Wound Healing Potentials of Ocimum Sanctum Leaves Extracts in Wistar Albino Rats

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Abstract-
Objective: *Effects of topical application of aqueous (OSAE), ethanol (OSEE), petroleum ether (OSPE) and benzene extracts (OSBE) of Ocimum sanctum leaves on the rate of haemostatic indices, wound healing and histology of healed wound were assessed.*
Methods: *Among seven groups of adult male Wistar albino rats, one group had no wound on their skins (Group I) and remaining of six groups were experimentally wounded in the nape of the dorsal neck. A thin layer of blank placebo was applied topically to the wounds of control rats (Group II). Wounds of experimental animals [(OSAE, Groups IV), (OSEE, Groups V), (OSPE, Groups VI) and (OSBE, Groups VI)] were treated with placebo containing 10% O.sanctum leaves extracts, respectively. A thin layer of standard Povidone Iodine ointment was applied topically to wounds of Group III animals as reference.*
Results: *The haemostatic analysis showed that the decrease was significant in the different extracts preparations groups but not significant in Groups II and III when compared independently with the control group I. Macroscopically, wounds treated with placebo containing different extracts or Povidone Iodine ointment have been significantly accelerated the rate of wound healing compared to placebo-treated wounds. Histological analysis of healed wounds has confirmed this effect. Wounds treated with placebo containing different extracts or Povidone Iodine ointment showed markedly less scar width at wound enclosure and granulating tissue contained markedly more collagen and proliferating fibroblasts, but with the absence of inflammatory cells compared to wounds treated with blank placebo.*
Conclusions: *the findings of significant effect on haemostatic indices and increased rate of wound healing together with the histological findings suggest that O.sanctum leaves extract is very effective in accelerating the wound healing process.*

Keywords- Ocimum sanctum, Wound healing, Haemostatic indices, Povidone Iodine, Placebo

I. INTRODUCTION

Wound is defined simply as the disruption of the cellular and anatomic continuity of a tissue [1]. Healing is a complex and intricate process initiated in response to an injury that restores the function and integrity of damaged tissues. The process of wound healing consists of integrated cellular and biochemical events leading to reestablishment of structural and functional integrity with regain of strength of injured tissue. There are four distinct stages involved in wound healing namely – inflammatory stage, debridement stage, proliferation stage and maturation/remodelling stage. When an injury occurs, the vascular integrity of the injured area is disrupted leading to extravasations of blood into the surrounding tissue or plasma when the damage is minor. The inflammatory stage is directed at preventing further loss of blood by platelet adhesion/accumulation at the site leading to coagulation which results to the formation of thrombus. The debridement stage occurs from the third to the sixth day after injury and involves the appearance of neutrophils to clear contaminating organisms. The proliferation or repair stage is characterized by endothelial budding in the nearby blood vessels forming new capillaries that penetrate and nourish the injured tissue. The maturation stage commences from the tenth day to several months depending on wound severity during which the number of capillaries decreases and wound changes from pink to white [2].

Plants play an essential role in the health care needs of native populations in India and use of preparations and infusions of plants to treat diseases has been practiced but their effectiveness should be scientifically validated to increase the credibility of their use. Reports about medicinal plants affecting various phases of the wound healing process, such as coagulation, inflammation, fibroplasia, collagenation, epithelisation and wound contraction are abundant in the scientific literature [3]. The genus *Ocimum L.* includes approximately 150 species, possessing a great variation in plant morphology and biology, essential oil content, and chemical composition [4]. The plants of genus *Ocimum* belonging to family Labiatae are very important for their

unique properties and are known to have medicinal properties [5]. Studies reveal that *O.sanctum* has a unique combination of pharmacological actions including anti-stress, immunoregulatory, hypoglycemic, antibacterial, anti-fungal, anti-inflammatory, anti-aging, anti-carcinogenic, antioxidant and cyclooygenase inhibitory reported for this plant [6]. Further, distillations of the leaf different extracts yield are known to be possess antibacterial, antioxidant, antifertility, antigenotoxic, antiinflammatory and hepatoprotective properties [7-11]. In light of its use as wound healing agent in folklore medicine, our present study incorporates the haemostatic indices and evaluation of wound healing effects of aqueous, ethanol, petroleum ether and benzene extracts of *O.sanctum* leaves in Wistar albino rats.

