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Research Article

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Analgesic, anti-inflammatory and anti-arthritic activity of *Euphorbia thymifolia* Linn phytosterol fraction

P. Bigoniya*, A. Singh Thakur, S. Singh

Department of Pharmacology and Pharmacognosy, Radharaman College of Pharmacy, Bhadbhada Road, Ratibad, Bhopal, India.

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ABSTRACT

Euphorbia thymifolia Linn. (family Euphorbiaceae) is an annual herb belonging to genus Euphorbia having many medicinal uses. The present study was carried out to evaluate the analgesic, anti-inflammatory and anti-arthritic activity of the phytosterol fraction of *E. thymifolia* (ETTS). Phytosterol was separated from the petroleum ether extract of dried aerial part, by refluxing with 5% alcoholic KOH for 4 hr followed by filtration. LD_{50} was calculated as 283 mg kg^{-1}, i.p and a dose level of 20, 30 and 40 mg kg^{-1} was chosen for *in vivo* studies. Analgesic activity was evaluated in mice using morphine and naloxone on Hot Plate instrument to assess the involvement of opioid receptor in analgesic activity. Anti-inflammatory and anti-arthritic activity was evaluated against carrageenan induced paw edema and Complete Freund's Adjuvant induced arthritis in rats by estimating TNF-α and knee joint Histopathology. ETTS showed significant ($P < 0.01$) increase in hot plate reaction time which was reversed in presence of naloxone. ETTS at 40 mg kg^{-1} decreased ($P < 0.01$-0.001) paw volume, arthritic index and TNF-α expression. ETTS showed repair of articular surfaces with small areas of erosion and irregularities with invasion of connective tissue. *E. thymifolia* total sterol possesses moderate analgesic, and potent anti-inflammatory and anti-arthritic activities at 40 mg kg^{-1}. Centrally acting mechanism may be involved in analgesic response modulating the opioid receptors having morphine like action as the effects of ETTS was blocked by naloxone. ETTS led to marked repair of articular surfaces and reduction of tissue edema and cellular infiltration.

Key words: *E. thymifollia*, Analgesic, Anti-inflammatory, Anti-arthritic, Phytosterol, TNF-α.

INRODUCTION

Herbal and natural products of traditional origin have been used by human kind since the advent of human race. Every culture, including western culture has evolved indigenous system of traditional healing[1]. Plants are a great source of medicines useful in the treatment of various diseases. Traditional medicine has not only played a vital role in providing healing but has also contributed to the discovery of most pharmaceutically active substances which have been used in the commercial production of drugs.

Euphorbia thymifolia Linn (family Euphorbiaceae) is an annual herb belonging to genus Euphorbia having many medicinal uses. Plant is commonly found in waste lands, along roadsides and wall sides under humid condition. *E. thymifolia* is a monocious, prostate, annual herb with numerous adventitious roots and stems containing white latex. The leaves are opposite, distichous and simple. The fruits are acutely 3-lobed, almost sessile capsule. This plant is reported to contain diterpenes, triterpenes, steroids and flavonoids[2]. This plant is being used from ancient times as a antibacterial to treat ringworms, in snakebite, to treat dermatitis, eczema and skin inflammation. *E. thymifolia* is widely used in Africa in the form of decoction or infusion against dysentery, enteritis, diarrhea and venereal diseases. The dried leaves and seeds are slightly aromatic and are used as a stimulant, astringent, anthelmintic and laxative[3]. The plant contains quercetin, essential oil, tannins and different phytosterols[4].

The ethyl acetate extract of *E. thymifolia* have been reported to exhibit *in vitro* anti-herpes simplex virus (HSV)-2 activity by Yang *et al*.[5]. Rahmatullah *et al*.[6] studied the antihyperglycemic and antinociceptive activities of methanolic extract of the whole plant of *E. thymifolia*. Looking into the rich presence of triterpenoids and steroids this study has been undertaken to evaluate the analgesic, anti-inflammatory and anti-arthritic activities of *E. thymifolia*.

