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Estimation of the novel antipyretic, anti-inflammatory, antinociceptive and antihyperlipidemic effects of silymarin in Albino rats and mice

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ABSTRACT

Objective: To evaluate the other pharmacological actions of silymarin in Albino rats and mice such as antipyretic, anti-inflammatory, antinociceptive and antihyperlipidemic effects.

Methods: Rats were injected intramuscularly with pyrogenic dose of brewer's yeast for the antipyretic test of silymarin. Another group of rats injected with 0.1 mL of 1% carrageenan solution in saline at the subplanter area of the right hind paw for the anti-inflammatory test of silymarin. Another group of mice tested by hot plate method for determination of antinociceptive effect of silymarin. Hyperlipidemia was induced using high fat diet for 2 months to estimate the antihyperlipidemic activity of silymarin.

Results: Silymarin showed a significant antipyretic effect of both doses (50 and 100 mg/kg) compared with control untreated group. Moreover, silymarin elucidated a significant anti-inflammatory effect of both doses reflected on the decrease of the rat paw edema every hour interval for 4 h after administration in comparison with control positive group. By the same taken, both doses of silymarine revealed a significant antinociceptive action in hot plate method at 30 and 60 min post administration. Besides, it lowered significantly the serum levels of prostaglandin E2, tumor necrosis factor alpha and interleukin 1 beta after 2 h of silymarin administration in carrageenan induced rat paw edema besides the significant decrease of total cholesterol, triglycerides, low density lipoprotein and significantly elevated high density lipoprotein after 2 weeks of silymarin administration.

Conclusions: These outcomes delivered a new vision into the possible pharmacological mechanisms by which silymarin advances antipyretic, anti-inflammatory, antinociceptive and antihyperlipidemic effects.

1. Introduction

Silymarin is a flavonolignan that has been presented fairly recently as a hepatic protective agent. It is extracted from the seeds and fruit of *Silybum marianum* with its active component silibinin. Silymarin is used for the treatment of various liver disorders manifested by progressive necrosis and functional deficiency [1,2]. Furthermore, it is able to afford liver protection against damages (ischemia, radiation, iron load and viral hepatitis) [3].

Silymarine, is a well-known natural antioxidant product and it had been used in many oxidative stress models in rats, besides its immunomodulatory effects on prostaglandin E2 (PGE2), tumor necrosis factor alpha (TNF-α) and interleukin 1 beta (IL-1β) [4]. So, in this work we examined the antipyretic effect of silymarin, which may show another property of this magic plant especially that many authors didn't evaluate this effect before on Silymarin.

A significant anti-inflammatory effect of silymarin has been described lately. Silymarin exhibits many effects such as inhibition of neutrophil migration, downregulation of Kupffer cells, decline of leukotriene production and utilization of prostaglandins [3,5,6]. The anti-inflammatory effect of silymarin is not well known up till now; it could be associated to the decrease of the production of nuclear factor-kappa (NF-κB), which controls the synthesis of several genes that plays an important role in the inflammatory progression [4,7].

High percentage of the phenolic compounds are known to have antinociceptive properties [8–10]. So, in this study we evaluated the antinociceptive effect of silymarin at two doses level (50 and 100 mg/kg) using hot plate method, which up till now no authors estimates it directly.

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The effect of silymarin on cellular permeability is linked with changes of membrane lipids (cholesterol and phospholipids), this may effect lipoprotein excretion and uptake [11]. Silymarin reduces plasma levels of low density lipoprotein (LDL) cholesterol in hyperlipidaemic rats [11]. Also, it is able to normalize the increase in plasma lipids observed and to antagonize the reduction in serum free fatty acids induced by thioacetamide. It is known that silibinin is able to provoke the increase in lipids and triglycerides produced in the liver and to activate fatty acid β-oxidation [11], it was evident that silymarin increases LDL binding to hepatocytes, an important factor for the reduction of LDL in plasma [11]. Moreover, silymarin may reduce triglyceride production in the liver [11].

So, we aimed by this study to spotlight on the possible antipyretic effect of silymarine and on the evaluation of PGE2, TNF-α and IL-1β which they play a major role in the inflammation process.

