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Comparative Evaluation for the Anti-Inflammatory & Anti-Oxidant Properties of Hydromethanolic Extract of Leaves, Flowers and Stems of Combretum Indicum Linn in Albino Wistars Rats

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KEYWORDS
Antioxidant, X-rays, *Combretum ndicum*, Biochemical, rutin, anti-inflammatory

ABSTRACT:

Combretum indicum plant indicated as herbal medication for many of diseases and disorders due to presence of various pharmacologically active phytoconstituents such as pelargonidin 3-glucoside, quisqualic acid, pelargonidin,Trigonelline and rutin. In this study, ratio of 50:50 Hydro-methanolic Extract of leaves, flowers and stems were taken and comparative evaluation was done by performing 21 days of CFA induced chronic anti-inflammatory model in female albino wistar rats. Preliminary phytochemical examination confirmed about the presence of phytocomponents such as carbohydrates, Tannins, flavonoids, glycosides, oils & fats, amino acids & protein which are already mentioned. 400 mg. kg.body weight selected extracts of these three parts of *C.indicum* were given to animals after examined acute oral toxicity. Paw volume, paw diameter, Body weight, arthritis score were examined on 0, 7th , 14th and on 21st days of model. Examination of blood components (RBCs, WBCs, PLTs, Hb, RF factor and CRP and Biochemical markers (SGPT, SGOT and ALP) were done with the blood, for enzymatic activity (SOD, CAT, GPx, GSH) antioxidant study done with blood. Histopathological evaluation & radiograpgy of animals paws were done in the end of the study. Improvement like condition in these parameters, confirmed the explanation of anti-inflammatory and antioxidant activity of this extract with compare to other, leaves extract was found more effective.

1. Introduction

Inflammation is a self-defencing and self-protecting physiological function for the survival of healthy life and to defend the cells and tissues of the host from the external stimulus by activation of proinflammatory mediators any abnormality and defect in innate immune response, leading to acute and chronic inflammation. [1-2] When any external stimulus triggers cells and tissues of the host, the initial changes that occur to eliminate the stimulus are called acute inflammation. [3] When the innate immune response fails due to some diseases, genetic hereditary, degeneration of neurons, or abnormality in immune functioning then the inflammation leads to chronic inflammation. [4-5] Rheumatoid arthritis is chronic arthritis that develops from the production of autoantibody and progressive disability of firstly smaller joints and then the larger joints due to abnormal functioning of cartilage, destruction of bone weakening of ligaments and tendons. [6-7] In current the physiological homeostasis of host will become abnormal due to changes of life styles and due to environmental risk factors, which leads to various arthritic diseases.[8] Nowadays herbal plants have a good source of phytoconstituents and have targeted effects,

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lesser side effects, more therapeutic effects and bioavailability. Various list of Herbals plants which having phytoconstituents such as flavonoids and phenols containing active constituents which targeted on rheumatoid arthritis are rutin, gallic acid, quisqualic acid, quercetin, trigonelline, and pelargonidin. [9-10]

In the present study, *Combretum indicum* was taken for the activity against inflammation for its efficacy against CFA-induced inflammation. *Combretum indicum* is a widely distributed and cultivated Plant in India and also in Africa, Indo Malaysia has a synonym of *Quisqualic indica* and is commonly knowns as Rangoon creeper, and belongs to the family Combrataecae.[11] Combretum indicum contains various phytoconstituents like rutin, hesperidin and quercetin which exhibit anti-inflammatory activity. [12] It also contains phytoconstituents polyphenols, flavonoids and phenolic compounds such as trigonelline, L-asparagine, L-proline, and quisqualic acid, which exhibit various therapeutic efficacy such as Anti-cholinesterase activity, anti-filarial activity, Antihyperlipidemic activity, Anti-bacterial activity, Antifungal activity, Anti-microbial activity, Antioxidant activity, Antiviral activity, Anti-pyretic activity, anti-inflammatory activity, Anti-malarial activity, Anti-diabetic activity, Anti-diarrhoeal activity etc. [13]

Materials & Methods Materials:

Chemical required:

Inflammation-inducing agents: - 0.1ml CFA was chosen for induction of chronic arthritis which were brought from Sigma-Aldrich chemical private limited, Bangalore, India.

Standard drug: - 10mg/kg Diclofenac was chosen as the standard drug as given in previous research paper. Diclofenac was obtained as a gift sample from Elam Pharma Pvt Limited, Ankleshwar, India.

Methods

Authentication Collection and drying of plant material

Combretum indicum was identified and authenticated by a head of BSI, Central region, Prayagraj. Uttar Pradesh, India. Selected aerial parts of plants (Leaves, flowers, and stems) were collected in the month of November (2022) from the local area of Naini Prayagraj. After collection plant material was rinsed with tap water to remove dust particles and soil. After washing material was dried firstly in air and at room temperature for one month.

Preparation of extract

The dried material was weighed and grinded into a small coarse powder. For extraction, Coarse powders of each part of leaves, flowers, and stems were defatted with petroleum ether by hot continuous extraction method at a temperature of 45 to 50 degrees Celsius, and then hydroalcoholic extraction of residue was done by the continuous hot method by taking a solvent in a ratio of methanol and water (50:50). The yield for HMELCI, HMEFCI, and HMESCI were found to 15gm/180gm, 15gm/180gm, 10gm/180g respectively. [14]

HMELCI-Hydromethanolic extract of leaves of combretum indicum, **HMEFCI-** Hydromethanolic extract of flower of combretum indicum, **HMESCI-** Hydromethanolic extract of stem of combretum indicum.

Phytochemical Study:

Preliminary Phytochemical screening:

Preliminary phytochemical examinations for the various components such as alkaloids, various glycoside, carbohydrates, amino acids & protein, oil & fats, tannins, phenolic compounds and flavonoids compound present in the HMELCI, HMEFCI, and HMESCI were done by using various reagent and standard procedure given in the standards book and research paper. [15-16]

Fluorescence Analysis:

Small drops of extracts were taken & various different solvents such as phenol, ethanol, Sulphuric acid, glacial acetic acid, ammonia solution, iodine solution, methanol, sodium hydroxide, ferric chloride solution and conc.nitric acid were taken and extracts were mixed with these solvents on the glass slide one by one, and examined the emitted colour in UV light of 254, 366, and 765 nm the under the UV chamber.[15]

Qualitative evaluation by TLC:

Extracts were dissolved in the methanol with 1mg/mL ratio for sample preparation. Slurry of silica gel G was

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prepared by dissolving the silica gel+ water with ratio of 1:2 ratio. A 0.25 mm thick TLC plate was prepared and spotting of the sample was done with microcapillary. The solvent systems such as (chloroform, methanol and ethyl acetate) in the ratio of 7:3:3 was prepared according to hit and trial and with the help of a research paper. Rf values were calculated on the basis of the distance traveled by solute and solvents. Examination of bands of phytoconstituents will be done by the 365 nm UV light under the UV chamber. [17-18]

Quantitative Analysis for Total Phenolic and Total Flavonoid Contents:

For the Phenolic components, the Folin-Ciocalteu reagent was used to determine the total phenolic content of the HMELCI, HMEFCI, and HMESCI. and a UV spectrophotometer was used to measure it at 760 nm. 10µg, 20µg, 30µg, 40µg, 50µg of each extract were taken and mixed with 5 mL of methanol in test tube. Then after centrifugation,0.2 mL sample +0.6ml (distilled water and Folin & ciocalteu reagent with 1:1 ratio) +1ml of 85% NaHCO3 and makeup the volume up to 3 ml and examined under 760 nm of UV light. For the flavonoid components, 10µg, 20µg, 30µg, 40µg, 50µg of each extract taken in test tube and mixed 5 mL of methanol then 0.2 ml of sample from each tube+2ml of 6% $AlCl_3$ solution, examined for the absorbance at 358 nm. TPC and TFC were calculated by using the formula RDV. 100/W, where value of R was obtained by the graph on the basis of absorbance in selected UV light, D is dilution factor, 3 for TPC and 4 for TFC and W=1.[16-19]

Pharmacological Study:

Experimental Animal required

For protocol, 36 Female Albino wistar rats were selected, and these animals were approved by the CPCSEA. After an Institutional meeting of CPCSEA, rats were procured from the organization, Chakraborty Enterprises Kolkata, which was approved by the CPCSEA and reg. no:1443/Po/Bt/S/11/CPCSEA.

