Ameliorative effect of seed extract of *Pterocarpus santalinus* on coragen induced haematological alterations and serum biochemical changes in Charles Foster rats

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In the present study, the ameliorative effect of an aqueous extract of *Pterocarpus santalinus* against coragen induced haematological changes, biochemical alterations and oxidative damage in Charles foster rats was undertaken. Coragen administration (1000 mg kg⁻¹ body weight orally for 6 weeks) was associated with significant rise in serum levels of alkaline phosphatase, urea, uric acid and creatinine and enhanced lipid peroxidation which is evident by significant increase in malondialdehyde (MDA) levels. Furthermore, significant changes in the haematological indices (red blood cell (RBC) count, haemoglobin percentage, haematocrit, mean corpuscular volume of RBCs, mean corpuscular haemoglobin concentration, mean corpuscular haemoglobin and white blood cell (WBC) count) were observed. Treatment with aqueous seed extract of *P. santalinus* (300 mg kg⁻¹ body weight orally for 30 days) attenuated the oxidative stress and improved haematological as well as biochemical alterations evoked by Coragen. Thus, *P. santalinus* possesses ameliorative effect against coragen induced toxicity.

Key words: *Pterocarpus santalinus*, coragen, lipid peroxidation, oxidative damage, MDA.

INTRODUCTION

Pesticides are widely used by farmers for agricultural purposes. It has conferred immense benefits to mankind by improving health and nutrition. Pesticides fall into numerous chemical classes, which have widely differing biological activities and thus differing potential to produce adverse effects in living organisms, including humans (Timothy and Ballantyne, 2004).

Ryanodine receptor-targeting insecticides (Ryanoids) are a novel class of insecticides acting selectively on ryanodine receptors of a broad spectrum of lepidopteran species. Ryanoids are synthetic analogues with the same mode of action as ryanodine, a naturally occurring insecticide extracted from *Ryania speciosa*. They bind to calcium channels in cardiac and skeletal muscle, blocking nervous transmission. Ryanodine receptors are intracellular channels in insects, responsible for the
control of calcium ion release (Ebbinghaus et al., 2006). Ryandine receptor plays an important role in controlling the release of calcium ions, universal intracellular transmitter, from intracellular stores. The flow of Ca\textsuperscript{2+} is regulated by ryandine receptors, which mediate in several metabolic and physiological cellular processes such as neurotransmission, hormones secretion, muscles excitation-contraction coupling (Copping and Duke, 2007). A class of antranilidiamides (antranilodihamides) were being developed; with representatives Chlorantraniliprole and Cyazypyr. Two insecticidal preparates containing Chlorantraniliprole were being promoted; Altacor and Coragen. Coragen 18.5 SC is a concentrated suspension of Chlorantraniliprole, being applied for use in apples against codling moth (Cydia pomonella), apple fruit moth (Argyresthia conjugella) and free leaf living larvae (Kar et al., 2013).

Pesticides are known to increase the production of reactive oxygen species (ROS), which in turn, generate oxidative stress in different tissues (Heikal et al., 2012; Heikal and Soliman, 2010; Heikal et al., 2011; Rai and Sharma, 2007). Many studies have implicated oxidative damage as the central mechanism of toxicity (Celik et al., 2009; Halliwell and Gutteridge, 2002; Kalender et al., 2010). Oxidative damage primarily occurs through production of reactive oxygen species (ROS), including hydroxyl radicals and hydrogen peroxide that are generated during the reaction and react with biological molecules, eventually damaging membranes and other tissues. High oxidative stress depletes the activity of antioxidant defense system and thus promotes free radicals generation. Oxygen free radicals could react with polyunsaturated fatty acids which lead to lipid peroxidation (LPO) (Gandhi et al., 2012; Messarah et al., 2010).

