Effect of saponin on mortality and histopathological changes in mice

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ABSTRACT We evaluated the acute toxicity and histopathological effects of saponin (extracted from the plant Citrullus colocynthis) on mice in order to assess its safety. The median lethal dose (LD50) of the saponin was 200 mg/kg. The histological changes were confined to the small intestine, liver and kidney, whereas the stomach, large intestine and heart appeared normal. The changes in the small intestine included haemorrhage and erosion of the mucosa. In addition, hepatorenal damage resulted from necrosis of liver cells and renal tubules.

Effet de la saponine sur la mortalité et changements histopathologiques chez les souris RESUME Nous avons évalué la toxicité aiguë et les effets histopathologiques de la saponine (extraite de la plante Citrullus colocynthis) sur les souris afin d’évaluer son innocuité. La dose létale médiane (DL50) de la saponine était 200 mg/kg. Les changements histologiques étaient confinés à l’intestin grêle, au foie et aux reins tandis que l’estomac, le gros intestin et le coeur apparaissaient normaux. Les changements survenus dans l’intestin grêle comprenaient l’hémorragie et l’érosion de la muqueuse. De plus, les atteintes hépato-rénale étaient dues à la nécrose des cellules hépatiques et des tubules rénaux.

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Introduction

Colocynth (Citrullus colocynthis) is a well known medicinal plant that grows naturally in the western Iraqi desert and in many other tropical and subtropical countries [1]. Its fruit has been recommended for indigestion and diabetic people in traditional medicine. Cases of acute toxic colitis after ingestion of colocynth have been reported [2,3]. Toxicity studies on small ruminants suggest that the fruit causes organ damage in the liver, kidney and gastrointestinal tract [4,5]. According to Wasfi, a dose of 800 mg/kg of the ethanolic extract of the leaves killed 60% of the treated rats [6]. Pharmacological examination of the surviving animals and histopathological observations suggested hepato renal damage.

In a study of our third author, saponin, purified from the rind extract of the colocynth fruit, proved to have antidiabetic effect on rabbits and mice [6]. Therefore, we attempted to evaluate its acute toxicity and histopathological effects in order to assess its safety.

Materials and methods

Plant material

Citrullus colocynthis plants were collected in October 1997 from the Albarjesia desert, west of Basra. The plant was kindly authenticated by Professor A.A. Alwan and a dry voucher specimen was deposited in the Herbarium of Basra, Department of Biology, College of Science, University of Basra.

The saponin was isolated according to a method described by Otsuka et al. [7]. In brief, the rind (epicarp and outermost layer of mesocarp) of the fruit was sun-dried, powdered and defatted in a Soxhlet with petroleum ether at 40-60 °C for 16 hours. The residue was added to absolute methanol and left overnight under reflux at 70 °C. It was then filtered and the filtrate evaporated to dryness. The yield was dissolved in distilled water, extracted in a separatory funnel with 1-butanol three times and dried by evaporation. Finally, the extract was dissolved in absolute methanol and saponin compounds were precipitated by adding ether. A yellowish-brown dry powder of pure saponin was collected.

Animals

Adult BALB/c mice (3 months old), of both sexes and weighing 28–30 g, were used in this study. They were supplied by the Animal House, Department of Biology, College of Science, University of Basra. They were kept under controlled conditions of temperature (20–30 °C), humidity (50%) and light (12:12-hour light–dark cycle). They were fed with pellets (supplied by the Animal House) and allowed to drink tap water ad libitum. All mice were apparently healthy. An acclimatization period of 7 days was allowed before experimentation.

Acute toxicity

We used 70 mice divided into 7 groups of 10 animals, 5 males and 5 females each. Groups 1–6 received 0.5 mL of saponin solution containing 50, 100, 150, 250, 350 and 600 mg/kg respectively. Group 7 was used as a control and received 0.5 mL of saline solution. The different doses were given by forced administration with a buccogastric cannula. The animals were kept under observation for 48 hours after dosing to check for symptoms, behavioural changes and death.

Histology

The experimental animals were dissected immediately after death and their stomachs, small and large intestines, liver, heart and
Table 1 Numbers of the mice that showed symptoms after different doses of saponin

<table>
<thead>
<tr>
<th>Symptom</th>
<th>50</th>
<th>100</th>
<th>150</th>
<th>250</th>
<th>350</th>
<th>600</th>
<th>0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anorexia</td>
<td>–</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>6</td>
<td>8</td>
<td>–</td>
</tr>
<tr>
<td>Abnormal gait</td>
<td>–</td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>5</td>
<td>9</td>
<td>–</td>
</tr>
<tr>
<td>Twitches</td>
<td>–</td>
<td>1</td>
<td>3</td>
<td>5</td>
<td>7</td>
<td>9</td>
<td>–</td>
</tr>
<tr>
<td>Bilharophtooci</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Reduced activity</td>
<td>–</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>10</td>
<td>–</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>–</td>
<td>1</td>
<td>3</td>
<td>6</td>
<td>8</td>
<td>10</td>
<td>–</td>
</tr>
</tbody>
</table>

There were 10 animals per dosage

Results

The symptoms and behavioural changes of the experimental animals during the observation period (48 hours) are shown in Table 1. The mortality caused by the different doses of saponin is shown in Table 2. Beside direct observation, comparison between Tables 1 and 2 shows that severe diarrhoea was the most serious symptom; after developing it, all the experimental animals died. The animals that survived had some symptoms, including mild diarrhoea, but were able to recover. There were no differences between the males and females regarding the symptoms and mortality. Mice mortality after different doses of saponin was plotted against probability values [8]. The median lethal dose (LD₅₀) of the saponin was found to be 200 mg/kg body weight.

