Imperial Journal of Interdisciplinary Research (IJIR)
Vol-2, Issue-6, 2016
ISSN: 2454-1362, http://www.onlinejournal.in

Acute Toxicity And Anti-Arthritic Activity On The Bark Of *Sterculia foetida* Linn.,

Kavitha M , Dr. Vadivu R* & Dr. Radha R
Department of Pharmacognosy, College of Pharmacy, Madras Medical College

Abstract: *To investigate the anti-arthritic acitivity of ethanolic extract of Sterculia foetida Linn., on Wistar albino rats by Complete Freund's Adjuvant model. The rats were selected and divided into five groups. Arthritis was induced to the animals in intra plantar region by using 0.1ml of Complete Freund's Adjuvant and then treated for 211 days. The various parameters like changes in the body weight, haematological parameters, paw volume, radiographical and histopathology of bone were studied. The results were reported that arthritic control group showed significantly increased paw volume were as ethanolic extract of Sterculia foetida Linn., at the dose of 200mg/kg and 400mg /kg treated group showed significant reduction in the paw volume which is compared to the normal group and standard (Diclofenac sodium) treated group. Radio graphical and histopathological studies revealed that the anti-arthritic activity of bark of Sterculia foetida by preventing cartilage and bone destruction of the adjuvant induced arthritis rats. The study conclude that Sterculia foetida showed better anti-arthritic activity with significant decrease in paw volume, normalize the hematological, radio graphical and histopathological parameters.*

Key Words: *Sterculia foetida Linn., Arthritis, paw volume, hematological, radio graphical and histopathological.*

INTRODUCTION[1-5]

Rheumatoid arthritis is a chronic auto-immune disorder that affects the joints, characterized by inflammation of the synovial membrane, pain and restricted joint movements. It is a systemic inflammatory disease in which the destruction of cartilage leads to bone deformity and loss of joint function and severe joint pain. While there are many treatments like non-steroidal anti-inflammatory drugs (NSAIDs) and disease modifying anti-rheumatoid arthritis drugs (DMARDs) available for rheumatoid arthritis. But these drugs associated with severe adverse reactions.

In recent years, ethanobotanical and traditional uses of phytoconstituents from plant origin received much attention as they are very effective and safe for human use. Man was completely depending on the natural medicine because of reduced side effects. Herbal medicine is a major component in all indigenous traditional medicine and a common element in Ayurveda, Siddha, Homeopathy and Naturopathy systems of medicine. The plant Sterculia foetida is easily and abundantly available in India. It has many medicinal properties such as anti-diabetic, anti-obesity, anti-microbial, anti-fertility etc., this plant also used in the treatment of rheumatism on folkloric claims but not scientifically proven. Hence in the present study an attempt was made to evaluate the anti-arthritic activity on the bark of *Sterculia foetida* Linn., in rats by Complete Freund's adjuvant induce arthritis model.

COLLECTION AND AUTHENTICATION OF THE PLANT BARK

The fresh bark of *Sterculia foetida* was collected in the month of june and authenticated by Dr. D. Aravind. M.D.(S), Msc. Medicinal plants, National Institute of Siddha (Govt. of India), Tambaram. A voucher specimen is deposited in the Department of Pharmacognosy, Madras Medical College, Chennai.

EXTRACTION METHOD

Fresh bark was collected, dried in shade, coarsely powdered and successively extracted with solvents of increasing polarity like n-hexane, chloroform, ethyl acetate and ethanol by continuous percolation process using soxhlet apparatus. After extraction each extracts were concentrated by using rotary vacuum evaporator. It is dried and the percentage yield was calculated. Appearance and consistency of the extract was noted.

ACUTE TOXICITY STUDY (OECD 423 GUIDELINES)[6]

PROCEDURE:

Acute toxicity study was carried out as per the OECD guidelines. Adult Wistar albino rats of either sex weighing between 150-200g 0f 3 animals were selected for study. All the animals were fasted

overnight provided with water ad libitum. Following period of fasting the ethanolic extract of the roots were administered at a dose of 2000mg/kg body weight orally. After administration special intense observation were taken for first four hours and followed by 14 days observation were carried out.

