http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

OPEN ACCESS Pakistan Journal of Biological Sciences

ISSN 1028-8880
DOI: 10.3923/pjbs.2021.706.715

Research Article
Evaluation of the Wheat Germ Oil Topical Formulations for Wound Healing Activity in Rats

[1]Raghda Zakaria, [1]Raad J. Musa, [1]Jabar Faraj, [1]Zead H. Mahmoud Abudayeh and [2,3]Hamdoon A. Mohammed

[1]Department of Applied Pharmaceutical Sciences, Faculty of Pharmacy, Isra University, Amman, Jordan
[2]Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, Qassim University, Buraydah, Kingdom of Saudi Arabia
[3]Department of Pharmacognosy, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt

Abstract

Background and Objective: Wheat Germ Oil (WGO), the flour-milling by-product of wheat has essential constituents for skin health care as vitamin E, B-complex, squalene and unsaturated fatty acids. Incorporate WGO into polymers of the cream and ointment bases and evaluate the wound healing potential of these WGO formulations in the rat-animal model. **Materials and Methods:** WGO creams and ointments were prepared in two concentrations, 10 and 20% and evaluated for storage stability, homogeneity and compatibility using Fourier Transform Infrared (FT-IR) spectrometry. An amount of 0.5 g of the WGO formulations was applied daily to the injured area of the rats back. Wounds were observed for any clinical changes and healing compared to the control animal group. **Results:** The WGO was compatible with the cream and ointment bases and physically stables over 60 days of storage. The formulations of WGO have induced dose-dependent wound healing properties however the ointment formulations were demonstrating wound healing activity significantly better than the creams at all the intervals of the treatment. Within three weeks, 20% WGO ointment has induced a 90%reduction in the wound size diameter. Also, wounds recovered by 50% in 10 and 14 days of treatment with 20% WGO ointment and cream, respectively. **Conclusion:** The results revealed that WGO is a potential wound-healing agent from the scope that WGO is a common cosmetic ingredient and available at affordable prices.

Key words: Wheat germ oil (WGO), wound healing, burns, wound size reduction, wheat germ oil creams, wheat germ oil ointments, phytosterols, tissue Rich-collagen

Citation: Zakaria, R., R.J. Musa, J. Faraj, Z.H.M. Abudayeh and H.A. Mohammed, 2021. Evaluation of the wheat germ oil topical formulations for wound healing activity in rats. Pak. J. Biol. Sci., 24: 706-715.

Corresponding Author: Zead H. Mahmoud Abudayeh, Department of Applied Pharmaceutical Sciences, Faculty of Pharmacy, Isra University, Amman, Jordan Tel: +962797318037 Fax: +96264711505

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Wheat Germ Oil (WGO), is obtained from the wheat germ, the by-product of the flour milling industry of common wheat (*Triticum aestivum*, family Poaceae)[1]. A large quantity of wheat germ and WGO have recovered annually as by-products of the wheat flour milling industry. The large availability of the WGO at affordable prices gave an economical value to their uses in the medical and cosmetic preparations[2]. The WGO is a rich natural source of vitamin E[3] and contains many other health-beneficial bioactive compounds, including polyunsaturated fatty acids[4], policosanol[5], which used as a dietary supplement and phytosterols (plant sterols) with cholesterol-level lowering potential in humans[6]. WGO contains essential constituents for skin health and management including squalene[7] which used in skin and hair cosmetic preparations as one of the natural secreted components of the skin surface polyunsaturated lipids and constituting 12%of the skin sebum composition[8]. WGO also contains numbers of B vitamins, i.e., B_1, B_2, B_3 and B_6 and folic acid which have a role in skin health a blood circulation[9]. Minerals such as phosphorous, magnesium, potassium, zinc, calcium, sodium, iron and copper are also highly represented in the WGO[10,11]. Further to the previous component, WGO also contains several other dietary constituents such as oleic, palmitic, stearic, arachidonic and linoleic fatty acids in addition to flavonoids and glutathione[12-14]. The WGO, therefore, has been reported for their use as antioxidant potential diet[15-17]. Also, the unsaturated fatty acids of WGO are precursors for the prostaglandins hormones which play role in the healing and diminish inflammatory processes[18]. Moreover, WGO has been used in dermatological and cosmetic preparations due to their antioxidant activity[19-21]. The oil has also used for the treatment of dry skin disorder produced in response to the dietary deficiencies of the poly-unsaturated fatty acids[22]. The WGO has a bright dark Yellowish-brown colour with a slightly Wheat-like smell. Besides, the consistency of the WGO makes it a valuable emollient for dry and dehydrated skin[23].

Skin is the largest body's organs and intact skin is essential for human health as an outside protective barrier, however, skin lesion or loss of skin integrity produced by injuries or burns might lead to severe infections, disability and death[24,25]. Therefore, protocols for wound healing and skin protection have been extensively reported[26,27]. The intact skin consists of two main layers, the upper tissue layer composed of keratinized stratified epidermis followed by the nourishment inner tissue Rich-collagen layer[28]. The enrich of WGO with the vitamin E, unsaturated fatty acids and other antioxidant constituents make it supportive materials for restoring the collagen of the skin connective tissues and help in the treatment of skin lesions and aging[29]. WGO is a common oil used in cosmetic preparations that demonstrated antimicrobial activity against *Streptococcus epidermidis* and *Pseudomonas aeruginosa*[30]. These organisms reported as a regular cause of postoperative infection and have been isolated from some skin ulcers[31,32]. The nutritional and biological values of WGO in addition to its regular uses in the skin and cosmetic preparations are imperative points for testing the wound healing properties of the WGO.

