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Pharmacological Evaluation Of Anti Inflammatory Activity of Ficus Dalhousiae in Rats

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ABSTRACT

Title: The aim of the current study was to evaluate Anti-Inflammatory activity of methanolic extracts of Ficus dalhousiae.

Objective: Inflammation is a well orchestrated response to undesirable stimuli including injury and infection. Inflammation acts as a major cause of development of many diseases in humans including renal disorders, immune disorders, cardiovascular, dental, and renal disorders.Hence the study was carried out with the aim of evaluating anti-inflammatory activity.

Methods:The anti-inflammatory activity was carried out with methanolic extracts on rats using egg albumin model and croton oil model. In Vitro studies were also carried out using membrane stability test and albumin denaturation inhibition test respectively, rats were fed with Ficus dalhousiae at concentrations of 250 mg/kg b.w and 500kg/mg b.w p.o. Change in paw volume was determined, GSH and LPO were estimated as well, proinflammatory cytokines like interleukins and TNF were estimated as well as vitamin C levels.

Results; The administration of Ficus dalhousiae at concentration of 250 and 500 mg/kg inhibited inflammation and lessened oedema in egg albumin induced oedema test. Oral administration of methanolic extracts of Ficus dalhousiae showed anti-inflammatory biological activity in mouse ear edema induced by croton oil by decreasing the ear edema weight . In agreement with this, the percentage inhibition of the extract of Ficus dalhousiae significantly inhibited the ear edema by 23.5% and 51.39% at 250 and 500 mg/kg doses, respectively.

Interpretation and conclusion: Results from study suggest that Ficus dalhousiae possess anti-inflammatory activity and hence useful in development of natural anti-inflammatory agents.

Keywords: Ficus Dalhousiae, Anti-inflammatory, Albumin

I. INTRODUCTION

Inflammation is defined as a biological response of body tissues to harmful stimuli, such as pathogens, damaged cells, or irritants, and is a protective response involving immune cells, blood vessels, and molecular mediators.When your body encounters an offending agent (like viruses, bacteria or toxic chemicals) or suffers an injury, it activates your immune system. Your immune system sends out its first responders: inflammatory cells and cytokines (substances that stimulate more inflammatory cells).[1]

These cells begin an inflammatory response to trap bacteria and other offending agents or start healing injured tissue.[2] The result can be pain swelling,bruising or rednes But inflammation also affects body systems you can't see. Inflammatory reaction, typica lly is characterised by redness, swelling, heat, and pain, it is the most important defence mechanism against invading pathogens.However, persistent or over-Inflammation leads to tissue damage and possibly failure of organs due to excessive production of microscopic derived mediators. Pro- inflammatory cytokines (e.g, TNF-α,IL-1β andIL-6) are produced in large quantities by activated macrophage/monocytes that stimulate cellular response via increasing prostaglandins and reactive oxygen species. Accumulation of these mediators may result in collateral damage to normal cells, resulting in diseases like atherosclerosis, bowel disease, septic-shockEtc.For 1000 years, the medications were totally of natural origin and extracted from inorganic materials, plant and animal products. Primary remedies can commonly have combined these components with mysticism, witchcraft, astrology, or religion ;however, it is assured that those medica tions, which were in effect, were successi vely verified and recognized, leading to the early herbalist .Herbal medicine continues to be an accepted form of treatment in the Orient, and Plant drugs based on traditional practice represent a huge portion of the

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pharmaceutical products in modern western countries. First, concerns have been raised that modern pharmaceutical practice too often involves costly drugs that produces unacceptable side effects.The experience shows that natural substances can apparently address several modern health concerns with fewer side effects and third, experience shows that modern medicine and traditional herbal medicine can be combined.

II. MATERIALS AND METHODS

Collection of Sample: Specimen has beenbeen identified and authenticated by Dr PP Baruah, department of botany Guwahati university. Reg No Herb/Bot/Gu/2022/50 .

PREPARATION OF EXTRACT

Method of Extraction; Each 100 gm of powder was subjected to extract with 1000 ml of methanol **INVIVO STUDIES; Egg Albumin Induced inflammation;** Egg albumin induced paw in rats , The paw edema method by winter et al, 1962 was used. Young male wistar albino adult rats were used. The acute inflammation of the hind paw was induced in each of the rats by injecting**.**0.1ml/kg body weight of fresh egg albumin into the sub plantar surface of the right hind paw

Croton Oil Induced Inflammation;Croton oil, indomethacin, and ficus dalhousiae extract were dissolved in acetone and applied topically in the bundle and underside of the ear. All groups (n= 6) had croton oil applied in the right ear (1 mg/20µL acetone), whereas the left ear received only acetone. Posterior to the croton oil application, indomethacin (10 mg/kg) was applied in the right ears, as well as ficus dalhousiae.

In-vitro Studies Denaturation of albumin The assay was carried out by adopting the methods described by Kumari et al. with some modifications in which the volume of each component in the reaction mixtures was reduced by half. The plant extracts and positive standard (indomethacin) were prepared at a concentration of 0.1% each (1.0 mg/ml). A reaction vessel for each mixture was prepared consisting of 200 µl of egg albumin, 1400 µl of phosphate buffered saline, and 1000 µl of the test extract. Distilled water instead of extracts was used as a negative control. Afterward, the mixtures were incubated at 37°C for 15 min and then heated at 70°C for 5 min. After cooling, their absorbances in a reflux condenser for 3 cycles of 7 hrs each until the volume reduced to half. Extract was filtered through whatman filter paper No.1 and evaporated to dry ness to get constant weight.

