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Research Article

Prophylactic effect of Hydroalcoholic extract of *Colocasia esculenta* leaves in CFA and Formaldehyde induced arthritic rats

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ABSTRACT:

Adjuvant induced arthritis is a chronic crippling, skeleton muscular disorder having nearest approximation to human rheumatoid arthritis for which there is currently no medicine available effecting a permanent cure. Even modern drugs used for the amelioration of the symptoms, offer only temporary relief and also produce severe side effects. This work was aimed at the scientific validation of the ethno-pharmacological claim about Colocasia esculenta and its anti-arthritic property. In the present study, prophylactic antiarthritic activity of hydroalcoholic extract of Colocasia esculenta leaves is done by Complete Freund's adjuvant induced arthritis model and formaldehyde induced arthritis model. Paw volume, paw diameter and loss in body weight during arthritis condition were corrected on prophylactic treatment with hydroalcoholic extract of Colocasia esculenta leaves and Diclofenac. Serum parameters such as SGOT, SGPT and ALP were also estimated for assessing the anti- arthritic potential of hydroalcoholic extract of Colocasia esculenta leaves. The results of the current investigation concluded, hydroalcoholic extract of Colocasia esculenta leaves possess a significant anti-arthritic activity against Complete Freund's adjuvant induced arthritis and formaldehyde induced arthritis model and justifying its prophylactic role in arthritic condition. The observed antiarthritic activity may be due to the presence of phytoconstituents such as alkaloid and flavonoids.

Keywords: *Adjuvant, Rheumatoid Arthritis, Colocasia esculenta, Diclofenac, Complete Freund's adjuvant (CFA), Formaldehyde.*

INTRODUCTION:

Rheumatoid arthritis (RA) is a chronic, systemic inflammatory disorder that may affect many tissues and organs, but principally attacks flexible (synovial) joints. The process produces an inflammatory response of the capsule around the joints (synovium) secondary to swelling (hyperplasia) of synovial cells, excess synovial fluid and the development of fibrous tissue (pannus) in the synovium.

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This hyperplasia leads to degeneration of cartilage, erosion of bone and ultimately functional loss of joints [1]. The current treatment of rheumatoid arthritis is intended to minimize the associated pain and inflammation using nonsteroidal anti-inflammatory drugs (NSAIDs) as well as to decelerate the progress of the disease by using disease-modifying antirheumatic drugs (DMARDs). DMARDs suppress the immunological processes involved in the progression of rheumatoid arthritis. Drugs that have the effects of both DMARDs and NSAIDs may be more effective in the treatment of rheumatoid arthritis, but there is a scarcity of such drugs acting through multiple mechanisms. Hence, the treatment of rheumatoid arthritis involves the combined use of NSAIDs and DMARDs. Even though

various categories like immunosuppressants, NSAIDs, steroidal anti-inflammatory drugs are being used till now, but due to severe adverse effects of these drugs, the development of new antiarthritic drugs are aimed towards the discovery of safe, potent drugs with minimal side effects. Hence, they commonly prefer complementary and alternative medicines [2, 3].

The plant products and their combinations are running well now in the market for the treatment of inflammatory and autoimmune diseases due to their lower side effects, more efficacies and less cost. Now it is a growing concern all over for the development of new safe, potent, less toxic anti-arthritic drug. Hence, there is a need to explore for more naturally available alternatives, so that their therapeutic values can be assessed and expanded [4]. Most of anti-inflammatory plants show anti-arthritic activity and presence of phytochemicals such as alkaloids, flavonoids, steroids are responsible for anti-arthritic activity [5]. Recent studies conclude that hydroalcoholic extract of *Colocasia esculenta* leaves shows presence of phytochemicals such as alkaloids, flavonoids, steroids [6]. Also, *Colocasia esculenta* plant have traditional claim for use in inflammatory disorder [7]. But no pharmacological work has been done on evaluation of its anti-arthritic activity. So the present study was carried out to evaluate prophylactic effect of hydroalcoholic extract of *Colocasia esculenta* leaves in CFA and Formaldehyde induced arthritic rats.

