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Effect of Beauty and Freckle Removing Soup Combined with Reduced Glutathione on Oxidative Stress and Disease-Related Factors in Patients with Chloasma

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ABSTRACT

The objective of this study was to discuss the effect of Beauty and Freckle Removing Soup (BFRS) combined with reduced glutathione on oxidative stress (OS) and disease-related factors in patients with chloasma. Patients with chloasma were selected in West Anhui Health Vocational College between January 2021 and June 2022. Eighty patients were randomly separated into a combination group (Group J) and glutathione group (Group G). Patients in the Group G were treated with reduced glutathione. For patients in the Group J, on the basis of Group G, they were treated with BFRS. After a 2-month treatment, the scores of TCM symptoms, chloasma area, total score of skin lesions, loss of trans epidermal water shunt, MDA level, SIL-2, TNF-α, MSH and DLQI in group J were remarkably lower than group G. The skin cuticle water content, SOD level, and GSH-Px level were remarkably superior to those of group G. It was concluded that BFRS combined with reduced glutathione performs excellent on treating chloasma, which is able to enhance the status of patients with chloasma significantly.

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INTRODUCTION

Melasma is a facial pigmentation disease with high difficulty in treatment, and its pathogenesis is relatively complex, mainly including factors including sun exposure, genetics, endocrine disorders, etc., which may lead to chloasma (Chen *et al*., 2022). In addition, oxidative stress (OS) reaction has a certain correlation in the pathogenesis of chloasma (Pan *et al*., 2022). Chloasma belongs to the category of face dust and liver spot in traditional Chinese medicine. Generally, the pathogenesis of chloasma is skin malnutrition (Huang *et al*., 2022). In the treatment of chloasma, many scholars have given their own opinions. Shan and Song (2020) treated patients with chloasma with Taohong Siwu decoction plus or minus tranexamic acid, and discussed the effect of this treatment on the level of sexual hormones and OS response of patients with chloasma. Cao *et al*. (2020) analyzed the pathogenic factors and pathogenesis of chloasma from the perspective of traditional Chinese medicine and western medicine, providing theoretical basis for the treatment of chloasma. Zhang *et al*. (2022) discussed the effect and mechanism of miRNA, and analyzed the significance of miRNA in the pathogenesis of chloasma. Chen *et al*. (2020) treated chloasma with *Bletilla striata* polysaccharide cream and Honghua Xiaoyao tablets. The clinical experimental results showed that the treatment was effective and could effectively treat chloasma. Reduced glutathione has the function of reducing pigment state and inhibiting OS reaction. BFRS has the function of removing freckles, so both are more commonly used in the treatment of chloasma. However, at present, there are few relevant research results on the treatment of chloasma with BFRS and reduced glutathione. Based on this, the study adopts the BFRS combined with reduced glutathione to treat patients with chloasma, and discusses the effect of this treatment method, providing a new way for the chloasma's treatment.

MATERIALS AND METHODS

Subjects

Eighty patients with chloasma were selected in West Anhui Health Vocational College between January 2021

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and June 2022. The patients were randomly divided into two groups, namely, group J and group G. The patients were all female, aged from 40 to 60 years old. The chloasma type is epidermal type and mixed type, and the course of disease is 1 to 13 years. There was no significant difference between the two groups. The inclusion criteria met the diagnostic criteria of western medicine for chloasma, conforming to TCM diagnostic criteria for chloasma, no sunburn in the near future, no mental illness, able to communicate normally, have not taken photosensitive drugs recently. The exclusion criteria were pregnant or lactating women, recent experience in treating chloasma from other places, suffer from serious endocrine system disease, and the patients who could not communicate normally due to mental, language, hearing and other disorders.

Plan of work

Patients in the Group G were treated with reduced glutathione for injection (Atomoran) (GYZZ H19991067, Chongqing Yaoyou Pharmaceutical Co., Ltd.). For specific treatment 1.8g of reduced glutathione for injection was added to every 100mL of normal saline, and was given through intravenous drip. The patients were treated 7 days a week, once a day for one month.

Patients in Group J, were treated with BFRS based on the therapy methods of the patients in Group G.

The BFRS was prepared by mixing 10 g of orange peel, 10 g of safflower, 10 g of red peony, 10 g of peach kernel, 12 g of acacia bark, 15 g of motherwort, 15 g of ligusticum chuanxiong, 20 g of angelica, 20 g of raw rehmannia and 20 g of salvia miltiorrhiza, in 400 ml of water to decoct. It was given twice a day, seven days a week, for one month.

