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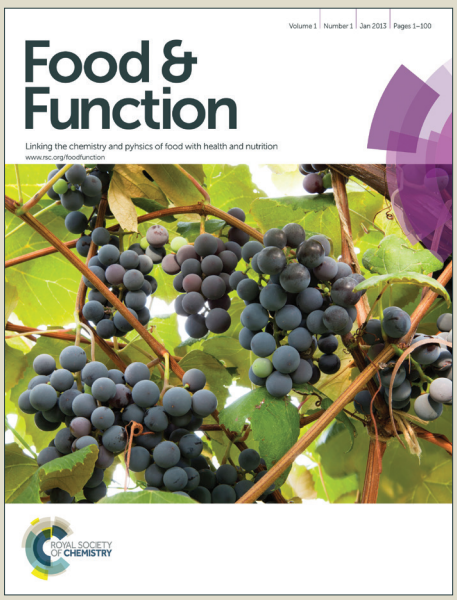

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Protective effects of apigenin on furan-induced toxicity in mice

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Running title: Protective effect of apigenin against furan

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Abstract

Furan, a food contaminant formed by heating, is possible carcinogenic to humans. In this study, we discussed the effect of administration of apigenin on furan-induced toxicity by determining the ROS content, oxidative damage, cytokines, DNA damage, and the liver and kidney damage of mice model. Our data showed that the administered apigenin of 5, 10, and 20 mg kg^{-1} bw d^{-1} could decrease the toxicity induced by furan in different extent. On the one hand, apigenin has ability to increase the oxidative damage indexes of glutathione (GSH), glutathione S-transferase (GST), superoxide dismutase (SOD) activities but decrease myeloperoxidase (MPO) activities, and maleic dialdehyde (MDA) content in the liver and kidney of mice treated by furan. On the other hand, it could decrease cytokines of tumor necrosis factor α (TNF-α), interleukin (IL)-1β, interleukin (IL)-6 content, increase interleukin (IL)-10 in the serum of furan-treated mice. Meanwhile, different concentrations of apigenin could decrease the ROS content, DNA damage index of 8-hydroxy-desoxyguanosine (8-OHdG) content, and decrease the liver and kidney damage indexes of aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactic dehydrogenase (LDH) activities, as well as blood urea nitrogen (BUN) and creatinine content in furan-treated mice..The protective effects of apigenin against furan-induced toxicity damage were mainly due to its excellent ability to scavenge free radicals and lipid oxidation inhibition ability. This is important when considering the possibility of using apigenin as a dietary supplement in diets for a beneficial application in the chemoprevention against furan toxicity.

Keywords: Furan, apigenin, radicals, antioxidant effect, protective effect, toxicity

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Introduction

Food processing involves many reactions, and many contaminants are generated during the heating of foods. Furan is just a typical food contaminant which could have harmful effects on the health of the human population. In 2004, the US FDA reported that furan occurred in a number of canned and jarred foods which especially underwent heat-processed.[1] Besides, almost all of the baby food sold in jars and cans contained detectable furan. US FDA (2004) internet data showed that furan concentrations in staple foods were below 18.8 ng g^{-1}, and in supplement foods were up to 108 ng g^{-1}.[1] In some certain foods, the level of furan is even higher than 5000 ng g^{-1}, especially in roasted coffee powder.[2] In our previous study, we found that furan was detected in almost all analyzed samples in 191 selected food products obtained from the Chinese markets, and the higher contents of furan were detected in traditional Chinese liquor (61.63 ng g^{-1}), coffee (71.36 ng g^{-1}), tea (68.28 ng g^{-1}) and pickle (85.63 ng g^{-1}).[3] In recent years, the formation mechanisms of furan have been raised by some researchers. The potential mechanisms about the formation of furan may be due to the thermal degradation and rearrangement of sugars, amino acids, and the oxidation by the reactive oxygen species (ROS) or lipoxygenase of polyunsaturated.[4-8] However, up to now, no conclusive remarks have been established regarding the main mechanism and major precursor of furan formation in different kinds of food processed at high temperatures.[9]

The toxicity of furan *in vitro* and *in vivo* has also been studied by some researches. The International Agency for Research on Cancer (IARC) has classified furan as a possible human carcinogen (Group 2B).[10] Furan has also been known as being both hepatoxic and carcinogenic in rats and mice by the National Toxicology Program (NTP).[11] In 90 days of gavaging experiments about the lower dose of furan, which was close to the estimated human exposures, a NOAEL of

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0.12 mg kg^{-1} bw was derived in the current study.[12] Selmanoğlu et al. investigate the effects of orally administered furan on liver and kidney in growing Wistar male rats for 90 days by determination of biochemical, morphological, histopathological and histomorphometrical examinations. Its results shown that furan may cause effects on the liver and kidney, and also could cause severe histopathological changes in the kidney in growing male rats.[13] As for the mechanistic aspects of the carcinogenicity of furan, studies have suggested that furan could act by both genotoxic and non-genotoxic mechanisms. More studies will be necessary in order to draw more precise conclusions concerning this issue.[9]

Since the toxicity of furan has harmful effects on the health of human, it is important to control or reduce the furan toxicity to humans. Recently, some natural bioactive components play important roles in controlling toxicity of the contaminants in mouse model. Zhang et al. and Wu et al. found that allicin could effectively reduce the toxicity induced by acrylamide; salidroside could protect the mice from the damage induced by furan toxicity.[14-16] These results of recent studies as well as our previous study have stimulated our interest in investigating the protective effects of some natural bioactive components against furan-induced toxicity *in vivo* in mouse.

