doi.org/10.36721/PJPS.2021.34.4.REG.1385-1392.1

Evaluation of wound healing potential and biochemical estimation of sage oil nanoemulsion on animal model

Muhammad Sarfaraz Alam[1]*, Mohammad Daud Ali[2], Sarfaraz Ahmad[3], Nuzhat Banu[2], Muhammad Sajid Ali[1], Nawazish Alam[3], Muhammad Ali[4], Muhammad Salahuddin Ansari[5], Muhammad Shamim[6] and Muhammad Ali Mujtaba[7]

[1]Department of Pharmaceutics, College of Pharmacy, Jazan University, Jazan, Saudi Arabia
[2]Department of Pharmacy, Mohammed Al-Mana College for Medical Sciences, Dammam, Saudi Arabia
[3]Department of Clinical Pharmacy, College of Pharmacy, Jazan University, Jazan, Saudi Arabia
[4]Department of Pharmacognosy, College of Pharmacy, Jazan University, Jazan, Saudi Arabia
[5]Department of Pharmacy Practice, College of Pharmacy, Shaqra, Saudi Arabia
[6]Department of Pharmacy, Teerthanker Mahaveer University, Uttar Pradesh, India
[7]Department of Pharmaceutics, Faculty of Pharmacy, Northern Border University Rafha, Saudi Arabia

Abstract: The study was aimed to design a nano emulsion formulations of Sage oil and to determine its effectiveness in healing the wound using rats as a model. Sage oil nanoemulsion (o/w) was formulated by a spontaneous emulsification method and tested for physicochemical parameters. The wound creation methods namely; circular excision and linear incision were utilized in the present study. Many specifications like tensile strength, DNA, total protein, Hexosamine and Uronic acid, were estimated from the tissues collected from incised wounds. The antioxidant and antimicrobial activity of the oil was estimated from the wound tissue homogenate. Finally epithelialization period and concentration of TNF-α were also measured. A Significant rise in collagen content by 77.52% and tensile strength by 56.20% were noticed in comparison to control. Reduction in period of epithelialization was noticed by 42.85% in comparison to control. The treatment groups confirmed significant antimicrobial activity in comparison to control. It was evident from the results that Sage oil nano emulsion could be the accelerator in wound healing process and it may be devoid of other drawbacks which would be possible with synthetic drug.

Keywords: Sage oil nano emulsion, tensile strength, epithelialization period, TNF-α, wound healing.

INTRODUCTION

Wounds are severe injuries on the skin that may affect the people's quality of life over the globe. Healing of any wound is a string of linked events during which there occurs the replacement of destructed tissue by newly formed tissue. The wound healing process happens to flow in a series of five-step that starts with inflammatory phase in which blood clotting occurs, fibroplasia and formation of new blood vessels in neovascularization, formation of granulation tissue during proliferative phase, re-epithelialization and concludes with the generation of new extracellular matrix and remodeling of granular tissue (Mori *et al*., 2016; Martinotti and Ranzato 2015). A lot of plants are having medicinal value and many constituents are investigated for the process of wound healing (Paul Victor *et al.,* 2017; Pereira and Bartolo 2016), this is attributed to the presence of several biomolecules and other active ingredients such as triterpenes, tannins, flavonoids and alkaloids (Noundou *et al.,* 2016; Francis *et al.,* 2020). These active phyto-constituents are reported to up-regulate the wound healing by accelerating the steps of wound healing process.

The wound healing activity is accelerated in different stages by the traditional use of plant materials (Qiu *et al.,* 2020). *Salvia officinalis* or S. *officinalis* (sage) has application in medical treatments since ancient times. Sage volatile oil has proven its therapeutic effects in digestive, respiratory, cardiac, blood circulation, endocrine diseases and metabolic conditions (Ghorbani and Esmaeilizadeh 2017). Treatment with Sage had shown a potential therapeutic benefit in humans by showing a beneficial and favorable effect in declining the bone loss (Szpyrka and Słowik-Borowiec 2019). Additionally, reports suggested that sage oil shrinks the injured skin area and reduce the duration of time required for the injury to recover e.g. broken skin, muscle tears, or fractured bone (Karimzadeh and Farahpour 2017). It is also used in wound healing, inflammatory condition and other skin disorder (Scrima *et al.,* 2020; Xu *et al.,* 2016). It also has a strong effect against infections of bacteria and fungi (Xu *et al.,* 2018).

