TO STUDY THE EFFECT OF SILYMARIN AGAINST LIPOPOLYSACHHARIDES INDUCED NEUROINFLAMMATION IN MICE

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ABSTRACT

Aim of the study: To study the effect of Silymarin against Intracerebral (i.c.) LPS induced Neuroinflammation in mice. Material and methods: The effect of Silymarin was examined against Intracerebral (i.c.) LPS induced Neuroinflammation in mice. The behavioural study was performed by using models Morris water maze. Various biochemical parameters such as proinflammatory cytokines (TNF-α) as indicator of Neuroinflammation, Acetyl cholinesterase (Ach E) activity as marker of cholinergic activity and malonaldialdehyde (MDA) and glutathione (GSH) as marker of oxidative stress were estimated. LPS was used in dose of (5µg/5µl) for Neuroinflammation in mice was used. Silymarin was administered in two different doses of 25mg/kg and 50mg/kg. Result: Silymarin exhibit a significant (p<0.05) decrease in latency time (LT) in Morris water maze model indicating memory enhancing activity of the drug in dose dependent manner. Also, (TNF-α) were significantly (p<0.05) reduced in mice brain as well as brain indicating antineuroinflammatory activity of drug. MDA level and Ach E activity was reduced significantly (p<0.05) in treated group in comparison to LPS treated group indicating anti oxidant effect of Silymarin. Conclusion: In conclusion, the present study indicated that Silymarin could ameliorate memory impairment induced by lipopolysachharide. Intracerebral microinjection of LPS ((5µg/5µl) significantly induced Neuroinflammation in mice and cause release of cytokines (TNF-α), free radical generation and oxidative stress in the brain regions. Treatment with Silymarin in two different doses (25mg/kg and 50 mg/kg) effectively attenuated LPS induced Neuroinflammation, oxidative stress and cognitive impairment.
KEYWORDS: Silymarin, LPS, MDA, GSH, Ach E, Intracerebral (i.c.) and Latency time (LT).

1. INTRODUCTION
Flavonoids are a group of polyphenolic substances present in most plants and are frequently consumed in human diet. It has been reported that flavonoids exert beneficial effects in experimental models of memory impairment due to their strong antioxidant potential (Bastianetto et al., 2002). Silymarin is a mixture of flavonoids present in milk thistle (Silybum marianum) belonging to family Asteraceae and used as a hepatoprotective in the treatment of liver disease. Silymarin has protective effects on the central nervous system against ethanol-induced Brain injury and memory impairment. Further Silymarin has been reported to protect inflammation and oxidative stress induced neurotoxicity. (Repetto et al., 2002).

Figure: 1 Silymarin (Shaker et al., 2010)

Silymarin, a flavonolignan from ‘milk thistle’ (Sylilbum marianum) plant is used from ancient times as a hepatoprotective drug. Along the hepatoprotective action silymarin owns also other actions as antioxidant, antilipidperoxidative, antifibrotic, anti-inflammatory, immunomodulatory, liver regenerating, etc. The milk thistle’s beneficial effects in gastrointestinal disorders and its antitumoral activities (Craig, 1999).

Neuroinflammation, Inflammation is a cardinal host defense response to injury, tissue ischemia, autoimmune responses or infectious agents. Classical signs of swelling, redness, heat and pain are witnessed in all tissues except that of brain as manifestations of
inflammation. These symptoms are caused as a result of increased amount of blood flow to the site of injury to get more nutrients and immune cells to an area in need (Koster et al., 1971). Invasion of circulating immune cells (lymphocytes and macrophages), induction or activation of inflammatory mediators such as cytokines, kinins, reducing and oxidizing species aid the repair and removal of injured or infected cells (Eming et al., 2007). Acute Neuroinflammation is more of a physiological response either to injury or insult to the CNS. Before "Neuroinflammation" became a commonly used term, endogenous CNS tissue responses to injury were referred to as ‘reactive gliosis’. Reactive gliosis entails accumulation of enlarged glial cells, notably microglia and astrocytes, appearing immediately after CNS injury has occurred. Chronic inflammation is often associated in the understanding of CNS disease as opposed to acute inflammation which is linked with CNS injury. It is proposed that chronic inflammation is a causative factor to the pathogenesis of neurological diseases and disorders (Kaminska et al., 2009). The immune cells and pro-inflammatory chemicals involved in neuroinflammation would underlie the mechanisms of diseases and neurodegeneration. The activation, or over activation, of immune cells involved in neuroinflammation and release of pro-inflammatory substances would result in reduced neuroprotection and neuronal repair, and increased neurodegeneration, leading to neurodegenerative diseases.

