Single and Combined Effect of Garlic and Carbon Tetrachloride on Serum and Brain Acetylcholinesterase Activity in Rat

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Abstract: Present experiment is aimed to study the effect of garlic on acetylcholinesterase activity in serum and brain of normal and carbon tetrachloride intoxicated rat. Twenty adult male rats of Wistar strain were divided into four groups, with five rats in each group. One group was left as control and to the second group only a single dose of 5 ml CCl₄ solution/kg body weight [intraperitoneal injection of 20% CCl₄(v/v) solution in olive oil] was given and then left untreated. To the third group only garlic was fed orally for 15 days at the dosage of 4g/kg body weight. The fourth group was also treated with a single dose of 5 ml CCl₄ solution/kg body weight and from the next day garlic was administered orally at the dose of 4g/kg body weight for 15 days. The result revealed that CCl₄ had insignificant inhibitory effect on serum and brain AChE level and garlic significantly accelerated the enzyme activity both in serum and brain of normal and carbon tetrachloride intoxicated rat. Therefore carbon tetrachloride showed mild inhibitory effect which is insignificant and garlic singly or in combination significantly enhanced AChE activity both in serum and brain.

Key words: Allium sativum, acetylcholinesterase, carbon tetrachloride, brain, serum.

I. Introduction

The enzyme acetylcholinesterase (AChE), rapidly split acetylcholine (ACh), the neurotransmitter at the synapses of all pre and post ganglionic fibres of parasympathetic and few post ganglionic fibres of sympathetic nerves and at the neuromuscular junction into choline and acidic component [1] thereby rapidly terminating the action. The enzyme is present in the motor end plate, synaptic junction, brain, spinal cord, red blood corpuscles and blood serum. Anything which interferes with the action of AChE causes serious disturbance of neurojunctional and neuromuscular activities. Exposure to certain toxic agents leads to convulsion, paralysis and perhaps death [2],[3].

The enzyme acetylcholinesterase had been estimated by Sastry and Murty and were they showed undernutrition had lowering effect and rehabilitation caused elevation of enzyme activity in rat brain[4]. Ellis et al. [5] administered carbon tetrachloride (CCl₄), a hepatotoxic substance in rabbit which did not show any change in plasma and liver AChE activity, whereas rat plasma and liver showed a decrease. Various workers have done experimental studies on the inhibitory effect of organophosphate, lead acetate, carbamate, etc on the AChE activities [6], [7]. Yassin had shown the efficacy of garlic on the enzyme activity in lead intoxicated rabbit[8]. Saluya and Kumar [9] studied on the inhibitory chronic effect of copper sulphate on AChE in rat stomach.

However, practically no attempt has been made to study the effect of garlic (Allium sativum Linn.) on AChE activity in the brain and serum of carbon tetrachloride intoxicated rat. Keeping this into our consideration an experimental design has been made to investigate the effect of garlic on AChE level of brain and serum in normal and carbon tetrachloride intoxicated rats under hepatotoxic stress.

II. Materials and Methods

Healthy male albino rats of Wistar strain of an average body weight 90g have been selected for experimentation. Animals were kept in clean polypropylene cages covered with chromate plate grill and maintained in normal husbandry conditions for 7 days with stock diet and water ad libitum for acclimatization. After 7 days animals were divided into four groups with 5 rats in each group. Throughout the experimental period animals were given stock diet [10] and water ad libitum.

A. Preparation of garlic paste and mode of feeding

Garlic was purchased from the local market and the cloves were cleaned, peeled and macerated to homogenous mass in a motor and pestle. Required amount was prepared freshly every day, mixed with known quantity of...
water and given orally by a feeding needle. The dosage was 4 g/kg body weight /day for 15 days time period [11].

B. Administration of CCl₄

A single dose of 5 ml CCl₄ solution/kg body weight (intaperitoneal injection of 20% CCl₄ (v/v) solution in olive oil) was given before commencement of the experiment [12]. Treated rats were given 4 g garlic /kg body weight per day for 15 days. Treatment is expressed against each group as follows:

- Group I- Untreated, control (C)
- Group II- CCl₄ injected on first day, then left untreated for 15 days (CCl₄)
- Group III- Garlic treatment from 2nd day for 15 days (G)
- Group IV- CCl₄ treatment on day 1, then fed garlic from day 2 for 15 days (GCCl₄)

All the animals were handled with utmost human care and autopsied on the 17th day, after 12 hours of fasting with ether anesthesia. Immediately after autopsy blood was collected directly from the heart and serum was separated by standard method. Brain was dissected out carefully and the whole brain was homogenized in ice cold normal saline in Potter Elvehjem homogenizer to obtain a 10% homogenate. It was centrifuged and the supernatant was used as the enzyme source. Both serum and brain AChE was determined by the method of Huns and Robert [13].

III. Statistical Analysis

Data were analyzed by Analysis of Variance (ANOVA). Statistical analysis was also done by student’s “t” test [14].

