

Research Article**Evaluation of *Anogeissus latifolia* Roxb on complete Freund's adjuvant induced arthritis in albino rats****P. Mumthaj*, Natarajan P.**

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Received: 26 April 2022

Revised: 21 June 2022

Accepted: 29 June 2022

Abstract

Objective: The aim of the present research work is to evaluate the anti arthritis activity of *Anogeissus latifolia* Roxb in complete Freund's adjuvant (CFA) induced rats. **Material and methods:** The ethanolic extract of *Anogeissus latifolia* Roxb (EEAL) was administered by the oral in the dose of 200 and 400mg/kg for a period of 21 days respectively. The biochemical, hematological parameters and histopathology of the joint tissue were performed. The EEAL attenuated the arthritis factors induced by CFA. **Results and conclusion:** The research investigation of both extracts was showed better protection against arthritis. In this study both extracts, at the 400mg/kg p.o. dose have more potent effect than the 200mg/kg p.o. The present study was concluded that EEAL of both concentrations (200 and 400mg/kg p.o.) provides a significant effect in dose dependent manner.

Keywords: Complete Freund's adjuvant, arthritis, Sub plantar, *Anogeissus latifolia*

Introduction

Traditional medicine is the synthesis of therapeutic experience of generations of practicing medical practitioner of indigenous system of medicine. Herbal drugs constitute only those traditional medicines which most use herbal plant formulation for therapeutic use. The initial recorded evidence of their use in Indian, Chinese, Egyptian, Greek, Roman and Syrian texts dates back to about 5000 years (Kamboj, 2000).

There are several reasons for patients choose to herbal therapies. Frequently cited is a "sense of control, a psychological comfort from taking action," which helps explain why many people taking herbs have diseases that are life threatening or incurable viz. diabetes, cancer, arthritis or AIDS. When patients use home remedies for acute, often self-limiting conditions, such as cold, sore throat, or bee sting, it is often because hospital care is not immediately available, too comfortable, costly or time-consuming. In rural areas, there are additional cultural factors that more interesting to use of botanicals, such as the environment and culture, a "man earth relationship." People

believe that where an area gives rise to a particular disease, it will also support plants that can be used to cure it (Winslow and Kroll, 1998).

Rheumatoid arthritis (RA) is a chronic, symmetrical, inflammatory autoimmune disease that earlier stage affects small joints, progressing to larger joints, and eventually the skin, eyes, heart, kidneys, and lungs. Often, the bone and cartilage of joints are destroyed, and tendons and ligaments turn to weak (Lee et al., 2017). The onset of this disease is ordinary from the age of 35 to 60 years, with remission and exacerbation. It can also afflict young children even before the age of 16 years (Fox, 2002; McInnes, 2011). The aim of the present research work is to evaluate the anti rheumatic activities of *Anogeissus latifolia* Roxb in albino rat.

Materials and methods**Taxonomical identification**

The plant species for the proposed study was authentic by Dr. N. Senthil kumar Head and Associate professor of Botany, Ayya Nadar Janaki Ammal College, Sivakasi, Virudhunagar Dist. The plant specimen was certified as *Anogeissus latifolia* Roxb of Family Combretaceae.

Extraction of plant material

The leaves parts were collected, shadow dried and then size reduced to small particles manually. The dried material was coarsely powdered before extraction. The extracts were

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DOI: <https://doi.org/10.31024/ajpp.2022.8.3.2>2455-2674/Copyright © 2022, N.S. Memorial Scientific Research and Education Society. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

prepared by 2000 ml Soxhlet apparatus continuous extracted with ethanol for 4 days. The temperature was maintained at 40-60°C. After the extraction process the crude extract was collected (Kou, 2005).

