Safety of Topical Formulations Containing *Hyptis suaveolens* Bioactive Compound

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Introduction

Hyptis suaveolens (L.) Poit (Labiatae), called in Thai as " Maeng Luk Kha", is well-known among Northern Thais for its anti-itching property. Part of the plant used for folklore remedies is leaves. The major chemical constituents containing in *H. suaveolens* oil were sabinene, β-caryophyllene,1, 8-cineole (Peerzada, 1997, Azevedo *et al.* 2001). *H. suaveolens* was reported to be of therapeutic value as a carminative, antiseptic, sudorific and galactagogue (Saluja and Santani, 1993). According to its dermal effect, there are some evidence which support this activity. Iwu *et al.* (1990) demonstrated that essential oil isolated from *H. suaveolens* inhibited the growth of both gram-negative and gram-positive bacteria as well as some fungi, *Candida albican* and *Aspergillus niger*. Fungiotoxicity of *H. suaveolens* oil against *Aspergillus flavus* has also been reported by Mishra and Dubey (1994). Additionally, marked antimicrobial activity of the oil against *Staphylococcus aureus, Trichophyton mentagrophytes* and *Trichophyton rubrum* was reported (Titawan *et al.*, 2004). Thus, the oil was initially prepared as topical cream for therapeutic use in skin infection. It has also been found that topical creams containing 10%, 20% and 30% of *H. suaveolens* oil possessed antimicrobial activity against *S. aureus, T. mentagrophytes* and *T. rubrum* (Titawan *et al.,* 2004). For safety reason, the toxicity of *H. suaveolens* cream needs to be investigated before application in human. Therefore, this study was carried out in order to assess possible toxicity of *H. suaveolens* cream in various concentrations from repeated exposure by the dermal route over a certain period of time in rats.

Material and methods

Collection of oils

Fresh aerial parts of *H. suaveolens* were collected at the flowering stage from Chiang Mai. The plant was identified and the voucher specimen was deposited at the Herbarium of Faculty of Pharmacy, Chiang Mai University, Thailand. The volatile oil was isolated through hydrodistillation for 3 hours. The oil was stored at 4 degree Celsius after dehydration.

Animals

Male and female Wistar rats, weighing 170-250 g were purchased from the National Laboratory Animal Center, Mahidol University, Nakornpathom, Thailand. The animals were housed in the animal facility of Faculty of Pharmacy, Chiang Mai University and kept at standard condition. The animals received food and water *ad libitum*. They were acclimatized for at least 5 days prior to experiment.

Repeated-dose dermal toxicity 28-day study

The animals were divided into 5 groups (n = 6-7) for each sex. Group 1 served as control and received cream containing 30% olive oil. Other three groups received cream in various concentrations of *H. suaveolens* oil, 3% (group 2), 10% (group 3) and 30% (group 4). The fifth group received cream in a concentration of 30% *H. suaveolens* oil and was kept to observe for reversibility or delayed toxic effects for 14 days post-treatment. Formulation of the cream has been reported by Titawan *et al.* (2004). Each concentration of the cream was tested in at least 12 animals

(6 male and 6 female rats). Body weight of the animals was measured weekly and recorded. Additional observations on behavior and applied skin appearance of the animals were made each day.

Twenty-four hours before the treatment, fur was shaved from the dorsal area of the trunk of the test animals for approximately 10 percent of the total body surface area according to OECD guideline (1987). Each concentration of the cream (0.1 g cream/100 g of rats) would then be applied uniformly over the clear skin of the animals once daily for 28 days. On the next day after completion of treatment, animals in group 1-4 were sacrificed. Blood samples and organs including brain, liver, lung, kidney and heart were collected for examination of hematology, clinical biochemistry and histopathology. For the fifth group, animals were sacrificed in the next 14 days after follow-up observation and blood samples as well as organs were collected for similar examination.

Statistical analysis

All data are expressed as the mean +/- S.E. The data were analyzed by one-way ANOVA and the significant level was set at p</= 0.05.

Results and Discussion

Effect of *H. suaveolens* cream on body weight of the rats was investigated each week for 4 weeks except in the fifth group that follow-up observation was made for 14 days post-treatment. There was no significant difference of average body weight between control and treated groups in both male and female rats during the study period. This result suggests that *H. suaveolens* oil which is the active ingredient in the cream may not affect food consumption or metabolism of the animals.