The current work designated to incorporate the wheat germ oil into the polymers of the cream and ointment bases and evaluate the wound healing potential of these WGO formulations in the animal model. The study was intended based on the huge availability of the WGO at a cheap price and the common use of this oil in cosmetic preparation.

MATERIALS AND METHODS

Study area: Preparation of the WGO formulations and *in vivo* wound healing evaluation was carried out at the Department of Applied Pharmaceutical Sciences, Faculty of Pharmacy, Isra University, Amman, Jordan. The FTIR-based compatibility of the formulations was carried out at the Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, Qassim University, Saudi Arabia. All the experiments were performed during 2020.

Materials: All chemicals and reagents were of the highest purity. Paraffin oil, beeswax, glycerol, borax, vitamin E and zinc oxide were purchased from Sigma-Aldrich (Germany). White soft paraffin, tween 80 and wheat germ oil were purchased from Tedia (USA).

Wheat germ oil cream preparation: The cold cream was formulated by the regular literature methods according to US Pharmacopeia[33]. The aqueous phase containing 2 g of the borax and 10 mL of the glycerol in distilled water (200 mL) was prepared and heated for 70°C on the water bath. The oily phase was also prepared by mixing the beeswax (32 g) and paraffin oil (100 g) at 70°C on a water bath. The cream base was formed by adding the aqueous phase dropwise to the oily phase with continuous round stirring using a glass rod and allowing the mixture to cool until room temperature. The WGO cream was prepared in two concentrations, 10 and 20%. The accurately weighed WGO was added dropwise to the previously prepared cold cream with continuous stirring then I mL of the tween 80 was added to the preparation under the stirring process.

Wheat germ oil ointment preparation: The ointment base and ointment formulations the WGO and zinc oxide were formulated according to the literature method and followed the instruction of the US pharmacopeia[34]. The ointment base was formulated by melting the bees was (32.5 g) and mixed with the white soft paraffin (617.5 g) and the mixture was kept on the water bath at 75°C until complete liquefaction. The mixture then allowed cooling with continuous stirring until the uniform ointment base obtained. Zinc oxide ointment was prepared by levigate 20 gm of the zinc oxide powder in 5 mL of the mineral oil and mixed with 80 g of the ointment base previously prepared. WGO ointment was formulated by mixing the WGO with the ointment base in a proportion of 10:90 and 20:80 (W/W) to prepare 10 and 20% WGO ointment, respectively.

Characterization of the cold cream and zinc oxide ointment loaded wheat germ oil

Compatibility study: The compatibility of the prepared cream and ointment bases with the WGO was determined by the Fourier Transform Infrared (FT-IR) analysis[35]. The physical mixture of the final product of the WGO (20%) with the cold cream or ointment polymers in addition to the WGO and polymers were subjected to FT-IR analysis. The samples were scanned in a range from 400-4000 cm^{-1} and recorded on an FT-IR (Bruker, OPTIK GmbH, Type: Tensor 27, Germany), adapted with attenuated total reflection (ATR) technique.

Homogeneity test of the final WGO formulations: The homogeneity of the formulated WGO ointments and creams were tested by the visual appearance and texture sensitivity[35]. A small quantity of the formulated ointments and creams pressed in-between the thumb and the index finger to feel the consistency of the formulated preparations and noticed whether homogeneous or not.

Stability storage: Three samples of the formulated WGO creams and ointments were stored for up to 60 days at room temperature (a temperature range of 25±2°C) and the humidity established at 60±5% RH (RH-stands for "Relative Humidity"). The samples were tested after the storage period for any changes in the odour, colour and texture. The samples were also examined visually for any separation or crystallization. the Assessment against the product's stability measurement will take place at selected time points.

Animal study: Twenty-two male Sprague rats weighing between 180-250 g were used in this study. Rats were obtained from animal house, faculty of pharmacy, Al-Isra University, Amman-Jordan. The protocol of the study was approved by the ethical committee of the Faculty of Pharmacy, Al-Isra University, Jordan. Rats were housed under standard laboratory conditions at room temperature of 32±2°C, humidity (55%) and were exposed to 12 hrs light/dark cycle. Animals were feed with a standard diet and water (*ad libitum*) and kept one week for acclimatization before the initiation of the experiment.

Animal experimental design: The animals were divided into five groups; Group 1, 2 and 3 (2 rats each) represent the negative control group of animals which shaved and burned without medication applied on the skin (G_1), rats treated with the cream base (G_2) and animals treated with the ointment base (G_3) containing zinc oxide, respectively. Besides, groups 4, 5, 6 and 7 (4 rats each) represent the 20% WGO cream treated group (G_4), 20% WGO ointment treated group (G_5), 10% WGO cream treated group (G_6) and 10% WGO ointment treated group (G_7), respectively. The animals were anaesthetized and the dorsal side of the animals was shaved by using an electrical clipper. The shaved area was sterilized with 70% alcohol. Burns inflections were only limited to the loin area and induced by a 100 g cylindrical Stainless-steel rod (2 cm bottom diameter) which was previously heated to 100°C in a boiling water bath and rested on its weight for 20 sec on each rat. The temperature of the rod was monitored using a thermocouple. The skin was pulled upwards, away from the underlying viscera, creating a flat surface. Wounds were evaluated for size, morphology and depth[36]. Accurately, 0.5 g of the formulated WGO cream and ointment or cream and ointment bases were applied daily to the injured area of the rats back using a sterile smooth wooden stick. The wounds were observed for any clinical changes and healing compared to the negative control group.

