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Curcuma's extraction attenuates propranolol-induced psoriasis like in mice by inhibition of keratin, proliferating cell nuclear antigen and toll-like receptor expression

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Abstract: Curcuma was the dried rhizomes of *Curcuma kwangsiensis* S.G. Lee et C.F. Liang (Chinese name: e zhu), have been used in China for thousands of years. There are some reports have shown that curcumin, the major component of curcuma, has a good curative effect on psoriasis, but the mechanism is still unknown, so the present study was designed to investigate the effect of curcuma's extraction on psoriasis-like mouse, and to explore the mechanisms of therapy. First, we observed that curcuma's extractions effect on mitosis of mouse vaginal epithelial cells; then making psoriasis like model and measuring the score of skin damage on days 7 and 14; finally, we observed the expression of immune factors (CK14, CK16, CK17, PCNA, TLR-2, TLR-4, TLR-9) in propranolol induced psoriasis like rats. Curcuma's extraction prohibited the mitosis of mouse vaginal epithelial cells; curcuma's extractions have a significantly efficacy and dose dependent inhibition on imiquimod induced psoriasis like rats; and the expression of immune factors (CK14, CK16, CK17, PCNA, TLR-2, TLR-4, TLR-9) was decreasing in the curcuma's extraction treated groups compared with normal groups. Our research proved that curcuma's extractions have a significantly efficacy on psoriasis like rats, thus, curcuma's extractions can be a potential novel treatment for psoriasis. Furthermore, the expression of immune factors was decreasing after treatment with curcuma's extraction suggest us cytokines has strong relation with the mechanism of therapy for psoriasis. Our results contribute towards validation of curcuma in the treatment of psoriasis and other joint disorders.

Keywords: Psoriasis, curcuma's extraction, immune factors.

INTRODUCTION

Psoriasis (Gupta *et al*., 2015) is an immune-mediated chronic inflammatory disease that is associated with both genetic and environmental factors, the etiology of psoriasis is multifactorial and not fully known, it affects above 2% of the population in the world. The pathological characteristics of psoriasis are epidermal keratinocyte excessive proliferation and incomplete differentiation. More than 80% of cases, psoriasis presents as symmetrical, sharply demarcated, erythematous, dry, scaling, pruritic plaques affecting the skin. According to recent studies (Zheng *et al*., 2007; Torti and Feldman, 2007), under the background of multi genes, the disease result from T-cell mediated autoimmune reaction. Many kinds of immune cells are closely related to the pathogenesis of psoriasis.

At present, there is lack of novel and safer drugs for effective treatment of psoriasis. So, the extraction of therapeutic plant or herbal formulations may be an alternative and complementary source for modern medicine in the development of new drugs.

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Curcuma kwangsiensis S.G. Lee et C.F. Liang. (Zingiberaceae) widely distributed in southwest regions of China, has been used in traditional Chinese medicine for the treatment of inflammation and promoting blood circulation. Its roots are one of the most important crude drugs frequently in prescriptions of traditional Chinese medicine for the treatment of "Oketsu" (Sasaki *et al*., 2003), curcuma was the dried rhizome of *Curcuma kwangsiensis*, which is used as a spice, food preservative, coloring agent, and in the traditional systems of medicine (Singh *et al*., 2013; Yuan *et al*., 2005), the major components of curcuma are curcuminoids such as curcumin and essential oil like curcumol.

Although, there is no report show that curcuma has therapeutic activities on psoriasis, there have been some reports show that curcumin has a good curative effect on the treatment of psoriasis; Kang *et al.* (2016) were investigated the effects of curcumin on inflammatory factors secretion in T cells and psoriasis developed in keratin (CK) 14-vascular endothelial growth factor (VEGF) transgenic mouse model, their results showed that 10 μM of curcumin significantly inhibited secretion of inflammatory factors including interleukin (IL)-17, IL-22, IFN-γ, IL-2, IL-8 and TNF-α in T cells by 30–60%

in vitro and more than 50% of T cells proliferation was inhibited by using 100μM, their data proved that curcumin has a great potential to treat psoriasis, with high efficacy and safety; Sun J *et al* (Sun *et al*., 2013) were investigated the effect of a curcumin gel on an IMQ-induced psoriasis-like mouse model, their results showed that curcumin inhibited the increase in skin thickness and inflammation in IMQ-treated mouse ear skin. In all, curcumin has a good treatment in psoriasis.

In fact, the essential oil (Singh *et al*., 2013; Yuan *et al*., 2005; Al-Reza *et al*., 2010) not only dominates the traditional use of this medicinal plant but also has been increasingly recognized to possess multiple therapeutic activities for treatment of viral infection, tumor, inflammation and substantial pharmacological properties. one of the active components of essential oil-curcumol also exhibits some characteristics (Yuan *et al*., 2005; Ding *et al*., 2014; Wang and Chen, 2011) such as anti-hepatic fibrosis, prevent tumors and anti-proliferation. Carrion *et al.* (2015) conducted a phase IV randomized, double-blind, placebo-controlled, pilot clinical trial to investigate the safety and efficacy of curcuma extract together with local phototherapy in patients with plaque psoriasis, which suggested that orally administered curcuma with visible light phototherapy have a good therapy on psoriasis.

So, the present study was designed to investigate the effect of curcuma's extraction on propranolol-induced psoriasis-like mouse model and to explore the mechanisms of efficacy. In summary, our study aims to lay the experimental foundation for the clinical application of curcuma.

MATERIALS AND METHODS

Chemicals and reagents

All chemicals and solvents used were analytical grade.
Glycerol monostearate (PubChem CID24699), stearic acid (PubChem CID5281), white Vaseline, cetylalcohol (PubChem CID2682), ethyl paraben (PubChem CID8434), methanol (PubChem CID887), ethanol (PubChem CID702), glycerol (PubChem CID753), sodium dodecyl sulfate (PubChem CID3423265), saline, colchicines (PubChem CID6167), estradiol benzoate (PubChem CID222757), paraformaldehyde (PubChem CID712), neutral gum paraffin (PubChem CID5284361).

Medicines

The dried rhizome of *Curcuma kwangsiensis,* which was bought from neautus Co., Ltd (Sichuan, China), and identified by Professor Zhu-Yun Yan, Department of Medicinal Botany and Traditional Chinese Medicine of Chengdu University of TCM. A voucher specimen (130111) was deposited in Teaching hospital of Chengdu University of TCM.

Curcuma extract by alcohol

Firstly, 50g of curcuma powder was dissolved in 70ml of methanol, ultrasonic for 10min, three times. Then taking the supernatant A into the centrifugal tube (1500 r/min, 10min) and supernatant B was extracted by centrifugation. The precipitations were dissolved in 250ml of methanol, ultrasonic for 10min. After 4h, supernatant C was collected and supernatant D was extracted by centrifugation, collected the supernatant B, D and merged them. Methanol was collected by rotary evaporator; the water bath temperature was 45^{o}C. The production was washed by 50% ethanol, and ethanol was evaporated by water bath, the reddish brown viscous liquid was curcuma's extraction (fig. 1).

For confirmation the composition of curcuma and curcuma's extraction, we conducted a fingerprint using high performance liquid chromatography (0.05% phosphoric acid aqueous solution (A) - acetonitrile (B) as mobile phase; wavelength was 216nm; column temperature was 30^{o}C; flow rate was 1ml/min;), all analyses were performed using a Waters e2695 series HPLC instrument (Water Technologies, USA). The results as figs. 2, we ensured that the composition of curcuma was not changed after extracting by alcohol. We also can get the major substances of curcuma from the fingerprints, such as germacrone (216nm), demethoxycurcumin and curcumin (423nm).

The preparation of cream base

70g of glycerol monostearate, 140g of stearic acid, 85g of white Vaseline, 20g of cetylalcohol, 1g of ethyl paraben, 8ml of ethanol were mixed, mixture was heated to 70^{o}C, got oil phase; 85g of glycerol, 10g of dodecyl sodium sulfate were dissolved in appropriate amount of purified water, heated to 70^{o}C, got aqueous phase. Oil phase was slowly added into aqueous phase, stirred mixture constantly, got the cream base.

The preparation of curcuma's extraction cream

The curcuma's extraction was added into cream base for several times, so that we got three kinds of curcuma's extraction cream with different concentration. The concentrations were 2.0g/kg, 1.0g/kg, 0.5g/kg (the curcuma concentration in the 1g of curcuma's extraction cream equal to that in 2.0g, 1.0g, 0.5g curcuma raw material)

Halometasone cream

The cream was produced by Bright Future Pharmaceutical Laboratories Limited (Batch number is 1011554, specification is 0.5mg/g and each production has 10g).

5% of Imiquimod cream was produced by Sichuan Provincial Ming-Xin Pharmaceutical Limited (Batch number is 101112) propranolol hydrochloride tablets were produced by Shandong Provincial Health Pharmaceutical Limited (Batch number is 1003014, specification is 10mg/tablets and each bottle has 100 tablets).