Many insecticides are hydrophobic molecules that bind extensively to biological membranes, especially phospholipid bilayers, and they may damage membranes by inducing lipid peroxidation (Heikal et al., 2011; Celik et al., 2009; Kalender et al., 2010). Oxidative stress is initiated by free radicals, which seek stability through electron pairing with biological macromolecules in healthy human cells and cause protein and DNA damage along with lipid peroxidation (Mishra et al., 2011). Herbal medicines derived from plant extracts are being increasingly utilized as adjunct treatment options for a wide variety of clinical diseases. Many phytochemicals have been found to play an important role as potential antioxidants and antimicrobials (Mishra et al., 2011).

\textit{Pterocarpus santalinus} (Linn Fabaceae), commonly known as “Red sanders” is a small to medium-sized deciduous tree, 7.5 m high, with an extremely hard dark purple heartwood with a bitter flavor. In the traditional system of medicine, the decoction prepared from the heartwood is attributed various medicinal properties, it has been used as a anti-pyretic, anti-inflammatory, anthelmintic, tonic, hemorrhage, dysentery, aphrodisiac, diaphoretic as well as to induce vomiting, to treat eye diseases, mental aberrations and ulcers (Kirtikar and Basu, 1987). The wood in combination with other drugs is also prescribed for snake-bites and scorpion-stings (Warrier et al., 1995). Decoction of the heartwood has been reported as a central nervous system (CNS) depressant and also shown to have anti-inflammatory activity for induced hand paw edema in rats when prepared in formalin (3%). Heartwood contains pterocarp, santalin A, B, pterocarpin, isopterocarponol, pterocarpodiolones with β-eudesiol and crytomeridol (Yoganarasimhan, 2000). In addition, Auron glycosides viz., 6-OH-1-Methyl-3',4',5'-trimethoxyaurone-4-O-rhamnoside and 6,4'-dihydroxyaurone-4-O-neohesperidoside, and isoflavone glycoside 4',5'-dihydroxy-7-O-methyl isoflavone 3'-O-beta-D-glucoside (Krishnaveni and Rao, 2000) are also present. However, the species has remained unexplored for many pharmacological activities claims. The phytochemical investigation of the seed extract has not been demonstrated so far. Hence, the present investigation was carried out to analyze the therapeutic effect of seed extract of \textit{P. santalinus} on alterations in haematological and biochemical parameters caused upon sub-chronic exposure to Coragen.

**MATERIALS AND METHODS**

**Animals**

Charles Foster rats (n = 30), weighing 180 to 200 g of 8 weeks old, were obtained from animal house of Mahavir Cancer Institute and Research Centre, Patna, India (CPCSEA Regd-No. 1129/bc/07/CPCSEA). The research work was approved by the (IAEC, Institutional Animal Ethics Committee) with IAEC No. IAEC/2011/12/01. Food and water to rats were provided ad libitum (prepared mixed formulated food by the laboratory itself). The experimental animals were housed in conventional polypropylene cages in small groups (2 each). The rats were randomly assigned to control and treatment groups. The temperature in the experimental animal room was maintained at 22±2°C with 12 h light/dark cycle.

**Chemicals**

Coragen 18.5 (SC 18.5% w/w) was obtained from M/S Krishi Seeds Niketan, Patna, India. Other reagents used were of analytical grade and were prepared in all glass-distilled water.

**Preparation of seed extract of \textit{P. santalinus}**

In the present study, dry seeds of \textit{P. santalinus} were procured from a tree at local garden at Patna, Bihar, India. The identity of the medicinal plant was confirmed by Dr. Ramakant Pandey (Botanist), Department of Biochemistry, Patna University, Patna, Bihar, India. The collected seeds of \textit{P. santalinus} were shade dried and were ground to fine powder. The powder was then soaked in 70% ethanol for 48 h and finally extracted with absolute ethanol using soxhlet apparatus for 6 to 8 h and the residue was concentrated and dried at 37°C. The ethanolic extract dose was calculated after LD\textsubscript{50} estimation which was 3.000 mg kg\textsuperscript{-1} body weight and finally made to the 1/10th dose that is 300 mg kg\textsuperscript{-1} body weight.
Experimental design

