CHAPTER 6

ANTIDOTE TO CLEISTANTHUS COLLINUS POISONING
INTRODUCTION

An efficient agent to combat the toxicity of *C. collinus* is yet to be made known. Management of emergency cases of poisoning due to *C. collinus* is only symptomatic. Death is reported to occur in humans in a day or two; sometimes within two hours, if the ingestion is large. Mode of action has been obscure.

Our studies on the biological effects following oral/iv administration of *C. collinus* in rats and rabbits revealed that certain key enzymes are inhibited even in sub-acute dose. Histological examination of various organs following lethal dose of *C. collinus* extract showed stomach to be the primary target organ where severe reaction in the gastric mucosa was observed.

It became evident on going through the literature that enzymes like LDH are susceptible to sulfhydryl inhibitors and a number of compounds are known to belong to this class. Earlier workers have showed that compounds rich in thiol content are capable of neutralizing the inhibitory action. Hence in the present study the possible role of a thiol containing compound, cysteine as antidote to *C. collinus* poisoning.
was explored, incidentally explaining the mechanism of poisoning.

**MATERIALS and METHODS**

Swiss albino rats (130-150 g) were given food and water *ad libitum* till 16 h prior to experiment. Rats (60 nos) were divided into three groups and were given orally lethal dose (10.5 g/kg) of *C. collinus* extract. The first group of rats (20 nos) at the end of 2 h received orally cysteine (500 mg/kg; aqueous solution); the second group (20 nos) at the same period received cysteine (500 mg/kg) intraperitoneally; and the third group (20 nos) received simultaneously equal volume of water (1 ml) instead of cysteine. A separate group of animals (6 nos each) received cysteine alone (500 mg/kg) orally or intraperitoneally. Another set that received water alone served as control.

The different groups of animals were kept in separate cages and mortality rate observed. Animals that died were processed for histological examination of various organs. In the case of animals that survived following cysteine treatment, peripheral blood was drawn.
after 24 h and the serum enzyme activities were estimated following standard procedures reported in Chapter 4.

RESULTS and DISCUSSION

Table I lists the observed mortality rate in cysteine-treated and untreated animals following oral administration of lethal dose of *C. collinus*. The effect of varying the dose of cysteine administered is provided in Table II. The serum activity of lactate dehydrogenase and alkaline phosphatase in cysteine-treated animals is provided in Table III. Histological pictures of stomach of cysteine-treated and untreated rat poisoned with *C. collinus* extract are compared in Figures la-lf.

As provided in Table I, animals that received treatment with cysteine showed high survival rate. Oral administration was found to be more effective in neutralizing the toxic effects of *C. collinus* than intraperitoneal route.

As shown in Table II increasing the dose of cysteine above 500 mg/kg virtually had no extra effect on the survival rate. It was also found that treatment
TABLE I

EFFECT OF TREATMENT WITH CYSTEINE IN CLEISTANTHUS COLLINUS POISONING

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of animals used</th>
<th>Number of animals survived</th>
<th>Mortality rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. collinus</td>
<td>20</td>
<td>10</td>
<td>50</td>
</tr>
<tr>
<td>C. collinus + cysteine (oral)</td>
<td>20</td>
<td>18</td>
<td>10</td>
</tr>
<tr>
<td>C. collinus + cysteine (intraperitoneal)</td>
<td>20</td>
<td>11</td>
<td>45</td>
</tr>
<tr>
<td>Administered cysteine alone (oral)</td>
<td>6</td>
<td>6</td>
<td>Nil</td>
</tr>
<tr>
<td>Administered cysteine alone (intraperitoneal)</td>
<td>6</td>
<td>6</td>
<td>Nil</td>
</tr>
</tbody>
</table>

Rats belonging to the first three groups received orally 10.5 g/kg of C. collinus extract. Second and third group of animals received subsequently treatment with cysteine (500 mg/kg) at the end of 2 h.
TABLE II

