



ANTI-INFLAMMATORY, ANTI-ARTHRITIC AND ANALGESIC ACTIVITY OF ETHANOL FRACTIONS OF SAPONIN ISOLATED FROM ROOTS OF *CHLOROPHYTUM BORIVILIANUM* SANT. AND FERN.

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Abstract:

Background: *Chlorophytum borivilianum* Sant. & Fern. (Liliaceae) has therapeutic application in Ayurvedic system of medicine. Roots of *Chlorophytum borivilianum* traditionally used for treatment of inflammatory disorders. The saponins is one of the major and important constituent present in these roots. Several studies have found evidence to support the belief that saponins have potent anti-inflammatory and analgesic properties.

Aim: The aim of the present study was to evaluate anti-inflammatory, antiarthritic and analgesic activity of the fractions of saponin from isolated from roots of *C.borivilianum*.

Material and Methods: The ethanol fraction of isolated saponins of *C. borivilianum* (EFS) at dose 3, 10 and 30 mg/kg p.o was evaluated in laboratory animals using carrageenan- induced paw edema, histamine-induced paw edema, cotton pellet induced granuloma, Freund's adjuvant-induced arthritis, Eddy's Hot plate method, tail flick method. Effect of EFS 30 mg/kg, on bone deformation was evaluated by X-ray radiography.

Result: The results showed that the EFS produced significant inhibition in carrageenan- induced inflammation, histamine-induced inflammation, cotton pellet induced granuloma and Freund's adjuvant induced arthritis in rats. The maximum effect observed at 10 and 30 mg/kg p.o. It also increases reaction time significantly indicating analgesic activity.

Conclusion: EFS at 10 and 30 mg/kg possess anti-inflammatory and anti-arthritis activity produced by inhibition of prostaglandin, inhibition of granuloma formation with mild central analgesic activity.

Keywords: *Chlorophytum borivilianum*, saponin, analgesic, anti-inflammatory, paw oedema

I. INTRODUCTION

Inflammation plays a vital role in various diseases, such as rheumatoid arthritis, atherosclerosis and asthma, which all show a high prevalence globally. Arthritis has a high prevalence and represents the prototype of an autoimmune inflammatory joint disease leading to progressive destruction of articular structures, particularly cartilage and bone¹. Mild to severe pain is associated with inflammatory condition and arthritis. The current available treatment of inflammatory disorders extensively use nonsteroidal anti-inflammatory drugs (NSAIDs) and corticosteroids. Although use of modern drugs for inflammation has a relieving effect, it is still unsatisfactory due to its severe side effects.^{2,3}

Several natural products and their derived formulations have been used in therapeutic applications for inflammatory disorders and related diseases.⁴ Many traditional healing herbs and their parts possess medicinal value and can be used to prevent, alleviate or cure several human diseases.⁵ The Indian traditional system of medicine 'Ayurveda' utilizes more than 1000 medicinal plants, from which therapeutic agents were derived and scientifically proven for their efficacy and safety parameters. *Chlorophytum borivilianum* Sant. & Fern. (Liliaceae) known as 'Safed Musli' is a traditional herb with assorted Ayurvedic relevance. It has therapeutic application in Ayurvedic system of medicine.⁶

C. borivilianum is a potential herb traditionally used in India and China to treat arthritis, oligospermia, diabetes and dysuria. In ayurvedic literature it is celebrated as a 'Divya Aushad' (enlightened medicine) with unparalleled medicinal properties. In 'Raja Ballabh Nighantu' it is recommended as rejuvenator and is helpful in neurological (vatic) disorders.⁷ It possess antiviral, anticancer, immunomodulatory, antistress, aphrodisiac, antimicrobial,⁸ improvement in male sex health,⁹ anthelmintic¹⁰ and hepatoprotective activity.¹¹ Among all the species of *Chlorophytum* present in India, *C. borivilianum* produces the maximum root tuber along with the highest saponin content.¹² Roots of these species contain saponins¹³ about 2% to 4%,^{11,14} which include borivilianosides A–D,¹⁵ borivilinoside E–H,¹⁶ chlorophytoside-I,¹⁷ furostanol and spirostanol saponins.¹³

Our preliminary research on crude saponins of *C. borivilianum* proved anti-inflammatory activity.¹⁸ Present study was aimed to further fractionation of saponins and evaluation of the anti-inflammatory anti-arthritic and analgesic activity of these fractions.

II. MATERIAL AND METHOD:

a. PLANT MATERIAL:

Roots of *C. borivilianum* were procured from crude drug supplier M/s. Gokuldas Goverdhandas (237, Budhawarpeth Pune). The plant material was identified and authenticated by Dr. R. B. Bhagat, Head, Department of Botany, Poona District Education Association's Arts, Commerce and Science College, Pirangut Tal. Mulshi Dist. Pune (NO. A.P.C.P./28/813/92/2/12-13).

b. PREPARATION OF EXTRACT:

Roots of *C. borivilianum* cut into small pieces, shade-dried, and coarsely powdered. Extraction of roots was carried out following previously published methods.²⁰ Coarse powder (1000g) was subjected to successive extraction with petroleum ether and water by cold maceration process for 72h at room temp (30 ± 2 °C). macerates (Extracts) were obtained by filtration and dried on water bath (45 ± 5 °C) to yield water extract 316.30g (31.63%). Ethanol and acetone (1:5) was slowly added to water extract to precipitated saponins. The light yellow color precipitate of saponin was separated, dried on water bath (yield 252.4 g (25.2%)) and confirmed

with test for saponins. The dried precipitate of saponin was further fractionated by solid-liquid fractionation with ethanol (yield 2.62%), acetone (yield 2.39%) and methanol (yield 4.92%) successively.

c. ANIMALS:

Healthy adult male Wistar rats weighing 200-250 g and Swiss albino mice weighing 25–30 g were obtained from National Institute of Biosciences, Manikbaug, Pune, and Serum Institute, Hadapsar, Pune, respectively and were housed in polypropylene cages in standard environmental conditions (Temperature $25\pm 2^{\circ}\text{C}$; relative humidity $55\pm 10\%$; and 12:12light:dark cycle.). The animals were fed on a standard pellet diet and water *ad libitum*.

Animals were acclimatized to the laboratory condition for at least 8 days prior to the experiment.

The experimental protocol (JSCOPR/04/IAEC/01/2014/02) was approved by the Institutional animal Ethical Committee (IAEC) of Jayawantrao Sawant College of Pharmacy and Research, Hadapsar, Pune, constituted under Committee for Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Government of India. The care of the laboratory animals was taken as per the CPCSEA guidelines.

d. METHODS:

i. Acute toxicity study (OECD AOT425, 2010):

Acute toxicity of extracts was performed at limit test 2000 mg/kg using Wistar rat (250–200 g). Ethanol fraction of saponin (EFS) 2000mg/kg was administered orally to overnight fasted rats. After administration of EFS rats were observed for behavioral changes during the first 24h (with special attention given during the first 4h), and daily thereafter, for total 14 days for sign of toxicity and/or mortality if any. The LD₅₀ was calculated by using OECD 425 software¹⁹. The preliminary doses were selected on the basis of literature review and results of acute toxicity study.

ii. CARRAGEENAN INDUCED PAW EDEMA:

Wistar rats of either sex (200-250g) were selected and divided into 5 groups, 6 animals in each group. Food was withheld with water *ad libitum* for 12 h before the administration of drugs. Animals were treated orally as follows: control group received vehicle, 3 test groups received three different doses of test drug 3, 10, 30 mg/kg p.o. and 5th group received marketed drug (Diclofenac 10 mg/kg p.o) for comparison. All animals received injection of 0.1 ml of 1% carrageenan in sub-plantar region 1h after drug treatment. Paw volume was measured using Plethysmometer (Orchid Scientific PLM02, Nashik, India), at an interval of 0, ½, 1, 2, 4, 6, 8 & 24 h after carrageenan injection.²¹

iii. HISTAMINE INDUCED PAW EDEMA:

Procedure was same as carrageenan-induced paw edema. In this method instead of carrageenan, injection of 0.1 ml of 1% histamine in sub-plantar region was used to induce edema. Paw volume was measured using Plethysmometer (Orchid Scientific PLM02, Nashik, India), at an interval of 0, ½, 1, 2 h after histamine injection.²¹

iv. COTTON PELLETT INDUCED GRANULOMA:

Swiss albino mice of either sex (25-30 g) were selected and divided into 5 groups, 6 animals in each group. Food was withheld 4h before experiment, with free access to water. On 1st day animals received the treatment as follows: control group received vehicle, 3 test groups received three different doses of test drug 3, 10, 30 mg/kg p.o. and 5th group received marketed drug (Diclofenac 10 mg/kg p.o) for comparison, same treatment was continued for 14 days. Animals were anesthetized with anesthetic ether one hour after administration first dose. Sterile cotton pellet of 10mg was implanted into one in each scapula region of mice by making small subcutaneous incision. On 15th day animals were sacrificed by giving excess anesthesia and cotton pellets were removed surgically, weighed and kept in hot air oven (60°C) until the constant weight is achieved. Net wet weight and dry weight of pellet and percent change of wet weight and dry weight were determined.²¹ The level of inhibition of granuloma tissue development was calculated = $[Tc - Tt / Tc] \times 100$ Where: Tc = weight of granuloma tissue of control group; Tt = weight of granuloma tissue of treated group.

v. Freund's adjuvant induced arthritis:

Wistar rats of either sex (200-250g) were selected and divided into 5 groups, 6 animals in each group. Animals receive treatment as follows: control group received vehicle, 3 test groups received three different doses of test drug 3, 10, 30 mg/kg p.o. and 5th group received marketed drug (indomethacin 2 mg/kg p.o.) for comparison and same treatment was continued for 12 days, from days 13 to 21, dosing was stopped. All animal received injection of 0.1 ml of complete Freund's adjuvant (CFA) in sub-plantar region of the left hind paw 1 h after drug treatment on first day. Volume of right and left paw was recorded before and after drug treatment on first day and consequently on 7th and 14th Day using plethysmometer (Orchid Scientific PLM02, Nashik, India). Body weight of animals was recorded on 1st and 21st day.²¹ Rats were scored for arthritis (arthritis index) daily by a set visual criterion. The body weight of all the animals was recorded using electronic balance. The joint thickness was measured in millimeters with the help of Vernier caliper and X-ray was taken to record the bone deformation^{22,23,24}

vi. Eddy's Hot plate method:

Mice were divided into five groups consisting of six mice in each group and treated as control group received vehicle, three test groups received test drug 3, 10, 30 mg/kg p.o respectively,

and 5th group received Diclofenac sodium 10 mg/kg. Food was withheld with water *ad libitum* 12 h before the administration of drugs. Individual animal was placed on the Eddy's hot plate maintained at 55 ± 1 °C. The reaction time in control and treated animals was recorded at 0, 30, 60, 120, and 180 min after the treatment.²⁵

vii. TAIL FLICK METHOD:

Mice were divided into five groups consisting of six mice in each group. First group received vehicle and served as control group, three test groups received test drug 3, 10, 30 mg/kg p.o respectively, and 5th group received fentanyl citrate 0.2mg/kg. Food was withheld with water *ad libitum* 4h before the administration of drugs. Individual mouse was placed in the analgesiometer cages leaving the tail 1.5 cm from its origin exposed on the radiant heat of nichrome wire loop maintained at 55 ± 1 °C, current intensity 3 Amp, gives constant radiant heat source. The cut-off reaction time was fixed at 10 sec to avoid any tissue damage. The reaction time in control and treated animals was recorded at 0, 15, 30, 60, 120, and 180 min after the treatment.²¹

viii. STATISTICAL ANALYSIS:

Data are presented as mean \pm standard error of mean (SEM). Analysis of variance (ANOVA) followed by Dunnett's t test was used to calculate statistical differences among treatment groups. Statistical analysis was conducted using InStat software (Version 3.06, GraphPad Software Inc., San Diego, CA, USA) Statistical difference considered significant at $P < 0.05$ compared to control group.

III. RESULTS:

a. Acute oral toxicity study:

The administration of ethanol fraction of saponins (EFS) to animals did not produce any behavioral changes and mortality. LD50 was found to be more than 2000 mg/kg calculated by (AOT425 statp gm, Version: 1.0), Acute Oral Toxicity (OECD Test Guideline 425) Statistical Program. On the basis of the AOT 425 report, doses of 3, 10 and 30 mg/kg were selected for evaluating pharmacological activity.

b. **Carrageenan induced paw edema:** Subcutaneous injection of Carrageenan (0.1 ml of 1% solution) in sub-plantar region induced edema in rat paw in control group. Animals treated with EFS 3, 10 and 30 mg/kg significantly ($P < 0.01$) inhibited inflammation induced by carrageenan at 2, 6 and 24 h compared to control group. Effect observed at 6 and 24h is comparable with effect of Diclofenac sodium (10 mg/kg) (Figure 1).

c. **Histamine induced paw edema:** Subcutaneous injection of histamine (0.1 ml of 1% solution) induced edema in paw of rats. Oral administration of EFS 3, 10 and 30 mg/kg significantly reduced paw edema ($P < 0.01$) at 2h and 3h compared to control group. The result

indicates that the EFS inhibit second phase of inflammation without affecting first phase (histamine release).

d. **Cotton pellet induced granuloma:** Subcutaneous implantation of 10 mg sterile cotton pellet into scapula region increased wet weight and dry weight of cotton pellet on 14th day. Administration of EFS 3, 10, 30 mg/kg p.o. significantly ($P<0.001$) decrease wet weight of cotton pellet with percentage inhibition of 47.80 %, 47.80 % and 49.73 % respectively on 14th day compared with wet weight of cotton pellet in control animals. Similar significant reduction was observed in dry weight of cotton pellet with percentage inhibition 45.83%, 51.59% and 37.69% respectively. Diclofenac sodium (10 mg/kg p.o.) significantly ($P<0.001$) decreased both wet weight and dry weight of cotton pellet with inhibition of 54.01 % and 56.71% respectively, compared with wet weight and dry weight of cotton pellet in control animals (Table 1).

e. **Freund's adjuvant induced arthritis:** A significant increase in the paw volume was observed in CFA treated animals up to 7th day, which can be attributed to its inflammatory response. Administration of EFS at a dose of 3, 10 and 30 mg/kg/day for a period of 21 days to arthritic animals, suppressed the chronic phase of inflammation significantly ($p<0.01$) compared to control group of animals. The animals treated with indomethacin 2 mg/kg/day significantly reduced the inflammation (Figure 3).

In control group the inflammation of tibiotarsal joint was observed on 7th day and which showed progressive increase till 21st day. EFS 30 mg/kg p.o treatment significantly ($p<0.01$) reduced the joint inflammation and its progression. Lower dose 3 and 10 mg/kg significantly ($p<0.05$) reduced inflammation till 10th day. Indomethacin 2 mg/kg significantly inhibited the inflammation of joints (Figure 4).

A continuous loss in body weight was observed throughout the period of 21 days in CFA treated control group. However, there was no significant alteration in body weight was observed in the indomethacin and EFS (3, 10 and 30 mg/kg) treated group compared to their weight on 1 day.

EFS 3, 10 and 30 mg/kg was significantly ($P<0.001$) protected loss of body weight compared to body weight of rat in control group on respective day from 7th to 21st day (Table 3).

Control animals showed severe lesions consisting of polyarthritis and ankylosis (Fig. 5). The rats treated with 3, 10 and 30 mg/kg EFS showed less severe lesions than controls.

f. **Eddy's Hot plate method:** Antinociceptive activity of EFS (3, 10 & 30 mg/kg) was evaluated using Eddy's Hot Plate Test. The basal reaction time for licking hind paw was recorded. EFS 3, 10 & 30 mg/kg significantly ($P<0.01$) increased reaction time at 30 min to 120 min interval. The highest nociception was exhibited at a higher dose of EFS 30 mg/kg, which is comparable to that of Diclofenac 10 mg/kg (Figure 6).

g. **Tail Flick Method:** Central analgesic activity was evaluated using tail flick method. Administration of EFS 3 mg/kg significantly ($P<0.05$) increased Reaction time whereas higher dose 10 and 30 mg/kg showed more significant ($P<0.01$) increase in reaction time compared to control group. Standard drug, fentanyl 0.2 mg/kg i.p produced more significant effect compared to EFS.

