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Research Article

Wound healing and anti-inflammatory activity of extract of Ficus racemosa linn. bark in albino rats

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ABSTRACT

Background: F. racemosa is an indigenous plant having anti-secretory, anti-diabetic, anti-ulcer etc. properties. It is used widely in the ayurvedic medicines.
Methods: The experimental models of wound and inflammation were used to assess the wound healing and anti-inflammatory properties of F. racemosa. The significance of differences was analyzed using students' 't' test.
Results: In the strength of 10% local application it could apparently enhanced the process of healing. At the dose of 20 mg/100 gm intraperitoneally it could show inhibition of carageenan induced acute inflammation at 3rd, 5th and 7th hour and at the dose of 30 mg/100 gm intraperitoneally, formalin induced subacute inflammation was inhibited till 4th day. The results were found statistically significant.
Conclusions: Aqueous extract of F. racemosa has got wound healing and anti-inflammatory activity. It is likely that the duration of action may be shorter.

Keywords: Ficus racemosa, Anti-inflammatory, Wound healing, Indigenous

INTRODUCTION

It is almost axiomatic that injury is followed by inflammation. It can be characterized by a vascular and cellular response designed to defend the body against alien substance and dispose of dead and dying tissue preparatory to the repair process. The word healing refers to the body's replacement of destroyed tissue by living tissue.

Ficus racemosa is the plant found in many parts of India. It is known by various names; as '*Umbro'* (Gujarati), *'Gular'* (Hindi), '*Udumbar'* (Sanskrit) etc. F. racemosa is mentioned in Ayurveda for the therapy of acidity (Amplapitta). The bark is also used for the treatment of various disorders like diabetes, asthma, dysentery, menorrhagia, etc. in Ayurveda.[1]

The bark powder is applied on ulcers and it forms one of the ingredients of 'Panchvalkal Kwath' which is commonly used in dressing wounds and ulcers.[2] The research work has been done to see the anti-ulcer and anti-secretory property.[3,4] There are data regarding its etanolic extract of leaves having hypotensive and vasodilator property.[5] The alcoholic extract of stem bark possess anti-protozoal activity against E. histolytica and has also shown hypoglycemic activity in albino rats.[6] However limited work has been done to see its wound healing and anti-inflammatory activity.

The present study was undertaken to detect its wound healing property and anti-inflammatory activity of its aqueous extract on experimental external wounds and inflammatory models in the albino rats.

METHODS

Plant and Extract

The plant of F. racemosa was obtained from the local source as per the standard guidelines. The bark was cut and dried for 10 days at room temperature. The powder was obtained by fine grinding.

200 gm of powder was added to 500 ml of distilled water and heated in water bath for 75^0C for 5-10 minutes; the powder was heated thrice in 24 hours. The content of beaker was filtered by Wattman's filter paper and filtrate was collected. After one hour, the remaining powder on the filter paper was taken back into the same beaker and immersed with another 200 ml of distilled water and

heated by the same way for 24 hours and same procedure was repeated and the filtrate was collected in another beaker. Again after one hour, the powder on the paper was taken back and the same procedure was repeated. So, total 900 ml of distilled water was added in 200 gm of powder. Same procedure was done on another 200 gm of powder. So, total 400 gm of powder was taken and total 1800 ml of distilled water was added.

All the filtrate was collected in the large beaker and in that 4 ml of Chloroform was added as a preservative. The beaker was put on the water bath at 100^0C. So, water got evaporated and a mass of aqueous extract was gained after three days.

Weight of the aqueous extract was calculated by comparing weight of the beaker with the extract (222 gms) and without the extract (182 gms). So, it was 40 gms. So, from 400 gms dried powder 40 gms (10 %) aqueous extract was collected.

The study was done to evaluate wound healing property and anti-inflammatory activity.

Animals and Models Used

Wistar albino rats (150-300 gms) of either sex, properly fed, kept in separate cages and procured from Gujarat Ayurved university, Jamnagar. All the animals were provided with food and water during the experiments.

Models

Wound healing: (i) incised wounds (ii) full thickness excised wound.

