Section A-Research paper
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Assesment of Wound Healing Activity of Polyherbal Formulation in *Wister* Rats

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Abstract:
Finding new antimicrobials from alternative sources, such as plants, has become necessary due to the current situation in which various antibiotic resistances has emerged in human pathogenic organisms. It is anticipated that plant extracts will be helpful against drug-resistant microbial infections because they have targets different from those that antibiotics use.The goal of the current study was to prepare a polyherbal formulation using *Ocimum sanctum* extract, *Citrus Limon* peel extract, *Aloe vera* gel powder, and Honey, as well as to assess its effects as an antioxidant and wound healer. The pH, Spreadability, Skin Irritation Study, Viscosity, Stability, and wound Healing Activity of the Prepared Ointment Formulations of different concentration were tested. The developed polyherbal formulation's ability to promote wound healing was tested using the wound contraction, antioxidant markers and histopathology of healed skin as the evaluation criteria**.**
Keywords: Polyherbal, Ointment, Extracts, Phytoconstituents and Wound healing.

Introduction:

Recent years have seen a rise in the usage of herbal remedies all across the world, or what we can refer to as a "Return to Nature." Since ancient times, people have recognised the benefits of using medicinal plants as a rich source of therapeutic compounds for the treatment and prevention of ailments and diseases (Thiem and Goslinska; 2004).

Wound: Physical, mechanical, or thermal forces that cause the normal skin to break or open are referred to as wounds. To put it another way, a wound is a laceration, contusion, or abrasion that compromises the integrity of the skin's epithelium. Wound healing is very complex process involved four major steps mainly: Heamotasis, Inflammation, Proliferative and Maturation steps (Filleur *et al*., 2005 and Obara *et al*; 2002).

Several Ayurvedic herbal plants have a substantial impact on wound healing. There has been a lot of research done on using medicinal herbs to control wound healing. Numerous herbal

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extracts have been demonstrated to aid the body's natural repair processes and play a significant part in wound healing (Guo & Dipietro; 2010).

Roots, petals, and leaves are just a few of the many plant parts used to make herbal remedies. Different extraction techniques are needed because of the large number of chemical ingredients and the possible medical benefits of each component. You can use fresh or dried plant material, depending on the herb. The continued use of herbal remedies, which have been a staple of healthcare since the dawn of time, has a huge negative influence on global trade (Mosihuzzaman; 2012).

Material and Method:

Collection and Authentication: *O. sanctum* & *A. vera* leaves from healthy plants were gathered from Sonipat, Haryana, Honey was collected in fresh from Sonipat, Haryana, and Lemon was obtained from Hisar. The herbarium of collected plant materials was prepared and authenticated from CSIR-NIScPR, Herbarium and Museum, New Delhi.

Processing and Extraction: *O. sanctum and C. limon* plant material was collected, cleaned, andwashed using distilled water. After being spread out in the shade on sheets of filter paper at roomtemperature for about two weeks in the case of O. sanctum and one month in the case of *C. lemon* peel. The dry material was then crushed mechanically for 5 minutes to produce a powder. The gel from *Aloe vera* leaves was extracted after they had been collected and cleaned with distilled water. The gel was dried and ground to form powder and stored in a container with a tight lid (Seifunnisha & Shanthi; 2020). Honey was used as such. Honey was used in purifiedform. The extraction of *Ocimum sanctum* and *Citrus limon* peel was done by soxhelet apparatus using 70%ethanol (Borah &Biswas; 2018). The percentage yield was calculated using formula asgiven below:

$$\text{Percentage yield} = \frac{\text{Weight of the dried extract}}{\text{Weight of the powder before extraction}} \times 100$$

