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Anti-inflammatory, Wound Healing and *in vivo* Antioxidant Properties of the Bark of *Ficus amplissima* Smith

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ABSTRACT

Background: Herbal preparations of *Ficus amplissima* had been considered as effective, economical and safe ethnomedicines for various ailments in Indian traditional system of medicine. The present study was aimed to explore scientifically the antioxidant, anti-inflammatory and wound healing potential of *Ficus amplissima* bark. **Materials and Methods:** In the present study, the methanol extract of *F. amplissima* bark were studied for enzymatic antioxidant activity through different assays, anti-inflammatory by using egg albumin induced inflammation and cotton pellet induced granuloma models (100 and 200 mg methanol extract) and wound healing activity by incorporating the two doses (1 and 2% (w/w)) of methanol extract and simple ointment base B.P. in concentration of 5% (w/w) using excision and incision wound models in rats. In case of the excision wound model, wound contraction and period of epithelization was studied while incision wound model was evaluated by determining tensile strength. **Results:** *F. amplissima* bark expressed the potent anti-inflammatory and *in vivo* antioxidant activity, where, 200 mg methanol extracts showed higher activity. Treatment of wound with ointment containing 2% (w/w) methanol extract was exhibited significant ($p<0.001$) wound healing activity. **Conclusion:** The methanol extract of *F. amplissima* bark exhibited better anti-inflammatory, wound healing and *in vivo* antioxidant activity most likely due to phenols constituents.

Key words: *Ficus amplissima*, kal-itchchi, anti-inflammatory, wound healing, antioxidant

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INTRODUCTION

Wound infection is one of the most common diseases in developing countries because of poor hygienic conditions (Kumar *et al*., 2006). Wounds are the physical injuries that result in an opening or breaking of the skin and appropriate method for healing of wounds is essential for the restoration of disrupted anatomical continuity and disturbed functional status of the skin (Singh *et al*., 2006). In other words, wound is a break in the epithelial integrity of the skin and may be accompanied by disruption of the structure and function of underlying normal tissue and may also result from a contusion, haematoma, laceration or an abrasion (Enoch and Leaper, 2005). Healing of wounds starts from the moment of injury and can continue for varying periods of time depending on the extent of wounding and the process can be broadly categorized into three stages; inflammatory phase, proliferate phase and finally the remodeling phase which ultimately determines the strength and appearance of the healed tissue (Sumitra *et al*., 2005). Medicinal plants have been used since time immemorial for treatment of various ailments of skin and dermatological disorders especially cuts, wounds and burns (Kumar *et al*., 2007).

Ficus amplissima Smith (Syn: *Ficus tsiela* Roxb.) belongs to the family Moraceae, commonly known as kal-itchchi. It is widely distributed all over India, northern Australia and other parts of Asia. The ethnobotanical views on *F. amplissima*, suggest that bark is used as an indigenous and ayurvedic herbal medicines (Herbal formulations) for diabetes with ailments of throat-haws up cheesy laumps (Oudhia, 2012). Previously the methanol extract of *F. amplissima* bark reported that, powerful antioxidant effect and anti-inflammatory activity against edema produced by carrageenan and histamine models in rats and bioactive chemical constituents reported from bark of the *F. amplissima* include Lup-20(29)-en-3-yl acetate, lupeol, Myristic acid, 1, 3, 4, 5-tetra hydroxyl cyclo hexane carboxylic acid, Stearic acid, Phytol, sitosterol, Lanosterol acetate (Murugan *et al*., 2012). Until now, there is no report on the possible wound healing properties of *F. amplissima* bark. Hence, the present study designed at evaluation of anti-inflammatory, wound healing and *in vivo* antioxidant activity of the plant.

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MATERIALS AND METHODS

Collection and identification of plant material: The fresh bark parts were collected during the month of October 2009 from Ramapuram village, Sathyamangalam, district of Erode, Tamil Nadu, India. The taxonomic identity of the plant was confirmed by Dr. A. Rajendran and voucher specimen (No.: 006147) was deposited at Botany Department Herbarium, Bharathiar University, Coimbatore, Tamil Nadu. The plant materials were washed under running tap water to remove the surface pollutants and the different parts of bark were separated mechanically. The separated parts were air dried under shade. The dried sample was powdered and used for further studies.

