PERTUBATIONS IN GLUTATHIONE AND ADENOSINE TRIPHOSPHATASE IN ACUTE ORAL TOXICOSIS OF CLEISTANTHUS COLLINUS: AN INDIGENOUS TOXIC PLANT

G. SARATHCHANDRA*, P. BALAKRISHNAMURTHY

*Toxicology Unit, Central University Laboratory, Madhavaram Milk Colony, Madras - 600 051 and Fredrick Institute of Plant Protection and Toxicology, Padappai.

Manuscript Received: 9-8-1996 Revised: 13-9-1996 Accepted: 23-9-1996.

SUMMARY
Objectives: Cleistanthus collinus a toxic plant, is frequently implicated in suicidal and homicidal poisoning. Its exact mode of toxicity is unravelling which is very essential to develop suitable strategy to antagonise toxicity. In consonance with the prevailing situation of collinus the present study was undertaken to characterise the precise mode of action.

Methods: C.collinus leaf extract (20% w/v) was administered at 24h LD50 dose orally to 18h starved rats (8g Kg\(^{-1}\)) and rabbits (10gKg\(^{-1}\)) which sacrificed after 180 minutes and vital organs were assayed for glutathione and ATPase.

Results: Glutathione profile revealed its depletion in various organs of rats (64.95% in liver, 51.60% in kidney, 15.60% in heart, 25.20% in brain and 27% in skeletal muscle) and in rabbits (42.60% in liver, 32.50% in kidney, 17.30% in heart, 13.50% in brain and 48.60% in skeletal muscle) as compared to that of the controls in the respective species. A similar trend of inhibition of ATPase activity was observed in the vital organs of rats (P<0.001, P<0.01) as well as in the case of rabbits (P<0.01).

Conclusion: It can be deduced from the present profile that C.collinus during its assault causes a definite depletion/inhibition of thiol/thiol containing enzymes which is responsible for the manifestation of toxicity and the present finding could pave way for the selection of thiol compounds as probable antidotes to combat C.collinus toxicosis.

KEY WORDS: Cleistanthus collinus  arylnapthalene lignan lactone   glutathione   adenosine triphosphatase

INTRODUCTION
Cleistanthus collinus (popularly known in Hindi as: Garari, Tamil: Oduvan, Telugu: Vadise, Malayalam: Nilapala) a shrub of the Euphorbiaceae family is abundant in many parts of India, Malaysia and Africa. All parts of the plant C.collinus are reported to be toxic; paste or decoction of the leaves is commonly used for poisoning humans and animals. It is also used as cattle and fish poison and also for procuring criminal abortion\(^{1}\).

It contains highly toxic arylnapthalene lignan lactone glycosides namely cleistanthin A, cleistanthin B and their genin diphyllin. Collinusin, another lignan lactone is also known to occur in the leaves. Isolation, structural elucidation and the toxicity of these compounds have been reported\(^{2-6}\).

Our earlier investigation on acute oral toxicity profile of C.collinus in rats and rabbits revealed an inhibition of certain thiol dependent enzymes namely lactate dehydrogenase and cholinesterase. there was a significant inhibition of these enzymes in tissues (liver and brain) as well as in serum samples of C.collinus intoxicated animals\(^{7}\).

The above observation prompted the probing of thiol status in C.collinus intoxicated animals, by assessing glutathione and ATPase activity which could pave way for the precise mode of action and ultimately for antidote strategy.

MATERIALS AND METHODS
Collection of leaves
Cleistanthus collinus leaves were collected from Tiruchirapalli (Thoralur), Tamil Nadu state of India during the month of April. They were sundried and aliquoted in polythene bags.

Preparation of C.collinus leaf extract
Sundried leaves of C.collinus were powdered well using a blender. An aqueous extract of the leaf powder was prepared freshly everytime prior to the
study. A 20% w/v aqueous solution of the *C. collinus* leaf extract was boiled, filtered and then concentrated to obtain 1g/ml which was used for oral administration. Extract so prepared was used for better forensic simulation.

**Animals**

Albino Wistar rats and white himalayan rabbits were obtained from Laboratory Animal Medicine Department, Tamil Nadu Veterinary and Animal Sciences University, Madras. Animals were maintained at an ambient temperature of 27°C and fed with pellet feed (rat, rabbit/Hindustan lever, Bombay, India) and tap water ad *libitum*. The animals were acclimated for 5 days and housed in separate cages. A 12 h light/dark cycle was maintained throughout the study.

