1O, 20O-diacetyl kamebakaurin protects against acetaminophen-induced hepatotoxicity in mice

Hiroki Yoshioka¹, Tsunemasa Nonogaki¹, Hiroyuki Ohnishi², Nobuyuki Fukuiji¹, Masae Yoshikawa¹, Ming-Yu Gui³, Yong-Ri Jin³, Xu-Wen Li³, Yoshiyuki Adachi⁴, Naohito Ohno⁴, Koichi Takeya⁴, Yukio Hitotsuyanagi⁴, Nobuhiko Miura⁵, and Yutaka Aoyagi¹

¹College of Pharmacy, Kinjo Gakuin University, 2-1723 Omori, Moriyamaku, Nagoya, Aichi 463-8521, Japan; ⁄Department of Health Science, School of Allied Health Science and Graduate School of Medical Sciences, Kitasato University, 1-15-1 Kitasato, Minami-ku, Sagamihara, Kanagawa 252-0373, Japan; ²Department of Chemistry, JiLin University, No.2 Xinmin street, Changchun, JiLin 130021, People’s Republic of China; ³School of Pharmacy, Tokyo University of Pharmacy & Life Sciences, 1432-1 Horinouchi, Hachioji, Tokyo 192-0392, Japan; and ⁴Division of Health Effects Research, Japan National Institute of Occupational Safety and Health, 6-21-1, Nagao, Tamaku, Kawasaki, Kanagawa 214-8585, Japan

(Received 12 July 2018; and accepted 8 August 2018)

ABSTRACT

The present study aimed to investigate the protective effects of kamebakaurin (KA) and 1O, 20O-diacetyl kamebakaurin (Ac₂KA) on acetaminophen (APAP)-induced hepatotoxicity and compare the hepatoprotective mechanisms of the two chemicals. Seven-week-old male C57BL/6J mice were orally administered KA, Ac₂KA, or an ethanol/olive oil emulsion once per day for 7-days. Twenty-four hours after the final administration, the mice were fasted and then intraperitoneally injected with 450 mg/kg APAP or saline. At 16 h after injection, the mice were euthanized and blood samples were collected for plasma analysis. Pretreatment with KA and Ac₂KA significantly attenuated APAP-induced hepatic injury. The protective effect of Ac₂KA was stronger than that of KA. These two chemicals attenuated oxidative stress, inflammatory cytokine production, c-jun N-terminal kinase activation, and receptor-interacting protein (RIP)-3 activation. Ac₂KA also decreased APAP-induced RIP-1 activation and nuclear factor kappa B (NF-κB) p65 translocation. Moreover, Ac₂KA repressed mRNA expression of Cyp1a2/2e1 in the liver. Our results showed that KA and Ac₂KA exerted protective effects against APAP-induced hepatotoxicity. The responsible mechanisms may be related to the chemicals’ antioxidant activity and the inhibition of c-jun N-terminal kinase activation and RIP-3 activation. The effects of Ac₂KA included those of KA, as well as RIP-1 inactivation, NF-κB inhibition, and Cyp inhibition.

Hepatic diseases are among the most serious health problems faced globally. As for drug-related liver injury, acetaminophen (APAP) overdose is well-known to induce fulminant hepatitis. It is also the main reason for the acute liver injury in industrialized countries. APAP is recognized as a safe and antipyretic drug and popular analgesic at therapeutic doses. However, APAP is reported to induce severe hepatic injury in human and laboratory animals (13). The predominant mechanism of APAP-induced hepatic injury has been reported in detail (9) and it occurs in multiple steps. First, APAP is metabolized to N-acetyl-p-benzoquinone imine (NAPQI) by CYP2E1 and CYP1A2. NAPQI depletes glutathione in hepatocytes and binds to various mitochondrial proteins. Mitochondrial glutathione depletion and covalent binding can increase the generation of mitochondrial...
reactive nitrogen and oxygen species, which then activate c-Jun-N-terminal kinase (JNK) (16). Activated JNK binds to the mitochondria, which induces further increase in the generation of reactive nitrogen and oxygen species. These processes cause additional oxidative stress and inhibit ATP synthesis. Insufficient ATP induces necrosis of hepatocytes. Peripheral to these essential events, a number of inflammatory mediators, such as chemokines and cytokines, are induced to modify the toxicity of APAP.

