SAR Journal of Pathology and Microbiology

Abbreviated Key Title: *SAR J Pathol Microbiol*

Home page: https://sarmedjournals.com/sarjpm/

OPEN ACCESS

ISSN 2707-7756 (P)

Research Article

Wound healing activity of the essential oils of *Rosmarinus officinalis* and *Populus alba* in a burn wound model in rats

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Article History: | Received: 12.02.2020 | Accepted: 22.02.2020 | Published: 28.02.2020 |

Abstract: **Background and objectives:** The present study evaluates the effect of essential oils of *Rosmarinus officinalis* and *Populus alba* on experimentally cutaneous heat-induced burn injury in rat. **Materials and Methods:** For this purpose, the acute toxicity test was carried out on Wistar rats that received intraperitoneally gradual doses (0.5to 7 ml /Kg) of the both essential oils. Then, Second-degree burn wounds were induced in five groups (N=5 in each). The percentage of wound healing were assessed on 5, 10, 15, 20 and 25 days. In the other hand, wound surface was monitored and photographed during the period of treatment. **Results and Conclusion:** The essential oil of *Populus alba* and even *Rosmarinus officinalis* have no acute toxicity in rats. The treated rats with essential oils of *Rosmarinus officinalis* and *Populus alba* presented a high level in healing percentage in comparison to the other groups (4.99 ± 2.3, 4.22 ± 0.19, 36.48 ± 1.12, 29.99 ± 0.8 and 84.82 ± 6.41% for the untreated, placebo, *Rosmarinus officinalis, Populus alba* and Madecassol® group respectively). Moreover, the results of wound surface at the 25 day presented a value of 6.65 ± 0.16, 6.7 ± 0.01, 4.44 ± 0.07, 4.9 ± 0.05 and 1.06 ± 0.44 cm^2respectively for the Untreated, Placebo, *Rosmarinus officinalis, Populus alba* and Madecassol® groups. These essential oils have a marked healing effect during the proliferative phase of wound healing process induced in rats.
Keywords: Burns, Essential oil, Healing, *Populus alba*, *Rosmarinus officinalis*, Wounds.

INTRODUCTION

A burn is an injury of skin surface, which can be deep and extended due to the action of heat, electricity, chemicals, or radiation (radioactivity) (Rouquette, C. 2002). Burns was defined as one of the most common forms of injury. The destructive outcomes of burns include physical disabilities as well as mental and emotional disorders (Ezzati, A. et al., 2009). Wound treatment depends on their importance and severity; they may require systemic treatment, in addition to local treatment. In this purpose, modern scientific methods should be used to exploit the therapeutic effects of herbal compounds (Chaudhari, M., & Mengi, S. 2006). Thus, in contrast, of conventional medicines, the non-toxic nature means of herbal preparations can make them effective to be administered over long periods (Upadhyay, N.K. et al., 2011; Vinothapooshan, G., & Sundar, K. 2010). Indeed, the medicinal plants are characterized by the biosynthesis of essential oils" known for their therapeutic effects in traditional medicine. Among the herbal remedies used, some have been tested experimentally in an attempt to highlight their potential healing (Bensegueni, A. 2007).

Rosmarinus officinalis L. (Rosemary) belongs to the family *Labiatae* or *Lamiaceae*. It occurs as a shrub, under shrub or herbaceous that may grow up to 2 m (Atik bekkara, F. et al., 2007). It is mainly native to the Mediterranean Region (Žegura, B. et al., 2011; Faria, L.R.D. et al., 2011; Pengelly, A. et al., 2012).

In folk medicine, Rosemary is used to treat different diseases such as: depression, insomniac and arthritic pains (Zargari, A. 1995). It participates in many physiological changes (increase of the synthesis of prostaglandin E_2, decrease of leucotrien B_4production in the polymorph leucocytes and prevention of complement system in humans) (Al-Sereiti, M. R. et al., 1999). Many chemical studies showed that rosemary contained a number of components responsible for the antioxidant property. Especially the three phenolic diterpenes: carnosic acid, carnosol and rosmarinic acid. Which is useful for burn wound healing (Batop,T. 1999).