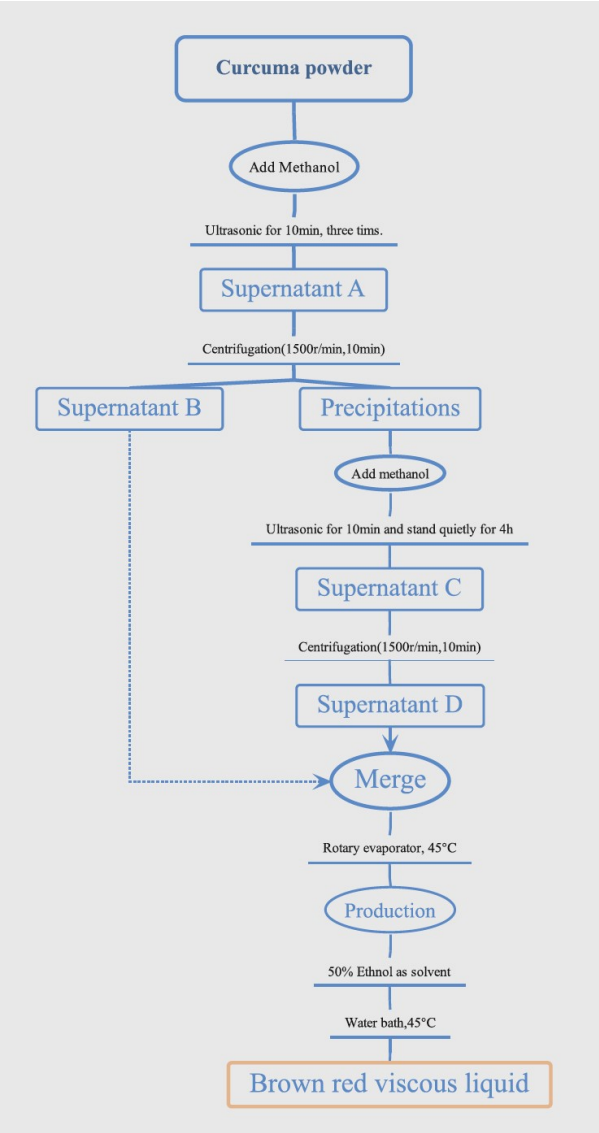
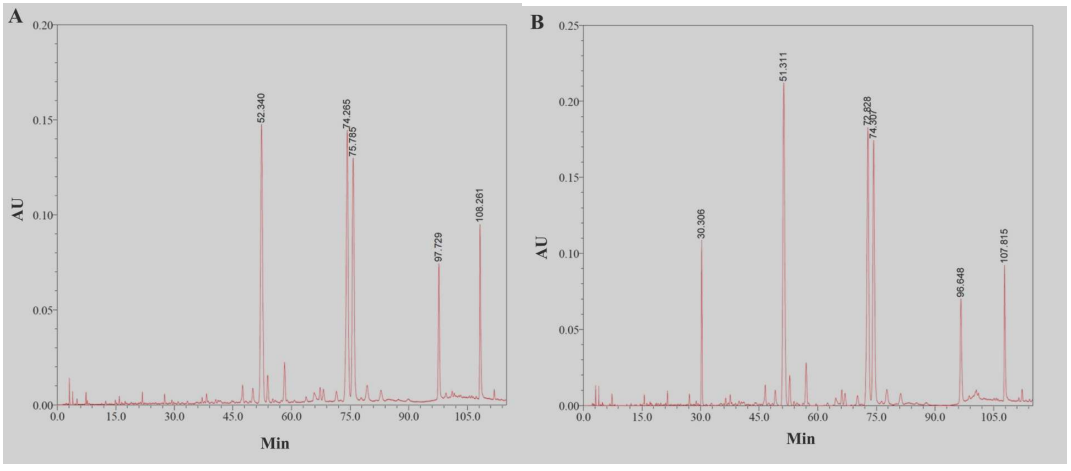


Fig. 1: Flow chart of Curcuma extract by alcohol.



Fingerprint was conducted a using high performance liquid chromatography, according to the fingerprint. It was ensured that the compositions of curcuma were not changed after extracting by alcohol.

Fig. 2: Fingerprint analysis of curcuma (A) and curcuma's extraction (B) by using HPLC.

The preparation of propranolol cream

Propranolol hydrochloride tablets was grinded into powder, and cream base was added into propranolol for several times, so that we got propranolol cream with the concentration is 50mg/g (1g of propranolol cream has 50mg propranolol).

Animals

Male and female, Kun-Ming mice (18-22g body weight) and albino guinea-pig (280-320g body weight) were bought from animal research laboratory of Sichuan Provincial People's Hospital (Chengdu, China). The animals were housed in plastic cages at room temperature, every cage has 5 of mouse, received food and water ad libitum. They were accustomed to the laboratory environment for 3 days before the experiments. Mice had access to food and water except during tests.

Experimental study groups (n=10 /group, except for the immune expression study in which there were 8 /group) were randomly assigned, and the researchers were blinded for the behavior tests. The animal experimental protocols were approved by Sichuan Provincial People's Hospital and carried out in accordance with the Animal Care Guidelines of the National Institutes of Health.

Estrogen induces mitosis of mouse vaginal epithelial cells

The female mouse (18-22g body weight) were randomly divided into six groups: negative control group, model control group, halometasone cream 0.5mg/kg group, curcuma's extraction cream 2.0 native medicine g/kg group, curcuma's extraction cream 1.0 native medicine g/kg group, curcuma's extraction cream 0.5 native medicine g/kg group, the number of mice in each group were 10.

Except the negative control group were intraperitoneally injected with 0.10ml/10g saline, other groups were intraperitoneally injected with 1.0mg/kg estradiol benzoate, once a day, for 3 consecutive days (after 3 days, the injection ran on alternate, until the test ending), which making mice vaginal epithelium in a proliferative state.

Psoriasis model

Making model by 5% Imiquimod cream

The mouse (18-22g body weight) were randomly divided into six groups negative control group, model control group, halometasone cream 0.5mg/kg group, curcuma's extraction cream 2.0 native medicine g/kg group, curcuma's extraction cream 1.0 native medicine g/kg group, curcuma's extraction cream 0.5 native medicine g/kg group, with 10 mice in each group. All of them were cut their back's hair. (hair removal area is about 2cm*3cm)

Starting from the second day of hair cutting, except the negative control group, the rest of the five groups were wiped 5% Imiquimod cream in the hair removal area, the negative control group give an equal amount of cream base. The dose was according to every 1kg weight wipes 200mg cream, once a day, for 14 days.

Making model by propranolol cream

The guinea pigs were randomly divided into seven groups

Blank control group, model control group, cream base group, halometasone cream 0.5mg/kg group, curcuma's extraction cream 2.0 native medicine g/kg group, curcuma's extraction cream 1.0 native medicine g/kg group, curcuma's extraction cream 0.5 native medicine g/kg group, with 8 rats in each group.

Except the blank control group was wiped with cream base in binaural dorsal skin, the rest of the group were treated with propranolol cream in binaural dorsal skin, the coated area is $0.1g/cm^2$, 3 times a day and continuously wiping for 4 weeks.

Immunohistochemical staining

Specimens

After four weeks, except negative control group and model control group, binaural dorsal skin of each group was wiped with cream base, halometasone cream (as a positive control group), curcuma's extraction cream 2.0 native medicine g/kg group, curcuma's extraction cream 1.0 native medicine g/kg group, curcuma's extraction cream 0.5 native medicine g/kg group, 3 times a day for 2 weeks. After two weeks of treatment, the guinea pigs were sacrificed, the left ears of guinea pig were made into specimens. The tissue specimens were fixed in neutral-buffered formalin and embedded in paraffin wax.

Staining

Histologic sections were stained with hematoxylin and eosin (H&E) for morphological assessment by light microscopy. For further study, paraffin sections were stained immunohistochemically with cytokeratin, proliferating cell nuclear antigen and toll-like receptor markers, the dilutions, sources of primary antibodies and secondary antibodies used in our study are listed in table 1.

Immunohistochemical staining (IHS) was performed on 4-mm thick paraffin sections by using the streptavidinbiotin-complex technique. The method was in accordance with Histological and Histochemical Methods: Theory and Practice. 4th ed (Szunyogova and Parson, 2016).

Measure

Measuring the content of CK14, CK16, CK17, PCNA, TLR-2, TLR-4, TLR-9 in specimens by ELISA, the methods were according to the kit instructions. The kits used in our study are listed in table 2.

STATISTICAL ANALYSIS

All data were presented as the mean of at least three independent experiments ± standard deviations (S.D.). Statistical analysis was performed by ANOVA followed by Student–Newman–Keuls (SNK) tests for multiple comparisons between treatment groups using SPSS 18.0 software. P<0.05 were considered statistically significant, P<0.01 were presented remarkably significant.