Acute Toxicity Study: - Study was performed to calculate the lethal dose of selected parts of extract according to OECD guidelines 423. Mainly 15 rats were taken and divided into five groups and there are three rats in each group. Before given an extract dose, rats were starved for 24 hours then the drug was given by oral gauge at an ascending order (5mg/kg, 50mg/kg, 300mg/kg, 2000mg/kg and 5000mg/kg) and the parameters are evaluated to check survival state of rats at frequencies of 30min, two hours, eight hours, twelve hours, and 24 hours. On a given low dose if no mortality was detected then the high dose was given and continued at a 5000mg/kg dose. [20-21]

Experimental induction of inflammation Induction of Inflammation by CFA:

For the induction of chronic inflammation, Complete Freund adjuvant was chosen as an antigen. CFA is a suspension of desiccated mycobacterium tuberculosis in paraffin oil and mannide monooleates after induction which causes inflammation, tissue necrosis, and ulceration. By using a 28-gauge insulin syringe 0.1 ml CFA was injected subcutaneously on the sub plantar region of the rat's hind paw.[22-23]

Experimental design

Female Wistar rats were divided into six groups and there are six rats in each group-

Group 1 : for 21 days, 1% gum acacia was administered by oral gauge for taken this group of rats as normal control group (NCG)

Group 2 : 0.1 ml CFA was given as antigen on 0th day taken this group of rats as arthritic control group (ACG)

Group 3: 0.1ml CFA + 10mg of Diclofenac was administered for 21 days and taken this group of rats as diclofenac treated group.(DTG)

Group 4: 0.1ml CFA + 400mg of HMELCI was administered for 21 days,

Group 5: 0.1ml CFA + 400mg of HMEFCI was administered for 21 days.

Group 6: 0.1ml CFA + 400mg of HMESCI was administered for 21 days.

Protocol of the experiment was completed by performing a 21-days Antigen-induced Arthritis model and the parameter such as paw volume, paw diameter, body weight & arthritic score was evaluated for anti-inflammatory activity on days 0^{th}, 7^{th}, 14th, 21st. On 21th day blood was taken from retro-orbital route for

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haematological parameter and for biochemical parameter and Anti-oxidant estimation was also done. After blood withdrawal one rats from every group was sacrifice by cervical disclocation method and the rats paw was dissected by incision for histopathological & radiography study of rat paw joints & tissue.[24-26]

Parameter evaluation

Paw volume determination

A Plethysmometer is a handy tool to measure the edema and volume displacement. So that this tool was used to measure the small changes on rat's paw after the antigen-induced inflammation and after the standard drug treatment with HMELCI, HMEFCI, and HMESCI.

Estimation of Paw Diameter:

The diameter of the rat's paw was measured with vernier caliper to identify the increase of inflammation after antigen induction and reduction in inflammation after the dosing with standard drug and HMELCI, HMEFCI, and HMESCI. Paw diameter is calculated and compared before and after the dosing.

Estimation of body weight

During inflammatory conditions, body weight should be decreased so that this parameter is included to check the body weight of all groups of rats by digital weighing balance on the given days in the protocol.

Estimation of arthritis score

Arthritis score was given to rats according to the condition of severity of inflammation controls group of rats having no inflammation they given a score 0 for slight inflammation 1 score was given, score 2 was given for moderate inflammation and for high inflammation score 3 was given.

Estimation of hematological parameter

Blood samples were taken on 21st day via a retro-orbital puncture route in the end of the Protocol from various selected groups and specimens were forwarded to the laboratory to evaluate the parameters such as RBC, WBC, H.B, PLC, ESR, C-REACTIVE PROTEIN and a RA factor.

For the antioxidant estimation:

At the end of the protocol of 21 days, specimens of blood and paw of rats were separated and send to Ticken laboratory, New Delhi for the evaluation of enzymatic activities of SOD, CAT, GPx and GSH.[27]

Estimation of Biochemical parameters:

Blood Sample was taken from the retro-orbital route on the day 21 and forwarded to laboratory for the liver function testing by a parameter such as SGOT, SGPT, ALP, and total protein.

Histopathological study:

On day 21th one rat from each group was sacrificed by cervical dislocation method and the rat's paws were dissected by incision and taken as a tissue sample for the histopathology study.

Radiography of rats legs from knee to hind paws.

One rat from each group was sacrificed by cervical dislocation metho. Rat's legs, from the knee to the hind paw were dissected and taken out for an x-ray to evaluate the cartilage and bony destruction.[28]

Results:

Table: 1. Preliminary Phytochemical examination:

Sr. N0.	Test	HMELCI	HMEFCI	HMESCI
1	Alkaloids	+ve	+ve	+ve
2	Glycoside	+ve	+ve	+ve
3	Saponin	+ve	+ve	-ve
4	Flavonoids	+ve	+ve	+ve
5	Carbohydrates	+ve	+ve	+ve
6	Amino acids & protein	+ve	-ve	-ve
7.	Oils & fats	+ve	+ve	+ve

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8	Tannin & phenolic	+ve	+ve	-ve
9.	Sterols	+ve	+ve	+ve

Qualitative investigation by TLC:

TLC was done on these three parts of *C.indicum* Linn. using the selected solvent of Chloroform: Methanol: Ethyl acetate (7:3:3) by the help of standard book & hit and trial and the research paper in which solvent system for phytoconstituents of anti-arthritic activity are already mentioned. Examination of Rf values were done by using UV chamber under the Uv. light at the 365 nm. Rf value of HMELCI was 0.58. 0.42 for HMEFCI & 0.88 for the HMELCI.

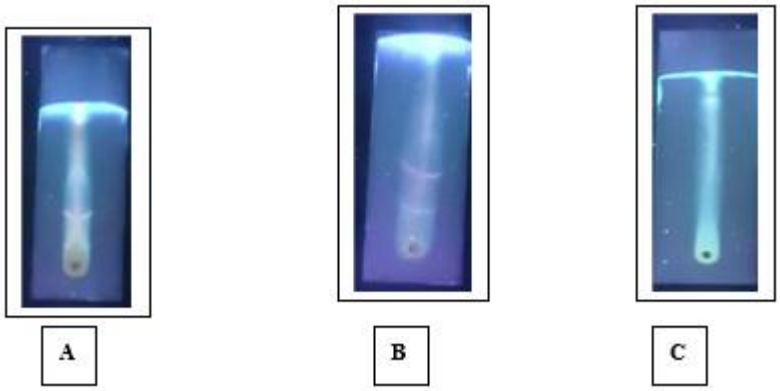

Fig:1. TLC plate for (A) HMELCI, (B) HMEFCI, and (C) HMESCI

Quantitative measurement of Total phenolic contents (TPC) and Total Flavonoid contents (TFC):

The Outcome of TPC for the (HMELCI) was obtained to 299.7 mg/gm dry weight of leaves then (HMEFCI) to 295.5 mg/gm dry weight of flower extract and 294 mg/gm dry weight of (HMESCI). TFC for the (HMELCI) was obtained to 394 mg/gm dry weight of leaves extract powder, 390.8 mg/gm dry weight of (HMELCI) and 348.8 mg/gm dry weight of (HMESCI)

Pharmacological Evaluation and Comparison:

Study for Acute Oral Toxicity:

When Selected extracts of various three parts of C. indicum have been given orally to selected female Wistar rats in increasing sequences of doses (5 mg/kg, 50 mg, 300mg/kg, and 2000mg/kg), no motility and mortality were seen. 400 mg/kg B.W. dose of these extracts was selected for the anti-inflammatory activities.

