Neurotoxic Effects of Carambola in Rats: The Role of Oxalate

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Background and Purpose: Carambola (star fruit) has been reported to contain neurotoxins that cause convulsions, hiccups, or death in uremic patients, and prolong barbiturate-induced sleeping time in rats. The constituent responsible for these effects remains uncertain. Carambola contains a large quantity of oxalate, which can induce depression of cerebral function and seizures. This study was conducted to investigate the role of oxalate in carambola toxicity in rats.

Materials and Methods: The effects on barbiturate-induced sleeping time and death caused by intraperitoneal administration of carambola juice were observed in Sprague-Dawley rats. To obtain a dose-dependent response curve and evaluate the lethal dose, rats were treated with serial amounts of pure carambola juice diluted with normal saline in a volume of 1:1. To test the role of oxalate in the neurotoxic effect of carambola, either 5.33 g/kg carambola after oxalate removal or 5.33 g/kg of pure carambola juice diluted with normal saline were administered intraperitoneally, while the control group was given normal saline before pentobarbital injection. The effects of carambola and oxalate-removed carambola on barbiturate-induced sleeping time were compared with those of saline. To assess the lethal effect of oxalate in carambola, we gave rats chemical oxalate at comparable concentrations to the oxalate content of carambola.

Results: Carambola juice administration prolonged barbiturate-induced sleeping time in a dose-dependent manner. The sleeping time of rats that received normal saline and 1.33 g/kg, 2.67 g/kg, 5.33 g/kg, and 10.67 g/kg of carambola juice were 66 ± 16.6, 93.7 ± 13.4, 113.3 ± 11.4, 117.5 ± 29.0, and 172.5 ± 38.8 minutes, respectively. The three higher-dose groups had longer sleeping times than controls ($p < 0.05$ or 0.005). This effect was eliminated after the removal of oxalate from carambola juice. Four of eight rats in the 10.67-g/kg group and all rats in the 21.33 g/kg and chemical oxalate groups died after seizure. Lethal doses of carambola juice were rendered harmless by the oxalate removal procedure.

Conclusions: Oxalate is a main constituent of carambola neurotoxicity. This finding suggests that patients with carambola intoxication should be treated for oxalate toxicosis.

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Key words:
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Carambola (star fruit), a member of the oxalidaceae family, is a popular fruit in many tropical countries such as Taiwan, Thailand, and Brazil. Its botanical name is *Averrhoa carambola*. There are sweet and sour types, and the sour type contains far more oxalate than the sweet type [1]. Because of the tart taste of sour carambola, it is not usually consumed as fresh fruit, while the sweet type is. Sour carambola is prepared as salted and diluted juice. However, sweet carambola still contains more oxalate than other fruits [1–3]. Traditional herbalists have used preparations of carambola for many symptoms including headache, vomiting, cough, and restlessness. However, carambola has been reported to contain a central acting depressant as indicated by its effects on barbiturate-induced sleeping time and by its association with convulsions, hiccups, or

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death in uremic patients [4–6]. Nevertheless, the component responsible for these manifestations remains uncertain. Carambola contains a large quantity of oxalate [1, 2, 7], which can induce depression of cerebral function and seizures [8]. Furthermore, there was a report that a patient presented with neurologic complications including muscle fasciculation, cramping, pupil dilatation, and seizure after ingesting *Oxalis corniculata*, a small plant containing high levels of oxalate and in the same family as carambola [9]. This report suggests that oxalate may have a role in carambola neurotoxicity.

In this study, we set up a rat model to investigate the role of oxalate in carambola intoxication. The effects of intraperitoneal administration of carambola juice and oxalate-removed carambola juice on barbiturate-induced sleeping time and death were investigated.

Materials and Methods

Sour carambola was collected from a local farm and homogenized. Insoluble residues were removed by passing the mixture through filter paper. The filtered carambola juice was kept at 4°C until use. Oxalate was removed from the juice by adding 10% calcium gluconate in a volume of 1:1, and the precipitate was collected by passing the mixture through filter paper. The filtered juice was called oxalate-removed carambola juice. The precipitate was air-dried and then ground to analyze its composition using an infrared spectrophotometer (Jasco IR-700, Tokyo, Japan). Pure carambola juice was diluted with normal saline in a volume of 1:1 to render the potassium concentration and volume similar to that of the oxalate-removed carambola juice. Both the pure carambola juice and the oxalate-removed carambola juice were analyzed for electrolyte content (288 Blood Gas System and 664 Fast 4 System, Ciba Corning Diagnostics Corp, Medfield, MA, USA) and pH (Φ 72 pH Meter, Beckman, Fullerton, CA, USA), and high-performance liquid chromatography was used to determine the oxalate concentration. A filtration column (ALLtech Econosil C18 10U, 250 mm x 4.6 mm; ALLtech Associates Inc, Deerfield, IL, USA) was connected to a high-performance liquid chromatograph (Varian 2550 HPLC; Varian Inc, Palo Alto, CA, USA). A 0.01 M KH_2PO_4 and 1% methanol buffer was used at a flow rate of 0.6 mL/minute, and the absorbance was measured at 220 nm. The measurement accuracy was 0.001 g/dL. Oxalic acid standards were purchased from Sigma (St. Louis, MO, USA). Chemical oxalic acid powder was diluted with normal saline to make its concentration similar to that in pure carambola juice.