On the other hand, *Populus alba* (White Poplar) is a woody angiosperm higher plant belonging to the family *Salicaceae*. It is a common species of the Mediterranean forests. According to several Mediterranean floras, this species is considered as cultivated or sub-spontaneous around the western Mediterranean Basin (Dickmann, D.I., & Kuzovkina, J. 2008). White poplar wood is a very poor fuel, which produces little heat to combustion. From the bark, we extract salicin (Jean-Claude, R, et a., 2008). The findings for *Populus alba* were that there's just not enough information to know very well the therapeutic activities of this plant. This study aims to investigate the effect of *R. officinalis* and *P. alba* essential oils on wound healing compared to Madecassol® drugs on cutaneous induced burn in rat.

MATERIALS AND METHODS

Chemicals

Cyclohexane, glycerol, thiopental, Xylocaine 2%, bentonite, Zinc Oxide, salicylic acid, sodium tetraborate, formaldehyde, and Copper sulfates were obtained from Sigma-Aldrich Chemical Company. The ointment Madecassol® was used for the control study.

Plant material

The aerial parts of *R. officinalis* and *P. alba* were collected and identified in the Mascara region (western of Algeria) during May 2013.

Animals

Twenty-five male Wistar rats were divided into five groups (N=5 for each). The rats were housed in standard conditions of temperature (22 ± 3 °C), humidity (60 ± 5%) and a 12 h light/dark cycle. They have a granules food and water *ad-libitum*. This work was realized with respect for the welfare of animals (W.H.O. 2015).

Preparation of essential oils

The essential oils (EO) were extracted by hydrodistillation. The extract was prepared using 100 g of leaves and flowers of each plant, after boiling and stability of the temperature stabilizes, the distillate was collected. Then, cyclohexane was used for three successive washes(10, 10, 20 ml).After agitation, the organic phase was concentrated and stored at +4 °C after the calculation of the extraction yield.

Acute toxicity test

50 Wistarratswith150±5g (females)and 220±8 g(males)were used. The use of the both sexes of rats can explain the difference between the hormonal and behavioral characteristics.

According to (Tahraoui, A. et al., 2010), the rats of five groups received by intra-peritoneal injection gradual doses (0.5 to 7 ml /Kg) of the both essential oils. The control group was received the saline solution (9‰ NaCl).

After the EO administration, the rats were monitored in the first, sixthand24th hour, for any death or change in behavior. This monitoring of clinical signs of toxicity included the observation of the weight changes in rats of different experimental groups throughout the period of acute toxicity (Adjoungoua, A.L. et al., 2008). In the 15^{th}day, the number of dead rats was calculated and converted into a percentage.

Formulation, characteristics and wound healing activity

Drugs administered percutaneously pose fewer problems than other ways; this was the reason that we choose the most classic formulation represented by the ointment.

Formulation and preparation of ointment

The objective was to develop two ointments one based on the essential oil of *R. officinalis* and the other based on the essential oil of *P. alba*. The pharmaceutical preparation was done in accordance with the standards of good manufacturing practices herbal medicines described by (Bettiol, F. 1995). The basic Excipients were glycerol, H_2O, bentonite, Zinc Oxide, salicylic acid, sodium tetraborate, formaldehyde, Copper sulfates and the active ingredients was the essential oil of *R. officinalis* and *P. alba*. To have a better consistency ointment, various tests were performed to determine the necessary quantities of the components.

Examination of macroscopic characteristics

Examination of macroscopic characteristics was the first approach to the quality of the preparation to determine the changes seen in terms of visual, olfactory (Ouattara, M. 1992). The color and odor have been appreciated, homogeneity by observing the dispersion of the extract in the excipients, after spreading a thin layer on a rigid flat surface using a spatula.

Microbiological control of the preparation

Since the using essential oils formulations developed in this work have not undergone any sterilization treatment, it was important to check if the preparations comply with microbiological requirements specified in the Pharmacopoeia. Microbiological analysis was applied; a count of the total aerobic mesophilic flora, yeasts/molds and looking for some pathogens (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans*). As of fecal contamination germ, a search for *Escherichia coli* was useful (Kahkeshani, N. et al., 2013).

