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EVALUATION OF ANTI-INFLAMMMATORY ACTIVITY OF METHANOLIC EXTRACT OF *CESTRUM NOCTURNUM* AND *TECTONA GRANDIS*

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ABSTRACT:-

The methanolic extract of *Cestrum nocturnum* bark given by oral route in rats showed a significant and dose-dependent anti-inflammatory activity by paw edema method used. The better activity was found at a dose of 30 mg/kg when compared to reference drug. The phenyl butazone was used as reference drug (100mg/kg). and methanolic extract of *Tectona grandis* leaves given by oral route in mice showed a significant and dose-dependent anti-inflammatory at a dose of 250 mg/kg when compared to reference drug. The Ibuprofen was used as reference drug (10mg/kg). From these results it is clear that the methanolic extract of the fruits of *Tectona grandis* posses' anti-inflammatory activity and this justify the folklore use of this plant.The study indicates the potential of these extracts as anti-inflammatory drugs. The activity may be attributed to the inhibition of the COX-2 enzyme or inhibition of the activation of transcription factors. The study validated the methanolic extract of *Tectona grandis* fruit towards management of pain and inflammation. The exact role of individual phytoconstituents needs to be illustrated using suitable bio-analytical techniques to extrapolate exact mechanism of their action

KEYWORDS

Anti-inflammatory, transcription, Phytoconstituents, paw edema

1. INTRODUCTION

Inflammation is tissue response to the injury. The inflammatory response is triggered whenever body tissues are injured by physical trauma (a blow), intense heat, irritating chemicals, or infection by viruses, fungi, or bacteria. Inflammation is not a synonym for infection. Even in cases where inflammation is caused by infection, the two are not synonymous: infection is caused by an exogenous pathogen, while inflammation is the response of the organism to the pathogen. In the absence of inflammation, wounds and infections would never heal and progressive destruction of the tissue would compromise the survival of the organism. However, an inflammation that runs unchecked can also lead to a host of diseases, such as hay fever, atherosclerosis, and rheumatoid arthritis. It is for this reason that inflammation is normally closely regulated by the body[5].

The agents causing inflammation may be as under:

Physical agents: heat, cold, radiation, mechanical trauma.

Chemical agents: organic and inorganic poison.

Infective agent and their toxins: from bacteria, viruses.

Immunological agents: cell-mediated and antigen-antibody reactions.

The inflammatory response has several beneficial effects:

1. Prevents the spread of damaging agents to nearby tissues.
2. Disposes of cell debris and pathogens.

Inadequate regulatory measures, weak quality control systems and largely uncontrolled distribution channels may have been contribution to the occurrence of such events. So pharmacovigilance of herbal medicines is required. We are confident that with conscious and systematic efforts, we can achieve excellent growth in this field which would help in earning valuable foreign exchange to the country[1-4].

Inflammation begins when a stimulus, such as infection, physical stress, or chemical stress, produces cellular damage.This damage initiates the activation of transcription factors that control the expression of many inflammatory mediators. Among the more important inflammatory mediators are the eicosanoids, biological oxidants, cytokines, adhesion factors, and digestive enzymes (proteases, hyaluronidase, collagenase, and elastase). Only the first three of these are therapeutic targets for anti-inflammatory drugs. The inflammatory response changes with time and can be divided into phases. The rapid phase occurs within seconds to minutes and consists of

vasodilation, increased blood flow, edema, and pain. The acute phase is characterized by induction of inflammatory genes by NF-B and other transcription factors. During this phase, moderate amounts of inflammatory mediators are produced. The chronic phase occurs over months to years and is marked by dramatically increased production of inflammatory mediators. The secondary chronic phase of inflammation occurs after years of oxidative damage has degraded blood vessels and tissues. Such chronic inflammation appears to play a leading role in many disease states, such as arteriosclerosis and cancer[6].

Types of Inflammation:

A. Acute inflammation

B. Chronic inflammation

Acute inflammation is the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leukocytes from the blood into the injured tissues.. There are five cardinal signs:

- Rubor (redness),
- Calor (increased heat),
- Tumor (swelling),
- Dolor (pain), and
- Function laesa (loss of function).

