ABSTRACT

Zingiberaceae members are well known medicinal herbs in all systems of traditional medicine. Turmeric is one of the important ingredient in cultural, medicinal and in industrial use especially in India. Mainly Curcuma longa (Turmeric) is a major spice in every dish of Indian food. Apart from culinary uses its medicinal significance as antibiotic, antipyretic against cold and cough, antiarthritic, antidiarrhoeal and also as antidiabetic. Wild turmeric C. neilgherrensis was distributed along the Seshachalam hill regions of Tirumala, Kadapa and in Nellore districts. Many tribes like Yanadi, Erukula, Nakkala and Irula tribes were habituated along these hill regions. The significant traditional medicinal uses of C.neilgherrensis rhizome, root and leaf extracts were tested scientifically and proved its antimicrobial, phytochemical, antidiarrhoeal, antidiabetic, antiulcer, anthelmintic and anti-inflammatory activities. Arthritic problems are one among the major health disorders proven to be multiple reasons and living styles all over the globe. Majority of the chemical drugs are only suppressants than the curative effects. Hence search for alternate source leads for the natural plant based drugs. Rhizome extracts of C.neilgherrensis were tested against acetic acid induced analgesic rats with aqueous and methanol extracts between 100-1000 gms/kg b.wt and found effective in reducing the mean writhing effects and inhibition percentage at 500 mg by 9.1 with 76.24% which is almost equal to the Diazepam treated rats showing 8 number of writhings with 79.11% inhibition of analgesic effect. Hence rhizome extracts also screened and found as effective against CFA induced arthritic rats with aqueous and methanol extracts at 500 mg/kg b.wt and observed the reduction in the volume of rat hind paw to that of normal rats and standard drug Diclofenac treated rats than the CFA induced arthritic rats. And also there is no any negative effect on the body weight loss or gain than the normal rats. It also proved in maintaining the haematological and biochemical
parameters which were affected in the arthritis rats also observed in the radiological studies in the protection and prevention of inflammation. The same was observed with the rhizome extracts of *Alpinia galanga*, *C.longa*, *C.zedoaria* and with *Zingiber officinale*. Hence *C.neilgherrensis* rhizome crude extracts may be recommended as analgesic and anti-arthritic drug to that of the other Zingiberaceae species which possessed curcuminoids and essential oils.

**KEY WORDS:** Wild turmeric, acetic acid induced, CFA induced, haematological, biochemical, radiological, diclofenac.

**INTRODUCTION**

Rheumatoid arthritis (RA) is one of the major autoimmune disease, which is associated with systematic inflammatory disorders that are characterized by progressive joint pain, destruction and deformity of joints.[1-2] It has worldwide prevalence of about 1% of the adult population. It affects woman more than men with an annual incidence of 3 per 10,000 adults between 20-45 years.[3-4]

Recently, it has been reported that toxins of bacterial and fungal pathogens may exacerbate the inflammatory response of the joints and bones. *Mycobacterium tuberculosis* and *M. leprae* are the most severe and most common bacteria that causes joint and bone diseases.[5] The radiographic studies of adjuvant-induced arthritic animals have shown pathological bony changes similar to the changes observed in clinical RA conditions.[6-8] The main categories of drugs that are used to treat rheumatoid arthritis are analgesics, Disease-Modifying Antirheumatic Drugs (DMARDs), Non-Steroidal Anti-Inflammatory Drugs (NSAIDs), corticosteroids and immunosuppressive drugs.[9-10]

Turmeric has medicinal association in the day-to-day life as a wound healer, against stomachache, flatulence, poisonous insect/ snake/ scorpion bite, ulcers, common cold, pimples, bronchitis, anti inflammatory, antidiabetic, sinusitis, eye diseases; dye yielding and in also worshipping god. Later on this plant has acquired great importance all over the world with its Antiaging, Anticancer, Anti-Alzheimer Antioxidant, Antimicrobial, Anti inflammatory and also a variety of other medicinal properties.[11] In the traditional veterinary medicine *Curcuma* plays an important role on the rural poultry, treating skin diseases of camel and buffalo,[12], Mastitis in cattle.[13] *C.aromatica* extracts shows effective anti-inflammatory activity on cattle.[14] Turmeric poultice is applied on broken legs of chicken and
domestic animals[15]; Used to cure Raniket disease of Birds, prevent hair fall, scabies, heal cuts, wounds of ring worm infection, itching, eczema, boils, urticaria and chronic skin eruptions of domestic animals.[16]

Chemo protective role of Curcumin in human colon cancer.[17] Essential oil of turmeric is found to be effective in Ayurvedic medicine of Indian system.[18] C. longa is the major source of Curcuminoids and volatile oils.[19] Curcumin also inhibits sperm motility and acts as novel intravaginal contraceptive and antidiabetic.[20-21] The main bioactive constituents of Curcuma species are volatile oils consisting of Curcuminoids and Oleoresins. The crude form of turmeric powder, fresh ground turmeric with the water; ether, chloroform and methanolic extracts plays an important role in the bioprotective activity of various ailments.[22]