MATERIALS AND METHODS:

Collection and Authentication of Plant Material:

The leaves of ***Colocasia esculenta*** were collected from Walchandnagar region of Maharashtra in the month of September-October 2011 and authenticated by Botanical Survey of India, Pune and herbarium voucher specimen No: BSI/WRC/Tech./2012/KUCCOES3.

Preparation of Hydroalcoholic Extract of *Colocasia esculenta* leaves:

Leaves of *Colocasia esculenta* were shade dried and coarsely powdered by using grinder mixer. The powdered material was macerated in sufficient quantity of distilled water and alcohol (1:1) and kept for 3 days. During maceration it was shaken twice daily [8]. On third day it was filtered and the filtrate obtained was evaporated on water bath (40-50^{0}C) to obtain solid reddish coloured dry mass. The extract was then preserved in the desicator and then used for phytochemical and pharmacological studies.

Phytochemical screening of the extract

The extract of *Colocasia esculenta* Linn. was subjected to qualitative analysis for the various phytoconstituents like alkaloids, carbohydrates, glycosides, phytosterols, saponins, tannins, proteins, amino acids and flavonoids [9].

Experimental animals

Male rats of Wistar strain weighing between 150-200 gm were used for the experiments. All the animals were obtained from animal house of R.D's College of Pharmacy, Bhor. All the protocols of animal experiments were approved by the Institutional Animal Ethics Committee (Approval no-RDCOP/IAEC/2011-2012/03) in accordance to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), ministry of Social Justice and Empowerment, Government of India, New Delhi. Albino rats and mice used for this work were obtained from the Yash farm and National Toxicological Centre, Pune. The animals were housed in Poly propylene cages and maintained at 24°C ± 2°C under 12 h light/ dark cycle and were feeded *ad libitum* with standard pellet diet and had free access to water. The animals were given standard diet supplied by Pranav Agro Industries Ltd. Sangli. The composition of the diet are Energy 3615 (Kcal/Kg), Crude Protein 22.05%, Crude Oil 4.5%, Crude Fibre 4.10%, Ash 11.10%, Sand Silica 0.75%.

Acute oral toxicity study (AOT):

Healthy adult Swiss albino mice (20-30 gm) were subjected to acute oral toxicity studies as per Organization for Economic Co-operation and Development (OECD) guidelines 2001 (AOT-423). Animals were observed individually after dosing at least once during the first 30 min, periodically during the first 24 h, with special attention given during the first 4 h, and daily thereafter, for a total of 14 days.The changes in skin, fur, eyes, mucous membranes, respiratory, circulatory, autonomic, central nervous system, somatomotor activity and behaviour pattern were noted (OECD guidelines, 2001)

Prophylactic effect of hydroalcoholic extract of *Colocasia esculenta* in complete Freund's adjuvant induced arthritic rats:

Freund's adjuvant induced Arthritis model was used to assess the anti-arthritic activity in albino wistar rats. Animals were randomly divided into six groups of six animal each (n=6). Wistar rats were made arthritic by single subplanter injection of 0.1 ml of Complete Freund's Adjuvant (CFA). Drug treatment was started from the initial day i.e. from the day of adjuvant injection (0 day) and continued till 21st day.

Group No.	Group title	Treatment	Route of Administration
I	Normal control	Distilled water 5 ml/kg	Per Oral.
II	Arthritic Control	Complete Freund's adjuvant (CFA) 0.1ml	Sub Planter.
III	Diclofenac	10 mg/kg	Per Oral.
		CFA 0.1ml	Sub Planter.
V	HECE 200	HECE 200 mg/kg	Per Oral.
		CFA 0.1ml	Sub Planter.
VI	HECE 400	HECE 400 mg/kg	Per Oral.
		CFA 0.1ml	Sub Planter.

Paw volume and Paw diameter was measured on 0th, 4th, 8th, 14th and 21st day by using plethysmometer and verneir caliper respectively [4, 10]. The body weights of the animals were measured by digital balance (CE, th-750) to access the course of the disease at the initial day before induction and at the end of 21st day. The rats were anaesthetized under light ether anaesthesia and blood was collected by retroorbital puncture for estimation of serum parameter such as SGOT, SGPT and ALP by using various diagnostic kits.

