

INFLUENCE OF THE FRUIT AND LEAF EXTRACT OF *PSIDIUM GUAJAVA* LINN. ON WOUND HEALING IN WISTAR RATS

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Abstract: Leaf extract of guava (*Psidium guajava* Linn.) has been reported to promote wound healing in traditional medicine but there is paucity of information in literature regarding similar activity of fruit extract. Therefore, the present study was planned to investigate the influence of leaf and fruit extract of *Psidium guajava* Linn. on re-sutured incision, excision, and dead space wounds in male Wistar rats. Wounds were inflicted under light ether anesthesia aseptically. Control animals received vehicle and other groups received aqueous extract of either leaf or fruit orally in the dose of 500mg/kg/day for a period of 10 days in the incision and dead space wounds, whereas treatment continued till complete wound closure in excision wound model. On the 10th day after estimation of breaking strength of the resutured incision wounds, animals were sacrificed and granulation tissue from dead space wounds were used to estimate the breaking strength and hydroxyproline content. Quantification of granulation tissue and histopathological slides were also carried out. Wound closure rate, epithelization time and scar features were studied in the excision wounds from the day of extract administration till complete closure of wounds. Only aqueous fruit extract significantly ($P < 0.01$) promoted the healing process in all three wound models studied. Histopathological slides revealed increased collagen content and granulation tissue in fruit extract treated group as compared to that of control. In contrast, the leaf extract delayed excision wound healing and had no effect on incision and dead space wounds. These findings merit, clinical evaluation of *Psidium guajava* fruit.

Key words: *Psidium guajava*, Wounds healing, Wistar rats

INTRODUCTION

Extensive research on wound healing has lead to identify several factors including drugs that influence the healing process. Drugs like famotidine [1], desipramine, clomipramine [2] have been reported to promote wound healing while others (cyclophosphamide, 5-fluorouracil, colchicine, amitriptyline) have retarded the healing process [3]. Nevertheless, due to their adverse reactions, drugs have limited role in day to day clinical practice to foster the healing process. Therefore, relatively safer and easily available plant products have been explored for their favorable influence on healing process. Several plant products used in traditional medicine to hasten wound healing e.g. *Moringa oleifera* [4] and

Bryophyllum pinnatum [5] have been reported to promote the healing in experimental animals.

Psidium guajava, is a common plant, grown all over India for its delicious fruits ‘gauva’. Its leaves have been used to enhance external wound healing in traditional medicine, but there is no information about rest of the plant parts. Therefore in present investigation, the fruits extract is explored for its influence, if any, on wound healing. The leaf extract is also studied simultaneously for comparative purpose.

MATERIALS AND METHODS

Animals: Healthy, male adult, Wistar rats weighing

150-250 g (procured from Central Animal House of the Institute) were housed individually and maintained on standard pellet diet (Amrut brand) with water *ad libitum* maintained at 12 h of light and dark cycle in the departmental laboratory for acclimatization. The study protocol was approved by the institutional animal ethics committee and ethical norms were strictly followed during experimental procedures. After careful depilation (without injuring the skin) at the wounding site the animals were starved overnight prior to the day of experimentation and were divided into control and treatment groups (n=6, in each) for each wound model.

Extracts: The leaves and fruits (white pulp) collected locally, were authenticated by the botany department of R. L. Science Institute, Belgaum. They were washed and dried in shade before subjecting for aqueous extraction. Soxhlet extraction was carried out using distilled water as solvent. The extract was obtained in liquid form and then it was allowed to evaporate at room temperature so as to obtain the powder extract, which was stored in air tight container till further use. The powder was reconstituted with distill water to obtain a strength of 200 mg/cc. The treatment groups were feed with either the fruit or leaf extract in dose of 500 mg/kg/day (dissolved in water). Food and water was withheld for 2 h prior to and after administration of the extract. Animals in control groups were administered with equal volume of distil water. The treatment every 24 h was continued for a period of 10 days in case of rats with incision and dead space wounds, while in excision wound groups till the complete epithelialization.

Wound models: (1) Excision wounds were inflicted as described by Morton and Malone [6] by excising the full thickness circular skin (approximately 500mm²) from the nape of the neck under ether anesthesia. Wound closure rate was assessed by tracing the wound on polythene paper on wounding day (0), followed by 4th, 8th, 12th, 16th day and subsequently on every alternate day/daily till complete closure. Falling of the scab without any raw area indicated time for complete epithelization and the same was noted. Similarly, scars were traced on complete epithelization to assess wound contraction by noting scar size and shape. Round/oval, large scars indicated poor contraction while stellate shaped or linear scars indicated enhanced wound contraction.

(2) Re-sutured incision wounds were inflicted with

two 6 cm long Para vertebral parallel incisions under light ether anesthesia as described earlier [7]. Sutures were removed on 8th day and breaking strength was measured on 10th day post wounding by continuous water flow technique as described by Lee [8]. Three readings were taken on each wound and the mean of six such readings in each animal was used for statistical analysis. Subsequently, the animals were sacrificed by overdose of anaesthesia.

