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Research Article

Evaluation Of Antiulcer And Antiarthritis Activity Of Methanolic Extract Of Tectona Grandis (Bark)

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ABSTRACT

The use of herbal supplements has become increasingly popular in recent years. It has been documented that as many as 31% of patients use herbal supplements concurrently with the prescribed conventional drugs and 70% of them do not report the use of these products to their healthcare providers. Simultaneous administration of herbs and drugs may mimic, The plant Tectona Grandis (Bark) was collected from Specimens were collected from different intertidal locations in the Tamilnadu coastal area, India. plant was identified and authenticated in the Department of studies in Botany JNTUH University, The complete course of experiment was carried out using healthy male Wistar rats weighing between 150-200 gm. They were housed in standard laboratory condition at room temperature along with 12 h light/dark cycle. Animals were observed individually during first 30 min, periodically during 48 hours with special attention given during first 4 hours (short-term toxicity) and daily thereafter for total of 14 days (short-term toxicity). LD 50 was found greater than 2 000 mg/kg, in Limit test, drug substance could be classified in the hazard classification as Class 5, Nontoxic in the Globally Harmonized System. A dose range of 100, 200 and 300 mg/kg was selected for EETG. Ethanolic extract of Tectona Grandis at 150 and 300 mg/kg were given the animals in the group. Ranitidine (50mg/kg) was used as the standard drug. After 1 hour of drug treatment all the animals were placed vertically in individual restraint cages in water at 220 for one hour. The rats were divided into six groups of 8rats each. The methanolic leaf extract was dissolved in normal saline using 10% tween 20 so as to achieve an oral dose of 2 g and 4 g kg body weight for groups 1 and 2, respectively.

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The extract was administered one day before arthritis induction and daily thereafter for the whole period of experiment (30 days). The third group was a positive control group treated with Indomethacin at a daily dose of 50 mg kg. On the basis of the results obtained in this study we conclude the potent anti-arthritic effect of Tectona Grandis (Bark) ethanolic extract may be through maintenance of synovial membrane and vascular permeability, thereby inhibiting cytokines and leukotriene CFA induced knee joint inflammation. In turn protecting synovial membrane and destruction of cartilage. The extract and indomethacin may propose the inhibitory effect on phospholipase A2 and prostaglandin. The results obtained in the present study indicate that Tectona Grandis (Bark) is having a potent anti-arthritic property.

INTRODUCTION

Herbal medication in addition referred to as herbal treatment or phytomedicine refers to employ a plant's seed, berries, roots, leaves, bark or flowers for healthful functions. Herbalist contains a long tradition of use outside of typical medication. It's turning into additional thought as improvement in analysis and internal control in conjunction with advances in clinical analysis show the worth of flavored medication in treat and prevent sickness. Plants have been in the treatment of various diseases from the time immemorial. The use of plant as a source of medicine lies deep in the root history mankind. Many of thousands of plant species growing throughout the world have medicinal use congaing active constituents that have direct actions on the body[1].

There used in herbal and conventional medicine and offer benefits that pharmaceutical drugs often lacks helping combat illness and support the bodies efforts to re gain good health hence herbal drugs are valuable as well as precious gift from nature to mankind[2]. Today herbal medicines are coming in to prominent because of the efficacy of the conventional medicine such as antibiotic, which have developed resistance to the many of the infectious organisms. Throughout the world have received greater attention in recent times because of it diversity of curing disease safety and well tolerated compared to the conventional medicine[3].

The herbs with natural combination of constituents as a whole are natural remedies[4]. This has proved to be more effective and safer than conventional medicines. The ability of herbal medicines to affect the body systems depends on the chemical constituents that scientist first started, extracting and isolating chemicals, from plants in the 18 century[5]. Research in to isolated plant constituents is of great importance, it has given rise to many of the world, most muscle relaxant, in existence is derived from curare and strongest pain killer of all morphine comes from poppy and cocaine coca undoubtedly, the plant kingdom still holds many species of plant containing substances of medicinal values, which have yet to be discovered[7].

Bengali (Segun, saigun); Burmese (kyun); English (teak wood, Indian oak, teak tree); Filipino (dalanang, djati); French (teck); Tamil (tekku, tekkumaram, tek) Tectona grandis is a large, deciduous tree reaching over 30 m in height in favourable conditions. Crown open with many small branches; the bole is often buttressed and may be fluted, up to 15 m long below the 1st branches, up to 1 m dbh. Bark is brown, distinctly fibrous with shallow, longitudinal fissures[9].