Presence of numerous bioactive constituents of the essential oil from this plant has gained a considerable attention (Winska *et al.,* 2019). The efficacy of many pharmaceuticals and herbals can be improved by the utilization of nanotechnological approaches. Moreover the benefits of the herbal formulation in accelerating a wound healing process can be increased by nanosizing of these

*Corresponding author: e-mail: sarfarazpharma1981@gmail.com

pharmaceuticals that shall improve its chemical action by controlling the delivery of active constituents to the site of injury (Arana *et al.,* 2015). Nano emulsions are the structures that make it possible to reduce the size of volatile oil to nanometer size range. After applying nanoemulsions on the wounded sites, a film is formed as droplets on the wounded site after evaporation of their water content. Nanoemulsions are one of the advanced delivery systems for the drug, it is made up of lipids and is considered stable. The components of these nanoemulsions are oil, surfactant, co-surfactant, and water. The droplet size of these components is in the scale of nanometers (Boche and Pokharkar 2017). This kind of nanoemulsion system has large interfacial areas, protect the compounds from adverse environmental conditions, and improve their stability (Perinelli *et al.,* 2020). The colloidal system, Oil-in-water (o/w) Nanoemulsions is among the top efficient systems available for encapsulation and delivery of hydrophobic compounds, like essential oils (Cheong *et al.,* 2018).

Many studies conducted earlier on this plant had reported various beneficial effects of this plant but none had studied the wound healing potential of sage oil nanoemulsion. Nanoemulsion became very popular in last three decade for the topical application due its high penetration capacity. The objective of the present study is to evaluate the capability of sage oil nanoemulsion in healing the wound in a scientific approach by using advanced techniques using a standard animal model. Further, we aim to measure the impact of TNF-α in healing the wound at molecular level. In view of these facts regarding medicinal values of the plant, we chose to examine the wound healing efficiency of Sage oil nanoemulsion on rats.

MATERIALS AND METHODS

Materials

Pepsin, calf thymus DNA, glucuronic acid, chloramine-T, L-hydroxyproline and bovine serum albumin were purchased from Merck Lab. Chemical India. Folin's Phenol reagent and *p*-dimethylaminobenzaldehyde were purchased from Loba Chemie, Mumbai, India. Tween 40, Methyl cellulose and IPA were also purchased from Merck Lab. Chemical India. A kit of protein G column was employed for purification of Anti-TNF- α mAb (Kirkegaard & Perry Lab., Gaithersburg, MD) from the culture supernatants of hybridoma cells (clone MP6-XT2.2-11). Sage oil was procured from Kanta Enterprises Private limited, India.

Preparation of optimized nanoemulsion

Selection of Tween 40 as surfactant and Isopropyl alcohol as co surfactant was based on their solubility in Sage oil. Surfactant combined with co-surfactant was prepared in different ratio and is known as Smix. Pseudo ternary phase diagrams were created for each Smix for finding suitable Smix ratio. For the preparation of nanoemulsion Smix (1:1) was selected. Smix ratio containing minimum percentage of surfactant and maximum oil percentage was selected for the preparation of nano-emulsion. Optimized nanoemulsion was formulated by spontaneous emulsification method. In brief, 45% (v/v) mixture of Tween-40 and Isopropyl alcohol (1:1 v/v) were added slowly in 20% (v/v) Sage oil (oil phase). In the next step the measured quantity of distilled water (35% v/v) was then added slowly drop by drop to obtain transparent and clear nanoemulsion of Sage oil.

Characterization of nanoemulsion

Optimized nanoemulsion of Sage oil was characterized for Droplet size, thermodynamic stability, Viscocity, polydispersity index (PDI), pH and refractive index (RI). The size of droplet, PDI and Zeta potential (ZP) were measured by photon correlation spectroscopy (PCS), using a Zetasizer 1000 HS (Malvern Instruments, UK). Light scattering was observed at 25°C at an angle of 90°. Brookfield DV III ultra V6.0 RV, cone and plate rheometer at 25±0.3°C was used to determine the viscosity, Abbes refractometer was used for measuring RI of nanoemulsion. pH of the prepared nanoemulsion was observed by using the Martini pH-meter. The conductivity was measured using a conductivity meter. Our previously published article of nanoemulsion explains the method in detail (Sarfaraz *et al.,* 2016).

Experimental animals

Wistar rats male (3 months) weighing from 180-210g was selected for wound healing study. Animals were kept in standard laboratory conditions in cross-ventilated animal house at relative humidity 44–56%, and light and dark cycle of 12:12 h for acclimatization. The animals were fed with standard diet and water during the experiment. This research was done in line with the ethical guidelines for conducting any investigations in laboratory animals and approved by the Institutional Animal Ethics Committee (IAEC), Teerthanker Mahaveer University (TMU), Moradabad, U.P, India. Institutional guidelines for animal research were followed in all experimental procedures and treatment of the rats.

Wound healing activity

In this study wound excision was done on the rat model. 18 rats were split into 3 groups with 6 in each. The Diethyl ether was used to anaesthetize rats and before wound in the hairs present at the back side of the rats were removed. By using anesthesia 4 cm^2 full thickness open excision wound was created by removing a portion of skin (Dwivedi *et al.,* 2016).

