Effect of *Cassia nomame* on Small Intestine Movement, Diuresis, and Anti-inflammation in Rats

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Abstract: **Objective** To investigate the preliminary pharmacological screening of *Cassia nomame*. **Methods** The effect of aqueous extract from *C. nomame* on gastrointestinal motor function was investigated by assessing the intestinal transit rate (ITR) of charcoal modeled into gastrointestinal motility dysfunction (GMD) by the administration of dopamine, atropine, or noradrenaline to the rats, respectively. Diuresis was studied *in vivo* by estimating the urine output. The anti-inflammatory activity was expressed as the percentage of swelling reduction by comparison on the mean thickness of ear swelling in mice. **Results** The ITR in these GMD animals was significantly retarded compared to that in normal animals. The retardation, however, was significantly inhibited by the ig administration of *C. nomame* (2 g/kg) for all GMD animals. The results suggested that *C. nomame* had the potential for development into a prokinetic agent that could prevent or alleviate GMD in patients. *C. nomame* increased urine output and suppressed significantly ear swelling induced by dirnethyl benzene in mice. **Conclusion** *C. nomame* could increase the gastrointestinal contractile activity of rats and has the effects of diuresis and anti-inflammation.

Key words: *Cassia nomame*; diuresis; ear swelling; gastrointestinal motility dysfunctions; intestinal transit
DOI: 10.1016/S1674-6384(13)60038-6

Introduction

Cassia nomame (Sieb.) Kitag. (CN) is an annual plant in the Legume family, and it is also used as a raw material for a diuretic, constipation, nephritis, or antidote in a traditional remedy. Especially, it is used to treat postpartum women with constipation. Hatano *et al* (1997) reported the flavan dimers and related phenolic constituents isolated from CN had the lipase-inhibitory activity. With a view to find the pharmacological rationale for these reports and traditional uses of CN, the aqueous extract from CN was evaluated for its effect on gastrointestinal transit, diuresis, and anti-inflammation in the present study.

Materials and methods

Plant materials and extract preparation

The whole herb of *Cassia nomame* (Sieb.) Kitag. was collected in August 2010 from Liaoning province, China, and identified by Prof. WANG Wei-ning, Liaoning Institute for Food and Drug Control. The sample of plant was deposited in our laboratory for future use. CN (2 kg) was boiled in distilled water at 100 ℃ for 2 h, and the aqueous extract was filtered, concentrated in vacuo, and lyophilized to give a powder, and the yield of extract was about 20.4%.

Animals

All animals used in the study were obtained from the Animal Center, the China Medical University. The SD rats weighing 150—200 g and Kunming mice weighing 25—30 g were used in the study, respectively. The animals were reared under the standard laboratory conditions [(22 ± 2) ℃, (60 ± 10)% relative humidity, and 12-h light-dark cycle] and had free access to food and water throughout the experiment.

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Received: April 18, 2013; Revised: May 28, 2013; Accepted: June 12, 2013

Dopamine-induced gastrointestinal motility

Fifteen rats were starved for 18 h and divided into control, model, and CN + Dopamine (DA) groups. In the CN + DA group, the rats were ig administered with CN (2 g/kg) once daily for 5 d. In the control and model groups, distilled water (10 mL/kg, once a day) was given instead of CN for 5 d. This experiment was carried out from the day 6 by the method of Chatterjee (1993). DA was used to induce the gastrointestinal motility of the rats. In the model and CN + DA groups, DA (ip 0.2 mg/kg) was given 40 min after the administration of CN or distilled water. Then 2 mL of charcoal meal (10% charcoal) was given orally to each rat. Twenty minutes later, the animals were killed by cervical dislocation and the abdomens were opened. The distance traveled by the charcoal meal from the pylorus to the caecum was measured and expressed as the percent of the total length (Devi, Boominathan, and Mandal, 2002).

Atropine-induced gastrointestinal motility

This experiment was carried out in the same way as described above for DA-induced gastrointestinal motility whereas atropine (1 mg/kg) was used instead of DA to reduce the gastrointestinal motility.

Noradrenaline-induced gastrointestinal motility

This experiment was carried out in the same way as described above for DA-induced gastrointestinal motility whereas noradrenaline (NA, 1 mg/kg) was used instead of DA to reduce the gastrointestinal motility.

Diuresis

This experiment was carried out in the same way as described above for the loss of righting reflex test. Prior to diuresis testing, distilled water (30 mL/kg) was ig given to each mouse. Then each mouse was administered with either water or 2 g/kg CN. Urine was collected over 2 h in stainless steel pans located beneath the grid floor of the chamber. All rats were normally hydrated at the beginning of the session, but neither food nor drinking water was available during the session.