Phenolic gingerols and related compounds are responsible for the pungent taste of ginger, have been a major focus of research related anti-inflammatory activity.[23-29] The ability of the individual pure compounds was observed to inhibit COX-1 and/or COX-2 activity in-vitro studies. Ginger use for arthritis treatment is being promoted in the United States.[30-32] Primarily in western societies for its antiemetic and carminative uses, ground ginger has been used. Also used as anti-inflammatory drug.[27, 33]

Hence, there is an immense need to find out the antiarthritic herbal drugs as that of turmeric and ginger. Curcuma neilgherrensis is a wild turmeric rhizome paste along with white egg, black gram and onion is used to cure bone fractures, inflamations and chronic inflammations (arthritis) by the tribes and herbalists of Talakona area along the Seshachalam hills. Rhizome powder paste is boiled and applied on the rheumatic swellings. Powder mixed with gingelly or coconut oil used to massage around the arthritic pains. A pinch of powder taken orally with hot milk for one week against bronchial disorders. Steaming with half spoon rhizome powder along with camphor to avoid cold and cough. A pinch of rhizome powder with water daily in the morning with an empty stomach to overcome diabetic conditions.[34] The present study aimed on the clinical evaluation of rhizome extracts against Complete Freund’s Adjuvant (CFA) induced arthritis rats has been carried out to prove its herbal uses more effectively against arthritic disorders.

MATERIAL AND METHODS
Material Collection: C. neilgherrensis rhizomes were collected during June -September from Talakona forest area of Seshachalam Hills. The taxonomical identity of the plant was
determined and authenticated the voucher specimens DC: 921, DC: 922 by Dr. N. Yasodamma, deposited in the Herbarium, Department of Botany (SVUTY) as per the standard method.[38] Rhizomes are thoroughly washed and dried under shade at 28 ± 2°C for about 30 days. The dried material was ground well into fine powder in a mixer grinder and sieved to give particle size of 50-150mm. The powder was stored in air sealed polythene bags at room temperature until further use.

**Extract preparation:** Shade dried rhizome powder was subjected to soxhlet extraction with methanol. Simultaneously aqueous extract also prepared. The above obtained semisolid extracts were preserved in air tight bottles at 4°C until further use.

**Animal selection:** For antiarthritic activity male Wistar albino rats weighing between 150 - 250 g were purchased from Sri Venkateswara Traders, Bangalore. The animals were acclimatized to standard laboratory conditions (temperature 25±2°C) and maintained on 12 hours light; 12 hours dark cycle. They were fed with ad libitum. The experiment was conducted according to the ethical norms approved by Institutional Animal Ethical Committee (CPCSEA/IAEC/ SVU/NY-BK/dt: 19/11/2011).

**Acute oral toxicity study (AOT):** Acute oral toxicity study was performed as per Organization for Economic Co-operation and Development (OECD-425) guidelines. Wistar rats (n=6) were used for the study. The animals were kept fasting for overnight providing only water, after which the leaf and rhizome aqueous and methanol extracts were administered orally at the dose level of 1000, 2000, 3000 and 5000 mg/kg body weight by intragastric tube and animals were observed individually after dosing at least once in 30 min during the first 24 hrs, with special attention given during the first 4 hrs, and daily thereafter, for a total of 14 days. Observations also made on the changes of skin, fur, eyes, behaviour pattern, body weight, food consumption and fluid intake were recorded daily. If mortality was observed in 2-3 animals, then the dose administered was assigned as toxic dose. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. Mortality was observed in 50% of the animals and then the LD$_{50}$ was calculated. If no mortality then it is considered of there is no toxicity and the 1/10$^{th}$ (500 mg) and 1/5$^{th}$ (1000 mg) as LD$_{50}$ value 5000 mg/kg b.wt. If the toxicity is seen it has been considered as the 1/25$^{th}$ and 1/100$^{th}$ of LD$_{50}$ value has to be considered as recommended safe doses for experimental studies to be evaluate as the standard drug doses. All the observations were made as per the OECD Guidelines and standard methods.[36-37]
ANALGESIC ACTIVITY

Acetic acid induced writhing method: Peripheral analgesic activity was evaluated using acetic acid-induced writhing test. In this method albino Wistar rats (Male) were divided in to fourteen groups. The group I represents control rats received 2% Tween 80 (10 ml/kg p.o). Group II received the standard drug diazepam at a dose of 10mg/kg. Group III-XIV received the rhizome aqueous and methanol extracts at dose levels 100, 250, 500 and 1000 mg/kg, each respectively by oral route. All the groups received 1 ml of 0.7% Acetic acid administrated intraperitoneally to the experimental rats to create pain sensation. Then, the rats were observed for specific contraction of body referred as 'writhing' for the next 20 min. Full writhing was not always completed by the animal, because sometimes the animals start to give writhing, but they do not finish it. This incomplete writhing was taken as half-writhing. Accordingly, two half-writhings were considered as one full writhing. The number of writhes in each treated group was compared to that of control group and Diazepam (10 mg/kg) a reference drug (positive control) treated rats analgesic activity.