IV. Results

Weekly body weight records of all the experimental animals (Table-1) were made. No behavioral changes in the animals were seen throughout the experimental period. Weekly record of the body weight showed that the body weight gain was normal. There was significant weight increment in all the animals which showed that there was a normal weight gain both in control and treated animals.

Table 1: Initial and final body weight of experimental animals

<table>
<thead>
<tr>
<th>Groups</th>
<th>Experiment</th>
<th>Body weights(g)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Initial (Mean ± SE)</td>
<td>Final (Mean ± SE)</td>
</tr>
<tr>
<td>I</td>
<td>C</td>
<td>90.00 ± 2.89</td>
<td>116.60 ± 1.87</td>
</tr>
<tr>
<td>II</td>
<td>CCl₄</td>
<td>90.50 ± 1.75</td>
<td>120.00 ± 2.50</td>
</tr>
<tr>
<td>III</td>
<td>G</td>
<td>91.60 ± 1.66</td>
<td>123.60 ± 3.85</td>
</tr>
<tr>
<td>IV</td>
<td>GCCl₄</td>
<td>90.00 ± 2.01</td>
<td>125.00 ± 2.89</td>
</tr>
</tbody>
</table>

Figure in the parenthesis indicates number of animals. SE- standard error, C-control, CCl₄-carbon tetrachloride treated, G-Garlic treated, GCCl₄- Garlic treatment in CCl₄ intoxicated rats.

Findings of Table 2, on AChE level of brain and serum show a similar propensity of responsiveness towards (CCl₄) or garlic or CCl₄ and garlic together. Garlic increased the enzyme level of both brain and serum in rat significantly. The mild and insignificant inhibitory effect of CCl₄ is counteracted by garlic in both brain and serum of rats. The brain AChE activity showed more responsiveness to the action of garlic.

Table 2: Acetylcholinesterase activity in serum and brain of experimental animals

<table>
<thead>
<tr>
<th>Groups</th>
<th>Experiment</th>
<th>AChE activity in serum</th>
<th>AChE activity in brain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean ± SE (units/ml)</td>
<td>Mean ± SE (units/mg tissue)</td>
</tr>
<tr>
<td>I</td>
<td>C</td>
<td>1585.36 ± 3.40</td>
<td>79.20 ± 0.50</td>
</tr>
<tr>
<td>II</td>
<td>CCl₄</td>
<td>1571.00 ± 7.09</td>
<td>NS</td>
</tr>
<tr>
<td>III</td>
<td>G</td>
<td>1711.85 ± 0.80</td>
<td>P &lt; .001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NS</td>
<td>P &lt; .001</td>
</tr>
<tr>
<td>IV</td>
<td>GCCl₄</td>
<td>1605.17 ± 2.08</td>
<td>P &lt; .05</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P &lt; .001</td>
</tr>
</tbody>
</table>

P < .05
Figure in the parenthesis indicates number of animals in each group. C-control, CCl₄-carbon tetrachloride treated, G-Garlic treated, GCCl₄- Garlic treatment in CCl₄ intoxicated rats, NS-not significant, SE-standard error, ¹ CCl₄ vs G, ² CCl₄ vs GCCl₄, ³ G vs GCCl₄.

V. Discussion

In the present study we find out the effect of garlic homogenate at the dosage of 4g/kg body weight per day for 15 days on serum and brain AChE activity in normal and carbon tetrachloride treated rats. It has been observed that the enzyme activity has increased significantly (P< .001) in garlic treated rats. Only CCl₄ treatment decreased the enzyme level insignificantly. However, the combined effect of CCl₄ and garlic showed an increased AChE value which is also significantly raised but lower compared to that of only garlic fed rats. Carbontetrachloride is a strong hepatotoxic compound due to the formation of trichloro methyl free radicals (CCl₃) that bind covalently to the neighboring lipid, initiating lipid peroxidation that leads to severe membrane damage. But in the present study CCl₄ practically did not alter the enzyme concentration which is partly in conformity with the observation of Ellis et al. [5] who administrated CCl₄ in rabbit which did not show any change in plasma and liver AChE activity but in their study they found that rat plasma and liver showed a decrease. They attributed it to the species specific difference of the ester hydrolyzing enzyme in liver and plasma of these two animal species. Various scientist have studied on the inhibitory action of organophosphate compounds, lead acetate, carbamate, etc, on AChE activity [6]. Heavy metals induced central cholinergic system and their possible mechanism have been been studied by Saxena et al.[15]. The effectiveness of garlic on the AChE activity of central nervous system has studied by Yassin [8] in lead intoxicated rabbits. Widespread use of AChE inhibitor specially pesticide produce large number of human poisoning events worldwide. The main known neurotoxic effect of these substances are AChE inhibition which causes cholinergic over stimulation that results in neuro psychological sequale as both short and long term effects [16],[17]. Therefore, our finding on acceleration of AChE activity as a result of garlic treatment alone or in combination or with CCl₄ intoxication can be an effective remedy in case of enzyme inhibition or neuropsychological deficit observed in old age. Also there may be an enormous scope for further study on the efficacy of garlic in counteracting the effect of pesticide and other poisoning that cause enzyme inhibition.

References