Chronic inflammation:

Chronic inflammation is an inflammatory immune response of prolonged duration that eventually leads to tissue damage.

Chronic inflammation is differentiated from acute inflammation by extended duration, lasting anywhere from a week to an indefinite time frame.

The exact nature of chronic inflammation depends on the causative agent and the body's attempts to ameliorate it.

Can be caused by one of the following ways:

1. Chronic inflammation following acute inflammation
2. Recurrent attacks of acute inflammation

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3. Chronic inflammation starting de novo

Some synthetic Anti-inflammatory drugs and advantages.

Drug	Adverse effects
Aspirin	Salycylism-dizziness, tinnitus, vertigo, reversible impairment of hearing and vision, excitation and mental confusion, hyperventilation and electrolyte imbalance. Aspirin in children raise the serum transminase, liver damage.
Phenylbutazone	Nausea, vomiting, epigastric distress, peptic ulcer, diarrhoea, CNS side effects, Edema, Hypersensitivity like rashes, serum sickness, hepatitis, stomatitis, Bone marrow depression, agranulocytosis, Stevens-Johnson syndrome, Goiter and hypeothyrodism occurred on long term use.
Indomethacin	Gastric irritation, nausea, anorexia, gastric bleeding, diarrhoea, frontal headache, dizziness, ataxia, mental confusion, hallucination, Hypersensitivity reactions, Epileptics and kidney disease in pregent women and children.
Ibuprofen	Gastric discomfort, nausea, vomiting, gastric erosion, occult blood loss, CNS side effects like dizziness, blurring of vision, Hypersensitivity reactions.
Mephenamic acid	Diarrohea, epigastric distress, Skin rashes, dizziness,
Diclofenac sodium	Epigastric pain, nausea, headache, dizziness, rashes, gastric ulceration, bleeding, kidney damage[88,89]

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TABLE.1. List of some synthetic Anti-inflammatory drugs and their disadvantages

Some herbal Anti-inflammatory drugs and their uses

Plant	Uses
Vatsnabh(*Aconitum ferox*)	Cardiac stimulant, Anti-rheumatic, Anti-inflammatory
Guggul(*Commiphora weightii*)	Hypocholesteremic, Hypolipidemic, Anti-inflammatiry, Anti-rheumatic
Amla(*Embelica officinalis*)	Anti-inflammatory, Diuretic, Laxative, Hepatoprotective , Anti-oxidant, Anti-fungal
Kutki (*Picrorhiza kurroa*)	Hepatoprotective, Immunomodulatory, Anti-inflammatory, Jaundice, In periodic Fever, In Nausea and anorexia, Dyspepsia, In bronchial asthma
Isabgol(*Plantago ovate)*	Aphrodiasic, Anti-inflammatory, Diarrhea, Demulcent, Laxative, Emollient
Kuth(*Saussurea costus*)	Anti-inflammatory, Anti-arthritic, Cytotoxic, Antioxidant, Aphrodisiac, Carminative, Anti-septic
Guduchi(*Tinospora cordifolia*)	Anti-cancer, Anti-malarial, Anti-periodic, Anti-allergic, Anti-spasmodic, Anti-inflammatory, Anti-leprotic, Anti-oxidant
Ashwgandha(*Withania somnifera*)	Sedative, Anti-rheumatic, Diuretic, Anti-inflammatory, Anti-stress, Anti-tumor, Immunomodulator,Hypotensive
Bilwa(*Semicarpus anacardium*)	Anti-inflammatory.
Termeric(*Curcuma longa)*	Anti-inflammatory, antiarthritic agent

Aloes(*Aloe barbadensis*)	Anti-inflammatory, pugative, antiwrinkle, wound healing.
Liquorice(*Glycyrrhi glabra)*	Expectorant, demulcent, antigastric agent, antispasmodic, ant-inflammatory, in addision's diseases.
Silymarin(*Silybumm arianum)*	In liver diseases, hepatoprotective, anti-inflammatory, antioxidant, anti-depressant.
Psoraleafruit(*Psoral ea corylifolia)*	Use in leucoderma, leprosy, psoriasis, inflammatory diseaces, also used as diutretics, anthelmintic, laxatives,
Peppermint oil(*Mentha peperita)*	Carminative, stimulant, antiseptic, spasmolytic, smooth muscles relaxant, ani-inflammatory, anti-ulcer[90].