2. Materials and methods

2.1. Animals

Adult male Albino Sprague Dawely rats and mice weighing 80–90 g and 18–20 g respectively were obtained from the National Research Center Animal Laboratory (Giza, Egypt) and were accommodated in standard polypropylene cages and kept under constant environmental conditions and equal light–dark cycles. The animals were fed commercially available normal standard diet and water *ad libitum*. The investigations were carried in accordance to the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health and Institutional Animal Ethical Committee and complies with the guidelines from the Canadian Council on Animal Care.

2.2. Plant material

Silymarin was purchased from Sigma–Aldrich Co. (St Louis, MO, USA).

2.3. Drugs and chemicals

ELISA technique was carried out using the corresponding ELISA rat kits for the assessment of TNF-α, IL-1β and PGE2 levels in serum (Pierce, Rockford, USA).

2.4. Standard drugs used as a control

Paracetamol was purchased from Misr Company for pharmaceutical industries, Giza, Egypt. While, indomethacin was obtained from El Kahera pharmaceutical industrial company, Giza, Egypt. Aspirin from was obtained Bayer Limited Egypt LLC, Cairo, Egypt. Atorvastatin was purchased from Alexandria pharmaceutical company, Alexandria, Egypt. Synchron CX5 auto-analyzer (Beckman Instruments INC, Brea, USA) was used for measuring serum total cholesterol, triglycerides, LDL and high density lipoprotein (HDL). Plant material and drugs were given orally.

2.5. Antipyretic test

Rats were divided into 4 groups ($n = 6$). Body temperature of each rat was measured from the rectum using thermometer. Rats were injected intramuscularly with pyrogenic dose of Brewer's yeast (1 mL/100 g body weight of 44% yeast suspension in saline) [12,13]. Temperature was measured after 18 h from injection and considered as zero time. Two doses of silymarin (50 and 100 mg/kg), paracetamol (50 mg/kg) (positive control) and saline (negative control) were administered once orally by gastric tube to each group rats respectively and the temperature was measured after 1 and 2 h interval [12–14].

2.6. Anti-inflammatory test

This test was carried out by carrageenan induced rat paw edema [15]. Rats were divided into 4 groups ($n = 6$), 2 doses of silymarin (50 and 100 mg/kg), indomethacine (20 mg/kg) (positive control) and saline (negative control) were administered once orally by gastric tube [16]. All rats were injected with 0.1 mL of 1% carrageenan solution in saline at the subplanter area of the right hind paw after 1 h from silymarin and indomethacine administration. Each rat its paw thickness was measured by planimeter and blood samples were collected from retro orbital plexus vein of anesthetized rats and centrifuged at 5000 r/min at 4 °C for 10 min using Hermle cooling bench top centrifuge Z 323 K (Japan) to separate serum samples for estimation of PGE2, TNF-α and IL-1β using plate reader (Tecan Spectra Classic Plate Reader, USA) before carrageenan administration. After administration of carrageenan the paw thickness of each rat was measured by planimeter every 1 h for 4 h, after 4 h serum samples were collected again for estimation of PGE2, TNF-α and IL-1β. Edema rate, inhibition, potency were calculated as follows [17]:

$$\text{Edema}\,(\%) = \frac{\text{Control thickness mean} - \text{Treated thickness mean}}{\text{Control thickness mean}} \times 100$$

$$\text{Inhibition}\,(\%) = \frac{\text{Control edema}\,(\%)\ \text{mean} - \text{Treated edema}\,(\%)\ \text{mean}}{\text{Control edema}\,(\%)\ \text{mean}} \times 100$$

$$\text{Potency}\,(\%) = 100 - \frac{\text{Inhibition}\,(\%)\ \text{indomethacine} - \text{Inhibition}\,(\%)\ \text{treated group}}{\text{Inhibition}\,(\%)\ \text{indomethacine}} \times 100$$

2.7. Antinociceptive test

This test was carried out by hot plate method for determination of silymarin analgesic effect [18]. Mice were adapted for 3 consecutive days before the test by putting them in hot plate device adapted at room temperature for 15 min [13]. Latency to show analgesic response, like licking the paws or jumping out the hot plate was recorded at 30 and 60 min after administration of 2 doses of silymarin (50 and 100 mg/kg), aspirin (200 mg/kg) (positive control) and saline (negative control) orally by gastric tube [19]. Response time for thermal pain was recorded before administration of silymarin and drug (0 time).