Control (C) (group 1) received topically 50 µL of mixture of 45% (v/v) Smix (1:1) and 55% (v/v) pure water once a day, for duration of 12 days. The group 2 received 50 µL

of the Sage oil (SO) applied topically in a day for duration of 12 days. The group 3 received 50 µL of the Sage oil nanoemulsion (SONE) applied topically in a day for duration of 12 days. The developed granulated tissues were extracted on 3, 6, 9 and 12 days after creating the wound and analysed. Sutures were removed on 7th day after wound creation. Tissue pieces in dumbbell shape were detached from the wound on 10th day post wounding and used for measurements of tensile strength.

Biophysical parameters

Wound healing potential was determined by measuring the portion of wound contracted in terms of percentage at interval of 3, 6, 9 and 12 days. % wound contraction = [(Wound area of control wound area of treated group) /Wound area of control] x 100. To determine this transparent graph paper was used to draw the portion of wound and its area was measured by planimetry the interval of 3, 6, 9 and 12 days. The total number of days required to close the wound completely without any remaining of raw wound is called epithelization period. The method similar to that followed by Schönborn *et al.*, was utilized for measuring the tensile strength of wound tissue (Schönborn *et al.,* 2020).

Biochemical estimations

An adopted method was used in separating nucleic acids (Ali *et al*., 2017). The pellets from the wound were firstly treated with 10% trichloroacetic acid (TCA), and then the contents were centrifuged. Finally it was treated with 3 ml of absolute alcohol to separate the lipid content. To separate the nucleic acids, the above lipid free contents was re suspended in 5 mL of 5% TCA and heated at 90°C for 15 min. DNA was estimated using earlier method by collecting the supernatant after centrifugation (Farkas *et al.,* 2017). Attwood *et al* described the method for estimation of total protein contents (Attwood *et al.,* 2020). Pellets were suspended in 0.1M Tris-HCl (pH 7.4) again and by hydroxyproline index the total collagen in granulation tissue was estimated (MacAlister *et al.,* 2016; Lin *et al.,* 2019). In another similar study Uronic acid and Hexosamine were estimated (Kumar and Kumar 2017).

Estimation of antioxidant activity

Antioxidant potential of the Sage oil was tested and ESR measurements were done at in X-band at room temperature for free radical analysis using a Varian type ESR spectrophotometer Model E112 with 100 KHz field modulation. To determine the capacity of sage oil to donate hydrogen or free radical scavenging, 2, 2-diphenyl-1 picrylhydrazyl (DPPH) free radical scavenging assay has been widely used. The estimation of the DPPH free radical scavenging capability of nanoemulsion as per the reported method by (Ali *et al.,* 2019; Luo *et al.,* 2019). 10µL of methanol is kept as a blank. A test sample of 10µL of sage oil Nano emulsion was added to 500µL of 50 µM DPPH in methanol, homogenously mixed and quickly transferred to the ESR spectrophotometer cell and analyzed after 60 sec.

Measurement of cytokine concentrations

Pro-inflammatory cytokines (TNF-α) concentration was measured by enzyme linked immunosorbent assay (ELISA) kits. To counter balance TNF-α biological activity, mAb was injected against cytokine in an amount of 400µg once a day earlier to and 3 days later the wound formation into peritoneal cavity of rat. IgG of rat was considered as the control antibody (Ab). TNF-α (25µg/kg) was injected intraperitoneally once a day before on the day of wound formation. It was obtained from a B-cell lymphoblastoid cell line (BALL-1) in human (Yu *et al*., 2019). PBS was used in homogenization of wound tissue and as well a control vehicle. ELISA kit was employed to measure the concentration of TNF-α in the supernatant liquid. The result displayed the values for each wound with a noticing limit 5.1pg/mL.

Antibacterial activity

If this inflammatory phase remains extended for a longer time and there in no clearance of bacteria, it may make the wound severe that may take a long time to heal. Therefore, for appropriate wound healing to occur it mandates that the wound is completely free of bacterial (Karahan *et al.,* 2018; Ustuner *et al.,* 2019). A reported Eldesouky *et al.* method was used for the estimation of antibacterial activity (Almarbi *et al.,* 2016). Nutrient agar plates were used that contained 1 mm^2 wells, these plates were instilled with the 10 µL of sage oil nanoemulsion. At the same time test microorganisms were streaked on the agar plates and incubated for the whole night at a temperature of 37°C. The development of zones of inhibition indicates a significant antibacterial activity. In another set of plates same amount of control vehicle was inoculated to set as controls.

STATISTICAL ANALYSIS

The data was entered, coded, and analyzed using Graph Pad Instat. Exe software. The experimental data was expressed as mean ± Standard Error of the Mean (SEM). Finally, one-way analysis of variance (ANOVA) followed by Tukey's Kramer multiple comparison test was employed and P value < 0.05 was considered statistically significant.

RESULTS

Characterization of nanoemulsion

Optimized nanoemulsion of Sage oil was evaluated for their particle size and found to be 235 nm with polydispersity index 0.343. The viscosity and pH were found to be 32.92±1.13 mP and 5.89±0.024 respectively. The Conductivity and refractive index were measured as 329±1.35 µs and 1.373±0.041 respectively.