Image – A

Image-B

MATERIALS AND METHODS

Plant material and preparation of extract:

The plant *Tectona Grandis* (Bark) was collected from Specimens were collected from different intertidal locations in the Tamilnadu coastal area, India. The plant was identified and authenticated in the Department of studies in Botany JNTUH University, Telangana, India. The TG bark were shade dried on a laboratory table for 6 days and reduced to powder by using dry grinder. This powder (100g) was then packed into Soxhlet apparatus and extracted using 95% ethanol (40-50°C). The extraction was carried out for 40h. The extract obtained was dried at 45 °C in hot air oven till green colour semisolid mass was obtained. The yield obtained was 4.5% and the semisolid extract was stored in a refrigerator at 4 °C until further use[12].

Experimental animals

The complete course of experiment was carried out using healthy male Wistar rats weighing between 150-200 gm. They were housed in standard laboratory condition at room temperature along with 12 h light/dark cycle[18]. The animals were provided with standard pelleted diet obtained commercially from the manufacturer (Vivo Biotech, Turkapally) and water ad libitum. After seven days of acclimatization period, they were randomly selected for different experimental groups. Ethical clearance was obtained from Institutional Animal Ethics Committee Surabi Dayakar Rao College of Pharmacy before conducting the experiment[23].

Drugs and Chemicals:

Haloperidol
Ethanol
Acetone
Complete Freund's adjuvant

Instruments:

Haematology cell counter (ERMA, Japan)
Plethysmograph

Acute toxicity studies:

Acute oral toxicity of ethanolic extract of *Tectona Grandis (Bark)* was determined according to the guidelines of Organization for Economic Co-operation & Development (OECD) following the Up & Down method (OECD guideline No. 425) and Fixed dose method (OECD guideline No. 420)[24]. Based on these methods a Limit test was performed to categorize the toxicity class of the compound. The animals (nulliparous and non-pregnant female Wistar albino rats) were fasted overnight with free access to water, weighed and a single dose of the test substance was administered orally. Animals were observed individually during first 30 min, periodically during 48 hours with special attention given during first 4 hours (short-term toxicity) and daily thereafter for total of 14 days (short-term toxicity). LD 50 was found greater than 2 000 mg/kg, in Limit test, drug substance could be classified in the hazard classification as Class 5, Nontoxic in the Globally Harmonized System. A dose range of 100, 200 and 300 mg/kg was selected for EETG[26].

Experimental study design:

Stress ulcers by cold water immersion.

Purpose:

Cooling of rats in water during the restraint period accelerates the occurrence of gastric ulcers and shortens the time of necessary immobilization (Takagi et al 1964, West 1982).

Procedure:

Albino rats of either sex were divided into four groups of six animals each. All the rats were overnight fasted before the study, but animals had

free access to water. Animals in the control group received only distilled water. Ethanolic extract of *Tectona Grandis*at 150 and 300 mg/kg were given the animals in the group. Ranitidine (50mg/kg) was used as the standard drug. After 1 hour of drug treatment all the animals were placed vertically in individual restraint cages in water at 22^0 for one hour. Then they are removed and dried, and injected intravenously via tail with 30 mg/kg Evans blue[28]. Ten minutes later, they are sacrificed in CO2 anaesthesia, and their stomachs were removed. Formal saline (2% v/v) is then injected into the totally ligated stomachs for storage overnight. The next day, the stomachs are opened along the greatest curvature, washed in warm water, and examined under a 3-fold magnifier. The lengths of the longest diameter of the lesions are measured and summated to give a total lesion score for each animal[30].

Animal grouping, extract dilution and application:

The rats were divided into six groups of 8rats each. The methanolic leaf extract was dissolved in normal saline using 10% tween 20 so as to achieve an oral dose of 2 g and 4 g kg body weight for groups 1 and 2, respectively. The extract was administered one day before arthritis induction and daily thereafter for the whole period of experiment (30 days). The third group was a positive control group treated with Indomethacin at a daily dose of 50 mg kg. The fourth group was a negative untreated control group. The fifth group was also a negative untreated control. However, arthritis was induced in this specific group one month earlier than the other groups and was used exclusively for gross examination of the articular surfaces. The sixth group was a normal non-arthritic group used for comparison with other groups in two experiments only; gross examination of the articular surfaces and evaluation of kidney and liver functions[36].

