中国药理学与毒理学杂志 2007 年 12 月; 21(6):476 - 481
Chin J Pharmacol Toxicol 2007 Dec; 21(6):476 - 481

Protective effect of ethyl acetate extract of *Pongamia pinnata* roots on ethanol-induced gastric mucosal injuries in rats

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Abstract: **AIM** To investigate the therapeutic effect of ethyl acetate extract from *Pongamia pinnata* roots (PREA) on ethanol-induced gastric lesions. **METHODS** The experimental gastric mucosal injuries were prepared by ig ethanol to rats, and the protective effect of PREA was evaluated by calculating lesion index, observing pathological changes, and measuring the contents of nitric oxide (NO) and malondialdehyde (MDA), and the activity of superoxide dismutase (SOD) from gastric mucosal tissue. In addition, gastric secretary and gastric wall adherent mucus were studied with the pylorus-ligation rat model. **RESULTS** Compared with the model control group, PREA (50, 150 and 450 $mg \cdot kg^{-1}$, ig) dose-dependently prevented the gastric mucosal damages induced by ethanol, its inhibition rates were 28.7%, 57.7% and 78.7%, respectively. The pathomorphology lesions of mucosal tissue were obviously ameliorated. PREA obviously antagonized the ethanol-induced elevation of MDA content, and reduction of NO level and SOD activity of gastric mucosa. PREA significantly reduced gastric juice volume, free acidity, total acidity and total acid output, but didn't affect the pepsin activity. Moreover, PREA obviously increased adherent mucus quantity of stomach wall, as well as free mucus quantity dissolved in gastric juice of pylorus-ligation rat. **CONCLUSION** PREA has protective effect on ethanol-induced gastric mucosal injuries, which suggests that PREA may be used for protection or treatment of human ethanol-induced gastric lesions.

Key words: *Pongamia pinnata* roots; ethyl acetate; ethanol; gastric mucosa

CLC number: R285
Document code: A
Article ID: 1000-3002(2007)06-0476-06

Ethanol-induced gastric lesion is a common disease in clinics that affects billions of people health in the globe. But there is not a drug that can prevent or treat it thoroughly because of its complex pathogenesis and unknown etiology. Therefore, the screening and development of drugs from Chinese medicinal plants for anti-gastric ulcer induced by ethanol is still in progress. *Pongamia pinnata* (Linn.) Pierre (*P. pinnata*) is a medium sized glabrous tree popularly known as poongaoil pongamia seeded in China. *P. pinnata* grows in the seaside and the bank of rivers and streams of all over Hainan Province of China, and planted as avenue trees in gardens. Different parts of *P. pinnata* have been used in medicine for bronchitis, whooping cough, rheumatic joints and inflammatory diseases. The flavonoids of *P. pinnata* were one of the main chemical ingredients[1]. It was reported that 7 flavonoids were isolated from ethanol extracts by the ethyl acetate extract method, and 70% ethanol extracts had anti-gastric ulcer effects in pylorus-ligated models[2]. In our lab the effective fractions from *P. pinnata* roots on experimental gastric ulcer had been screened and found the ethyl acetate extract from *P. pinnata* roots (PREA) was the effective fraction.

Received date: 2007-01-08 **Accepted date**: 2007-08-10
Foundation item: The project supported by Natural Science Foundation of Hainan Province (30530)

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So in this research the effect of PREA on ethanol-induced gastric mucosal lesions in rats was evaluated.

1 MATERIALS AND METHODS

1.1 Animals and reagents

Male and female Sprague-Dawley (SD) rats, 180 – 220 g, were purchased from the Center of Experimental Animal of Guandong Province. The certificate number is SCXK (Yue) 2003-0002. They were housed in polyprolene cages and maintained normal laboratory conditions at 21 – 23 ℃ on a 12 h light-dark cycle and fed a standard rodent chow and water.

Alcian blue 8GS (F20051221) was purchased from Shanghai Chemical Reagent Company, China National Medicine Group. Detection kits for nitric oxide (NO), malondialdehyde (MDA), superoxide dismutase (SOD) and pepsin were from Nanjing Jiancheng Bioengineering Institute. Sodium carboxymethyl cellulose (CMC-Na) was from Guoyao Chemical Co., batch number F2005-718. Cimetidine (Cim) was from Hainan Drug Manufactory Ltd., batch number 050701.