Table 1: Effect of EFS on cotton pellet-induced granuloma in mice.

Treatment group	Weight of cotton pellets	Inhibition (%)	Weight of cotton pellets	Inhibition (%)
	(mg) (wet)		(mg) (dry)	
Control	155.5 ± 12.74		52.16 ± 2.57	
Diclofenac (10)	71.5 ± 6.03**	54.01	22.58 ± 0.84**	56.71
EFS (3)	81.16 ± 8.34**	47.80	28.25 ± 1.76**	45.83
EFS (10)	81.16 ± 6.12**	47.80	25.25 ± 1.11**	51.59
EFS (30)	78.16 ± 4.75**	49.73	32.5 ± 2.02**	37.69

Data represented as Mean ± SEM; (n=6); Data analyzed by ANOVA followed by Dunnett's test. Results considered significant at ** $P<0.001$ compared to control group.

Table 2. Effect of EFS on body weight in CFA induced arthritis in rat

Treatment	1day	3 rd day	7 th day	10 th day	14 th day	21 st day
Control	251.66	243.33	223.33	200.00	183.33	146.66
	±6.54	±4.21	±4.21	±4.47	± 6.14	±6.14
Indomethacin (2)	250.23	248.33	241.66	243.33	246.66	248.33
	± 6.54	±4.77	±1.66 [#]	±4.21 ^{##}	±3.33 ^{##}	±3.07 ^{##}
EFS (3)	251.25	245.00	240.00	231.66	238.33	235.00
	± 4.77	±4.21	±2.58 [#]	±4.01 ^{##}	±3.07 ^{##}	±5.00 ^{##}
EFS (10)	250.66	246.66	240.00	236.66	240.00	238.33
	±4.77	±4.21	±4.47 [#]	±4.21 ^{##}	±3.65 ^{##}	±4.01 ^{##}
EFS (30)	250.48	245.00	240.00	238.33	243.33	245.00
	±4.01	± 3.41	±2.58 [#]	±4.77 ^{##}	±3.33 ^{##}	±2.23 ^{##}

Data represented as Mean ± SEM; (n=6); Data analyzed by ANOVA followed by Dunnett's test . Results considered significant at * $P<0.05$ and ** $P<0.01$ compared to control group.

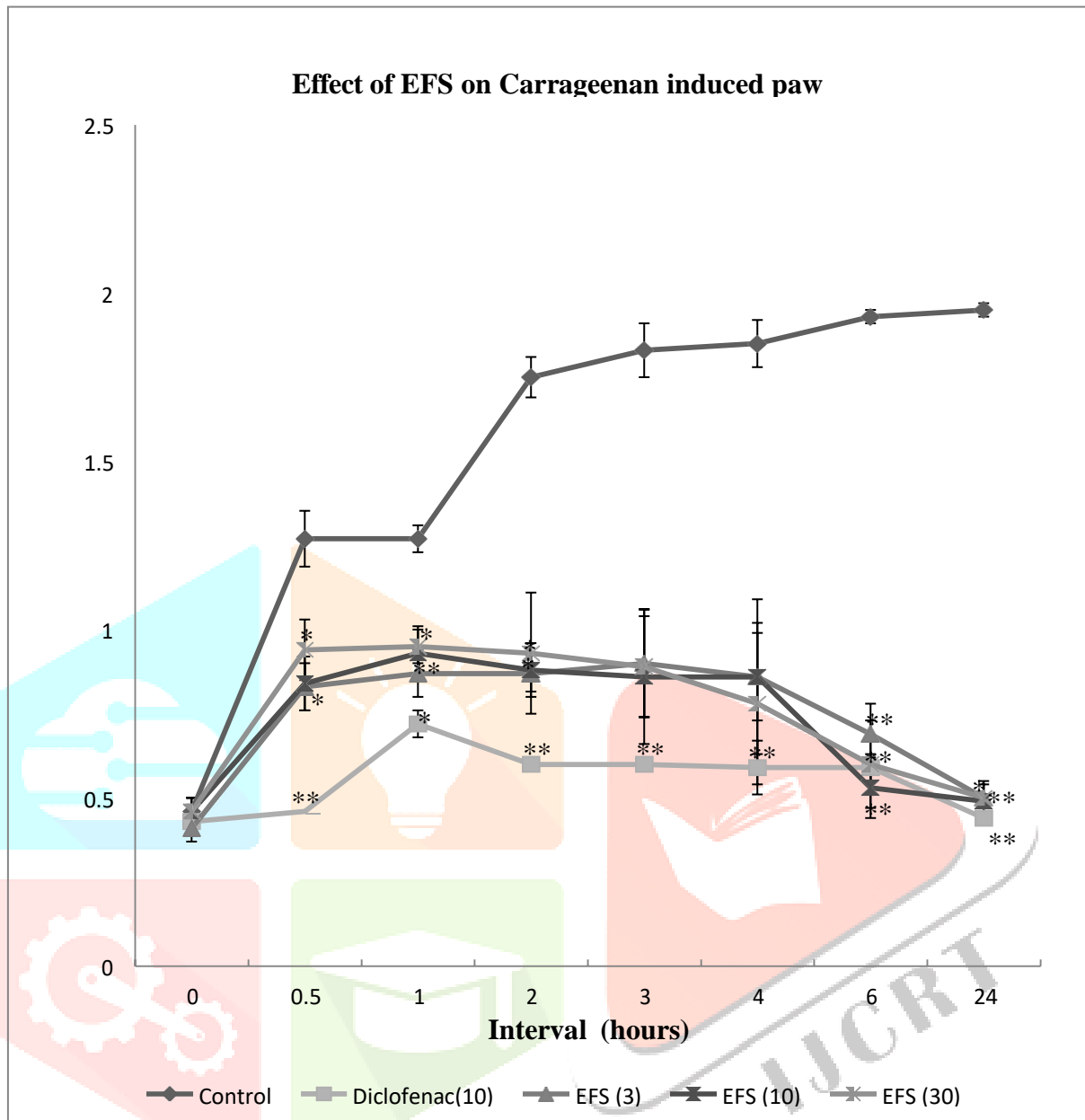


Figure 1: Effect of EFS on carrageenan induced paw oedema in rats
Data represented as Mean \pm SEM; (n=6); Data analyzed by ANOVA followed by Dunnett's test.
Results considered significant at *P<0.05, **P<0.01 compared to control group.

