Effect of Dichloromethane Extract of *Kielmeyera coriacea* Stems on Hepatic Catabolism of L-Alanine in Rats

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SUMMARY. The present study evaluates the effect of the treatment with dichloromethane (DcM) extract of *Kielmeyera coriacea* stems on hepatic catabolism of L-alanine (5 mM) in male and female Wistar rats. The rats were daily treated by gavage with DcM extract of *K. coriacea* (5 or 25 mg/kg) or an equal volume of vehicle (controls) during 90 days. After this period of treatment the animals were fasted (12 h), anaesthetized and submitted to liver perfusion. Livers from male and female rats treated with DcM extract (5 or 25 mg/kg) showed lower gluconeogenesis in comparison with livers from control animals. In addition, lower urea production was obtained in livers from female rats treated with DcM extract (25 mg/kg). However, the treatment with DcM extract (5 or 25 mg/kg) did not influence the hepatic production of pyruvate and L-lactate when compared with the control group. The results indicate that DcM extract of *K. coriacea* inhibits the gluconeogenesis, suggesting inhibition of the energetic metabolism in the liver.

RESUMEN. "Efecto del Extracto Diclorometánico (DcM) de Tallo de *Kielmeyera coriacea* en el Catabolismo Hepático de L-alanina en Ratas". En el presente estudio se evaluó el efecto del tratamiento con extracto diclorometánico (DcM) del tallo de *Kielmeyera coriacea* en el catabolismo hepático da L-alanina (5 mM) em ratas Wistar machos y hembras. Las ratas fueron tratadas diariamente con exctrato DcM de *K. coriacea* (5 o 25 mg/kg) o igual volumen de vehículo (control) durante 90 días. Después de este período de tratamiento, los animais em ayunas (12 h) fueron anestesiados y sometidos a perfusión de hígado. Los hígados de ratas machos y hembras tratados con extracto DcM (5 ou 25 mg/kg) mostraron menor gliconeogénesis em relación a los hígados de los animales control. Además se obtuvo menor producción hepática de urea en ratas tratadas con extracto DcM (25 mg/kg). Mientras tanto, el tratamiento com el extracto DcM (5 ou 25 mg/kg) no influenció la producción hepática de piruvato ni de L-lactato en comparación con el grupo control. Los resultados indican que el extracto DcM de tallo de *K. coriacea* inhibe la gliconeogénesis, sugiriendo la inhibición del metabolismo energético hepático.

INTRODUCTION

Kielmeyera coriacea Mart. is a tree popularly known in Brazil as "Pau Santo" or "Saco de Boi" that belongs to Clusiaceae family. The crude aqueous extract of this native specie of Central Brazilian plateau is traditionally used to treat several tropical diseases, including schistosomiasis, leishmaniasis, malaria, and fungal or bacterial infections [1]. Phytochemical investigation of hydroethanolic (HE) and dichloromethane (DcM) extracts of *K. coriacea* leaves and stems resulted in the isolation of 10 xanthones, two triterpenes and one biphenylic compound [2].

The biphenylic compound and four xanthones exhibited activity against *Cladosporium cucumerinum* while two prenylated xanthones inhibited *Candida albicans* growth [2]. In addition, antimicrobial activities against *Bacillus subtilis* by one xanthone and *Staphylococcus aureus* by the biphenylic compound aucuparin were reported [3]. Considering that *K. coriacea* extracts are rich in xanthones [2], and xanthones can impair the mitochondrial energy metabolism [4,5], this effect could explain the popular use of this plant against protozoan, fungal and bacterial diseases [1].

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Our previous studies showed that the chronic administration of the HE extract of stems of *K. coriacea* promoted antidepressant [7], ansiolitic [8] and antiulcer activity [9] in rats. A more recent work reports that the antidepressant like-effect was mediated by a serotonergic mechanism [10]. In addition, the possibility of an impairment of mithocondrial metabolism, like compounds from other plants [11-13] must be considered. To verify this possibility the effect of HE extract on hepatic energy metabolism was investigated. The results showed that HE extract inhibits hepatic gluconeogenesis in isolated perfused liver by acting as mitochondrial uncoupler and inhibitor of enzymatic activities of the respiratory chain [14].

On the other hand, preliminary studies of acute toxicity with DcM extract orally administered found high LD_{50} values , suggesting a wide margin of safety for compounds present in the extract. In agreement, the chronic toxicity study also showed that oral DcM extract can be continuously used with safety [15].