Evaluation of wounds: Wounds healing evaluation was performed through all the following procedures:

- **Wound photos:** Photographic representation for wound healing progress monitored by using an iPhone camera and a photo taken at (0, 1, 2, 3) weeks during the study
- **Wound size:** The progressive changes in wound diameter were measured using vernier calliper in millimetre every week interval until complete wound closure was recorded and the wound diameter reduction percentage was calculated from the equation[37]:

$$\text{Wound reduction (\%)} = \frac{w0 - wt}{w0} \times 100$$

where, w0 has wound diameter on day zero, wt is wound diameter on day 't.' the percentage of wound closure on day 7, 14 and 21 recorded and used as an indicator of wound healing change during the study[37].

Calculation of the time taken for 50% of wound closure (wc50) was calculated by a plot of percentage of wound closure against time during the study period.

- **Wound edge assessment:** Observation of Maceration, Dehydration, Undermining and Thickened/rolled edges[38]
- **Wound bed:** By evaluating the exudate and infection that includes many symptoms includes: Increased pain, Erythema, Local warmth, Edema, Increased exudates and delayed healing[39]

Progressive changes in the wound area were monitored periodically every 3 days during the study period.

Statistical analysis: Statistical analysis was performed using the student "t" test which was used to determine the significance between the mean of treatment with cold cream, zinc oxide ointment and different percentages of WGO creams and ointments for wound healing compared with a control group. One-way analysis of variance (ANOVA) and Tukey test were used for the statistical analysis of data difference mean among different study periods within each group. A probability value of $P<0.05$ was considered significant.

RESULTS

Stability and homogeneity of the WGO-formulations: The physical stability and homogeneity of the formulated WGO cream and ointment were evaluated; three samples of the small amount of cream and ointment were stored for 60 days at the normal room temperature and relative humidity of 60%. The formulated preparations were also tested after the storage period for their homogeneity. The examination of the stored WGO cream and ointment samples demonstrated that there was no change in the consistency of the WGO cream and ointment. Besides, there was no change in the formulations' original colour. Cracking and separation in the WGO creams and ointment were not recognized throughout the storage period which revealed the stability and compatibility of the formulated WGO cream and ointment.

FT-IR based compatibility of the WGO-formulations: The FT-IR spectrum showed in Fig. 1 was similar to the reported IR-spectrum of the WGO which showed an absorption peak at 1757 cm^{-1} for the carbonyl group of the oil triglycerides. The stretch absorption at 2980 and 1204 was assigned to the methyl and -C-O- stretching vibrations. However, the CH_2

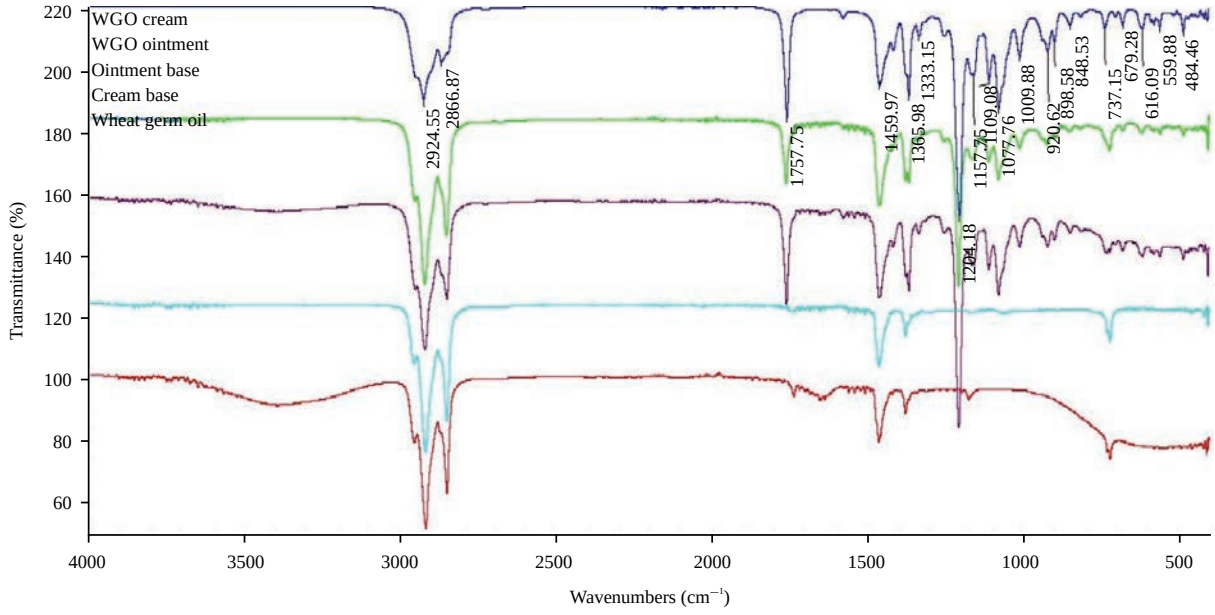

Fig. 1: Fourier Transform Infrared (FT-IR) spectra of the wheat germ oil and formulated wheat germ oil ointment and cream

Table 1: Reduction of skin-wound diameter induced by wheat germ oil creams and ointments during the period of wound healing

