Running title: Antioxidant, antinociceptive, and anti-inflammatory activities of Xanthii Fructus

Antioxidant, antinociceptive, and anti-inflammatory activities of Xanthii Fructus extract

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1. Introduction

Xanthii Fructus (XF) which is well known as “Cang-Erzi” in Taiwan is the dried fruit of *Xanthium strumarium* L. Aqueous extract of this fruit has been used for treatment of various diseases for treating sinusitis, headache due to rheumatism and skin pruritus (Hsu et al., 1985; Hsu et al., 200). XF was reported to have inhibitory effects on mast cell-mediated allergic reaction (Hong, et al., 2003), anti-inflammatory actions in lipopolysaccharide-stimulated inflammatory responses (An, et al., 2004) and prevent of ß-cell damage in type 1 diabetes (Song, et al., 2009). However, despite many studies on XF, the effects of AXF on anti-inflammatory and antinociceptive activities have rarely been examined.

The large number of studies has indicated that the reactive oxygen species scavenging inhibition and anti-inflammatory activities seen in herbs may be attributed to the various natural phenolic components with antioxidant effects and reducing activity present in it, such as caffeic acid and caffeoylquinic acids (Han et al., 2007). Thus, it was necessary to explore total polyphenolic contents (Taga et al., 1984) in the aqueous extract of Xanthii Fructus (AXF).

The inflammation process of λ-Carrageenan (Carr) induced edema is a well known model to assess the contribution of natural products against biochemical changes associated with acute inflammation. However, Carr induced inflammatory

process is a complicated function of release of biochemical from tissues and migrating cells (Huang et al., 2010). Previous studies suggested that the oxidative and nitrative stress as well as tumor necrosis factor α (TNF-α) are important mediators of inflammatory metabolism after Carr treatment (Rocha et al., 2006). In the present study, the antioxidant and antinociceptive effects of AXF were examined. We also evaluated the anti-inflammatory effects of AXF on paw edema induced by Carr in mice and investigated the underlying mechanisms *in vivo*.

2. Materials and methods

2.1. Materials

1, 1-Diphenyl-2-picrylhydrazyl (DPPH), *N*-(1-naphthyl) ethylenediamine dihydrochloride, sulfanilamide, 2, 2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), thiobarbituric acid (TBA), λ-Carrageenan (Carr) and indomethacin (Indo) were purchased from Sigma–Aldrich (St. Louis, MO, USA). The samples of Xanthii Fructus were obtained from Sun Ten Pharmaceutical Co., Ltd. in Taipei, Taiwan. The identification of samples was verified by Dr. J.J. Yang (Department of Pharmacy, Chia-Nan University of Pharmacy and Science, Taiwan).

2.2. Preparation of AXF

A 0.1 kg sample of pulverized Xanthii Fructus (XF) was extracted with water (1 L) at 100 ℃ for 60 min and then centrifuged at 10,000×g for 20 min. The extraction was repeated three times. The extracts were then combined and filtered through a No. 1 filer paper. The filtrates were collected, concentrated with a vacuum evaporator until the volume was below 10 mL and then freeze-dried. The yield obtained was 7.9 % (7.9 g; *w/w*). This sample was named as the aqueous extract of Xanthii Fructus (AXF).

2.2.1. Determination of total polyphenols

Total polyphenolics were determined as gallic acid equivalents (Taga et al., 1984). Different concentrations of AXF solutions were added with 2 mL sodium carbonate (20% (*w/v*)) individually to a 10 mL volumetric flask. After 5 min, 0.1 mL Folin–Ciocalteu reagent (50% (*v/v*)) was added and the volume were made up to 10 mL with H_2O. After incubation at 30 ℃ for 1 h, the absorbance at 750 nm was measured and compared to a gallic acid calibration curve.

2.2.2. Determination of DPPH radical inhibition

The effect of samples on the DPPH radical was estimated according to previously described (Hatano et al.,1988). The samples were added to a methanolic

solution (1 mL) of DPPH radical (final concentration of DPPH was 0.2 mM). The mixture was shaken vigorously and allowed to stand at room temperature for 30 min; the absorbance of the resulting solution was then measured spectrophotometrically at 517 nm.