Arthritis induction:

All Rats were anesthetized by intraperitoneal injection of 5% chloralhydrate at a dose of 0.25 mg g body weight. Then, to induce arthritis, 0.25 mL of CFA was injected into the planter region of the right hind paw of each rat after.

Measurement of paw volume and ankle diameter:

Measurements of the right and left paw volumes were done right before arthritis induction and on alternate days thereafter for 30 days using a plethysmometer. Rats were otherwise observed daily. Ankle diameter was determined using a caliper only twice: right before arthritis induction and at the end of the experiment (30 days after arthritis induction).

Gross examination of the articular surfaces:

All rats(extract-treated and controls) were sacrificed by overdose of ether 30 days after CFA administration. Right hind limb joints were prepared by removing all the surrounding muscles, capsules and ligaments for gross examination of the articular surfaces of the hip, knee and ankle joints. By this time, arthritis has been induced in the fifth group for two months. The aim of including this group was to evaluate the progression of arthritis and severity of articular damage compared with the other groups. In addition, joints of normal (non-arthritic) untreated rats were prepared for comparison with other groups. To compare the extent of adjuvant-induced arthritis in treatment and control groups, a scoring system for severity of arthritis in rats modified after was followed. The scoring system is illustrated in the following Table1[22].

Determination of serum inflammatory markers:

Leukocyte Count, Packed Cell Volume (PCV), Erythrocyte Sedimentation Rate (ESR), Rheumatoid Factor (RF) and c-reactive protein (CRP) were examined in the serum of the treated and untreated control groups (except groups 5 and

6) 30 days after CFA administration and before sacrifice of the rats.

Statistical analysis:

ANOVA test was applied to test the significance of differences between the results of extract-treated and positive and negative control groups. Dunnett's test was used to determine which means differ from negative control group at each point of time. The difference was considered significant at the conventional level of significance ($p<<1.00$) [35-39]

RESULTS AND DISCUSSION

Phytochemical evaluation of *Tectona Grandis*(Bark):

The extract of *Tectona Grandis (Bark)* was subjected to phytochemical screening for the presence of different phytoconstituents, and the extract was found to contain proteins, glycosides, alkaloids, tannins, phenolic compound, steroid reducing sugars and saponin glycosides (triterpenoidsaponins).

Stress ulcers by cold water immersion:

Placing the animals vertically in individual restraint cages in water at 22^0 C resulted in marked gross mucosal lesions in stomach. On gross examination these lesions were characterized by multiple haemorrhagic red bands (patches) of different sizes along the longitudinal axis of the glandular stomach (score 6, Table 10). Animals pre-treated with *A. aspera* showed significant protection against stress-induced gastric damage. *A. aspera* treated rats showed mild ulcers with interstitial haemorrhage (score 2). The intensity of lesions and haemorrhage was significantly reduced with *Tectona Grandis (Bark)* Morphometric evaluation was also carried out to evaluate the extent of ulcer. The size of the ulcer was significantly reduced in animals pre-treated with *Tectona Grandis* treated rats (Table 10). On microscopic examination, control rats showed total mucosal ulceration, haemorrhage and segmental mucosal necrosis of gastric epithelium characterized by many apoptotic bodies. Only patchy mucosal epithelial loss was seen in *Tectona Grandis* treated rats (Figure-12).

Table-10: Effect of *Tectona Grandis (Bark)* on stress induced ulcer.

Treatment	Gastric lesions		
	Length (mm)	Inhibition %	Score
Control	84.8±6.47	-	5.9±0.08
Ext of *T.G* (100mg)	27.4±2.55*	67.68	3.06±0.08*
Ext of *T.G* (200mg)	22.5±2.76*	73.46	2.2±0.08*
Ranitidine	18.43±2.71*	78.26	1.91±0.13*

The data are expressed as Mean± SEM. *P<0.001 as compared to control.

