Protective effect of Liankoushengji Powder on rats with Traumatized

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Abstract. Purpose: Study on the effect of modified liankou Shengji powder on treating skin injury rats. Materials and Methods: Divided sixteen male SD rats were randomly into four groups: model group, normal group, modified liankoushengji powder group and Yunnan Baiyao powder group. The other three groups except the normal group were modeled as follows to form a mechanical damage animal model: the left three sides of the back were surgically cut into square full-thickness skin defects, deep into the skin, with a length of about 1 cm on the side. After the mold-building was successful, modified liankoushengji powder and Yunnan Baiyao powder were mixed with the appropriate amount of Vaseline into a paste and then applied to the affected area of rats for drug intervention. All rats in the model group were coated with Vaseline twice a day for 4 days. To observe the body temperature and the growth wounds by glare projection of all rats. Then to calculate the wound healing rate. And to detect Vascular Endothelial Growth Factor (VEGF) by ELISA. Results: In modified liankoushengji powder group, the body temperature was no observable change comparing with the normal group (p > 0.05). But the changes of the increased new capillary formation and the enhanced wound healing rate were significant differences comparing with the normal group (p < 0.01). The enhanced concentration of VEGF was statistically significant (p < 0.05). With little change in body temperature, there was no remarkable difference between the modified liankoushengji powder group and the Yunnan Baiyao powder group (p > 0.05). The changes of the increased new capillary formation and the enhanced wound healing rate were significant differences in the modified liankoushengji powder group comparing with the Yunnan Baiyao powder group (p < 0.05). The enhanced concentration of VEGF was statistically significant (p < 0.05).
concentration of VEGF was statistically significant (p<0.05). Conclusion: Modified liankou Shengji powder has a certain role in the treatment of wound model rats.

1. Introduction
In daily life, injuries caused by various causes are often seen. Trauma which causes great damage and pain often takes a long time to repair. The scar left after healing has great harm to the patient's body and mind. Therefore, research on wound repair has become one of the hot spots in the medical field.

At present, the main methods of wound repair include the application of nanotechnology, the application of genetic engineering and nutritional adjuvant therapy [1]. But now the promotion and popularization of these technologies is difficult and extravagant. As far as the technology of Western medicine is concerned, Chinese medicine has a unique advantage in the process of wound repair.

The composition of the mouth is divided into 1 frankincense, 1 drug, 1 stone, which is found in the "Doctor Yi Jian" volume ten. In this experiment, we added Sanqi and used it as the first ideal choice drug for treatments. This combination not only enhances the efficacy of the medicines during wound repair, but also speeds up muscle growth. Thereby achieving the purpose of promoting wound healing and inhibiting scar formation. This experiment investigated the effect of modified liankoushengji powder on wound healing in rats.

2. Experimental animal
SD male rats, SPF level. Body mass (180–200)g, raving room temperature (22±2)°C, relative humidity 50%–60%, Day and night, natural light and dark alternate lighting, rats were given standard feed and drinking water.

3. Material experiment

3.1. Experimental drugs and reagents
Sanqi powder (purchased from the Third Affiliated Hospital of Henan University of Traditional Chinese Medicine); Ophicalcitum, frankincense, myrrh (what is an ingredient of the Liankoushengjisian) (purchased from the Third Affiliated Hospital of Henan University of Traditional Chinese Medicine); VEGF kit (Nanjing Built Biotechnology Co., Ltd.); Liankoushengjisian (The Third Affiliated Hospital of Henan University of Traditional Chinese Medicine, Ingredients: Ophicalcitum, frankincense, myrrh).

3.2. Experimental equipment
Electronic scale Water bath; Microplate reader; Electric heating constant temperature blast drying oven; Strong light flashlight.

4. Model preparation and evaluation
Modeling method: Using a rat full-thickness skin resection model, 16 rats, divided into 4 groups, In addition to the normal group, each rat was cut with a surgical scissors on both sides of the back to cut a square full-thickness skin defect of about 1 cm in diameter, deep to the skin, an animal model of mechanical damage is formed. If the wound area and depth are appropriate and the internal organs are not damaged, the model is successful [2].

5. Drug preparation and administration

5.1. Drug preparation
Mixing modified liankoushengji powder and Yunnan baiyao separately with Vaseline to make ointment, then spreaded them evenly on the upper gauze, and Vaseline contained 0.018g/cm².
5.2. Experimental groups
We divided 16 rats into 4 groups, they were normal group, model group, Yunnan baiyao group and modified liankoushengji powder group.

5.3. Give drugs
(1) Normal group: normal feeding did not give drugs. (2) Model group: disinfected the wound with medical alcohol and washed the wound with saline, then covered the wound with a gauze and wrapped with sterile dry gauze and changed dressing twice daily. (3) Yunnan baiyao group: the method was the same with normal group, then spreaded the Yunnan baiyao Vaseline cream evenly on the gauze. It was about 2mm thick. The following method was the same as the previous one. (4) Modified liankoushengji powder group: spreaded the modified liankoushengji powder Vaseline cream evenly on the gauze, other methods was the same as the Yunnan baiyao.