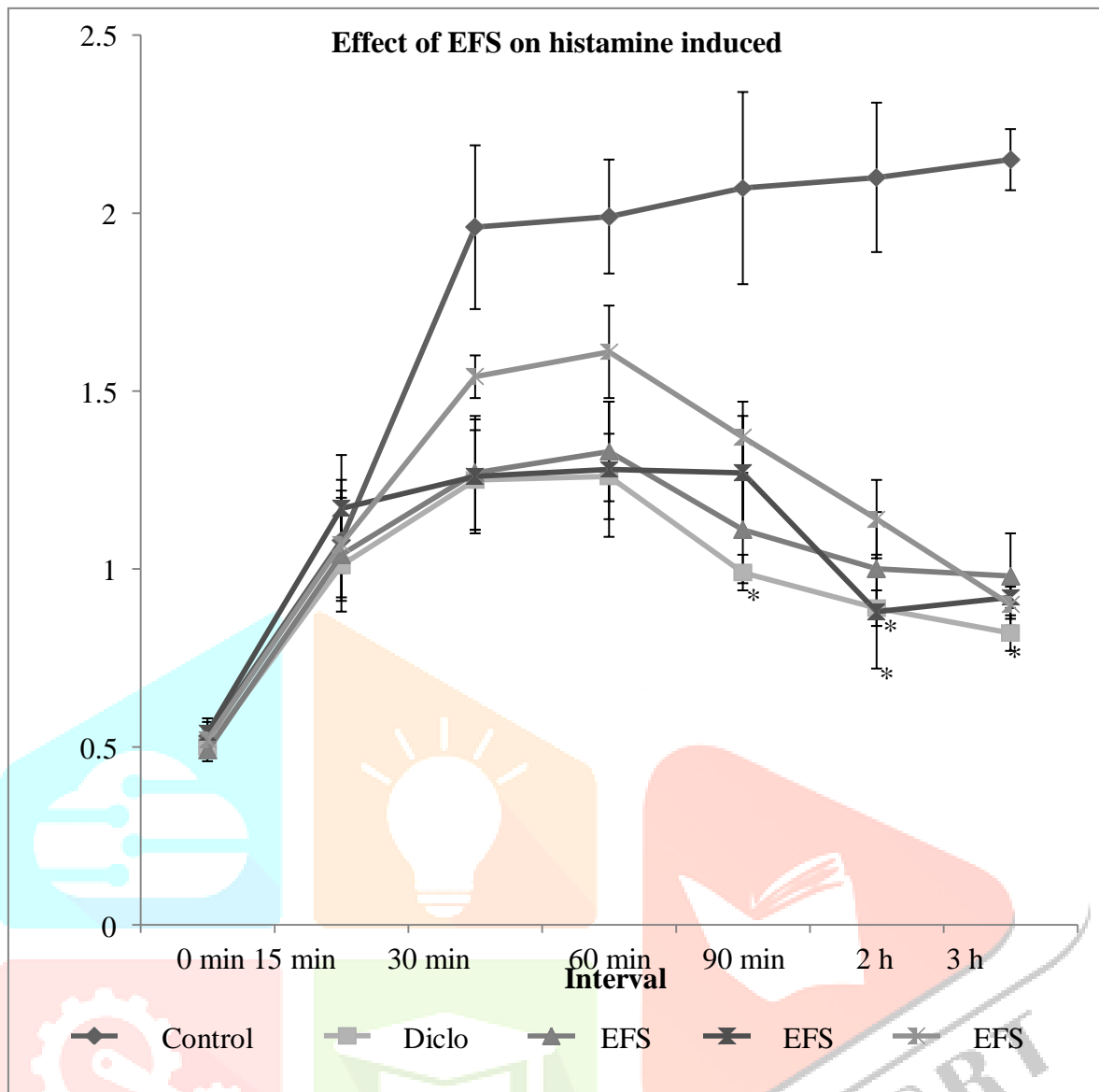


Figure 2: Effect of EFS on histamine induced paw oedema in rats. Data represented as Mean ± SEM; (n=6); Data analyzed by ANOVA followed by Dunnett's test. Results considered significant at *P<0.05, **P<0.01 compared to control group.

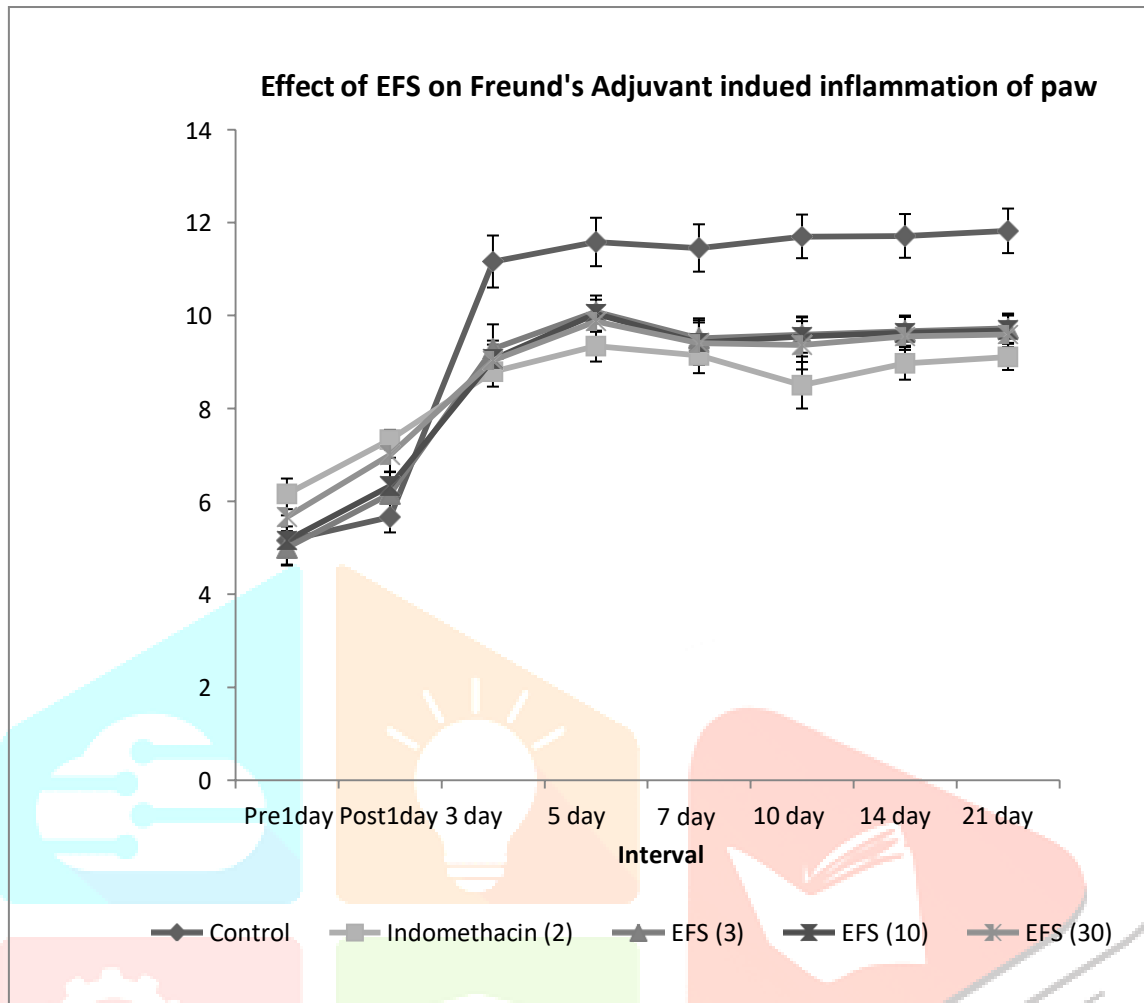


Figure 3: Effect of EFS on Freund's Adjuvant induced inflammation of paw
Data represented as Mean \pm SEM; (n=6); Data analyzed by ANOVA followed by Dunnett's test.
Results considered significant at *P<0.05 and **P<0.01 compared to control group.