ANTI-ARTHRITIC ACTIVITY

Complete Freund’s adjuvant (CFA) induced arthritis

Complete Freund’s adjuvant was used to induce arthritis in rats to investigate the anti-arthritic effect of aqueous and methanol extracts of rhizome. Albino Wister rats (Male) were divided in to nine groups. The group I represented normal rats given normal food and water. The group II represents control arthritic rats received 2% Tween 80 (10ml/kg p.o). Group III arthritic rats received the standard drug Diclofenac sodium at a dose of 50mg/kg. Group IV-IX arthritic rats received the aqueous and methanol rhizome extracts at daily doses of 250, 500 and 1000 mg/kg upto 21st day by oral route after 30 min of 0.1ml Complete Freund’s Adjuvant (CFA) (Sigma, U.S.A) injected in to the sub plantar region of right hind paw.[38-39]

Anti-arthritic effect of the extracts as well as Diclofenac sodium was evaluated by measuring injected paw volume on 0th, 7th, 14th and 21st day by using sliding Vernier calipers. The mean change of injected paw volume with respect to initial paw volume was calculated and also compared with control and standard drug treated rats and calculated the anti-arthritic effect.

Changes in body weights: The weight of each rat was recorded on day 1 and at weekly intervals (1, 7, 14 and 21st day) throughout the course of the study and calculated the mean change in the growth of body weights.[40-41]
Blood sample collection

On 21\textsuperscript{st} day to evaluate haematological and biochemical studies blood samples were collected between 8.00 -10.00am by the ophthalmic venous plexus located in the orbital sinus of the rat using a micro-capillary pipette. About 1ml of blood was collected from each rat into a labelled clean sample bottles containing 1ml of EDTA solution as anti-coagulant and without EDTA containing bottles separately.\textsuperscript{40-41}

Haematological parameters

Haematological analysis such as RBC (Red Blood Cells), WBC (White Blood Cells), Hb (Haemoglobin) and ESR (Erythrocyte Sedimentation Rate) were performed in total blood using Routting method.\textsuperscript{42}

Biochemical parameters

Blood collected into the sample tubes without EDTA solution was allowed to clot before centrifugation at 3000rpm for 10min. to obtain serum. Serum was separated and used for the bio-chemical analysis such as total protein and albumin \textsuperscript{43}, creatinine \textsuperscript{44}, BUN (Blood Urea Nitrogen) \textsuperscript{45}, ALP (Alkaline Phosphatase), AST (Aspertate Amino Transferase) \textsuperscript{46} and ALT (Alanine Amino Transferase).\textsuperscript{47}

Radiological Data

On the 21\textsuperscript{st} day the experimental animals were anesthetized with diethyl ether and placed in digital x-ray machine for the radiographic analysis of the joints. X-ray was taken at the joints for the confirmation and evaluation of the severity of arthritis in CFA induced rats and in drug treated rats.\textsuperscript{48-49}

Statistical analysis

All the data are expressed as mean ± SD. The data obtained from the various groups were statistically analyzed using \textbf{one way ANOVA} followed by Dunnett’s test. These values $p^*<0.05$ (significant); $p^{**}<0.01$ (more significant); $p^{***}>0.05$ (not significant) were considered to indicate a significant difference between the groups.
RESULTS

ANALGESIC ACTIVITY

Table 1: Effect on Acetic acid induced writhing activity

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Dose (mg)</th>
<th>Mean Writhings (30 min)</th>
<th>Inhibition %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10 ml</td>
<td>38.3±0.47</td>
<td>-</td>
</tr>
<tr>
<td>Diazepam</td>
<td>10</td>
<td>8.0±0.0**</td>
<td>79.11%</td>
</tr>
<tr>
<td>Aqueous</td>
<td>100</td>
<td>14.2±0.04**</td>
<td>62.92%</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>12.2±0.08**</td>
<td>68.14%</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>9.1±0.12**</td>
<td>76.24%</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>7.3±0.12**</td>
<td>80.93%</td>
</tr>
<tr>
<td>Methanolic</td>
<td>100</td>
<td>15.4±0.09**</td>
<td>59.79%</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>13.2±0.04**</td>
<td>65.53%</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>11.7±0.08**</td>
<td>69.45%</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>9.7±0.04**</td>
<td>74.67%</td>
</tr>
</tbody>
</table>

