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POLYHERBAL FORMULATION OF *AZADIRACHTA INDICA* LEAVES AND *ARTOCARPUS HETEROPHYLLUS* LEAVES EXTRACTS FOR WOUND HEALING IN ANIMALS

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ABSTRACT

Polyherbal leaves from *Azadirachta indica* (neem tree) and *Artocarpus heterophyllus* (Kathal) were used to study the effects of wound healing on adult male wistar rats. 18 Wistar rats in both sex groups, ranging in weight from 150 & 200 g on average, were used for this research. The rats were distributed into three distinct groups: control, standard, and test group. The polyherbal extracts of *Azadirachta indica* and *Artocarpus heterophyllus* were applied to a 20 mm^2 excision created on the dorsolateral side of the rats. Frequent saline was administered to the control animals. Each animal's wounds were attended to, measurements were taken, and morphometry was assessed every four days. On day 16, and the day when the epithelium completely regrows, wound biopsies were collected from each group using a random selection process.

The paraffin wax method was used to treat the tissue. Haematoxylin and eosin was used to stain slides in order to evaluate the histological characteristics of the end scar tissue profile, granulation, fibroblast, and neovascularization. Day 8 results demonstrated a considerable ($p<0.050$) constriction in the wound. Among the experimental group, the mean percentage of wound contraction was 8.15 ± 0.36, whereas in the control group it was 15.56 ± 0.74. At day 12, the experimental group's mean percentage contraction of the wound was 4.23 ± 0.67, whereas the control group was 9.78 ± 0.39. On day 12, the mean percentage wound contraction was not statistically significant ($p>0.050$Compared to the control category, the experimental group experienced a day of full wound closure was considerably ($p<0.05$) higher in the test drug treated group. *Azadirachta indica* leaf extracts used in aqueous form enhance wound healing activity by stimulating neovascularization and inflammation.

1. INTRODUCTION

The process of wound healing is a multifaceted phenomenon that involves various phases, such as inflammation, granulation, fibrogenesis, and neo-vascularization, to restore the skin's impaired functional status and interrupted anatomical integrity. Day 12 saw no statistically significant mean percentage wound contraction ($p>0.050$). When comparing the Azadirachta indica extract group to the control group, the experimental group's day of full wound closure was considerably ($p<0.05$) higher. When applied topically, aq. extracts of *Azadirachta indica* leaves promote wound contraction, inflammation, and neovascularization, all of which aid in the healing process. In accordance to a WHO report, between 70-80 percent of the world's population receives their primary healthcare from non-conventional sources, primarily herbal sources. This is particularly true in developing nations where the majority of people cannot afford the expense of seeing a western-style doctor or the cost of their prescription drugs. The fundamental principle of optimal wound healing is minimising tissue damage while preserving sufficient tissue perfusion and oxygenation, appropriate nutrition, & a wet wound healing environment.

Many plant preparations and their plant-based elements are recognized as potential alternatives to medications that promote wound healing because of the availability of a variety of active compounds, their ease of accessibility, and their low toxicity. The Vedic literature "Sarangdhar Samhita" has also emphasized the idea of polyherbalism, which maintains that products containing a combination of plant extracts are more beneficial than those holding individual extracts.(Periyanayagam K and Karthikeyan V, n.d.) "Medicinal plants that are antibacterial, antioxidant, and anti-inflammatory have been shown to slow down the healing process of wounds. Less expensive medication results from polyherbalism since it shortens the duration of treatment or lowers the cost of some anti-inflammatory and antibacterial medications. Plant extracts are more powerful than pure plant principles because they contain all of the ingredients instead of just one active ingredient. They also have fewer side effects. *Azadirachta indica* and *Artocarpus heterophyllus* leaves are utilized in this work to create a polyherbal extract that can be applied to wounds. (Baliga et al. 2011)

Neem, or *Azadirachta indica* (Meliaceae), is a tropical tree that is widely grown around the world and is considered to be the most valuable traditional medicinal plant native to India. The limonoids, azadirachtin and other compounds found in neem tree seeds have a strong insecticidal property against a range of insect pests while posing little risk to humans. Traditional medicine has employed neem oil, bark, & leaf extracts to treat respiratory disorders, constipation, intestinal helminthiasis, leprosy, and other ailments (Dev et al. 2019). The plant has been reported to have anti-inflammatory and antipyretic, neuropsychological, anti-microbial, anti-mycotic, cardiovascular, immunomodulatory, along with anti-hyperglycaemic effects. Rheumatism, indolent ulcers, and persistent syphilitic sores are all treated

with this herb. Numerous skin ailments can be treated with neem oil; biliary disorders, blood disorders, itching, ulcers, & burning sensations can all be treated with neem fruit, bark, leaf, root, and flower (James and Friday 2010).

