

Antinociceptive Effect of Silymarin in Experimental Animals

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Abstract

Background: Silymarin is a polyphenolic flavonoid derived from milk thistle (*Silybum marianum*) that has anti-inflammatory, cytoprotective, anticarcinogenic and antioxidant effects. It has been used medicinally to treat liver disorders including acute and chronic viral hepatitis, toxin/drug induced hepatitis, and alcoholic liver disease.

Objective: To evaluate the antinociceptive effect of silymarin in experimental animal model of pain.

Methods: The efficacy and dose response effect of silymarin (125, 250, and 500mg/kg) were assessed against control using tail flick test in mice as a model of nociceptive pain. In this model, all doses of silymarin were given intraperitoneally 15 min before immersion of tail in hot water 50°C, and Tail Flick

Latency was measured before, and after (15, 30, 60 and 120 min) administration of silymarin.

Result: Silymarin in 250 and 500mg/kg significantly increase Tail Flick Latency after 15, 30, 60 and 120 min in a dose dependent manner that the maximum effect seen after 120 min compared to baseline value.

Conclusion: Silymarin as a herbal drug produce a significant antinociceptive effect in experimental animal model of pain, and beside its better standardization, quality control, and safety profile, in addition to its availability and relative low cost, represent a good alternative choice for management of pain.

Keywords: Silymarin, milk thistle, pain

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Introduction:

Silybum marianum, commonly known as milk thistle (Family: Asteraceae/Compositae) is one of the oldest and thoroughly researched plants in the treatment of liver diseases. The plant itself grows in different areas world wide, with large purple flowering heads; the leaves are characterized by milky veins, from which the plant derives its name⁽¹⁾. The extracts of milk thistle is being used as a general medicinal herb from as early as 4th century B.C.⁽²⁾. It became a favored medicine for hepatobiliary diseases in 16th century, and in the 1960s the biologically active principals of the seed and fruit extracts were studied and the chemical structure was elucidated⁽³⁾. The safety and efficacy of this herbal drug has been analyzed by a systematic approach in a review by Saller *etal.* in 2001⁽⁴⁾. Accumulating pharmacological data indicate that plants in general are a substantial source of active compounds capable of exerting potential therapeutic activity in the organism⁽⁵⁾. Many herbal drugs may have new medicinal values, and are in use for the treatment of several conditions. In folk medicine, various drugs are used in single and/or in combined forms, for treating different types of inflammatory and arthritic conditions with considerable success⁽⁶⁾.

Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effect. Study of plants that may have new therapeutic uses in addition to their known traditional use represent a new search strategy in herbal medicine⁽⁷⁾.

Silymarin is obtained from *Silybum marianum* (milk thistle), which is an edible plant that has been used medicinally for centuries as a herbal medicine for the treatment of liver related disorders⁽⁸⁾.

Silymarin is widely prescribed by herbalists and has almost no known side effects; in addition to that, silymarin may prove superior to polyherbal formulation for its better standardization, quality control, safety profile, easy availability and low cost⁽⁹⁾.

Silymarin consist of four flavonolignan isomers; silybin, isosilybin, silydianin and silychristin, among them, silybin being the most active and commonly used. Silymarin offer good protection in various conditions of liver diseases in lab animals; it acts by many mechanisms that may include antioxidative, antilipid peroxidative, anticarcinogenic, antifibrotic, anti-inflammatory, membrane stabilizing, immunomodulatory and liver regenerating mechanisms⁽¹⁰⁾.

Although many drugs available to treat pain disorders, they produce various systemic side effects or exhibit tolerance upon chronic use. Many plant products have been claimed to be

free from side effect and are less toxic than synthetic drugs.

Silymarin is a herbal formulation and has been studied for its antioxidant, hepatoprotective and anticarcinogenic properties but not analgesic activity; thus the objective of this study is to identify the potential analgesic effect of silymarin using experimental animal model of nociceptive pain utilizing standard method like tail flick test.

Methods:

Animals: 28 albino mice obtained from animal house in college of veterinary medicine – Baghdad University, weighing 18-27gm were used in the experiments. The animals were housed in standard stainless steel cages at room temperature with 12-12 hour light dark cycle. The mice were randomly distributed into 4 groups of seven as control and test subjects. All animals had access to food and water freely through out the experiments. All experiments were performed considering all ethical circumstances. For antinociception recording, mice were allowed to acclimatize for 30 minutes before intraperitoneal injection.

Preparation of doses: The doses of 125, 250, and 500mg/kg of silymarin as crude powder (Luna Co, Egypt) was dissolved in 98% dimethyl sulfoxide to produce the required strength.

In the present study, animals were allocated into 4 groups, seven of each, the first group treated with vehicle only and served as control; second, third and fourth groups treated with silymarin 125,250, and 500mg/kg respectively.

Measurement of antinociception: In this study, the pain sensitivity of mice was measured with

hot water tail flick test (11), the pain threshold was measured during the mid –light period. The tail flick latency was determined by placing the distal part of the tail in a beaker containing water maintained at 50°C. Baseline tail flick latency was the value before administration of any drug. Following drug administration tail flick latency was measured at selected time intervals of 15 min, 30 min, 60min and 120 min respectively.

Statistical analysis: Data were expressed as the mean \pm SD; and analyzed statistically with student t-test, P-value \leq 0.05 were considered significant.

