Acute and Sub Acute Toxicity of *Pterocarpus Santalinus* Heartwood Extracts in Rats

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ABSTRACT

The present study was to evaluate the safety of the *Pterocarpus santalinus* heart wood extract through acute and sub acute toxicity study in rats. For acute toxicity study 50-2000mg/kg ethanol extract and chloroform extract of *Pterocarpus santalinus* heart wood were administered orally and obvious toxic symptoms and mortality was studied up to 72hrs. In sub acute study, effect of multiple weekly dosing of 100, 400 and 750mg/kg of ethanol extract and chloroform extract of *Pterocarpus santalinus* heart wood was investigated in rats for six weeks and the evaluation was done by the studies of hematological parameters, biochemical estimations of hepatorenal parameters, histological studies of the tissue. Both the extracts of *Pterocarpus santalinus* heart wood were found to be well tolerated up to 2g/kg in acute toxicity study. The sub acute toxicity studies showed no significant alteration on any of the parameters. Hence the results suggest that ethanol extract of *Pterocarpus santalinus* heart wood and chloroform extract of *Pterocarpus santalinus* heart wood is safe and can be used in the treatment of diseases without any toxicity.

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Keyword

*Pterocarpus santalinus* heartwood, hematological parameters, hepatorenal and antioxidant status

INTRODUCTION:

Medicinal plants and herbal preparations have recently received considerable attention and have been found to be promising choice over modern synthetic medicines in a numerous of studies. In developing countries, all over the world, 80% of population continues to use traditional medicine in primary medical problems1. Research carried out in last few decades has validated several such claims of use of traditional medicinal plants. Human beings has recognised the need for better control of the present use and the future development of chemicals which should chemically tested and retested before reaching of
chemicals primary and cumulative toxicity and its mutagenic, teratogenic and carcinogenic potential which can be obtained from animal studies.

Toxicology is defined as any harmful effect of a chemical or drug on a target organism. Acute and sub acute toxicity has been defined by various experts. Toxicity can be acute, sub acute, or chronic: Acute toxicity involves harmful effects in an organism through a single or short term exposure. Sub acute toxicity is the ability of toxic substance to cause effects for more than one year but less than the lifetime of the exposed organism. Sub acute or chronic toxicity is the ability of a substance or mixture of substances to cause harmful effects over an extended period, usually upon continues or continuous exposure sometimes remains for the entire life of the exposed organism².

The purpose of toxicity testing is to provide adequate database to make decision concerning the toxicology properties of chemicals and commercial products and to decide whether a drug or chemicals will be safe or not.

_Pterocarpus santalinus_ belongs to the family Fabaceae commonly called as Red Sandal wood or Rakthachandan. It is mentioned in the literature that a combination of red sandal wood, mustard and brown sugar in equal quantity prevents conception in women³.

_Rakthachandan_ has number of activities which include astringent, tonic and diaphoretic. A paste of the wood is used as cooling external application for inflammations and headache. It is said to be useful in bilious affections and skin diseases. A decoction of the seeds is used as an astringent tonic in chronic dysentery. Drinking water in _Rakthachandan_ wooden cups twice a day used for the treatment of diabetes⁴. A histological stain prepared from the heart wood has been found to be an excellent nuclear stain for various cells of animal and plant origin⁵,⁶.

Despite of the popular use and extensive pharmacological studies, the toxicity profile, especially on its chronic use, has not been yet explored. The present investigation was therefore carried out to study the
MATERIALS AND METHODS

Plant material

The *Pterocarpus santalinus* heartwood was collected in Andhr pradesh from (Turipathi), India and authenticated by Dr. Madhav shetty and retained in our laboratory for further studies.

Preparation of plant extract

500g of the heartwood powder of *Pterocarpus santalinus* was defatted by extraction with petroleum ether and successively extracted further with chloroform and ethanol in a Soxhlet extractor. The extracts will be dried under vacuum. The yield was 350g with respect to 1Kg of dried powder and used for oral administration.