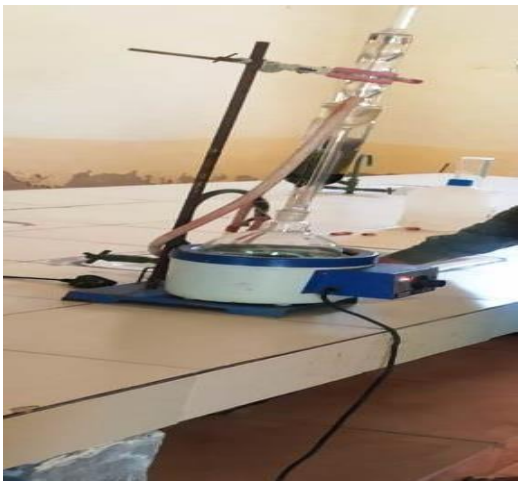

Fig.1 Extraction of *O. sanctum* leaves

Fig. 2 Extraction of *C. limon*peel

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The extracts/plant material were chemically analysed in order to identify phytoconstituents like phenolic chemicals, flavonoids, saponins, alkaloids, glycosides, sterols, carbohydrates, protein & amino acids, acidic compounds, gums & resins, etc. (Harbone; 1973 & Evans; 2002).

Preparation of Polyherbal Ointment:

Polyherbal ointment was prepared by first preparing ointment base by using the formula as given in British Pharmacopoeia, 1996. The different concentration of extract and honey was mixed with sufficient quantity of ointment base to form the required quantity of ointment.

Table1: Formulation of Polyherbal Ointment

S.No.	**Ingredients**	**Formulation**		
		Formulation A (F-A)	**Formulation B (F-B)**	**Formulation C (F-C)**
1.	Lemon Peel Extract	0.3 g	0.5 g	0.7 g
2.	*O. sanctum* Extract	0.3 g	0.5 g	0.7 g
3.	*A. vera* Extract	0.3 g	0.5 g	0.7 g
4.	Honey	0.3 g	0.5 g	0.7 g
5.	Simple Ointment base	q.s to 10 g	q.s to 10 g	q.s to 10 g

Pharmacological Activity: Pharmacological Activity was assessed in *wister* rat animals after approval from animal ethical committee.

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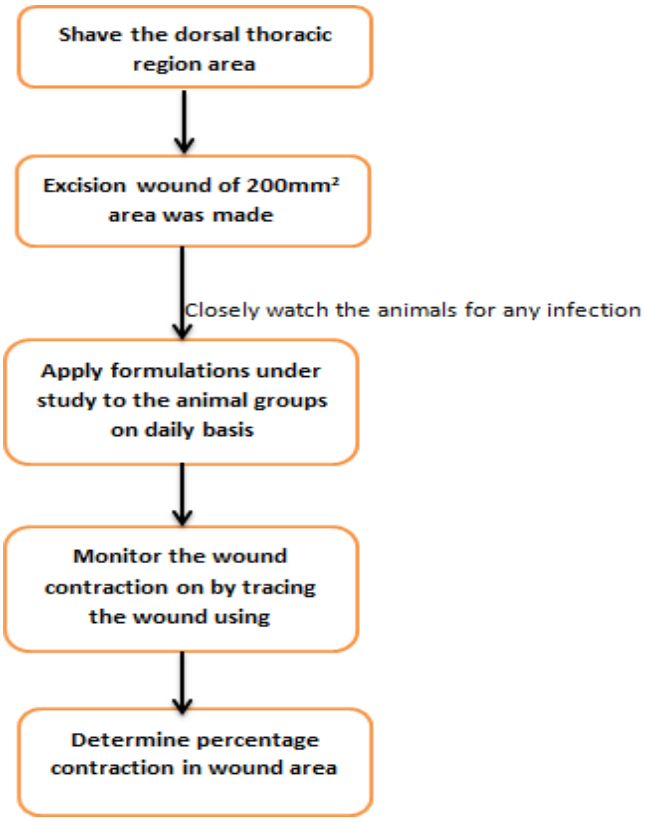

Fig. 3: Flowchart showing determination of Pharmacological Study

Determination of Antioxidant Markers:

Superoxide Dismutase (SOD):Prepare 10% w/v tissue homogenate in 0.1 M phosphate buffer or 0.15 M HCl, then 15 minutes of centrifuging at 15,000 rpm and 4 °C Consider the supernatant (0.1 ml) as the sample. Add 0.1 ml of the sample, 1.2 ml of sodium pyrophosphate buffer (pH 8.3, 0.052 M), 0.3 ml of 300 M Nitroblutetrazolium, and 0.2 ml of NADH (750 M) to the mixture.90 seconds of 90 °C incubation followed by 0.1 ml of glacial acetic acid addition. Add 4.0 ml of n-butanol and stir. 10 minutes after allowing to stand, centrifuge and separate butanol layer. Take the optical density at 560 nm (butanol is used as a reference) (Wen *et al*; 2013).