Currently, N-acetylcysteine (NAC) is the most effective medication prescribed to treat APAP-induced liver injury (10). However, clinical studies have reported that NAC often shows undesirable side effects, such as vomiting (18). Therefore, it is essential to develop side effect-free chemicals or compounds that offer maximum protective effects against APAP-induced hepatic injury. Presently, many researchers have been focusing on the development of drugs from natural products and plant extracts with the ability to prevent hepatic injury without any side effects.

Kamebakaurin (KA) is an ent-kaurane diterpenoid isolated from Rabdosia excise. KA is used in Asian folk medicine for the treatment of fever and arthralgia. Several investigations showed that KA significantly decreases chemokines and cytokines in lipopolysaccharide-stimulated microglial cells in vitro by inactivating nuclear factor-kappa B (NF-κB) signaling (11). Because APAP-induced hepatic injury is frequently associated with the inflammatory response, we hypothesized that KA protects against APAP-induced hepatic injury through the inhibition of inflammatory response. Our previous investigation indicated that KA prevents APAP-induced hepatic injury through the inhibition of lipid peroxidation and inflammatory response (21). However, our previous report was only a phenomenological study and did not focus on the mechanism. It is important to disclose the underlying mechanism by which KA attenuates APAP-induced hepatic injury. Moreover, we synthesized 10, 20O-diacetyl kamebakaurin (Ac2KA) from KA (1) and found in our preliminary tests that the in vitro NF-κB inhibitory activity of this new compound was much stronger than that of KA (data not shown). Thus, Ac2KA may more strongly prevent APAP-induced hepatic injury than KA.

Therefore, we investigated the protective mechanisms of Ac2KA and/or KA against APAP-induced hepatic injury.

MATERIAL AND METHODS

Animal treatment. Male 6-week-old C57BL/6J mice were purchased from CLEA Japan (Tokyo, Japan). Following arrival at our facility, the mice were maintained under standard conditions of controlled temperature (23 ± 2°C), humidity (55 ± 5%), and light (12-h light/dark cycle) with free access to food and water. Experiments started using 7-week-old mice. Following the experiment, any surviving mice were sacrificed by pentobarbital. These experiments were approved by the Institutional Animal Care and Experiment Committee of Kinjo Gakuin University.

Preparation of KA and Ac2KA. KA was isolated as previously reported (21). Ac2KA was synthesized from KA, as previously described (1). An ethanol solution (100 mg/mL) was employed in the following assay. The structures of KA and Ac2KA are described in Fig. 1.

Experimental protocol. The mice were randomly divided into 4 groups of 6–8 mice each. KA + APAP and Ac2KA + APAP groups received a 7-day pre-treatment with 50 mg/kg KA once per day or with Ac2KA in an olive oil/ethanol emulsion, respectively, whereas the control and APAP groups, as negative controls, were injected with olive oil/ethanol emulsion. Prior to APAP injection, the mice were fasted for 16 h, and the APAP group, the KA + APAP group, Ac2KA + APAP group were i.p. injected with 450 mg/kg APAP in an emulsion of polyethylene glycol and saline emulsion. The control group received the polyethylene glycol and saline emulsion. Sixteen hours after the final administration, mice from each group were sacrificed and bled to obtain the plasma. This plasma was stored at −80°C until analysis. In addition, we rapidly removed the mouse livers and fixed them in formalin solution for histological assay. The other pieces of the liver specimen were snap-frozen in liquid nitrogen and subsequently stored at −80°C.

