ANTI-INFLAMMATORY EFFECT OF SILYMARIN IN INFECTED RATS AND EVALUATION OF SERUM IL-8

Oruba K. Al-Bermai”, Sama J.Al-Zuwaini*, Nisreen Kaddim radi* and Wurood Alwan Kaddim*

*College of science for Women, University of Babylon, Babil, Iraq

ABSTRACT
Silymarin extracted from milk thistle (Silybum marianum) was used for many deceases to cure acute and chronic liver infection in human that caused by viral infection, exposure to poisons and alcohols. Eighty-six white rats (albino) were used that were divided into two groups. First group had been treated with standard anti-inflammatory drugs and with different doses of Silymarin 125, 250, 500 mg/kg. After 30 min, the experimental inflammation was induced by inoculation of egg albumin in paw of rats then the thickness of edema was measured after 1hr, 2hr and 3hr. The second group contains thirty rats to study the effective dose of silymarin 250 mg/kg in two models of administration. First model: rats were injected with silymarin in a pre-infection mode (as a protectant). The second model: Silymarin was injected in a post infection mode as treatment. The two groups of rats were killed, and their serum was collected for IL-8 evaluation by ELISA. The result showed that the activity of silymarin against inflammation depended on the dose in comparison with standard compound (acetyl salicylic acid, dexamethasone, meloxicam) and this activity was increased with dose quantity until 250mg/kg. The ELISA test showed that there is a significant difference between pre- and post-infection with the control group (p<0.05).

Keywords: Serum IL-8; Silymarin; Rats

INTRODUCTION
Inflammation it’s a physiological of an inflamed result from an attack of pathogen like bacteria of human and animal which develop immunological responses and finally lead to reduce damage and tissue repair (Nathan, 2002). This operation requires many different types of immune cells incorporation with several inter mediators and markers regulated by specific system (Gouwy et al, 2005). Primary responses of an inflammation induced by macrophage activation which is found in the same area of the inflammation through the interaction of specific receptors of antigen presenting cell with pathogen leading to induce the intimidate of immune system like cytokines, chemokines, protease and the activation of leukocyte cascade of inflammatory and immune responses controlled by genetic mediator that activate phagocytosis and apoptosis to make balance between anti-inflammatory response and pro-inflammation response which is necessary to prevent greater damage of tissue or spread of infection for long period (Lawrence et al, 2002). Silymarin was extracted from seed of Silybum marianum and used as herbal drug to cure several diseases like liver infection, gall-bladder infection and as antitoxin such as poisoning with mushroom, alcohols, chemical material and environmental pollutants. It is considered as the best medical herb used for curing liver infection (Levy, 2004; Dhiman et al, 2005 Mayer, 2005). Silymarin have an important role by acting as anti-oxidant agent that cleave and remove bonds of many compounds like ketyl radicals, phenylglyoxylic (Luper, 1998).

IL-8 was originally recognized as a neutrophil-chemo attractant protein. Indeed, IL-8 was not noted in normal adult plasma when the researcher performed the intravenous injection with LPS, A massive elevation of plasma IL-8 level after 2 h was noted.

IL-8 has a major character during inflammation like chemotactic activity against T cell and basophils. IL-8 increases neutrophils adhesion to endothelium and stimulates them to carry out phagocytosis. Additionally, IL-8 has immunomodulatory effect by stimulating the matrix metalloproteinase-9 expression, release of TNF-related apoptosis-inducing ligand (TRAIL) and prime respiratory burst in neutrophils (Jundi and Greene, 2015).

The present study includes two investigations: First, the experimental rates were injected with different doses with silymarin to determine the anti-inflammatory effect of Silymarin. While the second investigation of this study is using the effective dose of Silymarin (250 mg/kg) in two models of administration: First, Silymarin was injected in a pre-infection with E. coli, as a protectant. The second model is: Silymarin was injected as a post-infection treatment (serum was then collected from the two groups of rats for evaluating IL-8 by ELISA.

The aim of current study was to determine the anti-inflammatory dose of Silymarin that might be used in the future to cure different types of infections caused by different types of pathogens.

MATERIALS AND METHODS
1- Silymarin extract and drugs: Silymarin pure extract powder (Lona company, Egypt) was disso-
ived in 98% of dimethyl sulfoxide (DMSO) to make 250mg/ml as a standard solution and then we have diluted it to get different concentrations. DMSO and diethyl ether (Merck, Germany) and Dexamethasone (USA, Acetylsalicylic acid (France) and egg albumin powder (Sigma, USA).

