Original Article

Evaluation Of Wound Healing And Anti-Inflammatory Effect Of Topical Formulations Of Boswellia Serrata Using Adult Zebra Fish And Macrophage Raw 264.7 Cells

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Abstract

A large majority of the population in the world have employed medicinal plants for their therapeutic needs. There is an urgent need to find scientific evidence of the therapeutic nature of medicinal plants to be used in general practice. Inflammation is a common risk factor in the pathogenesis of conditions such as infections, arthritis, type 2 diabetes mellitus, obesity and cancer. The aim of the present study is to investigate the anti-inflammatory effect of the Boswellia serrata by formulating topical agents of gel and ointment using adult zebra fish model and macrophage RAW 264.7 cells The study has employed the utilization of adult zebrafish to evaluate the wound healing efficiency of the ointment and gel formulation of the B.serrata extract. In vitro anti-inflammatory activity was conducted by albumin denaturation assay, anti-proteinase activity, and membrane stabilization assay which showed a significant % inhibition when compared with standard Aspirin. In this study, we investigated the anti-inflammatory activities of B.serrata extract in lipopolysaccharide (LPS)-induced RAW 264.7 macrophages. It was seen that the ethanolic extracts of B.serrata significantly repressed the production of inflammatory mediators such as nitric oxide and the expression of pro-inflammatory cytokines in the LPS-stimulated RAW 264.7 cells. Caudal fin transection model was employed for induction of inflammatory wound healing using zebrafish. The parameters for evaluation of wound healing efficacy is the measurement of regenerative capacity of the fin and neutrophil estimation. Histopathological examination of wound was also performed. Upon treatment of 500 µg of ointment, the fishes shows a maximum amount of fin regeneration by comparison with gel preparation. Utilisation of the 500 µg of ointment also revealed a high amount of neutrophil count at the side 24 hours after the infliction of wound and a reduction in the inflammatory mediators after one day.

Keywords: Boswellia serrata, Macrophage RAW 264.7 cells,

INTRODUCTION

Boswellia serrata belongs to the family *Burseraceae.* It has various synonyms such as *Boswellia glabra Roxb., Boswellia thurifera Roxb. Ex fleming, Bursera thurifera. Boswellia serrata* is frequently found in India's tropical dry deciduous woods, where it exhibits a vast array of phenological reactions associate with the vegetative & reproductive stages.[1]

B.serrata is an important plant used in traditional Indian medicine. *Boswellia serrata* (Burseraceae) is one of Ayurveda's oldest & most revered herbs. Kundur, the old gum resin of *Boswellia serrata* was a key element in modern choice perfumes in the Unani system of medicine. For millennia, the gum has been utilized in the Indian system of medicines (Siddha, Ayurveda & Unani) to treat illness, particularly skin illness & rheumatism.[2]

Several biologically active substances including pinene dipentene, phellandrene, verbenol, Myrcene, etc. are isolated from the *Boswellia* plants family. Since ancient times, folk medicine practitioners have relied on the medicinal properties of *Boswellia serrata's* gum-resin extracts for the treatment of chronic inflammatory problems.[3] The resinous part of *B.serrata* possesses diterpenes, triterpenes, monoterpenes, tetracyclic triterpenic acids, and four primary pentacyclic triterpenic acids like acetyl-β-boswellic acid, β-boswellic acid, acetyl-11-keto-β-boswellic acid, as well as 11-keto-β-boswellic acid effective in inhibiting pro-inflammatory enzymes. Therefore, the current work was undertaken to identify the role of *B.serrata* ethanolic extract against inflammation based on the traditional claims.[4]

Injury and microorganism infections like viruses and bacteria may cause inflammation, which acts as the first-line defense system for the body. During the healing of injured tissues, inflammation is a crucial player in the mechanism.[5] An important involvement of macrophages is to regulate the immune system's inflammatory response. IL-1β (Interleukin-1 beta), IL-6 (Interleukin-6), COX-2 (cyclooxygenase-2), as well as TNF-α (tumor necrosis factor-α) are cytokines or proteins expressions increased in macrophages during inflammation.[6]

It is true that the physiological processes of cells are dependent on NO (nitric oxide), but excessive NO generation by iNOS (inducible nitric oxide synthase) leads to the catastrophic failure of significant physiological activities. NO has also been shown to have a dose-dependent effect on prostaglandin endoperoxide H synthase 2 (cyclooxygenase-2). Prostaglandin (PG) H synthase (EC 1.14.99.1), an enzyme that changes arachidonic acid into prostaglandin and hence has an important involvement as a mediator in inflammatory reactions, is also known as cyclooxygenase (COX). COX-2 has been shown to have detrimental consequences on chronic inflammatory disorders, and its selective antagonists are beneficial in a variety of tests and clinical interventions.[7]