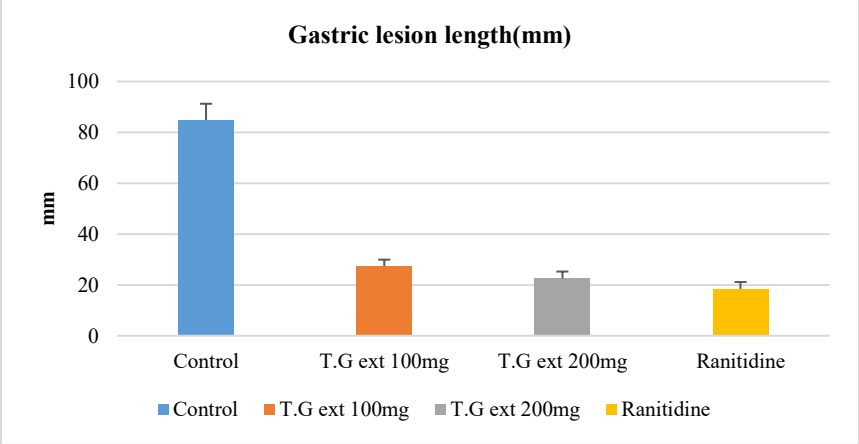

Figure-6: Histogram showing the effect of *T.G* on stress induced gastric lesions.

Restraint stress has been one of the most popular stressors in experimental medicine. It elicits the purest form of psychological frustration accompanied by vigorous struggling which means muscular exercise. In the cold restraint model, stress in the control group clearly produced a mucosal damage characterized by multiple haemorrhage red bands of different sizes on the glandular stomach. Exposure of the animals to the cold restraint stress may have caused severe imbalance in the normal physiological conditions that might have resulted in a stressful condition leading to ulcers. Pre-treatment with test drugs (*Tectona Grandis (Bark)* extract 100 and 200 mg/kg) produced a significant decrease in the intensity of gastric mucosal damage induced by the stress as compared with control. A significant increase in the ulcer index and mean score in the control group was observed, however both the parameters were significantly decreased in the treated groups (*Tectona Grandis (Bark)* extract 100 and 200 mg/kg). Further, examinations of the stomach images provide supportive evidence for the anti-ulcer activity of *Tectona Grandis (Bark)* extract 100 mg/kg and *Tectona Grandis (Bark)* extract 200 mg/kg treated groups which showed signs of recovery from the cold restraint stress induced ulcers. The reduction in the ulcer index and mean score may be attributed to the anti-ulcer activity of the *Tectona Grandis (Bark)* due to presence of antioxidants like phenolic compounds. Stress-induced ulcer is probably mediated by the release of histamine. It not only increases gastric secretion, often called the “aggressive factor”, but also causes disturbances of the gastric mucosal microcirculation and an abnormal motility, and reduces mucus production, known as the “defensive factor”. Moreover, stress-induced ulcers in animal models may be partially or entirely prevented by vagotomy, since increased vagal activity has been suggested as the main factor in stress-induced ulceration. The vagus nerve stimulates stomach acid secretion via interaction of its chemical mediator (acetylcholine) with the muscarinic receptor. The activation of the muscarinic receptor gives rise to sequential events that result in increased gastric acid secretion. These receptors are located on the cell membranes of parietal cells and histamine secretory cells. Therefore, the increase in acid secretion is a consequence of acetylcholine action on the histamine cell and parietal cell activity.

Stress-induced ulcers also involve damage by reactive oxygen species (ROS) apart from acid and pepsin related factors. Exposure to cold restraint is a well-known intensive stress response wherein both cold exposure and immobilization individually and synergistically are responsible for the generation of reactive oxygen species (ROS) e.g. superoxide anion, hydrogen peroxide, hydroxyl radicals etc. that cause lipid peroxidation, especially in membranes and result in tissue injury. Elevation in the levels of end products of lipid peroxidation in stress control rat stomachs was observed. The increase in MDA levels in the stomach suggests enhanced peroxidation leading to tissue damage and failure of the antioxidant defence mechanisms to prevent formation of excessive free radicals. Pre-treatment with *Tectona Grandis (Bark)* extract 100 mg/kg and *Tectona Grandis (Bark)* extract 200 mg/kg significantly reversed these changes. Hence, it is likely that the mechanism of ant-ulceration of *Tectona Grandis (Bark)* is due to its antioxidant effect.