All the data are expressed as mean ± SEM, n=6, * p<0.05 and **p<0.01 when compared with control group; One way ANOVA followed by Dunnett’s test

Effect on acetic acid induced Analgesic activity (Table-1; Figure-1, 2)

Analgesic activity of aqueous and methanol extracts of rhizome between 250 to 1000 mg showed more effective equally to that of diazepam at 10 mg with 8.0 writhings showed 79.11% of activity compare to that of control acetic acid induced rats 38.3 number of writhings. Aqueous extracts treated between 250-1000 mg showed with 12.2 to 7.3 writhings ranging 68.14 to 80.93%; methanol extracts with 13.7 to 9.7 writhings ranging 65.53 to 74.67% of inhibition of pain was observed.
ANTIARTHRITIC ACTIVITY

Table 2: Effect on Paw volume of Arthritic rats (mm & %)

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Dose</th>
<th>0th</th>
<th>7th</th>
<th>14th</th>
<th>21st</th>
<th>% of Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td></td>
<td>3.1 ± 0.03</td>
<td>3.1 ± 0.03</td>
<td>3.1 ± 0.03</td>
<td>3.1 ± 0.03</td>
<td>-</td>
</tr>
<tr>
<td>Control (CFA)</td>
<td>100</td>
<td>7.5 ± 0.01**</td>
<td>6.6 ± 0.03**</td>
<td>4.4 ± 0.03**</td>
<td>3.3 ± 0.03**</td>
<td>60.71</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>250</td>
<td>7.6 ± 0.03**</td>
<td>5.5 ± 0.04**</td>
<td>4.2 ± 0.01**</td>
<td>3.7 ± 0.03**</td>
<td>55.95</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>7.5 ± 0.03**</td>
<td>5.2 ± 0.01**</td>
<td>4.7 ± 0.01**</td>
<td>3.2 ± 0.03**</td>
<td>61.90</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>7.0 ± 0.03**</td>
<td>5.2 ± 0.03**</td>
<td>4.1 ± 0.03**</td>
<td>3.1 ± 0.01**</td>
<td>63.09</td>
</tr>
<tr>
<td>Aqueous extract</td>
<td>250</td>
<td>7.4 ± 0.01**</td>
<td>5.2 ± 0.03**</td>
<td>4.1 ± 0.03**</td>
<td>3.4 ± 0.03**</td>
<td>59.52</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>7.1 ± 0.08**</td>
<td>5.1 ± 0.04**</td>
<td>3.8 ± 0.03**</td>
<td>3.2 ± 0.01**</td>
<td>61.90</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>6.7 ± 0.01**</td>
<td>4.8 ± 0.01**</td>
<td>3.6 ± 0.01**</td>
<td>3.1 ± 0.01**</td>
<td>63.09</td>
</tr>
<tr>
<td>Methanolic extract</td>
<td>250</td>
<td>7.4 ± 0.01**</td>
<td>5.2 ± 0.03**</td>
<td>4.1 ± 0.03**</td>
<td>3.4 ± 0.03**</td>
<td>59.52</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>7.1 ± 0.08**</td>
<td>5.1 ± 0.04**</td>
<td>3.8 ± 0.03**</td>
<td>3.2 ± 0.01**</td>
<td>61.90</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>6.7 ± 0.01**</td>
<td>4.8 ± 0.01**</td>
<td>3.6 ± 0.01**</td>
<td>3.1 ± 0.01**</td>
<td>63.09</td>
</tr>
</tbody>
</table>

All the data are expressed as mean ± SEM, n=6, * p<0.05 and **p<0.01 when compared with control group; One way ANOVA followed by Dunnett’s test.

Figure-3: Effect of on Paw Volume of Arthritic Rats (mm)

Nor: Normal; Con: Control; Dic: Diclofenac; Aq: Aqueous; Me: Methanol

Table 3: Effect on body weights in arthritic rats (g/rat)