2. MATERIAL AND METHODS

2.1 Animals: Adult Swiss albino mice weighing 22–30 g were used. The animals were obtained from the Laboratory Animal Services Division of Indian Institute of Toxicology and Research Centre (IITR), Lucknow. The animals were kept in polycrystal cage (22.5×37.5 cm) and maintained under standard housing conditions (room temperature 24-27 °C and humidity 60-65%) with a 12 hours light and dark cycle. Food and water were available ad libitum but food was not allowed from 1 h prior to the behavioural study. All procedures described were reviewed and approved by the Institutional Animal Ethics Committee (IAEC), Indian Institute of Toxicology and Research Centre (IITR) Lucknow, India. (IAEC Approval No. Hygia/M.Pharm/26/2014-15.

2.2 Materials
Lipopolysaccharides, Donepezil, SodiumChloride, Potassium chloride, Magnesium chloride, Calcium chloride, Chloral hydrate, Phosphate buffer, Orthophosphoric acid, n- butanol, EGTA, EDTA, Triton, Sodium dihydrogen phosphate, Disodium hydrogen orthophosphate dehydrate, TBA, TCA, DTNB, Silymarin.
2.3 Intracerebral (i.c.) administrations of LPS
The mice were anesthetized with chloral hydrate (300 mg/kg, i.p.). LPS was dissolved in freshly prepared artificial CSF (aCSF) and administered in a volume of 10μl. LPS was injected (1μg/1ml) Intracerebral (i.c.). (Haley et al., 1957).

2.4 Experimental design for Morris water maze
The treatment was given for 5 days and after it to administer the lipopolysachharides intracerebrally (5μg/5μl, i.c.) in 4th ventricle of brain. After 2 hours, to performed Morris water maze.

<table>
<thead>
<tr>
<th>S No.</th>
<th>GROUP</th>
<th>TREATMENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>aCSF treated mice</td>
<td>aCSF (5μl, i.c.) + drug vehicle (p.o)</td>
</tr>
<tr>
<td>2</td>
<td>LPS treated mice</td>
<td>LPS (5μg/5μl, i.c.) + drug vehicle (p.o)</td>
</tr>
<tr>
<td>3</td>
<td>LPS + Silymarin</td>
<td>LPS (5μg/5μl, i.c.) + Silymarin (25 mg/kg, p.o.)</td>
</tr>
<tr>
<td>4</td>
<td>LPS + Silymarin</td>
<td>LPS (5μg/5μl, i.c.) + Silymarin (50 mg/kg, p.o.)</td>
</tr>
<tr>
<td>5</td>
<td>LPS + donepezil</td>
<td>LPS (5μg/5μl, i.c.) + donepezil (4mg/kg, i.p.)</td>
</tr>
</tbody>
</table>

2.5 Evaluation of spatial memory by Morris Water Maze test
The Morris water maze consisted of a large circular black pool of 120 cm diameter, 50 cm height, filled to a depth of 30 cm with water at 26 ± 2°C. A black colour round platform of 8 cm diameter was placed 1 cm below the surface of water in a constant position. The water was coloured with non-toxic black dye to hide the location of the submerged platform. The pool was divided into four hypothetical quadrants.

