Toxic Effects of the Pericarp of the Enterolobium contortisiliquum (Vell.) Morong Fruit on Chicks

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ABSTRACT
The aim of this study was to determine whether the pericarp of the Enterolobium contortisiliquum fruit, which contain saponins, may cause damage to the gastrointestinal tract and liver of Gallus gallus domesticus chicks. Chicks were fed the seedless pods of E. contortisiliquum at concentrations of 0% (control), 5%, or 10% of their total daily food intake for 5 consecutive days. On day 6, the animals underwent pathological examination. Necropsy revealed that all birds fed with the seedless pods of E. contortisiliquum at a concentration of 10% showed congested livers and enlarged ceca. Lesions of the digestive tract were characterized by necrosis of the epithelium of intestinal crypts, villous atrophy, and mononuclear inflammatory infiltrate in the lamina propria. In the liver, apparent and diffuse hepatocellular necrosis was observed along with disarray of the hepatic architecture and sinus congestion. The results of this study revealed that chicks provide a good experimental model for evaluating the toxic effects of E. contortisiliquum pods. The observed effects may be attributed to the presence of triterpenic saponins in the pericarp of broad beans.

INTRODUCTION
Enterolobium contortisiliquum (Vell) Mor is a plant of the Fabaceae-Mimosoideae (Leguminosae-Mimosoideae) family, commonly known as the pacara earpod tree (timbaúba, timbaúva, ximbaúva, timburi, orelha-de-negro, orelha-de-macaco, tamboril, and...
The tree can reach up to 30 m in height, is widely distributed across South America, being found in Uruguay, Argentina, Brazil, and Venezuela.\(^1\) Natural cases of cattle poisoning by \textit{E contortisiliquum} have been reported.\(^2-4\) Suspected toxicity of the broad beans has also been reported in two goats.\(^5\) Clinical signs of this toxicity include anorexia, depression, hepatogenous photosensitization, and miscarriages.\(^2-4\) The experimental replication of cattle poisoning by broad beans resulted in photosensitization in some studies,\(^3\) but only in severe diarrhea in other experiments conducted in both cattle,\(^2,4,6,7\) and sheep.\(^8\) The abortive activity of the broad beans was experimentally replicated in guinea pigs only.\(^9\)

The toxic compound of \textit{E contortisiliquum} broad beans has not yet been identified. A compound named enterolobin was isolated from its seeds, and some authors consider this the responsible component for the plant’s toxic activity.\(^1\) Enterolobin is a protein with a molecular weight of 51.3 to 59.8 kDa, depending on the extraction process, and is responsible for hemolytic,\(^10\) cytolytic,\(^11,12\) and pro-inflammatory activity.\(^13\)

Several other compounds have been isolated from \textit{E contortisiliquum} seeds, including a Kunitz-type trypsin inhibitor with two polypeptide chains,\(^14,15\) a 60-kDa endopeptidase that is not inhibited by the trypsin inhibitor or by cysteine proteases,\(^16\) a 23-kDa serine protease inhibitor which inhibits trypsin, chymotrypsin, and plasma kallikrein,\(^17\) a 60-kDa thiol protease inhibitor, which inactivates papain and bromelain,\(^17,18\) and a 35-kDa protein with arylamidase activity.\(^19\) Moreover, the seeds also show phospholipase D activity.\(^10\) The phytochemical analysis of the pericarps of the \textit{E contortisiliquum} fruit, on the other hand, revealed the presence of several triterpenic saponins, including 3β-O-β-D-glucopyranosyl-21β-E-cinamoiloxiolean-12-en-28-oic acid,\(20\) enterolosaponins A and B,\(21\) and contortisiliosides A-G.\(22\)

The aim of this study was to determine whether the pericarp of the \textit{E contortisiliquum} fruit, which contain saponins, might cause damage to the gastrointestinal tract and liver, and whether \textit{Gallus gallus domesticus} (Linnaeus, 1758) chicks can be used as an experimental model for the evaluation of this toxicity.

**MATERIALS AND METHODS**

Ripe \textit{Enterolobium contortisiliquum} (Vell.) Morong fruits were collected from five trees present in the Toxic Plant Bed at the Veterinary School of the UFMG, in Belo Horizonte, MG. The fruits were cleaned and cut open with knives specific for seed removal. Following drying, the seedless pods were ground. Saponin concentration in the pods was assayed using a spectrophotometric technique,\(^23\) and using Saponin from quililaja bark (Sigma-Aldrich, Saint Louis, MO, USA) as a standard. Saponin concentration was determined to be 21.9% in the ground pods used in this study.

The ground pods of \textit{E contortisiliquum} were mixed into the feed (Bichos & Sitios, IRCA Nutrição e Avicultura S.A., Carpina, PE). Eighteen (18) chicks were randomly

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**Table 1: Body weight, weight gain, and feed intake by chicks fed with 0% (control), 5%, and 10% seedless \textit{E contortisiliquum} fruit in the feed.**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>0%</th>
<th>5%</th>
<th>10%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (day 1), g</td>
<td>106.3 ± 6.82</td>
<td>110.0 ± 8.37</td>
<td>105.5 ± 4.80</td>
</tr>
<tr>
<td>Weight (day 5), g</td>
<td>152.7 ± 9.75</td>
<td>152.3 ± 9.08</td>
<td>147.5 ± 4.92</td>
</tr>
<tr>
<td>Weight gain, g</td>
<td>46.3 ± 4.93</td>
<td>42.3 ± 3.07</td>
<td>42.0 ± 3.62</td>
</tr>
<tr>
<td>Feed intake (group), g</td>
<td>1402</td>
<td>1016</td>
<td>707</td>
</tr>
</tbody>
</table>
distributed across three groups of six animals each, and were fed the *E contortisiliquum* pods at concentrations of 0% (control), 5%, and 10% total daily dietary intake. The experimental feeds were supplied for 5 consecutive days. Throughout the experimental period, water and feed were supplied ad libitum. On day six, the animals were euthanized by cervical dislocation. Each bird underwent necroscopic evaluation, and fragments were collected from the liver, heart, spleen, kidneys, proventriculus, gizzard, cecum, rectum, and brain for evaluation by microscopy. The collected fragments were fixed in formalin for subsequent routine histological processing and staining with hematoxylin and eosin (H&E).