EFFECT OF DOSE OF CYSTEINE ADMINISTERED AFTER TWO HOURS OF CLEISTANTHUS COLLINUS POISONING

<table>
<thead>
<tr>
<th>Amount of cysteine given (mg/kg)</th>
<th>Number of animals used</th>
<th>Mortality rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>10</td>
<td>40</td>
</tr>
<tr>
<td>500</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>1000</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

Rats were given orally 10.5 g/kg of C. collinus extract and treated with cysteine (oral) at the end of 2 h.
TABLE III

SERUM ENZYME ACTIVITY OF RATS (POISONED WITH CLEISTANTHUS COLLINUS) TREATED WITH CYSTEINE

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Enzyme activity, IU per litre</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Lactate dehydrogenase</td>
<td>462±8.6</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>115±9.2</td>
</tr>
</tbody>
</table>

* Rats were given orally 10.5 g/kg of C. collinus extract and received at the end of 2 hours, treatment with cysteine orally. Enzyme assays were carried out after 24 h in the case of survivals. Control group received only water. Values are average of six experiments ± SD.
The glandular mucosa resting on muscularis mucosa, the submucosa containing vessels, lymphatics, loose fibrous tissue, lymphoid cells etc and the outer muscle coat are shown (vide Chapter 4).

Squamoid metaplasia occurring with still some glandular mucosa persisting like an erosion—?epidermidization (vide Chapter 4)
FIGURE 1c
SECTION OF STOMACH OF RAT ADMINISTERED ACUTE DOSE OF *CLEISTANTHUS COLLINUS* EXTRACT

(Haematoxylin-eosin x 100)

A different region of stomach is shown. The entire submucosa is cellular with a necrotic mucosa catarrh presenting hyperkeratosis (vide Chapter 4).

FIGURE 1d
SECTION OF STOMACH OF RAT ADMINISTERED ACUTE DOSE OF *CLEISTANTHUS COLLINUS* EXTRACT

(Haematoxylin-eosin x 100)

A different field showing a lymphomatous like aggregation in the submucosa (vide Chapter 4).
FIGURE 1e

SECTION OF STOMACH OF RAT (POISONED WITH CLEISTANTHUS COLLINUS) TREATED WITH CYSTEINE ORALLY

(Hematoxylin-eosin x 100)

The sub mucosa zone is free from any cellular infiltrate. The mucosa is almost normal in appearance.

FIGURE 1f

SECTION OF STOMACH OF RAT (POISONED WITH CLEISTANTHUS COLLINUS) TREATED WITH CYSTEINE ORALLY

(Hematoxylin-eosin x 100)

A different field showing substantia propria, the sub mucosa etc that are free from any cellular infiltrate. The mucosa is normal, also the muscle coat.
with cysteine to \textit{C. collinus} poisoned rats had maximum effect up to two hours but not beyond.

Cysteine treatment (after \textit{C. collinus} poisoning) resulted in restoring the serum activity of LDH and alkaline phosphatase comparable to the control (Table III). However no significant change was noted for transaminases.

Few toxic agents exert their toxic action by interrupting the activity of specific enzymes. The poisoning of any single enzyme involved in a main metabolic chain will render the whole chain inoperative and will have a profound or even fatal effect upon the organism. Such an inactivation of an essential enzyme has been called a 'biochemical lesion'. The most obvious example is the toxicity of cyanide which is primarily due to inhibition of cytochrome oxidase resulting in cessation of aerobic oxidation process and thus causing death in few minutes (1).

In certain instances instead of a single specific enzyme, involvement of multiple enzyme systems is encountered, the well-known example being the sulf-hydryl inhibitors (2). Many compounds like fungal
lactones patulin and penicillic acid (3) and metals like arsenic, mercury and copper (4) are known to interact with the -SH group of the enzymes either by alkylation, mercaptide formation or oxidation.

