Evaluation of Gastroprotective Potential of the Ethanol Extract From *Murdannia loriformis* in rats

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**Summary.** *Murdannia loriformis* (ML) has been used in traditional medicine for the treatment of various diseases. The objective of this study was to examine gastroprotective activity of the ethanol extract of ML. For the gastroprotective study, rats received ML extract (100-400 mg/kg) via oral route before induction of gastric ulcer using three different inducer models; EtOH/HCl, indomethacin, and restraint water immersion stress. ML extract significantly inhibited gastric ulcer formation induced by EtOH/HCl (p<0.001), indomethacin (p<0.01) and stress (p<0.001). Like misoprostol, ML extract, increased the amount of mucus content in gastric wall mucus assay (p<0.001). Moreover, the extract was equivalent to cinetidine for reducing gastric acid secretion in pylorus ligation model (p<0.05). Therefore, all of these results indicated that ML extract possessed an effective gastroprotective effect via promoting mucus production and inhibiting gastric acid secretion.

**Industrial relevance.** In recent years, Thai government has had a policy for medicinal plant research and development in order to support the alternative health care. *M. loriformis* has long been used to treat a broad spectrum of disorders. The ethanol extract of this plant has already been proved to possess anti-inflammatory, analgesic, and antipyretic activities in rats without ulcerogenic effect. This research aims to investigate the gastroprotective activity of the ethanol extract of ML. The results from this study might give useful information in supporting one of its folklores uses.

**Keywords.** *Murdannia loriformis*; ethanol extract; gastroprotective activity; gastric ulcer; rat models

**INTRODUCTION**

Gastric and duodenal ulcers are the types of peptic ulcer disease (PUD), which occur in the gastrointestinal tract. The risk factors of PUD are *Helicobacter pylori* infection, non-steroidal anti-inflammatory drugs (NSAIDs), cigarette smoking, alcohol abuse and increasing of age (Everhart et al., 1998; Ramakrishnan and Salinas, 2007). Among these, the most important factors are *H. Pylori* infection and NSAIDs using (Hawkey, 1996; Hippisley-Cox et al., 2005; Marcus et al., 2013; Musumba et al., 2009; Papatheodoridis et al., 2006; Ramakrishnan and Salinas, 2007).

NSAIDs are widely used in many patients to relieve inflammation, especially in chronic inflammatory diseases such as osteoarthritis (OA) and rheumatoid arthritis (RA). Prolonged use of these drugs may produce many side-effects such as gastric erosion, peptic ulcer and bleeding (Musumba et al., 2009). Therefore, medicinal plants possessed anti-inflammatory activity and without mucosa side-effect will be a valuable substitute agent for patients suffering from arthritis.

*Murdannia loriformis* (Hassk) Rolla Rao et Kammathy is a herb in the family Commelinaeaceae (Figure 1). In Thailand, it is called “Ya Pak King”, and widely used for treating chronic bronchitis and as a remedy for cancers in early stage as well as other diseases including cold, throat infections, pneumonia, flu and to relieve inflamed wound (Saralamp et al., 1996). The previous studies reported that, *M. loriformis* possesses anti-mutagenic, anti-proliferative, antitumour and immunomodulator activity (Intiyot et al., 2002; Jiratchariyakul et al., 2006; Koontongkaew et al., 2009; Rearungchom, 1993; Viniksetkumneun et al., 1996) and stimulated T-lymphocyte proliferation (Jiratchariyakul et al., 2006). The phytochemical study revealed that, *M. loriformis* contains phytosterol glucoside (G1a), glycosphingolipid (G1b), amino acid, flavonoids, plant membrane lipid (Jiratchariyakul et al., 1996; Jiratchariyakul et al., 1998), syringic acid, and isovitexin (Jiratchariyakul et al., 2006). The glycosphingolipid compound, 2,β-O-D-glucopyranosyl- 2-(2’-hydroxy-Z-6’-eneosamide) sphingosine, is also found in the ethanol extract of *M. loriformis*. This compound showed immunomodulatory effect and increase the expression of CD 3,4 molecules in T lymphocytes. (Jiratchariyakul et al., 2006). Moreover, the study using in vivo experiments revealed that *M. loriformis* exhibited anti-inflammatory, antipyretic and analgesic effects in rat models (Somja, 2005). Interestingly, *M. loriformis* showed anti-inflammatory activity without side effect on gastric mucosa.