Moreover, we recently reported that the chronic treatment with DcM extract, as previously demonstrated to HE extract [7,10] was able to promote antidepressant effect. But, it must be emphasized that the effective dose of DcM extract which promoted antidepressant effect was lower (5.0 mg/kg) than that observed with HE extract, i.e., 60.0 mg/kg [16]. Therefore, in the present work we investigated if the DcM extract (5.0 mg/kg) also inhibits hepatic metabolism, as mentioned above to HE extract. Moreover, to simulate a possible therapeutic utilization, DcM extract was orally administered by gavagen during 90 days and the effect on the liver catabolism of L-alanine in male and female rats were investigated. The reason to study the effect of DcM extract on gluconeogenesis in livers from male and female rats was based in the following facts: (1): liver is the organ which receives the maximal amount of the active compounds via portal vein after oral administration; (2): gluconeogenesis depends on energetic mitochondrial metabolism and an impairment of mitochondrial function certainly affects these metabolic pathways; (3): by using male and female rats will be possible to detect if gender could influence the liver responsiveness to DcM extract.

MATERIALS AND METHODS
Plant material and DcM extract

K. coriacea Mart. was collected in the biological reserve of the experimental station of Mogi Guaçu (São Paulo, Brazil) in April, 2003. A voucher specimen (number SP 298463) was deposited in the Herbarium of the Botanical Institute of São Paulo, São Paulo state, Brazil. Species identification was done by Dr. Maria Claudia Young of the same institute.

The dried and crushed stems (1.0 kg) of *K. coriacea* were exhaustively extracted with 38 L of ethanol/water (9:1) at room temperature for 7 days, yielding 167.3 g of extract after evaporation of the solvents and lyophilisation. The HE extract was submitted to vacuum chromatography (silica gel) and eluted with DcM and stored at -20 °C until further use.

The HPLC-UV analysis of DcM extract used in this work identified several compounds with preponderance of xanthones. Phytochemical investigation of this extract as reported before [2] resulted in the identification of kielkorin; 1,3,7-trihydroxy-2-(3-metylbut-2-enyl)-xanthone; 1,3,5-trihydroxy-2-(3-metylbut-2-enyl)-xanthone (Fig. 1).
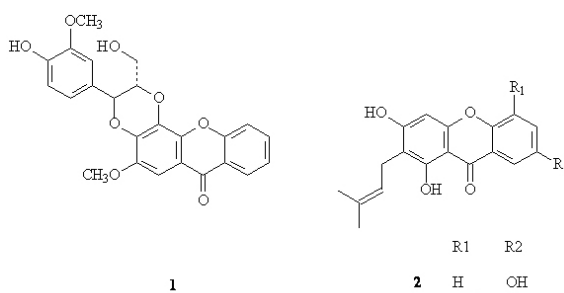

Figure 1. Main xanthones from DcM extract of *K. coriacea* (1) Kielcorin, (2) 1,3,7-Trihydroxy-2-(3-methyl-but-2-enyl)-xanthone and (3) 1,3,5-Trihydroxy-2-(3-methylbut-2-enyl)-xanthone.

Animals and treatment

Male and female Wistar rats weighing about 200 g were employed. The manipulation of the animals was approved by the Ethical Committee of the State University of Maringá (approval number 011/2006). Experimental groups received 5 or 25 mg/kg per day of DcM extract of stems of *K. coriacea* dissolved in 5% dimethylsulfoxide (DMSO) administered by gavage during 90 days (DcM group). Control group received 5% DMSO (C Group). For comparative purpose a group which received 2,4-dinitrophenol (30 mg/kg) dissolved in 5% DMSO or 5% DMSO (C group) were included. These substances were orally administered immediately before the anesthesia and liver perfusion experiments.

Liver perfusion experiments

After 90 days of treatment the rats were fasted (12 h) and anesthetized by an i.p injection of sodium thiopental (40 mg/kg) and submitted to laparotomy. The livers were perfused *in situ* using Krebs Henseleit bicarbonate buffer, pH 7.4, saturated with O_2/CO_2 (95/5%) as described elsewhere [17,18]. The perfusion fluid was pumped to a temperature controlled (37 °C) membrane oxygenator prior to entering the liver via portal vein. The liver perfusion experiments was performed in an open system, without recirculation of the perfusate. A constant flow rate in each experiment was adjusted according to the liver weight (4 mL/g of tissue fresh weight x min).

