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ORIGINAL ARTICLE

The Inhibitory Effects of Different Kinds of Ginsenosides on Skin Pigmentation in Melasma Mice Model

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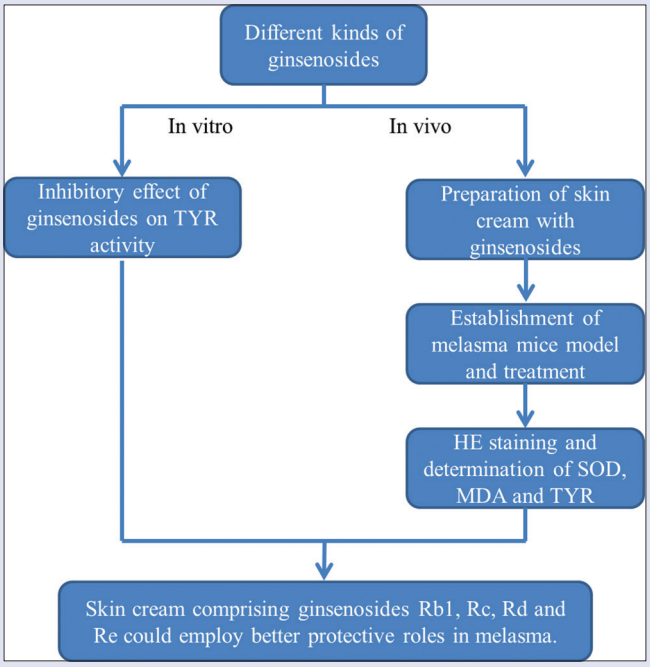
ABSTRACT

Objectives: The study was aimed to compare the effects of seven different kinds of ginsenosides and total saponins on melasma and to explore their related mechanisms. **Materials and Methods:** The inhibitory rate of ginsenosides on tyrosinase (TYR) activity was measured *in vitro*. The skin cream comprising different kinds of ginsenosides was prepared and the melasma mice model was established. After successful modeling, different skin creams were applied to the backside of mice for 30 days and these mice were separated into 11 groups. Afterward, the melanocytes and melanin in the epidermal cells were analyzed under the microscope. Finally, the activity of superoxide dismutase (SOD), TYR and the content of malondialdehyde (MDA) in melasma mice were determined by different assay kits. **Results:** The outcomes of *in vitro* experiment showed that ginsenosides could affect TYR activity with different concentrations. In the melasma mice model, ginsenosides and total saponins inhibited the growth of melanocytes and the synthesis of melanin. Furthermore, with the increasing use of skin cream comprising ginsenosides and total saponins, the activity of SOD showed an increase trend, while MDA content and TYR activity exhibited a decrease trend. Thirty (30) days of treatment with ginsenosides (except for Rg1 and Rg2) and total saponins significantly increased the SOD activity and decreased the MDA and TYR levels compared with those in melasma mice without treatment ($P < 0.05$). **Conclusion:** Skin cream comprising ginsenosides Rb1, Rc, Rd, and Re could employ better protective roles in melasma by suppressing oxidative stress and inhibiting the synthesis of melanin.

Key words: Ginsenosides, melanin, melasma, oxidative stress, tyrosinase

SUMMARY

- Ginsenosides have a potential effect on averting skin pigmentation and inhibiting tyrosinase activity
- It is the first report to compare the effects of seven different kinds of ginsenosides and total saponins on melasma
- Ginsenosides may play protective roles in oxidative stress of melasma by distressing SOD and MDA levels.



Abbreviations used: TYR: Tyrosinase; SOD: Superoxide dismutase; MDA: Malondialdehyde; UV: Ultraviolet; PBS: Phosphate buffer salt; HE: Hematoxylin-eosin.

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INTRODUCTION

Melanin, an essential protective substance for human skin to resist ultraviolet (UV) rays, is related to many skin illnesses, such as melasma, freckles, and senile lentigines.[1] Melasma, caused by the accumulation of abnormal melanin, is an irregular light-to-dark brown-colored macula on the skin that unprotected to sunlight, especially that on the face.[2] It is predominantly common among women in their 30s and 40s, especially among Asians.[3] Melasma has a great influence on the appearance of patients, causing psychological, emotional distress, and reducing the life quality of patients. Chronic ultraviolet (UV) exposure is typically thought to be associated with the development of melasma.[4] Due to the recurrence of melasma, its treatment is often interesting.[2] Therefore, it is imperative to explore a novel therapeutic strategy to manage melasma for a long time.