On the 5th day of treatment, Lipopolysachharide injected, after 2 hours of lipopolysachharides spatial learning and memory of animals were tested in Morris water maze. Trials were given for 5 consecutive days in order to train mice in the Morris water maze. The mice were given a maximum time of 90s (cut-off time) to find the hidden platform and were allowed to stay on it for 30s. The experimenter put the mice on platform herself that failed to locate the platform. The animals were given a daily session of 3 trials per day. Latency time to reach the platform was recorded in each trial. Mean latency time of all three trials is shown in the results. A significant decrease in latency time from that of 1st session was considered as a successful learning (Tota et al., 2010).

2.6 Estimation of biochemical parameters
All the biochemical and molecular studies were performed at the end of behavioural studies.
2.6.1 Preparation of brain tissue
The mice were decapitated under ether anaesthesia. The skull was cut open and the brain was exposed from its dorsal side. The whole brain was quickly removed and cleaned with chilled normal saline on the ice. A 10% (w/v) homogenate of brain samples (0.03M sodium phosphate buffer, pH-7.4) was prepared by using a homogenizer at a speed of 2000 rpm. The homogenized tissue preparation was used to measure acetyl cholinesterase, malondialdehyde, Acetyl cholinesterase, and glutathione and tumour necrosis factor – alpha. (Tota et al., 2010).

2.6.2 Estimation of glutathione in mice brain
GSH, a natural antioxidant level was determined by its reaction with 5-5’-dithiobis 2- nitro benzoic acid (DTNB) at 412 nm. The processed tissue sample was treated with equal volume of 5% TCA. The mixture was centrifuged at 3.000 rpm for 10 minutes, 0.05 ml of supernatant, 0.1 ml phosphate buffer (pH-8.4), DTNB and 0.05 ml of distilled water was added. Then absorbance was measured spectrophotometrically at 412 nm within 15 minutes in UV spectrophotometer (Tota et al., 2010).

2.6.3 Estimation of malondialdehyde in mice brain
MDA, measure of lipid per oxidation was measured spectrophotometrically. After homogenization, tissue homogenate was mixed with the 30% TCA, 5N HCl followed by the addition of 2% TBA in 0.5 N NaOH. The mixture was heated for 5 minutes at 90°C and centrifuged at 6000 rpm for 10 minutes. The pink colour of the supernatant was measured at 532 nm, using UV spectrophotometer (Colado et al., 1997).

2.6.4 Estimation of Acetyl cholinesterase in mice brain
Cholinergic marker Acetyl cholinesterase was estimated in the whole brain according to the method of Hlman. Brain homogenate was incubated for 5 minutes with 2.7 ml of phosphate buffer and 0.1 ml of DTNB. Then 0.1 ml freshly prepared acetylcholine iodide (PH – 8) was added and absorbance was read at 412Nm (Ellman et al., 1961).

2.6.5 Estimation of Tumor necrosis factor-alpha
Equilibrate all materials and prepared reagent to room temperature. Prior to use, mix all reagents thoroughly taking care not to creat any foam within the vials. Determined the number of micropipette strips required to test the desired number of samples, plus appropriate number of well needed for controls and standards. Remove sufficient micropipette strips from
the pouch. Added 100 µl of each standard sample including blank controls to the appropriate wells. Add 50 µl of biotinylated anti-TNF-α to all wells. Cover and incubate for 3 hours at room temperature (18-25°C). Wash three times. Add 100 µl of streptavidin–HRP. Incubate 30 minutes at room temperature. Wash three times. Add 100 µl of ready to use chromogen TMB substrate solution into each well and incubate in dark for 10-20 minutes at room temperature. Avoid direct exposure to light by wrapping the plate in aluminium foil. Add 100 µl H₂SO₄ reagent into each well within one hour. Read absorbance of each well on ELISA at 450 nm.

