THE EFFECTS OF KEM CON ONG AND KEM TRI BONG CREAMS ON THERMAL BURN IN RATS

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The present study evaluated the healing effect of topical administration of KEM CON ONG and KEM TRI BONG creams on thermal burns in rats, and their systemic toxicity in burned rats. Thermal burns were induced on the dorsal region of rats using a standard burning technique. The burned areas were covered topically with silver sulfadiazine, KEM CON ONG, or KEM TRI BONG twice a day for 21 days. The results indicated that treatment with KEM CON ONG and KEM TRI BONG significantly reduced the size of the wounded area, increased the hydroxyproline content in animals' skin tissues, and improved the histopathological structure of skin tissues. KEM TRI BONG exerted better effects than KEM CON ONG on thermal burns in rats. Besides, our study demonstrated that topical administration of KEM CON ONG and KEM TRI BONG creams caused no significant change in the general status, haematological parameters, renal and hepatic functions. Additionally, they did not alter the histology of liver and kidney in animals. In conclusion, the topical application of KEM CON ONG and KEM TRI BONG creams exerted healing effects on the burned skin, and did not cause systemic toxicity in a rat model.

Keywords: Burn, KEM CON ONG, KEM TRI BONG, healing, rat.

I. INTRODUCTION

Burn injuries are still considered an important health problem affecting both genders and all age groups in developed and developing countries, resulting into physical and psychological scars and cause chronic disabilities.[1] Burns can be defined as tissue lesions that occur as a result of exposure to thermal origin such as flames, hot surface and liquids, extreme cold, chemicals, radiation or friction. Thermal burns can be classified depending on the lesion severity into superficial or the depth of burn.[2] Research on burns has generated sustained interest over the past few decades. In current burn therapy, silver sulfadiazine was presented as the gold standard in topical burn treatment and has also antibacterial properties. Many authors reported association with toxicity to keratinocytes and fibroblasts, which may delay wound healing process and had some serious cytotoxic activities on the host cells. Many reports are also available on the resistance of several bacteria to silver sulfadiazine.[3] Therefore, there is a need for new agents for treatment of burn wounds in health care practice with less adverse problems and better efficacy.

Now-a-days, phytotherapy has never stopped gaining in popularity. The use of complementary traditional medicine which include herbal medicines in the treatment of various diseases has expanded rapidly in

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both developed and developing countries, attributable to affordability, accessibility and efficacy.[4] For centuries, the medicinal plants have been extensively used in burn wound repair. Cream KEM CON ONG and KEM TRI BONG consist of a mixture of sesame oil and some herbal plants. However, there are no systematic scientific studies to evaluate the healing effect on experimental burn models. Therefore, the present study demonstrated the healing effect of topical administration of KEM CON ONG and KEM TRI BONG creams on thermal burns in rats, and their systemic toxicity in burned rats.

II. METHODS

1. Preparation of KEM CON ONG and KEM TRI BONG creams

KEM CON ONG and KEM TRI BONG creams were provided by Nhat Nhat Pharmaceutical Co.,Ltd according to the principles of Good Manufacturing Practice, Good Laboratory Practice and Good Storage Practice. KEM CON ONG consists of a mixture of 10% sesame oil and leaves of *Psychotria rubra*, *Camellia sinensis*, *Piper betle*, *Aloe vera*, *Prunus persica*; bark of *Phellodendron amurense*, *Choerospondias axillaris*; roots of *Angelica dahurica*. The ingredients of KEM TRI BONG cream are 2.5% sesame oil and tuber of *Alocasia macrorrhizos*; radix of *Curcuma longa*, walnut oil (*Juglans regia)*, honey, camphor (*Cinnamomum camphora)*. The expected indication of KEM CON ONG and KEM TRI BONG is to treat burned lesion.

2. Experimental animals

Male and female *Wistar* rats (8 - 10 weeks age and 180 - 220g weight) were used for the study. Rats were housed in the laboratory animal room (25 ± 1ºC under 65 ± 5% humidity and 12h dark-light cycle (from 7:00 - 19:00)). Commercial laboratory food and tap water were given *ad libitum*. Rats were kept for one week to acclimatize before starting the experiment.

