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Research Article

Preliminary Investigations on *Diospyros malabarica* Leaves Extracts for Antiinflammatory and Wound Healing Activity

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ABSTRACT

The present study aimed to screen different extracts of *Diospyros malabarica* leaves for antiinflammatory and wound-healing effect in mice. Phytochemical screening of different extracts i.e. petroleum ether, chloroform, ethyl acetate, ethanol and aqueous extracts, were performed using different chemical test. All extracts were subjected to study antiinflammatory effects using xylene-induced edema and wound healing activity using an incision wound model. Antiinflammatory potential was recorded by observing the mice's percent inhibition of edema, nitric oxide (NO) estimation, and myeloperoxidase (MPO) action. The wound healing effect was observed by measurement of tensile strength, protein level and hydroxyproline level in the wound tissues. Observation of phytochemical study was confirmed that ethanolic extract of *D. malabarica* leaves contains glycosides and flavonoids as major chemical constituents. Antiinflammatory effect was confirmed by significant (p<0.05) percent inhibition of ear edema, significant reduction of NO level and MPO level of mice by ethanol extract of *D. malabarica* leaves. Wound healing potential was observed by the significant increase in tensile strength of wound tissue after treatment with ethanolic extract. A significant (p<0.05) increase in protein and hydroxyproline level of tissue were observed after treatment with ethanolic extract of *D. malabarica*. In conclusion, the potent antiinflammatory and wound healing effects of ethanolic extract of *D. malabarica* may be observed due to the presence of glycoside and flavonoid components.

Introduction

The body uses inflammation as a defense mechanism to get rid of harmful stimuli and start the healing process for the tissue, but if it goes unchecked, it can cause diseases like rheumatoid arthritis and atherosclerosis to develop.[1] It is characterized by pain, heat, swelling, redness, and occasionally loss of function.[2] The process of inflammation is a chain of events that occurs as part of the body's defensive mechanism against various endogenous and external stimuli. Vascular and cellular alterations are the two main categories for inflammation's main ingredients. The vascular changes include a rise in blood flow, temporary constriction of blood vessels, and temporary dilatation of arterioles and venules; a rise in permeability causes the release of chemical mediators, swelling, and a rise in viscosity. Leukocytes migrate from circulation to bacterial destruction during cellular changes. These alterations could be observed to investigate a compound's antiinflammatory properties.[3,4] Globally, it is acknowledged that inflammatory illnesses are the main factor contributing to morbidity among people.[5] An inflammatory state is linked to an immune system that is active, including immune cells and biomolecules.[6] An organism will become inflamed as a protective measure against bacteria, parasites, and viruses invasion. Acute inflammation is characterized by redness, heat, swelling, discomfort, and functional loss. Swelling, redness, and pain are each related to altered vascular permeability, faster blood flow, and nerve fiber sensitization.[7] In a normal state, the potential of tissue death is balanced by

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the protective powers of the inflammatory cascade. On the other hand, prolonged inflammation is often characterized by considerable tissue healing after an inflammatory response.[8] If uncontrolled, chronic inflammation is also connected to several stages of carcinogenesis and is known to increase the chance of developing various malignancies.[9]

The repair process by which tissue healing occurs is known as wound healing epithelial, endothelial, inflammatory, platelet, and fibroblast cells momentarily come together outside of their regular domains and interact to restore resemblances of their used discipline in this continual sequence of inflammation and repair. It results in resuming their normal function.