Effect of hydroalcoholic extract of *Colocasia esculenta* in formaldehyde induced arthritis:

Animals were randomly divided into five groups of six animal each (n=6). Rats were injected with 0.1 ml 2 % (v/v) of formaldehyde solution in the planter surface of the left foot, on the first and third day of the test. Drug treatment was started from the initial day i.e. from the day of formaldehyde injection (0day) and continued till 10th day.

Group No.	Group title	Treatment	Route of Administration
I	Normal control	Distilled water 5 ml/kg	Per Oral.
II	Arthritic Control	Formaldehyde (2%) 0.1ml	Sub Planter.
III	Diclofenac	Diclofenac 10 mg/kg	Per Oral.
		Formaldehyde (2%) 0.1ml	Sub Planter.
IV	HECE 200	HECE 200 mg/kg	Per Oral.
		Formaldehyde (2%) 0.1ml	Sub Planter.
V	HECE 400	HECE 400 mg/kg	Per Oral.
		Formaldehyde (2%) 0.1ml	Sub Planter.

The rat paw volume and paw diameter was recorded on 0th, 3rd, 6th and 10th by using Plethysmometer (UGO Basile, Italy 7140) and verneir calliper (Malik tools, Mumbai.) respectively [11].

STATISTICAL ANALYSIS:

The values were expressed as mean ± SEM (n=6). The statistical significance was assessed using student t-test, one-way or two-way analysis of variance (ANOVA) followed by Dunnet's test or Bonferroni post-tests and P<0.05, P<0.01 and P<0.001 were considered to be statistically significant.

RESULT:

Prophylactic effect of hydroalcoholic extract of *Colocasia esculenta* in complete Freund's adjuvant induced arthritic rats:

Sub planter injection of Complete Freund's adjuvant in arthritic control group showed significant increase in paw volume on 4th, 8th, 14th and 21st day as compared with normal control group. Administration of Diclofenac (10mg/kg) showed significant decrease in paw volume on 8th, 14th and 21st day as compared with arthritic control group with p<0.001 respectively. Administration of HECE (200 mg/kg) showed significant decrease in paw volume on 8th, 14th and 21st day as compared with arthritic control group with P<0.05, P<0.01 and P<0.001 respectively. Administration of HECE (400 mg/kg) showed significant decrease in paw volume on 8th and 14th, 21st day as compared with arthritic control group with P<0.01 and P<0.001 respectively.

Sub planter injection of Complete Freund's adjuvant in Arthritic control group showed significant increase in paw diameter on 4th, 8th, 14th and 21st day as compared with normal control group. Administration of Diclofenac (10mg/kg) showed significant decrease in paw diameter on 8th, 14th and 21st day as compared with arthritic control group with P<0.001 respectively. Administration of HECE (200 mg/kg) showed significant decrease in paw diameter on 8th, 14th and 21st day as compared with arthritic control group with P<0.05, P<0.01 and P<0.001 respectively. Administration of HECE (400 mg/kg) showed significant decrease in paw diameter on 8th and 14th, 21st day as compared with arthritic control group with P<0.01 and P<0.001 respectively.

Sub planter injection of Complete Freund's adjuvant in arthritic control group showed significant increase in SGPT, SGOT and ALP while significant decrease in total protein level as compared with normal control group. Administration of Diclofenac (10mg/kg) showed significant decrease in SGPT, SGOT and ALP level as compared with arthritic control group with P<0.001 respectively. Administration of HECE (200 mg/kg) showed significant decrease in SGPT, ALP and SGOT level as compared with arthritic control group with P<0.01 and P<0.05 respectively. Administration of HECE (400 mg/kg) showed significant decrease in SGPT, ALP and SGOT level as compared with arthritic control group with P<0.001 and P<0.01 respectively.

Sub planter injection of Complete Freund's adjuvant in arthritic control group showed significant decrease in body weight when compared with normal control group. Administration of Diclofenac (10mg/kg) showed significant increase in body weight as compared with arthritic control group with P<0.001. Administration of HECE (200 mg/kg) and HECE (400 mg/kg) showed significant increase in body weight as compared with arthritic control group with P<0.01 respectively.