2.2.3. Determination of ABTS radical inhibition

This assay determined the capacity of samples to scavenge the $ABTS^{\bullet+}$ as previously described (Arnao et al., 2001). The $ABTS^{\bullet+}$ was generated by reacting 1 mM ABTS with 0.5 mM hydrogen peroxide and 10 units/ml horseradish peroxidase in the dark at 30 ℃ for 2 h. After 1 ml $ABTS^{\bullet+}$ was added to samples, the absorbance at 734 nm was recorded after 10 min.

2.2.4.. Determination of reducing activity

The reducing power of samples was determined as previously described (Oyaizu, 1986). Potassium ferricyanide (2.5 mL, 10 mg/mL) was added to samples in phosphate buffer (2.5 mL, 200 mM, pH 6.6) and the mixture was incubated at 50 ℃ for 20 min. Trichloroacetic acid (2.5 mL, 100 mg/mL) was added to the mixture, which was then centrifuged at 1000g for 10 min. The supernatant (2.5 mL) was mixed with distilled water (2.5 mL) and ferric chloride (0.5 mL, 1.0 mg/mL), and then the

absorbance at 700 nm was read. Higher absorbance of the reaction mixture indicated greater reducing activity.

2.2.5. Determination of liposome oxidation

Lecithin (580 mg) was sonicated in an ultrasonic cleaner (Branson 8210, Branson ultrasonic Corporation, Danbury , CT, USA) in phosphate buffer (58 mL, 10 mM, pH 7.4) for 2 h in ice-cold water bath. The sonicated solution, $FeCl_3$, ascorbic acid and samples (0.2 mL) were mixed to produce a final concentration of 3.12 μM $FeCl_3$, and 125 μM ascorbic acid. The mixture was incubated for 1 h at 37℃ by the thiobarbituric acid (TBA) method (Tamura et al., 1991). The absorbance of the sample was read at 532 nm against a blank, which contained all reagents except lecithin. A lower level of absorbance indicated a stronger protective activity.

2.3. Animals

Imprinting control region (ICR; 6-8 weeks male) mice were obtained from the BioLASCO Taiwan Co., Ltd. The animals were kept in plexiglass cages at a constant temperature of 22 ± 1 ℃, relative humidity 55±5% with 12 h dark-light cycle for at least 2 weeks before the experiment. They were given food and water *ad libitum*. All experimental procedures were performed according to the NIH Guide for the Care and

Use of Laboratory Animals. The control groups were given 0.1 mL/10 g saline intraperitoneally using a bent blunted 27-gauge needle connected to a 1 mL syringe. All tests were conducted under the guidelines of the International Association for the Study of Pain (Zimmermann, 1983). This study was approved by the ethics committee of the Institutional Animal Care and Use Committee (IACUC) of China Medical University.

2.3.1. Evaluation of Acute Toxicity

Different concentrations of AXF were orally administered to 5 groups of 10 mice in order to estimate acute toxicity. Signs of toxicity during the first hour were observed and recorded. Ten control animals were given a vehicle (saline). After the acute phase, animals were observed for 14 days, keeping a record of the toxicity and mortality (Rivera et al., 2004). Food and water were provided throughout the experiment. If the mice died, they would be checked for autopsy and biochemical profiles.

2.3.2. Acetic acid-induced writhing response

After a 2-week adaptation period, male ICR mice (18 to 25 g) were randomly assigned to six groups (n = 8) including a normal control, an Indo positive control and four AXF-treated groups. Control group received 1% acetic acid (10 ml/kg body

weight) and the positive control group received Indo (10 mg/kg, i.p.) 25 min before intraperitoneal injection of 1% acetic acid (10 ml/kg body weight). AXF-treated groups received AXF (0.1, 0.5, and 1.0 g/kg, p.o.) 55 min before intraperitoneal injection of 1% acetic acid (10 mL/kg body weight). Five minutes after the i.p. injection of acetic acid, the number of writhings during the following 10 minutes was recorded (Sheua et al., 2009).

2.3.3. Formalin test

The antinociceptive activity of the drugs was determined using the formalin test (Dubuisson and Dennis, 1977). Control group received 5% formalin. Twenty micro-liter of 5% formalin was injected into the dorsal surface of the right hind-paw 60 min after administration of AXF (0.1, 0.5, and 1.0 g/kg, p.o.) and 30 min after administration of Indo (10 mg/kg, i.p.). The mice were observed for 30 min after the injection of formalin, and the amount of time spent licking the injected hind paw was recorded. The first 5 min post formalin injection is referred to as the early phase and the period between 15 min and 40 min as the late phase. The total time spent licking or biting the injured paw (pain behavior) was measured with a stop watch. The activity was recorded in 5 min intervals.