***Corresponding author.**
Dr. Papiya Bigoniya
Principal, Radharaman College of Pharmacy, Fatehpur Dobra, Bhadbhada Road, Ratibad, Bhopal-462 002, M.P., India.

MATERIAL AND METHODS

Plant material

E. thymifolia was collected in the month of September-October 2010 from the fields of village Samashgarh, Dist Bhopal (M.P). It was authenticated by Dr. Ziaul Hasan, Asst. Professor of Department of Botany, Safia College of Science, Bhopal, M.P and Voucher specimen (no. 183/Bot/Safia/10) was preserved.

Animal

Swiss albino mice (25-32 g) and Wistar albino rats of (150-200 g) of both sexes were used in the study. All the experiments were performed between 9:30 to 16:30 hours to overcome diurnal and circadian variations. Animals were housed in polypropylene cages, paddy husk bedding with free access to water and fed with standard commercial pellet chow (Hindustan Lever) at a temperature of 24 ± 2°C relative humidity of 65 ± 5% and 12:12 light: dark cycle. Experimental protocol used in this study were reviewed by Institutional animal ethics committee of Radharaman College of Pharmacy (Approval no. IAEC/RCP/OCT 2010/08) and were in accordance with the guidelines of IAEC.

Separation of Phytosterol

Arial plant part was harvested, dried at temperature below 45°C, size reduced and stored in air tight container. Phytosterol was separated from the petroleum ether extract of dried aerial part of plant, refluxed with 5% alcoholic KOH for 4 hr and then filtered. Filtrate was then extracted with di-ethyl ether, dried, yield calculated and tested for the presence of steroid. Separated total phytosterol was designated as *E. thymifolia* total sterol (ETTS) and used for *in vivo* studies[7].

Preparation of ETTS dose
Dose of ETTS was prepared by suspending 1 g of total sterol in 10 mL of 5% Tween 80 by triturating continuously to get homogenous suspension being of 100 mg mL^{-1} with final concentration of 50 µl mL^{-1} of Tween 80. Suspension was stored in airtight bottles in a cool place.

Acute toxicity studies (LD_{50})
The acute toxicity study of ETTS was performed on Swiss Albino mice. The animals were fasted for 12 hour prior to the experiment and were administered intraperitoneally at 500 mg kg^{-1} dose considering the parenteral route of administration of enriched phytosterol and observed for mortality up to 48 hr (short term toxicity). Based on the short-term toxicity outcome, a main test was performed to determine exact LD_{50} by graphical method as per OECD guideline 425[8].

Analgesic activity

Hot plate analgesia in mice
The hot plate test method was employed for the assessment of possible centrally mediated analgesic effect of ETTS. Over night fasted mice were screened by placing them one by one on hot plate maintained at 55 ± 1°C (max cut off time 15 sec). Animals with basal reaction time below 8 sec were selected and divided in to 5 groups each containing 6 animals, group I served as negative control; group II standard, morphine sulphate (4 mg kg^{-1}, i.p), whereas group III, IV and V animals received 20, 30 and 40 mg kg^{-1} doses of ETTS respectively. Each animal was individually placed gently on eddy's hot plate to observe latency to exhibit nociceptive responses such as paw licking or jumping off was noted at 15, 30, 45, 60 and 90 min after administration of test drugs or vehicle[9, 10].

Hot plate analgesia in mice in presence of naloxone
Eddy's hot plate test were repeated using opioid antagonist i.e naloxone (2 mg kg^{-1}, i.p) along with morphine, 20 and 30 mg kg^{-1} dose of ETTS following same protocol to assess the involvement of opioid receptor in analgesic activity of ETTS[11, 12].