Apigenin is a 4', 5, 7-trihydroxy flavones, widely found in a variety of fruits, vegetables, beans and tea. Apigenin possesses various pharmacological functions such as controlling apoptosis gene, inhibiting the expression of proto-oncogenes, inhibiting cancer cell proliferation, as well as inhibiting cancer cell invasion and metastasis.[17-20] Yang et al. investigate the protective effect of apigenin on acetaminophen-induced mouse acute liver injury and its potential mechanisms, which concluded that apigenin could protect against acetaminophen-induced acute liver injury in mice, and the mechanisms might be associated with enhancing hepatic GSH content via increment of

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GR activity.[21] However, no study has evaluated the protective effects of apigenin treatment against furan-induced toxicity damage using a mouse model. The present study tried to reveal the protective effect of apigenin from ROS content, oxidative damage, cytokines, DNA damage, and liver and kidney damage in furan-treated mouse in the absence or presence of apigenin to test the protective effect of apigenin against furan-induced toxicity. The antioxidant activity of apigenin was also evaluated in our present study. The dose of furan was chosen according to the 2-year study conducted by NTP (1993), and the doses of apigenin were on the basis of pre experiment we did before.[11]

Materials and methods

Materials

Furan (CAS: 110-00-9, purity>98.0%) and apigenin (520-36-5, purity>99%) were purchased from Sigma-Aldrich (St. Louis, Mo., U.S.A.). Apigenin was dissolved in normal saline to give a final concentration of 0.5, 1 and 2 mg mL^{-1}, respectively. Furan was diluted in normal saline to give a final concentration of 5 mg mL^{-1}. The dosing volumes of apigenin and furan solutions were based on each animal's body weight, on a basis of a volume of 0.2 mL for a mouse of 20 g.

Animals and experimental design

Fifty male healthy BALB/c mice (weighing 20 ± 5g) aged 4 to 5 weeks were utilized in this study, and they were provided by Laboratory Animals Center of Jilin University (Changchun, China). The research was carried out in accordance with the Guideline for Animal Experimentation of Jilin University (ChangChun, China). Animals were housed (10 mice each cage) in an air-conditioned room at 22 ± 2 °C and 30 ± 10% relative humidity. The animals were observed for general condition for 7 days during the quarantine and acclimation period to confirm

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that there were no abnormalities.

After a quarantine period of 7 days, 50 mice were randomly divided into five groups, each consisting of ten animals. Group I was treated with saline by oral gavage for 14 consecutive days and was denoted as the control group. Group II was treated intragastrically by gavage with saline for 7 consecutive days. On the 8th day, after the oral gavage saline, the mice were intraperitoneal injected with furan solution (8 mg kg^{-1} bw d^{-1}) for another 7 days and denoted as the Group furan. The dose of furan was chosen according to the 2-year study conducted by NTP (1993).[11] Groups III, IV, and V were treated intragastrically by gavage with apigenin (5, 10 and 20 mg kg^{-1} bw d^{-1}), respectively, for 7 days (once daily). On the 8th day, Groups III-V were adiministered intragastrically by gavage with apigenin (5, 10 and 20 mg kg^{-1} bw d^{-1}) and intraperitoneal injected with a single dose of furan (8 mg kg^{-1} bw d^{-1}) for another 7 days. The bodyweight of the animals was measured daily and the doses of furan and apigenin were recorded according to the body weight of the animals.

On the 15th day, the animals were sacrificed within 24h after the last treatment, the whole blood of mice was collected into heparinized test tubes and centrifuged at 2500 × g for 15 min at 4 °C to separate serum, and the serum was stored at -70 °C freezer for further analysis. The kidney and liver were excised immediately from the mice, washed thoroughly with ice-cold normal saline. The tissues were homogenized with 10% pre-chilled normal saline in a tissue homogenizer, and then centrifuged at 2500 × g for 10 min at 4 °C. The supernatant was used for subsequent biochemical analyses.