Panax ginseng C. A. Mey (Araliaceae family), has been employed as herbal remedy for thousands of years in China, East Asia, and Southeast

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Asia.[5] Different kinds of ginsenosides were distinguished in ginseng roots and played key roles in the pharmacological activity, including anti-fatigue, anti-cancer, cardio-protective, immunomodulatory, and antioxidant properties.[6-8] In northern China, there was an interesting phenomenon that the hands of workers who involved in ginseng cleaning work continued delicate all the time and were much smoother than others in autumn and winter. Hence, it was theoretical that some ingredients in ginseng may have a protective effect on the skin. Besides, Lee *et al*. reported that ginseng seed had an excellent inhibitory effect on the production of melanin and indicated low cytotoxicity when applied to melanocytes.[9] Another study of Wang *et al*. confirmed that ginsenoside Rb1 could conquer the melanin production in mice induced by B16 melanoma cells.[10] However, the effects and mechanisms of different kinds of ginsenosides on melasma are still uncertain.

Therefore, in this study, a melasma animal model was recognized and skin cream-containing different kinds of ginsenosides was produced. Afterward, different skin creams were applied to melasma mice. The effects of seven different kinds of ginsenosides and total saponins on melasma were compared and their related mechanisms were discovered. These findings would help to improve our understanding of the development of melasma and provide a theoretical basis for the treatment of melasma.

MATERIALS AND METHODS

Reagents and chemicals

Ginsenosides Rb1 (purity 99.23%), Rb2 (purity 99.54%), Rc (purity 99.33%), Rd (98.51%), Re (purity 99.68%), Rg1 (purity 98.75%), Rg2 (purity 98.93%), and total saponins (98.12%) were purchased from Jilin University (Jilin, China). Stearic acid (B25060), stearyl alcohol (C22123), isopropyl palmitate (S61528), olive oil (C41653), triethanolamine (S64111), and glycerin (AL12708) were purchased from Shanghai Yuanye Bio-Technology Co. Ltd. (Shanghai, China). HR-S1 emulsifier, methylparaben, propyl ester, and nitrogen were obtained from Guangzhou Kafin Biological Technology Co. Ltd. (Guangzhou, China). Tyrosinase (TYR) and 3,4-dihydroxy-L-phenylalanine (L-DOPA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Phosphate buffer salt (PBS) and 4% paraformaldehyde were obtained from Beijing Chemical Co., Ltd (Beijing, China). TYR assay kit was purchased from Shanghai Yuming Biotechnology Co. Ltd. (Shanghai, China). Superoxide dismutase (SOD) assay kit and malondialdehyde (MDA) assay kit were purchased from Shanghai Xinfan Biotechnology Co. Ltd. (Shanghai, China).

Inhibitory effect of ginsenosides on tyrosinase activity *in vitro*

The assay of TYR activity was performed by the method of Therdphapiyanak *et al*.,[11] with some alteration. Briefly, seven ginsenosides (5 mg) were dissolved in 5 mL phosphate-buffered saline (PBS) (pH 6.8, 1/15 mol/L) and then diluted into different concentrations of 0.125, 0.25, 0.5 and 1 mg/mL with PBS. Afterward, 50 μL different concentrations of ginsenoside samples were added to 100 μL PBS, followed by 50 μL TYR solution (200 U/mL). After pre-incubated at 25°C for 10 min, 50 μL L-DOPA (0.03%) were added to the mixture and then incubated at 25°C for 20 min. The optical density (OD) at 475 nm was measured using an UV spectrophotometer. The inhibitory rate (IR) of TYR activity was determined as follows:

$$\%\,IR = \frac{(A1 - A2) - (A3 - A4)}{(A1 - A2)} \times 100$$

Where A1 denote the OD_{475nm} of the mixture with 150 μL PBS, 50 μL L-DOPA and 50 μL TYR solution; A2 denotes the OD_{475nm} of the mixture with 200 μL PBS and 50 μL L-DDOPA; A3 denotes the OD_{475nm} of the mixture containing 100 μL PBS, 50 μL L-DOPA, 50 μL TYR solution and 50 μL test samples and A4 denote the OD_{475nm} of the mixture containing 150 μL PBS, 50 μL L-DOPA, and 50 μL test samples.

Preparation of skin cream

The production process of skin cream is complex. First, stearic acid (5%), stearyl alcohol (2%), isopropyl palmitate (6%), olive oil (0.5%), HR-S1 emulsifier (0.3%), methylparaben (0.15%), and propyl ester (0.05%) were added to distilled water (40%) and then mixed well until entirely dissolved. The mixture was oil phase A. Phase B was the mixture containing nitrogen (1%), triethanolamine (0.3%), glycerin (8%), and distilled water (33.5%). Subsequently, 3% ginsenosides were added to phase B and heated to 80°C, and the mixture was phase C. Afterward, phase C and HR-S1 emulsifier (0.2%) were added to phase A gradually. Meanwhile, the mixture was stirred in high speed for 5 min and defoamed after cooling down to 65°C–70°C. The mixture was ground into a paste of <5 μm in fineness and stirred strongly with high speed to form a milky semi-finished product. Finally, the milky semi-finished product was filled after vacuum degassing.

