Special Issue:
Advancements in Animal Health and Production in Low and Middle-Income Countries

The Effect of Nano Saffron Extract on Opened Wounds in Rats

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Abstract | Wound healing is a complex procedure, especially in animals where self-care is naturally limited and exposure to environmental aggravators is high. The present study aims to demonstrate the role of Nano-saffron in natural therapy and alternative medicine. To illustrate this, thirty male Wistar rats, housed in plastic cages, were randomly divided into three groups with each group containing 10 rats. Group 1 (G1) served as control, while group 2 (G2) was treated with Vaseline, and group 3 (3) was treated with 5% Nano-saffron. Rats were anesthetized with IM injection of ketamine (50mg / K.G BW), xylazine (10mg/K.G BW) mixture. A surgical incision was performed at the dorsal region about 10 mm of their back, and treatments were topically applied once a day for seven consecutive days. Thereafter, biopsies were taken on the 7th, 14th, and 21st days and examined for histopathological evaluation of the healing process. Histopathologic evaluation revealed the superiority of healing in group 3, treated with Nano saffron, highlighting its wound healing potential. Histological changes following wound injury in Nano saffron treated skin indicated complete healing of all skin layers, including the epidermis, reticular dermis, papillary dermis, hypodermis, and sebaceous glands. Collectively, Nano saffron demonstrated impressive wound healing capabilities and may offer an economical and safer option for post-wound care agents.

Keywords | Open wound, Nano saffron, Healing, Rat model

Received | June 24, 2025; **Accepted** | July 26, 2025; **Published** | August 07, 2025
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Citation | Mohammed MA, Diwan MF (2025). The effect of nano saffron extract on opened wounds in rats. J. Anim. Health Prod. 13(s1): 49-55.
DOI | https://dx.doi.org/10.17582/journal.jahp/2024/13.s1.49.55
ISSN (Online) | 2308-2801

INTRODUCTION

Wound is a breakdown of the skin with or without loss of underlying connective tissue caused by disease, like an ulcer, or injury, like surgery, chemicals, cold, heat, or pressure (Wilkins and Martin, 2013). Wounds can be classified according to various factors including cause, wither acute or chronic, location, depth, and contamination (Morteza *et al.*, 2022). Knowing the class of wound is essential for diagnosis, selecting therapy, and determining treatment regimens (Muhamed, 2016).

Wound healing is a natural physiological and biological process in response to tissue injury resulting in the restoration of tissue integrity (Alistair and Clare-Ellen, 2011). It is highly coordinated, multilayered process that begins as an inflammatory response (Bharati *et al.*, 2022), followed by proliferative phase, and finally a remodeling phase (Oztuk and Aylin, 2011). The participation of numerous cell types, cytokines, mediators and the vascular system (Angela *et al.*, 2024). Various conventional approaches are being used to promote wound healing. Recent developments in wound care technology include Nano-therapies, stem cell therapy, and bio-engineered skin grafts (Praveen *et al.*, 2022).

Nanoparticles are materials with dimensions less than 100 nm with various shapes (Dowling *et al*., 2004). It can result in stimulating numerous molecular and cellular processes that aid in creating a wound microenvironment with antibacterial, angiogenic, and anti-inflammatory effects (Anamik *et al*., 2022). The use of nanoparticles in products for topical applications improves the penetration of these materials compounds deep into skin layers (Magdalena and Jolanata, 2022).

The saffron plant belongs to the Iridaceae family. This herbaceous perennial plant can reach heights of 10 cm to 25 cm, developing from its bulbs (Shirin *et al*., 2018). Crocus sativus flowers begin to appear in autumn, and they contain a thin style that ends with a single stigma composed of three filaments with an intense red color, which are the plant parts that interest humans (Ibtissam *et al*., 2019). The main bioactive components in saffron are crocetin, crocin, saffranal, and picrocrocin (Loriana *et al*., 2020). Saffron has a long history in medicinal use; recent studies validate its potential to lower the risk of many diseases (Tibor, 2022). It exhibits anti-inflammatory activity (Poma *et al*., 2012) and antioxidant activity (Christodoulou *et al*., 2015). In wound healing, saffron has various mechanisms, including the enhancement of fibroblasts, epithelialization, and wound closure (Mojtaba *et al*., 2021).