Healing an injury is a dynamic, complex process. The individual's fluctuating state of health has an impact on the wound environment. Understanding the fundamental concepts of wound healing can be framed by knowing the physiology of the typical wound healing trajectory through the four sequential phases of hemostasis, inflammation, granulation, and maturation.[10]

A species of presently selected plant belonging to the Ebenaceae family called *Diospyros malabarica* is found all over India and other tropical parts with different temperatures in the world. Round, yellow, and ripe, the *D. malabarica* tree produces fruit throughout the months of July and August of any year.[11] Different species of Diospyros are used medicinally in Indian systems of traditional medicine like Ayurveda and Unani to treat fever, diabetes, snake bites, diarrhea, ulcers etc.[12] Leaves are reported as diuretic, carminative, laxative, and ophthalmic and are useful in dyspepsia, flatulence, burns, tubercular glands, scabies and wounds. *D. malabarica* bark is bitter, astringent and febrifuge. The unripe fruit is a more powerful astringent.[13]

It has been reported to contain a number of bioactive substances, including gallic acid, flavonoids, anthocyanins, saponins, alkaloids, sitosterol, betulinic acid, natural vitamin C, triterpenes and tannins, which are primarily responsible for their antiviral, antihelminthic, antiprotozoal and antioxidant activity.[14]

Review of literature revealed that *D. malabarica* has a positive impact on many ailments and is frequently used as a folk medicine in tropical and subtropical climates. It has mostly been used for treating rheumatism and hepatitis and has also been recognized as an antioxidant. *D. malabarica* leaves are used as diuretic, carminative, laxative, and ophthalmic and are useful in dyspepsia, flatulence, burns, tubercular glands, scabies and wounds.[15] The present study consists of preliminary phytochemical and pharmacological screening of different extracts of *D. malabarica* leaves for antiinflammatory and wound healing activity on experimental animal models.

Material and Methods

Identification and Collection of Plant Materials

D. malabarica leaves were taken from the area of Raisen (M.P.) throughout the duration of August and September months. Collected crude material was submitted to Jawaharlal Nehru Krishi Vishwavidyalaya, Jabalpur (M.P.) a plant specimen was identified and verified. Plant materials were collected, dried in shade for grinding and powdering, and stored for further additional steps, such as solvent extraction.

Extraction and Phytochemical Screening

The dried powdered plant material of *D. malabarica* (leaves) was taken for extraction (150 g) and extracted successively with petroleum ether, chloroform, ethyl acetate, ethanol and aqueous extracts. After complete extraction with each solvent, respective extracts were obtained. After the extraction procedure was finished, the solvent was entirely evaporated using a vacuum evaporator while operating at reduced pressure, yielding completely dried extract for subsequent use.

Through several chemical assays, phytochemical investigations were done through qualitatively screening using various chemical tests[16-18] for various extracts collected from *D. malabarica* aerial parts[16-18] for qualitative detection of the presence of different chemical constituents.

Antiinflammatory Activity

Animal selection

For antiinflammatory investigations, healthy swiss albino mice stain of either male or female, their weight measured between 95 to 100 g were used. They may be of either sex. Prior to the experiment, they were maintained in individual cage in the animal house for a week at a controlled temperature of 25°C, with relative humidity of 44 to 56%. We have maintained proper light and dark cycles throughout whole experimentation with 10 to 14 hours. The animal care and experimental protocols were in accordance with CPCSEA/IAEC. The animals were grouped and prior to dosing, polyacrylic cages should be used for at least 7 days to give time for acclimation to the laboratory environment. Al animals have freely allowed to food and water in whole duration. However, they went 48 hours without eating or drinking before the operation. The animals were place in individual group wise as per the following protocol, each group contained five animals. All extracts were prepared using a suspending agent (2% Tween-80) as a suspension by trituration. Following the fasting of the animals, and weighing properly, were given a calculated dose of extract orally. Do not allow all animals to the food for 3 to 4 hours immediately after dosing in mice.

Group I was control and saline solution (10 mL/kg p.o) was given to each animal in the group
Groups II was given orally petroleum ether extract of *D. malabarica* (PEEDM) to each animal of group (200 mg/kg; p.o.)
Groups III, was given orally chloroform extract of *D. malabarica* (CEDM) to each animal of group (200 mg/kg; p.o.)
Group IV was given orally, ethyl acetate extract of *D. malabarica* (EAEDM) to each animal of group (200 mg/kg, p.o.)
Group V was given orally, ethanol extract of *D. malabarica* (EEDM) to each animal of group (200 mg/kg; p.o.)
Group VI was given orally, aqueous extract of *D. malabarica* (AEDM) to each animal of group (200 mg/kg; p.o.)
Group VII was given orally, Indomethacin (5 mg/kg; p.o.) to each animal of group