2.3.4. Determination of carrageenan (Carr) induced edema

Carr-induced hind paw edema model was used for determination of anti-inflammatory activity (Winter et al., 1962). After a 2-weeks adaptation period, male ICR mice (18 to 25 g) were randomly assigned to five groups (n = 6) including Carr, positive Indo control and three AXF-treated groups. Carr group received 1% Carr (50 μL). AXF at doses of 0.1, 0.5, and 1.0 g/kg were orally administered 2 h before the injection with 1% Carr (50 μL) in the plantar side of right hind paws of the mice. And Indo (10 mg/kg) was intraperitoneally administered 90 min before the injection with 1% Carr (50 μL) in the plantar side of right hind paws of the mice. Paw volume was measured immediately after Carr injection at 1, 2, 3, 4 and 5 h intervals using a plethysmometer (model 7159, Ugo Basile, Varese, Italy). The degree of swelling induced was evaluated by *a* minus *b*, where *a* was the volume of the right hind paw after Carr treatment and *b* was the volume of the right hind paw before Carr treatment. Indo was used as a positive control.

In the later experiment, the right hind paw tissue and liver tissue were taken at the 5 h. The right hind paw tissue was rinsed in ice-cold normal saline, and immediately placed in cold normal saline four times their volume and homogenized at 4 ℃. Then the homogenate was centrifuged at 12,000×*g* for 5 min. The supernatant was obtained and stored at −20 ℃ refrigerator for MDA assays. The whole liver tissue was rinsed

in ice-cold normal saline, and immediately placed in cold normal saline one time their volume and homogenized at 4 ℃. Then the homogenate was centrifuged at 12,000*g* for 5 min. The supernatant was obtained and stored in the refrigerator at −20 ℃ for the antioxidant enzymes (CAT, SOD, and GPx) activity assays.

2.3.5. Determination of tissue lipid peroxidation

MDA was evaluated by the thiobarbituric acid reacting substances (TRARS) method (Ohishi et al., 1985). Briefly, MDA reacted with thiobarbituric acid in the acidic high temperature and formed a red-complex TBARS. The absorbance of TBARS was determined at 532 nm.

2.14. Determination of nitric oxide in serum

Serum NO production was indirectly assessed by measuring the nitrite levels as mentioned above. Serum samples were diluted 4 times with distilled water and deproteinized by adding zinc sulfate (300 mg/mL) to a final concentration of 15 mg/ml. After centrifugation at 10,000*g* for 5 min at room temperature, 100 μL of supernatant was applied to a microplate, followed by 100 μL of Griess reagent. After 10 min of color development at room temperature, the absorbance was measured and compared to a sodium nitrite calibration curve.

2.3.6. Measurement of tumor necrosis factor (TNF-α) in serum

Serum levels of TNF-α were determined using a commercially available ELISA kit (Biosource International, Inc., Camarillo, CA) according to the instructions of the manufacturer. TNF-α was determined from a standard curve.

2.3.7. Determination of antioxidant enzyme activity in liver

The following biochemical parameters were analyzed to check the hepatoprotective activity of AXF by the methods given below. Total SOD activity was determined by the inhibition of cytochrome *c* reduction (Flohe and Otting 1984). The reduction of cytochrome *c* was mediated by superoxide anions generated by the xanthine/xanthine oxidase system and monitored at 550 nm. One unit of SOD was defined as the amount of enzyme required to inhibit the rate of cytochrome *c* reduction by 50%. Total CAT activity estimation was based on the previously reported (Armstrong & Browne, 1994). In brief, the reduction of 10 mM H_2O_2 in 20 mM of phosphate buffer (pH 7) was monitored by measuring the absorbance at 240 nm. The activity was calculated by using a molar absorption coefficient, and the enzyme activity was defined as nanomoles of dissipating hydrogen peroxide per milligram

protein per minute. Total GPx activity in cytosol was determined as previously reported (Flohe & Gunzler, 1984). The enzyme solution was added to a mixture containing hydrogen peroxide and glutathione in 0.1 mM Tris buffer (pH 7.2) and the absorbance at 340 nm was measured. Activity was evaluated from a calibration curve, and the enzyme activity was defined as nanomoles of NADPH oxidized per milligram protein per minute. The protein concentration of the tissue was determined by the Bradford dye-binding assay (Bio-Rad, Hercules, CA).