6. Observation index
1) Rat body temperature
2) Observation of strong light projection on the wound surface: On the fifth day of the experiment, the wounds of SD rats were opened from the deep fascia before the rats were sacrificed. The light flashlight was used as the light source to make the wound surface transparent to observe the blood vessel and take photos.
3) Wound healing rate: healing rate (%) = (original surface area - current wound area) / original surface area × 100%. To evaluation of wound healing rate.
4) VEGF: The tissue around the wound was weighed and then make homogenate, centrifuged at 3000r/min for 20min, and the supernatant was aspirated. According to the kit instructions.

7. Result

7.1. Changes of body temperature of rats after injury
During the 2-4 days of the experiment, compared with the model group, the body temperature of each drug group increased to different degrees without statistical difference.

| Group            | Day2       | Day3       | Day4       |
|------------------|------------|------------|------------|
| The normal       | 37.08±0.50 | 37.08±0.72 | 37.64±0.74 |
| Model            | 37.18±0.80 | 37.02±0.60 | 37.14±0.47 |
| Yunnan baiyao    | 37.34±0.53 | 37.04±0.21 | 37.28±0.39 |
| liankou Shengji powder | 37.12±0.31 | 37.04±0.50 | 37.26±0.34 |

Note: compared with the normal group, * means P<0.05, ** means P<0.01

7.2. Observation results of wound bright light projection
After 4 days of administration, under strong light projection, it could be seen that there was a certain amount of capillary aggregation in the model group, the flavoured modified liankoushengji powder group and the Yunnan baiyao group. Compared with the model group, the capillary concentration in the Yunnan baiyao group and the modified liankoushengji powder group was relatively high. The concentration of capillaries was higher in modified liankoushengji powder group than in Yunnan baiyao group. See below 2.
7.3. Changes of wound healing rate after trauma in rats
Compared with the model group, on the second and fourth days, the wound healing rate in the modified liankoushengji powder group was significantly increased, with significant statistical difference (P < 0.01). On day 4, the wound healing rate of Yunnan baiyao group increased, with statistical difference (P < 0.05).

| Group                    | Day1  | Day2  | Day3  | Day4  |
|--------------------------|-------|-------|-------|-------|
| Model                    | 12.80±6.26 | 22.80±4.44 | 31.80±9.76 | 44.40±3.44 |
| Yunnan baiyao            | 13.00±5.61 | 30.40±7.30 | 41.40±8.45 | 53.00±3.67## |
| liankou Shengji powder   | 24.8±12.68 | 40.20±11.21## | 53.40±8.88## | 62.60±6.58## |

Note: compared with the model group, ## indicates P<0.01 and # indicates P<0.05

7.4. VEGF concentration in rat traumatic tissue
Compared with the model group, there was no significant difference in VEGF concentration in Yunnan Baiyao group (P > 0.05), but there was significant difference in modified liankoushengji powder (P < 0.05). See table 3.

| Group                    | VEGF          |
|--------------------------|---------------|
| Model                    | 179.385±28.612 |
| Yunnan baiyao            | 154.615±84.192 |
| liankou Shengji powder   | 256.384±64.046## |

Note: compared with the model group, # means P < 0.05.

8. Discussion
It has indicated that the body temperature of the three drug groups enhanced at different degree after 2-4 days, which was no statistical differences as compared with the model group .The results showed that the inflammatory reaction of the drug group was not distinct, and it was suggested that the modified liankoushengji powder could promote blood circulation for removing blood stasis and promoting tissue regeneration by activating the muscles to adjust the wound's local microcirculation .To observe the number of deep fascial capillaries in the lower part of the wound in rats with a strong light flashlight by the deep fascial glare projection experiment. One of the crucial factors is vascularization which affects wound healing, and with the increasing number of capillaries in the deep fascia of the lower part of the wound, the recovery degree of the wound is higher [3]. It is the effective and direct evaluation index that wound healing rate is of wound healing. As the number of deep fascial capillaries in the wound increased, the wound healing rate enhanced significantly in the modified liankoushengji group, which
compared with the model group. That indicated the modified liankoushengji powder was of obvious curative effect in treating wound healing.

VEGF is a glycoprotein which works on the cells of vascular endothelial. Proliferation of endothelial cell and inducing angiogenesis would be promoted significantly under its action [4]. In the modified liankoushengji powder groups, study proved that the concentration of VEGF was increased strongly which compared with the model groups, manifesting that the powder played a significant role in traumatic rats. It is speculated that the possible reason is that Chinese medicines of the prescription have the functions of promoting blood circulation, relieving pain, detumescence and promoting granulation, especially frankincense and myrrh. Furthermore, modified liankoushengji powder may act on releasing VEGF for promoting the concrescence of the skin wound.

It concluded that the modified liankoushengji powder was of obvious curative effect in treating injury model rats.

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