3. Methods

Evaluation of the healing effect of KEM CON ONG and KEM TRI BONG creams on thermal burns in rat

The rats were randomly divided into five groups of ten animals as follows:

- Group 1 (normal control rats): received vehicle (sterile distilled water).

- Group 2 (vehicle-treated burned rats): received vehicle (sterile distilled water).

- Group 3 (silver sulfadiazine-treated burned rats): topically applied silver sulfadiazine (Satyam Pharm & Chemicals Pvt., Ltd, India) at dose 0.3 g/time, twice a day for 21 days.

- Group 4 (KEM CON ONG-treated burned rats): topically applied KEM CON ONG at dose 0.3 g/time, twice a day for 21 days.

- Group 5 (KEM TRỊ BONG-treated burned rats): topically applied KEM TRI BONG at dose 0.3 g/time, twice a day for 21 days.

Thermal burns were formed on the back of each rat with using a standard burning technique.[5] The rats were anaesthetized with single intraperitoneal injection of 250 mg/kg chloralhydrate. The dorsal regions of the rats were shaven with an electric shaver and sterilized with 70% alcohol. Burn wounds were created at dorsum of the rats using a 200g cylindrical stainless-steel rod (2.5cm diameter), which was pre-heated to 100°C in boiling water. Temperature was monitored using a thermometer. The cylindrical stainless-steel rod was immersed in boiling water until thermal equilibrium was achieved, then it was placed without pressure for 35s on the back of the rats. All animals were resuscitated immediately with lactated Ringer's solution (2 ml/100g body weight) intraperitoneally. Following the burning, each animal was placed in a separate cage. Immediately after burn, burned areas were

covered with silver sulfadiazine, KEM CON ONG or KEM TRI BONG. These applications were repeated every day during 21 days.

Measurement of size of the wounded area

Size of the wounded area was observed and measured at day 7, 14, and 21 by using a digital camera, with one camera lens and from a constant focal distance. The area of wound was measured in a blind manner using ImageJ Basics software ver 1.38, which were recognized as a software for measuring the area in medical experimental research by World Health Organization.

Determination of the hydroxyproline content

At the end of the experiment, rats were anesthetized with chloral hydrate (250 mg/kg, i.p) and the skin samples were collected from each rat. The concentration of hydroxyproline in the skin was evaluated according to Stegemann H. and Stalder K method.[6]

Histopathological examinations

At the end of the experiment, the rats were anaesthetized and the burned skin tissue samples were collected for histopathological examinations. Histopathologic evaluation carried out randomly in 30% of each group size at Histopathology Department - Military Hospital 103.

Evaluation of systemic toxicity of topical administration of KEM CON ONG and KEM TRI BONG creams in burned rats

Blood samples were collected from each rat. The systemic effects were quantified through general conditions, body weight changes of rats; evaluation of hematopoietic function; liver damage; liver function and kidney function.[7] Follow-up parameters were checked at the time points before applying the test drugs, after 10 days and after 21 days. At the end of the experiment, rats were euthanized after blood collection and the internal organs (heart, liver, spleen, kidney, and lungs) were removed and observed for any gross lesions. The liver and kidneys of 30 percent of the animals of each group were preserved in 10% buffered formaldehyde solution for histopathological studies. Histopathology studies were performed at the Department of Pathology, Hospital 103.

Statistical analysis

SigmaPlot 12.0 (SYSTA Software Inc, Richmond, CA, USA) was used for statistical analysis. Obtained data were expressed as the mean ± SD and analysed by a one-way-ANOVA followed by the post hoc Student-Newman-Keuls test for multiple comparisons. Statistically significant differences were considered when the p value was less than 0.05.