ROS assay

The ROS content of mice in the serum was detected by the commercial ELISA kits, which was

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described by the reference of Zhang et al. (2013).[15]

Oxidative damage assay

The oxidative damage was evaluated by the activities of GSH, GST, SOD, MPO, and the content of MDA in the serum, which were detected by the commercial kits obtained from Beijing Dingguo Changsheng Biotechnology Co., Ltd. (Beijing, China) according to the manufacturer's instructions. The detail of the methods was described by the reference of Zhang et al. (2013). [15]

Cytokine assay

The cytokines in the serum of treated mice were evaluated by the changes of cytokine content, TNF-α, (IL)-1β, (IL)-6 and (IL)-10, which was described by the reference of Zhang et al. (2013).[15]

DNA damage assay

The contents of DNA damage was evaluated by the change of 8-OHdG in the serum by the commercial ELISA kits, which was obtained in Beijing Dingguo Changsheng Biotechnology Co., Ltd. (Beijing, China). The method was also described in the reference of Zhang et al. (2013). [15]

The damage of liver and kidney assay

The activities of AST, ALT, LDH and the content of BUN and creatinine, as well as protein content were determined by commercial kits obtained from Beijing Dingguo Changsheng Biotechnology Co., Ltd. (Beijing, China) according to the manufacturer's instructions.

Antioxidant activity analysis of apigenin

The antioxidant analysis of apigenin was evaluated by scavenging ABTS radical, hydroxyl radical (•OH), DPPH radical, and superoxideanion ($•O_2^-$), which were followed by the method of Yuan et al (2013). [16] The lipid oxidation inhibition assay of apigenin was measured using the method of Muñiz-Márquez et al. (2013). [22]

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Statistical analysis

Statistical analysis was performed using SPSS 11.5 software (Chicago, USA). The significance of difference was calculated by one-way ANOVA test, and the results with $p < 0.05$ were considered to be statistically significant. Graphs were drawn with OriginPro 8.0 software (OriginLab Corporation, Northampton, MA, USA).

Results

Effect of apigenin on the ROS content

We examined the effect of apigenin on the ROS content of furan-treated mice by using an ELISA kit. As shown in Fig. 1, the lowest ROS content (68.78 ± 0.56 U mL^{-1}) was observed in the control group. The ROS content was significantly increased in the furan-treated group compared with those in the control group ($p < 0.05$). The highest level of ROS (181.71 ± 3.55 U mL^{-1}) was observed when the mice were treated with furan alone. The ROS content then decreased accordingly with the increase of apigenin concentrations at the range of 5 to 20 mg kg^{-1} bw d^{-1}. The administration of apigenin at concentrations of 5, 10, and 20 mg kg^{-1} bw d^{-1} significant reduced the ROS content by 15.0%, 30.9%, and 50.5%, respectively, compared to the group treated by furan. But the ROS content in the groups treated with 20 mg kg^{-1} bw d^{-1} apigenin was still markedly higher than that in control group ($p < 0.05$).

Effect of apigenin on the oxidative damage in the liver and kidney

The effect of apigenin and furan on the oxidative damage in the liver and kidney of mice was evaluated by the indexes of GSH, GST, SOD, MPO, as well as the content of MDA, which were shown in Fig. 2 (A-E). The treatment of furan caused significant reduction of GSH, GST, and SOD activities in the livers and kidneys of male Balb/C mice, respectively, when compared to

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control group ($p < 0.05$). Enhancement of GSH level was observed in the groups treated with 5 mg kg^{-1} bw d^{-1}, 10 mg kg^{-1} bw d^{-1}, and 20 mg kg^{-1} bw d^{-1} of apigenin, respectively, compared with those treated by furan alone ($p < 0.05$). As for the activity of GST and SOD, similar trend was found tothe change of GSH. The administration of 20 mg kg^{-1} bw d^{-1} of apigenin, results in a significant elevation of GST activity compared to furan group, which increased by 45.42% and 78.6% in liver and kidney respectively, and also the SOD activity increased by 114.52% in liver and 108.40% in kidney. Increase of MPO activity and MDA content were observed in the group treated by furan compared with that of controlgroup ($p < 0.05$).However, treatment with different doses of apigenin have decreased significantly MPO activity and MDA content both in liver and kidney compared tothat of furan-treated mice ($p < 0.05$) in different extent.

Effect of apigenin on the cytokines

Four cytokines were detected for evaluating the effect of apigenin on the immunologic injury induced by furan, which were interleukins (IL)-6, (IL)-10, (IL)-1β, and TNF-α. As shown in Table 1, administration of furan showed significant increase of the content of (IL)-6, (IL)-1β, and TNF-α, and significant reduction of (IL)-10 content compared to the control group ($p<0.05$). Through the administration of apigenin with the dose of 5, 10, and 20 mg kg^{-1} bw d^{-1}, the content of (IL)-6 was effectively reduced ($p < 0.05$), while the treatment of 20 mg kg^{-1} bw d^{-1} of apigenin effectively reduced the (IL)-6 level from 82.08±0.94 pg mL^{-1} to 46.67±0.89 pg mL^{-1} close to that of the furan-treated group ($p < 0.05$). Meanwhile, the apigenin concentration of 20 mg kg^{-1} bw d^{-1} was also found to be very effective in causing drasticly decrease on the level of (IL)-1β and TNF-α ($p < 0.05$)..After treated with 20 mg kg^{-1} bw d^{-1} apigenin, the level of TNF-α and (IL)-1β in serum decreased by 30.97% and 43.80% respectively compared to furan group. On the

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contrary, the (IL)-10 levels of the groups treated with 5, 10, and 20 mg kg^{-1} bw d^{-1} of apigenin showed values of 109.16 ± 5.57, 123.21 ± 3.56, and 144.64 ± 3.41 pg mL^{-1}, respectively, which were significantly higher than those in the furan treated group ($p < 0.05$). When the mice were treated with 20 mg kg^{-1} bw d^{-1} of apigenin, its level of (IL)-10 was nearly back to the level of the control group.