Establishment of melasma animal model and treatment

A total of 176 female ICR mice weighing 19-22g were purchased from Jilin University, Jilin. All mice were maintained in room temperature (24°C ± 2°C) and humidity (50% ± 5%) conditions, with a 12 h light/dark cycle. After fed adaptively for 3 days, these mice were randomly divided into 11 groups (16 mice for each group): control group, melasma model group (MD group), MD + B group, MD + Rb1 group, MD + Rb2 group, MD + Rc group, MD + Rd group, MD + Re group, MD + Rg1 group, MD + Rg2 group and MD + total saponins group. Before modeling, all mice were anesthetized with 2% pentobarbital sodium (75 mg/kg) and then their back hair was detached. Afterward, in addition to the mice in the control group, all mice in other groups were injected with 0.4% progesterone (0.02 g/kg/day) and exposed to UV for 3 h every day. The step was repeated six times a week for 4 weeks to create the melasma animal model. The mice in the control group were injected with the equivalent dose of saline solution and exposed to white light. During the experiment, the mice were fed with food and water freely. After successful modeling, the mice in the MD group were not treated; the mice in the control group and MD + B group were applied with skin cream without ginsenosides, while the mice in other groups were applied with skin cream comprising different kinds of ginsenosides twice a day (about 0.4 g/time) for 30 days. The skin cream in different groups was prepared according to the formulation of Table 1. All animal experiments were approved by the Animal Ethics Committee of Jilin Agricultural Science and Technology College (IACUS No. 2019052301).

Collection of tissue samples

After every 7 days of the administration, 4 mice in each group were chosen to collect the back-skin tissues. Then tissues were washed in PBS and frozen in liquid nitrogen and stored at −80°C for subsequent study. At the end of the experiment, rest of the mice were killed by cervical dislocation and the back-skin tissues of mice in each group were obtained. Afterward, the epidermal tissues (1.5cm × 1.0 cm) were collected from the skin tissues and fixed with 4% paraformaldehyde for histopathological examination.

Histopathological analysis of epidermal tissues

The pathological changes of epidermal tissues were detected by histopathological analysis based on the method of Mao *et al*.[12]

Table 1: The formulations of skin cream in different groups

Ingredient (%, m/m)	CK	MD+B	MD+Rb1	MD+Rb2	MD+Rc	MD+Rd	MD+Re	MD+Rg1	MD+Rg2	MD+total saponins
Stearic acid	5	5	5	5	5	5	5	5	5	5
Stearyl alcohol	2	2	2	2	2	2	2	2	2	2
Isopropyl palmitate	6	6	6	6	6	6	6	6	6	6
Olive oil	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
HR-S1 emulsifier	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Methylparaben	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Propyl ester	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Nitrogen	1	1	1	1	1	1	1	1	1	1
Triethanolamine	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Glycerin	8	8	8	8	8	8	8	8	8	8
Distilled water	76.5	76.5	73.5	73.5	73.5	73.5	73.5	73.5	73.5	73.5
Rb1	0	0	3	0	0	0	0	0	0	0
Rb2	0	0	0	3	0	0	0	0	0	0
Rc	0	0	0	0	3	0	0	0	0	0
Rd	0	0	0	0	0	3	0	0	0	0
Re	0	0	0	0	0	0	3	0	0	0
Rg1	0	0	0	0	0	0	0	3	0	0
Rg2	0	0	0	0	0	0	0	0	3	0
Total saponins	0	0	0	0	0	0	0	0	0	3

Table 2: The inhibitory rate of different kinds of ginsenosides on tyrosinase activity *in vitro*

Concentrations ($mg.mL^{-1}$)	Rb1	Rb2	Rc	Rd	Re	Rg1	Rg2	Total saponins
	Inhibitory rate (%)							
1	26.20±0.03[b]	22.84±0.21	23.21±0.15[a]	50.69±0.14[a]	42.62±0.11[a]	27.42±0.05[b]	−27.5±0.14[a]	52.31±0.17[a]
0.5	27.13±0.01	30.45±0.14[a]	28.96±0.14[a]	36.04±0.06[a]	33.38±0.03[b]	27.21±0.14[b]	−13.35±0.13[b]	35.14±0.20[b]
0.25	27.83±0.13[b]	28.54±0.13[b]	37.62±0.23	35.72±0.01[a]	29.14±0.13	26.61±0.31[b]	−11.6±0.07[b]	34.31±0.04
0.125	33.23±0.05[a]	30.01±0.04[a]	38.54±0.12[b]	33.65±0.09	14.15±0.11[a]	24.81±0.25[c]	−11.19±0.04[b]	30.24±0.06[b]

Briefly, the epidermal tissues were fixed with 4% paraformaldehyde and embedded in paraffin. The 5-μm sections were cut and stained with hematoxylin-eosin. Slides were scanned and images were taken under an optical microscope (Olympus Corporation, Tokyo, Japan). The melanocyte number density and OD analysis were analyzed using Image-Pro Plus 6.0 software (Media Cybernetics, Maryland, USA) and melanin statistics were also calculated in different skin tissue layers.