The screening for anti-gastric ulcer activity found that *M. loriformis* reduced gastric ulcer formation in ETOH/HCl-induced gastric ulcer model. Therefore, it is interesting to evaluate whether *M. loriformis* is effective to prevent gastric ulcer. The objective of this study was to investigate gastric ulcer protective activity of the ethanol extract from *M. loriformis*. 

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Figure 1: *Murdannia loriformis* (Hassk.) Rolla Rao et Kammathy. Source photo: www.healthdd.info

**MATERIALS AND METHODS**

**Drugs, chemicals, and reagents.** Indomethacin (99%) and cimetidine were purchased from Sigma Chemical Company (St. Louis, U.S.A.). Misoprostol was purchased from Pfizer, Thailand. Absolute ethanol was purchased from Merck, Germany. All other reagents used in this experiment were of analytical grade. All test drugs and reagents were freshly prepared before use.

**Preparation of the ethanol extract from *M. loriformis*.** The powder of dry whole plant was purchased from Abhaibhubejhr's hospital, Prachinburi, Thailand. The plant material was authentic and it was proved to be identical to the voucher specimen (QBG. No. 25135) which was kept in the herbarium section of Queen Sirikit Botanical Garden. The ethanol extract, designated as ML extract, was prepared as follows: 1 kg of *M. loriformis* was overnight macerated in 10 liters of 80% ethanol at room temperature. The extraction process was repeated 3 times. The ethanol extract was filtered through Whatman’s filter paper No. 4. After filtration of extract, pooled and concentrated under reduced pressure condition (using a rotating evaporator, 55°C) and lyophilized. The yield of the ethanol extract was 14.1% of dry powder.

**Laboratory Animals.** Male Sprague-Dawley rats weighing between 250-300 g were obtained from the National Laboratory Animal Center, Mahidol University, Salaya, Nakorn Pathom, Thailand. All rats were kept in an animal room maintained under environmentally controlled conditions of 24 ± 1°C and 12 h light-12 h dark cycle. They were free access to drinking water and standard pelleted diet (082 C.P. MICE FEED, S.W.T. Co., Ltd., Samut Prakan, Thailand). The rats were acclimatized at least one week before starting the experiments. All rats used in this study were starved for 48 h but allowed free access to water. The water was withdrawn 1 h before starting all of experiments. All procedures were approved by the Animal Ethics Committee of the Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand (NO. 08/2556).

**Gastroprotective activity studies.** In the models of ethanol/hydrochloric acid (EtOH/HCl) and indomethacin-induced gastric ulceration, the rats were divided into six groups of six rats, while in stress-induced gastric ulcer model, the rats were divided into five groups of six rats as follows:

<table>
<thead>
<tr>
<th>Group (n=6/group)</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Models: EtOH/HCl- and indomethacin-induced gastric ulcer</strong></td>
<td></td>
</tr>
<tr>
<td>Gr. 1 Control group</td>
<td>vehicle</td>
</tr>
<tr>
<td>Gr. 2 Reference group (1)</td>
<td>cimetidine at the dose of 100 mg/kg</td>
</tr>
<tr>
<td>Gr. 3 Reference group (2)</td>
<td>misoprostol at the dose of 0.1 mg/kg</td>
</tr>
<tr>
<td>Gr. 4-6 Treated groups</td>
<td>ML extract at the doses of 100-400 mg/kg</td>
</tr>
</tbody>
</table>

| Model: restraint water immersion stress-induced gastric ulcer |
| Gr. 1 Control group | vehicle |
| Gr. 2 Reference group | cimetidine at the dose of 100 mg/kg |
| Gr. 3-5 Treated groups | ML extract at the doses of 100-400 mg/kg |

All test substances were orally administered in an equal volume of 5 mL/kg body weight of the rats. The control groups received only vehicle in the same volume and same route of administration.