		7 days		14 days		21 days		28 days	
Groups of animals	Number	Wound diameter (cm)	Reduction in wound size (%)	Wound diameter (cm)	Reduction in wound size (%)	Wound diameter (cm)	Reduction in wound size (%)	Wound diameter (cm)	Reduction in wound size (%)
Control	2	2	0	2± 0.1	5	1.8±o.1*	10	1.1±0.1*	45
Treated with cold cream	2	2	0	1.8±0.1	10	1.3±0.2*	35	0.7±0.1*	65
Treated with zinc oxide ointment	2	1.9	5	1.7±.1*	15	1.1±0.1*	45	0.5±0.1*	75
Treated with cream containing 20% WGO	4	1.4±0.1*abc	30	1±0.2*abc	50	0.3±0.1*abc	85	0*abc	100
Treated with ointment containing 20% WGO	4	1.2±0.1*abc	40	0.9±0.2*abc	55	0.2±0.1*abc	90	0*abc	100
Treated with cream containing 10% WGO	4	1.8±0.1	10	1.5±0.1*a	25	0.7±0.1*ab	65	0*abc	100
Treated with cream containing 10% WGO	4	1.7±0.1*a	15	1.4±0.1*a	30	0.5±0.1*abc	75	0*abc	100

N: Number of animals in each group. The diameter of the wound on day zero was 2 cm, ±: Denoted for the difference in wound size reduction among the animal in each group. *Percentage of wound size reduction was calculated according to the following equation: Wound reduction (%) = w0-wt/w0×100. Data expressed as Mean±SEM, N: Number of animals within the group, a: Significant difference between treatment with 10-20% of wheat oil cream and ointment as compared with a normal control group using student t-test ($p<0.05$). b: Significant difference between treatment with 10-20% of wheat oil cream and ointment as compared with a cold cream group using student t-test ($p<0.05$). c: Significant difference between treatment with 10-20% of wheat oil cream and ointment as compared with zinc oxide ointment group using student t-test ($p<0.05$). *Significant difference between means among different study periods within each group using ANOVA and Tukey test ($p<0.05$)

binding vibrations were recognized at 1459, 1365 and 737 cm^{-1} (Fig. 1). Noteworthy, no changes in the WGO diagnostic FT-IR peaks were recognized in the spectra of the WGO cream and WGO ointment which indicate no chemical interaction has been raised due to the physical mixture of the WGO with the cream and ointment polymers.

Wound healing properties of the WGO formulations: The results in Table 1 showed Dose-dependent and time sequence wound healing properties expressed by the reduction percentage of the wound diameter induced in response to the application of the 10 and 20% WGO ointment and creams. The results were recorded and compared to the untreated group of animals and animals treated with the cold cream and zinc oxide ointment (Table 1). The results in Table 1 also revealed better-wound healing activity of the WGO ointment over the WGO cream, i.e., after one week of the daily application of the WGO formulations, the 10 and 20% WGO ointment induced a 15 and 40% reduction in the wound size diameter, respectively, compared to 10 and 30% reduction in the wound size diameter by the WGO cream at similar concentrations. Moreover, the reduction in the wound size induced by the zinc oxide cream after one week of the application was 5 compared to 0% improvement for the application of the cold cream and non-treated group. After two weeks of the treatment, wounds sizes were greatly reduced to reach 55 and 50% reduction in diameter size by the 20% WGO ointment and cream, respectively. The 10% formulation of the WGO was also significantly reduced the wounds diameter sizes by 30 and 25% for the WGO ointment and cream, respectively after two weeks of the application. The untreated wounds and the wounds treated by the cold cream and zinc oxide ointment were also showed some improvement calculated by 5, 10 and 15% reduction in the wound diameter sizes, respectively. The observations after three weeks of the treatment revealed that wounds of the WGO treated animals were recovered by 65% in the animals treated with 10% WGO cream to 90% in the animals treated with 20% WGO ointment. However, complete recovery of the treated animals was reached in the fourth week of the application of the WGO ointments and creams.

Statistical calculations were also represented in Table 1; the results revealed no statistical differences, within the same group, in the wound healing (reduction in the wound size) between untreated group and groups subjected to cold cream and zinc oxide ointment application during the first two weeks of the treatment. However, significant variations were calculated within these groups in the third and fourth week of the treatment. Statistically, the wound size in the group of

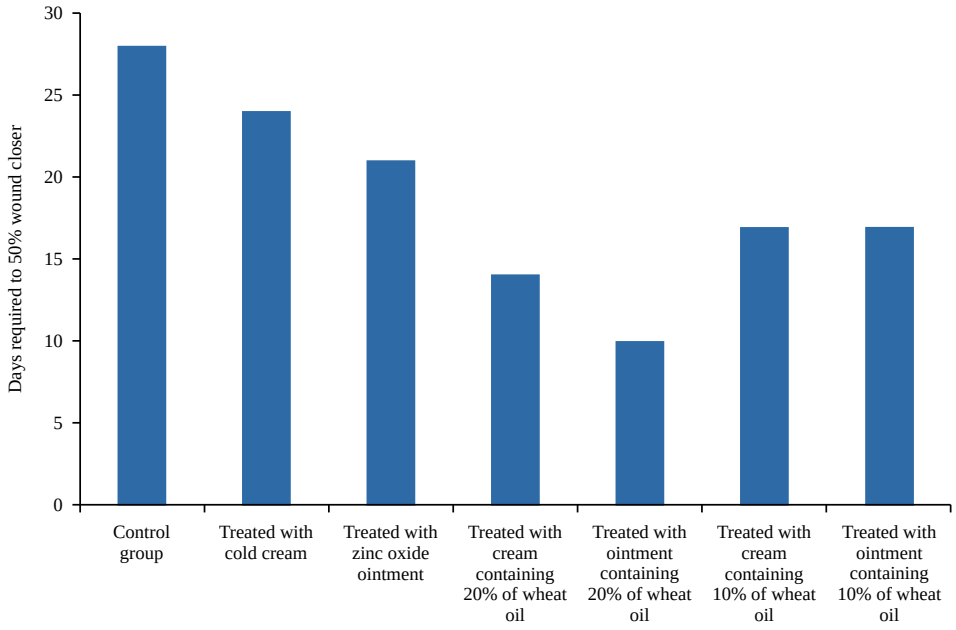

Fig. 2: Approximate time required to 50% wound closer within the control and treated animal groups