RESULTS

Curcuma's extraction cream prohibited the mitosis of mouse vaginal epithelial cells

After injection of estrogen, as shown in fig. 3 and table 3, the mitotic index of mouse vaginal epithelial cells increased significantly (*P*<0.01), compared with the negative control group, suggesting estrogen induces mitosis of mouse vaginal epithelial cells was success.

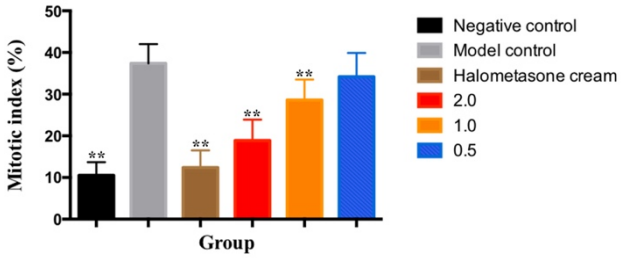


Fig. 3: The effect of curcuma's extraction cream in the mitosis of mouse vaginal epithelial cells. The negative control group and model group were vaginally injected with 10μl/10g cream base emulsion matrix, other groups were vaginally injected with 10μl/10g the drug emulsion matrix, once a day, for 10 consecutive days. Until 14th day, after an hour of injection, mice were intraperitoneally injected with 2.0mg/kg colchicine, killed the mice and collected the vaginal tissues after 5 hours of injection. The results represent three repeated experiments average value(mean) ± standard deviation (S.D.). Compared with model control group,*P<0.05, **P<0.01.

Then, beginning from the 4th day, except the negative control group and model control group were vaginally injected with 10μl/10g cream base emulsion matrix, other groups were vaginally injected with 10μl/10g the drug emulsion matrix, once a day, for 10 consecutive days. Until 14th day, after an hour of injection, mice were intraperitoneally injected with 2.0mg/kg colchicine and after 5 hours, killing the mice and collecting the vaginal tissues. The tissues were fixed by 10% formaldehyde, then made pathological sections and counted the numbers under light microscope through HE staining. The total number of meiosis in every 300 basal cells have converted into the number of nuclear mitosis in every 100 basal cells.

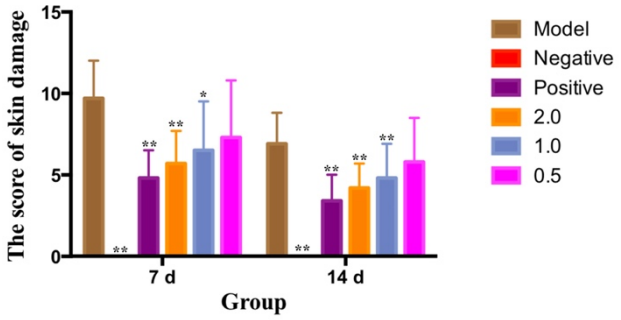


Fig. 4: the effect of curcuma's extraction cream to skin damage. Imiquimod-induced psoriasis like in mice were treatment with different concentrations of curcuma's extraction cream. The standard to score of the skin damage according to psoriasis area and severity index (PASI) In 7th and 14th days was separately estimated the score of skin damage through three clinical signs including erythema, induration and desquamation, every symptom on a scale from 0 to 4 (0: no; 1: light; 2: middle; 3: serious; 4: very serious). The results represent three repeated experiments average value (mean) ± standard deviation (S.D.). Compared with model control group,*P<0.05, **P<0.01.

As shown in fig. 3 and table 3, treatment with different concentration extraction cream, the mitotic index was significantly reduced, especially curcuma's extraction cream 2.0 native medicine g/kg group and curcuma's extraction cream 1.0 native medicine g/kg group, had remarkably significant (P<0.01) compared with the model group. In addition, the mitotic index of curcuma's extraction cream 0.5 native medicine g/kg group also had a weakly decreasing trend, although there was no significant difference with the model control group (P>0.05).

The results show us curcuma's extraction cream has a strong inhibition on mitotic of mouse vaginal epithelial cells increased. We did some deeply tests as follow to explore the efficacy of curcuma's extraction.

Curcuma's extraction cream relieve skin's damage

Mice was continuously wiped 5% Imiquimod for 14 days, the symptoms were appeared at 7th day, like skin erythema, scales and thicken, are similar with psoriasis. Symptoms were improved at 14th day, but the test analysis showed that there still has significant different with the negative control group (P<0.01), means that 5% Imiquimod cream induced psoriasis like model was success.

Next, except negative control group and model control group, binaural dorsal skin of each groups was wiped with 10μl/10g of halometasone cream (as a positive control group), curcuma's extraction cream 2.0 native medicine g/kg, curcuma's extraction cream 1.0 native medicine

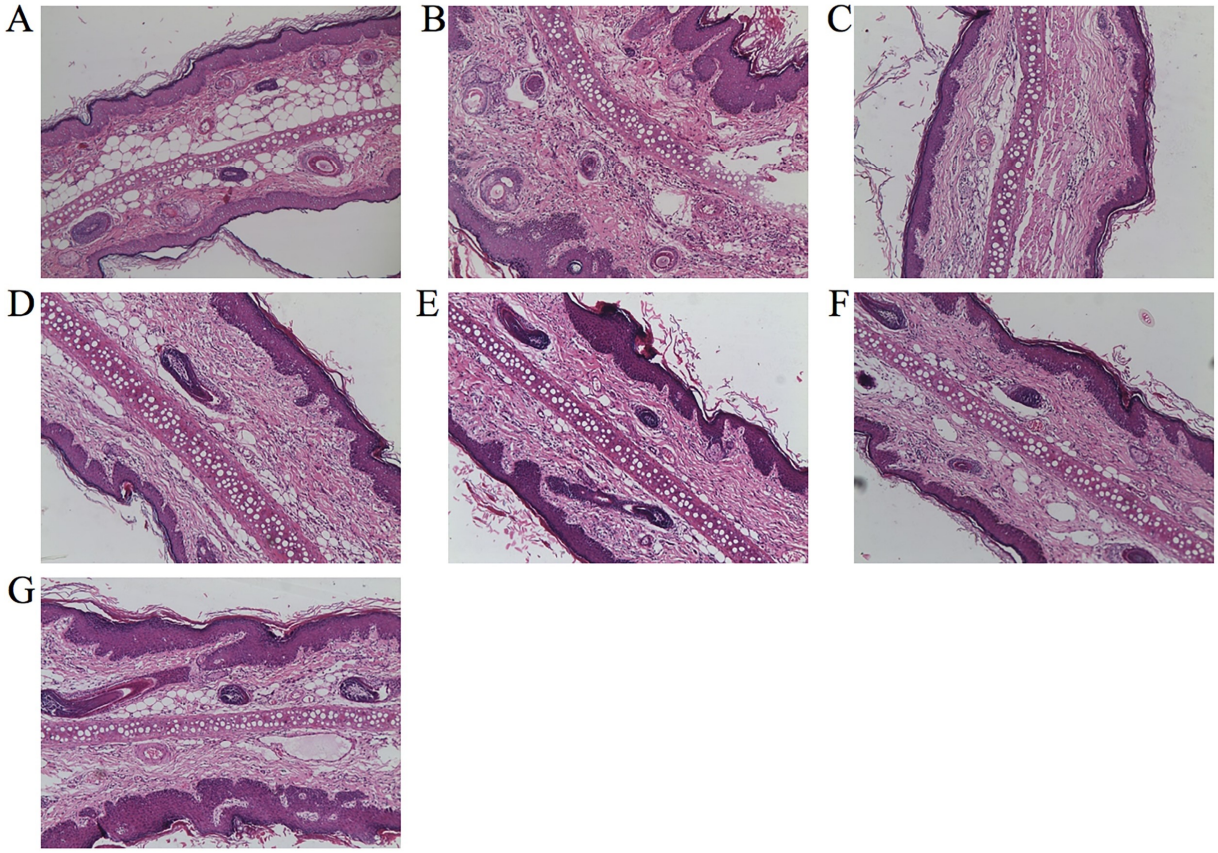


Fig. 5: Pathological imaging of all groups. Propranolol-induced psoriasis like in mice were treatment with different concentrations of curcuma's extraction cream. The left ear of guinea pig was made into specimen and stained, we observed it through optical microscope.

g/kg, curcuma's extraction cream 0.5 native medicine g/kg, once a day, for 14 days.

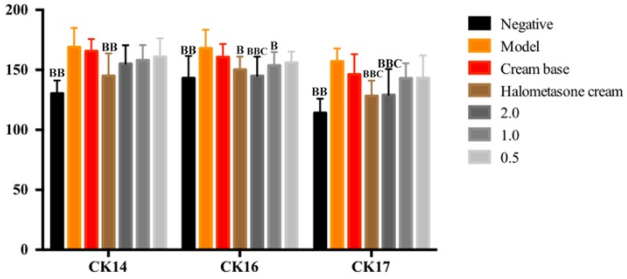


Fig. 6: Curcuma's extraction cream inhibited the expression of keratin (N=8). After treatment with different concentrations of curcuma's extraction cream, we tested the expression of CK14, CK16, CK17 in mice. The results represent three repeated experiments average value (mean) ± standard deviation (S.D.). Compared with model control group, $^{B}P<0.05$, $^{BB}P<0.01$; compared with cream base group, $^{C}P<0.05$, $^{CC}P<0.01$.

