EVALUATION OF ANTINEPHROTOXIC POTENTIAL OF AZIMA TETRACANTHA LAM. AND TRIBULUS TERRESTRIS LINN.

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Received: 3 Feb 2012, Revised and Accepted: 6 March 2012

ABSTRACT

The present study evaluates the Antinephrotoxic effect of leaf powder of Azima tetracantha Lam. and fruit powder of Tribulus terrestris Linn. Renal injury was induced by injecting 100mg/kg b.w of ferrous sulphate. Biochemical examinations were carried out for the evaluation of oxidative stress and nephrotoxicity. The animals treated with ferrous sulfate alone showed significantly higher level of urea, creatinine, Gamma glutamyl transferase (GGT), sodium, chloride, bicarbonate, TBARS, and lower level of super oxide dismutase, catalase, reduced form of glutathione, vitamin C, vitamin E, and potassium, when compared with control group. Oral administration of Azima tetracantha Lam. and Tribulus terrestris Linn. powders restore the above said changes near to control. The findings suggested that the probable mechanism of nephroprotection by Azima tetracantha Lam. and Tribulus terrestris Linn. against ferrous sulphate induced renal injury could be due to its antioxidant and free radical scavenging activity.

Keywords: Antinephrotoxic, Azima tetracantha Lam., Tribulus terrestris Linn. Ferrous sulphate.

INTRODUCTION

Nephrotoxicity is one of the most common kidney problems and occurs when body is exposed to a drug or toxin that causes damage to kidneys. Drug induced nephrotoxicity is one of the top priorities in the world. About one third of the cases of nephrotoxicity in United States are due to drug. When damage occurs, kidney was unable to rid body of excess urine and wastes. Electrolytes such as Na, K, Cl, and Mg will be elevated. Many toxic metals alter the afferent arterioles leading to a drop in renal blood flow and Glomerular Filtration Rate. The mode of action of renal toxins can be divided into three stages based on molecular and biochemical process.

1. The first step is interaction with cellular materials eg. Protein or DNA entailing complexation, covalent binding or involvement of radical species.

2. In the second step, there is depletion of thiol and lipid peroxidation with adverse effects on calcium homeostasis and oxidative phosphorylation.

3. The final step consists of cell death by disturbance of essential cellular process2.

The cause of chronic iron toxicity is same in both primary and secondary iron overload. The body’s limited iron storage or transport capacities have been chronically exceeded, exposing tissues to highly reactive iron complexes3. Transferrin saturation is most acute in transfusional iron overload. Saturation of intracellular storage protein leads to accumulation of labile iron in cells. Both processes lead to the deposition of non transferrin bound iron within the tissues. The major part of the cellular iron is safely bound in ferritin as well as in haem or iron, sulphur cluster containing proteins. However, a small part (0.2–3%) is loosely attached to proteins or lipids or weakly bound to low molecular mass ligands like phosphates or citrate, forming a transit iron pool that keeps iron available for the synthesis of iron containing proteins. This pool of iron is chelatable iron. In the presence of hydrogen peroxide, redox active iron participates in the Fenton reaction, which leads to a decomposition of hydrogen peroxide to highly reactive hydroxyl radicals. The hydroxyl radicals oxidises lipids, proteins and DNA, thus leads to cell damage4.

Demand for medicinal plants is increasing in both developing and developed countries. Research on medicinal plants is one of the leading areas of research globally5. In India, Indian system of medicine such as Siddha, Ayurveda, Unani etc claim to have preparations, which can alleviate various diseases from headache to cancer. The goals of using plants as sources of therapeutic agents are (a) to isolate bioactive compounds for direct use as drugs, e.g., digoxin, (b) to produce bioactive compounds of novel or known structures as lead compounds for semisynthesis to produce patentable entities of higher activity and/or lower toxicity, e.g., metformin. Azima tetracantha Lam. is a popular herb in Indian traditional medicine used as anti-arthritis, antimicrobial, hepato and nephroprotective agent. Whole plant extract of Azima tetracantha Lam. contain flavonoids, aminocids, tannins, saponins and alkaloids, which may be responsible for the above activities5. In East Africa the pounded roots of Azima tetracantha Lam. are applied directly to snake bites and an infusion is taken orally as a treatment. In India and Sri Lanka the root, root bark and leaves are added to food as a remedy for rheumatism. The plant is considered diuretic and is also used to treat dropsy, dyspepsia, chronic diarrhoea and as a stimulant tonic. In western India juice of the leaves is applied as eardrops against ear ache and crushed leaves are placed on painful teeth6.