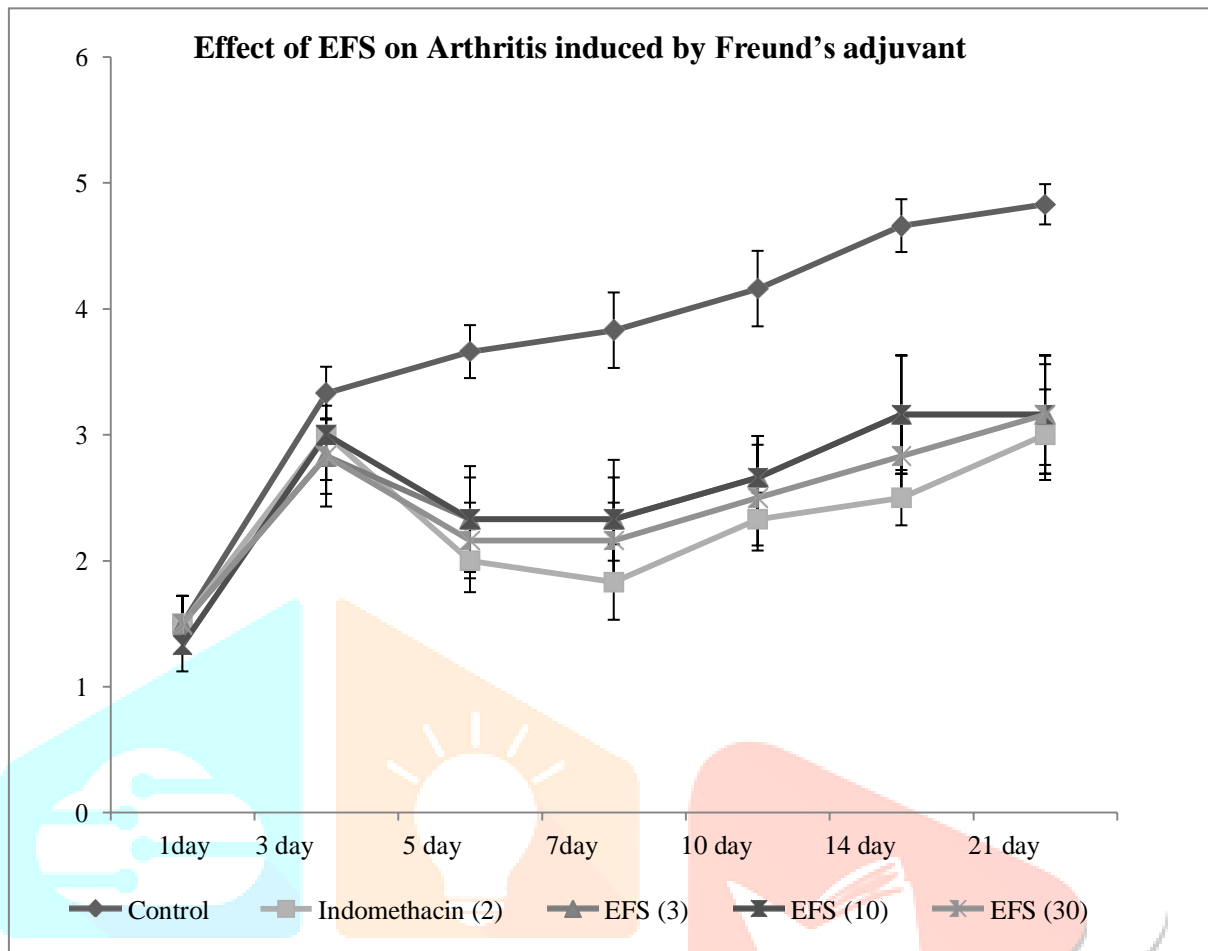
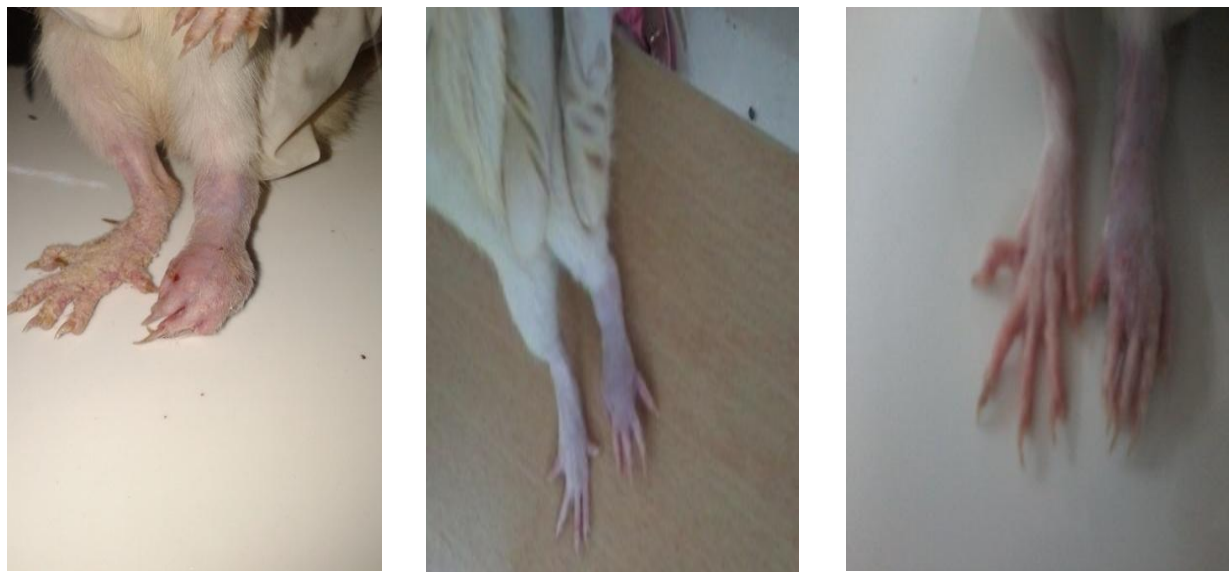


Figure 4: Effect of EFS on Arthritis induced by Complete Freund's adjuvant (CFA)
Data represented as Mean \pm SEM; (n=6); Data analyzed by ANOVA followed by Dunnett's test.
Results considered significant at *P<0.05 and **P<0.01 compared to control group.

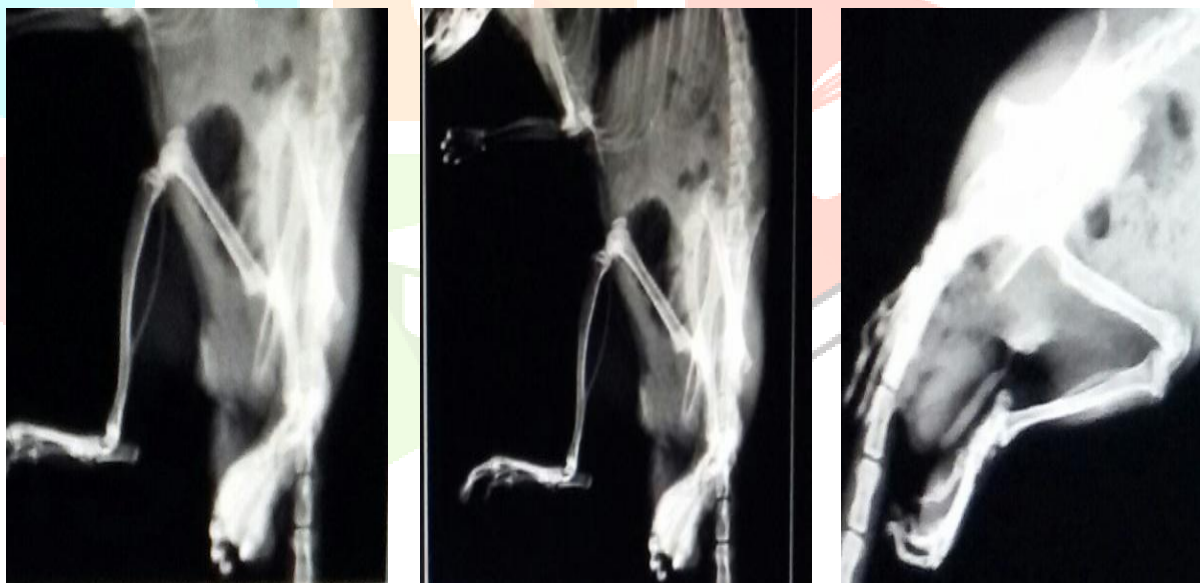


Control

EFS 30

Indomethacin (2)

Figure 5A. Effect of EFS on Arthritis induced by Complete Freund's adjuvant



Control

EFS 30

Indomethacin (2)