Healing a wound is a multi-step process that involves restoring the injured tissue's structural and functional characteristics. When there is no impairment, wound healing proceeds in a predictable three-phase fashion: remodeling, proliferation as well as inflammation. When an injury happens, the first cellular manifestation is inflammation, which begins shortly after hemostasis and occurs within a few hours after the injury. Many effector activities are carried out by neutrophils drawn to the wound site from the blood vessel network, such as the formation of ROS (Reactive Oxygen Species), degranulation, phagocytosis, the release of proinflammatory cytokines. The number of neutrophils at the wound site reaches a maximum after 24h and steadily decreases over the next few days. The extracellular matrix collapses and necrotic centers occur as a result of persistent inflammation. Deregulation of the immune system, which leads to unresolved inflammation, slows or prevents wound healing in the elderly and diabetics.

Apoptosis is induced and cleared by wound macrophages, which are responsible for releasing cytokines that stimulate an inflammatory response as well as for inducing and removing dying cells, correspondingly. The wound inflammation is reduced by clearing the neutrophil population by apoptosis, which starts the process of healing. Repelled from the inflamed region, a portion of this population migrates backward through the transendothelial to reach the vascular lumen, as shown by research on reverse migration in Zebrafish embryos. An important gold-standard metric for evaluating a substance's wound healing capability is its ability to infiltrate neutrophils before and after an inflammatory response has begun.[8]

A new method of histopathological analysis that is cost-effective, more efficient, and faster has been discovered in the study, which quantifies the traditional medicinal plant *Boswellia serrata* extracts' wound healing potential in the context of inflammation in an experimental adult Zebrafish wound model. This model comprises of injury made by transection of tail and it provides rapid, consistent findings in a short time. Despite substantial research into the principles underlying skin physiology and function, little is known about the healing mechanism itself due to a lack of appropriate model systems.

In the present study, evaluation of anti-inflammatory potential using ethanolic extract of *Boswellia serrata* (EEBS) using *in-vitro* methods and using LPS-stimulated RAW264.7 macrophages. *B.serrata* extract was formulated into gel and ointment and it was investigated for its wound healing efficacy using the adult zebrafish model.

METHODOLOGY

Sigma provided the Lignocaine. Solvents as well as chemicals utilized in the experiment were all procured from SISCO Research Laboratories and were of the highest quality.

PREPARATION OF THE PLANT EXTRACTS

The *Boswellia serrata* powder was taken and dried. 50g of powder was taken and macerated with 200ml of ethanol for the period of 72hrs. By using evaporation to concentrate the extract, a sterile container was utilized to keep the residue.

PHYTOCHEMICAL SCREENING

The assays have been performed qualitatively for a multitude of phytochemicals using standard plant analytical approaches, and the results were presented.

IN-VITRO ANTI-INFLAMMATORY ACTIVITY DETERMINATION

Albumin denaturation assay:

The protein denaturation assay for *B.serrata* were performed by the method of Muzushima and Sakat et al. The procedure was conducted with a few slight modifications. [10] The reaction consisted of a mixture with 100 µl of the test solutions, 100 µl of BSA (Bovine Serum Albumin 5%). pH was adjusted with the help of glacial acetic acid. The test tubes are then incubated for 20 minutes at 37ºc. It was heated for 10 minutes at 70 ºc. The tubes were allowed to be cooled for 10 minutes and the turbidity measurement was carried out at 660nm. Distilled water and the sample was used as a blank. Aspirin was used as a positive control.[9]

% inhibition of protein denaturation was calculated by % inhibition standard formula

Antiproteinase Activity:

Evaluation of antiproteinase activity was performed using methodology of Oyedeopo et al. The procedure was conducted with slight modifications. The reaction utilized a mixture with 0.06mg of trypsin, 1 ml of 20mM Tris HCl buffer. 1 ml of test extracts was prepared with different concentrations. The reaction mixture were incubated for 5 minutes at 37ºc. 1 ml of casein (0.8%) was added and incubated for 20 minutes. 2 ml of Perchloric acid (70%) was added for the reaction termination. The suspension obtained was centrifuged and absorbance of the supernatant was taken 210nm. Buffer was utilized as blank. [10]

% inhibition of proteinase inhibitory activity calculated using % inhibition standard formula

MEMBRANE STABILIZATION:

Red Blood Cells (RBCs) Suspension Preparation

Fresh human blood were collected and taken in a centrifuge tubes with heparin. The test tubes were centrifuged for 10 minutes at 3000 rpm. It was washed for 3 times with normal saline and the volume of blood was measured. They are made up to 10% suspension using normal saline.