A total of 30 rats (10 male and 20 female), were randomly assigned to control and treated groups. Coragen at concentration of 1000 mg kg\(^{-1}\) body weight (5 ml/1000 g body weight suspended in 10 ml distilled water) was administered orally, once a day, for a period of 42 days. This was followed by administration of aqueous seed extract at 300 mg kg\(^{-1}\) body weight orally daily for a period of 30 days. No treatment was administered to control group and was designated as healthy control.

Haematological analysis

Rats were anaesthesized with diethyl ether and blood samples were drawn from the heart of each animal. Two blood samples were taken with or without ethylenediaminetetraacetic acid (EDTA). The one with EDTA was used for haematological analysis and the other for the preparation of serum for the biochemical assays. Blood samples with anti-coagulant EDTA were analysed for blood parameters namely red blood cell (RBC) counts, white blood cell (WBC) counts, haemoglobin percentage, haematocrit (Ht), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) using an improved Neubauer’s Chamber (Depth 0.1 mm, 1/400 mm\(^2\)). Haemometer was used for the measurement of haemoglobin percentage (Berberian and Enan, 1989).

Biochemical evaluation

The sera obtained from different treatments were subjected to certain biochemical analyses. Serum alkaline phosphatase (ALP; EC 3.1.3.1) activity was measured by Mod. Kind and King’s method (Kind and King, 1954; Varley, 1975). The kidney function assays involved the determination of serum urea by Mod. Berthelot method (Berthelot, 1859; Fawcett and Scott, 1960), serum uric acid by Uricase/PAP method (Trinder, 1969; Fossati and Prencipe, 1980) and measurement of serum creatinine by alkaline picate method (Bones, 1945; Toro, 1975).

Lipid peroxidation

Thiobarbituric acid reactive substances (TBARS), as a marker for LPO, were determined by the double heating method (Draper and Hadley, 1990). The principle of the method was a spectrophotometric measurement of the color produced during the reaction to thiobarbituric acid (TBA) with malondialdehyde (MDA).

Statistical analysis

Results are presented as mean ± SD and total variation present in a set of data was analysed through one-way analysis of variance (ANOVA). Difference among mean values has been analysed by applying Dunnett’s t-test. Calculations were performed with the Graph Pad Prism 5.0 Program (GraphPad software, Inc., San Diego, USA). The criterion for statistical significance was set at p < 0.05.

RESULTS

Morbidity and mortality

The rats after sub-chronic Coragen exposure (1000 mg kg\(^{-1}\) body weight) have shown signs of toxicity such as nausea, nose bleeding, bulging of eyes, lack of body coordination, blackening of tongue and general body weakness. However, the recovery was achieved after the administration of aqueous seed extract of P. santalinus.

Haematological analysis

Data of haematological parameters as studied after 14 and 21 days of Coragen exposure showed significant decrease in the erythrocyte count (RBCs), haemoglobin percentage, haematocrit percentage and leukocyte count (WBCs), but increase in the MCV, MCH, MCHC in comparison with control group (Kumar et al., 2013).

Table 1 depicts the changes in the haematological parameters of Charles Foster rats exposed to Coragen at 1000 mgkg\(^{-1}\) body weight daily for 42 days and therapeutic effect of P. santalinus (300 mg kg\(^{-1}\) body weight).