3. RESULTS

3.1 Effect of Silymarin against LPS-induced Neuroinflammation in Morris water maze (No. of animals =6)

The memory function was assessed after LPS (5µg/5µl) administration in the Morris water maze. In control groups the escape latency time (LT) in comparison of 4th and 5th day was significantly lower than that of 1st and 2nd day. The escape latency time (LT) in comparison of 1st day to 5th day significantly decreased. There was significant increase in latency time in LPS (p<0.001) treated mice indicating memory impairment. Treatment with Silymarin (25 mg/kg and 50 mg/kg) showed dose dependent reduction in latency time in comparison control group indicating significant improvement in memory. Donepezil (5 mg/kg) (used as a positive control) reversed the memory impairment in mice produced by Lipopolysachharides.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control</th>
<th>LPS (5µg/5µl)</th>
<th>Donepezil (5mg/kg)</th>
<th>Silymarin (50mg/kg)</th>
<th>Silymarin (25mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st day</td>
<td>46.6±17.72851</td>
<td>81.6±4.97996</td>
<td>38.4±7.004284</td>
<td>45.5±1.805547</td>
<td>39.78±6.61879</td>
</tr>
<tr>
<td>2nd day</td>
<td>44.8±18.52566</td>
<td>77±8.944272</td>
<td>36.4±6.93974</td>
<td>36±1.224745</td>
<td>37.08±7.444891</td>
</tr>
<tr>
<td>3rd day</td>
<td>42.2±17.936</td>
<td>79.2±6.797052</td>
<td>33.6±6.66033</td>
<td>27.6±1.077033</td>
<td>34.94±7.012674**</td>
</tr>
<tr>
<td>4th day</td>
<td>39.4±17.86897</td>
<td>75.6±5.504544</td>
<td>32.2±6.7483337</td>
<td>22±0.707107</td>
<td>30.74±6.381348**</td>
</tr>
<tr>
<td>5th day</td>
<td>37.6±17.86897</td>
<td>77.6±4.505552</td>
<td>30.8±6.688797</td>
<td>17±0.707107</td>
<td>27.06±4.39244</td>
</tr>
</tbody>
</table>

*Table No. 2* Latency Time for Morris water maze

Table No. 2 Comparison of latency time (LT) to reach hidden platform. Mice were subjected to five consecutive water maze sessions with three trials per day session. Results were expressed as mean latency time (s) ±S.E.M. (n=6). Data were analyzed by one-way ANOVA followed by Tukey’s test for multiple comparisons. **Significant difference (**p<0.05 and *p<0.05) in latency time (LT) in comparison to day 1.
3.2 Effect of Silymarin against LPS induced Neuroinflammation on Morris water maze

![Morris water maze graph]

Figure: 2 Comparison of latency time (LT) to reach hidden platform. Results were expressed as mean latency time (s) ±S.E.M. (n=6). Data were analyzed by one-way ANOVA followed by Tukey’s test for multiple comparisons. **Significant difference (**p<0.05 and *p<0.05) in latency time (LT) in comparison to session 1.

3.3 Estimation of Glutathione (GSH) in mice brain

The glutathione (GSH) was estimated in mice brain on 6th day after LPS (5µg/5µl) administration. GSH levels were significantly decreased (p<0.05) in brain of LPS treated groups in comparison to control group. Treatment with Silymarin (25 mg/kg and 50 mg/kg) showed dose dependent restore in GSH level in comparison to LPS treated group indicating increase in memory function and reduced stress condition.