Plasma biochemical analysis. Plasma ALT activities were measured using the Transaminase CII Test Wako (Wako Pure Chemicals, Osaka) according to the manufacturer’s instructions. Each plasma sample (1.5 μL) was mixed with substrate mixture (75 μL), incubated at 37°C for 15 min, and then quenched through the addition of stopping solution (150 μL). The absorbance of blue pigment formed by the reaction was measured at 555 nm (22, 24).

Plasma levels of tumor necrosis factor (TNF) α and interleukin (IL)-6 were determined using commercially available ELISA kits, according to the manufacturer’s instructions (eBioscience, San Diego, CA).
Ac₂KA protects APAP-induced hepatotoxicity

**Hepatic glutathione levels** were measured using a GSSG/GSH quantification kit (Dojindo Laboratories, Kumamoto, Japan) according to the manufacturer’s instructions, as previously described (21).

**Western blotting analysis.** Liver sections (0.1 g) were homogenized with 900 μL ice-cold phosphate-buffered saline containing protease inhibitor (Nacalai Tesque) and centrifuged at 18,000 × g for 20 min at 4°C. The resulting supernatant of each sample was collected and measured for protein level using a BCA protein kit (Nacalai Tesque). Protein samples (30 μg) were subjected to sodium dodecyl sulfate-polyacrylamide electrophoresis using a 10% gel and transferred to a polyvinylidene difluoride membrane.

**Histopathological findings.** A portion of the left liver from each mouse was fixed in a formalin solution, dehydrated, and embedded in paraffin. Sections of 4-μm thickness were cut from the paraffin-embedded blocks. These sections were dewaxed in xylen and rehydrated in a graded ethanol series. After rehydration, the sections were stained with Mayer’s hematoxylin solution (Nacalai Tesque, Kyoto, Japan). After rinsing in running tap water, the sections were stained with 0.1% eosin solution (Wako Pure Chemical) containing acetic acid. Finally, the sections were dehydrated, cleared, and mounted with a cover glass. Histopathological features of each slice were examined under a light microscope (14).

**Measurement of total malondialdehyde levels and glutathione in the liver.** Total malondialdehyde levels in the liver were examined via a colorimetric microplate assay (Oxford Biochemical Research, Oxford, MI) according to the manufacturer’s protocol, as previously described (21).

**Evaluation of total antioxidant level and free radical-scavenging activity.** Total antioxidant levels were examined by a colorimetric total antioxidant assay kit (TA02, Oxford Biochemical Research). Various concentrations (0.001 to 100 μM) of test samples were used for further steps. The antioxidant level was determined by the reduction of Cu²⁺ to Cu⁺ to permit the assessment of the combined action of all antioxidants present in the sample. To detect the generated Cu⁺, a stable complex formed by a reaction between Cu⁺ and bathocuproine was detected at 450 nm. The obtained absorbance values were compared to the standard curve of uric acid as a reductant.

The free radical-scavenging activity of KA and Ac₂KA was measured by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. Various concentrations (0.001 to 100 μM) of test samples were mixed individually

---

**Fig. 1** Structure of KA and Ac₂KA. (A) and (B) indicate KA and Ac₂KA, respectively.
with 100 μM DPPH (Santa Cruz) in ethanol. The reaction mixtures were incubated at 37°C for 20 min, and the absorbance was determined at 517 nm. The percentage of DPPH free radical-scavenging activity was calculated based on the absorbance.

