SILYMARIN: A FLAVOLIGNAN WITH ANTIDEPRESSANT ACTIVITY

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Abstract:
The aim of the present study was to determine the effect of Silymarin (a flavolignan) in experimental model of depression. Silymarin was administered in 3 doses (150, 200, 250 mg/kg; i.p.) to male Swiss mice (25-30 g) for 15 days daily. On 15th day immobility time was determined using Forced swim test (FST). The two effective doses of Silymarin (200 and 250 mg/kg) from FST study were selected, and explored for their mechanism of action against reserpine-induced depression model. The behavioural parameters such as catalepsy, ptosis and hypothermia were estimated. These behavioural deficits were further, integrated with biogenic amine (brain nor-epinephrine, dopamine, serotonin) and oxidative stress (brain TBARS and GSH) parameters. Silymarin significantly ameliorated the depression by restoring behavioural, biochemical and neurochemical alterations against reserpine-induced depression.

Keywords: Depression, Forced swim test, Reserpine, Silymarin

Introduction
Depression, a debilitating psychiatric disorder, is predicted to be the second most prevalent human illness by the year 2020.[1] Earlier, depression was considered to be an old-age disease. However, current trends reveal an increased percentage of younger populations being affected from its consequences. The common symptoms of major depression include depressed or irritable mood, decreased interest in pleasurable activities, significant weight loss or gain, insomnia or hypersomnia, psychomotor agitation or retardation, fatigue or loss of energy, feeling of worthlessness or excessive guilt, poor concentrating power, and enhancement in suicidal tendencies.[2] Various antidepressants, ranging from monoamine-oxidase inhibitors to recently developed dual reuptake inhibitors, are prescribed for alleviating the symptoms of depression.[3] Despite the availability of these blockbuster molecules, approximately 30% of depressed patients do not respond to the existing drug therapies and the remaining 70% fails to achieve complete remission. Moreover, antidepressants are associated with a plethora of side effects and drug-drug/drug-food interactions. In this context, novel alternative approaches (such as St. John’s Wort, valerian, ginseng, saffron, lavender, Brahmi, Onion, Garlic, Satawari, Turmeric and Liquorice) are being tried to find more efficacious and safer drugs for the treatment of major depression.[4]

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Silymarin is a flavonolignan which is obtained from the seeds of 'milk thistle' (*Silybum marianum* L. Gaertn), family Asteraceae. Silymarin is actually a mixture of 3 flavonolignans (silybin, silydianin and silychristin), with silybin being the most active compound. Reports in the literature suggested that silymarin (and other flavonoids) can also influence the metabolism of brain amines in humans neuronal and neuroendocrine cell lines and the antioxidant capacity of flavonoids also may play a positive role in specific brain pathologies.[5,6] Therefore, the present study investigates the probable antidepressant-like activity of Silymarin in mice.

**MATERIALS AND METHODS:**

**Drugs and Chemicals**

Silymarin was purchased from Sigma-Aldrich (Mumbai, India) and administered to animals by suspending in phosphate buffer saline. Reserpine was purchased from Fluka Analytical, India and administered by dissolving in 50 µL glacial acetic acid and diluted to the correct concentration with distilled water. All other chemicals used in the present study were of analytical grade.

**Experimental Protocol**

Male Swiss mice (25-30 g) were procured from the Disease Free Small Animal House, Lala Lajpat Rai University of Veterinary and Animal Science Hisar, Haryana, India. The experimental protocol was approved by the Institutional Animal Ethics Committee and the care of the laboratory animals was taken as per the guidelines of CPCSEA, Ministry of Forests & Environment, Government of India. Silymarin was administered to different groups of mice (n=6) for 15 successive days daily in three doses (150, 200 and 250 mg/kg; *i.p.*). The doses of Silymarin were decided on the basis of previous studies.[7] On 15th day, antidepressant activity of Silymarin was evaluated by forced swim test (FST) activity. The two more effective doses of Silymarin (200 and 250 mg/kg, *i.p.*) in FST study were selected and explored for their mechanism of action against reserpine induced depression model.[8] Ptosis score (0 to 4 where, eyes closed = 4, eyes ¾ closed = 3, eyes ½ closed = 2, eyes ¼ closed = 1, eyes open = 0), cataleptic score (catalepsy more than 60 s = 5, between 30 and 60 s = 4, between 10 and 30 s = 3, between 5 and 10 s = 1, less than 5 s = 0) and hypothermia were measured after reserpine administration. After behavioural studies, the animals were sacrificed and brain norepinephrine, dopamine and serotonin content[9, 10], TBARS[11] and GSH[12] were measured. Brain monoamine (norepinephrine, dopamine and serotonin) levels were estimated at International Testing Centre, Panchkula, Haryana, India.