Antiarthritic activity:

The anti-arthritic activity was evaluated according to the ability of the treatment to inhibit CFA–induced paw swelling, ankle swelling, skin lesions and articular deformity. CFA, administrated subcutaneously in the right paw, caused a significant increase in the volume of not only the right paw, but the left paw as well, with fluctuation in paw volume all over the experimental period.

Significantly, the extract inhibited paw swelling compared with the untreated control dose-dependently in both the right and left paws, which indicates an anti-inflammatory effect of the extract. The effect of indomethacin was intermediate between the high (100mg kg) and low (200mg kg) doses of the extract. All the experimental groups showed a significant increase in the ankle diameter 30 days after CFA administration in comparison with the diameter before the adjuvant administration. However, neither treatment was associated with impedance of ankle swelling[165].

In this investigation, lesions developed at the paws and ankles of untreated rats and were in most cases supportive, whereas in the extract and indomethacin-treated rats the lesions developed over the paws only and none was supportive. In addition, functional disability manifested as difficulty in movement and dragging of the paw was observed in most of the control untreated rats, but not in the extract or indomethacin-treated rats. The disability was accompanied with generalized hardness and darkness of the paws. Gross examination of the articular surfaces: From gross examination of the articular surfaces of the joints we can conclude that both doses of the extract (100mg and 200mg kg), as well as ibuprofen, were effective in protecting the joints against deformity. The extract effectively inhibited redness, roughness and erosion of the tested joints (ankle, hip and knee) (Fig. 3-7 and Table 2). Although the extract could not inhibit ankle swelling, it did inhibit deformity of its articular surfaces.

Determination of serum inflammatory markers:

Both doses of the extract (100mg and 200mg kg) caused a significant increase in the PCV compared with untreated control group ($p<<1.00$) (Table 3). There was no significant difference in the erythrocyte sedimentation rates, WBC counts, or CRPs between the treated or control groups at the end of the experimental period. RF was negative in both treated and untreated rats.

Table no 1: Symptoms and Diagnosis of arthritis

	1987 criteria		2010 criteria	
Criteria	**Description**	**Score**	**Description**	**Score**
Morning stiffness	In and around joints, for at least 1 hour	1	Clinical synovitis/swelling in at least 1 joint not explained by another Disease	NA
Joint involvement	Physician observed soft tissue swelling or fluid in 3 of 14 possible joints	1	1 large joint 2-10 large joints 1-3 small joints (with or without large joint) 4-10 small joints (with or without large joint) >10 joints (at least 1 small)	0 1 2 3 5
Arthritis of hand joints	At least 1 swollen hand or wrist area 1 NA NA Symmetric arthritis Simultaneous bilateral Involvement	1	NA	NA
Symmetric arthritis	Simultaneous bilateral Involvement	1	NA	NA

Rheumatoid nodules	Subcutaneous nodules over bony prominences, extensor surfaces, or in juxta articular regions observed by physician	1	NA	NA
Serology	Positive RF serum test	1	Negative RF and negative ACPA Low-positive RF or ACPA High-positive RF or ACPA	0 2 3
Radiographic changes	Erosions or unequivocal bony decalcification in or adjacent to the involved joints, but not consistent with osteoarthritis	1	NA	NA
Acute phase reactants	CRP and ESR	NA	Normal CRP and ESR Abnormal CRP or ESR	0 1
Duration of symptoms	First 4 criteria must be present for at least 6 weeks	NA	<6 weeks >6 weeks	0 1
Criteria score required		>4/7		>6/10

Table 4: scoring system for severity of arthritis in rats;

Score	Characteristics of the joint
0	No changes (normal)
1	Slight redness, roughness and swelling
2	Moderate redness, roughness and swelling
3	Severe redness, swelling and roughness
4	Very severe redness, swelling and roughness

Table 5: Ankle diameter in mm (mean ± SD of 8 rats) of arthritic control rats and rats treated with *Tectona Grandis (Bark)* or indomethacin(mm).

	100mg kg	200mg kg	Untreated	Indomethacin-
Day	Extract	Extract	Control	Treated
Zero	6.125±0.14	6.125±0.16	6.28±0.14	6.28±0.14
Thirty	6.26±0.14	7.63±0.19	7.91±0.18	7.78±0.21

Table no: 6 Measurement of articular surface.