Comparison & Evaluation of Anti-inflammatory/ anti-arthritic Effect of HMELCI, HMEFCI & HMESCI on Physical parameters:
Effects on Paw Volume (PV):

Table.2: Comparison of Antiarthritic Effect of HMELCI, HMEFCI & HMESCI by measurement of rat paw volume (ml) in CFA induced arthritis.

Days	Group I	Group II	Group III	Group IV	Group V	Group VI
0th	1.05±0.12	3.20±0.34^{Z}	3.35±0.31	3.26±0.35	3.23± 0.31	3.21± 0.26
7th	1.11±0.15	3.43±0.22^{Z}	2.96±0.29	2.81±0.36	2.95±0.31	2.98±

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						0.25
14th	1.18±0.02	3.61±0.23^{Z}	2.18±0.20	2.15±0.34^{C}	2.56±0.30^{C}	2.71± 0.28^{C}
21st	1.18±0.18	3.95±0.15^{Z}	1.71±0.17	1.70±0.30^{C}	2.15±0.20^{C}	2.41± 0.25^{C}

Group I: Normal control group; Group II: Arthritic control group; Group III: Diclofenac treated group; Group IV: HMELCI; Group IV: HMEFCI; Group VI :HMESCI

The data represent as mean ± SD of 6 animals in each group ^{Z}p < 0.0001 compared to Normal control group. ^{c}p<0.001, compared with arthritic control group.

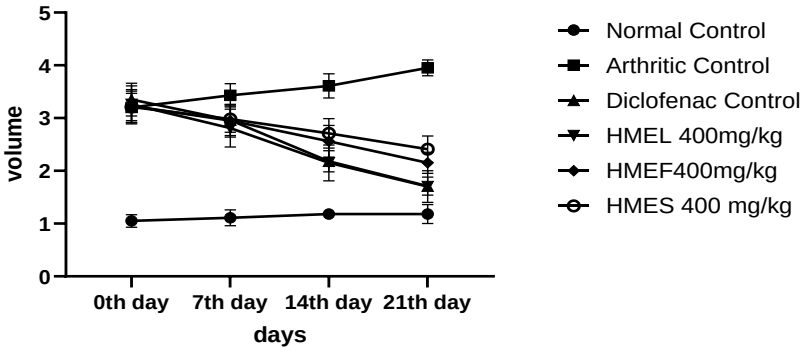

Figure.2: Effect of HMELCI, HMEFCI, and HMESCI on paw Volume

Description:

Various selected doses of extracts were found to be effective in CFA induced Inflammation after dosing with 400mg/kg Body weight Volumes were decreased by dosing with diclofenac and also with HMELCI, HMEFCI, and HMESCI. Leaves extract shows its effect to decrease paw volume (ml) 3.35±0.31 - 1.70±0.30 (ml) (p<0.001), flower extract showed their effect to decrease 3.23± 0.31- 2.15±0.20(ml) (P<0.001), and stem extract intended to decrease 3.21±0.26 - 2.41±0.25 (ml) (P<0.001).

Effects on Paw Diameter (PD) (mm):-

Table. 3 : Comparison of Antiarthritic Effect of Extract of Various Groups by Measurement of Paw Diameter in (mm):

Days	**Group I (NC)**	**Group II (CFA induced)**	**Group III (CFA+D.)**	**Group IV (CFA+ Leaves Extract)**	**Group V (CFA+ Flower Extract)**	**Group VI (CFA+ Stem Extract)**
0th	3.43±0.12	8.08±0.47^{Z}	8.20±0.20	8.14±0.42	8.19±0.46	8.30±0.55

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7th	3.48±0.23	8.31±0.41^{Z}	7.63±0.23	7.68±0.44	7.83±0.42	8.03±0.65
14th	3.43±0.11	8.8 ± 0.25^{Z}	6.94±0.21	6.99±0.41	7.42±0.35	7.71±0.63
21st	3.43±0.12	9.44±0.23^{Z}	5.81±0.17	5.96±0.42^{C}	6.77±0.35^{C}	7.20±0.57^{C}

Group I: Normal control group; Group II: Arthritic control group; Group III: Diclofenac treated group; Group IV: HMELCI; Group IV: HMEFCI; Group VI :HMESCI

The data represent as mean ± SD of 6 animals in each group ^{Z}p < 0.0001 compared to Normal control group. ^{c}p<0.001, compared with arthritic control group.

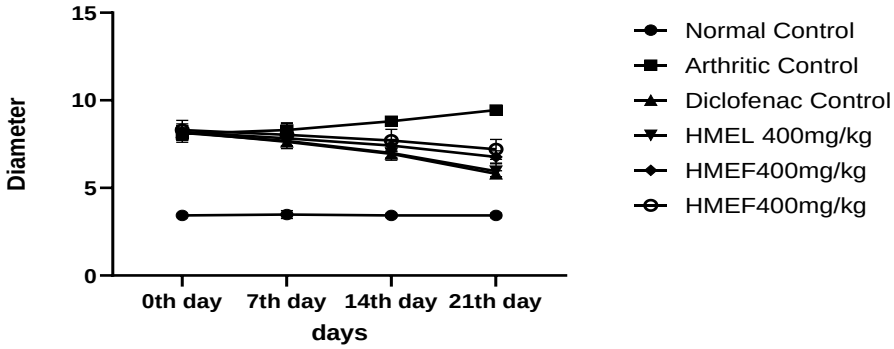

Figure.3: Effect of HMELCI, HMEFCI, and HMESCI on paw diameter (PD) (mm)

Description:

The outcome effect of the selected 400mg/kg dose of taken extracts was positive and they show a tendency to decrease PD. (PD) were decreased by dosing with diclofenac and also with a selected extract of *C.indicum.* (HMELCI) shows its effect to decrease 8.14±0.42 - 5.96±0.42(mm) (p<0.0001), (HMEFCI) showed their effect to decrease 8.19±0.46 - 6.77±0.35 (mm) (P<0.0001), and (HMESCI) intended to decrease 8.30±0.55 -7.20±0.57(mm) (P<0.001).

Effects on Body weight:

Table.4: Comparison of Antiarthritic Effect of Extract of Various Group by Measurement of Body Weight (gm)

Days	Group I	Group II	Group III	Group IV	Group V	Group VI
0th	126.6 ±2.35	151.66 ±3.72	121.66±41	115.83 ±3.43	123.33 ±4.71	109.16 ±3.43

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7th	127.5 ±2.50	148.33 ±3.72	124.16 ±3.43	119.16 ±4.48	125±2.88	110.83 ±3.43
14th	126.66 ±4.71	144.16 ±3.43	128.33 ±3.72	122.5 ±2.50	126.66 ±2.35	111.66 ±3.72
21st	128.33 ±3.72	139.16 ±4.48	131.66 ±4.71	126.66 ±2.35	129.16 ±1.86	113.33 ±2.35

Group I: Normal control group; Group II: Arthritic control group; Group III: Diclofenac treated group; Group IV: HMELCI; Group IV: HMEFCI; Group VI :HMESCI

The data represent as mean ± SD of 6 animals in each group $^{z}p < 0.0001$ compared to Normal control group. $^{c}p<0.0001$, compared with arthritic control group