Evaluation of the wound healing activity

Its purpose was the appreciation of the accelerating potential of the new formation of dermal tissue after application of an ointment based on essential oil of *R. officinalis* and *P. alba* on superficial wounds.

A burn was performed on one side of each rat under the ethical rules on laboratory animals and then treated with ointments of the EO of *R. officinalis* and *P. alba*. The comparison was released with a group of rats receiving a reference cream (Madecassol®) and a group treated with ointment without essential oil.

Burn induction and treatment

50 Male rats (220 - 315 g) were divided into05groupsof05rats each. The first group presented the Operated and untreated rats (OU). The second group was for the Operated rats and treated with ointment without extract (OPl). The third group was for the Operated rats and treated with ointment of *R. officinalis* (OR). Then, the fourth group was for the Operated rats and treated with ointment of *P. alba* (OP). The last group presented the Operated rats and treated with Madecassol® (OM).

The rats were given a general anesthesia with thiopental (1g) in the basic dose of 40 mg / kg by intraperitoneal injection. This anesthesia was complemented by local administration of Xylocaine 2% (10 mg / kg) for a total in sensitivity of the animal. The induction was reached after 3 to 5 min and anesthesia can stay for 2 h. At the end of hair removal, the rats were transported to a place with sterile conditions. The flank of the animal was disinfected with cotton soaked in surgical alcohol (Lodhi, S. et al., 2006).

The punch with a surface of 7cm² (3cm length,2.3cm width) was heated in contact with the flame for 40 sec and placed on the side of the animal for 5 seconds without exerting pressure. Each group was treated to test the healing effect, compared to a control group, corresponding to the spontaneous physiological healing. The treated zone was covered with sterile compress and was fixed by the adhesive tape. Rats were placed in individual cages with clean bedding. The ointment was used daily on the burnt areas of five groups with a local application of 0.5 g of different preparations.

Assessment of wound healing activity of essential oils
Body weight and rectal temperature

The body weight of rats was measured every 02 weeks (0, 14 and 28 days) using a Sartorius balance, BP 610, accuracy: 0.01g). The assessment of the temperature was performed using a rectal thermometer with an accuracy of 1/100°C.

Rate of wound healing

It was determined as the percentage of reduction in the wound area every 4 days from second day of treatment with a digital caliper (accuracy 0.03mm/0.001), by taking picture with a digital camera during the trial period (25 days).The percentage of contraction (PC) was calculated by the formula (Rouabah, Y. 2010).

Percentage of contraction (%) = (Initial Surface-Healed surface/Initial surface) ×100.

Statistical analysis

The values were presented as mean ± SD. Data were analyzed using ANOVA test. The result was considered statistically significant if the P values was less than0.05 ($p < 0.05$).

RESULTS

Extraction yields and characteristics of essential oils from two plants

The essential oil yield was 1.29 ± 0.03% for the Rosemary and 0.9 ± 0.08% for white poplar. The extraction yields were widely variable. The extraction yield of *R. officinalis* was higher than that quoted by (Atik bekkara, F. et al., 2007) and (Brulé, C.H., & Pecout, W. 1995) where the quantities obtained by these two works were respectively 0.8% and 0.9%.

Acute toxicity

After administration of rosemary and white poplar oil with gradual doses, the observations showed no severe symptoms of pain, despite some common signs such hypoactivity, but they were reversible. They were appeared in rats for a short time and the rats were returned to their activity.

Evaluation of the weight of the rats

The variation of the weight of the rats was a very important parameter. Regular monitoring of rats during the14 days period has led us to obtain the values for the figure 1. Distinctly, a difference was noted between the growth of the control rats and those treated.