Dimethylbenzene-induced ear swelling in mice

The mice were randomly divided into two groups (n = 10), and the mice in each group was ig administered once a day for 5 d with vehicle (control) or CN (2 g/kg), respectively. Swelling was induced on the inner surface of the right ear by topical application of dimethylbenzene (25 μL/ear) 1 h after the last administration. Thirty minutes after swelling induction, mice were sacrificed and the plugs (8 mm diameter) were removed from both treated (right) and untreated (left) ear at the same location. Swelling was measured as weight difference between the plug from treated (right) and untreated (left) ears in the same mouse. Anti-inflammatory activity was expressed as percentage of swelling reduction by comparing ear swelling in the treatment group with the control group.

Light microscopy assay

The ears were fixed in 4% paraformaldehyde (PFA) for 24 h. The samples were incubated in 50% ethanol for 1 h, routinely dehydrated and embedded, and the sagittal sections (4 μm thick) were cut. The slices were dewaxed, and stained with haematoxylin and eosin (HE) for histological examination. The HE-stained tissue slices were examined under the optical microscopy (Olympus, BX60 Japan) at 100-fold magnification.

Statistical analyses

The values were expressed as $\bar{X} \pm s$. Normal distribution of all the data was examined using the Shapiro-Wilk normality test. One-way ANOVA was used followed by Fisher's least-significant difference (LSD) for the homogeneity testing of variance (Levene's test), and the data were analyzed by Dunnett's T3 for the heteroschedasticity of variance test. $P < 0.05$ was considered statistically significant. All the statistical procedures were performed using SPSS 13.0 software for Windows (SPSS Inc., USA).

Results

Effect of CN on DA-induced gastrointestinal motility in rats

The movement of charcoal meal was significantly retarded in the DA group compared to the control group. CN significantly inhibited the retardation of the movement of charcoal meal in the DA-induced GMD (Table 1).

Effect of CN on atropine-induced gastro-intestinal motility in rats

The movement of charcoal meal was significantly retarded in the atropine group compared to the control group. CN significantly inhibited the retardation of movement of charcoal meal in the atropine-induced GMD (Table 2).

Table 1 Effect of CN on DA-induced gastrointestinal motility in rats ($\bar{X} \pm s$, $n = 5$)

Groups	Dose / (mg·kg^{-1})	Movement of charcoal meal / %	Inhibitory rate / %
control	—	52.0 ± 1.30	—
DA	0.2	14.8 ± 1.36***	71.54
CN + DA	2000	26.2 ± 2.56$^{\#\#\#}$	49.62

$^{***}P < 0.001$ *vs* control group; $^{\#\#\#}P < 0.001$ *vs* DA group

Table 2 Effect of CN on atropine-induced gastrointestinal motility in rats ($\bar{X} \pm s$, $n = 5$)

Groups	Dose / (mg·kg^{-1})	Movement of charcoal meal / %	Inhibitory rate / %
control	—	53.4 ± 0.93	—
atropine	1	18.6 ± 9.11***	65.17
CN + atropine	2000	53.2 ± 1.83$^{\#\#\#}$	0.37

$^{***}P < 0.001$ *vs* control group; $^{\#\#\#}P < 0.001$ *vs* atropine group

Effect of CN on NA-induced gastrointestinal motility in rats

The movement of charcoal meal was significantly retarded in the NA group compared to the control group. CN significantly inhibited the retardation of the movement of charcoal meal in the NA-induced GMD (Table 3).

Table 3 Effect of CN on NA-induced gastrointestinal motility in rats ($\bar{X} \pm s$, $n = 5$)

Groups	Dose / (mg·kg^{-1})	Movement of charcoal meal / %	Inhibitory rate / %
control	—	52.4 ± 0.68	—
NA	1	5.80 ± 0.49***	88.93
CN + NA	2000	21.00 ± 0.95$^{\#\#}$	59.92

$^{***}P < 0.001$ *vs* control group; $^{\#\#}P < 0.01$ *vs* NA group

Effect of CN on diuresis in mice

Administration of 2 g/kg CN caused large increases in urine output of rats in CN groups, which is significantly greater than that in the control group (Fig. 1).

Inhibition on dimethylbenzen-induced ear swelling in mice by CN

CN (2 g/kg) revealed the significant inhibition ($P < 0.05$) on ear swelling in mice induced by dimethylbenzene compared with the control group (Fig. 2).