Heat Induced Haemolysis

Heat induced hemolysis were performed using 1ml of test extracts along with 10 % prepared RBC suspension. Aspirin was used a standard. Incubation was done at 56ºc for a period of 30 minutes. Absorbance was read for the supernatant at 560 nm. [11]

Percentage inhibition of Haemolysis calculated using % inhibition standard formula.

Hypotonicity-Induced Haemolysis

Varying concentrations of the extracts ranging from 100-500µg/ml along with the reference sample and control were used. Diclofenac sodium was the standard used. It is mixed along with 2 ml of hyposaline, 1ml of phosphate buffer along with 0.5ml of HRBC Suspension. The mixtures are then incubated for 30 minutes at 37 ºC. It is then centrifuged at 3000rpm and the resulting supernatant was obtained. The supernatant was decanted and then absorbance was read at 560nm. [12]

% hemolysis protection was estimated by % Protection standard formula

ANTI-INFLAMMATORY EFFECT OF B.SERRATA ON MACROPHAGE RAW 264.7 CELLS

The ethanolic extracts of *Boswellia serrata* are tested for their anti-inflammatory activity on Macrophage RAW 264.7 cells. [13]

CELL CULTURE

Macrophage RAW 264.7 cells which were procured from NCCS, India were cultured in Phenol red-free DMEM. It was supplemented along with 100 µg/ml of streptomycin, 100 unit- s/ml penicillin and then along with 10% heat-inactivated foetal bovine serum. The cells were kept with 5% CO_2 at 37°C. They were washed with DMEM Medium and it was then detached along with 0.25% Trypsin-EDTA solution. They were then washed again with DMEM Medium at the density value of 2×10^6 cells/ml. The cell viability were performed using Trypan Blue. The cells were seeded at 2 × 105 cells/100 µl in 96-well plates and then it was incubated for a period of 1 hour for the TNF- α, IL-1β, and NO production assays.

The cells were then activated along with 5 µg/ml of LPS in the presence or absence of the test compounds i.e Ethanolic extracts of *Boswellia serrata* in different concentrations, dexamethasone and indomethacin for a period of 48 hours. The resulting supernatant were removed and it was then assayed with a commercial ELISA Kit.

LPS-INDUCED CYTOKINE PRODUCTION ASSAY

TNF-α and IL-1β were measured using the instructions of the ELISA kit. The 96 well plate were precoated with capture antibody. Several washings and blocking was performed and addition of 100 µl of working standards were added. It was then incubated for a period of 2 hours. 100 µl of the working detector solution which consists of biotinylated anti-mouse cytokine monoclonal antibody were added and it was then incubated for a period of 1 hour.

Washing was again done after which 100 µl of avidin-horseradish peroxidase conjugate was added. The culture was incubated for 30 minutes. Addition of 100 µl of substrate solution was done along with incubation for 15 minutes. It was then mixed with the 50 µl of stop solution and absorbance was red at 450nm. The inhibition of the cytokine production was calculated using the formula of standard calculation of cytokine.

NITRITE ASSAY

This assay consists of determining the presence of nitrite using Griess reagent. 50 µl of supernatant obtained from the test culture along with 5% (v/v) phosphoric acid and 50 µl of 1% (w/v) sulphanilic acid was mixed in a well plate. It was then

incubated for a period of 10 minutes at room temperature. It was then followed with the addition of 50 µl 0.1% (w/v) N-1-naphthylethylenediamine HCl in distilled water and again incubated for 10 minutes. The absorbance was measured at 540nm. The concentration of NO was calculated in comparison with the standard $NANO_2$. Dexamethasone and indomethacin were utilized as a standard. The inhibition of NO production was calculated using the formula of standard calculation of nitrite.

PREPARATION OF THE DRUG FORMULATION

Two types of pharmaceutical formulations using EEBS were prepared. The first is in the form of a gel, while the second is in the form of an ointment.

PREPARATION OF GEL FORMULATION:

The gel formulation was made by mixing the extract in two different concentrations of plant extract of *Boswellia serrata*.[14]

a) 0.25g (250µg) of gel formulation was made by mixing the extract with 250µg of petroleum ether and 2.25g of petroleum jelly.
b) 0.5g (500µg) of gel formulation was made by mixing the extract with 500µg of petroleum ether and 4.5g of petroleum jelly.