MATERIALS AND METHODS

The work was conducted to evaluate the effect of Nano-saffron in treatment of open wounds in rats, thirty adult male rate were randomly divided into three groups and each group contain 10 rats (G1 not treated, G2 treated with Vaseline and G3 treated with Nano-saffron), all are anesthetized (by IM injection mix of ketamine 50mg/kg BW and xylazine 10mg/kg BW) to make surgical incision at the dorsal region about 10 mm of their back. The treatment began on the first day of the surgical procedure and continued for 7 consecutive days. The specimens were collected and preserved in a 10% buffered formalin solution then sent to the histological laboratory for evaluation at 7, 14 and 21 days.

A 90-gram sample of saffron was used to obtain 25 grams of saffron extract by using soxhlet device. Then, the saffron extract powder was converted Nano particles by the ultrasonic method (Jinllto and Kenneth, 2010). 10 g of saffron extract was dissolved in 100 ml of ethanol, and then the solution was filtered three times (two times with filter paper and the third time with a syringe filter (22 mm). The filter solution was treated with ultrasonic waves by mean of ultrasonic cell disruptor device (ultrasonic cell disruptor: UCD-150; Ultrasonic power 150 W, Probe 6mm) (Faithful Intelligent Ultrasonic processor) using wave energy 50 watts for 30 min where the saffron particles reached to 62 nm in size (Qais *et al*., 2021), the treated solution was poured in a petri dishes and placed in oven at 50 °C to dry. The dried extract was scraped and collected from the petri dish, ground, and then made into a 5% ointment of Nano saffron 5 gm. of Nano saffron was crushed with 95 gm. of Vaseline in a glass mortar and pestle. Several tests were made to assess the Nano saffron structures, including, Scanning Electron Microscopy, UV vis. absorption spectroscopy, and FTIR.

RESULTS

UV-spectrophotometer

UV-Vis Spectroscopy can be described as an analytical technique used for identifying the absorbance of colloidal solutions and the optical properties of prepared compounds over a range of wave lengths (200 nm to 800nm). Approximately 2 mL of the prepared nanoparticles has been added to a 1 cm path-length quartz cuvette to record the UV–vis spectrum. showed UV–vis spectrum of synthesized nanomaterial with maximum absorption at 440 nm.

FTIR analysis

The Fourier Transform Infrared spectrometer (FTIR) spectrum of synthesized nanoparticles is presented. A vast vibration peak at 3391.68 cm^{-1} corresponds to the O-H stretch of the alcohol group. The absorption peaks at 2353.12 and 2319.29 cm^{-1} correspond to the strong O=C=O stretching of carbon dioxide. The strong band at 1712.59 cm^{-1} represents carboxylic acid (C=O stretch) present in the nanomaterial. The medium band at 1663.17 cm^{-1} relates to the imine/oxime (C=N stretching). The small vibrational peak at 1374.24 cm^{-1} corresponds to the medium C–H bending mode attributed to the alkane group (methyl group). Other bonds present in the sample were observed at 1230.16 cm^{-1} and 1160.52 cm^{-1}, corresponding to strong C-O stretching in alkyl aryl and ester groups, respectively. The prominent vibration peak at 1074.56 cm^{-1} represents a strong C-O stretching of alcohol.

SEM image analysis

FE-SEM can be described as an analysis that provides information about the morphology of the surface and particle size of prepared samples. It displays FE-SEM analysis of prepared NPs. The formed nanomaterial is uniform in shape with a smooth surface morphology. The diameter of the NPs is about 49.18±3.24 nm for 100 particles.

Clinical evaluation

All animals throughout the experiment time (21 days) post-wounding (PW). Clinically have been seen energetic, healthy, active, and with good healing processes at the site of injury. No infections developed in any of the experimental animals' wounds, and no deaths were recorded. Primarily, all of the wound areas increased in size within 4 hours after wound creation and continued to swell and increase in size for the following 24 hours- PW with inflammatory signs' exaggeration. The entire wound was swollen, and the wound edges were elevated and red. From the second day, PW showed a thick scab on the wound that persisted for longer than 7 days. The wound size decreased gradually until the 21st day, when it began to form scar tissue.

Surface area

The initial wound surface area (mm^2) on the first day of the experiment was (93.1). Skin wounds were measured on days 0, 7, 14, and 21 post wound injury. The average area of wound on the 7th day in G1, G2, and G3 was 60.38, 62.40, and 49.99, respectively (Table 1; Figure 2). There were significant differences between G2 and G3, while they recorded a significant difference in comparison to G1 and G2.