Xylene-induced ear edema

According to the outlined animal protocol, Swiss albino mice were fed various extracts orally once a day for two weeks, and xylene (0.05 mL) was administered to the right ear's posterior area at the same time. The left ear is still not being treated. Both ears were cut off, and ear lobe weights were taken, three hours following xylene treatment. Edema was measured by calculating the weight of both ears and then the difference in the weight of the right treated and left untreated ears.[19] The percentage inhibition (%) of edema of each group of animals was measured using following formula:
The difference of both ears was used to calculate the percent proportion of edema. This method was followed in all treated group of animals.

$$\text{Percentage edema} = \frac{\text{Measure weight of right ear} - \text{Measure weight of left ear}}{\text{Measure weight of left ear}} \times 100$$

Determination of nitric oxide (NO)

Various researchers report that reactive oxygen species and nitric oxide molecules are generated after any injury or edema occurs, and ultimately both combined induce tissue damage. This causes an inflammatory response that increases the formation and expression of various types of inflammatory components such as cytokines, prostaglandins E2 (PGE2), arachidonic acid derivatives and active oxygen species. In addition, NO is a strong vasodilator that contributes to the inflammatory process and causes edema to occur.[20]
Some specific reactions, like the Griess reaction, are used mainly to detect nitrite, a byproduct or derivative of nitric oxide reaction with oxygen, were used to indirectly measure NO levels in mouse ears.[21] In brief, 50 µL of each tissue supernatant were combined with 50 µL of 1% sulfanilamide mixed with 5% phosphoric acid and stand for incubation at 22°C in the dark for 5 minutes. After that, 50 µL of 0.1% naphthyl ethylenediamine dihydrochloride solution was mixed and absorbance was taken at 540 nm using microplate reader (ELISA, Micro Lab, Ahmedabad, India). Based on a sodium nitrite standard curve, the amount of nitrite was determined and nitrite content was stated as nmol per ear.

Myeloperoxidase (MPO) enzyme estimation

The Krawisz *et al.* (1984)[22] method was used to measure tissue MPO activity 24 hours after applying xylene to the mouse ear. Briefly, 10 mL of ice-cold solution of 50 mM potassium phosphate buffer (pH 6) mixed with solution of 0.5% hexadecyltrimethylammonium bromide was used to punch and mince 6 mm ear tissue. The homogenate was sonicated and centrifuged at 4°C for 20 minutes at 12000 g (Remi Centrifuge, India). Mixing 0.1 mL of the homogenate supernatant with 2.9 mL of 50 mM phosphate buffer solution in 0.0005% hydrogen peroxide, the MPO activity was expressed with spectrophotometrically absorbance at 460 nm. At room temperature, the change in absorbance per minute was used to perform MPO estimation. Observation of activity was expressed as percentage of MPO against the control group of animal administered with a vehicle.

Wound Healing Activity

Experimental animals

For the investigation on wound healing, either sexed, healthy Swiss albino mice (weighing 95–100 g) of either sex were chosen. Prior to the experiment, they were maintained in the animal house for a week at a controlled temperature of 25°C, with relative humidity of 44–56%. All animals were properly allowed for light and dark cycles for 10 and 14 hours. The animal care and experimental protocols strictly followed with CPCSEA/ IAEC guidelines. Experimentation started with selection of animals at random pattern, marked with a marker to enable for individual identification, and kept in its separate cage for at least 7 days prior to dosing to give it time to get used to the lab environment. For the experiment, the rats were kept in groups in polyacrylic cages. All groups of animals have access to food and water unlimited.
Administration of extracts doses were prepared by triturating with suspending agent Tween-80 (2%) and applied topically twice in a day.
Following seven groups were made for whole experimentation and each group contained 5 animals:
Group I was controlled and treated with vehicle given topically twice in a day to each animal in the group.
Groups II was given topically, petroleum ether extract of *D. malabarica* (PEEDM) twice in a day to each animal of group.
Groups III, was given topically, chloroform extract of *D. malabarica* (CEDM) twice in a day to each animal of group.
Group IV was given topically, ethyl acetate extract of *D. malabarica* (EAEDM) twice in a day to each animal of group.