<table>
<thead>
<tr>
<th>Blood Parameters</th>
<th>Control</th>
<th>Coragen treated 1000 mg kg(^{-1}) body weight (42 days)</th>
<th>P. santalinus treated 300 mg kg(^{-1}) body weight (14 days)</th>
<th>P. santalinus treated 300 mg kg(^{-1}) body weight (30 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC (10(^{12})/mm(^3))</td>
<td>7.46 ± 0.49</td>
<td>1.64 ± 0.42</td>
<td>2.70 ± 0.38</td>
<td>3.21 ± 0.45</td>
</tr>
<tr>
<td>Hb (%)</td>
<td>93.36 ± 0.17</td>
<td>32.17 ± 4.02</td>
<td>53.33 ± 3.56</td>
<td>71.67 ± 3.78</td>
</tr>
<tr>
<td>Haematocrit (%)</td>
<td>272.5 ± 4.04</td>
<td>92.50 ± 3.27</td>
<td>156.8 ± 4.71</td>
<td>209.5 ± 3.94</td>
</tr>
<tr>
<td>MCV (fL)</td>
<td>362.3 ± 3.39</td>
<td>641.8 ± 3.19</td>
<td>639.8 ± 3.02</td>
<td>707.0 ± 3.62</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>127.5 ± 2.88</td>
<td>214.7 ± 3.10</td>
<td>214.8 ± 2.89</td>
<td>235.1 ± 2.97</td>
</tr>
<tr>
<td>MCHC (g/L)</td>
<td>33.77 ± 3.09</td>
<td>18.10 ± 2.85</td>
<td>4.57 ± 0.58</td>
<td>4.64 ± 0.76</td>
</tr>
<tr>
<td>WBC (10(^{12})/mm(^3))</td>
<td>7500 ± 3.78</td>
<td>3600 ± 3.45</td>
<td>5551 ± 2.64</td>
<td>10501 ± 3.33</td>
</tr>
</tbody>
</table>

Data represent mean ± SD, n=6/group, p < 0.05.
for 30 days. There was a significant increase in haemoglobin percentage, RBC count and WBC count after *P. santalinus* administration. But, the other parameters showed a somewhat different trend. *P. santalinus* administration led to an increase in haematocrit percentage as compared to that after Coragen treatment, however the value was still less than the control. There was a slight decrease in MCV level after 14-day *P. santalinus* treatment but it was followed by gradual increase after 30 days *P. santalinus* treatment. MCHC showed a decreasing trend after *P. santalinus* administration. The same trend was observed in case of MCH.

Biochemical analyses

Figures 1 to 5 depicts the effects produced on selected functional indices of rat liver and kidney, respectively, following the repeated administration of *P. santalinus* extract. *P. santalinus* administration resulted in significant decrease in serum content of alkaline phosphatase when compared with those treated with Coragen (Figure 1). The alkaline phosphatase concentration compared favorably with the control by the 30th day of administration, a trend which was sustained throughout the experimental period as is evident from the value after 30 days exposure. The administration of *P. santalinus* seed extract led to a gradual decrease in serum lipid peroxidation, as evident from decrease in MDA level. However, the result was not comparable with that of the control group (Figure 2). The urea level was gradually decreased after administration of seed extract. However, it showed a vast difference as compared to that with control (Figure 3). The other two parameters denoting kidney function, that is, serum uric acid and creatinine levels showed a similar trend. Initially, there was an increase in levels of uric acid and creatinine after seed extract administration. However, after 30-day dosage, the levels gradually decreased and were favorably comparable with the control (Figures 4 and 5).

DISCUSSION

In the present study, the protective effect of an aqueous extract of *P. santalinus* against hepatotoxicity, nephrotoxicity and oxidative damage induced by sub-chronic exposure to Coragen was undertaken. The experimental models of Coragen was previously validated as a model of other pesticide induced toxicity on some haematological parameters (Saka et al., 2011; Mongi et al., 2011; Dahamma et al., 2011; Ambali et al., 2010, 2011; Bhardwaj et al., 2010; Mansour et al., 2007; Rahman and Siddiqui, 2006; Adhikari et al., 2004; Raizada et al., 2001;
**Figure 2.** Effect of *P. santalinus* seed extract on lipid peroxidation after repeated dose. Values are mean ± SD for 6 animals in each group.

