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ORIGINAL RESEARCH ARTICLE- EXPERIMENTAL **OPEN ACCESS**

A PRELIMINARY STUDY ON *VRANAROPANA KARMA* (WOUND HEALING ACTION) OF *CURCUMA LONGA* LINN

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ABSTRACT:

Background: Wound healing is a complex process of tissue repair that occurs in response to an injury. Plants and plant-derived elements are well explored in the treatment of various types of wounds. Curcumin is a natural polyphenolic substance that has been used since ancient times in Ayurveda for its healing properties, as it reduces inflammation and acts on the various healing stages of the wound. **Materials & Methods:** *Curcuma longa* Linn. has been told in various mentions by our acharyas for its different actions on the body. It has been widely used in the wound management since ages due to its antimicrobial, anti-inflammatory and quick wound healing effect. This activity was elicited in comparison with Kamilosan (German remedy) in Wistar Albino rats. **Results:** The present study emphasises on the effect of *Curcuma longa* L aiding in wound healing through quickening of formation of granulation tissue with complete laying of collagen. Massive proliferation of mature connective tissue was seen on 8th day. Complete healing with matured skin formation was observed on the 12th day. **Conclusion:** The study thus proves that *Curcuma longa* has a very good effect on wound healing through therapeutic properties including antioxidant, analgesic, anti-inflammatory, antiseptic activity, anti-carcinogenic activity, anti-tumour, antiviral, antibacterial, antifungal making it more acceptable than any other topical drugs.

Keywords: Wound healing, *Curcuma longa*, Antimicrobial, Ayurveda

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INTRODUCTION

Wound healing associates a well-orchestrated complex of biological and molecular events that involve cell migration, cell proliferation, and extracellular matrix deposition. Although these processes are similar to those driving embryogenesis, tissue and organ regeneration, and even pathological conditions [1], certain differences exist between adult wounds and these systems. Wound management at various times turns to be a challenge in clinical practice. In low-income countries, an even higher incidence, due to traumatic injuries and ulcers, is expected [2]. Recently, the World Health Organization (WHO) has recognized the unmet need for an interdisciplinary approach facing this global challenge, which has been accordingly addressed by the Association for the Advancement of Wound Care (AAWC) Global Volunteers program [3].

Despite accumulation of huge literature on Antimicrobial and wound healing actions of several drugs and their related biochemical events, there is paucity of information regarding the factors that help in accelerating wound healing action. The modern system of medicine tend to reduce the body to its components by isolation of the targeted organ, in present scenario these medicines stand first in the drugs which are rejected as many are sources for iatrogenic diseases. In Ayurveda treatment takes into cognizance the entire body with lesser side effects on the same. However, the concept of wound healing is dealt in depth in *Ayurvedic* treaties where it is discussed under two major pharmacological actions namely *Vrana Shodhana* and *Vrana Ropana* referring to Antimicrobial Action and wound healing acceleration respectively. Most of the synthetic drugs employ in modern science, often find constraints due to the non-judicious administration resulting in upsurge of iatrogenic diseases like Nephrotoxicity, Ototoxicity, Asphyxia etc. Hence the global interest has taken a stride on natural medicines especially of herbal origin. However, ancient literature especially *Charaka Samhita, Sushruta Samhita*[4,5], *Astanga Hridaya* have been a treasures of information among herbal medicines. In these treatises more than 280 plants are documented, some having upshot either in *Vrana Shodhana* or *Vrana Ropana* actions and a few showing both the effects. Among various such Plants mentioned in *Samhita, haridra* is a frequently used drug of choice. *Rajani, Nisha* are the Synonyms of *haridra*, and *Curcuma longa* is the botanical name of the plant. Recent studies provide the evidence of anti-cancer [6], anti-aging [7], and wound healing agent [8] action of curcumin, the active ingredient of

the Drug. This investigation is an earnest attempt in understanding of antimicrobial and wound healing property of *Curcuma longa* L represented by albino rats.

MATERIALS AND METHODS

Preparation of Trial Drug: The trial drug; *Curcuma longa* L., was Collected and was subjected to pharmaceutical process to obtain the form of *Ghanasatva*. The dried rhizomes of *Curcuma longa* were subjected to *Rasakriya* procedure to obtain *Ghanasatva*. Thus obtained *Ghanasatva* was later mixed in neutral petroleum jelly and homogenized maintaining 15% and 20% concentrations for comparative evaluation.