II. MATERIALS AND METHODS

2.1. Plant material

Plant materials *Ocimum sanctum* (Tulsi) was collected from local and was authenticated by department of Botany, Government First Grade College, Hubballi. The voucher specimen no (GFGC/2011/41) was deposited at the Herbarium of the Botanical department. The leaves collected fresh in bulk were washed with running tap water to remove adhering dust and debris followed by rinsing with the distilled water. The collected leaves were spread on paper and dried under shade which took about some days (30days) for complete drying. Then the dried leaves were grinded and then used for different extractions process in the departmental lab.

2.2. Animals

Wistar albino rats weighing 200-220g of either sex were obtained from the rat colony maintained in the department and were acclimatized for 10 days under standard housing conditions (26°±2°C; 45-55% RH with 12:12 h light/dark cycle). During experimental time, they were housed in standard metal cages and were maintained on a standard diet and water was given ad libitum. The animals were maintained under standard conditions in the animal house approved by Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) and all procedures described were reviewed and approved by the Institutional Animal Ethical Committee (IAEC, Ref: CAF/Ethics/558/2017) were obtained before undertaking animal experimentation.

2.3. Preparation of the extract

Powdered leaves were extracted using solvents: petroleum ether (OSPE), ethanol (OSEE) and benzene extract (OSBE) using Soxhlet apparatus, and successive extraction method. Cold maceration technique was used for obtaining aqueous extract (OSAE). Aqueous successive extract of *O.sanctum*, yield: 20–25% for leaves, were prepared. The *O.sanctum* leaf powder (100 g) was refluxed with 750 ml of double distilled water for 1 h at 75–80ºC. It was then cooled and filtered. This was repeated in three trials. The extracts were pooled and evaporated using lyophilizer. Ethanol successive extract of *O.sanctum*, yield: 10–15% for leaves, were prepared. The powders were weighted (75g) and extraction was done in 95% ethanol (700ml) as solvent by soxhlet apparatus. Then the extracts were collected (300ml) and evaporated to dryness in water bath at 60-75°C. The extract was stored at 4°C for further use. Petroleum ether successive extract of *O.sanctum*, yield: 1–5% for leaves, were prepared. *O.sanctum* leaf powder (100 g) was extracted by petroleum ether using soxhlet apparatus at 60-75°C. The petroleum ether extract was filtered and concentrated to dry mass by using vaccum distillation. Benzene successive extract of *O.sanctum*, yield: 1.75 –1.85% for leaves, were prepared. The powders were weighted (100g) and subjected to soxheltation process to get the benzene extract. After soxhlet extraction, the extracts obtained were filtered and then the extract was concentrated using rotary vacuum evaporator. The extracts were taken in round bottom flask which was heated at appropriate temperature on a water bath. The vapors of the solvent rise in the condenser and after condensation the solvent droplets was collected in the collecting flask. The resultant sticky mass was collected and extract thus obtained could dry and stored in a desiccator at 4ºC [12,13].

2.4. Acute Toxicity Studies

Acute oral toxicity study was performed as per OECD-404 guidelines [14]. Fifty animals (25 males and 25 females) were assigned equally into 9 groups labelled as vehicle (distilled water, Group 1); low (2 g/kg; Groups 2, 4, 6 and 8) and high (5 g/kg; Groups 3, 5, 7 and 9) dosages of OSAE, OSEE, OSPE and OSBE preparations, respectively. The animals were fasted overnight prior dosing. Food was withheld for a further 3 to 4 hours after dosing. Observations were done on mortality and behavioral changes of the rats following treatment for 24 hours. The acute toxicity LD_{50} was calculated at the statistical mean of the dose that resulted in 100% lethality and that cause no lethality at all.

2.5. Preparation of the treatment mixture

The semisolid mass of different extracts of *O.sanctum* were homogeneously mixed with placebo in a concentration of 10% (w/w) each as procedure described with little modification [15]. The mixtures were kept at 4°C and brought to a room temperature before application.