Animal weight and weight gain results are shown as the mean plus the standard error (SEM). The comparisons between the groups were performed using analysis of variance (ANOVA), followed by Tukey’s test with a preset statistical significance level of P<0.05. Analysis of the data was carried out with the help of R software, version 3.0.0.

**RESULTS**

The body weight, weight gain, and feed intake are shown in Table 1. There was no statistically significant difference in body weight and weight gain between the birds of the different groups. On the other hand, feed intake was lower in the groups treated with *E contortisiliquum* than in the control group.

Throughout the experimental period, no birds showed clinical signs of toxicity, except for increased water intake demonstrated by the animals fed with the plant pods at either concentration.

Necropsy revealed that all birds fed with *E contortisiliquum* at a concentration of 10% showed congested livers and enlarged ceca. None of the animals from the control group or those fed with *E contortisiliquum* at a concentration of 5% showed macroscopic lesions.

Microscopic evaluation of the livers of birds that received *E contortisiliquum* (at either concentration) revealed apparent and diffuse hepatocellular necrosis with hepatocyte strand breaks, disarray of the hepatic architecture, and sinus congestion (Fig 1).

**Figure 1:** Liver of chick that received feed with 10% seedless *E. contortisiliquum* pods, showing apparent and diffuse hepatocellular necrosis and sinus congestion. H&E staining, 40× magnification.

**Figure 2:** Large intestine of chick that received feed containing 10% pericarp of *E. contortisiliquum* pods, showing necrosis of the epithelium of the crypts and villous atrophy. H&E staining, 10× magnification.
These lesions were more severe in birds that received 10% pods in the feed than in those that received 5% pods. During microscopic evaluation of the digestive tract, necrosis of the epithelium of the intestinal crypts and villous atrophy was observed (Fig 2). These lesions were accompanied by mononuclear inflammatory infiltrate in the lamina propria. Lesions in the digestive tract were moderate in birds that received 10% *E. contortisiliquum* and mild in those that received 5%. No lesion was observed in any other organs.

**DISCUSSION**

The main factor influencing intake of the broad beans in large quantities is food scarcity, since apparently the beans have low palatability and animals will ingest them only when they are hungry. In this study, feed intake was lower when the pod bran was added to the feed. This observation strengthens the theory that broad beans have low palatability.

The main site for lesions caused by *E. contortisiliquum* is the digestive tract. These lesions were characterized by necrosis of the epithelium of intestinal crypts, villous atrophy, and mononuclear inflammatory infiltrate in the lamina propria. Similar lesions in the digestive tract have also been found in both natural and experimental cases in cattle, and experimentally in sheep and guinea pigs.

The liver is an important target organ for lesions caused by *E. contortisiliquum*. Experimental replication of pod poisoning in cattle resulted in areas of necrosis induced by coagulation of hepatocytes. Microscopic analysis of the liver in experiments conducted with guinea pigs, revealed periportal hemorrhagic necrosis induced by ingestion of broad beans. In this study, chicks showed apparent and diffuse hepato-cellular necrosis with disarray of the hepatic architecture and sinus congestion.

Enterolobin is a protein isolated from *E. contortisiliquum* with hemolytic, cytolytic, and pro-inflammatory activity, which some authors consider to be responsible for the plant’s toxic activity. Another compound isolated from this plant is a Kunitz-type trypsin inhibitor, which combines with the active sites of bovine trypsin and could, therefore, potentially be responsible for the effects on the digestive tract in poisoned animals. However, since enterolobin, the trypsin inhibitor, and other compounds with enzymatic activity are present only in the seeds of *E. contortisiliquum*, the occurrence of lesions caused by the pericarp of the fruit demonstrates that the toxic ingredient is likely the triterpenic saponins. Furthermore, at least a dozen different triterpenic saponins have been isolated and identified in the pericarps of pods of *E. contortisiliquum*.

Saponins are glycosides often found in plants, and are known to be responsible for causing digestive and hepatic lesions in several different species. According to their chemical structure, they are classified as steroidal or triterpenic saponins. Photosensitizing plants that contain saponins include *Panicum* spp, *Brachiaria* spp, *Tribulus terrestris*, *Narthecium ossifragum*, *Agave techeguilla*, and *Nolina texana*. In all of these plants, the saponins are steroidal. Only one experimental study was able to replicate the photosensitization effect after administration of *E. contortisiliquum*, whereas several other studies were unsuccessful. It is possible that significant variation exists in the saponin composition of the pods, and they may even contain other unidentified steroidal saponins.

The results of this study revealed that chicks could serve as a good experimental model for studying toxicity induced by *E. contortisiliquum* pods. The observed toxic effects may likely be attributed to the presence of triterpenic saponins in the pericarp of broad beans.

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REFERENCES