2- Study design: Eighty-six albino rats of both genders (purchased from the animal house of Babylon University) were used. Their weight ranged from 108-220g. All rat groups kept under the same condition during the experiment period. Rats were starved a night before starting the experiment (10). They were divided into two groups: first group included 56 albino rats, divided into 7 subgroups, each consist of 8 rats first group (control group) was given 2ml/kg of DMSO and the other three standard groups were given 100 mg/kg of Acetylsalicylic acid and 10mg/kg of meloxicam and 1mg/kg of Dexamethasone subsequently, while the three test groups were given silymarin in three doses 125, 250 and 500 ml/kg subsequently. Second group: includes thirty albino rats divided into three subgroups each contain ten albino rats: first group to investigate the protective effect of silymarin against E. coli infection, second group to investigate the curing effect of silymarin in infected rats. Third group were using as a control where the albino rats injected with normal saline only.

Edema thickness measurement: After 30 min of treatment, inflammation was induced in rats by injection of 0.1 ml egg albumin (as inflammation inducer) in the right paw of rat (Okoli et al., 2000; Duffy et al., 2001; Rang et al., 2003; Klaewklad, et al., 2017) and then the increasing volume of dermal layer at rat paw was measured before using a caliper and after 1, 2 and 3hrs of inflammation inducer injection. The result analyzed statistically using Anova table and t-test, p-values <0.05 is significant.

**Total IL-8 ELISA**: The quantitative determination of total IL-8 in serum of infected rats was performed by ELISA according to the manual procedure (My Biosource, USA).

**RESULTS**

Table 1. Figure 1, 2 and 3 shows that silymarin reduced acute inflammation at rat which is induced by E. coli. the used of Silymarin and dexamethasone, acetylsalicylic acid and meloxicam lead to reduce the inflammation at rat’s paw in comparison with the control group after 1hr, 2hr and 3hr from inflammation induction. The test group which intake silymarin at dose 125mg/kg, showed significant difference in comparison with the rest of groups during the three hr. of test (p<0.05), while there was no significant difference between silymarin at dose 250 and 500mg/kg and meloxicam, dexamethasone during the second and third hr. of the test. Silymarin at 250 and, 500mg/kg showed significant difference in comparison with acetylsalicylic acid during the second hr. and third hr. of the test (p<0.05). Silymarin at dose 500mg/kg did not show any significant difference during the second hr. of the test. The effective dose of silymarin against acute inflammation induced in rats is shown in Figure 1.

The effect of silymarin on acute inflammation at rat paw increased after dose duplication from 125 to 250mg/kg, while increasing of silymarin concentration to 500mg/kg did not show any significant difference in its anti-inflammatory activity.

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>Mean increase in paw thickness (mm)</th>
<th>% of inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1h</td>
<td>2h</td>
</tr>
<tr>
<td>Acetyl salicylic acid 100mg/kg</td>
<td>1.92 ± 0.06*a</td>
<td>1.34 ±0.04*a</td>
</tr>
<tr>
<td>Dimethyl sulfoxide 2 ml/kg</td>
<td>3.07± 0.06</td>
<td>2.28 ± 0.05</td>
</tr>
<tr>
<td>Dexamethazone 1mg/kg</td>
<td>1.79 ± 0.06*b</td>
<td>1.37 ± 0.06*</td>
</tr>
<tr>
<td>Meloxicam 10mg/kg</td>
<td>1.99 ± 0.05*a</td>
<td>1.48 ± 0.05*b</td>
</tr>
<tr>
<td>Silymarin 125 mg/kg</td>
<td>2.25 ± 0.06*c</td>
<td>1.76 ± 0.05*</td>
</tr>
<tr>
<td>Silymarin 250 mg/kg</td>
<td>2.06 ± 0.07*a</td>
<td>1.51 ± 0.06*b</td>
</tr>
<tr>
<td>Silymarin 500 mg/kg</td>
<td>1.86 ± 0.05*b</td>
<td>1.45±0.04*</td>
</tr>
</tbody>
</table>

Data were expressed as mean ± SEM; number of animals = 8 in each group; *P< 0.05; values with non-identical superscripts (a, b, c) among different groups are considered significantly different (P< 0.05).
Fig 1: Silymarin effect on acute inflammation induced by egg albumin in rat after 1hr.