As shown in fig. 4 and table 4, after using different concentrations of curcuma's extraction cream, the symptoms have significantly improved, skin erythema have reduced and skin become smoothly, especially curcuma's extraction cream 2.0 native medicine g/kg group and curcuma's extraction cream 1.0 native medicine g/kg group had remarkably significant ($P< 0.01$) compared with the model group; In addition, skin lesion in curcuma's extraction cream 0.5 native medicine g/kg group also had a weakly decreasing trend, while there was no statistically significant compared with the model control group ($P>0.05$).

The result proved that curcuma's extraction cream has a strong efficacy on psoriasis and has a dose dependent trend, but the therapy mechanism of curcuma's extraction still not clear, we assume that it is related to the expression of immune factors. Thus, we explored the change of the expression of immune factors in rats.

Histopathological effect

Observation without apparatus

Firstly, we observed the symptoms on propranolol cream induced psoriasis like model (see making model methods in *Psoriasis model*) and changing after treatment with difference concentration of curcuma's extraction cream.

Table 1: Immunohistochemical reagents, manufactures, and dilutions. Paraffin sections were stained immunohistochemically with cytokeratin, proliferating cell nuclear antigen and toll-like receptor markers, the dilutions, sources of primary antibodies and secondary antibodies used in our study are listed below:

Antigen	Clone	Manufacturers	Dilution
CK14	Rabbit monoclonal	Abcam	1/2000
CK16	Rabbit polyclonal	Abcam	1/500
CK17	Rabbit monoclonal	Abcam	1/200
PCNA	Rabbit polyclonal	Abcam	1/200.
TLR2	Rabbit polyclonal	Abcam	1/200
TLR4	Mouse monoclonal	Abcam	1/100
TLR9	Mouse monoclonal	Abcam	5 μg/mL
Secondary antibody	Rabbit	Zhongshan goldenbridge biotechnology	1/1000

CK, cytokeratin; PCNA, proliferating cell nuclear antigen; TLR, toll-like receptor

The ear skin in negative control group were smoothly, no erythema and any abnormal; In model group, the skin were appeared visible dark red or reddish-brown scales and different degrees of infiltration, so the method of making model was success; In treatment group, the skin lesions were certainly improved, becoming smoother and the scales were regression.

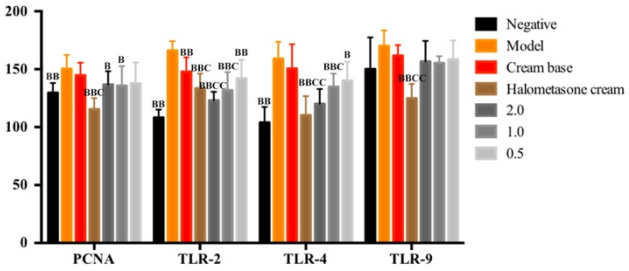


Fig. 7: Curcuma's extraction cream inhibited the expression of immune factors (N=8). After treatment with different concentrations of curcuma's extraction cream, we tested the expression of PCNA, TLR2, TLR4, TLR9 in mice. The results represent three repeated experiments average value (mean) ± standard deviation (S.D.). Compared with model control group, $^{B}P<0.05$, $^{BB}P<0.01$; compared with cream base group, $^{C}P<0.05$, $^{CC}P<0.01$.

Table 2: ELISA kits manufacture. Measuring the content of CK14, CK16, CK17, PCNA, TLR-2, TLR-4, TLR-9 in specimens by ELISA, the methods were according to the kit instructions. The kits used in our study are listed in table 2.

Antigen	Manufacturers
CK14	Abcam
CK16	Abcam
CK17	Abcam
PCNA	Abcam
TLR2	Abcam
TLR4	Abcam
TLR9	Abcam

CK, cytokeratin; PCNA, proliferating cell nuclear antigen; TLR, toll-like receptor

The result shown that curcuma's extraction cream also has a strong efficacy on psoriasis like induced by propranolol cream.

Observation by optical microscope

Then, histologic sections were stained with hematoxylin and eosin (H&E) for morphological assessment by light microscopy (see the detail in ***Immunohistochemical staining***).

In negative control group, as shown in figs. 5A, structure of skin epidermis and dermis were intact, which had clear boundaries, intact of basement membrane, epidermal cells were no edema, necrosis and exfoliation, papillary layer of dermis and reticular layer was clear, and blood vessel had not congestion, expansion, edema and hyperemia. Besides, the structure of epidermal stratum basales, stratum spinosum, stratum granule, stratum corneum and stratum lucidum were also intact.

In model group, as shown in figs. 5B, epidermis was thicker obviously and acanthosis, moderately keratinization and occasionally focal lesions parakeratosis and focal epidermal necrosis were appeared in epidermis, part of spinous cells were swelling including perinuclear space increased, dermis elongated, the number of mononuclear cells and lymphocytes infiltration in epidermis and dermis were increasing.

In cream base group and halometasone cream group, as shown in figs. 5C, 5D, symptoms were similar with model group, but relatively light.

While in treatment groups (curcuma's extraction cream 2.0 native medicine g/kg group, curcuma's extraction cream 1.0 native medicine g/kg group, curcuma's extraction cream 0.5 native medicine g/kg group), the lesion had improved, as shown in figs. 5E,5F,5G. The above results shown again that make psoriasis like model was success and curcuma's extraction cream also has a strong efficacy on psoriasis like model.

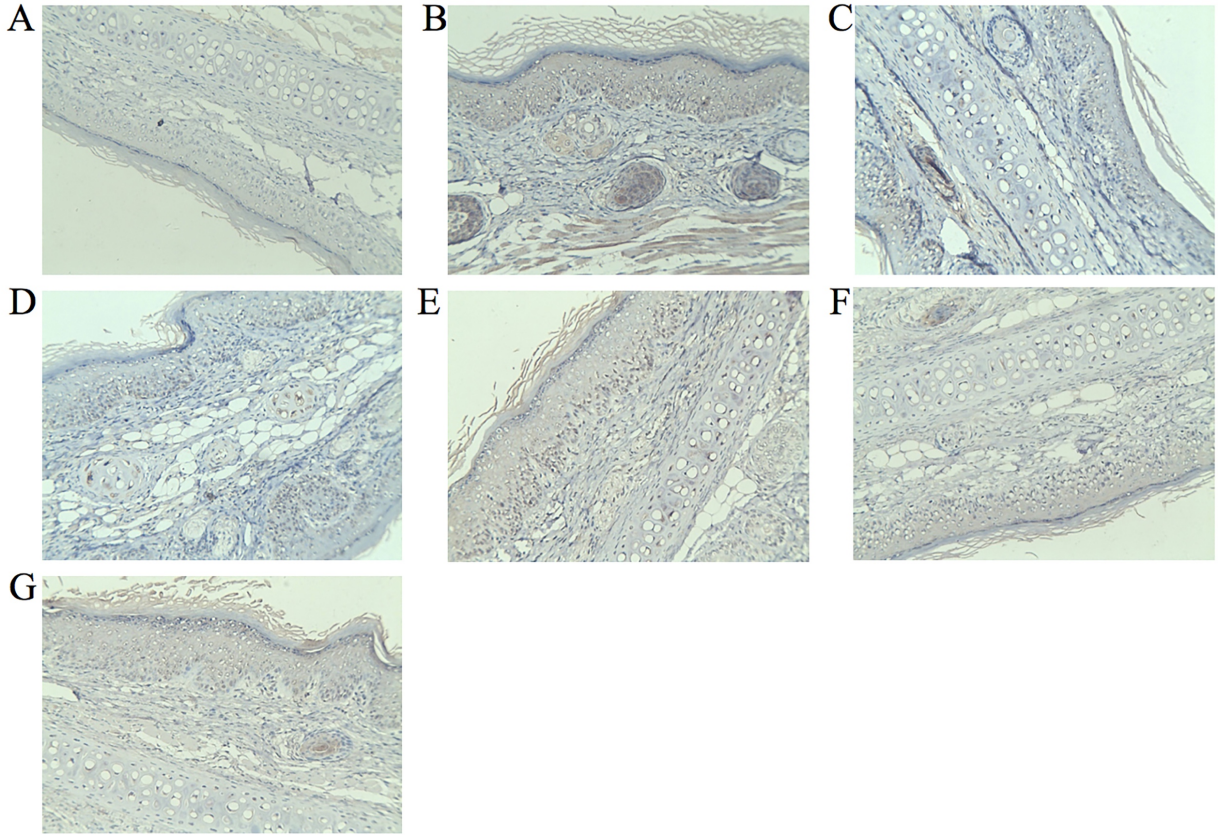


Fig. 8: Pathological imaging of all group's keratin 14.

