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EVALUATION OF WOUND HEALING ACTIVITY OF FOUR SAMPLES OF *SARJARASA* BY EXCISION WOUND MODEL IN ALBINO RATS

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ABSTRACT

Sarjarasa is the oleoresin obtained from the tree *Vateria indica*. There is confusion in the trade regarding the source of this drug and different samples are sold in the name of *Sarjarasa* which in turn badly impacts on the quality of finished products. Hence the present study was undertaken to evaluate three market samples, through organoleptic & experimental methods and compared with the established standards of the genuine sample procured from the original plant. The four samples were subjected for experimental evaluation by excision wound model, and their efficacy as wound healing agent was determined. Among market samples, Chennai sample was found pure, superior and exerted highly significant wound healing activity which is equivalent to original sample. Bangalore and Kerala samples seem to be adulterated and shown less efficacy on comparison with the genuine sample.

Key words – *Sarjarasa* samples, *Vateria indica*, evaluation, wound healing activity

INTRODUCTION

Correct identity of the exudates, is a challenging work due to their amorphous nature, and almost similar looking features. The availability of the genuine sample is crucial owing to scarcity of the source species, high cost and more over it is easier to adulterate. Unfortunately some of the trade and pharmaceutical people took undue advantage of this problem by doing commercial exploitation of niryasa by substitution and adulteration. Different market samples of the one exudate show divergent features and qualities which directly afflicts the quality of the finished product.

In the current scenario of GMP i.e. Good Manufacturing Practices, the quality control of herbal crude drugs and their bio-constituents is of paramount importance in justifying their acceptability by the consumer. With the advent of new analytical methods and sophisticated instrumental technology now it is possible to suggest practically demonstrable rigid quality assurance profiles for herbal drugs and their bio-active constituents[1].

Sarjarasa is the oleoresin obtained from the tree *Vateria indica* belonging to the family Dipterocarpae[2]. It is a huge tree with white flowers and found in the evergreen forests of western India and Karnataka. *Sarjarasa* occurs in rough irregular, solid brittle masses, breaking into angular pieces, 1.5 cm thick, pale white or light yellow in colour, odour fragrant, taste bland astringent[3,4].

There is confusion in the trade regarding identity of this drug and different samples were sold in the name of *Sarjarasa*[5]. Hence the present study has been undertaken to evaluate three different market samples of *Sarjarasa* by means of organoleptic & experimental methods and to match with the established standards of the genuine sample procured from the original plant.

The oleo gum resin of *Sarja* ie *Sarjarasa* is well-known for potent wound healing activity(*Bhavaprakasha Nighantu, Vatadi varga*), used successfully in the management of *vrana, dusta vrina, vranashotha, vipadika, kushtha*[6] etc. Hence in this study, experimental model of wound healing activity has been selected to evaluate the efficacy of three market samples of *Sarjarasa* and to compare with freshly collected sample.

OBJECTIVES-

1. Study of four samples of *Sarjarasa* with reference to their appearance and organoleptic characters.
2. Evaluation of wound healing activity of four samples of *Sarjarasa* by experimental model in albino rats using excision wound method.

MATERIALS AND METHODS

Collection of the samples

Market samples of *Sarjarasa niryasa* were collected from three different raw drug traders The first sample of *Sarjarasa* (S-1) collected from Chennai (Car street, Murugan temple, near Broad way). The second sample of *Sarjarasa* (S-2) procured from Bangalore (Mamul pet, Avenue Rd, K.R. market) and the third sample of *Sarjarasa* (S-3) procured from Kerala (Market Rd, Trissore). The original sample(S-4) was collected by tapping the stem bark of the mature tree *Vateria indica* belonging to Dipterocarpae in Udupi.

Organoleptic evaluation

The samples were subjected to organoleptic examinations, the characters like appearance, size, shape, colour, odour, taste, solubility, were noted.

Experimental evaluation by Excision Wound Model[7]-

Materials / Requirements

Animals – Wister strain Albino Rats 36 in number

Top pan weighing balance, electronic digital weighing balance (10mg), mortar and pestle $KMNO_4$ Stain, trial samples, tila taila, gas stove, vessels, dropper, sterilizer, scissor, toothed forceps, iodine tincture, cotton, anaesthetic ether, marker pen, trace paper, $1mm^2$ graph sheet.

Selection of the Animals

Wister strain albino rats of either sex with a body weight between 150 – 200 gms, bred in the animal house at SDM Ayurvedic College, Udupi, were used as experimental models. The animals were housed in group of six in each cage under controlled environment conditions as per the guidelines of CPCSEA. The animals were housed under standard laboratory conditions (12hr light/ dark cycle, at 25 ± 2˚ C). They were allowed free access to a standard dry rat pellet diet and water ad libidum.

