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Anti-Inflammatory Activity of Silibinin in Animal Models of Chronic Inflammation

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Abstract Attenuation of the chronic inflammatory response is a beneficial strategy to combat several human diseases. Traditional medicine offers many plant extracts and pure natural compounds as treatment options of a wide variety of disorders including acute and chronic inflammation. The present study was designed to evaluate the anti-inflammatory effect of silibinin in experimental animal models of chronic and granulomatous inflammations. Forty-eight rats were used to induce chronic inflammation in the hind paw with formalin and granulomatous inflammation with sterile cotton pellets. The anti-inflammatory activity of silibinin (300 mg/kg, P.O) was evaluated in the two models, and compared with that produced by dexamethasone (1 mg/kg, P.O). Silibinin decreased significantly the formation of exudate and granulation tissue compared with the vehicle, but still significantly lower than that produced by dexamethasone. In conclusion, our data suggest that silibinin inhibits the production of edema and granulation tissue in experimental animal models of chronic inflammation, and could be a potential choice for the treatment of chronic inflammatory disorders.

Keywords: *silibinin, chronic inflammation, granuloma, rats*

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1. Introduction

Inflammation is part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants [1]. This is related to cytokines and pro-inflammatory mediators secreted from macrophage. High concentrations of TNF-α, IL-1, and IL-6 are particularly destructive and are implicated in some of the pathologic responses that occur in chronic inflammatory diseases such as rheumatoid arthritis [2]. Inflammation has been recognized as a major risk factor for various human diseases. The chronic inflammatory responses predispose to pathological progression of chronic illnesses, characterized by infiltration of inflammatory cells, excessive production of cytokines, dysregulation of cellular signaling and loss of barrier function; among these chronic illnesses rheumatoid arthritis is considered as the most common inflammatory disease and is a major cause of disability [3]. Attenuation of the chronic inflammatory response is a beneficial strategy to combat several human diseases. Although steroidal and non-steroidal anti-inflammatory drugs are currently used to treat acute inflammation, these drugs have not been entirely successful in curing chronic inflammatory disorders because such compounds are accompanied by unexpected side effects [4,5]. Therefore, there is an urgent need to find safer anti-inflammatory compounds, and traditional medicine offers many plant extracts and pure natural compounds as treatment options of a wide variety of disorders including acute and chronic inflammation [6]. Among the natural active constituents, flavonoids are a family of polyphenolic substances whose members have exhibited a broad spectrum of biological activities including anti-inflammatory, anticancer, antimicrobial, antiviral, immunomodulatory, and antithrombotic activities [7,8,9,10]. Silibinin is a chemically defined flavonoid and the main active component of silymarin, a polyphenolic complex from *Silybum marianum*, which has anti-inflammatory, hepatoprotective and anticancer properties [11,12,13]. In spite of the presence of extensive medical literature that examining biological activity of silibinin, still insufficient data were reported on its anti-inflammatory effects in granuloma and rheumatoid arthritis. Therefore, the present study was designed to evaluate the anti-inflammatory effect of silibinin in experimental animal models of chronic and granulomatous inflammations.

2. Materials and Methods

2.1. Animals

Forty eight 10 weeks old, male Sprague-Dawley rats were housed in the animal house, School of Pharmacy,

Faculty of Medical Sciences, University of Sulaimani in well ventilated plastic cages, at an ambient temperature 25 ± 2°C and humidity of 55 ± 5% under 12hr dark-light cycle. Experimental protocols met the Guidelines for Animal Experimentation and approved by the Ethical Committee of the Faculty of Medical Sciences, University of Sulaimani. The animals were randomly allocated in to 2 groups of twenty-four rats in each group.

