Anti-Arthritic Activity Of Methanol Leaf Extract Of Chikadoma In Complete Freud's Adjunct-Induced Arthritic Rats

***[1]Ohadoma S.C, [1]Lawal B.A.S, and [2]Chukwu L.C.**

[1]Department of Pharmacology, College of Medical Sciences, University of Calabar, Nigeria

[2]Department of Pharmacology, Chukwuemeka Odumegwu Ojukwu University, Igbariam, Nigeria.

***Corresponding Author:** Dr. Ohadoma SC. Department of Pharmacology, College of Medical Sciences, University of Calabar, Nigeria.

E-mail: chodraf@yahoo.com, Phone: +2348035081946

***Abstract*—Background: One of the chronic multi-system diseases of unknown etiology is rheumatoid arthritis. This disease affects people in their prime of life with an unpredictable course, predominantly between the ages of 25 and 60 years.**

Objective: The present study was targeted at evaluating the anti-arthritic activity of methanol extract of Chikadoma leaves (MECL) in complete Freund's adjuvant (CFA) – induced arthritis in rodents.

Materials and Methods: By sub-cutaneous injection of 0.1 ml of CFA in rats, arthritis was induced. Arthritic rats were divided into four groups and one group for normal rats comprising 6 animals each. Groups III and IV of the arthritic rats received 200 and 400 mg/kg of Chikadoma extract respectively. Groups I and II of the arthritic rats received dexamethasone (1 mg/kg) as a standard drug and Tween-20 (2 ml/kg) respectively, while Group V of normal animals received Tween-20 (2 ml/kg). Group II and V served as Disease control and Normal control respectively. On 7, 14, 21 and 28 day, paw volumes were measured using a plethysmometer.

Results: The MECL (200 and 400 mg/kg orally) showed significant ($p<0.05$, $p<0.01$) reduction in paw volume, change in body weight in CFA rats at 28 day when compared with arthritic control rats. All data in statistical analysis were expressed as mean ± SEM. One-way ANOVA followed by Dunnett's test was employed to compare the mean values of test groups and control. Conclusion: The results generated from the present study revealed the potential anti-arthritic activity of methanol extract from the leaves of Chikadoma.

Keywords—Chikadoma leaves, Lupinus arboreus, anti-arthritic activity, complete Freund's adjuvant, dexamethasone.

Introduction

Rheumatoid arthritis (RA) is characterized by articular inflammation, rheumatoid pannus and by the formation of an inflammatory and invasive tissue that eventually leads to the destruction of joints. It is a systemic autoimmune disorder of unknown cause, affecting 1-15% of the population all over the world [1]. Analgesics and anti-inflammatory drugs including steroids are indicated for suppression of the symptoms.

The underlying immune processes are inhibited using disease- modifying anti-rheumatic drugs ($DMARD_S$), recent advances in therapies such as anti – CD 20 therapy (rituximab) and abatacept as well as anti-tumor necrosis factor (TNF)-α therapy (infliximab, etanercept and adalimumab) [2].

However, numerous adverse effects are associated with all these agents, hence researchers in recent times, have directed attention toward traditional system of medicine for the exploration and discovery of drugs that are long-acting anti-inflammatory with minimum adverse effects.

Chikadoma (Fabaceae) is an ethnomedicinal plant whose botanical and morphological description, even the phytochemical compositions can be influenced by source or geographical location. However, quinolizidine alkaloids have been registered constantly as the chemotaxonomical markers of the plant genus [3,4,5]. It is found in many countries including USA (Northern California) where it is known as "coastal bush". The English name is yellow bush[6] and in Nigeria, Chikadoma,-named after a lead researcher Dr. **Chika** Oha**doma**, who pioneered extensively work on the novelty study of this plant botanically referred to as *Lupinus arboreus*[5].

The various parts of Chikadoma have been used in the folk medicine for the treatment of deformities of the skin, scabies, scald heads and other cutaneous distempers as well as exert antimicrobial, antinociceptive and anti-inflammatory effects [7,5]. The above literatures, informed our choice of this plant to evaluate anti-arthritic activity.

Materials and methods

Plant materials, preparation of extract, determination of the acute toxicity and phytochemical

constituents are in accordance to earlier documented reports [8,9].

Plant materials

Fresh leaves of Chikadoma were collected from Owerri, Imo State, Nigeria. The plant was authenticated by Dr. Osuala F.N. of Pharmacognosy Department, Madonna University, Elele, Nigeria, and Voucher specimen of the leaf was deposited at the Herbarium.

Preparation of the extracts

The leaves were air-dried at room temperature for 28 days and pulverized into fine powder. The powdered leaf (2 kg) was extracted with absolute methanol (Sigma Aldrich, Germany) by cold maceration for 18 h. The mixture was filtered to obtain methanol extract which was evaporated using a rotary evaporator (RV 05 Basic 1B, IKA, Staufen, Germany) and the concentrated methanol extract stored in a refrigerator.