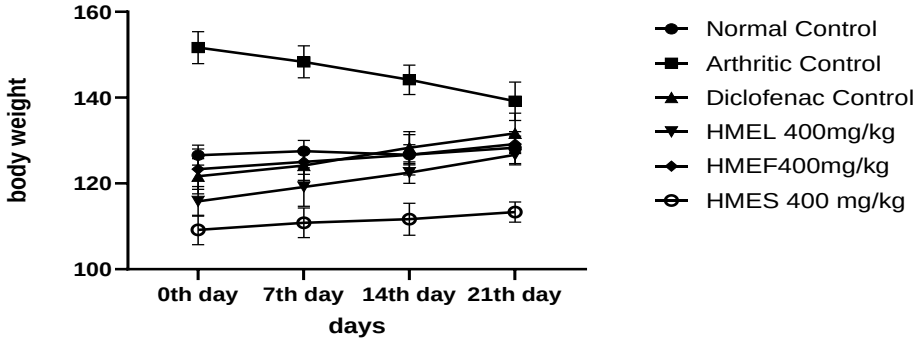

Figure.4: Effect of HMELCI, HMEFCI, and HMESCI on Body weight(gm)

Description:

The outcome effect of the selected 400mg/kg dose of taken extracts was positive and they show a tendency to increase body weight (in gm) in CFA induced Inflammation. Body weight were enhanced by dosing with diclofenac and also with a selected extract of *C.indicum.* (HMELCI) shows its effect to enhance, 115.83±3.43 - 126.66 ±2.35(gm) ($p<0.001$), (HMEFCI) showed their effect to enhance 123.33±4.71 - 129.16±1.86(gm) ($P<0.0001$), and (HMESCI) intended to enhance, 109.16±3.43 -113.33±2.35(gm) ($P<0.01$).

Arthritis score:

Table. 5: Arthritis score of various selected group in CFA induced model:

Days	Group I	Group II	Group III	Group IV	Group V	Group VI
0th	0	0	0	0	0	0
7th	0	2	2	2	2	2
14th	0	3	2	2	3	3
21st	0	3	1	1	2	2

Group I: Normal control group; Group II: Arthritic control group; Group III: Diclofenac treated group; Group IV: HMELCI; Group IV: HMEFCI; Group VI :HMESCI

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Description:

CFA induced groups of animals showed highly inflammatory like condition, therefore 3 Arthritis score was to that group on last day (21st day) of model. Medication with standard drug & (HMELCI) were provided 2. A score on last day due to exhibit high recovery and healing like condition. For the medication with (HMEFCI&HMESCI) group were given 2 arthritis score due to moderate recovery.

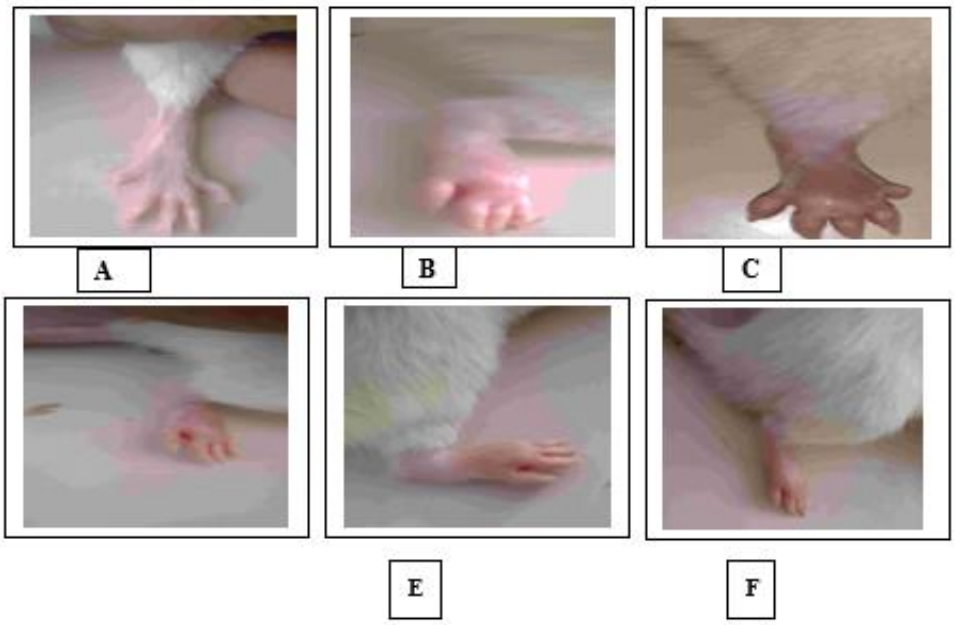

Fig 5.1: conditions of rats paw of various group on 0th day. A. Positive control, B.Inflammatory,C. Diclofenac treated, D. 400mg/kg HMELCI, E.400mg/kg HMEFCI, F.400mg/kg HMELCI

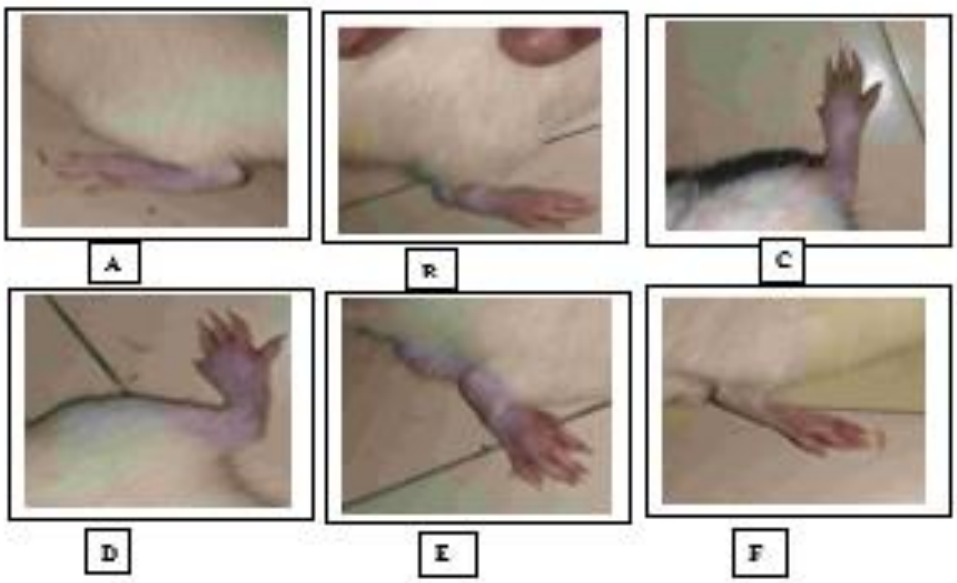

Fig 5.2: conditions of rat's paw of various group on 21st day. A. Positive control, B.InflammatoryC. Diclofenac treated, D. 400mg/kg HMELCI, E.400mg/kg HMEFCI, F.400mg/kg HMELCI

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Comparison & Evaluation of Effect of HMELCI, HMEFCI & HMESCI on hematological and biochemical components:

Table. 6: Comparison & Evaluation of Effect of HMELCI, HMEFCI & HMESCI on hematological parameters

Group	RBC (Mil. /mcL)	WBC (thousand /microlitre)	PLT (Lakh/Cub)	Hb (Gram/dl)	Rf Value (IU/mL)	CRP (mg/L)
Group I	5.8±0.42	6.2±0.45	2.84±0.34	13.98±0.22	0.00±0.00	1.42±0.22
Group II	4.1±0.30^{Z}	13.4±0.72^{Z}	8.8±0.24^{Z}	8.98±0.45^{Z}	48.06±0.22^{Z}	8.98±0.4^{Z}
Group III	6.4±0.46^{C}	8.2±0.32^{C}	3.85±0.45^{C}	14.02±0.28^{C}	29.34±0.18^{C}	2.98±0.3^{C}
Group IV	5.8±0.63^{C}	9.4±0.36^{C}	3.98±0.8^{C}	13.8±0.32^{C}	30.18±0.3^{C}	3.3±0.47^{C}
Group V	5.2±0.35	12.4±0.24	4.8±0.32	12.98±0.18	33.12±0.43	3.9±0.33
Group VI	5.1±0.25	11.4±0.35	4.22±0.43	12.45±0.35	34.4±0.45	4.02±0.38

Group I: Normal control group; Group II: Arthritic control group; Group III: Diclofenac treated group; Group IV: HMELCI; Group IV: HMEFCI; Group VI :HMESCI

RBC: Red blood cells; WBC: White blood cells; PLT: Platelet count; HB: Haemoglobin; Rf: Rheumatoid factor; CRP: C-reactive protein

The data represent as mean ± SD of 6 animals in each group ^{Z}p < 0.0001 compared to Normal control group. ^{c}p<0.001, compared with arthritic control group.