ANTI-ARTHRITIC ACTIVITY[7-11]

ADJUVANT INDUCED ARTHRITIS MODEL

This is one of the most commonly used animal models for evaluating anti-arthritic activity. Arthritis is induced in rats by intra plantar injection of 0.1ml of Complete Freund's Adjuvant (CFA) in left hind paw except for the vehicle control. The adjuvant contained heat killed *mycobacterium tuberculosis* in sterile paraffin oil. The test drug and the standard drug were administered orally for a period of 21 days and the paw volume was measured periodically using plethysmograph.

Test drug - Ethanolic extract were given at the dose of 200mg/kg and 400mg/kg for 21 days.

Standard drug – Diclofenac sodium were given at the dose of 15mg/kg for 21 days.

EXPERIMENTAL ANALYSIS

A total of 30 adult Wistar albino rats weighing (150-200g) were divided into 5 groups of 6 animals in each group

S.NO	GROUP	NAME OF THE GROUP	TREATMENT
1	I	Positive control	Treatment with vehicle for 21 days
2	II	Arthritic control	Treated with CFA
3	III	CFA + Standard	Treatment with Diclofenac sodium 15mg/kg for 21 days
4	IV	CFA + Test drug 1	Treatment with ethanol extract 200mg/kg
5	V	CFA + Test drug 2	Treatment with extract 200mg/kg

PARAMETERS STUDIED

CHANGES IN BODY WEIGHT

Body weight changes were observed every week.

PAW VOLUME MEASUREMENT

Paw volume of all the animal groups were measured by using plethysmograph at 0, 7, 14 and 21 days. The percentage inhibition of paw volume can be determined using this formula.

$$= \frac{(Vc - V0) - (Vt - V0) \times 100}{(Vc - V0)}$$

where, Vc - is the paw volume after induction

V0 - is the paw volume before induction

Vt - is the paw volume after treatment.

SECONDARY LESIONSs

Secondary lesions developed in ears, fore limbs, hind limbs and tails were scored.

HEMATOLOGICAL PARAMETERS

At the end of the experimental period (22^{nd} day) the blood was collected from the animal through retro- orbital plexus of all the groups and the hematological parameters such as hemoglobin content, RBC count, WBC count, Hemoglobin and ESR were studied.

RADIOGRAPHICAL STUDIES

At the end of the experimental period, X-ray were taken for the hind limbs of experimental animals and examined for soft tissue swelling, bony erosions and narrowing of the spaces between the joints.

HISTOPATHOLOGICAL STUDIES

At the end of the experiment, animals were sacrificed by cervical decapitation. The proximal inter phalangeal joints were removed and washed with saline and stored in 10% formalin for the evaluation of histopathological changes like soft tissue swelling, bone demineralization, pannus formation, cartilage erosion and joint space narrowing.

RESULTS

ACUTE TOXICITY STUDIES

OBSERVATION	30 mins	4 hrs	24 hrs	14th day
Body weight	-	-	-	-
Pre terminal deaths	-	-	-	-
Cage side observation	+	+	+	+
Motor activity	+	+	+	+
Convulsions	-	-	-	-
Pilorection	-	-	-	-
Righting reflex	+	+	+	+
Lacrimation	-	-	-	-
Salivation	-	-	-	-
Respiration	+	+	+	+
Skin color	+	+	+	+
Diarrhea	-	-	-	-
Loss of corneal reflex	-	-	-	-
Loss of pinnal reflex	-	-	-	-
Grooming	-	-	-	-
Sedation	-	-	-	-
Excitation	+	+	+	+
Aggression	+	+	+	+

NOTE: +, - Indicates presence or absence

Since there was no death and behavioral changes observed, the extract was considered as safe to administer. From this 1/10th and 1/5th of the dose (200mg and 400mg) were selected for further studies.

