TÜBİTAK

Original Article

Turk J Med Sci
2012; 42 (Sup.1): 1207-1213

E-mail: medsci@tubitak.gov.tr
doi:10.3906/sag-1112-17

Histopathological effects of the food contaminant furan on some endocrine glands of prepubertal male rats

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Aim: To evaluate the toxicity of furan on the thyroid gland, adrenal gland, and pancreas of prepubertal male rats.

Materials and methods: Furan was administered orally to 3–4-week-old male rats at doses of 2, 4, and 8 mg kg^{-1} day^{-1} by dissolving in corn oil for 90 days. The rats were sacrificed by cervical dislocation at the end of the experiment. Their endocrine glands including the thyroid gland, adrenal gland, and pancreas were removed and weighed. Tissues were examined morphologically and histopathologically under a light microscope.

Results: Organ and relative organ weights of rats did not change with furan administration. Histopathological changes were observed such as congestion in the pancreas and mononuclear cell infiltration, hyperplasia, and fibrosis in the adrenal gland.

Conclusion: Furan administration caused histopathological changes in a dose-dependent manner in prepubertal male rats.

Key words: Adrenal gland, furan, histopathology, pancreas, toxicology

Introduction

After the discovery of the heat-induced food contaminant acrylamide in carbohydrate-rich foods, a wide range of heat-induced food contaminants such as furan began to attract attention (1). Furan (C_4H_4O) is the main compound of many chemicals used in some industrial sectors. This chemical is a volatile, colorless liquid with a low boiling point (2,3). Furan and its derivatives occur naturally in many kinds of foods that undergo heat treatment such as canned and jarred foods including baby foods and infant formula, and beverages such as coffee at the highest level (4). Furan is formed by different routes: thermal degradation of carbohydrates only or with some amino acids (this reaction is called the Maillard reaction), thermal degradation of certain amino acids or ascorbic acid and related compounds, and thermal degradation of polyunsaturated fatty acids and carotenoids (5,6). It has been reported that ascorbic acid has the highest potential for furan production (7,8).

Furan is classified as carcinogenic in rodents such as mice and rats and "possibly carcinogenic to humans" by the IARC (9). It is metabolized by CYP2E1 to cis-2-butene-1,4-dialdehyde, which is a cytotoxic metabolite and binds to proteins and nucleosides irreversibly (10,11). The toxicity of furan is attributed to its cytotoxic metabolite cis-2-butene-1,4-dialdehyde, which induces cell proliferation and uncoupling of mitochondrial oxidative phosphorilation (12,13). In the NTP's 2-year study, B6C3F1 mice were orally administrated furan at doses of 8 and 15 mg kg^{-1} day^{-1} and F-344 rats at doses of 2, 4, and 8 mg kg^{-1} day^{-1} in both males and females. In that study, hepatocellular adenomas, carcinomas, and benign pheochromocytomas of the

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adrenal gland increased significantly (3). It has been reported that furan caused a significant increase in micronucleated cells in the splenocytes of mice that were administrated 2, 4, 8, and 15 mg kg^{-1} day^{-1} furan for 4 days (14). It has also been reported that furan caused some histopathological changes in the liver and kidney of male rats, changes in serum enzyme levels, and also dose-dependent increases in liver TNF-α levels at doses of 2, 4, and 8 mg kg^{-1} day^{-1} (15).

Toxicological research is needed because industrialized countries are suffering from the total burden of disease, attributed to environmental factors (16). Various kinds of chemicals are known to possibly influence the endocrine systems of mammalian animals, including the thyroid gland, adrenal glands, pancreas, hypothalamus, hypophysis, and reproductive organs (17). It has been reported by the EPA that children, representing the sub-population, may be affected by environmental contaminants rather than adults, since children are more exposed to chemicals and may be more vulnerable to the toxic effects of xenobiotics (18).

In this respect, the purpose of the present study was to evaluate the prospective effects of furan on endocrine glands such as the thyroid gland, adrenal gland, and pancreas of rats in the prepubertal period.

