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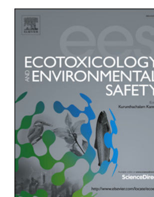

Differential susceptibility of kidneys and livers to proliferative processes and transcriptional level of the genes encoding desmin, vimentin, connexin 43, and nestin in rats exposed to furan

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ABSTRACT

In this study, we aimed to assess the differential toxic impact, induced by furan exposure, on the liver and kidney tissues by estimating reactive oxygen species (ROS) level, total antioxidant capacity (TAC), oxidative damage, and the tissue injury markers in a male rat model. To explain such impacts, 20 rats were assigned into two groups: a control group, where rats were administered corn oil as a vehicle, and a furan-administered group, where furan was orally administered to rats at a dose of 16 mg/kg b wt/day (five days per week over eight weeks). The transcriptional levels of intermediate filament proteins (desmin, vimentin, nestin, and connexin 43) were assessed by using quantitative real-time polymerase chain reaction (PCR), and the cell proliferation markers (proliferating cell nuclear antigen [PCNA] and proliferation-associated nuclear antigen [Ki–67]) were recognized by immunohistochemical analysis. Furthermore, the ultrastructural changes of liver and kidney were monitored using electron microscopy. Our findings showed that furan exposure could induce hepatic and renal damage to different extents. Furan can increase the ROS content, oxidative damage indices, and liver tissue injury indices but not kidney injury indices. Furthermore, it decreases the TAC in the serum of exposed rats. In addition, furan exposure was associated with changes in the mRNA expression pattern of intermediate filament proteins in both kidney and liver tissues. Moreover, furan enhances the expression of PCNA and Ki–67 in the liver tissues but not in the kidney tissues. The ultrastructure evaluation revealed the incidence of glomerular podocyte degeneration and hepatocyte injury. These results conclusively demonstrate that the deleterious effects of furan are caused by promoting fibrosis and hepatocyte proliferation in liver tissues and triggering podocyte injury in the kidney tissues.

1. Introduction

Food processing including thermal and non-thermal treatments involves various reactions that generate several undesirable contaminants, which can be converted to more dangerous metabolites under the enzymatic action (Høie et al., 2015). For example, the thermal degradation of carbohydrates during food processing is observed during non-enzymatic (Maillard) reactions through which a large amount of furanic compounds is formed. Furan, a substantial chemical contaminant, is found in a broad range of cooked foods, and its formation in the food depends on the type of thermal processing (Perez Locas and Yaylayan, 2004) and non-thermal processing, such as UV-C disinfection treatment (Fan and Geveke, 2007). Furan is used as a synthetic intermediate in some industrial processes including pesticides, stabilizers, solvents, resins, and pharmaceuticals. In addition, it can be emitted from domestic and accidental combustion activity as a component of incomplete combustion of wastes, such as plastics and accidental building and vehicle fires, and is present in engine exhaust and cigarette smoke (Hayes and Marnane, 2002). Tobacco products are the major sources of furan exposure for the general public. Mainstream cigarette smoke contains up to 65 mg furan per cigarette (IARC International Agency for Research on Cancer, 2004),

The food safety international agencies have conducted surveys to collect data regarding furan levels in food and to estimate the dietary exposure to furan (EFSA (European Food Safety Authority), 2004; FDA, 2004). They depicted that numerous heat-treated foods contained detectable levels of furan, almost all of the jarred and canned baby foods, where the highest levels of furan over 100 ppb were recorded in

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vegetables, particularly beans, squash, and sweet potatoes. In addition, furan levels are detectable in cooked and canned meats, beer, and bakery products such as toasted bread and rusks. The high levels of furan are detected in roasted coffee beans, probably because of the roasting process in which the high temperatures exceed most of the other food processing strategies (Huault et al., 2016; Becalski et al., 2016). Hence, coffee is the major source of dietary furan exposure for adults. Furan levels in food are reported to range from a few micrograms per kilogram to 7000 μg/kg (EFSA, 2010). Carbohydrates, ascorbic acid, and polyunsaturated fatty acid components are known to be the highest contributors to the formation of natural furan in food and drinks through a thermal degradation process (Yaylayan, 2006). Thus, the mitigation of furan from foods is probably more challenging compared with that of other contaminants because of their health benefits, which make them desirable food components.

The mean daily dietary consumption of furan in the U.S. has been evaluated to be 0.25 mg/kg b.wt. in adults (Morehouse et al., 2008), 0.41 mg/kg b wt. in children during the first year of life, and 0.9 mg/kg b.wt. in babies consuming just formula (DiNovi and Mihalov, 2007). In Europe, adults have been estimated to receive a dietary level of 0.78 mg/kg b.wt. Also, dietary intake levels may be as high as 3.5 mg/kg b.w./day (EFSA (European Food Safety Authority, 2009)), raising concern that the presence of furan in food may present a potential risk to human health

In 1995, the International Agency for Research on Cancer (IARC Internacional Agency for Research on Cancer, 1995) announced furan as “possibly carcinogenic to humans” (Group 2B), and thus, it has attracted more consideration worldwide. The exposure to furan for long term (two years) led to hepatocellular adenomas and carcinomas occurrence in experimental animals, and promoted high incidence of cholangiocarcinomas in rats (National Toxicology Programme (NTP), 1993; Moro et al., 2012).