Articular surface	100mg kg Extract	200mg kg Extract	Inomethacine Treated	Untreated Control	Untreated control with arthritis for 2 months	Normal non-arthritic
Talus at Ankle	1	0	0	2	3	0
Tibia at Ankle	0	0	1	1	2	0
Tibia of femur at hip	0	0	0	2	3	0

Table-no 7: Right hind paw volume

Days	control	Extract 150g/kg	Extract250g/kg	Standard indomethacin
0 days	6.13±0.165	6.125±0.144*	6.26±0.142*	6.29±0.144*
30days	7.912±0.1831	7.56±0.191**	7.36±0.2011*	7.80±0.212*

Table no 8 Left hind paw volume

Days	control	Extract 150g/kg	Extract250g/kg	Standard indomethacin
0 days	7.0±0.184	7.82±0.182*	7.4±0.157*	6.8±0.56**

30 days	6.23±0.383	7.62±0.2008*	6.05±0.2322*	6.35±0.2244*

*p<1.00&**p<0.59 when compared to arthritic control Results are expressed as mean ± S.E.M., n= no of animals in each group.

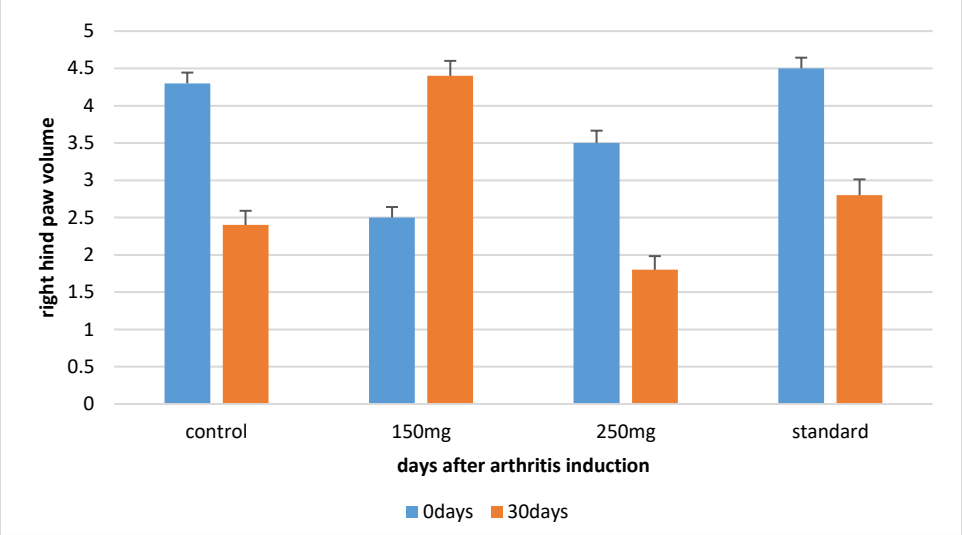

Fig-no 23: The ethanolic leaf extract of *Tectona Grandis (Bark)* inhibited CFA-induced swelling of right hind paws of rats. Data are expressed as mean ± SEM (n = 8). Significance (p<1.00) is shown for the difference between the high dose of the extract (4 g kg) and the untreated control. Zero time is the adjuvant administration.