Table 1: Surface area (mm^2).

P. G.	0 day	7 days	14 days	21 days
G1	93.1 ± 0 Aa	60.38 ± 0.878 Ab	43.007 ± 0.735 Ac	29.68 ± 0.785Ad
G2	93.1 ±0 Aa	62.40 ± 0.686 Bb	40.19 ± 0853 Bc	26.62± 0.673 Bb
G3	93.1 ±0 Aa	49.99 ± 0.978Db	10.9 ± 0.652 Dc	5.06 ± 0.362 Dd
	2.70 < LSD (P 0.05)			

Mean± standard error. Means with different capital letters in the same column and small letters in the same row denote a significant difference at P<0.05.

Histopathologic assessment of wound healing

The histopathologic assessment reveals the following changes in every group for 3 weeks.

On the 7th day post-wound injury

G1: Histopathological changes after wound injury after one week showed that there is a Thick crust attached closely to the edges of the wound, profuse purulent exudate, and wide scar tissue with profuse granulation tissue in the dermis. Profuse purulent exudate which formed from dead neutrophils and tissue debris and thick crust above the edges of wound Thin and branched network of collagen fibers in the dermis (Figure 1A).

G2: Histopathological changes following wound injury, one week after, showed a Thick crust with purulent exudate and complete sloughing of the epidermal layers. They are High granulation tissue with profuse fibrosis, a thin and branched network of collagen, and wide scar tissue (Figure 1B). Showed was thick crust above the edges of the wound. There were profused granulation tissue with few blood vessels, newly formed, and fibrosis. Additionally, a branched network of collagen is present (Figure 1C).

G3: Histopathological changes observed one week after wound injury showed marked downward hyperplasia of the stratum basal in the epidermis. There were Presence of purulent exudate above the site of wound characterized by dead neutrophils and tissue debris. That was hyperemia and inflammatory cells in the dermis. There is hyperemia, as well as the presence of inflammatory cell, primarily macrophages, in the dermis (Figure 1D).