Group V was given topically, ethanol extract of *D. malabarica* (EEDM) twice in a day to each animal of group.
Group VI was given topically, aqueous extract of *D. malabarica* (AEDM) twice a day to each group animal.
Group VII was given topically, Povidine iodine ointment twice in a day to each animal of group

Incision wound model

Before creating wounds, all animals were anesthetized. Hair was removed from each back side of the animal and two paravertebral long incisions were cut through the skin, about 1.5 cm from the midline. Throughout the experiment, no local nor systemic antimicrobials were applied. Both edges of incised skin were sutured by using black silk surgical thread (No. 000) and a curved needle (No. 11). They were maintained together and sewn together. The continuous threads on both edges were tightened to ensure a complete closure of wound edges. Following stitching, the incision was left uncovered while simple ointment base, a sample medicine, and a normal drug ointment were applied every day for up to nine days. On the ninth day, sutures were removed once the wounds had fully healed, and a tensiometer was used to evaluate the tensile strength of animal skin.[23]

Tensile strength measurement

Any resistance to break in the skin tissue under tension is known as tensile strength of skin tissue. It reveals how much the restored tissue is able to withstand stress before breaking, and it may also partially reveal the healed tissue. The recently healed tissue, including the scar, was removed to evaluate the tensile strength. The 'Tensiometer' is the name of the measurement device.[24] It comprises of a hardwood board measuring 6 x 12 inches with one arm fastened on either side of the board's maximum allowable distance. The board is set against a table's edge. On the top of one arm is a pulley with a bearing. A fishing line (20 lb test mono filament) is linked at the top of one arm with an alligator clamp that is 1-cm wide so that it can reach the board. A one litre polyethylene bottle was connected with the other end and second alligator clamp is fastened. The animals were given ether anesthesia under an open mask prior to testing. The sutures are taken out of the mice's patched wounds a day before the tensile strength test is measured. Then, in the center of the board, a stack of paper towels was placed on top of the animal. The quantity of towels might be changed to place the wound at the same level as the posts' points.

Protein Content Determination

Using the Lowry (1951) method, the protein content of skin tissues was assessed.[25] At initial step, sample of tissue lysate was mixed with sodium tartrate, sodium carbonate solution and copper sulphate with manual mixing to determine the amount of tissue protein. Folin-Ciocalteau reagent was applied after the mixture had stood for 10 minutes, and the mixture turned bluish in 20 to 30 minutes. The absorbance was taken at 650 nm using spectrophotometer. The calculation was done from a standard curve of protein.

Hydroxyproline Measurement

The gelatin solution in water was put into a small beaker (20 to 30 mL) after the tubes were taken out and given time to cool. Take two separate portions of 1-mL, 6N hydrochloric acid, the solution in the residual was absorbed and then transferred to stoppered tubes. The autoclave was heated for 6 to 12 hours at 15 lb of pressure to achieve hydrolysis. Sample of tissue hydrolysate was diluted and filtered with water to 10 mL after chilling. To prepare sample for the colorimetric, 1-mL portions of diluted hydrolysate was further mixed with 5.0 mL assay buffer, and 2.5 mL of chloramine T reagent was added. This mixture of reagents was stand for 20 minutes at 25°C, room temperature. Taking 2.5 mL of freshly prepared dimethylamino- benzaldehyde reagent solution was added to previous mixture and mixed thoroughly all tubes at 60°C for 15 minutes. This whole content was cool in tap water for 1 to 2 minutes.[26] This whole content was used to take absorbance at 557 nm immediately using UV visible spectrophotometer (Shimadzu).