Animals

Adult female wister rats of weight 125-150g were used for acute toxicity study and adult male rats of weight 125-150g were used for sub acute toxicity. The animals were kept in polypropylene cages with Husk bedding and maintained under standard laboratory conditions. Standard pellet diet and water were given *ad libitum*. The rats were acclimatized to laboratory proviso for one week before commencement of experiment. The toxicity studies conducted as per internationally accepted protocol drawn under OECD No 420 guidelines.

Phytochemical analysis

Preliminary phytochemical screening of the extracts was carried out using standard methods.
Acute toxicity study

Healthy adult female wister rats were starved overnight and were divided into five groups (n=6). Group I-IV animals were orally fed with ethanol extract and chloroform extract of Pterocarpus santalinus heartwood in increasing dose levels of 50, 500, 1000, 2000mg/kg respectively, while group V (untreated) serve as control. The animals were observed continuously for first 2h for any gross change in behavioral, neurological and autonomic profiles or any other symptoms of toxicity and mortality if any and next 24h for any lethality or death⁸.

Sub acute toxicity study

Adult male Wistar rats were starved overnight and were divided in to four groups (n=6). Group I- III animals were orally fed with ethanol extract and chloroform extract of Pterocarpus santalinus heartwood in increasing dose level of 100, 400, 750mg/kg respectively, while group IV (untreated) served as control for 28 days. The animals were observed for sign and symptoms, behaviour alteration, food and water intake and body weight changes. All animals were observed twice daily for mortality during the 28 days period study. The weight of Pterocarpus santalinus heartwood treated animal was recorded on day 0 and at weekly intervals throughout the course of study. The group means body weights were calculated⁹.

At the end of the experiment, after 24h of the last dose and 18h fasting, animals were sacrificed and blood was collected from orbital sinus and taken into heparinized tube for hematological studies and non-heparinized centrifuge tube for biochemical analysis. Liver tissue was collected from the animals for the evaluation of in vivo antioxidant status and part of the liver tissue was taken for the histological studies¹⁰,¹¹.

Hematological studies

Red blood cells (RBC), white blood cells (WBC), Hemoglobin (Hb), Erythrocyte sedimentation rate (ESR), Platelet, clotting time and packed cellular volume (PCV) were performed using routine method¹².
Biochemical estimation

The effect of ethanol extract and chloroform extract of *Pterocarpus santalinus* heartwood treatment on the biochemical parameters of the experimental rats were evaluated by the estimation of blood glucose\(^1\), Cholesterol\(^2\), Serum glutamate Pyruvate (GPT)\(^3\), Glutamate oxaloacetate transaminase (GOT)\(^4\), Alkaline Phosphatase\(^5\), Total Bilirubin\(^6\), Urea and BUN\(^7\), Total Protein and albumin\(^8\) and Creatinine\(^9\) were estimated in serum. Urine samples were also collected at the end of the study period and analyzed for Specific Gravity, pH, glucose, Proteins Ketones and Occult Blood were performed.

Histological studies

At the end of 28 days, rats were sacrificed and Liver was removed and subject to Histological examination. The tissues were washed in normal saline and fixed immediately in 10% formalin for a period of at least 24h, dehydrated with alcohol and entrenched in paraffin, cut into 4-5μm thick sections for microscopic observation\(^15\).

Statistical analysis

Values were presented as mean ± S.E.M. Data were statistically evaluated by one-way analysis of variance (ANOVA) followed by key multiple comparison tests. \(P < 0.01\) was considered as statistically significant.

RESULTS

Preliminary phytochemical screening of *Pterocarpus santalinus* heartwood revealed the presence of various compounds; chloroform extract contains glycosides and ethanol extract contains flavonoids, alkaloids, tannins, phenols, saponins and sterols.