Isolation of total RNA and real-time RT-PCR assay. Total RNA was extracted from the liver sections using ISOGEN II reagent (Nippon Gene, Tokyo, Japan). qRT-PCR was performed with One Step SYBR PrimeScript PLUS RT-PCR kit (Perfect Real Time, Takara Bio) using an Applied Biosystems 7300 (Applied Biosystems, Foster City, CA). The PCR reaction volume was 20 μL and the reaction mixture contained 0.4 μM primers, 0.4 μL ROX Dye, sample RNA (30 ng), 2 × One Step SYBR RT-PCR Buffer 4, TaKaRa Ex Taq HS Mix, and PrimeScript PLUS RTase Mix. PCR conditions were as follows: 42°C for 5 min, 95°C for 10 s, and 40 cycles of 95°C for 5 s and 60°C for 31 s. Gene expression was normalized to GAPDH mRNA levels. The oligonucleotide sequences of the primers were as follows: sense, 5’-TGGTGAAGGTGGTTGAAC-3’, and antisense, 5’-GTCTCTTGGATGGCAACAATCTCC-3’ for the mouse GAPDH (NM_001289726); sense, 5’-CATTCTTGTGTTCAGGATACAAG-3’, and antisense, 5’-GATACTTAGGGAAACCTCCGC-3’ for the mouse Cyp2e1 (NM_021282); sense, 5’-GACACCTCACTGAATGGCTTC-3’, and antisense, 5’-ACACAAAGGGGTCTTTCCACTG-3’ for the mouse Cyp1a2 (NM_009993) (20).

Statistical analysis. Statistical analyses of the differences between two groups were evaluated using the two-tailed Student’s t-test. Statistical analyses of multiple comparisons were estimated by using one-way analysis of variance with the Tukey’s post-hoc test. All statistical analyses were performed using the SPSS 24.0 software (SPSS, Inc., Chicago, IL, USA). P < 0.05 was considered statistically significant.

RESULTS

Effects of KA and Ac₃KA pretreatment on markers of hepatic function in APAP-treated mice

In our initial animal study, we measured plasma ALT and AST activities. Exposure to APAP led to a significant increase in ALT activity at 16 h (Fig. 2A). Pretreatment with KA significantly decreased APAP-induced ALT activity, and Ac₃KA pretreatment was more effective against APAP-induced hepatotoxicity than KA. The same trend was observed in AST activities (Fig. 2B).

Effect of KA and Ac₃KA pretreatment on plasma TNF-α and IL-6 levels in APAP-treated mice

We determined the plasma levels of the pro-inflammatory cytokines, TNF α and IL-6. APAP administration significantly increased plasma TNF α levels. This increase was significantly attenuated by pretreatment with KA and Ac₃KA (Fig. 2C). A similar trend was observed in plasma IL-6 levels (Fig. 2D).

Changes in hepatic malondialdehyde and glutathione levels in APAP-treated mice pretreated with KA and Ac₃KA

We measured liver malondialdehyde level (Fig. 2E) as a marker of lipid peroxidation. APAP administration significantly increased hepatic malondialdehyde levels, whereas pretreatment with KA slightly attenuated the APAP-induced upregulation. Ac₃KA pretreatment more strongly attenuated hepatic malondialdehyde levels than KA. Moreover, APAP significantly decreased hepatic glutathione levels. However, pretreatment with KA and Ac₂KA restored glutathione levels to those comparable with that of the control (Fig. 2F).

Effects of KA and Ac₃KA pretreatment on the hepatic structure of APAP-treated mice

We evaluated liver histopathology using H&E staining (Fig. 3). The liver of APAP-treated mice showed severe necrosis around the central veins (Fig. 3B). The liver of mice pretreated with KA also showed necrosis around the central veins; however, the necrotic area was smaller than that in the APAP-treated group (Fig. 3C). In the mice pretreated with Ac₃KA, no necrotic area was observed, although a slight injury, such as infiltration of inflammatory cells, was detected (Fig. 3D).

We evaluated the hepatoprotective effects of KA and Ac₃KA by TUNEL assay. APAP-treated mice showed DNA fragmentation and extensive DNA damage around the central veins (Fig. 3F). In contrast, pretreatment with KA decreased the number of TUNEL-positive cells (Fig. 3G). Furthermore, TUNEL-positive cells were hardly detected in mice pretreated with Ac₃KA (Fig. 3H).

