

In Vivo Evaluation of the Gastroprotective Activity of Methanolic Extract from *Tabernaemontana Pandacaqui* (Apocynaceae) Leaves on Ethanol-Induced Ulcerations on Male Swiss Mice

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Abstract: *Tabernaemontana pandacaqui*, commonly known as kampupot or banana bush, is a shrub that produces numerous white flowers. Its leaves are elliptical or narrowly elliptical. It is known to be used in Thai folklore medicine since it possesses an abundant source of indole alkaloids. In this study, the methanolic extract of *Tabernaemontana pandacaqui* (Apocynaceae) was evaluated for its gastroprotective activity in different groups of male Swiss mice using Ethanol ulcer models with Omeprazole (30mg/kg) as the standard drug. Ulcerations were induced through the administration of 95% Ethanol (1mL/200g). The adult Swiss mice were randomly distributed into 6 groups, where 5 of them were administered with the methanolic plant extract in a single dose by oral gavage at doses 50 mg/kg, 100 mg/kg, and 150 mg/kg, and 1 group was administered with normal saline solution as the vehicle. Microscopic analysis was performed by isolating a portion of the stomach from each experimental group, and were placed in a 10% formalin immersed in paraffin. The sections were examined by a pathologist without the knowledge of the experimental groups for presence of any negative features. Efficacy was assessed by the determination of the mean ulcer index and the percentage of ulcer protection, as well as determining the volume and pH of the gastric juice. The histopathological changes were quantified according to an arbitrary scale following a gross examination of gastric lesions in the isolated stomach by a 10x magnifier lens to assess the formation of ulcers, and were counted using scoring of ulcers. The mean ulcer score of each test animal was expressed as ulcer index. It was found that as the dose increases, the gastric pH also increases. There is also an observed decrease in the volume of the gastric juice, as well as a decrease in the mean ulcer index of the different dose levels induced to the test animals. Omeprazole at 30mg/kg BW inhibited ulcer formation by 90%, while the low dose group, intermediate dose group, and high dose group inhibited ulcer formation by 50%, 70%, and 80% respectively. Prevention of gastric ulcer seems to correlate with the blockade of H⁺/K⁺ ATP-ase. As seen in the statistical analysis using one-way Anova Kruskal-Wallis test, there is a significant difference in the ulcer score of the six groups (p=0.002), indicating that the ulcer group had the most severe ulcer level, followed by low dose. The ulcer inhibition of omeprazole, high dose and intermediate dose do not differ (p=0.276). In conclusion, the methanolic extract of *Tabernaemontana pandacaqui* may exhibit a gastroprotective activity against ethanol-induced gastric ulcer model in Swiss mice, however further studies are recommended to be conducted to validate the effectivity of the plant extract.

Keywords: Gastroprotective agent, Indole alkaloids, Omeprazole, Oral gavage, *Tabernaemontana pandacaqui*, Ulcerations.

1. INTRODUCTION

Peptic ulcers are sores in the lining of the stomach, esophagus, and small intestine that develop as a result of erosion from stomach acid (Higuera, 2012). Acidity is still considered necessary for the formation of ulcers and its suppression is still the primary treatment, infection of the stomach by a bacterium called *Helicobacter pyloricus* and chronic use of nonsteroidal anti-inflammatory medications or NSAIDs are the two most important initiating causes of ulcers.

Tabernaemontana pandacaqui, commonly named as the windmill bush or banana bush, is a plant that is from a genus of *Tabernaemontana L.* It is largely distributed in the northern part of Thailand, a country in Southeast Asia. In Thai folklore, it is believed to have medicinal properties treating various sicknesses for the plant contains indole alkaloids. The stems also contain alcohol extract which is significant for its anti-inflammatory and other therapeutic abilities (Sultana *et al.*, 2015).

This study will evaluate the gastroprotective activity of the methanolic extract from the leaves of *Tabernaemontana pandacaqui* through extraction following orogastric intubation on different control groups of adult Sprague-Dawley rats. Scoring of ulcerations will then be performed to further appraise the pharmacologic effect of the methanolic extract. A review of related and existing literature will also be performed to validate the results. Postliminary recommendations will be winnowed from conclusions issuing from the data analysis.