Selection of animal model

Albino rats 150-250g were selected for experimental study. The rats were kept in properly numbered large polypropylene cages with stainless steel top grill having facilities and given a standard diet and water and *ad libitum* throughout the experimental period. The animals were maintained in 12hr. light and dark cycle at 22°C ($\pm 3^\circ\text{C}$) in a well ventilated animal house under natural conditions, they were acclimatized to laboratory condition for 10 days prior to the commencement of the experiment. Paddy husk was used as bedding materials and changed once a week (CPCSEA 423). The study was conducted in accordance with the ethical committee. SBCP/2021-2022/CPCSEA/IAEC/I(1)/F16/132

Statistical analysis

Values were expressed as mean \pm SEM. The mean difference in paw volume difference, biochemical parameters and haematological parameters were analysed using One way ANOVA followed by Dunnett's test. The values were considered significant at $P < 0.01$ and $P < 0.05$. Analysis was performed using Graph Pad prism statistical software (Version 9.3.1).

Table 1. Animal Protocol

Groups	Drug and dose
I	Normal Control(normal saline 5ml/kg.p.o)
II	Diseased Control(CFA 0.1 ml on sub plantar region)
III	Standard (CFA 0.1 ml on sub plantar region + Indomethacin 10 mg /kg.i.p)
IV	Low dose (CFA 0.1 ml on sub plantar region + EEAL dose 200mg/kg.p.o)
V	High dose (CFA 0.1 ml on sub plantar region +EEAL dose 400mg/kg.p.o)

Table 2. Effect of EEAL on mean paw volume change in Complete Freund's adjuvant (CFA) induced arthritis in albino rats

Drug treatment	Mean change in paw volume							
	0 Days	3 days	6days	9 days	12 days	15 days	18 days	21 days
Healthy control (Normal saline 5ml/kg)	0.00 \pm 0.03*	0.00 \pm 0.04**	0.00 \pm 0.03**	0.00 \pm 0.02**	0.00 \pm 0.01***	0.00 \pm 0.03*	0.00 \pm 0.03*	0.00 \pm 0.01*
Negative control +CFA 0.1 ml in s.p r region	0.13 \pm 0.07	0.18 \pm 0.04	0.22 \pm 0.03	0.28 \pm 0.04	0.35 \pm 0.02	0.38 \pm 0.04	0.34 \pm 0.03	0.31 \pm 0.03
CFA 0.1 ml s.p region +Indomethacin 10mg/kg .p.o	0.16 \pm 0.04*	0.13 \pm 0.03*	0.14 \pm 0.04***	0.12 \pm 0.04*	0.10 \pm 0.01**	0.09 \pm 0.02*	0.04 \pm 0.03*	0.03 \pm 0.02**
CFA 0.1 ml s.p region + EEAL 200 mg/kg p.o	0.15 \pm 0.01*	0.16 \pm 0.06**	0.14 \pm 0.07*	0.13 \pm 0.03**	0.11 \pm 0.01***	0.10 \pm 0.02*	0.09 \pm 0.03**	0.06 \pm 0.01***
CFA 0.1 ml s.p region + EEAL 400mg/kg p.o	0.18 \pm 0.07***	0.16 \pm 0.04**	0.13 \pm 0.02*	0.12 \pm 0.05**	0.10 \pm 0.02**	0.09 \pm 0.01***	0.07 \pm 0.02***	0.06 \pm 0.02*

Values are expressed as Means \pm SEM, (n=6) on the end of the experiment, statistical significance (P) calculation by one way ANOVA followed by Dunnett's test, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ calculated by comparing drug treated groups with negative control group.

Anti arthritis activity in Freund's adjuvant induced arthritis

Arthritis was induced by the injection of 1000 μ l of CFA, (Containing 1mg/ml of heat-killed *Mycobacterium tuberculosis*, 0.85 ml paraffin oil and 0.15 ml of mannide monooleate.) into the sub planter region of right hind paw of rat. The day of CFA injection was considered as day 1. The oral administration of the EEAL, Indomethacin (10mg/kg, i.p) of all the groups starts from day 12, once daily until day 21. Anti arthritic activity of Ethanolic extract was evaluated the arthritic score on day 0, 3, 6, 9, 12, 15, 18 and day 21. The last day, all the animals were sacrificed under ether anaesthesia the blood was collected by retro-orbital route for haematological parameters. The joints were also cut for histology (Balamurugan and Natarajan, 2018).