Hepatoprotective effect of KA and Ac₃KA through RIP and JNK inactivation

APAP-induced hepatotoxicity is thought to involve primary hepatocyte necrosis, with RIP1 and RIP3 emerging as key regulators of necrotic cell death (2, 20). Therefore, we examined the hepatoprotective effect of KA and Ac₃KA on RIP1 and RIP3 expression in APAP-treated mice. No obvious expression
Ac₂KA protects APAP-induced hepatotoxicity

Figure 2: Effect of KA and Ac₂KA pretreatment on hepatic injury marker levels, inflammatory cytokine levels, and oxidative stress. Mice were pretreated with KA, Ac₂KA, or vehicle by oral gavage once daily for 7 days. On day 7, the mice were fasted for 16 h and intraperitoneally injected with 450 mg/kg APAP. The parameters were measured 16 h after APAP administration. Panels (A–D) indicate plasma ALT, AST, TNFα, and IL-6, respectively. Panels (E) and (F) indicate hepatic malondialdehyde (MDA) and glutathione (GSH) levels, respectively. Data are presented as the mean ± SD (n=6–8 per group). *P < 0.05 and **P < 0.01 versus control group, and *P < 0.05 and **P < 0.01 versus APAP group.
Fig. 3 Ac2KA pretreatment protects animals from acute APAP-induced hepatotoxicity, as assessed by H&E staining and TUNEL staining. (A–D) H&E-stained liver sections of mice from the control, APAP, KA + APAP, and Ac2KA + APAP groups, respectively. (E–H) TUNEL-stained liver sections of mice from the control, APAP, KA + APAP, and Ac2KA + APAP groups, respectively. The area inside a dotted circle (B and C) is the area of necrosis. Panel (B) shows severe necrosis and panel (C) shows mild necrosis. Black arrows, TUNEL-positive cells; CV, central vein; bar, 50 μm.
of RIP1 was observed in the control group, whereas RIP1 expression was evident in the APAP-exposed mice (Fig. 4A). RIP1 protein levels were attenuated by pretreatment with KA and, to a greater extent, by pretreatment with Ac2KA. In addition, both KA and Ac2KA pretreatment caused a similar decrease in APAP-induced RIP3 expression.

Several studies have reported the key role of JNK1/2 activation in APAP-induced hepatotoxicity (3, 7). The present study showed that APAP increased the phosphorylation of JNK1/2 (Fig. 4B). The APAP-induced hepatic JNK1/2 phosphorylation was inhibited by pretreatment with either KA or Ac2KA.

**Effect of KA and Ac2KA pretreatment on p65 localization in APAP-treated mice**

Next, using immunohistochemistry, we evaluated the effect of KA and Ac2KA on APAP-induced p65 activation. Almost all of the p65 in the control group was localized in the cytoplasm of hepatocytes (Fig. 5A). On the other hand, APAP increased the level of nuclear p65 in the mouse liver (Fig. 5B). However, pretreatment with KA almost completely prevented APAP-induced p65 localization in the nucleus (Fig. 5C). Moreover, in mice pretreated with Ac2KA, p65 was hard to be detected in the nucleus (Fig. 5D).

**Hepatoprotective effect of KA and Ac2KA evaluated by radical-scavenging activity, antioxidant capacity, and CYP-inducting ability**

To further investigate the protective effects of KA and Ac2KA against APAP-induced hepatic injury, we measured the free radical-scavenging activity (Fig. 6A) and total antioxidant level (Fig. 6B). The radical-scavenging activity of KA and Ac2KA was slightly increased in a dose-dependent manner. Total antioxidant levels in mice pretreated with KA and Ac2KA were increased in a dose-dependent manner, while dose-dependent levels of two chemicals were the same.

