Sanguinarine: its potential as a liver toxic alkaloid present in the seeds of *Argemone mexicana*

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Summary. The alkaloid sanguinarine reported to be responsible for several outbreaks of epidemic dropsy in the tropics was examined for its hepatotoxic potential in rats. The studies showed that a single i.p. dose (10 mg/kg) of sanguinarine not only increased the activity of SGPT and SGOT substantially but also caused a significant loss of microsomal cytochrome P-450 and benzphetamine N-demethylase activity. Furthermore, the treated rats exhibited considerable loss of body and liver weight, peritoneal edema and slightly enlarged livers with fibrinous material. Microscopic examination of the liver tissue showed progressive cellular degeneration and necrosis further substantiating that sanguinarine is a potential hepatotoxic alkaloid.
Key words. Argemone mexicana; sanguinarine; hepatotoxicity; epidemic dropsy.

Sanguinarine (fig. 1) is an alkaloid predominantly found in the seeds of the plant *Argemone mexicana,* which are common contaminants of grains and mustard seeds in the tropics[1]. The toxic alkaloid as a contaminant of mustard oil and grains has been reported to cause numerous outbreaks of human poisoning known as epidemic dropsy which is characterized by edema of the legs, congestive heart failure, hepatomegaly, ataxia and glaucoma[2]. Biochemically, snaguinarine has been shown to block oxidation of pyruvate and to inhibit Na-+K-ATPase[3]. Further, it produces type II binding spectrum with rat liver microsomes[4] and prolongs pentobarbital sleeping time in male rats[5]. Tandon et al.[1] suspected sanguinarine to be injurious to liver, but evidence to support the speculation is insufficient. This prompted us to carry out the following studies.

Materials and methods. Sanguinarine (as sanguinarine nitrate) was purchased from Aldrich Chemical Corp, Milwaukee, WI, USA. Male Sprague-Dawley rats (Southern Animal Farms, Prattville, AL, USA) weighing 200–250 g were used in these studies. They were housed in a temperature- and light-controlled room with free access to water and Purina rat chow. Sanguinarine (10 mg/kg dose) dissolved in distilled water was administered i.p to each animal in a group of five. The respective control group was similarly given distilled water alone. The animals were sacrificed by decapitation at 24 h following the treatment and blood samples were collected to prepare serum for the determination of the activities of serum glutamic oxalacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT). Activities of these enzymes were determined using kits purchased from Sigma Chemical Co. (St. Louis, MO, USA). Immediately after decapitation, livers of the animals were collected, perfused and used for the isolation of microsomes following the published procedure[6]. Benzphetamine N-demethylase activity and cytochrome P-450 concentration in the microsomes were also determined using procedures reported previously[6]. Protein in the microsomes was estimated by the Biuret method modified to include deoxycholate[7].

In a similar experiment, control and treated animals were sacrificed 24 h post-treatment, their livers weighed and subsequently fixed in 10% buffered formalin for 48 h. The tissues were dehydrated through an alcohol series, embedded in paraffin, sectioned and stained with hematoxyline and eosin. The tissue sections were examined microscopically for pathologic lesions by a veterinary pathologist (Dr Y. Cho).

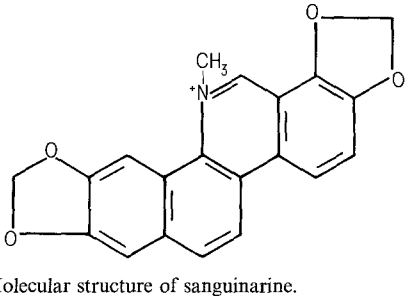

Figure 1. Molecular structure of sanguinarine.

Results and discussion. The effect of a single dose of sanguinarine (10 mg/kg, i.p.) on body and liver weight is shown in table 1. 24 h after sanguinarine administration poisoned animals lost weight markedly as compared to the control rats. Similarly, the treated animals exhibited a significant decrease in liver weights although the livers appeared to be slightly enlarged with fibrinous material on the surface. Endothoracic cavity and livers of the control rats were normal. By contrast, there was a clear incidence of ascites and indication of hepatitis in the treated rats providing evidence of toxic liver injury. However, these experimental observations on acute toxicity of sanguinarine do not concur with those seen in poisoned human patients studied by Tandon et al.[8]. These researchers examined 11 cases of epidemic dropsy caused by ingestion of argemone oil-contaminated mustard oil and found that one of the patients had ascites and only 24% of them showed soft nontendered hepatomegaly. These differences may be attributed, among other factors, to the amount of sanguinarine taken and the route and duration of exposure.