Statistical Analysis

Data were represented as mean ± SD for all statistical analysis. Each treatment group was containing six animals. All data was analyzed statistically using mean values and ANOVA as well as by the multiple comparisons test (Tukey's). GraphPad Instat Software executed a statistical analysis of the results. Presented data were considered statistically significant if $p<0.05$.

Results and Discussion

Phytochemical Screening

The dried powder of aerial parts of *D. malabarica* leaves were used for extraction successively using various solvents in increasing polarity order i.e. petroleum ether, chloroform, ethyl acetate, ethanol and water. Extraction with different organic solvents was done by using soxhlet apparatus and aqueous extract was obtained by maceration process.

Results of phytochemical screening of *D. malabarica* leaves extracts were showed that petroleum ether extract showed the presence of terpenoids, fatty acids and sterols. Chloroform extract showed presence of alkaloids. Additionally, ethyl acetate extract confirmed the presence of glycosides and flavonoids. Ethanolic extract confirmed the presence of glycosides, flavonoids, amino acid and proteins. Aqueous extract was showed positive test for carbohydrates and tannins.

Antiinflammatory Activity

Various phlogistic agents e.g. arachidonic acid, histamine, croton oil, xylene, formalin, oxozolone and phorbol myristate acetate are commonly applied to induce inflammation in the experimental animals. Present study consist of preliminary screening of various extracts of *D. malabarica* leaves for antiinflammatory potential by using xylene-induced edema and wound healing activity using incision model on experimental animals.

The xylene-induced ear edema model is a convenient *in-vivo* method for investigating antiinflammatory potential or effectiveness, particularly in cases of edema and fluid buildup indicative of an early inflammatory reaction in mice.

In present study, different extracts i.e petroleum ether extract, chloroform extract; ethyl acetate extract; ethanol extract and aqueous extracts of *D. malabarica* were given orally to experimental mice as per given protocol for measurement of percentage inhibition in the xylene-induced ear edema of mice. Ethanol extract of *D. malabarica* (EEDM) at the dose of 200 mg/kg; p.o. showed significant percent inhibition (57.47%) of ear edema and it was comparable with the standard Indomethacin (5 mg/kg; p.o.) group of treatment (Table 1 and Fig. 1). Standard Indomethacin (5 mg/kg; p.o.) group of treatment showed percent inhibition (60.91%). Other extract, petroleum ether extract, chloroform extract; ethyl acetate extract and aqueous extracts of *D. malabarica* does not showed any significant percent of inhibition.

Applying xylene to the ear causes neurogenic edema. It has a tenuous connection to substance P. Substance P is an undecapeptide found in both the central and peripheral nervous systems and plays a major physiological role as a neurotransmitter or neuromodulator. Substance P participation in neurogenic inflammation is suggested by the peripheral vasodilatation and plasma extravasations caused by its release from sensory neurons. Thus, it may result in the mice's ears bulging.[27]

Results of present study confirmed that nitric oxide level of test ear was increased significantly compared to the control mice ear. Control mice was treated only with saline solution. These results confirmed nitric oxide release's participation in acute inflammation produced by xylene application on the ear in the model.

Different extracts of *D. malabarica* leaves (petroleum ether, chloroform; ethyl acetate; ethanol extract and aqueous extracts) were given orally to the respective animal groups along with xylene treatment. Results were showed that ethanol extract of *D. malabarica* (EEDM; 200 mg/kg; p.o.) treated group of animals were showed significant reduced level of NO and MPO as 26.31 ± 1.64 and 12.48 ± 0.88, respectively (Table 2 and Fig. 2). It was comparable to Indomethacin (5 mg/kg; p.o.) group of animals.

These observations are concluded that the acute inflammation was reduced by application of ethanol extract of *D. malabarica* may have capacity to obstruct the pathways involved in NO production may be connected, at least in part. Since xylene plays a crucial part in the regulation of inflammatory dermal reactions through activating various intracellular signaling pathways. Various enzymes regulate these pathways, e.g., inducible nitric oxide synthase (iNOS). The production of NO encourages vasodilatation, which directly contributes to the development of inflammation and the inflammatory reactions.[28]

Monocytes and macrophages have substantially lower concentrations of MPO, another enzymatic molecule found in neutrophils. It is well known that the quantity of neutrophils on the inflamed tissue directly relates to the amount of MPO activity.[29] The drug's inhibition of MPO activity reduces the production of such reactive oxygen species such as hypochlorous acid, which are directly connected with antiinflammatory properties of plant extracts.