Extraction of plant material: The powdered bark material was packed in small thimbles separately and extracted successively with organic solvents such as petroleum ether, chloroform, acetone and methanol in the increasing order of polarity using soxhlet apparatus. Each time before extracting with the next solvent, the thimble was air dried. The different solvent extracts were concentrated by rotary vacuum evaporator (Yamato RE300, Japan) and then air dried. The dried extract obtained with each solvent was weighed. The percentage of yield was calculated in terms of the air dried weight plant material (1 mg mL^{-1} of respective organic solvents) the extract obtained was used for the assessment of further investigation.

Animals: The healthy Wistar albino male rats, weighing 150-200 g, were housed under standard environmental conditions of temperature and humidity (25±0.50°C) and 12 h (light/dark cycle) were utilized for the studies. The animals were fed with standard pellet diet and water *ad libitum*. The animal studies were performed in the institute with due permission from Institutional Animal Ethical Committee (KMCRET/M.Sc./4/2010-11, India).

Preparation of test samples for bioassay: For the anti-inflammatory test model, samples were given orally to test animals after suspending in a mixture of distilled H_2O and 0.5% sodium carboxymethyl cellulose (CMC). The control group animals received the same experimental handling as those for the test groups except the drug treatment which was replaced with appropriate volumes of the dosing vehicle. Indomethacin (10 mg kg^{-1}) in 0.5% CMC was used as a reference drug.

Acute dermal study was carried out to find out the therapeutic dose of the methanol extract. The acute dermal toxicity was studied by applying the Ointments containing the highest concentrations of 2% (w/w) methanol extract of *F. amplissima* bark on the shaved back of the rats. The OECD (Organization for Economic and Co-operation Development) guidelines No. 402 (OECD, 1987) were followed for the study.

Anti-inflammatory activity: Male Wistar albino rats (150-200 g) were housed for this study. Each rat was marked as H (head), B (body), BT (body-tail) and N (unmarked). A mark was made on the left hind paw just beyond tibio-tarsal junction, so that every time the paw is dipped in the mercury column up to the fixed mark to ensure constant paw volume. They were fasted overnight but had given free access to water. The initial paw volume of each rat was noted by mercury displacement method.

Egg albumin induced inflammation and cotton pellet induced granuloma models: To study the anti-inflammatory property of metnanol extract of *Ficus amplissima* bark against egg albumin induced hind paw edema in rats, the methodology by Okokon and Nwafor (2010) was followed. The animals were divided into four groups each comprising six rats namely:

- **Group I:** Control (0.6% CMC)
- **Group II:** Oral feeding of Indomethacin (10 mg kg^{-1})
- **Group III:** Oral feeding of bark extract, dose 1 (100 mg kg^{-1})
- **Group IV:** Oral feeding of bark extract, dose 2 (200 mg kg^{-1})

After 1 h, 0.1 mL of 1% (w/v) egg albumin (suspended in normal saline) was injected in the plantar region of the left paw of control as well as plant extract treated group. Paw thickness of left leg of control and plant extract treated rats were noted at every 1 h after the administration of egg albumin. The reading was taken for total of 4 h.

To study the anti-inflammatory property of methanol extract of *F. amplissima* bark against cotton pellet granuloma model (Winter and Porter, 1957) the animals were divided into four groups each comprising six rats namely:

- **Group I:** Control (0.6% CMC)
- **Group II:** Oral feeding of Indomethacin (10 mg kg^{-1})
- **Group III:** Oral feeding of bark extract, dose 1 (100 mg kg^{-1})
- **Group IV:** Oral feeding of bark extract, dose 2 (200 mg kg^{-1})

After shaving the fur, the animals were anaesthetised. Sterile pre-weighed cotton pellets (50±1 mg) were

implanted in the axilla region of each rat through a small incision. The plant extracts and standard were administered to the respective group of animals for 7 consecutive days from the day of cotton pellet implantation. On the 8th day, the pellets were removed and incubated at 37°C for 24 h and dried at 60°C to constant weight. The weight of the granulomatous tissue was calculated by the difference between the initial and the final dry weight of the cotton pellets.

Wound healing activity: The animals were grouped into three major groups viz., control, standard and test with six animals in each group. The control group was treated with simple ointment base B.P. The standard group was treated with Betadine (Win Medicare containing 5% (w/w)) ointment. The test groups were treated with ointments with different concentrations of methanol extracts viz., 1% (w/w) and 2% (w/w) incorporated in simple ointment base 0.5% (w/w) in both the models.