Determination of superoxide dismutase, malondialdehyde, and tyrosinase

The skin tissues at different times were re-suspended in PBS and then the samples were centrifuged at 3000 rpm for 10 min at room temperature. The supernatant was collected for a successive experiment. According to the manufactures' directives, the activity of SOD, the contents of MDA and TYR were measured using SOD assay kit, MDA assay kit, and TYR assay kit, respectively.

Statistical analysis

All data were presented as mean ± standard deviation. Statistical analyses were performed using SPSS 17.0 (SPSS Inc., Chicago, IL, USA) statistical package and significance was verified by one-way ANOVA. $P < 0.05$ was taken as a criterion of statistical significance.

RESULTS

Inhibitory effect of different kinds of ginsenosides on tyrosinase activity *in vitro*

The TYR inhibition of various ginsenosides was determined by measuring the OD_{475nm} value *in vitro*. With the increasing concentration of ginsenosides Rb1 and Rc, the IR of TYR activity decreased slowly [Table 2]. When the concentration of Rb1 and Rc was 0.125 mg/mL, the IRs were up to 33.23% ± 0.05%, 38.54% ± 0.12%, respectively [Table 2]. Furthermore, it is clear that the IRs of TYR activity was certainly correlated with the concentrations of ginsenosides Rd, Re, Rg1, and total saponins [Table 2]. When the concentration of Rd, Re, Rg1, and total saponins was 1 mg/mL, the IR was the highest and the inhibitory effect of total saponins was better than that of Rd, Re, Rg1 [Table 2]. For ginsenoside Rb2, when its concentration was 0.5 mg/mL, the IR was higher (30.45% ± 0.14%), while its concentration was 1 mg/mL, the IR was lower [22.84% ± 0.21%, Table 2]. However, it is obvious that ginsenoside Rg2 exhibited no inhibition on TYR activity, but had a significant promoting effect. When the concentration of Rg2 was 1 mg/mL, Rg2 is the better promoter to enhance TYR activity [Table 2]. Taken together, ginsenosides could affect TYR activity *in vitro* with different concentrations and could be an auspicious candidate as the effective whitening agent.

Analyses of melanocytes in epidermal cells of mice

A microscope with ×10 objective field and ×40 objective lens was used to detect the melanocytes in epidermal cells of mice [Figure 1]. The size and color of melanocytes in MD group and MD + B group were larger and deeper than those in CK group and there was a moving propensity toward to the skin surface in MD group and MD + B group [Figure 1a and b]. In addition to the skin cream-containing with Re, the morphology of melanocytes treated with skin cream comprising with other ginsenosides and total saponins was restored compared with the MD + B group, especially the morphology of melanocytes in Rd group was alike with that in CK group [Figure 1a and b]. As shown in Figure 1b, the darker