We also measured the effect of KA and Ac2KA on hepatic Cyp2e1 (Fig. 6C) and Cyp1a2 mRNA expression (Fig. 6D) because Cyp2e1 and Cyp1a2 are essential in APAP activation. KA pretreatment slightly increased Cyp2e1 and Cyp1a2 mRNA levels, whereas Ac2KA pretreatment significantly decreased both mRNA levels.

**DISCUSSION**

The present study showed that KA pretreatment inhibited APAP overdose-induced acute toxicity in the mouse liver. The effect of Ac2KA, a KA derivative, was more potent than that of KA. Because APAP-induced hepatic injury occurs in several steps, KA and Ac2KA may exert protective effects against multiple steps of APAP action.

Initially, we hypothesized that KA and Ac2KA were antioxidants, as the antioxidant natural products, which are known to prevent APAP-induced hepatic injury (6, 8). Several investigations have reported that ent-kaurene diterpenoid compounds exert antioxidant activities (4, 19). In our current study, KA and Ac2KA showed antioxidant activities; therefore, the protective mechanism of KA and Ac2KA against APAP-induced hepatic injury might be attributed to their antioxidant activities. However, since both chemicals induced the same activities, the difference in their hepatoprotective effects might be due to other mechanisms.

Second possible explanation for the hepatoprotective mechanism of KA and Ac2KA was necrosis-re-
Ac 2 KA through both RIP1 and RIP3 inhibition.

Third explanation for the hepatoprotective mechanism of KA and Ac 2 KA involves mitogen-activated protein kinases (MAPKs). There are three major MAPKs (ERK1/2, p38, and JNK) and each consists of three classes of serine/threonine kinases. The involvement of MAPK in APAP-induced hepatic injury has been shown in several studies (12, 25), and the involvement of JNK is already well-established. JNK is the transactivator of activating transcription factor and activator protein-1. Activated activator protein-1 regulates ROS-induced hepatocyte death. We observed increased levels of phosphorylated JNK by APAP administration. In addition, pretreatment with KA and Ac 2 KA suppressed this increase in JNK phosphorylation, although these levels of suppression were comparable. Collectively, our results provided evidence that pretreatment with KA and Ac 2 KA attenuated the activation of JNK and the transactivation of activating transcription factor and activator protein-1. Thus, the differences in the protective effects of KA and Ac 2 KA against APAP-induced hepatic injury may be explained by the level of RIP1 inhibition. Our current study suggested that KA exerted a protective effect against APAP-induced necrosis through RIP3 inhibition and Ac 2 KA through both RIP1 and RIP3 inhibition.

Fourth explanation for the hepatoprotective mechanism of KA and Ac 2 KA is NF-κB inhibition activity, since KA is known to suppress NF-κB (11). The relation between KA and Ac 2 KA and NF-κB inhibition has been reported previously. In the present study, we observed decreased levels of phosphorylated NF-κB by pretreatment with KA and Ac 2 KA, although these levels of suppression were comparable. Collectively, our results suggested that KA and Ac 2 KA attenuated the activation of NF-κB and the transactivation of activating transcription factor and activator protein-1. Thus, the differences in the protective effects of KA and Ac 2 KA against APAP-induced hepatic injury may be explained by the level of NF-κB inhibition.

Fig. 5 Effect of KA and Ac 2 KA pretreatment on hepatic p65 expression and localization. Liver specimens were isolated, fixed, and processed by standard methods. The expression and localization of p65 (green) were detected using mouse anti-p65 monoclonal antibody and anti-mouse IgG-FITC. Arrows indicate p65 localization in the nucleus.
Ac2KA protects APAP-induced hepatotoxicity

Ac2KA protects APAP-induced hepatotoxicity by inhibiting NF-κB activation. Fifth possible protective mechanism of KA and Ac2KA was inhibition of CYP1A2 and CYP2E1, which prevent the formation of APAP metabolites (toxic NAPQI). We used real-time RT-PCR to investigate the effect of KA and Ac2KA on Cyp2e1 and Cyp1a2 mRNA expression, and showed that Ac2KA significantly attenuated Cyp2e1 and Cyp1a2 gene expression. In contrast, the level of these Cyps was not modulated by KA. These differences might provide an additional hypothesis for the protective mechanisms of KA and Ac2KA against APAP-induced toxicity.