Thiol group (-SH) is extremely reactive and many enzymes have been shown to require -SH group for their activity. Enzymes of importance in the metabolism of carbohydrates, fats and amino acids require essentially the -SH group for carrying out their functional activity. The role of -SH groups in thiol enzymes is twofold. In oxidation-reduction enzymes, they act as electron mediators between substrate and coenzyme (NAD or flavin); in the hydrolytic and transfer enzymes, they act as links between protein and metal or between protein and ATP. Thiol group (soluble as well as fixed, attached to the side chain of the proteins) possesses important function in the performance of the processes leading to cell division and cell growth. Actomyosin formation and perhaps muscle contraction and fibrin formation in blood coagulation require fixed -SH group (5).

The inhibitory action of mycotoxins patulin and penicillic acid is attributed to the presence of
lactone ring with \( \alpha, \beta \) unsaturation that interacts with the -SH and possibly -NH\(_2\) group of the enzymes either by addition or substitution reactions (3,6,7).

It is also known that compounds like cysteine, glutathione and thioglycollic acid neutralize the -SH inhibitory action (4,8). The antidote effect of cysteine and its derivatives is well-known (8,9).

Cysteine is also known to prevent teratogenic effects of thiram (10). Though many thiol containing compounds are known to be effective, cysteine is preferred due to its high LD\(_{50}\) values (9).

As described in Chapter 4 studies on biological alteration in _C. collinus_ toxicity showed that certain enzymes are inhibited, probably through interaction with the enzymes. This action of _C. collinus_ was further confirmed in a separate study (Damodaran et al, unpublished data) which showed that activity of acetylcholinesterase of brain, adenosine triphosphatase (total as well as Na\(^+\) - K\(^+\) ion dependent) of liver, kidney, heart and brain, creatine kinase of liver, muscle and brain is inhibited significantly in rabbits following _C. collinus_ administration. These enzymes like LDH are known to
be inhibited by sulfhydryl inhibitors (11-14).

The inhibitory action of _C. collinus_ can be attributed to the presence of carbonyl group in the lactone ring of its active constituents. This is supported by the observation that serum activity of lactate dehydrogenase in _C. collinus_ poisoned animals treated with cysteine was comparable to the normal value.

In such cysteine-treated experimental animals, restoration of serum alkaline phosphatase activity was also noted. An explanation for this appears obscure. This is because cysteine is known to inhibit alkaline phosphatase (15); whereas in the present situation cysteine has blocked the inhibition (by _C. collinus_) of the enzyme. It is hence to be presumed that the mode of action of _C. collinus_ to cause inhibition of this enzyme and that by which cysteine blocks the inhibition are in different perspectives.

The insignificant effect of cysteine treatment on the inhibition of transaminases may however be
constituents with the amino group of the enzymes. It may be noted here that similar explanation is offered in the toxicity of patulin which inhibits many thiol containing enzymes including LDH, aldolase and alcohol dehydrogenase; however in that case cysteine blocks the inhibition of LDH but not of aldolase and alcohol dehydrogenase which observation has been attributed to the interaction of patulin with amino group of the enzymes (8).

The antidotal effect of cysteine is further illustrated from the histological pictures also. The near normal restoration of the stomach is evident (Figure 1e and 1f).

From the preceding discussion it appears that death in C. collinus poisoning is very likely due to the combined effect of (a) irritant action on stomach and (b) inhibition of enzymes. It is interesting to note that patulin, a fungal lactone also acts in a similar way-inhibiting the enzyme activity as well as producing gross and microscopic lesions in stomach (16). Amaryllis
Abrus, Ricinus and water hemlock are also known to produce irritant effect (17).

It is now evident that cysteine as an antidote reduces greatly the mortality rate and prevents considerably the biochemical and histological lesions due to C. collinus. The use of cysteine (oral) as an antidote is thus recommended in emergency cases of poisoning due to C. collinus.

CONCLUSION

Irritant action on stomach and inhibition of certain key enzymes appear to be the most probable mode of action of C. collinus. Oral administration of cysteine (500 mg/kg) is recommended as antidote to C. collinus poisoning.
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