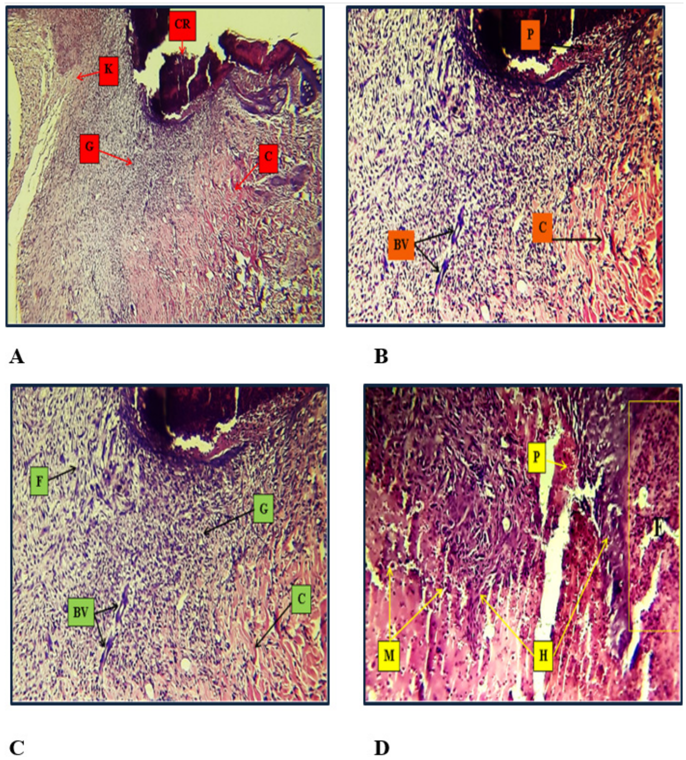


Figure 1: (A) Control group (G1) 7 days post wound shows profuse purulent exudate (P) which formed from dead neutrophils and tissue debris and thick crust above the edges of wound. Thin and branched network of collagen fibers (C) and newly formed blood vessels (BV) in the dermis. 10X H & E. **(B)** Positive control group (G2) 7 days post wound Shows thick crust (CR) with purulent exudate and complete sloughing at epidermal layers. High granulation tissue (G) with profuse fibrosis, thin and branched network of collagen (C) and wide scar tissue (K). 4X H & E. **(C)** Positive control group (G2) 7 days post wound shows thick crust above the edges of wound. Profuse granulation tissue (G) with few blood vessels newly formed (BV) and fibrosis (F). Also there is branched network of collagen (C). 10X H & E. **(D)** Treatment group (G3) 7 days post wound shows there is marked downward hyperplasia (H) of stratum basale in the epidermis. Presence of purulent exudate (E) beneath the epidermis characterized by dead neutrophils and tissue debris. Hyperemia (P) and inflammatory cells (M) in the dermis. 10X H & E.

On the 14th day post-wound injury

G1: Histopathological changes that follow wound injury after the second week showed Complete sloughing of epidermal layers with slight hyperplasia on both edges of the wound. They are profuse granulation tissue with wide scar tissue. Few hyperplastic areas are present on the edge of the wound, which contains profuse granulation tissue with high fibrosis and newly formed small blood vessels (Figure 2A).

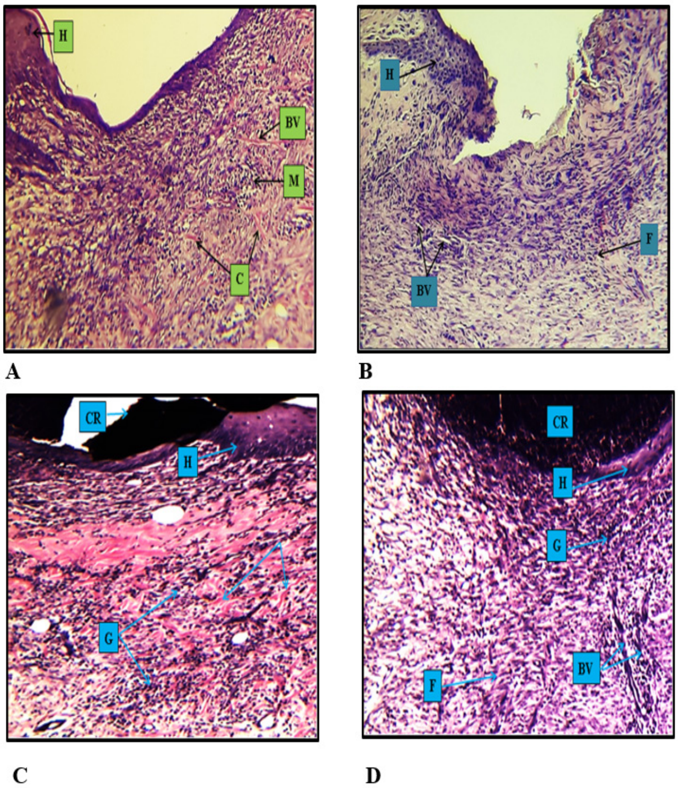


Figure 2: (A) Control group (G1) 14 days post wound shows few hyperplasia (H) on the edge of the wound with profuse granulation tissue which contain high fibrosis (F) and newly formed, small blood vessels (BV). 10X H & E. **(B)** Positive control (G2) 14 days post wound shows thin and few hyperplasia (H) of stratum basale above the edges of wound. Infiltration of inflammatory cells mainly macrophages (M), new formation of small blood vessels (BV) with fibrosis and thin network of collagen fibers (C) in the dermis. 10X H & E. **(C)** Treatment group (G3) 14 days post wound shows presence of thick crust (CR) attached closely with the epidermal layers. Hyperplasia (H) and proliferation of basal cells in the epidermis. In the dermis, there is high granulation tissue (G) with fine irregular network of collagen fibers (C). 10X H & E. **(D)** Treatment group (G3) 14 days post wound shows thick crust (CR) above the epidermis and hyperplasia (H) of stratum basale. Profuse granulation tissue (G) with small new blood vessels formation (BV) above the site of wound and profuse fibrosis (F). 10X H & E.

G2: Histopathological changes following wound injury after the second week showed wide scar tissue with complete sloughing of crust and a few purulent exudates above the epidermis. In the dermis, there is profuse fibrosis and few, newly-formed blood vessels with a small amount of collagen fibers. Thin and little hyperplasia of the stratum basal above the edges of the wound. They are Infiltration of inflammatory cells, mainly macrophages, new formation of small blood vessels with fibrosis, and a thin network of collagen fibers in the dermis. With higher magnification, there is hyperplasia of basal cells in the stratum basal, accompanied by infiltration of macrophage and the formation of new small blood vessels (Figure 2B).