2.8. Antihyperlipidemic test

Hyperlipidemia was induced using high fat diet for 2 months [20]. This diet formula was prepared by mixing normal fat diet

with atherogenic components (0.15% thiouracil, 0.4% choline chloride, 20% lactose, 2% sodium cholate, 20% hydrogenated vegetable oil, 20% sucrose and 5% cholesterol). Rats were divided into 4 groups (n = 10), 2 groups were received 50 and 100 mg/kg of silymarin respectively for 2 weeks, 1 group was received atorvastatin (1 mg/kg) (positive control) twice a week for 2 weeks [21] and 1 group was received saline (negative control) for 2 weeks. All drugs were administered orally using gastric tube and all groups received normal fat diet during 2 weeks of treatment. After 2 weeks of treatment and after overnight fasting, blood samples were collected from retro-orbital plexus vein of anesthetized rats and centrifuged to separate serum samples for estimation of total cholesterol, triglycerides, HDL and LDL using Synchron CX5 auto-analyzer (Beckman Instruments INC, Brea, USA).

2.9. Statistical analysis

The differences between groups were tested for significance using ANOVA, followed by Tukey *post hoc* test determined by SPSS software program, version 21. Values are expressed as mean ± SE. The level of statistical significance was taken at $P < 0.05$.

3. Results

Table 1 revealed the antipyretic effect of silymarin (50 and 100 mg/kg) was significant in comparison with control positive group. Both doses of silymarin showed a dose dependent effect manner, which was appeared in lowering the body temperature (100 mg/kg silymarin more effective than the 50 mg/kg dose). But, paracetamol (standard drug) showed a more powerful effect than the two doses of silymarin.

The anti-inflammatory effect of both doses of silymarin (Table 2) was elucidated in the significant decline of rat paw edema% compared with positive control group which is reflected also on the percentage of inhibition and the percentage of potency. Both doses of silymarin showed a dose dependent effect manner (100 mg/kg silymarin more effective than the 50 mg/kg dose). In addition, the significant decrease in PGE2, TNF-α and IL-1β 4 h after administration of carrageenan in comparison with positive control group (Table 3). Both doses of silymarin showed a dose dependent effect manner (100 mg/kg silymarin more effective than the 50 mg/kg dose). Moreover, indomethacine (standard drug) was more potent in its anti-

Table 1
Antipyretic effect of silymarin in Albino rats.

Groups	Body temperature (°C)		
	Zero time	1 h	2 h
Control (1 mL saline)	38.50 ± 0.13	39.02 ± 0.04	39.20 ± 0.11
Paracetamol (50 mg/kg)	38.70 ± 0.10	37.96 ± 0.08*	37.60 ± 0.05*
Silymarine (50 mg/kg)	38.75 ± 0.12	38.40 ± 0.07*	38.15 ± 0.16*
Silymarine (100 mg/kg)	38.70 ± 0.10	38.20 ± 0.08*	37.80 ± 0.11*

Values are means ± SE of 6 animals. As compared with normal control (*) group (One-way ANOVA followed by Tukey *post hoc* test) at $P < 0.05$.

Table 2
Anti-inflammatory effect of silymarin in Albino rats (%).

Groups	Edema	Inhibition	Potency
Control (1 mL saline)	15.16 ± 0.01	–	–
Indomethacine (20 mg/kg)	3.36 ± 0.30*	77.84	100.0
Silymarine (50 mg/kg)	11.14 ± 0.01*	26.52	34.1
Silymarine (100 mg/kg)	7.12 ± 0.30*	53.03	68.1

Values are means ± SE of 6 animals. As compared with control positive (*) group (One-way ANOVA followed by Tukey *post hoc* test) at $P < 0.05$.

Table 3
Effect of silymarin on PGE2, TNF-α and IL-1β levels in serum after 4 h of carrageenan administration in Albino rats (ng/L).

Groups	PGE2	TNF-α	IL-1β
Normal	201.65 ± 18.61	9.81 ± 0.78	38.51 ± 2.95
Control (1 mL saline)	710.18 ± 45.66†	55.41 ± 2.85†	101.75 ± 7.81†
Indomethacine (20 mg/kg)	386.85 ± 19.75*†	18.33 ± 1.01*†	53.48 ± 4.03*†
Silymarine (50 mg/kg)	592.55 ± 33.41*†	41.46 ± 3.11*†	82.86 ± 6.66*†
Silymarine (100 mg/kg)	442.00 ± 26.20*†	28.11 ± 1.95*†	67.91 ± 5.38*†

Values are means ± SE of 6 animals. As compared with control positive (*) and normal (†) groups (One-way ANOVA followed by Tukey *post hoc* test) at $P < 0.05$.

Table 4
Antinociceptive effect of silymarin in Albino mice (seconds).

Groups	Response time		
	Zero time	30 min	60 min
Control (1 mL saline)	11.18 ± 0.36	11.23 ± 0.37	11.35 ± 0.33
Aspirin (200 mg/kg)	11.41 ± 0.31	15.33 ± 0.22*	17.90 ± 0.30*
Silymarine (50 mg/kg)	11.55 ± 0.46	13.70 ± 0.20*	16.26 ± 0.15*
Silymarine (100 mg/kg)	11.38 ± 0.44	14.23 ± 0.14*	17.76 ± 0.15*

Values are means ± SE of 6 animals. As compared with normal control (*) group (One-way ANOVA followed by Tukey *post hoc* test) at $P < 0.05$.

Table 5
Antihyperlipidemic effect of 2 weeks of administration of silymarin on total cholesterol, triglycerides, HDL and LDL after 2 months of high fat diet in Albino rats (mg/dL).

Group	Parameters	Time	
		Zero	2 weeks
Control (1 mL saline)	Cholesterol	175.66 ± 2.9	170.59 ± 2.1
	Triglycerides	161.44 ± 1.8	158.88 ± 2.3
	HDL	15.05 ± 0.9	15.41 ± 0.8
	LDL	132.32 ± 2.2	128.21 ± 1.9
Atorvastatin (1 mg/kg)	Cholesterol	181.28 ± 5.4	108.49 ± 4.2*
	Triglycerides	156.12 ± 3.4	96.76 ± 1.9*
	HDL	14.08 ± 0.3	30.77 ± 1.1*
	LDL	128.84 ± 4.1	71.75 ± 0.7*
Silymarin (50 mg/kg)	Cholesterol	168.44 ± 3.9	144.61 ± 5.6*
	Triglycerides	157.88 ± 5.4	120.91 ± 1.6*
	HDL	15.55 ± 0.4	22.52 ± 0.4*
	LDL	129.07 ± 3.5	101.11 ± 1.4*
Silymarin (100 mg/kg)	Cholesterol	172.35 ± 5.1	120.38 ± 5.5*
	Triglycerides	163.41 ± 6.1	130.13 ± 4.5*
	HDL	13.96 ± 0.7	27.62 ± 0.2*
	LDL	135.86 ± 4.1	90.22 ± 2.1*

Values are means ± SE of 10 animals. As compared with zero time (*) (One-way ANOVA followed by Tukey *post hoc* test) at $P < 0.05$.

Table 6
Silymarin effect on body weight in Albino rats (g).

Groups	Body weight		
	Zero time	2 months	2 weeks Post treatment
Control (1 mL saline)	82.71 ± 2.32	286.73 ± 8.68*	278.26 ± 6.77
Atorvastatin (1 mg/kg)	79.52 ± 1.43	293.65 ± 9.40*	190.35 ± 4.11†
Silymarine (50 mg/kg)	80.93 ± 3.11	281.88 ± 5.33*	253.64 ± 3.56†
Silymarine (100 mg/kg)	81.44 ± 2.22	288.59 ± 7.86*	221.75 ± 2.85†

Values are means ± SE of 10 animals. As compared with zero time (*) group, 2 months (†) group (One-way ANOVA followed by Tukey *post hoc* test) at $P < 0.05$.

inflammatory effect (rat paw edema and inflammatory cytokines) than the two doses of silymarin.