2.6. Experimentally induced wounds

Forty- two Wistar albino rats were indiscriminately divided into 7 groups of 6 rats each during the experiment and each rat was housed individually (one rat per cage). Group I: The animals in this group had no wound on their skins. For the rest groups, the animals were anesthetized by using combination of 1 ml of ketamine (50 mg/kg, intraperitoneally) and xylazine (10 mg/kg, intraperitoneally). The animals were treated humanely during the inducement of the experimental wound. Then skin shaved by electrical shaver and disinfected with 70% alcohol. An area of uniform wound 2.00 cm in diameter was excised from the nape of the dorsal neck of all rats with the aid of round seal as described [16].

2.7. Topical application of vehicles

A thin layer of blank placebo was applied topically to the wounds of control rats (Group II twice a day. The semisolid mass of *O.sanctum* different extracts were homogeneously mixed with blank placebo in a concentration of 10% (w/w) and thin layers of the mixtures were applied topically twice a day to the wounds of aqueous extract of *O.sanctum* treated animals (OSAE, Groups IV), ethanol extract of *O.sanctum* treated animals (OSEE, Groups V), petroleum ether of *O.sanctum* treated animals (OSPE, Groups VI) and benzene extract of *O.sanctum* treated animals (OSBE, Groups VII), respectively. Wounds of Group III rats were treated with a thin layer of positive (standard) control treated with Povidone Iodine ointment twice daily. The wound was observed daily until complete wound enclosure occurs.

2.8. Blood Collection

The blood samples were collected by cardiac puncture from the heart of the rats for haematological analysis of the thrombin and prothrombin time after the last day of treatment of the animals with semisolid mass of different extracts of *O.sanctum*. Also, the blood samples for clotting and bleeding time were done using the tail vein of the rats.

2.9. Determination of bleeding time

This was determined using a modified Duke method [17]. A skin puncture was made quickly using disposable lancet and the stopwatch was started as soon as bleeding started. The puncture was dabbed with filter paper every 15 sec until the paper no longer stained red with blood. Bleeding time was then taken as the time when the blood stopped flowing from the puncture.

2.10. Determination of Thrombin time

After separating the plasma from the whole blood by centrifugation, bovine thrombin (Sigma Chemical Co.,) is added to the sample of plasma. Clot formation is detected optically or mechanically by a coagulation instrument. The time between the addition of the thrombin and the clot formation is recorded as the thrombin clotting time.

2.11. Determination of clotting time

Blood was taken directly from the heart to avoid contamination with tissue thromboplastin (1.2 ml from each rat). 0.2 ml of blood was then delivered into six glass test tubes that had formerly been warmed and maintained at 37°C and the tubes without delay positioned in a 37°C water bath to mimic the temperature of the internal environment. The stopwatch was started immediately the blood was delivered into the glass test tubes and the tubes were continually tilted at 40 sec intervals (awaiting blood in them stopped flowing when tilted at an angle of 90°), starting with the first, to see and note the time when the blood clotted. The clotting time was taken as the average of the times blood clotted in the six tubes.

2.12. Determination of prothrombin time

Blood was collected into sample vials containing 3.2% sodium citrate (as specified in the prothrombin time PT, test kit used) in the ratio 1:9 with the blood sample. The blood was then centrifuged at 1000 g for 15 min to obtain platelet poor plasma. Thromboplastin PT-S was placed in a water bath at 37°C; and 0.1 ml of test plasma was also put into a test tube and placed in the water bath to prewarm to 37°C. 0.2 ml of warmed thromboplastin PT-S was then forcibly added to the test plasma and the stopwatch was started. The tube was tilted repeated until a clot was formed, and the time taken for clot to form was noted. This was repeated for all the blood samples (five in each group). Precaution was taken to perform test within 3 h of blood collection since the labile factor deteriorates quickly at room temperature.

2.13. Histological evaluation of healed wounds

The skin specimens from wounds healed areas of control and treated were fixed in 10% buffered formalin and processed by paraffin tissue processing machine. The healed skin was assessed by taking a 5µm section followed by staining with hematoxylin and eosin [18].

2.14. Statistical analysis

All values were expressed as mean ± SEM and the statistical significance of differences among groups in term of rate of haemostatic indices and wound healing were evaluated

using one-way analysis of variance ANOVA using the Graph Pad Prism software method, followed by either Dunnet test by comparing all treated groups against controls. A value of $P \leq 0.05$ is considered to indicate a significant difference between experimental and controls.