EXPERIMENTAL ANIMALS;Wistar

Albino rats of male sex weighing (150-200 g) was used in this study.The animals were housed in polypropylene cages in groups of six rats per cage and kept under controlled environmental conditions. Care of animals done according to guidelines of IAEC(Institutional Animal Ethics Committee IAEC/09/21-22/02/18/12/21

5.2.4 ACUTE TOXICITY : Acute oral toxicity study was performed as per OECD-423 guidelines category IV (acute toxic class method,[72] (Reported by Syed s et al; Antipyretic
and analgesic effects of ficus dalhousiae; RJPT
were measured at 680 nm (colorimeter).The inhibition percentage of protein denaturation was calculated using the following formula:% Denaturation inhibition =(1-D/C)×100%. Where D is the absorbance reading of the test sample, and C is the absorbance reading without test sample (negative control.)

Membrane stability test;Anti-inflammato ry activity of methanolic extract of Ficus dalhousiae was evaluated by using in vitro human red blood cell stability method. Blood sample was collected from a fresh volunteer, who hasn't had Anti-inflamma tory or contraceptive drugs for at least since a week. I collected blood mixed with sterilised Alsever solution. Alsever solution was prepared by 2% dextrose, 0.8% sodium citrate, 0.05% citric acid and 0.42% sodium chloride dissolved in distilled water.Blood sample was centrifuged at 3000 rpm and packed cell was washed with isosaline and a 10% (V/V) suspension of isosaline was made. Three different solutions of ficus dalhousiae were mixed with 1 ml phosphate buffer, 2ml hyposaline and 0.5ml HRBC suspension. Indomethacin was used as a contrastable drug and instead of hyposaline 2ml water was used as control.The haemoglobin content in supernatant was calculated using a spectrophotometer at 560 nm spectrum. The result was estimated by the following equation ;Percentage of hemolysis = OD of test/ OD of control x 100.

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The percent of membrane protection was calculated by the following equation-Percentage of protection =100- OD of test/ OD of control x100.

Estimation of skin protein and vitamin C acid levels: Skin protein levels was carried out based on lowest methods of protein estimation.Vitamin C levels was estimated using
HPLC method.

Estimation of TNF and IL-6;TNF-α and IL-6 Assay. IL-6 was measured by enzyme-linked immunosorbent assay kit (Blossom Biotechnologies The capture antibody of IL-6 was added to each well of a 96-well plate overnight. Next day, a second set of biotinylated antibodies was incubated with sample tis- sues or standard antigens in the plate before streptavidin was added. The colour of the reaction converted from purple to yellow and was recorded at 450 nm. TNF-α was detected using the same method as IL-6. Each sample was presented as pg/mg in TNF-α and IL-6 concentration.

Estimation of GSH and LPO; GSH was determined by method of Moron et al.Aliquots of homogenate were mixed with equal volume of ice cold 5% TCA and precipitated proteins were removed by centrifugation. The supernatant was added to equal volume of 0.2 M phosphate buffer, PH 8.0 and measured at 412. And LPO was estimated using the thiobarbituric acid assay.

Hematologic Analysis: Complete blood count was performed by a Coulter veterinary automatic analyzer Aba- cus Junior Vet (Diatron Messtechnik, Budapest, Hungary). The assay included white blood cell count (WBC), the number of neutrophils (NEU), monocytes (MON), lympho- cytes (LYM), red blood cell count (RBC), haemoglobin concentration (HGB), haematocrit (HCT), mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), red blood cell distribution width (RDW), platelet count (PLT), thrombocytes (PCT), medium platelet volume (MPV), and platelet distribution width (PDW), was per- formed in the Haematology Laboratory of Faculty.

STATISTICAL ANALYSIS; The values were expressed as mean ± standard error mean. $p<0.05$ was considered significant, denoted by symbol (*). The data was analysed by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison using graph pad prism.

RESULTS: Egg albumin induced oedema results: Administration of extracts of Ficus dalhousiae on egg albumin induced oedema in rats caused a significant ($P<0.05$ – 0.001) dose-dependent anti-inflammatory effect against oedema caused by egg albumin.The effect was comparable to that of standard drug i.e.indomethacin (10 mg/kg).

Treatment	Dose	1hr	2hr	3hr	4hr	5hr
Disease Control: Egg albumin	0.1ml /kg	0.8±0.07	0.73±0.05	0.62±0.03	0.39±0.04	0.29±0.04
Test drug I: FDME 250 mg/kg	250 mg/Kg	0.7±0.05	0.55±0.04	0.42±0.04	0.030±0.03	0.021±0.03
Test drug II: FDME 500 mg/kg	500 mg/kg	0.61±0.07	0.53±0.05	0.37±0.07	0.030±0.03	0.18±0.04
Indomethacin	10 mg/kg	0.68 ±0.05	0.55±0.04	0.34± 0.04	0.27± 0.05	0.020±0.04

Table : Egg albumin induced oedema

Values are expressed as Mean ± SEM (n=6) .

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Skin Protein Quantification And Vitamin C Acid Levels;Skin protein quantification was carried out using Lowry's method and it was seen that Skin Proteins were depleted in the negative control group and recovered majorly with FDME 500 mg/kg and indomethacin group. Vitamin C is an important antioxidant which makes it important in inflammation and immunity. It helps to neutralise free radicals that cause oxidative damage to cells, Hence decrease in vitamin C levels leaves cells more prone to inflammation.

