Toxicology Reports 3 (2016) 1–3

Contents lists available at ScienceDirect

Toxicology Reports

journal homepage: www.elsevier.com/locate/toxrep

Evaluation of the anti-inflammatory activities of *Quillaja saponaria* Mol. saponin extract in mice

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ARTICLE INFO

Article history:
Received 2 September 2015
Received in revised form 7 November 2015
Accepted 14 November 2015
Available online 1 December 2015

Keywords:
Anti-inflammatory activity
Aqueous extract
Paw edema

ABSTRACT

Objective: Quillaja saponaria bark contains a high percentage of triterpene saponins and has been used for centuries as antiinflammatory and analgesic agent in Chilean folk medicine.

In the Present study the anti-inflammatory activities of the aqueous extract of commercially partially purified saponin from *Quillaja saponari*a Mol. in *in vivo* animal models.

Methods & materials:: Aqueous extract of the plant material was prepared by cold maceration. The anti-inflammatory activity of a commercial *Quillaja saponaria* Mol. (QS) saponin extract was investigated by carragenan induced mice paw edema model for acute inflammation (Winter, 1962) [16].

Results: The anti-inflammatory activity was evaluated by carragenan in paw edema model in swiss albino mice (18–20 g). The anti-inflammatory activity was found to be dose dependent in carragenan induced paw edema. QS was found to significantly ($p < 0.05$) reduce the carragenan induced mice paw edema (38.59%; 20 mg/kg bw) as compared to carragenan control. The percentage inhibition of standard anti-inflammatory drug indomethacin was (55%; 10 mg/kg, bw).

Conclusion: The results of the present study demonstrate that the aqueous extract of *Quillaja saponaria* saponins (QS) possess significant anti-inflammatory activity.

1. Introduction

Inflammation is a complex biological response of vascular tissues against aggressive agents such as pathogens, irritants, or damaged cells. Acute inflammation is the initial response and is characterized by the increased movement of plasma and innate immune system cells, such as neutrophils and macrophages, from the blood into the injured tissues. The standard signs of inflammation are expressed by increased blood flow, elevated cellular metabolism, vasodilatation, release of soluble mediators, extravasation of fluids and cellular influx [1]. Upon the presence of the inflammatory agent, cell membranes induce the activation of phospholipase A2 followed by release of arachidonic acid and inflammatory mediators such as cytokines, serotonin, histamine, prostaglandin and leukotrienes that increase vascular permeability, thus facilitating the migration of leukocytes to the site of inflammation [2]. Inflammation induced by carrageenan is acute, nonimmune, well-researched, and highly reproducible. Cardinal signs of inflammation—edema, hyperalgesia, and erythema—develop immediately following subcutaneous injection, resulting from action of proinflammatory agents—bradykinin, histamine, tachykinins, complement and reactive oxygen, and nitrogen species.

Many saponins tested have displayed significant antinociceptive, anti-inflammatory and antipyretic activities possibly due to their nonglycosidic moiety, the sapogenin, but also many diverse activities have also been reported such as antiallergic, antifungal, analgesic and others [3–6]. Moreover a variety of extracts have proved to be useful in animal models of inflammation [7–10].

The bark of *Quillaja saponaria* Mol., Quillajaceae (“soap bark”, “Seifenrinde”, “Panama bark”, “Bois de Panama”) has been used from times immemorial by the *Mapuche* people, the major ethnic group of south-central Chile, to wash hair and wool [11] and for the treatment of toothache and respiratory inflammations. The more or less pure saponins and specific fractions of the same are widely used as vaccine adjuvants [12]. The Chilean soapbark tree (*Quillaja saponaria* Mol.) grows in a wide range of habitats in the forests and scrubland of the Mediterranean climate zone of central Chile. In relation to the traditional use of quillay bark, *Mapuche* indigenous people used it as analgesic for the relief toothache and as detergent agent [13]. This species is well known for its content of triterpene saponins (between 8.5 and 16.4%) that are widely used in industry, in personal care products and as vaccine adjuvants. Related triterpene glycosides and their aglycones have been shown to possess

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http://dx.doi.org/10.1016/j.toxrep.2015.11.006

Table 1
Effect of aqueous bark extract of Quillaja saponaria at 1 mg/kg, 5 mg/kg, 10 mg/kg and 20 mg/kg *po* and indomethacin as compared to carragenan control in carragenan induced paw edema model using vernier callipers.