The most commonly used trituration method as mentioned in British Pharmacopeia (Anonymous, 2009) was followed for the preparation of ointment. The hard paraffin (0.5 g) and cetostearyl alcohol (0.5 g) were melted and then wool fat (0.5 g), yellow soft paraffin (8.5 g) was incorporated, stirred until all the ingredients were melted. Foreign particles were removed by decantation and the mixture was stirred thoroughly until cold. The extract (100, 200 mg) has added with the above simple ointment base; 1 and 2%, respectively.

Excision wound model: The rats were anesthetized by administering ketamine (0.5 mL kg^{-1} b.wt. intra peritoneal). An excision wound of circular area (approx.) 500 mm^2 and 2 mm depth of full thickness was made on the shaved back of the rats 30 min later the administration of ketamine injection. The wounding day was considered as day 0. The wounds were treated with topical application of the ointments as described above till the wounds were completely healed. The wounds were monitored and the area of wound was measured on 3, 6, 9, 12, 15, 18, 21 post-wounding days and the mean% wound closure. The period of epithelization was calculated as the number of days required for falling of the dead tissue remnants without any residual raw wound (Nayak *et al*., 2007). Wound healing rate (Kumar *et al*., 2008) was calculated using the following equation:

$$\text{Wound closure (\%)} = \frac{\text{Wound area on day 0} - \text{wound area on day n}}{\text{Wound area on day 0}} \times 100$$

where, n is the No. of days: 3rd, 6th, 9th, 12th, 15th, 18th and 21st day.

Incision wound model: Incision wounds of about 6 cm in length and 2 mm in depth were made with sterile scalpel on the shaved back of the rats 30 min later the administration of ketamine (0.5 mL kg^{-1} b.wt. i.p.) injection given for anesthetizing the rats. The parted skin was kept together and stitched with black silk at 0.5 cm intervals. Surgical thread (No. 000) and a curved needle (No. 9) were used for stitching. The continuous thread on both wound edges were tightened for good closure of the wounds. The wounds of animals in the different groups were treated with topical application of the ointments as mentioned before, for the period of 10 days. The wounding day was considered as day 0. When wounds were cured thoroughly, the sutures were removed on the 8th post-wounding day and the tensile strength of the skin that is the weight in grams required to break open the wound/skin was measured by tensiometer on the 10th day. Tensile strength was calculated using the following equation (Reddy *et al*., 2008):

$$\text{Tensile strength} = \frac{\text{Breaking strength (g)}}{\text{Cross-sectional area of skin (mm}^2\text{)}}$$

Antioxidant activity: Lipid peroxidation was assayed by the measurement of malondialdehyde (MDA) levels in liver on the basis of reaction with thiobarbituric acid (Ohkawa *et al*., 1979). The activity of superoxide dismutase (SOD) was determined in liver by monitoring the inhibition of the autoxidation of pyrogallol (Marklund and Marklund, 1974). Catalase (CAT) activity in liver was determined according to the standard method (Aebi, 1974). Proteins were determined according to Lowry *et al*. (1951) method using bovine serum albumin as a standard.

Histopathological examinations: A specimen sample of skin tissues from control, standard and treated groups were taken out from the healed wounds of the animals in excision and incision wound models for histopathological examinations. The thin sections were cut and stained with haematoxylin and eosin (McManus and Mowry, 1960) and observed under microscope for the histopathological changes such as fibroblast proliferation, collagen formation and angiogenesis.

Statistical analysis: Results obtained from the two wound models have been expressed as Mean±SEM (Standard Error Mean)/area and were compared with the corresponding control group (simple ointment B.P.) by applying ANOVA (Analysis of Variance) test (Mukherjee *et al*., 2000).

RESULTS

Anti-inflammatory activity

Egg albumin induced inflammation and cotton pellet induced granuloma models: Administration of *F. amplissima* bark methanol extract on egg albumin induced edema in rats caused a significant ($p<0.001$) dose dependent anti-inflammatory effect against edema (5.13±0.29). The anti-inflammatory effects are comparable to that of positive control i.e., indomethacin (10 mg kg^{-1}) and are shown in Table 1. The methanol extract at doses of 100 and 200 mg kg^{-1} was capable reducing the oedema formation induced by egg albumin at 4th h.

In the present investigation, the methanol extract of *F. amplissima* bark registered profound antiinflammatory activity against cotton pellet-induced granuloma in the experimental rats. The extract exhibited a significant ($p<0.01$) antiinflammatory effect in a dose dependant manner and the results were comparable to that of standard drug Indomethacin. The methanol extract at the dose of 200 mg kg^{-1} showed maximum granuloma inhibition (69.37%) which is comparable to that of the standard drug Indomethacin (75.69%).