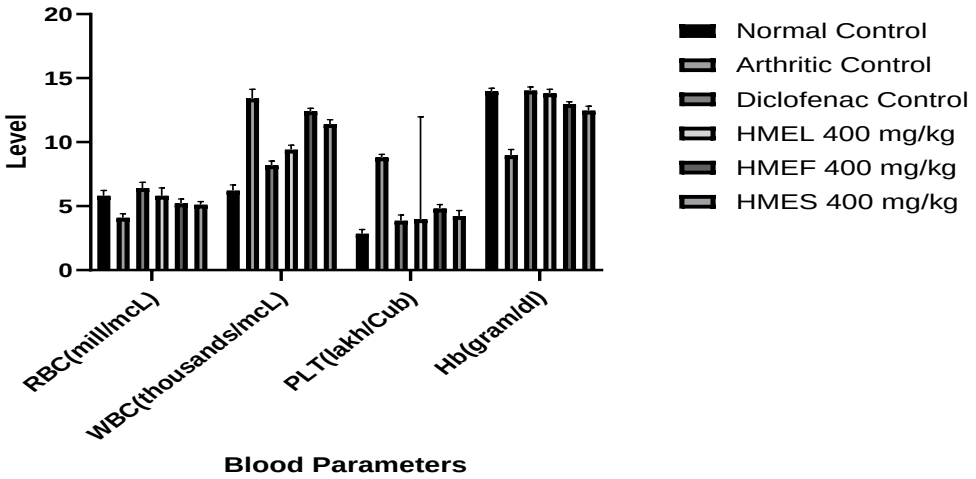

Figure.6: Effect of HMELCI, HMEFCI, and HMESCI on blood

Table. 7: Comparison & Evaluation of Effect of HMELCI, HMEFCI &HMESCI on biochemical parameters

Group	AST(IU/L)	ALT(IU/L)	ALP(IU/L)	Total Protein g/dL)	A/G ratio
Group I	148.12±1.18	30.42±0.45	70.42±1.08	13.98±0.22	1.28±1.08

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Group II	304.15±1.08^{Z}	104.4±0.88^{Z}	320.40±1.4^{Z}	8.98±0.45^{Z}	0.78±1.04^{Z}
Group III	120.54±1.11	58.84±0.34	90.45±0.96	14.02±0.28	1.22±1.05
Group IV	204.13±1.53^{C}	88.45±0.94^{C}	240.40±1.08^{C}	13.8±0.32^{C}	1.14±0.98^{C}
Group V	220.13±1.28	92.4±0.52	254.28±1.02	12.98±0.18	1.04±1.11
Group VI	228.24 ±1.11	98.46±0.72	288±1.16	12.45±0.35	1.02±0.46

Group I: Normal control group; Group II: Arthritic control group; Group III: Diclofenac treated group; Group IV: HMELCI; Group IV: HMEFCI; Group VI :HMESCI

AST: Aspartate phosphatase; ALT: Alanine amino-transferase; ALP: Alkaline phosphatase

The data represent as mean ± SD of 6 animals in each group ^{Z}p < 0.0001 compared to Normal control group. ᶜp<0.001, compared with arthrit control group.

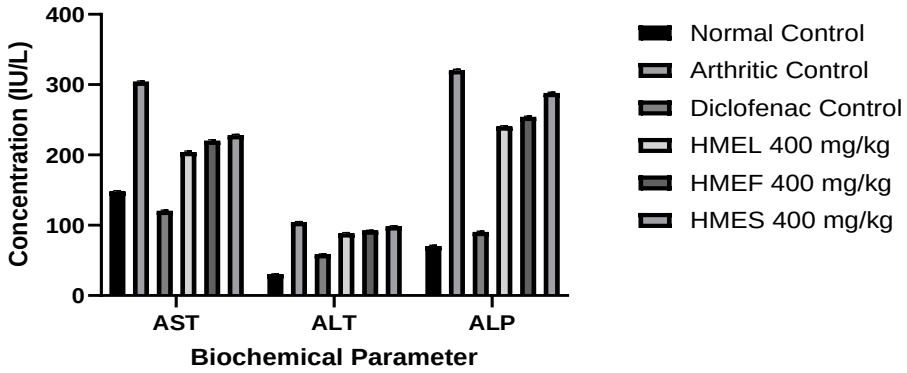

Figure.7: Effect of HMELCI, HMEFCI, and HMESCI on Biochemical parameter

Description:

In Fig.6, it can be seen that these selected 400mg/kg dose of extract helpful to enhance the RBC, Hb concentration and reduce the WBC, Platelets, Ra (RF) Value and CRP level in the CFA indued arthritic animals. In the animal, dosing with 400mg/kg BW HMELCI, RBC level (Mil. /mcL) enhanced 4.1±0.30 -5.8±0.63(p<0.001), Hb (in gm/dL) 8.98±0.45-13.8±0.32(P<0.001). WBC level (thousand /microlitre) 13.4±0.72-8.4±0.36(P<0.001), PLT level (Lakh/mcL) 8.8±0.243.98±0.8(P<0.001), Ra value (IU/mL) 48.06±0.22-30.18±0.3(P<0.001), CRP value (mg/L)8.98±0.4-3.3±0.47(P<0.001). HMEFCI RBC level was reached to 5.2±0.35(P<0.001), Hb was reached to 12.98±0.18(P<0.001), WBC was 10.4±0.24(P<0.001), platelet was 4.8±0.32(P<0.001), Ra value was 33.12±0.43(P<0.001), CRP was 3.9±0.33(P<0.001). Animals medicated with HMESCI RBC level was reached to 5.1±0.25(P<0.01), Hb level was 12.45±0.35(P<0.001)., WBC level was reached to 11.4±0.35(P<0.001)., PLT was 4.22±0.4, Ra value was obtained to 34.4±0.45(P<0.001)., CRP value was 4.02±0.38(P<0.001). Fig.7, help in comparison and evaluation of the effect of std drug, and 400mg/kg dose of various extract for biochemical components of the animals. Levels of SGPT, SGOT and ALP were increased due to reason of CFA induced inflammation. ALP was reached 70.42±1.08320.40±1.4,P<0.0001(U/L), ALT was reached, 30.42±0.45-104.4±0.88, (P<0.0001), (IU/L), AST was 148.12±1.18 -304.15±1.08, (P<0.0001), (IU/L). ALT of HMELCI showed the 88.45±0.94 (P<0.001), HMEFCI showed to 92.4±0.52 (P<0.001), the value was 98.46±0.72 (P<0.001) for HMESCI. ALP was seen to 240.40±1.08 (P<0.001), 254.28±1.02 (P<0.001), 288±1.16 (P<0.001) for the

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Extract of HMELCI, HMEFCI & HMESCI SGOT was seen to 204.13±1.53(P<0.001), 220.13±1.28(P<0.001),228.24 ±1.11 (P<0.001) for the Extract of Leaves, flowers and Stem.