The rats were selected randomly and grouped into six groups(A- F) of six rats each. The rats in each group were stained at their different body parts for correct identification.

Procedure -

Preparation of medicine:

10 g of *Sarjarasa* sample weighed and added to 40 ml of Tila taila taken in a

steel vessel and heated till *Sarjarasa* completely dissolved in taila. Then the mixture is filtered with a cora cloth and allowed to cool. On cooling the mixture of *Sarjarasa* and *taila* attains thick viscid form and hence it is heated indirectly in hot water bath daily before application on wounds.

Wounding (Excision wound)-

All procedures employed in present study were reviewed and approved by Institutional Animal Ethical Committee of SDM College of Ayurveda Udupi.

The wounding procedure was carried out in albino rats under ether anaesthesia taking all aseptic precautions. A handful of cotton swab is saturated with 5 ml of Diethyl ether(Anaesthetic Ether) and kept inside a transparent jar. The animal was placed inside the jar and closed with the lid. In about 5-8 minutes when the rat undergoes sufficient anaesthesia it is taken out and placed on the operation table.

An area of 300 mm^2 on the back of the rat was shaved using scissor and blade. A circular mark of 225 mm^2 was made in the shaved area and full thickness of skin was cut off along the edge of the circular mark using sterilized scissor. Immediately after wounding it was swabbed with cotton saturated with Iodine tincture.

Wound care

Each wounded rat was housed independently in separate cages to ensure protection from injury and contamination from other rats. The cages were cleaned and bedding was replaced daily to maintain good hygiene. Wounds were traced on transparent trace paper and the size determined using 1 mm^2 graph paper on the day of wounding(Initial size/day-1). The animals were inspected daily for their general health status and wound healing. To monitor the changes in the wound shapes, tracing of wound margin and size determination was done subsequently on 3^{rd}, 6^{th} , 9^{th} ,12^{th} ,15^{th} day of wounding and thereafter daily till complete healing. The markings on the trace paper were again retraced on a millimeter scale graph paper using carbon sheet and the wound size was determined in mm^2 . Percentage wound contraction and duration of complete epithelialisation were noted and compared.

Wound contraction-

The important parameter which indicates the wound healing is wound contraction. The approximation of the wound margin was recorded using trace paper and graph sheet at different days. The percentage of wound contraction ie size reduction is calculated using the formula –

% wound contraction = (Initial wound size ─ specific day wound size) × 100/
Initial wound size

The percentage wound contraction was recorded on 3^{rd}, 6^{th} ,9^{th} ,12^{th} ,15^{th} ,days and thereafter daily up to 21 days.

Period of complete healing (epithelialisation)

It was monitored by noting the number of days required for the scar or crust to fall of from the wound surface without leaving any mark in the wounded area.

Statistical analysis

The wound size and wound contraction observed in different groups (n=6) are presented as mean ± S.E.M. and analyzed for statistical significance using one way ANOVA followed by Dunnet's t test as post hoc test. The 'P' value lesser than 0.05 was considered significant. Computer stat soft-

ware package Sigma stat version 3.5 was used for analysis.

OBSERVATIONS AND RESULTS –

The organoleptic features such as colour appearance odour etc of four samples are depicted in table no. 1

Results of wound healing activity –

The progressive changes in wound size on specified days are depicted in table 3. Number of days required for falling of scab without any residual raw wound (period of epithelialization or healing) also shown in table 3. Percentage wound closure calculated and shown in table no. 4

The mean percentage reductions observed in the respective trial and taila groups were compared with the mean % reduction obtained in control group.

DISCUSSION

Organoleptic characters

Among four samples Chennai sample(S1) and the original sample(S4) were observed in white colour crystal like resinous exudates with balsamic odour. They were pure with no added physical impurities. On trituration with water gave milky white suspension. When heated with tila taila dissolved quickly, and yielded yellowish viscid emulsion of graded density. The sample 2 from Bangalore found in brownish yellow coloured large lumps with characteristic balsamic odour. A little amount of bark and other impurities were found in the sample. The Kerala sample was seen as smaller lumps with dark brown colour and found mixed with larger quantity of bark and other physical impurities. On triturating with water gave turbid dark brownish suspension. When heated with tila taila dark brown coloured thick viscid paste like mass formed.

Experimental evaluation

On analysis of % wound closure readings during 6^{th} and 9^{th} day, no statistically significant change in the wound closure among the groups observed. The differences in the mean values among the trial and control groups are not great enough to exclude the possibility that the difference is due to random sampling variability; and there is not a statistically significant difference as P values for 6^{th} and 9^{th} day readings are 0.151 & 0.128 respectively.