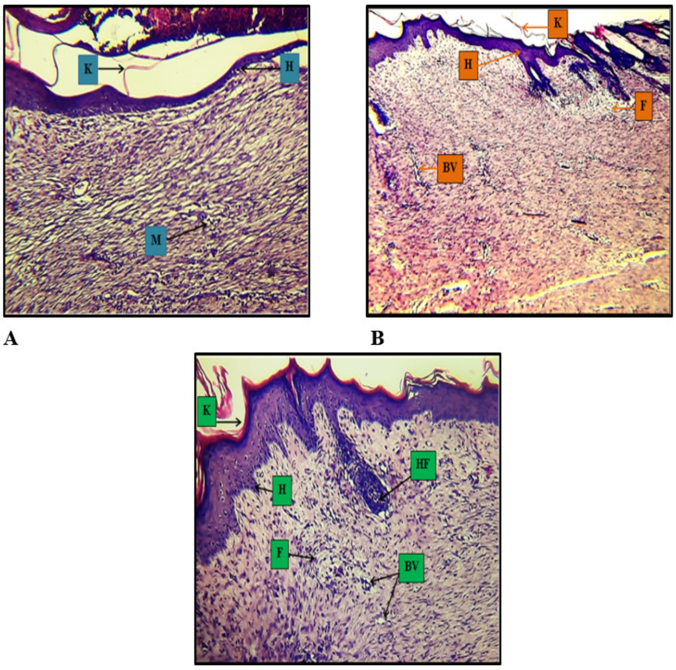
G3: Histopathological changes observed in the second week after wound injury revealed the presence of a thick crust closely adhered to the epidermal layers, as well as hyperplasia and proliferation of basal cells in the epidermis. In the dermis, there is high granulation tissue with a fine, irregular network of collagen fibers (Figure 2C). A thick crust is present above the epidermis, and there is hyperplasia of the stratum basale. They are profound granulation tissue with small new blood vessel formation above the site of the wound and profuse fibrosis (Figure 2D).

On the 21st day post-wound injury

G1: Histopathological changes that follow wound injury after the 3rd week showed thin and proliferating epidermal layers above the wound edges. Wide scar tissue in the dermis with profuse fibrosis horizontally on the wound edges, and small, newly formed blood vessels were found vertically on it. New and few hyperplasia of the stratum basal with a thin keratinized layer were observed. In the dermis, there is scattered infiltration of macrophages, accompanied by profuse fibrosis and a limited number of blood vessel formations (Figure 3A).

G2: Histopathological changes observed after the third week following wound injury revealed a thin keratinized layer with thick epidermal layers, wide scar tissue with severe and profuse fibrosis, and newly formed small blood vessels. Slightly downward hyperplasia of epidermal layers with a thin keratinized layer above it was observed. In the dermis, there is profuse fibrosis and newly formed blood vessels that are vertically oriented on the edges of the wound (Figure 3B).

G3: Histopathological changes following wound injury, observed after the third week revealed normal epidermal layers with a thin, keratinized layer above the epidermis, narrow scar tissue with profuse fibrosis, and newly formed hair follicles in the dermis. Normal epidermal layers with slight hyperplasia of the stratum basal were seen. A thin keratinized layer is closely attached to the epidermis, with newly formed blood vessels and profuse fibrosis, along with small hair follicles in the dermis (Figure 3C).



A B

C

Figure 3: (A) Control group (G1) 21 days post wound shows new and few hyperplasia of stratum basale (H) with thin keratinized layer above it (K). In the dermis, there is scattered infiltration of macrophages (M) with profuse fibrosis and few blood vessels formation. 10X H & E. **(B)** Positive control (G2) 21 days post wound shows thin keratinized layer (K) with thick epidermal layers (H). Wide scar tissue with severe and profuse fibrosis (F) and newly-formed small blood vessels (BV). 4X H & E. **(C)** Treatment group (G3) 21 days post wound shows normal epidermal layers with slightly hyperplasia (H) of stratum basale. Thin keratinized layer (K) closely attached with the epidermis. Newly-formed blood vessels (BV) and profuse fibrosis (F) with small hair follicles (HF) in the dermis. 10X H & E.

