Evaluation of Wound Healing Effect of Different Extracts of AZADIRACHTA INDICA in WISTAR Albino Rats

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Abstract— A study was conducted to evaluate the rate of haemostatic indices and wound healing activity of aqueous (AIAE), ethanol (AIEE), petroleum ether (AIPE) and benzene extracts (AIBE) of Azadirachta indica leaves in adult Wister albino rats by topical route of administration. Among seven groups of 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 [(AIAE, Groups IV), (AIEE, Groups V), (AIPE, Groups VI) and (AIBE, Groups VI)] were treated with placebo containing 10% A.indica leaves extracts, respectively. A thin layer of standard Povidone Iodine ointment was applied topically to wounds of Group III animals as reference. 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. In conclusion, the significant effect on haemostatic indices and increased rate of wound healing together with the histological findings in the extracts treated animals supports the claims made by traditional healers of the benefits obtained from the medicinal use of A.indica.

Keywords: Azadirachta indica, Wound healing, Haemostatic indices, Povidone Iodine, Placebo

I. INTRODUCTION

Wound is defined as contravene or disturbance in the continuity of normal tissue resulting in a variety of cellular and molecular events. 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 [1]. 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 [2].

The presence of various life sustaining constituents in plants have recommended scientists to examine these plants with a view to determine potential wound healing properties. Among the plants, *Azadirachta indica* (Neem, a *Meliaceae* family) is an omnipotent and one of the most versatile medicinal plants having a wide spectrum of biological activity [3]. Every part of the tree has been used as a home remedy against various human diseases. Various studies have been carried out and it is shown to have anti-inflammatory, anti-hyperglycaemic, anti-pyretic, immunostimulant, antiulcer, antioxidant, anti-ulcer, anti-malarial, antifungal, antibacterial, antiviral, antioxidant, antimutagenic, anticarcinogenic, antifertility and hepatoprotective activity [4-8]. In clinical studies of neem oil in the treatment of chronic, non-healing wounds exhibited 50% wound healing was observed in patients [9] and in addition the mean of clinical score of wound healing was not significantly different between Neosporin [10]. Study were made to evaluate the wound healing activity of different extracts of *A. indica* leaves using excision and incision wound models in laboratory rats and results revealed that extracts of plant significantly promoted the wound healing activity in both excision and incision wound models [11-13] by endorsing wound healing activity through increased inflammatory response and neovascularisation [14]. Of course, there are various reports which indicate wound healing properties of *A.indica*, but still there is limited publications regarding wound healing activity of *A.indica* different leaf extracts in comparison with some established wound healing agent to best of our knowledge. Consequently, the present study incorporates and aimed to explore on the rate of haemostatic indices and wound healing effects of aqueous, ethanol, petroleum ether and benzene extracts of *A.indica* leaves in Wistar albino rats.

II. MATERIALS AND METHODS

A. Plant material

Plant materials *Azadirachta indica* (Neem) was collected from local and was authenticated by department of Botany, Anjuman Arts, Sciences and Commerce College, Vijayapura. The voucher specimen no (AASCC/2009/27) 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.

B. 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.

C. Extracts Preparation

Powdered leaves were extracted using solvents: petroleum ether (AIPE), ethanol (AIEE) and benzene extract (AIBE) using Soxhlet apparatus and successive extraction method. Cold maceration technique was used for obtaining aqueous extract (AIAE). Aqueous successive extract of *A. indica,* yield: 20–25% for leaves, were prepared. The *A. indica* 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 *A. indica,* 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 *A. indica,* yield: 1–5% for leaves, were prepared. *A. indica* 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 *A. indica,* 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 was allowed to dry and stored in a desiccator at 4ºC [15, 16].

D. Acute Toxicity Studies

Acute oral toxicity study was performed as per OECD-404 guidelines [17]. 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 AIAE, AIEE, AIPE and AIBE 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.

E. Preparation of the treatment mixture

The semisolid mass of different extracts of *A. indica* were homogeneously mixed with placebo in a concentration of 10% (w/w) each as procedure depicted with slight revision [18]. The mixtures were kept at 4°C and brought to a room temperature before employment.

F. 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 [19].

G. Topical application of vehicles

A thin layer of blank placebo was applied topically to the wounds of control rats (Group 1) twice a day. The semisolid mass of *A. indica* 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 *A. indica* treated animals (AIAE, Groups IV), ethanol extract of *A. indica* treated animals (AIEE, Groups V), petroleum ether of *A. indica* treated animals (AIPE, Groups VI) and benzene extract of *A. indica* treated animals (AIBE, 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.