PREPARATION OF OINTMENT FORMULATION:

The ointment formulation was made by mixing the extract in two different concentrations of plant extract *Boswellia serrata*

a) 10% ointment formulation was made by mixing 0.25g (250µg) of plant extract with 2.25g of ointment base, which contains 0.15g of bees wax and 2.1g of white paraffin.
b) 20%ointment formulation was made by mixing 0.5g (500µg) of plant extract with 2.25g of ointment base, which contains 0.15g of bees wax and 2.1g of white paraffin.

PREPARATION OF WOUND MODEL

Adult zebrafish were collected in Kolathur, Chennai. Before the studies, the fish were allowed two weeks to acclimatize to the laboratory surroundings. Fish weighing less than 0.4g were used in the experiments. The fish was divided into six groups: control, standard, and four experimental groups, each with set of 10 fishes (ointment and gel formulation at 2 different concentration). With a sterile knife, the fish was anesthetized using tricaine and the caudal fin was transected 5mm from the posterior side. Before and after transection, the fish were photographed.

TREATMENT OF WOUND MODEL WITH OINTMENT AND GEL PREPARATION

The fin transected fish were given various amounts (250µg and 500µg) of gel and ointment formulation of the extract that had been developed earlier. With gloved hands, the medication formulation containing the different concentration of the extract was carefully administered to the fin transection region. The treated fish were then moved to a separate tank which are labelled individually. The study utilized triplicate values of measurement. The fish in the control group was just given the formulation bases.

EVALUATION OF WOUND HEALING POTENTIAL

Treatment of wound model with ointment and gel preparation

The fishes were given various amounts (250µg and 500µg) of gel and ointment formulation of the extract that had been developed earlier. Under aseptic settings, the medicine formulation including varying concentrations of the extract was applied with gloved hands to the transected fin. The treated fish were then shifted into a recovery tank, where they are separated into groups according to the concentration at which they had been treated. Throughout the trial, triplicates have been kept. The control group's fish were only fed the formulation bases.

FIN REGENERATION

For fin regeneration, the fish was evaluated for its fin regenerative capabilities three, five, and seven days after trauma. The growth of the fin was determined, and the outcomes were captured as well as reported.

NEUTROPHIL MIGRATION

At 24 hours and on day 7 of administration, the neutrophil population of the treated zebrafish wound model as well as the control zebrafish was assessed. The treated fish were euthanized using tricaine. Histological sections utilized hematoxylin, eosin stain for the presence of neutrophils. [15]

RESULTS

Phytochemical screening

The phytochemical screening of *Boswellia serrata* showed the indications of various phytoconstitutents listed in the Table 1.

Table 1. Phytochemical screening of *Boswellia serrate*

S.No	Phytoconstituents tested	Ethanol extract
1	Flavanoids	+
2	Saponins	+
3	Glycosides	+
4	Phenols	+
5	Alkaloids	+
6	Terpenoids	+

'+' indicates presence, '-' indicates absence

IN VITRO ANTI INFLAMMATORY ACTIVITY

Inhibition of Albumin Denaturation

Investigation was performed to analyse the anti-inflammatory activity of the plant extract. It was found that the EEBS was effective in inhibiting protein denaturation. (Table 2) The maximum inhibitory value of 57.14 % at a concentration of 400 µg/ml. The standard used for the comparison of anti-inflammatory activity showed a % inhibition of 68.57% at 100 µg/ml compared with that of the control.

Proteinase Inhibitory Action

EEBS showed significant anti-proteinase activity when it was studied at different concentrations. (Table 3) A maximum inhibitory value of 48.71% was seen at 500 µg/ml. Aspirin used as the standard showed a max % inhibition of 64.10 % at 100 µg/ml compared with that of the control.

MEMBRANE STABILIZATION

Heat Induced Haemolysis

Different concentrations of the extract was utilized to inhibit the heat-induced haemolysis. (Table 4). A maximum inhibitory value of 65.62% was seen at 500 µg/ml. Aspirin used as the standard showed a max % inhibition of 71.87 % at 100 µg/ml compared with that of the control

Hypotonicity Induced Haemolysis

The results of the haemolysis assay showed that the extract at a concentration of 500 µg/ml had a % inhibition of 65.62% (Table 5). This showed that the extract is useful for protection of the erythrocyte membrane. The erythrocyte membrane was lysed with the hypotonic solution for this activity. Diclofenac were used as the standard. It showed the maximum inhibition of 71.87 % at 100 µg/ml compared with that of the control.