Acute toxicity study

In acute toxicity study, ethanol and chloroform extract of *Pterocarpus santalinus* heartwood did not show any mortality or toxic effect up to the dose of 2g/kg during the observational period of 24h. It did not produce any significant changes in behaviour, breathing, sensory nervous system responses and GIT.
effects. These results showed that in single dose, there are no adverse effects in both chloroform and ethanol extract of *Pterocarpus santalinus* heartwood indicating that the medium lethal dose (LD$_{50}$) is higher than 2000mg/kg in rats. Accordingly both the extract was safe at a dose of 2000mg/kg. Accordingly one-fifth of the maximum tolerated dose ie, 400mg/kg was considered as the high dose of ethanol and chloroform extract of *Pterocarpus santalinus* heartwood and used for the sub acute toxicity study in the present investigation.

**Sub acute toxicity study**

In sub-acute toxicity study, ethanol and chloroform extract of *Pterocarpus santalinus* heartwood administration did not showed any significant changes in behaviour or locomotor activity, no sign of intoxication were observed during the 28 days period of the treated animals No difference in growth between the control group and treated groups. No change in fur coating, eyes and respiratory function.

**Table I: Changes in growth and body weight of rats following treatment with different doses of ethanol and chloroform extract of *Pterocarpus santalinus* heartwood.**

There was no significance difference in the food and water consumption between the treated and control group. *(Table: I)*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose mg/kg</th>
<th>Body weight (gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Initial</td>
</tr>
<tr>
<td>-</td>
<td></td>
<td>154 ± 0.1</td>
</tr>
<tr>
<td>extract of <em>Pterocarpus santalinus</em> heartwood</td>
<td>100</td>
<td>143 ± 1.7</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>145 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>750</td>
<td>155 ± 0.4</td>
</tr>
<tr>
<td>chloroform extract of <em>Pterocarpus santalinus</em> heartwood</td>
<td>100</td>
<td>140 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>148 ± 2.1</td>
</tr>
<tr>
<td></td>
<td>750</td>
<td>150 ± 1.9</td>
</tr>
</tbody>
</table>
All Values are expressed as mean ± SEM, n= 6, *P<0.05, **P<0.01 ***P<0.001 Vs control.

Effect of ethanol and chloroform extract of *Pterocarpus santalinus* heartwood on hematological parameter has been presented in (Table: II). RBC, WBC, Hb, ESR and PVC were not significantly different both in control and ethanol and chloroform extract of *Pterocarpus santalinus* heartwood treated animals. There was slightly change in Platelet and clotting time with control (Table: II). All the values were found to be within the normal range and there was no difference between the groups.

