flavonoids such as HP inhibit the expression of proinflammatory genes in response to inflammatory mediators such as TNF-α and IL-1β [30, 31]. Therefore, it was investigated as an anti-asthmatic agent in rat and mouse and was found to be effective in allergic asthma models in rats and mice [31, 32]. It was claimed that these effects of HP were due to its suppression of the AhR receptors. It was also determined that [25] Q binds directly AhR as a natural ligand. In this context, the immunoprotective effects of HP in TCDD toxicity may occur because Q and TCDD bind AhR competitively. Additionally, it was suggested that the production of ROS occurrs in inflammatory cytokine-stimulated cells (such as TNF-α and IL-1β), with TCDD treatment and ROS being mediators of TNF-α-induced cell injury [33, 34]. Therefore, it is thought that the protective effects of HP against the lipid peroxidation and histological damage induced by TCDD may be due to the fact that it decreases levels of inflammatory cytokines.

CONCLUSION

The current study revealed the hepatotoxic and immunotoxic effects of TCDD by determination of oxidative stress in liver tissue, histological alterations in liver tissue and serum cytokine changes in rats. The use of HP, in combination with TCDD, minimized its toxicity as revealed by the decreasing TBARS levels, reduced histological changes in tissue, lowered serum TNF-α and IL-1β levels , and increased antioxidant enzyme activity (SOD, CAT), and GSH levels. The beneficial effects of HP against TCDD-induced hepatotoxic and immunotoxic damage may be due to its antioxidant properties and its affinity for binding to ArH receptor competitively. Therefore, we suggest that HP, a citrus flavonoid, can prevent and protect against TCDD toxicity in terms of liver damage and cytokine levels.

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REFERENCES

1. Ciftci O. The Investigation of Effect Mechanism, Chemical Structure and Toxicokinetics Properties of Dioxins Compounds. *J Turgut Ozal Med Cent* 2010; 17: 413-22.

2. Hassoun EA, Vodhanel J, Abushaban A. The modulatory effects of ellagic acid and vitamin E succinate on TCDD-induced oxidative stress in different brain regions of rats after subchronic exposure. *J Biochem Mol Toxicol* 2004; 18: 96-203.

3. Ciftci O, Tanyıldızı S, Godekmerdan A. Protective effect of curcumin on immune system and body weight gain on rats intoxicated with 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD). *Immunopharmacol Immunotoxicol* 2010; 32: 99-104.

4. Ciftci O, Ozdemir I. Protective effects of quercetin and chrysin against 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) induced oxidative stress, body wasting and altered cytokine productions in rats. *Immunopharmacol Immunotoxicol* 2011; 33: 504-8.

5. Alsharif NZ, Hassoun EA. Protective effects of vitamin A and vitamin E succinate against 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)-induced body wasting, hepatomegaly, thymic atrophy, production of reactive oxygen species and DNA damage in C57BL/6J mice. *Basic Clin Pharmacol Toxicol* 2004; 95: 131-8.

6. Slezak BP, Hatch GE, Devito MJ, Diliberto JJ, Slade R, Crissman K, Hassoun E, Bırnbaum LS. Oxidative stress in female B6C3F1 mice following acute and subchronic exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). *Toxicol Sci* 2000; 54: 390-8.

7. Ciftci O, Vardi N, Ozdemir I. Effects of quercetin and chrysin on 2,3,7,8-tetrachlorodibenzo-p-dioxin induced hepatotoxicity in rats. *Environ Toxicol* 2011; May 4. doi: 10.1002/tox.20707. [Epub ahead of print]

8. Kerkvliet NI. Recent advances in understanding the mechanisms of TCDD immunotoxicity. *Int Immunopharmaco* 2002; 2: 277-91.

9. Prell RA, Dearstyne E, Steppan LG, Anthony T, Vella AT, Kerkvliet NI. CTL. Hyporesponsiveness Induced by 2,3,7,8-Tetrachlorodibenzo-p-dioxin: Role of Cytokines and Apoptosis. *Toxicol App Pharmacol* 2000; 166: 214-21.

10. Kim MJ, Pelloux V, Guyot E, Tordjman J, Bui LC, Chevallier A, Forest C, Benelli C, Clément K, Barouki R. Inflammatory pathway genes belong to major targets of persistent organic pollutants in adipose cells. *Environ Health Perspect* 2012; 120: 508-14.

11. Choi EJ, Ahn WS. Neuroprotective effects of chronic hesperetin administration in mice. *Arch Pharm Res* 2008; 31: 1457-62.

12. Menze ET, Tadros MG, Abdel-Tawab AM, Khalifa AE. Potential neuroprotective effects of Hesperidin on 3-nitropropionic acid-induced neurotoxicity in rats. *Neurotoxicology* 2012; 33: 1265-75.

13. Pradeep K, Park SH, Ko KC. Hesperidin a flavanoglycone protects against gamma-irradiation induced hepatocellular damage and oxidative stress in Sprague-Dawley rats. *Eur J Pharmacol* 2008; 587: 273-80.

14. Shagirtha K, Pari L. Hesperetin, a citrus flavonone, protects potentially cadmium induced oxidative testicular dysfunction in rats. *Ecotoxicol Environ Saf* 2011; 74: 2105-11.