Effect of apigenin on the DNA damage

To evaluate the effect of apigenin on the DNA damage in the serum of furan-treated mice, we assessed the changes of 8-OHdG content with different concentrations of apigenin. As shown in Fig. 3, after being treated with furan (8 mg kg^{-1} bw d^{-1}) for 7 days, a significant increase of 8-OHdG content was observed compared to the control group ($p<0.05$), which reached at 33.74 ± 1.04 ng mL^{-1}, nearly three times higher compared with the control group. After being treated with different concentrations of apigenin, the effect of furan on 8-OHdG content was exhibited significantly, with 8-OHdG value 24.35 ± 1.30 ng mL^{-1} for 5 mg kg^{-1} bw d^{-1}, 17.64 ± 0.63 ng mL^{-1} for 10 mg kg^{-1} bw d^{-1} and 14.29 ± 0.42 ng mL^{-1} for 20 mg kg^{-1} bw d^{-1}, respectively.

Effect of apigenin on the damage of liver and kidney

The effect of apigenin on the damage of liver and kidney in the serum of furan-treated mice was evaluated by the parameters of AST, ALT, LDH, BUN and creatinine. As shown in Table 2, pretreatment with the doses of 8 mg kg^{-1} bw d^{-1} of furan significantly elevated the activities of AST, ALT, LDH, and the levels of BUN and creatinine compared to the control group ($p<0.05$). The activities of AST and ALT have been considered as effective indicator of hepatic injury for a long time. In the current study, pretreatment with apigenin dramatically reduced the AST and ALT activities in the serum compared to the furan-treated group ($p<0.05$). As for the activity of AST,

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when the mice were treated with 20 mg kg^{-1} bw d^{-1} of apigenin, the activity of AST (12.79±0.94 U L^{-1}) was still significant higher than it incontrol group(8.98±0.37 U L^{-1}) (p<0.05). Meanwhile,the group treated with 20 mg kg^{-1} bw d^{-1} of apigenin showed the most effective protective effect from hepatic injury induced by furan, with the values of ALT activity of 14.53±0.65 U L^{-1}, nearly back to the level of the control group. The effect of apigenin on the damage of the kidney in the serum of furan-treated mice was evaluated by the changes of BUN, LDH, and creatinine. The results in Table 2 showed that treatment with 8 mg kg^{-1} bw d^{-1} of furan for 7 days, the activity of LDH and the levels of BUN and creatinine were significantly increased compared tocontrol group (p<0.05). Treatment with 20 mg kg^{-1} bw d^{-1} of apigenin for 14 days prevented the LDH activity to 26.3%, the BUN level to 23.2%, and the creatinine level to 36.8% in the serum, respectively.

Antioxidant activity of apigenin

The antioxidant activity of apigenin was evaluated using five methods, and the results were shown in Table 3. The ABTS radical scavenging ability, •OH-scavenging ability, DPPH radical scavenging ability, $•O_2^-$ scavenging ability and lipid oxidation inhibition increased with the increase concentration of apigenin. The IC_{50} values of various antioxidant assays were used to evaluate the antioxidant level of apigenin. The IC_{50} values of apigenin concentration for the DPPH radical, ABTS radical, •OH, and $•O_2^-$ were 6.42, 5.61, 0.004, and 0.01 mg mL^{-1}, respectively.

Discussion

Our study provides the first evidence of a potential protective effect of apigenin on the toxicity induced by furan in Balb/C mice modle. This protection is related to the concentration of apigenin ranging from 5 to 20 mg kg^{-1} bw d^{-1}.

In recent studies, the reactive oxygen species (ROS), such as superoxide anion ($•O_2^-$), hydrogen

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peroxide (H_2O_2), and hydroxyl radical (•OH), have been associated with many diseases in the human body.[23] In the current paper, we first studied the effect of furan and apigenin on the ROS in male mice model. The data revealed that treating mice with furan of 8 mg kg^{-1} bw d^{-1} for 7 days significantly increased the content of ROS in the serum (p<0.05, Fig.1), the ROS level was 2.6-fold higher than that in the control group. After treatment with apigenin, the ROS content was decreased with a clear dose-dependency trend. In the vitro experiment on the determination of antioxidant activity of apigenin, lower IC_{50} values indicated that apigenin had excellent antioxidant properties. Among the assays, the best inhibiting capacity of apigenin was observed in •OH (0.004 mg mL^{-1}). In lipid oxidation inhibition assay, the general ability of apigenin to prevent lipid oxidant was tested, and a 32.91 ± 1.25% lipid oxidation inhibition was observed, which showed the apigenin also could inhibit lipid oxidation effectively. That is to say, apigenin has great ability to free radical scavenging, such as •OH, and $•O_2^-$ free radical, and it is maybe an important reason for apigenin to decrease the ROS content induced by furan.