Morphological observation of CN on dimethylbenzen-induced ear swelling in mice

Histological assessment was used to estimate CN on dimethylbenzen-induced ear swelling in mice. The ear sections of the control mice (untreated) showed ear tissue swelling and gap widened. The ear sections of CN-treated mice revealed that the tissue space is very narrow and even into a narrow striated muscle fiber. The histopathological ear lesions were markedly ameliorated by pretreatment with CN (Fig. 3).

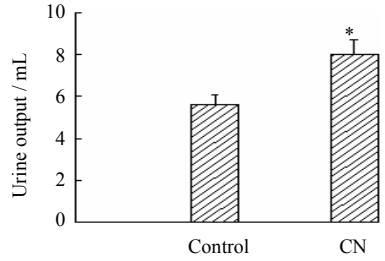

Fig. 1 Effect of CN on urine output

$^{*}P < 0.05$ *vs* control group

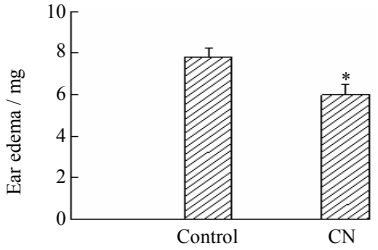

Fig. 2 Anti-inflammatory effects against dimethylbenzene-induced ear swelling in mice ($\bar{X} \pm s$, $n = 10$)

$^{*}P < 0.05$ *vs* control group

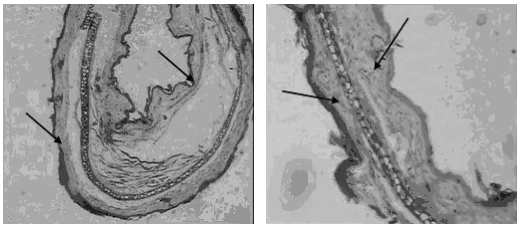

Fig. 3 Morphology of CN on dimethylbenzen-induced ear swelling in mice

Representative photographs of ear sections stained with HE showing the pathological changes in ear tissues under microscopy. Arrow indicates striated muscle fiber

Discussion

CN belongs to genus *Cassia* Linn., and its chemical constituents are similar to those of *C. mimosoides* L. var. *nomame* Makino. The constituents of *C. mimosoides* var. *nomame* are including anthraquinones, flavonoids, catechins, and so on (Wang *et al*, 2000; Hatano *et al*, 1997).

In the present studies, DA, atropine, and NA, which are known to be DA agonists, muscarinic receptor antagonists, and α-adrenergic receptor agonists, respectively (Yamazaki *et al*, 2000; Suchitra *et al*, 2003), have been utilized as pharmacological means to

inhibit GI motor functions. DA is an important neurotransmitter that its diverse inhibitory action to the dynamia in alimentary tract is mainly due to blocking acetylcholine release from neuron by the DA receptor of later ganglion cholinergic neuron (Guo and Ke, 2001). Studies showed that bound anthraquinones in *Rheum palmatum* L. were hydrolyzed by colorectal bacteria into free aglycones to stimulate the intestinal mucosa and plexus of intestinal muscle layers and to cause the purgative effect, and the anthracenones had the acetylcholine-like activity to activate muscarinic receptor to cause the peristaltic activity of intestine (Wu *et al*, 1983; Zhou *et al*, 1983). We found that CN could inhibit the intestinal transit rate (ITR) retardation. Therefore, muscarinic receptors, α-receptors, and DA receptors might be associated with the pharmacological action of CN. We also speculated that the bound anthraquinones in CN caused the same effects as the bound anthraquinones of *R. palmatum* stimulated the peristaltic activity of intestine and had the purgative effect.

Our studies also revealed that CN increased the urine output and caused the diuretic actions, some studies found that the diuretic effects of rhein, emodin, and aloe rhein might inhibit Na, K-ATPase activity (Zhou and Chen, 1988), and the result was consistent with that of CN. Our pharmacological studies on the extract from CN provide in part the scientific support for the traditional usage of *C. nomame*, particularly about gastrointestinal motility and diuretic actions.

Many natural ingredients, such as flavonoids and catechins, have the anti-inflammatory effect. For example, the flavoniods extracted from *Scutellaria baicalensis* Georg have the anti-inflammatory effect and the polyphenols-rich extract from tea has the anti-inflammatory effect (Yang *et al*, 2012; Chen *et al*, 2012). The current studies showed that CN could inhibit the ear swelling induced by dimethylbenzene due to the effective ingredients in CN.

In summary, our study demonstrated that CN could accelerate ITR in DA-, atropine-, and NA-treated rats, respectively, and showed the diuretic actions and anti-inflammation in mice. Further pharmacological investigations are required to demonstrate its action mechanisms, as well as future bioactivity-guided phytochemical work to identify its active constituents.

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