Anti-inflammatory activity

Carrageenan induced Paw edema in rats
Anti-inflammatory activity was assessed by the method described by Winter *et al*.[13]. Rats of either sex were divided into 4 groups containing six each. Group I served as negative control, group II received diclofenac (10 mg kg^{-1}, i.p.), group III and IV received ETTS 30 and 40 mg kg^{-1}, i.p respectively. Animals were treated with all the test drugs and subsequently 1 hr after treatment, 0.1 mL of 1% suspension of carrageenan in normal saline was injected into the subplantar region of left hind paw to induce edema. The paw volume was measured at 0, 1, 2, 3 and 4 hr after carrageenan injection using plethysmometer[14, 15].

Anti-arthritic activity

Complete Freund's adjuvant induced arthritis in rats
Rats were divided in five groups: Group I vehicle control, group II negative control, group III Methotrexate (0.3 mg kg^{-1}, i.p.), Group IV and V received 30 and 40 mg kg^{-1} of ETTS respectively.

Arthritis in animals of all the groups except group I, was induced by subcutaneous injection of 0.1 mL of Complete Freund's Adjuvant (CFA) at sub plantar region of right hind paw and at the base of tail at 0 day. Different doses of ETTS (30 and 40 mg kg^{-1}, i.p), vehicle and standard drug methotrexate (0.3 mg kg^{-1}, i.p) was administered continuously upto 28 days. On seventh day of treatment the CFA was again injected to animals of all groups except vehicle control and paw volume was measured at 0, 7th, 14th and 21st day. All animals were sacrificed on 28th day and blood samples were collected for Tumor Necrosis Factor (TNF-α) estimation and knee joint was processed for histological examination[16, 17].

Estimation of TNF-a
The level of TNF-α gets elevated in synovium as well as in blood in inflammatory disease, which can be detected through ELIZA. The blood samples collected were stored at 4°C for 30 min and centrifuged at 3000 g for 10 min, serum was collected and stored in deep freezer at 20°C until analysis. TNF-α estimation was done by using Ray bio (Ray biotech inc, norcross, GA) rat TNF-α ELISA kit and ELISA plate reader[18].

Knee joint Histopathology of rats
Histological samples from right hind leg were taken at 28th day and slides were prepared using hematoxylin and eosin staining. The hind paws were amputated above the knee joint and were fixed in 70% formaldehyde solution. The paws were then decalcified, embedded in paraffin and sectioned in a mid-sagittal plane. Un-inflamed decalcified joint at the same time were used as control sample[16].

Statistical Analysis
All results were expressed as Mean ± standard error mean (SEM), data were analyzed using one way ANOVA followed by Tukey-Kramer multiple comparison test. *P* value <0.05 was considered significant. The analysis was carried out using Instat Graph pad software version 4.

RESULTS

Acute toxicity studies (LD_{50})
Based on the OECD guidelines a limit test was performed to categorize the toxicity class (LD_{50}) of the compound. The limit test was performed at 500 mg kg^{-1} (i.p.), which showed mortality (40%). A main test was performed to determine the exact LD_{50} value following OECD up and down method. LD_{50} was calculated as 283 mg kg^{-1}, i.p from graphical representation log dose versus profile value.

Hot plate analgesia in mice
ETTS at 30 and 40 mg kg^{-1} dose showed significant (P<0.05-0.01) increase in hot plate reaction time compared to vehicle control group. ETTS at 20 mg kg^{-1} dose have non-significant analgesic effect, so the dose was not tested for rest of the experimental protocols.

Table 1: Effect of ETTS on hot plate reaction time of mice, alone and in combination with opioid antagonist naloxone.