Treatment	Protein levels (µg/ml)	Plasma Vitamin C(µM)
Normal Control: Vehicle	746.24 ±0.03	75.65±0.05
Disease Control: Egg albumin	329.46 ±0.08	46.74±0.03
Test drug I: FDME 250 mg/kg	546.29±0.12**	58.67±0.04**
Test drug II: FDME 500 mg/kg	674.56±0.04***	69.45±0.05***
Standard: Indomethacin	668.94±0.10***	64.56±0.05***

Table 05:: **Skin Protein Quantification And Vitamin C Acid Levels**
Values are expressed as Mean±SEM (n=6) .

Table 06: Statistical analysis: Protein levels

Tukey's multiple comparisons test	Mean Diff.	Summary	Adjusted P Value
Control vs. Disease control	418.0	***	<0.001
Control vs. FDME 250 mg/kg	79.19	***	<0.001
Control vs. FDME 500 mg/kg	194.7	***	<0.001
Control vs. indomethacin	71.26	***	<0.001
Disease control vs. FDME 250 mg/kg	-338.8	***	<0.001
Disease control vs. FDME 500 mg/kg	-223.2	***	<0.001
Disease control vs. indomethacin	-346.7	***	<0.001

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FDME 250mg/kg vs. FDME 500 mg/kg	115.6	***	<0.001
FDME 250 mg/kg vs. Indomethacin	-7.930	**	0.0047
FDME 500 mg/kg vs. Indomethacin	-123.5	***	<0.001

Values are expressed as Mean±SEM (n=6) ,using one way ANOVA followed by Tukey's multiple comparison test.

Table 07: Statistical analysis: Vitamin C acid levels

Tukey's multiple comparisons test	Mean Diff.	Summary	Adjusted P Value
Control vs.Disease control	28.48	***	<0.001
Control vs. FDME 250 mg/kg	10.37	***	<0.001
Control vs. FDME 500 mg/kg	16.59	***	<0.001
Control vs. indomethacin	7.037	***	<0.001
Disease control vs. FDME 250 mg/kg	-18.12	***	<0.001
Disease control vs. FDME 500 mg/kg	-11.90	***	<0.001
Disease control vs. indomethacin	-21.45	***	<0.001
FDME 250 mg/kg vs. FDME 500 mg/kg	6.222	***	<0.001
FDME 250 mg/kg vs. indomethacin	-3.328	**	0.002
FDME 500 mg/kg vs. indomethacin	-9.550	***	<0.001

Values are expressed as Mean ± SEM (n=6) ,using one way ANOVA followed by Tukey's multiple comparison test.

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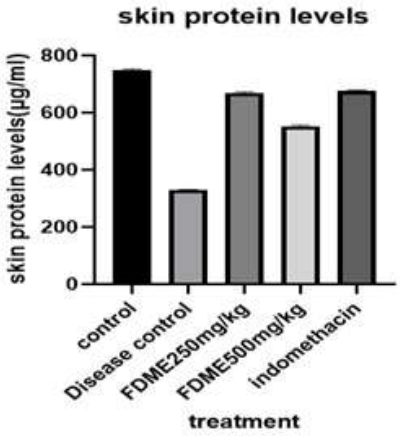

Figure 6: Skin protein levels

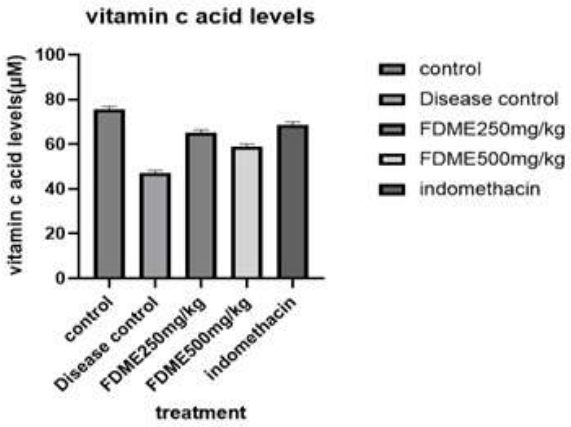

Figure 6: Vitamin C acid levels

Values are expressed as Mean±SEM (n=6).

TNF-alpha and IL-6

Prostaglandin and proinflammatory cytokines (as TNF-α and IL-1β) cause painful sensation.This noci-ceptive effect can be prevented by analgesic agents with central actions such as morphine as well as peripherally acting drugs like NSAID. In order to evaluate whether FDME acted centrally or peripherally in the suppression of pain, egg albumin inflammation test was conducted, after the experiment was carried out the paw skin was taken and tested for presence or absence of TNF and IL-6

Groups	**TNF-α (µg/ml)**	**IL-6 (µg/ml)**
Normal Control: Vehicle	324.47 ± 0.04	232.54 ±0.05
Disease Control: Egg albumin	645.36 ±0.08	629.24 ±0.05
Test drug I: FDME 250 mg/kg	401.35 ±0.04**	414.04 ±0.10**
Test drug II: FDME 500 mg/kg	364.72 ±0.14***	341.42 ± 0.04***
STD drug: Indomethacin 10 mg/kg	396.29±0.07****	394.65 ±0.05***

Table 08: TNF-alpha and IL-6 **;** Values are expressed as Mean ± SEM (n=6)

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Table 09: Statistical analysis: TNF-α concentrations

Tukey's multiple comparisons test	Summary	Adjusted P Value
Control vs. Disease control	***	<0.001
Control vs. FDME 250 mg/kg	***	<0.001
Control vs. FDME 500 mg/kg	***	<0.001
Control vs. indomethacin	***	<0.001
Disease control vs. FDME 250 mg/kg	**	<0.01
Disease control vs. FDME 500 mg/kg	***	<0.001
Disease control vs. Indomethacin	***	<0.001
FDME 250 mg/kg vs. FDME 500 mg/kg	*	0.0480
FDME 250 mg/kg vs. Indomethacin	***	<0.001
FDME 500 mg/kg vs. Indomethacin	***	<0.001

Table 6.3: Values are expressed as Mean ± SEM (n=6) ,using one way ANOVA followed by Tukey's multi comparison test.

