

Anti-inflammatory Effects of *Scoparia dulcis* L. and Betulinic Acid

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Abstract

The aims of this study intended to investigate the anti-inflammatory activity of the 70% ethanol extract from *Scoparia dulcis* (SDE) and betulinic acid based on λ -carrageenan-induced paw edema in mice. The anti-inflammatory mechanisms of SDE and betulinic acid were examined by detecting the levels of cyclooxygenase-2 (COX-2), nitric oxide (NO), tumor necrosis factor (TNF- α), interleukin-1 β (IL-1 β) and malondialdehyde (MDA) in the edema paw tissue and the activities of superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione reductase (GRd) in the liver. The betulinic acid content of SDE was detected by high performance liquid chromatography (HPLC). In the anti-inflammatory model, the results showed that SDE (0.5 and 1.0 g/kg) and betulinic acid (20 and 40 mg/kg) reduced the paw edema at 3, 4 and 5 hours after λ -carrageenan administration. Moreover, SDE and betulinic acid affected the levels of COX-2, NO, TNF- α and IL-1 β in the λ -carrageenan-induced edema paws. The activities of SOD, GPx and GRd in liver tissues were increased and the MDA levels in the edema paws were decreased. It is suggested that SDE and betulinic acid possessed anti-inflammatory activities and the anti-inflammatory mechanisms appear to be related to the reduction of the levels of COX-2, NO, TNF- α and IL-1 β in inflamed tissues, as well as the inhibition of MDA level via increasing the activities of SOD, GPx and GRd. The analytical result showed

that the content of betulinic acid was 6.25 mg/g extract.

Keywords: *Scoparia dulcis*; Betulinic acid; Anti-Inflammation; Cyclooxygenase-2;

Nitric Oxide; Malondialdehyde; Tumor Necrosis Factor- α .

Introduction

Scoparia dulcis L. (*Scrophulariaceae*), a small, much branched, glabrous leafy annual herb, is a **well-known** folk medicine used for hypertension in Taiwan (Chow *et al.*, 1974). In India, it is used to treat diabetes, toothache and gastric disorders (Satyanarayana, 1969). In recent years, some dietary supplements containing *S. dulcis* were used as health foods and drinks. Phytochemical investigations on *S. dulcis* have been reported to contain steroids, diterpenoids, triterpenoids, flavonoids and benzenoids (Kawasaki *et al.*, 1988; Hayashi *et al.*, 1993). Betulinic acid is one of the constituents isolated from *S. dulcis* (Mahato *et al.*, 1981), and has been reported to possess anti-HIV, anti-bacterial, antimalarial, anticancer, analgesic and anti-inflammatory activities (Yogeeswari and Sriram, 2005). There have been a number of studies that indicated the pharmacological activities of *S. dulcis*, such as anti-viral (Hayashi *et al.*, 1988), analgesic, anti-inflammatory (Freire *et al.*, 1993), anti-ulcerative (Sonia *et al.*, 2007) and anti-hyperglycemia effects (Pari and Venkateswaran, 2002). In our previous study, the hepatoprotective effect of *S. dulcis* was proved (Tsai *et al.*, 2010). Furthermore, even though the anti-inflammatory effect of *S. dulcis* have been reported, the mechanism was not clear enough.

Inflammation is a series of processes involving cytokines and various mediators, such as prostaglandins and leukotrienes. Pro-inflammatory mediators (e.g., TNF- α

and IL1- β) excite the production of many cytokines during the response to inflammation, including prostaglandins (PGs) and NO (Chao *et al.*, 2009). Previous studies indicated that the inflammatory response induced by λ -carrageenan was also proved to be associated with antioxidant enzyme activities (Lu *et al.*, 2007). Therefore, the aim of this study is to investigate the effects of the 70% ethanol extract from *S. dulcis* (SDE) and betulinic acid by the inflammation induced by λ -carrageenan in mice. We also examined the levels of cyclooxygenase-2 (COX-2), nitric oxide (NO), tumor necrosis factor (TNF- α), interleukin-1 β (IL-1 β) and malondialdehyde (MDA) in the edema paw and the activities of superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione reductase (GRd) in the liver to investigate the anti-inflammatory mechanisms of SDE and betulinic acid. Additionally, the content of betulinic acid was analyzed by HPLC.

Materials and Methods

Plant Materials

Whole plants of *S. dulcis* were collected from Taichung County, Taiwan as described by Flora of Taiwan. A voucher specimen (Number: TNM S100389) was deposited in the National Museum of Natural Science (TNM), Taichung, Taiwan. The plants were identified by Dr. Hsin-Fu Yen, Associate Researcher of the National

Museum of Natural Science, Taichung, Taiwan. It was authenticated in many aspects, including morphology (flowers, fruits, seeds and pollens), histological microscopic examination (leaves and stems) and ITS (Internal Transcribed Spacer) region of rDNA. The sequence data was submitted to the National Center for Biotechnology Information (NCBI) GenBank.