**EtOH/HCl-induced gastric ulcer.** The model EtOH/HCl-induced gastric lesion is commonly used to determine anti-gastric ulcer activity involving gastric mucosal protective factors. In this model, HCl causes severe damage to gastric mucosa, while ethanol produces necrotic lesions by direct necrotizing action leading to reduce defensive factors. It has been gastric cytoprotective agents are able to prevent ulcer formation in this model (Araki et al., 2000; Glavin and Szabo, 1992). One hour after test substances administration, the rats were orally administered with 1.0 mL HCl/EtOH (60 mL EtOH + 1.7 mL HCl + 38.3 mL H2O) (Mizui and Doteuchi, 1983) to induce gastric ulceration. One hour later, the rats were sacrificed with an overdose of ether. The stomachs were removed and determination of gastric ulcer.

**Indomethacin-induced gastric ulcer.** Indomethacin causes gastric ulceration by inhibiting COX enzyme resulting in the decrease of cytoprotective PG production (Selling et al., 1987; Vane, 1971). The gastric lesions in this model can be prevented by proton pump inhibitors (Cavallini et al., 2006), acid anti-secretory agents (Kurata et al., 1992) and gastric cytoprotective agents (Wilson, 1987). In this experiment, indomethacin [in 0.5% carboxymethylcellulose (CMC)] at the dose of 100 mg/kg was used to induce gastric damage (Djahanguiri, 1969; Nwafor et al., 2000). One hour after test drugs administration, the rats were orally administered with indomethacin. Five hours later, they were sacrificed with an overdose of ether and the stomach was removed for of gastric ulcer determination.

**Restraint water immersion stress-induced gastric ulcer.** This model has been widely accepted for studying stress ulcer (Takagi et al., 1964; Uramoto et al., 1990). The pathogenic mechanisms of stress-induced gastric mucosal lesions include
disturbance of gastric mucosal microcirculation (Kitagawa et al., 1979), abnormal gastric motility (Watanabe, 1966), and increase gastric secretion (Brodie et al., 1962). It has been reported that anti-secretory agents are capable to prevent stress-induced gastric ulceration in rats (Kitagawa et al., 1979). In this model, the rats were induced gastric ulcer by restrained individually in stainless steel cages and immersed up to their xiphoid in a water bath maintained at 22 ± 2°C. After 5 h of this exposure, the rats were sacrificed with an overdose of ether the stomach was removed for determination of gastric ulcer (Takagi et al., 1964).

**Evaluation of gastric ulcer.** The gastric lesions were assessed as previously described method (Chattopadhyay et al., 2006). The length in millimeter of lesion was determined by measuring each lesion along its greater diameter using a binocular magnifier (10X). An ulcer index (UI) was the sum of the total length in each group divided by the number of rats in that group.

**Gastric visible mucus secretion.** The rats were divided into 6 groups of normal rat, ML 400 mg/kg, control, misoprostol 0.1 mg/kg, cimetidine 100 mg/kg and ML 400 mg/kg respectively. The rats in groups one and two were not induced gastric damage. These two groups were set to study the effect of ML extract on gastric mucus production compared with normal group. The remaining four groups were induced gastric ulceration by EtOH/HCl. Gastric wall mucus was determined using the Alcian blue method (Corne et al., 1974). The mucus levels were quantified using Alcian blue standard curve. The results were expressed as microgram Alcian blue/g stomach.

**Pylorus ligation.** The rats were divided into three groups of control, cimetidine 100 mg/kg and ML 400 mg/kg. One hour after test substance administration, pylorus ligation was performed as the method described by Shay (Shay, 1945). Briefly, rats were lightly anesthetized by ether. The abdomen was opened and the pylorus was ligated. The abdomen was closed by suturing. The rats were sacrificed 5 h later by an overdose of ether. The stomach was removed and its content was subjected to measurement of total gastric juice volume, gastric pH and total acid output. The gastric juice was centrifuged and the total acidity of the supernatant was determined by titration with 0.1 N NaOH using phenolphthalein as an indicator.