**Table II: Effect of ethanol extract and chloroform extract of *Pterocarpus santalinus* heartwood on hematological parameters of control and treated rats.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Hb gm %</th>
<th>RBC 10⁶/cum m</th>
<th>WBC 10³/cu.m</th>
<th>ESR mm/1⁰ hr</th>
<th>Platelets (K/µL)</th>
<th>Clotting time (Sec)</th>
<th>PCV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol extract of <em>Pterocarpus santalinus</em> heartwood.</td>
<td>-</td>
<td>14.18 ± 1.46</td>
<td>5.43 ± 1.23</td>
<td>4.63 ± 0.34</td>
<td>4.15 ± 1.16</td>
<td>350 ± 15.13</td>
<td>150.40 ± 2.49</td>
<td>60 ± 2.33</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>11.12 ± 1.25</td>
<td>5.10 ± 1.70</td>
<td>5.10 ± 0.97</td>
<td>4.22 ± 1.28</td>
<td>400 ± 16.14</td>
<td>113.30 ± 2.12</td>
<td>50 ± 3.26</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>12.52 ± 1.20</td>
<td>4.96 ± 1.38</td>
<td>8.46 ± 0.26</td>
<td>4.00 ± 1.30</td>
<td>425 ± 17.18</td>
<td>110.30 ± 0.38</td>
<td>57 ± 2.16</td>
</tr>
<tr>
<td></td>
<td>750</td>
<td>13.30 ± 0.14</td>
<td>5.69 ± 1.65</td>
<td>7.93 ± 0.14</td>
<td>4.52 ± 2.18</td>
<td>450 ± 10.15</td>
<td>120.10 ± 1.20</td>
<td>55 ± 1.27</td>
</tr>
<tr>
<td>Chloroform extract of <em>Pterocarpus santalinus</em> heartwood.</td>
<td>100</td>
<td>11.70 ± 1.35</td>
<td>5.52 ± 1.30</td>
<td>4.88 ± 1.37</td>
<td>4.25 ± 1.18</td>
<td>415 ± 10.11</td>
<td>130.30 ± 2.30</td>
<td>62 ± 2.16</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>13.00 ± 0.52</td>
<td>5.60 ± 1.80</td>
<td>8.46 ± 0.26</td>
<td>4.14 ± 1.30</td>
<td>475 ± 12.50</td>
<td>125.10 ± 0.80</td>
<td>59 ± 216</td>
</tr>
<tr>
<td></td>
<td>750</td>
<td>15.12 ± 0.84</td>
<td>5.90 ± 1.50</td>
<td>7.93 ± 0.14</td>
<td>3.90 ± 1.38</td>
<td>459 ± 10.20</td>
<td>115.60 ± 2.20</td>
<td>52 ± 1.57</td>
</tr>
</tbody>
</table>
Values are mean ± SEM, (n=6), * p< 0.01 for ethanol and chloroform extract of *Pterocarpus santalinus* heartwood treated group vs normal control group.

The normal levels of SGPT, SGOT, ALP, total bilirubin and protein in serum are unaltered and renal parameters i.e urea, uric acid and creatinine are also unaltered as shown in (Table: III)

**Table III: Effect of ethanol extract and chloroform extract of *Pterocarpus santalinus* heartwood on biochemical parameters for hepatorenal functions in control and treated rats.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>SGOT (IU/L)</th>
<th>SGPT (IU/L)</th>
<th>ALP (IU/L)</th>
<th>Total Bil (mg/dl)</th>
<th>Uric acid (mg%)</th>
<th>Creatinine (mg%)</th>
<th>Urea (mg%)</th>
<th>Total proteins (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>55.34±1.78</td>
<td>49.11±1.99</td>
<td>98.55±1.45</td>
<td>2.06±0.67</td>
<td>10.33±1.55</td>
<td>1.57±1.99</td>
<td>45.55±5.30</td>
<td>5.67±2.32</td>
</tr>
<tr>
<td>Ethanol extract of <em>Pterocarpus santalinus</em> heartwood</td>
<td>100</td>
<td>60.74±1.33</td>
<td>57.32±3.85</td>
<td>103.12±3.11</td>
<td>2.50±1.67</td>
<td>7.06±1.16</td>
<td>2.00±1.27</td>
<td>40.80±4.10</td>
<td>6.57±4.10</td>
</tr>
<tr>
<td>400</td>
<td>63.5±1.6</td>
<td>55.0±1.43</td>
<td>110±2.50</td>
<td>2.45±1.12</td>
<td>7.56±1.47</td>
<td>2.20±2.00</td>
<td>38.18±1.90</td>
<td>7.07±2.4</td>
<td></td>
</tr>
<tr>
<td>750</td>
<td>65.0±1.7</td>
<td>62±2.34</td>
<td>115±3.11</td>
<td>2.10±1.16</td>
<td>7.77±0.39</td>
<td>1.80±1.38</td>
<td>35.30±2.34</td>
<td>7.11±1.11</td>
<td></td>
</tr>
<tr>
<td>Chloroform extract of <em>Pterocarpus santalinus</em> heartwood</td>
<td>100</td>
<td>65.57±2.77</td>
<td>55.17±3.87</td>
<td>100.27±1.87</td>
<td>2.35±1.34</td>
<td>9.45±4.34</td>
<td>2.25±0.33</td>
<td>46.65±0.42</td>
<td>6.00±2.78</td>
</tr>
<tr>
<td>400</td>
<td>60.71±1.23</td>
<td>60.47±1.20</td>
<td>97.83±0.57</td>
<td>2.50±1.40</td>
<td>8.50±2.50</td>
<td>2.47±1.67</td>
<td>50.60±1.47</td>
<td>6.50±1.12</td>
<td></td>
</tr>
<tr>
<td>750</td>
<td>70.50±2.65</td>
<td>61.44±1.67</td>
<td>110.44±1.67</td>
<td>2.90±1.30</td>
<td>9.15±2.24</td>
<td>2.50±1.54</td>
<td>55.43±2.11</td>
<td>7.09±2.56</td>
<td></td>
</tr>
</tbody>
</table>
Values are mean ± SEM, (n=6), *p<0.01 for ethanol and chloroform extract of *Pterocarpus santalinus* heartwood treated group vs normal control group.