TABLE.2. List of some herbal Anti-inflammatory drugs and theirs uses:-

2. MATERIALS AND METHODS

2.1. Selection of Plant Material

The proposed study we are focusing on the pharmacological screening of anti-inflammatory herbal drug *Cestrum nocturnum and Tectona Grandis Linn.*

2.2. Extraction of Plant Material

In this firstly the plant material is taken and it is dried under the shade. And then it is powdered with the help of crushing .when it is completely powdered and dried. It is weighed and subjected to the extraction process followed by soxhlet extraction.

2.3. Collection and Authentication of plant material

The plant material of research interest is collected from the healthy plant from Ujjain local region.

Identification: The plant material is identified by Dr. Anurag titov, department of botany government madhav science PG College Ujjain.

Drying: The plant materials were dried at room temperature under a well ventilated shade by distributing them homogeneously; the material had kept away from the direct sunlight because the ultraviolet radiation may produce chemical reaction giving rise to compound artifacts.

3. PHARMACOGNOSTICAL STUDIES

3.1 Determination of Foreign Matter

A thin layer of the original sample, weighing between 100 and 500 gm, was laid out. The sample was examined visually or using a 6x lens, and the foreign organic matter was manually removed as thoroughly as possible.

Following medicinal plants from local region will be evaluated for the possible biological activity.

1. *Cestrum nocturnum* bark extract is screened for anti-inflammatory activity.
2. *Tectona grandis* fruit extract is screened for anti inflammatory activity.

S. No.	Plant Used	Part of plant Taken	Method Used Activity Evaluated	Observation by study
1	*Cestrum nocturnum*	bark extract	Carageenan induced paw edema method for anti-inflammatory activity	Shows anti inflammatory activity
2.	*Tectona grandis*	fruit extract	Carageenan induced paw edema for anti inflammatory activity.	Shows anti inflammatory activity.

Table 3-The observation on the basis of experimental studies

3.2. Extractionand phytochemical screening of screened plants

The extract thus obtained, after standardization, may be used asmedicinal agent as such in the form of tinctures or fluid extracts or further processed to be incorporated in any dosage form such as tablets and capsules. This product contains complex mixture of many medicinal plant metabolites, such as alkaloids, glycosides, terpenoids, flavonoids and lignans[13].The general

techniques of medicinal plant extraction include maceration, infusion, percolation, digestion, decoction, hot continuous extraction (Soxhlet),aqueous-alcoholic extraction by fermentation, countercurrent extraction, microwave-assistedextraction, ultrasound extraction (sonication), supercritical fluid extraction, and phytonicextraction (with hydro fluorocarbon solvents). For aromatic plants, hydro distillation techniques(water distillation, steam distillation, water and steam distillation), micro distillation, thermomicrodistillation and molecular Distillation[13].The basic parameters influencing the quality of an extract are[10]:

1. Plant part used as starting material
2. Solvent used for extraction
3. Extraction procedure

Effect of extracted plant phytochemicals depends on[10]:

1. The nature of the plant material
2. Its origin
3. Degree of processing
4. Moisture content
5. Particle size

The variations in different extraction methods that will affect quantity and secondary metabolite composition of an extract depends upon[10]:

1. Type of extraction
2. Time of extraction
3. Temperature
4. Nature of solvent
5. Solvent concentration
6. Polarity

All the dried plant materials were powdered and weighted 70 grams and filled in soxhlet apparatus for extraction. First the extracts were defatted with Methanol for 72 hour. And the defatted extract then dried and again filled in apparatus for extraction with alcohol. Alcoholic extract were concentrated by recovering solvent. The percentage yield was noted for all the plant material and the extracts were assayed for possible biological activity.