2. METHODOLOGY

The *in vivo* evaluation of the gastroprotective activity of methanolic extract from *Tabernaemontana pandacaqui* leaves on ethanol-induced ulcerations on Swiss mice was conducted using experimental method. *Tabernaemontana pandacaqui* plant extract was prepared, and it was subjected to phytochemical screening by determining the presence of alkaloids using different qualitative tests. The methanolic extract of *Tabernaemontana pandacaqui* was evaluated for its gastroprotective activity in different groups of male Swiss mice using Ethanol ulcer models with Omeprazole (30mg/kg) as the standard drug. Ulcerations were induced through the administration of 95% Ethanol (1mL/200g). The adult Swiss mice were randomly distributed into 6 groups, where 5 of them were administered with the methanolic plant extract in a single dose by oral gavage, at different dose levels for the prevention of gastric lesions, and 1 group was administered with normal saline solution as the vehicle. Microscopic analysis was performed by isolating a portion of the stomach from each experimental group, and were placed in a 10% formalin immersed in paraffin. The sections were examined by a pathologist without the knowledge of the experimental groups for presence of any negative features. Efficacy was assessed by the determination of the mean ulcer index and the percentage of ulcer protection, as well as determining the volume of the gastric juice. The histopathological changes were quantified according to an arbitrary scale following a gross examination of gastric lesions in the isolated stomach by a 10x magnifier lens to assess the formation of ulcers, and were counted using scoring of ulcers. The mean ulcer score of each test animal was expressed as ulcer index.

3. RESULTS AND DISCUSSION

The presence of Indole alkaloids in the methanolic extract of *Tabernaemontana pandacaqui* was confirmed in different phytochemical screenings such as Tannic acid, Mayer's, Dragendorff's, Hager's and Wagners.

Table 1. Reagents and Positive Results

Reagents	Positive Result
Tannic acid	Precipitation
Mayer's reagent	White precipitate
Dragendorff's reagent	Orange precipitate
Hager's reagent	Yellow precipitate
Wagner's reagent	Reddish-brown precipitate

The gastroprotective activity may be associated with the increase in gastric pH, making it more basic, as well as decreasing the volume of gastric pH. The presence of inflammatory infiltrators predominantly neutrophils was also exhibited by the plant extract. It was found that as the dose increases, the gastric pH also increases. There is also an

observed decrease in the volume of the gastric juice, as well as a decrease in the mean ulcer index of the different dose levels induced to the test animals.

The Histopathological findings showed Omeprazole at 30mg/kg BW inhibited ulcer formation by 90%, while the low dose group, intermediate dose group, and high dose group inhibited ulcer formation by 50%, 70%, and 80% respectively.

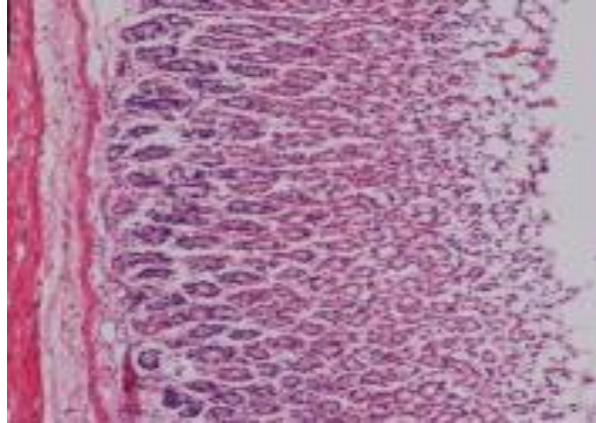


Figure 1. Histological appearance of Normal Control group

The Histological results showed the histological appearance of the gastric mucosa of the experimental mice in the normal control group displayed normal arrangement of the gastric epithelium and gastric gland in the mucosal region