Comparing the pairwise difference in median values of wound contraction between negative control and taila group on 12^{th} day , it is noted that there is improvement in taila group and maximum percentage of contraction observed in rats of C group which are treated with topical application of Chennai sample when compared with original sample. The Bangalore and Kerala samples also showed remarkable contraction.(table 3).

On analysis of 15^{th} day readings it was noted that all the trial groups treated with samples of Sarjarasa showed statistically significant improvement in different grades(F > C > D > E) where C- Sample 1; D-Sample 2; E- Sample 3; F – Sample 4, when compared with negative control and tila taila group. (table 3)

On reviewing the mean percentage reduction of wound size in different groups (table 2) following assumptions can be made

Wound healing is a natural physiological process which is a part of body's defense mechanism and in rats this process took an approximate duration of 17 to 21 days. During first few days (3^{rd}, 6^{th} , & 9^{th} day reading) no much recognizable change in the wound closure among the groups ob-

served. But from 9th day onwards changes in the wound size in different groups were remarkable.

On 15th day mean percentage closure in control was 88.01, and in taila group 92.16 and the difference in the means was found to be 4,15 . The closure in the group F (s4) where original sample was applied was maximum 97.32 which was highly significant. The reduction of wound size in group C(s1) was 96.39 and this change is nearer to that observed in original sample group. The changes in the wound size in D(s2) & E (s3) groups were 94.81 and 94.48.which are almost same.

From 17th day onwards complete healing was noticed in different groups (Table – 3) as follows. In Group B(taila) on 19th day ; in Group C(s1) & E(s3) on 18th day and in Group D (s2) and F(s4) on 17th day complete closure of wound(100%) noticed. It was also noticed in the present study that during the wound care no rat shown any type of complications like secondary infection, bleeding etc.

CONCLUSION

A comparative study of three market samples of *Sarjarasa* collected from Chennai, Bangalore and Kerala w.s.r. to the macroscopic and organoleptic characters revealed their genuine identity when compared with the original sample procured by taping the stem bark of the tree *Vateria indica* belonging to Dipterocarpae. Experimental evaluation of these four samples w.s.r. to the wound healing activity by excision wound method, showed comparatively greater efficacy of the original sample. Among three market samples, Chennai sample(S1) was found pure and superior in white colour crystal like resinous exudate with balsamic odour. There was no added physical impurities and the sample exerted highly significant wound healing activity which is equivalent to that shown by the original sample. Bangalore and Kerala samples found with impurities and shown less efficacy on comparison with the genuine sample.

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Table-1 Features of four samples of *Sarjarasa niryasa*

Features	Sample 1 (Cheennai)	Sample 2 (Bangalore)	Sample 3 (Kerala)	Sample 4 (original)
Appearance	Irregular crystalline	irregular larger pieces	irregular smaller pieces	Translucent harder pieces
Impurities	Absent	Traces of physical impurities	Present with woody materials	Absent
Colour	Dull white	brown	Brownish black	White
Aqueous suspension	Whitish brown emulsion	Turbid brown dark emulsion	Dark brownish black	White emulsion
Odour	Balsamic agreeable	Balsamic agreeable	Dull balsamic	Balsamic strong agreeable
Taste	Astringent bitter	Faint bitter	Bitter atringent	Astringent

Table No 2 (GroupWise drugs and dosage)

Group	Drug	Dose(topical application)
Group – A	Control group	-
Group -B	Tila taila	Quantity sufficient
Group -C	*Sarjarasa* Sample -1 (Chennai)	,,
Group -D	*Sarjarasa* Sample 2 (Bangalore)	,,
Group -E	*Sarjarasa* Sample -3 (Kerala)	,,
Group -F	*Sarjarasa* Sample -4 (original)	,,

Table 3 -Wound size in different groups at specified days (values in mm)^2

DAY >	1	3	6	9	12	15	16	17	18	19	20	21
A	187.17	139.00	113.00	73.50	44.17	21.17	10.17	6.33	3.00	1.33	0.50	0.33
B	224.33	178.17	131.67	73.33	41.83	18.17	7.50	2.83	1.67	0	0	0
C	240.00	167.50	122.33	71.33	14.83	8.67	4.17	2.83	0	0	0	0
D	192.50	126.00	83.50	41.50	21.50	10.00	2.83	0	0	0	0	0
E	211.33	158.83	113.00	49.33	21.50	11.67	5.17	1.67	0	0	0	0
F	186.67	124.50	89.17	45.33	17.50	5.00	0.33	0	0	0	0	0

Group A - control ; Group B – taila; Group C- Sarjaras Sample 1; Group D-Sample 2; Group E- Sample 3; Group F – Sample 4