1.2 Drug preparation

P. pinnata roots were collected in Haikou (Hainan Province, China) in March 20, 2006 and identified by CHENG Guo-Biao, a traditional Chinese materia medica (TCMM) specialist in Institute for Drug Control of Hainan Province, China. The voucher was deposited at Institute for Drug Control of Hainan Province, China. The powder of *P. pinnata* roots (2.41 kg) was recirculated 3 times with 70% ethanol (1∶8, *V/V*) at 60℃, 2 h each time. The ethanol mixtures were filtered and concentrated under reduced pressure at 60℃ with the instrument of turning evaporation. The powdered extract of ethanol extract was 188.0 g, and the yield was 7.8%. The powdered extract was extracted by using sequential petroleum ether and ethyl acetate for 3 times, and then the powdered extract of ethyl acetate (49.4 g), PREA, was obtained after recovering ethyl acetate. The yield of PREA was 2.0%. The main components of PREA were flavonoids, their content was 60.9%, determined by TCMM specialist in Institute for Drug Control of Hainan Province. PREA and Cim were dissolved in CMC-Na (5 g·L^{-1}) to make the suspensions just prior to drug administration.

1.3 Evaluation of gastric mucosa damage

The rats were randomly divided into 6 groups, including normal, model, PREA (50, 150 and 450 mg·kg^{-1}) and Cim (140 mg·kg^{-1}) groups. PREA or Cim was given (ig) to rats once a day for 5 d except the rats of normal and model groups, which were given (ig) 0.5% CMC-Na (15 mL·kg^{-1}) instead of PREA or Cim. All rats except that in normal group were given (ig) 0.8 mL absolute ethanol (99.5%)[4] 2 h after the last administration of drugs. One hour later all the animals were sacrificed, stomachs were removed and opened along the greater curvature to observe the lesions macroscopically. Scoring standard was as follows: intact gastric mucosa was defined as 0; point-shaped bleeding, point-shaped erosion and strip-shaped lesion of no more than 1 mm were all defined as 1; strip-shaped lesion with width more than 1 mm was doubly scored. Total scores of every rat were considered as lesion index. Inhibition ratio of lesion formation (%) = (A – B)/A × 100% (A, B were the lesion index of model group and drug-treated group, respectively).

1.4 Histological analysis

Strip-shaped tissue specimens from gastric antrum were quickly cut off under ice bath after the evaluation of gastric mucosal lesion. The specimens were fixed in 40 g·L^{-1} neutrally buffered formaldehyde, embedded in paraffin. Sections of 5 μm thick were cut, placed on adhesive-coated slides, and stained with hematoxylin and eosin (HE) for histopathological analysis (cooperated with Department of Histology and Embryology).

1.5 Measurements of NO and MDA contents and SOD activity

After the strip-shaped tissue specimens had been cut off from rat stomach, gastric mucous membranes were quickly stripped off from the rest of stomach on ice. Gastric mucosa homogenates of 10% were prepared with ice-cold saline. Homogenates were centrifuged at 2000 × *g* for 10 min at 4℃ and the supernatant was saved for measurement of NO and MDA contents and SOD activity according to the instructions of corresponding kits. Protein content was determined using total protein quantification kit with the Coomassie-blue method.

1.6 Determination of gastric secretion and gastric wall adherent mucus quantity

Grouping and administration of the experimental rats were the same as that of ethanol-induced models, but no normal group. From d 3 of the drug administration the rats were fasted, and 2 h after the fifth drug administration the pylorus of each rat was ligated under ether anesthesia[5]. The rats were kept being fasted and 5 h later the rats were sacrificed. The stomachs were removed and the volume of gastric juice was determined after centrifugation at 2000 × *g* for 10 min. Acidity was assessed by titration against 0.01 mol·L^{-1} NaOH. The activity of gastric pepsin was assessed by amino acid deoxidization method. Adherent mucus quantity of stomach wall and free mucus quantity dissolving in gastric juice were measured by Alcian blue method[6].

1.7 Statistical analysis

The results were expressed as $\bar{x} \pm s$. Statistical analysis was done with SAS system(8.0). Data were analyzed using F-test, and q-test to compare the results between every two groups. $P < 0.05$ was taken as significant.

