Indian J. Pharmac. 18 : 171-174 INDIAN JOURNAL OF PHARMACOLOGY

ANTIMICROBIAL AND INFECTED WOUND HEALING .RESPONSE OF SOME TRADITIONAL DRUGS

A.M. THAKER AND J.V. ANJARIA
Department of Pharmacology,
Veterinary College, Anand.

1. Significant inhibition of growth of staphylococcus aures, streptococcus pyogenes, corynebacterium spp., escherichia coli, and pseudomonas aeruginosa was observed in vitro by traditional drugs like Ocimum Sanctum, Azadirachta indica, Annona squamosa and Bergia odorata.
2. Healing of thewound by indigenous ointment formulation was comparable to that of nitrofurazone and little better than propamidine cream in mice infected by the oraganisms.The indigenous plant material understudy showed significant inhibition of pathogenic microorganisms and effective healing of infected wounds.

Key phrases: Traditional drugs, infected wound healing, antimicrobial.

Introduction

Folklore and ayurvedic literature claim that Azadirachta indica, Annona squamosa, ocimum sanctum and bergia odorata possess some antiseptic and antimicrobial activity. Rao et al. (1969) observed antiviral property of Azadirachta indica in tissue culture method. Asian livestock (1981) described leaves of Annona squamosa as an antiseptic. Since the plants are still used for wound dressing in indigenous form, this made the basis for scientific investigation to prove their efficacy. Hence the present work was undertaken to study effect of some traditional drugs on the wound healing.

Materials and Methods

Collection and extraction *of plants :*

The leaves of Azadirachta indica, Annona squamosa, Ocimum sanctum and Bergia odorata were collected from the College premises, dried in shade and pulverized to 1000 μ particle size. Powdered material was extracted serially with ether, chloroform, alcohol and water. After evaporating solvent, dry extract was isolated and used to determine in vitro. antimicrobial activity and wound healing property on mice.

In vitro antimicrobial activity :

Staphylococcus aureus, Streptococcus pyogenes, Corynebacterium spp., Escherichia coli and Pseudomonas aeruginosa isolated from animal wounds, as per the Department of Microbiology, were taken for antimicrobial activity testing. Suspension of the extract in propylene glycol (BDH) was used for further tests. 0.1 ml. of inoculum consisting of 6 hours broth culture and 0.1 ml of extract suspension in propylene glycol was added to a test tube consisting of 5 ml glucose broth, while in control 0.1. ml. propylene glycol was added instead of extracts. The tubes were incubated at 37°C

temperature for 24 hours. After incubation bacterial load was calculated by preparing 10 fold dilution in sterilised phosphate buffer saline (PBS pH 7.1) and their subsequent plating by Standard plate count (SPC) method (Seaman, 1963). Bacterial load of the broth culture treated with extract was compared with that of plain control.

Preparation of ointments :

Plain plant extract ointment was prepared by taking accurately weighed required quantum of extract and mixed with simple ointment (B.P. 1958).

Similar ointment formulation was prepared with extracts mixed with simple ointment with zinc oxide base (protected recipe).

Experiment on wound healing:

Twelve groups of healthy albino mice (five each) of same age group were acclimatized for a period of 6 to 8 days and were maintained on uniform diet and management throughout the experimental period.

The area of dorsal plane of thoracolumber region was prepared and skin depth rectangular wounds of 2.00cm X 0.5 cm were surgically induced. A loopful of inoculum of overnight bacterial culture comprising of Staphylococcus aureus, Streptococcus pyogenes, Corynebacterium spp., Escherichia coli and Pseudomonas aeruginosa organisms, previously isolated from animal wounds as indicated was applied for infecting the wound. Forty eight hours were left for infection to set in and then the treatment was started in the following manner.

Group I to IV : Treated with plain formulation of all the four plant extracts seperately.

Group V to VIII Treated with ointments prepared from all four plant extracts with zinc oxide base (protected recipe) separately.