IN-VIVO EVALUATION OF ANTI-ARTHRITIC ACTIVITY

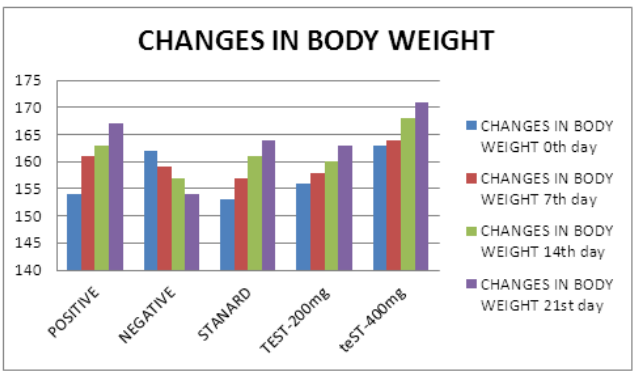
ADJUVENT INDUCED ARTHRITIS (Complete Freund's Adjuvant)

Changes in the body weight in (gm) Adjuvant-induced Arthritic Rats

Treatment	0 day	7th day	14th day	21st day
Group I	154 ±4.74	161 ± 9.58**	163 ± 9.3380**	167 ±10.30**
Group II (Arthritic control)	162 ± 6.408	159± 6.91*	157± 56.64*	154 ± 5.780*
Group III Diclofenac sodium	153 ± 8.846	157 ± 2.87***	161 ± 2.13***	164 ± 2.31b***
Group IV 200 mg/ kg b.w	156 ± 4.49	158 ±5.09***	160 ±6.33***	163 ± 4.81***
Group V 400mg / kg b.w	163 ± 5.79b	164 ±2.81***	168 ±4.979***	171 ± 5.42***

Values represent in the result are mean ± SD (n=6)

$^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ as compared to the arthritis control. The data was analyzed using one way analysis of variance (ANOVA) followed by Tukey HSD Test.



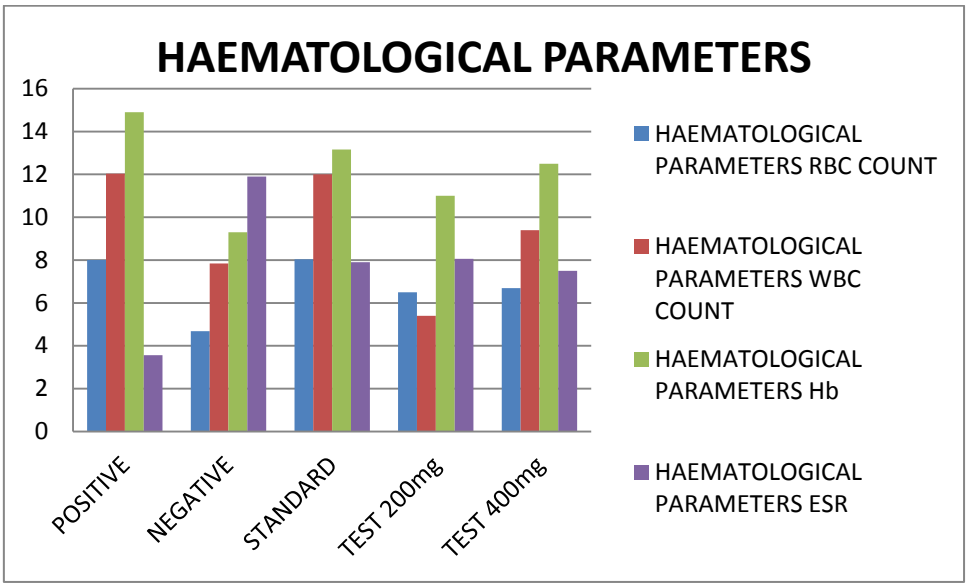
The body weight of the different groups of rats was noted. During the treatment the body weight of the animals in control, standard, test dose I and test dose II were increased except arthritis induced rats.