Results:

The baseline values of tail flick latency in the four groups of mice (n=7, each) were 5.25 \pm 3.37, 5.43 \pm 2.14, 4.65 \pm 1.8, and 4.96 \pm 2.4 seconds respectively. There were no significant differences between the mean baselines of tail flick latency in the vehicle treated and silymarin treated mice. As shown in table (1) and figure (1), silymarin produced dose dependent antinociception following its ip administration; when given to mice at 125mg/kg it results in non-significant increase in tail flick latency after 120 min from administration; in the groups with larger doses (250, and 500mg/kg) the significant increase in tail flick latency started 15min after silymarin administration, and reaching a peak after 120 min, the percentage increase in tail flick latency produced by ip administration of silymarin dose of 125,250, and 500mg/kg were 17.31%, 109.68%, and 127.22% respectively fig(1).

Table 1: Effect of silymarin (125,250 and 500mg/kg) on latency to withdrawal tail in tail flick test in mice.

Treatment	Latency to withdrawal tail (sec)				
	0 min	15 min	30 min	60 min	120 min
Control	5.25 \pm 3.37	4.63 \pm 1.7	5.82 \pm 2.02	4.78 \pm 2.5	4.88 \pm 2.1
Silymarin 125mg/kg	5.43 \pm 2.14	5.01 \pm 3.1	5.98 \pm 1.8	5.09 \pm 2.6	6.37 \pm 4.1
Silymarin 250mg/kg	4.65 \pm 1.8	7.81 \pm 3.7*	7.84 \pm 7.2*	8.05 \pm 3.3*	9.75 \pm 3.6*
Silymarin 500mg/kg	4.96 \pm 2.4	8.52 \pm 2.1*	8.79 \pm 4.6*	9.94 \pm 4.1*	11.27 \pm 3.5*

Results represent mean \pm SD; * P \leq 0.05 considered significant change as compared to control.

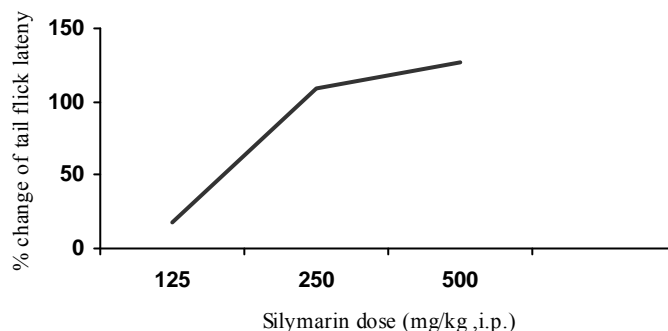


Figure (1): Dose –response line for silymarin given ip to mice.

Antinociception was determined by tail flick latency to hot water 50°C. Ordinates shows percentage changes to tail flick latency 120 min

after silymarin administration. All points represent mean \pm SD from seven mice.

Discussion:

Although the use of these drugs has a sound tradition, and their medicinal uses and general safety are well known to people, their use has yet to be rationalized in therapeutics, using the current methodology. Scientific studies are therefore required to assess their safety and efficacy⁽¹²⁾. It is very important to study herbal products for evaluating their acclaimed properties, as recently numbers of herbal products are being introduced in the market. In view of this, an attempt to study the herbal formulation of silymarin for its analgesic activity in experimental induced animal models of pain. Pain is associated with various clinical conditions like arthritis, cancer, vascular diseases and burns⁽¹³⁾. Silymarin was evaluated for its analgesic activity in animal models. A significant ($P \leq 0.05$) antinociceptive effect was observed for silymarin by tail flick test method.

In the present study, silymarin demonstrated a significant ($P \leq 0.05$) antinociceptive effect in a dose dependent manner in animal model of pain. The tail flick latency found to be suitable for the evaluation of centrally but not peripherally acting analgesics. It involves higher brain functions and consists of responses to nociceptive stimuli organized at a supraspinal level⁽¹⁴⁾. The nociceptors seem to be sensitized by sensory nerves. In this study, silymarin (250 and 500mg/kg) significantly increase latency to flick tail in experimental animals, the ability of the extract to show significant effect in this type of pain induction suggest that its analgesic effect may in part be related to its anti-inflammatory properties⁽¹⁵⁾. A number of studies have suggested that

silymarin is an anti-inflammatory, it regulates inflammatory mediators such as tumor necrosis factor (TNF)⁽¹⁶⁾, TNF-alpha, nitrous oxide, interleukin-6, and interleukin-1 receptor antagonist⁽¹⁷⁾. In addition to that, Fiebrich and Koch,(1979), reported that silymarin is an inhibitor of prostaglandin synthetase⁽¹⁸⁾, and non-competitive inhibitor of lipoxygenase⁽¹⁹⁾. Furthermore, Gupta et al, (2000), demonstrated the anti-inflammatory and anti arthritic activities of silymarin acting through inhibition of 5-lipoxygenase⁽²⁰⁾, on the other hand, DelaPuerta (1996), reported that silymarin produce anti-inflammatory effect in dose dependent manner, and that silymarin also produced a dose-dependent inhibition of leukocytes accumulation in inflammatory exudates⁽²¹⁾, besides its known antioxidant properties and its ability to act as a radical scavenger; these results suggest that silymarin exert an important antinociceptive effect which may be probably mediated via its anti-inflammatory effect by inhibition of various autacoids formation and release, in addition to its well defined antioxidant activity. Further studies are needed to elucidate the exact mechanism by which silymarin exert its antinociceptive effect. In conclusion, the present study indicate that silymarin has significant antinociceptive properties in experimental animal model of pain which is probably mediated via anti inflammatory effect and antioxidant effect as well as central inhibitory mechanism, which may find clinical use for the management of painful conditions.

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