III. RESULTS

3.1. Acute toxicity study

No mortality occurred amongst the Wistar Albino rats with dose levels of 2 g/kg and 5 g/kg of dosages of OSAE, OSEE, OSPE and OSBE preparations during the study period. Behavioral observation did not show evidence indicative of significant dosage toxicity. The results suggested that the oral LD50 of dosages of OSAE, OSEE, OSPE and OSBE preparations was greater than 5 g/kg.

3.2. Bleeding Time

The extract decreased bleeding time in rats of the experimental group in comparison with the control group I. The mean bleeding time in control group was 6.34±0.33 min, blank placebo (5.45 ± 0.49 min) and Povidone Iodine (5.29 ± 0.31min) were mean values obtained, respectively. Whereas the *O.sanctum* extracts preparations of OSAE (4.78± 0.23), OSEE (4.35± 0.42), OSPE (4.52± 0.30) and OSBE (4.62± 0.28) min were mean values obtained, respectively. The analysis showed that the decrease was significant in the semisolid mass of *O.sanctum* extract preparations group at $P \leq 0.05$ but not significant in blank placebo and Povidone Iodine groups when compared independently with the control group (Fig.1A).

3.2. Clotting Time

There was a decrease in clotting time of OSAE, OSEE, OSPE and OSBE preparation groups when compared to control group as shown in Fig.1B. The mean value of control was 6.92 ±0.08 min, blank placebo (6.39 ±0.49 min) and Povidone Iodine (6.52 ±0.51 min) were mean values obtained, respectively. Whereas the *O.sanctum* extracts preparations of OSAE (5.75±0.74), OSEE (4.67±0.55), OSPE (4.92±0.62) and OSBE (5.52±0.54) min were mean values obtained, respectively. The analysis showed that this decrease was statistically significant ($P \leq 0.05$).

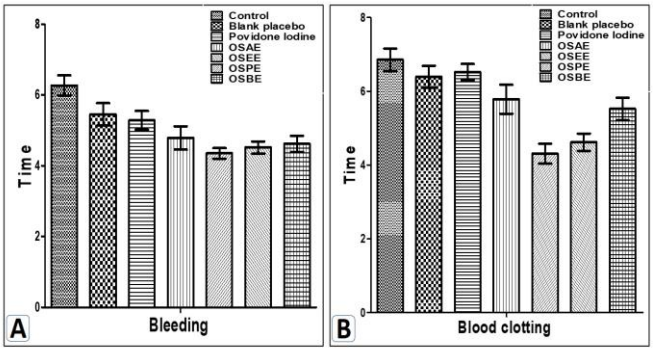

Figure 1: Graphic representation of bleeding time (A) and clotting time (B) in blank placebo group and treated with 10% *O.sanctum* leaf extract preparations or with positive control treated with Povidone Iodine ointment. Values are expressed as mean ± SEM (n=6).

3.3. Prothrombin Time

The mean value of prothrombin time was 13.85 ±0.81 min in control group, 11.92 ±0.12 min (blank placebo group) and 11.21 ±0.89 min (Povidone Iodine group) was obtained, respectively. Whereas the *O.sanctum* extracts preparations of OSAE (16.23 ±0.78), OSEE (15.50 ±0.52), OSPE (15.84 ±0.68) and OSBE (16.46 ±0.70) min were mean values obtained, respectively, as shown in Fig.2A. There were significant decreases ($P \leq 0.05$) in prothrombin time in the blank placebo and Povidone Iodine groups compared with the control group but there was no significant decrease ($P \leq 0.05$) in prothrombin time in all extract preparations groups compared with the control group.

3.4. Thrombin Time

The mean value of thrombin time was 19.21 ±0.06 min in control group, 19.15±0.75 min (blank placebo group) and 17.57±0.57 min (Povidone Iodine group) was obtained, respectively. Whereas the *O.sanctum* extracts preparations groups of OSAE (17.34±0.57), OSEE (16.74±0.39), OSPE (16.81±0.30) and OSBE (17.12±0.42) min were mean values obtained, respectively (Fig.2B). There was a significant decrease ($P \leq 0.05$) in prothrombin time in the semisolid mass of *O.sanctum* extract preparations groups compared with the control group.