The method of Muir and Winter was used to test the effect of carambola on barbiturate-induced sleeping time in male Sprague Dawley rats weighing 150 to 200 g [4, 10]. Intraperitoneal injections with carambola juice, oxalate-removed carambola juice, chemical oxalate, and normal saline were performed 15 minutes before the intraperitoneal administration of 66.7 mg/kg of pentobarbital sodium [4]. Sleeping time was recorded from the administration of pentobarbital to the regaining of a righting reflex. Mortality rates with various doses were also recorded. To obtain a dose-dependent response curve of the effects on barbiturate-induced sleeping time, rats were given a series of doses of carambola juice (1.33 g/kg, 2.67 g/kg, 5.33 g/kg, 10.67 g/kg, and 21.33 g/kg) and oxalate-removed carambola juice (5.33 g/kg, 10.67 g/kg, and 21.33 g/kg). There were six rats in most dose groups but eight in the 10.67 g/kg dose group. To test the role of oxalate in the neurotoxic effects of carambola, 5.33 g/kg of carambola juice, oxalate-removed carambola juice, chemical oxalate with comparable oxalate content, and normal saline (control) was intraperitoneally administered and then sleeping time, seizure, and death in each group were recorded. Rats were killed and pathologic examinations of the brain and kidneys were performed, including hematoxylin-eosin stain under light microscope, polarized microscopy, and transmission electronic microscopy.

Pentobarbital-induced sleeping time was expressed as mean ± standard deviation. Repeated ANOVA with Bonferroni test for comparison with controls was used to analyze the sleeping time data in different dose groups. One-way ANOVA with Bonferroni method for 2 x 2 comparisons was used to analyze the sleeping time data between different preparation groups. The mortality rates were expressed as proportions and analyzed by Fisher's exact test. A probability of less than 0.05 was considered significant.

Results

The oxalate concentration of pure carambola juice was 2.42 g/dL, which declined to 0.03 g/dL after removal of oxalate. The potassium concentration of carambola juice was diluted from 35 mmol/L to 17 mmol/L by the oxalate-reducing procedure or normal saline dilution. The calcium concentrations of diluted carambola juice and oxalate-removed carambola juice were both less than 0.25 mEq/L, and the pH values were 2.0 and 2.1, respectively. Magnesium was undetectable in carambola juice. Infrared spectroscopy showed that the precipitate formed after oxalate removal consisted of calcium

oxalate dihydrate and calcium oxalate monohydrate (Fig. 1).

The Table shows the sleeping time and mortality rates for rats that received the four different preparations and the dose-dependent response of pentobarbital-induced sleeping time to carambola juice. The three higher-dose groups had significantly longer sleeping times than controls ($p < 0.05$). Four of eight rats in the 10.67 g/kg group and all six rats in the 21.33 g/kg group died. Sleeping times were significantly longer with carambola juice than with other preparations.

Fifty percent of the rats that received carambola juice 10.67 g/kg and 100% of rats that received carambola juice 21.33 g/kg died. In contrast, all rats treated with oxalate-removed carambola juice survived, even at extreme doses. However, all rats that received chemical oxalate at comparable oxalate concentrations died. Before death, rats presented with hiccups, unstable gait, and/or convulsions 1 to 5 minutes after intraperitoneal administration of carambola juice or chemical oxalate. Autopsy revealed extensive calcium oxalate deposition in the kidney, while the brain was spared, as seen by transmission electron microscopy and polarized light microscopy (Fig. 2). Under polarized light, oxalate crystals are refractive and show all colors of the rainbow, with yellow predominant. Kidneys from rats treated with oxalate-removed carambola juice were free of calcium oxalate crystals, which was confirmed by alizarin red S stain [11] and polarized light microscopy.