indicate that sub acute treatment with ethanol and chloroform extract of *Pterocarpus santalinus* heartwood does not posses any significant adverse effect on hepatorenal functioning of the animals.

(Table: IV) explores the lipid profile and blood sugar level of normal ethanol and chloroform extract of *Pterocarpus santalinus* heartwood treated animals after the six week experimental period.

The results revealed that the extract does not adversely alter the lipid profile and blood sugar level of the animals after sub acute supplementation.

Histological observation of the liver tissue in both control as well as extracts treated rats showed no difference indicating that *Pterocarpus santalinus* heartwood extracts did not result any adverse effect on this organ. (Fig.I and Fig.II)

**Table IV: Effect of ethanol and chloroform extract of *Pterocarpus santalinus* heartwood on lipid profile and glucose level in control and treated rats.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Triglyceride (mg/dl)</th>
<th>Total cholesterol (mg/dl)</th>
<th>HDL (mg/dl)</th>
<th>LDL (mg/dl)</th>
<th>Glucose (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>110.21±1.55</td>
<td>140.79±3.22</td>
<td>70.11±1.53</td>
<td>30.45±2.40</td>
<td>88.98±2.76</td>
</tr>
<tr>
<td>Ethanol extract of <em>Pterocarpus santalinus</em> heartwood</td>
<td>100</td>
<td>120.34±4.52</td>
<td>145.98±1.77</td>
<td>77.14±3.22</td>
<td>36.26±2.51</td>
<td>90.27±2.78</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>130.20±1.13</td>
<td>150.0±2.34</td>
<td>75.45±1.50</td>
<td>42.15±1.24</td>
<td>100.50±2.40</td>
</tr>
<tr>
<td></td>
<td>750</td>
<td>125.10±1.72</td>
<td>162±1.30</td>
<td>95.95±3.10</td>
<td>47.18±1.68</td>
<td>95.17±1.09</td>
</tr>
<tr>
<td>Chloroform extract of <em>Pterocarpus santalinus</em> heartwood</td>
<td>100</td>
<td>125.44±2.28</td>
<td>141.77±1.09</td>
<td>85.55±3.65</td>
<td>35.67±2.11</td>
<td>105.43±1.11</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>130.75±1.23</td>
<td>160.71±1.88</td>
<td>97.83±4.22</td>
<td>44.50±3.10</td>
<td>98.10±3.40</td>
</tr>
<tr>
<td></td>
<td>750</td>
<td>110.50±2.15</td>
<td>161.54±1.22</td>
<td>75.14±1.67</td>
<td>47.45±2.10</td>
<td>109.75±2.75</td>
</tr>
</tbody>
</table>

Values are mean ± SEM, (n=6), *p<0.01 for ethanol and chloroform extract of *Pterocarpus santalinus* heartwood treated group vs normal control group.
Fig 1. Histopathological Studies of Liver in Sub acute Toxicity Studies of ethanol extract of **Pterocarpus santalinus** heartwood.