Animal Experiment: The animal experiment was conducted and Wister stained albino rats were the animal of choice for the experiment. To evaluate the wound healing action, four types of wounds were created at nape region, as different models namely

1) Circular wound of one cm, without damaging subcutaneous tissue;

2) Incision of half mm width by thirty mm length;

3) Wound of half cm width and thirty mm length was created by damaging subcutaneous tissue, challenged three times repeatedly with an interval of two hours;

4) Aforesaid type with a depth of half cm.

After crafting different wounds, inoculum containing Staphylococcus aureus was introduced with a dosage of 0.5 - 1 MC Farland's standard.

Methodology:

For antimycotic experiment, aqueous concentration of the drug was prepared from 5% to 20% wt vol. and soluble fractions were made. Cup plate method was employed using circular bore, due care was been taken to maintain fine edges of the well. The area around well as streaked with Candida albicans and later culture plates were incubated at 37^0 C for 24 to 48 hours to assess inhibition action, maintaining inoculum volume of 100 micro litre per 20 ml of SDA having a bore diameter of 10 mm, plate size of 100 mm, 125 microlitres of test agent and SDA as a test media

Grouping: For comparative evaluation of wound healing activity, albino rats of same age group, ranging between 100-150 gms, maintained under the similar rearing conditions were selected and categorised into four groups, each having twenty one test animals namely :

- GA- Control Group,
- GB- Treated with Kamilosan drug (German Remedies);
- GC- Treated with 15% concentration of the trial drug and
- GD- 20% Concentration of trial drug.

The animals were sacrificed on the 4th, 8th and 12th day of creation of wound in order to

obtain materials for biochemical and histopathological investigations. Before sacrificing, the degree of inflammation and area of the wound were noted down as a part of macroscopic assessment of healing.

Total duration of study: 15days

Procurement: Animal House, Hillside College of Pharmacy

Start date: 10/2/23, End date: 24/2/23

Observations: Mucopolysaccharide estimation was carried out using Newman and Loghan method. Collagen concentration was estimated based on Elson and Morghan method.

Comparative histopathological study was carried out for all the four groups for assessment of proliferation, differentiation and formation of granulation tissue.

Statistical tests: Various parameters were subjected to biostatistical analysis, encompassing computation of mean, standard deviation and statistical range. Besides 'l' test and 'f test were conducted to assess the significance of trial drug.

RESULTS

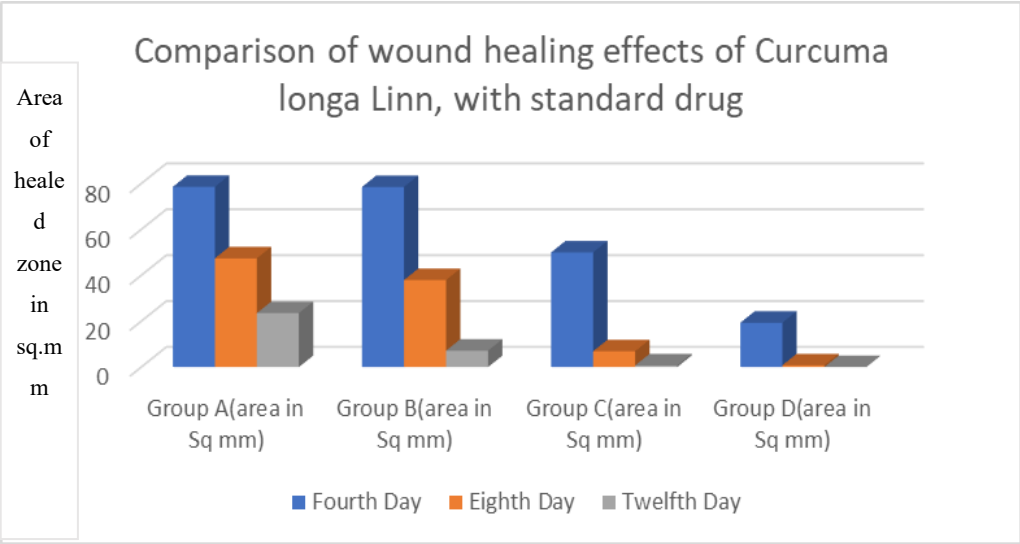
The investigation is centred on antimicrobial, antimycotic and wound healing acceleration phenomena. In vivo models for antimicrobial activity with Staphylococcus aureus failed, as the wound did not take up the infection. The in vitro culture platings of Candida albicans did not exhibit any inhibition zone, even on in easing the concentration of inoculum.