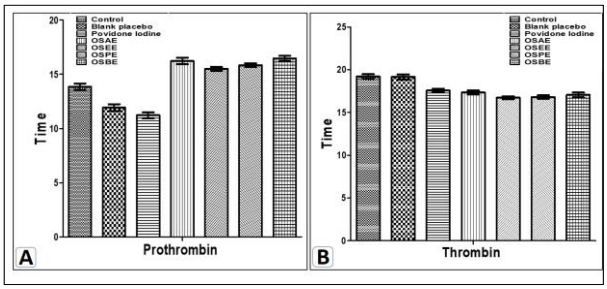

Figure 2: Graphic representation of prothrombin time (A) and thrombin time (B) in blank placebo group and treated with 10% *O.sanctum* leaf extract preparations or with positive control treated with Povidone Iodine ointment. Values are expressed as mean ± SEM (n=6).

3.5. Wound histopathology

Grossly, wounds treated with 10% OSAE, OSEE, OSPE and OSBE preparation groups or with Povidone Iodine ointment showed considerable signs of dermal healing and significantly ($P \leq 0.05$) healed faster than wounds treated with blank placebo. While for the days of complete wound healing (end scar), the mean value of healing time (days) was 21.17 ±0.48 in blank placebo group and 12.67 ±0.31 (Povidone Iodine group) were obtained, respectively. Whereas the *O.sanctum* extracts preparations groups of OSAE (15.17±0.74), OSEE (14.33±0.42), OSPE (14.83±0.40) and OSBE (15.50±0.36) days were mean values obtained, respectively. The difference in mean of the experimental animals is statistically insignificant when compared to the control group ($P \leq 0.05$). However, there were no significant differences between wounds treated with 10% semisolid mass of *O.sanctum* extract preparations groups or Povidone Iodine ointment in terms of rate of accelerating the wound healing process (Fig.3A-C).

Histological observations of wounds treated with *O.sanctum* extract preparations groups (Fig. 4C-F) or Povidone Iodine ointment (Fig. 4B), had higher wound healing acceleration than the blank placebo treated group (Fig. 4A). *O.sanctum* extract preparations and Povidone Iodine groups showed markedly less scar at wound enclosure and granulation tissue contained markedly increased collagen fibres, fibroblasts and proliferating blood capillaries, and absence of inflammatory cells. All extract treated groups exhibit the healing progression with clearly developed epithelialization, fibroblast infiltration, angiogenesis, mononuclear cell infiltration and hair follicles. Wounds treated with blank placebo showed less collagen fibre, fibroblasts and blood capillaries, and more inflammatory cells. There was comparatively disparity in histology of Povidone Iodine ointment and *O.sanctum* extract preparations treated wounds although Povidone Iodine showed much effectiveness in terms of accelerated rate of wound healing process.

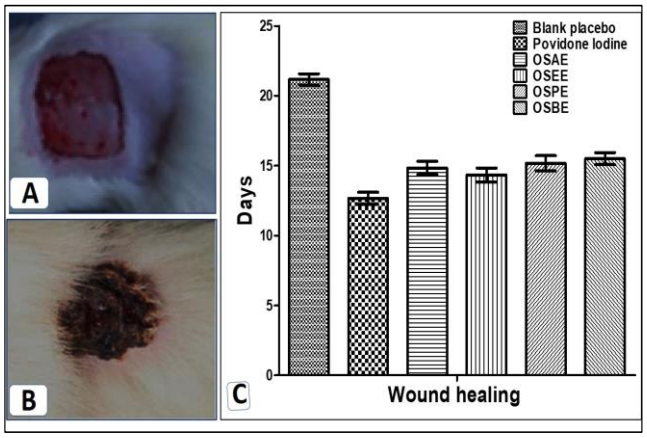

Figure 3: Photographic and graphic representation of wound healing observations on initial (A), complete wound enclosure occurs (B) and time required for wound healing (C) in blank placebo group and treated with 10% *O.sanctum* leaf extract preparations or with positive control treated with Povidone Iodine ointment. Values are expressed as mean ± SEM (n=6).