(3) Dead space wounds in each animal (under light ether anaesthesia) were inflicted by implanting two sterile cotton pellets (10 mg) and two cylindrical grass piths (25 mm x 3 mm) subcutaneously in the axillae or groin randomly. The granulation tissues were removed on 10th day post wounding under ether anaesthesia and later animals were sacrificed by over anaesthesia. Granulation tissues covering the cotton pellet were dried at 60°C overnight to record the constant dry weight, expressed as mg/100g body weight [9]. One of the granulation tissues over the grass piths was opened and trimmed to a rectangular piece for estimation of breaking strength and subsequent estimation of hydroxyproline content colorimetrically [10]. The other granulation tissue grown on the grass pith was preserved in 10 percent formalin for 24 hours for fixation before processing for paraffin sectioning. All the tissues after fixation were processed in a histokinite as per standard procedures laid down and the paraffin sections were cut in a standard rotary microtone at 5 microns thickness. The sections were then stained with Van Gieson stain for collagen and the collagen content was assessed by observing under the light microscope. All the surgical procedures were carried out aseptically and none of the animals were treated with local or systemic antimicrobial agents.

Statistical analysis: All the results were expressed in mean $\pm$ SEM. The data were analyzed by ANOVA followed by Dunnet's test, $p < 0.05$ was considered significant.

RESULTS

Re-sutured incision wounds: The fruit extract significantly ($p < 0.01$) increased wound breaking strength in contrast to leaf extract which insignificantly decreased breaking strength as compared to control groups. (Table 1).

Dead space wounds: Fruit extract significantly ($p < 0.01$) increased breaking strength of granulation

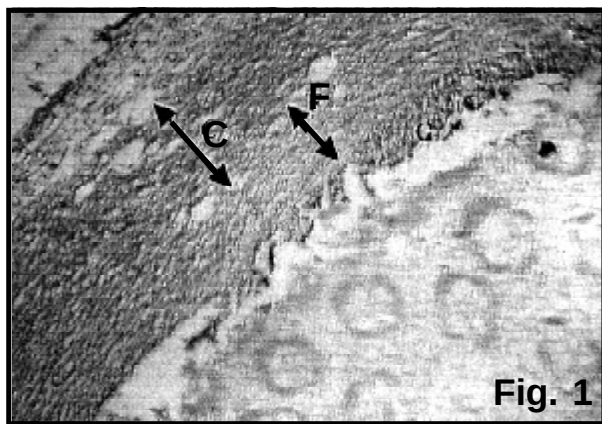


Fig. 1

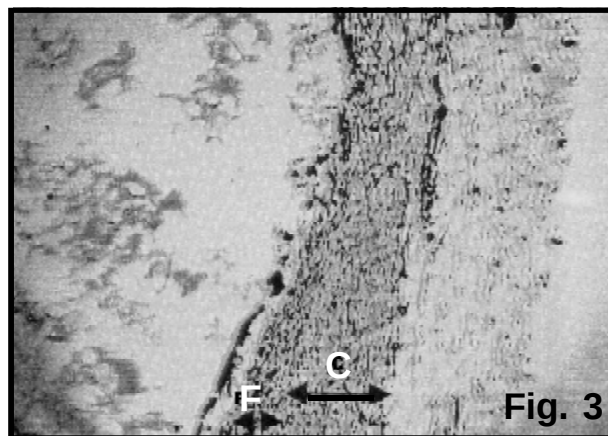


Fig. 3

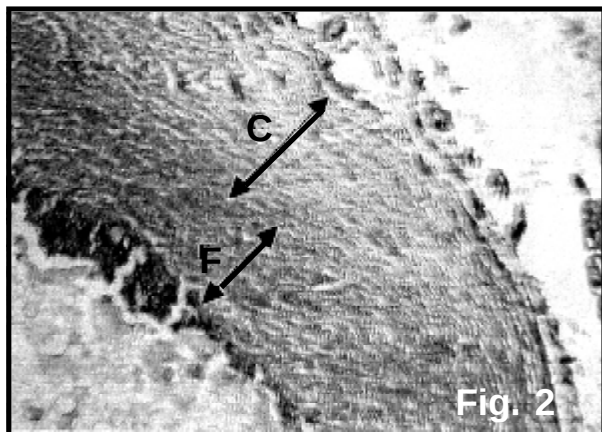


Fig. 2

Figs. 1-3 are microphotographs of granulation tissue stained with Van Gieson stain. **Fig. 1:** Control group 10 X. **Fig. 2:** Fruit extract treated - Marked increase in fibrocollagen content as compared to other groups 10 X. **Fig. 3:** Leaf extract treated 10 X. (C- Collagen, F- Fibrous tissue)

Histopathological studies revealed increased fibrocollagen and granulation tissue in fruit extract treated group (Fig. 2) as compared to that of control (Fig. 1), but a slight decrease in fibrocollagen content than the control was seen in leaf extract treated group (Fig. 3).

tissue as compared to control but insignificant reduction in breaking strength was seen in leaf extract treated group (Table 1).