Table 10: Statistical analysis: IL-6 concentrations

Tukey's multiple comparisons test	Summary	Adjusted P Value
Control vs. Disease control	***	<0.001
Control vs. FDME 250 mg/kg	***	<0.001
Control vs. FDME 500 mg/kg	***	<0.001
Control vs. indomethacin	***	<0.001

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Disease control vs. FDME 250 mg/kg	**	<0.001
Disease control vs. FDME 500 mg/kg	***	<0.001
Disease control vs. indomethacin	***	<0.001
FDME 250 mg/kg vs. FDME 500 mg/kg	*	0.0197
FDME 250 mg/kg vs. indomethacin	***	<0.001
FDME 500 mg/kg vs. indomethacin	***	<0.001

Table 6.3: Values are expressed as Mean±SEM (n=6) ,using one way ANOVA followed by Tukey's multiple comparison test.

TNF-α Level and IL-6 levels

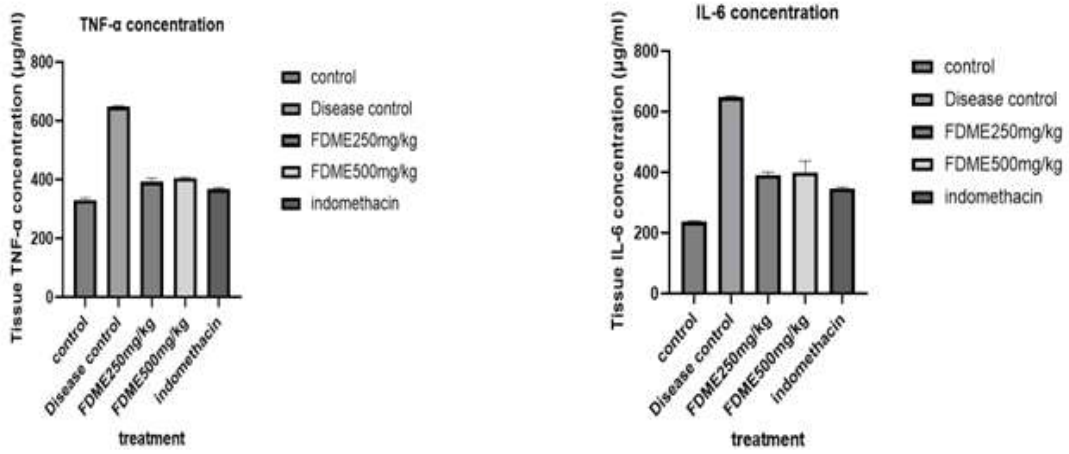

Fig 8 and 9; TNF-α and IL-6 concentrations in rat paw edema.
Values are expressed as Mean±SEM (n=6)

6.4 Antioxidant Enzyme Study
GSH(Glutathione Stimulating Hormones) AND LPO (Lipid Peroxidation)

Groups	**GSH (nmoles/min/mg of protein)**	**LPO (nmoles of MDA/g protein**
Normal Control: Vehicle	4.09 ±0.07	1.29 ±0.05
Disease Control: Egg albumin	2.41±0.03	2.66 ±0.04

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Test drug I: FDME 250 mg/kg	2.97 ±0.12	1.78 ±0.05
Test drug II: FDME 500 mg/kg	3.73 ±0.09	1.96 ±0.14
STD drug: Indomethacin 10 mg/kg	3.47 ±0.14	1.37 ±0.05

Table 11: GSH(Glutathione Stimulating Hormones) AND LPO (Lipid Peroxidation)
Values are expressed as Mean±SEM (n=6)

Table 12: **Statistical analysis:** GSH levels

Tukey's multiple comparisons test	**Summary**	**Adjusted P Value**
control vs. Disease control	***	<0.001
control vs. FDME 250 mg/kg	***	<0.001
control vs. FDME 500 mg/kg	***	<0.001
control vs. indomethacin	***	<0.001
Disease control vs. FDME 250 mg/kg	**	<0.047
Disease control vs. FDME 500 mg/kg	***	<0.001
Disease control vs. indomethacin	***	<0.001
FDME 250 mg/kg vs. FDME 500 mg/kg	*	<0.01
FDME 250 mg/kg vs. indomethacin	***	<0.001
FDME 500 mg/kg vs. indomethacin	***	<0.001

Table 5.9 : Values are expressed as Mean±SEM (n=6) ,using one way ANOVA followed by Tukey's multi comparison test.

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Table 13: **Statistical analysis:** LPO levels

Tukey's multiple comparisons test	**Summary**	**Adjusted P Value**
Control vs. Disease control	***	<0.001
Control vs. FDME 250 mg/kg	***	<0.001
Control vs. FDME 500 mg/kg	***	<0.001
Control vs. indomethacin	**	0.032
Disease control vs. FDME 250 mg/kg	**	<0.01
Disease control vs. FDME 500 mg/kg	***	<0.001
Disease control vs. indomethacin	***	<0.001

Values are expressed as Mean±SEM (n=6) ,using one way ANOVA followed by Tukey's multi comparison test.