The oxidative damage of furan was also evaluated by determining the activities of GST, SOD, MPO, and the changes of GSH and MDA levels in the liver and kidney of furan-treated mice. GSH is as an essential intracellular reducing substance for the maintenance of thiol groups on intracellular proteins and antioxidant molecules in living organisms.[24] Furan is metabolized by cytochrome P450 enzymes to its major metabolite cis-2-butene-1,4-dial (BDA),[25] which has been shown to react with cellular nucleophiles such as GSH and amino acids and to cause cross-links between thiols and amino groups.[26] GSH plays an important role in protecting several tissues and cell lines against injuries by oxidants and reactive electrophiles.[27] GSH activities both in the liver and in the kidney treated by furan were significantly decreased (p<0.05), showed that the GSH

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activities were maybe strained by the formation of cross-links between BDA and GSH. GST, previously known as ligandins, comprises a family of eukaryotic and prokaryotic phase II metabolic isozymes best known for their ability to catalyze the conjugation of the reduced form of GSH to xenobiotic substrates for the purpose of detoxification. GST activity is a sensitive factor that reflects the damage of the body. We observed a significant decrease in GST activity both in the liver and in the kidney of furan-treated mouse compared to the control group ($p<0.05$), showing that furan had damaged the liver hepatocyte and kidney to some extent. As GST increases the solubility of hydrophobic substances, it also plays an important role in the storage and excretion of xenobiotics. Compounds that increase the activity of GST, which metabolizes toxic compounds to non-toxic, protect the liver.[28] In the current study, GST activity was inhibited by the addition of furan in the body, showing that furan could induce the damage on the important detoxification enzymes and further cause damage to the body. However, the addition of apigenin has inhibited the decrease of GSH level and GST activity induced by furan, showing that apigenin paly a role on reduce the damage from furan. This results are consistent with the study of Yang et al., which research about the protective effect of apigenin on mouse acute liver injury induced by acetaminophen and its associate with increment of hepatic glutathione reductase activity.[21] Antioxidant enzymes such as SOD are capable of catalyzing the dismutation of superoxide ($\bullet O_2^-$) into oxygen and hydrogen peroxide. Thus, they are an important antioxidant defense in nearly all cells exposed to oxygen and often used to evaluate the oxidative stress of organism.[29] Furan with a concentration of 8 mg kg^{-1} bw d^{-1} for 7 days significantly decreased the activities of SOD in the liver and kidney of treated mice compared to the control group ($p<0.05$), shown that furan occurred the oxidative stress. Myeloperoxidase (MPO) is a peroxidase enzyme, which is an

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endogenous lysosomal enzyme that removes H_2O_2 and catalyzes the formation of toxic hypochlorous acid. The current study revealed that the MPO activities were significantly increased by furan in its current concentration. MPO and its oxidative products play key roles in the lipid peroxidation in liver damage. Lipid peroxidation generates a complex variety of products, many of which are reactive electrophiles. Some of these react with protein and DNA and cause toxicity and mutagenicity.[30] Some peroxidaious study showed the correlation between the levels of neutrophil and MDA, the latter was an indicator of free radical-mediated lipid peroxidation damage.[31] MDA is the end product of lipid peroxidation. Elevated liver MDA levels imply that enhanced peroxidation causes tissue damage and the breakdown of antioxidant defense mechanisms, thus preventing the formation of superabundant free radicals.[32] Our data showed the treatment with furan caused significantly increased MDA levels compared with the control group (Fig. 2). In our study, the food contaminant of furan has the ability to enhance the oxidative stress and ROS content in the mice，it could know from the changes of the abilities of GST, SOD, MPO, and the levels of GSH, MDA and ROS, which is consistent with the study of Cordelli et al. ,[29] they revealed from gene expression analysis that ROS/ oxidative stress production (Gstm1, Gstm3, Gyp4a10, and Cyp4a14) genes were significantly up-regulated in furan-treated mice with a concentration of 15 mg kg^{-1} bw d^{-1} for 28 days. Hickling et al [33] also found that furan-induced changes in the expression of various genes were associated with oxidative stress, DNA damage, and cell cycle control. After the mice were treated with apigenin at 5, 10, and 20 mg kg^{-1} bw d^{-1}, the activities of oxidative stress related enzymes, such as GSH, GST, and SOD, were increased with a dose-dependency trend. MPO enzymes are associated with the lipid peroxidation, which is regarded as one of the basic mechanisms of tissue damage caused by free radicals.[34] The general ability of apigenin to prevent

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lipid oxidant was (32.91 ± 1.25)% of lipid oxidation inhibition, showing the apigenin also have ability to inhibit lipid oxidation. This might be an effective inhibitor in reducing the MPO abilities and MDA formation. Singh et al.[35] also found that apigenin is able to quench the lipid peroxidation chain and is capable of shielding the membrane from free radicals which cause injuries.