Anti-inflammatory activity: (i) Carageenan induced hind paw edema (acute inflammation) and (ii) Formalin induced hind paw edema. (sub acute inflammation)

Incised Wounds

The animals were anaesthetized with a cotton pallet soaked in ether; the pallet and animal both were placed under a bells jar. Within 10 minutes the animals were anaesthetized. Their backs were shaved and cleaned with spirit and savlon. Two incised wounds of 1 cm each were made with a scalpel and a forceps. One was made between the forelimbs slightly on the right side to the vertebral column; and another between the hind limbs slightly on left side to the vertebral column. Here randomly, one of the wound was considered as control and another as treated. All the wounds were sutured with 4-0 silk. Two stitches were taken.

The control limbs were applied with the plain white paraffin jelly and the treated wounds were applied with the extract mixed with paraffin jelly. The placebo and the drug application was done once daily for five days. On the fifth day the stitches were removed and the tensile strength was measured. To see the tensile strength; at equidistance two threads were passed on both sides of all the wounds. One end was fixed with a fix object (tap) and the other end was anchored in the hook of a spring balance weighing up to 1 kg. The force was applied by pulling the spring balance and at the point where the wound totally gapped, was considered as the tensile strength of that wound.

Excised wounds[7]

The animals were anaesthetized by the same manner and their backs were shaved and cleaned with spirit and savlon. To full thickness excised wounds were made. The upper one just lateral to the vertebral column on the left side between the forelimbs and the lower one was made on the right side between the hind limbs. Full thickness of skin in the area of 1 square centimeter was excised with a scissors and scalpel. The wounds were cleaned and left opened on the day of operation. Here also the randomly the wounds were considered as control and treated. From the next day, the control wounds were applied with plain paraffin jelly and the treated with the extract mixed in jelly in strength of 5, 10 & 20 % in three different groups. The application was done daily till the wound was completely covered by the skin. The progressive decrease in wound area was monitored by tracing the wound margin on a tracing paper and complete healing was noted.

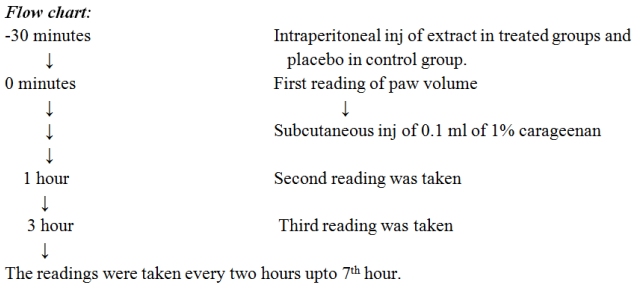

Figure 1: Study pattern of acute inflammation produced by 1% carageenan.

Acute inflammation

Carageenan induced hind paw edema[8]

The method described by winter et al. The animals were divided into two groups. One group was considered as control and another as treated. The treated ones were marked with carbol fuchsin on their heads for the identification. The treated groups were injected intraperitoneally with the extract dissolved in distilled water in the strength of 5, 10, 20 and 40 mgs/100 gms body weights of rats; and the control group was given intraperitoneal injection of distilled water in the equal volume. Paw volumes of all the rats were measured with the help of a plathysmometer. The readings were taken according to the flow chart (Figure 1). The injection of drug was given half an hour before the injection carageenan. For acute inflammation 0.1 ml of 1% carageenan made in the sterile normal saline was injected

into the subplanter space subcutaneously in the right hind paw of all the rats.

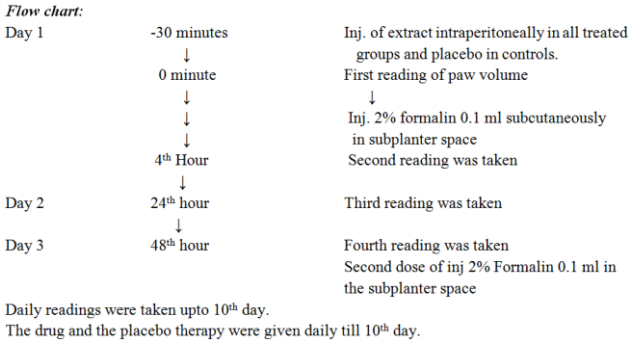

Figure 2: Study pattern of sub-acute inflammation produced by 2% formalin.