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Dose</th>
<th>0th</th>
<th>7th</th>
<th>14th</th>
<th>21st</th>
<th>Change in b.wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td></td>
<td>179.3 ± 0.19</td>
<td>186.0 ± 0.33</td>
<td>192.0 ± 0.33</td>
<td>217.3 ± 0.19</td>
<td>38.0</td>
</tr>
<tr>
<td>Control -CFA</td>
<td>100</td>
<td>180.0 ± 0.33**</td>
<td>190.3 ± 0.19**</td>
<td>202.0 ± 0.33**</td>
<td>209.3 ± 0.19**</td>
<td>29.0</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>250</td>
<td>178.6±0.19</td>
<td>183.3±0.19**</td>
<td>186.0±0.33**</td>
<td>198.6±0.38**</td>
<td>20.0</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>181.6±0.19**</td>
<td>193.0±0.33**</td>
<td>203.3±0.38**</td>
<td>216.6±0.38**</td>
<td>30.0</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>180.6±0.19**</td>
<td>197.0±0.33**</td>
<td>206.3±0.38**</td>
<td>216.0±0.33**</td>
<td>35.4</td>
</tr>
<tr>
<td>Aqueous extract</td>
<td>250</td>
<td>182.3±0.19**</td>
<td>190.6±0.38**</td>
<td>201.3±0.19**</td>
<td>211.1±0.34**</td>
<td>28.8</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>183.0±0.33**</td>
<td>195.0±0.33**</td>
<td>206.0±0.33**</td>
<td>217.3±0.19**</td>
<td>34.3</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>179.3±0.19*</td>
<td>193.0±0.33**</td>
<td>208.0±0.33**</td>
<td>216.3±0.19**</td>
<td>37.0</td>
</tr>
</tbody>
</table>

All the data are expressed as mean ± SEM, n=6, * p<0.05 and **p<0.01 when compared with control group; One way ANOVA followed by Dunnett’s test.
Plate-1: Anti-arthritic Activity of *C. neilgherrensis* Rhizome Aqueous and Methanol Extracts

Aqueous Extracts

- **A**: 250 mg; **B**: 500 mg; **C**: 1000 mg

Methanolic extracts ranging from 250-1000 mg/kg b.wt showed decreased arthritic effect 3.7 to 3.1 mm of paw volume dependent on drug concentration when compared to the CFA induced arthritis 8.4mm paw volume slowly by 21st day equally to that of the normal rats 3.1 mm. The standard drug Diclofenac treated rats showed 3.3 mm with 63.09 % of inhibition which is equal with methanol extracts 3.1mm; 60.71% and with aqueous extracts treated rats 3.7mm paw volume with 60.7 % of arthritis inhibition was observed.
Effect on Body weights of arthritic rats: (Table- 3; Figure-5, 6)
There is no effect on body weights and growth of the animals and also there is no any behavioral changes on rats with both the extracts upto 1000 mg. Methanol extract treated rats after 21\textsuperscript{st} day showed the gain in the body weight by 37 gm at 1000 mg with a weight of 216.3 gm; 35.4 gm gain with aqueous extracts 216.0 gm and with Diclofenac 29 gm gain 209.3gm compared to that of the normal rats as 38 gm 217.3 gm of weight showed no effect on the body weights and the growth of the rats.

Table 4: Effect on Haematological parameters of arthritic rats (g/rat)

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Dose</th>
<th>RBC (millions /mm\textsuperscript{2})</th>
<th>WBC (thousands /mm\textsuperscript{2})</th>
<th>Hb (g/dL)</th>
<th>ESR (mm/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td></td>
<td>7.8±0.00</td>
<td>11.1±0.01</td>
<td>14.2±0.008</td>
<td>3.2±0.004</td>
</tr>
<tr>
<td>Control -CFA</td>
<td></td>
<td>5.9±0.004</td>
<td>13.4±0.004</td>
<td>11.8±0.01</td>
<td>4.3±0.03</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>100</td>
<td>7.7±0.004**</td>
<td>11.2±0.01**</td>
<td>14.1±0.008**</td>
<td>3.9±0.03**</td>
</tr>
<tr>
<td>Aqueous extract</td>
<td>250</td>
<td>6.9±0.01**</td>
<td>12.1±0.01**</td>
<td>12.9±0.01**</td>
<td>4.1±0.01**</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>7.7±0.04**</td>
<td>11.4±0.01**</td>
<td>14.0±0.01**</td>
<td>3.8±0.01**</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>7.8±0.01**</td>
<td>11.2±0.01**</td>
<td>14.2±0.01**</td>
<td>3.4±0.01**</td>
</tr>
<tr>
<td>Methanolic extract</td>
<td>250</td>
<td>7.6±0.01**</td>
<td>11.3±0.01**</td>
<td>14.0±0.01**</td>
<td>3.8±0.01**</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>7.7±0.01**</td>
<td>11.2±0.01**</td>
<td>14.2±0.01**</td>
<td>3.4±0.01**</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>7.8±0.01**</td>
<td>11.1±0.01**</td>
<td>14.2±0.008**</td>
<td>3.2±0.03**</td>
</tr>
</tbody>
</table>

All the data are expressed as mean ± SEM, n=6, * p<0.05 and **p<0.01 when compared with control group; One way ANOVA followed by Dunnett’s test.
Effect on haematological parameters (Table- 4; Figure- 7)