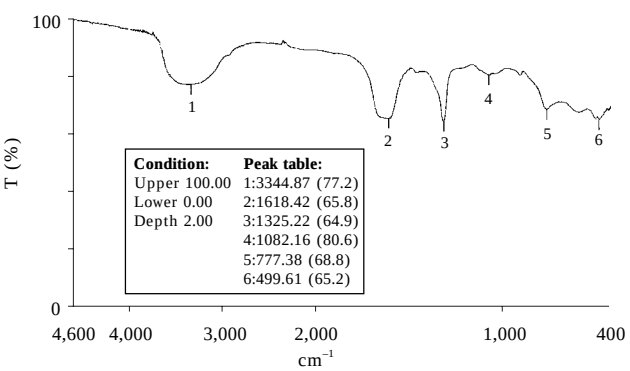

Fig. 1. *Infrared spectroscopy of the precipitate of carambola juice and 10% calcium gluconate. The tomography indicates the presence of oxalate monohydrate and calcium oxalate dihydrate. In the high frequency of the spectrum, the decreased intensities of the discrete bands from 3,477 to 3,047 indicates the presence of dihydrate crystals, while the monohydrate crystals are indicated by the band contour at 779.*

Discussion

Like most plants in the oxalidaceae family, carambola contains abundant amounts of oxalate. Because of the similar neurologic manifestations caused by ingestion of many high oxalate-containing plants [12], it is prudent to consider oxalate as a causal agent of carambola-associated encephalopathy in dialysis patients. Our results revealed that carambola dose-dependently prolongs barbiturate-induced sleeping time. This finding is compatible with a previous study [4]. This effect was eliminated after oxalate removal with 10% calcium gluconate, and lethal doses of carambola juice were also rendered harmless by oxalate removal. Further analysis of the precipitate produced in the oxalate removal procedure by infrared spectroscopy showed calcium oxalate monohydrate and calcium oxalate dihydrate. At autopsy, the kidneys showed oxalate toxicosis. Taken together, these findings suggest that oxalate plays a major role in the neurotoxicity of carambola. In contrast, hyperkalemia and severe acidemia-induced death were ruled out by similar potassium concentrations and pH values in diluted carambola juice and oxalate-removed carambola juice.

Oxalate has been shown to depress cerebral function, as shown in studies of oxalate toxicosis or ethylene glycol poisoning [8, 13, 14]. The immediate and intense precipitation of calcium and oxalate *in vitro* would suggest that brain embolism caused by calcium oxalate crystal is a reasonable possibility. However, pathologic examination of the brain tissue in our study showed no calcium oxalate crystals or other organic lesions. Therefore, the neurologic manifestations of oxalate intoxication might be caused by functional rather than structural changes. Oxalate-induced hypocalcemia is another possible mechanism of oxalate poisoning that has been demonstrated previously [8, 12–14]. However, hypocalcemia as the sole cause of intoxication is questionable because animals made hypocalcemic by dialysis survive and, although blood calcium concentrations can be maintained in animals with plant-induced oxalosis, the animals still die [8]. Previous studies have demonstrated that oxalate inhibits enzyme activities involved in energy metabolism, some of which need calcium and magnesium for complete function [8, 15, 16]. Thus, oxalate may interfere with energy metabolism directly or indirectly by influencing the availability of calcium. Moreover, intracellular hypocalcemia has been demonstrated *in vitro* with an oxalate precursor, oxalipaltin [17]. However, the exact mechanism responsible for oxalate neurotoxicity needs further clarification.

Table. Barbiturate sleeping time and mortality

		Carambola juice					Oxalate-removed carambola juice			Chemical oxalate
	Normal saline	1.33 g/kg	2.67 g/kg	5.33 g/kg	10.67 g/kg	21.33 g/kg	5.33 g/kg*	10.67 g/kg	21.33 g/kg	
n	6	6	6	6	8	6	6	8	6	6
Barbiturate sleeping time (min)†	66.0 ± 16.6	93.7 ± 13.4	113.3 ± 11.4‡	117 ± 29.0‡	172.5 ± 38.8§	NC¶	66.2 ± 9.4	NC	NC	NC¶
Mortality‖	0%	0%	0%	0%	50%	100%	0%	0%	0%	100%

*The effect of carambola juice (5.33 g/kg) on pentobarbital-induced sleeping time was significantly greater than that of the oxalate-removed carambola juice (5.33 g/kg) and normal saline.†Mean ± standard deviation. NC = not checked. ‡$p < 0.05$; §$p < 0.005$, *vs* control group. ¶Because of immediate death, the barbiturate sleeping time was not checked in these groups. ‖Mortality rates of rats treated with carambola juice at a dose of 10.67 g/kg and 21.33 g/kg were higher than those treated with carambola juice 5.33 g/kg and all oxalate-removed carambola juice groups; $p < 0.05$.