Chemicals

The following chemicals and reagents, betulinic acid, λ -carrageenan, indomethacin, Griess reagent, etc., were purchased from Sigma-Aldrich Chemical Co. The SOD, GPx, GRd and MDA activity assay kits were purchased from Randox Laboratory Ltd. The NO and COX-2 assay kits were purchased from Cayman Chemicals Co. Chemicals and enzyme immunometric assay kits for mouse IL-1 β and TNF- α were obtained from eBioscience Inc. All of the other reagents used were analytical grade.

Preparation of Plant Extracts

Whole plants of *S. dulcis* were cut into small pieces and air dried, then crushed **into** coarse powder. The coarse powder (1.5 kg) was extracted with 2L of 70% ethanol three times. The filtrates were evaporated under reduced pressure with a vacuum

rotary evaporator. The remaining solution was lyophilized and 108 g (7.2 % net gain) of crude extract was yielded. The extract was stored in a refrigerator before the experiment.

Experimental Animals

Male ICR mice (20~25 g) were purchased from BioLasco Charles River Technology, Taipei, Taiwan. They were raised in the animal center of China Medical University at 22 ± 1 °C, relative humidity 55 ± 5 %, with a light and dark cycle of 12 hours for at least one week before the experiment. Animals were provided with a rodent diet and clean water *ad libitum*. Animal tests used in this study were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals. The experimental protocol was approved by the Committee on Animal Research, China Medical University, under the code 99-134.

λ -Carrageenan-Induced Mice Paw Edema

The anti-inflammatory activities of SDE and betulinic acid were determined by the λ -carrageenan-induced edema test in the hind paws of mice. Male ICR mice (10 per each group) were fasted for 24 hours before the experiment with free access to water. 50 μ l of a 1% λ -carrageenan suspension in saline was injected into the plantar

side of right hind paws of the mice (Posadas *et al.*, 2004). Paw volume was measured at 1, 2, 3, 4 and 5 hours after the administration of the λ -carrageenan using a plethysmometer. The degree of swelling was evaluated by the delta volume ($a-b$), where a and b were the volume of the right hind paw after and before the λ -carrageenan treatment, respectively. Indomethacin (20 mg/kg, p.o.), SDE (0.1, 0.5 and 1.0 g/kg, p.o.) and betulinic acid (10, 20 and 40 mg/kg, p.o.) were administered at 2 hours after λ -carrageenan injection. The control group was given an equal volume of saline.

In the secondary experiment, the whole right hind paw tissues and liver tissues were taken at the third hour. The right hind paw tissue was rinsed in ice-cold normal saline, and immediately placed in its four volumes of cold normal saline and homogenized at 4°C. Then the homogenate was centrifuged at 12,000 rpm for 5 minutes. The supernatant was stored at -80°C for the COX-2, NO, TNF- α , IL-1 β and MDA assays. Additionally, the whole liver tissue was rinsed in ice-cold normal saline, and immediately placed in an equal volume of cold normal saline and finally homogenized at 4°C. Then the homogenate was centrifuged at 12,000 rpm for 5 minutes. The supernatant was obtained and stored at -80°C for the antioxidant enzymes (SOD, GPx, and GRd) activity assays.

COX-2 assay

COX-2 was examined according to manufacturer's instructions. The peroxidase activity of COX-2 was assayed colorimetrically by monitoring the appearance of oxidized N, N, N', N'- tetramethyl-*p*-phenylenediamine (TMPD) at 590 nm. The COX-2 activity was expressed as U/ml per mg protein. One unit was equal to nmol/min.

NO assay

NO was measured based on the method of Moshage *et al.* (1995). For nitrite determination, nitrate was converted into nitrite utilizing nitrate reductase; NO_2^- was measured by using the Griess reaction (Green *et al.*, 1982). The absorbance of the final product (purplish red) was determined at 540 nm. Values obtained by this procedure represent the sum of nitrite and nitrate.

TNF- α and IL-1 β assays

TNF- α and IL-1 β assays were measured by enzyme-linked immunosorbent assays (ELISA). Assays were performed according to manufacturer's instructions. The amount of TNF- α and IL-1 β were determined by reference to standard curves

(0-1000 pg/ml) constructed in each assay. The concentration of TNF- α and IL-1 β in each sample were expressed as picogram per milligram protein (pg/mg) for cytokine concentration.

MDA assay

MDA was evaluated by the thiobarbituric acid reacting substance (TBARS) method (Draper and Hadley, 1990). Briefly, MDA reacted with thiobarbituric acid in an acidic condition with high temperature (above 90°C) and formed a red-complex TBARS. The absorbance of TBARS was determined at 532 nm.