The details of wound healing areas during fourth, eighth and twelfth day of observation in control and the experimental / trial groups are recorded in table 1.

Table 1: Central tendency and dispersion of wound healing activity in different groups of Albino Rats.

No of days	Group A(area in Sq mm)		Group B(area in Sq mm)		Group C(area in Sq mm)		Group D(area in Sq mm)	
	IA X &S	HA X & S	IA X &S	HA X & S	IA X &S	HA X & S	IA X &S	HA X & S
Fourth Day	78.571	0.016	78.573	0.066	78.566	28.578	78.570	59.258
	±0.004	±0.04	±.0005	±0.0081	±0.008	±0.246	±0.00	±0.487
Eighth Day	78.570	31.170	78.573	40.636	78.566	71.658	78.570	77.795
	±0.004	±3.193	±0.005	±1.794	±0.008	±0.115	±0.000	±0.039
Twelfth Day	78.570	55.038	78.573	71.411	78.566	77.826	78.570	78.568
	±0.004	±0.366	±0.005	±0.060	±0.008	±0.045	±0.00	±4.246

IA-Initial Area, HA- Healed Area, X- Mean, S- Standard deviation.



Graph- Comparison of wound healing effects of Curcuma longa Linn, with standard drug

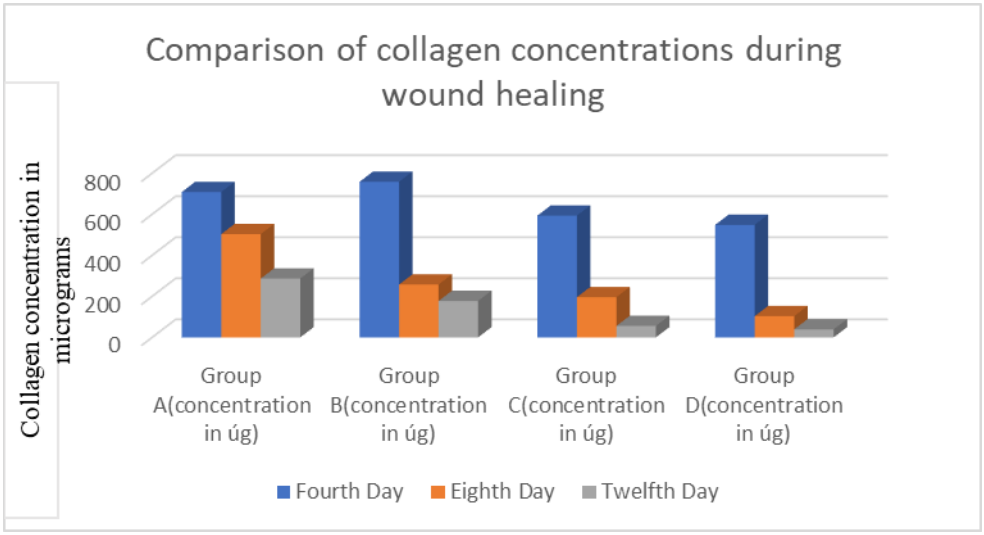
Note: Group A-Control, Group B- Standard, Group C- Trial drug 15%, Group D- Trial Drug 20%

Results of biochemical investigation on mucopolysaccharide and collagen concentrations, during different duration of healing are presented in table 2.