H. 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 *A. indica*. Also the blood samples for clotting and bleeding time was done using the tail vein of the rats.

I. Sample Analysis

1) Determination of bleeding time

This was resolved using a modified Duke method [20]. A skin puncture was made rapidly using disposable lancet and the stopwatch was started immediately bleeding started. The puncture was wiped with filter paper every 15sec awaiting 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) 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.

3) Determination of prothrombin time

Blood was assembled 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 achieve platelet poor plasma. Thromboplastin PT-S was positioned in a water bath at 37°C; and 0.1 ml of test plasma was also put into a test tube and positioned in the water bath to prewarm to 37°C. 0.2 ml of warmed thromboplastin PT-S was then compulsorily inserted to the test plasma and the stopwatch was started. The tube was tilted repeated in anticipation of a clot was formed and the time taken for clot to form was noted. This was repeated for all the blood samples (six in each group). Precaution was taken to perform test within 3 hour of blood collection since the labile factor declines quickly at room temperature.

4) 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.

J. 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 [21].

K. 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

A. 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 AIAE, AIEE, AIPE and AIBE 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 AIAE, AIEE, AIPE and AIBE preparations was greater than 5 g/kg.

B. Effect of semisolid mass of A. indica extract preparations on haemostatic indices

1) Bleeding Time

The semisolid mass of *A.indica* extract preparations decreased bleeding time in rats in comparison with the control group I. The mean bleeding time in control group was 6.34±0.33 mins, blank placebo (5.45 ± 0.49 min) and Povidone Iodine (5.29 ± 0.31min) were mean values obtained, respectively. Whereas the *A.indica* extract preparations groups of AIAE (4.52± 0.23), AIEE (4.35± 0.42), AIPE (4.62± 0.30) and AIBE (4.78± 0.28) min were mean values obtained, respectively. The analysis showed that the decrease was significant in the semisolid mass of *A. indica* 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).

2) Clotting Time

There was a decrease in clotting time of AIAE, AIEE, AIPE and AIBE preparation groups when compared to control group as shown in Fig.1B. The mean value of control was 6.92 ±0.18 min, blank placebo (6.39 ±0.49 min) and Povidone Iodine (6.52 ±0.51 min) were mean values obtained, respectively. Whereas the *A.indica* extracts preparations of AIAE (4.92±0.55), AIEE (4.67±0.70), AIPE (5.52±0.62) and AIBE (5.75±0.54) min were mean values obtained, respectively. The analysis showed that this decrease was statistically significant ($P \leq 0.05$).

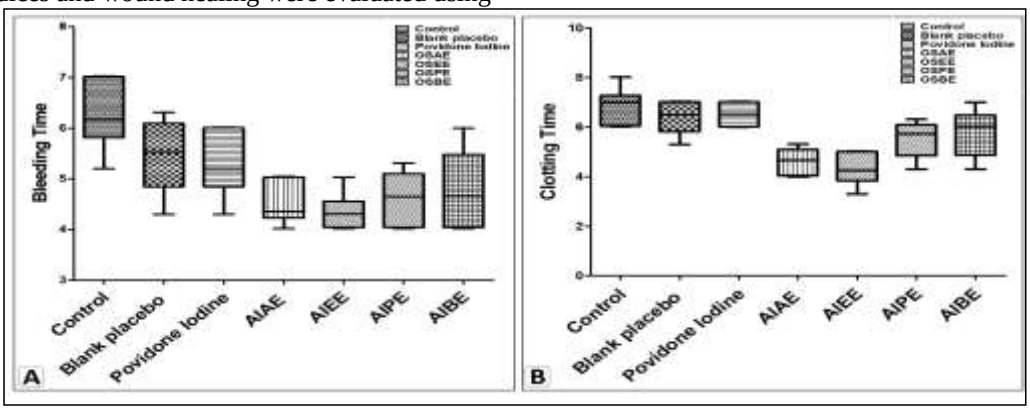

Fig. 1: Graphic representation of bleeding time (A) and clotting time (B) in blank placebo group and treated with 10% *A.indica* leaf extract preparations or with positive control treated with Povidone Iodine ointment. Values are expressed as mean ± SEM (n=6).