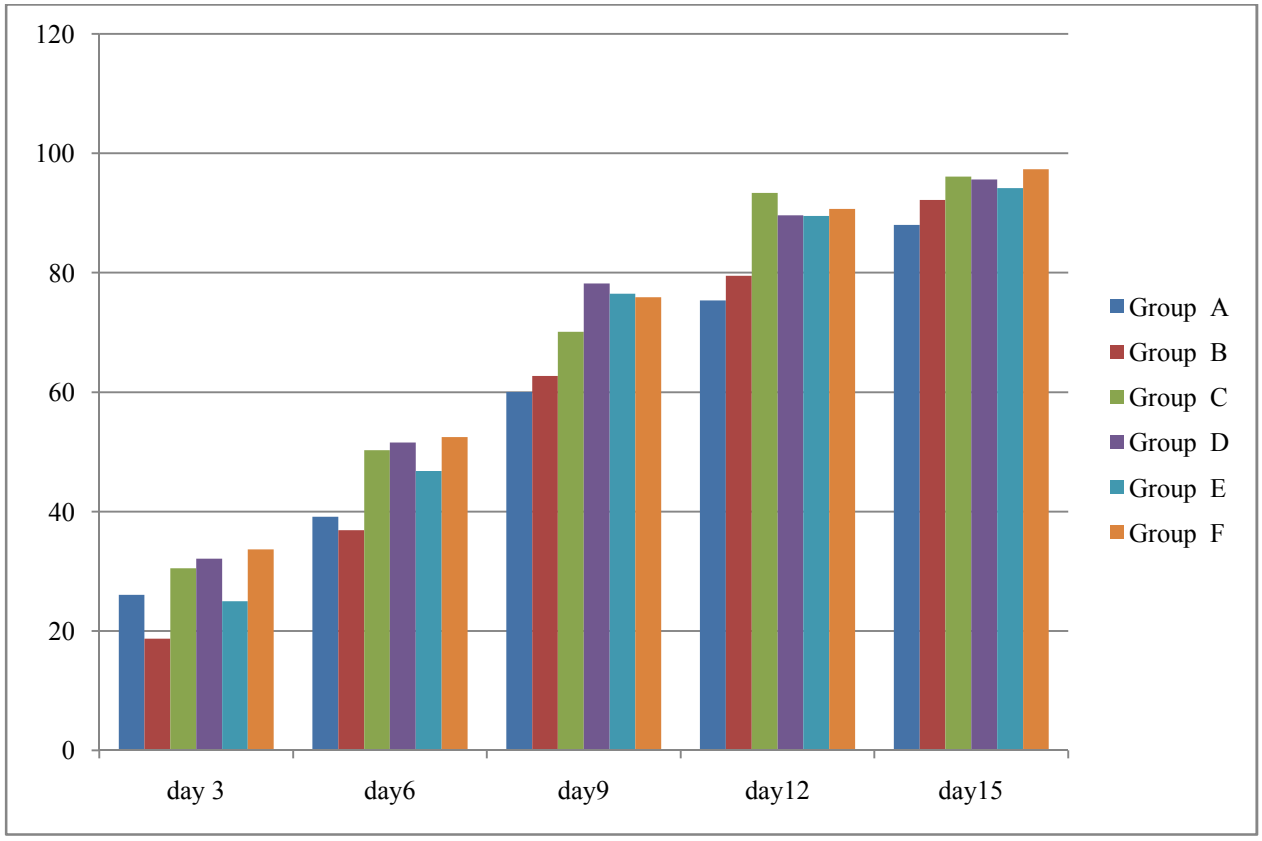
Table 4 Percentage wound contraction on specified days

Groups	6th day	9th day	12th day	15th day
A	39.148 ± 5.88	60.043 ± 6.71	75.341 ± 6.82	88.004 ± 3.96
B	36.884 ± 7.17	62.683 ± 9.53	79.453 ± 6.08	92.163 ± 1.12
C	50.264 ± 3.71	70.090 ± 4.89	93.375 ± 1.25	96.105 ± 0.72

D	51.546 ± 4.55	78.221 ± 2.91	89.619 ± 0.92	95.611 ± 0.84
E	46.796 ± 1.56	76.463 ± 2.11	89.479 ± 1.25	94.180 ± 1.05
F	52.448 ± 3.17	75.910 ± 3.97	90.701 ± 1.69	97.325 ± 0.67

Group A - control ; Group B – taila; Group C- Sarjaras Sample 1; Group D-Sample 2; Group E- Sample 3;
Group F – Sample 4

Fig 1 Wound contraction % in different groups at specified days



Group A - control ; Group B – taila; Group C- Sarjaras Sample 1; Group D-Sample 2; Group E- Sample 3;
Group F – Sample 4

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