Treatment (mg kg^{-1}, i.p.)	Reaction time (sec) Mean ± SEM 15 min	30 min	45 min	60 min
Vehicle control (1 mL/100 gm)	4.69 ± 0.33	4.82 ± 0.41	4.24 ± 0.36	4.66 ± 0.43
Morphine (4)	13.16 ± 1.63**	14.90 ± 1.68**	15.31 ± 2.74***	12.92 ± 1.57**
ETTS (20)	8.16 ± 1.40ns	10.21 ± 1.72ns	9.73 ± 1.81ns	7.85 ± 1.76ns
ETTS (30)	11.14 ± 1.68*	12.22 ± 2.87ns	12.80 ± 1.92*	10.70 ± 1.81*
ETTS (40)	12.65 ± 2.33**	13.41 ± 1.37*	13.67 ± 1.79**	12.24 ± 1.63**
Morphine + Naloxone (4 + 2)	6.51 ± 0.43ns,a	7.25 ± 1.05ns,a	6.81 ± 1.39ns,a	7.26 ± 0.86ns,ns
ETTS + Naloxone (30 + 2)	5.37 ± 0.61ns,b	5.73 ± 0.94ns,a	5.94 ± 0.65ns,b	6.45 ± 0.97ns,a
ETTS + Naloxone (40 + 2)	6.82 ± 0.59ns,a	7.62 ± 1.03ns,a	7.12 ± 0.42ns ,a	7.63 ± 0.74ns,ns

*ETTS = Euphorbia thymifolia total sterol, I.P. = intra peritoneal, No = 6, *P<0.05, **P<0.01, ***P<0.001 and ns = not significant compared to vehicle control. aP<0.05, bP<0.01 and ns = not significant compared to naloxone combined groups with the respective groups alone.*

Morphine administered after pretreatment of naloxone (2 mg kg^{-1}) showed significant (P<0.05) reversal of analgesic response. Likewise ETTS administration after naloxone also showed significant (P<0.05-0.01) inhibition of analgesic effect signifying involvement of opioid receptor in the analgesic effect of *E. thymifolia* total sterol.

Carrageenan induced Paw edema in rats

The results showed significant (P<0.01) anti-inflammatory activity of ETTS at 40 mg kg^{-1} after 3 hr on carrageenan induced paw edema compared with the vehicle control group. Diclofenac and ETTS at 10 and 40 mg kg^{-1} dose respectively inhibited 61.95 and 53.26% paw volume in the treated groups at 3 hr.

Table 2: Effect of ETTS on carrageenan-induced paw edema in rats.

Treatment (mg kg^{-1}, i.p.)	Paw Volume (mL) Mean ± SEM 0 hr	1 hr	2 hr	3 hr	4 hr	% Inhibition of paw volume at 3 hrs
Vehicle control (1 mL/100 gm)	0.26 ± 0.05	0.63 ± 0.09	0.82 ± 0.03	0.92 ± 0.02	1.21 ± 0.05	—
Diclofenac sodium (10)	0.24 ± 0.02	0.36 ± 0.02**	0.31 ± 0.04**	0.35 ± 0.06**	0.42 ± 0.06**	61.95
ETTS (30)	0.28 ± 0.01	0.54 ± 0.03*	0.49 ± 0.03*	0.52 ± 0.04**	0.59 ± 0.03**	43.47
ETTS (40)	0.26 ± 0.02	0.46 ± 0.02**	0.38 ± 0.05**	0.43 ± 0.03**	0.48 ± 0.07**	53.26

*ETTS = Euphorbia thymifolia total sterol, I.P. = intra peritoneal, n = 6, **P<0.01 and *P<0.05 compared to vehicle control group.*

Freund's complete adjuvant induced arthritis in rats

FCA was injected on the 0 day in sub plantar region of left hind paw and the paw volume, body weight and arthritic index was determined on 0, 7th, 14th and 21st day. The results suggested that ETTS at 40 mg kg^{-1} extreme significantly (P<0.001) decreased paw volume starting from 7th day. ETTS inhibited arthritic index significantly after 14 (P<0.01) and 21 (P<0.001) days of treatment.

Table 3: Effect of ETTS on Complete Fraud's Adjuvant induced arthritis in rats.