**Experimental protocol**

The experimental animals were segregated into two groups per species (rats 150-200 g, 6 nos.; rabbits 1-1.25 kg, 4 nos.). Group I acted as control and group II as test group which received *C. collinus* aqueous extract at 24 h LD50 with the help of an intubation needle. 24 h LD50 dose (values mean ± SD) for *C. collinus* rats = 7.4 ± 3.5 gkg⁻¹ and rabbits = 10 ± 1.5 gkg⁻¹ were obtained from our previous experiments. For practical purposes rats were adminstered 8 gkg⁻¹ and rabbits 10 gkg⁻¹. All the controls were administered vehicle (distilled water) as per the body weight; maximum volume administered in rats did not exceed 1 ml and 3 ml for rabbits. The experiments were conducted in duplicate.

Animals were sacrificed by cervical decapitation 120 minutes after the oral administration of *C. collinus* extract. Tissue samples of brain, heart, liver, kidney and skeletal muscle were collected from all the animals (including controls), rinsed in ice cold saline, blotted on a filter paper and weighed. One set of organs were homogenised in 20 volumes of ice cold 0.15 M potassium chloride for glutathione assay while another set of organs were homogenised in 10 volumes of deionised water for ATPase estimation. Homogenisation was carried out with the help of a teflon homogeniser. The homogenates were aliquoted and frozen.

Glutathione estimation was carried out as described by Ellman with modification of David et al. ATPase was assayed following the method of Fiske and Subbarow while Lowry’s method was employed for protein estimations. Statistical analysis of the data was done by one tailed Student’s ‘t’ test. P values < 0.05 were considered significant.

**RESULTS**

During the crisis of the toxic influence of *C. collinus* in rats and rabbits glutathione was depleted significantly in various tissues (Table 1). Monitoring of ATPase including Na, K and Mg, ATPase was found to be vulnerable to *C. collinus* toxic assault (Tables 2 and 3).

**DISCUSSION**

Glutathione comprises as much as 90% of the non-protein thiol content of mammalian cells and performs a pivotal role in maintaining their metabolic and transport functions. Mammalian cells have evolved a protective mechanism to minimize injurious results that are evident from xenobiotics. A major endogenous protective system is the glutathione cycle. Glutathione acts both as nucleophilic “scavenger” of numerous compounds and their metabolites via enzymatic and chemical mechanisms, converting electrophilic centres to thioether bonds. Glutathione depletion to about 20-30% of total glutathione levels can impair cell defence against the toxic actions which may lead to cell injury and death. Further, glutathione is considered to be
Table 2. *Cleistanthus collinus* toxic effect on adenosine triphosphatase activity (micromoles/hr/mg protein) in rats.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Control</th>
<th>Toxin</th>
<th>Control</th>
<th>Toxin</th>
<th>Control</th>
<th>Toxin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>67.65 ± 1.5</td>
<td>22.68 ± 1.0**</td>
<td>60.62 ± 1.0</td>
<td>18.42 ± 0.7**</td>
<td>7.03 ± 0.9</td>
<td>4.26 ± 0.6**</td>
</tr>
<tr>
<td>Kidney</td>
<td>77.20 ± 1.0</td>
<td>38.68 ± 1.0*</td>
<td>74.87 ± 0.6</td>
<td>37.85 ± 0.9*</td>
<td>4.66 ± 0.6</td>
<td>2.27 ± 0.5*</td>
</tr>
<tr>
<td>Heart</td>
<td>23.61 ± 1.0</td>
<td>15.58</td>
<td>19.30 ± 0.9</td>
<td>14.45 ± 1.0</td>
<td>4.31 ± 0.7</td>
<td>1.13 ± 0.05**</td>
</tr>
<tr>
<td>Brain</td>
<td>36.36 ± 2.0</td>
<td>24.97 ± 1.5</td>
<td>23.02 ± 1.0</td>
<td>20.33 ± 0.9</td>
<td>13.34 ± 1.0</td>
<td>4.63 ± 0.10**</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>78.80 ± 1.5</td>
<td>14.29 ± 1.0**</td>
<td>67.66 ± 2.0</td>
<td>12.80 ± 1.01**</td>
<td>11.14 ± 0.75</td>
<td>1.49 ± 0.08**</td>
</tr>
</tbody>
</table>

Values expressed as mean ± SE (n=6). *P < 0.01, **P < 0.001 when compared to respective control.