Tribulus terrestris Linn. (Zygophyllaceae) is an annual plant native of Mediterranean region. In India, it is called “Gokhru”. Tribulus terrestris Linn. is an important herb commonly used as folk medicine in many countries for different purposes6. Fruits of Tribulus terrestris Linn. has been shown to exhibit diuretic, antiurolithiatic, CNS(Central Nervous System) stimulant, antimicrobial and antifungal activities in rats5. Recently Bourke and Rogerson et al. have confirmed the antioxidant and antihypertensive activity in rat’s heart. Hence the present study was undertaken to investigate renal protective activity of Azima tetracantha Lam. and Tribulus terrestris Linn. against ferrous sulphate toxicity in experimental rats.

MATERIALS AND METHODS

Animals

Healthy young albino rats (130gm-150gm) were purchased from animal house, Manapparai, Tamilnadu. The groups of rats were kept separately in individual stainless steel hoppers. The test animals were characterised by strain, source, sex, weight and age. Every individual animal was provided conventional laboratory diet with an unlimited supply of drinking water. The experiment was designed and conducted in accordance with the guidelines of Institutional Animal Ethical Committee (IAEC).

Chemicals

Ferrous sulphate (AR) was purchased from Merck (India). Carnitine was procured from WINDLAS biotech limited, Delhi.
Plant materials
Azima tetracantha Lam. leaves were collected from Pattukkottai, Tamil Nadu and Tribulus terrestris Linn.fruits were collected from Needamangalam near Thanjavur, Tamilnadu.

Dose preparation and administration
Azima tetracantha Lam. leaves and Tribulus terrestris Linn.fruits were dried at 45°C for 48 h, powdered using electric grinder and stored in a container. Azima tetracantha Lam.100 mg mixed with 3 ml of water, Tribulus terrestris Linn.6mg mixed with 1ml of water were fed to the animals orally using an applicator.

Experimental protocol
Group I: Six rats were kept as control.

RESULTS AND DISCUSSION
According to the previous research findings, proximal tubules seem to be the major site of metal induced nephrotoxicity. Depending on, the severity of intoxication, functional defects are often accompanied with a plethora of structural damages in the proximal tubules epithelium, including loss of cell-cell contacts and detachment of cells from the basement membrane, blebbing, shortening and loss of microvilli, loss of basolateral invaginations, vesiculation of the cytoplasm, derangement of the cytoskeleton, swelling, vacuolation, fragmentation of mitochondria, swelling of lysosomes and whole cells, etc.

Iron sulphate can enter the proximal tubule cell via endolysosomal compartment at the brush-border membrane or via organic anion and cation transporters at the basolateral membrane and damage the nephrons58. The biochemical markers of nephrotoxicity are urea, creatinine and GGT. Their levels are significantly elevated in nephrotic condition due to metal induced damage to nephrons. In nephrotoxicity, the serum urea and creatinine accumulates because the rate of serum urea and creatinine production exceeds the rate of clearance due to defects in the glomerular filtration rate.

The present study shows the significant elevation (Table-1) in the levels of urea, GGT and creatinine in ferrous sulphate induced group compared to control. After treatment with herbal drugs viz, Azima tetracantha Lam. and Tribulus terrestris Linn.and standard drug of carnitine, there was a significant decrease in the levels near to normal compared to ferrous sulphate induced group.

The kidneys play important role in body function, not only by filtering the blood and getting rid of waste products, but also by balancing levels of electrolytes in the body, controlling blood pressure, and stimulating the production of red blood cells. The kidneys have the ability to monitor the amount of body fluid, the concentrations of electrolytes like sodium and potassium and the acid-base balance of the body51.

In kidney failure the functions of kidney decreases, the symptoms are related to the inability to regulate water and electrolyte balances and to clear waste products from the body. Chronic iron overload is associated with marked iron deposition in the glomeruli and proximal and distal tubules accompanied by glomerular hypercellularity, mesangial expansion, tubular atrophy, and interstitial fibrosis of the kidneys. Due to this there is severe alteration in the absorption of electrolytes and changes in the glomerular filtration rate.