Table 1: Effect of Sage oil and Sage oil nanoemulsion on circular excision wound model in rats at different days of treatment.

Formulation	Wound area of contraction ± SEM (%)				
	0 day	3rd day	6th day	9th day	12th day
Control	0.0± 0.00	28.91±1.49	52.98±2.35	69.91±4.13	76.01±6.09
Sage oil	0.0 ± 0.00	30.595±2.917	60.13±1.289	75.275±3.288	80.023±2.32*
Sage oil nanoemulsion	0.0± 0.00	36.55±2.17	79.13±1.29	98.25±4.28	100.9±5.03**

Table 2: Effect of Sage oil and Sage oil nanoemulsion on wound epithelization period in excision wound model in rats.

Formulation	Period of epithelization ± SEM (Days)
Control	21.40 ±0.86
Sage oil	16.01 ±1.01
Sage oil nanoemulsion	12.60 ±0.67 ***

Table 3: TNF-α concentration in the wound tissue homogenate was measured at 0 and 6 h and on days 1, 3, 6, 9 and 12 after wound creation. Each column represents the mean ± SEM of 3 homogenates, each of which was prepared from 6 wounds.

Cytokines	Time						
	0 h	6 h	Day 1	day 3	day 6	Day 9	Day 12
TNF-α (pg/ml)	61.93±1.92	83.19±2.93	89.93±4.11	72.91±4.11	62.01±1.97	62.80±2.01	62.82±1.91

Table 4: Statistical results

S. No.	Name of the activity	Comparison	p - value
1.	Tensile strength	C vs SO	ns $p > 0.05$
		C vs SONE	*** $p < 0.001$
		SO vs SONE	*** $p < 0.001$
2.	Wound contraction	C vs SO	ns $p > 0.05$
		C vs SONE	** $p < 0.01$
		SO vs SONE	* $p < 0.05$
3.	Period of epithelization	C vs SO	ns $p > 0.05$
		C vs SONE	*** $p < 0.001$
		SO vs SONE	* $p < 0.05$
4.	DNA content	C vs SO	ns $p > 0.05$
		C vs SONE	*** $p < 0.001$
		SO vs SONE	*** $p < 0.001$
5.	Hexosamine	C vs SO	ns $p > 0.05$
		C vs SONE	** $p < 0.001$
		SO vs SONE	* $p < 0.01$
6.	Uronic acid	C vs SO	ns $p > 0.05$
		C vs SONE	** $p < 0.01$
		SO vs SONE	* $p < 0.05$
6.	Antioxidant	C vs SO	ns $p > 0.05$
		C vs SONE	*** $p < 0.001$
		SO vs SONE	** $p < 0.01$
7.	Antimicrobial (*S.aureus)*	C vs SO	ns $p > 0.05$
		C vs SONE	*** $p < 0.001$
		SO vs SONE	*** $p < 0.001$
	(P. aeruginosa)	C vs SO	ns $p > 0.05$
		C vs SONE	*** $p < 0.001$
		SO vs SONE	*** $p < 0.001$

Wound healing activity

In this study control tensile strengths, Sage oil and Sage oil nanoemulsion were observed to be 8.95 ± 0.36 g/cm^2, 9.76±0.6743 and 13.98 ± 0.76 g/cm^2 respectively with $p < 0.05$. The results of wound contraction are shown in table 1. It was found that the wound contraction in the treated group rats was much faster.

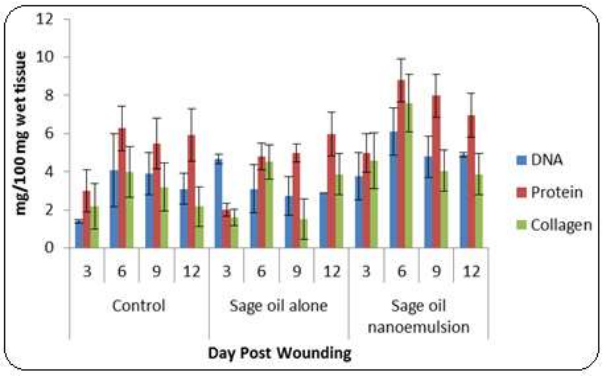

Fig. 1: DNA, protein and collagen contents in the granulation from control and experimental rats (all values are mg/ 100mg wet tissue) mean ± SEM, n = 6

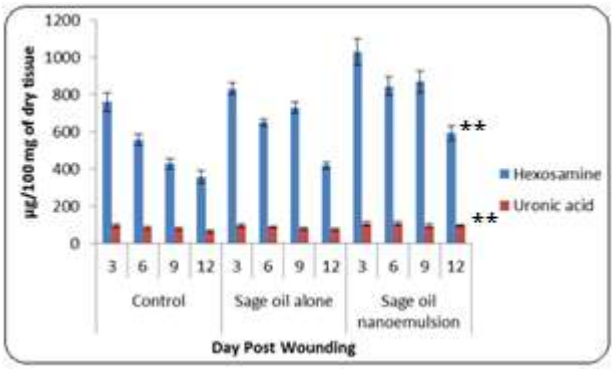

Fig. 2: Hexosamine and uronic acid content in dry granulation tissue (µg/100mg of dry tissue) mean ± SEM, n = 6

