

## Analgesic effect of silymarin in chicks

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### Abstract

There were no studies about the analgesic effect of silymarin in the chicken. This study examined antinociceptive effect of silymarin given intraperitoneally in 7-9 day-old chicks. The median effective dose of silymarin for the induction of analgesia to electric stimulation in the chicks was 65.3 mg/kg. Silymarin at 60, 120 and 240 mg/kg revealed analgesic effect to electric stimulation in chicks in dose dependent manner in comparison with the control group. The analgesic effect of silymarin at 120 and 240 mg/kg started at 15 min after injection and lasted after over 120 min of injection were as silymarin at 60 mg/kg the analgesic effect started at 15 min after injection and declined before 120 min of injection. The peak of analgesic effect for 60, 120 and 240 mg/kg were at 60 min after injection. These results indicate that silymarin have an analgesic property in the chicks model.

**Keywords:** Silymarin, Analgesia, Chicks, Electrostimulation

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### التأثير المسكن للسليمارين في أفراخ الدجاج

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### الخلاصة

لعدم وجود دراسات عن التأثير المسكن للسليمارين في الدواجن تم إجراء هذه الدراسة للكشف عن التأثير المسكن للسليمارين في أفراخ الدجاج بعمر 7-9 يوم عن طريق الحقن في الخلب و كانت الجرعة المسكنة الوسطية للسليمارين في إفراخ الدجاج عن طريق الحقن في الخلب باستخدام جهاز المحفز الكهربائي هي 65,3 ملغم/كغم و اظهر السليمارين بجرع 60 و 120 و 240 ملغم/كغم في الخلب تأثيراً مسكناً للألم المحدث بواسطة الجهاز المحفز الكهربائي و بشكل معتمد على الجرعة بالمقارنة مع مجموعة السيطرة و بدأ التأثير المسكن للألم للسليمارين بجرع 60 و 120 و 240 ملغم/كغم في الخلب بعد حفته بـ 15 دقيقة و استمر بعد ساعتين من حفته للسليمارين بجرع 60 و 120 و 240 ملغم/كغم و تلاشى التأثير المسكن للسليمارين بجرعة 60 ملغم/كغم بعد ساعتين من حفته و كانت ذروة التأثير المسكن للسليمارين بجرع 60 و 120 و 240 ملغم/كغم بعد ساعة من حفته. دلت نتائجنا على وجود التأثير المسكن للألم للسليمارين في نموذج أفراخ الدجاج.

### Introduction

Phytomedicine, also named Herbal medicine, refers to the use of seeds, berries, roots, leaves, bark, or flowers for medical purposes. Silymarin is got from *Silybum marianum* (milk thistle), a palatable plant that has been used medicinally for centuries as an herbal medicine for the treatment of liver disease (1). Silymarin is the active extract of seeds and fruits of the milk thistle (*Silybum marianum*)

and contains the flavonolignans isomers called silybin, isosilybin, silydianin, and silychristin. It has almost no known side effects (1). Silymarin clinically used in the treatment of many of liver disorders like hepatitis, chronic alcoholic liver disease, liver cirrhosis, ischemic injury, and radiation toxicity (2). Silymarin have many pharmacological actions as a free radical scavenger that affects various steps in arachidonic acid cascade via cyclooxygenase and lipoxygenase pathways (3). Besides,

silymarin modulates immune system through suppression of neutrophil immigration and mast cell immobilization (4). It also inhibits TNF- $\alpha$  -induced production of free radicals and lipid peroxidation, and modulates T-cell function (5,6). Increasing the concentrations of the endogenous antioxidant enzymes like glutathione peroxidase, glutathione reductase, superoxide dismutase and catalase (7,8). In addition, it's considered a liver protection for its efficiency to stabilize the cell membranes of hepatocytes, blocking the entry of toxic substances into these cells. Silymarin joins to receptors present on these membranes, inhibiting the binding of toxins in these sites, reducing drug-induced hepatocellular damage (9,10). It also stimulates the synthesis and activity of enzymes responsible for the hepatic biotransformation process, such as glutathione S-transferase (11,12). Due to the adverse effects of available synthetic medications in the long term treatment of painful conditions and inflammation, many studies have tested different plant extracts and their active compounds for antinociceptive and anti-inflammatory activities (13,14). There are no published reports of silymarin on its analgesic profile. Thus, the objective of the present work is to explore the potential of herbal medicine silymarin for its analgesic effects using the electric stimulation method to induce pain in chicks.