Iron overload also damages hepatocytes and causes hypovolemia, which in turn causes decreased tissue perfusion and metabolic acidosis which leads to changes in the concentration of serum electrolytes. Significant increase in serum sodium and decrease in potassium might also be due to changes in the absorption in the renal tubules55.

Elevation in chloride and bicarbonate may be seen in certain kidney diseases. The significant higher mean value of chloride gave the same pattern as the level of sodium because chloride is always in association with sodium. Our data showed that increased level of sodium, chloride, bicarbonate and decreased level of potassium (Table -1) in ferrous sulphate induced rats. On administration of herbal drugs and standard drug carnitine significantly altered the serum electrolytes near to normal compared to toxicity induced group of animals.

The oxidative stress due to diminished elimination and increased production of free radicals leads to the accumulation of reactive oxygen species. These highly reactive molecules directly or indirectly (via lipid peroxidation) induce further damage to the function/permeability of various intracellular organelles, leading to the cytoplasmic accumulation of Ca2+ ions. The elevated Ca2+ ion promotes a variety of intracellular reactions, including depolymerization of the cytoskeleton, thus contributing to the diminished endocytosis and intracellular vesicle recycling. Synergistic action of reactive oxygen species and Ca2+ ion leads to the damage of mitochondrial function, organelle swelling and release of cytochrome C and other proteins via ruptured membranes into the cytoplasm, where it promotes transformation of inactive procaspase into caspase. The final, executive caspase III translocates the 'cell death message' into the nucleus. The cell death may proceed by apoptosis, if the mitochondrial adenine triphosphate synthesis is still active, or by necrosis, if adenosine triphosphate is heavily depleted. The renal injury in Fe-loaded animals was accompanied by a significant elevation of plasma and renal tissue TBARS concentrations, which signify an increased reactive oxygen species activity leading to enhanced lipid peroxidation.

Superoxide radicals affect the lipid phase of the myelin by changing its structure into a more disordered state. The superoxide radical also inhibits acetylase activity and thereby affects the tricarboxylic acid cycle. Super oxide dismutase catalyzes the dismutation of the superoxide radical into hydrogen peroxide53. In iron toxic conditions the free radical production will be elevated due to enhanced lipid peroxidation. The level of Super oxide dismutase was found to be decreased in toxic condition indicating the presence of high level reactive oxygen species.

Table 1: Changes in the level of electrolytes and kidney markers

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium</td>
<td>5.4 ± 0.45</td>
<td>2.57 ± 0.76</td>
<td>4.32 ± 0.40</td>
<td>4.77 ± 0.34</td>
<td>5.5 ± 0.14</td>
</tr>
<tr>
<td>Chloride</td>
<td>98 ± 2.16</td>
<td>135.75 ± 1.70</td>
<td>119.75 ± 2.5</td>
<td>105 ± 5.71</td>
<td>104.25 ± 4.03</td>
</tr>
<tr>
<td>Bicarbonate</td>
<td>22.25 ± 5.73</td>
<td>27 ± 4.32</td>
<td>23 ± 0.81</td>
<td>20 ± 2.16</td>
<td>104.75 ± 1.70</td>
</tr>
<tr>
<td>GGT</td>
<td>10.6 ± 0.25</td>
<td>16.2 ± 0.80</td>
<td>11.85 ± 0.68</td>
<td>11.22 ± 0.28</td>
<td>110.7 ± 0.57</td>
</tr>
<tr>
<td>Creatinine</td>
<td>0.45 ± 0.05</td>
<td>1.82 ± 0.17</td>
<td>1.17 ± 0.09</td>
<td>0.82 ± 0.05</td>
<td>0.537 ± 0.22</td>
</tr>
<tr>
<td>Urea</td>
<td>35.25 ± 1.70</td>
<td>82.5 ± 3.87</td>
<td>40.25 ± 2.5</td>
<td>33.25 ± 2.21</td>
<td>36 ± 2.16</td>
</tr>
</tbody>
</table>


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The defensive antioxidant enzyme next to super oxide dismutase is catalase. Catalase traps the harmful hydrogen peroxide and converts it into water and oxygen. The activity of catalase was found to be decreased in iron induced rats. The inhibition of catalase activity during iron induced toxicity may be due to the increased generation of reactive free radicals, which can create an oxidative stress in the cells.