Comparison & Evaluation of In vivo anti-oxidants Effect of HME of leaves, Flowers and stems on Enzymatic activity:

Table. 8: Antioxidant activity of selected extract on enzymatic activity:

Group	SOD (U/mg)	CAT (µmol/min/ mg)	GPx(µmol/min/ mg)	GSH (mM/100g)
Group I	11.4±0.4	43.5±0.6	13.3±0.5	32±0.28
Group II	$4.6±0.5^{Z}$	$28±0.2^{Z}$	$7.5±0.4^{Z}$	$9.8±0.8^{Z}$
Group III	11.3±0.6	40.3±0.8	12.38±0.38	29±0.3
Group IV	$10.8±0.2^{C}$	$39±0.7^{C}$	$12.2±0.2^{C}$	$28.4±0.4^{C}$
Group V	$8.1±0.3^{b}$	$35.5±0.6^{b}$	$9.8±0.2^{b}$	$21±0.2^{b}$
Group VI	$7.4±0.6^{b}$	$34±0.1^{b}$	$8.6±0.3^{b}$	$20±0.43^{b}$

Group I: Normal control group; Group II: Arthritic control group; Group III: Diclofenac treated group; Group IV: HMELCI; Group IV: HMEFCI; Group VI :HMESCI
SOD: Superoxide dimustase; CAT: catalase; GPx:Glutathione peroxidase

The data represent as mean ± SD of 6 animals in each group $^{Z}p < 0.0001$ compared to Normal control group. $^{c}p<0.0001$, $^{b}P< 0.001$, compared with arthritic control group.

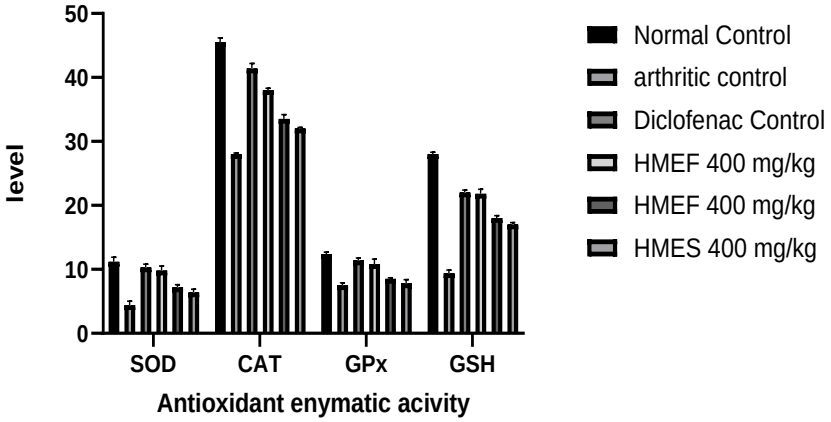

Figure.8: Effect of HMELCI, HMEFCI, and HMESCI on Antioxidant enzyme activity

Descriptions:

Table 8. Give the information that enzymatic activities of SOD, CAT, GPs and GSH in animal were reduced to 4.6±0.5 (P<0.0001), 28±0.2(P<0.0001), 7.5±0.4(P<0.0001),and 9.8±0.8(P<0.0001) after inflammatory induction.400mg/kg dose of HMELCI exhibit their effect for the SOD, CAT, GPXs and GSH to 10.8±0.2, 39±0.7, 12.2±0.2, 28.4±0.4 ;HMEFCI exhibit 8.1±0.3, 35.5±0.6, 9.8±0.2, 21±0.2. antioxidant activity of HMESCI was shown For SOD, CAT, GPs and GSH to 7.4±0.6, 34±0.1, 8.6±0.3, 20±0.43. Here, HMELCI show max. Antioxidant activity with compare to HMEFCI & HMESCI.

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Radiological Evaluation and Comparison:

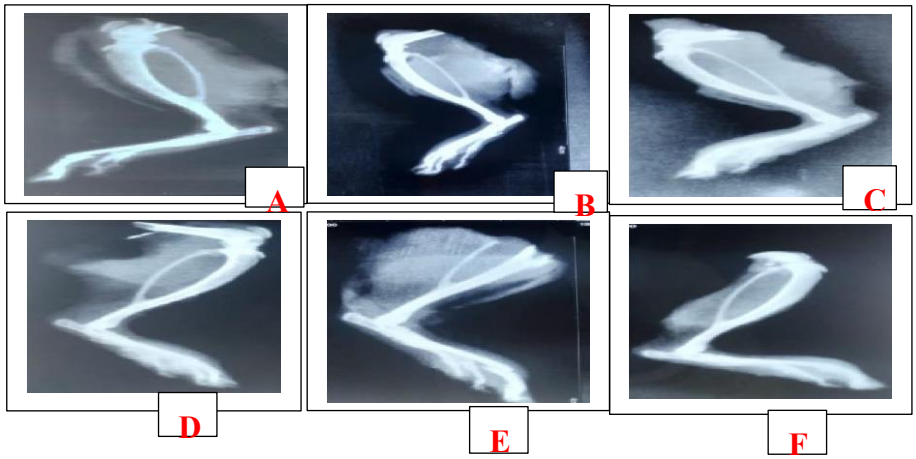

Fig.9: Xray report of CFA induced selected group of animals. A. Normal control, B. Arthritic control, C.CFA+10mg/kg Diclofenac., D. CFA+400mg/kg HMELCI, E. CFA+400mg/kg HMEFCI, CFA+ HMELCI.

Descriptions:

Fig, 9.0, show xray report for the effect of drugs in the joints and bones of animals. In positive control group, joint spaces, soft tissue, and size & shapes could be seen to normal, no erosion like condition could be seen (A). joint spaces seen to be closer, size of bone was altered, there are destruction can be seen in tarsals and metatarsals of the paw bone, soft tissue become more thick Arthritic control group (ICG). (B). standard medicated group show recovery as per standard with compare to ICG.(C) Leaves extract medicated group Exhibit recovery in CFA induced inflammation, similar to Diclo. medicated animals. joints of the tarsals and metatarsals could be seen in well define condition, no erosion like condition, size and mass of bone and soft tissues around the bone are normal (D). Leaves and (E) Stems extract, both also explained about recovery and effectivity but these are less effective to the leaves extract.

Histopathological Evaluation and Comparison:

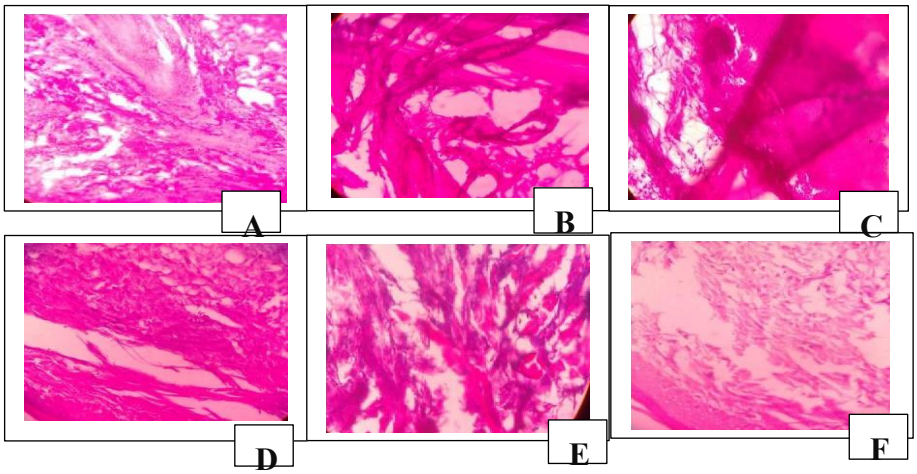

Fig.10: histopathological report of CFA induced selected group of animals. A. Normal control, B. Arthritic control, C. Diclofenac treated group, D. HMELCI, E. HMEFCI, (F) HMESCI.

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In Fig 10, The size of tissues and bone can be seen in normal conditions, (A). CFA induced inflammation exposed to show necrosis in the tissues, synovial cavity is packed with fluids. Concertation of macrophagic cells and fibrins was found to be enhanced out. (B). Diclo. medicated group possess its effectivity by improving the inflammation condition in reversing the inflammatory conditions. (C). show high effectivity in in recovering the inflammation and infection cause by CFA with compare to HMEFCI & HMELCI. Size of tissues was seen to be normal. Macrophagic cells, liquid in Synovial cavity and fibrin protein are found to normal conditions much similar to normal control. HMEFCI & HMELCI also show a tendency of recovery.