In conclusion, we showed that pretreatment with KA and Ac2KA inhibited APAP-induced hepatic injury and that the hepatoprotective effect of Ac2KA was more potent than that of KA. We considered that the broad hepatoprotective mechanisms of KA included antioxidant activity, MAPK inhibition, and...
RIP-induced necroptosis suppression. Moreover, the effects of Ac2KA included the inhibition of hepatic NF-κB translocation, and Cyp2e1 and Cyp1a2 expression, in addition to those of KA. Further investigations are needed because the present study evaluated only the effects of KA and Ac2KA pretreatment. If post-treatment with KA and Ac2KA has protective effects against APAP-induced hepatotoxicity, KA and Ac2KA might be suitable as alternatives of NAC. Therefore, our current findings have provided a contribution to the development of treatment against acute liver injury and disease.

Acknowledgments

This research financially supported by Kinjo Gakuin University Research Grant.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

1. Aoyagi Y, Ozawa K, Kobayashi T, Hasuda T, Gui M-Y, Jin Y-R, Li X-W, Fukaya H, Yano R, Hitotsuyanagi Y and Takeya K (2014) Efficient transformation of 7,14-dihydroxy-ent-kauranes to novel ent-abietaenes having cis-fused α-methylene γ-lactones under Mitsunobu reaction conditions and their cytotoxicities. Tetrahedron 70, 3030–3041.
2. Christofferson DE and Yuan J (2010) Necroptosis as an alternative form of programmed cell death. Curr Opin Cell Biol 22, 263–268.
3. Ding Y, Li Q, Xu Y, Chen Y, Deng Y, Zhi F and Qian K (2016) Attenuating oxidative stress by paeonol protected against acetaminophen-induced hepatotoxicity in mice. PLoS One 11, e0154375.
4. Ertas A, Ozturk M, Boga M and Topcu G (2009) Antioxidant and anticholinesterase activity evaluation of ent-kaurane diterpenoids from Sideritis arguta. J Nat Prod 72, 500–502.
5. Fukaya S, Nagatsu A and Yoshioka H (2018) The Kampo formula “Juzen-taiho-to” exerts protective effects on ethanol-induced liver injury in mice. Fundam Toxicol Sci 5, 105–112.
6. Ghosh A and Sil PC (2009) Protection of acetaminophen induced mitochondrial dysfunctions and hepatic necrosis via Akt-NF-kappaB pathway: role of a novel plant protein. Chem Biol Interact 177, 96–106.
7. Gunawan BK, Liu ZX, Han D, Hanana N, Gaarde WA and Kaplowitz N (2006) c-Jun N-terminal kinase plays a major role in murine acetaminophen hepatotoxicity. Gastroenterology 131, 165–178.
8. He CY, Liang BB, Fan XY, Cao L, Chen R, Guo YJ and Zhao J (2012) The dual role of osteopontin in acetaminophen hepatotoxicity. Acta Pharmacol Sin 33, 1004–1012.
9. Jaeschke H, Knight TR and Bajt ML (2003) The role of oxidant stress and reactive nitrogen species in acetaminophen hepatotoxicity. Toxicol Lett 144, 279–288.
10. Lauterburg BH and Mitchell JR (1987) Therapeutic doses of acetaminophen stimulate the turnover of cysteine and glutathione in man. J Hepatol 4, 206–211.
11. Lee JH, Koo TH, Hwang BY and Lee JJ (2002) Kaurane diterpene, kamebakaurin, inhibits NF-kappa B by directly targeting the DNA-binding activity of p50 and blocks the expression of antiapoptotic NF-kappa B target genes. J Biol Chem 277, 18411–18420.