Arthritis induced rats showed decrease in RBC from 7.8 to 6.9 m/mm²; Hb 14.2 to 14.0 g/dL than the normal rats and increased WBC 11.1 to 12.1 thousand/mm² and ESR levels 3.2 to 4.3 mm/hr; when compare with the standard drug Diclofenac treated rats RBC 7.7 m/mm² and Hb 14.1 g/dL; WBC 11.2 thousand/mm² and ESR 3.97 mm/hr. Methanolic extracts between 250-1000 mg showed RBC: 7.6 to 7.8 m/mm²; Hb 14.0 to 14.2 g/dL; WBC 11.3 to 11.1 thousand/mm² and ESR levels 3.8-3.2 mm/hr; with aqueous extracts RBC 6.9-7.8 million/mm²; Hb 12.9-14.2 g/dL; WBC 12.1 to 11.2 thousand/mm² and ESR levels 4.1 to 3.4 mm/hr. Hence rhizome extracts proved more effective in increasing RBC, Hb; decreased WBC and ESR levels to that of the normal rats and standard drug treated rats.

Table 5: Effect on biochemical parameters of arthritic rats

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Dose</th>
<th>ALP (mg/dL)</th>
<th>AST (mg/dL)</th>
<th>ALT (mg/dL)</th>
<th>Blood Urea (g/dL)</th>
<th>Creatinine (g/dL)</th>
<th>Total Protein (mg/dL)</th>
<th>Albumin (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td></td>
<td>120.3±0.19</td>
<td>78.3±0.19</td>
<td>28.0±0.33</td>
<td>22.0±0.33</td>
<td>0.7±0.004</td>
<td>7.2±0.09</td>
<td>4.8±0.04</td>
</tr>
<tr>
<td>Control - CFA</td>
<td>100</td>
<td>259.0±0.33</td>
<td>140.6±0.38</td>
<td>47.6±0.38</td>
<td>32.0±0.33</td>
<td>1.4±0.06</td>
<td>3.5±0.04</td>
<td>2.2±0.01</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>100</td>
<td>145.0±0.33*</td>
<td>85.0±0.33*</td>
<td>31.6±0.19*</td>
<td>23.9±0.11*</td>
<td>0.7±0.01*</td>
<td>7.2±0.05</td>
<td>4.5±0.02*</td>
</tr>
<tr>
<td></td>
<td>ALP (IU/L)</td>
<td>AST (IU/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------------</td>
<td>-----------------------------------------------------------------------------</td>
<td>-----------------------------------------------------------------------------</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aqueous extract</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>250</td>
<td>160.0±0.33*</td>
<td>105.0±0.19*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>500</td>
<td>144.0±0.19*</td>
<td>87.0±0.19*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1000</td>
<td>125.0±0.19*</td>
<td>76.0±0.19*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methanolic extract</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>250</td>
<td>145.0±0.33*</td>
<td>84.5±0.01*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>500</td>
<td>132.0±0.19*</td>
<td>80.0±0.19*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1000</td>
<td>125.0±0.19*</td>
<td>79.0±0.19*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

All the data are expressed as mean ± SEM, n=6, * p<0.05 and **p<0.01 when compared with control group; One way ANOVA followed by Dunnett’s test
**Figure 8: Effect of on biochemical parameters of arthritic rats**

**ALP:** Alkaline Phosphatase; **AST:** Aspartate Amino Transferase; **ALT:** Alanine Amino Transferase

**Con:** Control; **Dic:** Diclofenac; **Aq:** Aqueous; **Me:** Methanol

**Effect on Biochemical parameters of arthritis rats:** (Table 5; Figure 8)

There is an increased levels of ALP, AST, ALT, blood urea and creatinine in the arthritis induced rats compare to that of the normal rats as ALP 120.3 to 259.0 mg/dL; AST 78.3 to 140.6 mg/dL; ALT 28.0 to 47.6 mg/dL; blood urea 22.0 to 32.0 g/dL; and creatinine 0.7 to 1.4 g/dL; and observed decreased levels of total protein 7.2 to 3.5 mg/dL and albumin 4.8 to 2.2 mg/dL. But with aqueous and methanol extract treated rats at 1000 mg there is an effective control on ALP 125.0; AST 76.0 and 79.0; ALT 29.0 and 28.0; blood urea 22.0 g/dL and creatinine 0.7mg/dL levels decreased equally to that of normal rats and increased total proteins 7.1 and 7.2; albumin 4.7 and 4.8 mg/dL respectively.

**Radiological Analysis (Plate-2):** From the X-ray data the normal animals showed absence of soft tissue swelling and bone destruction. The CFA treated arthritic animals found with soft tissue swelling along with narrowing of the joint spaces and sign of bone destruction.
The treatment groups have shown prevention against bone destruction. Therefore the aqueous and methanol extracts at 500, 1000mg showing more significant activity compared to the standard drug Diclofenac treated rats in minimizing the arthritic effect.