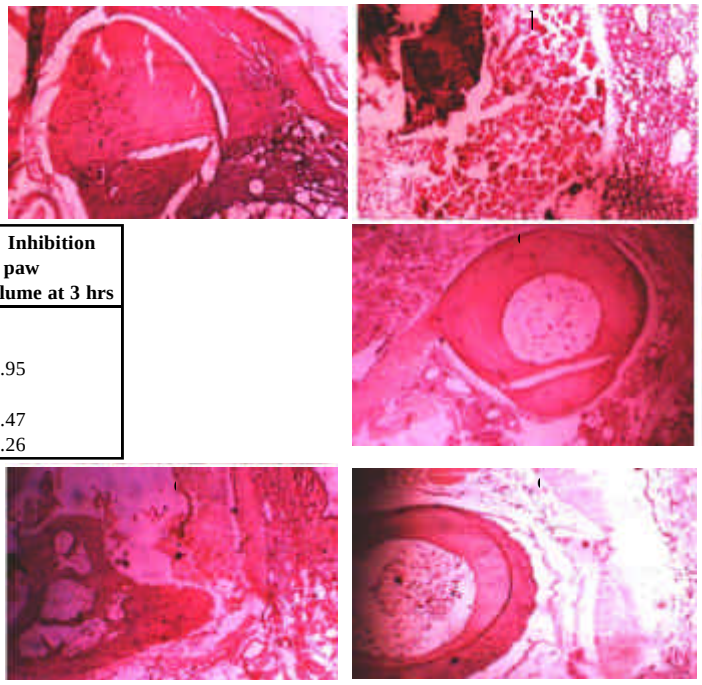
Treatment (mg kg^{-1}, i.p.)	Time in days (Mean ± SEM) 0 PV (mL)	AI	7 PV (mL)	AI	14 PV (mL)	AI	21 PV (mL)	AI
Vehicle Control	0.22 ±0.01	0	0.86 ±0.05	1.2 ±0.30	0.93 ±0.06	6.66 ±0.95	0.86 ±0.08	8.03 ±1.18
MTX (0.3)	0.25 ±0.03	0	0.31 ±0.09***	0.83 ±0.03ns	0.37 ±0.09***	2.24 ±0.51**	0.27 ±0.07***	1.33 ±0.61***
ETTS (30)	0.23 ±0.01	0	0.43 ±0.08**	1.2 ±0.17ns	0.42 ±0.07***	4.05 ±0.8 ns	0.45 ±0.03**	3.66 ±0.88*
ETTS (40)	0.20 ±0.01	0	0.38 ±0.06***	0.93 ±0.06ns	0.35 ±0.08***	2.66 ±0.66**	0.39 ±0.09***	2.16 ±0.79***

*MTX = Methoteraxte, ETTS = Euphorbia thymifolia total sterol, I.P. = intra peritoneal, n = 6, PV - Paw Volume, AI - Arthritic index. *P<0.05, **P<0.01, ***P<0.001 and ns = not significant compared to vehicle control.*

TNF-a estimation

The estimation of TNF-α showed that methotrexate and ETTS at 40 mg kg^{-1} caused respectively 70.03 and 62.30% decrease in TNF-α expression compared to arthritic control group.

Figure 1: Photomicrographs of adjuvant arthritic rat knee joint treated with different doses of ETTS.



(a) Normal joint of vehicle control rat; (b) CFA treated joint with cartilage erosion, severe synovial hyperplasia and infiltration of mononuclear cells (c) Diclofenac treatment showed mild synovial hyperplasia, infiltration of fewer mononuclear cells (d) ETTS 30 mg kg^{-1} treatment showing infiltration of mononuclear cells, cartilage erosion and articular surface degradation; (e) ETTS 40 mg kg^{-1} treatment normalized articular surfaces with small areas of inflammation and surface irregularities.

Table 4: Effect of ETTS on serum TNF-a level of Complete Fraud's Adjuvant induced arthritic rat.