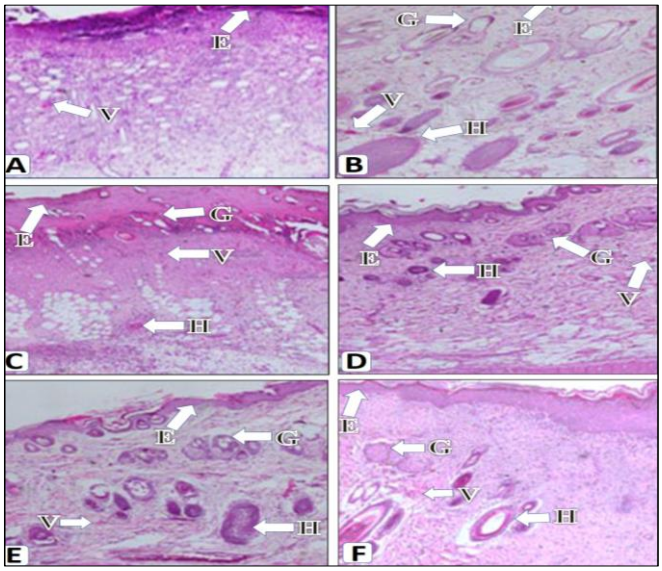

Figure 4: Histological sections of healed wounds treated with placebo containing 10% *O.sanctum* leaf extract preparations. Dressed in blank placebo revealed wide scar at the wound closure (A), treated with Povidone Iodine revealed wide scar at the wound closure (B), Placebo containing 10% aqueous (OSAE), ethanol (OSEE), petroleum ether (OSPE) and benzene (OSBE) extracts showing granulation tissue contains less collagen, fibroblast, and blood capillaries as well as more inflammatory cells (C-F). (H & E stains 80 × magnifications, Bar = 100 µm, E = Epidermis; G = Sebaceous gland; H = Hair follicle; V = Blood vessel).

IV. DISCUSSION

Plants have the immense potential for the management and treatment of wounds. Many plants are used by tribal and folklore and these plants owe their effects to direct effect on the wound healing process. Not many have been screened scientifically for the evaluation of their wound healing activity in different pharmacological models and patients, but the potential of most remains unexplored [19] and the exact step and mechanism in wound repair processes affected by the plants extract was not established [20]. Phytochemical screening and analysis for various chemical constituents of *O.sanctum* in different solvent extracts like aqueous, ethanol, methanol and benzene and the results show the occurrence of flavonoids, alkaloids, carbohydrate, glycosides, cardiac glycosides, anthraquinone, saponins, phenolic compounds, tannins, proteins, phenolics, amino acids, terpenoids, diterpenes and steroids [21-23].

Coagulation of blood is a complex process which is tightly regulated at cellular level. Haemostatic agents can speed up this process by affecting these main steps. Contrast to haemostatic agents, antithrombic agents retards the platelet aggregation whereas anti-coagulating agents could stop coagulation after the initial platelet aggregation step [24]. This study was undertaken to compare the wound healing property of different extracts (aqueous, ethanol, petroleum ether and benzene) of *O.sanctum* leaves by topical route of administration and find the effectiveness of these extracts on haemostatic indices in albino rats. Topical application of different extracts of *O.sanctum* leaves significantly accelerated the rate of haemostatic ability and wound healing process. Study has been shown that the aqueous extract of *O.sanctum* showed anticoagulation effects in human plasma by measuring the prothrombin time, activated partial thromboplastin time and thrombin time as basic coagulation tests and concluded that *O. sanctum* leaves may act as an anticoagulant and thus, potentially may replace the current conventional anticoagulant drugs [25]. Haemostatic ability was exhibited by decreasing bleeding, thrombin and clotting time which measures blood coagulation and this effect was as result of bioactive components [21,23] such as tannins which have been implicated in the haemostatic activity of plants where they arrest bleeding from damaged or injured vessels to form vascular plugs [26] or the absence or presence of these bioactive compounds in different extracts were based upon the chemical group type and polarity index of solvents [27].