Table 2: % Inhibition of Albumin Denaturation

TEST SAMPLE	CONCENTRATION (µg/ml)	ABSORBANCE	% INHIBITION
CONTROL		0.35±0.04	-
EEBS	100	0.24±0.06**	31.42
EEBS	200	0.19±0.02**	45.71
EEBS	300	0.20±0.04**	42.85
EEBS	400	0.13±0.04**	62.85
EEBS	500	0.15±0.05**	57.14
ASPIRIN	100	0.11±0.06**	68.57

Each value represents the mean ± SD. N=3, Experimental group were compared with control **p<0.01, considered extremely significant.

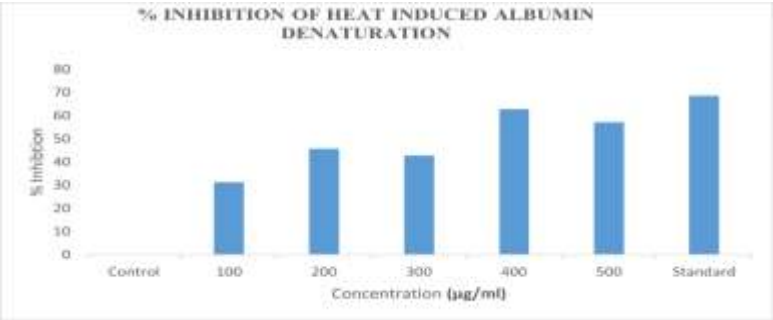


Figure 1: % Inhibition of heat-induced albumin denaturation

Table 3: % Inhibition of Proteinase Inhibitory Action

TEST SAMPLE	CONCENTRATION (µg/ml)	ABSORBANCE	% INHIBITION
CONTROL		0.39±0.05	-
EEBS	100	0.38±0.09**	5.1
EEBS	200	0.37±0.02**	10.2
EEBS	300	0.29±0.07**	25.64
EEBS	400	0.25±0.01**	35.89
EEBS	500	0.20±0.03**	48.71
ASPIRIN	100	0.14±0.01**	64.10

Each value represents the mean ± SD. N=3, Experimental group were compared with control **p<0.01, considered extremely significant.

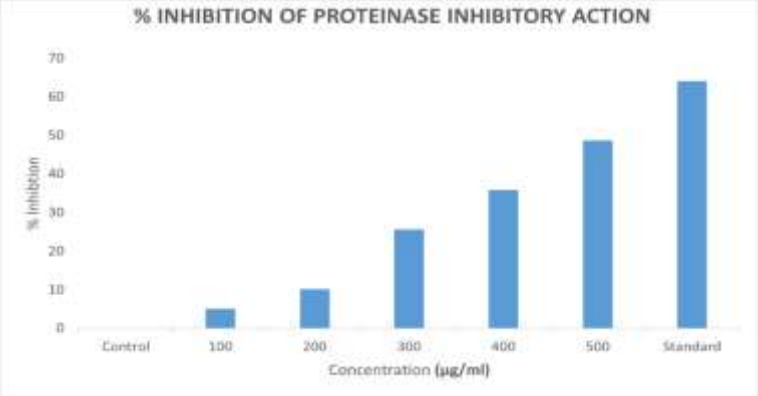


Figure 2:% Inhibition of proteinase inhibitory action

Table 4: % Inhibition of Heat Induced Haemolysis Of RBC

GROUPS	CONCENTRATION (µg/ml)	ABSORBANCE	% INHIBITION
CONTROL		0.32±0.01	-
EEBS	100	0.27±0.06**	15.6
EEBS	200	0.25±0.07**	21.8
EEBS	300	0.21±0.05**	34.37
EEBS	400	0.19±0.01**	40.62
EEBS	500	0.1±0.03**	65.62
ASPIRIN	100	0.09±0.06**	71.87

Each value represents the mean ± SD. N=3, Experimental group were compared with control **p<0.01, considered extremely significant.

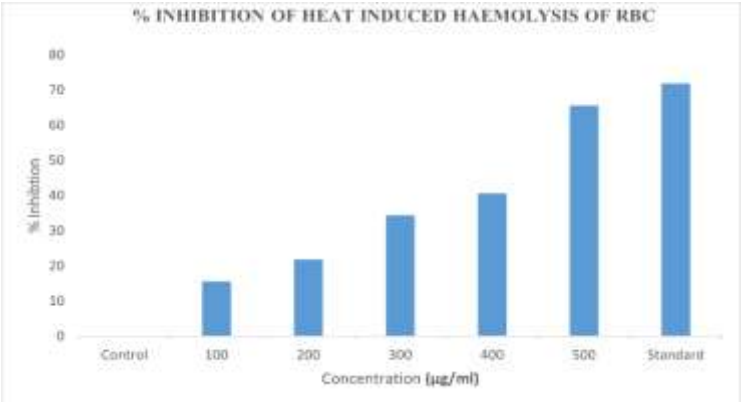


Figure 3:% Inhibition of heat-induced haemolysis of RBC

Table 5: % Inhibition Of Hypotonicity Induced Haemolysis Of RBC

GROUPS	CONCENTRATION (µg/ml)	ABSORBANCE	% INHIBITION
CONTROL		0.32±0.03	-
EEBS	100	0.27±0.02**	15.62
EEBS	200	0.21±0.09**	32.25
EEBS	300	0.15±0.04**	46.87