From additional observations, it has been found that 11 out of 14 female rats, receiving 30% *H. suaveolens* cream showed sign of erythema. The erythema could be observed within 24 hours of application and was limited to some extent. The occurrence of erythema may be due to irritation caused by *H. suaveolens* oil. There were no other abnormal signs and symptoms.

By examining effect of *H. suaveolens* cream on relative organ weight, there was no significant difference between control and treated groups in both male (groups 1-4) and every group of female rats. Exception was found in group 5 of male rats which received 30% *H. suaveolens* cream and was observed for 14 days after treatment. In the 5^{th} group, relative brain weight of the rats was significantly lower than that of control ($p< 0.05$). This result may be due to rapid growth of male rats in 14 days that have caused the reduction of relative brain weight. The effect of *H. suaveolens* oil on the brain was not taken into account because no abnormal signs and symptoms of central nervous system could be observed.

Tables 1 and 2 show effects of H. *suaveolens* cream on clinical biochemistry and hematological parameters of control and treated male and female rats, respectively. There was no significant difference in most of the parameters, e.g., aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (AP), hemoglobin, measured in both sexes of treated rats from those in control group. In contrast, the value of lactate dehydrogenase (LDH) in both male and female rats in the fifth group was significantly increased compared to that of control. Generally, the increase in LDH may indicate the abnormality of several organs, for example, heart, liver, kidney, lung, brain, etc. Additionally, the abnormality of organs mentioned above can cause the increase of some other enzymes, for example, AST, ALT, AP. Taken together with the result from histological examination, it is less likely that the increase in LDH was due to organ damage from treatment of *H. suaveolens* cream. There was a chronic toxicity study by Attawish *et al.* (2005) in which five doses (5, 50, 250 and 500 mg/kg/day) of water extract of *H. suaveolens* were orally

given to rats for 6 months and no significant toxic effects were observed. In that study, LDH measurement has not been performed. Therefore, further investigation should be carried out to obtain more information on the increase of LDH.

Parameter	Group				
	I, Control	II, 3% cream	III, 10 % cream	IV, 30% cream	V, 30% cream and 14 days follow-up
Calcium	8.6 ± 2.1	7.6 ± 0.9	8.1 ± 1.8	7.8 ± 1.6	7.6 ± 1.0
BUN	23.5 ± 5.4	22.3 ± 2.1	23.7 ± 2.1	22.0 ± 1.4	22.0 ± 3.9
Total protein	5.8 ± 0.4	5.8 ± 0.4	6.2 ± 0.2	6.0 ± 0.1	5.4 ± 0.4
Total bilirubin	0.6 ± 0.5	0.6 ± 0.4	0.6 ± 0.4	0.7 ± 0.5	0.5 ± 0.2
AST	57.8 ± 8.7	59.7 ± 10.6	55.7 ± 8.4	51.8 ± 6.6	99.8 ± 49.8
ALT	25.8 ± 5.6	29.0 ± 4.4	28.0 ± 8.5	21.2 ± 5.6	32.2 ± 6.4
AP	57.5 ± 9.2	57.7 ± 4.0	51.3 ± 3.1	53.2 ± 3.5	51.6 ± 12.3
LDH	718.0 ± 293.0	594.7 ± 20.1	694.7 ± 70.1	551.0 ± 55.4	2733.6 ± 1192.8*
Hematocrit (%)	41.3 ± 4.0	41.7 ± 3.8	42.7 ± 2.1	42.0 ± 3.0	41.3 ± 2.3
WBC (x 10^3/mm^3)	2.3 ± 0.4	2.4 ± 0.2	3.0 ± 0.4	2.4 ± 0.5	2.8 ± 0.5

Table 1 Clinical biochemistry and hematological value of control and treated male rats after 4 weeks of treatment (Abbreviation: BUN: Blood urine nitrogen; AP: Alkaline phosphatase; AST: Aspartate aminotransferase; LDH: Lactate dehydrogenase and ALT: Alanine aminotransferase); *: significantly different from control, p < 0.05