**Statistical analysis.** The data from the experiments were expressed as mean ± standard error of mean (S.E.M). Statistical comparison between groups were analyzed by using one way ANOVA and post hoc least-significant difference (LSD) test and p values less than 0.05 were considered significant.

**RESULTS**

**Effect of ML extract on EtOH/HCl-induced gastric ulcer in rats.** EtOH/HCl administration caused severe gastric mucosal damage and hemorrhage. The UI of the control group was 84.2 ± 11.83 mm. Misoprostol and cimetidine significantly reduced the UI to 1.4 ± 0.86 and 25.4 ± 3.56 mm, respectively (p<0.001). Likewise, the ML extract significantly inhibited gastric ulcer formation in a dose dependent manner. The UI of ML extract treated groups (100, 200, and 400 mg/kg) were 31.4 ± 3.88, 12.4 ± 7.28, and 2.7 ± 2.22 mm, respectively (p<0.001) (Figure 2-3).

**Figure 2.** Effect of ML extract on EtOH/HCl-induced gastric ulcer in rats. Values are expressed as mean ± S.E.M. (n=6). ***p<0.001 compared with the control group.
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**Figure 3:** Macroscopic photographs of rat stomach with the acute gastric ulcer lesions induced by EtOH/HCl.

**Effect of ML extract on indomethacin-induced gastric ulcer.** The petechiae lesions in stomach were found after oral administration of indomethacin. The UI of the control group was 7.02 ± 2.03 mm. Misoprostol and cimetidine showed gastric ulcer inhibitory effect in this model. The misoprostol and cimetidine treated groups showed the UI values of 1.58 ± 0.74 mm and 0.80 ± 0.48 mm, respectively. The ML extract also dose dependently and significantly inhibited gastric ulcer formation in this model. The UI of the ML extract at the high dose was 1.32 ± 0.57 mm, comparable to that of the misoprostol group (Figure 4-5).

**Figure 4:** Effect of ML extract on indomethacin-induced gastric ulcer in rats. Values are expressed as mean ± S.E.M. (n=6). *p<0.05, **p<0.01 compared with the control group.
Figure 5: Macroscopic photographs of rat stomach with the acute gastric ulcer lesions induced by indomethacin.

Effect of ML extract on restraint water immersion stress-induced gastric ulcer. In this model, hemorrhagic form of lesions was found in the glandular part of the stomach of the rats. The UI of control rats was 11.15 ± 0.72 mm. Cimetidine 100 mg/kg significantly reduced the UI in rats to 1.27 ± 0.35 mm ($p<0.001$). The UI of ML extract treated groups (100, 200, and 400 mg/kg) were 4.65 ± 1.05, 3.43 ± 1.04, and 2.70 ± 0.50 mm, respectively. Thus, the ML extract significantly inhibited gastric ulcer formation when compared with the vehicle control group ($p<0.001$) (Figure 6-7).

**Figure 6.** Effect of ML extract on restraint water immersion stress-induced gastric ulcer in rats. Values are expressed as mean ± S.E.M. (n=6). ***$p<0.001$ compared with the control group.
Effect of ML extract on gastric visible mucus secretion. The results of gastric wall mucus secretion is shown in Figure 8. Gastric wall mucus in the normal rats was 25.08 ± 0.99 µg alcian blue/g wet stomach. ML extract alone did not change gastric wall mucus level when compared with the normal rat. Oral administration of EtOH/HCl significantly decreased gastric wall mucus in the control rats when compared with the normal rats (p<0.05). ML extract (400 mg/kg) and misoprostol (0.1 mg/kg) significantly increased gastric wall mucus to 33.10 ± 1.88 and 32.65 ± 3.67 µg alcian blue/g wet stomach, respectively when compared with the control group (p<0.001). In this model, cimetidine was not effective in increasing gastric wall mucus level.