The leaves of *A. heterophyllus*, or jackfruit, are used to treat a variety of conditions including asthma, healing wounds, ringworm infestation, gall bladder stones, pustules, anti-syphilis, anthelmintic, lactogogue, ear pain, anti-ulcer, anti-cariogenic, adsorbent, anti-bacterial, anti-inflammatory, anaemia, dermatitis, cough, diarrhoea, fever, sedative, and digestive issues (Emamoke, Theodore, and Julius 2013a). The current work uses the excision wound healing model (WHEM) to examine the impact of the flavonoid fraction in EAAH on wound healing. Additionally, it was revealed that the intriguing natural triterpenoid ursolic acid, which has numerous advantageous properties like anti-inflammatory, hepatoprotective, antibacterial, and antiulcer properties, is present in the roots of *A. heterophyllus* (Poddar et al., n.d.).

2. MATERIALS AND METHODS

2.1. Plant collection & authentication

The leaves of *Artocarpus heterophyllus* and *Azadirachta indica* were gathered in Paltoopur, Jaunpur. Mr. Vinay Ranjan, Head of Office and Scientist-E, BSI Central Regional Centre, Prayagraj 212002, identified and verified the specimen.

2.2. Animal procurement

Wistar albino rats with weight averaging between 150 and 200 grams were used in this experiment. The Institutional Animal Ethics Committee (Approval number SIP/IAEC/015/03/24) approved the study protocol in compliance with the guidelines set out by the Committee for Control and Supervision of Experiments on Animals (CCSEA), India. After the animals were acclimated to the standard laboratory atmosphere of 25 ± 2 °C, 44–56% humidity range, and a 12:12 h light-dark cycle, they were fed a standard meal and had unrestricted access to water throughout the research.

2.3. Extraction of plant product

The leaves were gathered, crushed into small pieces, allowed to air dry at room temperature for approximately ten days, ground into a powder, and then sealed in an airtight container. After that, 100 grams of powder were macerated in 300 milliliters of 90% ethanol for two days at room temperature, stirring occasionally.(Gupta et al. 2019)The plant's ethanol extract was collected in a different container and concentrated using a rotatory vacuum evaporator at lowered pressure below 50 ºC.(Tamanam, Nagala, and Rapaka, n.d.) Japan's Shimadzu Company used a freeze dryer to dry the concentrated extract. Ultimately,

a residue with a blackish-green color that yielded 5.6% w/w was collected and stored at 4OC in a refrigerator.

2.4. Preparation of polyherbal gel

2 grams of carbopol-940 were uniformly mixed into 50 millilitres of distilled water in a 100-millilitre beaker, and the mixture was continuously swirled. The Carbopol was allowed to expand overnight in the beaker. A separate mixture was cooked in a water bath using 5 millilitres of distilled water, 0.2 millilitres of 0.5% methyl paraben, and 0.1 millilitres of 0.2% propyl paraben. Following cooling, the required amounts of sodium meta-bisulphide (0.2 gm), 5% propylene glycol-400 (5 ml), and polyherbal mixture (2 gm for 2% and 5 gm for 5% gel) were well mixed (Emamoke, Julius, and Theodore 2013b). The finished product was poured into a readymade Carbopol gel base and triethanolamine was added drop by drop while being agitated continuously to reach the correct skin pH. Enough water was added to the final composition to get the necessary gel consistency (Kalyani et al).

2.5. Phytochemical Screening

Various phytochemical screening assays were conducted on the plant extracts in order to identify and search for chemicals like glycosides, alkaloids, flavonoids, terpenoids, phenolics, and tannins (Omar and associates, 2011) (Nupur, 2014) (Jaradat, Elmarzugi, and Eid, 2017).