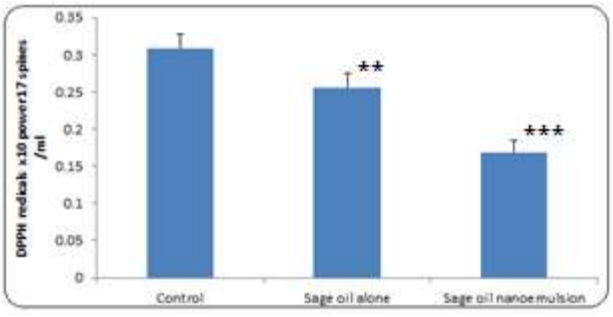

Fig. 3: Antioxidant activity of Sage oil nanoemulsion quantified through ESR measurements of DPPH radical spin reduction (× 10^{17} spins/mL) mean ± SEM, n = 6

Biophysical parameters

The results of effect of Control, Sage oil and Sage oil nanoemulsion on epithelization period are displayed in table 2. The time taken for the epithelization to complete was observed to be 21.40 ±0.86, 16.01 ±1.01 and 12.60 ±0.67 days for control Sage oil and Sage oil nano emulsion group, respectively.

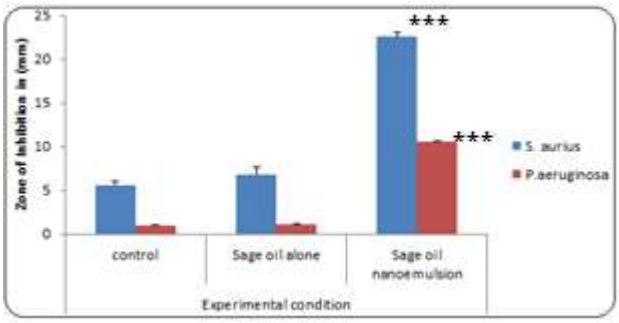

Fig. 4: Antimicrobial activity of control and treated groups against *S. aureus* and *P. aeruginosa* mean ± SEM, n = 6

Biochemical Estimations

Fig. 1 displays the results of total protein, collagen and DNA content in the granulation tissues of control, Sage oil and Sage oil nanoemulsion treated wounds fig. 2 shows the levels of Uronic acid and Hexosamine in the granulation tissues of control, Sage oil and Sage oil nanoemulsion group. They are found to be higher in Sage oil nanoemulsion group in contrast to control group and Sage oil treated group during wound healing process.

Estimation of antioxidant activity

Fig. 3 reveals the antioxidant effect of Sage oil nanoemulsion, estimated by erythrocyte sedimentation rate (ESR) measurement technique. Up to 55.45% reduction of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) spins was noted in treated group in comparison to the control.

Measurement of cytokine concentrations

The concentration of TNF-α from tissue homogenate of the wound was calculated at zero and 6th hour on day 1 and then 1st , 3rd , 6th , 9th and 12th days respectively after incision of wound as shown in table 3. Each column reflects the mean ± SEM value of 3 homogenates, that were prepared each from 6 wounds.

Antibacterial activity

A significant antimicrobial activity of Sage oil nanoemulsion has been observed as displayed in fig 4. The Control, Sage oil, Sage oil nanoemulsion showed antimicrobial activity against *S. aureus* and *P. aeruginosa*. The reason behind showing antimicrobial activity of control group may be because of it contain surfactant and alcoholic co-surfactant present in that.

DISCUSSION

Plant metabolic product appears to be auspicious agents for healing the wound and due to their easy availability,