Parameter	Group				
	I, Control	II, 3% cream	III, 10 % cream	IV, 30% cream	V, 30% cream and 14 days follow-up
Calcium	7.6 ± 1.3	8.8 ± 1.1	6.7 ± 1.2	7.0 ± 0.8	8.2 ± 1.0
BUN	22.2 ± 4.0	25.0 ± 4.0	23.7 ± 4.0	25.3 ± 4.3	28.0 ± 3.4
Total protein	6.4 ± 0.5	5.7 ± 0.4	5.7 ± 0.4	5.9 ± 0.4	5.8 ± 0.6
Total bilirubin	0.8 ± 0.3	0.5 ± 0.1	0.5 ± 0.1	0.6 ± 0.1	0.8 ± 0.2
AST	53.0 ± 10.0	51.3 ± 1.5	59.3 ± 10.8	54.0 ± 2.4	72.8 ± 28.4
ALT	21.4 ± 4.4	20.7 ± 0.6	22.0 ± 6.1	21.3 ± 1.0	26.4 ± 10.2
AP	32.0 ± 1.2	30.3 ± 1.2	40.3 ± 8.7	37.0 ± 10.1	39.2 ± 13.5
LDH	546.2 ± 65.8	570.0 ± 48.5	684.7 ± 52.0	563.5 ± 23.4	1594.8 ± 264.5*
Hematocrit (%)	38.0 ± 1.0	42.3 ± 4.9	42.3 ± 2.9	41.7 ± 4.1	41.5 ± 3.5
WBC (x 10^3/mm^3)	2.4 ± 1.0	2.8 ± 0.4	2.9 ± 0.6	2.4 ± 1.0	2.4 ± 0.7

Table 2 Clinical biochemistry and hematological value of control and treated female rats after 4 weeks of treatment; *: significantly different from control, p < 0.05

Histopathological examination

In addition to gross examination of the organs from control and treated rats, effect of 30% *H. suaveolens* cream on organs was examined histologically as shown in Figures 1 and 2. There was no sign of abnormality found in tissues of all organs.

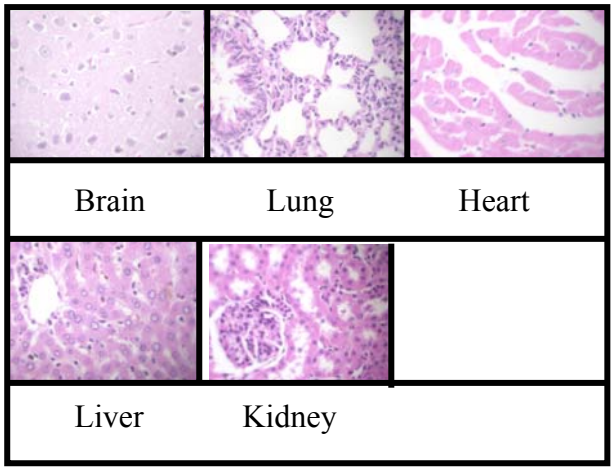

Figure 1 Histopathology of organs of rats in control group

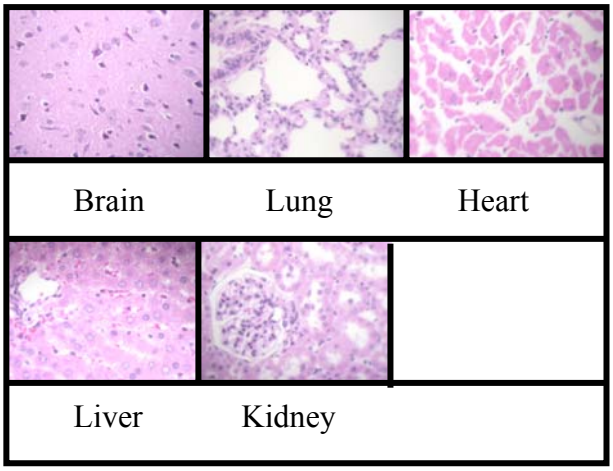

Figure 2 Histopathology of organs of rats in treated group that received 30% *H. suaveolens* cream for 4 weeks

Conclusion

Repeated-dose dermal toxicity 28-day study of *H. suaveolens* cream in various concentrations (3%, 10% and 30%) revealed that *H. suaveolens* cream in the concentrations of 3% and 10% produce no toxic effect, whereas further investigation should be carried out to obtain more information on the effect of 30% cream.

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