Figure 5B. Effect of EFS on Arthritis induced by Complete Freund's adjuvant

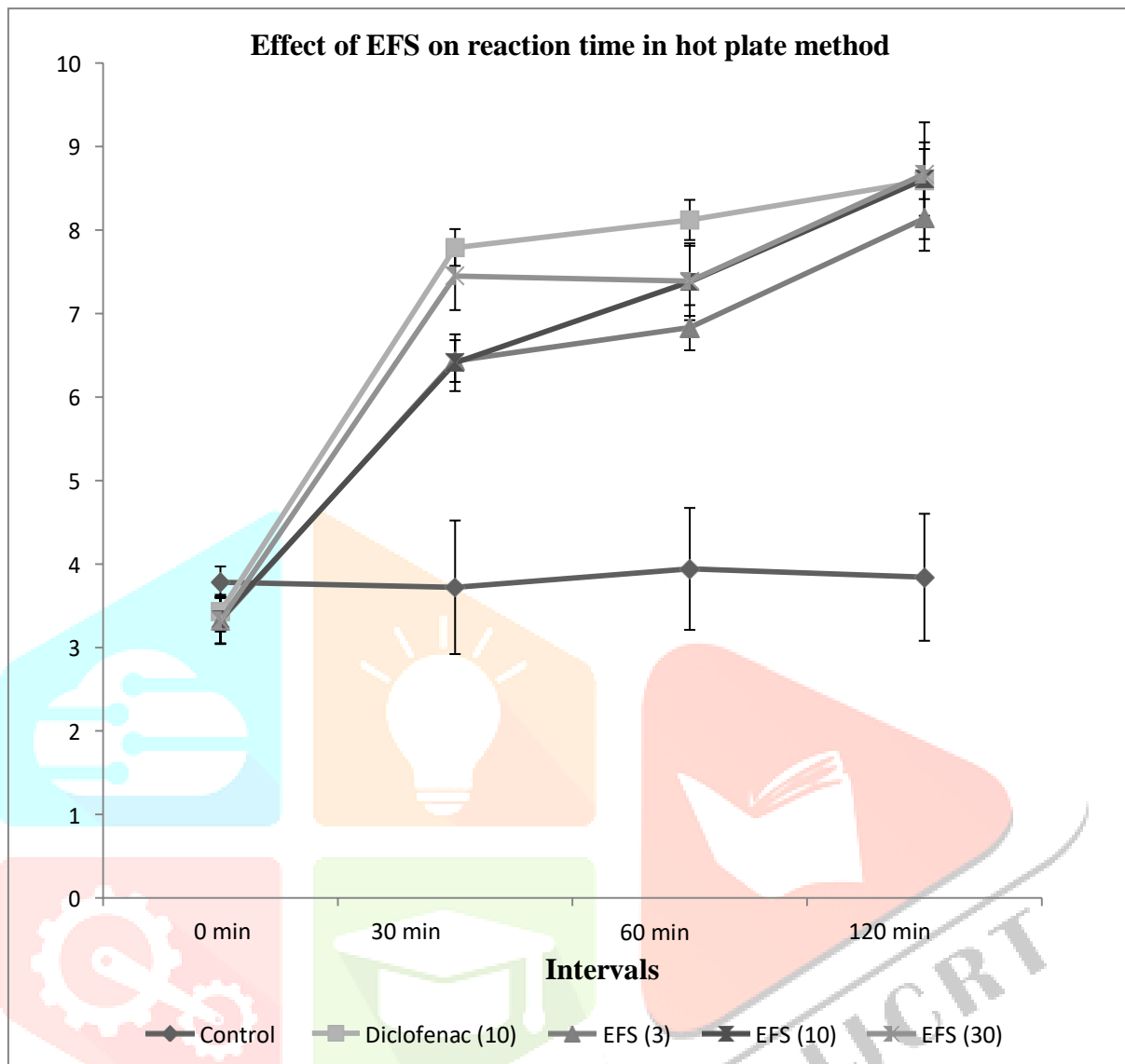


Figure 6: Effect of EFS on reaction time (sec) in hot plate method

Data represented as Mean \pm SEM; (n=6); Data analyzed by ANOVA followed by Dunnett's test. Results considered significant at *P<0.05 and **P<0.01 compared to control group.

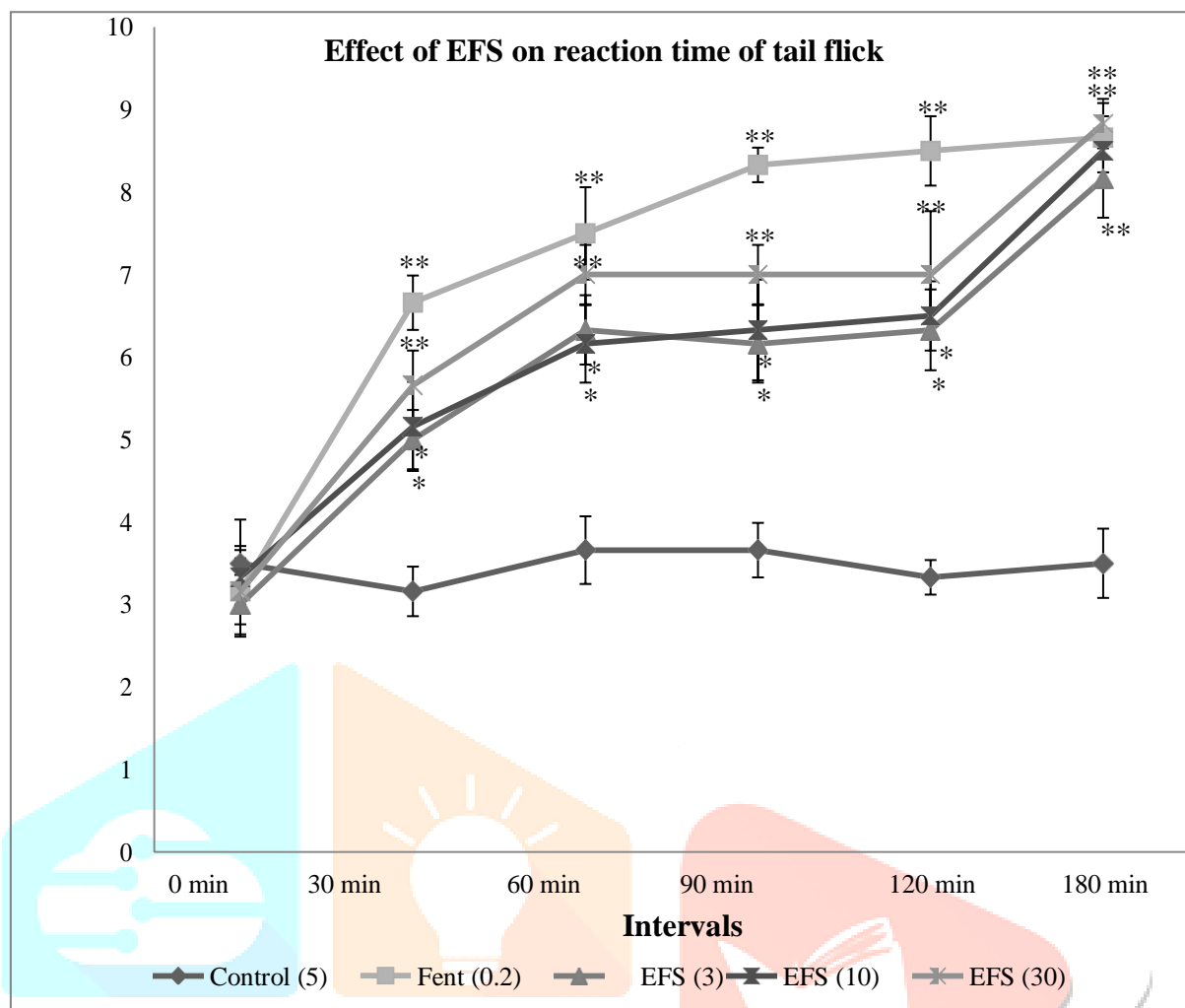


Figure 7: Effect of EFS on average reaction time in tail flick method

Data represented as Mean \pm SEM; (n=6); Data analyzed by ANOVA followed by Dunnett's test. Results considered significant at *P<0.05 and **P<0.01 compared to control group.

IV. DISCUSSION:

The roots of *Chlorophytum borivilianum* is traditionally used for treatment of inflammatory disorders. In spite of their famous legacy, the pharmacological effects have not been fully explored from the anti-inflammatory view point. The present study evaluated the anti-inflammatory, anti-arthritic and analgesics activity of fractions of saponins isolated from *C. borivilianum*.

Toxicity study revealed that LD50 of EFS is more than 2000mg/kg as well as it did not produced any alteration in normal behavior of animals.