Treatment (mg kg^{-1}, i.p.)	Concentration (ng mL^{-1})	% Inhibition compared to arthritic control
Vehicle control	0.03 ± 0.001	-
Arthritic control	15.65 ± 0.92***	-
CFA + Methotrexate (0.3)	4.69 ± 0.52***,a	70.03
CFA + ETTS (30)	8.94 ± 1.12***,a	42.88
CFA + ETTS (40)	5.91 ± 0.86***,a	62.30

*ETTS = Euphorbia thymifolia total sterol, I.P. = intra peritoneal, n = 6, **P<0.01 and ***P<0.001 compared vehicle control. aP<0.001 compared with standard methotrexate.*

Histopathology of Knee joint

Healthy articular surface was observed to be with smooth, uninterrupted surface and an even distribution of chondrocytes in the normal control animals. Sections of joints from control arthritic group revealed degradation of the articular surface, erosion of cartilage, hyperplasia and connective tissue with highly infiltrated inflammatory cells. The present results revealed that ETTS at 40 mg kg^{-1} dose reduced the damaging effect of adjuvant arthritis especially on the articular surfaces. Lesser protective effects were shown by the low dose of ETTS. Section of synovial joint treated with ETTS showed repair of articular surfaces with small areas of erosion with irregularities and invasion of connective tissue.

DISCUSSION

The study was conducted to evaluate the analgesic, anti-inflammatory and anti-arthritic activity of the phytosterol fraction of *E. thymifolia* on several experimental models. *E. thymifolia* was found

to be rich in steroidal content leading us to extract and enrich the phytosterols fraction. The steroids are the centrally active class of phytoconstituents which are responsible for many pharmacological activities. Phytopharmacological studies to explore the pharmacological effect of *E. thymifolia* steroids have not been reported till date. The estimated LD_{50} of *E. thymifolia* total steroid indicate its moderately toxic properties. A dose range of 20, 30 and 40 mg kg^{-1} were selected for ETTS to evaluate the analgesic, anti-inflammatory and anti-arthritic profile.

The results of current investigation showed significant analgesic, anti-inflammatory and anti-arthritic activities of *E. thymifolia* total sterol at 40 mg kg-1 dose on mice and rats. The proposed mechanisms of anti-nociceptive activity based on the pain models used in this study showed that they are likely to be mediated centrally (spinally and supraspinally) on the nervous system. In addition, the anti-nociceptive effect of the ETTS was abolished by naloxone in the same manner as for morphine in the hot plate algesia, indicating that the ETTS acts partly through opioid mediated mechanisms. The μ opioid receptor would probably be a binding site of ETTS.

Carrageenan injection into the rat paw provokes a local, acute inflammatory reaction that is a widely employed method for evaluating the potential anti-inflammatory agents[13]. Carrageenan induced inflammation consists of two phases, the early phase is related to the production of histamine, 5-hydroxytryptamin, bradykinins and cyclooxygenase products, while the delayed phase has been linked to neutrophil infiltration, as well as to the continuing of the production of arachidonic metabolites[19]. ETTS was capable of attenuating carrageenan-induced inflammation may be by inhibition of the local inflammatory mediators.

Freund's adjuvant induced arthritis develop chronic swelling in multiple joints with the influence of inflammatory cells, erosion of joint cartilage and bone destruction on rats having close similarities to human rheumatoid disease[20]. Determination of paw swelling is apparently simple, sensitive and quick procedures for evaluating the degree of inflammation and the therapeutic effectiveness of drugs as anti-inflammatory agents. The chronic inflammation involves the release of number of mediators like cytokines, GM-CSF, interferons and PGDF responsible for pain, bone and cartilage destruction leading to severe disability[21]. Daily administration of ETTS effectively inhibited the increase of hind paw volume and inflammation. The inhibition of the increase in hind paw volume was associated with inhibition of neutrophil infiltration and significantly lesser tissue destruction as assessed by histology. Results also showed that ETTS treatment decreased the local production of pro-inflammatory cytokines like TNF-α. In rheumatoid arthritis, the synovium is intensively infiltrated by macrophages which are the major source of TNF-α, IL-1β and inducible nitric oxide synthase mediated NO generation during immune response. The pro-inflammatory cytokines TNF-α, IL-1β, and IL-6 are shown to play an important role in the pathophysiology of arthritis development in animal models as well as in humans[22].

