HEPATOPROTECTIVE ACTIVITY OF Tephrosia purpurea ON CARBON TETRACHLORIDE INDUCED HEPATOTOXICITY

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ABSTRACT
Liver, the largest gland is a vital organ. It is the metabolic engine-room of the body. Drug induced liver injury is a major health problem. In the present study to investigate the hepatoprotective activity of Tephrosia purpurea on Carbon tetrachloride induced hepatotoxicity. Body weights of the animals were recorded and they were divided into 3 groups of 6 animals each as follows. Group 1: Normal control rats fed with standard diet and served as a control. Group 2: Rats were induced with hepatocellular damage by receiving suspension of Carbon tetrachloride (CCl₄) in olive oil (1:2,v/v, 1ml of CCl₄ i.p./kg body weight) was given every 72 hrs for 7 consecutive days. Group 3: Rats were treated with Tephrosia Purpurea orally (through intragastric tube) at the dose of 500 mg/kg body weight for every day in addition to CCl₄ was given every 72 hrs for 7 consecutive days. In the present study revealed that significant difference in CCl₄ intoxicated rats with respect to most of the biochemical parameters analyzed. Treatment with Tephrosia purpurea leaf restored the level of liver marker enzymes and oxidative stress markers. Hepatoprotective activity of Tephrosia purpurea leaf was documented.

INTRODUCTION
Liver, the largest gland is a vital organ. It is the metabolic “engine-room of the body”. Almost all the drugs, foods and water constituents are metabolized and detoxified in the liver, and as such it is often exposed to maladies resulting in a number of clinical syndromes. Many chemicals, foods, drugs and infections (parasitic, bacterial, viral or fungal) can cause variety of liver diseases such as hepatitis, jaundice, cirrhosis, liver cancer, etc. Because of variations in liver dysfunctions and difficulties encountered in reaching to a proper diagnosis, a physician is rarely able to provide specific treatment. At the most, supportive and symptomatic treatments are given but the multiplicity of deranged functions renders the treatment still more complicated. Besides this, modern (allopathic) drugs exhibit severe toxicity, thus there is a definite need to search alternate drugs having maximum therapeutic value with no or least toxicity” (Pandey Govind, 1980, 1990). Drug induced liver injury (dili) is a major health problem that challenges not only health care professional but also the paramedical industry and drug regulatory agencies according to the united states acute liver failure study group (Ostapowicz et al., 2002) DILI accounts for more than 50% acute liver failure including hepatotoxicity caused by over dose of acetaminophen (apap 39%) and idio syncratic liver injury triggered by other drugs (13%)
Carbon tetrachloride (CCl₄), a hepatotoxin, has been used extensively for decades to induce liver injury in various experimental models to elucidate the mechanisms behind hepatotoxicity (Hardin, 1954). Experiments tally induced
cirrhotic response in the rat by CCl₄ is shown to be superflcially similar to human cirrhosis of the liver (Tamayo, 1983). CCl₄ called also perchloromethane or tetrachloromethane, is a colourless, non-inflammable volatile liquid with a distinct odour and immiscible with water, is produced by chlorination of methane, ethane, propane or propene. The molecular weight of this compound is 153.82 Da. Although this compound has been prohibited in the US since 1970 to use as a dry cleaning agent owing to its profound toxicity, it is still produced in large quantities for various purposes globally because an interdict has not yet been imposed for overall use. However, a significant reduction in the manufacture of this compound has been observed in the last several decades. At its infancy this solvent is used in pharmaceutical preparations, such as anaesthetics. It has been known for a long time that inhalation of the vapour of this compound can depress the central nervous system activity and cause degeneration of liver and kidneys through exerting a destructive and poisonous effect to the cells and organs as many other well known toxins do.

Plant and plant products are being used as a source of medicine since long. According to World Health Organization (WHO) more than 80% of the world’s population, mostly in poor and less developed countries depend on traditional plant-based medicines for their primary healthcare needs. Medicinal plants are the nature’s gift to human being to make disease free healthy life. It plays a vital role to preserve our health. India is one of the most medicoculturally diverse countries in the world where the medicinal plant sector is part of a time-honored tradition that is respected even today. Here, the main traditional systems of medicine include Ayurveda, Unani and Siddha. The earliest mention of the use of plants in medicine is found in the Rigveda which was written between 4500 and 1600 BC. During British period due to Western culture our Traditional art of natural healing is disappeared slowly. Now it is reappearing due to realization of its importance in curing diseases without any side effect (Dahanukar et al., 2000). In the present study to investigate the hepatoprotective activity of Tephrosia purpurea on Carbon tetrachloride induced hepatotoxicity

MATERIALS AND METHODS

Animals

Male albino rats of Wistar strain approximately weighing 180-190g were used in this study. They were healthy animals purchased from the Indian Institute of Science, Bangalore. The animals were housed in spacious polypropylene cages bedded with rice husk. The animal room was well ventilated and maintained under standard experimental conditions (Temperature 27 ± 2°C and 12 hour light/dark cycle) throughout the experimental period. All the animals were fed with standard pellet diet and water were provided ad libitum. They were acclimatized to the environment for one week prior to experimental use. The experiment was carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India.

