Research Article

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NEUROPROTECTIVE EFFECT OF BIOFLAVONOIDS AGAINST CARBON TETRACHLORIDE INDUCED NEUROTOXICITY IN RATS

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ABSTRACT

The present study was undertaken to explore the neuroprotective potential of bioflavonoids against carbon tetrachloride (CCl₄) induced neurotoxicity in rats. Neurotoxicity was induced by CCl₄ (3ml/kg body weight) in wistar rats. Blood biochemical, hematological parameters and histopathological studies were carried out to assess the neuroprotective effect. Administration of CCl₄ has induced significant neurotoxicity in rats, which was evident from enhanced levels of potassium and decreased levels of sodium. Pretreatment with quercetin (50 mg/kg dose orally) significantly reversed CCl₄ induced neurotoxicity than the silymarin (50 mg/kg dose orally). From the obtained results it can be concluded that quercetin exerted a significant neuroprotective effect against CCl₄ induced neurotoxicity in rats than silymarin (p>0.001) for sodium and potassium in blood biochemical parameters, packed cell volume, mean corpuscular hemoglobin count and red blood cells as well in attenuation of pathological changes in brain tissues.

Keywords: Neurotoxicity, bioflavonoid, carbon tetrachloride, quercetin, silymarin

INTRODUCTION

Flavonoids comprise a large group of chemical compounds with a basic diphenylpropane structure. They were divided into several subclasses like flavonols, flavans, flavonoids and flavonones based on their functional groups. These compounds naturally occur in various plant parts (fruits, vegetables, nuts, seeds, flowers) and essential constituent of diet as well. Epidemiological studies indicated that diets rich in flavonoids were associated with reduced incidence of several chronic diseases including hepatic, renal diseases, asthma, type II diabetes and certain types of cancer. The neuroprotective properties of flavonoids were multi-faceted involving antioxidant and anti-inflammatory effects. The antioxidant property of flavonoids was thought, until relatively recently, to underlie the majority of their protective cellular effects. However, it is becoming increasingly apparent that flavonoids also influence cellular function by modulating the activity of many enzymes including the inhibition of protein kinases and lipid kinases¹.

Silymarin is a compound which is obtained from the plant Silybum marianum (milthistle) was an edible plant it has been used medicinally for centuries as a herbal medicine. It is a mixture of mainly three flavonolignans: silybin, sildianin, and silychristine, with silybin being the most active. This compound has been used to treat liver disorders. The compound consistent antioxidant activity, stimulation of ribosomal RNA polymerase and subsequent protein synthesis, lead to enhanced hepatocyte regeneration².³.

Quercetin a major representative of the flavonol subclass, has received the considerable attention because of its overwhelming presence in foods. Quercetin and its sugar-bond or glucosylated, forms represent to 60-75% of flavonoids intake. It displayed the ability to prevent the oxidation of low-density lipoproteins (LDL) by scavenging free radicals and chelating transition metal ions. As a result, quercetin may aid in the prevention of certain diseases viz., cancer, atherosclerosis and chronic inflammation⁴.

MATERIALS AND METHODS

Animals
Wister rats of either sex; weighing around 200-250 g in the present study were the in-house breed of Chalapathi Institute of Pharmaceutical Sciences, Guntur. They were exposed to an alternate light and dark cycle of 12 hour period. During this period the animals were provided with a standard diet and water ad libitum. The animals were acclimatized to the laboratory conditions for at least 5 days before the neurotoxicity test. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of Chalapathi Institute of Pharmaceutical Sciences, Guntur, Andhra Pradesh (Approval No. 09/IAEC/CPS/2016-17; dt 05/04/2016) and care of the animals was taken as per guidelines of the committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Forest, Environment, Climate Change, Government of India.

Drugs and reagents
Silymarin, quercetin was purchased from Sigma Aldrich, Bangalore, India. Carbon tetrachloride (CCl₄) and formalin were obtained from the Chalapathi Institute of Pharmaceutical Sciences. CCl₄ was administered intraperitoneally (i.p.) to induce neurotoxicity in wistar rats⁵.

Experimental groups
The efficacy of silymarin was compared with quercetin by evaluating in vivo neuroprotective activity in rats against CCl₄ induced neurotoxicity⁶,⁷,⁸.