Antioxidant Enzymatic Activity Measurements

The following biochemical parameters were analyzed to evaluate the antioxidant activities of SDE and betulinic acid by the methods given below. SOD enzymatic activity was determined in accordance with the method of Misra and Fridovich (1972) at room temperature. 100 μ l of liver homogenate supernatant was added to 880 μ l (0.05 M, pH 10.2, 0.1 mM EDTA) carbonate buffer. 20 μ l of 30 mM epinephrine (in 0.05% acetic acid) was added to the mixture at 480 nm for 4 minutes on a Hitachi U 2000 Spectrophotometer. The enzymatic activity was expressed as the amount of enzyme that inhibits the oxidation of epinephrine by 50% which is equal to one unit.

GPx enzyme activity was determined according to the method of Flohe and Gunzler (1984) at 37°C. A reaction mixture consisted of 500 µl phosphate buffer, 100 µl 0.01 M GSH (reduced form), 100 µl 1.5 mM NADPH and 100 µl GRd (0.24 units). 100 µl of supernatant was added to the reaction mixture and incubated at 37°C for 10 minutes. Then 50 µl of 12 mM *t*-butyl hydroperoxide was added to 450 µl of the tissue reaction mixture and measured at 340 nm for 180 seconds. The molar extinction coefficient of 6.22×10^{-3} was used to determine the enzymatic activity. One unit of activity was equal to the mM of NADPH oxidized/min per mg protein.

GRd enzyme activity was determined by the method of Carlberg and Mannervik (1985) at 37°C. 50 µl of NADPH (2 mM) in 10 mM Tris buffer (pH 7.0) was added in a cuvette containing 50 µl of GSSG (20 mM) in phosphate buffer. 100 µl of supernatant was added to the NADPH-GSSG buffered solution and measured at 340 nm for 3 minutes. The molar extinction coefficient of 6.22×10^{-3} was used to determine the GRd enzyme activity. One unit of activity was equal to the mM of NADPH oxidized /min per mg protein.

Phytochemical Analysis of SDE by HPLC

The HPLC profile was established for betulinic acid and SDE. The HPLC instrument used in this study contained a Hitachi L-7200 autosampler, a Hitachi

L-7100 HPLC solvent delivery pump, and a Hitachi L-7455 diode array detector. Chromatographic separation was performed with a LiChroCART RP-18 endcapped column (250 × 4.6 mm, i.d., 5 µm pore size, Merck, Germany). The mobile phase consisted of 0.2 % formic acid and acetonitrile (25:75, v/v), under isocratic conditions. The sample injection volume was 20 µl. The flow rate was 1.0 ml/min and the detection wavelength was 205 nm. Three injections were performed for each sample.

Statistical Analysis

All data were represented as mean ± SE. Statistical analyses were performed with SPSS software. Statistical analyses were carried out using one-way ANOVA followed by Scheffe's multiple range test.

Results

Effects of SDE and Betulinic Acid on λ-carrageenan-induced Mice Paw Edema

The results of λ-carrageenan-induced mice paw edema were represented in Table 1, it was observed that SDE (0.5 and 1.0 g/kg) and betulinic acid (20 and 40 mg/kg) significantly inhibited ($p < 0.01-0.001$) the development of paw edema induced by carrageenan after 3, 4 and 5 hours of treatment. A similar result was observed by treatment with indomethacin (20 mg/kg, $p < 0.01-0.001$).

Effects of SDE and Betulinic Acid on COX-2 level Measurements

As shown in Fig. 1, the activity of COX-2 increased significantly in the edema paw of mice after carrageenan administration on the third hour. However, COX-2 activities were decreased significantly by treatments with SDE (0.5 and 1.0 g/kg) and betulinic acid (20 and 40 mg/kg), as well as indomethacin at 20 mg/kg ($p < 0.05-0.001$).

Effects of SDE and Betulinic Acid on NO level Measurements

The results of NO level were shown in Fig. 2. The NO level in the edema paw induced by λ -carrageenan was significantly increased. There **was** a significant effect in the NO level when treating with SDE (0.5 and 1.0 g/kg) and betulinic acid (20 and 40 mg/kg) ($P < 0.01-0.001$).

Effects of SDE and Betulinic Acid on TNF- α and IL-1 β Levels

As the results **shown**, TNF- α level in the λ -carrageenan induced edema paws was raised significantly. The increased TNF- α levels were reduced by treatment with SDE (0.5 and 1.0 g/kg) and betulinic acid (20 and 40 mg/kg) ($P < 0.01-0.001$, Fig. 3). The increased IL-1 β levels were decreased by treatment with SDE at dose of 1.0 g/kg and betulinic acid at dose of 40 mg/kg ($P < 0.05$, Fig. 4).

Effects of SDE and Betulinic Acid on MDA level Measurements

As shown in Fig. 5, the levels of MDA in the edema paw induced by λ -carrageenan were significantly elevated. However, MDA levels were reduced by pretreatment with SDE 0.5 g/kg ($P<0.05$) and 1.0 g/kg ($P<0.01$), as well as betulinic acid (20 and 40 mg/kg) ($P<0.01$) and indomethacin (20 mg/kg) ($P<0.001$).