animals treated with cream containing 20% of wheat oil was found to significantly shrinkage among the four weeks of the treatment ($p<0.05$). Similarly, significant wound healing progress was recorded in the groups of animals that treated with 10 and 20% of the WGO ointments as compared to all other control groups (Table 1). The approximate time required for 50% wound closer showed in Fig. 2 and supports the wound healing activity of the formulated WGO creams and ointments especially at the higher concentrations (20%). For instance, wounds were recovered by 50% in approximately 10 and 14 days after treatment with 20% WGO ointment and cream, respectively (Fig. 2). However, the approximate days required to recover 50% of the wound in the control group of animals and animals treated with cold cream and zinc oxide ointment were 28, 24 and 21 days, respectively.

The differences between WGO cream and ointment wound healing effect at their both concentrations, 10 and 20%, are demonstrated clearly in Fig. 3a-c. The figure showed better activity to the WGO ointments over the WGO creams at all the intervals of the experiment (Fig. 3a-b). Besides, the superiority wound healing effect of the WGO formulation expressed in wound size reduction over the untreated animal group and animals subjected to the cold cream and zinc oxide treatment during the whole treatment plane were demonstrated in Fig. 3a, c and d. Fig. 3 also revealed that 20% of the WGO in the cream and ointment formulations are more active in the treatment of wounds in the experimental animals over the 10% formulations.

The progress in the animals' wounds' healing was also monitored through photographic representation for the burned wound skin. The data of Fig. 4 showed the wound shape before treatment at day 0 (Fig. 4a) and the wound closure after treatment for continuous four weeks with the 20% WGO cream (Fig. 4b) and ointment (Fig. 4c). The photographs which have been taken during the one-week intervals for successive four weeks of the wound treatment confirm the wound size-diameter reduction and revealed the potential use of the WGO formulation in the treatment of the animal wounds.

DISCUSSION

This study investigated the healing potential activity of the WGO topical preparations, creams and ointments, on the thermal-induced burns in rats' animal model. The evaluation criteria of the WGO formulations were measured using the reduction of the burn wound size, accelerate and improve the wound healing process, prevent the existence of the scar on the skin during the study period for 28 days. The treatment of burn wounds has always been one of mankind global worldwide concerns as they are the most severe form of trauma that has afflicted humanity since ancient times[40] and could induce a common universal problem that leads to horrible scarring; serious handicapping. The healing process, a natural body reaction to injury initiates immediately after wounding and occurs in four stages; the first phase is