1A. Section of liver treated with normal saline

1B. Section of liver treated with ethanol extract of *Pterocarpus santalinus* heartwood (100 mg/kg)

1C. Section of liver treated with ethanol extract of *Pterocarpus santalinus* heartwood (400 mg/kg)

1D. Section of liver treated with ethanol extract of *Pterocarpus santalinus* heartwood (750 mg/kg)
Fig 2. Histopathological Studies of Liver in Sub acute Toxicity Studies of chloroform extract of *Pterocarpus santalinus* heartwood.

2A. Section of liver treated with normal saline

2B. Section of liver treated with chloroform extract of *Pterocarpus santalinus* heartwood (100 mg/kg)

2C. Section of liver treated with chloroform extract of *Pterocarpus santalinus* heartwood (400 mg/kg)

2D. Section of liver treated with chloroform extract of *Pterocarpus santalinus* heartwood (750 mg/kg)

**DISCUSSION**

The purpose of the study was to look at the toxicity profile of the *Pterocarpus santalinus*, a 28 days study is considers a sub acute toxicity study, which is well accepted for eliciting any toxicity on long term feeding. It gives valuable information on the cumulative toxicity of a substance on the target organs.
or prolonged exposure. A wide verity of adverse effect can be detected from sub acute toxicity studies. The results from such studies can provide information, which will aid in selecting dose level. The use of herbal medicines has received a great attention as alternatives to synthetic pharmaceutical products in recent times, leading to the increase in their demand. Experimental screening method including a thorough toxicity study is therefore important to ascertain the safety and efficacy of these herbal drugs. Acute toxicity studies showed the lack of mortality and toxicity up to oral treatment of 2000mg extract/kg body weight which suggests that the ethanol extract and chloroform extract of *Pterocarpus santalinus* heartwood is practically nontoxic at single dose.

During the experimental period, there was no treatment related effect on hemoglobin concentration and RBC count in both the extracts. Insignificant change in WBC count was probably due to normal response to foreign bodies or stress associated with the chronic toxicity studies.

Decreased level of SGPT, SGOT and ALP reflects the structural and functional dysfunction of hepato cellular membrane or cell rupture, and thereby indicates liver damage. Bilirubin is formed from degeneration of hemoglobin by the action of reticuloendothelial systems through out the body. Increased bilirubin level reflects the depth of jaundice. The normal value of the hepatic biochemical parameters reveals the safety profile of the extracts on liver function even on its chronic use. The normal values of the renal biochemical parameters i.e urea, uric acid and creatinine suggest that the extracts do not produce any sort of disturbance in the renal function, as has been found in case of various plant extracts and hence is safe on its chronic use in various diseases.

The extracts exerted protective effect on the lipid profile of the animals which was evident by the unaltered values of LDL, HDL and cholesterol in the treated group.

The endogenous antioxidant status after the chronic use of the extracts was found to be quite equivalent to that of the normal rats. However the slight higher value of GSH was observed in the *Pterocarpus santalinus* heartwood extracts treated rats. This indicates the protective role of the extracts on the endogenous antioxidant system which may be beneficial especially in case of the oxidative stress of the various disease conditions.
Histological observations results showed the normal cellular architectures in the treated group of animals, without any necrosis or fatty infiltration, which can substantiate the safety profile of the extracts clearly.

CONCLUSION

Acute toxicity studies showed the lack of mortality and toxicity up to oral treatment of 2000 mg extract/kg body weight which suggests that the ethanol extract and chloroform extract of *Pterocarpus santalinus* heartwood is practically nontoxic at single dose, thus provides evidence for the total safety profile of the ethanol extract and chloroform extract of *Pterocarpus santalinus* heartwood suggesting its safe use in single dose treatment as well as for long term therapeutic application in case of various chronic diseases, without producing any toxic effects.

REFERENCE:


