Investigation of Arthritis Suppressing Potency of Chloris Paraguaiensis on Freund's Adjuvant Induced Arthritis in Rats

Somasundaram G¹, Jaikumar S*¹, Balaji subramaniam R²

¹Department of Pharmacology Sri Lakshmi Narayana institute of medical sciences Puducherry 605502, India
²Department of dentistry Sri lakshmi narayana institute of medical sciences Ossudu kudapakkam Pondicherry, India

ABSTRACT

Arthritis is a widespread disorder that occurs in elderly patients currently in the world. It is one of those disorders that affect the joints and is classified as an inflammatory disorder that is a result of immune system malfunctioning. Taking into consideration the side effects of immune suppressants that are used to treat arthritis; generally, plants are being investigated for the chemical constituents to treat the diseases. It is proved that moieties obtained from herbal sources are relatively safer and potent too. The economical methods of treating illness are using plants, and so the herbs are investigated for the treatment of arthritis. Chloris paraguayensis Steud., is one of the members of grass family which is grown wildly in tropical countries all around the world. It usually grows like a weed and can be employed for many pharmacological problems. Traditional claims are there to treat DM, Rheumatism and diarrhoea. Chemical constituents like flavonoids, tannins and phenols have been isolated from the plant earlier. Cloris was selected as a subject to investigate its anti-arthritis potential in vivo using experimental animals following the folklore claims that the plant can be used to treat arthritis. It may be due to the presence of flavonoids and polyphenol-rich chemical constituents in the plant. The exact mechanism of action is to be established and the extracts in this current study showed a significantly comparable activity with that of the standard drug indomethacin.

INTRODUCTION

Arthritis is a widespread disorder that occurs in elderly patients currently in the world. It is one of those disorders that affect the joints and is classified as an inflammatory disorder that is a result of immune system malfunctioning. The disease leads to the damage of the cartilage in the joints leading to the ankylosing joints. It can also be the cause for other disorders like pleuritis, scleritis characterized by nodular lesions and is commonly seen in subcutaneous tissue. Even though the origins of the diseases are not known clearly, immune problems play a significant role in determining the prognosis of the disease (Jain et al., 2011).

In the world currently, more than six crore people are affected by arthritis. Generally, women are affected by 3 times more than men. The average age group of the population are affected mostly by arthritis. The age is between 35-55, and other ages...
Table 1: Changes in the body weights of rats due to ethanolic extract of whole plant of Chloris paraguaiensis Steud

| Group                        | Initial  | 1st week  | 2nd week  | 3rd week  |
|------------------------------|----------|-----------|-----------|-----------|
| Control Group                | 189.23+8.07 | 192.42+8.09 | 195.21+7.52 | 198.54+6.36 |
| Inducing agent               | 164.89+9.76 | 151.28+5.11 | 155.73+6.48 | 156.56+7.01 |
| Standard drug                | 187.02+7.31 | 183.54+7.36 | 186.67+5.35 | 195.72+5.29 |
| CEE-250mg/kg                 | 194.45+5.025 | 186.89+4.672 | 190.92+4.521 | 191.34+4.654 |
| CEE-500mg/kg                 | 161.67+4.928 | 157.92+3.045 | 159.14+3.496 | 168.57+3.278 |

Table 2: Changes in the Paw Volumes of rats due to ethanolic extract of whole plant of Chloris paraguaiensis Steud

| Group                        | 0 week                  | 1st week                  | 2nd week                  | 3rd week                  | % Inhibition |
|------------------------------|-------------------------|---------------------------|---------------------------|---------------------------|--------------|
| Control Group                | 0.218+0.1442            | 0.229+0.0136              | 0.212+0.0149              | 0.216+0.0127              | —            |
| Inducing agent               | 0.234+0.1767            | 0.786+0.0253**            | 0.708+0.0190**            | —                         | —            |
| Standard drug                | 0.296+0.0278            | 0.585+0.0483**            | 0.465+0.0382**            | 0.402+0.0378**            | 57.28        |
| CEE-250mg/kg                 | 0.253+0.0341            | 0.702+0.0295**            | 0.598+0.0423**            | 0.516+0.0412**            | 43.63        |
| CEE-500mg/kg                 | 0.240+0.0182            | 0.73+0.0174**             | 0.532+0.0387**            | 0.491+0.0239**            | 46.25        |

are being affected too. There is some marker that is used to evaluate the disease extend and spread the synovial study can determine that. Still, in the lab, experimental rats were used to study the activity invivo accurately.

Taking into consideration the side effects of immune suppressants that are used to treat arthritis generally, plants are being investigated for the chemical constituents to treat the diseases. It is proved that moieties obtained from herbal sources are relatively safer and potent too. The cheapest methods of treating the disease are using plants and so the herbs are investigated for the treatment of arthritis (Shiddamallayya et al., 2010).

Chloris paraguaiensis Steud, is one of the members of grass family which is grown wildly in tropical countries all around the world. It usually grows like a weed and can be employed for many pharmacological problems. Traditional claims are there to treat DM, Rheumatism and diarrhoea. Chemical constituents like flavonoids, tannins and phenols have been isolated from the plant earlier. CNS stimulants like caffeine and theobromine are also isolated from the plant (Burris et al., 2012). So in this current research, the antiarthritic activity of the plant as investigated invivo using laboratory rats.

**MATERIALS**

**Plant Material**

Whole plants of Chloris were collected in the month of November 2019 and they are shade dried for five days. The plants were duly authenticated and the herbarium specimen is left in the college library. The dried powder is finely ground and extracted with ethanol using a cold maceration method for two days. The extract was filtered and evaporated using a rotary evaporator and stored using a desiccator. The percentage yield was calculated as 15.4% w/w and noted as (CEE).

Experimental animals used for the investigation are albino Wistar rats which weighed 130-170gm and were acclimatized for 2 days before the experiments. They were let free access to food and water. The experiments were performed on the rats were approved and as per the animal ethics committee permission.

**Arthritic Activity**

The anti-arthritic activity was performed by the procedure prescribed by Freund’s Adjuvant method in rats (Lokesh et al., 2010). The animals were divided into five groups of 5 animals in each group.

The groups planning is according to the below protocol

Group I: Normal Control Group-given with only CMC solution at 1% solution.

Freund’s Adjuvant (Difco-mycobacterium strain of bacteria suspended in paraffin at concentration of 5mg/ml) was administered in the sub plantar region during the initial stage of the experiment.
Table 3: Changes in the blood cell parameters due to ethanolic extract of whole plant of Chloris paraguaiensis Steud

| Group            | RBC (106 cells/mm³) | WBC (10³ cells/mm³) | Hb (gm %) | ESR (mm/hr) |
|------------------|---------------------|---------------------|-----------|-------------|
| Control Group    | 7.03 ± 0.082        | 8.05 ± 0.041        | 16.82 ± 0.045 | 4.22 ± 0.096 |
| Inducing agent   | 6.42 ± 0.491**      | 10.23 ± 0.057**     | 11.21 ± 0.056** | 7.84 ± 0.152** |
| Standard drug    | 7.26 ± 0.054**      | 8.52 ± 0.128**      | 14.04 ± 0.078** | 5.31 ± 0.104** |
| CEE-250mg/kg     | 6.37 ± 0.068**      | 9.47 ± 0.125**      | 12.33 ± 0.071** | 6.86 ± 0.149** |
| CEE-500mg/kg     | 8.06 ± 0.085**      | 8.89 ± 0.074**      | 13.06 ± 0.182** | 5.25 ± 0.063** |

Table 4: Changes in the Serum parameters due to ethanolic extract of whole plant of Chloris paraguaiensis Steud

| Group            | SGOT (IU/L) | SGPT (IU/L) | ALP (IU/L) |
|------------------|-------------|-------------|------------|
| Control Group    | 59.72 ± 4.132 | 47.82 ± 2.814 | 174.42 ± 3.673 |
| Inducing agent   | 103.81 ± 2.354** | 91.61 ± 2.254** | 198.65 ± 3.781** |
| Standard drug    | 74.64 ± 2.427** | 62.92 ± 2.468** | 181.20 ± 2.619* |
| CEE-250mg/kg     | 79.05 ± 2.126** | 70.74 ± 3.410** | 193.36 ± 3.245** |
| CEE-500mg/kg     | 86.67 ± 2.469** | 66.83 ± 2.956** | 187.93 ± 4.926** |

Group II: Positive Control Group: Freund’s adjuvant was administered with just CMC solution.

Group III: Negative Control group: Freund’s adjuvant was administered with Indomethacin as a standard drug at a dose of 10mg/kg body weight (Pathak et al., 2009).

Group IV: Extract doses CEE 250mg/kg body weight with Freund’s adjuvant was administered.

Group V: Extract doses CEE 500mg/kg body weight with Freund’s adjuvant was administered.

The weights were noted in regular intervals before and after the start of the experiment. The paw volumes were noted on the last day of the experiment and compared with the initial volume and normal control. Blood was withdrawn ethically from rats as per procedure and was subjected to centrifugation. The cellular blood parameters like RBC, WBC, Hb and ESR were estimated. The serum constituents like SGOT, SGPT and ALP were also estimated.

RESULTS AND DISCUSSION

The data corresponding to the antiarthritic activity of Chloris has been portrayed in the tables. The weight gain was also noted in regular intervals and there no significant change in the weights in the extract-treated groups (Table 1). So in fact, the weights did not influence the arthritis and the extracts facilitated the reduction of weights due to its anti-lipidemic property (Snekhalatha et al., 2013); (Schurgers et al., 2011).

The paw volumes noted are in (Table 2). There was a significant change in the volume of the paw as inflammation was induced by the bacterial strain which was similar to the arthritis features in human beings. So it was used as the model in this investigation. The extracts showed a dose-dependent antiarthritic activity at 250mg/kg and 500mg/kg, which is significant when compared to the standard drugs indomethacin. The percentage inhibition was also nearer to the standard drug with 45% inhibition. The activity helped in the normalization or prevention of joint deformation in the body due to the induction agent (Pramod et al., 2012).

Cellular constituents of the blood like RBC gives us an indication of the level of inflammation in the body. It was lowered with the induction of arthritis using mycobacterium and was significantly elevated with standard drug and extracts at 500mg/kg, which was comparable. It was similar in case of WBC, Hb and ESR also. The serum parameters like SGOT, SGPT and ALP were also elevated in the induction of arthritis (Table 3). It indicates that there was a serious inflammatory response in the body with the bacteria. The biomarkers used in the study displayed the values that are similar to the standard drug indomethacin Sutradhar et al. (2006). The indomethacin drug, and the extracts helped in lowering those values to a considerably low level which also in a dose based manner (Chillingworth and Donaldson, 2003); (Liu et al., 2009).

Overall it can be said that the extracts showed a better anti-arthritic activity compared to the standard drug.
indomethacin, which proves the traditional claims of the plant to treat rheumatism (Table 4).

CONCLUSIONS
Cloris was selected as a subject to investigate its anti-arthritis potential in vivo using experimental animals following the folklore claims that the plant can be used to treat arthritis. It may be due to the presence of flavonoids and polyphenol-rich chemical constituents in the plant. The exact mechanism of action is to be established and the extracts in this current study showed a significantly comparable activity with that of the standard drug indomethacin.

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Conflict of Interest
The authors declare that they have no conflict of interest for this study.

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REFERENCES
Burris, K. P., Harte, F. M., Davidson, P., Stewart, C., Jr, Zivanovic, S. 2012. Composition and bioactive properties of yerba mate. chilean journal of agricultural research, 72:2–5.

Chillingworth, N. L., Donaldson, L. F. 2003. Characterisation of a Freund’s complete adjuvant-induced model of chronic arthritis in mice. Journal of Neuroscience Methods, 128(1-2):45–52.

Jain, A., Choubey, S., Singour, P. K., Rajak, H., Pawar, R. S. 2011. Sidacordifolia( Linn) - An overview. Journal of Applied Pharmaceutical Science, 1(2):23–31.

Liu, Y. L., Lin, H. M., Zou, R., Wu, J. C., Han, R., Raymond, L. N., Reid, P. F., Qin, Z. H. 2009. Suppression of complete freund’s adjuvant-induced adjuvant arthritis by cobra toxin. Acta Pharmacologica Sinica.

Lokesh, K., Bhardwaj, K. S., Patil, M., Kaushik, Amit, sahu, Y., Prakash, V. K., Verma 2010. Anti-inflammatory properties of bark and leaf of Ficus benghalensis. International Journal of Drug Development & Research.

Pathak, N., Gohil, P., Patel, N. B., Kasture, S., Jivani, N., Bhalodia, Y. 2009. Curative Effect of Albizia lebbeck Methanolic Extract against Adju vant Arthritis- With Special Reference to Bone Erosion. International Journal of Pharmaceutical Sciences and Drug Research, 1(3):183–187.

Pramod, V., Pattar, M., Jayaraj 2012. Pharmacognostic And Phytochemical Investigation Of SidacordifoliaL -A Threatened Medicinal Herb. International Journal of Pharmacy and Pharmaceutical Sciences, 4(1):114–117.

Schurgers, E., Billiau, A., Matthys, P. 2011. Collagen-Induced Arthritis as an Animal Model for Rheumatoid Arthritis: Focus on Interferon-γ. Journal of Interferon & Cytokine Research, 31(12):917–926.

Shiddamallayya, N., Yasmeen, A., Gopakumar, K. 2010. Medico-botanical survey of kumar parvatha kukke subramanya. Indian Journal of Traditional Knowledge, 9(1):96–99.

Snekhala, U., Anburajan, M., Venkatraman, B., Menaka, M. 2013. Evaluation of complete Freund’s adjuvant-induced arthritis in a Wistar rat model. Zeitschrift für Rheumatologie, 72(4):375–382.

Sutradhar, R. K., Rahman, A. M., Ahmad, M., Bachar, S. C., AchintoSaha, Guha, S. K. 2006. Bioactive Alkaloid from Sidacordifolia.Linn. with Analgesic and Anti-Inflammatory Activities. Iranian Journal Of Pharmacology & Therapeutics, 2(4):744–749.