Wound healing activity

Excision wound study: The results of wound healing activity by excision wound model are presented in Table 2 and Fig. 1 (A: Initial day, B: Applying the ointment, C: 5th day, D: 10 day, E: 15th day, F: 21st day). In excision model, the percentage closure of wound area was significantly increased ($p<0.05$-0.001) by the curative effect of both the doses (1 and 2%). The doses decreased the epithelization period of *F. amplissima* bark methanol extract (18.02 and 14.83 days) as evidenced by shorter period for fall of escher when compared to standard (12.62 days) and control (27.00 days). In this model, 2% bark methanol extract treated groups demonstrated 94.23% contraction on 12th day, respectively. This was close to contraction value of the reference drug Betadine (100% on 12th day).

Histopathological studies: The histopathological evaluation revealed that the lesser epithelialization and lesser collagen formation in control group delayed the healing process (Fig. 2a). Original tissue regeneration was much greater in the standard and treated groups (Fig. 2b). Treatment of the wounds with 1 and 2% *F. amplissima* bark methanol extract was associated with enhanced formation of epidermis, deposition of connective tissue and faster re-modeling when compared to that of control and vehicle groups. In 2% treated groups, the histology of granulation tissue showed almost complete healing with more fibroblast within marked increase of collagen tissue and increased number of blood vessels (Fig. 2c, d).

Table 1: Anti-inflammatory effect of *Ficus amplissima* methanol extract of bark on egg albumin induced paw edema and cotton pellet-induced granuloma in rats

		Time intervals (h) and Paw thickness (mm)				
Treatment	Dose (mg kg^{-1})	0	1	2	3	4
Control	-	4.45±0.11	5.60±0.44	5.89±0.53	5.98±0.91	6.06±0.04
Indomethacin	10	5.04±0.55	5.55±0.56	4.88±0.59**	4.53±0.28**	4.45±0.68***
Methanol extract	100	4.33±0.36	6.11±0.35	5.61±0.16	5.06±0.35**	5.27±0.18*
Methanol extract	200	4.15±0.21	5.65±0.36	5.52±0.41	5.05±0.29**	5.13±0.29**
		Cotton pellet-induced granuloma in rats				
Treatments	Dose (mg kg^{-1})	Weight of the pellet (mg)				Inhibition (%)
Control	-	81.25±3.15				-
Indomethacin	10	15.43±1.75***				75.69
Methanol extract	100	52.25±0.80				34.23
Methanol extract	200	17.70±1.97***				69.37

Values are Mean±SEM, n = 6, Statistically significant at $p<0.001$*** when compared with control

Table 2: Effect of topical application of ointments containing methanol extracts of *F. amplissima* bark on wound contraction of excision wound

	Percentage of wound contraction (days)							Period of epithelialization
Groups	3	6	9	12	15	18	21	(days)
Control	12.61±2.31	26.33±2.5	37.32±2.21***	44.00±2.58***	56.83±2.16***	67.01±2.21***	73.80±2.51***	27.00±0.03
Betadine (5% w/w)	23.96±2.3	39.54±2.1*	76.62±2.8***	100.00±0.15***	100.00±0.00***	100.00±0.00***	100.00±0.00***	12.62±0.06
Ointment base	14.46±1.94	29.57±1.29	44.25±1.29*	57.32±1.26***	68.47±1.41***	74.12±1.70***	82.50±1.70***	23.39±0.82
1% extract of bark	15.33±2.54	31.01±2.78	56.26±2.08***	68.04±1.51***	81.24±1.07***	100.00±0.02***	100.00±0.0***	18.02±0.01
2% extract of bark	8.62±1.8	29.75±3.3	69.45±3.6***	96.23±1.8***	100.0±0.100***	100.00±0.00***	100.0±0.00***	14.83±0.07

Values are expressed as Mean±SE, n = 6 animals in each group, Treated groups are compared by student t-test with the control group, ***$p<0.001$, **$p<0.01$, *$p<0.05$