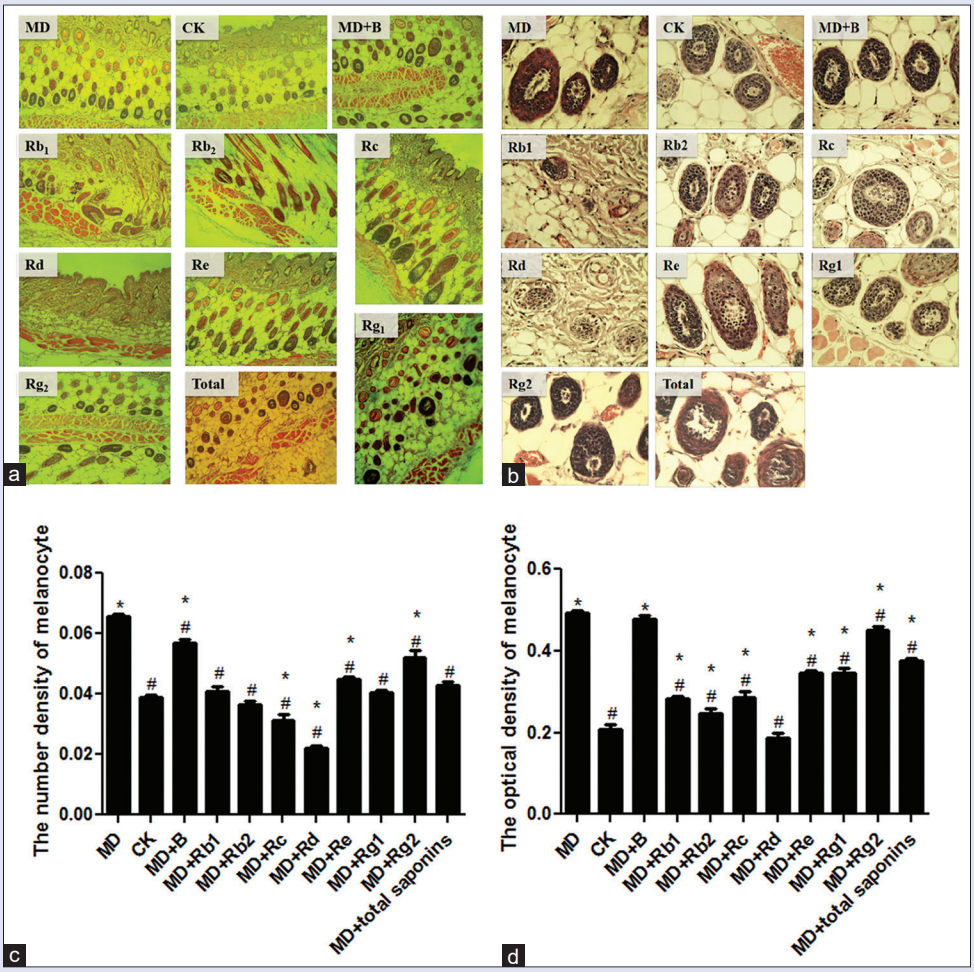

Figure 1: (a) The histopathologic changes of epidermal tissuesin different groups under a ×10 microscope. (b) Morphologic changes of melanocytes in different groups under a ×40 microscope. (c) The number density of melanocytes. (d) The optical density of melanocytes. **P* < 0.05, compared with the control group. #*P* < 0.05, compared with MD group. MD: Melasma model group; CK: Control group; MD + B: MD + B group; Rb1: MD + Rb1 group; Rb2: MD + Rb2 group; Rc: MD + Rc group; Rd: MD + Rc group; Re: MD + Re group; Rg1: MD + Rg1 group; Rg2: MD + Rg2 group; Total: MD + total saponins group

color of melanocytes in epidermal cells was detected in MD, MD + B, Rg2, and total saponins groups.

Furthermore, the number density and OD of melanocytes were determined. According to the number density analysis, the contents of melanocytes in MD group, CK group and MD + B group were, respectively, 0.0657 ± 0.009, 0.0387 ± 0.009, and 0.0567 ± 0.001, which indicated that the number of melanocytes in MD and MD + B group was suggestively increased compared with the CK group [*P* < 0.05, Figure 1c]. However, after treated with the skin cream with different ginsenosides and total saponins, the number of melanocytes in MD + Rb1, MD + Rb2, MD + Rc, MD + Rd, MD + Re, MD + Rg1, MD + Rg2, and MD + total saponins groups were significantly lower than that in the MD group [*P* < 0.05, Figure 1c]. The variation tendency of melanocytes OD was similar to that of melanocytes number density [Figure 1d]. These results showed that ginsenosides and total saponins had an inhibitory effect on the growth of melanocytes.

Analysis of melanin in spine cell layer and stratum corneum

Melasma is categorized by epidermal hyperpigmentation and augmented melanin production. The production ability of melanin in mice was also evaluated in the spine cell layer and stratum corneum [Figure 2a and b]. In the spine cell layer, the contents of melanin in MD and MD + B groups were, respectively, 30 ± 2.50 and 34 ± 0.81, which were significantly higher than that in the CK group [15.67 ± 1.25, *P* < 0.05, Figure 2c]. After applied with skin cream comprising of ginsenosides (except for Rg2) and total saponins, the contents of melanin in the spine cell layer were significantly decreased compared with the MD group [Figure 2c]. In particular, the contents of melanin in MD + Rb1, MD + Rc, MD + Re and MD + Rg1 groups were reduced to a similar level of that in CK group (*P* > 0.05). Besides, the change trend in the stratum corneum was similar to that in the spine cell layer [Figure 2d]. The contents of melanin in MD and MD + B groups were significantly higher than that in CK group (*P* < 0.05), while after administrated with skin cream-containing ginsenosides Rb1, Rb2, Rc, Rd, Re, Rg1 and total saponins, the contents were greatly reduced compared with the MD group [*P* < 0.05, Figure 2d]. In particular, the contents of melanin in MD + Rb1, MD + Rd, and MD + Re groups were decreased to a similar level of that in CK group (*P* > 0.05), while the contents in MD + Rc and MD + Rg1 groups were greatly lower than that in CK group [*P* < 0.05, Figure 2d].