Measurements of Antioxidant Enzymatic Activities

The results of antioxidant enzymes such as SOD, GPx and GRd at the 3rd hour following the intrapaw injection of λ -carrageenan in mice are presented in Table 2. SOD, GPx and GRd activities in liver tissue were decreased significantly after λ -carrageenan administration at the 3rd hour. Treatment with SDE at doses of 0.5 g/kg and 1.0 g/kg, betulinic acid at a dose of 40 mg/kg and indomethacin at a dose of 20 mg/kg increased the levels of SOD, GPx and GRd activities significantly.

Phytochemical Analysis of SDE by HPLC

The HPLC chromatographic profiles of betulinic acid and SDE are shown in Fig. 6. In the chromatogram of the standard, a peak of betulinic acid at the retention time of 17.68 min was detected. A similar peak was also observed in the SDE

chromatogram. According to the calibration curve, the content of betulinic acid in SDE was 6.25 mg/g of extract.

Discussion

The anti-inflammatory activities of SDE and betulinic acid were evaluated in λ -carrageenan-induced paw edema, an *in vivo* animal model of acute inflammation commonly employed for assessing the anti-edematous effect of various natural products and experimental compounds (Lai *et al.*, 2010). The cellular and molecular mechanism of the λ -carrageenan-induced inflammation is well characterized. In our study, SDE, betulinic acid and indomethacin revealed anti-inflammatory effects in λ -carrageenan-induced mice paw edema. These findings demonstrated that SDE and betulinic acid have *in vivo* anti-inflammatory effects in λ -carrageenan-induced acute inflammation.

It is **well-known** that the degree of the edema induced by λ -carrageenan was maximal 3 hours after injection (Kirkova *et al.*, 1992). Inflammation response induced by λ -carrageenan immediately induced the release of several inflammatory mediators such as histamine, serotonin and bradykinin, and then further the biosynthesis of prostaglandins (PGs) and nitric oxide (NO), which are produced by inducible isoforms of COX (COX-2) and nitric oxide synthase (iNOS), respectively (Seibert *et*

al., 1994). COX-2 is responsible for the biosynthesis of PGs under acute inflammatory conditions (Xie et al., 1991). NO, produced by inducible nitric oxide synthase (iNOS) during conversion of l-arginine to l-citrulline, is an important pro-inflammatory mediator in the pathogenesis of inflammation (Salvemini *et al.*, 1996). The COX-2 and NO levels in the edema paw tissues of mice were significantly diminished by treatment with SDE and betulinic acid. These findings demonstrated that the mechanisms of anti-inflammatory activities of SDE and betulinic acid in the model of λ -carrageenan-induced paw edema of mice might act through the inhibitions of COX-2 and NO levels.

Some inflammatory mediators, including TNF- α and IL-1 β , are involved in the development of many inflammatory disorders (Dinarello, 1997). TNF- α is a major pro-inflammatory cytokine which can induce immune responses by activating T cells and macrophages and can stimulate secretion of other inflammatory cytokines (Beutler and Cerami, 1989). IL-1 β is another pro-inflammatory cytokine, primarily released by monocytes, macrophages, fibroblasts and endothelial cells (Dung *et al.*, 2009). Because cytokines are critical to the inflammatory responses, modulation of their production can improve therapeutic benefits. In the present work, treatment of SDE and betulinic acid significantly decreased the TNF- α and IL-1 β levels in the edema paw tissues of mice. These results indicated that SDE and betulinic acid

possess anti-inflammatory activities.

Previous studies indicated that among the several models of acute inflammation, λ -carrageenan-induced inflammation was concerned with free radical and has been applied to research the free radical generation in liver tissue after inflammatory states (Lai *et al.*, 2009). The λ -carrageenan-induced inflammatory response has been linked to neutrophil infiltration and the production of neutrophil derived free radicals, such as hydrogen peroxide, superoxide and hydroxyl radicals, as well as the release of other neutrophil-derived mediators (Dawson *et al.*, 1991). MDA formation is a key event of oxidative stress and widely used as a marker of free radical mediated lipid peroxidation injury (Flemming *et al.*, 1997). Thus, inflammatory effect would result in the accumulation of MDA. Our results indicated that the production of MDA was reduced by treatment of SDE and betulinic acid. Glutathione is a known oxyradical scavenger and enhancement of the level of Glutathione is conducive to reduction of the production of MDA (Ko *et al.*, 2010). GPx and GRd are GSH-related enzymes and have anti-oxidative roles in cellular defense against reactive free radicals (Cuzzocrea *et al.*, 1999). SOD is evidenced as an effective anti-oxidative enzyme. The reaction of NO with superoxide anion forms peroxynitrite, a strong cytotoxic oxidant causing lipid peroxidation and cellular damage, which increases the production of PGs (Daniela, 1996). In this study, there is significantly raised in SOD, GPx and GRd

activities with SDE and betulinic acid treatment. Furthermore, we assume the suppression of MDA production was likely due to the increases of SOD, GPx and GRd activities. Also, the increase of SOD not only enhances the superoxide anion scavenging capacity but also prevents the peroxynitrite-mediated tissue inflammatory response.