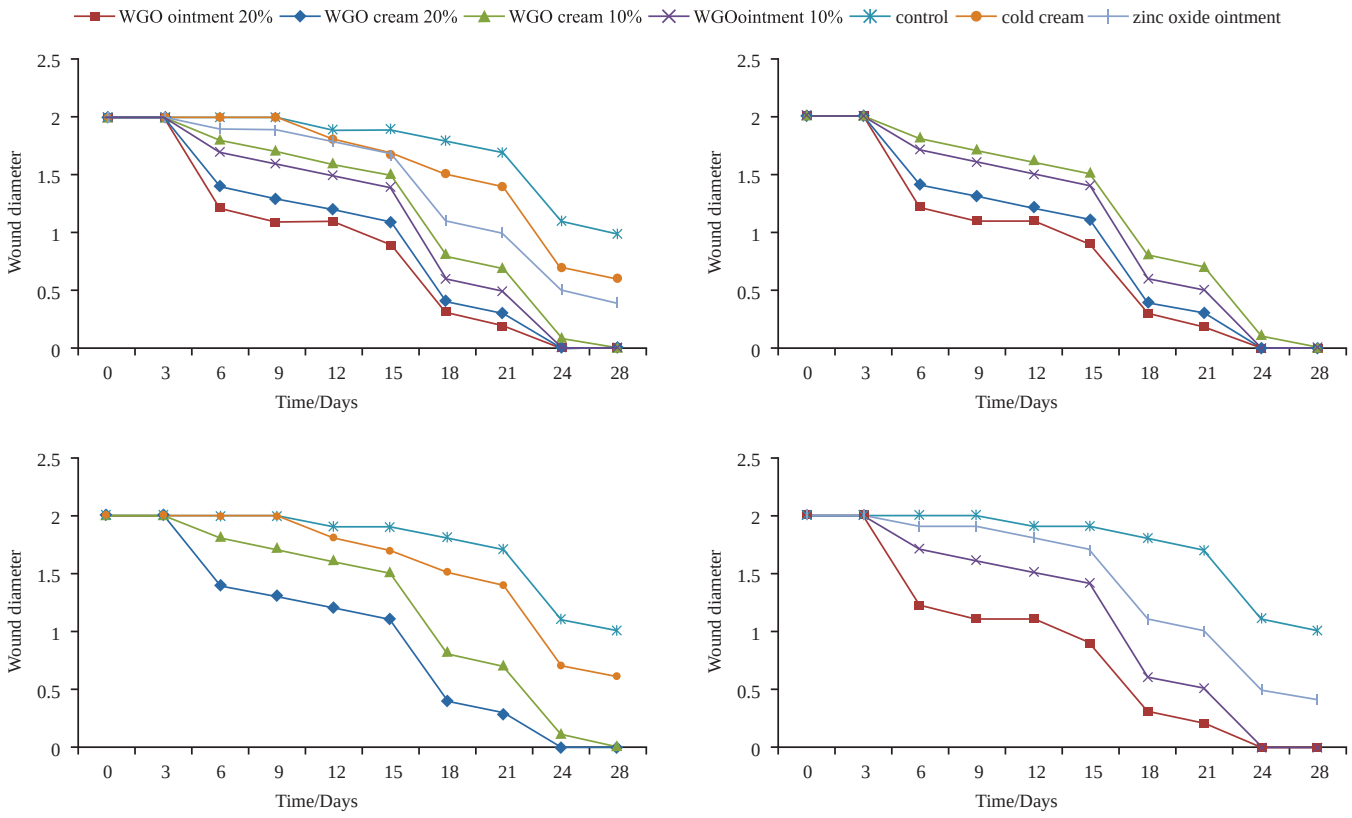

Fig. 3(a-d): Reduction in wounds diameter during the treatment with the WGO-formulations

Wound contraction percentage among the (a) groups of the animal during periods of study, (b) Animal treated with 10 and 20% of WGO creams and ointments, (c) Animal treated with 10 and 20% of wheat oil creams, control group, zinc oxide ointment group and (d) Animal treated with 10 and 20% of wheat oil ointment, control group, zinc oxide ointment group

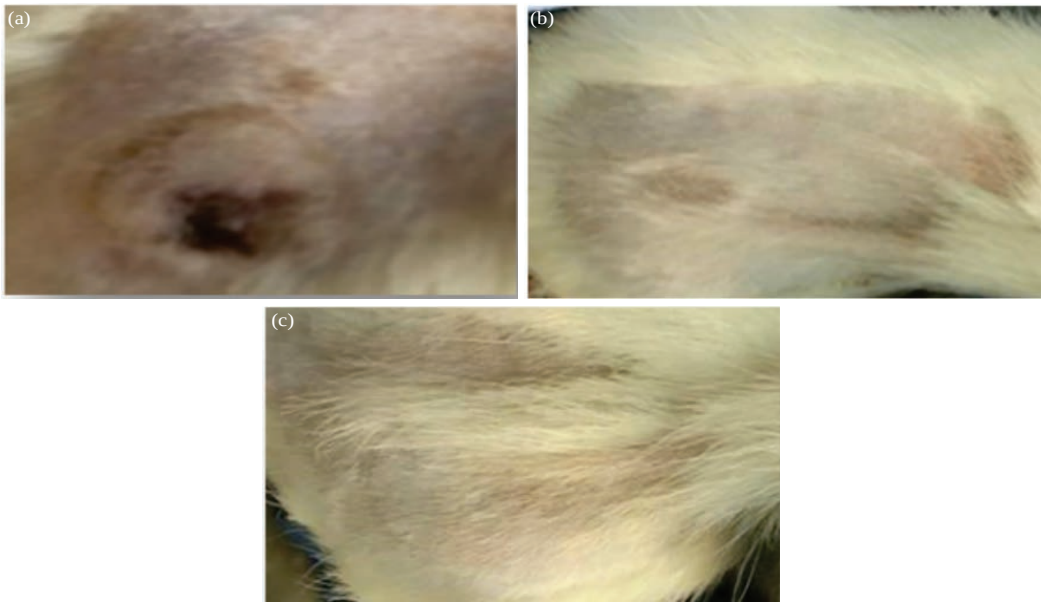

Fig. 4(a-c): Photographs of wound repair in rats model after four weeks of treatment with the WGO-formulations

Wound shapes, (a) Before treatment, (b) After four weeks treatment with 20% WGO cream and (c) After four weeks treatment with 20% WGO ointment

coagulation which controls excessive blood loss from the damaged vessels. The next stage of the healing process is inflammation and debridement of the wound followed by re-epithelialization which includes migration, proliferation and differentiation of squamous epithelial cells of the epidermis. In the final stage of the healing process collagen deposition and remodelling occurs within the dermis[41]. Topical preparations are a very important pharmaceutical dosage form for wound treatment. Besides, topical applications such as sprays, creams and ointments are one of the major approaches for the management of burn wounds, accelerate their healing process and reduce burn wound size and multiple agents have been used for this purpose[42,43].

The formulated WGO topical preparations were successfully prepared and were passed the stability and homogeneity testing over the 60 days of storage. FT-IR spectrum for the WGO and formulated WGO cream and ointment also confirmed the compatibility of the formulated WGO preparations. The characteristic peaks for the WGO in the FT-IR spectra of the WGO formulations indorsed that there were no chemical interactions between the WGO and the polymers that could be arising or depleting the chemical-functional groups in the WGO constituent's structures[35].