Cytokines play important roles in the normal physiology of cells. They are related with the immune response, inflammation, and tissue injury or repair. [36] Our data about cytokines including TNF-α，(IL)-1β, (IL)-6 and (IL)-10, showed that furan activated inflammatory cells and subsequently amplified the inflammatory response by releasing various cytokines. When the mice were intragastrically given apigenin, the contents of TNF-α, (IL)-1β and (IL)-6 were markedly decreased and the (IL)-10 level was increased in mice serum. These results suggested that apigenin could alleviate tissue injury caused by furan through suppressing inflammatory response. Furan could induce inflammatory response by increasing expression of cytokines and other inflammation-associated genes, such as (IL)-1β, (IL)-6, and (IL)-10, as was confirmed by our present study. [12, 34, 37] Gerritsen et al and Takano-Ishikawa et al [38,39] revealed that apigenin inhibited the expression of inflammation-related molecules, such as intercellular adhesion molecule-1, vascular cell adhesion molecule-1, and E-selectin, induced by TNF-α and IL-1α. Lee et al (2007) [40] studied that apigenin profoundly reduced the tumor necrosis factor-α (TNF-α)-induced adhesion of monocytes to HUVEC monolayer, further suggesting that apigenin has significant anti-inflammatory activity that is involved in blocking NO-mediated COX-2 expression and monocyte adherence. Funakoshi-Tago et al [41] found that apigenin significantly inhibited TNF-α-induced NF-κB transcriptional activation. Similarly, in the study of Rithidech et al, [42] apigenin at dosage of 10mg kg^{-1} bw d^{-1} significantly inhibited cytokines such as TNF, IL-1

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and IL-6 expression in vivo given to mice after irradiation. Man et al [43] demonstrated that all these anti-inflammatory effects induced by apigenin were likely attributed to its antioxidant properties. Our research showed that apigenin had great antioxidant abilities. The best inhibiting capacity of apigenin was observed in •OH and $•O_2^-$, which would contribute to the anti-inflammatory effect of apigenin on that induced by furan in mice model.

8-OHdG is a marker of oxidative damage, and mutations may arise from the formation of 8-OHdG involving G·C→T·A transversions.[44,45] We found that 8-OHdG levels were dramatically enhanced by furan with a concentration of 8 mg kg^{-1} bw d^{-1} for 7 days compared to the control group (p<0.05, Fig. 4). In the study of Hickling et al (2010), [33] there was a marked association between CYP2E1 expression and DNA oxidation (8-OHdG) in areas of centrilobular hepatocyte necrosis seen after a single dose of furan of 30 mg kg^{-1} bw d^{-1} daily doses per week for three months. After one month of recovery experiments from three-month treatment, 8-OHdG was still observed in areas of furan-induced cholangiofibrosis. The present study also demonstrated that apigenin could significantly decrease the level of 8-OHdG in furan-treated mice. We also found that the highest dose of apigenin (20 mg kg^{-1} bw d^{-1}) had the best protective effect ($p < 0.05$).

Furan is a typical hepatotoxicity compound, and the liver is the main target organ of furan-induced toxicity in rats and mice with a clear dose-dependency and probably acting by a genotoxic mechanism. [11] In our present study, we evaluated the hepatic enzymes, such as ALT and AST, as the biochemical markers for the detection of early acute hepatic damage. Their increased levels in serum indicated the increased permeability and damage and/or necrosis of hepatocytes. In our present study, furan caused a significant increase in the activities of AST and ALT (Table 1), which was attributed to the severe damage of the tissue membrane. This results are consistent with

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the study of Moser et al. and Hamadeh et al. [46,47] After an administration of apigenin, the activities of AST and ALT decreased in the serum of mice. Similarly, the increased level of LDH that is an intracellular enzyme in serum is an indicator of a cell damage.[48] The findings suggested apigenin was effective in preventing the furan-induced hepatocyte damage. We also evaluated the changes of BUN and creatinine levels of apigenin treated mice to study the kidney damage induced by furan. BUN is an indirect and rough measurement to the metabolic function of the liver and excretory function of the kidney. Furan could significantly increase the BUN content in the serum, which is consistent with the study of Gill et al ,[12] Treatment with apigenin with the concentration of 5, 10, and 20 mg kg^{-1} bw d^{-1} could restrain the increase of BUN level compared to the furan-treated group. Similarly, we found a dramatically increase of cretinine levels in the mice treated by furan compared to the control group (p<0.05). While in the study of Gill et al , [12] who founded that creatinine was not affected in females, whereas in males it showed an increase linear trend with the furan concentration of 0.0, 0.03, 0.12, 0.5, 2.0, and 8.0mg kg^{-1} bw d^{-1}. Apigenin significantly prevented the rises of creatinine levels in serum among the furan-treated mice, suggesting the apigenin potently protected against the kidney toxicity induced by furan.