HEAMATOLOGICAL PARAMETERS

Effect of Hematological parameters in Adjuvant-induced Arthritis in Rats

PARAMETERS	RBC count (pre cu.mm)	WBC count (pre cu.mm)	Hb (g.dL)	ESR (mm)
Group I (Control)	8.01±0.138	12.03± 1.15**	14.9±0.346**	3.56±0.342**
Group II (Arthritic control)	4.68±0.147	4.84±1.16*	9.3±0.257*	11.9±0.694*
Group III (Diclofenac sodium)	8.04± 0.264	12.0±0.41***	13.16±0.151***	7.9± 1.48***
Group IV (200 mg/ kg)	6.50±0.235	8.1±8.216***	11.0±0.258***	8.06±1.274***
Group V (400mg/kg)	6.69±0.102	9.4±0.755***	12.5±0.207***	7.5±0.843***

*p < 0.05, **p < 0.01, ***p < 0.001 as compared to the arthritis control. The data was analyzed using one way analysis of variance (ANOVA) followed by Tukey HSD Test.



The result indicates that arthritic group of animals showed decreased RBC, WBC and Hemoglobin levels. But the ESR levels were increased in arthritic control group. In the standard group all the blood levels are brought back to the normal levels. The group which is treated with 200mg/kg showed an improvement in the blood parameters. The group treated with 400mg/kg extract showed significant improvement of blood parameters which was comparable with that of the standard drug.

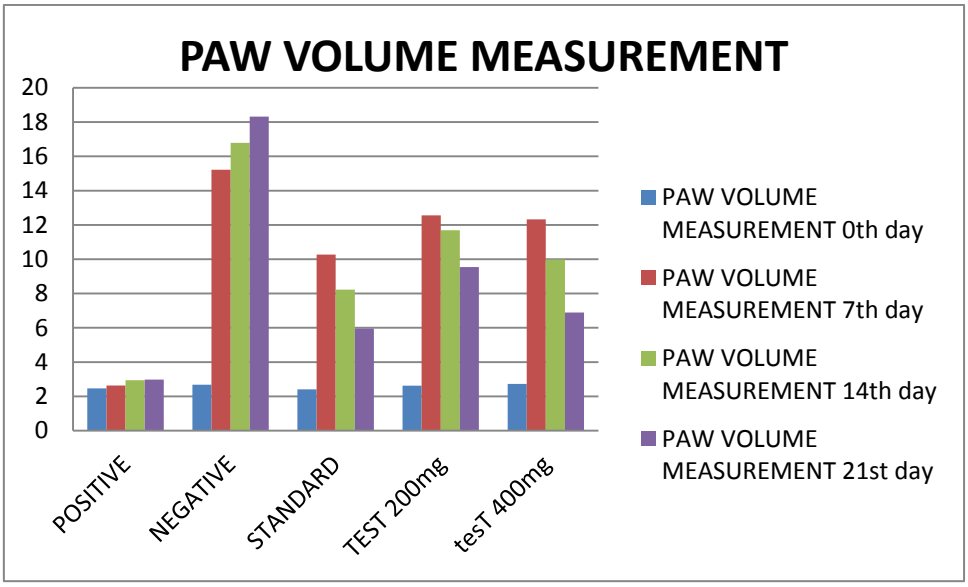
Measurement of paw volume using plethysmograph in

Adjuvant-induced Arthritis in Rats

Treatment	0 day	7th day	14th day	21st day
Group I (Control)	2.46±0.54	2.63 ±0.45**	2.94 ±.14**	2.98±0.32**
Group II	2.68 ± 0.21	15.22 ± 0.15*	16.79 ± 0.32*	18.32± .142*

(Arthritic control)				
Group III Diclofenac sodium	2.41±0.34	10.27±0.17***	8.23±0.14***	5.96 ± 0.13***
Group IV (200 mg/ kg b.w)	2.62±0.113	12.56±0.213***	11.69 ± 0.16***	9.54±0.019***
Group V (400mg/kg b.w)	2.73±0.21	12.32± 0.14***	9.98 ± 0.145***	6.89±0.018***

*p < 0.05, **p < 0.01, ***p < 0.001 as compared to the arthritis control. The data was analyzed using one way analysis of variance (ANOVA) followed by Tukey HSD Test.



Percentage inhibition of Paw volume in Adjuvant - induced Arthritis Rats.