Table 2: Central Tendency and Dispersion of Mucopolysaccharide and collagen Concentrations during Healing

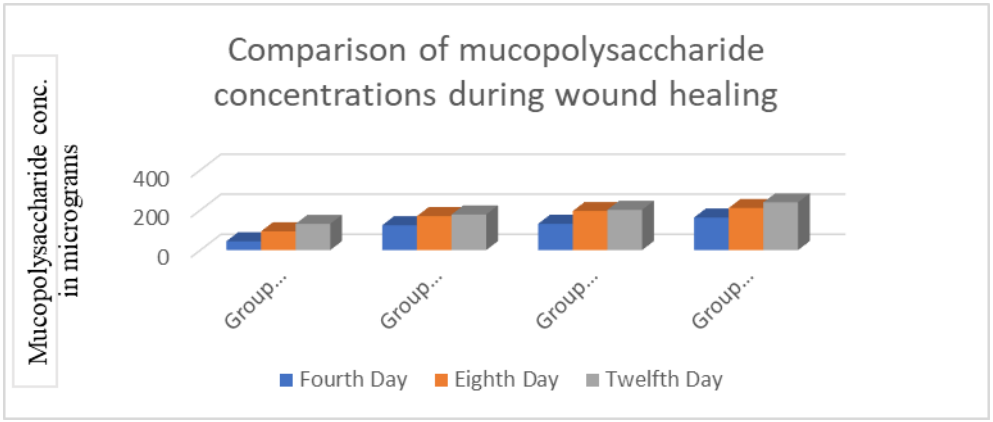
No of days	Group A(concentration in úg)		Group B(concentration in úg)		Group C(concentration in úg)		Group D(concentration in úg)	
	MP X &S	C X & S	MP X &S	C X & S	MP X &S	C X & S	MP X &S	C X & S
Fourth Day	708.330 ±157.650	44.130 ±13.430	758.330 ±67.280	124.330 ±28.177	593.330 ±114.484	131.550 ±19.005	548.330 ±88.633	162.876 ±9.630
Eighth Day	503.330 ±81.649	93.705 ±21.390	257.500 ±51.356	169.715 ±19.120	195.830 ±29.734	195.203 ±7.704	103.330 ±22.500	210.410 ±14.061
Twelfth Day	286.660 ±58.878	131.170 ±16.414	177.500 ±69.48	177.790 ±20.384	55.830 ±26.720	201.420 ±11.313	39.166 ±17.150	238.590 ±11.365

MP-Mucopolysaccharide, C- Collagen, X- Mean, S- Standard deviation.



Graph 2: Comparison of collagen concentrations during wound healing

Note: Group A-Control, Group B- Standard, Group C- Trial drug 15%, Group D- Trial Drug 20%



Graph 3 Comparison of mucopolysaccharide concentrations during wound healing

Note: Group A-Control, Group B- Standard, Group C- Trial drug 15%, Group D- Trial Drug 20%

Comparison of group A with the rest, for area of healed zone, mucopolysachride concentration and collagen concentration for various days of observation with student t' test are given in table 3.

Table 3: Student 't' Test details for various biological parameters

Biological Parameters	Student 't' test values						
	Type of Intervention	Fourth Day		Eighth Day		Twelfth Day	
AHZ	GA vs GB	1.3458	NS	6.322	S	105.72	S
	GA vs GC	279.79	S	30.997	S	151.22	S
	GA vs GD	284.48	S	35.71	S	157.35	S

MPC	GA vs GB	0.7136	NS	1.4595	NS	2.61	S
	GA vs GC	6.23	S	8.65	S	11.55	S
	GA vs GD	2.93	S	8.734	S	9.874	S
CC	GA vs GB	6.26	S	6.48	S	4.35	S
	GA vs GC	9.14	S	6.41	S	8.62	S
	GA vs GD	17.39	S	6.80	S	13.17	S

AHZ- Area of healed zone, MPC- Mucopolysaccharide concentration, CC- Collagen Concentration, GA- Group A (Control Group), GB- Group B (Standard Drug), GC- Group C (Trial Drug of 15% concentration), GD- Group D (Trial drug 20% Concentration), S- Significance at 0.05 P level for df 10, NS- Non- Significance at 0.05 P level for df 10.

Analysis of variance for the aforesaid parameters is presented in table 4.

Table 4: Analysis of Variance for different biological parameters.