**Plate-2: Radiological Studies of Anti-Arthritic Activity (on 21st day)**

<table>
<thead>
<tr>
<th>Normal</th>
<th>CFA</th>
<th>Diclofenac</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous -250 mg</td>
<td>Aqueous -500 mg</td>
<td>Aqueous -1000 mg</td>
</tr>
<tr>
<td>Methanol -250 mg</td>
<td>Methanol -500 mg</td>
<td>Methanol -1000 mg</td>
</tr>
</tbody>
</table>

Normal: Showing Normal Soft Tissue and bones.

CFA: Shows soft tissue swelling along with narrowing of the joint space and with bone destruction.

Diclofenac: Standard drug has no bone destruction and no swelling of the joint.

Aqueous/Methanol -250mg: Shows the mild swelling of the soft tissue and mild bone destruction.

Aqueous/Methanol-1000 mg: Shows most significant activity with normal soft tissue and bones.

*Aqueous 500, 1000mg and Methanol 500, 1000mg are showing more significant activity compared to the control Diclofenac.*

**DISCUSSION**

**Analgesic Activity:** Oral administration of *C.xanthorrhiza* rhizome crude extract on swiss albino mice at 150 and 300 mg/kg b.wt, exhibited 33.2 and 50.5% inhibition of acetic acid induced writhing effects of analgesic activity.\(^{[50]}\) Analgesic and anti-inflammatory properties of ethanolic rhizome extract of *C. mangga* were reported.\(^{[51]}\) Presence of O-coumaric acid, protocatechuic acid, syringic acid, and vanillic acid has been reported from the leaves
of *C. longa*. Anti-inflammatory and analgesic properties of protocatechuic acid have been shown in rat and mice models.[52-53] Methanolic extracts of *C. zedoaria* rhizome had mild analgesic property with writhing inhibition of 66.67%. Pet ether extracts of the rhizome and stem had shown effective analgesic activity with writhing inhibition of 70.24%, 71.43% respectively; the leaf extracts showed significant analgesic property with writhing inhibition of 91.67%.[54] Test on antinociceptive activity at 50, 100, 200 and 400 mg per kg b.wt with *C. zedoaria* rhizome extracts reduced the number of abdominal constrictions by 24.1, 27.6, 31.0, and 34.5%, respectively. The standard pain relieving (antinociceptive) drug Aspirin, reduced the number of writhings by 31.0 and 51.7% at 200 and 400 mg per kg b.wt respectively.[55]

**Arthritic Activity:** Application of standard Piroxicam gel was found to inhibit arthritic edema to an extent of 66.96%. Petroleum ether, chloroform and alcohol extracts of *Alpinia galanga* rhizome showed 48.69, 44.63 and 54.68% inhibition of arthritis respectively.[56]

Oral absorption and bio-disposition of essential oils and their terpene components are non-toxic but there is evidence of hepatocellular damage, anemia or occult gastrointestinal bleeding observed after one month of daily administration of crude or refined Turmeric Essential Oils (TEO). No peritonitis observed on necropsy. However, proteus bacteria was detected in the abdominal cavity of the crude TEO treated animals.[57] Isolated compounds shows cytotoxic and apoptotic effects on various cell lines. These compounds bind to the peroxisome proliferator-activated receptor γ (PPAR-γ) inhibition of inducible Prostaglandin-E2 (PGE2) and Nitric Oxide (NO) production.[58-62] TEO inhibits platelet aggregation.[63]

Compared activity between Isolated eight distinct fractions of TEO extract vs. the crude extracts in blocking COX-mediated PEG2 production *in vitro* studies shows the effective potency of the crude TEO extract than the isolated fractions. Hence the essential oils had the same potency to that of turmeric crude extracts; curcuminoinds and other isolated constituents.[64, 62, 65]

Treatment with crude TEO, 520 mg/kg/dL through articular and extra-articular against anti-inflammatory effects on the Streptococcal Cell Wall (SCW) model animals at the peak acute joint inflammation observed the decreased joint swelling at every time point. Analyzed on daily basis, showed relatively modest but statistically 21% inhibition of joint swelling; but leukocytosis or hepatic granulomatous inflammation were unaltered. However, it must also be stated that the safe long term use of dietary supplements containing higher doses of TEO
and/or curcuminoids than those consumed with culinary intake 4 mg/kg/dL curcuminoids or TEO in a typical Indian diet, given the 3% yield of curcuminoids.[66-68]

A profound anti-arthritic effect of C.longa curcuminoid extracts inhibit nuclear factor - kB (NF-kB) activation in rheumatoid arthritis rats, blocking multiple downstream signaling pathways critical to joint inflammation, including cyclo-oxygenase (COX) stimulated prostaglandin-E2 (PGE2) production.[67]