Phytochemical investigations have shown that *S. dulcis* contains diterpenoids, triterpenoids and flavonoids (Kawasaki *et al.*, 1988; Hayashi *et al.*, 1993). Betulinic acid is one of the triterpenoids isolated from *S. dulcis*. In our laboratory, we also have isolated this compound from *S. dulcis*. Betulinic acid has been confirmed to express anti-HIV, anti-bacterial, antimalarial, anticancer, analgesic and anti-inflammatory activities (Yogeeswari and Sriram, 2005). Our results of anti-inflammatory activity were in agreement with the previous reports. As mentioned above, the anti-inflammatory mechanism of betulinic acid in the paw edema mice induced by λ -carrageenan was determined in this study. Previous studies demonstrated that two triterpenoids in *S. dulcis*, glutinol and scoparinol, have analgesic and anti-inflammatory activities (Amhed *et al.*, 2001; Freire *et al.*, 1993). The content analysis of betulinic acid by HPLC also showed the presence of 6.25 mg/g in SDE. Therefore, betulinic acid may be another important active constituent with anti-inflammatory effect in SDE.

In conclusion, these results suggested that SDE and betulinic acid exhibited anti-inflammatory activities against λ -carrageenan-induced paw edema. The anti-inflammatory mechanisms of SDE and betulinic acid are considered to be closely related to the inhibition of the formation of PGs by suppressing TNF- α , IL-1 β and COX-2 levels and decreasing the levels of MDA and NO via increasing the activities of SOD, GPx and GRd activities. Betulinic acid may be one of biomarkers in SDE. Therefore, SDE has been shown to possess the potential to be developed into a pharmacological agent for the prevention or treatment of inflammatory disorders.

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Figure Legend

Figure 1. Effects of SDE, betulinic acid and indomethacin (Indo) on tissue COX-2 concentration of edema paw in mice. Each value represents as mean \pm S.E.M. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ as compared with the λ -carrageenan (Carr) group (one-way ANOVA followed by Scheffe's multiple range test).

Figure 2. Effects of SDE, betulinic acid and indomethacin (Indo) on nitrate/nitrite concentration of edema paw in mice. Each value represents as mean \pm S.E.M. ** $p < 0.01$, *** $p < 0.001$ as compared with the λ -carrageenan (Carr) group (one-way ANOVA followed by Scheffe's multiple range test).

Figure 3. Effects of SDE, betulinic acid and indomethacin (Indo) on the tissue TNF- α concentration of edema paw in mice. Each value was represented as mean \pm SEM. ** $p < 0.01$, *** $p < 0.001$ as compared to the λ -carrageenan (Carr) group (one-way ANOVA followed by Scheffe's multiple range test).

Figure 4. Effects of SDE, betulinic acid and indomethacin (Indo) on the tissue IL-1 β concentration of edema paw in mice. Each value was represented as mean \pm SEM. * p

< 0.05, ** p < 0.01 as compared to the λ -carrageenan (Carr) group (one-way ANOVA followed by Scheffe's multiple range test).

Figure 5. Effects of SDE, betulinic acid and indomethacin (Indo) on the tissue MDA concentration of edema paw in mice. Each value represents as mean \pm S.E.M. * p < 0.05, ** p < 0.01, *** p < 0.001 as compared with the λ -carrageenan (Carr) group (one-way ANOVA followed by Scheffe's multiple range test).

Figure 6. HPLC chromatographs of betulinic acid (a) and SDE (b).