Animals

Healthy adult Wister rats of both sexes weighing 200-300 g were used in this study. The animals were obtained from the Animal house, Department of Pharmacology and Toxicology, Madonna University, Elele,Nigeria. The animals were maintained under standard Laboratory conditions and had free access to water and standard pellets (Guinea Feeds Plc, Nigeria). The animals were transferred to work area and allowed two weeks of acclimatization.

Induction of Arthritis

Arthritis was induced in the rats by intraplantar injection of 0.1 ml of Complete Freund's adjuvant (Sigma Aldrich, Germany) in the left hind paw. The adjuvant contained heat-killed *Mycobacterium tuberculosis* in sterile paraffin oil (10 mg/ml). The paw volumes of all the animal groups were measured using a plethysmometer (Ugo Basile, 7140) at intervals of 0, 7, 14, 21, and 28 days after the injection of CFA [10], alongside the Body weights.

Experimental design

The CFA-induced arthritic animals were divided into four group comprising six rats each, and one group of normal non-arthritic rats. MECL was given at doses of 200 and 400 mg/kg for 28 days, as a suspension in Tween-20 at a dose of 2 ml/kg to different groups of rats.

Group I: Arthritic animals received Dexamethasone (1 mg/kg) serving as standard drug

Group II: Arthritic animals received Tween-20 at a dose of 2 ml/kg as a suspension in distilled water and served as Disease control.

Group III: Arthritic animals received MECL at a dose of 200 mg/kg, orally.

Group IV: Arthritic animals received MECL at a dose of 400 mg/kg, orally.

Group V: Normal animals received Tween-20 at a dose of 2 ml/kg as a suspension in distilled water and served as Normal control.

Statistical Analysis

All the data were statistically evaluated with ANOVA and the differences were determined by Dunnett's multiple comparison tests among groups using Graph pad prism 5.0. Values were considered to be significant when $p<0.05$. The results were expressed as mean ± SEM for six rats in each group.

Results

Effect of MECL on Body Weight

Body weight of all the rats in the normal control group appreciated till day 28, while that of all the rats in the arthritic control group was significantly reduced till day 28 as compared to the normal control group. In furtherance, body weight of all the rats in MECL-treated and Dexamethasone-treated groups increased significantly ($p<0.01$) as compared to the arthritic control group (Table 1).

Effect of MECL on Paw Volume

Intraplantar injection of CFA in animals induced severe inflammation and redness over a period of 24 h. Oral administration of MECL (200 and 400 mg/kg) alleviated the alteration in paw volume significantly ($p<0.5$, $p<0.01$) respectively, on day 28 when compared with arthritic control (Table II).

Table 1: Effect of MECL on body weight of CFA-induced arthritic rats

Groups	Treatment	Dose (mg/kg)	Change in body weight (g)				
			Day 0	Day 7	Day 14	Day 21	Day 28
I	Dexamethasone	1	205 ± 0.60	194 ± 0.32**	197 ± 0.41**	205 ± 0.29**	211 ± 0.64**
II	Disease control	-	204 ± 0.59	187 ± 0.59	179 ± 0.64	166 ± 0.55	164 ± 0.60
III	MECL	200	203 ± 0.46	191 ± 0.41*	193 ± 0.46*	198 ± 0.46*	209 ± 0.46*
IV	MECL	400	204 ± 0.29	194 ± 0.60**	195 ± 0.29**	202 ± 0.50**	213 ± 0.55**
V	Normal Control	Tween-20	205 ± 0.29	215 ± 0.54	217 ± 0.48	219 ± 0.48	223 ± 0.46

Mean ± SEM, n = 6, *p<0.05, ** p <0.01 as compared to the disease control group. Results analyzed by one-way ANOVA followed by Dunnett's test.

Table II: Effect of MECL on paw edema of CFA-induced arthritic rats

Groups	Treatment	Dose (mg/kg)	Displacement of mercury (ml) in different days				
			Day 0	Day 7	Day 14	Day 21	Day 28
I	Dexamethasone	1	0.25 ± 0.01	0.76± 0.02	0.48 ± 0.01**	0.41 ± 0.02**	0.38 ± 0.02**
II	Disease control	-	0.25 ± 0.01	0.83 ± 0.01	0.83 ± 0.01	0.76 ± 0.01	0.71 ± 0.02
III	MECL	200	0.23 ± 0.01	0.78 ± 0.02	0.73 ± 0.02*	0.61 ± 0.02*	0.58 ± 0.02*
IV	MECL	400	0.23 ± 0.01	0.75 ± 0.01	0.68 ± 0.02**	0.43 ± 0.03**	0.40 ± 0.02**
V	Normal Control	Tween-20	0.21± 0.00	0.23± 0.01	217 ± 0.00	0.21 ± 0.00	0.20 ± 0.01

Mean ± SEM, n = 6, *p<0.05, ** p <0.01 as compared to the disease control group. Results are expressed by one-way ANOVA followed by Dunnett's test. .