Table No. 3 Effect of Silymarin on GSH level in mice brain

<table>
<thead>
<tr>
<th>Group</th>
<th>Concentration (n mole/mg) as Mean ±SEM (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.65825±0.003705</td>
</tr>
<tr>
<td>Disease</td>
<td>0.25475±0.004498</td>
</tr>
<tr>
<td>Standard</td>
<td>0.61925±0.01894***</td>
</tr>
<tr>
<td>Silymarin 25 mg/Kg</td>
<td>0.4405±0.012507*</td>
</tr>
<tr>
<td>Silymarin 50 mg/Kg</td>
<td>0.5990±0.039239**</td>
</tr>
</tbody>
</table>

Table No. 3 Effect of Silymarin (25mg/kg and 50mg/kg) on GSH level in different parts of brain. Results were expressed as mean±S.E.M. and analyzed by one-way ANOVA followed by Tukey’s test for multiple comparisons. **Significant difference (**p<0.05) vs. contorol group and *Significant difference (*p<0.05) vs. LPS group.
Effect of Silymarin on GSH level in mice brain

![Glutathione Graph](chart)

Figure: 3 Effect of Silymarin on GSH level in mice brain. Graph shows GSH level calculated in µg/mg protein. Data were analyzed by one-way ANOVA followed by Tukey’s test for multiple comparisons. **Significant difference (\( ^* p < 0.05 \)) vs. aCSF group and *Significant difference (\( ^* p < 0.05 \)) vs. LPS group.

3.4 Estimation of Malondialdehyde (MDA) in mice brain

The malondialdehyde (MDA) was estimated in mice brain on 6th day after LPS (5µg/5µl) administration. MDA levels were significantly increased \( (p<0.05) \) in brain of LPS treated groups in comparison to control group. Treatment with Silymarin (25 mg/kg and 50mg/kg) showed dose dependent reduction in MDA level in comparison to LPS treated group indicating increase in memory function and reduced stress condition.

Table No. 4 Effect of Silymarin on MDA level in mice brain

<table>
<thead>
<tr>
<th>Group</th>
<th>Concentration (nmole/mg) as Mean ±SEM (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.6285±0.14779</td>
</tr>
<tr>
<td>Disease</td>
<td>1.073±0.00505###</td>
</tr>
<tr>
<td>Standard</td>
<td>0.6835±0.007858***</td>
</tr>
<tr>
<td>Silymarin 25 mg/kg</td>
<td>0.726±0.017325**</td>
</tr>
<tr>
<td>Silymarin 50 mg/kg</td>
<td>0.894±0.00275***</td>
</tr>
</tbody>
</table>

Table No. 4 Effect of Silymarin on MDA level in mice brain. Graph shows MDA level calculated in nmol/mg protein. Data were analyzed by one-way ANOVA followed by Tukey’s test for multiple comparisons. **Significant difference (\( ^* p < 0.05 \)) vs. aCSF group and *Significant difference (\( ^* p < 0.05 \)) vs. LPS group.
3.5 Effect of Silymarin on MDA level in mice brain

![Graph showing the effect of Silymarin on MDA level in mice brain.](image)

Figure: 4 Effect of Silymarin on MDA level in mice brain. Graph shows MDA level calculated in nmol/mg protein. Data were analyzed by one-way ANOVA followed by Tukey’s test for multiple comparisons. **Significant difference (\( ^{**}p<0.05 \)) vs. aCSF group and *Significant difference (\( ^{*}p<0.05 \)) vs. LPS group.

3.6 Estimation of Acetyl cholinesterase activity (Ach E) in mice brain

The Acetyl cholinesterase activity (Ach E) was estimated in mice brain on 6th day after LPS (5µg/5µl) administration. Acetyl cholinesterase (Ach E) activity levels were highly elevated (\( p<0.05 \)) in brain of LPS treated groups in comparison to control group. Treatment with Silymarin (25 mg/kg and 50 mg/kg) showed dose dependent reduction in Acetyl cholinesterase (AchE) in comparison to LPS treated group indicating significant (\( p<0.001 \)) decrease in Acetyl cholinesterase (Ach E) activity indicating increase in memory function and reduced stress condition.