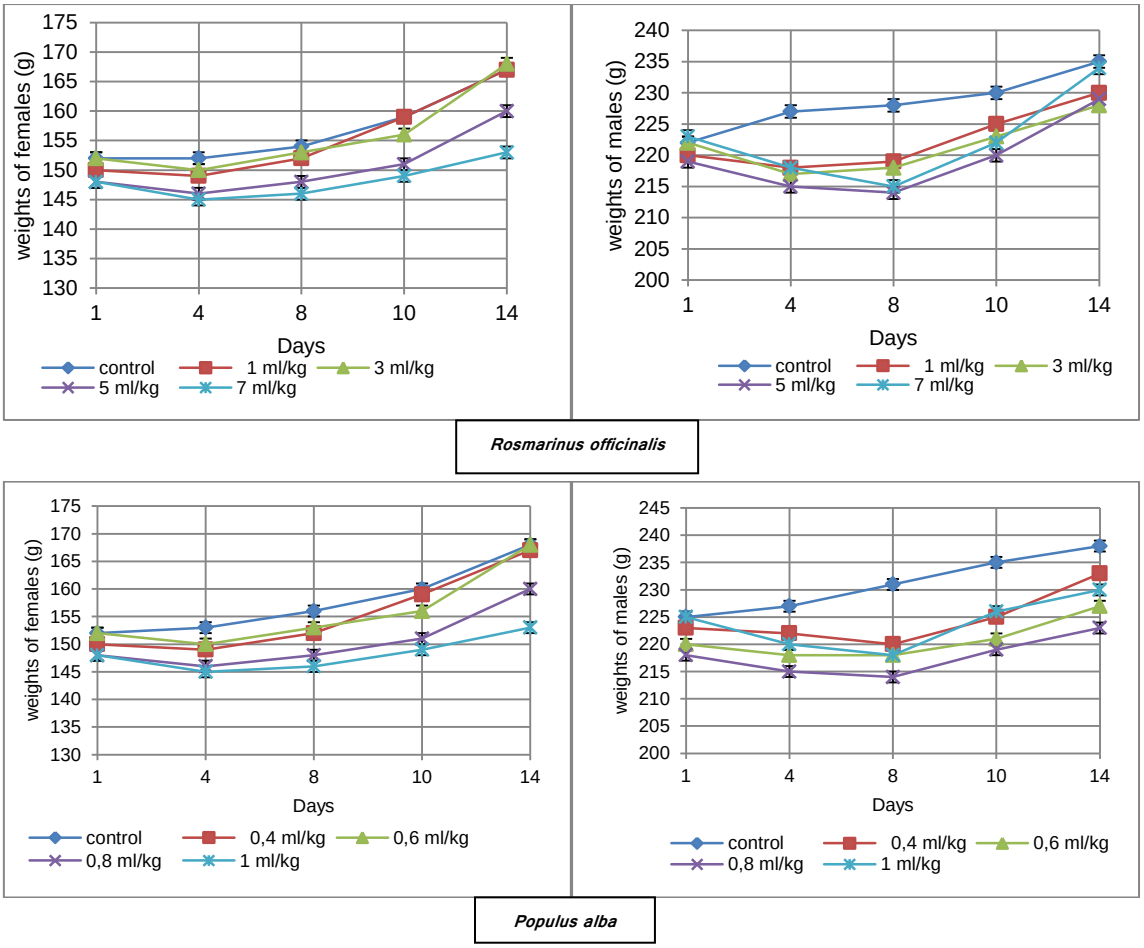


Figure 1. Evolution of the weight of rats after administration of EO (Expressed by sexes).

Assessment of wound healing activity of essential oils

The Both ointments have a grayish color due to the bentonite and have a specific smell of each essential oil. Based on the review, the two ointments appeared perfectly homogeneous without the presence of lumps. The pH of the ointment formulated with EO of *R. officinalis* was 6.4, while that formulated with EO of *P. alba* was 5.8. The enumeration of Total Mesophilic Aerobic Flora and yeasts/molds on preparations gave loads of 76 and5 CFU/ ml, respectively. A total lack of desired pathogen was well noticed. These results were consistent with the standards of the European Pharmacopoeia. This can be explained by the presence of some compounds such as sodium tetraborate by its bacteriostatic and fungistatic effect with the pH, which were 6.4 for the ointment of *R. officinalis* and 5.8 for the ointment of *P. alba*. These physicochemical factors were unfavorable for the growth of pathogens germs.