According to the findings, the MPO level of test ear

Table 1: Observation of effect of *D. malabarica* extracts on weight of ear in xylene induced ear edema model

Animal groups	*Measure weight of ear lobe (g) Mean ± SD*	*Percent Inhibition (%)*
Control (Saline solution)	0.87 ± 0.04	-
PEEDM (200 mg/kg; p.o.)	0.77 ± 0.11	11.90
CEDM (200 mg/kg; p.o.)	0.81 ± 0.07	6.89
EAEDM (200 mg/kg; p.o.)	0.49 ± 0.10	43.67
EEDM (200 mg/kg; p.o.)	0.37 ± 0.05*	57.47*
AEDM (200 mg/kg; p.o.)	0.44 ± 0.07	49.42
Indomethacin (5 mg/kg; p.o.)	0.34 ± 0.08*	60.91*

Data represented as mean ± S.D. (n = 5), *p<0.05 when data analysis and compared with control and reference group. PEEDM: petroleum ether extract of *D. malabarica*; CEDM: Chloroform extract of *D. malabarica*; EAEDM: Ethyl acetate extract of *D. malabarica*; EEDM: ethanol extract of *D. malabarica*; AEDM: Aqueous extract of *D. malabarica*

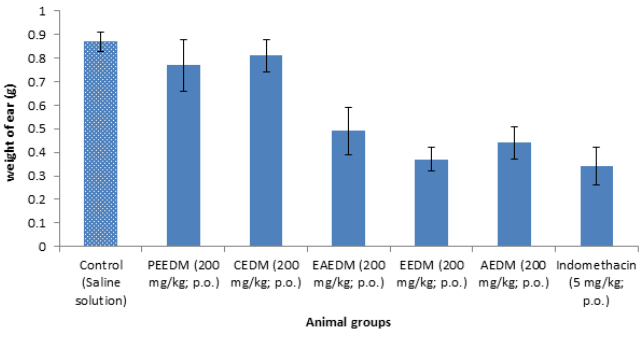

Fig. 1: Effect of different extracts of *D. malabarica* on weight of ear in xylene induced ear edema in mice. Data represented as mean ± S.D. (n = 5), PEEDM: petroleum ether extract of *D. malabarica*; CEDM: Chloroform extract of *D. malabarica*; EAEDM: Ethyl acetate extract of *D. malabarica*; EEDM: ethanol extract of *D. malabarica*; AEDM: Aqueous extract of *D. malabarica*

Table 2: Observations of effect of *D. malabarica* extracts on inflammatory parameters in xylene induced ear edema

Animal groups	*Inflammatory parameters*	
	Nitric oxide (NO) level (µmol/mg)	*MPO level (U/g)*
Control (Saline solution)	68.42 ± 2.67	37.64 ± 1.42
PEEDM (200 mg/kg; p.o.)	58.12 ± 2.51	27.12 ± 1.69
CEDM (200 mg/kg; p.o.)	54.82 ± 2.66	25.84 ± 1.20
EAEDM (200 mg/kg; p.o.)	41.57 ± 2.14	29.64 ± 1.55
EEDM (200 mg/kg; p.o.)	26.31 ± 1.64*	12.48 ± 0.88*
AEDM (200 mg/kg; p.o.)	42.84 ± 2.01	27.14 ± 1.67
Indomethacin (5 mg/kg; p.o.)	24.75 ± 1.34*	13.27 ± 0.57*