Materials and methods

Furan (purity 99.0%) was obtained from Fluka (Newport News, VA, USA). This study received approval from the Ethics Committee of Hacettepe University. The study was performed on 3–4-week-old male Wistar rats obtained from the Experimental Animals Laboratory of Hacettepe University. All animals were acclimated to our laboratory conditions for 1 week before the beginning of the experiments. The animals were kept at a constant 12 h light/12 h dark cycle under controlled conditions (20.8 ± 1.6 °C, 50.5 ± 7% humidity). The rats were randomly divided into 5 groups with 8 animals in each: control, vehicle control, and 3 experimental groups. The animals in the experimental groups were administrated furan (dissolved in corn oil) orally at doses of 2, 4, and 8 mg kg^{-1} day^{-1}. The body weights of rats in all groups were recorded weekly and daily food/water intake was recorded during the experiment. After 90 days of administration, the rats were sacrificed.

Histopathological examination

The thyroid gland, adrenal gland, and pancreas of each rat were removed, observed grossly, and weighed, consecutively. Relative organ weights (organ weight/body weight) were calculated. Tissues were fixed in Bouin's fixative, dehydrated in alcohol, and embedded in paraffin blocks. Slides were prepared at 5 μm thickness and stained with 2 different histological stains for light microscopic examination: hematoxylin & eosin and Heidenhain's azocarmine-aniline blue staining. Photographs of the histopathological findings were taken (Olympus BX51, Japan).

Statistical analysis

The data were analyzed using SPSS by analysis of variance (ANOVA) and incidences of histopathological findings were analyzed by Fisher's exact test. The statistical significance was assigned at the $P \leq 0.05$ level.

Results

The organ weights and relative organ weights of the endocrine glands were not changed by furan administration. The organ and relative organ weights of rats in the control and furan administration groups are shown in Table 1. There were no exposure-related histopathological changes in the thyroid gland of furan administered rats. However, some histopathological changes were observed in the pancreas and adrenal gland in rats in the furan administrated groups. The Langerhans islets of the pancreas of a rat in the control group are shown in Figure 1a and 2a. Vascular congestion in the Langerhans islets of the pancreas was observed in furan administered rats (Figure 1b, 2b). Additionally, acinar cell necrosis was observed in the exocrine part of the pancreas (Figure 2c). The adrenal gland of a rat in the control group is shown in Figure 3a and 4a. Mononuclear cell infiltration was observed in the adrenal gland of rats in the 4 and 8 mg kg^{-1} day^{-1} furan administration groups (Figure 3b). In addition, nodular hyperplasia (Figure 4b) and fibrosis in the cortex of the adrenal gland were observed in the 2 and 8 mg kg^{-1} day^{-1} furan treated rats (Figure 3c). The incidences of histopathological changes in the control and furan treated groups are shown in Table

Table 1. Organ and relative organ weights of rats in the control and furan administration groups.

	Control	Oil Control	Furan mg kg^{-1} day^{-1}		
			2	4	8
Final body weights	320.37 ± 14.32	311.87 ± 9.90	324.62 ± 11.63	311.00 ± 5.48	322.50 ± 8.25
Adrenal gland					
Absolute (g)	0.03 ± 0.004	0.03 ± 0.003	0.03 ± 0.003	0.03 ± 0.001	0.04 ± 0.001
Relative (×10^{-3})	0.10 ± 0.01	0.10 ± 0.01	0.10 ± 0.01	0.10 ± 0.01	0.10 ± 0.02
Pancreas					
Absolute (g)	0.57 ± 0.04	0.74 ± 0.10	0.59 ± 0.05	0.69 ± 0.06	0.55 ± 0.05
Relative (×10^{-3})	2.00 ± 0.11	2.00 ± 0.27	2.00 ± 0.18	2.00 ± 0.21	2.00 ± 0.09

Data were expressed as mean ± standard error.

2. Vascular congestion incidence in the Langerhans islets of the pancreas in the furan administered rats was increased significantly (P = 0.026).