2.6. Acute dermal toxicity

The acute skin toxicity test findings for the polyherbal extract were determined in accordance with OECD recommendation no. 402. Adult Wistar rats of both sexes were utilised. The 18 animals were divided into three distinct groups that included 06 animals each (Barua and collaborators, 2010). An adequate depilatory process was used a day before to the test to remove 10% of the body hair from the dorsal areas of the test animals. 500 mg/kg, 1000 mg/kg, and 2000 mg/kg of topical PHG (5%) were administered to the animals in Group I, Group II, and Group III, respectively. Every animal's fur, eyes, behaviors, and toxic dermal reactions were noted over the course of a 14-day period (Yusufu, Rabo, and Biu 2010). Introduction to the "OECD/OCDE 423 OECD GUIDELINE FOR TESTING OF CHEMICALS Acute Oral Toxicity-Acute Toxic Class Method" (2001)

2.7. Experimental Design

Excision wound model

A substantially modified variant of the procedure outlined was used to create an excision wound. The animals were anaesthetized with ketamine hydrochloride (100 mg per kg, through the abdomen, body

weight) after their dorsal regions were shaved with Veet depilatory cream. The target wound's area was marked, and the shaved off dorsal region was used to create an impression. A full thickness excision wound, encompassing a 20 mm2 circular area, was made along the marking using a surgical blade, pointed scissors, and toothed forceps (Islam and others, n.d.). Rats were exposed to the open aseptic environment without any clothing. From the day of the procedure (zero day) until full healing, a once-daily application of the standard medicine and 5% weight/weight of the prepared extract gel was made. The model depicts the process of wound contraction and epithelialization were assessed. Every fourth day following the creation of the wound, the percentage contraction of the wound was measured (Periyanayagam K and Karthikeyan V, n.d.)(Amadi et al., n.d.). All of the rats were put to sleep at the conclusion of the study, and specimen samples from the tissues of the healed wounds were taken from each group, leaving a 5 mm margin of normal skin surrounding the edges of the healed wounds. Tissue samples were kept in a 10% formalin solution and utilized for investigations into histopathology & biochemistry (Chidambara Murthy et al. 2004).

2.8. Grouping of animals

The following scheduled treatments were applied to the rats, which were split into three groups (n = 6).

Group-I	Control (diseased).
Group-II	Standard (topically administered 5% w/w povidone iodine ointment USP)
Group- III	PHG (5%) applied topically

3. RESULTS

3.1. Presence of phytochemicals

Following phytochemicals were found in the extracts of *Azadirachta indica* and *Artocarpus heterophyllus* (Shukla, Khurshid, and Kumar 2020) (S., Karigar, and Murthy 2020).

Table 01 Phytochemical testing report of AI & AH

S.N.	Test Performed	*Azadirachta indica*	*Artocarpus heterophyllus*
1.	Alkoloid	+	+
2.	Saponin	+	-

3.	Tannin & Phenolic compounds	+	+
4.	Flavanoids	+	+
5.	Glycoside	+	+
6.	Protein & Amino acid	-	+
7.	Carbohydrate	+	+
8.	Terpenoids	+	+

3.2. Evaluation of wound healing ability of polyherbal gel

Table 02: Effect of AI & AH leaves extract on wound area

Groups	**Treatment**	**Wound contraction (mm^2) on day**			
		4 days	**8 days**	**12 days**	**16 days**
Group 01 (Control)	-	18.98 ± 0.35	15.56 ± 0.74	9.78 ± 0.39	4.68 ± 0.68
Group 02 (Standard)	5% w/w povidone-iodine ointment USP	13.3 ± 0.74	6.65 ± 0.66	3.10 ± 0.58	0.46 ± 0.13
Group 03 (Test)	5% w/w Polyherbal gel of AI and AH	14.53 ± 0.73	8.15 ± 0.36	4.23 ± 0.67	1.10 ± 0.14

(Mean ± SD, n = 6) represented as the post-wounding day epithelization time in each group, with *P<0.05 and ** P<0.001 in comparison to the control.