Despite the popularity of carambola, the connection of carambola with neurotoxicity has rarely been reported in uremic patients [4–6], and never in healthy subjects. Depending on renal excretion for clearance, oxalate accumulation is inevitable in uremic patients when ingesting substantial amounts of oxalate. Normal people can easily excrete oxalate from the urine. Hence, carambola-related seizure and death has not been reported in people with normal renal function. We have measured different preparations of carambola juice [3], and found that fresh carambola contains the most abundant oxalate content among fruits [1–3]. In Taiwan, carambola juice is prepared with sour fruit, which is pickled with 8% salt for 3 months. Oxalate content decreased from 0.82 to 0.3 g/dL after the pickling process [7]; dilution before commercialization further decreases the oxalate content. Hence, most people ingest little oxalate from commercial carambola juice. This situation may explain the disagreement between the popularity of carambola juice and the rare incidence of carambola juice-associated neurotoxicity in uremic patients [5]. Vegetables, such as rhubarb and beet, contain a lot of oxalate; however, they have seldom been reported to be associated with neurotoxicity [12]. According to our previous studies, fasting can enhance oxalate absorption [18]. Because rhubarb and beet are usually cooked and consumed with other foods, this may precipitate oxalate to insoluble oxalate in the intestine and reduce oxalate absorption. In addition to the quantity of oxalate absorption and impaired excretion, there may be some other predisposing factors for the vulnerability to carambola neurotoxicity in uremic patients. Among these, the blood

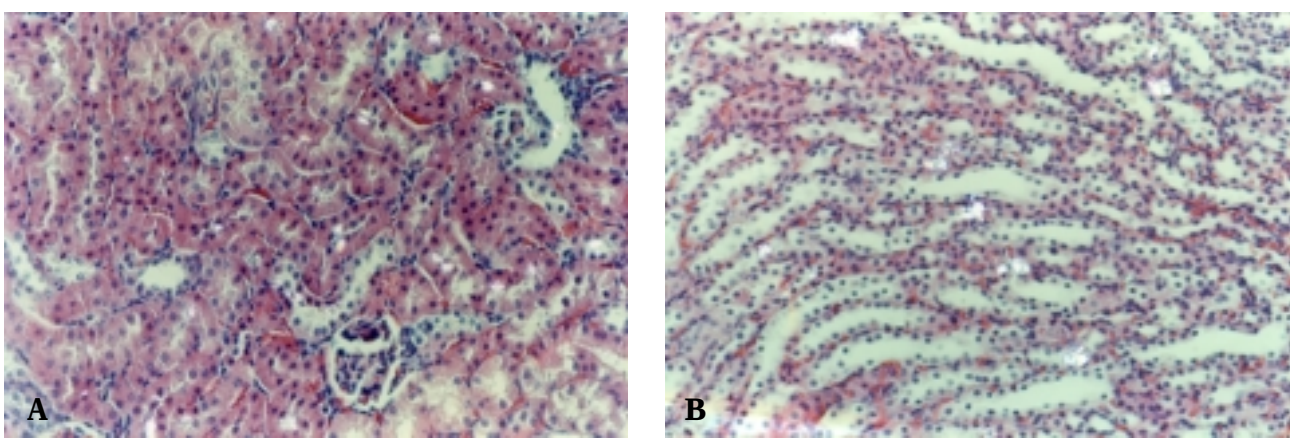

Fig. 2. *A) and B) Representative photomicrographs of renal pathology in rats that received 10.67 g/kg of carambola juice (hematoxylin-eosin stain with polarized microscopy; magnification x 150). There are extensive intra-luminal and intra-epithelial depositions of calcium oxalate crystals in the kidney.*

cerebrospinal fluid barrier dysfunction and decreased plasma protein-binding ratio noted in uremic patients may make some contribution [19, 20].

Based on our findings, we conclude that oxalate is one of the main constituents of carambola neurotoxicity. Accordingly, we suggest that uremic patients with carambola intoxication should be treated for oxalate toxicosis. The recommended measures for oxalate toxicosis include gastric lavage with water containing calcium or magnesium salts to precipitate oxalate and reduce absorption, administration of milk to counteract local corrosive effects, hemodialysis for oxalate removal, and administration of calcium gluconate to correct hypocalcemia [8, 12].

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