Treatment	7th day	14th day	21st day
Group I (Positive control)	**0**	**0**	**0**
Group II (negative control)	**0**	**0**	**0**
Group III (Diclofenac sodium)	32.52	50.98	67.46
Group IV (200 mg/ kg)	17.47	30.37	47.92
Group V (400mg/kg)	19.05	40.55	62.39

RADIOGRAPHY STUDY

The radiographic images of hind limb of rats were taken and discussed as follows.

Group I : Normal control group showed no changes in the joints.

Group II : Arthritic control group showed destruction of bones at joints, narrowing of joints and swelling of soft tissues.

Group III : Standard group (Diclofenac sodium) showed no destruction of bones at joint, swelling of soft tissues are reduced to normal.

Group IV : Test dose I (200mg/kg) showed mild destruction of bones and swelling of soft tissues.

Group V : Test dose II (400mg/kg) showed mild changes in the joint and the swelling of soft tissues is completely reduced.

RADIOGRAPHY OF BONE

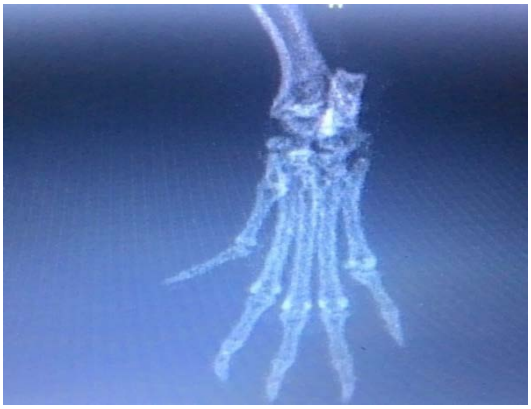


FIG NO: 1 POSITIVE CONTROL

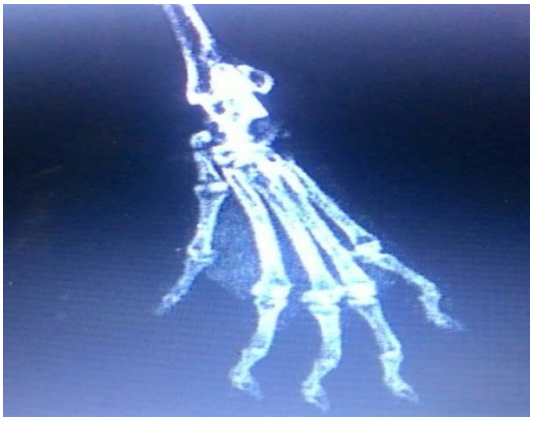


FIG NO: 2 NEGATIVE CONTROL

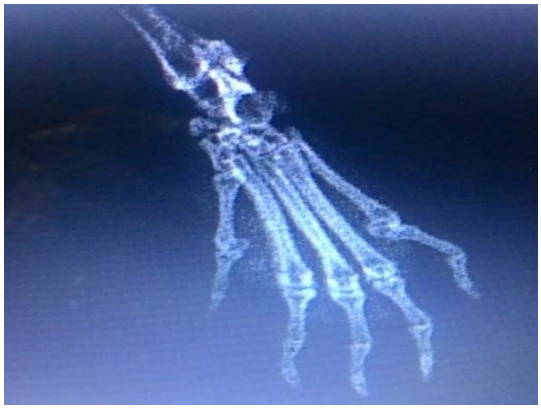


FIG NO: 3 STANDARD (DICLOFENAC SODIUM)