2.2. Formalin-Induced Chronic Inflammation

In this model, chronic inflammation was induced by injecting 0.1ml of 2% formaldehyde into the sub planter area of the right hand paw of ether-anesthetized rat. Both drugs including silibinin (300 mg/kg body weight), which is formulated as suspension in 5% cabroxy methyl cellulose (CMC) in distilled water as tested drug, dexamethasone (1 mg/kg body weight) as a standard drug, and the vehicle distilled water (0.2 ml/100 gm body weight), given to the control group. All were given 30 min prior to formaldehyde injection, and continued for seven consecutive days. Both drugs and the vehicle were given orally as once daily doses. Selection of silibinin dose was according to the outcome of previously reported data by Lu et al (2009) [14]. In this model, the increase in paw thickness was measured by the vernier caliper method. The paw thickness was measured before and 6 days after induction of inflammation, and presented as mean increase in paw thickness (mm) [15]. The ability of the drugs to suppress paw inflammation was expressed as a percentage of inhibition of paw edema and this percentage can be calculated according to the following equation [16]:

Percentage of inhibition (%) = (C – T) / C x 100

Where C = increase in paw thickness of control group of rats and, T = increase in paw thickness of treated group of rats.

2.3. Cotton Pellet-Induced Granulomatous Inflammation

The cotton pellets-induced granuloma was performed using the method of Winter and Porter [17]. Cotton pellets weighing 10 ± 1 mg were sterilized in an autoclave for 30 min at 120°C under 15 Ib pressure. Four pellets were implanted subcutaneously (S.C.), into the ventral region, two on either side, in each rat under light ether anesthesia. Both drugs (silibinin 300 mg/kg and dexamethasone 1 mg/kg) and the vehicle (distilled water 0.2 ml/100 gm) were given orally for seven consecutive days from the day of cotton pellet implantation. On 8th day, the animals were anesthetized and the pellets together with the granuloma tissues were carefully removed and made free from extraneous tissues. The wet pellets were weighed for wet weight and then dried in an incubator at 60°C for 18 hr until a constant weight was obtained (all the exudates was dried); after that the dried pellets were weighed again [18]. The exudate amount (mg) was calculated by subtracting the constant dry weight of pellet from the immediate wet weight of pellet. The granulation tissue formation (dry weight) was calculated after deducting the weight of cotton pellet (10 mg) from the constant dry weight of pellet, and considered as a measure of granuloma tissue formation. The percent inhibitions of exudate and granuloma tissue formation were determined as follows:

Exudate inhibition (%) = {1 − Exudate in treated group / Exudate in controls} x 100.

Granuloma inhibition (%) = {1 − granuloma in treated group / granuloma in controls} x 100.

3. Statistical Analysis

All the results were expressed as mean ± SD. Analyses were processed using Graph Pad Prism software for Windows (version 5.0, Graph Pad Software, Inc., San Diego, CA). The significance of difference among the studied groups was determined using one-way analysis of variance (ANOVA). Receiver operating characteristic (ROC) curve was used to assess the performance of the screening tests at different levels. Values with $P < 0.05$ were considered significant.

4. Results

Table 1. Anti-inflammatory activity of silibinin (300 mg/kg, P.O) in animal model of formalin-induced chronic inflammation

Treatment group	Mean paw thickness at baseline (mm)	Mean paw thickness after 7 days (mm)	Mean changes in paw thickness (mm)	Inhibition of paw edema (%)
Control	4.38 ± 0.2	7.67 ± 0.17^{a}	3.28 ± 0.33^{a}	-
Dexamethasone 1 mg/kg	4.49 ± 0.16	5.6 ± 0.25^{b}	1.11 ± 0.2^{b}	66.1 ± 6.2^{a}
Silibinin 300 mg/kg	4.7 ± 0.22	6.68 ± 0.29^{c}	1.98 ± 0.39^{c}	39.5 ± 11.9^{b}

Values are presented as mean ± SD; n = 8 rats in each group; values with non-identical superscripts (a, b, c) among different groups are considered significantly different (P < 0.05)

Table 2. Anti-inflammatory activity of silibinin (300 mg/kg, P.O) in animal model of cotton wool-induced granuloma