Figure 2 illustrated a demonstrative liver perfusion experiment. After a pre infusion period without L-alanine (15 min), L-alanine (5 mM) was dissolved in the perfusion fluid and infused during 30 min (15-45 min). Samples of the effluent perfusion fluid were collected at 5-min intervals and the concentration of glucose [19], urea [20], pyruvate [21] and L-lactate [22] was analysed. The differences in the glucose (gluconeogenesis),

urea (ureagenesis), pyruvate and L-lactate production during (15-45 min) and before (0-15 min) the infusion of the L-alanine allowed calculation of the areas under the curves (AUC), expressed as µmol/g. Thus, the AUCs shown in Tables 1 and 2 were obtained from similar experiments to that illustrated in Figure 2.

Statistical analysis

The differences in the liver glucose, urea, pyruvate and L-lactate production during (15-45 min) and before (0-15 min) the infusion of the L-alanine allowed calculation of the AUC, expressed as µmol/g. The GraphPad Prism program (version 4.0) was used to calculate the AUC. Data were analysed statistically by the unpaired student's t-test. P values < 0.05, 0.01 and 0.001 were considered to be significant. Results were reported as mean ± SEM.

RESULTS AND DISCUSSION

In this study the chronic oral administration DcM of extract of *K. coriacea* were done and the impact on liver metabolism was investigat-
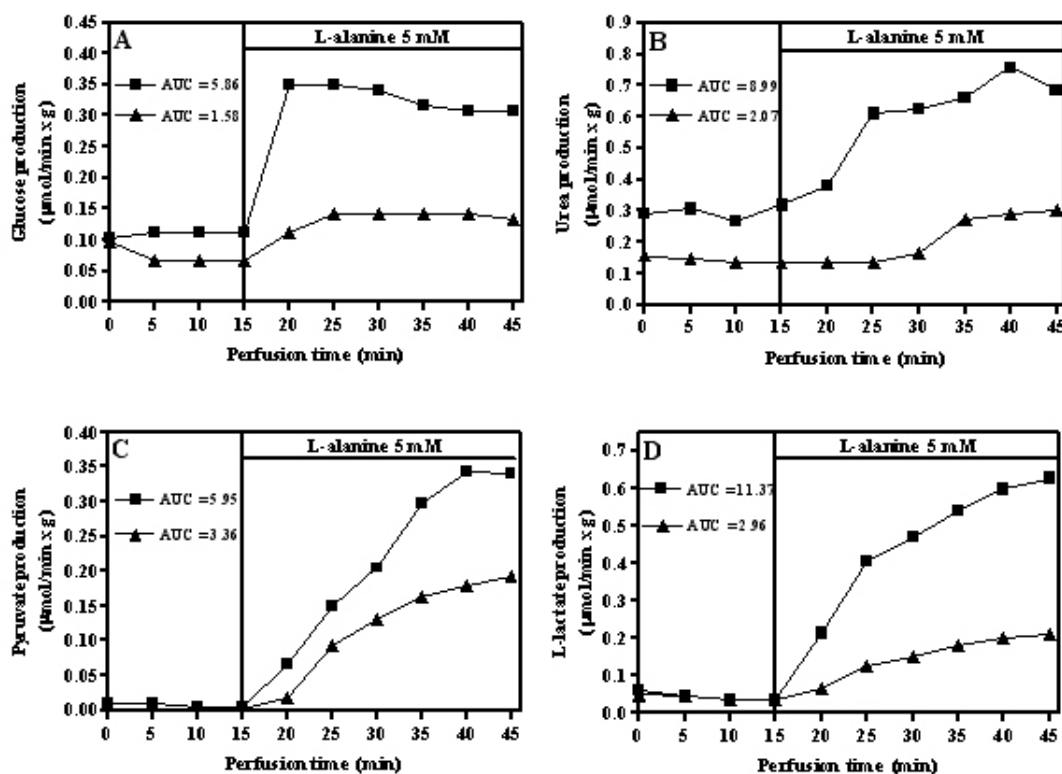

Figure 2. Demonstrative experiment of glucose, urea, pyruvate and L-lactate production from L-alanine in perfused livers of 12 h fasted rats. In these experiments livers from rats that received oral 2,4-dinitrophenol 30 mg/kg dissolved in DMSO (▲) or an equal volume of oral DMSO, i.e., control group (■) were compared. These substances were administered immediately before the anesthesia and liver perfusion experiments. The effluent perfusate was sampled at 5 minutes intervals and used for glucose (A), urea (B), pyruvate (C) and L-lactate (D) determinations. The areas under curves (AUC) were calculated as described in materials and methods.