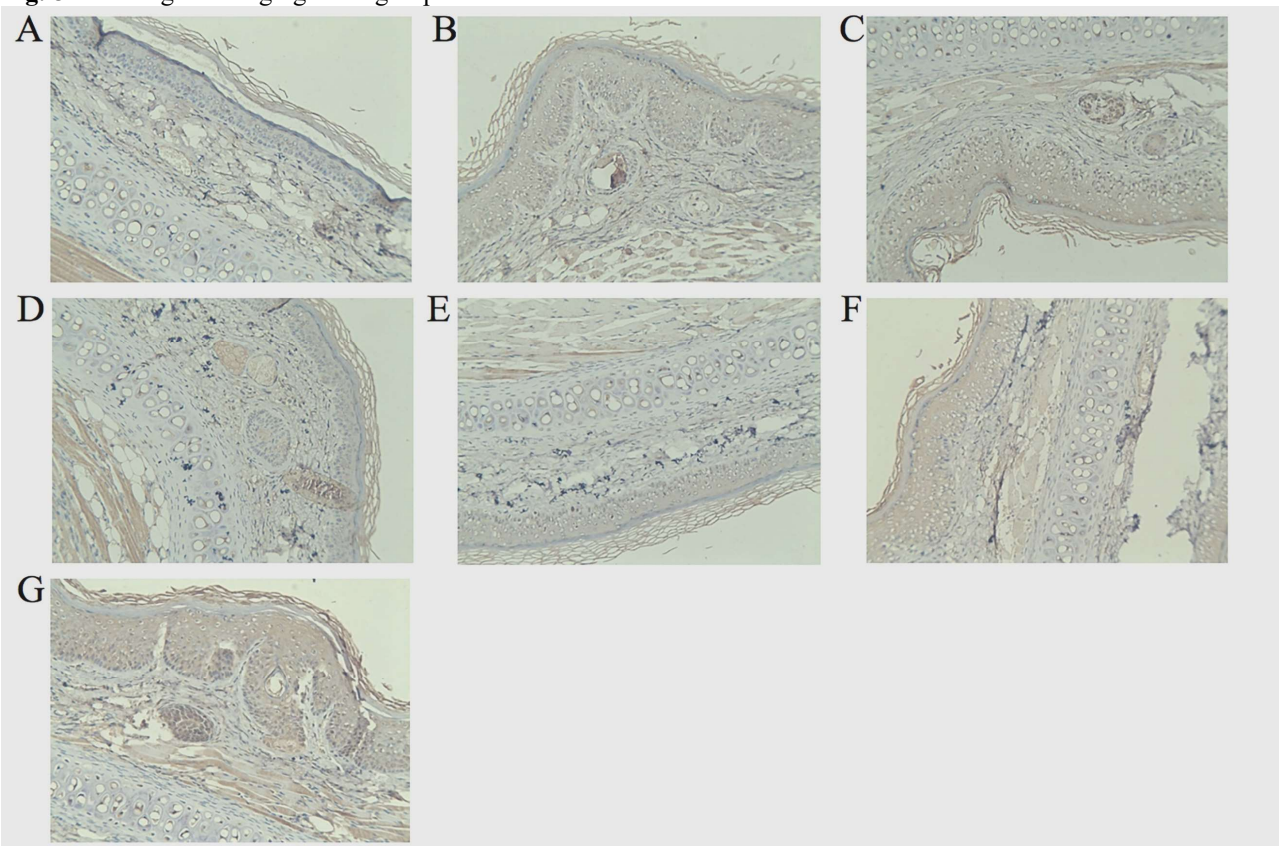


Fig.9: Pathological imaging of all group's keratin 16.

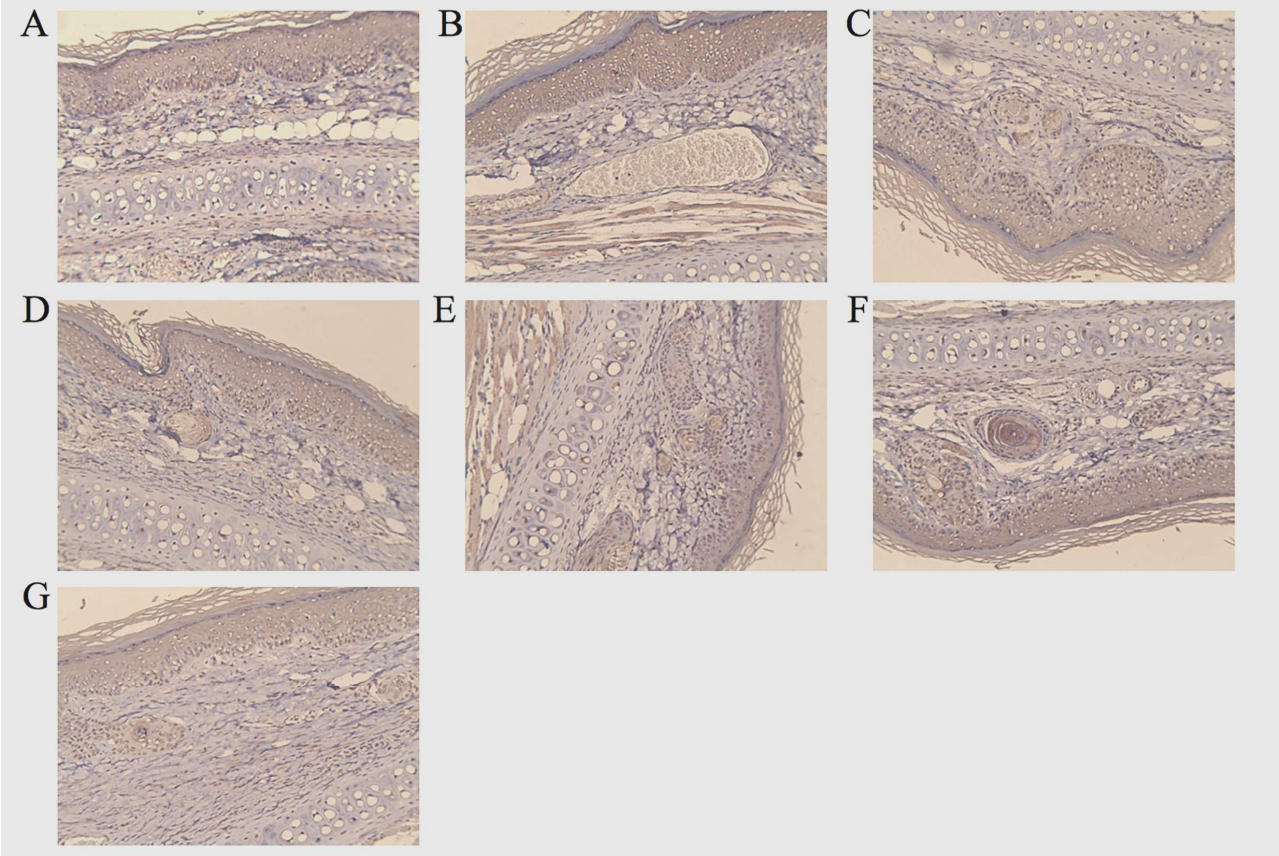


Fig. 10: Pathological imaging of all group's keratin 17.

