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Toxicological and Pharmacological Studies of *Picrasma crenata* (Vell.) Engler (Simaroubaceae) in Mice and Rats

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SUMMARY. The toxicity and the anti-inflammatory, anti-ulcer, anti-hyperglycemic, hypoglycemic and anti-hyperlipidemic effect of the hydroalcoholic extract from woods (without bark) of *Picrasma crenata* (Vell.) Engler (Simaroubaceae) were investigated. The extract showed low toxicity in mice. Additionally, in agreement with the popular use to treat gastric ulcer and diabetes, anti-ulcer and blood glucose lowering effect were detected in rats. However, anti-inflammatory, anti-hyperglycemic and anti-hyperlipidemic effects were not observed in these animals.

RESUMEN. "Estudios Toxicológicos y Farmacológicos de *Pricrasma crenata* (Vell.) Engler (Simaroubaceae) en ratones y ratas". Se investigó la toxicidad, actividad anti-inflamatória, anti-úlcera, anti-hiperglucemiante, hipoglucemiante y anti-hiperlipidémica del extracto hidroalcohólico de tallo (sin corteza) de *Picrasma crenata* (Vell.) Engler (Simaroubaceae). El extracto presentó baja toxicidad en ratones. Además, de acuerdo al uso popular de esta espécie en el tratamiento de la gastritis y de la diabetes mellitus, se observo um efecto anti-úlcera e hipoglucemiante en ratas. Sin embargo, no fue observado ningún efecto anti-inflamatório, anti-hiperglucémico ni anti-hiperlipidámico en estos animales.

INTRODUCTION

Picrasma crenata (Vell.) Engl. (Simaroubaceae) is a Brazilian tree used in the traditional medicine to treat diabetes and gastric ulcers [1]. Phytochemical investigation of Picrasma crenata (PC) obtained quassinoids, alkaloids, quinones, phenylpropanoids and lignanes [2]. In addition, we previously demonstrated the presence of a new stereoisomer, i.e., dihydronorneoquassim [3]. Some quassinoids received renewed attention based on their anti-cancer [4-5] and anti-ulcer [6] and apoptotic activity [7]. Since studies suggesting toxicity, anti-ulcer and anti-diabetic properties of the hydroalcoholic extract of dried wood without bark from PC were not demonstrated we decided to investigate these aspects. Thus, employing mice as experimental model, we determined the toxicity of PC. In addition, we expanded this study investigating the anti-inflammatory, anti-ulcer, anti-hyperglycemic, hypoglycemic and anti-hyperlipidemic potential of PC.

MATERIALS AND METHODS

Plant material

The aerial parts of PC were collected in Ortigueira, PR, Brazil (June, 2000) and a voucher specimen (HUM 8361) was deposited in the Herbarium of the State University of Maringá, PR, Brazil. The dried plant material was pulverized and the particle size of powder with moderate coarse was determined 8 and stored in a polyethylene bag under refrigeration.

Chemicals

Indometacin, and carrageenan were purchased from Sigma Chemical Company. Amilose was obtained from Isofar. All other reagents were of the highest purity obtainable.

Preparation of the extract

Dried wood without bark (2.0 kg) powder from PC was exhaustively extracted with ethanol/water (1:1 v/v) in the dark at room temperature, evaporated *in vacuum*. Finally, the extract (43 g) was lyophilized and stored.

Animals

Male Swiss mice (20-30 g) were used for toxicity evaluation and male Wistar rats (200-220 g) were employed to pharmacological studies. They were housed in plastic cages on groups of

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five and kept with controlled temperature (23 ± 2 °C) and light (12 h):dark (12 h) cycle. Food (Nuvilab(r)) and water were freely available. The treatment of the animals followed the Brazilian Law on the protection of animals.

Toxicity

The effect of the hydroalcoholic extracts of PC on kidneys, heart, lungs, liver and body weigh according to the method described by Brito [9] was investigated. The experimental groups received PC extract intragastrically (i.g) (2500 and 5000 mg/kg) or intraperitoneally (i.p) (1000 and 2500 mg/kg). The control groups received an equal volume of water + Tween 80 (i.g or i.p). The Swiss mice were observed during two weeks and killed under ether anesthesia. The kidneys, heart, lungs and liver were removed and weighted.

Anti-inflammatory activity

The effect of hydroalcoholic extracts of PC on exudates volume and leukocyte numbers 4 h after carrageenan injection (200 µg/pleural cavity) were measured in rats. To induce accumulation of an inflammatory exudates into the pleural cavity, carrageenan (200 µg) diluted in phosphate buffer was injected in a standard volume (0.25 mL) under light ether anesthesia. The injection was performed on the right side of mediastinum, between the second and third ribs, according to the method of Vinegar *et al.* [10]. Four hours later, the rats were killed and the skin and pectoral muscle were retracted leaving the chest wall exposed. A longitudinal incision was performed between the third and fifth ribs on each side of the mediastinum. The chest wall was opened and the inflammatory exudates collected by aspiration with a Pasteur pipette and transferred to a conical calibrated centrifuge tube. The total volume of exudates was determined and an aliquot (50 (L) was used to determine the total leukocyte number in a Neubauer chamber. For differential cell counts, red blood cells were lysed by the addition of Turk's solution. The remaining fluid was centrifuged at 500 rpm during 4 min and the cells diluted in plasma (0.1 mL) obtained from blood of rats. Exudate smears were prepared, air dried and fixed with Rosenfeld stain. The extract of PC was administered i.g 30 min before the procedures above described.