Discussion:

Inflammation is a self-defense and self-protecting physiological function for the survival of healthy life and to defend the cells and tissues of the host from external stimulus by activation of proinflammatory mediators any abnormality and defect in innate immune response led to acute and chronic inflammation.[29] Rheumatoid arthritis and other inflammatory diseases and disorders are one of the major world-wide problems of people in nowadays. This one is very common in females and old aged ones. Although so many synthetic and natural drugs are available but still new medicines and innovation in medication strategy are required for the medication and control from inflammatory disorders. Major problems with the synthetic medication are their define and undefine toxic effects. Therefore, herbal medication become more common as per need of people because rutin, gallic acid, quisqualic acid, quercetin, trigolleline and pelargonidin present in many of traditional plant and possess anti-inflammatory activity [30-31]

In this research, Leaves, Flowers and stems of *Combretum indicum* Linn have been selected for the comparative evaluation of effect of these parts. Rutin is major constituents of flavonoid category present in these parts of *C. indicum* and possess excellent anti-inflammatory activity [32]. In-vivo anti-inflammatory study was done by comparison and evaluation of anti-inflammatory effect of hydro-methanolic extract of these three parts by the help of CFA induced Inflammation model of 21 days in albino wistar rats. CFA is oily chemical, possess inflammatory reactions by boosting and activation of macrophases (proliferation of T cells) in the infected area and causes swelling and painful condition in animal.[33]

Preliminary phytochemical examination, TLC [17-18] and Quantitative analysis of TPC and TFC [16-19] confirmed the presence of various phytochemical required for the anti-inflammatory effect**.** No mortality and morbidity were seen after performing acute oral toxicity with dosing with max dose of 5000mg/kg as per OECD Guidelines**.**Therefore assuming, some level of safety and effectivity, 400mg/kg dose of hydro-methanolic extracts all parts of *C.indicum* were decided for dosing through route of oral cavity.[34]

Paw volume (PV) and Diameter (PD) were measured from 0^{th} to 21^{st} day of model and found to be reduce with the treatment of 400 mg/kg of HMELCI, HMEFCI, HMESCI with compare to Arthritic group of animals. These extracts show the tendency to reduce the CFA induced swelling and inflammatory condition. HMELCI (leaves extract) exhibit the max. improvement. CFA induced inflammation have tendency to disturb the proper metabolic process and reduced the body weight 400 mg/kg HMELCI, HMEFCI, HMESCI were shown to increase the body weight of animal's days by day (0^{th} to 21^{st}). 400 mg/kg HMELCI has found to show high effectivity with compare to HMEFCI, HMESCI.

The levels of WBCs, PLTs, Rf, CRPs in the blood increase due to activation of macrophagic cells and due activation of immunological reactions and the concentration level of RBCs and Hb are decrease in inflammatory conditions .400 mg/kg HMELCI, HMEFCI, HMESCI were shown to reduce these WBCs, PLTs, Rf, CRPs and shown to enhance the RBCs and Hb of animal's days by day (0^{th} to 21^{st}). 400 mg/kg HMELCI has found to show high effectivity with compare to HMEFCI, HMESCI. [35]

During the inflammatory condition in liver, infected cells and hepatocytes release the TNF-a and ROS (reactive oxygen species) which causes necrosis like conditions due to peroxidation of cell organelle and plasma which enhance the Level of SGOT & SGPT and ALP level in the blood. [] 400 mg/kg HMELCI, HMEFCI, HMESCI were shown to reduce these biochemical markers 400 mg/kg HMELCI has found to show high effectivity with compare to HMEFCI, HMESCI**.**[36]

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Body has tendency to protect the cells of the organ from released Free radicals due to any harmful factors and maintained the ROS. In inflammatory/arthritis disease, due to disturbance of maintained level of ROS in any organs, may damage the tissue of that organs. SOD have tendency to maintained the ROS by converting the free radicle of superoxide into the hydrogen peroxide. CAT help to maintain the detoxification process of Hydrogen peroxide and stimulate the destruction of hydrogen peroxide into safe inactive products. GPx have tendency to covert the lipid per oxide to non-reactive product and helpful in inhibiting the increasing the level of hydrogen peroxide. GSH revealed the defense mechanism and show protective effect against free radicals of outer cells. Level of these antioxidant protecting enzymes were reduced in the presence of CFA induced Inflammation. 400 mg/kg HMELCI, HMEFCI, HMESCI were shown to enhance this enzymatic activity of SOD, CAT, GPx and GSH. 400 mg/kg HMELCI has found to show high effectivity with compare to HMEFCI, HMESCI.[37]

CFA induced inflammation, have capacity to reducing the gap between bone of tarsals & metatarsales and causes destruction in tissue of small bone and enhance the thickness of soft tissue, present around the bones due to speed up the activity of fluid and WBCs in the inflamed area. 400 mg/kg HMELCI, HMEFCI, HMESCI were shown to improvement in these defects to previous and similar to the normal control animals. HMELCI found to show maximum recovery with compare to other. It shows their effect similar to the DMG.[38]

In Histopathology study, CFA induced inflammation exposed their activity by enhancing the movements of synovial fluids and macrophagic cells, near to injured areas. Concentration of a greater number of macrophagic cells can be seen in that area. 400 mg/kg HMELCI, HMEFCI, HMESCI were shown to recover inflammatory condition. Among all extract, HMELCI is highly effective.[39]

Conclusion:

Concluding the overall study of the comparative evaluation for the anti-inflammatory & antioxidant properties of leaves, flowers, and stems of *combretum indicum* linn, HMELCI, HMEFCI, HMESCI found to reduce the inflammation like condition significantly in selected animals. 21 days CFA induced inflammatory model was performed by taking 10 mg/kg diclofenac as standard drug and 400 mg/kg BW HMELCI, HMEFCI, HMESCI. Improvement was seen in all the defined parameters of Biochemical, physical and radiological and histopathological evaluation. Among all extract, HMELCI is highly effective then HMEFCI then HMESCI. This study will helpful in future research for the innovation and discovery of new herbal drugs and for new strategy of medication.

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References:

1. Antonelli, M. and Kushner, I. (2017), It's time to redefine inflammation. The FASEB Journal, 31: 1787-1791. https://doi.org/10.1096/fj.201601326R
2. Oronsky, B., Caroen, S., & Reid, T. (2022). What Exactly Is Inflammation *International Journal of Molecular Sciences*, *23*(23), 14905.
3. Kumar. Vinay, Abbas.K. Abdul, Fausto.Nelson, "Robins Basic Pathology", 8th Edition, 2007, Published by Saunders Elsevier Publication, page no. 24 to 52.
4. Rindfleisch, A. J., & Muller, D. (2005). Diagnosis and management of rheumatoid arthritis. *American family physician*, *72*(6), 1037-1047.
5. Mohan. Harsh, "Textbook of pathology", 7th edition, published by Jaypee brother medical publishers(p) ltd, page no 809 to 825.
6. Guo, Q., Wang, Y., Xu, D., Nossent, J., Pavlos, N. J., & Xu, J. (2018). Rheumatoid arthritis: pathological mechanisms and modern pharmacologic therapies. *Bone research*, *6*(1),
7. 'Tortora.Gehard.J.', 'Derrickson.Bryon', " Principles of Anatomy & Physiology", 15th edition, Published by wiley Publication, page no: 223 to 228.
8. Finckh, A., Gilbert, B., Hodkinson, B., Bae, S. C., Thomas, R., Deane, K. D., ... & Lauper, K. (2022). Global epidemiology of rheumatoid arthritis. *Nature Reviews Rheumatology*,

18(10), 591-602.