Fig. 1

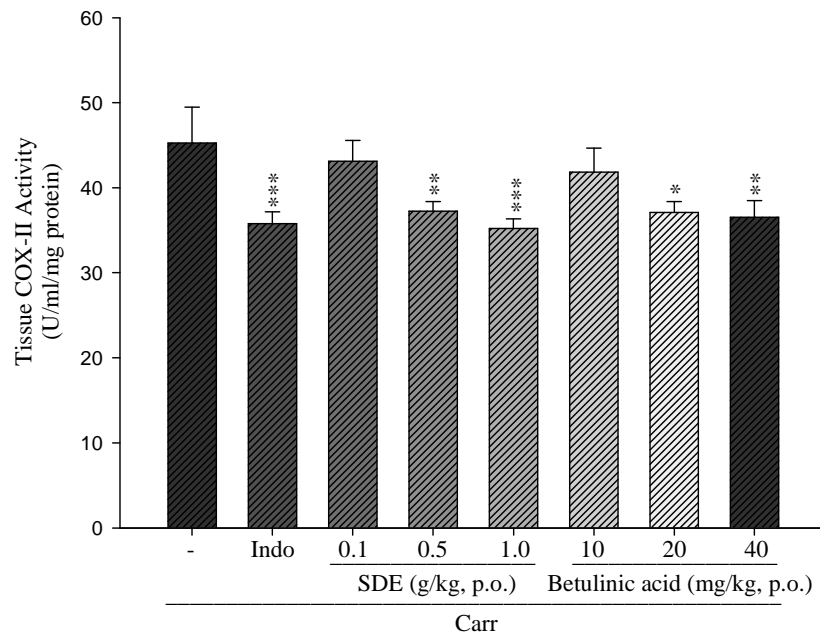


Fig. 2

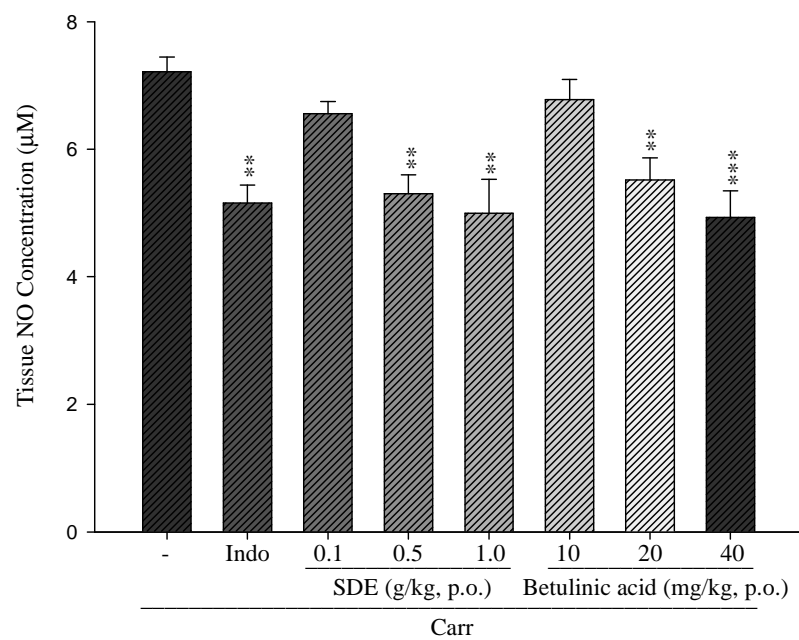


Fig. 3

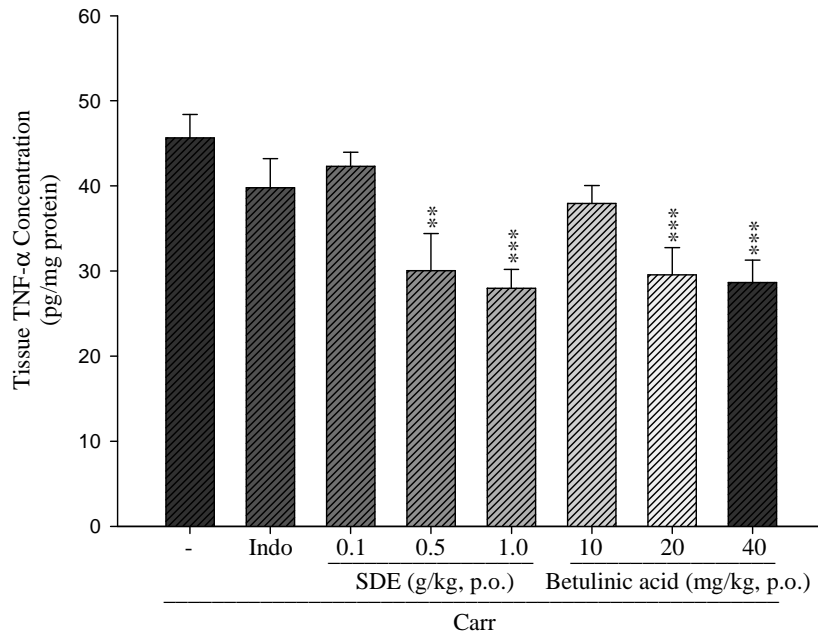


Fig. 4