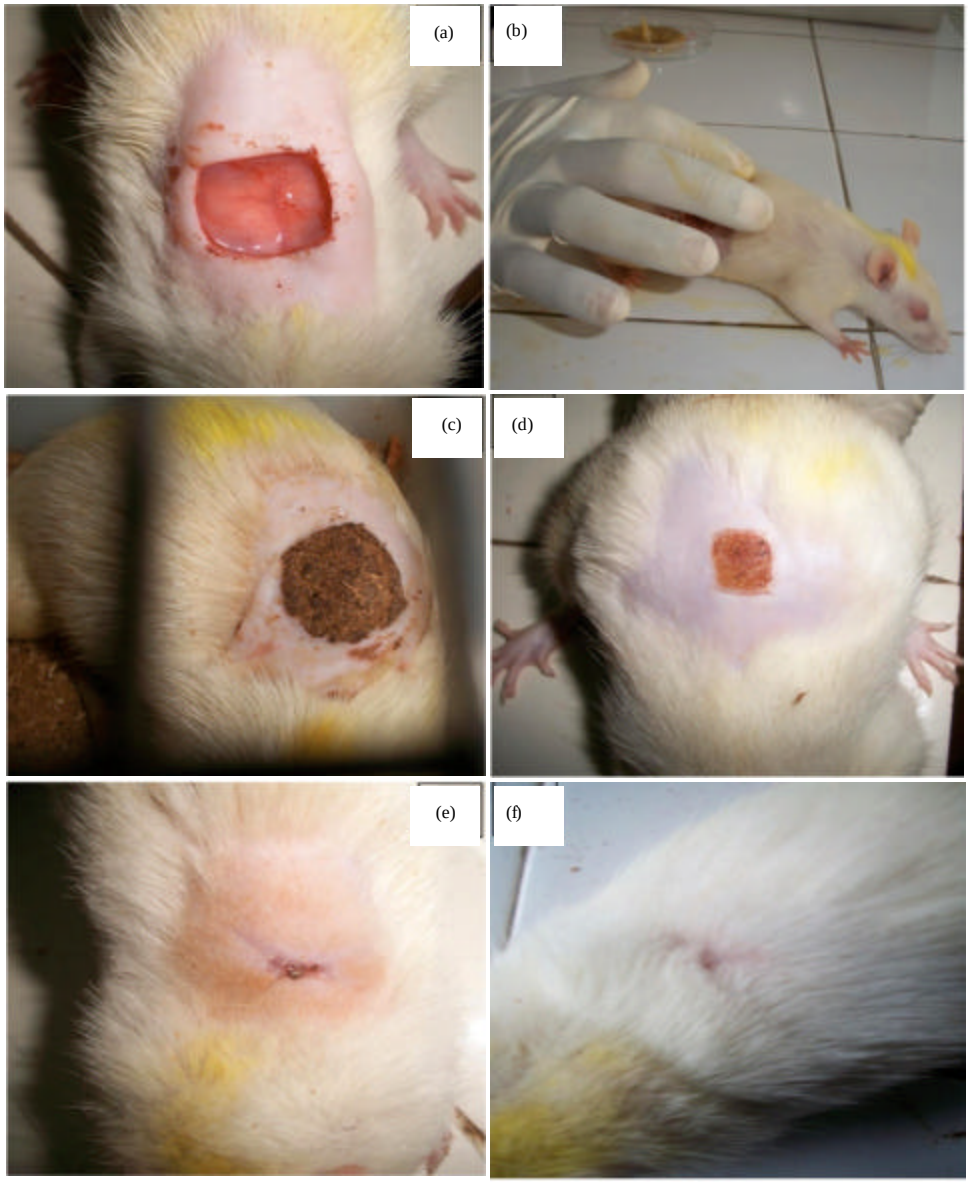

Fig. 1(a-f): Photographic representation of wound contraction on *F. amplissima* bark methanol extract of excision wound healing days (1-21 days), (a) Initial day, (b) Applying the ointment, (c) 5th day, (d) 10th day, (e) 15th day and (f) 21st day

Incision wound study: The effect of wound healing activity was evaluated by determining the tensile strength of the incision wound of different groups viz., control group treated with simple ointment base B.P., standard group treated with drug betadine and the test group treated with the extracts at different concentrations. The results are presented as mean weight in gram±SEM/area required to break open the restored wound (Table 3).