Tissue GSH Levels

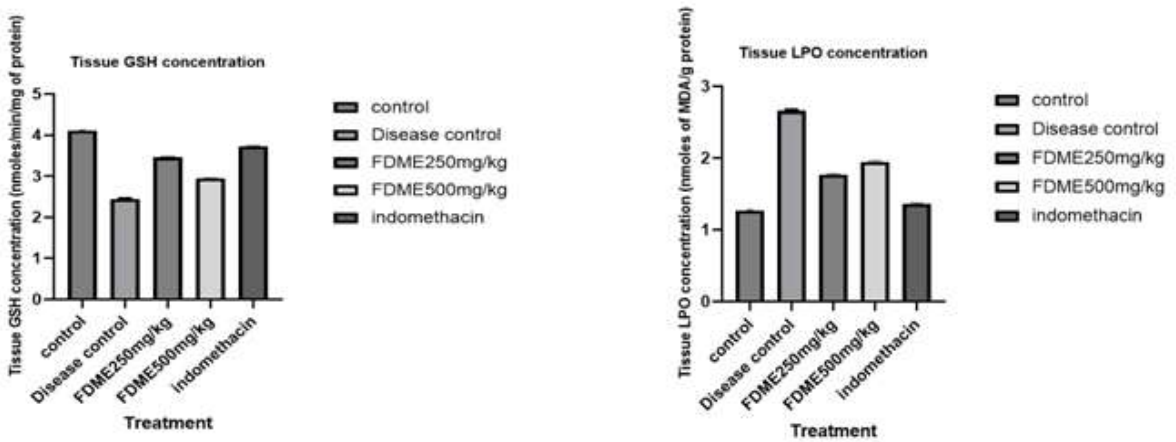

Figure 10 and 11: Results of Concentration of GSH and LPO on paw oedema are expressed as Mean ± standard error of mean (SEM). N = 6 using one way ANOVA

6.5 HAEMATOLOGY

Infection can lead Inflammation and white blood cells are produced in response, inflammation response itself can also be associAted with increase In WBC through antibody Production And cytokine release, lymphocyte orchestrate and amplify the function of other immune cells and contributes to host defence against pathogens and pathogenesis of inflammation.

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Test description	Normal Control	Diseases control: Egg Albumin	Test Drug I: FDME 500 mg/kg	Test Drug II: FDME 250 mg/kg	STD drug: Indomethacin	Units
HB(gm/dl)	2.55±0.05	2.75±0.07	2.44±0.08	2.54±0.07	2.45±0.07	gm/dl
WBC	155.67±0.13	166±0.03	149±0.08	155±0.07	149±0.08	c/cmm
PCV	12.56±0.33	13.1±1.3	12.2±0.06	12.58±0.03	12.3±0.09	%
MCV	14.6±0.09	14.8±0.05	14.4±0.08	14.55±0.02	14.3±0.09	fl
MCHC	7.78±0.05	8.01±0.06	7.6±0.07	7.9±0.07	7.5±0.06	gm/dl
RBC	2.17±0.06	2.19±0.5	2.16±0.15	2.18±0.12	2.15±0.06	m/cmm
Neutrophils	13.80±0.05	14.5±0.09	13.5±0.12	14±0.08	13.3±0.09	%
lymphocytes	11.7±1.03	12.1±0.4	11.1±0.05	11.5±0.03	11±0.03	%
Monocytes	0.86±0.01	0.9±0.7	0.85±0.09	0.87±0.07	0.84±0.07	%
Basophils	0.95±0.05	1.5±0.05	0.94±0.04	0.95±0.06	0.96±0.05	%
Eosinophils	0.15±0.04	0.17±0.08	0.14±0.05	0.16±00.01	0.15±0.03	%
Platelet	1.6±0.08	2.1±1.5	1.5±0.03	1.65±0.03	1.4±0.06	lakh/cmm
ESR	12.5±1.1	13±0.05	12.4±0.0	12.3±0.04	12.5±0.08	mm/hr

Table 14: Haematology LevelsValues are expressed as Mean±SEM (n=6)

6.6 Croton Oil Induced Ear Oedema

To test the anti-inflammatory effect of Ficus Dalhousiae methanolic extract , two doses of the extract (250 and 500 mg/kg) were applied on the ear after croton oil Was applied to induce Ear oedema. The application of Ficus Dalhousiae methanolic extract on-ear induced a significant change in ear weight for both 250 mg/kg (one-way ANOVA; $p< 0.05$) and 500 mg/kg(one-way ANOVA; $p< 0.01$) doses in on ear edema assay compared to the control group. Indomethacin induced a significant decrease in the change in ear weight compared with the control group (one-way ANOVA; $p< 0.001$**).**

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Treatment	DOSE	Change in ear weight	% inhibition
DC: Egg albumin	0.1ml/kg	0.0083 ±0.00028	0.00
Test drug I: FDME	250 mg/kg	0.0065 ±0.00014	23.5
Test drug II: FDME	500 mg/kg	0.0043 ±0.00021	51.39
STD drug: Indomethacin	10 mg/kg	0.0010±0.00107	89.05

Table 15: % Inhibition Results of change in weight are expressed as Mean ± standard error of mean (SEM). N = 6 in each group one way ANOVA.

Table 16: Statistical analysis: Croton oil test

Tukey's multiple comparisons test	Mean Diff.	Summary	Adjusted P Value
Disease control vs. FDME 250 mg/kg	0.1933	***	<0.001
Disease control vs. FDME 500 mg/kg	0.3750	****	<0.0001
Disease control vs. indomethacin	0.7017	****	<0.0001
FDME 250 mg/kg vs. FDME 500 mg/kg	0.1817	**	<0.01
FDME 250 mg/kg vs. indomethacin	0.5083	***	<0.001
FDME 500 mg/kg vs. indomethacin	0.3267	***	<0.001

Values are expressed as Mean ± SEM (n=6) ,using one way ANOVA followed by Tukey's multiple comparison test.