## **Materials and methods**

Day old Ross broiler chicks of both sexes were purchased from a certified local hatchery and they were maintained until the age of 7-9 days when the experiments were done. The chicks were housed in a room with a temperature of 32-35°C, constant lighting, and wood shavings as floor litter, with free access to drinking water and feed. The commercial powder of silymarin (175 mg, 21<sup>ST</sup> Century HealthCare, Inc.) was dissolved in warm distilled water to obtain the concentrations needed for injection intraperitoneally (i.p.) in a volume of 10 ml/kg body weight (15). Chicks in the control group were injected i.p. with physiological saline solution at 10 ml/kg. All doses of silymarin were freshly prepared before each experiment. All experiments complied with our institutional regulations addressing animal use, attention and humane care which are based on the guidelines of the National Research Council (16).

### **Determination of the median effective dose (ED<sub>50</sub>) of silymarin for the induction of analgesia in chicks**

The up-and-down method (18) was used to determine separately the individual ED<sub>50</sub> of silymarin for the induction of analgesia in chicks. Analgesia was assessed by the increase in pain threshold using an electric stimulator (SRI, Scientific and Research Instruments Ltd, UK) after setting the frequency at 50 Hz, the width at 5 ms and the pulse

amplitude at 10 volts. The electrodes of the stimulator were gently put in the free feather of the upper chest region, wetted with distilled water, under the wing. The response of the chick to pain after electric stimulation was in the form of distress calls and/or resisting with wing flapping (18,19). Each chick was subjected to a minimum voltage that caused aversive pain response before the silymarin injection and then 15 min after the injection. The increase or decrease in voltage that caused pain response was calculated for each chick. Usually, the latency for positive analgesic response was apparent within 2 s after the electrical stimulation. The choice of these doses based on preliminary experiments in chicks.

### **Dose dependent analgesic effect of silymarin in chicks**

Thirty-two chicks were randomly divided into four groups of eight birds each. The chicks were injected with either normal saline solution (control) or with silymarin at 60, 120 and 240 mg/kg. The highest dose of silymarin was almost two-fold of the analgesic ED<sub>50</sub> of the drug. For each chick, we measured the minimum voltage that caused aversive pain response at 0, 15, 30, 60 and 120 min after the injection. The increase in the voltage in each group was assessed statistically to determine the analgesic response of the chicks to silymarin.

### **Statistical Analysis**

Data were expressed as mean + SEM. Statistical analysis was done by using one-way analysis of variance (ANOVA) followed by Dunnett's test. P<0.05 were considered significant (17).

## **Results**

### **Determination of the median effective dose (ED<sub>50</sub>) of silymarin for the induction of analgesia in chicks**

The ED<sub>50</sub> value of silymarin determined by the up-and-down method for the induction of analgesia in the chicks was 65.3 mg/kg, i.p (Table 1).

### **Dose dependent analgesic effect of silymarin in chicks**

Silymarin produced a dose dependent analgesic effect following its intraperitoneally administration; when given to chicks at 60, 120 and 240 mg/kg in comparison with the control group which injected with normal saline only. In the groups with larger doses 120 and 240 mg/kg the significance of analgesic effect started at 15 min after injection and lasted over 120 min of injection were as the group with 60 mg/kg the analgesic effect started at 15 min after injection and declined after 120 min of injection. The peak of analgesic effect for 60,120 and 240 mg/kg were at 60 min after injection (Figure 1). The observations are given in table 2.

Table 1: Median effective dose (ED<sub>50</sub>) of silymarin injected intraperitoneally for induction of analgesia in 7-9 day-old chicks

Variable	Analgesia
ED <sub>50</sub> (mg/kg)	65.3
Range of the doses used (mg/kg)	60-80-100
Initial dose (mg/kg)	100
Last dose (mg/kg)	80
Number of chicks used	6 (xxoxox)
Increase or decrease in the dose (mg/kg)	20
Minimum-maximum voltage that caused pain	6-9 before silymarin injection 6-10 after silymarin injection

X: Positive response of analgesia, O: Negative response of analgesia, The ED<sub>50</sub> were determined by the up-and-down method (18).