Table 3: Effect of topical application of ointments containing methanol extracts of *F. amplissima* bark on tensile strength of the skin having incision wound

Group (n= 6)	Tensile strength (g) (Mean±SEM)
Control	320.82±3.60
Betadine (5% w/w)	653.00±4.81
1% methanol extract of bark	673.00±2.54**
2% methanol extract of bark	750.66±3.20**

n = 6 animals in each group, Treated groups are compared by Student t-test with the control group, **p<0.01, ***p<0.001

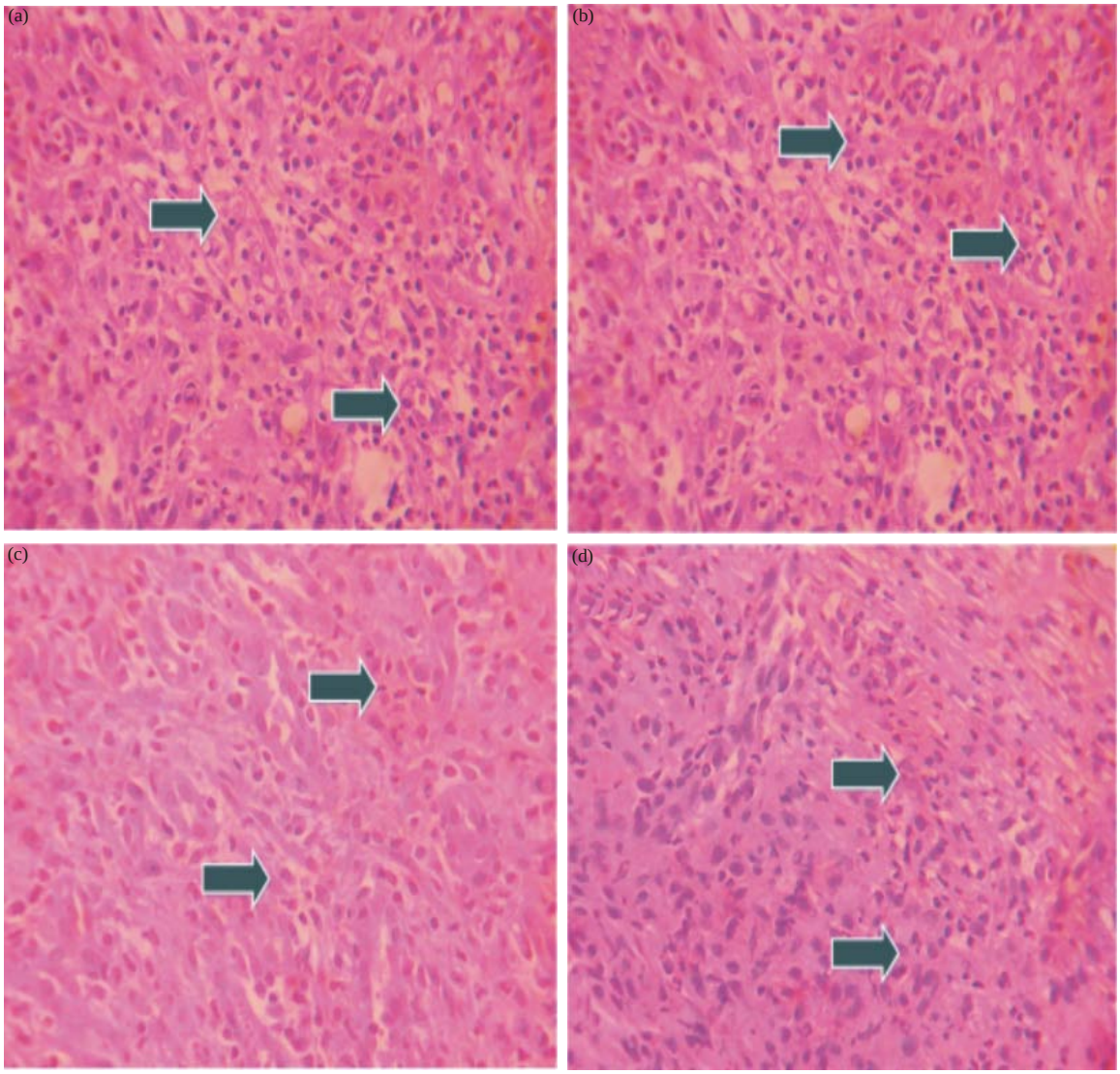

Fig. 2(a-d): Histopathological evaluation of wound tissues in excision model of *F. amplissima* bark (methanol extract), (a) Histological section of the granuloma tissue of control rats viewing incomplete healing with less epithelialization arrows showing macrophages and lesser collagen formation indicated incomplete healing of the wound, (b) Histological section of the granuloma tissue of standard rats viewing complete healing with elevated epithelialization arrows showing macrophages and foremost collagen formation indicated complete healing of the wound, (c) Histological section of granulation tissue of the rats treated with methanol extract of *F. amplissima* bark methanol extract (1%) showing (arrows) increased collagenation, lesser macrophages (arrows) and (d) Histological section of granulation tissue of the rats treated with methanol extract of *F. amplissima* bark methanol extract (2%) showing (arrows) increased collagenation with few macrophages (arrows)

The animals treated with ointment containing 2% (w/w) methanol extract indicated significantly high ($p<0.001$) tensile strength as compared to the control group.