Evaluation of the *in-vivo* wound healing activity

Measuring weight and rectal temperature

From 1st to14thday, there was a slight decrease in the weight of rats. After this time, a significant increase in body weight was noted especially in the different operated rats ($P < 0.05$).The values obtained after twenty-eight days (28 D) of the operation clearly showed a recovery in body weight of rats of different groups with an average increase recorded in the OM group .The results in the first period(14 day) clearly showed that the decrease in body weight was probably related to the direct effect of the trauma and other pathophysiological factors of the healing process (Figure 2).The results of the rectal temperature showed a slight difference of the recorded values ranging between 36°C and 38°C throughout the experiment. Whatever the nature of the treatment, temperature remained statically close to physiological values, with values ranging from 36.4±0.48°C and 38±0.36°C (figure 3).

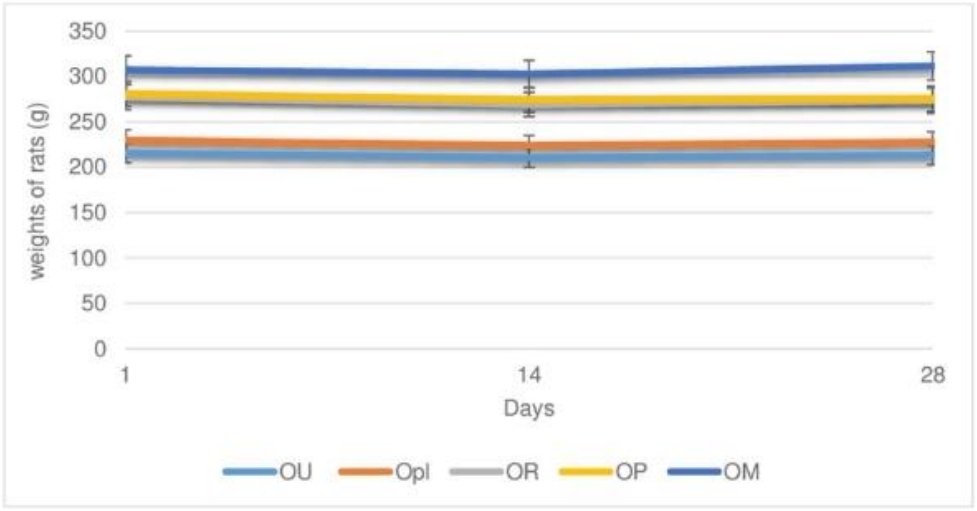


Figure 2. Evolution of weights of rats, according to the different treatments.
OU: Operated and untreated rats, OPl: Operated rats and treated with ointment without extract, OR: Operated rats and treated with ointment of *R. officinalis*, OP: Operated rats and treated with ointment of *P. alba*, OM: Operated rats and treated with Madecassol®.

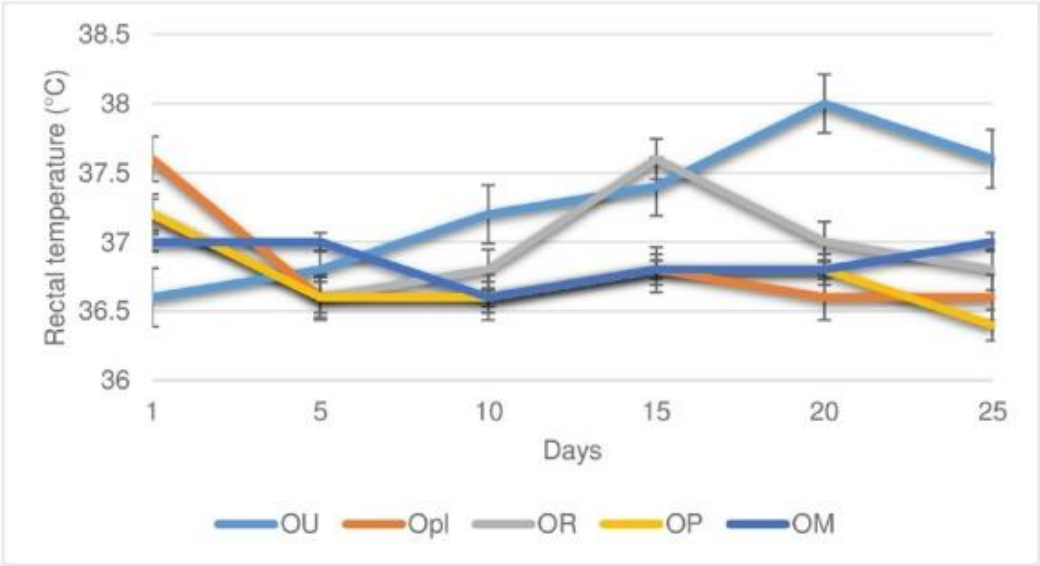


Figure 3. Evolution of rectal temperature of rats.