Discussion

Homeostasis is the ability of an organism to maintain a stable internal environment and this requires constant monitoring and adjustments as conditions change. This system of the body is called homeostatic regulation. Homeostasis should be in dynamic equilibrium since the internal and external conditions of the body are not stable. It is a way to understand the biology and physiology of animals by utilizing their endocrine system to regulate the process (19).

The endocrine system is an important system of the body that plays an essential and pervasive role in the regulation of metabolic processes such as maintenance of homeostasis, regulation of growth maturation, reactions to exterior stimulations (stress, infection, etc.), and regulation of reproduction (20). Various factors (environmental factors, chemicals, physiological factors, etc.) are known to predispose the endocrine glands to toxicity. Disorders of endocrine glands result in disease, the effects of which may proceed to many organs and functions (21). The present study was designed to evaluate the possible effects of a heat-induced food contaminant (furan) on the endocrine glands of prepubertal rats. It is known that organ and relative organ weights are crucial benchmarks for toxicological studies (22,23). Organ and relative organ weights of furan administered rats did not change significantly when compared with control groups.

As regards the histopathological examination of endocrine glands, we did not observe any changes in

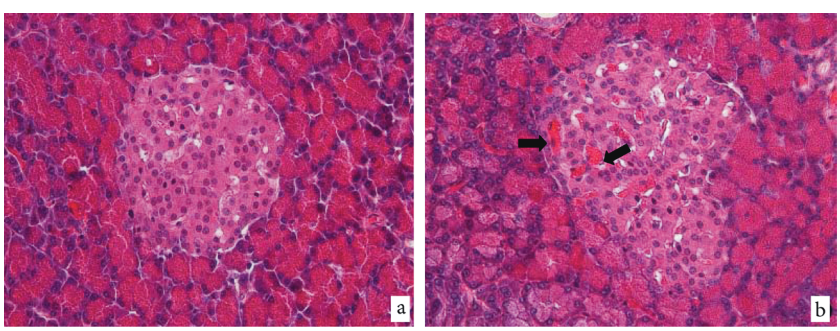

Figure 1. Hematoxylin & eosin staining, ×200, **a)** Islet of Langerhans in pancreas of rat in control group, **b)** Islet of Langerhans in pancreas of rat in 4 mg kg^{-1} day^{-1} furan administration group, vascular congestion (→).

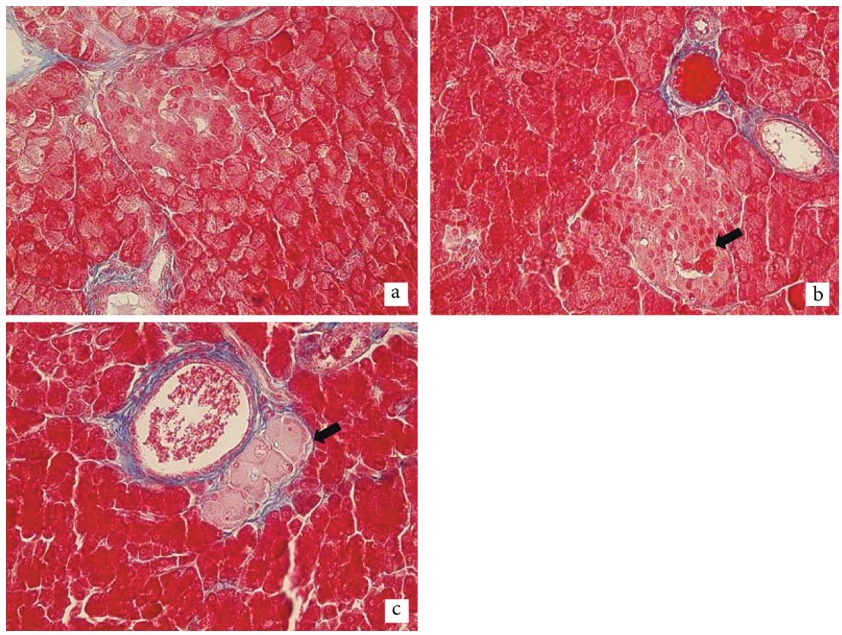

Figure 2. Heidenhain's azocarmine-aniline blue staining, ×200, **a)** Islet of Langerhans in pancreas of rat in control group, **b)** Islet of Langerhans in pancreas of rat in 8 mg kg^{-1} day^{-1} furan administration group, vascular congestion (→). Exocrine pancreas, **c)** Acinar cell necrosis (→) in 8 mg kg^{-1} day^{-1} furan administration group.