Data analysis showed *p<0.05 when compared against the control group and reference group using one-way ANOVA and Tukey's test. Data appeared as a mean ± SD. PEEDM: petroleum ether extract of *D. malabarica*; CEDM: Chloroform extract of *D. malabarica*; EAEDM: Ethyl acetate extract of *D. malabarica*; EEDM: ethanol extract of *D. malabarica;* AEDM: Aqueous extract of *D. malabarica*

(inflamed controls, treated just with saline solution) was much greater than it was in non-inflamed controls (left ear controls that weren't treated) and in groups that had received plant extract treatment. The findings demonstrate that xylene can cause neutrophil infiltration into mouse ear tissue. Treatment with plant extract and indomethacin significantly (p<0.05) reduce the MPO levels of test mice ear, which may help to mitigate the problem.

Wound Healing Activity

Wound healing activity of different extracts of *D. malabarica* i.e. petroleum ether, chloroform, ethyl acetate, ethanol and aqueous extract were studied using an incision wound model in mice. Healing effect was compared with povidone iodine ointment. Wound healing effects were observed by measurement of tensile strength and biochemical parameters (protein estimation, hydroxyproline level) after 9th day of treatment in incision wound of mice. Observations of study was confirmed that out of all extracts, ethanol extract was showed a significant increase in tensile strength of wound tissue, and it was comparable to the reference group of animals (Table 3, Fig. 3) after 9th day of treatment.

Results of protein content and hydroxyproline content of animal groups treated with different extracts of *D. malabarica* were shown (Table 4 and Fig. 4). A significant (p<0.05) increase in protein content (80.55 ± 3.62) and hydroxyproline content (64.85 ± 2.75) were observed in ethanol extract of *D. malabarica* treated group of animals, whereas standard group of animals showed protein content and hydroxyproline content as 82.34 ± 3.28 and 63.27 ± 2.67, respectively. Other extracts of *D. malabarica* i.e. petroleum ether, chloroform, ethyl acetate and aqueous extracts does not showed significant increase in protein content and hydroxyproline content.

Hydroxyproline is an important component of collagen

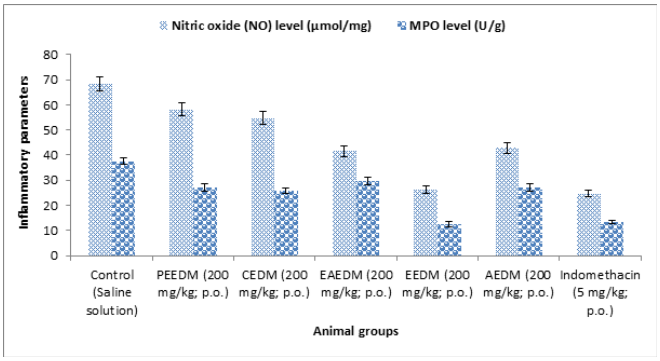

Fig. 2: Observation of different extracts of *D. malabarica* on inflammatory parameters i.e. level of nitric oxide (NO) and myeloperoxidase (MPO), after causing inflammation by application of xylene in mice ear. Data represent *p<0.05 when compared test ear against the control and reference group. All comparison were against each other using one-way ANOVA and Tukey's test. Data appeared as a mean ± SD. PEEDM: petroleum ether extract of *D. malabarica*; CEDM: Chloroform extract of *D. malabarica*; EAEDM: Ethyl acetate extract of *D. malabarica*; EEDM: ethanol extract of *D. malabarica*; AEDM: Aqueous extract of *D. malabarica*

Table 3: Effect of different extracts of *D. malabarica* on tensile strength of wound tissue in incision wound in mice

Animal groups	*Tensile strength (gm/cm^2)*
Control	285.46 ± 7.45
PEEDM	310.28 ± 8.65
CEDM	307.16 ± 8.42
EAEDM	452.82 ± 9.25
EEDM	756.46 ± 11.37*
AEDM	352.66 ± 8.20
Povidone iodine ointment	762.14 ± 11.96

Each value is the mean ± S.D. (n = 5) *P<0.05 when statically analyses and compared with control group by one way ANOVA followed by Tukey's test. All tabulated value represented as mean ± SD.