Conclusion

In conclusion, this study was first to investigate the effects of apigenin on the toxicity induced by furan in mice model. By evaluating the ROS content, oxidative damage, cytokines, DNA damage, and liver and kidney damage, our data demonstrated that apigenin possessed a powerful protective capacity from the toxicity and damage induced by furan. Taken together, these results strongly suggest that apigenin is an effective agent to protect the exogenous toxic compound, such as furan, but further investigation will be needed to clarify the exact mechanisms. At least we can

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clear that taking food rich in apigenin or supplementing with apigenin might be health beneficial for the individuals who are at risk of furan toxicity.

Acknowledgements

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Figure captions:

Fig. 1 Effects of apigenin on ROS content in the serum of furan-treated mice.

All values are expressed as means ± standard deviation (n = 8). Values in the same with different superscript upper case letters are significantly ($p < 0.05$) different.

Fig.2 Effects of apigenin on the activities of GSH, GST, SOD, MPO, and MDA content in the liver and kidney of furan-treated mice. (A) GSH; (B) GST; (C) SOD; (D) MPO; (E) MDA.

All values are expressed as means ± standard deviation (n = 8). Values in the same with different superscript upper case letters are significantly ($p < 0.05$) different.

Fig.3 Effects of apigenin on 8-OHdG content in the serum of furan-treated mice.

All values are expressed as means ± standard deviation (n = 8). Values in the same with different superscript upper case letters are significantly ($p < 0.05$) different.

Table captions:

Table 1 Effects of apigenin on the content of (IL)-6, (IL)-10, (IL)-1β, and TNF-α in the serum of furan-treated mice.

Table 2 Effects of apigenin on activity of AST, ALT and LDH, as well as levels of BUN and creatininein in the serum of furan-treated mice.

Table 3 Antioxidant activity of apigenin by scavenging free radicals.