9. Guardia, T., Rotelli, A. E., Juarez, A. O., & Pelzer, L. E. (2001). Anti-inflammatory properties of plant flavonoids. Effects of rutin, quercetin and hesperidin on adjuvant arthritis in rat. *Il farmaco*, *56*(9), 683-687.
10. Kumar, S., Bajwa, B. S., Kuldeep, S., & Kalia, A. N. (2013). Anti-inflammatory activity of herbal plants: a review. *Int J Adv Pharm Biol Chem*, *2*(2), 272-281.
11. Sahu, J., Patel, P. K., & Dubey, B. (2012). Quisqualis indica Linn: A review of its medicinal properties. *Int. J. Pharm. Phytopharmacol. Res*, *1*(5), 313-321.
12. Kaur, A., Nain, P., & Nain, J. (2012). Herbal plants used in treatment of rheumatoid arthritis: a review. *Int J Pharm Pharm Sci*, *4*(4), 44-57.
13. Dey, Y. N., Ota, S., Srikanth, N., Jamal, M., & Wanjari, M. (2012). A phytopharmacological review on an important medicinal plant-*Quisqualis indica*. *Ayu*, *33*(1), 27
14. Jaiswal, falguni & awani, rai & wal, pranay & wal, ankita & singh, shashi. (2021). To evaluate the anti-arthritic and immunomodulatory activity of hydroalcoholic and methanolic extract of quisqualis indica linn in rats. European journal of pharmaceutical and medical research. 8. 478.
15. Khandelwal K.R. "Practical Pharmacognosy technique and experiment" Nirali prakashan (2010).
16. Dutta, A., Biswas, S., Biswas, M., Ghosh, P., Ghosh, C., Das, S., & Chatterjee, S. (2019). Phytochemical screening, anti-oxidant and anti-microbial activity of leaf, stem and flower of Rangoon creeper: a comparative study. *Journal of Medicinal Plants Studies*, *7*, 123-130.
17. Anjaliv, P., Vivek, J., Jigar, P., Keyuri, B., Vaibhavi, S., Jayesh, D., ... & Devang, P. (2023). Establishment of quality parameters of Quisqualis indica leaves through sophisticated analytical techniques. *Bulletin of the Chemical Society of Ethiopia*, *37*(2), 289-298.
18. Chatwal, R Gurdeep, Anand. K. Sham., "Instrumental method chemical analysis" Himalaya publishing house, fifth edition (2018), page no. 256 to 298.
19. Chandra, S., Khan, S., Avula, B., Lata, H., Yang, M. H., ElSohly, M. A., & Khan, I. A. (2014). Assessment of total phenolic and flavonoid content, antioxidant properties, and yield of aeroponically and conventionally grown leafy vegetables an fruit crops: A comparative study. *Evidence-based complementary and alternative medicine*, *2014*.
20. OECD. Guidelines 423: Acute Oral Toxicity-Acute toxic class method. "OECD Guidelines for Testing chemicals (2001).
21. 'Kulkarni.S.K. "Handbook of experimental pharmacology", Published by Vallabh Prakashan.
22. Asquith, D. L., Miller, A. M., McInnes, I. B., & Liew, F. Y. (2009). Animal models of rheumatoid arthritis. *European journal of immunology*, *39*(8), 2040-2044.
23. Perumal, S. S., Ekambaram, S. P., & Dhanam, T. (2017). In vivo antiarthritic activity of the ethanol extracts of stem bark and seeds of Calophyllum inophyllum in Freund's complete adjuvant induced arthritis. *Pharmaceutical biology*, *55*(1), 1330-1336.
24. Kadhem, M. A. (2016). Anti-Arthritic activity of ethanolic extract of Lawsonia inermis in Freund'S adjuvant induced arthritic rats. *IOSR J Agric Vet Sci*, *9*, 1-6.
25. Vogel.H.gerhard, " Drug Discovery and Evaluation", second edition, 20022, springer Publication, page No: 1162 to 1164.
26. Mondal, P., Das, S., Mahato, K., Borah, S., Junejo, J. A., & Zaman, K. (2016). Evaluation of anti-arthritic potential of the hydro-alcoholic extract of the stem bark of Plumeria rubra in freund's complete adjuvant-induced arthritis in rats. *International Journal of Pharmaceutical Sciences and Research*, *7*(9), 3675.
27. Alam, M. N., Bristi, N. J., & Rafiquzzaman, M. (2013). Review on in vivo and in vitro methods evaluation of antioxidant activity. *Saudi pharmaceutical journal*, *21*(2), 143152.
28. Kaushik, S., Jain, P., Satapathy, T., Purabiya, P., & Roy, A. (2021). Evaluation of antiarthritic and anti-inflammatory activities of Martynia annua L. Ethanolic extract. *Clinical Phytoscience*, *7*, 1-11.
29. V. Krishna, "Textbook of pathology", 2004, published by orient Longman publication, page

no 56 to 75.
30. Kirtikar K. R and Basu B.D. “Indian medicinal plant”, Vol. IV, International book distributers (2007): 2609 to 2610.
31. Vivek-Ananth, R. P., Mohanraj, K., Sahoo, A. K., & Samal, A. (2023). IMPPAT 2.0: an enhanced and expanded phytochemical atlas of Indian medicinal plants. *ACS omega*, *8*(9), 8827-8845.
32. Rastogi Ram.P. and Mehrotra B.N. “Compendium of Indian medicinal plants”, vol II, central Drug Research Institute and national Institute of Science Communication and Information Resources (1991):40.
33. Yende, S. R., Sannapuri, V. D., Vyawahare, N. S., & Harle, U. N. (2010). Antirheumatoid activity of aqueous extract of Piper longum on freunds adjuvant-induced arthritis in rats. *Int J Pharm Sci Res*, *1*(9), 129-133.
34. Erhirhie, E. O., Ihekwereme, C. P., & Ilodigwe, E. E. (2018). Advances in acute toxicity testing: strengths, weaknesses and regulatory acceptance. *Interdisciplinary toxicology*, *11*(1), 5–12. https://doi.org/10.2478/intox-2018-0001
35. Ekambaram, S., Perumal, S. S., & Subramanian, V. (2010). Evaluation of antiarthritic activity of Strychnos potatorum Linn seeds in Freund's adjuvant induced arthritic rat model. *BMC complementary and alternative medicine*, *10*, 56. https://doi.org/10.1186/1472-6882-10-56
36. Li, Y., Kakkar, R., & Wang, J. (2018). *In vivo* and *in vitro* Approach to Anti-arthritic and Anti-inflammatory Effect of Crocetin by Alteration of Nuclear Factor-E2-Related Factor 2/hem Oxygenase (HO)-1 and NF-κB Expression. *Frontiers in pharmacology*, *9*, 1341. https://doi.org/10.3389/fphar.2018.01341
37. Devi, P & Kumari, S & Kokilavani, C. (2007). Effect of Vitex negundo leaf extract on the free radical scavengers in complete Freund's adjuvant induced arthritic rats. Indian journal of clinical biochemistry: IJCB. 22. 143-7. 10.1007/BF02912899.
38. Guha, Pokhraj & Paul, Subhashis & Das, & Halder, Biswajit & Bhattacharjee, Soumen & Chaudhuri, Tapas. (2014). Analyses of human and rat clinical parameters in Rheumatoid Arthritis raise the possibility of use of crude Aloe vera gel in disease amelioration. Immunome Research. 10. 10.4172/17457580.1000081.
39. umapathy, Snekhalatha & Mariamichael, Anburajan & Balasubramaniam, Venkatraman & Menaka, M.. (2013). Evaluation of complete Freund’s adjuvant-induced arthritis in a Wistar rat model. Zeitschrift für Rheumatologie. 72. 10.1007/s00393-012-1083-8.