Vitamin E is a chain breaking antioxidant. It can repair oxidizing radicals directly, and prevent the chain propagation step during lipid autoxidation. The reduced form of glutathione substrate is required radicals directly, and prevent the chain propagation step during lipid peroxidation. Vitamin C may counteract the free radicals through autooxidation. Reduced form of glutathione substrate is required radicals directly, and prevent the chain propagation step during lipid peroxidation. Vitamin C functions as an aqueous phase antioxidant. It circulates freely within the plasma and plays a crucial role in the recycling of vitamin E and other antioxidants. Vitamin C shows inhibition of lipid peroxidation. Vitamin C may counteract the free radicals through effective scavenging and blocking the conjugation of reactive metabolite to reduced form of glutathione substrate.

The higher level of iron intake develops kidney damage by enhancing lipid peroxidation in the kidney. Glutathione belongs to antioxidant defense systems and prevents harmful effects of free radicals by scavenging hydroxyl radicals and singlet oxygen. Therefore, reduced form of glutathione substrate may contribute to the decrease in antioxidant potential leading to oxidative stress and consequently to nephrotoxicity.

In the present study (Table-2), we observed that increased level of TBARS and decreased levels of antioxidants viz., Super oxide dismutase, Catalase, reduced form of glutathione, Vitamin C and E in ferrous sulphate induced group which may be due to increased reactive oxygen species generation. Administration of herbal drugs of Azima tetracantha Lam. and Tribulus terrestris Linn. improved the antioxidant status. The increased levels of antioxidants indicate the herbal drug administration helps in decreased utility of antioxidants by reacting with reactive oxygen species, thereby maintaining their concentration in kidney. Herbal drugs Azima tetracantha Lam. and Tribulus terrestris Linn. are able to ameliorate ferrous sulphate induced adverse effects by improving antioxidant status and reducing lipid peroxidation as well as protecting cellular membranes.

Table 2: Changes in the level of TBARS and Antioxidants

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Super oxide dismutase</td>
<td>34.87 ± 0.25</td>
<td>20.12 ± 0.28</td>
<td>35.2 ± 0.33</td>
<td>34.5 ± 1.29</td>
<td>36 ± 2.44</td>
</tr>
<tr>
<td>Catalase</td>
<td>59.13 ± 0.04</td>
<td>48.42 ± 0.17</td>
<td>67.2 ± 0.86</td>
<td>70.62 ± 0.38</td>
<td>63.6 ± 0.25</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>64 ± 2.16</td>
<td>29.5 ± 3.41</td>
<td>52.5 ± 2.38</td>
<td>58 ± 6.68</td>
<td>45.5 ± 3.41</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>144.75 ± 2.62</td>
<td>67.25 ± 3.50</td>
<td>134 ± 4.54</td>
<td>140 ± 3.55</td>
<td>122.5 ± 1.2</td>
</tr>
<tr>
<td>Reduced form of glutathione</td>
<td>72.5 ± 4.50</td>
<td>29.5 ± 3.41</td>
<td>56 ± 0.81</td>
<td>68.5 ± 5.32</td>
<td>42.5 ± 2.38</td>
</tr>
<tr>
<td>TBARS</td>
<td>0.32 ± 0.02</td>
<td>0.71 ± 0.05</td>
<td>0.44 ± 0.02</td>
<td>0.38 ± 0.04</td>
<td>0.35 ± 0.08</td>
</tr>
</tbody>
</table>

In conclusion, oral administration of aqueous extracts of Tribulus terrestris Linn. and Azima tetracantha Lam. are effective in reducing the kidney damage induced by ferrous sulphate. Further studies are warranted to isolate and characterise the antinephrotoxic principles from the leaves and fruits of Azima tetracantha Lam. and Tribulus terrestris Linn. respectively.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the Correspondent, Principal and Head of the Department, S.T.E.T Women’s College, Mannargudi, Tamil Nadu, India for providing the laboratory and animal house facilities.

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