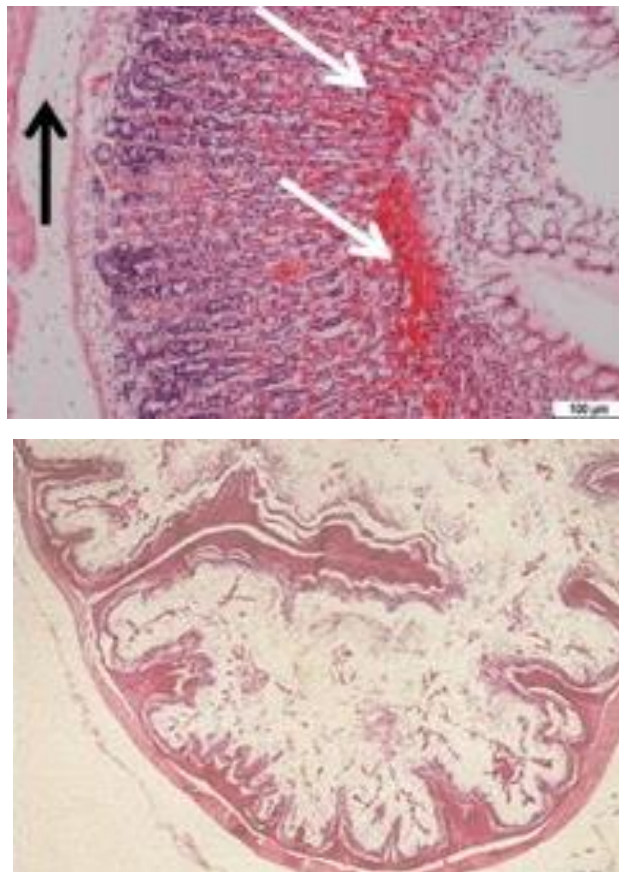


Figure2 . Histological Appearance of Ulcer control group and Low dose group at 50 mg/kg

Figure 2 shows the histological appearance of the ulcer control group, and the Low dose group at 50 mg.kg. It has an extensive disruption to the surface epithelium and hemorrhagic necrosis, penetrating deeply into the gastric mucosa, and extensive edema and leukocyte infiltration in the submucosa which indicates no significant difference between the two groups.

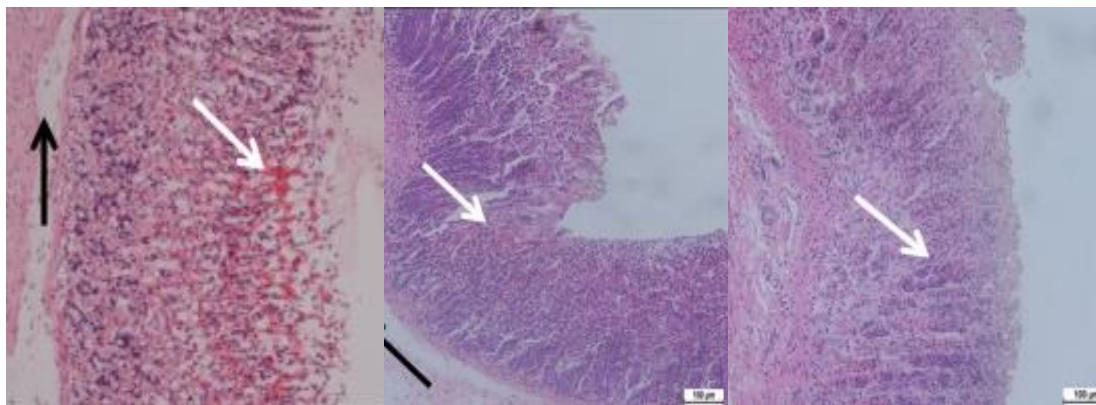


Figure 3. Histological Appearance of Omeprazole group, Intermediate dose group at 100 mg/kg, and High dose group at 150 mg/kg

The male Swiss mice pretreated with omeprazole at 30 mg/kg or that pretreated with *Tabernaemontana pandacaqui* methanolic extract at 100 and 150 mg/kg showed the improved histological appearance compared to the user control.

Table 2. Statistical Analysis using one-way Anova Kruskal-Wallis test

Groups	Ulcer Score	χ^2	p-value
Omeprazole	0 (0 to 0.5)	19.459	0.002
Control	3 (3 to 3)		
Normal	0 (0 to 0)		
Low Dose	1.0 (0.5 to 2)		
Intermediate Dose	1 (0 + 1)		
High Dose	0.5 (0 + 1)		

As seen in the statistical analysis using one-way Anova Kruskal-Wallis test, there is a significant difference in the ulcer score of the six groups ($p=0.002$), indicating that the ulcer group had the most severe ulcer level, followed by low dose. The ulcer inhibition of omeprazole, high dose and intermediate dose do not differ ($p=0.276$). Also, since it is a proven anti-inflammatory and antinociceptive agent, those certain properties may contribute to the prevention of gastric lesions and inflammation.

4. CONCLUSION

In conclusion, the methanolic extract of *Tabernaemontana pandacaqui* exhibits a gastroprotective activity against ethanol- induced gastric ulcer model in Swiss mice at doses 100 mg/kg and 150 mg/kg, however further studies are recommended to be conducted to validate the effectivity of the plant extract.

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