Chemicals:

Carbon tetrachloride Sodium hydroxide and Trichloro Acetic acid (TCAs) and Diethyl ether were purchased for Sigma chemical company, Mumbai All other chemicals and reagents used in this study was of analytical grade with high purity and were obtained from Glaxo laboratories and Sisco Research laboratories, Mumbai, India.

Plant material and preparation of extract

The leaves of Tephrosia Purpurea were collected from Tamil University, February 2015 at Thanjavur. The collected whole plant of Tephrosia Purpurea were cut into small pieces and shade dried at room temperature and makes a fine powder using grinder mixture. The powder material of Tephrosia Purpurea was macerated with 50% methanol at room temperature for 3 days. After 3 days, the supernatant was transferred into china dish. The supernatant was completely removed by keeping the china dish over a boiling water bath at 45°C. A semi solid extract was obtained after complete elimination of alcohol. The obtained residue was kept in the refrigerator for further use. The extract was made up to a known volume in distilled water just before oral administration.

Experimental design

Body weights of the animals were recorded and they were divided into 3 groups of 6 animals each as follows. Group 1: Normal control rats fed with standard diet and served as a control. Group 2: Rats were induced with hepatocellular damage by receiving suspension of Carbon tetrachloride (CCl₄) in olive oil (1:2,v/v, 1mL of CCl₄ i.p/kg body weight) was given every 72 hrs for 7 consecutive days. Group 3: Rats were treated with Tephrosia Purpurea orally (through intragastric tube) at the dose of 500 mg/kg body weight for every day in addition to CCl₄ was given every 72 hrs for 7 consecutive days.

Collection of blood and preparation of serum sample

At the end of the experimental period, the animals were killed cervical dislocation after an overnight fasting. The blood sample was collected. The blood was allowed to clot by standing at room temperature for 30 minutes and then refrigerated for another 30 minute. The resultant clear part was centrifuged at 3000 rpm for 10minutes and then the serum (supernatant) was isolated and stored at refrigerated until required for biochemical analysis.

Phytochemical analysis

Chemical tests were carried out on the aqueous extract and on the powdered specimens using standard procedures to identify the constituents as described by Sofowara (1993), Trease and Evansm(1989) and Harborne (1973).
phosphatase activity was measured by the method of Annon (1963). Protein was estimated by the method of Lowry et al. (1951). Albumin was estimated by the method of Rodkey (1965).

**Statistical Analysis:**

Values were expressed as mean ± SD for six rats in the each group and statistical significant differences between mean values were determined by student “t” tested and p< 0.05 were considered to be significant.

**RESULTS**

The present study was carried out on the plant sample revealed the presence of medicinally active constituents. The phytochemical characters of the *Tephrosia purpurea* investigated a summarized in Table 1. The present study was carried out to evaluate the Hepatoprotective activity of *Tephrosia purpurea* in rats. The observations made on different groups of experimental animals were compared as follows.

**Table 1. Phytochemical screening of Tephrosia purpurea**

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saponin</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>++</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>Polyphenol</td>
<td>++</td>
</tr>
</tbody>
</table>

(+)Presence (-) Absence

**Hepatoprotective activity**

The present study was carried out to evaluate the Hepatoprotective activity of *Tephrosia purpurea* leaf. The observations made on different groups of experimental and control animals were compared as follows.

Table I represents the levels of MDA and GSH in serum of normal and experimental rats. Group II CCl₄ intoxicated rats showed a significant increased in the level of MDA when compared to Group I rats. Group III CCl₄ intoxicated rats treated with *Tephrosia purpurea* leaf significantly decreased in the level of MDA when compared to group II.

Group II CCl₄ intoxicated rats showed a significant decreased in the level of GSH when compared to Group I rats. Group III CCl₄ intoxicated rats treated with *Tephrosia purpurea* leaf significantly increased in the level of GSH as compared to group II.

**Table I Effect of Tephrosia purpurea leaf on MDA and GSH in experimental rats**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (mg/dl)</td>
<td>5.90±1.20</td>
<td>9.61±1.82*</td>
<td>5.60±1.09**</td>
</tr>
<tr>
<td>GSH (mg/dl)</td>
<td>1.77±0.48</td>
<td>0.5 ±0.30*</td>
<td>1.61±0.47**</td>
</tr>
</tbody>
</table>

Values were expressed as mean ± SD for six rats in each group.