Blood sample collection

After experiment procedures the, animals were anaesthetised by Ketamine HCL (rats) then blood was collected from retro orbital route, serum was separated from blood by centrifuge under 3600 rpm at 15 min, after that hematological study was performed.

Results and discussion

Effect of ethanolic extract of *Anogeissus latifolia roxb* on change in paw volume (ml) in CFA induced arthritis in wistar rats

The mean difference in paw volume was found to be zero in normal control group. Negative control group is significantly increased when compared with normal control. The group of standard drug (Indomethacin 10mg/kg.p.o) group decreased the paw volume when its compared with negative control group. The group IV treated with EEAL-200 mg/kg, p.o and group V treated with 400 mg/kg, p.o Significantly decreased the paw volume when

compared with the negative control group (Table 2).

Effect on RBC level

In normal control group, the RBC level was found to be $8.41 \times 10^6/\text{mm}^3$. The negative control group was significantly decreased $4.23 \times 10^6/\text{mm}^3$ when its compared with normal control group. The standard (Indomethacin 10 mg/kg.p.o) treated group was significantly increased the level of RBC to $8.22 \times 10^6/\text{mm}^3$ when compared to negative control group. The EEAL 200mg/kg p.o treated group, 400mg/kg p.o treated groups were significantly increased the RBC level to 7.03 and $7.64 \times 10^6/\text{mm}^3$ when its compared with negative control group. The result was shown in table 3.

Effect on WBC level

In WBC level of normal control group was found to be $8.32 \times 10^3/\text{mm}^3$ and the negative control group was significantly increased to WBC $13.20 \times 10^3/\text{mm}^3$. The Standard Indomethacin 10mg/kg.p.o treated group was significantly decreased to WBC $8.13 \times 10^3/\text{mm}^3$ when compared to negative control group. The EEAL 200mg/kg p.o and 400mg/kg p.o treated groups was significantly decreased WBC to 9.61 and $9.18 \times 10^3/\text{mm}^3$ when its compared to negative control group. The result was shown in table 3.

Effect on Haemoglobin level

In normal control group of haemoglobin value was found to be 12.11 g%. The negative control group was significantly decreased to 7.6 g%. The Standard Indomethacin 10mg/kg.p.o treated group was significantly increased the level of haemoglobin to 11.98 g% when its compared with negative control group. The EEAL 200mg/kg p.o and 400mg/kg p.o treated groups were significantly increased the haemoglobin level to 9.4 and 10.1 g% when its compared to negative control group (Table 3).

Effect of ESR level

The level of ESR was found to be 37.21% in normal control group and the negative control group was significantly increased to 63.62%. The Standard Indomethacin 10mg/kg.p.o treated group was decreased the elevated level of ESR to 40.12%. when its compared to negative control group. The EEAL 200mg/kg

p.o and 400mg/kg p.o treated groups were significantly decreased the ESR level to 47.54% and 43.83% respectively, when its compared with negative control group (table 3).

Effect on SGOT level

Normal control serum SGOT level was found 62.85 U/L by normal group of animal. CFA induced arthritic group significantly increased SGOT level contain 118.00 U/L. Then the standard group treated by indomethacin 10mg/kg.p.o. this group serum SGOT significantly reduced the serum SGOT level 59.66 U/L. Then the EEAL (200mg/kg) and EEAL(400mg/kg) treated group of animal serum contain 73.33 U/L and 66.34 U/L. Significantly increasing the SGOT level in negative group when its compared with each other group. The result was shown in table 4.