Groups $n = 5$ (in mm)	0 hr	30 min	1 hr	2 hr	3 hr	4 hr
Carragenan (1% w/v)	1.8 ± 0.05	3.34 ± 0.06	3.9 ± 0.03	3.99 ± 0.05	3.95 ± 0.06	3.99 ± 0.02
Carragenan + Indomethacin (10 mg/kg bw)	1.8 ± 0.05	3.0 ± 0.05	2.96 ± 0.07 (24.1%)	2.25 ± 0.04 (43.6%)	2.2 ± 0.06[a] (44.3%)	1.8 ± 0.06[a] (55%)
Carragenan + Quillaja saponaria 1 mg/kg bw	1.85 ± 0.26	3.4 ± 0.05	3.5 ± 0.12 (10.25%)	3.30 ± 0.15 (17.29%)	3.25 ± 0.05[a] (17.72%)	3.2 ± 0.01[a] (19.79%)
Carragenan + Quillaja saponaria 5 mg/kg bw	1.85 ± 0.12	2.8 ± 0.01	3.3 ± 0.1 (15.38%)	3.25 ± 0.25 (18.54%)	3.2 ± 0.01[a] (18.98%)	3.0 ± 0.06[a] (24.8%)
Carragenan + Quillaja saponaria 10 mg/kg bw	1.8 ± 0.06	2.9 ± 0.05	3.25 ± 0.02 (16.66%)	3.28 ± 0.12 (17.79%)	3.05 ± 0.02[a] (22.78%)	2.77 ± 0.05[a] (30.57%)
Carragenan + Quillaja saponaria 20 mg/kg bw	1.85 ± 0.01	2.88 ± 0.02	3.2 ± 0.01 (17.94%)	3.25 ± 0.15 (18.54%)	3.0 ± 0.01[a] (24.05%)	2.4 ± 0.01[a] (40%)

Values are expressed as mean ± SD.
[a] $P < 0.05$-significant compared to carragenan treated group.

anti-inflammatory, antiallergic, antiviral, antifungal and cytotoxic properties [14].

Paw swelling, or footpad edema, is a convenient method for assessing inflammatory responses to antigenic challenges and irritants. Typically, test compounds are assessed for acute anti-inflammatory activity by examining their ability to reduce or prevent the development of carrageenan-induced paw swelling. In the present study attempts are made to validate the claims of folklore regarding the anti-inflammatory activities of this medicinal plant.

2. Methods & materials

2.1. Chemicals

Partially purified *Quillaja saponaria* Mol. Saponin was commercially obtained from Sigma–Aldrich (99% pure with sapogenin). Carragenan was obtained from Sigma Chemical Co., Indomethacin (Sigma–Aldrich), Sodium chloride (Merck) was purchased from Chaulia Chemicals,Midnapore (enlisted supplier). The vernier callipers was purchased from Precision India Ltd.

2.2. Animals

Male Swiss albino mice (18 ± 2 g) were obtained commercially from enlisted supplier of Vidyasagar University and maintained in standard laboratory conditions. They were given standard laboratory diet and water *ad* libitum. All animal experiments are approved by the University Animal Ethics Committee, Department of Physiology with Community Health, Vidyasagar University, Paschim Medinipur, India and were in accordance with the guidelines of the committee for the purpose of Control and Supervision of Experiments on Animal (CPCSEA), Government of India.

2.3. Aqueous extract

Aqueous extract was prepared by cold maceration. Extract was filtered and concentrated to dryness by a rotary evaporator.

2.4. Acute toxicity study

To assess the acute toxicity LD50 value of the aqueous extract was attempted using the up and down method described by Bruce [15].

2.5. Acute inflammation

Carrageenan-induced rat paw oedema is used widely as a working model of inflammation in the search for new anti-inflammatory drug. The anti inflammatory activity of the aqueous extracts of *Quillaja saponaria* Mol. was evaluated by carrageenan-induced rat paw oedema method [16].