Effect of hydroalcoholic extract of *Colocasia esculenta* in formaldehyde induced arthritis:

Sub planter injection of formaldehyde in arthritic control group showed significant increase in paw volume on 3rd, 6th and 10th day as compared with normal control group. Administration of Diclofenac (10mg/kg) showed significant decrease in paw volume on 3rd, 6th and 10th day as compared with arthritic control group with p<0.001 respectively.

Administration of HECE (200 mg/kg) showed significant decrease in paw volume on 6th and 10th day as compared with arthritic control group with P<0.01 respectively. Administration of HECE (400 mg/kg) showed significant decrease in paw volume on 6th and 10th day as compared with arthritic control group with P<0.01 and P<0.001 respectively.

Sub planter injection of formaldehyde in arthritic control group showed significant increase in paw diameter on 3rd, 6th and 10th day as compared with normal control group. Administration of Diclofenac (10mg/kg) showed significant decrease in paw diameter on 6th and 10th day as compared with arthritic control group with P<0.001 respectively. Administration of HECE (200 mg/kg) showed significant decrease in paw diameter on 6th and 10th day as compared with arthritic control group with P<0.05 and P<0.01 respectively. Administration of HECE (400 mg/kg) showed significant decrease in paw diameter on 6th and 10th day as compared with arthritic control group with P<0.01 and P<0.001 respectively.

DISCUSSION:

The Freund's complete adjuvant (FCA) induced arthritis model in rats is the most common model. This preclinical model predicted the activities of a number of compounds that are currently used in the treatment of rheumatoid arthritis are being tested in clinical trials.There are 4 phases of arthritis on the basis of biochemical markers of arthritis (1) Day 1-4 with acute local inflammation and systemic effects (liver), (2) Days 7-12 with remission of acute inflammation and periarthritis, (3) Days 12-28 with chronic inflammation, periarthritis and osteogenic activity,(4) Day 35 onwards (indefinitely) with permanent articular deformity and minimal (burnout) inflammation. A general increase in 5- HT synthesis within the whole central nervous system during the acute phase of the disease (2-3 weeks postinoculation) with a specific, further enhancement restricted to the spinal cord during the post acute phase (4-6 weeks postinoculation) [12].

The present study was carried out to see the efficiency of Indian herbal source against a chronic inflammatory disease i.e. arthritis. In the present study, rats were selected to induce arthritis because they develop a chronic swelling in multiple joints due to accumulation of inflammatory cells, erosion of joint cartilage and bone destruction. It has close similarities to human rheumatoid diseases [13]. The determination of paw swelling is apparently simple, sensitive and quick procedure for evaluating the degree of inflammation and the therapeutic effects of drugs. The freund's adjuvant model is chosen as it develop chronic swelling in multiple joints with influence of inflammatory cells with erosion of joint cartilage and bone destruction. Chronic inflammation involves the release of number of mediators like cytokines (IL-1B and TNF-alpha), GM-CSF, interferon's and PGDF. These mediators are responsible for the pain, destruction of bone and cartilage that can lead to severe disability [14].

However standard drug and Hydroalcoholic extract of *Colocasia esculenta* significantly suppressed the swelling of the paws and also decreases the paw volume in acute phase which may be due to the suppression of inflammatory mediator released due to induction of freund's adjuvant. Though the actual mechanism of suppressing inflammation is not known but it can be correlated with the presence of alkaloids and flavonoids in suppressing the inflammation and antioxidant activity.

As the incidence and severity of arthritis increased, the changes in the body weights of the rats also occurred during the course of the experimental period. Earlier findings suggest that changes in body weight during arthritic inflammation are due to alteration in metabolic activities of rats and also due to reduced absorption of glucose and leucine in rat intestine. In addition to this decreased food intake throughout the study period due to partial immobility accompanying hyperalgesia may also be one of the reasons [15]. But on the treatment with anti-inflammatory drugs, the decrease in absorption was nullified and it