The histopathological changes were confined to the small intestine, liver and kidneys, while the large intestine, stomach and heart appeared normal except for the presence of saponin residue. The changes were found to correlate with the increased dose of saponin and duration of exposure before death. The changes in the animals

kidneys were excised. The control animals were killed after 48 hours by decapitation and their organs were similarly removed. The organs were cut in appropriate sizes and fixed in 10% buffered formalin for at least 2 days. They were embedded in paraffin, cut in 5-μm-thick sections and stained with haematoxylin and eosin (H&E). The sections were examined and photographed by a phase contrast microscope (Olympus).
administered lower doses and those administered higher doses but which died early were either mild or completely absent. Focal or more extensive severe changes were observed in animals administered higher doses which died later.

Traces of saponin were found in only a few places of the submucosa of the stomach and large intestine and in heart cavities. In contrast, larger amounts of saponin appeared in the lumen and submucosa of the small intestine (Figures 1 and 2). Sections of the small intestine (Figure 3) showed acute erosion of the superficial or middle parts of some intestinal villi. Haemorrhage was evident in these parts and inside the lamina propria. Liver sections (Figures 4 and 5) showed small haemorrhage in many lobules and congestion of central veins and liver sinusoids. Moreover, destruction of the liver architecture due to the necrosis of liver cells was also seen. In addition to exudation and haemorrhage in the glomeruli, the kidney section (Figures 6 and 7) showed focal destruction of the renal tubules.

Discussion

The symptoms, histopathological changes and death of experimental mice shown in this study were obviously due to acute toxicity caused by the oral administration of saponin. Colocynth, from which the saponin was isolated, has been known to be a strong laxative since antiquity. According to Soulier, 0.6–1.0 g/day of colocynth extract constitutes an overdose capable of inducing bloody diarrhoea, while 2.4 g per day could be fatal [9].

The experimental animals could be classified into three categories: 1) animals that

Figure 1 Saponin residue in the lumen of the small intestine (H&E × 200)

Figure 2 Saponin in the submucosa of the small intestine (H&E × 200)
Figure 3 Section showing erosion in the mucosa of an intestinal villus and haemorrhage in the lamina propria (H&E × 400)

Figure 4 Section of liver showing blood congestion (H&E × 200)

Figure 5 Higher magnification showing necrosis of liver cells (H&E × 2000)

Figure 6 Section of kidney showing haemorrhage and destruction of renal tubules (H&E × 300)
died early within the first few hours of ingestion; 2) animals that died later within the observation period (up to 48 hours); 3) animals that exhibited mild symptoms but were able to survive. Possible causes of death are: heart failure, acute hypoglycaemia and hepatorenal damage. The immediate cause of death of animals that died early was probably heart failure. According to Kingsbury, at toxic levels, cardioactive glycosides produce cardiac irregularities and heart block [10]. Furthermore, in many lethal poisonings, heart failure can be brought about by malfunction of innervation or of the heart’s conductive tissues, or it may be a result of a more direct effect on the heart musculature. As time after ingestion goes on, the saponin has enough time to exert its action and death is likely to be due to acute hypoglycaemia rather than heart failure. This probably explains the death of the animals that died later, since hypoglycaemia was confirmed in these animals by blood test [5]. Hepatorenal damage, seen in the animals that died later, is another possible cause of death, although apparently overshadowed by the acute hypoglycaemia. The animals that survived escaped heart failure and acute hypoglycaemia and were also able to overcome hepatorenal damage by regeneration. Hepatorenal damage in these latter animals was confirmed by pharmacological and histopathological observations [7].

The histopathological changes observed in the small intestine in our study were generally similar to those described for acute toxic colitis after ingestion of colocynth in humans [2,3], goats and calves [4] and sheep [5]. The changes in the small intestine, liver and kidney were also similar to those described by Wasfi in rats treated with leaves and seed extracts of the plant [7]. We, however, investigated the effect of purified saponin.

In contrast to other organs and despite the malfunction mentioned above, the heart did not show any histological abnormalities. According to Kingsbury, once a toxin is in the blood, all organs are exposed to its effect unless a membrane barrier intervenes [10]. This may be correlated with the fact that saponin passes rapidly with blood through heart cavities, which are well protected by the endocardium, in contrast to its relative delay in the infiltrative tissues of the liver and kidney and the absorbent mucosa of the small intestine. The main absorption of saponin by the small intestine and its delay there probably reduce the possibility of any damage to the stomach and large intestine.

A further study on the effect of therapeutic doses of saponin on different organs is currently under way.
References