The Clinical studies proved the inhibition of swelling, with TEO on articular inflammation were confirmed at the molecular level by assembling the activation of genes. The early induction of chemokines are responsible for the intra-articular recruitment of inflammatory cells (neutrophil chemokines and monocyte chemokines, Monocyte chemotactic protein-1 (MCP-1)), and the key inflammatory cytokines (IL-1 beta), prevented by TEO treatment. Similarly, activation of an innate immune responsible for antibody-independent complement pathways (induced expression of mannose binding lectin and properdin), and the inducible expression of other downstream mediators of joint inflammation, such as COX-2, was blocked in the joints of SCW-injected animals treated with TEO.[69]

Between gingerol (Zingiber officinale) fraction vs. crude Dichloromethane (DCM) extract, only the DCM extract was effective in blocking the joint destruction and damage of articular cartilage that accompanies joint inflammation. Reactivation of infectious diseases made quiescent by protective granulomatous responses (Tuberculosis) are known risk of anti-inflammatory treatments in RA patients.[70] Gingerol fraction treatment was more efficacious than specific Tumor Necrosis Factor (TNF) blockade in the prevention and treatment of SCW-induced joint swelling.[71]

In the toxicity studies animals treated with ginger fraction exhibited mild abnormalities in liver and kidney function, and also had occult blood in their stool with evidence of hemorrhagic enteritis of the small intestine on necropsy. The antiarthritic effects of ginger involves additive and/or synergistic effects of multiple components, but not limited to the gingerols.[27,33]

Streptococcal Cell Wall (SCW) induced arthritis, was inhibited by a fraction containing only gingerols and their derivatives. Both crude and fraction were efficacious in preventing joint inflammation. However, the crude Dichloromethane extract (DCM), which also contained...
essential oils and more polar compounds, was more effective (when normalized to gingerol content) in preventing both joint inflammation and destruction.\[72\]

*In-vitro* bioactivity of each of the 11 fractions as the gingerol and gingerol derivative-containing fractions (fractions 4–9) were most potent in inhibiting PGE\(_2\) production. IC\(_{50}\) for the inhibition of PGE\(_2\) production by the crude extract was similar to that of the gingerol fraction. This finding suggests that the non-gingerol components of the crude DCM extract has no significant effect on PGE\(_2\) release when administered in isolation, may synergize with the gingerols to suppress prostaglandin-mediated inflammation.\[73-75, 27, 33\]

*Curcuma neilgherrensis* rhizomes are already reported as efficient antimicrobial agents as that of *Ampicillin* and *Nystatin* and also acts as anthelmintic agent to that of *Albendazole* which possess major secondary metabolites like phenolics, flavonoids, anthocyanidins, alkaloids, tannins, terpenoids and saponins in high quantities. Presence of protocatechueic, caffeic, melilotic, salicylic acids; myricetin, apigenin, kaempferol, quercetin, peonidin and cyanidin compounds supported and proved as potent inhibitor of ulcer index to 5.0 with 63.5% ulcer protection at 500 mg/kg body weight of aqueous rhizome extracts of on pyloric ligated gastric ulcers induced Albino rats equally to that of the standard drug *Omeprazole*.\[76-78\]

Methanol extracts of rhizome at 1000 mg/kg b.wt proved more effective to that of *Atropine* with 70% antidiarhoeal activity and 60 % of fluid inhibition may be due to the presence of tannins, terpinoids, glycosides, flavonoids and saponins. And it is also proved as the safe drug upto 5000 mg/kg b.wt without toxic and any behavioural changes.\[79\]

**CONCLUSION**

It was observed that there is an effective inhibition of rhizome extracts of *C.neilgherrensis* against acetic acid induced analgesic and against CFA induced arthritis rats. Both aqueous and methanol crude extracts between 250-500 mg/kg b.wt shown equally to that of the control rats and also with the standard drug Diazepam and Diclofenac treated rats. There is no side effects on body weights and other behavioural changes without any toxicity. Also noticed effective management in the blood profile and in biochemical activities. There is no damage to the inflammed tissues observed in the radiological studies. The same analgesic and anti-arthritic effect was also well correlated with *C.longa*, *C.zedoaria*, *A.galanga* and *Zingiber officinalis* rhizome crude extracts than the essential oils and isolated compounds like
curcuminoids. Hence it is recommended as safe drug upto 500 mg/kg b.wt on daily dosages against arthritis. Further studies also necessitated to design drug against analgesic and arthritic disorders.

ACKNOWLEDGEMENTS:
The authors are grateful to the University Grants Commission (UGC), New Delhi for Financial assistance. We are also indebted to the Department of Botany, S.V.U College of Sciences, Sri Venkateswara University, Tirupati, Andhra Pradesh, India for providing the space and facilities to complete the above Research work. The authors are grateful in this regard.

REFERENCES


45. Natelson S. Micro techniques of clinical chemistry for the routine laboratory, Thomas Spring field, Dilliners. 1957: 381.