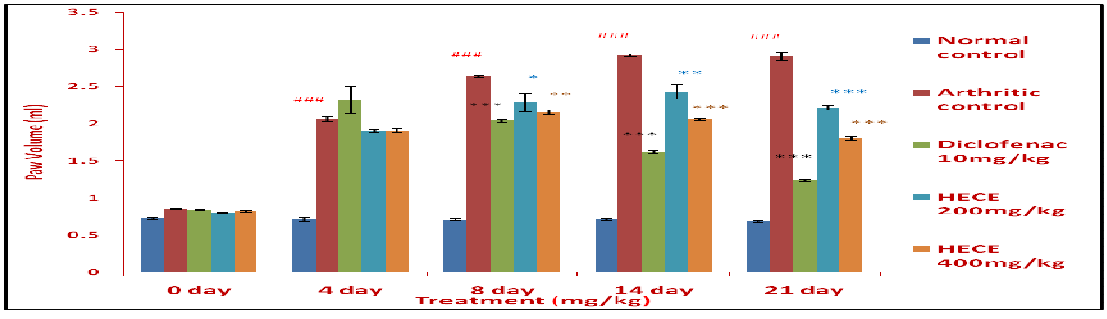
shows that the anti-inflammatory drugs correct the decreased/deranged absorption capacity of intestine during inflammation. The increased body weight during treatment of standard drug, Hydroalcoholic extracts of *Colocasia esculenta* may be due to the restoration of absorption capacity of intestine.

The activities of SGOT, SGPT and ALP increases significantly in arthritic rats, since these are good indices of liver and kidney impairment which is also considered a feature of CFA induced arthritis [16]. Earlier findings suggest that CFA administration in rats immunologically alters the hepatic biochemistry (Disruption of the hepatocyte, Disruption of the transport proteins, Cytolytic T-cell activation, Apoptosis of hepatocytes, mitochondrial disruption, Bile duct injury etc.). Elevated levels of serum ALP in CFA induced arthritic rats can be due to localized bone loss in the form of bone erosion and peri-articular osteopenia [17]. The extract also shows significant effect on various blood and serum parameters.

Formaldehyde induced arthritis is one of most commonly used acute model for assessing anti-arthritic potential of plant extract. The development of edema in the paw of the rat after injection of formaldehyde (0.1ml,2% w/v) is due to the release of histamine, serotonin and the prostaglandin like substances at the site of injection [11]. In present study, arthritic control group showed significant increase in paw swelling and paw diameter as compared to normal control group. This effect of formalin is due to localized inflammation and pain. The inflammatory effect of formalin is biphasic, an early neurogenic component followed by a later tissue mediated response [11]. However hydroalcoholic extract of *Colocasia esculenta* (200 and 400 mg/kg) showed significant decrease in paw volume and paw diameter as compared with arthritic control group. Inhibition of paw edema in formaldehyde induced arthritis may be due to the anti-inflammatory potential of HECE.

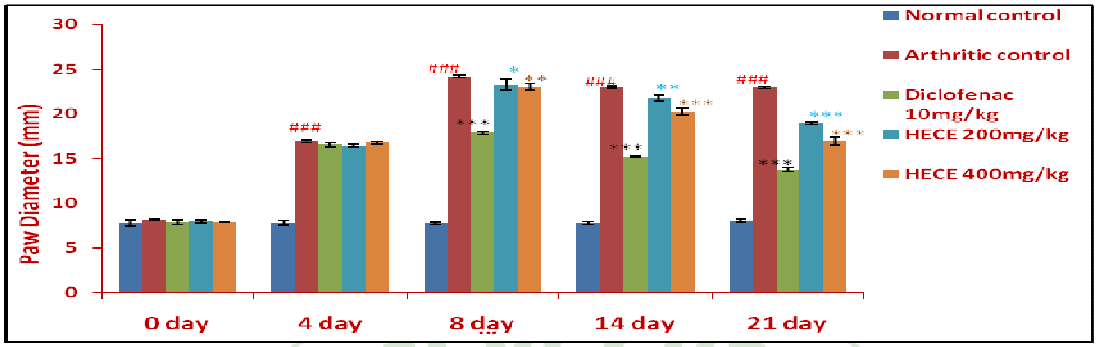
CONCLUSION:

On the basis of the results obtained in this study we conclude, and propose that possibly, the potent anti-arthritic effect of *Colocasia esculenta* extract may be through maintenance of synovial membrane, thereby inhibiting cytokines and leukotriene infiltration inhibition as evidenced in paw edema volume. In turn, protecting synovial membrane and improving health status through anti-inflammatory properties of HECE. The results of present study showed that the HECE produced significant reduction in paw volume, paw diameter, SGOT, SGPT and ALP at different time intervals and increase in body weight. In present study, the preliminary phytochemical analysis revealed and the HPTLC analysis confirmed the presence of alkaloids and flavonoids. It was earlier reported that the leaves of *Colocasia esculenta* showed anti-inflammatory activity [7]. Flavonoids, alkaloids and glycosides are responsible for antiarthritic activity [18]. Hence it was suggested that the presence of flavonoids, alkaloids, glycosides, etc. may be responsible for the antiarthritic effect of the HECE. From the results observed from the current investigation, it is concluded that the Hydroalcoholic extract of *Colocasia esculenta* possesses potentially useful antiarthritic activity since it give a positive result in controlling inflammation in adjuvant induced arthritic model in rats.



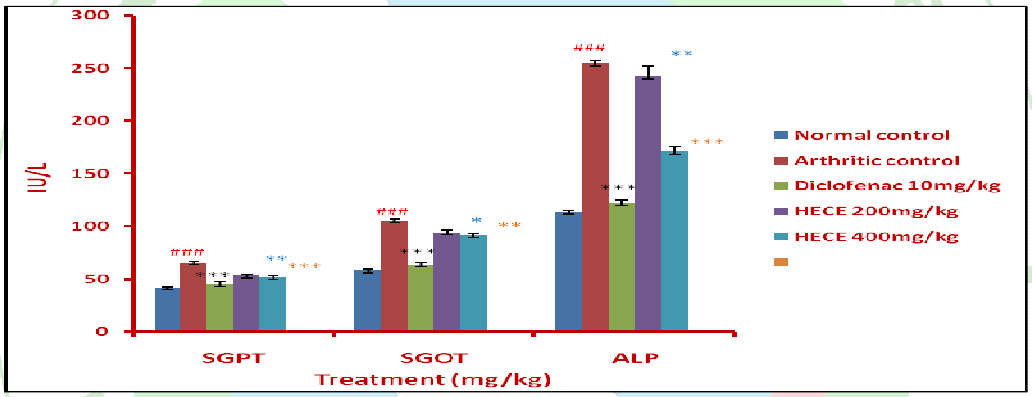
Values are expressed as mean ± SEM (n=6). *P<0.05, **P<0.01 and ***P<0.001 as compared with arthritic control (Two-way ANOVA followed by Bonferroni test). ### indicates significant induction when compared with normal control group.

Fig. 4.1 Effect of HECE on CFA induced arthritis paw volume (ml) (Prophylactic study)



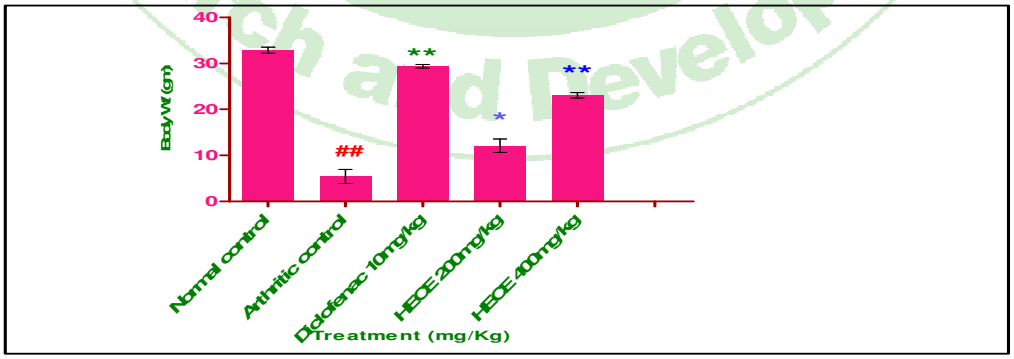
Values are expressed as mean ± SEM (n=6). *P<0.05, **P<0.01 and ***P<0.001 as compared with arthritic control (Two-way ANOVA followed by Bonferroni test). ### indicates significant induction when compared with normal control group.