The wound closer percentages over the treatment period which was extended to four weeks revealed the potential wound healing activity of the WGO formulations. Reports have been proved the anti-inflammatory of the WGO which might be the direct cause of its wound healing properties[18,29,44]. The oil has been also reported for the antioxidant activity and its beneficially effect on the immune system that also participates in the wound improvement processes[45]. The results showed in Table 1 and Fig 2-4 revealed that the ointment formulation is preferred over the cream formulation of the WGO as a healing preparation during the intervals of the wound treatment. The superiority of the WGO ointment formulation is attributed to the nature of the polymers which might extend the WGO release and make it available in the wound site for a longer time compared to the cream base.

CONCLUSION

The WGO formulated cream and ointment polymers formulated in two different concentrations, showed computability and constant stability during two months' storage time at room temperature. WGO cream and ointment formulation was significantly more effective in the treatment of animal wounds compared to the untreated animals or animals subjected to cold cream and zinc oxide ointment application. The higher concentration of the WGO cream and ointment (20%) were more effective and curing the wounds up to 90% within 21 days of the daily treatment without exceeding the irritation or inflammation compared to the 10% WGO formulations. The WGO ointments reproduce better healing properties than the cream formulation in all the treatment intervals of the wounds.

SIGNIFICANCE STATEMENT

This study describes the formulation and biological application of WGO in wound treatment. The study proved that WGO formulations have accelerated the wound closure and reduced the wound inflammation in the rats' model. The study also confirmed that ointment incorporated WGO was more effective than the cream formulation and WGO also help to recover the burning wounds more effectively in future.

ACKNOWLEDGMENT

The authors appreciate the institutional support from the Faculty of Pharmacy, Isra University and College of Pharmacy, Qassim University.

REFERENCES

1. Hertrampf, J.W. and F. Piedad-Pascual, 2000. Wheat and Wheat By-Products. In: Handbook on Ingredients for Aquaculture Feeds, Hertrampf, J.W. and F. Piedad-Pascual (Eds.)., Springer, Netherlands, pp: 531–542.
2. Mahmoud, A.A., A.A.A. Mohdaly and N.A.A. Elneairy, 2015. Wheat germ: An overview on nutritional value, antioxidant potential and antibacterial characteristics. Food Nutr. Sci., 6: 265-277.
3. Evans, H.M., O.H. Emerson and G.A. Emerson, 1974. The isolation from wheat germ oil of an alcohol, α-tocopherol, having the properties of vitamin E. Nutr. Rev., 32: 80-82.
4. Dunford, N.T. and M. Zhang, 2003. Pressurized solvent extraction of wheat germ oil. Food Res. Int., 36: 905-909.
5. Irmak, S. and N.T. Dunford, 2005. Policosanol contents and compositions of wheat varieties. J. Agric. Food Chem., 531: 5583-5586.
6. Leguizamón, C., C.L. Weller, V.L. Schlegel and T.P. Carr, 2009. Plant sterol and policosanol characterization of hexane extracts from grain sorghum, corn and their DDGS. J. Am. Oil Chem. Soc., 86: 707-716.
7. Malecka, M., 1991. The effect of squalene on the heat stability of rapeseed oil and model lipids. Food/Nahrung, 35: 541-542.
8. Pappas, A., 2009. Epidermal surface lipids. Dermato-Endocrinology, 1: 72-76.