EEBS	400	0.14±0.07**	56.25
EEBS	500	0.110.02**	65.62
DICLOFENAC	100	0.09±0.01**	71.87

Each value represents the mean ± SD. N=3, Experimental group were compared with control **p<0.01, considered extremely significant.

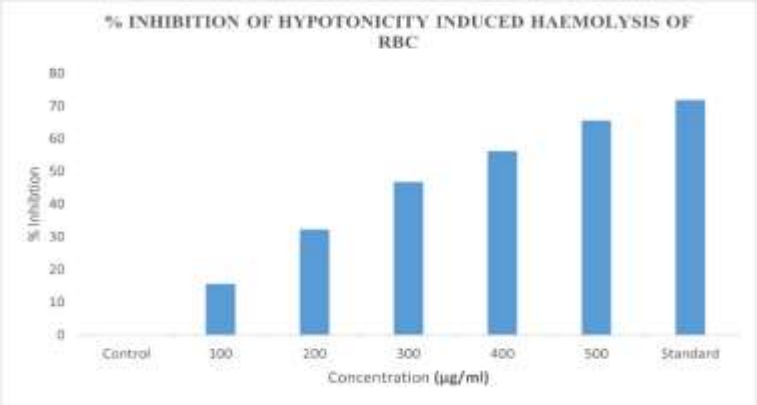


Figure 4:% Inhibition of Hypotonicity induced haemolysis of RBC

ANTI INFLAMMATORY EFFECT OF EEBS ON RAW 264.7 MACROPHAGES

EEBS at various concentrations was subjected to *in vitro* evaluation of anti-inflammatory activity using LPS induced RAW 264.7 cell line and compared with the standard Indomethacin and Dexamethasone. EEBS at 500 µg/ml was found to cause a reduction/inhibition of TNF- α when compared with that standard Dexamethasone and indomethacin. EEBS was also found to cause reduction/inhibition of IL-1 β showing a max inhibition of 55 µg/ml at a concentration of 500 µg/ml. The compound also inhibited NO to a value of 15 µg/ml compared to dexamethasone and indomethacin (10 µM). The results are shown in table 6.

Table 6 Effect of EEBS, Dexamethasone, Indomethacin on TNF- α production on LPS stimulated RAW 264.7 macrophages

Treatment	Concentration (µg/ml)	TNF-α (µg/ml)	IL-1β (µg/ml)	NO (µg/ml)
EEBS	25	2475	110	25
	50	2283	90	23
	100	2154	85	24
	250	2008	62	22
	500	507	55	15
Dexamethasone	0.38	2417	121	15
	1.96	2197	20	15
	3.92	1958	3	14
Indomethacin	0.36	1932	128	18
	1.79	1927	53	15
	3.58	1854	42	14
LPS	5	2495	140	28

EVALUATION OF WOUND HEALING ACTIVITY

Fin regeneration

On the first, third, fifth, and seventh days, the measured fin growth was compared to that of the control. The fish treated with the ointment formulation at 500µg recovered better than those treated with gel formulation, and the rate of recovery increased with cumulative concentration (Fig. 5). Fin regeneration was greatest on the 7th day in fish treated with 500µg of the gel formulation in comparison to the control fishes (Table 7).

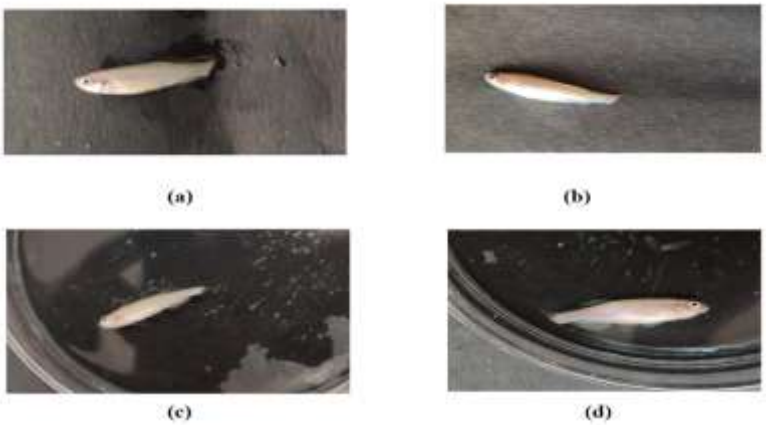


Figure 5. Transection of fin in zebrafish (a) Animal after transection, (b) untreated animals- Day 7 post-transection, (c) 500 µg ointment formulation- Day 7 post-transection and (d) 500 µg gel formulation -Day 7 post-transection.