***In vivo* antioxidant activity:** There is a definite role of free radicals in the pathogenesis of wound, the antioxidant activity was also studied. The results for antioxidant potential of *F. amplissima* bark methanol extract indicate that it possesses potent antioxidant activity by inhibiting lipid peroxidation, reduced glutathione and SOD (10.78±0.20) levels while increasing the CAT (253.12±14.98) activity (Table 4).

Table 4: Antioxidant activity of the *F. amplissima* methanol extract of bark

Sample concentration	TBARS (nmol mg^{-1} protein)	GSH level (μg mg^{-1} protein)	SOD (units g^{-1}) tissue	CAT (μg g^{-1} liver)
Control	1.48±0.07	6.36±0.75	31.29±1.8	116.33±13.66
F. amplissima methanol extract of bark (200 mg kg^{-1} b.wt.)	0.98±0.12**	4.59±0.96**	10.78±0.20**	253.12±14.98***

TBARS: Thiobarbituric acid reactive substances, GSH: Reduced glutathione, SOD: Superoxide dismutase, CAT: Catalase, values are Mean±SEM of six replicates, **$p<0.01$, ***$p<0.001$ statistically significant difference in comparison with control group

DISCUSSION

The present study establishes the anti-inflammatory activity of methanol extract of *F. amplissima* in Wistar albino rats, representing different phases of inflammation. The developments of edema in the paw of the rat after the injection of carrageenan and egg albumin were due to the release of histamine, serotonin and prostaglandin like substances. The egg albumin induced paw edema model in rats were known to be sensitive to cyclooxygenase inhibitors and has been used to evaluate the effect of non-steroidal antiinflammatory agents, which primarily inhibit the cyclooxygenase (COX) involved in prostaglandin synthesis (Seibert *et al*., 1994). On the basis of the results showed, egg albumin induced inflammation method, the methanol extract of bark at concentration of 200 mg kg^{-1} resulted meager ($p<0.01$) than the standard indomethacin but it provided better result when compared to the control. Results of methanol extract of *F. amplissima* bark indicates that the extract possibly exhibits its anti-inflammatory action by inhibiting the synthesis, release or action of inflammatory mediators including histamine, serotonin and prostaglandin known to mediate acute inflammation induced by phlogistic agents, that are likely also involved in egg albumin induced acute oedema (Li *et al*., 2008).

Cotton pellet granuloma model was used to evaluate chronic anti-inflammatory activity of methanol extract of *F. amplissima* bark. Chronic inflammation is a reaction arising when the acute response is insufficient to eliminate proinflammatory agents and induces a proliferation of fibroblasts and the infiltration of neutrophils and exudation. Chronic inflammation occurs by means of the development of proliferative cells. NSAIDs (Non Steroid Anti-inflammatory Drugs) cause a decrease in granuloma tissue arising as a result of cellular reaction, released by inhibiting granulocyte infiltration to the foreign body implanted (Ionac *et al*., 1996). Methanol extract of bark showed significant anti-inflammatory activity by reducing granulomatous tissue in cotton pellet granuloma method and found to be effective in chronic inflammatory condition. However, the inhibition effect was not found to be strong as indomethacin. Since cotton pellet-induced granuloma corresponds to the proliferative phase of inflammation, it is possible that, in this inflammatory model, the methanol extract of bark acts by inhibiting fibroblasts formation.