Biological parameters	No of Days	Source of variation	Sum of squares	d.f	Variance	F-Ratio	
AHZ	Fourth day	B.W.S	14495.7	3	G.V-4831.9	62952.25	S
		W.S	1.5351	20	S.V-0.076755		
	Eighth day	B.W.S	10994.91	3	G.V-716.96	170704761.9	S
		W.S	67.17345	20	S.V-0.0000042		
	Twelfth day	B.W.S	22751.02	3	G.V-7583.67	217233.01229	S
		W.S	0.698206	20	S.V-0.0349103		
MPC	Fourth day	B.W.S	46149.60	3	G.V-15383.2	1.24	S
		W.S	247722.44	20	S.V-12386.12		
	Eighth day	B.W.S	526674.69	3	G.V-175558.23	65.66	S
		W.S	53474.80	20	S.V-2673.74		
	Twelfth day	B.W.S	241011.10	3	G.V-80337.03	34.54	S
		W.S	46512.38	20	S.V-2325.61		

CC	Fourth day	B.W.S	48357.83	3	G.V-16119.27	59.58	S
		W.S	5410.54	20	S.V-270.52		
	Eighth day	B.W.S	51088.58	3	G.V-17029.59	47.49	S
		W.S	7171.10	20	S.V-358.55		
	Twelfth day	B.W.S	36426.23	3	G.V-12142.07	51.55	S
		W.S	4710.66	20	S.V-235.23		

df- Degree of freedom, AHZ- area of the healed zone, MPC- Mucopolysaccharide concentration, C.C-Collagen Concentration, G.V- Greater variance, S.V- Smaller variance, S- Significance at 0.05 P level for given degree of freedom.

Histopathological changes observed in four groups of animals occurring on fourth, eighth and twelfth days, showing details of multi focal haemorrhages in dermis, congested blood vessels, infiltration of neutrophils, eosinophils, proliferation of angioblasts and fibroblasts, formation of new capillaries, granulation tissues, regeneration of epidermis finally manifesting in wound healing are represented in plates 4a to 4d, 5a to 5d and 6a to 6d.

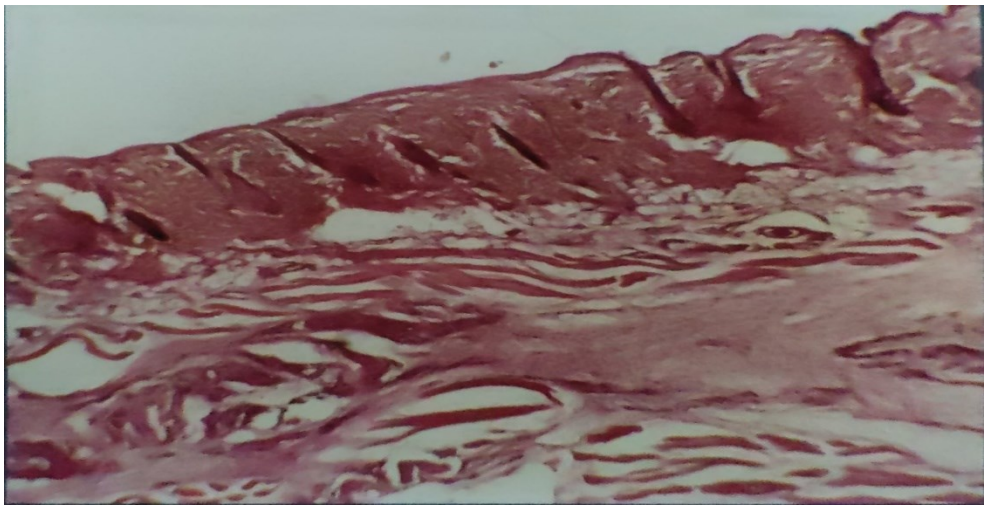
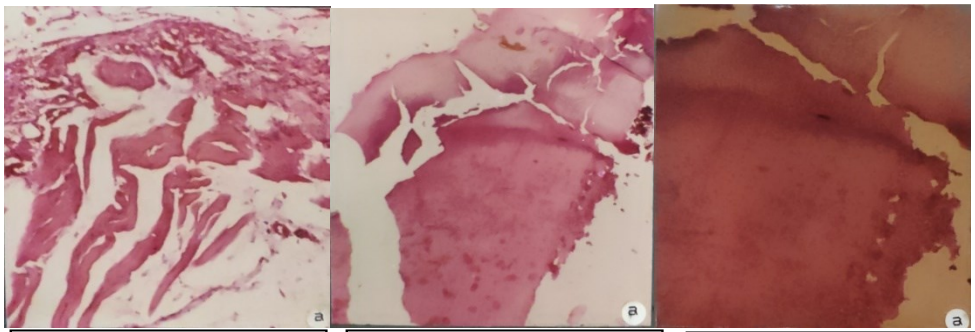