Several studies have focused on the genotoxicity mechanism by which furan exerts its carcinogenicity, as it is still inconsistent and contradictory, demonstrating that furan metabolites bind to DNA in the target organ and potentially induce genotoxic effects in the form of chromosomal aberrations and DNA strand breaks in vivo (Neuwirth et al., 2012). In addition, it has been observed that furan induces the exchange of sister chromatids, which is a sensitive endpoint for genotoxicity in vitro (Glatt et al., 2005). Hence, it suggests the contribution of furan genotoxicity in the induction of carcinogenicity including hepatocellular tumors and cholangiocarcinomas. In addition, Chen et al. (2010) revealed that the carcinogenicity of furan in rodents may arise from non-genotoxic changes. In addition, although furan exposure influences the expression of genes that control the cell cycle and cell death, it does not appear to affect the expression of genes involved in responses to DNA damage.

The toxic consequences of furan administration on different body systems including reproductive system (El-Akabawy and El-Sherif, 2016), immune system (Alam et al., 2017), and endocrine glands (Karacaoglu et al., 2012) have been evaluated in previously published studies. The deleterious effects of furan are mainly exerted because of the oxidation of cytochrome P450-catalyzed furan ring, which generated a reactive cis-butene-1,4-dial toxic metabolite, which can consequently bind irreversibly to various cellular components, including protein and DNA (Phillips et al., 2013).

Despite the existing data on chronic furan exposure toxicity and carcinogenicity, which evaluated long-term and low-dose exposure of furan to animals (de Conti et al., 2016; Von Tungeln et al., 2017), the potential hazards and molecular responses of high furan exposure for short term, resulting from increasing dietary consumption and industrial generation, are still unclear and subject for discussion. Therefore, we aimed at two major objectives: studying the differential toxic impact induced by furan exposure in the liver and kidney tissues through serum biochemistry assessment, pathology findings, and immunohistochemical recognition of proliferation-associated nuclear antigen (Ki–67) and proliferating cell nuclear antigen (PCNA) in liver and kidney tissues and explaining the observed furan-induced consequences through the analysis of transcriptional-mediated changes in the expression of intermediate filament proteins (desmin, vimentin, connexin 43, and nestin) in both the organs.

2. Materials and methods

2.1. Test compounds and chemicals

Furan (C_4H_4O ≥99% purity, molecular weight 68.07, CAS number 110–00–9) was purchased from Sigma-Aldrich Co., St. Louis, USA. For exposures, furan was diluted with corn oil and then kept in brown glass vials at 4 °C. Fresh solutions were prepared every week as needed.

2.2. Animals and experimental design

Twenty adult male albino rats (150–200 gm) were used. They were gotten from the Laboratory Animal Farm of the Faculty of Veterinary Medicine, Zagazig University, Egypt, and acclimated to the laboratory conditions for two weeks prior to use. The rats were kept in stainless steel cages with hardwood shavings bedding under conditions (at 21–24 °C, a 12 h light/dark cycle, 50–60%, humidity) and given standard diet and fresh water ad libitum throughout the investigation. All creatures were treated and handled as per the rules of the National Institutes of Health, USA, for the Care and Use of Laboratory Animals, affirmed by the Ethics of Animal Use in Research Committee, Cairo University, Egypt.

The animals were randomized into two groups, control, and furan-exposed groups, each containing 10 rats, each group having two replicates (5 rat/replicate). The rats of the control group orally received corn oil as a vehicle. Whereas, the rats of the furan-exposed group were orally administered furan at a dose of 16 mg/kg b.wt/day (five days per week over eight weeks; McDaniel et al., 2012). At the end of the experiment, the final body weight of each rat in both groups was measured for determining the changes in their body weight.

2.3. Blood sampling and tissue preparation

The whole blood was collected from the orbital vessels of control and treated rats and then centrifuged at 3000 rpm for 15 min for the separation of serum, which was stored at − 20 °C for the biochemical analysis of liver and kidney tissue injury markers and DNA damage marker. All rats of each group were euthanized by cervical dislocation under sodium pentobarbital anesthesia (60 mg/kg). The specimens of liver and kidney tissues were dissected and rinsed with sterile physiological saline and weighed to calculate the relative kidney weight (equivalent organ weight/body weight × 100). The specimens of kidney and liver tissues in each group were divided into three sets; a set of tissue was fixed in 10% neutral buffered formalin for histopathological and immunohistochemical studies. Another set was fixed with 3% glutaraldehyde solution for electron microscopy investigation. In addition, the final set was quickly frozen by liquid nitrogen and kept at − 80 °C for further gene expression analysis.

2.4. Biochemical analysis

2.4.1. Renal and hepatic tissue injury markers

Creatinine, urea, and lactate dehydrogenase (LDH) were quantitatively determined in serum by using colorimetric Spinreact Co. kits (Santa Coloma, Spain). Protein level, bilirubin content, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) levels were estimated using the kits provided by Diamond Diagnostics (Egypt). Alkaline phosphatase (ALP) level was measured by using the kits obtained from Spectrum Diagnostics (Egypt). These markers were quantitatively evaluated following manufacturers' instructions.

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