Anti-hyperlipidemic activity

The effect of PC extract on blood triglycerides levels after the administration of soy oil (SO) was investigated. Triglycerides were measured 30 min after the administration of SO (6.0 mL/kg) or SO (6.0 mL/kg) + PC (100 mg/kg). Control group were composed by rats that received water instead SO or PC. All substances were administered i.g. in 22-h fasted rats. The rats were killed by decapitation and the blood was collected to measure blood triglycerides levels [11].

Anti-hyperglycemic activity

Anti-hyperglycemic activity was measured as previously described [12]. 22-h fasted rats received i.g: amilose 1000 mg/kg (A) or A + lyophilized extract of *PC* (100 mg/kg). The rats were killed by decapitation and the blood was collected to measure glycemia [13] immediately before (0 min) and 30 min after the administration of A (Control group) or PC + A (PC + A group).

Anti-ulcer activity

Acute gastric mucosal lesions were obtained with 24 h fasted rats which received indomethacin or acidified-ethanol. Cimetidine (100 mg/kg) and saline were used respectively as reference drug and control. Indomethacin (20 mg/kg) was administered subcutaneously, according to the method of Aguwa & Mittal [14]. The rats were killed 7 h later. Acidified-ethanol induced gastric lesions were obtained according to the method of Mizui & Doteuchi [15] in rats which received i.g 1.0 mL/100 g of HCl (0.3 M) in ethanol 60%. The rats were killed 1 h later. PC extracts (500, 750 or 1000 mg/kg), cimetidine or saline were i.g administered i.g 30 min before indometacin or HCl-ethanol. After each treatment the rats were killed, the stomachs removed, opened and its inner surface was examined. The gastric lesions were counted and the mean ulcerative index was calculated by the method of Szelenyi & Thiemer [16].

Hypoglycemic activity

The rats received i.g lyophilized extract of PC (100 mg/kg) during 15 days. Control group received water. After this period the rats were starved during 22 h, killed by decapitation and the blood was collected to measure glycemia [13].

Statistical Analysis

ANOVA followed by Duncan test or student t test were applied. The results were expressed as mean ± SEM. The statistical method used in each experiment was particularized in the corresponding Tables.

RESULTS

PC extract i.g administered (2500 and 5000 mg/kg) did not show effect on kidneys, heart, lungs, liver and body weigh. In addition, one death and increased kidneys weigh ($p < 0.05$) were observed in mice treated with PC extract i.p administered (2500 mg/kg). However, the weigh of the body, lungs, liver and heart remained unchanged (Table 1).

In the carrageenan-induced pleurisy model (Table 2), the volume of exudate and the number of cells migrating into the pleural cavity was similar (control *vs.* PC group).

Moreover, PC did not show effects on the triglyceride elevation promoted by soy oil administration (Table 3) or glycemia elevation after amilose administration (Table 4).

The anti-ulcerogenic effects of the PC extract in the indomethacin and acidified-ethanol induced lesions are summarized in Table 5. Gastric lesions induced by indomethacin and acidified-ethanol were reduced ($p<0.05$) by i.g administration of the PC extract. The PC extract decreased ($p<0.05$) the indomethacin induced lesion at the dose of 1000 mg/kg. Similarly, in the acidified-ethanol model, the PC extract produced a dose-dependent reduction when compared with the control group.

Finally, fasted rats treated i.g during 14 days with PC (100 mg/kg) showed reduced ($p < 0.05$) glycemia (Table 6).

DISCUSSION

The toxicity of the hydroalcoholic extract of PC was low in mice, particularly to i.g administration, since in a dose about seven thousand times higher than that used in the traditional medicine, i.e., 5000 mg/kg, no death was ob-

Groups	Dose (mg/kg)	BW Day 1	BW Day 7	BW Day 14	Heart	Lungs	Kidneys	Liver
Control	-	40.1 ± 3.9	41.6 ± 4.5	42.6 ± 4.3	0.21 ± 0.03	0.31 ± 0.03	0.64 ± 0.08	2.97 ± 0.42
i.g	2500	41.7 ± 2.4	43.2 ± 2.2	44.0 ± 2.4	0.25 ± 0.04	0.35 ± 0.05	0.65 ± 0.05	3.06 ± 0.50
	5000	41.4 ± 4.2	42.2 ± 4.6	43.8 ± 5.2	0.26 ± 0.05	0.35 ± 0.06	0.70 ± 0.06	3.08 ± 0.47
i.p	1000	40.8 ± 4.9	42.6 ± 3.1	43.6 ± 3.1	0.25 ± 0.04	0.35 ± 0.05	0.70 ± 0.05	3.27 ± 0.23
	2500	39.8 ± 2.7	41.1 ± 2.2	43.7 ± 2.2	0.23 ± 0.04	0.31 ± 0.05	0.73 ± 0.05*	3.28 ± 0.24

Table 1. Effect of hydroalcoholic extract of *P. crenata* without bark (PC) on body weigh (BW) and weight of heart, lungs, kidney and liver in mice. PC was intragastrically (i.g) or intraperitoneally (i.p) administered. Values are the mean ± SEM of 10 mice. Controls mice received an equal volume of water + Tween 80. *p < 0.05 compared to the control group (ANOVA followed by Duncan test). N = number of animals.