less side effects, efficacy as crude drugs, and no toxicity. Earlier studies proved significant wound healing potential of various plant and their metabolites such as *Centella asiatica* (Oryan *et al*., 2016). Likely, the promising effect of *A. vera* in healing process was also reported (Yao *et al.*, 2017). The outcome of previous studies elated us for further study of other plant, which displayed medicinal values for shortening the time for wound healing. Therefore, studies that were to be conducted *in vivo* on Sage oil nanoemulsion for wound healing were planned systemically. Optimized Sage oil nanoemulsion pH was suitable for topical use and conductivity parameters confirmed that our nanoemulsion was o/w type. Tensile strength observation confirms that the Sage oil nanoemulsion possesses excellent wound healing property. Wound tissue treated with sage oil nanoemulsion treated displayed higher tensile strength, indicating the presence of larger collagen content at the location of the wound. Sage oil nanoemulsion facilitated the wound contraction symbolically from day 3rd to 9th in comparison to control group ($p<0.05$). Rats treated with optimized Sage oil nanoemulsion showed a significantly lower time for Epithelization as compared with rats in control group ($p<0.05$). These results guaranteed the strong potential of nanoemulsion for wound healing effects. There was a significant increase in collagen content of about 77.52% on day 6 after wound incision in the treated group. A Similar trend was observed with DNA content and total protein content. Cellular hyperplasia reveals that the treated wounds with Sage oil nanoemulsion had an elevated amount of DNA. Concurrently, there was also matrix proteins deposition in the granulation tissues, up regulation in the total protein content and expressive active synthesis. The regulation of healing process is due to the essential collagen component in extracellular matrix to a larger extent. Initially during wound healing process the type III collagen levels are specially increased (Pezzoli *et al*., 2018). Collagen content was quantified in granulation tissues and it has been observed that there is an improved generation of new collagens in treated group wound after treatment with Sage oil nanoemulsion. It has been reported earlier that in a newly developed collagen at a wound site, per unit area collagen content and tissue tensile strength increases (He *et al*., 2018; Seo *et al*., 2017). Matrix molecules like Uronic acid and Hexosamine, are engaged in new extracellular matrix synthesis. In the initial stages of wound healing, there has been a rise in the levels of these components, followed by normal levels (Naraginti *et al.,* 2016). Similar trend was observed in study of Sage oil nanoemulsion treated wounds, where there was a significant rise in the amount of hexosamine and uronic acid. One of the key aspects that play a significantly important role in wound healing is the control of inflammation and oxidation. The healing of the wound is usually complicated due to free radicals produced in response to damage to cutaneous tissue that may destroy lipids, proteins and extracellular matrix (ECM) elements (Lim *et al.,* 2018). Our investigation used ESR method for DPPH free radical reducing properties and the lipid peroxide. It was found that Sage oil nanoemulsion has promising antioxidant activity, which may help to prevent oxidative damage and up regulate the process of healing in wound. In the first few hours there was a sharp rise in TNF-α concentration. On day 1 it reached the peak concentration level and then the level dip slowly further for few more days until it reaches almost basal value on day 12. The results of the study shows that TNF-α is involved closely in the very early process in healing of wound. In wounded tissue TNF-α was released and reached to its highest value on first day. This elevation in TNF-α level was not significant by statistics even at the point of time where it reached at its highest level. The elevated TNF-α level identified just after creating wound (0 h), is believed to be the one responsible to an extent in regulating homeostasis in skin tissue (Xue and Jackson 2015). Thus, the elevated values of TNF-α initially on day 1 in wound tissue and later going back to basal value from day 3 to 9 leads to the inference that these were likely to be one of the factor responsible for wound healing process. They also give strong evidence that the sage oil nanoemulsion accelerated the process of healing the wound. Our research also pointed that Sage oil nanoemulsion has antimicrobial activity against *S.aureus* and *P. aeruginosa*. Infections after surgeries are mainly due to exposure of surgical wound to microorganisms. Many of the herbal products are well known to have inherent activity against bacteria. All these results further more reinforced the effectiveness of Sage oil nanoemulsion in wound healing process.

CONCLUSION

The present study confirms the potential of sage oil nanoemulsion in wound healing in rats. Sage oil nanoemulsion was formulated using aqueous phase titration method and studied for various physicochemical parameters. Sage oil nanoemulsion showed promising effect on various stages of the process of wound healing and the result was found statistically significant as compared to the control group. It is concluded that Sage oil nanoemulsion have a potential for wound healing and it would be free from other drawbacks that may be seen in synthetic drugs.

REFERENCES

Ali N, Rampazzo RCP, Costa ADT and Krieger MA (2017). Current nucleic acid extraction methods and their implications to point-of-care diagnostics. *Biomed. Res. Int.*, 2017: 9306564.

Ali S, Alam S, Ahmad S, Ali M, Ahsan W and Siddiqui MR (2019). Wound healing activity of alcoholic extract of *Tamarix aphylla* L. on animal models. *Biomed. Pharmacol. J.,* **12**(1): 41-48.

Al-Marby A, Ejike CE, Nasim MJ, Awadh-Ali NA, Al-Badani RA, Alghamdi GM and Jacob C (2016). Nematicidal and antimicrobial activities of methanol extracts of 17 plants, of importance in ethnopharmacology, obtained from the Arabian Peninsula. *J. Intercult. Ethnopharmacol.,* **5**(2): 114-21.

Arana L, Salado C, Vega S, Aizpurua-Olaizola O, Arada I, Suarez T, Usobiaga A, Arrondo JLR, Alonso A, Goñi FM and Alkorta I (2015). Solid lipid nanoparticles for delivery of *Calendula officinalis* extract. Colloids. *Surf. B. Biointerfaces.*, **135**(11): 18-26.

Attwood PV (2020). A quantitative method for the measurement of protein. Histidine phosphorylation. *Methods. Mol. Biol*, Humana, New York, **2077**: 51-61.