9. Abdou, H.M., N.A. Mohamed, D.A. El Mekkawy and S.B. El-Hengary, 2017. Vitamin E and/or wheat germ oil supplementation ameliorate oxidative stress induced by cadmium chloride in pregnant rats and their fetuses. Jordan J. Biol. Sci., 10: 39-48.
10. Brandolini, A. and A. Hidalgo, 2012. Wheat germ: Not only a by-product. Int. J. Food Sci. Nutr., 63: 71-74.
11. Ge, Y., A. Sun, Y. Ni and T. Cai, 2001. Study and development of a defatted wheat germ nutritive noodle. Eur. Food Res. Technol., 212: 344-348.
12. Zou, Y., M. Yang, G. Zhang, H. He and T. Yang, 2015. Antioxidant activities and phenolic compositions of wheat germ as affected by the roasting process. J. Am. Oil Chem. Soc., 92: 1303-1312.
13. Huang, J.N., G.H. Song, Q. Sun, C. Zhan and H. Wei, 2010. Extraction of glutathione and wheat germ protein from defatted wheat germ. Food Sci., Vol. 22.
14. Alessandri, C., P. Pignatelli, L. Loffredo, L. Lenti and M. del Ben *et al*., 2006. Alpha-linolenic acid-rich wheat germ oil decreases oxidative stress and CD40 ligand in patients with mild hypercholesterolemia. Arterioscler. Thromb. Vasc. Biol., 26: 2577-2578.
15. Krings, U. and R.G. Berger, 2001. Antioxidant activity of some roasted foods. Food Chem., 72: 223-229.
16. Shahidi, F., 2000. Antioxidants in food and food antioxidants. Food/Nahrung, 44: 158-163.
17. Leenhardt, F., A. Fardet, B. Lyan, E. Gueux and E. Rock *et al*., 2008. Wheat germ supplementation of a low vitamin E diet in rats affords effective antioxidant protection in tissues. J. Am. Coll. Nutr., 27: 222-228.
18. Piras, A., A. Rosa, D. Falconieri, S. Porcedda, M. Dessì and B. Marongiu, 2009. Extraction of oil from wheat germ by supercritical CO_2. Molecules, 14: 2573-2581.
19. Yildirim, K. and A.M. Kostem, 2014. A technical glance on some cosmetic oils. Eur. Sci. J., 2: 425-435.
20. Badea, G., I. Lacatusu, N. Badea, C. Ott and A. Meghea, 2015. Use of various vegetable oils in designing photoprotective nanostructured formulations for UV protection and antioxidant activity. Ind. Crops Prod., 67: 18-24.
21. Zhu, K., H. Zhou and H. Qian, 2006. Antioxidant and free radical-scavenging activities of wheat germ protein hydrolysates (WGPH) prepared with alcalase. Process Biochem., 41: 1296-1302.
22. Berdick, M., 1972. The role of fats and oils in cosmetics. J. Am. Oil Chem. Soc., 49: 406-408.
23. Kan, A., 2012. Chemical and elemental characterization of wheat germ oil (*Triticum* spp. L.) cultivated in Turkey. Afr. J. Agric. Res., 7: 4979-4982.
24. Edwards, R. and K.G. Harding, 2004. Bacteria and wound healing. Curr. Opin. Infect. Dis., 17: 91-96.
25. Singer, A.J. and R.A.F. Clark, 1999. Cutaneous wound healing. N. Engl. J. Med., 341: 738-746.
26. Bolton, L., P. McNees, L. van Rijswijk, J. de Leon and C. Lyder *et al*., 2004. Wound-healing outcomes using standardized assessment and care in clinical practice. J. Wound Ostomy Cont. Nurs., 31: 65-71.
27. Thompson, P., D. Langemo, J. Anderson, D. Hanson and S. Hunter, 2005. Skin care protocols for pressure ulcers and incontinence in long-term care: A quasi-experimental study. Adv. Skin Wound Care, 18: 422-429.
28. Martin, P., 1997. Wound healing: Aiming for perfect skin regeneration. Science, 276: 75-81.
29. Ghafoor, K., M.M. Ozcan, F. Al Juhaimi, E.E. Babiker, Z.I. Sarker, I.A.M. Ahmed and M.A. Ahmed, 2017. Nutritional composition, extraction and utilization of wheat germ oil: A review. Eur. J. Lipid Sci. Technol., Vol. 119. 10.1002/ejlt.201600160.
30. Marchwińska, K. and K. Michocka, 2017. The antimicrobial properties of selected oils used as cosmetics compounds. Studia Oeconomica Posnaniensia, 5: 35-44.
31. Valencia, I.C., R.S. Kirsner and F.A. Kerdel, 2004. Microbiologic evaluation of skin wounds: Alarming trend toward antibiotic resistance in an inpatient dermatology service during a 10-year period. J. Am. Acad. Dermatol., 50: 845-849.
32. Basualdo, C., V. Sgroy, M.S. Finola and J.M. Marioli, 2007. Comparison of the antibacterial activity of honey from different provenance against bacteria usually isolated from skin wounds. Vet. Microbiol., 124: 375-381.
33. Jarrahi, M. and A.A. Vafaei, 2004. Effect of topical phenytoin cream on linear incisional wound healing in albino rats. Daru, 12: 156-158.
34. Ueda, C.T., V.P. Shah, K. Derdzinski, G. Ewing and G. Flynn *et al*., 2009. Topical and transdermal drug products. Pharmacopeial Forum, 35: 750-764.
35. Abdellatif, A.A.H., S.H. Alawadh, A. Bouazzaoui, A.H. Alhowail and H.A. Mohammed, 2020. Anthocyanins rich pomegranate cream as a topical formulation with anti-aging activity. J. Dermatolog. Treat., 31: 1-8.
36. Cai, E.Z., C.H. Ang, A. Raju, K.B. Tan and E.C.H. Hing *et al*., 2014. Creation of consistent burn wounds: A rat model. Arch. Plast. Surg., 41: 317-324.
37. Ahmad, M., M.N. Ansari, A. Alam and T.H. Khan, 2013. Oral dose of citrus peel extracts promotes wound repair in diabetic rats. Pak. J. Biol. Sci., 16: 1086-1094.
38. Dowsett, C., M.N. Gronemann and K. Harding, 2015. Taking wound assessment beyond the edge. Wounds Int., 6: 19-23.
39. Dowsett, C. and B. von Hallern, 2017. The triangle of wound assessment: A holistic framework from wound assessment to management goals and treatments. Wounds Int., 8: 34-39.
40. Garcia-Espinoza, J.A., V.B. Aguilar-Aragon, E.H. Ortiz-Villalobos, R.A. Garcia-Manzano and B.A. Antonio, 2017. Burns: Definition, classification, pathophysiology and initial approach. Gen Med., 5: 1-5.

41. Phillips, G.D., R.A. Whitehead and D.R. Kinghton, 1991. Initiation and pattern of angiogenesis in wound healing in the rat. Am. J. Anat., 192: 257-262.
42. Chang, R.Y.K., S. Morales, Y. Okamoto and H.K. Chan, 2020. Topical application of bacteriophages for treatment of wound infections. Transl. Res., 220: 153-166.
43. Leon-Villapalos, J., M.G. Jeschke and D.N. Herndon, 2008. Topical management of facial burns. Burns, 34: 903-911.
44. Kang, B.K., M.J. Kim, D.H. Jeong, K.B. Kim and N.Y. Bae *et al*., 2016. Anti-inflammatory effect of wheat germ oil on lipopolysaccharide-stimulated RAW 264.7 cells and mouse ear edema. Microbiol. Biotechnol. Lett., 44: 236-245.
45. Yuldasheva, N.K., N.T. Ul'chenko and A.I. Glushenkova, 2010. Wheat germ oil. Chem. Nat. Compd., 46: 97-98.