Image 1: Vertical section of normal skin of an albino rat



4a: Control rat-4th day | 5a: Control rat-8th day | 6a: Control rat-12th day

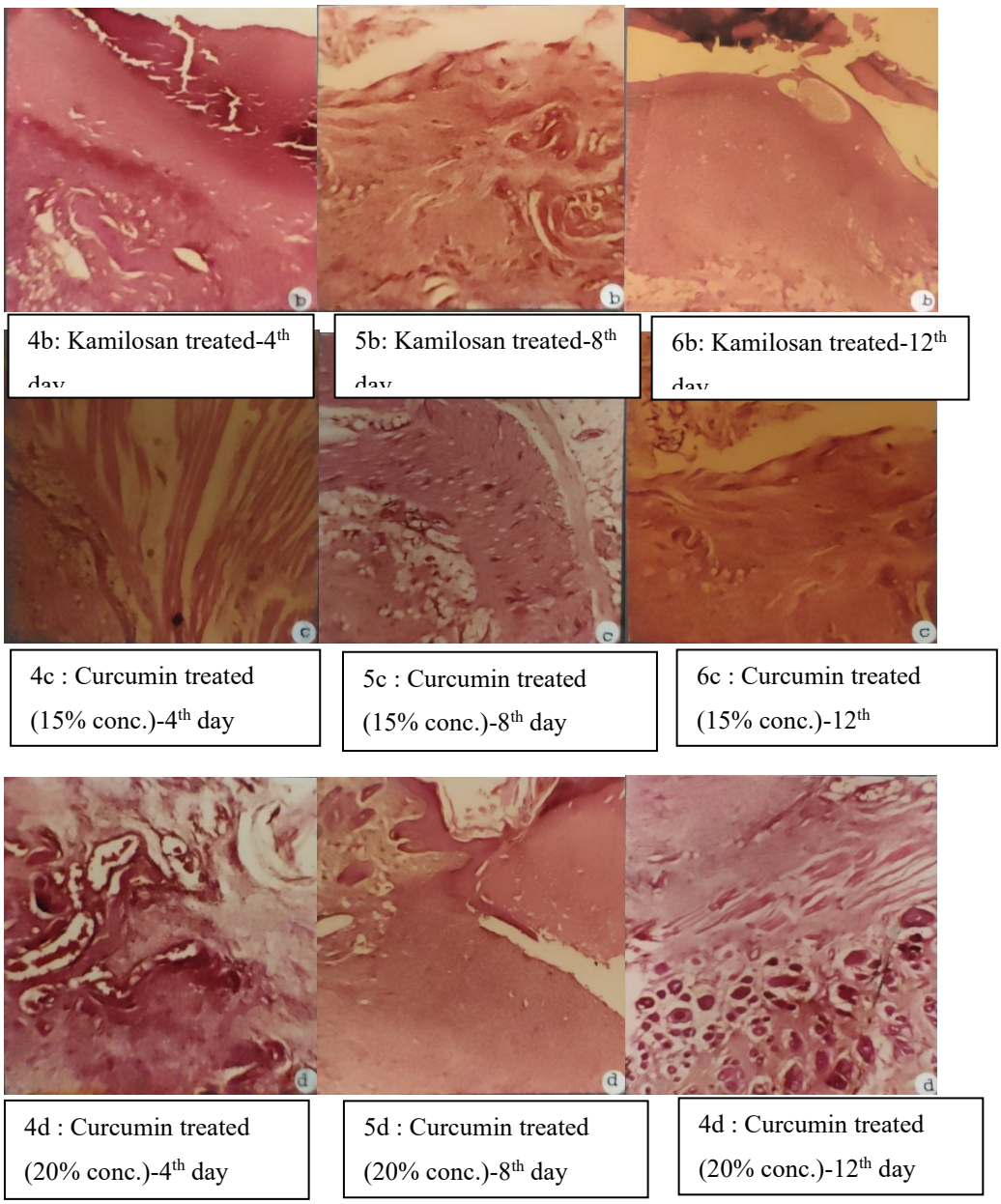


Image 2: Photo Plates: Showing Vertical section of wounded skin of an albino rat on different days of the four groups.