Group IX : Treated with M and B antiseptic cream containing propamidine isethionate 0.15% W/W in a water miscible base).

Group X : Treated with furacin vet. soluble ointment (SKF) containing veterinary nitrofurazone B. Vet C. (0.2% W/W with washable base).

Croup XI : Treated with simple ointment (B.P. 1958),

Group XII : Untreated control.

Such two sets of groups of mice (total 120) were kepr for study. One for 6th day wound healing observation and antoher for 12th day wound healing observation. Application of ointment was done once a day after cleaning with surgical cotton wool. After 6th day and 12th day treatment wound tissues were collected for histopathological study (Luna. 1963; Lille.1965).

Results and Discussion

Out of various extracts. chloroform extract was found to be more active on pilot trials, hence this fraction was taken for further study. The inhibitory effect of the extracts of plants under study was observed to a considerable extent upon test organisms. The mean concentration of organisms taken as difference between control and effect. due to inhibitory effect of various extracts upon various organisms is presented in Table 1. The percent inhibition of organisms as compared to that of control, is presented in Table 2.

The inhibitory effect of various extracts on the organisms is statistically significant ($P<0.05$) however, the effect on different types of organisms wich different extracts is variable (Table 2).

Histopathological *Examination* :

Treatment with zinc oxide base formulation (protected recipe) :

The skin wounds at sixth day of treatment with zinc oxide base formulation of all four plants revealed little necrosis and haemorrhages on the surface of wound. Large number of infiltrating macrophages below the necrotic area and growth of the granulation tissue was observed. These lesions indicated good growth of healing tissue.

The skin wounds at twelve day treatment revealed extensive deposition of collagen fibers. elongated fibrocytes and few capillaries. There war complete absence of hair follicles in healed area. Keratinization was also noticed on completely grown epidermis suggesting complete healing of wounds.

Nitrofurazone vet. and M and B antiseptic cream

The skin wound treated with nitrofurazone vet.

Table 1 : Mean inhibition values of some organisms due to in vitro inhibitory effect of some-plant extracts. (mean of 3 observations).

Name of organisms	Mean inhibition value (x 10^5) after treatment with extreact and incubation at 37°C for 24 hrs.			
	CAZI	CANS	cos	CBO
Staphylococcus aureus	5.55 ± 0.46	100.67 ± 98.12	553.36 ± 551.27	0.42 ± 2.28
Streptococcus pyogenes	2925.17 ± 1043.17	106.25 ± 40.66	23.61 ± 12.45	130.59 ± 56.56
Corynebacterium spp.	67.27 ± 39.63	0.05 ± 0.04	46.43 ± 45.78	0.06 ± 0.03
Escherichia coli	216.67 ± 55.37	790.37 ± 377.14	515.77 ± 185.78	105.72 ± 47.96
Pseudomonas aeruginosa	43.33 ± 29.63	1958.67 ± 1391.63	717.38 ± 486.42	2831.95 ± 2060.92

CAZI = Chloroform extract d Azadirachta indica.
CANS = Chloroform extract d Annona squamosa.
COS = Chloroform extract d Ocimum sanctum
CBO = Chloroform extract d Bergia odorata.

Note : The results of interation between extracts X organisms (analysis of variance) were statistically significant (P<0.05).

Table 2. : Percentage inhibition of various organisms by certain plant extracts.

Organisms	Inhibition by extracts			
	AZI	ANS	OS	BO
Staphylococcus aureus	92.64	97.33	99.23	73.68
Streptococcus pyogenes	99.82	91.06	97.44	89.64
Corynebacterium spp.	99.86	26.31	100.00	85.71
Escherichia coli	91.16	76.98	91.17	94.25
Pseudomonas aeruninosa	4.77	76.57	88.52	94.36

AZI = Azadirachta indica
ANS : Annona squamosa
OS : Ocimum sanctum
BO : Begia odorata

ointment revealed complete healing as characterized by deposition of collagen fibersand maturation of fibrous connective tissue. No infiltrating inflammatory cells were seen. There was complete absence of hair follicles in the healed area. Epidermis was completely covering the healed area. Keratinization of the epidermis was also noticed even at sixth day of treatment.