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 *A. indica* extracts preparations of AIAE (15.84 ±0.78), AIEE (15.50 ±0.52), AIPE (16.46 ±0.68) and AIBE (16.23 ±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.

4) Thrombin Time

The mean value of thrombin time was 19.21 ±0.26 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 *A. indica* extracts preparations of AIAE (16.81±0.39), AIEE (16.74±0.30), AIPE (17.12±0.57) and AIBE (17.34±0.42) min were mean values obtained, respectively, as shown in Fig.2B. There was a significant decrease ($P \leq 0.05$) in prothrombin time in the semisolid mass of *A. indica* extract preparations groups compared with the control group.

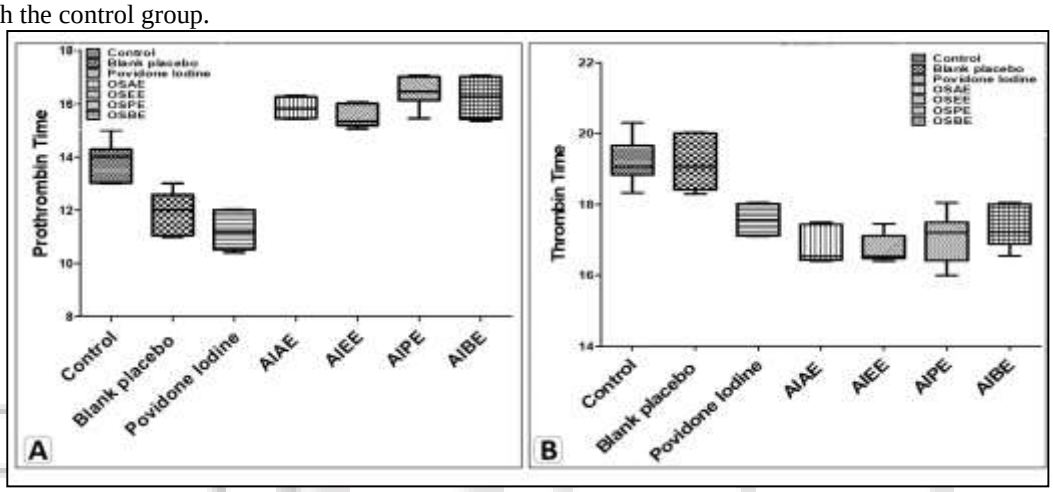

Fig. 2: Graphic representation of prothrombin time (A) and thrombin time (B) in blank placebo group and treated with 10% *A.indica* leaf extract preparations or with positive control treated with Povidone Iodine ointment. Values are expressed as mean ± SEM (n=6).

C. Effect of semisolid mass of A. indica extracts preparations on wound healing

Grossly, wounds treated with 10% AIAE, AIEE, AIPE and AIBE 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 *A.indica* extracts preparations of AIAE (14.83±0.42), AIEE (14.33±0.50), AIPE (15.17±0.36) and AIBE (15.50±0.40) 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 *A. indica* 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 *A. indica* 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). *A. indica* 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 *A. indica* extract preparations treated wounds although Povidone Iodine showed much effectiveness in terms of accelerated rate of wound healing process.

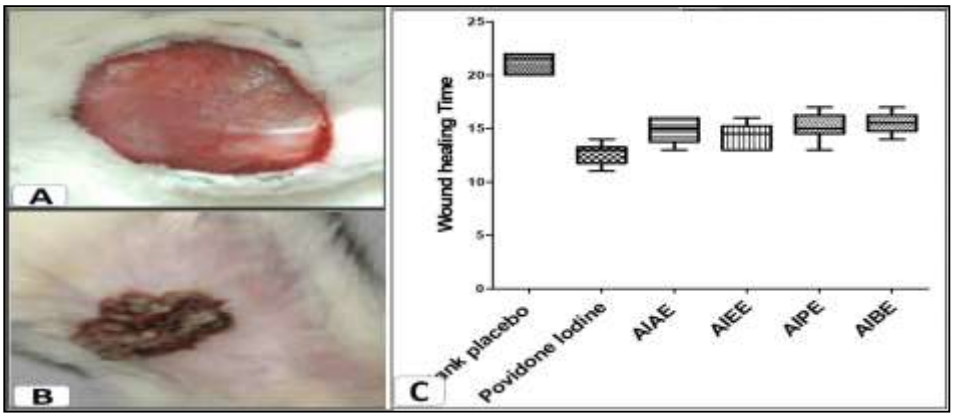

Fig. 3: Photographic and graphic representation of wound healing observations on initial (A), complete wound enclosure occurs (B) and time required for wound healing (C) in blank placebo group and treated with 10% A.indica leaf extract preparations or with positive control treated with Povidone Iodine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