Effect of ML extract on pylorus ligation. The pylorus ligation caused the decrease of gastric pH from gastric acid secretion as observed in the control group (Table 1). Cimetidine, the reference drug, significantly reduced gastric volume and total acidity as well as increased gastric pH when compared with those of the control group (p<0.01). ML extract (400 mg/kg) caused a significant reduction of gastric volume and total acidity (p<0.05). It also tended to increase gastric pH but with no statistical difference.
Table 1: Effect of ML extract on pylorus ligation model.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>Gastric volume (mL/100g)</th>
<th>Gastric pH</th>
<th>Total acidity (mEq/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>2.39± 0.22</td>
<td>1.68± 0.10</td>
<td>110.1 ± 11.9</td>
</tr>
<tr>
<td>Cimetidine</td>
<td>100</td>
<td>1.39± 0.28 **</td>
<td>4.6± 1.08 **</td>
<td>58.5 ± 14.0 **</td>
</tr>
<tr>
<td>ML</td>
<td>400</td>
<td>1.62± 0.24 *</td>
<td>2.9± 0.84</td>
<td>65.0 ± 15.2 *</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E.M. (n=6). One hour after oral drug treatment, the pylorus of each rats was ligated. Gastric juice volume, gastric pH and total acid output were measured five hours after pylorus ligation. *p<0.05, **p<0.01 compared with the vehicle-treated control group.

DISCUSSION AND CONCLUSION

The pathogenesis of gastric ulcer results from an imbalance of aggressive gastric luminal factor and defensive mucosal barrier function (Malfertheiner et al., 2009). The aggressive factors are gastric juice acid, pepsin, bile reflux, NSAIDs, H. pylori bacteria and alcohol. The defensive factors are mucosal blood flow, surface epithelial cells, prostaglandins (PGs), phospholipids or surfactant, mucus, bicarbonate secretion, gastric motility, mucosa impermeability against H+ ion, heat shock protein, and others (Syam et al., 2009).

PGs play a central role in maintaining mucosal integrity (Robert et al., 1979). This protective effect of PGs is the result of several actions, including their ability to inhibit acid secretion, stimulate both HCO3- and mucus secretion, increase mucosal blood flow, and modify the local inflammatory response induced by acid (Binder, 2009; Sung, 2010).

In the present study, ML extract significantly decreased ulcer formation induced by EtOH/HCl, indomethacin, and stress. In EtOH/HCl-induced gastric ulcer, the mucosa protective factors are reduced, therefore anti-ulcerogenic activity of ML extract is probably due to the increase in mucosal resistance such as gastric blood flow or stimulation of PG production. ML extract also inhibited gastric lesion induced by indomethacin. Indomethacin induces gastric ulcer by inhibiting COX resulting in the decrease of cytoprotective system and PG production. It is likely that anti-gastric ulcer activity of ML extract may be due to an increased production of endogenous PGs and reduction of other aggressive factors. However, this explanation needs to be further explored. ML extract inhibited gastric ulcer formation in the stress-induced ulcer model as well. The pathogenic mechanisms of stress-induced gastric mucosal lesions involve the disturbance of gastric mucosal microcirculation (Kitagawa et al., 1979), an increase in gastric secretion (Brodie et al., 1962), and an abnormal gastric motility (Watanabe, 1966). Thus, it is possible that ML extract exerted anti-gastric effect by inhibiting gastric acid secretion.

Gastric wall mucus plays a major role as a defensive factor against gastrointestinal damage. ML extract increased the amount of gastric wall mucus in EtOH/HCl-induced gastric ulcer, suggested that ML extract increased defensive factor similarly to a reference drug, misoprostol. ML extract also significantly reduced gastric acid secretion in the pylorus ligation model and thereby leading to the reduction of gastric ulcers.

Taken together, anti-gastric ulcer activity of ML extract is probably mediated through the reduction of aggressive factor (gastric acid) and increasing defensive factor (mucus). However, ML extract was a crude extract that contained many phytochemical agents. In the present study, the search for active compound(s) exerted gastroprotective activity was not included. Further phytochemical study of ML extract is warranted to identify the active compounds and the future study will be performed to elucidate the mechanism of action.

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