The activity of superoxide dismutase

SOD plays an important role in protecting cells from oxidative damage and continuing the balance of oxygen free radicals in the body.[13] It is clear

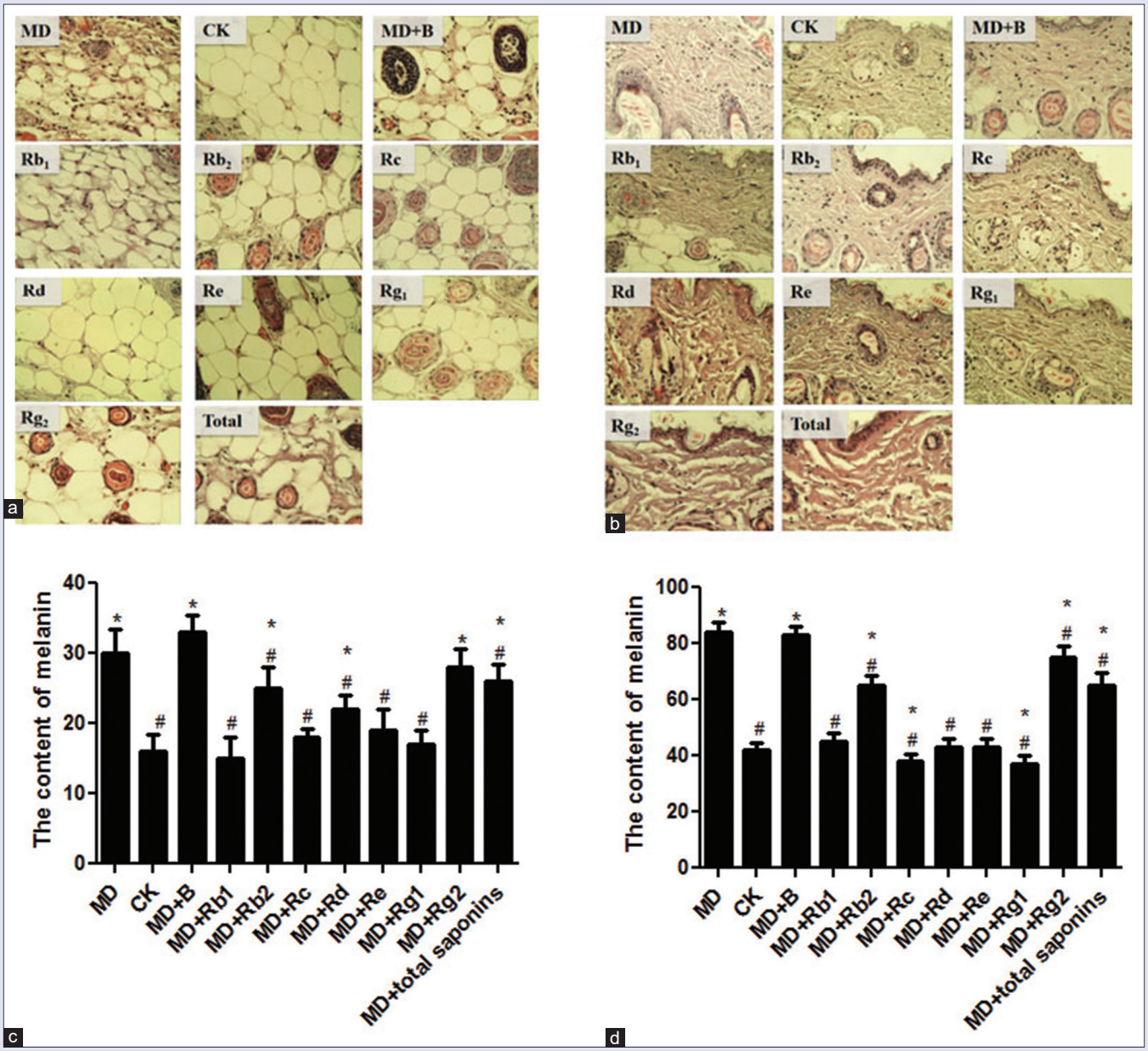

Figure 2: (a) Morphologic changes of spinal cell layer in epidermal tissues under a ×40 microscope. (b) Morphologic changes of stratum corneum in epidermal tissues under a ×40 microscope. (c) The content of melanin in spinal cell layer. (d) The content of melanin in stratum corneum. **P* < 0.05, compared with the control group. #*P* < 0.05, compared with the melasma model group. MD: Melasma model group; CK: Control group; MD + B: MD + B group; Rb1: MD + Rb1 group; Rb2: MD + Rb2 group; Rc: MD + Rc group; Rd: MD + Rc group; Re: MD + Re group; Rg1: MD + Rg1 group; Rg2: MD + Rg2 group; Total: MD + total saponins group

that the activity of SOD in mice skin displayed an increasing tendency with the increase of time after applied with the skin cream comprising ginsenosides and total saponins [Figure 3a]. Furthermore, after treatment for 30 days, the activities of SOD in MD group and MD + B group were severally 31.89 ± 1.05 U/mg pro and 42.98 ± 2.00 U/mg pro, which were suggestively lower than that in CK group [70.38 ± 0.62 U/mg pro, $P < 0.05$, Figure 3a]. However, after applied with skin cream comprising ginsenosides Rb1, Rb2, Rc, Rd, Re, Rg1, Rg2, and total saponins for 30 days, the activities of SOD were 77.37 ± 1.52 U/mg pro, 64.38 ± 1.77 U/mg pro, 57.10 ± 1.00 U/mg pro, 48.40 ± 0.70 U/mg pro, 61.22 ± 1.57 U/mg pro, 46.59 ± 1.21 U/mg pro, 36.46 ± 0.50 U/mg pro and 55.50 ± 1.35 U/mg pro, respectively [Figure 3a]. The results showed that ginsenosides and total saponins could improve the activity of SOD in melasma mice, especially ginsenosides Rb1, Rb2, and Re.