Subcutaneous injection of carrageenan 0.1ml (1% solution) into the rat paw produces inflammation resulting from plasma extravasations, increased tissue fluid and plasma protein exudation along with neutrophil extravasations due to the metabolism of arachidonic acid. The carrageenan induced inflammation is biphasic event; first phase begins immediately after the injection of carrageenan and diminishes in 2nd h. The second phase begins at the end of the first phase and remains through 2nd h up to 6th h.²⁶ In first phase there is a release of histamine, serotonin, and bradykinin affecting vascular permeability, which is subsequently sustained by

the release of prostaglandins and NO in late/second phase. In addition, neutrophil infiltration, release of free radicals viz. hydrogen peroxide, superoxide, and hydroxyl radicals from neutrophils play a role in the late phase of carrageenan-induced inflammation. The inflammatory edema reached its maximum level at the 3rd hour and after that it started declining produced by inducible isoform of cyclooxygenase (COX-2) and nitric oxide synthase (iNOS), respectively.²⁷ In the present research, EFS inhibited inflammation at 6th and 24th h indicating inhibition of second phase of inflammation. Anti-inflammatory activity of the EFS was related to the inhibition of one or more intracellular signaling pathways involved in the effects of several inflammatory mediators. The suppression of the action in the second phase may be explained by an inhibition of cyclooxygenase pathway.^{28,29,30} Diclofenac sodium (10 mg/kg p.o.) at 2 h to 24 h suppressed the paw edema produced by carrageenan supporting its well proven cyclooxygenase pathway inhibition.^{31,32}

Histamine exists in bound form in granules (mast cell or basophils) and in free form during inflammatory process.³² Histamine induced paw edema is a well established model to study inflammation and neutrophil infiltration in paw tissue.³³ Subcutaneous injection of histamine produces rapid rise in edema and peak was observed at 30 min.³² Several reports have confirmed that histamine alone and in association with chemo-attractants such as platelet activating factor, interleukin 8 and leukotriene B4 involve in the regulation of neutrophil recruitment.³³ In the present study oral administration EFS (3, 10, 30 mg/kg p.o.) did not show inhibition of edema at 1 h indicates test drug did not affected the first (histamine release) phase. Reduction in paw edema was observed from 2h, indicating inhibition of second phase i.e. prostaglandin release without affecting histamine, which support the findings in carrageenan model.

The cotton pellet granuloma method is used for studies of chronic inflammation and considered as a typical feature of established chronic inflammatory reaction³⁴ and proliferative phase of inflammation.^{35,36} The subcutaneous implantation of sterile cotton pellet has been divided into three phases, transudative phase, exudative phase, and proliferative phase.^{37,38} In chronic inflammation monocyte infiltration and fibroblast proliferation take place. This proliferation becomes widespread by proliferation of small vessels or granuloma. Non-steroidal anti-inflammatory drugs decrease the size of granuloma which results from cellular reaction by inhibiting granulocyte infiltration/inflammation, preventing generation of collagen fibers and suppressing mucopolysaccharides.³⁹ Administration of EFS (3, 10, 30 mg/kg p.o) significantly decreased wet weight and dry weight of the cotton pellets, suggesting that EFS reduces both exudative and proliferative phase of granuloma formation. This outcome supports the earlier study by Panda et al., (2011)³⁷ reporting similar effect of methanolic extract of *C. borivianum* on proliferative phase of granuloma formation. Diclofenac sodium significantly decreases both wet weight and dry weight of the cotton pellet granuloma by inhibiting proliferation of fibroblast and fluid accumulation in chronic inflammation.

Freund's adjuvant induced arthritis in rats is most commonly used model to evaluate arthritic activity. Freund's adjuvant develops chronic inflammation in multiple joints due to accumulation of inflammatory cells with erosion of joint cartilage and bone destruction.⁴⁰ The change in paw volume could be divided into four phases of arthritis on the basis of biochemical markers of arthritis, first phase from 1st to 4th day with acute local inflammation and systemic effects, second phase span from day 7th to 10th with remission of acute inflammation and peri-arthritis, third phase from day 10th to 21st with chronic inflammation, peri-arthritis and osteogenic activity, finally fourth phase from day 21th onwards with permanent articular deformity and minimal (burn-out) inflammation.⁴¹ Administration of EFS (3, 10 and 30 mg/kg p.o) suppressed paw edema in acute and chronic phase of adjuvant induced arthritis however, the secondary inflammation of joints was reduced at dose 10, 30 mg/kg p.o indicating only higher doses are effective in arthritic phase. It has been reported earlier by Deore⁴² et al., 2010 that, water extract of *C. borivilianum* exhibit anti-arthritic activity due to presence of saponins.

As the incidence and severity of arthritis increased, the loss of the body weight occurred during arthritic condition due to alterations in the metabolic activities of diseased rats.⁴³ The anti-inflammatory drugs correct the decreased absorption capacity of intestine during inflammation.⁴⁴ EFS treatment inhibited the loss of body weight indicating prevention of alteration in metabolic activity.

The analgesic activity was determined by hot plate and radiant heat tail-flick model in mice using analgesiometer.^{45,46,47} In the present study, EFS increased the reaction time in hotplate suggesting its analgesic activity; the probable mechanism could be by inhibition of prostaglandin synthesis. Prostaglandins play significant role in different phases of inflammatory reactions and elicit pain by direct stimulation of sensory nerve endings and also sensitize sensory nerve endings to other pain provoking stimuli.

In tail flick method the procedure is based on the observation that morphine like drugs selectively prolongs the reaction time of the typical tail withdrawal reflex of mice.⁴⁸

EFS 3, 10 and 30 mg/kg increased the pain threshold. The higher dose 30 mg/kg showed more significant effect indicating central analgesic activity.

This suggests ethanol fraction of saponin isolated from *C. borivilianum* has potential for therapeutic use in treatment of inflammation and pain condition. Moreover the saponin fraction showed positive test for steroids this indicates that the steroid saponin is responsible for the anti-inflammatory and analgesic activity. This validates the traditional claim of *C. borivilianum* for treating the inflammation and pain.

V. CONCLUSION:

It is concluded that ethanol fraction of saponin (EFS) obtained from *C. borivilianum* possesses anti-inflammatory, anti-arthritic and analgesic effect. The more prominent effect observed at 10 and 30 mg/kg. Anti-inflammatory and anti-arthritic activity is probably due to inhibition of

prostaglandin and reduction in the proliferative granuloma formation whereas analgesic effect is due to inhibition of prostaglandins and central mechanism.

CONFLICT OF INTERESTS:

The authors declare no conflict of interests.

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REFERENCES:

1. Strietholt S, Maurer B, Peters MA, Pap T, Gay S. 2008. Review: Epigenetic modifications in rheumatoid arthritis. *Arthritis Res Ther* 2008;10:219-225
2. Laar MV, Pergolizzi JV, Mellinghoff HU, Merchante IM, Nalamachu S, O'Brien J, Perrot S, Raffa RB. 2012. Pain Treatment in Arthritis-Related Pain: Beyond NSAIDs. *Open Rheumatol J*, 6:320-330
3. Muluye RA, Bian Y, Alemu PN. 2014. Anti-inflammatory and Antimicrobial Effects of Heat clearing Chinese Herbs: A Current Review. *J Tradit Complement Med*, 4:93-98
4. Hussein SZ, Yusoff KM, Makpol S, Yusof YA. 2012. Gelam honey inhibits the production of pro-inflammatory mediators NO, PGE2, TNF- α , and IL-6 in carrageenan-induced acute paw edema in rats. *Evid Based Complement Alternat Med*, 1-13.
5. Kumar GP, Kumar R, Chaurasia OP, Singh SB. 2011. Current status and potential prospects of medicinal plant sector in trans-Himalayan Ladakh. *J Med Plant Res*, 5(14):2929-40.
6. Purohit SD, Dave A, Kukda G. 1994. Micropropagation of safed musli (*Chlorophytum borivilianum*), a rare Indian medicinal herb. *Plant Cell Tissue Organ Cult* 39:93-96.
7. Singh A, Chauhan HS. 2003. Safed musli (*Chlorophytum borivilianum*) distribution, biodiversity and cultivation. *J Med Arom Plant Sci*, 25:712-719.
8. Deore SL, Khadabadi SS. 2009. Screening of antistress properties of *Chlorophytum borivilianum*. *Pharmacologyonline*, 1:320-328.
9. Rath SK, Panja AK. 2013. Clinical evaluation of root tubers of Shweta Musali (*Chlorophytum borivilianum* L.) and its effect on semen and testosterone. *Ayu*, 34(3):273-275.
10. Panda SK, Das D, Tripathy NK. 2011. Anti-inflammatory potential of *Chlorophytum borivilianum* Sant. & Fern. root tubers. *J Global Trends Pharm Sci*, 2(2): 242- 251.
11. Sharma SK, Kumar M. 2011. Hepatoprotective effect of *Chlorophytum borivilianum* root extract against arsenic intoxication. *Pharmacologyonline*, 3: 1021-1032.
12. Attele AS, Wu JA, Yuan CS. 1999. Ginseng pharmacology: Multiple constituents and multiple actions. *Biochem Pharmacol*, 58(11):1685-1693.
13. Marais W, Reilly J. 1978. *Chlorophytum* and its related genera (Liliaceae). *Kew Bulletin*, 32:653-663.
14. Thakur M, Chsuhsn NS, Bhargava S, Dixit VK. 2009. A comparative study on aphrodisiac activity of some Ayurvedic herbs in male albino rats. *Arch Sex Behav*, 38(6):1009-1015
15. Acharya D, Mitaine-Offer AC, Kaushik N, Miyamoto T, Paululat T, Lacaille-Dubois MA. 2008. Furostane-type steroidal saponins from the roots of *Chlorophytum borivilianum*. *Helv Chim Acta*, 91:2262-2269.
16. Acharya D, Mitaine-Offer AC, Kaushik N, Miyamoto T, Paululat T, Mirjolet JF, Duchamp O, Lacaille-Dubois MA. 2009. Cytotoxic spirostane-type saponins from the roots of *Chlorophytum borivilianum*. *J Nat Prod*, 72(1):177-181.
17. Singh D, Pokhriyal B, Joshi YM, Kadam V. 2012. Phytopharmacological aspects of *Chlorophytum borivilianum* (safed musli): A review. *Int J Res Phram Chem*, 2(3):853-859
18. Lande AA, Ambavade SD, Swami US, Adkar PP, Ambavade PD, Waghmare AB. 2015. Saponins isolated from roots of *Chlorophytum borivilianum* reduce acute and chronic inflammation and histone deacetylase. *J Integr Med*, 13(1):25-33.
19. OECD test guidelines 425. Acute oral toxicity. Statistical programme (AOT 425 Stat pgm) version:1.0, 2010 (http://www.oecd-library.org/environment/test_no_425-acute-oral-toxicity-up_and_down-procedure_978926407149-en).
20. Thakur M, Bhargava S, Dixit VK. 2007. Immunomodulatory activity of *Chlorophytum borivilianum* Sant. F.