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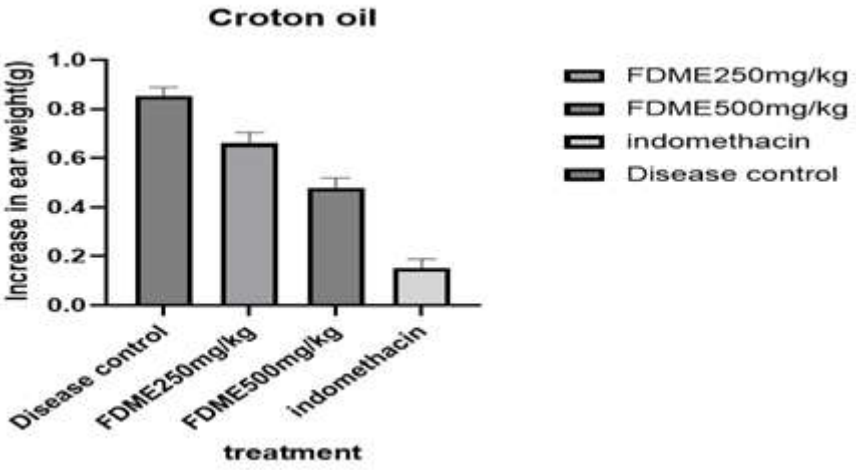

Figure 12: Results of change in ear weight are expressed as mean±standard error of mean (SEM). N = 6 in each group one way ANOVA.

6.7 In Vitro Tests: Membrane Stability Test

The extract at concentration range from 100 µg/mL to 300 µg/mL protects the human erythrocyte membranes against lysis induced by hypotonic solution. At concentration of 100 µg/mL, the extract inhibited 27.38% of RBC haemolysis as compared with 40.84% produced by Indomethacin at 100 µg/mL. Since human red blood cell membranes are similar to lysosomal membrane components, the prevention of hypotonicity induced HRBC membrane lysis was taken as a measure of anti-inflammatory activity of drugs. The results obtained demonstrated that Methanolic extract of Fruits of Ficus dalhousiae can significantly and dose-dependently inhibit HRBC haemolysis.

Concentration (µg/ml)	Absorbance	Prevention of lysis (%
Indomethacin 100	0.158	40.76
FDME 300	0.163	36
FDME 200	0.153	29.68
FDME 100	0.190	27.38

Table 17: Data representing absorbance and percentage prevention of lysis

The investigation suggested good ability of the Methanolic fruits extract to resist the cell lysis in small concentrations as compared to the standard drug Indomethacin at 100 µg/mL, though not more than indomethacin. Even the highest concentration of the extract at 300 µg/mL was able to prevent lysis of 36% which was yet 4.76% less than that of indomethacin, when used in only 100 µg/mL.

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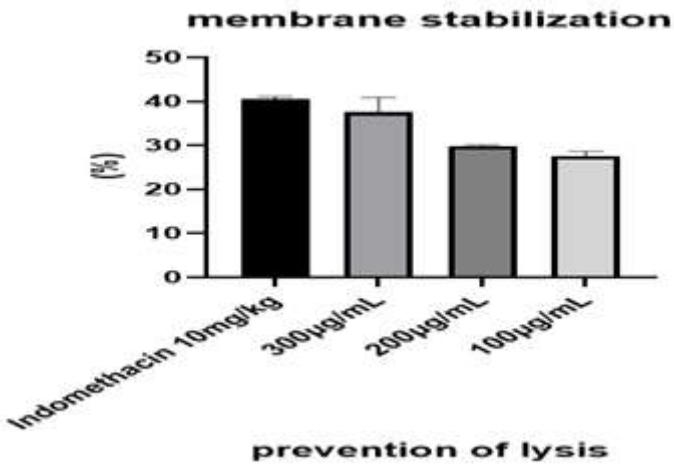

Figure 13: Bar chart above represents the percentage of membrane protection of the Methanolic fruits extract with a reference to Indomethacin 100 µg/mL as standard

6.8 Inhibition of Albumin Denaturation

Anti-inflammatory activity of FIcus Dalhousiae was evaluated against denaturation of egg albumin method. The highest inhibition rate was observed in both indomethacin and Ficus Dalhousiae extracts at the concentration of 1000 µg/ml. There was significantly higher inhibition With FDME 500 mg/kg as compared to Indomethacin at the concentrations of 0.01 µg/ml and 0.1 µg/ml.

CONCENTRATION (µg/ml)	% Inhibition Egg albumin denaturation		
	Standard	FDME 250(mg/kg)	FDME 500(mg/kg)
0.1	14.26±0.63	17.09±0.27	17.70±0.66
1	15.30±0.93	20.09±0.57	18.80±0.24
10	23.63±1.39	22.09±1.27	24.50±1.22
100	24.84±0.75	23.09±2.37	25.67±0.89
1000	26.09±2.17	25.09±2.19	27.93±3.17

Table 18: % Inhibition Egg albumin denaturationResults are expressed as Mean ± Standard error of mean (SEM).

