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)

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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 μg/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

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. 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.

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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, anti-hypertensive 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.
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 Malaty-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 given together with TCDD, and decreased cytokine values were shown.[7] The pos-

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 Microsystems Imaging Solutions Ltd., Cambridge, UK).

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 and GSH and TBARS levels are given in table 1. It was shown that TBARS levels were sig-
**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 in the formation of TBARS and a decrease 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.

<table>
<thead>
<tr>
<th></th>
<th>Necrotic cells</th>
<th>Congestion</th>
<th>Cell infiltration</th>
<th>Vacuolization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.14 ± 0.42a</td>
<td>0.20 ± 0.48a</td>
<td>0.39 ± 0.49a</td>
<td>0.43 ± 1.49a</td>
</tr>
<tr>
<td>TCDD</td>
<td>2.80 ± 0.48b</td>
<td>2.43 ± 0.60b</td>
<td>2.09 ± 0.65b</td>
<td>2.41 ± 0.83b</td>
</tr>
<tr>
<td>HP</td>
<td>0.67 ± 0.78c</td>
<td>0.31 ± 0.56a</td>
<td>0.56 ± 0.63a</td>
<td>0.99 ± 0.92c</td>
</tr>
<tr>
<td>TCDD+HP</td>
<td>1.63 ± 0.68d</td>
<td>1.43 ± 0.60c</td>
<td>1.77 ± 0.73c</td>
<td>1.81 ± 0.85d</td>
</tr>
</tbody>
</table>

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

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

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.
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 cachectin, 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 occurs 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 AhR 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.


REFERENCES