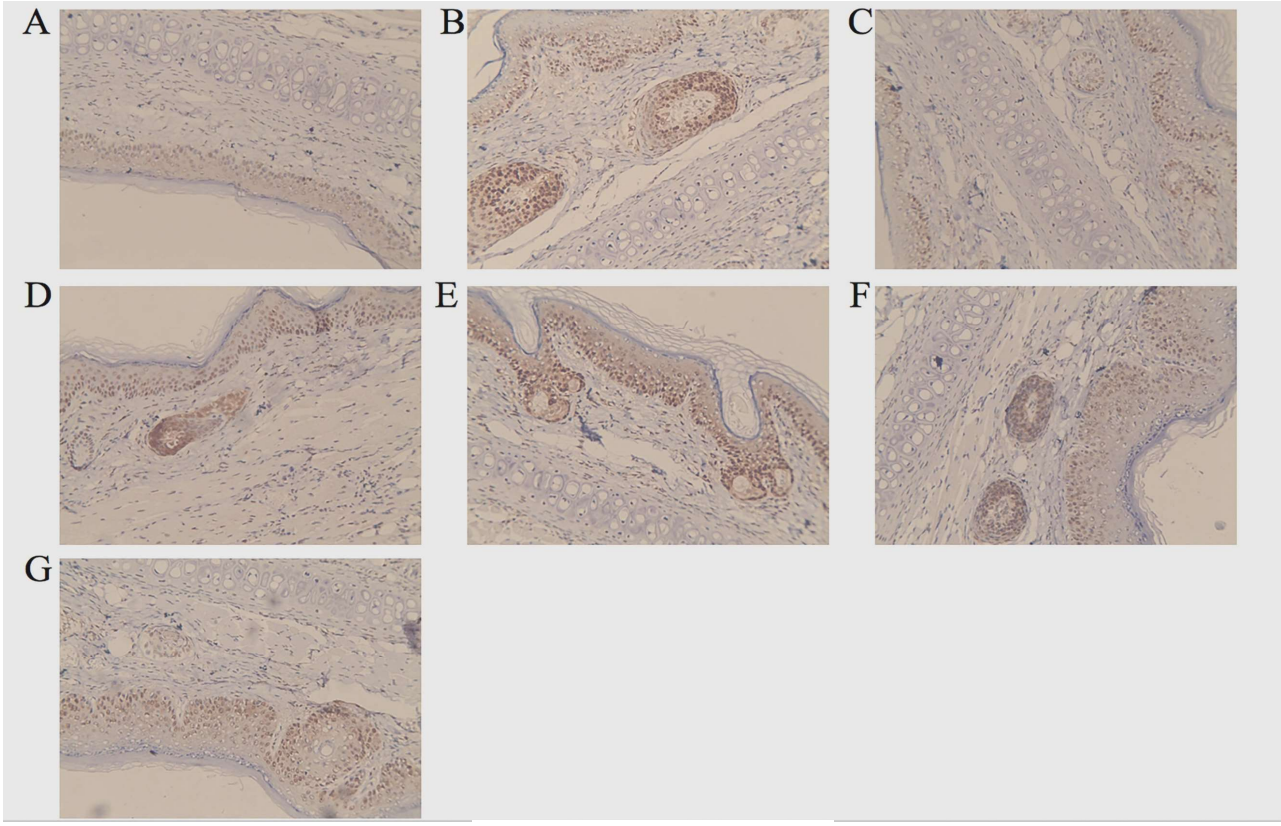


Fig. 11: Pathological imaging of all group's proliferating cell nuclear antigen.

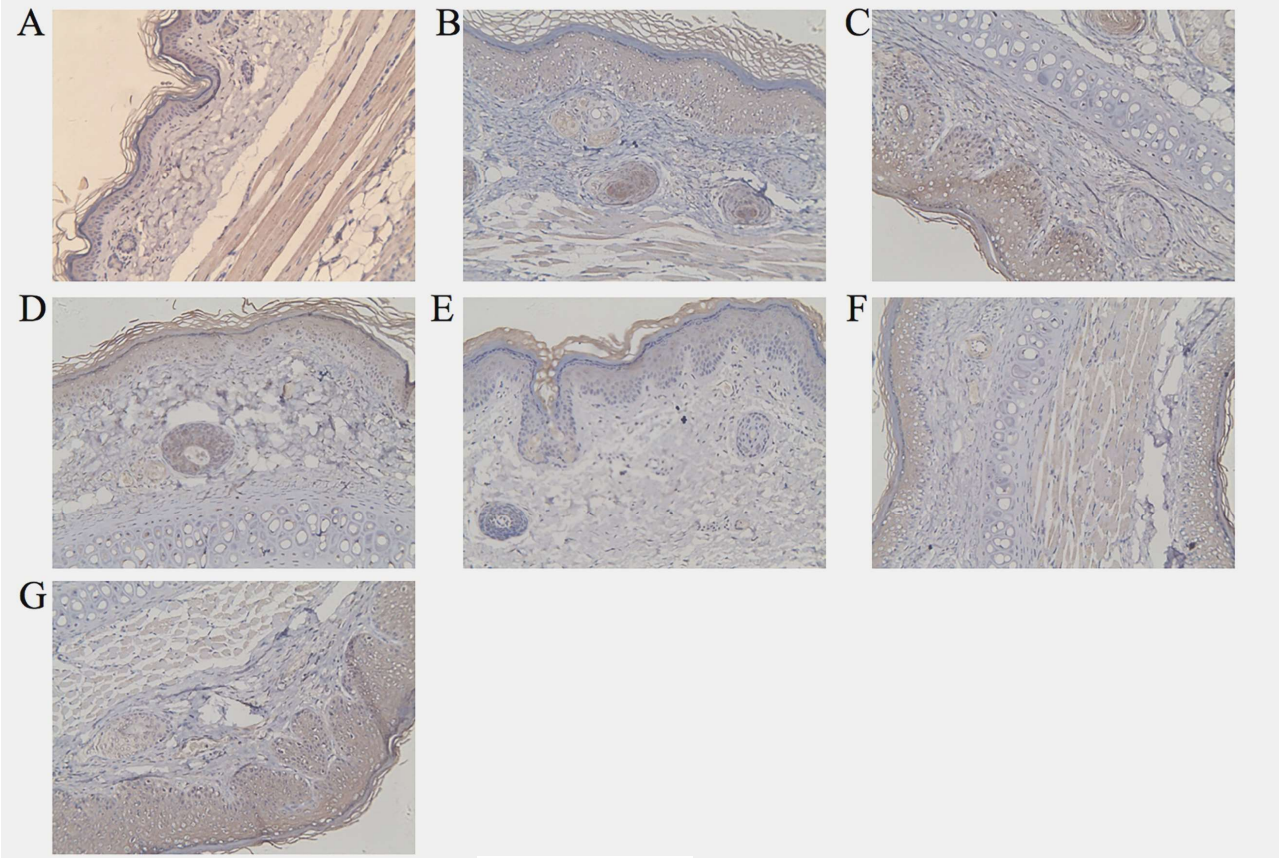


Fig. 12: Pathological imaging of all group's toll-like receptors 2.