Four groups, each comprising of five wistar rats, were employed in the study. Group I (Control group): Rats were administered normal saline (0.9% w/v), orally for 14 days. Group II (CCl₄ treated control group): Rats were administered CCl₄ (3 ml/kg, orally for 4 days, starting 24 h before the neurotoxicity test), and then administered normal saline orally for 12 days. Group III (Quercetin treated group): Rats were administered quercetin (50 mg/kg, orally for 14 days) started 24 h before the neurotoxicity test. Group IV (Silymarin treated group): Rats were administered silymarin (50 mg/kg, orally for 14 days) started 24 h before the neurotoxicity test.
i.p.) on the day 14. Group III (Silymarin + CCL₄ treated group): Rats were treated with silymarin (50 mg/kg, orally) for 14 days. On the 14th day silymarin was administered 60 min prior the administration of CCL₄. Group IV (Quercetin + CCL₄-treated group): Rats were treated with quercetin (50 mg/kg, orally). The remainder of the procedure was similar to that of Group III. The blood samples were collected by cardiac puncture under deep anesthesia (terminal blood sampling using a 23G needle vertically into the sternum) produced by the administration of ketamine and xylazine. After sufficient exsanguination, the brain of the treatment animals was isolated after cervical dislocation. The blood samples and brain were immediately subjected to hematological, blood biochemical and histopathological studies respectively. The carcass of the dissected animal was disposed by deep burial as per the Standard Operating Procedures of CPCSEA, Government of India. The histopathological brain study samples were prepared by the hematoxylin and eosin staining techniques.

Statistical analysis

The results of the study expressed as mean ± standard error of means (S.E.M.). The data of neurotoxicity studies were statistically analyzed by one-way analysis of variance (ANOVA) followed by Tukey’s multiple range test using Graphpad Prism version 6.0. A p-value (<0.05) was considered to be statistically significant.

RESULTS

Various pharmacological studies employed in the present study did not show any significant mortality. Further, no significant difference was observed between the results obtained from rats of either sex.

Effect on blood biochemical and hematological parameters

The treatment group relatively to control group received significant impact on blood biochemical and hematological parameters. The neurotoxic agent CCl₄ drastically reduced the sodium levels where as increased potassium level. In turn the lymphocytes count was also decreased. Silymarin and quercetin treatment groups showed significant neuroprotective effect (p<0.05) against CCl₄ group (Table 1 & 2). Administration of silymarin (50 mg/kg, orally) to CCl₄ treated animals showed significant difference in sodium, total count, differential count and in PCV. Treatment with quercetin (50 mg/kg, orally) significantly decreased the CCl₄ induced rise in blood biochemical and hematological parameters (Table 1 & 2).

Table 1: Blood biochemical parameters of various treatment groups

<table>
<thead>
<tr>
<th>S.No</th>
<th>Parameter</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Sodium (Na⁺) (mmol/lit)</td>
<td>135.4±1.5</td>
<td>116.6±5.44</td>
<td>134.8±1.88</td>
<td>132.4±2.29</td>
</tr>
<tr>
<td>2.</td>
<td>Potassium (K⁺) (mmol/lit)</td>
<td>7.24±0.2</td>
<td>9.8±0.09</td>
<td>10±0</td>
<td>8.84±0.13</td>
</tr>
</tbody>
</table>