Subacute inflammation

Formalin induced inflammation of hind paw[9]

The animals were divided into control and treated groups. The treated groups were daily injected intraperitoneally with the extract in the strength of 10, 20 and 30 gms/100 gms body weights of rats. The control group was given equal volume of distilled water intraperitoneally. The injection of drug/placebo was given half an hour prior to the injection formalin. To produce subacute inflammation 0.1 ml of 2% formalin was injected subcutaneously in the sub planter spaces right hind paws. The paw volumes were measured according to the flow chart (Figure 2).

All the data thus obtained were plotted on the graph and compared the inhibition of inflammation and they were analysed statistically by students' 't' test.

RESULTS

In incised wounds the tensile strength on 5th day was measured. Average tensile strength of the treated group is greater than that of the control group. Apparently it is higher, but the results are not significant statistically.

Table 1: Effect of aqueous extract of F. racemosa 10% ointment applied locally on incised wounds.

No.	Tensile strength (gms) Control	Tensile strength (gms) Treated
Mean	406.25	418.75

It could accelerate the process of healing at the dose level of 10% local application. The doses slightly lower (5%) and slightly higher (20%) could not show promising results.

Table 2: Effect of aqueous extract of F. racemosa ointment applied locally on excised wounds.

Groups	Dose In %	Mean healing In days Control	Mean healing In days Treated
A	5	13.16	14
B	10	15.07	12.57
C	20	10.2	10.8

Table 3: Effect of aqueous extract of F. racemosa on Acute Inflammation produced by 1% carageenan.

Groups	1st Hour	3rd Hour	5th Hour	7th Hour
Group A				
Control	04.47	14.92	22.38	50.74
Treated	05.97	05.97	13.43	14.92*
Group B				
Control	13.23	39.70	47.05	91.00
Treated	09.75	20.73	32.92	28.04*
Group C				
Control	32.87	73.97	69.86	57.53
Treated	38.46	12.30*	12.30*	18.46*
Group D				
Control	26.22	40.98	54.09	65.57
Treated	01.42	04.28*	17.14*	28.57

Doses of F. racemosa extract:

Group A: 5mg/100 gms Group C: 20 mg/100 gms

Group B: 10 mg/100 gms Group D: 40 mg/100 gms

*Indicates the results differ statistically significantly by students unpaired 't' test from the control groups.

The healing effect was seen in three groups of animals. The strength of the extract was 5, 10 & 15% respectively, which was applied locally on the wounds. In 5% and 20% the drug did not facilitate the healing process; however in the concentration of 10% ointment, it decreased the total number of days of complete healing. So, apparently it indicates that it facilitated the healing but could not reach upto the statistically significant levels.

F. racemosa is used in the dressing of external wounds. So, we tried to see the wound healing and anti-inflammatory activity in various concentrations. There were data available regarding anti secretory activity and anti-ulcer activity in the dose level of 20 mg/100 gms. So, we tried in the doses lower and higher than that dose. Our results indicate that at the dose level of 20 mg/100 gms it shows anti-inflammatory activity. The effect on incised wounds and tensile strength on local application was apparently visible from the results, but was not statistically significant.

The effect on excised wound with local application in 5%, 10% & 20% dose was seen; in which 5% and 20% did not show any positive response, but at the dose level of 10% did potentiate the process of healing. But the data were not statistically significant.

Table 4: Comparison of results of F. racemosa aqueous extract on acute inflammation produced by 1% carageenan.

Groups	0 minute	7th Hour	t_5	*p* value
Group A				
Control	0.67±0.1699	1.01±0.1699	4.92	< 0.01
Treated	0.67±0.054	0.77±0.054	4.09	< 0.01
Group B				
Control	0.68±0.123	1.3±0.123	12.02	< 0.001
Treated	0.82±0.1516	1.05±0.1516	3.71	< 0.01
Group C				
Control	0.73±0.347	1.15±0.347	1.702	> 0.1
Treated	0.65 ± 0.230	0.77 ± 0.230	1.30	> 0.1
Group D				
Control	0.61±0.163	1.01±0.163	6.06	< 0.01
Treated	0.70±0.129	0.90±0.129	3.19	< 0.05

Doses of F. racemosa extract:

Group A: 5mg/100 gms Group C: 20 mg/100 gms

Group B: 10 mg/100 gms Group D: 40 mg/100 gms

Table 5: Effect of aqueous extract of F. racemosa on sub-acute inflammation produced by 2% formalin.