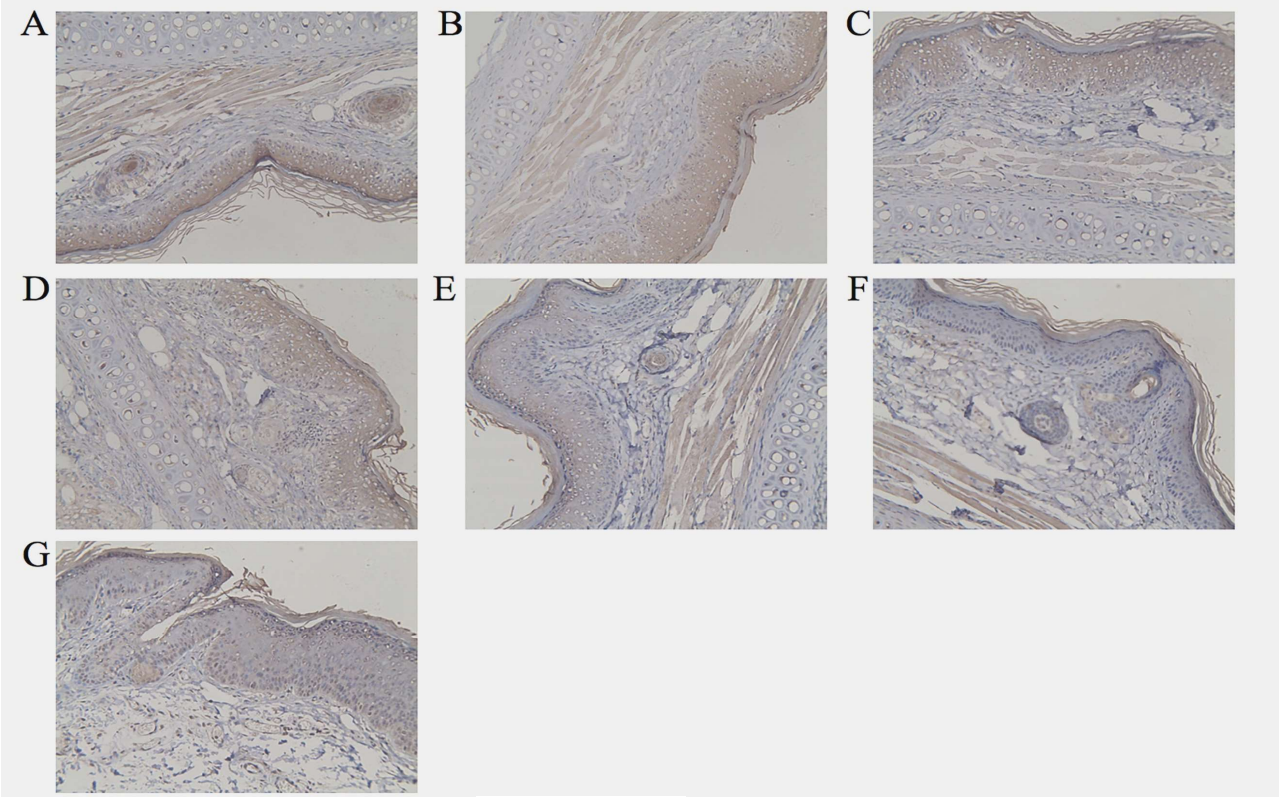


Fig. 13: Pathological imaging of all group's toll-like receptors 4

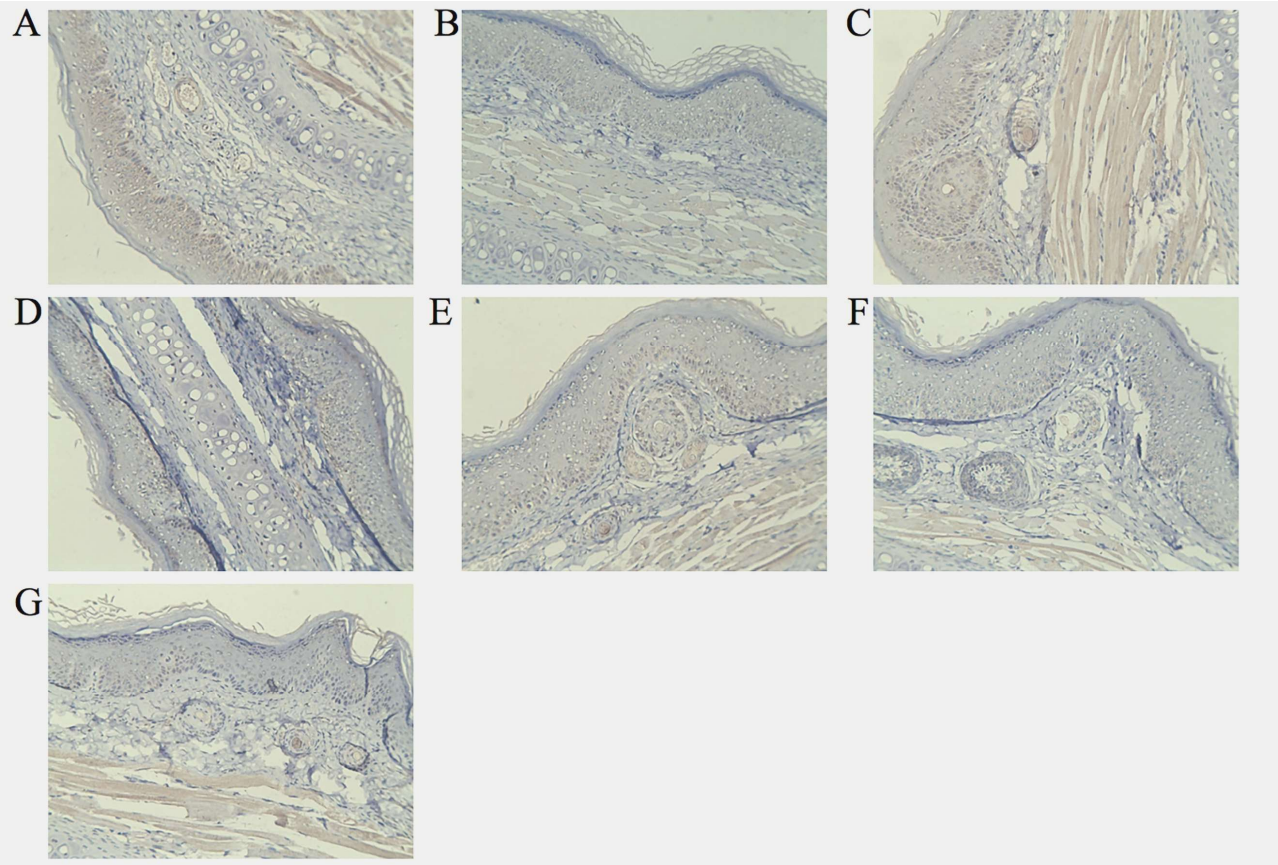


Fig. 14: Pathological imaging of all group's toll-like receptors 6

Table 3: The effect of curcuma's extraction cream in the mitosis of mouse vaginal epithelial cells (X±S)

Group	Dose (g/kg)	Animal (n)	Mitotic index (%)
Negative control	——	10	10.5±3.2**
Model control	——	10	37.4±4.6
Halometasone cream (positive control)	0.0005	10	12.4±4.1**
Curcuma's extraction cream	2.0	10	18.9±5.0**
Curcuma's extraction cream	1.0	10	28.6±4.9**
Curcuma's extraction cream	0.5	10	34.2±5.7

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

Table 4: the effect of curcuma's extraction cream to skin damage (X±S)

Group	Dose (g/kg)	Animal (n)	The score of skin damage	
			7 d	14 d
Negative control	——	10	0.0±0.0**	0.0±0.0**
Model control	——	10	9.7±2.3	6.9±1.9
Halometasone cream (positive control)	0.0005	10	4.8±1.7**	3.4±1.6**
Curcuma's extraction cream	2.0	10	5.7±2.0**	4.2±1.5**
Curcuma's extraction cream	1.0	10	6.5±3.0*	4.8±2.1**
Curcuma's extraction cream	0.5	10	7.3±3.5	5.8±2.7

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

Table 5: Curcuma's extraction cream inhibited the expression of keratin (N=8) (X±S)

Group	CK14	CK16	CK17
Negative	130.4±10.8^{BB}	143.1±18.6^{BB}	114.1±11.9^{BB}
Model	169.0±16.0	168.0±15.5	157.0±10.9
Cream base	165.7±10.0	160.6±11.0	146.2±16.9
Halometasone cream	145.0±18.7^{BB}	150.2±10.8^{B}	128.3±12.7^{BBC}
Curcuma's extraction cream 2.0	155.1±15.5	144.9±16.1^{BBC}	129.0±21.7^{BBC}
Curcuma's extraction cream 1.0	158.1±12.6	153.7±11.2^{B}	143.0±12.5
Curcuma's extraction cream 0.5	160.9±15.4	156.0±9.3	143.2±18.9

Table 6: Curcuma's extraction cream inhibited the expression of immune factors (N=8) (X±S)

Group	PCNA	TLR-2	TLR-4	TLR-9
Negative	129.6±8.5^{BB}	108.2±6.9^{BB}	103.9±13.6^{BB}	150.1±27.3
Model	150.3±12.0	166.1±8.0	159.0±14.7	170.1±13.3
Cream base	144.7±10.9	147.8±12.5^{BB}	150.4±21.0	161.6±9.2
Halometasone cream	115.4±9.6^{BBC}	133.5±12.7^{BBC}	110.2±16.5^{BBCC}	124.8±12.5^{BBCC}
Curcuma's extraction cream 2.0	136.7±11.6^{B}	123.0±7.5^{BBCC}	120.0±12.9^{BBCC}	156.7±17.7
Curcuma's extraction cream 1.0	135.6±17.1^{B}	131.8±15.7^{BBC}	134.7±11.5^{BBC}	155.2±5.9
Curcuma's extraction cream 0.5	137.5±18.2	142.0±16.1^{BB}	140.0±16.4^{B}	158.3±16.5

Compared with model control group, $^{B}P<0.05$, $^{BB}P<0.01$;
Compared with cream base group, $^{C}P<0.05$, $^{CC}P<0.01$.

Curcuma's extraction cream inhibited the expression of immune factors

Curcuma's extraction cream change the expression of keratin

Next, as shown in figs. 6 and table 5 and figs. 8 A-G, figs. 9 A-G, figs 10 A-G: In model group, the expression of CK14, CK16 and CK17 were significantly increased, compared with negative control group, there was statistical significance (P<0.01). After treatment, the expression of CK14, CK16 and CK17 were all decreased in different degrees.

In positive control group, compared with the expression of CK14, CK16 and CK17 in the model group, there was statistically significant (P<0.01); and compared with the cream base group, the expression of CK17 had statistical significance (P<0.05).

In curcuma's extraction cream 2.0 native medicine g/kg group, compared with the expression of CK16, CK17 in model control group, there was significantly decreased (P<0.01).

In curcuma's extraction cream 2.0 native medicine g/kg group, compared with the expression of CK16 in the model group, there was significantly decreased (P<0.05); and compared with the cream base group, there was also significantly decreased (P<0.05).

All in all, the data shown us that curcuma's extraction cream have decreased the expression of CK14, CK16, CK17, which may result in improving the symptoms of psoriasis.

Curcuma's extraction cream change the expression of proliferating cell nuclear antigen

How about curcuma's extraction cream influence on proliferating cell nuclear antigen? As shown in figs. 7 and table 6 and figs. 11 A-G

In model group, the expression of PCNA was significantly higher than that in negative group (P<0.01)

After treatment, the expression of PCNA was decreased with different degrees. In halometasone cream group, compared with the model group, the expression of PCNA was decreased (P<0.01); In curcuma's extraction cream 2.0 native medicine g/kg group and curcuma's extraction cream 1.0 native medicine g/kg group, compared with model control group, the expression of PCNA were both decreased (P<0.05).

The data shown us that high concentration of curcuma's extraction cream has powerfully inhibit on the expression of PCNA than halometasone cream, which means curcuma's extraction may has highly curative effect on psoriasis than halometasone cream.

Curcuma's extraction cream change the expression of toll-like receptors

We found the same trend in the expression of toll-like receptors, as shown in figs. 7 and table 6 and s figs. 12 A-G, figs. 13 A-G, figs. 14 A-G. In model group, the expression of TLR-2 and TLR-4 were significantly higher than that in negative group (P<0.01).

After treatment, the expression of TLR-2, TLR-4 and TLR-9 were decreased in different degrees.

