ACUTE TOXICITY TEST OF MEDICINAL PLANTS AND HERBAL REMEDIES OF APHTHOUS ULCER

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ABSTRACT: Four medicinal plants, Quercus infectoria (FAGACEAE), Kaempferia galanga (ZINGIBERACEAE), Coptis chinensis (RANUNCULACEAE) and Glycyrrhiza uralensis (LEGUMINOSAE), and two aphthous ulcer preparations with these plants were tested using the brine shrimp lethality test and in vivo acute toxicity (Wistar rat and Swiss albino mouse). In cytotoxicity test, Q. infectoria showed the highest toxicity at LD50 8.82 µg/ml (24 h) and 6.26 µg/ml (48 h) compared with the other plants. In vivo test, the high dose of aphthous powder (2-8 g/kg) and aphthous gel (3-12 mg/kg) did not cause histopathologic effect on rodent liver and kidneys. The results suggest that these plants and their preparations should be safe for the human ulcer treatments.

Keywords: Aphthous ulcer, Quercus infectoria (FAGACEAE), Kaempferia galanga (ZINGIBERACEAE), Coptis chinensis (RANUNCULACEAE), Glycyrrhiza uralensis (LEGUMINOSAE), Toxicity test

INTRODUCTION: An aphthous ulcer or canker sore is a kind of mouth ulcer which presents as a painful open sore inside the mouth. The disease is caused by a break in the mucous membrane. Although the exact pathophysiology remains obscure, many factors can contribute to the pathogenesis of these lesions, such as immunologocal factors, local trauma, smoking, stress, hormonal state, family history, food hypersensitivity and infection. Aphthous ulcer is normally healed without treatment within 1 to 2 weeks. Pain can be treated with several pain-relieving gels and herbal remedies. In Thai traditional medicine, in general the mixed medicinal plants will be prepared to cure this disease from the four kinds of plants, Quercus infectoria (FAGACEAE), Kaempferia galanga (ZINGIBERACEAE), Coptis chinensis (RANUNCULACEAE) and Glycyrrhiza uralensis (LEGUMINOSAE). The plant materials in this formulation showed the multi-pharmaceutical activity. Q. infectoria, C. chinensis and G. uralensis were used in traditional East Asian medicine to treat different skin disorders and anti-inflammatory activity. All of these plants showed the antimicrobial activity. The essential oil of K. galanga had antibacterial effect on Escherichia coli, Staphylococcus aureus, Bacillus cereus and Listeria monocytogenes. Glycyrhrizin was the main compound of G. uralensis which showed antimicrobial activity on the disc diffusion method. The rhizome extracts from G. uralensis had antifungal activity against Candida albicans with MIC valves of 1.56 mg/ml. For the safety to use these plants and preparations (gel and powder forms), the medicinal plants need to be evaluated for their toxicity. The aim of this study was to test the acute toxicity of four medicinal plants, Quercus infectoria (FAGACEAE), Kaempferia galanga (ZINGIBERACEAE), Coptis chinensis (RANUNCULACEAE) and Glycyrrhiza uralensis (LEGUMINOSAE), and the aphthous powder and with brine shrimp lethality test and animal toxicity test.

Plant Materials and Chemicals

Plant materials

The dried and pulverized galls of Q. infectoria, rhizomes of K. galanga, roots of G. uralensis, and roots of C. chinensis were kindly provided from Khaolaor Bhaesaj Ltd., Part, Thailand. The voucher specimens were deposited in the
Pharmacognosy Department Herbarium, at the Faculty of Pharmacy, Srinakharinwirot University, Nakhonnayok province, Thailand. Animals, brine shrimp were obtained from SK Trading Company Limited, Thailand and Wistar rats including Swiss albino mice were obtained from the National Laboratory Animal Centre, Mahidol University, Salaya, Nakhon Pathom province. Chemicals, formalin, hematoxylin & eosin were purchased from Samchai Chemical Company Limited, Thailand.

Formulation of Aphthous powder and gel

Aphthous powder was kindly provided from the manufacturer, Khaoaor Bhaesaj Ltd., Part, Thailand. “Ya-Kwad-Samarn-Lin Khaoaor Bhaesaj” has been popularly known in Thailand more than 70 years and is still the best seller of the manufacturer. Referring to the label, the mixed powder (650 g) consisted of 150 g of galls of Q. infectoria, 25 g of roots of G. uralensis, 110 g of rhizomes of K. galanga, and 25 g of roots of C. chinensis. Aphthous gel was developed in Faculty of Pharmacy, Srinakharinwirot University by using carbopol 934P as the gelling agent and consisted of 10% w/w of aphthous powder.