Groups	0 min	4 hr	2nd day	3rd day	4th day	5th day	6th day	7th day	8th day	9th day	10th day
A	0.47	0.66	0.62	0.60	0.71	0.68	0.62	0.57	0.53	0.49	0.48
B	0.52	0.68	0.69	0.65	0.96	0.85	0.67	0.60	0.57	0.57	0.52
C	0.50	0.61	0.54	0.52	0.68	0.65	0.58	0.53	0.51	0.49	0.48

Group A: Control

Group B: 10 mg/100 gms

Group C: 20 mg/100 gms

Table 6: Effect of aqueous extract of F. racemosa on sub-acute inflammation produced by 2% formalin.

Groups	0 min	4 hr	2nd day	3rd day	4th day	5th day	6th day	7th day	8th day	9th day	10th day
A'	0.51	0.85	0.79	0.74	0.88	0.82	0.76	0.70	0.68	0.64	0.64
D	0.50	0.71	0.69	0.62	0.67	0.59	0.58	0.55	0.54	0.53	0.51

Group A': Control

Group D: 30 mg/100 gms

Table 7: Comparison of results of F. racemosa aqueous extract on sub-acute inflammation produced by 2% formalin.

Groups	Paw volume on 4th day	t_9	*p* value
A	0.71	12.96	< 0.001
B	0.96		
A	0.71	0.63	> 0.1
C	0.68		
A'	0.88	4.822	< 0.01
D	0.67		

In case of carageenan induced acute inflammation the results were showing anti-inflammatory activity. We tried in 5, 10, 20 & 40 mg/100 gms body weight of rats. In the dose of 5 mg/100 gms the drug could partly inhibit inflammation; but could not suppress it. In the dose of 10 mg/100 gms the drug could inhibit the inflammation significantly at 7th hour. At the dose level of 20 mg/100 gms the drug could significantly inhibit the inflammation from 3rd hour up to 7th hour. This dose level was quite significant in reducing the number of gastric ulcers.[3]

By increasing the dose up to 40mg/100 gms, the inhibition of inflammation started early and intended inhibition was produced at 3rd and 5th hour, which could be significant statistically. However, this inhibition could not remain significant at 7th hour.

In Formalin induced sub acute inflammation the drug in the dose of 10mg/100 gms could not inhibit the inflammation, but at the dose level of 20 mg/100 gms

apparently showed from the results, and that was more on the 4th day; however it also could not reach upto statistically significant levels. But by increasing the dose up to 30 mg/100 gms it could inhibit the inflammation which was visible on the results as well as it was statistically significant.

DISCUSSION

Data shows that when used in incised wound there was a slight increase in the tensile strength in the treated group. It could accelerate the process of healing at the dose level of 10% local application. The doses slightly lower (5%) and slightly higher (20%) could not show promising results. In acute inflammation the drug could significantly inhibit inflammation at 3rd, 5th & 7th hour in the dose level of 20mg/100 gms. In the lower dose (5mg/100 gms & 10 mg/100 gms) the results were significant only at 7th hour. In the higher dose (40mg/100 gms) the drug showed inhibition of inflammation in 3rd and 5th hour. At 7th hour, it could inhibit the inflammation but failed reach up to statistically significant level. In sub-acute inflammation the drug could inhibit the inflammation at the dose of 20 mg/100 gms which was seen apparently from graph. However it could not reach upto statistically significant level. By increasing the dose upto 30 mg/100 gms the drug could inhibit the sub acute inflammation apparently as well as significantly.

So, the results are suggestive that aqueous extract of F. racemosa has got wound healing and anti-inflammatory activity. It is likely that the duration of action may be shorter.

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