Post-treatment observation

After the treatment, the therapeutic efficacy of chloasma in the study was divided into four grades, namely obvious, effective and ineffective. Among them, obvious significant effect means that the color spots on the patient's face have obviously disappeared, the area of fading is more than 60%, and the color spots have significantly faded or disappeared. Effective means that the color spots on the patient's face have subsided to a certain extent, with the subsided area between 30% and 60%, and the color of the color spots has faded to a certain extent. Ineffectiveness means that the color spot on the patient's face has not reached the standard of effective and obvious effect. The effective rate of treatment is: total number of invalid patients/ total number of patients. The following criteria were used to determine the thrapentic efficency of chloasma.

(i) Melasma area

During the treatment, the area of chloasma on the patient's face was measured with a ruler. The treatment effect was evaluated according to the reduction of the patient's chloasma area.

(ii) Total score of skin lesions

During the treatment, the total score of skin lesions was used to evaluate the degree of skin lesions of patients. The total score of the skin lesions is 6 points, the lower score means better treatment effect.

(iii) Skin lesions

During the treatment, the facial skin lesions of patients in Group J and Group G were evaluated by the water content of the cuticle and the loss of the trans epidermal water diversion. The measurement of both was realized by skin moisture loss tester.

(iv) TCM syndrome integral

During the treatment, TCM syndrome integral is used to evaluate the clinical symptoms of patients. The total score of TCM syndrome score is 30. The lower the score of the patient means less severe clinical symptoms, and better the treatment effect.

(v) OS reaction

During the treatment, the OS reaction of the patient is reflected by the indicators of the patient's serum malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) to evaluate the treatment effect. The levels of these indicators were determined by enzyme-linked immunosorbent assay.

(vi) Disease related factors

During the treatment, the patient's serum levels of soluble interleukin-2 (SIL-2), tumor necrosis factor-α (TNF-α) and melatonin stimulating hormone (MSH) were determined by enzyme-linked immunosorbent assay.

(vii) Quality of life index score (DLQI)

DLQI was used to evaluate the quality of life of patients. The number of problems included in DLQI is 10, and the score of each problem is 0 to 3 points, which corresponds to the severity of the problem. The total score of DLQI is 30 points. The quality of life increases as the score decreases.

Statistical analysis

All data generated during the experiment were processed and analyzed by software SPSS 19.0. For measurement data, it is expressed in the form of mean ±

standard deviation, using 't' test. For count data χ^2 test was used. The difference is significant when $P<0.05$.

RESULTS

Table I shows the results of TCM, chloasma and skin lesions scores of the patients who participated in the study. During the therapy, the TCM syndrome score of Group J and Group G continued to decline, and the decline was significant after one month of treatment. The decline of TCM syndrome score in Group J was more significant than Group G after treatment ($P<0.05$). Before treatment, there was no remarkable variation in chloasma area and skin lesions between Group J and Group G. During the therapy, the total scores in Group J and Group G continued to decrease, and the decrease was significant after one month of treatment ($P<0.05$). The total scores of chloasma area and skin lesions in Group J decreased more significantly than Group G after treatment ($P<0.05$).

Table I. Effect of beauty and freckle removing soup on TCM, Chloasma and skin lesions scores for the patients before and after treatment.

Before treatment		Treatment time (weeks)							
		1	2	3	4	5	6	7	8
TCM									
Group J	12.58±2.40	12.46±2.33	10.55±1.56	9.84±2.03	9.03±2.16	8.43±1.05	7.86±0.89	7.46±1.52	6.53±1.35
Group G	12.63±2.17	12.36±2.15	12.01±1.87	11.55±0.96	11.12±1.25	10.86±1.33	10.45±1.08	9.83±0.92	9.42±0.63
	t 0.624	0.713	0.962	2.412	2.896	2.405	3.504	4.478	6.532
	P > 0.05	>0.05	>0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Chloasma (cm^2)									
Group J	3.73±1.02	3.67±1.03	3.58±0.88	3.26±0.44	2.91±0.32	2.67±0.53	2.34±0.46	2.00±0.32	1.82±0.45
Group G	3.72±0.98	3.69±1.05	3.61±0.83	3.54±0.86	3.42±1.01	3.26±0.63	3.10±0.22	3.01±0.15	2.84±0.12
	t 0.654	0.143	0.857	2.133	6.081	6.252	6.354	7.443	7.582
	P > 0.05	>0.05	>0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Skin lesions									
Group J	4.56±0.59	4.32±0.64	4.01±0.35	3.75±0.46	3.23±0.28	2.94±0.57	2.76±0.52	2.52±0.33	2.14±0.36
Group G	4.53±0.64	4.40±0.62	4.27±0.53	4.14±0.63	3.86±0.62	3.71±0.25	3.58±0.33	3.45±0.42	3.38±0.43
	t 0.752	0.144	1.834	2.415	3.082	6.306	6.557	6.932	7.853
	P >0.05	>0.05	>0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