2.3.8. Histological examination

For histological examination, biopsies of paws were taken 5 h following the interplanetary injection of Carr. The tissue slices were fixed in (1.85% formaldehyde, 1% acetic acid) for 1 week at room temperature, dehydrated by graded ethanol and embedded in Paraffin (Sherwood Medical). Sections (thickness 5 μm) were deparaffinized with xylene and stained with H & E stain. All samples were observed and photographed with BH2 Olympus microscopy. Every 3~5 tissue slices were randomly chosen from Carr-, Indo- and AXF-treated (1.0 g/kg) groups. The numbers of neutrophils were counted in each scope (400 x) and thereafter obtain their average count from 5 scopes of every tissue slice.

2.3.9. Protein Lysate Preparation and Western blot Analysis of iNOS and COX-2

Total protein was extracted with a RIPA solution (radioimmuno-precipitation assay buffer) at -20℃ overnight. We used BSA (bovine serum albumin) as a protein standard to calculate equal total cellular protein amounts. Protein samples (30 μg) were resolved by denaturing sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) using standard methods, and then were transferred to PVDF membranes by electroblotting and blocking with 1% BSA. The membranes were probed with the primary antibodies (iNOS, COX-2 and β-actin) at 4℃ overnight, washed three times with PBST, and incubated for 1 h at 37 ℃ with horseradish peroxidase conjugated secondary antibodies. The membranes were washed three times and the immunoreactive proteins were detected by enhanced chemiluminescence (ECL) using hyperfilm and ECL reagent (Amersham International plc., Buckinghamshire, U.K.). The results of Western blot analysis were quantified by measuring the relative intensity compared to the control using Kodak Molecular Imaging Software and represented in the relative intensities.

2.4. Statistical analysis

Data are expressed as mean ± S.E.M. Statistical evaluation was carried out by one-way analysis of variance (ANOVA followed by Scheffe's multiple range test).

Statistical significance is expressed as $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$.

3. Results

3.1. The contents of total phenols and the antioxidant activities of AXF

The antioxidant activities observed in Xanthii Fructus were recognized as the phenolic components including caffeic acid, 1,3,5-tri-O-caffeoyl quinic acid, potassium 3-O-caffeoyl quinate and 1,5-tri-O-caffeoyl quinic acid present in Xanthii Fructus (Sheu et al., 2003). The contents of total phenols in the AXF were expressed in gallic acid equivalents (Table 1). The results showed that the AXF contained amounts of total phenols equal to 18.2 mg gallic acid/mL at 0.2 mg dry extract/mL. In other words, the levels of total phenols in the AXF were 91.0 mg/g extract.

Table 1 also shows ABTS scavenging, DPPH scavenging, reducing power, and liposome protection of the AXF. AXF showed 70.6-76.4% and 35.2-79.1% scavenging activity on ABTS radicals and DPPH radical scavenging in the range of 0.05-0.2 mg/mL. Reducing activity of natural products can usually achieved by terminating the radicals' chain reaction. The reducing power of AXF was a concentration-dependent manner and increased by 2.95-fold in the range of 0.05-0.2 mg/mL. Lipid peroxidation producing toxic aldehyde is a harmful process. Liposome protection was used as an index to assay the protective action of the AXF on lipid

molecules. AXF in the range of 0.05-0.2 mg/mL exhibited a dose-dependently protective effect, 6.6-25.0%, on the liposome damage induced by the Fe^{3+}/H_2O_2 reaction (Table 1). Further, the correlation between the DPPH inhibition, reducing activity as well as liposome protection, respectively, and the levels of total phenols was determined by linear regression analysis. The correlation coefficients (r^2) for DPPH inhibition, reducing activity as well as liposome protection were above 0.9. These observations implied that the antioxidant properties of AXF were well correlated with the levels of total phenols in AXF (data not shown).

3.2. Evaluation of Acute Toxicity in Mice

When AXF was studied by oral administration in mice, no signs of toxicity were observed. In the LD_{50} experiment, no mice died under the dose of 10 g/kg.