Table 7. Growth of caudal fin after application of ointment and gel formulation

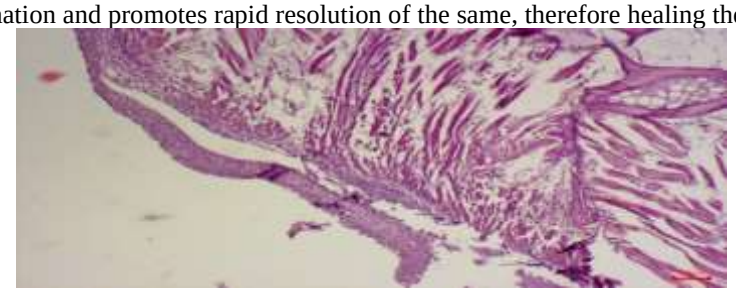
S.No	Treatment	Concentration(µg)	Fingrowth (Mean±S.D.) (mm)
1)	Control	Formulation base	0.9±0.1
2)	Gel Formulation	200 500	1.2±0.3 1.3±0.2
3)	Ointment Formulation	200 500	1.2±0.1 1.5±0.1
4)	Standard (Povidone)	5%w/w	1.7 ±0.2

Values were expressed as mean ± S.D. for the determinations

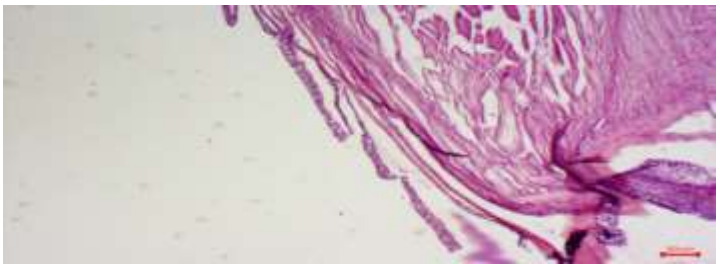
NEUTROPHIL MIGRATION

It was discovered that the neutrophil levels in fin regions increased during 24 hours and thereafter declined on the 7th day of monitoring (Fig. 6). The fish in the control group recruited fewer neutrophils than the fish given the extracts. Fig. 5 indicates that the fish treated with ointment formulation recruited more neutrophils at the injury area in 24h after injury than the fish treated with the gel formulation, the number rising with concentration. Neutrophil numbers dropped dramatically on the 7th day in comparison to control, which demonstrated a far smaller fall in neutrophil numbers and, therefore, a potentially lower resolution of inflammatory conditions. In comparison to the gel-treated group, treatment with the ointment formulation of 500µg was successful in resolving inflammation. For the determinations, statistical analysis was undertaken and the data were presented as Mean±S.D. [16]

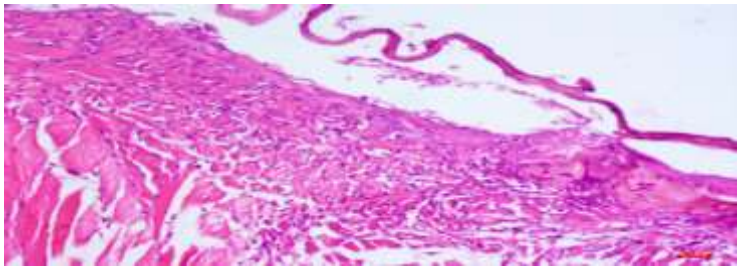
According to the findings, adult Zebrafish might act as a suitable alternate experimental model for determining the efficacy of a plant extract in accelerating the healing process after injury. In the early following of injury, the extract stimulates initial inflammation and promotes rapid resolution of the same, therefore healing the injury.