Cotton pellet granuloma weight (mg % of body weight) was increased significantly ($p < 0.01$) in fruit extract treated group (79.72 ± 1.86) as compared to controls (32.28 ± 1.01), while it was decreased in leaf extract treated group (30.15 ± 1.05) which was not significant. Hydroxyproline content was significantly ($p < 0.01$) increased only in fruit extract treated group as compared to that of control (Table 1).

Excision wounds: The rate of wound closure in fruit extract treated group was significantly ($p < 0.01$) more on 4th, 8th, 12th, 16th day as compared to that of control (Table 2). In contrast, the leaf extract significantly ($p < 0.01$) decreased the percentage of wound closure from 8th day onwards as compared to that of control (Table 2).

The time for complete epithelization was 17.83 ± 0.40 days in control group, while it was significantly ($p < 0.01$) decreased in fruit extract treated group with a mean value of 15.17 ± 0.16 days. However, time for epithelization (20.83 ± 0.30 days) was significantly

Table 1: Effect of various extracts of *Psidium guajava* Linn. on resutured incision and dead space wounds. * $P < 0.01$

Groups (n=6)	Resutured incision Wound breaking strength (g)	Granuloma Breaking strength	Granuloma dry Weight (mg % of body weight)	Hydroxyproline ($\mu\text{g} / 1 \text{ g}$ of wet granulation tissue)
	Mean $\pm$ SEM			
Control	187.0 ± 8.699	203.3 ± 12.29	32.28 ± 1.01	6.56 ± 0.41
Fruit extract	$234.2 \pm 7.171^*$	$245 \pm 5.627^*$	$79.72 \pm 1.862^*$	$13.79 \pm 0.576^*$
Leaf extract	169.5 ± 2.187	190.7 ± 3.169	30.15 ± 1.057	5.78 ± 0.455

Table 2: Effect of various extracts of *Psidium guajava* Linn. on excision wounds. * $P < 0.01$, ** $P < 0.05$

Groups	Wound closure (% of original area) in mm^2 on day					
	4 th	8 th	12 th	16 th	Days for complete closure	Scare area (mm^2)
	(Mean $\pm$ SEM)					
Control	12.39 ± 2.41	57.23 ± 4.31	79.99 ± 1.08	93.33 ± 1.715	17.83 ± 0.401	44.50 ± 3.344
Fruit extract	$23 \pm 2.031^*$	$71.30 \pm 0.90^*$	$94.12 \pm 0.54^*$	100**	$15.17 \pm 0.16^*$	$28.67 \pm 1.14^*$
Leaf extract	13.25 ± 1.20	$44.49 \pm 1.74^*$	$72.87 \pm 1.45^*$	$84.12 \pm 2.28^*$	$20.83 \pm 0.3^*$	$53.67 \pm 1.2^{**}$

($p < 0.01$) increased in leaf extract group. The mean area, $28.67 \pm 1.14 \text{ mm}^2$ of stellate/ linear scars in fruit extract treated group was significantly ($p < 0.01$) reduced as compared to $44.50 \pm 3.34 \text{ mm}^2$ of control group. In leaf extract treated group scars were significantly ($p < 0.05$) larger with mean area of $53.67 \pm 1.28 \text{ mm}^2$ as compared to controls, indicating poor wound contraction.

DISCUSSION

The results of the present study clearly indicate that fruit extract has enhanced the healing of all types of wounds studied while leaf extract failed to do so. The finding of the present study differs from an earlier report in which intraperitoneal administration of fruit extract at dose of 25 mg/kg decreased granuloma formation in rats [11]. The discrepancy could be explained on the basis of difference in the dose and route of administration of the fruit extract used in this study.

There are no reports regarding the influence of systemically administered aqueous extract of leaf and fruit of *Psidium guajava* on re-sutured incision and excision wounds. However, in traditional medicine leaves have been reported to promote the healing of open wounds when dressed with it. This prohealing activity of leaves on open wounds could be attributed to their antimicrobial activity since, the leaf extract have been reported to possess antimicrobial activity [12]. Lack of prohealing activity of leaf extract in open wounds as observed in the present study could be explained on the basis of method of treating. In the present study, the extract has been feed orally while in traditional medicine leaves were applied to the wounds. Moreover, such reports on prohealing activity of leaves are not based on scientific evaluations.

Earlier reports that leaf extract has anti-inflammatory activity [13] support our findings as anti-inflammatory drugs suppress healing [8]. Prohealing activity of fruit extract as observed in the present study is corroborated by other findings of the study such as markedly increased fibrocollagen (histopathological studies) and significantly ($p < 0.01$) increased hydroxyproline content of granulation tissue.

Fruit extract has been reported to contain vitamin C, Zn, quercetine etc. [14]. Quercetine rich leaves of *Bryophyllum pinnatum* [5] and Zn containing fruits

of *Moringa olifera* [4] have been reported to promote wound healing. Thus, the prohealing activity of *Psidium guajava* fruit extract could be attributed to its constituents like quercetine and Zn.

The finding of the present study establishes the prohealing activity of *Psidium guajava* fruit pulp. If the finding of the present study could be extrapolated to human situation, consumption of the fruit may promote healing of post operative and clinical wounds. However, this needs to be confirmed clinically.

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