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Calculation of percentage yield:

Plant Material	Solvent	Wt. of powered leaves taken	Wt. of extract obtained	% yield
*Cestrum nocturnum*Bark	Methanol	70 gm.	22 gm.	31.42%
*Tectona Grandis*fruit	Methanol	70 gm.	22 gm.	31.42 %

Table 4- Calculation of percentage yield of Plant Extracts

The table shows that extraction of 70 gm powered material of *Cestrum nocturnum, and tectona grandis*in methanol gives the quantity of extracts in grams. The percentage yield obtained was shown in the table.

Formula:

$$\% \text{ yield} = \frac{\text{Practical yield}}{\text{Theoretical yield}} \times 100$$

3.3.Preliminary phytochemical investigations

The methanolic extract obtained was tested for presence of various phytoconstituents with the help of different chemical tests.

Procedure followed:

Powdered material of plants of *Cestrum nocturnum, pithecellobium dulse, Mimospus hexandra roxb, Morus indica and tectona grandis* were soaked in respective solvent for 4 days at room temperature and were then analyzed for its phytochemical contents.

Test For	*Cestrum nocturnum*	*Tectona Grandis*	Test For	*Cestrum nocturnum*	*Tectona Grandis*
Alkaloids	+	-	**Proteins**	-	+

(Mayer's test)			(Xanthoprotic test)		
Carbohydrates (Molisch's test)	+	+	**Steroids and Triterpenoids** (Libermann-Burchard test)	+	-
Flavonoids (Lead acetate test)	+	+	**(Phenoliccompounds)** (Ferric chloride test)	+	+
Glycosides(Saponins) (Froth formation test)	+	+	**Amino acids** **(**Ninhydrine test)	-	+

Where (+) indicates Presence and (–) indicates absence of respective phytochemicals.

Table 5 - Results of phytochemical tests

4. EXPERIMENTAL WORK

4.1. Evaluation of anti-inflammatory activity of leaves of *Cestrum nocturnum*[14, 15]

Materials and method:

In this method wister male/female albino rats (150-200 gm) of either sex were used for study. They were housed in standard polypropylene cages under room temperature (24 ± 2^{O}C); relative humidity (60 % – 70 %) and exposed to 12:12hours light: dark cycle.

Group 1- Carrageenan (0.05 ml of 1% solution).

Group 2- Phenylbutazone as Standard (100 mg/kg).

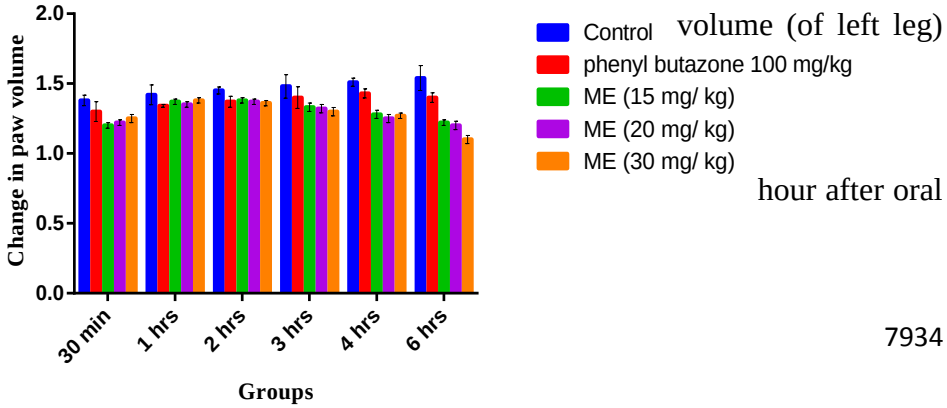
Group 3- Methanolic Extract of *Cestrum nocturnum* (15 mg/kg).

Group 4- Methanolic Extract of *Cestrum nocturnum* (20 mg/kg).

Group 5- Methanolic Extract of *Cestrum nocturnum* (30 mg/kg).