Treatment group	Weight of exudate (mg)	Inhibition of exudate %	Weight of granuloma (mg)	Inhibition of granuloma %
Control	157.5 ± 43.1^{a}	-	35.1 ± 10.2^{a}	-
Dexamethasone 1 mg/kg	73.6 ± 8.6^{b}	53.3 ± 5.2^{a}	12.2 ± 2.8^{b}	65.3 ± 5.8^{a}
Silibinin 300 mg/kg	105.3 ± 12.8^{c}	33.3 ± 7.6^{b}	21.3 ± 4.3^{c}	39.5 ± 10.3^{b}

Values are presented as mean ± SD; n = 8 rats in each group; values with non-identical superscripts (a, b, c) among different groups are considered significantly different ($P < 0.05$)

In Table 1, silibinin significantly decreased paw thickness by 39.5% compared to control. Meanwhile, dexamethasone produces 66.1% decrease in the thickness of paw edema compared with control, and found to be

significantly greater than that produced by silibinin ($P < 0.05$). The data presented in Table 2 clearly shows that silibinin significantly attenuated both exudates and granuloma by 33.3% and 39.5% respectively compared to control group. Meanwhile, dexamethasone produces 53.3% inhibition of exudates and 65.3% inhibition of granuloma compared to controls, which was significantly greater than that produced by silibinin. Figure 1 indicates that in both models, silibinin produces equivalent anti-inflammatory activity in terms of both edema and granuloma, where non-significant differences were reported between the percent changes in the markers of chronic inflammation of the two utilized models. Utilizing different markers of chronic inflammation, the validity of the two models of chronic inflammation in detecting the anti-inflammatory activity of silibinin were evaluated, and the sensitivity and specificity of the two methods using ROC curve revealed a non-significant ($P > 0.05$) overlap for AUC in both methods regarding the edema and granuloma as indicators of chronic inflammation, with AUC value of 0.64 and 0.457 respectively (Figure 2 and Figure 3).

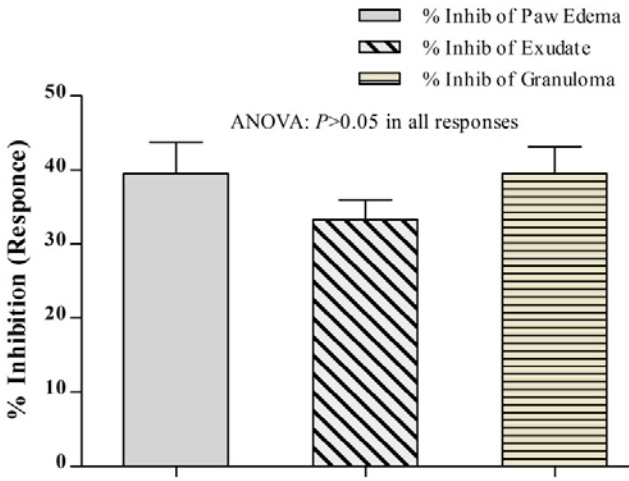

Figure 1. Comparison between the anti-inflammatory response (% inhibition to the orally administered 300 mg/kg silibinin) in two different models of chronic inflammation in rats; n = 8 rats in each group

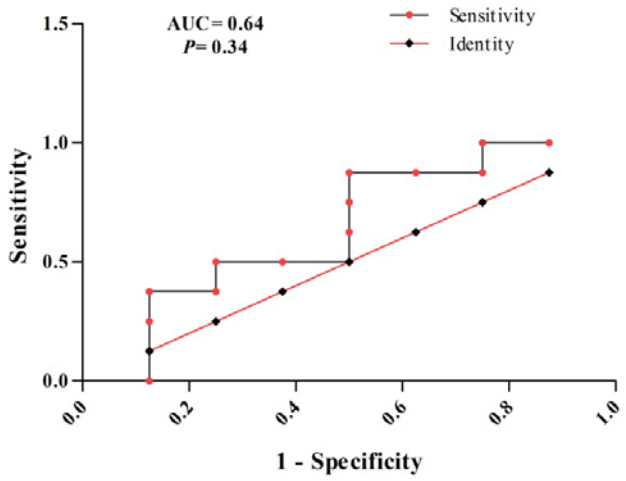

Figure 2. ROC curve illustrating the sensitivity and specificity for different values of % inhibition of exudate formation corresponding to the inhibition of paw edema in two different models of chronic inflammation in rats