Toxin group of rats received aqueous *C. collinus* leaf extract (9g kg⁻¹) and controls received comparable volume of vehicle only.

Table 3. Adenosine triphosphatase activity (micromoles/hr/mg protein) in acute *Cleistanthus collinus* toxicosis in rabbits.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Control</th>
<th>Toxin</th>
<th>Control</th>
<th>Toxin</th>
<th>Control</th>
<th>Toxin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>44.50 ± 1.0</td>
<td>34.75 ± 1.0</td>
<td>24.88 ± 1.0</td>
<td>21.75 ± 1.0</td>
<td>22.25 ± 2.0</td>
<td>10.88 ± 1.0</td>
</tr>
<tr>
<td>Kidney</td>
<td>81.25 ± 0.5</td>
<td>55.80 ± 0.5*</td>
<td>62.00 ± 2.0</td>
<td>46.13 ± 1.0</td>
<td>19.25 ± 1.0</td>
<td>9.75 ± 0.90*</td>
</tr>
<tr>
<td>Heart</td>
<td>42.25 ± 0.9</td>
<td>24.90 ± 0.8*</td>
<td>31.28 ± 1.0</td>
<td>15.75 ± 0.9*</td>
<td>10.98 ± 0.70</td>
<td>9.15 ± 0.80</td>
</tr>
<tr>
<td>Brain</td>
<td>44.00 ± 1.0</td>
<td>34.00 ± 0.9</td>
<td>32.88</td>
<td>25.30 ± 0.7</td>
<td>11.13 ± 0.60</td>
<td>8.70 ± 0.60</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>76.13 ± 2.0</td>
<td>51.13 ± 1.5</td>
<td>72.50 ± 2.0</td>
<td>49.40 ± 2.0</td>
<td>3.63 ± 0.05</td>
<td>17.00 ± 0.06*</td>
</tr>
</tbody>
</table>

Values expressed as mean±SE (n=4). *P < 0.01 when compared to respective control.

Toxin group of rabbits administered *C. collinus* aqueous extract (10g kg⁻¹) and controls received a comparable volume of vehicle only.

A crucial factor in maintaining the structural integrity of cell membranes largely through reactions that protect membranes against free radical formation. Glutathione and glutathione dependent enzyme systems are known to provide major protection against toxic agents produced by xenobiotics.

Macromolecules that are involved in membrane transport process and in maintaining structural activity are ATPases. The loss of ATPase activity has been advocated to result from the oxidation of -SH (thiol) groups.

In the present study, acute lethal dose of *C. collinus* produced varied but significant depletion of glutathione and ATPases in all the five tissues (liver, kidney, skeletal muscle, brain and heart), suggesting the involvement of glutathione mediated detoxification mechanism in various tissues of rats and rabbits.

In our earlier study on acute toxicity profile of *C. collinus* in rats and rabbits, there was a significant decrease in the activity of lactate dehydrogenase and cholinesterase along with elevated levels of transaminase activity in various tissues. Histopathological lesions included degenerative changes like focal hepatic necrosis, glomerular degeneration and cerebral gliosis. These findings could be correlated with glutathione depletion and ATPase inhibition observed in the present profile.

Similar interaction has been documented in toxic fungal lactone, penicillic acid for its inhibitory action of ATPase and concomitant reduction in the amount of free reactive sulphydryl.

Studies on the structure activity relationship with respect to the toxic action of sesquiterpene lactone (phytoxin) on a number of biological systems have revealed that its biological activity (lactones) could
be related to the presence of functional groups that could form covalent bonds with critical biological nucleophiles. It has been well established that these lactones can efficiently alkylate thiols and amines in the biological system. Thus the toxic action of these lactones could be ascribed to general cytotoxic effects on a number of cellular, tissue and organ systems. The toxicity of \textit{C. collinus} leaves has been attributed to the presence of highly toxic arylamphathalene lignan lactones namely cleistanthin A and cleistanthin B. The chain of biochemical perturbation observed in \textit{C. collinus} toxicosis could be attributed to the lignan lactone interaction with thiols as seen in sesquiterpene lactone poisoned animals.

Taking into holistic view of the crisis induced in \textit{C. collinus} poisoning, it is possible to characterise the toxicity to depletion/inhibition of thiol/thiol dependent enzymes. For the first time, the systematic characterisation of the most probable mode of action of \textit{C. collinus} has been reported to involve inhibition of specific thiol status. This finding could pave the way for the selection of antidote strategy to combat \textit{C. collinus} poisoning.

REFERENCES