**Figure 3.** Effect of *P. santalinus* seed extract on serum urea after repeated dose. Values are mean ± SD with 6 animals in each group.
Figure 4. Effect of *P. santalinus* seed extract on serum uric acid after repeated dose. Values are mean ± SD with 6 animals in each group.

Figure 5. Effect of *P. santalinus* seed extract on serum creatinine after repeated dose. Values are mean ± SD with 6 animals in each group.
Neskovic et al., 1991). Coragen is an insecticidal preparate containing chlorantraniliprole as the active substance. The ryanodine receptor targeting insecticide, coragen, is an antranilc diamide acting selectively on ryanodine receptors (RyRs). The compound has extremely high affinity to the open-form ryanodine receptor, a group of calcium ion channels found in skeletal and heart muscle cells. At nanomolar concentration, it locks the receptor in a half-open state, whereas, it fully closes them at micromolar concentrations. The effect of binding at nM level is that it causes release of calcium ions from calcium stores in the sarcoplasmic reticulum leading to massive muscular contractions. This is true for both mammals and insects. However, the mammalian toxicity being proposed for chlorantraniliprole is low (Lahm et al., 2007). P. santalinus is a highly valued woody plant, whose bark extract has a blood glucose level lowering effect in experimental animals (Varma and Vijayamma, 1991). Methanol and aqueous extracts of heartwood of PS have shown anti-hepatotoxicity in CCl4-induced hepatotoxicity (Rane and Gramarc, 1998). Himoliv, a polyherbal Ayurvedic formulation containing P. santalinus as one of the ingredients has been reported to possess hepatoprotective activity (Bhattacharya et al., 2003).

Liver plays an important role in metabolism to maintain energy level and metabolic stability of the body (Guyton and Hall, 2006). It is also the site of biotransformation by which a toxic compound has been transformed in less harmful form to reduce toxicity (Hodgson, 2004). The present study has shown the therapeutic effect of aqueous extract of P. santalinus on liver of coragen intoxicated rats. The toxic effects of organophosphorus insecticides are to conjugate with the natural complement of enzyme in the body, thereby inactivating them. Phosphate enzymes act by hydrolyzing phosphor mono ester including 3 and 5 phosphoproteins, these may also be involved in the transfer of phosphate (Hanafy et al., 1991). Phosphatases are involved in many different processes that require mobilization of phosphate ion or dephosphorylation as part of anabolic, catabolic or transfer processes. It was found, that the increased level of phosphatases may be to indicate metabolic activity, perhaps to meet the stress induced by prolonged exposure to the pesticides. These enzyme changes are indicative of the cellular toxicity and tissue damage induced by these pesticides in the rats probably by altering the specific molecular pathways (Khalid et al., 2013). Alkaline phosphatase activity is related to hepatocyte function. An increase in its activity is due to increased synthesis in presence of increased biliary pressure (Kumar et al., 2006).

Urea and creatinine are waste products of protein metabolism that need to be excreted by the kidney, therefore, marked increase in serum urea and creatinine, as noticed in this study, confirms an indication of functional damage to the kidney (Garba et al., 2007).

Urea level can be increased by many other factors such as dehydration, antiuretic drugs and diet, whilst, since creatinine is more specific to the kidney, therefore kidney damage is the only significant factor that increases serum creatinine level (Nwanjo et al., 2005). Therefore, significant increase in urea and creatinine levels noticed in this study are a classical sign that the kidney was adversely affected by coragen administration. Under our experimental conditions, coragen had a poison effect on adult rats, increasing serum uric acid levels. Impairment in kidney function could probably occur as a result of kidney oxidative damage. In fact, uric acid in blood is the most important antioxidant (Ames et al., 1981). This compound is the end product of purine catalolism and can reduce oxidative stress by scavenging various reactive oxygen species (ROS) (Mahjoubi et al., 2008). Kidney dysfunction and nephrotoxicity induced by coragen in present investigation are consequences of oxidative stress. Treatment of coragen-intoxicated rats with aqueous extract of PS normalized the levels of urea and creatinine.