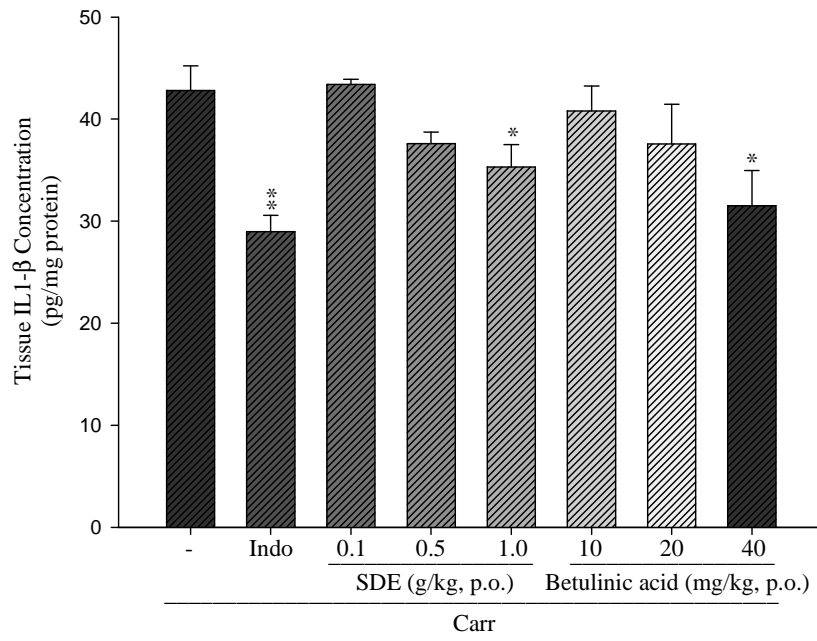


Fig. 5

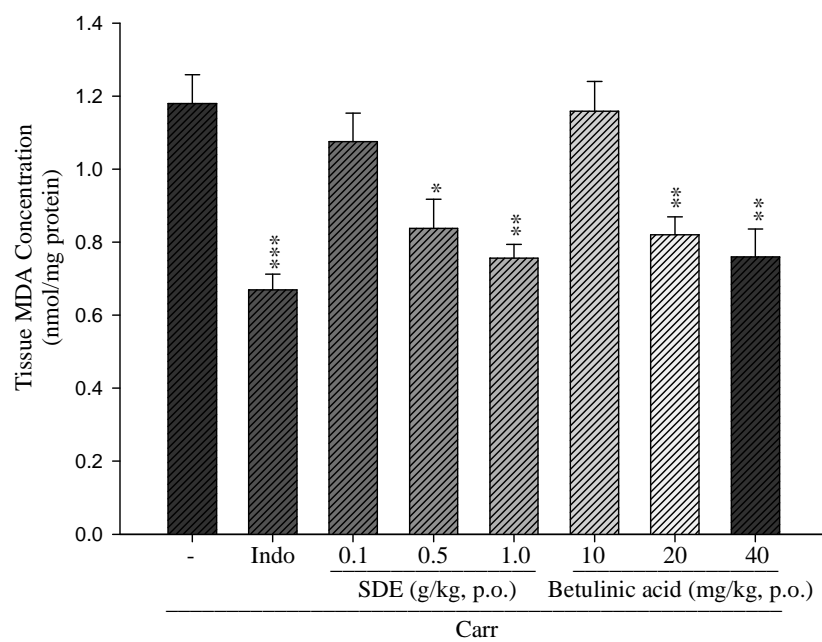
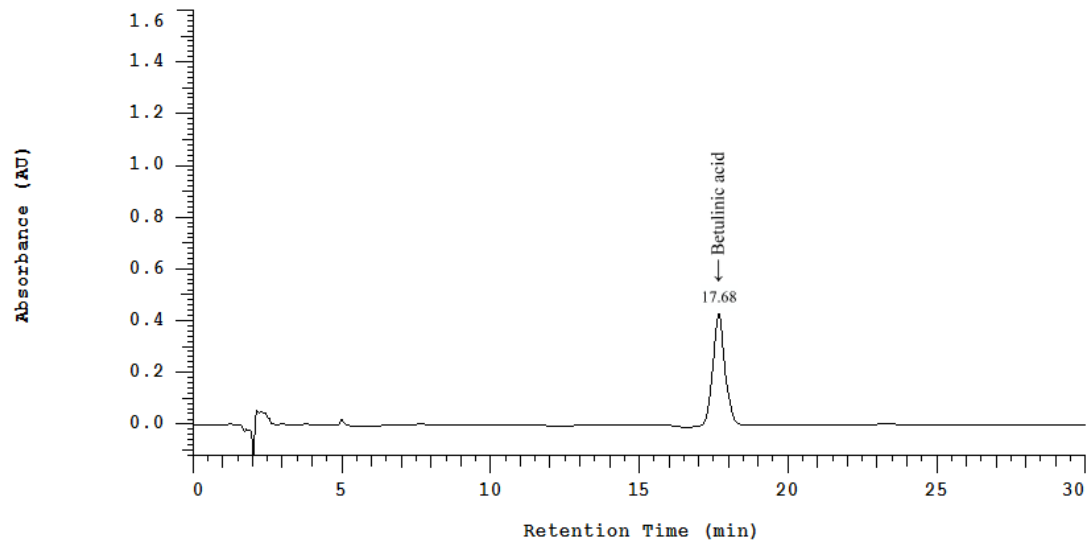


Fig. 6

(a)



(b)