Procedure:

Phenyl butazone aqueous suspension and alcoholic extract were administered orally using intragastric tube.The initial paw volume (of left leg) of each rat was measured using plethysmometer.One hour after oral



administration of phenyl butazone and test extracts all the rats were challenged by an injection of 0.05 ml of 1% *w/v* solution of carrageenan into the planter side of the left hind paw.The paw volume was measured by using plethysmometer immediately after injection 30min, 1hr, 2hrs, 3hrs, 4hrs, 6hrs after challenged with carrageenan. The percent increase in the paw volume before and after sub plantar injection of carrageenan was calculated. The mean percent difference of control group was compared with drug treated group to calculate the percent inhibition.

Fig 1: Graph for anti-inflammatory activity of methanolic extract of *C. nocturnum*

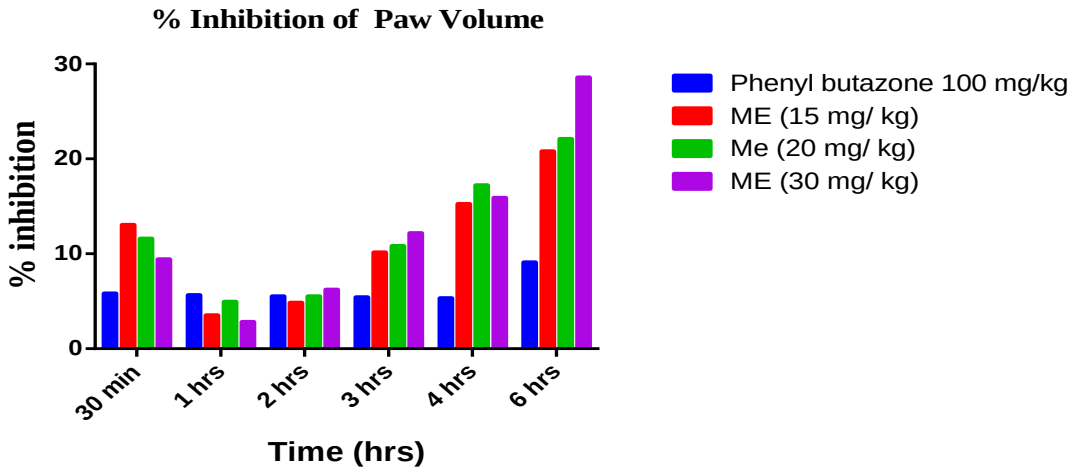


Fig 2: Graph for % inhibition of paw volume of methanolic extract of *C. nocturnu*

Groups and Doses Given	**Mean difference in paw edema volume (ml) ± SEM (% inhibition)**						
	Dose	**30 min**	**1hr**	**2hrs**	**3hrs**	**4hrs**	**6hrs**
Control Carageenan (1%W/V)	0.05 ml	1.38± 0.038	1.42± 0.070	1.45± 0.025	1.48± 0.084	1.51± 0.029	1.54± 0.089
Phenyl butazone	100mg/kg p.o	1.30± 0.070 (5.80%)	1.34± 0.010 (5.63%)	1.37± 0.04 (5.52%)	1.40± 0.077 (5.41%)	1.43± 0.032 (5.30%)	1.40± 0.035 (9.09%)
Methanolic Extract	15mg/kg p.o	1.20± 0.028 (13.04%)	1.37± 0.025 (3.52%)	1.38± 0.026 (4.83%)	1.33± 0.035 (10.14%)	1.28± 0.030 [a] (15.23%)	1.22± 0.026[a] (20.78%)
Methanolic Extract	20mg/kg p.o	1.22± 0.025 (11.59%)	1.35± 0.027 (4.93%)	1.37± 0.027 (5.52%)	1.32± 0.030 (10.81%)	1.25± 0.035 [a] (17.22%)	1.20± 0.035 [a] (22.08%)
Methanolic Extract	30mg/kg p.o	1.25± 0.036 (9.42%)	1.38± 0.025 (2.82%)	1.36± 0.025 (6.21%)	1.30± 0.035 (12.16%)	1.27± 0.024 [a] (15.89%)	1.10± 0.036 [a] (28.57%)

Each value is representing ± SEM of four observations (n=5) a=p< 0.05, compared to phenyl butazone. Data was analyses by one way ANOVA followed by Sidak-Bonferroni Multiple comparison test.