<table>
<thead>
<tr>
<th>Group</th>
<th>Concentration (nmole/mg) as Mean ±SEM (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.48075±0.012154</td>
</tr>
<tr>
<td>Disease</td>
<td>0.9815±0.007354###</td>
</tr>
<tr>
<td>Standard</td>
<td>0.5835±0.009042###</td>
</tr>
<tr>
<td>Silymarin 25mg/kg</td>
<td>0.358±0.012013881**</td>
</tr>
<tr>
<td>Silymarin 50mg/kg</td>
<td>0.4955±0.0259966324***</td>
</tr>
</tbody>
</table>

Table no.4 Effect of Silymarin on Ach E activity in mice brain. Results were expressed as mean±S.E.M. and analyzed by one-way ANOVA followed by Tukey’s test for multiple comparisons. **Significant difference (\( ^{**}p<0.05 \)) vs. control group and *Significant difference (\( ^{*}p<0.05 \)) vs. LPS group.
Effect of Acetyl cholinesterase level in mice brain

![Acetylcholinesterase graph]

**Figure:** Effect of silymarin on AchE activity in different parts of brain. Results were expressed as mean±S.E.M. and analyzed by one-way ANOVA followed by Tukey’s test for multiple comparisons. **Significant difference (**$p<0.05$) vs. control group and *Significant difference (*$p<0.05$) vs. LPS group.

3.7 Effect of Silymarin on Tumor necrosis factor – alpha

The TNF-$\alpha$ level were estimated in mice brain on 6$^{th}$ day after LPS (5$\mu$g/5$\mu$l) administration. TNF-$\alpha$ levels were highly elevated ($p<0.05$) in brain of LPS treated groups in comparison to control group. Treatment with Silymarin (25 mg/kg and 50 mg/kg) showed dose dependent reduction in TNF-$\alpha$ in comparison to LPS treated group significantly ($p<0.05$) which shows neuroinflammatory of Silymarin.

**Table No.6** Effect of Silymarin on TNF-$\alpha$ in mice brain

<table>
<thead>
<tr>
<th>Group</th>
<th>Concentration (nmole/mg) as Mean ±SEM (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.658±0.020932</td>
</tr>
<tr>
<td>Disease</td>
<td>1.215±0.140614###</td>
</tr>
<tr>
<td>Standard</td>
<td>0.8065±0.008026###</td>
</tr>
<tr>
<td>Silymarin 25mg/kg</td>
<td>0.4735±0.024693116*</td>
</tr>
<tr>
<td>Silymarin 50mg/kg</td>
<td>0.558±0.022852334**</td>
</tr>
</tbody>
</table>

**Table No. 6** Effect of Silymarin on TNF-$\alpha$ level in mice brain. Results were expressed as mean±S.E.M. and analyzed by one-way ANOVA followed by Tukey’s test for multiple comparisons. **Significant difference (**$p<0.05$) vs. control group and *Significant difference (*$p<0.05$) vs. LPS group.
3.8 Effect of TNF-α level in mice brain

![Graph showing TNF-α levels](image)

Figure: 6 Effect of Silymarin on TNF-α level in mice brain. Results were expressed as mean±S.E.M. and analyzed by one-way ANOVA followed by Tukey’s test for multiple comparisons. **Significant difference (**p<0.05) vs. control group and *Significant difference (*p<0.05) vs. LPS group.

4. DISCUSSION AND CONCLUSION

Neuroinflammation is a neurodegenerative disease. Neuroinflammation is acute and chronic disorder of central nervous system. Proinflammatory mediators released by activated glial cells during Neuroinflammation have been proposed to contribute to neuropathological phenomenon, underlying cognitive deficits in Alzheimer’s disease. In response to proinflammatory cytokines reactive oxygen and nitrogen species are released which are responsible for the condition of oxidative stress. Neuronal damage and death can be initiated by free radical injury. Free radicals are formed during normal metabolism and free radical injury occurs within living cells when the generation of reactive oxygen species exceeds intrinsic antioxidant ability. This situation is referred as oxidative stress and memory impairment which causes Neuroinflammation.