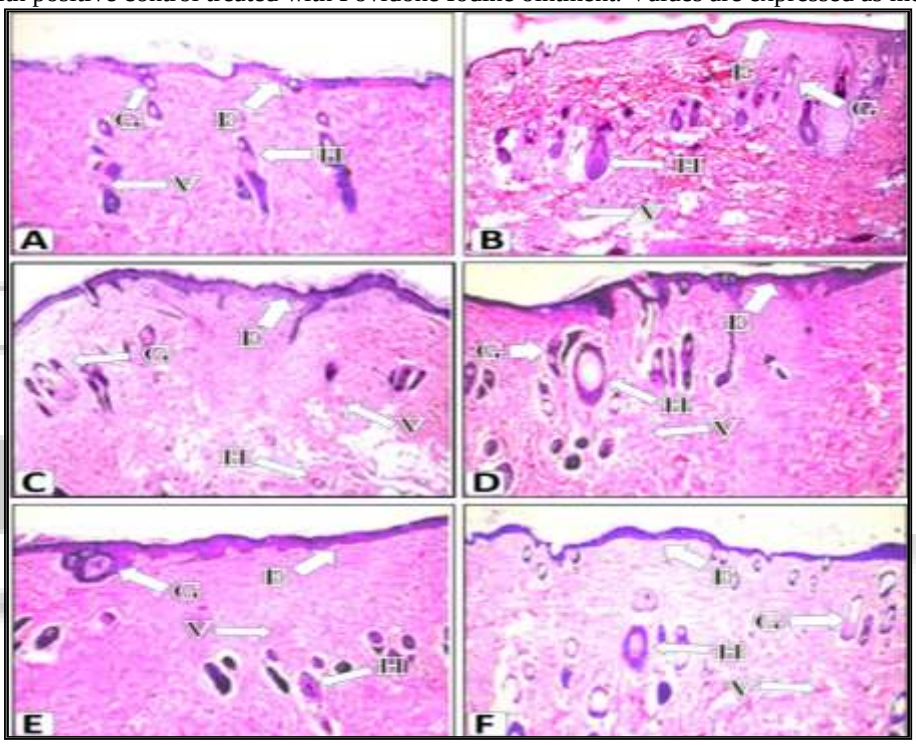

Fig. 4: Histological sections of healed wounds treated with placebo containing 10% *A.indica* leaf extract preparations. Dressed with blank placebo revealed wide scar at the wound closure (A), treated with Povidone Iodine revealed wide scar at the wound closure (B), Placebo containing 10% aqueous (AIAE), ethanol (AIEE), petroleum ether (AIPE) and benzene (AIBE) 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

Many plants and various preparations thereof have been used traditionally in relation to wound treatment, especially due to their immense potential to affect the wound healing process. Plant obtained extracts and/or isolates persuade healing and tissue regeneration through manifold connected mechanisms, which habitually have a synergistic consequence on the overall healing competence [22]. Not many have been screened scientifically for the evaluation of their wound healing activity in different pharmacological models, but the potential of most remains unexplored [23] and the precise step and mechanism in wound repair processes influenced by the plants extract was not established [24]. More than 135 compounds have been isolated from different parts of neem and several reviews have also been published on the chemistry and structural diversity of these compounds mainly of the following families: flavonoids, catechins, anthocyanins, quercetins, saponins, tannins, limonoids, gallic acid and other minor polyphenols; all known to have biological effects [25, 26]. Qualitative phytochemical analysis of aqueous, ethanolic, hexane, chloroform, butanol, ethyl acetate, and methanol crude extracts of neem leaves indicating high levels of alkaloids, carbohydrates, reducing sugars, flavonoids, glycosides, tannins, phenolic compounds, saponins, proteins, amino acids, triterpenoids, and steroids [27,28].

