ASSESSMENT OF HEPATOPROTECTIVE ACTIVITY OF ROOTS AND BARKS OF ACHYRANTHES ASPERA IN CARBON TETRACHLORIDE-INDUCED HEPATOTOXICITY IN RATS

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ABSTRACT

Carbon tetrachloride (CCl4) is a colorless liquid organic compound which is used for household work. Mainly liver and kidney of human are the major organs for CCl4 toxicity. Liver can damage after 24 hours of CCl4 ingestion and can cause severe fatal symptoms including painful swollen liver, hemorrhages, hepatic coma leading to death. We examined the effect of CCl4 on rat liver. Serum glutamic pyruvic transaminase (SGPT) and serum bilirubin levels are considered as one of the safest therapeutic agents to treat hepatic inflammation [13]. The concept of herbal medicine and their use has been started since the ancient time. Based on research, direct use of herbs and extract of plants are widely using as drugs by ensuring safety and effectiveness to treat various types of diseases. In modern times herbs are the major source where we can synthesize and extract active metabolites [7]. In our study we have chosen Achyranthes aspera which is a perennial herb, 1-2m in height commonly known as Rough Chaff Tree. This plants usually found as a weed on roadsides in South Asian region including Bangladesh [8].

1. INTRODUCTION

Based on a study, liver is one of the vital organs in human body having a major role for maintaining, regulating homeostasis of the body by metabolizing protein, fat and carbohydrate [1]. Study showed, continuous exposure of harmful metabolites, poisons and drugs can lead to various acute and chronic liver diseases like hepatitis, cirrhosis, hepatotoxicity [2]. According to research, there are more than 100 types of environmental poisons which can cause liver disease. Carbon tetrachloride (CCl4) is considered as one of the major poisons which cause severe hepatotoxicity after ingestion and inhalation in animal body [3]. Though a few data were found to identify the toxic effect of CCl4 in human, but current research reviled that this toxin may also cause harmful effect on human liver. After administration of CCl4, rats increase oxidation of protein in the liver as a result oxidized protein accumulate in the liver. Oxidative damage to hepatocellular proteins may contribute to the pathogenesis of CCl4 induced liver injury. According to a scholar, an enzyme which is known as serum glutamic pyruvic transaminase normally present in liver. This enzyme is released into the blood when any unusual physiological, pathological changes and damaged occur in liver. As a result, the level of SGPT increases in the blood then normal value [4-6]. Serum glutamic oxaloacetate transaminase (SGOT) is another biomarker to identify liver diseases. It is released into the blood when cells that contain are damaged. Bilirubin is the yellow breakdown product of normal heme catabolism the elevated levels of bilirubin may indicate certain diseases including hepatotoxicity.

2. MATERIALS AND METHOD

2.1 Chemicals

Plant extract was prepared by following a standard process in the Daffodil International University medicinal chemistry laboratory. SGPT and Bilirubin reagent was collected from pharmacology research laboratory of Daffodil International University. Carbon tetrachloride and dexamethasone API were collected from the research laboratory of University of Rajshahi. Olive oil was purchased from surgical laboratories.
2.2 Preparation extract from plants

After collection, barks and roots were grinded individually. The powdered barks about 230 gm and root weight 97 gm were taken separately in two colored reagent bottles. Almost 2-liter methanol was used to soak the sample. The sealed bottles were kept in dry and cool place for 1.5 weeks with occasional stirring and shaking. Both root and bark extract then filtered through cotton first then with Whatman No.1 filter paper. To obtain crude methanolic extract of roots and barks rotary evaporator was used under reduced pressure and 45°C - 50°C.

2.3 Test Animals

Male Wister albino rats, 3 to 5 weeks old and weighing between 75-140 g of both sexes were used in the experiments. Total number of rats (24) was collected from the Department of Pharmacy, Jahangirnagar University, Savar, Dhaka. All the rats were divided into six groups (1 control and 5 CC1 induced) each group having four rats. All the animals were kept in room temperature 22±3ºC and relative humidity 65±5%. A 12:12 hour, light: dark cycle was followed. All the experimental procedure and protocol used in the study were followed as international animal ethics committee for the purposive control.

2.4 Stock Solution

The Stock Solution was prepared by the ratio of Carbon tetra chloride: olive oil (1:3)

2.5 Instrument

Hypodermic syringes (1cc, 3cc) and insulin syringe were used. Butterfly needle tube and mouth gages were used for convenient administration of CCl4.

2.6 Administration of CCl4

0.5 ml/kg body weight dose, were administered intra-peritoneal. All doses were single dose.

2.7 Collection of serum

After completing carbon tetra chloride treatment, the rats were sacrificed and 2-3ml of blood was collected directly from heart by syringes, centrifuged at 4000 rpm for 30minutes and the serum was obtained.

2.8 Liver function test

To assess liver damage by CCl4, the determination of enzyme levels was largely used. Based on a study, serum SGPT, bilirubin is the most important marker to diagnose hepatic damage because SGPT, SGOT and bilirubin release when liver is damaged by any toxins or pathological and physiological abnormalities [14]. In this study, an increase in the activities of SGPT and bilirubin in serum evidenced the CCl4 induced hepatocellular damage [15,16]. When CCl4 induced rats are treated with dexamethasone phosphate injection there was a significant decrease of SGPT and bilirubin level.

2.9 Statistical analysis

All the analysis for this experiment were performed by one-way ANOVA followed by Scheffe’s post-hoc test. Values were expressed as mean ± S.D. of four animals of each groups. Here we found p-value is < 0.05 which is significant.