Boche M and Pokharkar V (2017). Quetiapine nanoemulsion for intranasal drug delivery: evaluation of brain-targeting efficiency. *AAPS. Pharm. Sci. Tech.*, **3**(18): 686-696.

Cheong AM, Tan CP and Nyam KL (2018). Effect of emulsification method and particle size on the rate of *in vivo* oral bioavailability of Kenaf (*Hibiscus cannabinus* L.) Seed oil. *J. Food. Sci.,* **83**(7): 1964-1969.

Dwivedi D, Dwivedi M, Malviya S and Singh V (2016). Evaluation of wound healing, anti-microbial and antioxidant potential of *Pongamia pinnata* in wistar rats. *J. Tradit. Complement. Med.,* **7**(1): 79-85.

Farkas K, Hassard F, McDonald JE, Malham SK and Jones DL (2017). Evaluation of molecular methods for the detection and quantification of pathogen-derived nucleic acids in sediment. *Front. Microbiol*., **8**(53): 1-12.

Francis NA, Gillespie D, White P, Bates J, Lowe R, Sewell B, Phillips R, Stanton H, Kirby N, Wootton M, Thomas-Jones E, Hood K, Llor C, Cals J, Melbye H, Naik G, Gal M, Fitzsimmons D, Alam MF, Riga E, Cochrane A and Butler CC (2020). C-reactive protein point-of-care testing for safely reducing antibiotics for acute exacerbations of chronic obstructive pulmonary disease: The PACE RCT. *Health. Technol. Assess*., **24**(15): 1-108.

Ghorbani A and Esmaeilizadeh M (2017). Pharmacological properties of *Salvia officinalis* and its components. *J. Tradit. Complement. Med.,* **7**(4): 433-440.

He J, Yang B, Dong M and Wang Y (2018). Crossing the roof of the world: Trade in medicinal plants from Nepal to China. *J. Ethnopharmacol.,* **224**(10): 100-110.

Karahan A, AAbbasoglu A, Işık SA, Çevik B, Saltan Ç, Elbaş No and Yalılı A (2018). Factors affecting wound healing in individuals with pressure ulcers: A retrospective study. *Ostomy. Wound. Manage,* **64**(2): 32-39.

Karimzadeh S and Farahpour MR (2017). Topical application of *Salvia officinalis* hydroethanolic leaf extract improves wound healing process. *Indian. J. Exp. Biol.*, **55**(2): 98-106.

Kumar P and Kumar V (2017). Estimation of uronic acids using diverse approaches and monosaccharide composition of alkali soluble polysaccharide from Vitex negundo Linn. *Carbohydr. Polym.,* **165**(6): 205-212.

Lim JH, Lee BY, Kim JW, Han YJ, Chung JH, Kim MH, Kwak DW, Park SY, Choi HB and Ryu HM (2018). Evaluation of extraction methods for methylated cell-free fetal DNA from maternal plasma. *J. Assist. Reprod. Genet.,* **35**(4): 637-641.

Lin AH, Zitnay JL, Li Y, Yu SM and Weiss JA (2019). Microplate assay for denatured collagen using collagen hybridizing peptides. *J. Orthop. Res.,* **37**(2): 431-438.

Luo S, Jiang X, Jia L, Tan C, Li M, Yang Q, Du Y and Ding C (2019). *In vivo* and *in vitro* antioxidant activities of methanol extracts from olive leaves on *Caenorhabditis elegans*. *Molecules.*, **24**(4): 704.

MacAlister CA, Ortiz-Ramírez C, Becker JD, Feijó JA and Lippman ZB (2016). Hydroxyproline O-arabinosyl transferase mutants oppositely alter tip growth in *Arabidopsis thaliana* and *Physcomitrella patens. Plant J*., **85**(2): 193-208.

Martinotti S and Ranzato E. Propolis (2015). Propolis: A new frontier for wound healing? *Burns & Trauma*., **3**(9): 22.

Mori HM, Kawanami H, Kawahata H and Aoki M (2016). Wound healing potential of lavender oil by acceleration of granulation and wound contraction through induction of TGF-β in a rat model. *BMC Complement. Altern. Med.*, **26**(16): 144.

Naraginti S, Kumari PL, Das RK, Sivakumar A, Patil SH and Andhalkar VV (2016). Amelioration of excision wounds by topical application of green synthesized, formulated silver and gold nanoparticles in albino Wistar rats. *Mater. Sci. Eng. C. Mater. Biol. Appl.*, **62**(5): 293-300.

Noundou XS, Krause RW, van Vuuren SF, Ndinteh DT and Olivier DK (2016). Antibacterial effects of *Alchornea cordifolia* (Schumach and Thonn.) Müll. Arg extracts and compounds on gastrointestinal, skin, respiratory and urinary tract pathogens. *J. Ethnopharmacol*., **179**(17): 76-82.