Large numbers of xenobiotics have been identified to have potential to generate free radicals in biological system (Ahmed et al., 2000; Kehrer, 1993). Free radicals have become an attractive means to explain the toxicity of numerous xenobiotics. Some of these free radicals interact with various tissue components, resulting in dysfunction and the question of whether oxidative stress is a major cause of injury remains equivocal. In this study, we have investigated the effects of administration of aqueous extract of PS on lipid peroxidation induced by Coragen. To our knowledge, there is no information concerning protective action of PS extract on oxidative injury induced by Coragen.

Lipid peroxidation has been extensively used as a marker of oxidative stress. Oxidative stress is an outcome of a multi-step process spanning from perturbations in the balance between the levels of oxidants/prooxidants and antioxidants (both enzymatic and non-enzymatic) to tissue damage leading to onset of several disease states and finally to apoptosis. Several factors (called as risk factors) are thought to be associated with potentiating of the impact of pesticides-induced oxidative stress in living systems and hence play crucial role in the evaluation of safety or toxicity of the pesticide concerned (Agrawal and Sharma, 2010). The increase in the levels of thiobarbituric acid-reactive substances (the marker of extent of lipid peroxidation) in serum of rats due to coragen administration produced oxidative stress due to the generation of free radicals and subsequently altered the antioxidant defense system in erythrocytes (John et al., 2001). Oxidative damage in tissues due to the formation of reactive oxygen species (ROS) can be counter-balanced by the different oxidative systems of the host. These defenses appear to be inducible by nutrients or non-nutrients in the diet (Jaiswal et al., 2013). The protective effect of P. santalinus seed
extract may be in part due to santalin A, B, the main constituents of *P. santalinus*. Hence, the hepatoprotective and nephroprotective activities of *P. santalinus* may be attributed to its components including alkaloid, triterpenoid, saponins and flavonoidal constituents (Metha et al., 1999; Tran et al., 2001; Vijayan et al., 2003; Xiong et al., 2003; Dhanabal et al., 2007).

The decrease in RBC count and Hb % can be correlated with the increased arterial O$_2$ saturation of blood, which acts indirectly as stimulus for bone marrow erythrocyte production (Zabulyte et al., 2007). The significant decrease in the RBC, haemoglobin and haematocrit may be a consequence of severe haemorrhage which results in the dilution of blood caused due to the influx of cells and fluids from body stores (Celik and Suzek, 2009; Kalender et al., 2006). In the severe anaemic condition, there is immense decrease in the number of red blood cells leading to impaired synthesis of haemoglobin due to iron deficiency or impaired production of erythrocyte due to deficiency of folic acid and vitamin B$_12$ (Murray et al., 2007). The decrease in WBCs count was due to the possible reason of their getting used up while encountering a variety of inflammation injury and subsequent infections resulting due to the Coragen treatment. This decrease is possibly due to the failure or suppression or destruction of stem cells in the bone marrow, which leads to decrease in the number of leucocytes denoting marked decrease in the cellularity of bone marrow (Chandrasoma and Taylor, 1991; Uzun and Kalender, 2013).

The aqueous seed extract of *P. santalinus* normalized the haematological indices to a much greater extent. This therapeutic role may be attributed to its phytochemical constituents determining the capability of *P. santalinus* extract to repair the damaged stem cells thus leading to increased blood cell synthesis.

### Conclusion

In the present scenario, we cannot check the flow of pesticides from entering it into the human food chain but can control its deleterious effect by administration of suitable antidotes. In the present study, coragen caused severe haemato-toxicity as well as hepato-renal toxicity. But *P. santalinus* played the vital role to combat the deleterious effect of coragen. Thus, *P. santalinus* is the best antidote against coragen induced toxicity.

### Conflict of interest

The authors have no conflict of interest.

### REFERENCES