Table 4 showed the significance of antinociceptive activity of both doses of silymarin compared to the normal control group after 30 and 60 min of silymarin administration, which is reflected on the increase of the response time to thermal pain. Both doses of silymarin showed a dose dependent effect manner (100 mg/kg silymarin more effective than the 50 mg/kg dose). However, aspirin (standard) drug represented more obvious antinociceptive activity than the both doses of silymarin.

Both doses of silymarin showed a significant anti-hyperlipidemic effect (Table 5), which was reflected on the significant decrease in cholesterol, triglycerides and LDL serum levels in comparison with zero time group. While silymarin showed a significant increase in HDL serum level in hyperlipidemic rats in comparison with zero time group. By the same way, both doses of silymarin showed a dose dependent effect manner (100 mg/kg silymarin more effective than the 50 mg/kg dose). But the effect of atorvastatin (standard drug) was stronger than the both doses silymarin.

The significant decline in the body weight of the treated hyperlipidemic animals' was contributed to the effect of both doses of silymarin (Table 6) compared with the 2 months high fat diet group. And both doses of silymarin showed a dose dependent effect manner (100 mg/kg silymarin more effective than the 50 mg/kg dose). However, atorvastatin (standard drug) was more effective than both doses of silymarin.

4. Discussion

Silymarin, an extract from the milk thistle plant seeds, has been used for centuries against liver diseases. Silymarin is a blend of flavonolignans: silybin-A, silybin-B, isosilybin-A, isosilybin-B, silychristin, silydianin and isosilychristin and one flavonoid named taxifolin. Silymarin has antioxidant, immunomodulatory, anti-proliferative and anti-viral activities although its mechanism of action is incompletely known till date [22–24]. Earlier studies have contained within inadequate data and consumption of changeable doses, making it difficult to compare results between readings.

In our study, silymarin showed a novel significant antipyretic effect against pyrogenic dose of brewer's yeast. This pathogenic dose is caused by massive production of PGE2 which is responsible for fever production [25]. Silymarin dose-dependently suppressed the TNF-α, IL-1β and PGE2. Consistent with these results, the mRNA expression of IL-1β and cyclooxygenase-2 was also completely blocked by silymarin. Moreover, activity of NF-κB was also inhibited by silymarin as stated by another author [26].

Also, silymarin elucidated an anti-inflammatory action against carrageenan induced inflammation. Edema occurred through 2 phases, the first one occurred due to the release of histamine and cyclooxygenase ingredients, while the second one, occurred due to the release of prostaglandins, proteases and lysosomes [13,27]. Silymarin prevents the release of inflammatory mediators, such as TNF-α, IL-1β, PGE2 and nitric oxide [26]. Authors found that silymarin in a dose-dependent manner declined the production of inducible nitric oxide synthase. It was suggested that the inhibitory effect of silymarin was arbitrated to the inhibition of NF-κB activation [28,29].

Antinociceptive effect of silymarin was clear against normal control group like the same effect if its anti-inflammatory effect due to its inhibition of prostaglandins (PGE2 and prostaglandin F2) (pain inducers) [30–32].

The two doses of silymarin exhibited a significant anti-hyperlipidemic activity reflected on the significant decline of cholesterol, triglycerides, LDL and body weight and a significant increase if HDL in comparison with zero time group in a dose dependent manner. This action due to the influence of the increased cellular permeability which is closely associated with changes of membrane lipids [33]. This proposes that silymarin may stimulate lipoprotein secretion and uptake. Also, silymarin and silibinin decrease the synthesis and turnover of phospholipids in the liver. Furthermore, silibinin is able to inhibit phospholipid synthesis [11]. In addition, silibinin induces phosphatidylcholine synthesis and increases the activity of cholinephosphate cytidyltransferase in rat liver in normal conditions [33]. Silymarin, also stimulate fatty acid β-oxidation and may reduce triglyceride synthesis in the liver [1].

This experiment was planned to clarify the antipyretic, anti-inflammatory, antinociceptive and antihyperlipidemic effects of silymarin in two doses forms to prove which dose is more powerful and if its action is in dose dependent manner or not, especially silymarin used in worldwide and in Egypt as a natural liver support in many cases of liver diseases. So, we made this study to evaluate other pharmacological actions and benefits of silymarin.

Conflict of interest statement

We declare that we have no conflict of interest.

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