O.sanctum plant has been tested and found to be a remarkable antioxidant and the leaves possess significant antioxidant activity [10]. A methanol extract and an aqueous suspension of *O sanctum* leaves were found to have antiinflammatory, analgesic and immunostimulatory properties [28]. Flavonoids isolated from *O sanctum* scavenged free radicals in vitro and showed anti lipoperoxidant activity in vivo at very low concentration [29]. The free radical scavenging activity of plant flavonoids help in the healing of wounds [7]. Low levels of antioxidants accompanied by raised level by markers of free radical damage play a significant role in wound healing in rats [30]. Free radical scavenging activity is a major mechanism by which *O sanctum* products protect against cellular damage [31]. It is likely that the antioxidant property of *O.sanctum* extract could be linked to its wound healing acceleration. Topical applications of compounds with antioxidant properties significantly improve wound healing and protect tissues from oxidative damage [32, 33]**.**

Ethanolic extract of *O. sanctum* significantly decreased the anti-healing effect of dexamethasone in all wound models like incision, excision and dead space wound model and it was reported that the plant has various actions like free radical scavenging effect, metal chelation and immune modulation [34]. Rats treated with the aqueous extracts of *O. sanctum* showed significant increase in the activity of SOD catalase and GSH with a decrease in MDA level in granulation tissue [35], these enzymes are known to quench the superoxide radical and thus prevent the damage of cells caused by free radicals [36]. Better wound healing, seen under the influence of this plant extract, may be because of the presence of flavonoids, which is responsible for the free radical scavenging activity which is believed to be one of the most important components of wound healing [37].

The present study noticed that the *O.sanctum* extract preparations in 10% (w/w) concentration blank placebo were equipped for delivering critical wound healing action. Evidence from literature of phytochemical analysis, the *O.sanctum* extract revealed presence of flavonoids, tannins, saponins, triterpinoids, phenolic compounds and alkaloids. Triterpinoids [38] and flavonoids [39] are known to promote the wound healing process mainly due to their astringent and antimicrobial property, which seems to be responsible for wound contraction and increased rate of epithelisation. Thus, wound healing property of *O.sanctum* may be attributed the phytoconstituents present in it, which may be due to their individual or additive effect that fastens the process of wound healing. As a positive control, povidone iodine is a uniquely effective antiseptic and used widely for the prevention and treatment of infection [40]. The result proves that povidone iodine indeed increases the rate of wound healing by being a disinfectant to the wound. Semisolid mass of *A.indica* extract preparations and povidone iodine accelerate the rate of wound healing. The results show that extract preparations have the

relatively same wound healing rate compared to povidone iodine. Thus, *O.sanctum* leaves extract can be made an alternative to povidone iodine because both groups give the same outcome. In our study on the effect of semisolid mass of *O.sanctum* extract preparations on haemostatic indices and wound healing, we found significantly improved wound-healing activity has been observed with the OSEE recovering effect than OSPE, however, preparations of OSAE and OSBE exhibited similar effect in wound healing rate and epithelialisation when compared to rest of the groups. It is due to the presence of alkaloids, carbohydrate, glycosides, phenolic compounds, tannins, proteins, amino acids, flavonoids, terpenoids, saponins and steroids, triterpinoids and flavonoids in ethanol extract.

V. CONCLUSIONS

Keeping in view the tremendous pharmacological activities and a wealth of available literature, *O.sanctum*, may be utilized to alleviate the symptoms of a variety of diseases as evident from pre-clinical data. However, with pharmacological and toxicological studies point of view; it is one of the plant different extract preparations tried in this study and since topical route of *O.sanctum* administration has showed almost similar effect in their leaves extract preparations, topical route can be preferred as wound healing agent. Thus, it appears that different mechanisms like free radical scavenging as well as immune modulation may act at different levels to bring about the wound healing effects of this medicinal plant. Topical route is more advantageous as it has minimal systemic toxicity and is convenient to use. The present study demonstrated that *O.sanctum* leaves extracts, as topical application of wounds, significantly accelerate the wound healing process and histological observations suggest that *O.sanctum* has potential in the management of wound healing and suggests further studies are needed to isolate the active compound(s) responsible for generating wound healing activity and its exact mechanism(s) of action.

VI. ACKNOWLEDGMENTS

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Conflict of interest statement

The authors declare no conflict of interests.

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