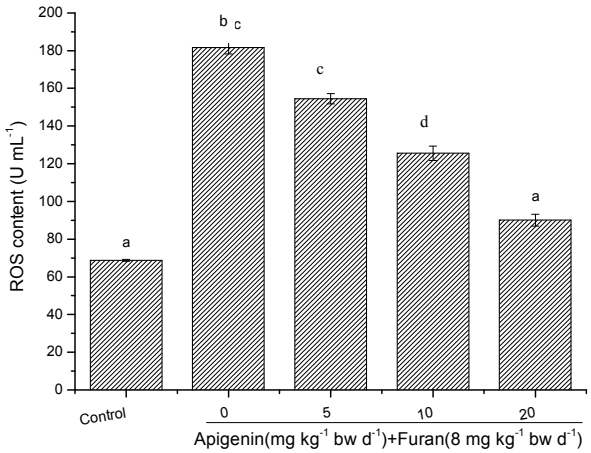

Fig. 1

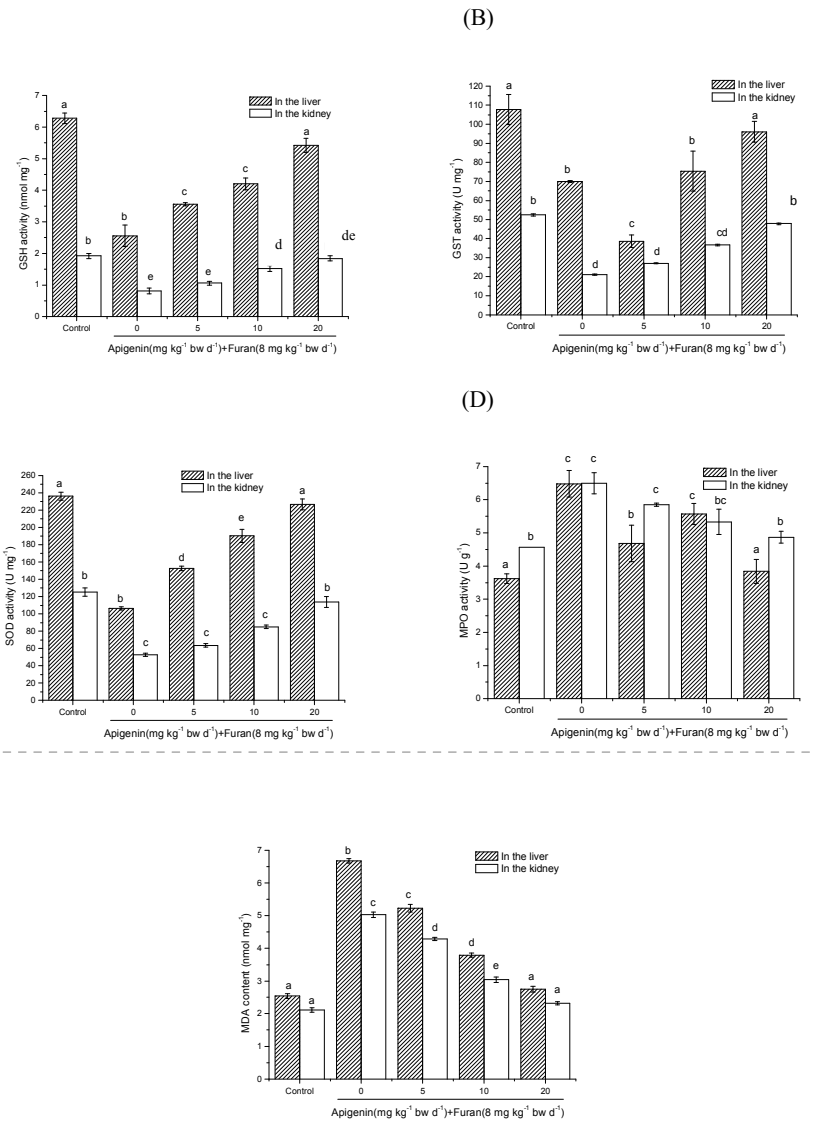

Fig. 2

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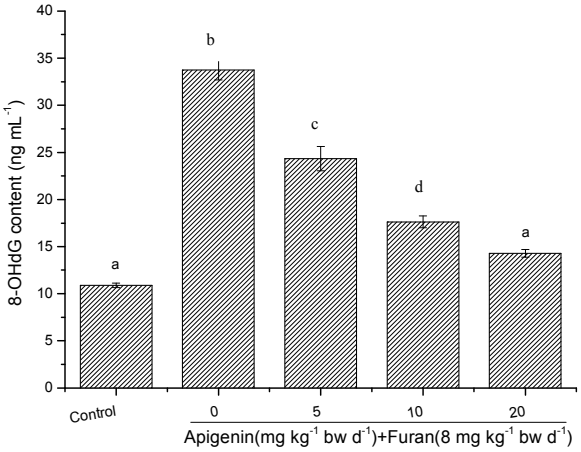

Fig. 3

Table 1

Groups	IL-6 (pg mL^{-1})	IL-10 (pg mL^{-1})	IL-1β (pg mL^{-1})	TNF-α (pg mL^{-1})
Control	32.46±0.67^{a}	152.30±3.42^{a}	37.99±1.41^{a}	188.75±0.73^{a}
Furan (8 mg kg^{-1} bw d^{-1})	82.08±0.94^{b}	103.99±4.18^{b}	93.33±2.05^{b}	351.50±2.05^{b}
Furan (8 mg kg^{-1} bw d^{-1}) +Apigenin (5 mg kg^{-1} bw d^{-1})	71.42±1.03^{b}	109.16±5.57^{b}	84.62±2.73^{b}	302.95±2.35^{c}
Furan (8 mg kg^{-1} bw d^{-1}) + Apigenin (10 mg kg^{-1} bw d^{-1})	58.87±1.08^{c}	123.21±3.56^{c}	62.15±0.52^{c}	272.21±1.34^{d}
Furan (8 mg kg^{-1} bw d^{-1}) +Apigenin (20 mg kg^{-1} bw d^{-1})	46.67±0.89^{ac}	144.64±3.41^{a}	52.45±1.08^{c}	242.63±2.23^{e}

All values are expressed as means ± standard deviation (n = 8). Values in the same column with different superscript upper case letters are significantly ($p < 0.05$) different.

Table 2

Groups	AST ($U L^{-1}$)	ALT ($U L^{-1}$)	LDH ($U L^{-1}$)	BUN ($mg L^{-1}$)	Creatinine ($\mu mol L^{-1}$)
Control	8.98±0.37 [a]	12.45±0.82 [a]	350.14±1.76 [a]	87.41±0.62 [a]	60.18±0.35 [a]
Furan (8 mg kg^{-1} bw d^{-1})	27.01±0.88 [b]	30.80±1.37 [b]	489.81±1.65 [b]	119.20±0.39 [b]	101.75±1.36 [b]
Furan (8 mg kg^{-1} bw d^{-1}) +Apigenin (5 mg kg^{-1} bw d^{-1})	21.95±0.53 [c]	25.84±0.53 [b]	423.90±2.30 [c]	113.69±0.70 [b]	87.18±1.80 [c]
Furan (8 mg kg^{-1} bw d^{-1}) + Apigenin (10 mg kg^{-1} bw d^{-1})	17.40±1.02 [c]	19.63±0.35 [c]	391.18±1.04 [d]	103.49±0.81 [c]	76.45±1.39 [c]
Furan (8 mg kg^{-1} bw d^{-1}) +Apigenin (20 mg kg^{-1} bw d^{-1})	12.79±0.94 [d]	14.53±0.65 [a]	361.20±1.15 [a]	91.49±1.28 [a]	64.35±1.00 [a]

All values are expressed as means ± standard deviation (n = 8). Values in the same column with different superscript upper case letters are significantly ($p < 0.05$) different.

Table 3

Free radical	Concentration apigenin (mg mL^{-1})	Scavenging Rate (%)	IC_{50} (mg mL^{-1})
DPPH	1	43	6.42
	5	44	
	10	52	
	15	72	
ABTS	2	17	5.61
	4	47	
	6	59	
	8	60	
•OH	0.001	9	0.004
	0.002	38	
	0.004	49	
	0.006	71	
$•O_2^-$	0.03	6	0.01
	0.06	11	
	0.09	23	
	0.12	65	