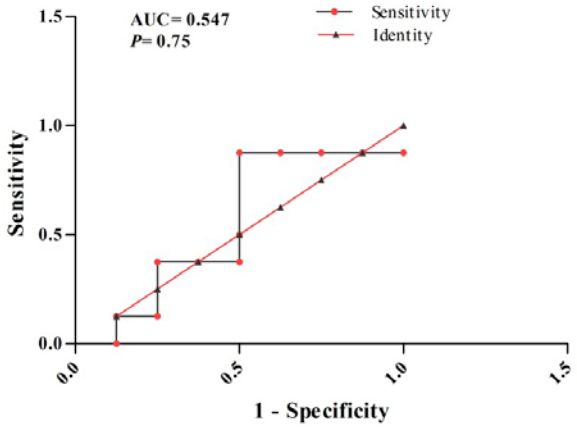

Figure 3. ROC curve illustrating the sensitivity and specificity for different values of % inhibition of granuloma formation corresponding to the inhibition of paw edema in two different models of chronic inflammation in rats

5. Discussion

Silibinin is a flavonolignan found milk thistle; it contains antioxidant properties and protects the liver from damage and toxins. However, the activity of silibinin on inflammatory diseases has not been well investigated. Numerous studies have proposed that flavonoids act through a variety mechanisms to prevent and attenuate inflammatory responses and serve as possible cardioprotective, neuroprotective and chemo preventive agents [19]. Therefore, in the present study, we investigated whether silibinin have a therapeutic effect on chronic inflammatory disorders. Interestingly, silibinin attenuated the formation of edema and granulation tissue after induction of inflammation in rats. In agreement with our data, it was reported that silymarin (the total extract of milk thistle) produces inhibition of edema in rat model of chronic inflammation [20]. Moreover, silymarin, the extract that contain 80% silibinin, is found to be effective in ameliorating the inflammatory processes and decrease the serum levels of the inflammatory cytokines in patients with knee osteoarthritis, when used alone or adjuvant with piroxicam or meloxicam [21]. The inhibitory effect of silibinin on formalin-induced edema and cotton pellet-induced granuloma could support its anti-proliferative effect, because this model also used to evaluate agents with the probable anti-proliferative activity. The anti-proliferative actions of silibinin converge on inhibition of signaling pathways that regulate the cell cycle including protein kinase B (Akt) and cyclin-dependent kinases [22]. However, it is important to emphasize that the anti-proliferative action of silibinin has been described chiefly with one component of silymarin, silibinin, at relatively high doses (100-300 μmol/l or ca. 50-150 μg/ml) [23]. Whether flavonoids from daily food intake really affect an inflammatory response is not clearly established [24,25]. No experimental data available that clearly describes the relation between flavonoid intake and incidence or severity of inflammatory disorders, such as RA and OA was available. Accordingly, the exact therapeutic dose of silibinin cannot be exactly characterized, and this is considered an important limitation of the study. On the other hand, the pharmacological effect produced by the flavonoid is quite different, and treatment with a certain flavonoid may affect, at least in part, some inflammatory responses in many situations [26,27]. The results of the present study are comparable with those observed with quercetin, another potent flavonoid that prevents further recruitment of inflammation cells to the site of inflammatory response [28]. All these including the present study, proved that several flavonoids including silibinin really inhibit the expression of pro-inflammatory molecules [29] in experimental animal models and human studies. These findings suggested that modulation of pro-inflammatory mechanisms is certainly one of the major actions, or mechanisms of flavonoids that may explain their anti-inflammatory activity. In conclusion, our data suggest that silibinin inhibits the production of edema and granulation tissue in experimental animal models of chronic inflammation. Silibinin could be a potential choice for the treatment of chronic inflammatory disorders.

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