Table-6-Observations of paw volume and % inhibition of methanolic extract of bark of *C.nocturnum* by paw edema method:

4.2. Evaluation of Anti-inflammatory activity of fruits of *Tectona grandis*[19]

Method and Procedure

In this method wister male/female albino rats (150-200 gm) of either sex were used for study. They were housed in standard polypropylene cages under room temperature (24 ± 2^{O}C); relative humidity (60 % – 70 %) and exposed to 12:12hours light: dark cycle. Wister male/female albino rats (150-200 gm) of either sex were fasted overnight, and segregated into four groups of five animals each.The animals were weighed and marked.

Group 1- Carrageenan (0.05ml of 1% solution).

Group 2- Ibuprofen as Standard (10 mg/kg).

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Group 3- Methanolicextract of *Tectona grandis* (250 mg/kg).

Group 4- Methanolicextract of *Tectona grandis* (500 mg/kg).

Procedure:

The dried extracts were formulated as a suspension in distilled water. Ibuprofen aqueous suspension and methanolic extract were administered orally using intragastric tube.A ring was marked on the left paw of each animal so that constant length of paw could be dipped each time.The initial paw volume (of left leg) of each rat was measured using plethysmometer.One hour after oral administration of ibuprofen and test drug all the rats were challenged by an injection of 0.05 ml of 1% *w/v* solution of carrageenan into the planter sideof the left hind paw.The paw volume was measured byusing plethysmometerimmediately after injection 30min, 1hr, 2hrs, 3hrs, 4hrs, after challenged with carrageenan.The percent increase in the paw volume before and after sub plantar injection of carrageenan was calculated. The mean percent difference of control group was compared with drug treated group to calculate the percent inhibition

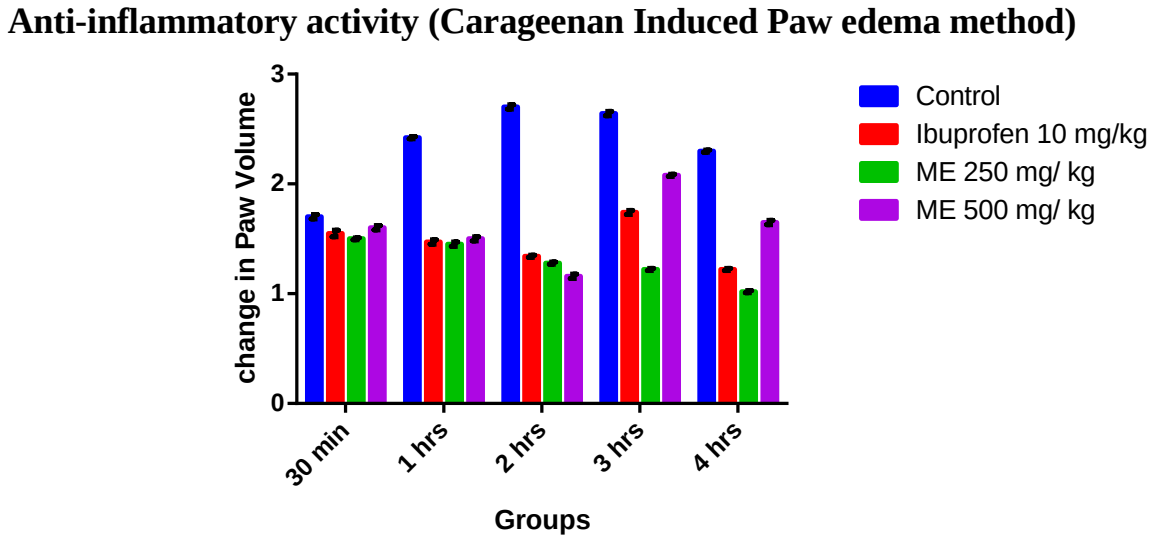

Fig 3: Change in Paw Volume of groups by Carageenan induced paw edema method *(Tectona grandis)*