	Values of AUC (µmol/g)		
	C	**DcM 5**	**DcM 25**
Glucose	4.95 ± 0.55	1.84 ± 0.22***	1.52 ± 0.44***
Pyruvate	2.93 ± 0.29	3.15 ± 0.51	3.22 ± 0.28
L-lactate	3.93 ± 0.90	2.51 ± 0.60	2.27 ± 0.55
Urea	44.39 ± 3.34	41.83 ± 5.11	37.91 ± 2.79

Table 1. Effect of DcM extract of *K. coriacea* stems on glucose, pyruvate, L-lactate and urea production from L-alanine (5 mM) in perfused livers of male rats. Values are mean ± standard error of 4-6 liver perfusion experiments. AUC = area under the curve. *** p < 0.001 *vs* C group (unpaired Student t-test).

	Values of AUC (µmol/g)		
	C	**DcM 5**	**DcM 25**
Glucose	3.71 ± 0.77	2.80 ± 1.13	1.76 ± 0.26*
Pyruvate	5.20 ± 0.57	3.48 ± 0.66	5.25 ± 0.59
L-lactate	5.37 ± 1.29	4.78 ± 1.52	3.89 ± 0.99
Urea	47.93 ± 1.92	45.69 ± 5.92	39.06 ± 1.17**

Table 2. Effect of DcM extract of K. coriacea stems on glucose, pyruvate, L-lactate and urea production from L-alanine (5 mM) in perfused livers of female rats. Liver perfusion experiments were performed as described in Figure 2. Values are mean ± standard error of 4-6 liver perfusion experiments. AUC = area under the curve. * p < 0.05 *vs* C group (unpaired Student t-test); ** p < 0.01 *vs* C group (unpaired Student t-test).

ed. In addition, considering that an antidepressant effect of DcM extract of *K. coriacea* [16] was observed after chronic treatment with 5 mg/kg we investigated if this dose also shows inhibitory effect on hepatic gluconeogenesis. Moreover, for comparative purposes the dose of 25 mg/kg was used. Therefore, hepatic gluconeogenesis from the most important gluconeogenic substrate, i.e., L-alanine [23] in livers from male and female rats treated during 90 days by gavage with DcM extract (5 and 25 mg/kg) were investigated.

L-alanine cross the liver cell membrane and release the amine group for the ureagenesis during its conversion to pyruvate. From the cytosol, pyruvate enters the mitochondria where it is carboxylated and then leaves the mitochondria as aspartate or malate. In the cytosol these compounds are converted to oxaloacetate, then to phosphoenolpyruvate and after several steps they are converted to glucose by microsomal glucose-6-phosphatase and released from the hepatocyte [24]. Since this complex pathway depends on mitochondrial energy metabolism, the production of glucose from L-alanine can be used as a marker of the impact of oral chronic treatment with extract of *K. coriacea* on liver metabolism.

In agreement with the results obtained by Zagoto *et al.* [14] the treatment with DcM extract of *K. coriacea* (5 or 25 mg/kg) also decreased (P < 0.001) the hepatic gluconeogenesis from L-alanine in male (Table 1) rats. But to female rats (Table 2) the inhibition (P < 0.05) of hepatic gluconeogenesis was observed only with 25 mg/kg. Another interesting observation was different liver ureagenesis responsiveness to the *K. coriacea* treatment (25 mg/kg) in female rats, considering that the inhibition of urea production was more intense (P < 0.05) in this group (Table 2) if compared with male rats (Table 1). Moreover, L-lactate and pyruvate production in male (Table 1) and female (Table 2) rats treated with 5 or 25 mg/kg were not different (P > 0.05).

The results obtained are compatible with an inhibition of mitochondrial energetic metabolism enough to generate a significant decrease of glucose production. To confirm this proposition in part of the experiments rats which received oral 2,4-dinitrophenol, a classical mitochondrial uncoupler [25], used here as positive control, showed lower glucose (Figure 2A), urea (Figure 2B), pyruvate (Figure 2C) and L-lactate (Figure 2D) production.

Finally, it must be considered that the inhibitory effect of DcM extract on gluconeogenesis, particularly in male rats was obtained with the same dose (5 mg/kg) necessary to obtain antidepressant effect, i.e., 5 mg/kg [16, 26]. In view fact that DcM extract contain xanthones, triterpenes and biphenyl derivatives [2] the identifica-

tion of the molecule with antidepressant effect and the demonstration of the absence of toxicological effect will be necessary to open the possibility of the development of a new therapeutic compound. Therefore future studies will test new compounds from DcM extract.

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