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 [29]. Report from neem of total 9 crude extracts as well as their sulphated derivatives results shown their level of fibrinolytic and antimicrobial activities which depends on both the protein and carbohydrate contents [30] and another study with the fractionated acetone water neem leaf extract demonstrated a dose-related prolongation in Prothrombin Time (PT) and Activated Partial Thromboplastin Time (APTT) values by fractionated acetone water neem leaf extract [31]. The assessment of effectiveness of *A.indica* leaves extracts on haemostatic indices in present study and results found a significant accelerated the rate of haemostatic ability in albino rats. Reports have shown the beneficial effect of Tannins and flavonoids in haemostasis and thrombosis inhibiting the platelet function [32,33]. Polysaccharide compounds present in plants also might contribute to blood coagulation activities. Haemostatic ability of *A.indica* extract preparations was exhibited by decreasing bleeding, thrombin and clotting time which measures blood coagulation and this effect was as result of bioactive components such as tannins or flavonoids which have been implicated in the haemostatic activity of plants where they arrest bleeding from damaged or injured vessels to form vascular plugs [34] or the absence or presence of these bioactive compounds in different extracts were based upon the chemical group type and polarity index of solvents [35].

The potential effects of various parts of the *A.indica* that are seen when using different extracts can certainly be attributed cellular and molecular mechanisms, these mechanisms include free radical scavenging, detoxification, DNA repair, cell cycle alteration, programmed cell death mitigation and autophagy, immune surveillance, antioxidant, anti-inflammatory, anti-angiogenic, and anti-metastatic activities and the ability to modulate of various signaling pathways [36,37]. The free radical scavenging activity of plant flavonoids help in the healing of wounds. 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. It also explained that terpenoids, source of *A. indica*, which play an important role in wound healing [38] because the terpenoid strengthen the skin, increase the concentration of antioxidants in wounds, and restore inflamed tissues by increasing blood supply [39]. Research has shown that *A.indica* is rich in a wide range of compounds with pharmacological potential, found to be a remarkable antioxidant and the leaves possess significant antioxidant activity [27]. Furthermore, in another study, it has been observed that relatively methanolic extracts of neem leaves possess significantly more antioxidant properties than chloroform extracts [40]. Aqueous extract of leaves showed significant reduction in the longest diameter wounds [9] and are supposed to act biochemically through inflammatory response and neovascularisation, which is a crucial step in wound healing process [41] and considered as one of the factors which accelerate wound healing by *A.indica* leaves [14].

Stimulation of epithelial cell proliferation and angiogenesis are important processes for the increased wound healing. Evidence from literature of phytochemical analysis, the *A.indica* extracts revealed presence of flavonoids, tannins, saponins, triterpinoids, phenolic compounds and alkaloids. Triterpinoids [42] and flavonoids [43] are known to endorse the wound healing process mainly due to their astringent and antimicrobial property, which seems to be accountable for wound contraction and increased rate of epithelisation. The present study noticed that the *A.indica* extract preparations in 10% (w/w) concentration blank placebo were equipped for delivering critical wound healing action. Neem contains many active ingredients such as nimbidin, nimbin, and nimbidol with anti-inflammatory, antibacterial, antifungal, and antiviral properties that may help it accelerating the wound healing process. In addition, neem contains an excellent amount of amino acid, vitamin and mineral that is very important in wound healing processes in proliferation phase [4]. Thus, wound healing property of *A.indica* may be attributed the phytoconstituents present in it, which may be due to their individual or preservative effect that fastens the process of wound healing.

This study was undertaken to compare the wound healing property of semisolid mass of *A.indica* different extracts preparations in (10% concentration blank placebo by topical route of administration and find the effectiveness of these extracts on haemostatic indices in albino rats. As a positive control, Povidone iodine is a uniquely effective antiseptic and used widely for the prevention and treatment of infection [44]. 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, neem leaves extract can be made an alternative to Povidone iodine because all extracts treated groups give the comparatively outcome. Topical application of different extracts of leaves significantly accelerated the rate of haemostatic ability and wound healing process. In results, it was found significantly improved wound-healing activity has been observed with the AIEE better effect than AIAE, however, preparations of AIPE and AIBE 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

The present study demonstrated that extracts *of A.indica* leaves, as topical application of wounds, significantly accelerate the wound healing process and histological observations suggest that *A.indica* has potential in the management of wound healing. It showed remarkable speed up in haemostatic indices and better wound healing as compared to standard Povidone Iodine ointment. Therefore,

we may interpret that 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 and observation of such response may be due to the presence of various phytoconstituents which were found to be present in the *A.indica* leaves. Further studies are needed to isolate the active compound(s) responsible for generating wound healing activity and its exact mechanism(s) of action.

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