3.3. Histopathological analysis

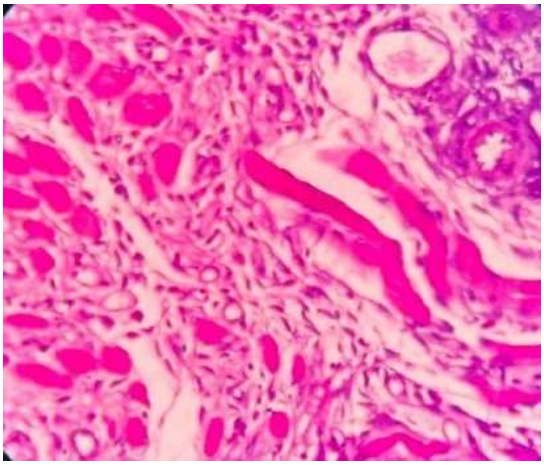

Fig 1: Test

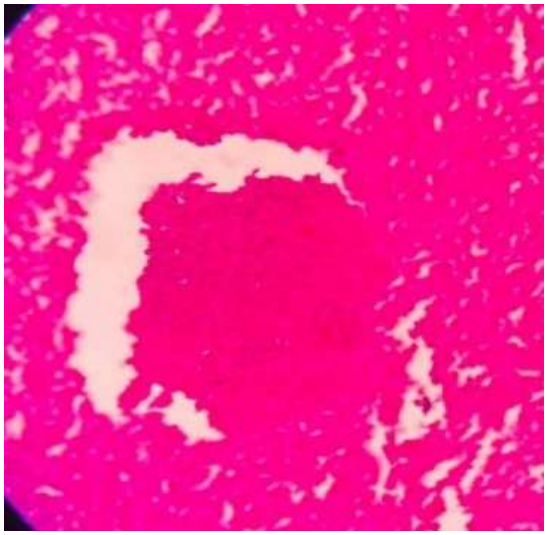

Fig 2: Control

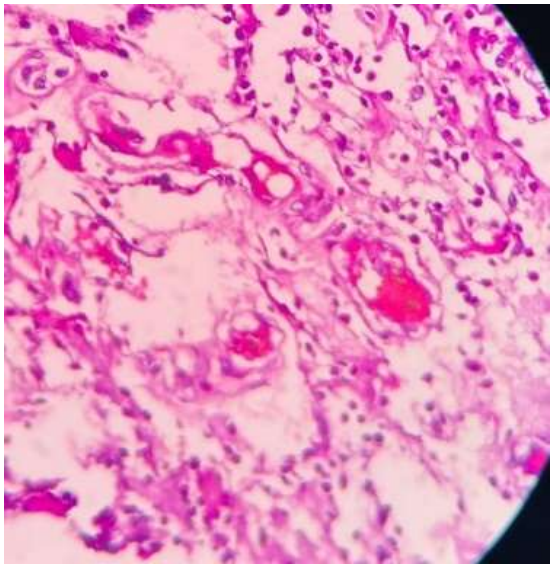

Fig 3: Standard

4. DISCUSSION

The purpose of this research work was to assess the ability of ethanolic extracts of *Azadirachta indica* along with *Artocarpus heterophyllus* leaves to cure wounds via excision wound models in Albino Wistar rats. Studies on the wound healing properties of *Artocarpus heterophyllus* and *Azadirachta indica* leaves show that the flavonoids, phenolic, and tannin constituents in *Artocarpus heterophyllus* leaves are important in the wound healing process, whereas nimbidin, a compound found in the plant's seeds and leaves, is thought to be the main factor in *Azadirachta indica* (neem) wound healing. It was determined that the gel formulation containing both *Azadirachta indica* and *Artocarpus heterophyllus* extracts had a considerably higher wound-contracting ability than the control. According to the results of the excision wound model, there was not a significant rise in wound contraction in each of the groups within the first four days, as compared to the control group. From day '0' to day 16, the excision wound model demonstrated a noticeable ($P<0.01$) raise in the mean percentage of wound closure. The standard drug (Povidone Iodine)-treated group experienced this increase at 93.46%, whereas the test drug-treated group experienced this rise at 78.19%.

In comparison to the control group, the test group showed a greater proliferation of connective tissues and angiogenesis on day 16 in histological investigations. While a blood clot was visible at the location of the 16-day-old excision wound in the control grp, the test drug-treated group showed a higher degree of epithelialization and fibroblastic deposition (Figs. 1, 2, and 3). The enhanced wound healing activity of the test plants was shown by enhanced angiogenesis in the groups treated with the test drugs.

5. CONCLUSION

The results obtained revealed that polyherbal gel formulations containing ethanolic extracts of *Artocarpus heterophyllus* and *Azadirachta indica* demonstrated wound healing efficacy by promoting neovascularization and an enhanced inflammatory response. To completely understand the mechanism underlying the wound-healing properties of *Artocarpus heterophyllus* and *Azadirachta indica*, more research including isolated ingredients is necessary.

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