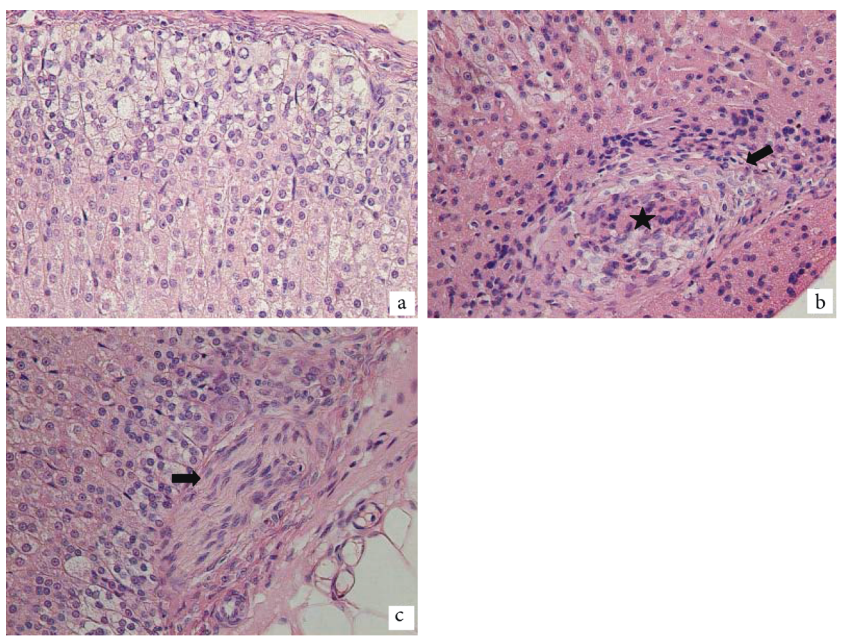

Figure 3. Hematoxylin & eosin staining, ×200, **a)** Adrenal cortex of rat in control group, **b)** Adrenal cortex of rat in 4 mg kg^{-1} day^{-1} furan treatment group, mononuclear cell infiltration (→) and nodular hyperplasia (*), **c)** Adrenal cortex of rat in 2 mg kg^{-1} day^{-1} furan administration group, fibrosis (→).

Table 2. Incidence of histopathological changes in rats in the control and furan administration groups.

	Control	Oil Control	Furan mg kg^{-1} day^{-1}		
			2	4	8
Adrenal gland					
Mononuclear cell infiltration	0 / 8	0 / 8	0 / 8	3 / 8	1 / 8
Nodular hyperplasia	0 / 8	0 / 8	2 / 8	0 / 8	2 / 8
Fibrosis	0 / 8	0 / 8	3 / 8	0 / 8	3 / 8
Pancreas					
congestion in Langerhans islet	0 / 8	0 / 8	1 / 8	5 / 8[a,b]	5 / 8[a,b]

Data were expressed as a number of affected / number of examined rats.
[a] Significantly different from control group
[b] Significantly different from oil control group
[c] Significantly different from 2 mg kg^{-1} day^{-1} furan administration group