Oryan A, Mohammadalipour A, Moshiri A and Tabandeh MR (2016). Topical application of aloe vera accelerated wound healing, modeling and remodeling: an experimental study. *Ann. Plast. Surg.,* **77**(1): 37-46.

Paul-Victor C, Dalle Vacche S, Sordo F, Fink S, Speck T, Michaud V and Speck O (2017). Effect of mechanical damage and wound healing on the viscoelastic properties of stems of flax cultivars (*Linum usitatissimum* L. cv. Eden and cv. Drakkar). *PLoS One*., **12**(10): e0185958.

Pereira RF and Bartolo PJ (2016). Traditional Therapies for Skin Wound Healing. *Adv. Wound Care (New Rochelle)*., (5) (5): 208-229.

Perinelli DR, Palmieri GF, Cespi M and Bonacucina G (2020). Encapsulation of flavours and fragrances into

polymeric capsules and cyclodextrins inclusion complexes: An update. *Molecules*, **25**(24): 5878.

Pezzoli D, Di Paolo J, Kumra H, Fois G, Candiani G, Reinhardt DP and Mantovani D (2018). Fibronectin promotes elastin deposition, elasticity and mechanical strength in cellularised collagen-based scaffolds. *Biomaterials*, **180**(10): 130-142.

Qiu H, Liu S, Wu K, Zhao R, Cao L and Wang H (2020). Prospective application of exosomes derived from adipose-derived stem cells in skin wound healing: A review. *J. Cosmet. Dermatol.*, **19**(3): 574-581.

Sarfaraz Alam M, Ali MS, Zakir F, Alam N, Intakhab Alam M, Ahmad F, Siddiqui MR, Ali MD, Ansari MS, Ahmad S and Ali M (2016). Enhancement of anti-dermatitis potential of clobetasol propionate by DHA [Docosahexaenoic Acid] rich algal oil nanoemulsion gel. *Iran. J. Pharm. Res.*, **15**(1): 35-52.

Schonborn K, Willenborg S, Schulz JN, Imhof T, Eming SA, Quondamatteo F, Brinckmann J, Niehoff A, Paulsson M, Koch M, Eckes B and Krieg T (2020). Role of collagen XII in skin homeostasis and repair. *Matrix. Biol.*, **94**(12): 57-76.

Scrima M, Melito C, Merola F, Iorio A, Vito N, Giori AM and Ferravante A (2020). Evaluation of wound healing activity of *Salvia haenkei* hydroalcoholic aerial part extract on *in vitro* and *in vivo* experimental models. *Clin. Cosmet. Investig. Dermatol.*, 2020(13): 627-637.

Seo GY, Lim Y, Koh D, Huh JS, Hyun C, Kim YM and Cho M (2017). TMF and glycitin act synergistically on keratinocytes and fibroblasts to promote wound healing and anti-scarring activity. *Exp. Mol. Med.*, **49**(3): e302.

Szpyrka E and Słowik-Borowiec M (2019). Consumer health risk to pesticide residues in *Salvia officinalis* L. and its infusions. *J. Environ. Sci. Health. B.*, **54**(1): 14-19.

Ustuner O, Anlas C, Bakirel T, Ustun-Alkan F, Diren Sigirci B, Ak S, Akpulat HA, Donmez C and Koca-Caliskan U (2019). *In vitro* evaluation of antioxidant, anti-inflammatory, antimicrobial and wound healing potential of *Thymus Sipyleus* Boiss. Subsp. *Rosulans* (Borbas) *Jalas*. *Molecules*, **24**(18): 3353.

Winska K, Mączka W, Lyczko J, Grabarczyk M, Czubaszek A and Szumny A (2019). Essential oils as antimicrobial agents-myth or real alternative? *Molecules*., **24**(11): 2130.

Xu J, Wei K, Zhang G, Lei L, Yang D, Wang W, Han Q, Xia Y, Bi Y, Yang M and Li M (2018). Ethno-pharmacology, phytochemistry and pharmacology of Chinese Salvia species: A review. *J. Ethnopharmacol*., 28(225): 18-30.

Xu YM, Ding GH, Huang J and Xiong Y (2016). Tanshinone IIA pretreatment attenuates ischemia/ reperfusion-induced renal injury. *Exp. Ther. Med*., 12(**4**): 2741-2746.

Xue M and Jackson CJ (2015). Extra cellular matrix reorganization during wound healing and its impact on abnormal scarring. *Adv. Wound. Care* (New Rochelle), **4**(3): 119-136.

Yao CH, Yeh JY, Chen YS, Li MH and Huang CH (2017). Wound-healing effect of electrospun gelatin nanofibres containing *Centella asiatica* extract in a rat model. *J. Tissue. Eng. Regen. Med.*, **11**(3): 905-915.

Yu C, Xu ZX, Hao YH, Gao YB, Yao BW, Zhang J, Wang B, Hu ZQ and Peng RY (2019). A novel micro current dressing for wound healing in a rat skin defect model. *Mil. Med. Res.*, **6**(1): 22.