Groups	Dose (mg/kg)	Exudate volume (ml)	Leukocyte number (cell/10^9/L)
Control	-	0.80 ± 0.02	62440 ± 2917
PC	500	0.82 ± 0.12	60940 ± 6913
PC	1000	0.80 ± 0.06	63940 ± 5587

Table 2. Acute effect of hydroalcoholic extract of *P. crenata* wood without bark (PC) on exudates volume and leukocyte numbers 4 h after carrageenan injection (200 µg/pleural cavity) in rats. PC was intragastrically administered.Values are the mean (SEM of 8 rats. Control rats received an equal volume of vehicle solution.

Groups	Blood triglycerides
Control (6)	76.87 ± 1.83
SO (5)	129.50 ± 14.21*
PC + SO (6)	149.80 ± 8.77*

Table 3. Acute effect of extract of *P. crenata* wood without bark (PC) on blood triglycerides levels after oral administration of soy oil (SO). Triglycerides were measured 30 min after the administration of SO (6.0 mL/kg) or SO (6.0 mL/kg) + PC (100 mg/kg). Control group were composed by rats that received water instead SO or PC. All substances were intragastrically administered. Values are the mean ± S.E.M of 5-6 rats. *P < 0.05 *vs.* control; Student's t-test.

Groups	Glycemia (0 min)	Glycemia (30 min)
A (Control)	90.68 ± 3.54	137.40 ± 3.35
PC + A	93.02 ± 3.52	138.10 ± 3.14

Table 4. Acute effect of extract of *P. crenata* wood without bark (PC) on the elevation of glycemia promoted by the administration of amilose (A) in 22 h fasted rats. Blood glucose levels were measured immediately before (0 min) and 30 min after the administration of (A) (1000 mg/kg) or (A) (1000 mg/kg) + PC (100 mg/kg) in rats. PC and A were intragastrically administered. Values are mean ± S.E.M. of 6 rats. P > 0.05 *vs.* control; Student's t-test.

Groups	Treatment	Dose (mg/kg)	Ulcer index	Reduction (%)
	Control (20)		26.3 ± 1.7	
	Cimetidine (10)	100	1.0 ± 0.4 *	96
Indomethacin	PC (10)	500	21.3 ± 2.2	19
	PC (10)	750	24.1 ± 3.4	8
	PC (10)	1000	17.8 ± 2.5 *	32
	Control (20)		46.3 ± 4.0	
	Cimetidine (10)	100	26.0 ± 2.0 *	44
Acidified ethanol	PC (10)	500	35.5 ± 4.1	23
	PC (10)	750	33.3 ± 3.5 *	28
	PC (10)	1000	19.3 ± 3.5 *	58

Table 5. Acute effect of hydroalcoholic extract of *P. crenata* without bark (PC) on gastric lesion induced by indomethacin or acidified-ethanol in rats. PC was intragastrically administered.Values are the mean ± SEM of 10-20 rats. ( ) number of animals. Control rats received an equal volume of vehicle solution (0.9% NaCl). * P<0.05, compared to the control group (ANOVA followed by Duncan test).

Groups	Glycemia
PC	87.32 ± 3.0 *
Control	96.77 ± 1.6

Table 6. Effect of the treatment (100 mg/kg) with extract of *P. crenata* wood without bark (PC) or water (Control) during 15 days on glycemia in 22 h fasted rats. PC was intragastrically administered. Values are the mean ± S.E.M of 6 rats. *P < 0.05 *vs.* control; Student's t-test.

served. Moreover, the hydroalcoholic extract of PC exhibited lower toxicity than i.p *Quassia amara* in mice [17], which showed LD50 of 1000 mg/kg.

Gastric lesions induced by indomethacin or acidified-ethanol were reduced (p<0.05) by i.g administration of the PC extract. The effect of PC extract at the dose of 1000 mg/kg was comparable to those produced by cimetidine, the reference drug included in the study (Table 5). These results are in agreement with the anti-ulcer effect of quassinoids in rats (Tada *et al.*) [6] and with the popular use to treat gastric ulcer.

Finally, to verify the anti-diabetic effect of the chronic treatment with PC, fasting glycemia was measured after 14 days of treatment. In agreement with Ikegame 18, rats treated i.g during 14 days with PC (100 mg/kg) showed reduced (p < 0.05) glycemia (Table 6). Because gluconeogenesis is crucial to glycemia maintenance during fasting, as we previously demonstrated to *Stevia rebaudiana* [19], this possibility must be considered. However, it will be necessary future studies to confirm this suggestion.

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