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6.9 HISTOPATHOLOGY: No Inflammation, tissue destruction, and swelling Phenomenon were observed in the paws of normal rats. On the other hand, the Egg albumin control group displayed enlarged cavities in the paw tissue. Edematous condition was Reduced by treatment with 10 mg/kg of indomethacin and 500/ 250 mg/kg of FDME. The severity of rat paw edema was also graded and summarised as seen below

<table>
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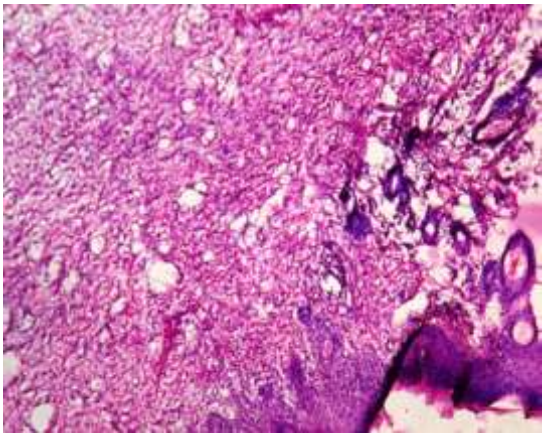
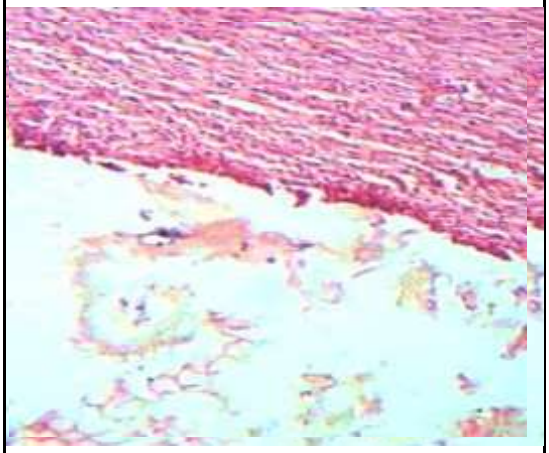
<td> 
Showing fully formed epidermis layer, no inflammation cells.more no. of collagen fibre and blood vessel
Normal Control</td>
<td> 
Showing dense acute inflammatory infiltrate with granulation tissue in the wall of the lesion.

Disease Control</td>
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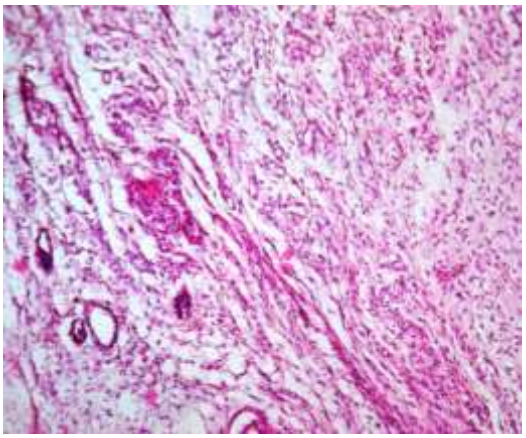
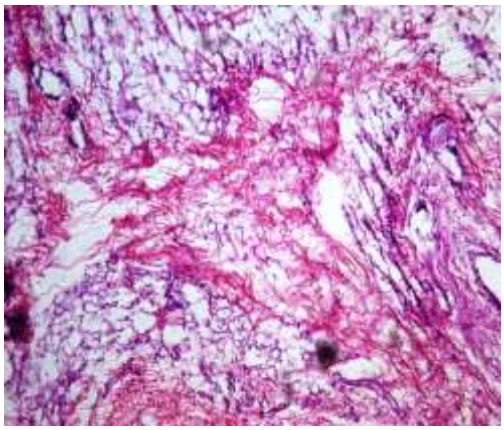
<td> 
Showing moderate acute inflammatory infiltrat
e with reduced fibroblastic activity in the wall of lesion.

STD drug: Indomethacin</td>
<td> 
Showing moderate acute inflammatory infiltrate
with reduced fibroblastic activity in the wall of lesion.

Test drug: FDME 250 mg/kg</td>
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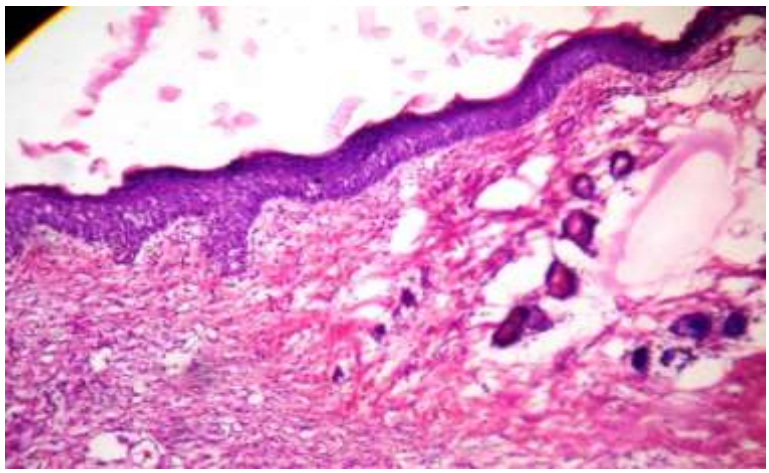

Showing moderate acute Inflammation on infiltrate with reduced fibroblastic activity in the wall of lesion