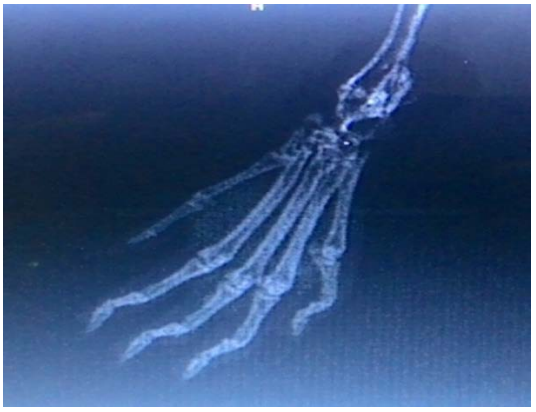


FIG NO: 4 TEST DOSE 200mg/kg

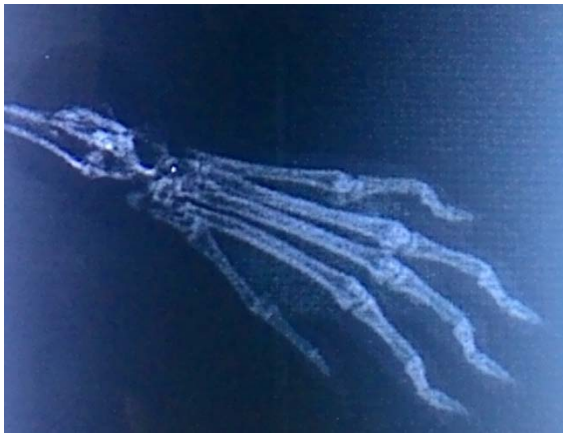


FIG NO: 5 TEST DOSE 400mg/kg

HISTOPATHAOLOGICAL EXAMINATION OF BONE

Group I : Normal control group showed normal cartilage.
Group II : Arthritic control group showed erosion of bone and formation of edema.

Group III : Standard group (Diclofenac sodium) showed normal cartilage and no formation of edema occurs.

Group IV : Test dose I (200mg/kg) showed mild erosion of bones.
Group V : Test dose II (400mg/kg) showed normal cartilage and absence of edema.

HISTOPATHOLOGY OF BONE

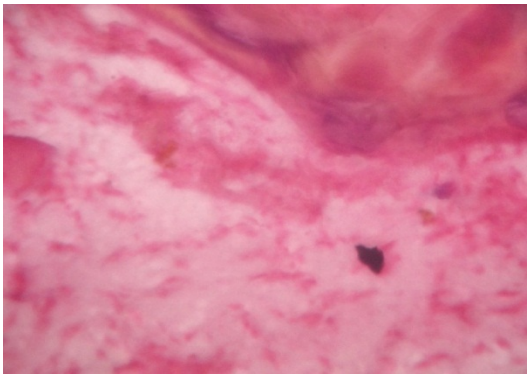


FIG NO: 6 POSITIVE CONTROL

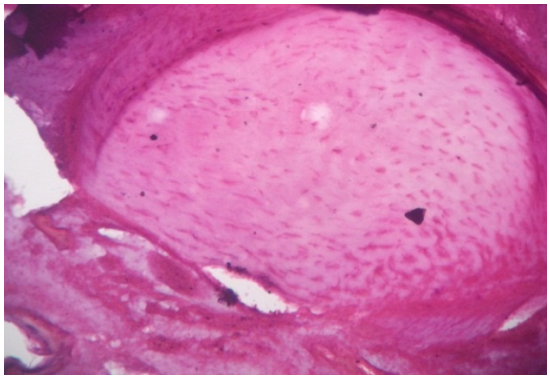


FIG NO: 7 NEGATIVE CONTROL

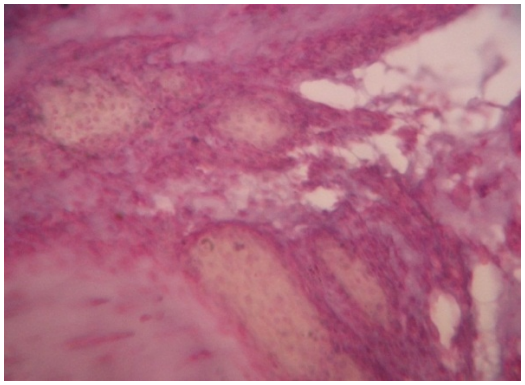


FIG NO: 8 STANDARD (DICLOFENAC SODIUM)