III. RESULTS

1. Healing effects of KEM CON ONG and KEM TRI BONG creams on thermal burns in rat

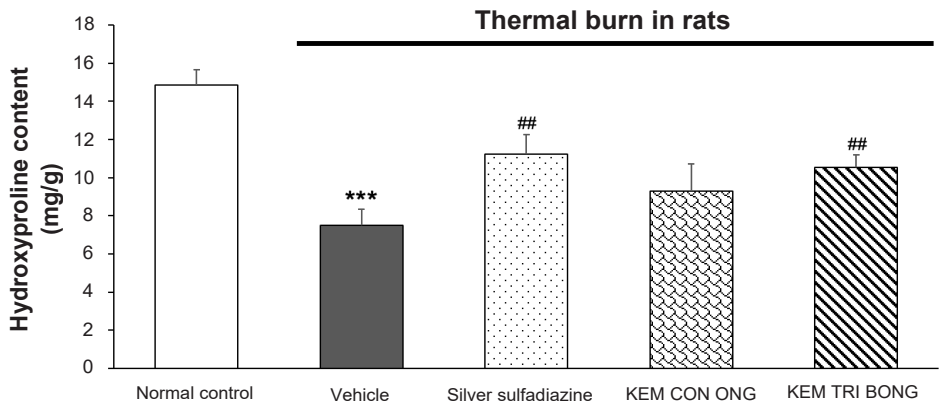
Table 1. Effects of KEM CON ONG and KEM TRI BONG on size of the wounded area in rats

Groups	Size of wounded area (cm^2)		
	After 7 days	After 14 days	After 21 days
Vehicle-treated burned rats	4.309 ± 1.007	3.291 ± 0.955	1.595 ± 0.517
Silver sulfadiazine-treated burned rats	3.773 ± 0.880	2.442 ± 0.471$^{\#}$	0.978 ± 0.369$^{\#\#}$
KEM CON ONG-treated burned rats	3.921 ± 0.580	2.796 ± 0.836	1.105 ± 0.352$^{\#}$
KEM TRI BONG-treated burned rats	3.695 ± 0.914	2.164 ± 0.636$^{\#\#}$	0.760 ± 0.292$^{\#\#\#\$}$

$^{\#}p < 0.05$, $^{\#\#}p < 0.01$: compared to vehicle-treated burned rats

$^{\$}p < 0.05$: compared to KEM CON ONG-treated burned rats

According to the Table 1, the results indicated that after 14 days of administration, silver sulfadiazine and KEM TRI BONG markedly reduced the wounded area compared with the vehicle-treated model group ($p < 0.05$). The burned area of KEM CON ONG-treated rats decreased compared with the vehicle-treated rats, but the difference was not statistically significant ($p > 0.05$). After 21 days of administration, compared with the vehicle-treated group treatment of silver sulfadiazine, KEM CON ONG and KEM TRI BONG significantly reduced the size of wounded area ($p < 0.05$). The burned area of KEM TRI BONG was significantly smaller than that of KEM CON ONG $p < 0.05$).



****p < 0.001 compared to normal control group*

##p < 0.01 compared to vehicle-treated burned rats

Figure 1. Effects of KEM CON ONG and KEM TRI BONG on hydroxyproline content in skin tissues

As shown in figure 1, the content of hydroxyproline in rats' skin of the vehicle-treated group was significantly lower than the normal control group ($p < 0.001$). Compared with the vehicle-treated model group, treatment of silver sulfadiazine and KEM TRI BONG were significantly increased the amount of hydroxyproline in the skin tissue. In addition, the content of hydroxyproline in the skin tissue of KEM CON ONG-treated rats decreased compared with the vehicle-treated rats, but the difference was not statistically significant ($p > 0.05$).

2. The microscopic representative images of burned wounds in *Wistar* rats

Image 1. The microscopic representative images of burned wounds in *Wistar* rats

The skin biopsy of normal control group demonstrates the properly stratum epidermis with keratinization, clear basal lamina, skin-dependent components in dermis layer, the

loose connective tissue and small blood vessels. Skin structure is normal. In the vehicle-treated group, the rat skin tissue showed large ulcerated area, surface covered with necrotic substance erythrocyte fibrin, many inflammatory cells, neutrophils, macrophages. On the 21st day, burn healing was better in silver sulfadiazine, KEM CON ONG and KEM TRI BONG-treated groups than the vehicle-treated group.

3. Evaluation of systemic toxicity of topical administration of KEM CON ONG and KEM TRI BONG creams in burned rats

General status

During the experimental period, there was an increasing in body weight in each group of animals. No significant difference was seen as compared to that of control group. None of the animals in all treated groups showed any macroscopic or gross pathological changes when compared to the control group.