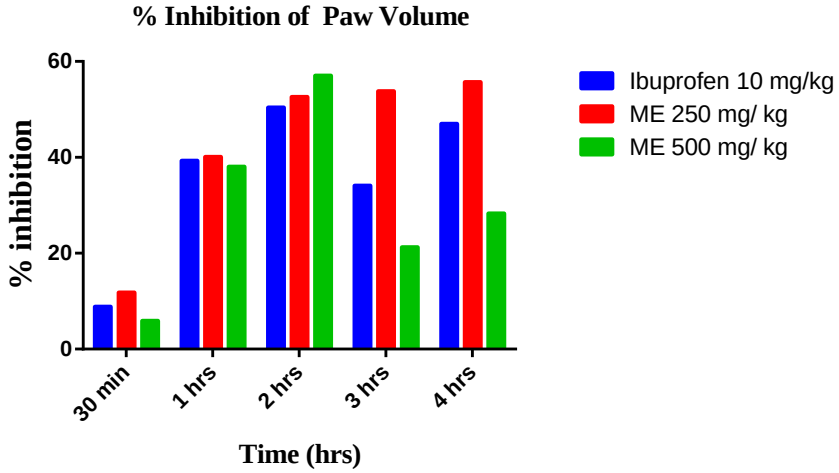

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Fig 4: % inhibition of paw volume of groups by carageenan induced paw edema method *(Tectona grandis)*

Groups and doses given	**Mean difference in paw edema volume (ml) ± SEM (% inhibition)**					
	Dose	**30 min**	**1hr**	**2hrs**	**3hrs**	**4hrs**
Control Carageenan (1%W/V)	0.05 ml	1.70±0.025	2.42±0.017	2.70±0.024	2.64±0.020	2.30±0.017
Ibuprofen	10mg/kg p.o	1.55±0.036 (8.82 %)	1.47±0.020 (39.26%)	1.34±0.019 (50.37 %)	1.74±0.022 (34.09 %)	1.22±0.013 (46.96 %)
Methanolic Extract	250mg/kg p.o	1.50±0.018 (11.76 %)	1.45±0.020 (40.08 %)	1.28±0.019 [a] (52.59 %)	1.22±0.016 [a] (53.79 %)	1.02±0.014 [a] (55.65 %)
Methanolic Extract	500mg/kg p.o	1.6±0.021 (5.88 %)	1.5±0.021 (38.02 %)	1.16±0.022 [a] (57.04%)	2.08±0.013 (21.21 %)	1.65±0.023 (28.26%)

Table-7 -Observations of paw volume and % inhibition of methanolic extract of fruits of *Tectona grandis* by paw edema method

5. CONCLUSION

There was a dose dependent inhibitory activity in carrageenan induced paw inflammation at all assessment times. Phenyl butazone, a COX-inhibitor at the dose of 100 mg/kg, p.o.significantly reduced the paw edema. This indicates action against release of histamine, serotonin and kininsin early phase, while later phases are suspected to be arachidinate metabolites producing an edema dependent on mobilization of neutrophils.The result of the present study indicates that methanolic extract of *Cestrum nocturnum* bark (20 and 30 mg/kg, p.o.) and phenyl butazone play a crucial role as protective factors against the carrageenan-induced acute inflammation.

In conclusion, this study demonstrated that the methanol extract of *Cestrum nocturnum* bark has a dose dependant antiinflammatory activity.

Tectona grandis has been claimed to be useful in treatment of pain and inflammation and the current literature survey revealed that no systematic approach has been made towards documentation of this claim. In this investigation the anti inflammatory activities of *Tectona*

grandis fruitextracts were studied using paw edema model which is one of the most widely used primary test to screen new anti-inflammatory agents and measure the ability of a compound to reduce local edema induced in the rat foot pad by injection of an irritant agent. This edema depends on the participation of kinins and polymorphonuclear leukocytes with their proinflammatory factors including prostaglandins. The development of edema in the paw of the rat after the injection of carrageenan has been described as a biphasic event. The initial phase, observed around 1 hour, is attributed to the release of histamine and serotonin; the second, accelerating phase of swelling is mainly due to the release of prostaglandin-like substances. In the present study all the doses of methanolic extract of fruits of *Tectona grandis* significantly decreased the inflammation at 3rd and 4th hours after treatment which was statistically equipotent to that of reference standard ibuprofen.