the thyroid glands of rats in the furan administration groups. It is known that the thyroid gland plays a crucial role in the synthesis, storage, and secretion of thyroid hormone (24,25). Thyroxine (T4) and triiodothyronine (T3) are thyroid hormones that are produced in the thyroid gland and they play an essential role in the regulation of body temperature, in protein synthesis, and in energy production and regulation. Thyroid hormones are known to affect directly many organs in the body. Some problems may occur in other parts of the body in the presence of some changes in these hormones and also the thyroid gland (26). In this case we may suggest that furan did not cause any disturbance in thyroid gland histology. In a study in which furan was administered to male and female mice at doses of 30 and 60 mg kg^{-1} for 13 weeks, no histopathological changes were observed in the thyroid gland (3). This result is consistent with the NTP study. However, in a 2-year NTP study it was reported that furan caused adenoma, C-cell adenoma, C-cell carcinoma, and follicular cell carcinoma in the thyroid gland of rats in furan administration groups at doses of 2, 4, and 8 mg kg^{-1} (3). The differences between these results may be attributed to exposure time, exposure doses, and species of animals.

Congestion in the islets of Langerhans was observed in the pancreas of rats in the furan administration groups. The islet of Langerhans is an important unit that controls the metabolism such as glucose levels by secreting the polypeptide hormones insulin and glucagon (27). The endocrine cells of the pancreas secrete hormones to the extracellular

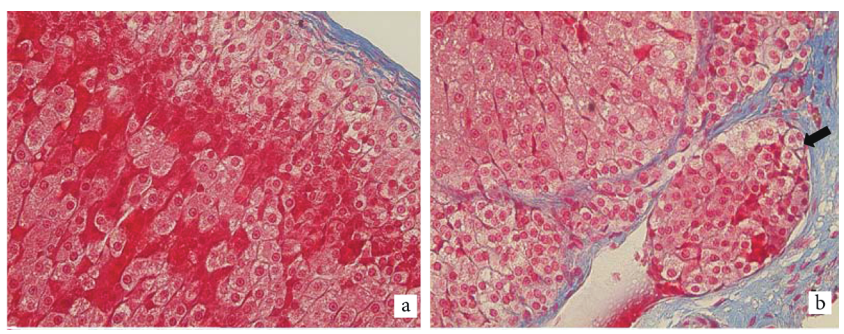

Figure 4. Heidenhain's azocarmine-aniline blue staining, ×200, **a)** Adrenal cortex of rat in control group, **b)** Adrenal cortex of rat in 2 mg kg^{-1} day^{-1} furan administration group, nodular hyperplasia (→).

space and then they are carried to the blood and sites of action. It is known that islets of Langerhans receive 20% of pancreatic flow although it represents only 1%–2% of the total pancreatic volume (27). Congestion may be due to an increase in blood flow to the islets of Langerhans in the furan administration groups and this may explain the increasing function of β-cells in the pancreas. However, it is not known if furan caused changes in pancreatic hormone levels.

The adrenal gland consists of 2 parts, an outer cortex and an inner medulla, which both secrete hormones essential for life. Catecholamines are secreted under the control of the sympathetic nervous system by the adrenal medulla (28) and steroid hormones are produced by the adrenal cortex. Failure of adrenal function may result in some disorders involving the electrolyte and carbohydrate metabolism (29). In this study, furan administration caused some histopathological changes in the cortex of the adrenal gland such as mononuclear cell infiltration, fibrosis, and hyperplasia. Adrenal injury may influence the hormonal activity of the adrenal cortex such as changes in serum corticosterone levels. In the NTP study in which both sexes of mice were administered 2, 4, and 8 mg kg^{-1} day^{-1} furan for 2 years, it was reported that furan caused increased benign pheochromocytomas of the adrenal gland and this was evidence of the carcinogenic activity of furan (3). In the present study the effects of furan were not so serious, and so the administration time may be an important factor affecting the results of toxic effects. It has been reported that furan caused decreases in testosterone levels in a dose-dependent manner and also histopathological changes were observed in the reproductive organs of male rats (30).

According to this subchronic toxicological study, we may suggest that furan has some slight effects on male rat endocrine glands. However, this study needs further supportive experiments such as hormone level detection and molecular analysis for evaluating the mechanisms underlying toxicity caused by furan.

Acknowledgment

This research was partially supported by the Scientific and Technological Research Council of Turkey (TÜBİTAK 107T122 project).

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