Effect on hematopoietic function

Table 2. Effect of KEM CON ONG and KEM TRI BONG on hematopoietic function

Parameters	Groups	Before of treatment	After 10 days	After 21 days
RBC (T/L)	Normal control	7.76 ± 0.95	7.72 ± 0.81	8.12 ± 0.87
	KEM CON ONG	7.85 ± 0.81	7.87 ± 0.90	8.35 ± 0.77
	KEM TRI BONG	8.18 ± 0.46	8.03 ± 0.58	8.61 ± 0.66
Hemoglobin (g/dl)	Normal control	10.28 ± 0.78	9.98 ± 0.92	10.55 ± 1.26
	KEM CON ONG	10.64 ± 0.90	10.06 ± 0.74	10.62 ± 0.69
	KEM TRI BONG	9.91 ± 0.89	10.09 ± 0.70	10.36 ± 1.15
Hematocrit (%)	Normal control	40.96 ± 4.43	40.91 ± 4.11	42.27 ± 3.61
	KEM CON ONG	42.11 ± 3.78	40.23 ± 5.36	43.11 ± 2.93
	KEM TRI BONG	44.00 ± 3.62	41.18 ± 5.04	45.21 ± 3.94
Mean Corpuscular Volume (fl)	Normal control	53.50 ± 1.18	53.00 ± 1.49	52.60 ± 1.26
	KEM CON ONG	53.00 ± 1.70	52.00 ± 1.41	51.80 ± 1.40
	KEM TRI BONG	53.70 ± 1.83	52.60 ± 1.84	52.70 ± 1.25
White blood cell (G/l)	Normal control	6.09 ± 1.19	6.64 ± 1.36	6.61 ± 1.29
	KEM CON ONG	6.43 ± 1.06	7.07 ± 1.58	7.57 ± 1.60
	KEM TRI BONG	6.22 ± 1.06	6.98 ± 1.28	7.66 ± 1.47
Platelets (G/l)	Normal control	533.30 ± 102.16	549.80 ± 99.50	598.30 ± 98.30
	KEM CON ONG	553.80 ± 106.06	575.50 ± 98.92	663.10 ± 97.46
	KEM TRI BONG	573.90 ± 96.96	570.70 ± 102.92	642.20 ± 99.39

As shown in Table 2, there were no significant difference in red blood cells count, hematocrit, hemoglobin level, platelet count, total WBC count between KEM CON ONG and KEM TRI BONG-treated groups and normal control group ($p > 0.05$)

Effect on liver damage

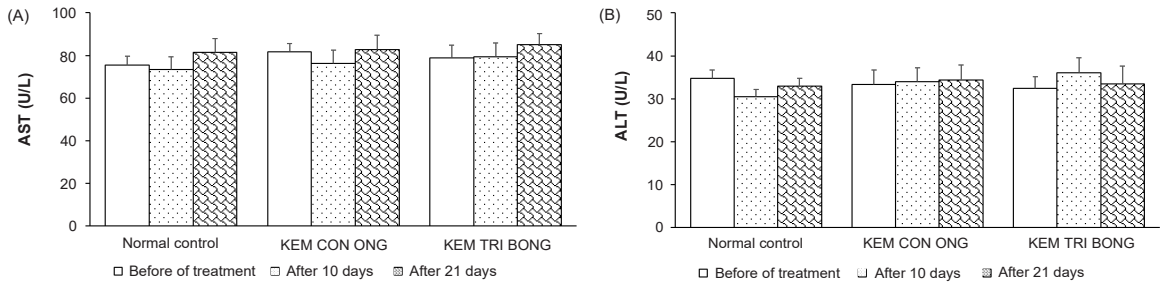


Figure 2. Effect of KEM CON ONG and KEM TRI BONG on AST and ALT level

Figure 2 demonstrates that KEM CON ONG and KEM TRI BONG did not cause statistical difference in AST and ALT level when comparing the treated groups to the normal control group ($p > 0.05$).

Effect on liver function

Table 3. Effect of KEM CON ONG and KEM TRI BONG on liver function

	Groups	Before of treatment	After 10 days	After 21 days
Total bilirubin (mmol/L)	Normal control	10.13 ± 0.77	10.24 ± 0.74	10.04 ± 0.92
	KEM CON ONG	10.45 ± 0.88	9.86 ± 0.62	9.57 ± 0.76
	KEM TRI BONG	10.10 ± 0.82	10.32 ± 0.91	9.54 ± 0.58
Albumin (g/dL)	Normal control	2.54 ± 0.21	2.60 ± 0.20	2.58 ± 0.23
	KEM CON ONG	2.69 ± 0.22	2.52 ± 0.24	2.61 ± 0.14
	KEM TRI BONG	2.66 ± 0.17	2.57 ± 0.11	2.54 ± 0.31
Total cholesterol (mmol/L)	Normal control	1.32 ± 0.18	1.25 ± 0.14	1.42 ± 0.24
	KEM CON ONG	1.30 ± 0.13	1.27 ± 0.15	1.44 ± 0.14
	KEM TRI BONG	1.31 ± 0.14	1.36 ± 0.23	1.34 ± 0.10

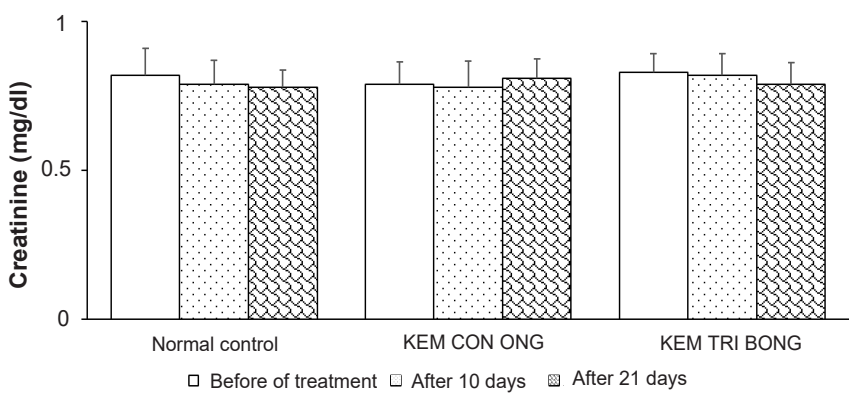


Figure 3. Effect of KEM CON ONG and KEM TRI BONG on creatinine level

The effect of KEM CON ONG and KEM TRI BONG on the total bilirubin, albumin and total cholesterol of the normal control group and treated groups are presented in Table 3. No statistical difference was observed between groups ($p > 0.05$).

Effect on kidney function

Figure 3 demonstrates that after topical treatment of KEM CON ONG and KEM TRI BONG did not cause statistical difference in creatinine level when comparing the treated groups to the normal control group ($p > 0.05$).

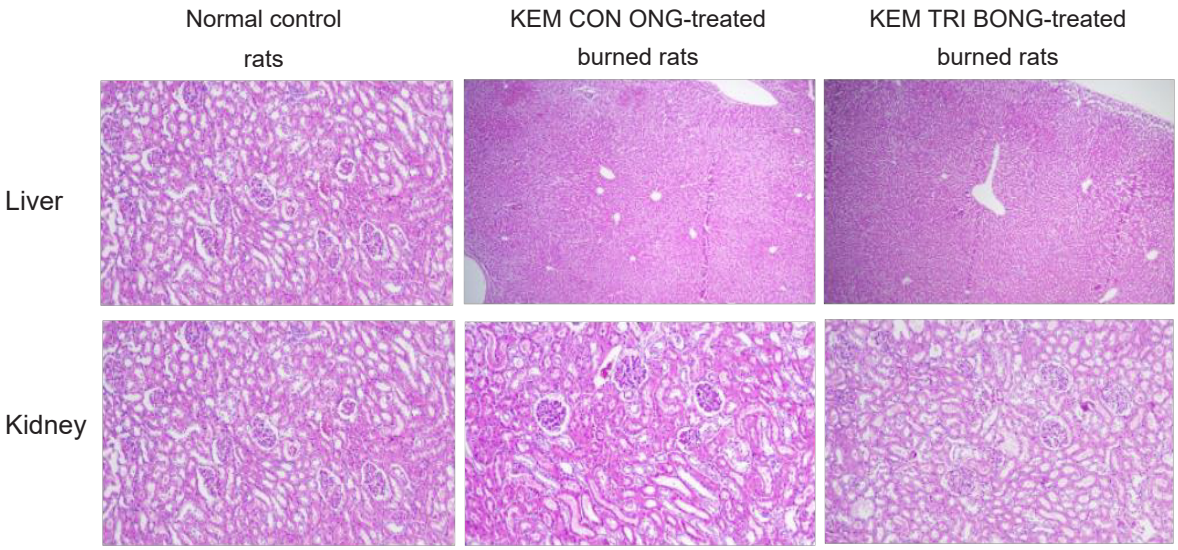


Image 2. Histopathological examnination of liver and kidney

Histopathological examnination

No gross lesion or change in size was observed when subjected all experimental rats to a full gross necropsy which examined of the hearts, livers, lungs, kidneys and abdominal cavities. There were no significant differences in histopathological examinations of livers and kidneys between KEM CON ONG and KEM TRI BONG-treated rats and normal control group.

IV. DISCUSSION

In this study, we evaluated the effect of topical administration of cream KEM CON ONG and KEM TRI BONG on thermal burn wounds in rats. KEM CON ONG and KEM TRI BONG significantly reduced the size of the wounded area, increased the hydroxyproline content in animals' skin tissues, and improved the histopathological structure of skin tissues.

Cream KEM CON ONG and KEM TRI BONG consist of a mixture of sesame oil and some herbal plants. To date, there are no systematic scientific studies to evaluate its influence on experimental burned models. It was reported that individual use of some herbal plants in KEM CON ONG cream exerted healing effects on the burned skin. The extract from the bark of *Choerospondias Axillaris* increased epithelialisation of second degree burn wounds.[9] Jia-Ru Wu reported that *Phellodendron amurense* decreased endothelium and macrophage cell numbers under an inflammatory state and was recommended for healing efficacy of burn wounds.[10] Additionally, *Angelica dahurica* extract, *Piper betle* leaf extract exhibited effects in the treatment of skin lesions, including anti-inflammatory activity, antimicrobial activity, cell stimulation properties, and the promotion of wound healing.[11,12] Moreover, some ingredients

of KEM TRI BONG also exhibited healing effects on the burned skin. Linoleic acid, the main component in Walnut oil, can promote full-thickness burning wounds by stimulating cell proliferation and differentiation.[13] Moreover, *in vitro* and *in vivo* studies have highlighted a broad range of activities provided by honey in burn treatment. These include anti-infectious, anti-inflammatory, antiexudative, antioxidant actions.[14] In addition, the mixture of of sesame oil, camphor and honey had a significant effect on epithelization and neovascularization. So, the mixture exerted the healing effect on second degree burn wound injuries in rat.[15] Sesame, one of the components of test drugs, has been studied for its effects on burns. Sesame belongs to Pedaliaceae family and contains about 5.1% lignans such as samin and seminole, which is responsible for its unique physiological and biochemical properties such as antioxidant, anti-mutagenic, and anti-inflammatory activities. Sesame oil stimulates fibroblast production in wounds.[16] The current study results showed that a combination of sesame oil and herbal plants at different concentrations accelerated the healing process of burn wounds in rats especially at concentrations 2.5% sesame oil. This study also showed the advantages of using multiple herb materials simultaneously on the wound sites during treatment. So, KEM CON ONG and KEM TRI BONG contain ingredients that have healing effect on the burned skin. The two creams have a common ingredient which is seasame oil but in different concentration. Our results indicated that KEM TRI BONG cream with 2.5% of seasame oil exerted better effects than KEM CON ONG on thermal burns in rats. Thus, KEM TRI BONG was chosen to be applied in clinically burn-treating concept.

In addition, we also evaluated the systemic toxicity of topical administration of KEM CON ONG and KEM TRI BONG creams on thermal burns in rats. Long-term topical application can also affect the systemic effects, especially applied on open wounds. Overall, the findings of this study indicated that topical administration of KEM CON ONG and KEM TRI BONG creams caused no significant change in the general status, haematological parameters, renal and hepatic functions. Additionally, they did not alter the histology of liver and kidney in animals.

V. CONCLUSION

The present study demonstrated that the topical application of KEM CON ONG and KEM TRI BONG creams exerted healing effects on the burned skin, and did not cause systemic toxicity in a rat model. KEM TRI BONG exerted better effects than KEM CON ONG on thermal burns in rats.

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