In halometasone Cream group, the expression of TLR-4, TLR-9, compared with the model group, were significantly decreased (P<0.01), and compared with the cream base group, there were significantly decreased (P< 0.01), too.

Compared with the model group, the expression of TLR-2, TLR-4 were significantly decreased (P<0.01) in the curcuma's extraction cream 2.0 native medicine g/kg group, the expression of TLR-2, TLR-4 were decreased (P<0.05) in the curcuma's extraction cream 1.0 native medicine g/kg group , the expression of TLR-4 were decreased (P<0.05) in the curcuma's extraction cream 0.5 native medicine g/kg group. And compared with the cream base group, the expression of TLR-2, TLR-4 in the curcuma's extraction cream 2.0 native medicine g/kg group, the curcuma's extraction cream 1.0 native medicine g/kg group were significantly decreased (P< 0.01).

So, the data shown us that curcuma's extraction cream inhibited the expression of TLR-2, TLR-4, although have no influence on TLR-9, which still means that curcuma's extraction has highly curative effect on psoriasis.

DISCUSSION

Psoriasis is a common inflammatory and chronic skin disease that affects above 2% the population in the world, the etiology of RA or treatment is not yet fully clear. The disease is characterized by significantly epidermal proliferation and unusual differentiation caused by keratinocytes' immune activation, always followed by a series of inflammatory response and immune confusion, and was involved in both innate and acquired immune system. This hyper-proliferation is triggered by various cytokines, which is secreted by activated resident immune cells, an infiltrate of T cells, dendritic cells, as well as the keratinocytes themselves (Ishitsuka *et al*., 2013; Elder, 2009; Batacsorgo *et al*., 1993).

The main pathological of psoriasis are parakeratosis, epidermal hyper-proliferation and the increasing of cell mitosis. Proliferation of vaginal epithelial cells induced by estrogen can be used to simulate the psoriasis characteristics of epidermal hyperplasia, which is commonly used in the preliminary pharmacodynamical screening of psoriasis.

In addition, there has no mature model to simulate the pathological changes of psoriasis epidermal hyperplasia in skin cells, only HaCaT immortalized human epidermal cells (HaCaT cells) can be used to explore the mechanism of psoriasis, which relevant research is being carried out. So, in this study we choose mouse vaginal epithelial cells used in studying the effect of curcuma's extraction cream on the mitosis.

In our study, the result shown that the mitotic index was significantly reduced in the groups that treatment with curcuma extraction cream, so it proved that curcuma's extraction can inhibit mice vaginal epithelial mitosis, curcuma's extraction may have the effect of anti-psoriasis.

We assessed psoriasis-like induced by Imiquimod in King-Ming mice and propranolol in guinea pig. We expect to evaluate the pharmacodynamics efficacy of curcuma's extraction cream in treatment of psoriasis through two different mechanism models.

Imiquimod is an agonist of TLR, can bond with TLR7 of epidermal plasmacytoid dendritic cells and macrophages, which will induce a large number of secretion of interferon alpha (IFN-alpha), a series of interleukin and so on, finally cause a series of histological changes, like hyperkeratosis, parakeratosis, acanthosis, angiogenesis and infiltration of inflammatory cells (Kan *et al*., 2012), the histopathological changes are similar to psoriasis, so we often use imiquimod to make psoriasis-like model of mice.

Propranolol can block beta adrenergic receptor in keratinocytes cells and reduce cyclic adenosine monophosphate (cAMP) levels, will lead to a series of histological changes, such as epidermal hyperkeratosis, parakeratosis, acanthosis and the cuticle of polymorph nuclear cell infiltration, the histological changes are similar to psoriasis, so we often use propranolol to make psoriasis-like model of guinea pig.

Daily application of IMQ on mice skin can triggers Th1 and Th17 cell-mediated adaptive immunity and mice rapidly exhibit thick skin with erythema and scales like psoriasis (Fits *et al*., 2009). Our results shown that wiping 5% Imiquimod for 7 days, the symptoms which were similar with psoriasis were appeared, after using curcuma's extraction cream, the symptoms of skin lesions were relieved. It explained that curcuma's extraction can alleviate psoriasis.

Psoriasis not only has connected with environment, but also involves deeply genetic factor. Inflammatory cytokines in psoriasis patients were higher than normal people, and the serum concentrations of a subset of cytokines also has the same trend in psoriasis people.

Psoriasis will cause cell differentiation, which is associated with proliferating nuclear cell antigen. Proliferating nuclear cell antigen is a nuclear protein synthesized or expressed only in proliferating cells, which is essential for eukaryotic cell DNA synthesis (Kawahira, 1999), so if the tissues have proliferating cells, PCNA expression will increase. The expression of PNCA is same with DNA synthesis, began to rise at the G1 phase,

got peaked at S phase, fell to lowest at M phase, so PCNA can indirectly reflect the proliferation state of cells, PCNA is currently recognized to assess the proliferation state index of cells (Miracco *et al*., 2000). Research showed the expression of PCNA in the patients with psoriasis were significantly enhanced, which was related to hyper-proliferation and abnormal differentiation of psoriatic epidermal cells (Kawahira, 1999).

Our result shown that the expression of PCNA in mouse were significantly decreased after treatment with curcuma extraction cream, it illustrated that curcuma extraction can inhibit cell proliferation.

As early as 1960 (Bernerd *et al*., 1992), Rothberg found that patients with psoriasis compared to the normal people the expression of epidermal keratin are differences, the current more consistent view is mainly focused on the changes of CK5, CK14, CK10, CK16 and CK17. Holland (Holland *et al*., 1989) and Thewes (Thewes *et al*., 1991) found in the lesions of psoriasis CK14 level compared with normal skin was changed, the expression in psoriatic lesions were increased. In additions, CK16 is characteristic of hyperplasia in epidermis, and it in the edge of skin lesions of psoriasis also have high expression, Thewes (Thewes *et al*., 1991) confirmed the skin which did not affected by psoriasis also have the expression of CK16, it can well explain the phenomenon of Kobner, so CK16 can be used as markers for psoriasis (Wislincenus, 1989; Stoler, 1988; Mare *et al*., 1990). CK17 in normal skin have no expression, while in skin lesions of psoriasis have high expression. Hence, CK17 is considered to be a hallmark of psoriasis (Fu and Wang, 2012).

We find the expression of CK14, CK16, CK17 in psoriasis-like model of guinea pig increase. Similarly, other studies (Thewes *et al*., 1991; Cstelijins *et al*., 1999; Van *et al*., 2007; Jong *et al*., 1995) also proved that the expressions of CK14, CK16, CK17 were remarkably higher in injured skin. The result also shown that after treatment, the expression of CK14, CK16 and CK17 were all decreased, so it proved that curcuma extraction can inhibit the level of CK14, CK16, CK17, which may be one of mechanisms for curcuma extraction anti-psoriasis.

There is affluence evidence supporting that genetic variant has a great significant for psoriasis in innate immunity field (Tsoi *et al*., 2012). TLR, the full name is Toll-like receptors, are expressed on immune cells, including monocytes, macrophages and others. TLRs have an important position in autoimmune disease, is a bridge to connect auto-inflammatory and infection (Liu *et al*., 2014).

Plenty of studies have investigated the expression of TLR in psoriasis. Early studies have discovered that the expression of TLR2 and TLR4 are remarkable increasing in psoriatic patients (Garcia *et al*., 2013), and through enhancing TLR2 expression of psoriatic patients could further upregulate TLR4 expression in epidermal and dermal dendritic cells (Begon *et al*., 2007). TLR2 is relate to the deep skin layers while TLR4 is relate to the superficial skin layers (Panzer *et al*., 2014). TLR4 is guessed to be an incentive of cell apoptosis and play an important role in the autoimmune system, especially associated with TLR2. Besides, a new study has proved that the expression of TLR4 on peripheral blood mononuclear cells of psoriasis were much more higher than that in normal people (Garcia *et al*., 2013).

Our results shown that TLR-2 and TLR-4 in the skin lesions of guinea pigs were higher than that in the blank group ($P<0.05$), after treatment with curcuma's extraction cream, the expression was remarkably decreased($P<0.05$), which may be one of anti-psoriasis mechanisms for curcuma extraction.

CONCLUSIONS

Our study proves that curcuma extraction may have effect on psoriasis. The beneficial effect of this agent maybe partly due to the expression of keratin, PCNA, TLR2, TLR4 and TLR-9 were decreased, and cause the balance between pro-inflammatory cytokine and anti-inflammatory were changed. So, from anti-inflammatory and immunomodulatory perspective, our investigation has provided a novel direction for curing psoriasis.

The results show that curcuma's extraction can inhibit the mouse vaginal epithelial cell mitosis and improve some symptoms of psoriasis. The change and improvement may connect with the change of immune factors expression, such as keratin, proliferating cell nuclear antigen, toll-like receptors, which were significantly decreased after treatment with curcuma's extraction.

Ethics approval

The animal experimental protocols were approved by Sichuan Provincial People's Hospital and carried out in accordance with the Animal Care Guidelines of the National Institutes of Health.

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