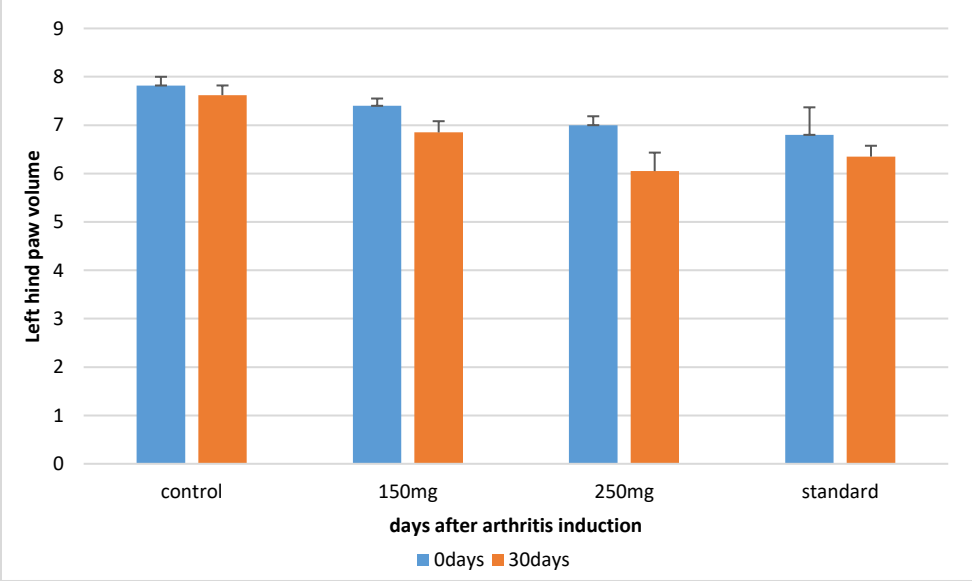

Fig-no24: The ethanolic leaf extract of *Tectona Grandis (Bark)* inhibited CFA-induced swelling of left hind paws of rats. Data are expressed as mean ± SEM (n = 8). Significance (p<0.50) is shown for the difference between the high dose of the extract (4 g kg) and the untreated control. Zero time is the adjuvant administration

The ethanolic leaf extract of *Tectona Grandis (Bark)* is safe at high doses acutely and sub acutely. It has significant anti-arthritic effect, as it inhibited the CFA-induced paw swelling, skin lesions and articular deformity. These results support the use of *Tectona Grandis (Bark)* as an herbal medicine for the treatment of inflammatory disorders and rheumatoid arthritis.

The results of the paw volume and the transfer of inflammation from the site of CFA administration to the other paw can be explained by a phenomenon that called the migratory phenomenon of the adjuvant-induced arthritis and our result was consistence with findings of the fluctuation in paw volume observed in these Figs. 1-2 has also been shown before and is an expected phenomenon with inflammatory diseases that frequently exhibit flares and remissions. Suggested that the process involved in the development of secondary lesion does not appear to be infectious, but rather a generalized immunological response to the constituents of the tubercle bacilli.

Gross examination of the articular surfaces:

The mechanism by which the extract exhibited anti-arthritic activity could be similar to that of *Tectona Grandis (Bark)* which was shown to suppress the activation of nuclear factor (NF-kB). In chronic inflammatory diseases, NF-kB is elevated and is responsible for enhanced expression of many pro-inflammatory mediators[183-191].

CONCLUSION

Human race is largely dependent on the plant kingdom which is not only providing a source of vital nutrients, but also caters to the needs of humans by providing remedies to different types of ailments. This has led to the evolution of plant science dealing with the usage of plants in treating and controlling many different diseases by trial and error. Herbal medicine is referred to use of plant products to treat or prevent a disease. Herbal medicine is also known as a subset of larger term "Complementary and alternative medicine" (CAM). Long before the advent of modern medicine herbs were the mainstream remedies for nearly all ailments. Now a days due to the adverse effects of modern medicine, people have been turning in increasing numbers to the use of herbal medicine as both an alternative and adjunct to modern drugs.

Medicinal plants have their values due to the presence of chemical constituents, commonly known as secondary metabolites, present in various plants. These substances are alkaloids, glycosides, flavonoids, essential and fatty oils, resins, gums, mucilage, tannins etc. of large use. These active principles may be present in the various parts of the plant, viz. roots, seeds, leaves, wood etc. The present study was carried out to determine the anti-ulcer and antiarthritic, activities of Ethanolic extract of *Tectona Grandis.*

The present antiulcer study suggests that the gastro protective activity of ethanolic extract of *Tectona Grandis (Bark)* is due to its antioxidant activity, which helps in the prevention of formation of reactive oxygen species, which causes lipid peroxidation leading to tissue damage and failure of the antioxidant defence mechanism.

In conclusion, further investigations and isolation of the active phytoconstituents will lead to use this plant *Tectona Grandis (Bark)* as a potential therapeutic agent to treat arthritis, and ulcers, On the basis of the results obtained in this study we conclude the potent anti-arthritic effect of *Tectona Grandis (Bark)* ethanolic extract may be through maintenance of synovial membrane and vascular permeability, thereby inhibiting cytokines and leukotriene CFA induced knee joint inflammation. In turn protecting synovial membrane and destruction of cartilage. The extract and indomethacin may propose the inhibitory effect on phospholipase A_2 and prostaglandin. The results obtained in the present study indicate that

Tectona Grandis (Bark) is having a potent anti-arthritic property.

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