REFERENCES:

1. Mukherjee P.K., (2003): GMP for Indian Systems of Medicine. Business Horizons, New Delhi; 99-112.
2. Perumal S.R., and Ignacimuthu S. (1998): J. Ethnopharmacol. 62; 173-182
3. Puspangadan P., and Atal, C.K. (1984): J. Ethnopharmacol. 11; 59-77
4. Rabe, T., and Staden, J.V., (1997): J. Ethnopharmaco/. 56: 81-87.
5. Craig C. R., Stitzel R. E., Modern Pharmacology with Clinical Applications, 5th Edn, 424-425,2005
6. Mohan H., Textbook of Pathology, Jaypee publication, Delhi, 3rd Edn, 134-190, 1998
7. Hart B.L., (1988). "Biological basis of the behavior of sick animals". Neuroscience and biobehavioral reviews12 (2): 123–137.
8. Johnson R.W., (2002). "The concept of sickness behavior: a brief chronological account of four key discoveries". Veterinary immunology and immunopathology87 (3–4): 443–450.
9. Kelley, K.W., Bluthé, R.M., Dantzer, R., Zhou J.H., Shen W.H., Johnson R.W., Broussard S.R., (2003). "Cytokine-induced sickness behavior". Brain, behavior, and immunity. 17 Suppl 1: S112–118.
10. Ncube N.S., Afolayan A.J., Okoh A.I., Assessment Techniques of Antimicrobial Properties of Natural Compounds of Plant Origin: current methods and future trends. African Journal of Biotechnology 2008; 7 (12): 1797-1806.
11. Remington J.P., Remington: The Science and Practice of Pharmacy, 21 edition, Lippincott Williams & Wilkins, 773-774.

12. Das K., Tiwari R.K.S., Shrivastava D.K., Techniques for Evaluation of Medicinal Plant Products as Antimicrobial Agent: Current methods and future trends. Journal of Medicinal Plants Research 2010; 4 (2): 104-111.
13. Handa S.S., Khanuja S.P.S., Longo G., Rakesh D.D., Extraction Technologies for Medicinal and AromaticPlants. International centre for science and hightechnology, Trieste, 2008, 21-25.
14. Vogel Gerhard H., Drug Discovery and Evaluation Pharmacological Assays, Second Edition, Springer-Verlag Berlin Edn., New York, 1994; 759-762,772-774.
15. Patil, M.B.,Jabalpure, S.S., Pramod, H.J., Manvi, F.V.; Anti-inflammatory activity of *Anacardium occidentale* leaves, Indian J. of Pharm. Sc. 2003; 65(1): 70-72.
16. Eddy N.B., Leimback D.; Synthetic analgesic. II.Dithienylbutenyland dithienylbutylamines. JPharmacol Exp Ther.1953; 107: 385.
17. Koster R, Anderson M, De-Beer E.J.; Acetic acid analgesic screen. Federation Proc.1959; 18: 418.
18. Purnima A. Et al, Anti-inflammatory, Analgesic and Antipyretic Activities of *Mimusops elengi* Linn, Indian J Pharm Sci. 2010; Jul-Aug 72(4): 480–485.
19. Gijtenbeek J.M.M., Vanden Bent M.J. and Vecht C.J.J.; Neurol 1999; 246;339-346.
20. Chatterjee G.K., Burman T.K.,Nagchaudhuri A.K., Pal S.P.; Anti-inflammatory and antipyretic activities of *Morus indica*,Planta Medica 1983; Jun; 48 (2):116-9.
21. Khan M.A. et. al., "A comparative study on the antioxidant activity of methanolic extracts from different parts of *Morus alba*(Moraceae)", BMC Res Notes, 2013 Jan 19; 6-24.
22. Walter F.,Medical Physiology: A Cellular And Molecular Approaoch. Elsevier/Saunders. (2003), p. 1300. ISBN 1-4160-2328-3.
23. Steffer A., Stephen J.H., Nancy J., "The role of TNF-a in fever: opposing actions of human and murine TNF-oa and interactions with IL-fl in the rat". Brftish Journal of Pharmacology, (April 25, 1996), 2012-12-