While,on treatment with M and B antisepticcream. the wound tissue at 6th and 12th day of treatment showed slower healing process as evident by the incomplete epithelial covering of hairless healing area.

Treatment with *plain formulation*

Skin tissues of the wounds treated with plain formulations of all four plants revealed more progressive healing at sixth and twelve day treatment. This was characterised by extensive proliferation of fibrous connective tissue with small number of monocytes. The epithelial layer was found to cover half of the wound.

Simple ointment treated wounds :

Sections of the skin at sixth day treatment revealed haemorrhages with little amount of fibriqonecrotic material and granulation tissue while at twelve day treatment abundant collagenous deposition with granulation tissue and fibrino necrotic material was seen.

Untreated infected control wounds :

Sections of the skin wound at sixth day treatment revealed interference in wound healing, showing severe necrosis and haemorrhages with large number of infiltrating neutrophiis. While at twelve day treatment presence of large number of infiltrating macrophages and moderate amount of granulation tissue indicated delayed and incomplete wound healing.

The results of the present experiment indicated that plant extracts under study have significant ($p < 0.05$) inhibitory effect upon thegrowthof various pathogenic organisms to the extent of 75% to 99Xexcept that of the inhibition of Corynebacteriurn spp.. was 26.31% by Annona squamosa and 4.77% inhibition of Pseudomonas aeruginosa by Azadirachta indica.

In vitro experiments for healing of wounds infected with the same pathogenic microorganisms corborated with the findings of the in vivo experiments. The healing with four indigenous drug formulations with zinc oxide base was comparable to nitrofurazone vet. treated wounds. All 'ointments of indigenous formulations were equieffective. The untreated infected wounds showed much delayed and incomplete healing even on the twelveth day. The healing of the wounds with the plain formulation was slower as compared to formulation with zinc oxide base. It is suggestive that extract formulation with zinc oxide base seems to have synergistic effect on healing compared to plain formulations. The proved antimicrobial activityof traditional drugs on organisms specialty like Corynebacterium and othe studied, may go long way in formulating some intramammar preparations for controlling the problematic Corynebacterium mastitis in milking animals. It may contribute to the preparation of cheap, effective, economic routine would healing remedies for the farmers from the natural resources. WHO and FAO have already initiated the systematic work in this field.

References

ASIAN LIVESTOCK (1980) Revival of traditional veterinay medicines Vol. VI (4) : 33. Summarised report RAPA 43. A preliminary study on tradifional system of veterinary medicine.

BRITISH PHARMACOPIA (1958) General medical council. P. 462. London. Lille, R.D. (1954) Histopathological technique and practical histochemistry P. 107-141 New York, Toronto, Sydney. London. The Blackiston Division, McGraw Hill Book Co.

LUNA. L.C. (1960) Manual of Histological and special staining technique. Armed forces Institute of pathology. 2nd edition p. 28-31 New York: McGraw Hill Book Company.

RAO. A.R.; SUKUMAR S.; PARAMSIVAM. T.\'.; KANALAKSHI, T.V. PARASHURAMAN, A.R. and SHANTHA M. 1969) Study of antiviral activity of tender leaves of margosa tree (Melia azadirachta)on vaccinia virus, A preliminary report. Ind. J. Med. Res. (3) 495 SEAMAN, A. (1963) Bacteriology for dairy student. First edition. p, 131-133. Bombay. B.I. Publication.

Accepted after revision : November TO, 1985

Dr. A.M. Thaker
Assistant research Scientist
Department of Pharmacology.
Vetrinary college,
Haryana Agri. University *Hissar 125004*