The content of malondialdehyde

With the increasing use of skin cream, the contents of MDA were decreased slowly. Compared with the CK group, the contents of MDA in other groups were all significantly higher after administrated with skin cream for 7 days [$P < 0.05$, Figure 3b]. However, after treatment for 30 days, the contents of MDA in MD + Rb2, MD + Rc, MD + total saponins groups were 1.226 ± 0.013 nmol/mgprot, 1.132 ± 0.003 nmol/mgprot and 1.380 ± 0.003 nmol/mgprot, respectively; which were close

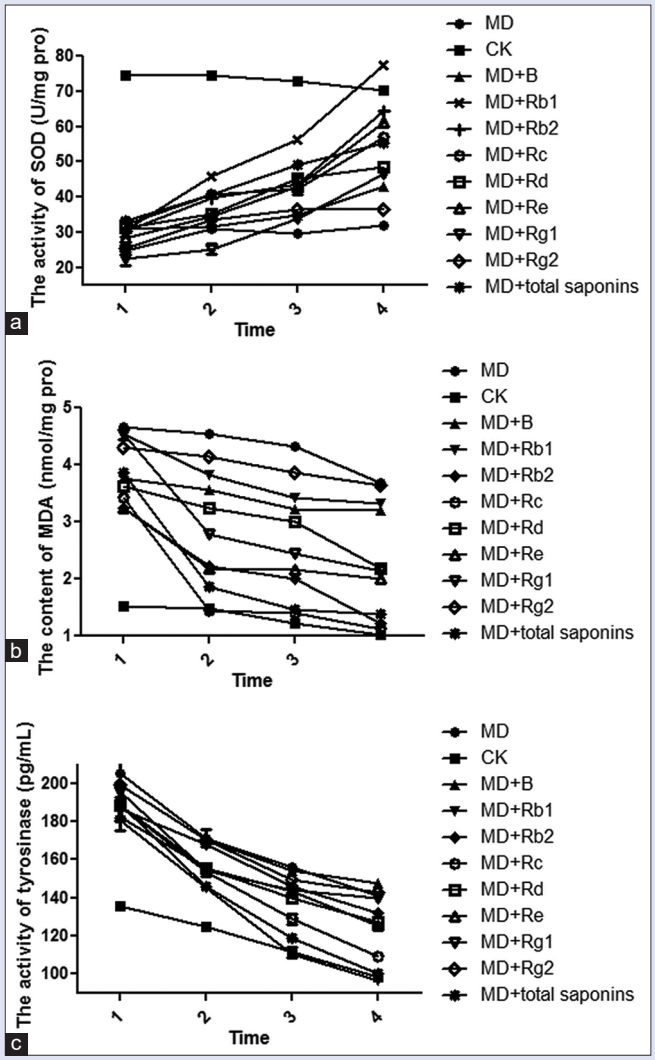

Figure 3: (a) The activity of SOD in different groups with different times. (b) The activity of MDA in different groups with different times. (c) The activity of TYR in different groups with different times. MDA: Malondialdehyde; SOD: Superoxide dismutase; TYR: Tyrosinase

to that in CK group [1.017 ± 0.011 nmol/mgprot, $P > 0.05$, Figure 3b]. Besides, the contents of MDA in MD + Rb1 and MD + Rg2 group were 3.33 ± 0.006 nmol/mgprot and 3.64 ± 0.015 nmol/mgprot, which were similar to that in MD and MD + B groups [$P > 0.05$, Figure 3b]. These results established that ginsenosides Rb2, Rc, and total saponins had better inhibitory effects on MDA content.

The level of tyrosinase

The activity of TYR was determined to assess the melanin production capacity. It is obvious that ginsenosides and total saponins inhibited the activity of TYR with the increasing use of skin cream. When the skin cream comprising ginsenosides and total saponins were used for 7 days, the inhibitory effects of ginsenosides and total saponins on TYR activity were not significant compared with the MD + B group [$P > 0.05$, Figure 3c]. However, after administration for 30 days, the activities of TYR in MD + Rb1 and total saponins groups were 96.54 ± 0.18 pg/mL and 100.44 ± 1.57 pg/mL, which were close to the TYR level in the CK group [98.70 ± 0.10 pg/mL, $P > 0.05$, Figure 3c]. For ginsenosides Rg1 and Rg2, the TYR activities in MD + Rg1 and MD + Rg2 groups were severally 140.01 ± 1.10 pg/mL and 142.84 ± 2.46 pg/mL, which were alike with the level in MD group [141.02 ± 0.56 pg/mL, $P > 0.05$, Figure 3c].