The present study is demonstrated that Neuroinflammation induced by Intracerebral administration of lipopolysachharides in mice brain.

Cholinergic system in the hippocampus plays an important role in memory formation and retrieval. Pivotal role of cholinergic system in memory is further underlined by use of acetylcholine esterase inhibitors in Alzheimer to prevent memory decline. In present study, cholinergic system also got affected in LPS induced memory deficit because of enhancement
of activity of Ach E resulting in increased degradation of acetylcholine. The Acetyl cholinesterase activity (Ach E) was estimated in mice brain on 6th day after LPS (5µg/5µl) administration. Acetyl cholinesterase (Ach E) activity levels were highly elevated in brain of LPS treated groups in comparison to control group. Treatment with Silymarin (25 mg/kg and 50 mg/kg) showed dose dependent reduction in Acetyl cholinesterase (Ach E) in comparison to LPS treated group indicating significant decrease in Acetyl cholinesterase (Ach E)activity.

Polyunsaturated fatty acids are present in the brain phospholipids, which are the major constituents of the neuronal membrane. Lipid peroxidation indicates degeneration of neuronal membrane. MDA is an end product of lipid peroxidation and is a measure of free radical generation. Recently, it was observed that lipid peroxidation in the brain occurs in early stage of AD (Williams et al. 2006). Therefore, an elevated level of MDA suggests neuronal degeneration. Glutathione is an essential tripeptide, an antioxidant found in all animal cells. It is the principal intracellular non-protein thiol and plays a major role in the maintenance of the intracellular redox state. The level of GSH decreases with an increase in the generation of free radicals (Dringen et al. 2000). In the present study, LPS caused a significant increase in MDA level and decrease in GSH levels in the brain regions indicating increased oxidative stress. LPS induced oxidative stress was effectively prevented by donepezil and in present study also there was significant increase in GSH level and MDA levels were reduced significantly in Silymarin treated group in a dose dependent manner indicating antioxidant activity.

This is a concept that the balance between pro and anti-inflammatory cytokines affects the outcome of certain diseases such as neurodegenerative disorders (O'Shea et al.2002). In this study we used LPS to induce Neuroinflammation as it activates glial cells to synthesize and secrete the proinflammatory cytokines-interleukins (IL-1, IL-6), TNF-α and IFNγ, which are involved in pathology of Neuroinflammation. TNF-α was estimated in different brain regions (striatum, cerebral cortex, hippocampus and hypothalamus) following LPS administration as an indicator of Neuroinflammation. TNF-α levels were reduced significantly in silymarin treated group in a dose dependent manner indicating anti-neuroinflammatory activity of drug.

In conclusion, the present study indicated that Silymarin could ameliorate memory impairment induced by lipopolysachharide. The effect of Silymarin may be attributed to its strong antioxidative action and its effect on brain energy metabolism. Therefore, its widespread use as dietary supplement should be encouraged to ward off age associated
memory disorders like AD. The results showed that Silymarin effective in preventing memory loss and there is a benefit of drugs. This drug have completely different profile regarding the mechanism of action, so the it may be useful if given for a longer period of time and this warrants further highly specified animal experiments and also needs to be evaluated in Neuroinflammation disease. The effect of Silymarin drug, as shown in my study, may hold great clinical significance. Intracerebral microinjection of LPS significantly induced Neuroinflammation in mice and caused release of cytokines (TNF-α), free radical generation and oxidative stress in the brain regions. Treatment with Silymarin effectively attenuated LPS induced Neuroinflammation, oxidative stress and cognitive impairment.

Addition of a cost effective drug like Silymarin not only lowers the overall cost of the therapy but it may also improve the memory retention in neurodegenerative disorders like Neuroinflammation.

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