**RESULTS**

**Effect of silymarin on the Forced swim test (FST) activity in mice:**

Administration of silymarin (150, 200 and 250 mg/kg; *i.p.*) for 15 days to separate groups of mice reduced the immobility time as compared to control mice (Figure 1). On the basis of FST studies, the more effective doses of silymarin i.e. 200 and 250 mg/kg (p<0.001) were selected for further studies.
Effect of Silymarin on ptosis, catalepsy and hypothermia:
Reserpine control mice showed higher ptosis score (Figure 2), enhanced cataleptic score and reduction in body temperature (Table 1) as compared to control mice. Silymarin (200 and 250 mg/kg; i.p.) administration for 15 successive days to mice before reserpine administration prevented the rise in ptosis score (p<0.05), prohibited the enhancement in catalepsy score (p< 0.05) and ameliorated the reduction in body temperature as compared to reserpine control mice.

Effects of silymarin on brain norepinephrine dopamine and serotonin levels:
The level of brain norepinephrine, dopamine and serotonin levels were found to be significantly lower in reserpine treated mice compared to control mice. However, pre-administration of silymarin in reserpine mice significantly prevented the decline in the norepinephrine, dopamine and serotonin levels as compared to reserpinised mice (Table 1).

Effects of silymarin on brain TBARS and GSH levels:
Reserpine treated mice showed enhanced levels of brain TBARS levels and reduced GSH levels as compared to control group. However, pre-administration of silymarin to reserpine induced depressive mice markedly reduced the rise in brain TBARS levels and increased the GSH levels (Table 1).

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**Figure 1:** Effect of Silymarin on immobility time of mice in the forced swim test.
Values are expressed as Mean ± SEM, (n=6). *, *** represents p < 0.05 and p < 0.001 respectively compared to Control. (One way ANOVA followed by Tukey’s test). SIL = Silymarin.
Figure 2: Effect of Silymarin on degree of ptosis.
Values are expressed as Mean ± SEM, (n=6). Superscripts *, ** indicate statistical significance at (p < 0.05, p < 0.01) respectively in comparison to Reserpine. (One way ANOVA followed by Tukey’s test). RES = Reserpine, SIL = Silymarin.

Table 1: Effect of Silymarin on catalepsy score, body temperature, brain norepinephrine, dopamine, serotonin, TBARS and GSH levels of mice

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Reserpine</th>
<th>Sil 200 + Res</th>
<th>Sil 250 + Res</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catalepsy (s)</td>
<td>0.33 ± 0.210***</td>
<td>4.83 ± 0.166</td>
<td>3.50 ± 0.223**</td>
<td>2.83 ±0.401***</td>
</tr>
<tr>
<td>Body temperature (°C)</td>
<td>36.33 ± 0.240***</td>
<td>29.33 ± 0.251</td>
<td>32.66 ± 0.055**</td>
<td>32.78 ± 0.054**</td>
</tr>
<tr>
<td>Norepinephrine (ng/g tissue)</td>
<td>463.6 ± 0.57***</td>
<td>121.8 ± 0.45</td>
<td>196.3 ± 2.05**</td>
<td>253.7 ± 0.53**</td>
</tr>
<tr>
<td>Dopamine (ng/g tissue)</td>
<td>671 ± 0.20**</td>
<td>29.31 ± 1.50</td>
<td>56.9 ± 1.25**</td>
<td>105.5 ± 1.35***</td>
</tr>
<tr>
<td>Serotonin (ng/g tissue)</td>
<td>408.9 ± 0.39***</td>
<td>11.7 ± 0.71</td>
<td>39.6 ± 0.64**</td>
<td>42.8 ± 0.55**</td>
</tr>
<tr>
<td>Brain TBARS (nmol/g)</td>
<td>0.30 ± 0.007***</td>
<td>0.62 ± 0.007</td>
<td>0.52 ± 0.006**</td>
<td>0.42 ± 0.005***</td>
</tr>
<tr>
<td>Brain GSH (µmol/g)</td>
<td>2.35 ± 0.131***</td>
<td>1.25 ± 0.061</td>
<td>1.65 ± 0.061**</td>
<td>1.96 ± 0.076***</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SEM, (n=6). *, ** and *** represents p < 0.05, p< 0.01 and p < 0.001 respectively as compared to Reserpine