Swiss albino mice (18 ± 2 g) obtained from commercial suppliers were used. Antiinflammatory activity was measured using carrageenan induced rat paw oedema assay. The rats were divided into 7 groups of 5 animals each (plant extract was dissolved in sterile distilled water and administered intra peritoneally at different dose levels). **Group I** was treated with carragenan (1%w/v) in saline in the subplanter region of the right hind paw. Rats of **Group II** were given normal saline and treated as negative control. Rats in **Group III** were administered Indomethacin(10 mg/kg, *bw*) and considered as standard. Rats from **Group IV** to **Group VII** were given increasing doses of aqueous extracts of *Quillaja saponaria* (1 mg/kg, 5 mg/kg, 10 mg/kg, 20 mg/kg bw). Acute paw edema was induced by injecting 0.1 ml of 1% (w/v) carrageenan solution, prepared in normal saline. After 1 h, 0.1 ml, 1% carrageenan suspension in 0.9% NaCl solution was injected into the sub-plantar tissue of the right hind paw. The linear paw circumference will be measured at hourly interval for 4 h. The perimeter of paw was measured by using vernier callipers. Measurements were taken at 0–4 h after the administration of the carrageenan.

The anti-inflammatory activity was calculated by using the relation

$$\%\text{inhibition of edema} = \frac{T - T_0}{T} \times 100$$

T, Thickness of paw in control group; T_0, Thickness of paw edema in the test compound treated group.

2.6. Statistical analysis

Results of antiinflammatory activity were expressed as Mean increase in paw diameter ± SD. Results were analyzed using one way ANOVA. Differences were considered as statistically significant at $P < 0.05$ are compared to control.

3. Results

3.1. Antiinflammatory activity

The aqueous extract of *Quillaja saponaria* Mol. and standard drug as compared to carragenan control (at 3rd and 4th hour) in carragenan induced paw edema model using vernier callipers.

Aqueous extract administered at a dose of 20 mg/kg, showed 17.94%, 18.54%, 24.05% and 40% inhibition at 1–4th hour respectively while Indomethacin at a dose of 10 mg/kg *po* prevented carragenan induced paw edema with a percentage inhibition of 24.1%, 43.6%,44.4%, 55% at 1–4th hour respectively (Table 1).

4. Discussion

Inflammation is a part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells or irritants. It is characterized by redness, swollen joints, joint pain, its stiffness and loss of joint function. Inflammation is currently treated by NSAIDs. Unfortunately these drugs cause increased risk of blood clot resulting in heart attacks and strokes. Therefore, the developments of potent anti-inflammatory drugs from the natural products are now under considerations. Carragenan induced acute inflammation is one of the most suitable test procedure to screen antiinflammtory drugs. The time course of edema development in carragenan induced edema is represented by a biphasic curve [17]. The first phase of inflammation occurs within an hour of injection of carragenan which occurs partly due to trauma of injection and partly due to serotonin and histamine component. Carragenan induced paw edema is sensitive to cycloxygenase inhibitors and are used to evaluate the effect of non steroidal anti-inflammatory agents, which primarily inhibit the cycloxygenase involved in prostaglandin synthesis [18]. It plays a major role in the second phase of anti-inflammatory reaction, which is measured at the 3rd hour. As shown there is a significant percentage inhibition ($p < 0.05$) of paw edema at the 4th hour (40% at 20 mg/kg, bw). The percentage inhibition of standard anti-inflammatory drug indomethacin was (55%; 10 mg/kg, bw). Therefore it can be inferred that the possible inhibitory effect of aqueous extract of *Quillaja saponaria* Mol. in carragenan induced inflammation may be due to inhibition of cycloxygenase leading to inhibition of prostaglandin synthesis.

5. Conclusion

The Chilean Soap bark tree (*Quillaja saponaria* Mol.) is exceptionally rich in natural triterpenic saponins. In ancient tradition of the indigenous *Mapuche* people of Central Chile, aqueous extracts of milled bark played an important part in their personal and medicinal care. Earlier topical anti-inflammatory activity of quillaic acid from *Quillaja saponaria* Mol. and some derivatives [19] has been reported. Antinociceptive activity of *Quillaja saponaria* Mol. saponin extract, quallic acid and derivatives in mice has been studied [20]. This study reports the anti-inflammatory activity (*in vivo*) of commercial partially purified *Quillaja saponaria* Mol saponin in carragenan induced paw edema model. This study aimed to corroborate the presumed anti-inflammatory activity of this plant and validate the folklore claims.

Acknowledgements

Author is grateful to the Head, Department of Human Physiology with Community Health, Vidyasagar University for his encouragement and support. Author is indebted to UGC for financial support received in the form of UGC-BSR Start Up grant(S.No:20-4[17]/2012[BSR] dt 08/03/2013). Author is thankful to all the tribal informants who have participated in this survey and provided their valuable knowledge relevant to this work.

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