Wound healing is stepwise process, which consists of different phases such as hemostasis, inflammation, proliferative and remodeling or maturation. The genetic response regulating the body's own cellular resistance mechanisms contributes to the wound and its repair (Charles *et al*., 1995). Hence, in the present study, excision and incision wound models were used to evaluate the effect of methanol extract of bark ointment on various phases. In incision wound, the increase in tensile strength of treated wounds may be due to the increase in collagen concentration and stabilization of the fibres (Udupa *et al*., 1995). Increase in blood vessels and role of antioxidants were experimentally proved (White and Heckler, 1990). In excision wound, the methanol extract of bark showed faster healing with earlier wound contraction compared with control groups. The earlier wound contraction rate of the methanol extract of bark may be due to stimulation of interleukin, an inflammatory a chemokine which affects the function and recruitment of various inflammatory cells, fibroblasts and keratinocytes. It may increase the gap junctional intracellular communication in cultured fibroblasts and induces a more rapid maturation of granulation tissue (Moyer *et al*., 2002). Methanol extract of *F. amplissima* bark increased cellular proliferation and collagen synthesis at the wound site as evidenced by increase in total protein and total collagen contents reflected by hydroxyproline content of granulation tissues. The glycosaminoglycans are a major component of the extra cellular matrix of skin, joints, eyes and many other tissues and organs. Inspite of its simple structure, it demonstrates remarkable visco elastic and hygroscopic properties which are relevant for dermal tissue function. Biological activities in skin are due to its interaction with various binding proteins. Due to an influence on signaling pathways, hyaluronic acid and hydroxyproline is involved in the wound-healing process and scarless fetal healing. In clinical trials, topical application of hyaluronic acid has improved the healing of wound (Weindl *et al*., 2004). In addition, the muco-polysaccharide hyaluronic acid protects granulation tissue from oxygen free radical damage and thereby stimulates wound healing (Bayliss, 1984). Among the glycosaminoglycans, hydroxyproline, dermatan sulfate and dermatan have also been implicated in wound repair and fibrosis. Their ability to bind and alter protein-protein interactions has identified them as

important determinants of cellular responsiveness in development, homeostasis and disease (Trowbridge and Gallo, 2002). From this study, hydroxyproline content was significantly increased ($p<0.01$) when compared with control. Since *F. amplissima* bark extract of methanol has increased the levels of these compounds considerably, it is likely that the observed increase in tensile strength was not only due to increased collagen synthesis but also due to its proper deposition and alignment. Molecular oxygen plays a central role in the pathogenesis and therapy of chronic wounds. Overproduction of Reactive Oxygen Species (ROS) results in oxidative stress thereby causing cytotoxicity and delayed wound healing. Therefore, elimination of ROS could be an important strategy in healing of chronic wounds (Dissemond *et al*., 2002). Therefore, estimation of antioxidants like SOD, catalase and glutathione in granulation tissues is also relevant because these antioxidants hasten the process of wound healing by destroying the free radicals (Halliwell *et al*., 1988). The significant alteration in the antioxidant profile accompanied by the elevated levels of MDA (Malonaldehyde), a marker of free radical damage, may be attributed to impaired wound healing in immunocompromised rats (Gupta *et al*., 2002). While numerous attempts have been made to identify prognostic biomarkers of wound healing in skin, these have met with limited success. The results showed that methanol extract of bark ointment possesses a distinct prohealing stroke. This was demonstrated by a significant increase in the rate of wound contraction and by enhanced epithelialization period. Significant increase ($p<0.01$) in tensile strength and hydroxyproline content were observed, which was auxiliary supported by histopathological studies. This indicated newly formed fibroblasts cells, collagen fibres and blood vessels. Recent studies with other plant extracts have shown that phytochemical constituents like flavanoids (Tsuchiya *et al*., 1996), triterpenoids (Scortichini and Rossi, 1991) and tannins (Rane *et al*., 2003) are known to promote the wound-healing process. Preliminary phytochemical screening of *F. amplissima* bark extract of methanol showed the presence of alkaloids, flavonoids and tannins. *Ficus* species are rich source of polyphenolic compounds, flavanoids which are responsible for strong antioxidant properties that help in prevention and therapy of various oxidative stress related diseases such as neurodegenerative and hepatic diseases. The wound healing actions of methanol extract bark *F. amplissima* may probably be due to the phytoconstituents present in the plant or could be a function of either the individual or the additive effects of the phytoconstituents. Hence, the results obtained from data concludes that methanolic extract ointment of *F. amplissima* methanol extract of bark has properties that render it capable of promoting wound healing activities such as stimulating wound contraction and increasing tensile strength of incision as compared to control.

CONCLUSION

F. amplissima showed potent *in vivo* antioxidant, anti-inflammatory and wound healing activities suggesting that and ethno pharmacological approach in selecting the plant for study may be useful. In addition, the probability of success will be more, if the chosen species is used medicinally in the traditional system for the treatment of skin disease. The wound healing studies on methanol extract of *F. amplissima* bark indicate that the phenols constituents/tannins play an important role in wound healing process. It can be proposed that, the high content of phenolic compounds, saponins, tannins and flavonoids in the methanol extract of *F. amplissima* bark can be responsible for anti-inflammatory and wound healing activity, almost certainly due to astringent property of tannins. Thus, the folklore claim for the external use of *F. amplissima* bark methanol extract on the wounds can be justified by the present study.

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