Evaluation of the healing process

During the period of healing and with a specific time interval, the wounds were regularly measured and photographed (Figure 5). Measuring the area of each wound was performed on the treated and untreated rats. Visual observation of wounds showed the presence of signs of infection at the wound of OU and OPl unlike those treated with essential oils of *R. officinalis*, *P. alba* and Madecassol®. After the fifth day, the average of the surface reduced in the OR, OP, OM groups. This inductive effect of healing was significantly greater in OM group compared to the OR and OP groups ($P < 0.05$) (Figure 4).

The measure of wound area presented a significant decrease in all treated groups (OR, OP, OM)compared to OPl group from the 10 to 25 days ($P < 0.05$).At the 25 day, the results presented a value of 6.65 ± 0.16, 6.7 ± 0.01, 4.44 ± 0.07, 4.9 ± 0.05 and 1.06 ±0.44 cm^2 respectively for the 05 groups (Figure 05). The percentage of wound healing in the groups 01, 02, 03, 04 and 05 were 4.99 ± 2.3, 4.22 ± 0.19, 36.48 ± 1.12, 29.99 ± 0.8 and 84.82 ± 6.41% respectively. The rate of healing in the treated groups was higher than the OPl group (Figure 5).

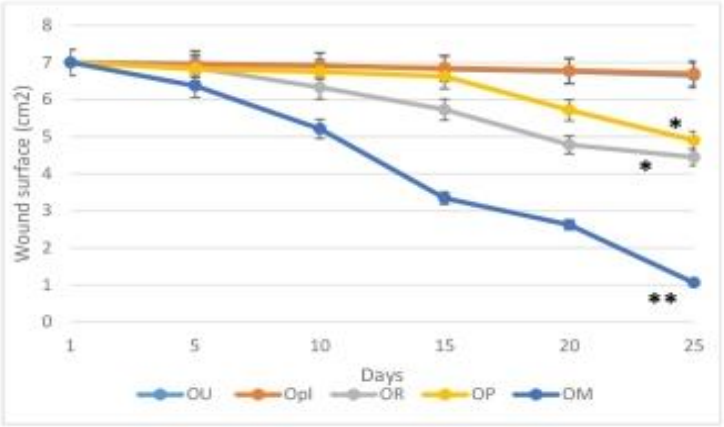


Figure 4. Evolution of the surface of the wounds (cm^2) in the treated and untreated groups.

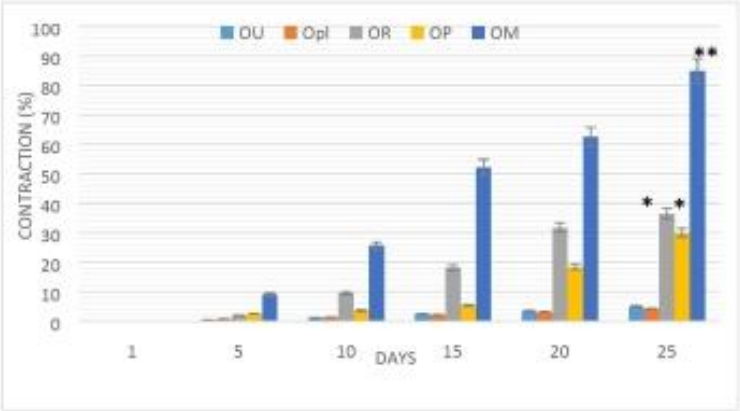
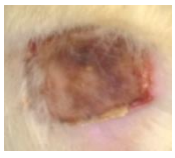
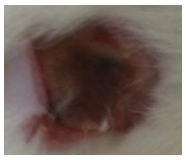
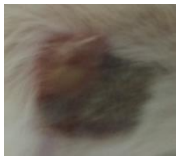
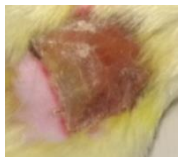


Figure 5. The evaluation of contraction (%) of woundsin05groups.