Table 2: Hematological parameters of various treatment groups

<table>
<thead>
<tr>
<th>S.No</th>
<th>Parameter</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Hemoglobin (mg/dl)</td>
<td>13.56±0.21</td>
<td>16.4±0.39</td>
<td>13.41±0.76</td>
<td>10.55±1.12</td>
</tr>
<tr>
<td>2.</td>
<td>Total Count(mm³)</td>
<td>3800± 291.54</td>
<td>2440±156.84</td>
<td>1540±166.13</td>
<td>3500±164.31</td>
</tr>
<tr>
<td>3.</td>
<td>Differential Count (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a)</td>
<td>Polymorphs</td>
<td>30±4.29</td>
<td>36.6±1.28</td>
<td>31.6±2.16</td>
<td>36±1.92</td>
</tr>
<tr>
<td>b)</td>
<td>Lymphocytes</td>
<td>62.8±4.72</td>
<td>55±2.05</td>
<td>60.6±3.39</td>
<td>55±1.58</td>
</tr>
<tr>
<td>c)</td>
<td>Eosinophils</td>
<td>2.2±0.86</td>
<td>4.4±1.02</td>
<td>2.6±0.51</td>
<td>5.8±0.86</td>
</tr>
<tr>
<td>d)</td>
<td>Monocytes</td>
<td>2.4±0.81</td>
<td>5±0.71</td>
<td>2.4±0.81</td>
<td>3±0.63</td>
</tr>
<tr>
<td>4.</td>
<td>Packed Cell Volume (%)</td>
<td>42.4±0.92</td>
<td>50.2±0.86</td>
<td>38.2±2.31</td>
<td>31±3.57</td>
</tr>
<tr>
<td>5.</td>
<td>Mean Corpuscular Hemoglobin Count (%)</td>
<td>31±0.32</td>
<td>32.8±0.37</td>
<td>34.4±0.51</td>
<td>30.8±0.37</td>
</tr>
<tr>
<td>6.</td>
<td>Platelet Count (lakhs/mm³)</td>
<td>2.94±0.22</td>
<td>4.86±0.57</td>
<td>3.44±0.65</td>
<td>3.36±0.71</td>
</tr>
<tr>
<td>7.</td>
<td>Red Blood Cells (million/mm³)</td>
<td>7.3±0.26</td>
<td>8.87±0.17</td>
<td>7.22±0.44</td>
<td>5.46±0.72</td>
</tr>
</tbody>
</table>

Figure 1: Histology slides of the isolated brain of various treatment groups showing haematoxylin and eosin stained cells
Histopathological studies
In relation with group I, group II animals showed significant damage in brain tissue. The significance of p value is <0.05. The group II showed foci of necrosis and inflammation, whereas in group I normal parenchyma was observed. From the figure (c) necrosis was observed in group III animals. The group IV animals showed significant recovery of brain tissue when compared with group III (Figure 1).

DISCUSSION
Carbon tetrachloride induced neurotoxicity employed in the present study is one of the most widely accepted models to evaluate neurotoxicity of the animals. In the present study, CCl4 induced neurotoxicity showed a significant increase in observed blood biochemical parameters except sodium (p<0.002) when compared to control group. The hematological parameters showed increased levels except lymphocytes (p <0.271) and total count (p<0.003).

Pretreatment with silymarin attenuated CCl4 induced neurotoxicity when compared to positive control group which is evident from significant increase in potassium (0.0001) and hematological parameters. The total count showed a significant decrease in total count (p<0.0003) and packed cell volume showed significant decrease (p=0.001)

In hematological parameters silymarin showed no significant difference against quercetin except mean corpuscular hemoglobin count (p=0.0002). In blood biochemical parameters the silymarin showed significant difference in potassium (p< 0.0001).

Pretreatment with quercetin showed significant difference in sodium (p<0.0504) and potassium (p< 0.0001). Group IV showed a significant decreased in observed hematological parameters except hemoglobin (p=0.9916), platelet count (p< 0.1413) and differential count (p=0.4419) when compared with CCl4 group.

Therefore, from the above findings, it is evident that quercetin abolished CCl4 induced neurotoxicity. The neuroprotective properties of flavonoids are multi-faceted involving antioxidant and anti-inflammatory effects. The antioxidant property of flavonoids was thought, until relatively recently, to underlie the majority of their protective cellular effects. However, it is becoming increasingly apparent that flavonoids also influence cellular function by modulating the activity of many enzymes including the inhibition of protein kinases and lipid kinases.

Therefore, it may be concluded that quercetin exerts its beneficial effect in CCl4-induced neurotoxicity by virtue of its ability to prevent the damage of cell membranes and secondary free radicals, antioxidative, and anti-inflammatory actions. Nevertheless, further studies are needed to explore the full potential of quercetin in neuroprotective effect.

CONCLUSION
From the obtained results it can be concluded that quercetin exerted a significant neuroprotective effect against CCl4 induced neurotoxicity in rats than silymarin (p<0.001) for most of the blood biochemical parameters, mean corpuscular hemoglobin count as well in attenuation of pathological changes in brain tissues.

REFERENCES

Cite this article as:


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