Fig 2: Silymarin effect on acute inflammation induced by egg albumin in rat after 2hr.

Fig 3: Silymarin effect on acute inflammation induced by egg albumin in rat after 3hr.

From Figure 4 and 5, the result showed that there is a significant difference in the level of IL-8 between post-infection and control and pre-infection and control group. There was no significant difference between pre- and post-infection in IL-8 level (Figure 6).

Figure 4: The level of interleukin 8 at rat serum for control group and Silymarin post-infection group. \( P=0.000009 \)

Figure 5: The level of interleukin 8 at rat serum for control group and Silymarin pre-infection, \( P=0.00006 \)
DISCUSSION

The liver infection causes serious problem in recent days, so we have to find the solution for this problem, one of these solutions is to find a successful and safe therapy that can reduce hepato inflammation in human like Silymarin, in this study. We have used rats with bacterial inflammation as live model to express anti-inflammatory action (Amos et al, 2002). The acute inflammation is represented by making edema at rat paw induced by egg albumin and increase blood vessel permeability leading to activation for several marker of inflammation like histamine, HT-5, prostanoid, kinins (Ialenti et al., 1992).

According to Marsha-Lyn et al., (2002), the inflammation occurs in three distinct stages. Early stage, including work of histamine and HI-5 (lasts for 2 hr) then intermediate stage, which depends on the activation of bradykinin then final stage, completed by synthesis of prostanoid. Many studies that used silymarin as anti-inflammatory agent at lab samples showed that inhibition of inflammation was made by the suppression of neutrophils migration to the area of inflammation and leading to release of ROS, RNS and proteolysis enzyme and increase in the permeability of endothelial layer (De la et al., 1996).

Other studies on Silymarin that used to treat infected rat with inflammation induced by papaya latex and carrageenan injected at rat ears, showed that Silymarin had decreased the inflammation at dose 25, 50 and 100 mg/kg while it had no effect on inflammation induced by carrageenan and decreased the inflammation in rat ear by 36% (Gupta et al., 2000).

Silymarin plays many biological roles like work as antioxidant agent, anti-inflammatory, anticancer, work to increase the cell content of GSH and activated SOD and decrease lipid peroxidation and leading to protect the stability of cell membrane of mast cell (Chlopikova et al., 2004; Paril, 2004). The action of silymarin can be explained in the first stage of inflammation (initiated by histamine and HI-5) by the suppression of mast cells and inhibition of the release of intermediates which are responsible to initiate early stage of acute inflammation at rat paw and prevent TNF-α activation of NF-KB which organize the expression of different genes that are contributed in the inflammation (Monna, 1999; Baumann, 2004; Kang et al., 2004).

The other important role of silymarin for suppressing third stage of acute inflammation is done by inhibiting the synthesis of PG and gene expression of COX and suppress all stages of inflammation initiation and diffusion by inhibiting migration of leukocyte to the site of infection and enhancing the resolving stage of inflammation by increasing the ability of macrophage phagocytosis and remove all inflammatory cells. Silymarin, in comparison with non-steroidal anti-inflammatory agent, has the same action in inhibiting the diffusion of inflammation by suppressing the synthesis of PG but it has negative reaction on the resolving phase of inflammation by inhabiting the synthesis of 15d-PGJ2 which is considered as an important anti-inflammatory mediator (breakdown stage) (Giles, 2001; Gilroy et al., 2003).

In this study we estimated level of inflammation pro and post-infection by measuring the level of Interleukin -8 in rat serum because of its role as a pro-inflammatory cytokine that participate in acute inflammation responses to infection as reported by many researches (Zahler et al., 1999). Responses of inflammation are initiated by pathogen infection (bacteria, viruses) resulted in release of
cytokines and chemokines that are activated by nuclear kappa (NFκB) to reduce migration of leukocyte, limit the replication and spreading of pathogen and activation of TNF-α and IL-8 and finally reduce the inflammation (Henri et al., 2002; Mihm et al., 2004; Jundi and Greene, 2015).

**Conclusion**

Silymarin extract has anti-inflammatory effect as indicated by reducing edema thickness and decrease serum IL-8 level in in infected rats with *E. coli*.

**REFERENCES**


Giles K.M., Glucocorticoids augmentation of macrophage capacity for phagocytosis of apoptotic cells is associated with the reduced p130expression, loss of paxillin/pyk2 phosphorylation and high levels of active Rac. *J. Immunol.* 167: 976-986 (2001).