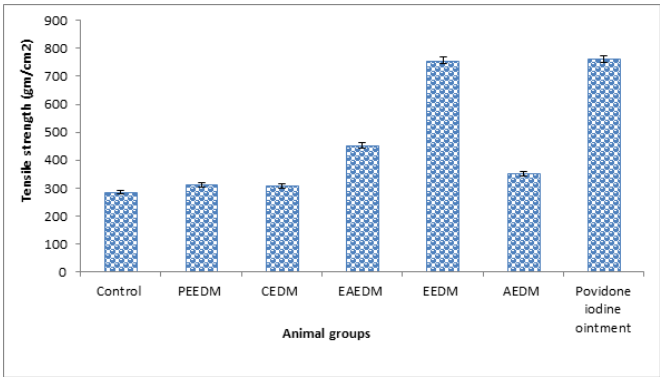

Fig. 3: Observation of tensile strength measurement after treatment with different extracts of *D. malabarica* in incision wound model. Data represented as mean ± S.D. (n = 5)

and can be used as a key component for estimating collagen content in any sample. In addition to being employed as a marker of collagen synthesis, the estimate of hydroxyproline is a recognized technique for biochemically

Table 4: Effect of different extracts of *D. malabarica* on biochemical parameters of incision wound in mice

Animal groups	*Hydroxyproline level (mg/g tissue)*	*Protein level (mg/g tissue)*
Control	22.54 ± 1.20	33.28 ± 1.88
PEEDM	30.42 ± 1.64	40.22 ± 1.79
CEDM	28.05 ± 1.52	37.95 ± 1.62
EAEDM	32.62 ± 1.66	42.58 ± 2.75
EEDM	64.85 ± 2.75	80.55 ± 3.62
AEDM	27.10 ± 1.85	38.62 ± 1.44
Povidone iodine ointment	63.27 ± 2.67	82.34 ± 3.28

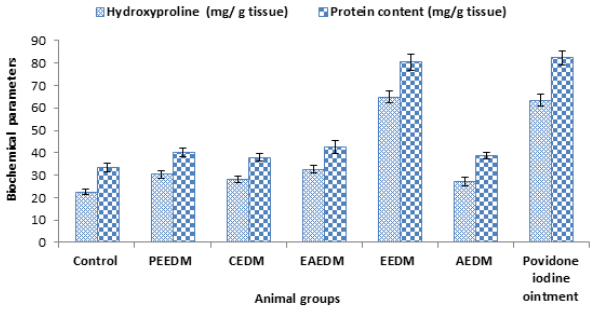

Fig. 4: Effect of different extracts of *D. malabarica* on hydroxyproline and protein content of incision wound tissue

assessing the overall collagen content of a sample.[30] The main function of collagen is to provide strength and elasticity to the skin tissue. This strength can be represented as the tensile strength of healed skin tissue. A significant improvement in tensile strength indicates to increase in collagen in the extract-treated group of incision wound as compared with the control group of animal wounds. According to certain theories, the amount of protein in granulation tissue reflects the rates of protein synthesis and cell proliferation. Increased collagen production results in an increase in protein content.[31] The present study showed that protein content of extract treatment group was significantly increased. This increase in protein content implies that the treatment with respective extracts may able to stimulate cell proliferation. *D. malabarica* already been reported to contain higher flavonoid content[32,33] components that may be responsible for antiinflammatory and wound healing potential.

Conclusion

Results of the present study were observed that during the early stages of inflammation, ethanol extract of *D. malabarica* leaves was significantly inhibiting ear edema, suggesting that the ethanolic extract may be preventing the production of histamine and serotonin. This indicated that the ethanolic extract might exert its antiinflammatory effects via preventing histamine production, release, or activity. The wound-healing effect may also correlate with the antiinflammatory effect of *D. malabarica* extract. The antiinflammatory and wound healing effect of *D. malabarica* leaves may be due to the presence of flavonoids or flavonoid glycosides in ethanol extract.

Conflict of Interest

The authors do not have any conflict of interest in the present research work.

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