Reduced glutathione (GSH):

2.5 ml of 5% TCA and 0.5 g of the tissue were combined to create a homogenate. For 10 minutes, the protein precipitate was centrifuged at 1000 rpm. The estimated amount of reduced glutathione was 0.1 ml of the supernatant.

The 0.2M sodium phosphate buffer (pH 8.0) was used to dilute the supernatant (0.1ml) to 1.0ml. Also generated was standard reduced glutathione with quantities ranging from 2 to 10 nmoles. After adding two ml of freshly made DTNB solution, the amount of yellow colour that had emerged after 10 minutes was measured in a spectrophotometer at 412 nm. The amounts are given in nmoles GSH/g sample (Sedlak&Linsay; 1968).

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Histopathology of Healed Skin:

To examine the healing markers, a portion of the healed (10-day-old) regenerated tissue was removed and cleaned with ordinary saline solution. The tissues were dehydrated with 90% ethanol after the section had been fixed in 10% formalin solution. They were then thinly sliced into 7-m-thick sections, stained with hematoxylin-eosin dye, and examined under a light microscope for keratinization, epithelization, fibrosis, collagen formation, and neovascularization. The sections were carefully mounted on the slides with Canada balsam and covered with a cover slip before being viewed and examined under a microscope (Karodi *et al*; 2009 and Yeo *et al*; 2000).

Result and Discussion:

Pharmacological Activity:

Percentage Wound Contraction:

Table 2: Percentage Wound Contraction Data (mm)

S.No	Group	Days				
		3	**9**	**15**	**21**	**24**
I	**Control**	14.82± 0.98	33.86± 1.75	67.49± 1.03	96.43± 0.77	99.44± 0.35
II	**Marketed Formulation (Betadine Ointment)**	15.86± 0.37	59.20± 0.51**	99.02± 0.21**	100**	100**
III	**F-A**	11.37± 2.16	35.01± 0.28	81.49± 0.49**	99.33± 0.10**	100**
IV	**F-B**	7.34± 0.92*	35.50± 0.43	84.68± 0.47**	99.83± 0.10	100**
V	**F-C**	16.43± 0.36	54.77± 3.00**	97.50± 0.55**	100**	100**

*P<0.050, **P<0.001

Antioxidant Markers:

Table 3: Antioxidant Markers Data

Group No.	**Treatment**	**SOD (unit/mg tissue)**	**GSH (nmol/mg tissue)**
I	Control	13.85±0.46	0.61±0.01
II	Marketed Formulation	33.15±0.32	1.6±0.02
III	Formulation A	18.61±0.32	1.14±0.05
IV	Formulation B	21.46±0.43	1.68±0.11
V	Formulation C	29.07±0.23	2.38±0.03