Table 2: Effect of silymarin (60, 120 and 240 mg/kg) on electrostimulation in chicks

Groups	The increase in voltage caused pain after			
	15 min	30 min	60 min	120 min
Control	0.0±0.26	0.25±0.25	0.0±0.33	0.0±0.28
Silymarin 60 mg	1.13±0.23*	2.13±0.48*	3.00±0.27*	0.13±0.13
Silymarin 120 mg	1.75±0.25 <sup>a</sup>	2.38±0.38 <sup>a</sup>	3.38±0.53*	1.25±0.37*
Silymarin 240 mg	3.62±0.18 <sup>a</sup>	3.75±0.31 <sup>a</sup>	4.38±0.38 <sup>a</sup>	2.13±0.30 <sup>ab</sup>

n = 8, the observations are mean ± SEM, \*P<0.05, as compared to control, a P<0.05, as compared to silymarin at 60 mg/kg, b P<0.05, as compared to silymarin at 120 mg/kg.

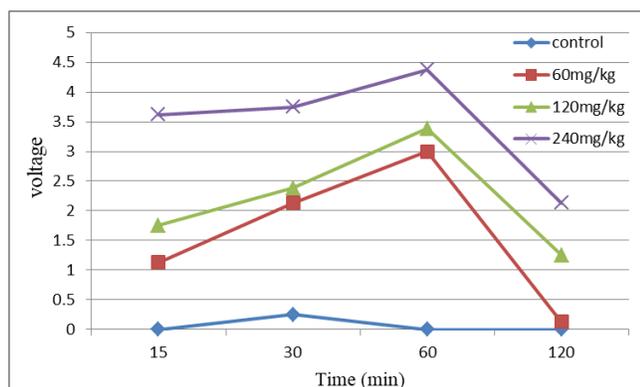


Figure 1: Analgesic effect of silymarin at 0 (control group), 60, 120 and 240 mg/kg i.p. after 15, 30, 60 and 120 min of injection. Calling of chicks was the indicator for pain sensation after raised the voltage of electrostimulator.

## Discussion

In the present study, silymarin demonstrated a significant (P<0.05) analgesic activity at different doses. We demonstrated the analgesic effect of silymarin by electric stimulation experimental protocols (19,20). It is very important to study herbal products for assaying their unknown properties, as recently numbers of herbal products are being introduced in the market. In view of this, an attempt to study the silymarin for its analgesic activity in

the chicks model. Pain and hyperalgesia are related to diverse clinical cases like inflammatory conditions, cancer, vascular diseases and burns (21). Several studies explored the analgesic effect of silymarin in mice and rats using different techniques for assaying pain and analgesia, different routes of administration and different time for assaying the pain and analgesia (22-25). The ED<sub>50</sub> for analgesic effect of silymarin in chicks model was 65.3 mg/kg i.p. This dose of silymarin helps us to choose the doses of our study. Analgesic effect of silymarin was clear against control group, changes in the dose and time of silymarin administration may be involved in its analgesic effect, silymarin at 60 mg/kg showed disappearance of analgesic effect after 120 min of injection whereas silymarin at 120 and 240 mg/kg showed persistent analgesic effect over 120 min. However, a similar argument about the analgesic effect in mice and rats was previously demonstrated (22-25). Our finding indicated that the peak of analgesic effect of silymarin was 1hour after intraperitoneally injection for all doses of silymarin Figure1. The exact mechanism of action of the silymarin as analgesic drug was unclear, several studies attributed the analgesic effect to the inhibitory effect of Tumor necrosis factor - $\alpha$ , Interleukin 1 $\beta$ , cyclooxygenase 2 and prostaglandin E<sub>2</sub> these inflammatory mediators suppress by block the mRNA expression of it by silymarin (5,26). Other researchers suggested that the silymarin have antinociceptive effect due to its inhibition of prostaglandins (PGE<sub>2</sub> and prostaglandin F<sub>2</sub>) (pain inducers) (27-30). In

addition, Moreover, different receptors for neurotransmitters, responsible in the pain transmission, are not the same in these animal models. Therefore, various analgesic activity profiles of different models may be seen.

## Conclusion

Our present study indicates that silymarin has significant analgesic effect on the chicks model thus, it can be concluded that silymarin has analgesic effect which are may be mediated by suppression of prostaglandin synthesis and other autacoid mediated inflammatory response as well as central inhibitory mechanism, which may find a clinical benefit for the control of painful condition in our animal model

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