Preparation of plant powders

The galls of Q. infectoria, rhizomes of K. galanga, roots of G. uralensis, and roots of C. chinensis were washed thoroughly, then chopped into small pieces, dried in the hot air oven at 50°C and ground to powder, passed through sieve with mesh no.80 and kept in the well-closed desiccators. The plant materials and preparations were dissolved in dimethylsulfoxide (DMSO) at the stock concentration of 0.2 mg/ml for in vitro assays.

Brine shrimp lethality test

One gram of dry brine shrimp cysts was incubated in a hatching container with the aeration artificial sea water (38 g/ml) at 28-30°C under the light source. After 18-24 h, the phototropic nauplii were collected with a pipette and accumulate in a vial. Ten shrimps were transferred each well containing 100 μl artificial sea water in a 96 microwell-plate. The tested samples were added into each well to make 200 μl. The toxicity was determined after 24 and 48 h. The dead (non-motile) shrimps were counted under a binocular stereomicroscope. The mortality percentage of each concentration was calculated using the following formula: % death = (the summation of dead shrimps / the summation of controlled survival shrimps x 100). The LD_{50} was calculated using the Probit analysis program.

Animal toxicity test

Wistar rats and mice of both sexes were used in the experiment to compare their histopathological changes. Totally, 90 Wistar rats weighing 168-205 g and 180 Swiss albino mice weighing 17-24 g were acclimatized for 7 days before the treatments. Standard diet was provided and water was available ad libitum. Ten rats (5 of each sex) and 20 mice (10 of each sex) were administrated with the aphthous powder diluted in sterile distilled water in the concentrations of 2, 4 and 8 ml/kg. For the aphthous gel treatment groups the subjects were administrated with the aphthous gel in 3, 6 and 12 ml/kg and 12 ml/kg for the gel base. The animals control groups were treated with sterile distilled water. The administration was done by using the stomach tubes for introduction both diluted aphthous powder and gel directly into the animal stomachs. All animals were treated once daily for 14 days and were observed for the changing behavior. After treatments, all sacrificed animals were dissected for their livers and kidneys for histopathological examination. The experiment protocol was submitted to KU Animal care guideline committee.

Statistical Analysis

Animal toxicity results were expressed as mean ± standard error of mean (S.E.M.). The data obtained from acute toxicity studies was analyzed using Student’s t-test. P values less than 0.05 were considered significant.

Histopathological examination

The liver and kidney tissues of all treated animals in both sexes were fixed in 10% buffered formalin for at least 24 h. All samples were cut into small blocks. These blocks of tissues were then routinely processed and embedded in the
paraplast plus. These paraffin blocks were sectioned into 5 µm thick and stained with hematoxylin & eosin. The stained tissues were examined for histopathological alterations under the microscope.

RESULTS AND DISCUSSION:

Brine shrimp lethality test

The cytotoxicity assay tested against brine shrimp with different medicinal herb preparations exhibited the low level toxicity. The table 1 shows the calculated LD50 of these powder preparations. In brine shrimp cytotoxicity test, *Q. infectoria* showed the highest toxicity at LD50 8.82 µg/ml (24 h) and 6.26 µg/ml (48 h) compared to other plants. While the finished product shows very low cytotoxic effects with brine shrimp lethality testing.

Histopathological examination

Both sexes of rats and mice showed no sign of toxicological effect or death during 14 days of the experimental period after a single oral dose administration of the aphthous powder and gel at 2, 4 and 8 ml/kg and 3, 6 and 12 ml/kg and 12 ml/kg, respectively as compare to gel base. Toxicity evaluation was further carried out by observing body weight gain of the animals as summarized in Table 2 and 3. The body weight of the female and male rats receiving medicinal plant powers and their products showed slightly increase during the first period (7 d) as compared to the second period (7-14 d). However, the mean body weight gain obtained from this study was not affected by the product, suggesting that the products might not have altered food intake. Reductions in the body weight are a valuable indicator in evaluating the toxicity of preparation. The result of body weight of both mice and rats obtained from this study contradicts those obtained by Moore and Persaud14); Adebajo et al.15) and Grance et al.16). Including the behavioral observation, all animals did not alter their responses in behavioral expression and did not show any toxicological signs during the experimental period.

The liver, known to be a key organ in the metabolism and detoxification of xenobiotics, is vulnerable to damage induced by a huge variety of chemicals12,13). Under the microscopic examination, the liver of mice administrated with both aphthous powder and gel in all concentrations and also in the control groups showed normal cellular architecture with binucleated formation mostly around the central veins. The hepatocytes still arranged in cords. The hepatocyte vacuolation with small vacuoles in the
Figure 3 Microscopic pictures of rat liver tissues administrated with the aphthous powder in 14 days. (A) Binucleated hepatocytes (arrow) were observed and also vacuoles (v) in cytoplasm were clearly noticed in the control group. (B, C, D) Binucleated hepatocytes were also observed in the groups of 2, 6 and 12 g/kg of body weight treatments, respectively, including the vacuoles (v) in cytoplasm of the hepatocytes. (bar = 50 μm)