2 RESULTS

2.1 Effect of PREA on gastric lesions induced by ethanol

In model group the large band like hemorrhagic erosions in the glandular stomach was evident(Fig 1, Tab 1). PREA (50, 150 and 450 mg·kg^{-1}) dose-dependently prevented the

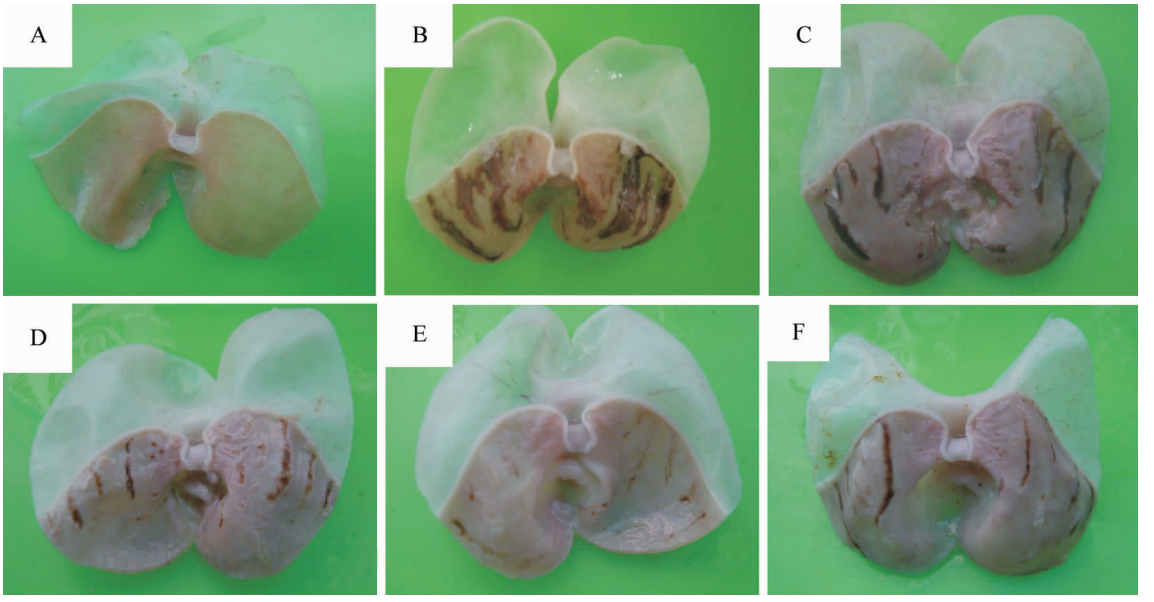

Fig 1. Effect of ethyl acetate extract from *Pongamia pinnata* roots (PREA) on gastric lesions induced by ethanol in rats. Drugs were given (ig) once a day for 5 d. Two hours after the last administration, 99.5% ethanol 0.8 mL was ig to all rats except normal group. One hour later the rats were sacrificed and gastric lesions were observed macroscopically. A: normal; B: model; C: PREA 50 mg·kg^{-1}; D: PREA 150 mg·kg^{-1}; E: PREA 450 mg·kg^{-1}; F: cimetidine (Cim) 140 mg·kg^{-1}.

Tab 1. Effect of PREA on gastric lesions induced by ethanol in rats

Group	Dose/mg·kg^{-1}	Lesion index	Inhibition rate/%
Normal		0 ± 0	
Model		119 ± 19	
PREA	50	85 ± 15 **	28.7
	150	50 ± 19 **	57.7
	450	25 ± 14 **	78.7
Cim	140	37 ± 11 **	69.2

See Fig 1 for the treatments. Total scores of every rat were considered as lesion index. The scoring standard and inhibition rate calculation see MATERIALS AND METHODS 1.3. $\bar{x} \pm s$, n = 8. $^{**}P < 0.01$, compared with model group.

gastric mucosal damages compared with model group. The effect of PREA 450 mg·kg^{-1} was likely stronger than that of Cim 140 mg·kg^{-1}.

2.2 Effect of PREA on histopathological changes

The structure of gastric mucosa from normal group was distinct: intact and uninterrupted epithelia, gastric fundal gland lining up in order and no hyperemia. In the model group, it found the focal and diffuse mucosal epithelia damage, edema, split of epithelial lamina propria of mucosa tissue and irregular gland arrangement. Gastric mucosal capillaries were severely dilated and congested, and a lot of red blood cells were exudated out. However, pathomorphological lesions of mucosa tissue in the groups pretreated by PREA (50, 150 and 450 mg·kg^{-1}) and Cim (140 mg·kg^{-1}) were obviously ameliorated at different degrees. Especially there were only dilated capillaries, slight edema and a small quantity of necrosis tissues in PREA 450 mg·kg^{-1} group.

2.3 Effect of PREA on NO and MDA contents and SOD activity of gastric mucosa

NO content and SOD activity of gastric mucosa homogenates in model group significantly decreased compared with that in normal group. PREA 50, 150 and 450 mg·kg^{-1} dose-dependently inhibited ethanol-induced decrease of NO level and SOD activity. MDA content in gastric mucosa of model group obviously increased compared with that of normal group. PREA 50, 150 and 450 mg·kg^{-1} significantly prevented the increase of MDA content compared with that of model group (Tab 2).

2.4 Effect of PREA on gastric secretion

As shown in Tab 3, PREA (150 and 450 mg·kg^{-1}) significantly reduced the gastric juice, free acidity, total acidity and total acid output. However, all doses of PREA showed no effect on pepsin activity.

Tab 2. Effect of PREA on nitric oxide (NO) and malondialdehyde (MDA) contents and superoxide dismutase (SOD) activity of gastric mucosa damaged by ethanol in rats

Group	Dose/mg·kg^{-1}	NO /μmol·g^{-1} protein	MDA /mmol·g^{-1} protein	SOD activity /mmol·min^{-1}·g^{-1} protein
Normal		3.5 ± 1.2	2.9 ± 0.6	43.8 ± 8.8
Model		2.5 ± 0.7 *	6.1 ± 1.7 **	29.1 ± 10.3 **
PREA	50	4.0 ± 1.3 $^{\#}$	3.7 ± 1.0 $^{\#\#}$	40.8 ± 9.4 $^{\#}$
	150	5.2 ± 1.4 $^{\#\#}$	2.8 ± 0.5 $^{\#\#}$	52.1 ± 12.8 $^{\#\#}$
	450	5.6 ± 1.6 $^{\#\#}$	2.4 ± 0.3 $^{\#\#}$	63.0 ± 13.5 $^{\#\#}$
Cim	140	4.8 ± 1.2 $^{\#\#}$	3.0 ± 0.8 $^{\#\#}$	46.4 ± 12.1 $^{\#\#}$

See Fig 1 for the treatments. $\bar{x} \pm s$, n = 8. $^{*}P < 0.05$, $^{**}P < 0.01$, compared with normal group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, compared with model group.

Tab 3.　Effect of PREA on gastric secretion in pylorus-ligation rats

Group	Dose/ mg·kg^{-1}	Gastric juice /mL	Free acidity /mmol·L^{-1}	Total acidity /mmol·L^{-1}	Total acid output /mmol·h^{-1}	Pepsin activity /mg·min^{-1} · L^{-1}
Model		6.2 ± 0.9	84 ± 9	112 ± 15	0.14 ± 0.03	46 ± 10
RREA	50	5.9 ± 1.0 *	81 ± 13	107 ± 13	0.12 ± 0.03	45 ± 9
	150	5.2 ± 0.7 *	56 ± 12 **	81 ± 16 **	0.08 ± 0.01 **	43 ± 12
	450	4.6 ± 1.2 **	37 ± 12 **	67 ± 15 **	0.06 ± 0.03 **	45 ± 10
Cim	140	3.7 ± 1.0 **	41 ± 9 **	68 ± 14 **	0.05 ± 0.02 **	33 ± 8 *

The treatments were the same as Fig 1 except the pylorus-ligation rat model used. $\bar{x} \pm s$, $n = 8$. * $P < 0.05$, ** $P < 0.01$, compared with model group.

2.5　Effect of PREA on gastric mucus secretion in pylorus-ligation rats

Tab 4 showed that PREA (150 and 450 mg·kg^{-1}) significantly increased adherent mucus quantity of stomach wall. PREA 450 mg·kg^{-1} also increased free mucus quantity dissolving in gastric juice.

Tab 4.　Effect of PREA on gastric mucus secretion in pylorus-ligation rats

Group	Dose/ mg·kg^{-1}	Adherent mucus of stomach wall/mg	Free mucus dissolving in gastric juice/mg
Model		0.73 ± 0.27	0.54 ± 0.11
RREA	50	0.77 ± 0.25	0.57 ± 0.09
	150	1.04 ± 0.27 *	0.59 ± 0.10
	450	1.21 ± 0.19 **	0.64 ± 0.06 *
Cim	140	0.77 ± 0.19	0.56 ± 0.05

See Tab 3 for the treatments. $\bar{x} \pm s$, $n = 8$. * $P < 0.05$, ** $P < 0.01$, compared with model group.

3　DISCUSSION

Ethanol-induced gastric mucosal damage in rats is often used as an experimental model when screening drugs for anti-ulcer activity because it represents the most common cause of gastric ulcer in man. Studies in the past have shown that the 70% ethanol extracts from *P. pinnata* roots prevented gastric mucosa lesion with pylorus ligated models[3]. Prabha *et al*[7] reported that methanolic extract of *P. pinnata* roots showed significant protection against gastric mucosal damage induced by aspirin, but not against ethanol-induced gastric ulceration. In this study it was demonstrated that PREA significantly decreased ethanol-induced gastric mucosa lesion index and obviously relieved pathological changes. These results did not agree with the results of Prabha *et al*. It was possible that the method of building model and the drug dosage were different.

Endogenous NO has a dual action in the gastrointestinal tract: protective effect by constitutive nitric oxide synthase (eNOS)/NO and proulcerogenic effects by iNOS/NO[8]. Therefore, it was suggested that decreasing of NO content was closely related to gastric mucosal injury. Our experiment results showed that PREA previous administration could antagonize the decrease of NO level induced by ethanol. Gastrointestinal mucosal can produce a great amount of oxygen-derived free radicals, which have high toxicity, when gastric mucosa undergoes chemical substance stimulation, ischemia, and disorders of energy metabolism as well as neurohumor regulation[9]. Gastric mucosa is damaged by the oxygen-derived free radicals by the products of lipid peroxidation, such as MDA. SOD is the first line of defense against oxygen-derived free radicals. Our results showed that MDA contents obviously increased and SOD activity significantly decreased in ethanol-induced gastric mucosa and PREA obviously antagonized the changes.

Ethanol-induced gastric mucosal injury has been also suggested to be due to impairments in

defensive factors such as gastric mucus[10]. When tested on pylorus-ligated rats, PREA increased gastric mucus content, inhibited gastric secretary volume and reduced free acidity and total acidity, which suggested its modulation on gastric secretion. However, PREA did not have any significant effect on gastric pepsin activity.

In conclusion, PREA has protective effects on ethanol-induced gastric mucosal injuries and may be useful in the protection or treatment of gastric damage by ethanol in man. However, further studies are needed to clarify the exact mechanism of gastric protective effect of PREA.

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水黄皮根乙酸乙酯萃取物对大鼠乙醇型胃黏膜损伤的保护作用

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摘要: 目的　研究水黄皮根乙酸乙酯萃取物(PREA)对乙醇致胃黏膜损伤的治疗作用。**方法**　建立乙醇致大鼠胃黏膜损伤模型,通过观察胃组织病理学改变、计算胃黏膜损伤指数、检测胃黏膜组织一氧化氮(NO)、丙二醛(MDA)含量和超氧化物歧化酶(SOD)活性评价 PREA 对乙醇型胃黏膜损伤的保护作用。采用幽门结扎模型,观察 PREA 对大鼠胃液分泌和胃黏液分泌的影响。**结果**　与模型组比较,PREA 可剂量依赖性地降低乙醇所致胃黏膜损伤指数,明显改善胃黏膜损伤的病理变化,抑制乙醇引起的胃黏膜 MDA 含量升高及 NO 水平和 SOD 活性降低,并显著减少胃酸分泌、抑制游离胃酸酸度和总酸度,对胃蛋白酶活性没有明显影响。另外,可显著抑制幽门结扎模型大鼠胃腔游离黏液以及胃壁结合黏液的分泌。**结论**　PREA 对乙醇型胃黏膜损伤具有明显的保护作用,提示 PREA 可能成为预防或治疗乙醇所致胃损伤的药物。

关键词: 水黄皮根; 乙酸乙酯; 乙醇; 胃黏膜

基金项目: 海南省自然科学基金资助项目(30530)

(本文编辑　齐春会)