Test drug: FDME 500 mg/kg

Figure 14: Histopathology of egg albumin induced paw oedema

III. DISCUSSION;

The use of herbal medicine has become an integral part of life. Plants having medicinal value in the form of chemical substances that produce a definite physiological action on the human body are called phytochemicals. Since ancient times these phytochemicals have been used to cure the disease in herbal and homoeopathic medicines. These are non-nutritive substances, have protective or disease preventive properties (Kirtikar et al., 1987). The rationale of this Research is to provide comprehensive information on Ficus dalhousiae, a rare plant species of medicinal importance, highlighting the plant's Phyto-pharmacological profile along with a critical appraisal of its ethnobotanical and traditional uses. Ficus is a genus of about 850 species of woody trees, shrubs, vines, epiphytes, and hemi epiphytes in the family Moraceae. The Moraceae family represents a vital group of plants which have innumerable medicinal properties. Ficus dalhousiae is one among the rare species of Ficus belonging to the Moraceae family with immense medicinal value.This species is endemic to peninsular area and a very rare species, first described this species as Urostigma dalhousiae based on Wights' collection from India and later Miquel named it as Ficus dalhousiae. Subsequently recorded this species from the Nilgiri Mountains in the altitudinal range of 605-1370 m wherever it grows. It is widely used in Ayurveda, Siddha, Unani and folk medicine. The whole plant and its parts like leaf, stem, stembark, root, root-bark, flowers, fruits and seeds are well known to have medicinal properties and have a long history of use by indigenous communities in India. The medicinal value of this plant is mainly for the treatment of a large number of human ailments. The leaves and the stem bark of the plant are used in liver and skin diseases. Bark paste is used in the treatment of leprosy. Fruit are used as cardiotonic. Phytochemical screening of Ficus dalhousiae different plant parts shows the Presence of various phytoconstituents responsible for its medicinal value. The pharmacological activities of Medicinal plants are due to the presence of secondary metabolites such as flavonoids, sterols, alkaloids, phenols, glycosides, saponins etc. As the plant contains tannins, flavonoids and phenolic compounds, there is a possibility of antioxidant property in the plant. The medicinal properties of

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plants have been explored. Inflammatory reaction, typically is characterised by redness, swelling, heat, and pain, it is the most important defence mechanism against invading pathogens. However, persistent or over inflammation leads to tissue damage and possibly failure of organs due to excessive production of macroscopic-derived mediators. Pro-inflammatory cytokines (e.gTNF-α, IL-1β and IL-6) are produced in large quantities by activated macrophage/monocytes that stimulate cellular response via increasing prostaglandins (PGs) and reactive oxygen species. Accumulation of these mediators may result in collateral damage to normal cells, resulting in diseases like atherosclerosis, bowel disease, septic-shock etc.Paw oedema was induced using egg albumin treated using various concentrations of Ficus dalhousiae and indomethacin. Egg white contains allergen protein, which was suspected to contribute to edema formation. However, egg white contained proteins with unique pro- and/or anti-inflammatory properties and therefore needed further research to determine the protein fraction that plays a role as an edema inductor. The results of the study showed that the egg white could induce edema of the rat's paw. Edema formation began in the 1st h and reached the peaks in the 2nd h after the subcutaneous injection of egg white. A number of leukocyte cells were also found in the inflamed paw tissues. Oral administration of methanolic extracts of Ficus dalhousiae showed an anti-inflammatory biological activity in mouse ear edema induced by croton oil by decreasing the ear edema weight. In agreement with this, the percentage inhibition of the extract of Ficus dalhousiae significantly inhibited the ear edema by 23.5% and 51.39% at 250 and 500 mg/kg doses, respectively. The Presence of flavonoids and terpenoids in FDME, could be responsible for the decrease in inflammation. Flavonoids belong to the group of natural polyphenolic compounds with more than 4000 identified varieties. A Large number of epidemiological, in-vitro, and in vivo studies have documented the anti-inflammatory properties of a wide variety of flavonoids in different chronic In-Vitro studies were also carried out using Membrane stabilisation test and albumin denaturation inhibition test, which at the end of study showed and proved anti-inflammatory activity of Ficus dalhousiae. In albumin denaturation test; The assay was carried out by adopting the methods described by Kumari et al. with some modifications in which the volume of each component in the reaction mixtures was reduced by half. The plant extracts and positive standard (indomethacin) were prepared at a concentration of 0.1% each (1.0 mg/ml). A reaction vessel for each mixture was prepared consisting of 200 µl of egg albumin, 1400 µl of phosphate buffered saline, and 1000 µl of the test extract. Distilled water instead of extracts was used as a negative control. Afterward, the mixtures were incubated at 37°C for 15 min and then heated at 70°C for 5 min. After cooling, their absorbances were measured at 680 nm (colorimeter).

Results showed that the highest inhibition rate was observed in both indomethacin and Ficus dalhousiae extracts at the concentration of 1000 µg/ml. There was significantly higher inhibition With FDME 500 mg/kg as compared to Indomethacin at the concentrations of 0.01µg/ml and 0.1 µg/ml Membrane stability test was also carried out for the extract from the fruits of Ficus dalhousiae and the fruits extract showed maximum protection and minimum hemolysis of the HRBC at 27.38% and 40.84% respectively.

in suppressing the release of inflammatory mediators. Further investigation is required to identify the active principle responsible for anti-inflammatory activity.

IV. CONCLUSION:

Since inflammation is caused due to infection or injuries and can be seen in various scenarios or Disease conditions, it's important that new solutions be found to aid in management of inflammation as many conventional anti-inflammatory drugs today have more severe adverse effects. The current study, as investigated, shows that Ficus dalhousiae miq plant can be used in management of inflammation. The results obtained from various tests and assays carried out, proves and show that FDME at doses of 500mg/kg and 250 mg/kg exhibit anti-inflammatory activities. It may be due to the presence of phytoconstituents such as terpenoids, tannins which play a vital role

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