3. RESULTS

Table 1: Level of SGPT on CCl4 induced rat compared with controlled group (37ºC)

<table>
<thead>
<tr>
<th>SL</th>
<th>SGPT level in controlled group U/L</th>
<th>SGPT level in CCl4 induced rat U/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>52±1</td>
<td>121±3</td>
</tr>
<tr>
<td>2</td>
<td>59.5±1.5</td>
<td>275±2</td>
</tr>
<tr>
<td>3</td>
<td>82±0.1</td>
<td>198±3.2</td>
</tr>
<tr>
<td>4</td>
<td>71±2</td>
<td>312±4</td>
</tr>
</tbody>
</table>

Table 2: Level of Bilirubin (mg/dl) CCl4 induced rat compared with controlled group

<table>
<thead>
<tr>
<th>Bilirubin level in controlled group</th>
<th>Bilirubin level in CCl4 induced rat</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 1.7±0.02</td>
<td>9.2±1.041</td>
</tr>
<tr>
<td>2 3.6±0.1</td>
<td>15±6.4±0.1</td>
</tr>
<tr>
<td>3 2.5±0.08</td>
<td>10±0.023</td>
</tr>
<tr>
<td>4 2.2±0.031</td>
<td>13±3.034</td>
</tr>
</tbody>
</table>

Table 3: SGPT and bilirubin in CCl4 induced rat treated with Dexamethasone

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>SGPT level U/L</th>
<th>Bilirubin level mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>11.42±0.034</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>12.38±0.42</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>106.87±0.07</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>96.25±0.31</td>
</tr>
</tbody>
</table>

Effect of CCl4 (0.5 ml per kg body weight) on liver enzyme, Bilirubin on rat. From the above chart it has been seen that there is a significant difference of SGPT level among control group and CCl4 induced group of rat. The result was expressed in mean ± SD where p< 0.05
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Effect of Dexamethasone (2, 4, 6, and 8 ml/kg body weight) on liver enzyme SGPT on rat. From the above chart it has been observed that there is a significant difference of SGPT level among CCl4 induced group of rat and after administration of Dexamethasone in CCl4 induced rat. The result was expressed in mean ± S.D.

Table 5: Effect of Plant Extract on SGPT, Bilirubin level of CCl4 induced rat (Bark)

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>SGPT level U/L</th>
<th>Bilirubin level mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>223.2</td>
<td>6.05±0.02</td>
</tr>
<tr>
<td>2</td>
<td>167.82</td>
<td>4.04±0.03</td>
</tr>
<tr>
<td>3</td>
<td>190.4</td>
<td>7.5±1</td>
</tr>
</tbody>
</table>

After administration of bark extract in a dose dependent manner (1mg/kg, 20 mg/kg, 30 mg/kg) there was no significant change occur.

4. DISCUSSION

Based on a research, liver damage induced by carbon tetrachloride is commonly used model for the screening of hepatoprotective activity of different active compounds [17]. In modern research many plants were tested for potential antioxidant and hepatoprotective liver damage in experimental animal model [18]. CCl4 hepatotoxicity model is widely using for this kind of studies. CCl4 metabolically activated to form trichloromethyl free radical (CCl3) which bind with cellular lipids and proteins which cause the structural change of endoplasmic reticulum and other membrane. Study showed, loss of activation of metabolic enzyme, reduction of protein synthesis and loss of glucose 6-phosphatase activation cause liver damage [19]. In this study (table 1 and table 2) it has been found that after administration of CCl4 1mg/kg body weight the level of SGPT and bilirubin increased. Various studies indicate that Achyranthes aspera has so many pharmacological effects on rats including hepatoprotective activity on paracetamol and sodium nitrate induced rats on the other hand this study indicates that with the increase of plant extract dose the level of SGPT and bilirubin decreased in a significant rate (Figure 5 and 6) [20-22]. By reviewing literature, we have seen amelioration effects against CCl4-induced hepatocarcinogenesis in Swiss albino rats where the whole plant extract of Achyranthes aspera was used [23]. We used the methanolic extract of root and bark of Achyranthes aspera separately. From the above figures and tables, it can be said there is a significant difference of bilirubin and SGPT level among control group and CCl4 induced group of rat after treating with methanolic extract of roots of Achyranthes aspera. But there is no significant change occur by treating with bark of Achyranthes aspera in CCl4 induced hepatotoxicity [24]. It has been reported that the methanolic extract of leaf of this plant shows a hepatoprotective activity in rifampine induced albino rats where the level of SGPT, SGT and total bilirubin decreases with the increase of dose but in this study the effect of roots and barks has been examined separately [25,26].

5. CONCLUSION

Present study demonstrated that carbon tetrachloride induction causes prominent increase of the liver enzymes such as SGPT, which may be due
to the decrease of antioxidant system in serum in CCl4 induced rat and oxidative stress is an important mechanism of organ damage. The detailed mechanisms are worthy of further investigation and knowledge. We chose the natural source such as Achyranthes aspera (Family: Amaranthaceae) depending upon its potential activities through literature review. A positive effect has been observed for plant extract against CCl4 induced hepatotoxicity. In conclusion, we can say methanolic extract of roots of Achyranthes aspera is a prominent source to treat CCl4 induced liver damage. By comparing with standard (dexamethasone) is has been revealed that herbal products specifically plant source is a great source for treating hepatic diseases. We will extend our research to find out the active compounds which are mainly responsible for hepatoprotective activity.

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REFERENCES