* Significantly different from Group I (p<0.001)
** Significantly different from Group II (p<0.001)

Table II represents the levels of protein in serum of normal and experimental rats. Group II CCl₄ intoxicated rats showed a significant decreased in the level of protein when compared to Group I rats. Group III CCl₄ intoxicated rats treated with *Tephrosia purpurea* leaf significantly increased in the level of protein when compared to group II.

**Table II Effect of Tephrosia purpurea leaf on Protein in experimental rats**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein (gm/dl)</td>
<td>7.6 ± 0.17</td>
<td>6.45± 0.26*</td>
<td>7.18±0.41**</td>
</tr>
<tr>
<td>Albumin (gm/dl)</td>
<td>3.86±0.67</td>
<td>2.67± 0.63*</td>
<td>4± 0.52**</td>
</tr>
</tbody>
</table>

Values were expressed as mean ± SD for six rats in each group.

* Significantly different from Group I (p<0.001)
** Significantly different from Group II (p<0.001)

Table III represents the activity of SGOT and SGPT in serum of normal and experimental rats. Group II CCl₄ intoxicated rats showed a significant increased in the activity of SGOT when compared to Group I rats. Group III CCl₄ intoxicated rats treated *Tephrosia purpurea* leaf significantly decreased in the activity of SGOT when compared to group II.

Group II CCl₄ intoxicated rats showed a significant increased in the activity of SGPT when compared to Group I rats. Group III CCl₄ intoxicated rats treated with *Tephrosia purpurea* leaf significantly decreased in the activity of SGPT as compared to group II.

**Table III Effect of Tephrosia purpurea leaf on SGOT and SGPT in experimental rats**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>SGOT (IU/l)</td>
<td>56 ± 5.7</td>
<td>78± 5.6*</td>
<td>42± 2.4**</td>
</tr>
<tr>
<td>SGPT (IU/l)</td>
<td>38 ± 3.7</td>
<td>50± 3.9*</td>
<td>24± 1.7**</td>
</tr>
</tbody>
</table>

Values were expressed as mean ± SD for six rats in each group.

* Significantly different from Group I (p<0.001)
** Significantly different from Group II (p<0.001)
Table III Effect of *Tephrosia purpurea* leaf on SGOT, SGPT and Acid phosphatase activities in experimental rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>SGOT (IU/dl)</td>
<td>36.30±4.51</td>
<td>60.99±4.60*</td>
<td>38.86±4.51**</td>
</tr>
<tr>
<td>SGPT (IU/dl)</td>
<td>33.18±2.83</td>
<td>58.84±6.06*</td>
<td>31.36±5.32**</td>
</tr>
<tr>
<td>Acid phosphatase (IU/dl)</td>
<td>8.82 ± 1.01</td>
<td>15.61±0.62*</td>
<td>7.37±0.91**</td>
</tr>
</tbody>
</table>

Values were expressed as mean ± SD for six rats in each group.
* Significantly different from Group I (p<0.001)
** Significantly different from Group II (p<0.001)

**DISCUSSION**

Ample experimental and epidemiological studies support the involvement of oxidative stress in the pathogenesis and progression of several chronic diseases (Tewari *et al*., 2000). It is now known that oxygen, indispensable for maintaining life, sometimes becomes toxic and results in the generation of most aggressive agents such as reactive oxygen species (ROS). The high reactivity of ROS may trigger a host of disorders in body resulting in tissue damage and necrosis in many instances (Prasad Varier *et al*., 1999). It has been hypothesized that one of the principal causes CCl$_4$ induced liver injury is LPO by free radical derivatives of CCl$_4$. Thus the antioxidant activity or the inhibition of the generation of free radicals is important in the protection against CCl$_4$–induced hepatopathy (Castro *et al*., 1974). CCl$_4$ mediated oxidative stress was taken here as the experimental model for hepatotoxicity and oxidative stress.

Malondialdehyde (MDA) is the major aldehyde resulting from the peroxidation of biological membrane polyunsaturated fatty acid. MDA, a secondary product of lipid peroxidation is used as an indicator of tissue damage by series of chain reactions (Ray and Husain, 2002). The study of lipid peroxidation is attracting much attention in recent years due to its role in diseases process membrane lipids are particularly susceptible to lipid peroxidation due to the presence of polyunsaturated fatty acids. It has been implicated in the pathogenesis of a number of diseases and clinical conditions. These include atherosclerosis, cancer etc., Experimental and clinical evidence suggests that aldehyde products of lipid peroxidation can also act as bioactive molecule in physiological and pathological conditions. It is now generally accepted that lipid peroxidation and its product play an important role in liver, kidney, heart and brain toxicity (Lakshmi *et al*., 2005). MDA is one of the indicators of oxidative stress. In this context a marked increase in the concentration of MDA was observed in CCl$_4$ intoxicated rats when compared to control rats. Administration of *Tephrosia purpurea* significantly decreased the levels of MDA in CCl$_4$ intoxicated rats.