Macroscopic pictures of wound area in various groups (Figure 6) during the period of treatment showed a decrease in the surface of the wounds. Morphological assessment indicated that the wound healing effect of two essential oils was specifically observed in the period 10 to 20 days after wounds.

Groups / Days	OU	OPl	OR	OP	OM
02					
06					

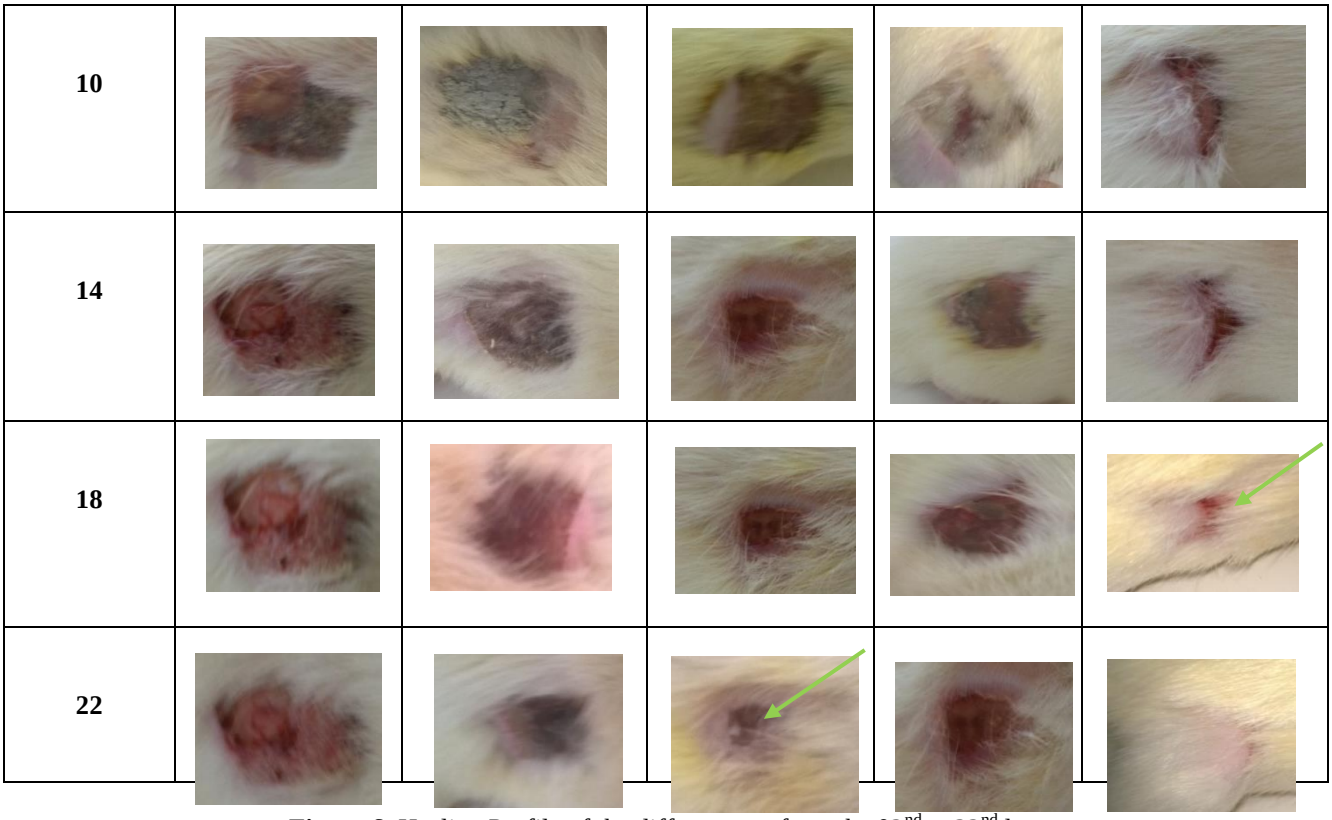


Figure 6. Healing Profile of the different rats from the 02nd to 22nd day.