DISCUSSION

Melasma results from a local change in pigmentation, extremely affecting people's mental and emotional health.[3] Therefore, it is required to develop an alternative strategy for the long-term treatment of melasma. Some studies have shown that ginsenosides isolated from ginseng have a variety of biological activities, including antioxidation, immunoregulation, and anti-aging.[14-16] In the present study, skin cream was prepared with different kinds of ginsenosides and melasma animal models established to explore the effects of different skin cream on melasma and their related mechanisms. It was found that ginsenosides could affect TYR activity *in vitro* with different concentrations. The *in vivo* experiment showed that ginsenosides and total saponins lessened the symptom of melasma via inhibiting melanocyte growth and melanin formation. Furthermore, ginsenosides and total saponins could also impact the activity of SOD, TRY, and the content of MDA to improve melasma.

Melanin is an important factor that determined skin pigmentation in many animals, including humans. The interactions between keratinocytes and melanocytes have been shown to be vital in the regulation of melanogenesis.[17] Keratinocytes could control the growth and activity of melanocytes through various soluble factors and cell adhesion molecules.[18] Some studies have established that the increased melanocytes could promote the synthesis of melanin, which was associated with melasma.[19,20] In our study, the growth of melanocytes and melanin synthesis was inhibited by ginsenosides and total saponins in the melasma mice and ginsenosides Rb1, Rc, Re, and Rg1 had improved inhibitory effects on melanin formation. A study of Lee *et al.*[9] stated that the ethanol extract of ginseng seed had an excellent inhibitory effect on melanin production and showed low cytotoxicity when applied to melanocytes. Another study has also indicated that ginsenosides Rh6, R4, and R13, isolated from the leave of hydroponic *Panax ginseng*, showed 23.9%, 27.8%, and 35.2% melanogenic inhibitory activity, respectively, when their concentration was 80 μM.[21] Combining with our findings, it was speculated that ginsenosides Rb1, Rc, Rd, and Re may have improved effects on melasma via inhibiting melanocyte growth and melanin formation.

Further to explore the underlying mechanism of ginsenosides on melanin production, the activity of SOD, TYR, and the content of MDA was determined. Among them, SOD can precisely remove superoxide anion free radicals in living organisms and eliminate the damage of exogenous oxygen free radicals to the organism, thus protecting cells from oxidative damage.[22,23] MDA, which is the final oxidation product of lipid peroxidation by free radicals, can lead to the cross-linking polymerization of macromolecules such as proteins and nucleic acid.[24] Hence, the content of MDA was measured to assess the degree of lipid peroxidation and cell damage in the body.[25,26] In this study, the activity of SOD was decreased in the melasma mice, while the activity in other groups was significantly enhanced after treatment for 30 days (except for the skin cream-containing ginsenosides Rg2). The contents of MDA in melasma mice were obviously increased and after applied with skin cream containing ginsenosides (except for Rb1 and Rg2) and total saponins, the contents were reduced. A study of Li *et al.*[27] has confirmed that ginsenoside Rg1 could suppress oxidative stress reaction through increasing SOD activity, decreasing the content of MDA, and improving D-galactose-induced aging. Therefore, we speculated that ginsenosides and total saponins may prevent the occurrence and development of melasma by inhibiting oxidative stress.

In addition, TYR is the vital enzyme for the synthesis of melanin, which occurs in melanocytes in the basal cells of the epidermis.[28] Alam *et al*.[29] reported that jineol from *Scolopendra subspinipes mutilans* exhibited a significant inhibitory effect on TRY activity, thus inhibiting the melanin production in melan-a cells. Another study displayed that silymarin could have an effect on melasma by decreasing the expression of TYR protein using western blot method.[30] In our study, the activity of TYR was enhanced and melanin synthesis was increased in the melasma mice. However, the activity was inhibited by ginsenosides (except for Rg1 and Rg2) and total saponins, which showed that ginsenosides Rb1, Rb2, Rc, Rd, Re, and total saponins may confine the activity of TYR and prevent the synthesis of melanin, so as to improve melasma.

CONCLUSION

Skin cream comprising ginsenosides Rb1, Rc, Rd, and Re could employ better protective roles in melasma compared with skin cream with other ginsenosides. The protection mechanisms may be intricate in the suppression of oxidative stress reaction by enhancing the activity of SOD, decreasing the content of MDA, and in the synthesis of melanin by controlling the growth of melanocytes and the activity of TYR. Our findings will provide new insights into the therapeutic strategy of ginsenosides for melasma and improve our understanding of the existence and development in melasma.

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Conflicts of interest

There are no conflicts of interest.

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