Cytokines have multiple effects in arthritic joints viz expression of adhesion and chemo attractant molecules, facilitation of leukocyte influx and activation[23]. ETTS showed effectively lowed expression of TNF-α in experimental arthritis, as a possible anti-inflammatory mechanism. *E. thymifolia* exhibited superoxide radical and hydroxyl radical scavenging activities which may be associated with reduced formation and release of inflammatory cytokines[24]. ETTS led to marked repair of articular surfaces and reduction of tissue edema and cellular infiltration. These findings correlate with the measured biochemical parameters and the results of paw edema. Steroidal glycoside from different plant sources i.e. *Yucca schidigera, Agave Americana, Trigonella foenum* and *Coriandrum sativum* are report to have anti-arthritic and anti-inflammatory properties[25,26,27]. *E. thymifolia* total sterol attenuated most of the biochemical and pathophysiological aspects of rheumatic arthritis. *E. thymifolia* phytosterol effects prove it to be a moderate analgesic and potential anti-inflammatory herb.

CONCLUSION

The analgesic activity possibly may be through a centrally acting mechanism modulating the opioid receptors having morphine like action as the effects of ETTS was blocked by naloxone, a non selective and pure opioid antagonist thus suggesting the involvement of the central opioid system in mediating the anti-nociceptive activity. The anti-inflammatory activity of ETTS was comparable with the standard drug diclofenac sodium which might be due to the inhibition of local inflammatory mediators released due to carrageenan injection. The observation of physiological parameter, estimation of TNF-α and knee joint histopathology suggests that ETTS posses anti-arthritic potential possibly in both primary and secondary phases of arthritis induced in rats by CFA by lowering level of TNF-α and also protecting the animals from secondary lesion as there was low arthritic index along with protection of knee joints. Depending on the results obtained in these studies we conclude that isolated phytosterol fraction of *E. thymifolia* has potent analgesic, anti-inflammatory and anti-arthritic effects.

REFERENCES

1. Atkinson DC, Boura AL, Hicks R. Observations on the pharmacological properties of inflammatory exudate. Eur. J. Pharmacol., 8, 1969, 348-353.
2. Schmelzer GH, Gurib-Fakim A. Medicinal plants. Plant Resources of Tropical Africa, 3, 2008, 294-296.
3. Kirtikar KR, Basu BD. Indian Medicinal Plants. 2nd edn., International Book Distributors, Dehradun, 2006, pp. 2199-2201.
4. Mosango DM. *Euphorbia thymifolia* L. In: Schmelzer GH, Gurib-Fakim A. (Editors). Prota 11(1): Medicinal plants/ Plantes médicinales 1. [CD-Rom]. PROTA, Wageningen, Netherlands, 2008.
5. Yang CM, Cheng HY, Lin TC, Chiang LC, Lin CC. *Euphorbia thymifolia* suppresses herpes simplex virus-2 infection by directly inactivating virus infectivity. Clin. Exp. Pharmacol. Physiol., 32, 2005, 346-349.
6. Rahmatullah M, Hasan SK, Ali Z, Rahman S, Jahan R. Antihyperglycemic and antinociceptive activities of methanolic extract of *Euphorbia thymifolia* L. whole plants. J. Chinese Integr. Med., 10, 2012, 228-32.
7. Singh S, Ahmad I, Matsumoto T. Science and Technology. Nihan University, 27, 1988, 3231-3234.
8. Diener W, Mischke U, Kayser D, Schlede E. The Biometric Evaluation of the OECD Modified Version of the acute-Toxic Class Method (Oral). Arch. Toxicol., 69, 1995, 729-734.
9. Silva J, Abebe W, Sousa SM, Durate VG, Machado MIL,

Matos FJA. Analgesic and Anti-inflammatory effect of essential oils of *Eucalyptus*. J. Ethnopharmacol., 89, 2003, 277-283.