DISCUSSION

The extraction yield was influenced by many factors such as the type of soil, the equipment used, the operating pressure, regularity the heating, the distillation cooling, the method and distillation time (Shah, A.H. et a., 1989). The absence of severe signs or mortality of rats indicated that the essential oils of *R. officinalis* and *P. alba* devoided of acute toxicity in rats. The change in body weight of the operated rats was due to the process of organism's defense against the trauma. That required the involvement of multiple organic and biological systems and functions such as protein synthesis (collagen) and the division of different stem cells involved in post-traumatic pathophysiological process (Almeida, M.C. et al., 2006). The stability of the rectal temperature can be explained by the resistance of these rats to trauma, to the nature of the inflammatory process and of the immune system (Benderdour, M. et al., 1998).

The visual observation of treated wounds can be interpreted by the antimicrobial effect of these essential oils and even active principle of the Madecassol®.

In addition, the wound healing process can be strengthened with the action of certain ingredients of the ointment (the effect of sodium tetraborate in the synthesis of the extracellular matrix) (Gérard, G., & Hugues, C. 1999), as well as the salicylic acid for its anti-inflammatory, antipyretic and analgesic effect and the copper sulfates for its anti-inflammatory action (MacKay, D., & Miller, A.L. 2003). According to the physiopathological studies, this period represented the proliferative phase of the healing process, and it was characterized by the formation of granulation tissue and the phenomenon of re-epithelialization (Rodgers, K.E. et al., 2003; Rinastiti, M., & Santoso, A.L. 2006; Nayak, B.S., & Pinto Pereira, L.M. 2006). The wound healing process required three stages: inflammation, proliferation and remodeling of the extra cellular matrix. The proliferative phase was characterized by many operations such as collagen deposition, epithelialization, angiogenesis and wound contraction (Hasani-Ranjbar, S. et al., 2008). It was noticed that free radicals generated at the site of injury presented one of the factors which causing damage to cellular membranes, nucleotides, lipids and proteins (Hasani-Ranjbar, S. et al., 2009; Pharmacopée Française. 1965). Therefore, the wound healing effects of Rosemary and white poplar can mainly come from their antioxidant potential. The healing effect of Rosemary and white poplar essential oils was investigated with several mechanisms such as increasing rate of re-epithelialization and neovascularization, scavenging of destructive free radicals, inflammation reduction and control of infection. Therefore, medicinal plants can show positive effects according to their antioxidant and anti-inflammatory constituents. It was noted that wounds surfaces in rats treated with rosemary and white poplar tend to heal faster than similar wounds treated without essential oils. These results indicated that these

plants provided a new natural alternative for the treatment of burn wounds in rats.

The present study showed that the essential oils of the two plants (*R. officinalis*, *P. alba*) were devoid of acute toxicity in rats. The treated rats with essential oils of *R. officinalis* and *P. alba* presented a high level in healing percentage comparing to the other groups (4.99 ± 2.3, 4.22 ± 0.19, 36.48 ± 1.12, 29.99 ± 0.8 and 84.82 ± 6.41% for the untreated, placebo, *Rosmarinus officinalis*, *Populus alba* and Madecassol® group respectively). Moreover, the results of wound surface at the 25 day presented a value of 6.65 ± 0.16, 6.7 ± 0.01, 4.44 ± 0.07, 4.9 ± 0.05 and 1.06 ± 0.44 cm^2 respectively for the Untreated, Placebo, *Rosmarinus officinalis*, *Populus alba* and Madecassol® groups. Essential oils have both a protective activity marked during the proliferative phase of wound healing process.

CONCLUSION

According to this study, the use of essential oils of *R. officinalis* and *P. alba* in order allowed us to achieve the best condition for wound healing in the shortest possible time. It was suggested to introduce these essential oils during the early stages of burn treatment due to the antioxidant, anti-inflammatory activities of its bioactive compounds.

Acknowledgment

The authors are gratefully acknowledged the support of directorate of University of Mascara. The author also wishes to acknowledge the reviewers for their constructive comments on the manuscript.

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