15. Youdim KA, Dobbie MS, Kuhnle G, Proteggente AR, Abbott NJ, Rice-Evans C. Interaction between flavonoids and the blood–brain barrier: in vitro studies. *J Neurochem* 2003; 85: 180-92.

16. Hassoun EA, Li F, Abushaman A, Stohs SJ. The relative abilities of TCDD and its congeners to induce oxidative stress in the hepatic and brain tissues of rats after subchronic exposure. *Toxicology* 2000; 145: 103-13.

17. Turkez H, Geyikoglu F, Yousef MI. Beneficial effect of astaxanthin on 2,3,7,8-tetrachlorodibenzo-p-dioxin-induced liver injury in rats. *Toxicol Ind Health* 2012 Feb 6. [Epub ahead of print] DOI: 10.1177/0748233711434959

18. Turkez H, Geyikoglu F, Yousef MI, Togar B, Vancelik S. Propolis alleviates 2,3,7,8-Tetrachlorodibenzo-p-dioxin-induced histological changes, oxidative stress and DNA damage in rat liver. *Toxicol Ind Health* 2012 Mar 14. [Epub ahead of print] doi: 10.1177/0748233712440139

19. Kern PA, Fishman RB, Song W, Brown AD, Fonseca V. The effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on oxidative enzymes in adipocytes and liver. *Toxicology* 2002; 171: 117-25.

20. González-Molina E, Domínguez-Perles R, Moreno DA, García-Viguera C. Natural bioactive compounds of Citrus limon for food and health. *J Pharm Biomed Anal* 2010; 51: 327-45.

21. Pari L, Shagirtha K. Hesperetin protects against oxidative stress related hepatic dysfunction by cadmium in rats. *Exp Toxicol Pathol* 2012; 64: 513-20.

22. Nandakumar N, Balasubramanian MP. Hesperidin protects renal and hepatic tissues against free radical-mediated oxidative stress during DMBA-induced experimental breast cancer. *J Environ Pathol Toxicol Oncol* 2011; 30: 283-300.

23. Mimura J, Fujii-Kuriyama Y. Functional role of AhR in the expression of toxic effects by TCDD. *Biochim Biophys Acta* 2003; 1619: 263-8.

24. Alsharif NZ, Grandjean CJ, Murray WJ, Stohs SJ. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD)-induced decrease in the fluidity of rat liver membranes. *Xenobiotica* 1990; 20: 979-88.

25. Yang YL, Hsu HT, Wang KH, Wang CS, Chen CM, Ko WC. Hesperidin-3′-o-methylether is more potent than hesperidin in phosphodiesterase inhibition and suppression of ovalbumin-induced airway hyperresponsiveness. *Evid Based Complement Alternat Med* 2012; 2012: 908562.

26. Czepiel J, Biesiada G, Gajda M, Szczepański W, Szypuła K, Dabrowski Z, Mach T. The effect of TCDD dioxin on the rat liver in biochemical and histological assessment. *Folia Biol (Krakow)* 2010; 58: 85-90.

27. Patterson RM, Stachlewitz R, Germolec D. Induction of apoptosis by 2,3,7,8-tetrachlorodibenzo-p-dioxin following endotoxin exposure. *Toxicol Appl Pharmacol* 2003; 190: 120-34.

28. Kalliolias GD, Gordon RA, Ivashkiv LB. Suppression of TNF-{alpha} and IL-1 Signaling Identifies a Mechanism of Homeostatic Regulation of Macrophages by IL-27. *J Immunol* 2010; 185: 7047-56.

29. Fan F, Yan B, Wood G, Viluksela M, Rozman KK. Cytokines (IL-1beta and TNFalpha) in relation to biochemical and immunological effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in rats. *Toxicology* 1997; 15;116: 9-16.

30. Park HH, Lee S, Son HY, Park SB, Kim MS, Choi EJ, Singh TS, Ha JH, Lee MG, Kim JE, Hyun MC, Kwon TK, Kim YH, Kim SH. Flavonoids inhibit histamine release and expression of proinflammatory cytokines in mast cells. *Arch Pharm Res* 2008; 31: 1303-11.

31. Wei D, Ci X, Chu X, Wei M, Hua S, Deng X. Hesperidin suppresses ovalbumin-induced airway inflammation in a mouse allergic asthma model. *Inflammation* 2012; 35: 114-21.

32. Kim SH, Kim BK, Lee YC. Antiasthmatic effects of hesperidin, a potential Th2 cytokine antagonist, in a mouse model of allergic asthma. *Mediators Inflamm* 2011; 2011: 485402.

33. Rier SE, Coe CL, Lemieux AM, Martin DC, Morris R, Lucier GW, Clark GC. Increased tumor necrosis factor-alpha production by peripheral blood leukocytes from TCDD-exposed rhesus monkeys. *Toxicol Sci* 2001; 60: 327-37.

34. Kern PA, Fishman RB, Song W, Brown AD, Fonseca V. The effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on oxidative enzymes in adipocytes and liver. *Toxicology* 2002; 171: 117-25.