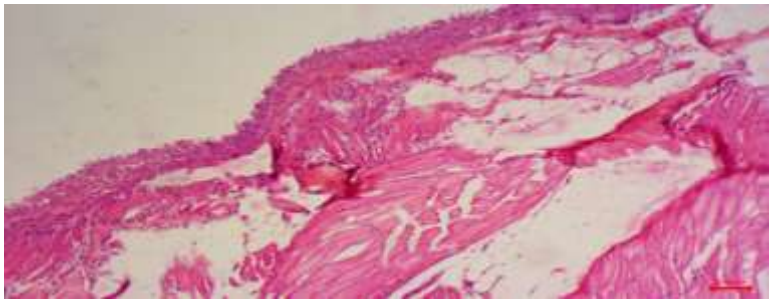
a. 250 µg of gel formulation showing re epithelialization



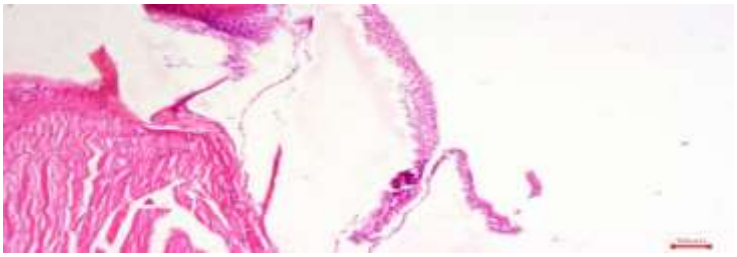
b. 500 µg gel formulation showing re epithelialization



c.250 µg ointment formulation showing reepithelialisation formation , mnc in muscle area



d. 500 µg of ointment formulation showing re epithelialisation and mnc



e. Standard group showing reepithelialisation

Figure 6 Histopathological examination of the caudal fin section

The results reveals that utilization of zebrafish can be an alternative screening model for evaluating the efficacy of wound healing drugs. The application of gel and ointment preparations helps in rapid resolution of the injury.

DISCUSSION

In the present study, the anti-inflammatory activity of EEBS has been determined which consists of assays such as albumin denaturation assay, anti-proteinase activity, and membrane stabilization assay. Over the course of years, novel anti inflammatory drugs were introduced but they had definite side effects of the CVS. Therefore there is a need for the development of ant inflammatory agents which are natural and safe. The aim of these studies should focus on reduction of the side effects and improvement of the therapeutic index.[17]

Protein denaturation plays a vital role in inflammatory conditions. Anti- inflammatory agents such as salicylic acid showcases a thermal induction of protein denaturation. EEBS can be utilized for the inhibitory action for the release of lysosomal contents. These contents are usually released from the neutrophils found at the site of inflammation. [18]
Proteinases are a group of enzymes which are involved in inflammatory arthritic conditions. These are released from lysosomal granules consisting of neutrophils. They cause tissue destruction in inflammatory conditions. Studies have shown the role of polyphenols, flavonoids which may serve as a therapeutic medium for causing anti-inflammatory action. The presence of such phytochemicals can be responsible for its therapeutic action. [19]

The zebrafish is a kind of vertebrate that can spontaneously regenerate. Treatment with gel and ointment formulations of *B. Serrata* extract increased the model organism's regeneration. Because of the synergistic effects of the many phytoconstituents in this well-known traditional plant, it has healing properties. Phytochemical screening reveals the presence of flavonoids, terpenoids, phenols, glycosides. [20]Multiple biological pathways have been proposed to improve wound healing by polyphenolic substances, including flavonoids, which are believed to reduce inflammation by inhibiting the generation of free radicals as well as reactive oxygen species. Their healing impact has been linked to a reduction in acute inflammation caused by a time-dependent regulating of pro-inflammatory chemokine expression as well as repair-regulating chemokine expression. Various polyphenol-collagen composites have been studied and shown to be efficient in wound dressing applications. [21]

The anti-inflammatory and wound-healing properties of glycosides have been identified. The anti-microbial characteristics of terpenoids, flavonoids, as well as phenols may have performed an important role in preventing persistent

inflammation by reduction of bacterial infection surrounding the wound. [22] Wound macrophages may be targeted by the extract and its phytoconstituents when they first become inflammatory or/and by apoptotic signals when inflammation progresses to the final stages. [23]

The constructed model's molecular details will be probed further using modern methodologies in the next investigations. As a result, the plant extract demonstrated its wound-healing ability by reducing inflammation in the injury area.[24]
For qualitative and semi-quantitative reflection of molecular wound healing capabilities in terms of regeneration as well as inflammation, the adult Zebrafish fin regeneration system is a suitable substitute model. Inflammation and regeneration are crucial events in the natural healing mechanism.

CONCLUSION

Experimental results show that Zebrafish may be utilized to be wound inflammation model to test for wound healing characteristics of diverse bioactive fractions derived from synthetic origin or plants.

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