10. Aiyelero OM, Ibrahim ZG, Yaro AH. Analgesic and Anti-inflammatory properties of the methanol extract of *Ficus Ingens* in rodents. Nig. J. Pharm. Sci., 8, 2009, 79-86.
11. Segato FN, Castro-souza C, Segato EN, Morato S, Coimbra NC, Sucrose ingestion causes opioid analgesia. Braz. J. Med. Bio. Res., 30, 1997, 981-984.
12. Ridtitid W, Sae-wong C, Reanmongkol W, Wongnawa M. Antinociceptive activity of the methanolic extract of *Kaempferia galanga* Linn. J. Ethnopharmacol., 118, 2008, 225-230.
13. Winter CA, Risley EA, Nuss W. Carrageenan-induced edema in hind paws of the rat as an assay for anti-inflammatory drugs. Proc. Soc. Exp. Biol. Med., 111, 1962, 544–547.
14. Brooks RR, Carpenter JI, Jones SM, Zieglerm TC, Pong SF. Carrageenan induced paw inflammation model and its response to NSAIDS. J. Pharmacol. Meth., 25, 1991, 275-283.
15. Patel SS, Verma NK, Chatterjee C, Gauthaman K. Screening of *Caesalpinia pulcherrima* Linn Flowers for Analgesic and Anti-inflammatory Activities. Int. J. Appl. Res. Nat. Prod., 3, 2010, 1-5.
16. Helyes Z, Szabo A. Nemeth J, Jakab B, Pinter E, Banovolgyi A, Kereskai L, Keri G, Szolcsanyi J. Antiinflammmatory and Antiarthritic effect of somatostatin released from capsaicin-sensitive sensory nerve terminals in a CFA induced chronic arthritic model in rat. Arthr. Rheum., 50, 2004, 1677-85.
17. Cynthia AD, Eric W, Eric BG. Anti-arthritic effect of an ethanolic extract of *Capparis erythrocarpos* Isert roots in freund's adjuvant induced arthritis in rats. J. Pharmacol. Toxicol., 6 , 2011, 201-217.
18. Liu YL, Lin HM, Zou R, Wu JC, Han R, Raymond LN, Reid PF, Qin ZH. Suppression of CFA induced Adjuvant arthritis by Cobratoxin. Acta Pharmacol. Sin., 30, 2009, 219-227.
19. Dawson J, Sedgwick AD, Edwards JC, Lees PA. Comparative study of the cellular, exudative and histological responses to carrageenan, dextran and zymosan in the mouse. Int. J. Tiss. Rea., 13, 1991, 171–185.
20. Singh S, Majumdar DK. Effect of fixed oils of Ocimum sanctum against experimentally induced arthritis and joint edema in laboratory animals. J. Pharmacog., 34, 1996, 218-222.
21. Eric GB, Lawrence JL. Rheumatoid arthritis and its therapy. The Textbook of therapeutic drugs and disease management. 6th edn., Williams and Wilkins Company, Baltimore, 1996, pp. 579-595.
22. Ivashkiv LB. Cytokine expression and cell activation in inflammatory arthritis. Adv. Immunol., 63, 1996, 337-376.
23. Cannnetti CA, Leung BP, Culshaw S, McInnes IB, Cunha FQ, Liew FY. IL-18 enhanced collagen-induced arthritis by recruiting neutrophil via TNF-α and leukotriene B_4. J. Immunol., 171, 2003, 1009-1015.
24. Lin CC, Cheng HY, Yang CM, Lin TC. Antioxidant and antiviral activities of *Euphorbia thymifolia* L. J. Biomed. Sci., 9(6), 2002, 656-64.
25. Cheeke PR, Piacente S, Oleszek W. Anti-inflammatory and anti-arthritic effects of *yucca schidigera*. J. Inflamm., 3, 2006, 6.
26. Ammar NM, Sya O, Mohammad DA. Study of the anti-inflammatory activity of some medicinal edible plants growing in Egypt. J. Islamic Acad. Sci., 10, 1997, 113-122.
27. Peana AT, Moretti MD, Manconi V, Desole G, Pippia P. Anti-inflammatory activity of aqueous extracts and steroidal sapogenins of *Agave americana*. Planta. Med., 63, 1997, 199-202.

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