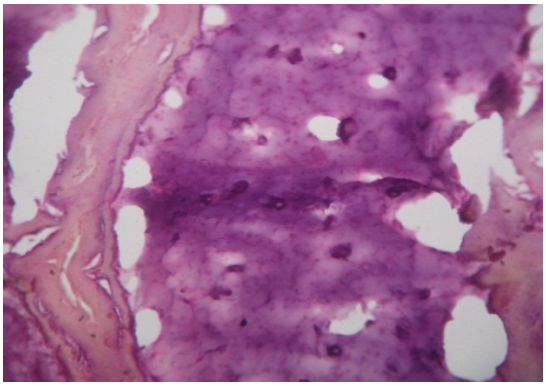


FIG NO: 9 TEST DOSE 200mg/kg

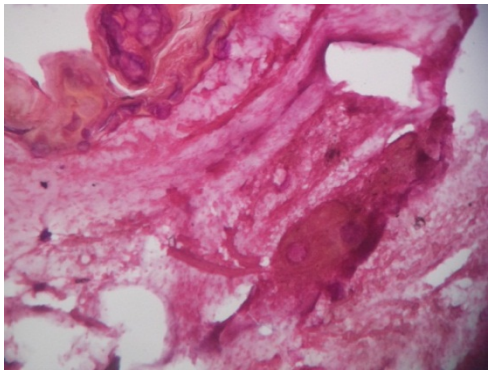


FIG NO: 10 TST DOSE 400mg/kg

DISCUSSION

Acute toxicity studies were carried out in the Wistar albino rats as per the OECD guidelines. The Ethanol extract was found to be safe up to the dose of 2000mg/kg. Therefore $1/10^{th}$ of the dose (200mg/kg) and $1/20^{th}$ of the dose (400mg/kg) were selected.

In-vivo anti-arthritic activity was carried out for ethanolic extract in Wistar albino rats by using complete freund's adjuvant induced arthritis model. Ethanolic extract of dose 200 and 400mg/kg were given orally to the rats. It is compared with standard Diclofenac sodium. Various parameters were studied for the evaluation of anti-arthritic activity. The ethanolic extract at the dose 400mg/kg showed significant anti-arthritic effect on the arthritis induced rats which were comparable with that of the standard.

CONCLUSION

The current investigation was concluded that the plant *Sterculia foetida* Linn., possess a significant anti-arthritic activity against adjuvant induced arthritis rats. Ethanolic bark extract showed the maximum percentage inhibition of paw volume, increased body weight, improved haematologic parameters, histopathological and radio graphical parameters and compared with standard Diclofenac sodium.

Further studies are focused on *in-vivo* pharmacological activity of isolated compound and its structural activity relationship.

REFERENCE

1. www.nhp.gov.in>world–arthitis-day_pg World Arthritis Day / National Health Portal Of India.
2. http://googleweblight.com/?lite_medicinenet.com Treatment for rheumatoid arthritis.
3. www.healthline.com>osteoarthritis>herbaltreatment.in
4. Introduction of *Sterculia foetida* Linn., https://en.m.wikipedia.org/wiki/ Sterculia_foetida.
5. List of Philippines' Medicinal Plants in English and Tagalog, available at http://www.stuartxchange.com/completelist.html.
6. Rekha Rajenderan and Ekambaram Krishnakumar, Anti-Arthritic Activity of *Premna serratifolia* Linn., wood against Adjuvant Induced Arthritis, Avicenna J Med Biotech , 2(2), 2010, 101-106.
7. Jubie S, Jawahar N, Ruby Koshy, Anti-arthritic activity of bark extracts of *Alangium salviifolium Wang,* (1), 2008, 433-436.
8. Divya Singh, Rahul Nainwani, Amit Gupta, In- vivo Anti-inflammatory and Anti-arthritis activity of hydro alcoholic extract of *Pongamia pinnata* (L)., pierre seed, *IJPPS*, 2(12), 2013 798-803
9. Vijayalaxmi A, Varudha Bakshi, Nazia Begum, Anti-arthritic and Anti-inflammatory activity of Beta caryophyllene against complete Freund's Adjuvant induced Arthritis in Wistar Rats, *JBRR, imedPub Journals*, 1(9), 2015, 1-10.
10. Sarpate Rupali, Tupkari Suresh, Evaluation of Anti-arthritic Potential of the Genus Strobilanthus, *IJDDR*, 4(2), 2016, 230-236.
11. Jitendra Bhangale, Sanjeev Acharya, Anti-arthritic activity of *Cynodon dactylon* (L.) Pers, *IJEB,* 52, 2014, 215-222.