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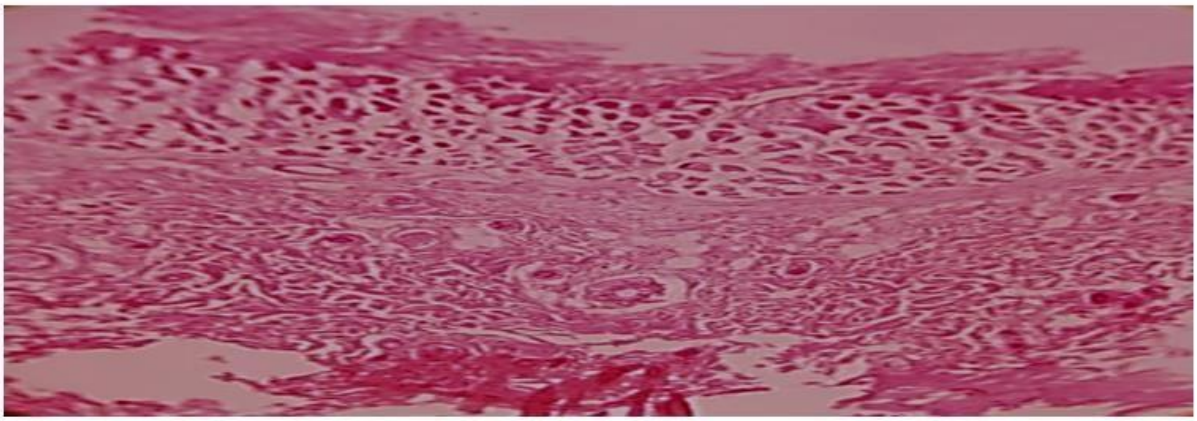
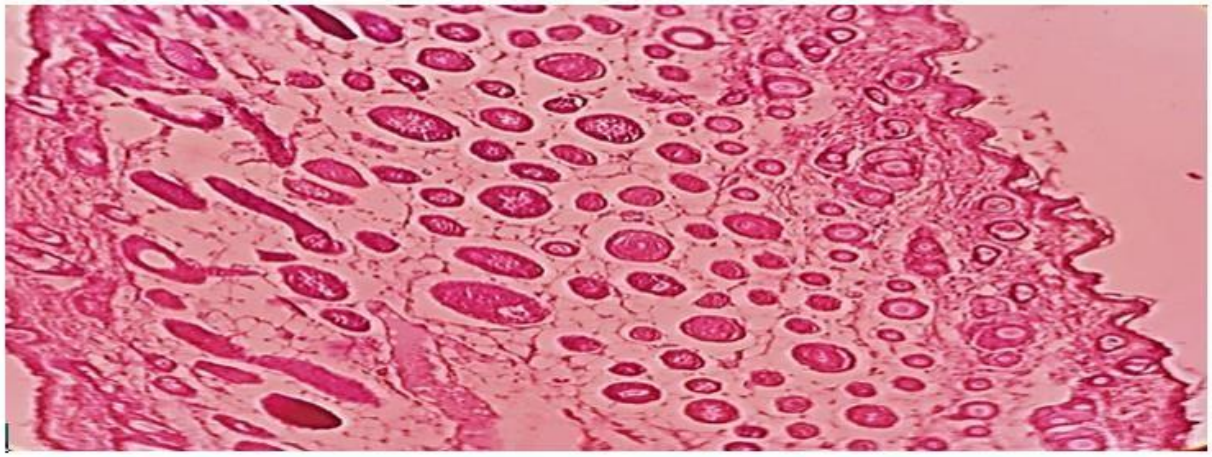
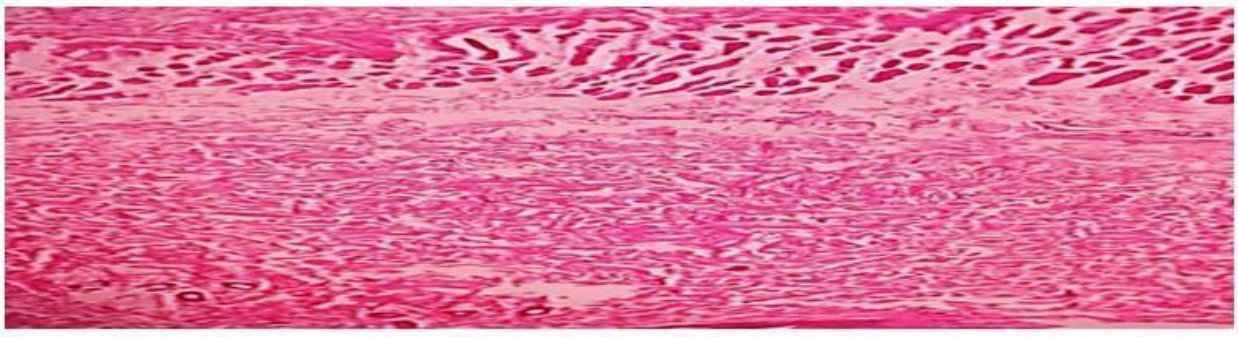
Histopathology of Skin:



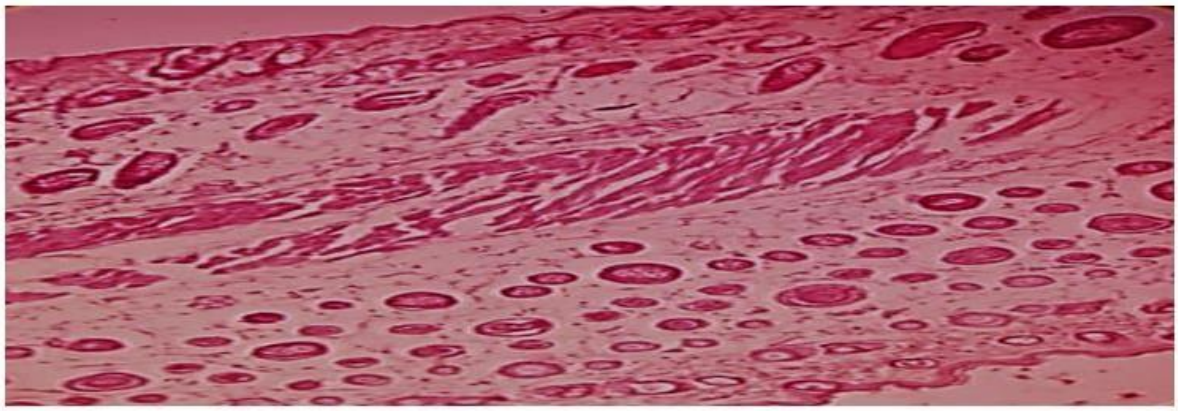
Group-I control (More inflammatory cell infiltration)



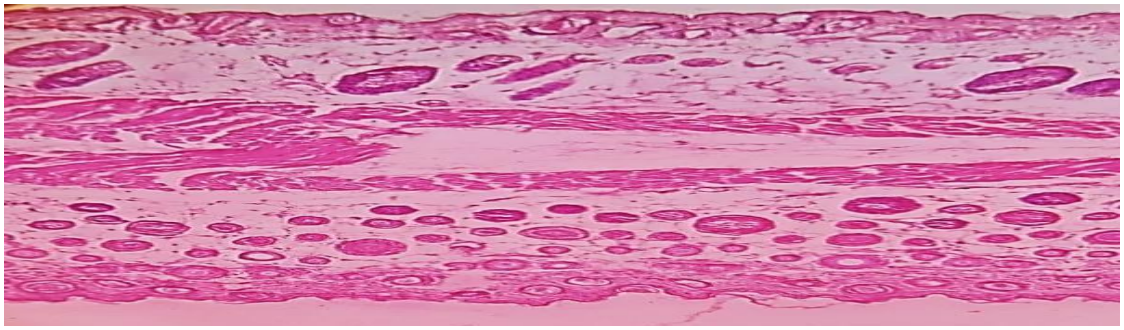
Group- II Marketed Formulation treated Group
(Less inflammatory infiltration, angiogenesis)



Group III Treated with F- A
(More fibroblast, angiogenesis)



Group IV Treated with F- B
(More fibroblast, angiogenesis)



Group-V Treated with F-C
(Increased Fibroblast, complete re-epitheliazation)

Fig 5: Histoptahology of skin

Conclusion:

Based on literature review and analysis of the published data different methods/procedures are envisagedto avail phytoconstituents considering wound healing property due to disadvantages with the current approaches of synthetic agents. When compared to current approaches, whichcarry the risk of serious side effects and recurrent disease attacks, natural and herbal therapies were once thought to be a classic approach, but their use has significantly increased in recent years.

The current study investigated the anti-inflammatory and possible wound healing effects of a multi-herbal ointment combining *ocimum sanctum*, *aloe vera*, *citrus limon*, and honey as phytoconstituents against excision wound models in *wistar* rats. It is necessary to confirm the positive effects and understand the additional mechanisms underlying the wound healing activity of these chosen medicinal plants based on the results of pre-clinical investigations that showed their promising activity.

Excision wound model results showed that Polyherbal Formulations decreased the area of the wound that contracted and the time it took for the epithelium to form in a dose-dependent way.

In the excision wound model, the F-C treated group showed the greatest healing (100%) after 21 days, which was almost as high as the 100% healing rate of the standard treated group.

Localized damages and an increase in free radical activity followed every skin injury. Despite the fact that GSH and SOD are naturally occurring antioxidant enzymes, any damage reduced their antioxidant power. In comparison to the control, F-A, and F-B treated groups, the SOD level of F-C was determined to be 29.07 unit/mg tissue. The GSH level of the F-C group was discovered to be 2.38 nmol/mg tissue, which is also significantly greater than the levels in the control, F-A, and F-B treated groups. The elevated levels of SOD and GSH in F-C further supported the substance's putative antioxidant ability.

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