GSH is a major non-protein thiol in living organism, which plays a central role of co-ordinating the body’s antioxidant defense process. It is implicated in the cellular defense against xenobiotics and naturally occurring deleterious compounds such as free radicals. Glutathione status is a highly sensitive indicator of cell functionality and viability. Perturbation of GSH status of a biological system has been reported to lead to serious consequences (Pastore *et al*., 2003). Decline GSH content in serum and liver of CCl$_4$ intoxicated rats, and its subsequent return towards near normalcy in CCl$_4$ and *Tephrosia purpurea* treated rats reveal antioxidant effect of *Tephrosia purpurea*. Explanations of the possible mechanism underlying the hepatoprotective properties of drugs include the prevention of GSH depletion and destruction of free radicals (Fraga *et al*., 1987).

The diagnosis of organ disease / damage is aided by measurement of a number of non-functional serum enzymes characteristic of that tissue or organ. The amount of enzyme released depends on the degree of cellular damage, the intracellular concentration of the enzymes and the mass of affected tissue. The concentration of the enzymes released reflects the severity of the damage. SGPT and SGOT are enzymes normally present in the liver, heart, muscles and blood cells. They are basically located within hepatocytes. So when liver cells are damaged or die transaminases are released into blood stream, where they can be measured they are therefore of index of liver injury (Vasudha *et al*., 2006). Administration of *Tephrosia purpurea* to CCl$_4$ intoxicated rats restored the level of SGOT and SGPT offering the maximum hepatoprotection with respect to different liver marker enzymes. This confirms the liver protective activity of *Tephrosia purpurea*. Further, *Tephrosia purpurea* has significantly increased the level of liver protein, which indicates hepatoprotective activity. Stimulation of protein synthesis accelerates the regeneration process and the production of liver cells.

Proteinuria, most often reflecting loss of the normal glomerular impermeability to filtration of plasma proteins, is an early sign of kidney disease. Impairment of the normal capacities of various segments of the renal tubules to reabsorb water and electrolytes to effectively maintain the volume and composition of body fluids within normal limits ma also be key manifestations of kidney dysfunction. Proteinuria is a hallmark of kidney disease. Thus detection of proteinuria is necessary for the recognition of most kidney diseases (Cohen and Lemann, 1991). In the present study, we observed the decreased level of protein in CCl$_4$ intoxicated rat as compared with control rats. This is due liver dysfunction. Administration of *Tephrosia purpurea* significantly increased in the level of protein in CCl$_4$ intoxicated rats.

Serum albumin, the major plasma protein synthesized in the liver, is a clinically useful marker of hepatic synthetic function (Friedman *et al*., 1996). Albumin’s protective effect has been attributed to its nonspecific binding of redox-active transition metal ions capable of catalyzing reactions that yield hydroxyl or hydroxyl-like radicals (Strubelt and Younes,
1994). Some evidence suggests that albumin may act more directly as a free radical scavenger or as a participant in scavenging reactions. Joe et al. (1999) reported that serum albumin acts as a major physiologic antioxidant. Studies with the albumin suggested that low serum albumin concentration was associated with a greater loss of muscle mass (Castaneda et al., 1993). In the present study, we observed the decreased level of albumin in CCl₄ intoxicated as compared with control rats. This may be due to impairment of antioxidant defense and liver function. Administration of Tephrosia purpurea leaf significantly increased the level of albumin in CCl₄ intoxicated rats.

In the present study revealed that significant difference between CCl₄ intoxicated rats and Tephrosia purpurea leaf treated rats with respect to most of the biochemical parameters analyzed. Oxidative stress markers such as MDA and GSH were analysed in experimental rats. The increased MDA content and decreased GSH content were observed in CCl₄ intoxicated rats as compared to control rats. This result indicates that the increases in oxidative stress in CCl₄ intoxicated rats. Supplementation of Tephrosia purpurea leaf restored the level of MDA and GSH content. Protein and albumin level were also analysed in experimental rats. The decreased in the level of protein and albumin in CCl₄ intoxicated rats as compared to control rat. This may be indicates the impairment of liver function in CCl₄ intoxicated rats. Treatment with Tephrosia purpurea leaf restored the level of protein and albumin content. Liver marker enzymes such as SGOT, SGPT and ACP were also analysed in experimental rats. The increased activity of these enzymes in CCl₄ intoxicated rats were observed as compared to control rats. This may be indicate the inflammation in the liver of CCl₄ intoxicated rats. Supplementation of Tephrosia purpurea leaf possess’ potential hepatoprotective activity.

REFERENCES


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