DISCUSSION

In this study, medicinal plants were used to enhance wound healing processes and reduce the bacterial burden (Edraki *et al*., 2014). After preparation of Nano saffron extract, the samples were analyzed for Nano tests to improve and analyze Nano saffron. UV-vis. The spectrum shows a maximum absorption 245 nm. As shown. This is accompanied by (Parida and Dash, 2015). All molecules give similar UV absorption spectra.

FT-IR analyze of Nano saffron sample show O=C=O stretching bond, strong stretch bond of C=O of carboxylic acid, C-N bond of imine/oxime, C-H peak of alkane, the largest vibration peak of C-O starching of alcohol, these features of bonds improve successful preparation of Nano saffron is agreed with (Tawanda *et al*., 2018). SEM analysis showed the surface morphology and particles size, saffron Nano particles were uniform in shape with smooth surface morphology, the diameter of 100 particles were 49.18±3.24nm indicating Nano size particles, Nano size increase the potency of these particles for penetration of body cells and enhanced the pharmaceutical efficacy, these result agreed with (Altammar, 2023).

During the treatment period, the animals exhibited energetic, healthy, and active behavior, with a good healing process. No infections developed in any wounds, and no deaths were recorded. After 4 hours of wound creation, the wounds increase in size and swelling within 24 hours post wound, which is a sign of inflammation as reported by (Montserrat *et al*., 2023).

After 7 days post wounding, the wound size decreased gradually till the 21th day becoming a small scar tissue as circular or liner in shape, the superiority of healing was obvious in G3 than other groups, indicate powerful healing characters of Nano saffron this is agreed with (Ghasemali *et al*., 2008). This result may be due to smooth transition from the exudative inflammation phase to the proliferative phase, characterized by reduced leukocyte infiltration, especially at the wound edges.

The finding of this study supports the efficacy of Nano saffron extract in promoting wound healing compared with the control and vaseline-treated group, the Vaseline treated group exhibit slightly improve wound healing compared to control group with thick epidermis and a thin keratinized layer. However, sever and extensive fibrosis in the dermis with only a few newly formed small blood vessels, suggest that Vaseline alone doesn't significantly promote angiogenesis or collagen deposition as confirmed by (Lee *et al*., 2015), their study highlighted the limitation of conventional topical treatments, which lack bioactive compounds that actively stimulate the wound healing process.

The histopathologic evaluation shows that wound healing in G3 is superior to that in other treated groups at the end of the study. G3 showed normal epidermal layers with slightly hyperplastic stratum basal, a thin keratinized layer closely attached to the epidermis, newly. They were Formed blood vessels profuse fibrosis with small hair follicles in the dermis. This result is satisfactory (Marc *et al*., 2024). Our results show that the Nano saffron was better at wound healing compared to the other groups. The enhance wound healing effects of Nano saffron can be attributed to multiple biological mechanisms, first saffron contain powerful antioxidants which reduce oxidative stress and promote fibroblast proliferation (Soheilifar *et al*., 2024), secondly, anti-inflammatory properties help regulate the secretion of inflammatory mediators such as interleukins and tumor necrosis factors, thereby minimizing prolong inflammation and facilitating tissue repair, thirdly, saffron stimulate collagen synthesis which play a crucial role in skin remodeling and scar tissue formation (Comino-Sanz *et al*., 2021).

CONCLUSIONS AND RECOMMENDATIONS

The 5% saffron extract nano particles ointment for treating skin wounds in rats significantly accelerate the healing process by enhancing cellular proliferation, wound contraction, maturation, and remodeling phase. Further experiments are suggested to assess the effect of Nano saffron on the healing process of chronic open wounds.

ACKNOWLEDGMENTS

The author is grateful to the University of Al-Qadisiyha, College of Veterinary Medicine for all the facilities to achieve this study.

NOVELTY STATEMENT

This study introduces a novel approach by using the Nano saffron extract in treating wounds in rat. The findings highlight the positive effect of the Nano saffron extract on wound healing process, suggest their potential utility in wound healing.

AUTHOR'S CONTRIBUTION

Muslim F. Diwan conceptualized and design the experiment. Mohammed A. Mohammed carried out the experimental work. All authors analyzed the data and drafted the manuscript. All authors reviewed and approved the final version of the manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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