Effect on SGPT level

Normal control serum SGPT level was found 44.55 U/L by normal group of animal. CFA induced arthritic group SGPT level have 79.87 U/L it significantly increased when its compared with normal control group. Then the standard group was treated by indomethacin 10mg/kg.p.o..this serum contain 47.92 U/L its significantly decreased when its compared with negative control group. The EEAL(200 mg/kg.p.o) and EEAL(400mg/kg) treated group of animal serum contain 56.47 U/L and 51.94 U/L Significantly decreasing the SGPT level when its compared with negative control group (Table 4).

ALP

Normal control serum ALP level was found 65.34 U/L by normal group of animal. CFA induced arthritic group ALP level contain 79.87 U/L significantly increased when its compared with normal control group. Then the standard group was treated by indomethacin 10mg/kg.p.o. this serum contain 47.92 U/L significantly decreased when its compare with negative control group. Then the EEAL (200 mg/kg.p.o) and EEAL (400mg/kg) treated group of animal serum contain 56.47 U/L and 51.94 U/L. Significantly decreasing the ALP level when its compared with negative control group. The result was shown in table 4.

Table 3. Effect of EEAL on Hematological parameters in Freund's adjuvant induced arthritis in albino rat

Drug and dose treatment	RBC ($10^6/\text{mm}^3$)	WBC ($10^3/\text{mm}^3$)	Haemoglobin (g%)	ESR %
Normal control (Normal saline 5ml/kg)	8.41± 0.07*	8.32± 0.653*	12.11±0.602**	37.21± 3.115 ***
Negative control +CFA 0.1 ml in s.p region	4.23± 0.05	13.10± 0.720	7.6± 0.607	63.62± 1.873
CFA 0.1 ml s.p region +Indomethacin 10mg/kg i.p	8.22± 0.06***	8.13± 0.684**	11.98± 0.69*	40.12± 5.010**
CFA 0.1 ml s.p region +EEAL 200mg/kg p.o	7.03± 0.06**	9.61± 0.7034**	9.4± 0.59	47.54± 3.351**
CFA 0.1 ml s.p region +EEAL 400mg/kg p.o	7.64± 0.05**	9.18± 0.72***	10.1± 0.68	43.83± 1.604**

Values are expressed as Means ± SEM, (n=3) on the end of the experiment; statistical significance (P) calculation by one way ANOVA followed by Dunnett's test, $p < 0.05$, $**p < 0.01$, $***p < 0.001$ calculated by comparing drug treated groups with negative control group

Table. 4 Effect of EEAL on the biochemical parameters in Complete Freund's adjuvant (CFA) induced arthritis in albino rats

Drug and dose treatment	Biochemical analysis				
	SGOT U/L	SGPT U/L	ALP U/L	Total protein mg/dl	Creatinine mg/dl
Normal control (Normal saline 5ml/kg)	62.85± 2.169*	44.55± 4.266**	63.34± 6.441**	6.58±0.295*	0.313± 0.0632*
Negative control +CFA 0.1 ml in s.p region	118.72±8.253	79.87±3.724	121.1±7.050	2.501± 0.323	0.650±0.057
CFA 0.1 ml s.p region +Indomethacin 10mg/kg .p.o	59.66± 4.361**	47.92± 4.783*	77.11± 8.186*	6.38± 0.4707**	0.385±0.0564*
CFA 0.1 ml s.p region +EEAL 200mg/kg p.o	73.33± 4.196*	56.47±4.322**	90.88±7.441***	6.12± 0.3683*	0.506± 0.0537*
CFA 0.1 ml s.p region +EEAL 400mg/kg p.o	66.34± 3.676*	51.97± 4.944**	85.37± 5.187**	5.99± 0.4328*	0.457±0.0521**

Values were expressed as Means ± SEM., (n=6) on the end of the experiment; statistical significance (P) calculation by one way ANOVA followed by Dunnett's test, $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ calculated by comparing drug treated groups with negative control group