Evid Based Complement Alternat Med, 4(4):419-23

21. Vogel, 2002: Vogel HG. Drug Discovery and evaluation: Pharmacological assays. 2nd ed. New York Spinger-verlag berlin Heidelberg; 2002:725-820.
22. Bodhankar SL, Thakurdesai PA, Singhal S, Gaur V. 2007. Anorexic effect of (r)- sibutramine: Comparison with (rs)-sibutramine [corrected] and (s)-sibutramine. Indian J Physiol Pharmacol, 51:175-178.
23. Badgular LB, Ghosh P, Gaur V, Bodhankar SL. 2009. Effect of petroleum ether extract of *Sphaeranthus indicus* linn. On complete Freund's adjuvant induced arthritis in laboratory rats. Pharmacologyonline, 2:281-291
24. Bendele A, McComb J, Gould T, McAbee T, Sennello G, Chlipala E, Guy M.1999. Animal models of arthritis: Relevance to human disease. Toxicol Pathol, 27:134- 142.
25. Turner RT. 1965. Screening methods in pharmacology, Academic press, New York; pp:100.
26. Vinegar R, Schreiber W, Hugo R. 1969. Biphasic development of carrageenan oedema in rats. J Pharmacol Exp Ther, 166(1): 96-103.
27. Seibert K, Zhang Y, Leahy K, Hauser S, Masferrer J, Perkins W. 1994. Pharmacological and biochemical demonstration of the role of cyclooxygenase-2 in inflammation and pain. Proc Natl Acad Sci U S A , 91:12013-12017
28. Lo TN, Almeida AP, Beaven MA. 1982. Dextran and carrageenan evoke different inflammatory responses in rat with respect to composition of infiltrates and effect of indomethacin. J Pharmacol Exp Ther, 221(1):261-67
29. Suleyman H, Gul HI, Gul M, Alkan M, Gocer F. 2007. Anti-inflammatory Activity of Bis (3-aryl-3-oxo-propyl) methylamine Hydrochloride in Rat. Biol Pharm Bull, 30(1):63-67.
30. Pereira LP, Silva KS, Silva RO, Assreuy AS, Pereira MG. 2012. Anti-inflammatory polysaccharides of *Azadirachta indica* seed tegument. Rev Bras Farmacogn 22(3):1-8.
31. Channa S, Dar A, Anjum S, Yaqoob M, Atta-Ur-Rahman. 2006. Anti-inflammatory activity of *Bacopa monniera* in rodents. J Ethnopharmacol, 1(2):286-289.
32. Rachchh MA, Yadav PN, Gokani RH, Jain SM. 2011. Anti-inflammatory activity of *Benincasa hispida* fruit. Int J Res Pharm Bio Sci, 2(3):98-106
33. Tamaddonfard E, Farshid AA, Hosseini L. 2012. Crocin alleviates the local paw edema induced by histamine in rats. Avicenna J Phytomed, 2(2):97-104
34. Ochi T, Motoyama Y, Goto T. 2003. The analgesic effect profile of FR122047, a selective cyclooxygenase-1 inhibitor, in chemical nociceptive models. Eur J Pharmacol, 391:49-54
35. Radhika P, Rao PR, Archana J, Rao NK. 2005. Anti-inflammatory Activity of a New Sphingosine Derivative and Cembrenoid Diterpene (Lobohedleolide) Isolated from Marine Soft Corals of *Sinularia crassa* tixier-durivault and lobophytum species of the Andaman and Nicobar Islands. Biol Pharm Bull, 28(7):1311-13
36. Kaneria MS, Naik SR, Kohli RK. 2007. Anti-inflammatory, antiarthritic activity of herbal formulation (DRF/AY/4012). Indian J Exp Biol, 45:278-84.
37. Panda SK, Das D, Tripathy NK. 2011b. A study on antipyretic activity of *Chlorophytum borivilianum* Sant. and Fern. root tubers. Int J Pharmaceut Res Dev, 3(3):153- 156.
38. Sireeratawong S, Itharat A, Lerdvuthisophon N, Piyabhan P, Khonsung P, Boonraeng S, Jaijoy K. 2012. Anti-inflammatory, analgesic, and antipyretic activities of the ethanol extract of *Piper interruptum* Opiz. and *Piper chaba* Linn. Int Scho Res Net Pharmacol, 1-6.
39. Ramprasath VR, Shanthi P, Sachdanandam P. 2004. Anti-inflammatory effect of *Semecarpus anacardium* Linn. Nut extract in acute and chronic inflammatory conditions. Biol Pharm Bull, 27(12):2028-31.
40. Rajendran R and Krishnakumar E. 2010. Anti-Arthritic Activity of *Premna serratifolia* Linn. Wood against Adjuvant Induced Arthritis. J Med Biotec, 2(2):101-6.
41. Chitme HR, Patel NP. 2009. Antiarthrititis activity of *Aristolochia bracteata* extract in experimental animals. Open Natural Products J, 2:6-15
42. Deore SL, Khadabadi SS. 2010. Effect of *Chlorophytum borivilianum* on adjuvant induced arthritis in rats. Ann Biol Res, 1(1):36-40.
43. Tripathy S, Pradhan D, Anjana M. 2010. Anti-inflammatory and antiarthritic potential of *Ammania baccifera* Linn. Int J Res Pharm Biomed Sci,1(3):1-8.
44. Kore KJ, Shete RV, Desai NV. 2011 Anti-arthritis activity of hydroalcoholic extract of *Lawsonia innermis*. Int J Drug Dev Res, 3(4):217-224.
45. D'Amour FE, Smith DL. 1941. A method for determining loss of pain sensation. J Pharmacol Exp Ther, 72:74-79.
46. Kulkarni SK. 1999. Handbook of Experimental Pharmacology. 3rd ed. New Delhi: Vallabh Prakashan, 168-70.
47. Geetha M, Saluja, Shankar AK, Mehta RS. 2004. Analgesic and anti-inflammatory activity of *Couroupita guianensis* Aubl. J Nat Rem, 4(1):52.
48. Ezeja MI , Omeh YS, Ezeigbo II, Ekechukwu A. 2011. Evaluation of the Analgesic Activity of the Methanolic Stem Bark Extract of *Dialium Guineense* (Wild). Ann Med Health Sci Res, 1(1):55-62.