Discussions

The results showed that methanol extract of Chikadoma leaves and dexamethasone increased the body weight but reduced the paw edema. CFA is used to initiate induction to arthritis which is a chronic inflammatory disease characterized by proliferation of fibroblastic, infiltration of the synovial lining by inflammatory cytokines as well as a paucity of apoptosis resulting in bone and joint destruction [11].

The CFA model is the authentic model of RA, which has been extensively employed in pre-clinical screening of new anti-arthritis compound and has successfully predicted effects in multiple new therapeutics. The joint pathology as observed in this animal model resembles the cartilage degradation, bone resorption, and cellular influx noticed in human RA[12,13]. Paw thickness and paw volume are physical indicators of the inflammation in both early and chronic phase of the disease. An increase of the paw footpad and tibiotarsal joint diameters after 14th day, is characteristics of the progression of arthritis, which can be attributed to the delayed immunological flare in the disorder [13]. The MECL-treated groups showed significant reduction in paw volume suggesting the anti-inflammatory tendency of the leaves because the determination of paw swelling is an apparently sensitive, quick, and simple procedure for evaluating the degree of inflammation and assessing therapeutic effectiveness of drugs [2] . The possible mechanism of action though not investigated in this work might be by a suppressive effect on Th-1 helper cells since T-cell proliferation remains an important mechanism of adjuvant diseases, specifically their differentiation into Th-1 helper cells [14].

Conclusion

From the outcome obtained in the present study, it may be concluded that Chikadoma (*Lupinus arboreus)* possesses significant anti-arthritic activity. Further research may be planned as an extension to prove Chikadoma as a potent anti-arthritic agent.

Conflict of interest statement:

We declare that the authors have no conflict of interest.

Source of support: Nil

References

[1] Liu M, Dong J,Yang Y, Yang X, Xu H. Anti-inflammatory effects of triptolide loaded poly (D, L-lactic acid) nanoparticles on adjuvant-induced arthritis in rats. J. Ethnopharmacol 2005; 219-225.

[2] Ohadoma SC. Clinical and natural product pharmacology made easy. 2nd ed., Nigeria: Reverend Publishers 2017; p.282 - 298.

[3] Kar A. Pharmacognosy and Pharmacobiotechnology. 2nd ed. New Delhi: New Age Publishers, 2007; p.449.

[4] Treasew GE. A textbook of Pharmacognosy. 9th ed. London: W.B. Saunders; 1996; p. 201.

[5] Ohadoma SC. Scientific basis for the therapeutic use of *Lupines arboreus.* European Journal of Pharmaceutical and medical Research. 2018; 5(3): 30-43.

[6] Pickart AJ, Miller LD. Yellow bush lupine invasion in Northern California coastal dunes. Ecological impacts and manual restoration techniques. Restore Ecol, 1998; 6:59-68.

[7] Pliny IO. The family of lupines. A collection of Miller Institute for Basic Research, Miller Publishers, USA, 2009.

[8] Ohadoma SC. Akah PA, Enye JC. Pharmacological assessment of the antinociceptive and anti-inflammatory effects of the methanol leaf extract and fractions of *Lupinus arboreus*. Asian J.Sc. Tech, 2015; 6(8):1663-1668.

[9] Ohadoma SC, Akah PA, Nkemnele CA, Ikeduba EN, Nwokoma EI. Determination of the acute toxicity and phytochemical constituents of the methanol leaf extract of *Lupinus arboreus* Journal of Science, Engineering and Technology, 2010; 17(3): 9738 – 9743.

[10] Newbould BB. Chemotherapy of arthritis induced in rats by Mycobacterial Adjuvant. Brit J Pharmacol Chemother 1963; 21: 127-136.

[11] Ghildiyal S, Gautam MK, Joshi VK, Goel RK. Anti-inflammatory activity of two classical formulations of Laghupanchamula in rats. J Ayurveda Integr Med 2013; 4: 23 – 27.

[12] Bendele A, McComb J, Gould T, McAbee T, Sennello G, Chlipala E, *et al.* Animal models of arthritis: Relevance to human disease. Toxicol Pathol 1999; 27: 134-142.

[13] Andersen ML, Santos EH, Seabra Mde L, da Silva AA, Tufik S. Evaluation of acute and chronic treatments with *Harpagophytum procumbens* on Freud's adjuvant-induced arthritis in rats. J Ethnopharmacol 2004; 91:325-330.

[14] Weiner HL. Induction and mechanism of action of transforming growth factor – beta – secreting Th-3 regulatory cells. Immunol Rev 2001; 182:207-214.