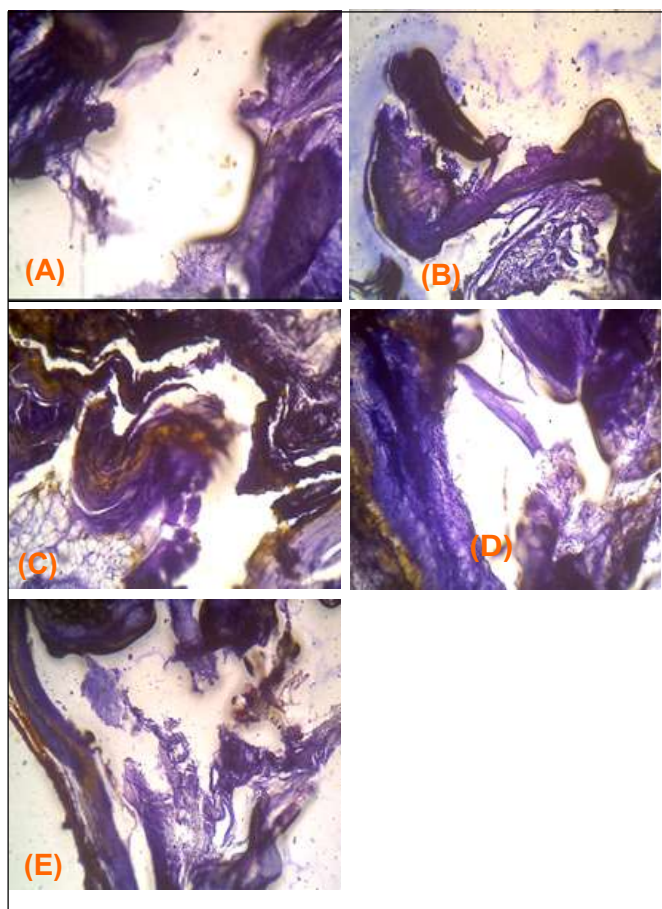


Figure. 1 Histopathological study of CFA induced arthritis joints: (A) **Normal group:** Bone and cartilage between the joints appeared normal. Synoviocytes surrounding the cartilage appeared normal. (B) **Negative group:** Severe pannus formation surrounding the surrounding the joints in which extensive proliferation of fibro vascular tissue or granulation tissue. (C) **Standard group:** Bone and cartilage between the joints appeared normal.No erosion noticed (D) **200mg/kg group:** Moderate pannus formation in which proliferation of fibro vascular tissue or granulation tissue. (E) **400mg/kg group:** Bone structure appeared normal no erosion noticed .Mild erosion noticed .Cartilage /synovial membranes appeared normal. Mild erosion present in the cartilage.

Total protein level

The normal control group, the serum total protein level was found 6.58 g/dl, the protein level of CFA induced group 2.501g/dl significantly decreased total protein level. Standard group treated by indomethacin 10mg/kg.p.o was found 6.38 g/dl significantly increasing when its compare with negative control group. EEAL (200 mg/kg) and EEAL (400 mg/kg) treated group was found 6.12 g/dl and 5.99 g/dl. Significantly increasing the total protein when its compared with Group The result was shown in table 4.

Creatinine level

The level of normal control group of creatinine was found to be 0.313 mg/dl. The creatinine level of negative control group was significantly increased to 0.650 mg/dl. when its compared with negative control group. Standard group treated by indomethacin 10mg/kg.p.o was decreased the creatinine level to 0.385 mg/dl when its compared with negative control group. The EEAL 200mg/kg p.o and 400mg/kg p.o treated groups were decreased the creatinine level to 0.506 mg/dl and 0.457 mg/dl respectively, when its compared with negative control group. The result was shown in table 4.

Conclusion

The present investigation, both extracts was showed better protection against arthritic. But in this both extracts, the 400mg/kg p.o. have more potent effect than the 200mg/kg p.o. From this research data I conclude that the effect produced by the extract was dose dependent manner only.

Acknowledgement

The authors are sincere thanks to Mr. S. Sreeram Ashok, Correspondent, Sankaralingam Bhuvaneshwari College of Pharmacy, Sivakasi, Tamil Nadu for providing the facilities to carry out the research work.

Conflict of interest

None

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