Group G, glutathione group; Group J, combination group; Beauty and freckle removing soup combined with reduced glutathione

Table II shows the changes of skin lesions and OS reaction scores for the patients. There was no discernible variation between the two groups in the levels of the variables. After the treatment, the skin cuticle water content of the two groups increased remarkably (P<0.05). The loss of transepidermal water shunt decreased significantly. The water content of skin cuticle in Group J was significantly superior to Group G after therapy(P<0.05). The loss of transepidermal water shunt was remarkably lower (P<0.05). MDA in Group J and Group G decreased significantly, and the Group J's index level decreased more than Group G (P<0.05). The SOD and GSH-Px were remarkably increased (P<0.05). The Group J's index level was remarkably superior to Group G (P<0.05). The levels of SIL-2, TNF-α and MSH in Group J and Group G decreased significantly after therapy. The levels of SIL-2, TNF-α and MSH in Group J decreased significantly. the DLQI score of Group J and Group G decreased significantly (P<0.05). Meanwhile, the Group J's DLQI score decreased remarkably than Group G.

Table III compare the clinical efficacy of two groups after therapy. Group J's effective rate of treatment is 17.5% higher than that in Group G, and the effective number of treatments in Group J is remarkably superior to Group G (P<0.05).

DISCUSSION

The above results show that the treatment effect of BFRS combined with reduced glutathione on chloasma is significantly better than that of only using reduced glutathione. This shows that the treatment of melasma with BFRS and reduced glutathione is better. The research results are consistent with the existing research results and verify the effectiveness of the research. Many studies have confirmed that OS reaction has a certain correlation

Table II. Changes of skin lesions and OS reaction scores for the patients at before and after the treatment.

Index	Time	Group J	Group G	t	P
SCM (%)	1	38.38±3.84	38.40±4.14	0.653	>0.05
	2	45.42±4.04	41.18±3.62	2.017	<0.05
	t	6.532	4.315	-	-
	P	<0.05	<0.05	-	-
TEW [g/(h·cm2)]	1	11.57±2.84	11.55±3.01	0.353	>0.05
	2	9.02±1.43	10.05±2.44	4.507	<0.05
	t	5.497	3.532	-	-
	P	<0.05	<0.05	-	-
MDA (μmol/L)	1	34.99±3.25	35.08±3.12	0.765	>0.05
	2	21.32±3.23	25.58±3.82	4.065	<0.05
	t	7.652	6.353	-	-
	P	<0.05	<0.05	-	-
SOD (U/mL)	1	23.68±3.62	23.56±3.75	0.652	>0.05
	2	27.24±3.53	25.03±2.64	6.532	<0.05
	t	8.436	2.103	-	-
	P	<0.05	<0.05	-	-
GSH-Px (g/L)	1	6.08±1.38	6.14±1.46	0.553	>0.05
	2	9.14±1.42	7.38±1.08	6.352	<0.05
	t	8.144	2.058	-	-
	P	<0.05	<0.05	-	-
SIL-2 (U/mL)	1	754.52±71.06	751.44±76.76	0.581	>0.05
	2	439.46+68.33	686.47±75.46	8.479	<0.05
	t	9.032	2.306	-	-
	P	<0.05	<0.05	-	-
TNF-α (ng/mL)	1	9.30±1.74	9.32±1.72	0.253	>0.05
	2	5.08±0.86	7.02±0.66	3.022	<0.05
	t	6.839	3.514	-	-
	P	<0.05	<0.05	-	-
MSH (pmol/L)	1	468.55±40.03	470.72±41.98	0.439	>0.05
	2	318.47±28.49	363.72±30.08	2.104	<0.05
	t	6.355	3.704	-	-
	P	<0.05	<0.05	-	-
DLQI	1	18.52±3.82	18.19±4.03	0.668	>0.05
	2	9.08±2.02	12.34±2.37	5.145	<0.05
	t	9.876	7.344	-	-
	P	<0.05	<0.05	-	-

SCM, Skin cuticle moisture content; TEW, Transepidermal water diversion loss; 1, Before treatment; 2, after treatment. MDA, malondialdehyde; SOD, superoxide dismutase; GSHPx, glutathione peroxidase; SIL-2, soluble interleukin-2; TNF-α, tumor necrosis factor alpha; MSH, melanocyte-stimulating hormone; DLQ1, quality of life index score.

Table III. Clinical efficacy of group J and group G.