3.2.1. Acetic acid-induced writhing response

The cumulative amount of abdominal stretching correlated with the level of acetic acid-induced pain (Fig. 1A). AXF treatment (0.1, 0.5 and 1.0 g/kg) significantly inhibited the number of writhing in comparison with control. The inhibition rates of the number of writhing compared with control are 25.49%, 37.25%, and 47.06%, respectively. This inhibiting effect of acetic acid-induced writhing by AXF (1.0 g/kg) was similar to that produced by a positive control Indo (10 mg/kg) ($P < 0.001$).

3.2.2. Formalin test

AXF significantly inhibited formalin-induced pain in the late phase; however, there was no inhibition in the early phase (Fig. 1B). AXF treatment (0.1, 0.5 and 1.0 g/kg) significantly inhibited the formalin-induced pain (late phase) in comparison with the normal controls. The inhibition rates of formalin-induced licking compared with the control are 35.29%, 37.50%, and 58.82%, respectively. This inhibiting effect of formalin-induced licking time by AXF (1.0 g/kg) was similar to that produced by a positive control Indo (10 mg/kg) ($P < 0.001$).

3.2.3. λ-Carrageenan (Carr)-induced edema

According to Fig. 2, Indo reduced the edema volumes in comparison to control group during the 5 h of Carr treatment. Further, in the range of 0.1–1.0 g/kg, AXF showed a concentration dependent inhibition on edema development after 5 h of Carr treatment. AXF at the concentration of 1 g/kg, the levels of edema volume were decreased to 68.9% of that observed in the Carr alone group. These data implied that AXF could exhibit an inhibitor of edema in acute inflammatory processes.

3.2.4. Effects of AXF on MDA, NO and TNF-α levels

Lipid peroxidation serves as a marker of cellular damage and has been recognized as resulting from inflammatory processes. As shown in Fig. 3A, MDA level in the edema paw induced by Carr was significantly increased. However, AXF treatment (0.1, 0.5 and 1.0 g/kg) significantly inhibited the Carr-induced MDA level in comparison with the Carr group. The inhibition rates MDA levels compared with the Carr group are 25.9%, 38.4%, and 45.5%, respectively.

The level of nitrite in serum is a regular index for intracellular NO and iNOS production in *vivo*. As shown in Fig. 3B and 3C, Carr increased the level of nitrite and TNF-α in serum in comparison to control group. Meanwhile, in the range of 0.1-1.0 g/kg, AXF could inhibit the level of nitrite to 70-45% of that observed in Carr group. AXF also could inhibit the level of TNF-α to 73-55% of that observed in Carr group. These data implied that AXF could exhibit an inhibitor of tissue NO and TNF-α production *in vivo* in Carr induced inflammatory processes.

3.2.5. Effects of AXF on the activities of antioxidant enzymes

Under healthy condition, free radicals are prevented by enzymes directly interacting with ROS (e.g. CAT, SOD, and GPx) in the liver. As shown in Table 2, Carr decreased the activities of CAT, SOD, and GPx in liver by 36%, 29%, and 29%, respectively, in comparison to control group. In the range of 0.1-1.0 g/kg, AXF could

increase the activities of CAT to 115%-137%, SOD to 104%-122%, and GPX to 109%-117%, respectively, of that observed in Carr along group. Indo also exhibited increase effects in the activities of CAT (126%), SOD (129%), and GPx (118%) in comparison to Carr group. These data implied that the protective effects of AXF might be attributed to its elevation in antioxidant enzymes activities of Carr induced mice.

3.2.6. Histological examination.

Further, paw biopsies of animals treated with the AXF (1.0 g/kg) showed a reduction in inflammatory response Carr-induced. Actually inflammatory cells were reduced in number and confined to near the vascular areas. Intercellular spaces did not show any cellular infiltrations. Collagen fibers were regular in shape and showed a reduction of intercellular spaces. Moreover the hypoderm connective tissue was not damaged (Fig. 4). Neutrophils were notified increased with Carr treatment ($p < 0.001$). Indo and the AXF (1.0 g/kg) could significantly decrease the neutrophils numbers as compared to the Carr-treated group ($p < 0.01$ or $p < 0.001$) (Fig. 4E).