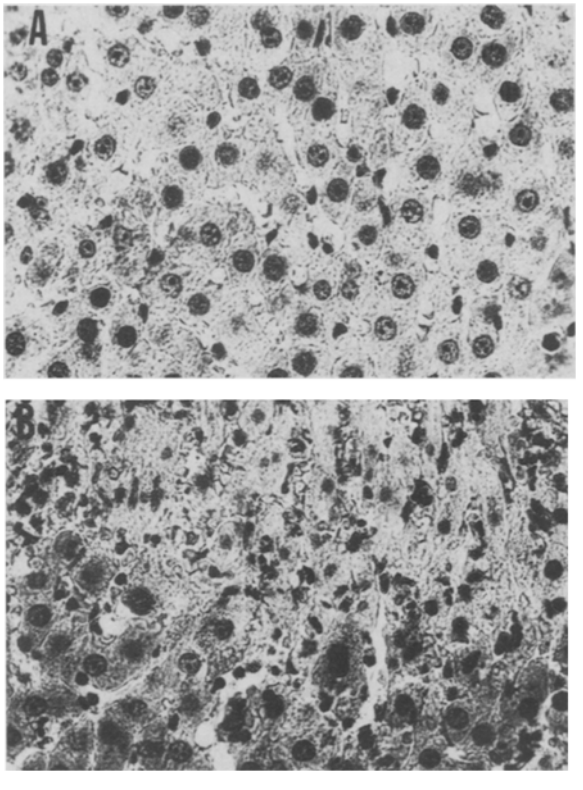

Figure 2. *A* Representative photomicrograph of liver section from a control rat. Note the normal appearance of the cell nuclei. × 120. *B* Photomicrograph of liver section from rat treated with sanguinarine. Cell swelling, progressive degeneration and necrosis are evident. × 120.

Table 1. Effect of a single dose (10 mg/kg, i.p.) of sanguinarine on body and liver weight

	Change in b.wt (%)	Liver weight (g/100 g b.wt)*
Control	+ 5.08	5.03 ± 0.12
Treated	− 7.22	3.75 ± 0.06**

* Values represent mean ± SD of five samples. ** Significantly different ($p < 0.05$) from corresponding control.

Table 2. Effect of sanguinarine on serum and liver microsomal enzymes

Treatment	SGPT (Karmen units)*	SGOT (Karmen units)*	Cyt. P-450 (nmoles/mg microsomal protein)*	Benzphetamine N-demethylase (nmoles HCHO/min/mg microsomal protein)*
Control	29 ± 4	163 ± 17	0.659 ± 0.01	2.87 ± 0.15
Sanguinarine	44 ± 8**	296 ± 32**	0.459 ± 0.05**	1.99 ± 0.21**

* Values represent mean ± SD of five samples. ** Significantly different ($p < 0.05$) from corresponding control.

Next, to assess the toxic effect of sanguinarine on liver, the activities of serum enzymes (SGPT and SGOT) and the hepatic microsomal enzymes were used as the indices of toxicity in these studies. As can be seen in table 2, a single dose of sanguinarine caused almost 50% and 100% increase in the activities of SGPT and SGOT, respectively, indicating sanguinarine-induced liver injury was substantial. According to Urbanek-Karlowska, the tests of liver microsomal enzyme activities as indicators of liver damage are more useful than those of blood serum[9]. Therefore, the concentration of cytochrome P-450 and the activity of benzphetamine N-demethylase in the liver microsomes from sanguinarine-treated animals were determined (table 2). The results of the determinations show that the alkaloid caused a significant loss of cytochrome P-450 and benzphetamine N-demethylase activity, confirming the evidence of liver injury provided by serum enzyme tests. The inhibition of liver microsomal enzymes and the subsequent liver damage may have occurred due to the binding of the alkaloid to cytochrome P-450[4].

The above noted biochemical data were further substantiated by histological changes in the liver tissues (fig. 2). Livers from rats treated with sanguinarine showed swollen hepatocytes with hydropic changes. The nuclei of the cells also showed swelling and degenerative changes, all leading to necrosis. These microscopic lesions correlate positively with the biochemical alterations, but the evidence is not sufficient to conclude that the two events are directly related although it is known that necrogenic toxicants such as carbon tetrachloride severely affect serum and microsomal enzymes. However, the studies provide an evidence of hepatotoxic potential of sanguinarine which is an important food contaminant responsible for severe outbreaks of epidemic dropsy in the tropics.

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The effects of acute and chronic morphine on regional distribution of cardiac output in brain

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Summary. Both acute and chronic administration of morphine resulted in an increase in the percent cardiac output received by brain. However, various brain regions were affected differently by the drug treatments. The greatest increases in percent cardiac output received after chronic administration of morphine occurred in pons and cerebellum, while the greatest increases after acute administration occurred in cortex and midbrain. The changes found are in contrast with earlier studies which suggest that morphine has no effect on cerebral blood flow.
Key words. Guinea pig brain; cardiac output; morphine; cerebral blood flow.

Opioids, including β-endorphin, enkephalins and morphine, have been shown to depress cardiovascular parameters, including heart rate and blood pressure[1], as well as inhibit the responsiveness of the cardiovascular baro- and chemoreceptor reflexes[2]. Evidence suggests that these responses are mediated through central nervous system (CNS) receptors. When naloxone, a potent opioid antagonist, is administered i.c.v., the doses which are necessary to reverse many of the cardiovascular opioid effects are as much as 100-fold lower than doses administered i.v.[3]. In addition, only the stereospecific (−) isomer of naloxone is effective in this reversal[4], suggesting a specific CNS receptor-mediated response.

Changes in regional cerebral blood flow (rCBF) have been shown to be correlated with changes in neuronal activity in specific brain regions[5] and with changes in regional metabolic activity[6]. Changes in neuronal activity have also been shown to correlate with the utilization of blood born energy substrates[7]. However, it has been assumed that morphine does not alter