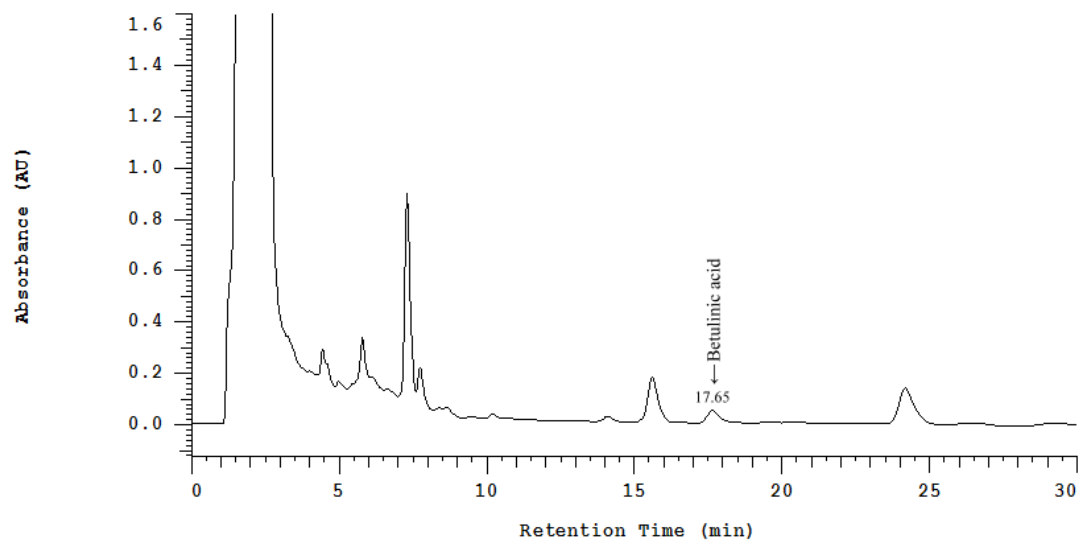


Table 1. Effects of the 70% ethanol extract of *S.dulcis* (SDE), betulinic acid (BA) and indomethacin (Indo) on the hind paw edema induced by λ -carrageenan in mice.

Groups	Change of edema volume (ml)				
	1h	2h	3h	4h	5h
Carr	0.52 ± 0.03	0.60 ± 0.01	0.74 ± 0.03	0.73 ± 0.04	0.81 ± 0.06
Carr + Indo	0.54 ± 0.05	0.59 ± 0.01	0.49 ± 0.05**	0.40 ± 0.04***	0.32 ± 0.02***
Carr + SDE 0.1	0.55 ± 0.03	0.64 ± 0.02	0.67 ± 0.02	0.69 ± 0.01	0.73 ± 0.02
Carr + SDE 0.5	0.58 ± 0.02	0.64 ± 0.04	0.53 ± 0.02**	0.45 ± 0.01**	0.47 ± 0.01**
Carr + SDE 1.0	0.53 ± 0.02	0.62 ± 0.04	0.44 ± 0.04**	0.35 ± 0.03***	0.38 ± 0.02***
Carr + BA 10	0.59 ± 0.03	0.72 ± 0.03	0.65 ± 0.05	0.70 ± 0.03	0.74 ± 0.03
Carr + BA 20	0.50 ± 0.07	0.66 ± 0.05	0.48 ± 0.06**	0.50 ± 0.06**	0.51 ± 0.04**
Carr + BA 40	0.57 ± 0.06	0.63 ± 0.07	0.41 ± 0.04**	0.41 ± 0.03***	0.46 ± 0.05**

Each value represents as mean ± S.E.M. ** $p < 0.01$, *** $p < 0.001$ as compared with the Carr (λ -carrageenan) group (one-way ANOVA followed by Scheffe's multiple range test).

Table 2. Effects of the 70% ethanol extract of *S.dulcis* (SDE), betulinic acid (BA) and indomethacin (Indo) on the liver SOD, GPx, and GRd activities in mice

Groups	SOD (U/mg protein)	GPx (U/mg protein)	GRd (U/mg protein)
Carr	38.19 ± 3.66	1.24 ± 0.03	0.069 ± 0.006
Carr + Indo	55.49 ± 3.12**	1.52 ± 0.02***	0.098 ± 0.006**
Carr + SDE 0.1	45.34 ± 4.92	1.21 ± 0.03	0.071 ± 0.001
Carr + SDE 0.5	53.14 ± 2.36*	1.43 ± 0.02*	0.095 ± 0.007**
Carr + SDE 1.0	59.66 ± 2.55**	1.56 ± 0.07***	0.098 ± 0.005**
Carr + BA 10	39.48 ± 4.07	1.22 ± 0.05	0.071 ± 0.001
Carr + BA 20	48.95 ± 4.15	1.34 ± 0.07	0.100 ± 0.002***
Carr + BA 40	53.78 ± 3.10*	1.55 ± 0.08**	0.104 ± 0.008***

Each value represents as mean ± S.E.M. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ as compared with the Carr (λ -carrageenan) group (one-way ANOVA followed by Scheffe's multiple range test).