Figure 4 Microscopic pictures of rat liver tissues administrated with the aphthous gel in 14 days. (A) Binucleated hepatocytes (arrow) were observed around the central vein (cv) and also the vacuoles (v) in cytoplasm were clearly noticed in the control group. (B, C, D) Binucleated hepatocytes were also observed in the groups of 2, 6 and 12 g/kg of body weight treatments, respectively, including the vacuoles (v) in cytoplasm of the hepatocytes. (bar = 50 μm)

Figure 5 Microscopic pictures of mice kidney tissues administrated with the aphthous powder and gel in 14 days. (A, B) Kidney tissue of control mice showed the normal architecture of glomeruli (G) as same as the epithelial linings of both proximal (P) and distal convoluted tubule (D). (C) Glomeruli (G) and proximal convoluted tubule (P) of mice administrated with the gel free product showed the same architecture as of the control group. (D) Mice administrated with 12 mg/kg aphthous powder also showed the normal architectures of both glomerulus (G) and proximal convoluted tubule (P). (E, F) Mice administrated with 6 and 12 mg/kg aphthous gel showed the same normal architectures of glomeruli (G), proximal (P) and also distal convoluted tubule (D) as of the control group. (bar = 50 μm)

Figure 6 Microscopic pictures of rat kidney tissues administrated with the aphthous powder and gel in 14 days. (A, B) Rats of the control group showed the normal architectures of glomeruli (G) and also the epithelial linings of both proximal (P) and distal convoluted tubule (D). (C) Glomeruli (G) and proximal convoluted tubule (P) of rat administrated with gel free product showed the same architecture as of the control group. (D) Rats administrated with 12 mg/kg aphthous powder also showed the normal architectures of glomerulus (G). (E, F) Rats administrated with 6 and 12 mg/kg aphthous gel showed the same normal architectures of glomeruli (G) and proximal convoluted tubule (P) with tiny areas of hemorrhage (H). (bar = 50 μm)
cytoplasm was also observed both in the control and treated groups (figure 1 and 2). There were no signs of morphological injury in the central vein and hemorrhage. Compared to the mice, liver of rats administrated with aphthous powder and gel in all concentrations showed noticeably similar appearances. These findings also showed the similar appearances in the liver of the rats in control groups of rats (figure 3 and 4). However, the occurrence of hepatic vacuolation observed in this study occurred whenever cells are unable to maintain hydroelectrolytic homeostasis. With the continuous accumulation of water in the cells, clear spaces appear within the cytoplasm, leading to vacuolar degeneration, which is reversible as soon as exposure to the toxic agent is discontinued[17].

Kidneys are particularly vulnerable to toxic agents given their high rate of perfusion and their ability to concentrate a range of substances in the tubular lumen[12, 13]. The physiological alteration may account for the histopathological changes. Nevertheless, no lesions were observed in the kidneys of both mice and rats from either treatments or concentrations. The glomerular architecture showed normal appearance as the control groups. The epithelial lining of both proximal and distal tubules also performed the normal architecture (figure 5 and 6). These results suggested that the aphthous powder and gel did not affect the physiological activities of the kidneys. In opposite to this result, the physiological and histopathological alterations caused by the toxic action were reported by other

Table 1 The LD50 of the medicinal plants and herbal remedies for aphthous ulcer

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<th>Sample</th>
<th>LD50 in 24 h</th>
<th>LD50 in 48 h</th>
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<tr>
<td>Quercus infectoria</td>
<td>8.82 μg/ml</td>
<td>6.26 μg/ml</td>
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<td>6.48 μg/ml</td>
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<td>Coptis chinensis</td>
<td>7.58 x 10^7 μg/ml</td>
<td>1.24 μg/ml</td>
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<td>1.96 μg/ml</td>
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<td>Kaempferia galanga</td>
<td>3.25 x 10^3 μg/ml</td>
<td>1.86 x 10^1 μg/ml</td>
<td>&gt;10 μg/ml</td>
<td>7.26 μg/ml</td>
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<tr>
<td>Glycyrrhiza uralensis</td>
<td>1.87 x 10^3 μg/ml</td>
<td>3.57 x 10^3 μg/ml</td>
<td>&gt;10 μg/ml</td>
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Table 2 Body weight of rats in acute toxicity of the aphthous powder and gel

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<td>Glycyrrhiza uralensis</td>
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Table 3 Body weight of mice in acute toxicity of the aphthous powder and gel

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<td>Kaempferia galanga</td>
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<td>Glycyrrhiza uralensis</td>
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From our study, these plants and their preparations could be safe for the ulcer human treatments. Both animal toxicity and brine shrimp lethality test results exhibited no toxicological sign or any other effect on the experimental animals during the test period (14 d). In conclusion, with these results, all of the tested plants could be safe to use as the aphthous ulcer treatments in human.

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REFERENCES:


