

Ginkgo paste improves tinea corporis in a guinea pig model

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Abstract: To investigate the effects of Ginkgo paste for external use on tinea corporis in a guinea pig model. The guinea pig tinea corporis model were induced by infection with *Trichophyton mentagrophytes* strains. And then, high and low doses of Ginkgo water-paste and alcohol-paste were administrated to the animals. The symptom, tinea corporis skin lesions and histopathological aspects of guinea pig were analyzed. High and low doses of Ginkgo alcohol-paste and Ginkgo water-paste could significantly reduced the tinea corporis symptom ($P < 0.01$), increased negative rate of strain culture ($P < 0.01$), and improved pathological changes of tinea corporis ($P < 0.01$). The best efficacy was observed in high dose ginkgo alcohol paste group. These results suggest that Ginkgo paste exhibits the efficacy to treat tinea corporis in a guinea pig model.

Introduction

Ginkgo is the dried ripe seed of *Ginkgo biloba* ginkgo plants, and has been used in Chinese medicine to treat lung, kidney diseases and cognitive impairment and dementia (Tan *et al.*, 2015). Oral administration of ginkgo can relieve asthma and improve polyuria and leucorrhea (Xiao *et al.*, 2013). Recent studies have shown that external use of ginkgo could treat enterobiasis. Tinea corporis is a clinical common disease mainly caused by fungal infection (Wu, 2013). In addition, generalized tinea corporis is easy to develop in the patients who have diabetes and other chronic metabolic diseases, have long-term oral taking of immunosuppressants, or have external use of corticosteroid hormone and glucocorticoid (Gao, 2009).

The herb have been used for anti-fungal infection and improved the symptom of the patients with tinea corporis (Shahi *et al.*, 2000). However, the efficacy of ginkgo on tinea corporis remains unclear. In this study, we aimed to explore the external use of ginkgo paste as a novel treatment for tinea corporis. We established guinea pig model of tinea corporis, and evaluated the efficacy of ginkgo paste.

Materials and Methods

Drugs and chemicals

Ginkgo was purchased from Zhengzhou Dongsheng Pharmaceutic and identified by Henan University of Chinese

Medicine. After removing the impurities, ginkgo was powdered and went through a 120 mesh sieve, and then made as water or alcohol paste with alcohol concentration of 60%. Tinea ointment (Xianyaogao) was produced by Heilongjiang Dragon Pharmaceutical Limited Company (No. 100501).

Strain and culture

The standard strain of *Trichophyton mentagrophytes* (Strain No. CMCC1F15a) was purchased from Strain Preservation Center at Institute of Dermatology, Chinese Academy of Medical Sciences, and inoculated in mold medium for culture. After 10 days, the colonies were removed to make suspension in sterile sodium chloride as described previously (Miao *et al.*, 2016; Cui and Tian, 2015).

Animals

All animal experiments were approved by Institute Committee of Animal Care and Use. Eighty guinea pigs (weight 200–250 g, half male and half female) were provide by Henan Kangda Laboratory Animal Limited Liability Company (Certificate No. 0006922). Animals were single-housed under standard laboratory conditions of food and water *ad libitum*, $22 \pm 2^\circ\text{C}$, a 12 h light:dark cycle (lights on at 08:00) and relative humidity 50–60% unless otherwise specified. All procedures were approved and performed in accordance with the guidelines of Henan University of Chinese Medicine.

Experimental design

Before the experiment, the fur was cut in each guinea pig on both sides of the back with the size about 3 cm × 3 cm.

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Then the hair was removed and there was no obvious damage in the hair removal area. Ten animals were randomly selected (half male and half female) as control group, For the remaining 70 guinea pigs, a surgical knife was used to scratch the skin until there was blood leakage in the hair removal area. Then *Trichophyton mentagrophytes* was inoculated on the wound at 1 ml each 1 cm² wound (Wu, 2013). After successful infection, the lesion was scored at 0–4 as described previously (Kong et al., 2012).

The 70 guinea pigs were randomly divided into 7 groups, model, tinea ointment, 60% alcohol, high dose of ginkgo water paste, low dose of ginkgo water paste, high dose of ginkgo alcohol paste, and low dose of ginkgo alcohol paste ($n = 10$). The corresponding drugs were applied to each group of guinea pigs. The high and low dose group contained crude ginkgo paste at 0.60 g/ml and 0.30 g/ml, respectively. Control group and model group were applied with 0.9% sodium chloride as the control. After drug use, all groups were covered with two layers of gauze, preservation film cover and then fixed with adhesive tape (Hu, 2013). The drug treatment lasted 6 h every day for 12 days. The healing of lesions was observed every day.

At the end of treatment, guinea pigs were sacrificed and four pieces of skins from the lesion sites were collected. One piece of skin was fixed in 10% formaldehyde, paraffin embedded and sectioned for HE staining, the other 3 pieces were soaked in 70% ethanol for 2–3 min, washed with saline and inoculated in potato glucose agar to observe the growth of *Trichophyton mentagrophytes* fungus. The efficacy of drug treatment was judged based on the lesions of the skin and the culture of *Trichophyton mentagrophytes* (Miao et al., 2014a; Zhang and Du, 2012).

Symptom scores

Scoring system: 0—no signs. 1—A small number of erythematous papules or lesions tend to heal, and new hairs grow. 2—The erythema was fused or scattered with redness around it. 3—The erythema was fully enlarged, with a lot of scales and thick skin. 4—Fastigium lesions with bleeding.

Histopathological examination

The Paraffin section procedure: After lesion skin fixed with 10% formalin for 24 h. The samples dehydrated by incubations in

different concentration of alcohol. And then, cleared with xylene. Afterwards, Embedded in paraffin at 56°C in a hot air oven for 24 h. Skin sections were processed for paraffin embedding and 4- μ m sections were prepared.

Histopathological examination: Skin sections were then stained with HE and examined under a light microscope (Olympus BX61, Tokyo, Japan). A scoring system ranging from ‘-’ to ‘+++’ points was used to evaluate the degree of severity of the observed histopathological changes, where ‘-’ = Skin epithelial tissue is intact, and subcutaneous tissues and hair follicles and other appendages are normal. ‘-’ = The squamous epithelium of the skin epithelium was slightly hyperplasia with a small number of inflammatory cells, and the adnexa such as hair follicles were normal. ‘++’ = The squamous epithelium of the skin epithelial tissue was obviously hyperplasia with thin inflammatory cells, and hair follicles and other appendages were damaged. ‘+++’ = Skin epithelial tissue squamous epithelium was obviously hyperplasia with a thick layer of inflammatory cells, hair follicles and other appendages were damaged.

Statistical analysis

Statistical analysis was performed using SPSS 21.0 for statistical software, the comparison among groups was analyzed by using single factor analysis of variance. The measurement data are expressed as the mean \pm S.E.M. Negative and positive rate was analyzed by chi-square test. Rank data were analyzed by Riddit test. $P < 0.05$ indicated significant difference.

Results

Effects of treatment on the symptoms of guinea pig tinea corporis model

As shown in Tab. 1, compared with control group, the 1th, 4th, 8th, 12th days model group exhibited significantly increased symptoms of tinea corporis ($P < 0.01$), indicating that the model was successful. On the first day after treatment, compared with the model group, there was no significant difference in symptoms of tinea corporis in other groups of guinea pigs ($P > 0.05$). On the 4th day after treatment, compared with the model group, tinea ointment

TABLE 1

Effects of drugs on guinea pig tinea corporis model symptom

Group	N	Time			
		1 d	4 d	8 d	12 d
Control group	10	0.0 \pm 0.0**	0.0 \pm 0.0**	0.0 \pm 0.0**	0.0 \pm 0.0**
Model group	10	3.4 \pm 0.5	3.2 \pm 0.4	2.7 \pm 0.5	2.1 \pm 0.3
Alcohol group	10	3.5 \pm 0.5	3.0 \pm 0.5	2.5 \pm 0.5	2.0 \pm 0.5
Tinea ointment group	10	3.4 \pm 0.5	2.7 \pm 0.5**	2.0 \pm 0.5**	1.1 \pm 0.3**
High dose of ginkgo alcoho-paste	10	3.4 \pm 0.5	2.8 \pm 0.4*	2.1 \pm 0.3**	1.2 \pm 0.4**
Low dose of ginkgo alcohol-paste	10	3.4 \pm 0.5	2.9 \pm 0.3	2.2 \pm 0.6*	1.4 \pm 0.5**
High dose of ginkgo water-paste	10	3.5 \pm 0.5	2.9 \pm 0.3	2.4 \pm 0.5	1.5 \pm 0.5**
Low dose of ginkgo water-paste	10	3.5 \pm 0.5	2.9 \pm 0.3	2.5 \pm 0.5	1.6 \pm 0.5**

Compared with model group, * $P < 0.05$, ** $P < 0.01$.

group had significantly reduced symptoms of tinea corporis ($P < 0.01$), and the high dose ginkgo alcohol-paste group also had reduced symptoms of tinea corporis ($P < 0.05$). On the 8th day after treatment, compared with the model group, tinea ointment group and the high dose ginkgo alcohol-paste group significantly reduced the symptoms of tinea corporis ($P < 0.01$), the low dose ginkgo alcohol-paste group also reduced the symptoms of tinea corporis ($P < 0.05$). On the 12th day of modeling, compared with the model group, each dose of ginkgo alcohol-paste and ginkgo water-paste significantly reduced the symptoms of tinea corporis ($P < 0.01$). Among them, high dose of ginkgo alcohol-paste group had the maximum effects to reduce tinea corporis symptom.

Effects of treatment on fungus culture of guinea pig tinea corporis model

As shown in Tab. 2, compared with control group, the model group had significantly lower rate of negative culture ($P < 0.01$). Compared with model group, tinea ointment group, each dose of ginkgo alcohol-paste and ginkgo water-paste had significantly increased rate of negative culture ($P < 0.01$). Among them, high dose of ginkgo alcohol-paste group had the maximum effects to increase the rate of negative culture.

Effects of treatment on the skin lesions of guinea pig tinea corporis model

Local histological images of guinea pigs in each group were shown in Fig. 1. In control group, the epithelial squamous epithelium, cutinized layer and the hair follicles and sebaceous glands of subcutaneous tissue in the skin of guinea pigs were normal (Fig. 1A). In model group, we observed squamous epithelial hyperplasia, cutinized layer thickening, and seriously destroyed hair follicles and sebaceous glands of subcutaneous tissue, infiltrated by a large number of inflammatory cells (Fig. 1B). In alcohol group, we observed squamous epithelial hyperplasia, cutinized layer thickening, and seriously destroyed hair follicles and sebaceous glands of subcutaneous tissue, infiltrated by a large number of inflammatory cells (Fig. 1C). In tinea ointment group, the cutinized layer of the squamous epithelium was covered by a large number of inflammatory cells, and hair follicles and sebaceous glands of subcutaneous tissue showed some damage (Fig. 1D). In high and low dose of ginkgo alcohol-paste groups, we observed mild hyperplasia of squamous epithelium in the skin, cutinized layer thinning, and reduced inflammatory cells, the hair follicles and sebaceous glands of subcutaneous

TABLE 2

Effects of drugs on fungus culture from the skin lesions of guinea pig tinea corporis model

Group	n	Negative	Positive	Total	Negative rate (%)
Control group	10	30	0	30	100.00**
Model group	10	6	24	30	20.00
Alcohol group	10	9	21	30	30.00
Tinea ointment group	10	21	9	30	70.00**
High dose of ginkgo alcohol-paste	10	20	10	30	66.67**
Low dose of ginkgo alcohol-paste	10	19	11	30	63.33**
High dose of ginkgo water-paste	10	15	15	30	50.00**
Low dose of ginkgo water-paste	10	15	15	30	50.00**

Compared with model group, * $P < 0.05$, ** $P < 0.01$.

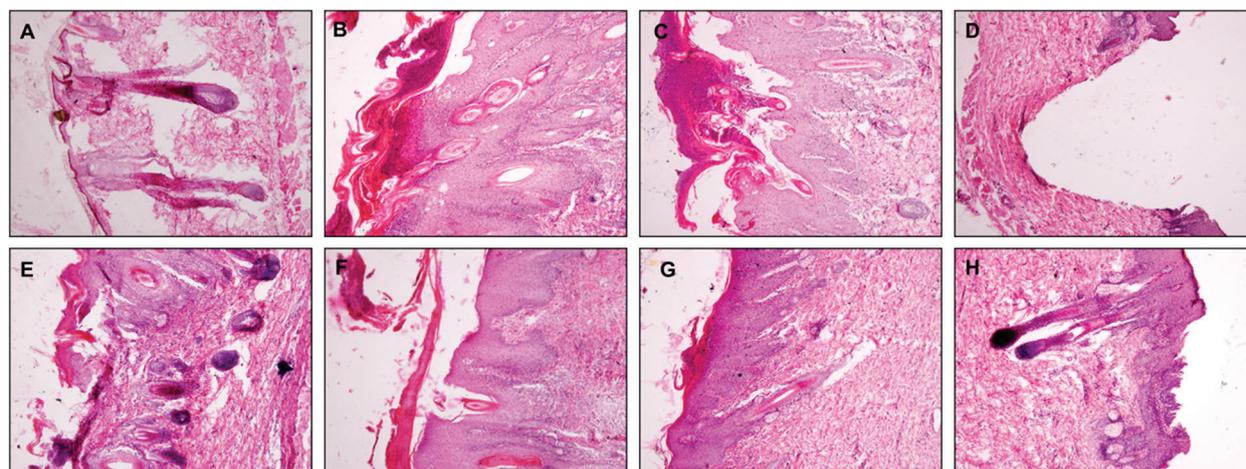


FIGURE 1. HE staining of skin lesions in guinea pig tinea corporis model.

(A) Control group; (B) Model group; (C) Alcohol group; (D) Tinea ointment group; (E) High dose of ginkgo alcohol-paste group; (F) Low dose of ginkgo alcohol-paste group; (G) High dose of ginkgo water-paste group; (H) Low dose of ginkgo water-paste group.

TABLE 3

Effects of drugs on fungus culture from the skin lesions of guinea pig tinea corporis model

Group	n	-	+	++	+++	P
Control group	10	10	0	0	0	**
Model group	10	0	0	1	9	
Alcohol group	10	0	0	4	6	
Tinea ointment group	10	0	3	7	0	**
High dose of ginkgo alcohol-paste	10	1	6	3	0	**
Low dose of ginkgo alcohol-paste	10	0	5	5	0	**
High dose of ginkgo water-paste	10	1	7	2	0	**
Low dose of ginkgo water-paste	10	0	4	6	0	**

Compared with model group, * $P < 0.05$, ** $P < 0.01$.

tissue were generally normal (Figs. 1E and 1F). In high dose of ginkgo water-paste group, the squamous epithelium of epithelial tissue, cutinized layer and the hair follicles and sebaceous glands of subcutaneous tissue were generally normal, but there was a large number of inflammatory cells in the subcutaneous tissue (Fig. 1G). In low dose of ginkgo water-paste group, the squamous epithelium of epithelial tissue and cutinized layer showed partial injury and defect, there was decreased infiltration of inflammatory cells, and the hair follicles and sebaceous glands of subcutaneous tissue were generally normal (Fig. 1H).

As shown in Tab. 3, compared with control group, the model group had significant pathological damage of tinea corporis ($P < 0.01$); Compared with the model group, the tinea ointment group, every dose of ginkgo alcohol-paste and ginkgo water-paste significantly improved pathological injury of tinea corporis ($P < 0.01$); but alcohol group did not improve injury symptoms of tinea corporis ($P > 0.05$).

Discussion

Tinea corporis is superficial mycosis caused by pathogenic fungus invasion in human epidermis. In the south of the Yangtze River Basin, the climate is wet and warm, and tinea corporis has high prevalence rate and recurrence rate. The genus *Trichophyton* and *Epidermophyton* is currently the main pathogen of tinea corporis in China, the common strains are *Trichophyton rubrum*, *Trichophyton mentagrophytes* and *Epidermophyton floccosum* (Tan et al., 2014; Liu et al., 2014). *Trichophyton mentagrophytes* belongs to the genus *Trichophyton*, can encroach the skin, hair and nail finger (toe), the tinea rash appears as circular or irregular shape, and can have pimples and blisters around, without leaving pigmentation after healing (Tang and Miao, 2014). *Trichophyton mentagrophytes* is the second pathogenic bacteria for infection of tinea corporis and cruris and tinea manus and pedis, after *Trichophyton rubrum*. The use of traditional Chinese medicine is cheap, and after boiling traditional Chinese medicine, direct wet compress or soak can shorten the course of treatment, and have no adverse reactions such as allergic reactions (Luo et al., 2016; Miao et al., 2014b). In this study, we used the inoculation of *Trichophyton mentagrophytes* strain in skin wound to make

the tinea corporis model, and the modeling method is simple and easy to operate (Zheng and Liu, 2011).

In order to judge curative effect of Ginkgo paste topical for tinea corporis, in this study we used common indicators such as lesion score, culture from the skin lesions and pathological changes of the skin section. The skin infiltrated by inflammatory cells can cause the pathological changes of skin squamous epithelium, cuticular layer, subcutaneous tissue. Thus the changes of skin pathological tissues can reflect the degree of infiltration of inflammatory cells in each layer of the damaged tissue and the healing of the skin. Our results showed that each dose of ginkgo alcohol paste and each dose of ginkgo water paste significantly reduced the symptoms of tinea corporis in guinea pigs, significantly increased the negative rate of strain culture, improved the pathological changes and reduced inflammatory cell infiltration. Among them, the large dose of Ginkgo alcohol paste group had the best effects.

Based on Chinese Materia Medica, external application of ginkgo can treat the ringworm sores in the head and underpart chancre. Ginkgo has anti-allergy, anti-inflammatory and antioxidant effects. It was reported that the ginkgo can be used to treat acne (Zhang, 2014). This is the first study to evaluate the effect of Ginkgo topical paste on guinea pig tinea corporis model. This study provides experimental support for external application of ginkgo for clinical treatment of tinea corporis.

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Conflicts of Interest: The authors declare that they have no conflicts of interest to report regarding the present study.

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