DISCUSSION
In the present study, administration of silymarin to mice for 15 successive days exhibited antidepressant activity as indicated by the enhancement in the immobility time in forced swim test. The presence of flavonoids may be responsible for the observed antidepressant effect of Silymarin; as Silymarin contains 72 % flavonoids such as silybin, silychristin and silydianin. Flavanoids can influence the metabolism of brain amines in human neuronal and neuroendocrine cell lines. Furthermore, Silymarin treatment was found to ameliorate the depression symptoms associated with reserpine administration. Reserpine is a well known monoamine depleting agent and intraperitoneal administration of reserpine provides a popular and widely used model for inducing depression in experimentally animals. Reserpine irreversibly blocks the vesicular monoamine transporter (VMAT). This normally transports free norepinephrine, serotonin, and dopamine from the cytoplasm of the presynaptic nerve terminal into storage vesicles for subsequent release into
the synaptic cleft. Unprotected neurotransmitters are metabolized by MAO in the cytoplasm and consequently never reach the synapse.\textsuperscript{[14]} Depletion of biogenic amines (NE, 5-HT, DA) in the brain has been observed to induce catalepsy, ptosis and hypothermia.\textsuperscript{[15]} In the present study, reserpine treated mice exhibited closed eyes (ptosis), a state of catalepsy and decrease in body temperature as compared to non-treated animals. Reserpine induced catalepsy, ptosis is primarily due to blockade of dopamine receptors in the striatum. Reversal of reserpine-inducehd hypothermia indicates the possibility due to the increase serotonin levels through 5-HT\textsubscript{3} receptor blockage.\textsuperscript{[16]} In the present study, silymarin was found to decrease the cataleptic and ptosis score and increase the rectal temperature of mice as compared to reserpine control mice, indicating that drug act by increasing the amount of biogenic amines at the synaptic cleft. Mice treated with silymarin exhibited enhanced brain norepinephrine, dopamine and serotonin levels. These observations are in agreement with study of Osuchowski et al; which has reported that intraperitoneal treatment with Silymarin treatment increased norepinephrine, dopamine and serotonin levels in specific brain areas.\textsuperscript{[6]} Furthermore, silymarin is reported to directly suppress the activities of monoamine oxidase (MAO) in the C6 astrocyte cell lines.\textsuperscript{[17]} MAO suppressive activity might be responsible for enhanced brain monoamine levels.\textsuperscript{[18]} In the present study, Silymarin treatment to mice reduced brain TBARS levels and enhanced GSH levels indicating antioxidant activity of Silymarin. These findings are in agreement with previous study which reported that Silymarin has free radical scavenging activity and it is reported to inhibit the lipid-peroxidation process.\textsuperscript{[19]} Therefore, the present study suggests a possible link between Silymarin and monoamine pathways. Silymarin might be useful in the management of depression owing to its property of enhancing monoamine neurotransmitter levels in the brain in addition to antioxidant activity. However, further studies are very much warranted using a longer treatment regimen.

**References:**


6. Osuchowski MF, Johnson VJ, He Q, Sharma RP. Alterations in regional brain neurotransmitters by silymarin, a natural antioxidant flavonoid mixture,


10. Lakshmana MK, Raju TR. Endosulfan induces small but significant changes in the levels of noradrenaline, dopamine and serotonin in the developing rat brain and deficits in the operant learning performance. Toxicology 1994; 91:139-50.