Fig. 4.2 Effect of HECE on CFA induced arthritis paw diameter (mm) (Prophylactic study)



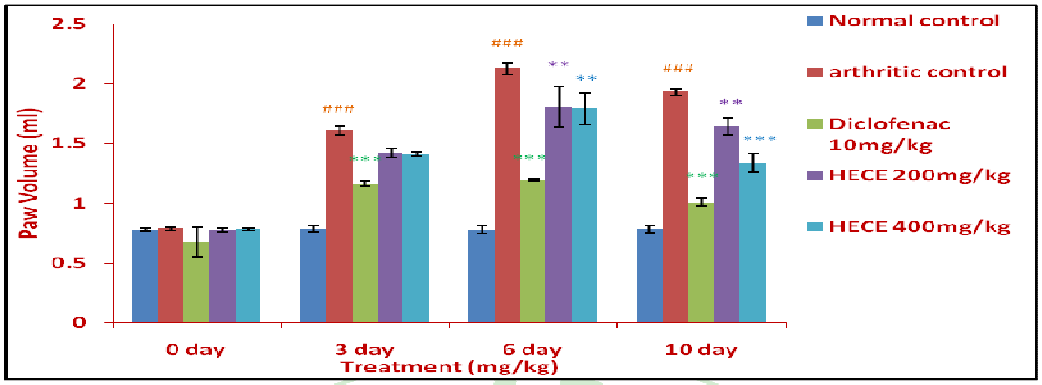
Values are expressed as mean ± SEM (n=6). *P<0.05, **P<0.01 and ***P<0.001 as compared with arthritic control (Two-way ANOVA followed by Bonferroni test). ### indicates significant induction when compared with normal control group.

Fig. 4.3 Effect of HECE on various Biochemical parameters in CFA induced arthritis (Prophylactic study)



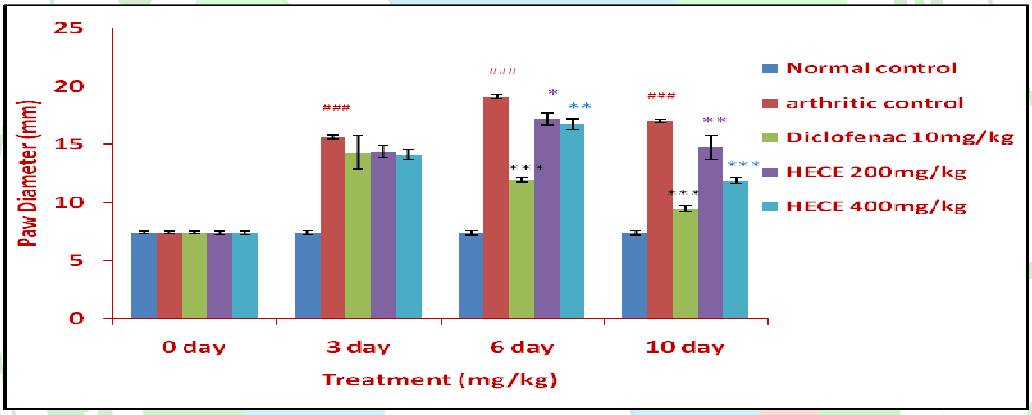
Values are expressed as mean ± SEM (n=6). *P<0.05, **P<0.01 and ***P<0.001 as compared with arthritic control (One-way ANOVA followed by Student's-t test). ### indicates significant induction when compared with normal control group.

Fig. 4.4 Effect of HECE on Body wt in CFA induced arthritis (Prophylactic study)



Values are expressed as mean ± SEM (n=6). *P<0.05, **P<0.01 and ***P<0.001 as compared with arthritic control (Two-way ANOVA followed by Bonferroni test). ### indicates significant induction when compared with normal control group.

Fig. 4.5 Effect of HECE on Formaldehyde induced arthritis paw volume (ml).



Values are expressed as mean ± SEM (n=6). *P<0.05, **P<0.01 and ***P<0.001 as compared with arthritic control (Two-way ANOVA followed by Bonferroni test). ### indicates significant induction when compared with normal control group.

Fig. 4.6 Effect of HECE on Formaldehyde induced arthritis paw diameter (mm).

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