3.2.7. Effects of AXF on Carr-induced iNOS and COX-2 protein expressions in Mice Paw Edema

The results showed that injection of AXF (1.0 g/kg) on Carr-induced for 5 h inhibited iNOS and COX-2 proteins expression in mouse paw edema (Fig. 5A). The intensity of protein bands were analyzed and showed an average of 54.6% and 52.4% down-regulation of iNOS and COX-2 protein, respectively, after treatment with AXF at 1.0 g/kg compared with the Carr-induced alone (Fig. 5B). In addition, the protein expression showed an average of 63.6% and 57.2% down-regulation of iNOS and COX-2 protein after treatment with Indo at 10 mg/kg compared with the Carr-induced alone.

4. Discussion

Xanthii Fructus, which is commonly used in Chinese medicine, was reported to have anti-inflammatory effects in RAW264.7 macrophage cell (An, et al., 2004). Therefore, in this study, we focused on the effects of the antioxidant, antinociceptive, and anti-inflammatory effects of AXF were examined with the objective of elucidating the signaling mechanism through which they exert their effects.

There are many studies suggested that natural components, such as the phenolics of plant extracts can exhibit antioxidants and regular cellular redox states. The protective activities of AXF were contributed to their phenolic components including caffeic acid, 1, 3, 5-tri-O-caffeoyl quinic acid, potassium 3-O-caffeoyl quinate, and 1,

5-tri-O-caffeoyl quinic acid. Caffeic acid, a member of polyphenols, has been shown to act as a potent antioxidant, anti-inflammatory component and exerts a protective effect in traumatic brain injury (Zhang, et al., 2007). Caffeoyl quinic acids exhibited significant activities such as anti-oxidant, anti-inflammatory, enzyme inhibition, liver-protecting activity, and anticoagulant activity (Hung, et al., 2006; Zhao, et al., 2006). In this study, AXF showed multiple biological activities including antioxidant, antinociceptive effects and decrease of Carr induced inflammatory paw edema. Apparently, these compounds in AXF contribute to its antioxidant and anti-inflammatory effects. These activities were closely correlated with its polyphenolic constituents though other unknown active components in the AXF could also play important roles in its protective effects.

In this study, these data implied that the total phenolics of AXF could exhibit contribution to the radical scavenging, reducing activities as well as lipid protection. In fact, the overproduction of NO could induce DNA damage as well as cytotoxicity. Previously paper evidenced that the inhibitory effects of AXF on LPS induced NO production could contribute to the decrease of oxidative stress and inflammation development (An, et al., 2004). Free radical has been proposed to play an important role in the Carr induced acute inflammatory response (Salvemini et al., 1996). Therefore, AXF decreased NO production *in vivo* could further lead to reduce the

edema response of Carr treatment. Additionally, TNF-α is a major mediator of Carr induced inflammation and can increase the further release of kinins and leukotrienes, which promote vasodilation and capillary permeability (Huang et al., 2010). AXF decreased TNF-α and NO productions in Carr induced mice, which suggested that AXF could decrease the release of cytokines and inhibit the inflammatory responses *in vivo*.

The Carr induced edema test is a useful model to determine the anti-inflammatory effects of natural products (Kumar & Kuttan, 2009). According to Fig. 2, the degree of swelling of the Carr-injected paws was maximal 3 h. after injection and the mean increase in volume at that time was about 100% in the control group. Statistical analysis revealed that AXF (1.0 g/kg) significantly inhibited the development of Carr-induced paw edema after 3 h. of treatment ($p < 0.01$). Additionally, in this study, there is a significant increase in CAT, SOD and GPx activities with AXF treatment. Furthermore, there is a significant decrease in the tissue MDA level with AXF treatment in Carr induced mice. The decrease of MDA production is probably due to the increase of CAT, SOD and GPx activities. Consequently, AXF could positively regulate cellular antioxidative activities against inflammatory oxidation and antioxidant system defect *in vivo*.

In conclusion, these data suggested that AXF showed antioxidant and

anti-inflammatory effects. The anti-inflammatory effects of AXF may be related to iNOS reduction and associated with the increase in the activities of antioxidant enzymes (CAT, SOD, and GPx). Although our study revealed the possible protective effects of AXF against oxidantive and inflammatory effects, these results are still not enough for the safety assessment of AXF. Further investigations of the physiological effects of Xanthii Fructus are still intensively required.

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