DISCUSSION

Ancient concept of treatment of wounds encompassed simultaneous antimicrobial and acceleration of wound healing processes which has been the main reason for choosing *Curcuma longa* L. Many investigators have contributed on antibacterial, antifungal and anti-inflammatory activities of *Curcuma longa* L. using petroleum ether extracts and its fractions or alcoholic extracts [9]. Usage of such organic solvents in treatment could be toxic on many occasions. Hence, in this investigation aqueous extracts of *Ghanasatva* is employed which would never be detrimental.

Antibacterial activity of *Curcuma longa* L. has been studied by numerous investigators. In this investigation Staphylococcus aureus that was being tried for antibacterial efficacy failed to induce sepsis in albino rats [10]. Hence, direct inoculations of the pathogen into the organism were tried out, that also being futile. This may be due to the inherent resistance of the animals for a common pyogen.

Antimycotic action of *Curcuma longa* L. is being studied by several researchers. In this investigation Candida albicans, which poses a major problem among infants causing oral thrush and other infections in adults was tried to cause infection [11]. However no sepsis could be induced. In ancient classics *Curcuma longa* L. has not been indicated as antifungal drug. It is reported that antifungal action is exhibited only against dermatomycosis and sub-cutaneous mycosis and seventy six extracts examined showed no activity against deep mycosis.

From table 2 and graph 2, it can be visualised that collagen concentration during healing gradually increases in accordance with increased duration. The least increment is seen in control group, where the natural resistance of the animal offers to the wound healing process. Comparison of the standard drug and the trial drug reveals higher efficacy of the latter.

Most of the modern research regarding treatment of any kind of wound would be application of either an antibacterial or antimycotic drug, which could be of synthetic or natural origin. Whenever these drugs are employed in highly refined concentrations, most of them cause iatrogenic effects. Moreover, these drugs have harmful effects on various intestinal flora, and get retained within the system having adverse reactions. Hence, it is imperative to embark upon a totally harmless, broad spectral host independent, antimicrobial drug, having synergetic action, without creating any resistance among microbes. Such a holistic approach is very well recorded in ancient treatises.

Many of the reports on the antibacterial and antifungal activity of *Curcuma longa* L. have attributed the activity due to the presence of curcumin. All these reports involve usage of highly refined compositions. In this study herbal extracts of the drug is made use of in the form of *Ghanasatva* which would offer the optimum dosage. This shows more concentration of water soluble active principles, besides volatile components and also curcumin, have efficacy in wound healing acceleration. The mode of preparation of *Ghanasatva* through *Rasakriya* process appears to be much simple and economical, but being efficacious.

Comparative evaluation of standard drug Kamilosan of herbal origin - aqueous extract of *Chammonella metricaria* flowers, fifteen and twenty percent aqueous extracts of *Curcuma longa* L for their wound healing effect in terms of area of healed zone is presented in Image 2. It can be reaffirmed that rate of healing is several times higher in GC and GD groups possessing *Curcuma longa* L. Comparison of healing on different durations reveals that control and standard drug have negligible effect on healing, while the trial drug has profound influence even on the fourth day. A steady increase in wound healing effect is very well seen on eighth and tenth day, maximum effect being shown by the trial drug.

Results depicted in figure 3 reveal that mucopolysaccharide concentration shows a steep decrease in GB, GC and GD groups, the least being represented by GD, when compared with control drug. Comparison of all the drugs over duration, emphasise that *Curcuma longa* L. has marked activity in production of collagen replacing mucopolysaccharide in the wounds.

CONCLUSION:

Ghana satva of *Curcuma longa* L was prepared to assess the antimicrobial and accelerating wound healing action. An insight into morphological wound healing action observed in this investigation, behaviour of physiological parameters namely collagen and mucopolysaccharide, supplemented by histopathological observations emphatically reveals the supremacy of low cost, but highly elite *Curcuma longa* L. extract over costly commercial products like kamilosan. Thus from this study it can be concluded that ancient concomitant concepts *ropana* and *shodana* actions of the potent drug – *Haridra* with reference to its swift wound healing property.

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