12. Ma JQ, Ding J, Zhang L and Liu CM (2014) Hepatoprotective properties of sesamin against CCl4 induced oxidative stress-mediated apoptosis in mice via JNK pathway. Food Chem Toxicol 64, 41–48.
13. McGill MR, Sharpe MR, Williams CD, Taha M, Curry SC and Jaeschke H (2012) The mechanism underlying acetaminophen-induced hepatotoxicity in humans and mice involves mitochondrial damage and nuclear DNA fragmentation. J Clin Invest 122, 1574–1583.
14. Miura N, Yoshioka H, Ashimori A, Ohtani K, Hasegawa T, Hwang GW, Ikeda M and Nonogaki T (2017) Multidirectional analyses of hepatic chronotoxicity induced by cadmium in mice. J Toxicol Sci 42, 597–604.
15. Osborn SL, Diehl G, Han SJ, Xue L, Kurd N, Hsieh K, Cado D, Robey EA and Winoto A (2010) Fas-associated death domain (FADD) is a negative regulator of T-cell receptor-mediated necroptosis. Proc Natl Acad Sci USA 107, 13034–13039.
16. Suzuki B, Ythanze MD, Johnson HS, Gaarde WA, Han D and Kaplowitz N (2014) Protein kinase C (PKC) participates in acetaminophen hepatotoxicity through c-jun-N-terminal kinase (JNK)-dependent and -independent signaling pathways. Hepatology 59, 1543–1554.
17. Sharma M, Gadang V and Jaeschke A (2012) Critical role for mixed-lineage kinase 3 in acetaminophen-induced hepatotoxicity. Mol Pharmacol 82, 1001–1007.
18. Smilkstein MJ, Knapp GL, Kulig KW and Ramnack BH (1988) Efficacy of oral N-acetylcysteine in the treatment of acetaminophen overdose. Analysis of the national multicenter study (1976 to 1985). N Engl J Med 319, 1557–1562.
19. Thirugnanasampandan R, Jayakumar R, Narmatha Bai V, Martin E and Rajendra Prasad KJ (2008) Antiacetylcholine-sterase and antioxidant ent-Kaurene diterpenoid, melissolidesin from Isodon wightii (Benthum) H. Hara. Nat Prod Res 22, 681–688.
20. Vandenabeele P, Galluzzi L, Vanden Bergh T and Kroemer G (2010) Molecular mechanisms of necroptosis: an ordered form of programmed cell death. Nat Rev Mol Cell Biol 11, 700–714.
21. Yoshioka H, Aoyagi Y, Fukushima N, Gui MY, Jin YR, Li XW, Hwang GW, Ohtani K and Miura N and Nonogaki T (2017) Suppressve effect of kamebakaurin on acetaminophen-induced hepatotoxicity by inhibiting lipid peroxidation and inflammatory response in mice. Pharmacol Rep 69, 903–907.
22. Yoshioka H, Nonogaki T, Fukushima N, Shinohara Y, Hwang GW, Ohtani K and Miura N (2017) Chronicity of bromobenzene-induced hepatic injury in mice. J Toxicol Sci 42, 251–258.
23. Yoshioka H, Usuda H, Fujii H and Nonogaki T (2017) Sasa veitchii extracts suppress acetaminophen-induced hepatotoxicity in mice. Environ Health Prev Med 22, 54.
24. Yoshioka H, Usuda H, Nonogaki T, Onosaka S (2016) Carbon tetrachloride-induced lethality in mouse is prevented by multiple pretreatment with zinc sulfate. J Toxicol Sci 41, 55–63.
25. Zhang YF, He W, Zhang C, Liu XJ, Lu Y, Wang H, Zhang ZH, Chen X and Xu DX (2014) Role of receptor interacting protein (RIP)I on apoptosis-inhibiting factor-mediated necroptosis during acetaminophen-evoked acute liver failure in mice. Toxicol Lett 225, 445–453.