Group	Significant effect (n, %)	Valid (n, %)	Invalid (n, %)	Efficiency (n, %)
J (n=40)	23 (57.5)	16 (40)	1 (2.5)	39 (97.5)
G (n=40)	19 (47.5)	13 (32.5)	8 (20)	32 (80.0)
χ^2	-	-	-	0.156
P	-	-	-	>0.05

in the pathogenesis of chloasma (Karrabi et al., 2021). In the body of patients with chloasma, the levels of SOD and GSH-Px are low, which leads to the lack of inhibition pathway of OS reaction, which leads to certain problems in the endocrine function of patients. In this case, the activity of enzymes in the patient's body that can promote the enhancement of the secretion level of melanocytes increases, thus enhancing the enhancement of the secretion level of melanin. These melanins gather to form color spots. Therefore, according to the measurement of the level of SOD and GSH-Px in patients, the degree of oxidative damage can be judged to a certain extent. In addition, a quantity of studies proved that when the level of MDA is high, it will promote the OS reaction, leading to the aggravation of chloasma (Lai *et al*., 2022). When the level of SOD is high, it will act as an antioxidant and weaken the OS reaction. Thus, it can inhibit the level of melanin secreted by melanocytes, reduce melanin aggregation, and reduce the area of pigmentation on the patient's face. Therefore, the levels of SOD, GSH-Px and MDA can reflect the body's OS response to a certain extent. For this reason, the above three indicators have been used in many studies to evaluate the OS response, so as to evaluate the therapeutic effect of chloasma. Reduced glutathione can neutralize oxygen free radicals in melanin, reduce melanin, and eliminate the area and color of facial skin spots in patients with chloasma. Some studies show that the BFRS contains unsaturated fatty acids such as tanshinone and ligustrazine, which can neutralize oxidation groups and eliminate melanin (Spierings, 2020; Hatem *et al*., 2022). Therefore, the combination of BFRS and reduced glutathione can more effectively reduce OS reaction. In the study, before treatment, there was no discernible variation in the MDA, SOD, GSH-Px and other OS indicators between the two groups. After treatment, the level of MDA in Group J and Group G decreased significantly, and the index level in Group J decreased more than Group G. The SOD and GSH-Px were remarkably increased, and the index level of Group J was remarkably superior to Group G. The above results show that the treatment method of BFRS combined with reduced glutathione can reduce the MDA in individuals with chloasma and enhance the SOD and GSH-Px. So as to inhibit OS reaction and achieve the

purpose of treating chloasma. The experimental results are consistent with the above research contents.

In previous studies on chloasma, it was considered that the higher the level of MSH factor, the higher the level of melanin synthesis and secretion in the patient's body, leading to the aggravation of the patient's condition. Therefore, MSH factor level is often used to evaluate the therapeutic effect of chloasma. The patient's body inflammatory reaction will lead to the aggravation of facial skin damage, and then promote the development of chloasma. There are many kinds of unsaturated fatty acids in the BFRS. These unsaturated fatty acids will neutralize oxidative radicals and inhibit OS reaction, thus reducing the degree of injury and inflammatory reaction of the body, and ultimately avoiding skin damage. In addition, ligustrazine, ligustrazine lactone and other components in BFRS can also reduce tyrosine activity, thereby inhibiting MSH level hence reducing the secretion of melanin (Zhao *et al*., 2021). The levels of SIL-2, TNF-α can directly reflect the degree of inflammation in the body. Therefore, MSH, SIL-2, TNF-α was used to assess the individual's inflammatory reaction and the improvement of the condition. It is confirmed that the MDA in group J and group G decreased significantly. The index level of group J decreased more significantly than Group G; The levels of SOD and GSH-Px were significantly increased. Compared with Group G, the index level of Group J increased more significantly. The experimental results are consistent with the previous research. Regarding OS, there are many reports on cellular and molecular levels (Tan *et al*., 2022; Long *et al*., 2023; Luo *et al*., 2022; Ma *et al*., 2022; Xu *et al*., 2022; Zamudio-Cuevas *et al*., 2022; Zhao *et al*., 2022).

There are a number of limitations in the present study. The number of patients included in the study is small, and there may be some chance, which affects the accuracy of the experimental results. Therefore, it is necessary to expand the quantity of samples included in the follow-up study to avoid the error impact caused by contingency.

CONCLUSION

The BFRS combined with reduced glutathione proposed in the study has a significant positive impact on OS reaction and disease-related factors in patients with chloasma, and has an excellent therapeutic effect on chloasma, with good practicability.

Funding

Not applicable.

IRB approval

This study was approved by the Advanced Studies Research Board of West Anhui Health Vocational College, Lu'an, 237005, China.

Ethical approval

The study was carried out in compliance with guidelines issued by Ethical Review Board Committee of West Anhui Health Vocational College. The official letter would be available on fair request to corresponding author. The patient was informed of the experimental content and signed the electronic informed consent form.

Statement of conflict of interest

The authors have declared no conflict of interest.

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