ejpmr, 2021,8(5), 65-71

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

ANTI-ARTHRITIC POTENTIAL OF AQUEOUS AND ETHANOL LEAF AND ROOT EXTRACTS OF ALAFIA BARTERI OLIV. *(APOCYNACEAE)* IN WISTAR RATS

[1,2]Kolawole, Ikeoluwa O., *[1]Ebuehi, Osaretin A.T. and [1]Odesanmi, Omolola S.

[1]Department of Biochemistry, College of Medicine, University of Lagos, PMB 12003, Lagos, Nigeria.
[2]Department of Biological Sciences, College of Basic and Applied Sciences, Mountain Top University, Ibafo, Ogun State, Nigeria.

***Corresponding Author: Prof. O.A.T. Ebuehi**

Department of Biochemistry, College of Medicine, University of Lagos, PMB 12003, Lagos, Nigeria.

Article Received on 25/02/2021 **Article Revised on 17/03/2021** **Article Accepted on 07/04/2021**

ABSTRACT

Alafia barteri is valued for its effectiveness in the traditional medicine system in Nigeria and other African countries, though its anti-arthritic activity is yet to be evaluated. This study is to evaluate the anti-arthritic potential of the aqueous and ethanol leaf and root extracts of *Alafia barteri* plant in Wistar rats using the CFA-induced arthritis model. 75 healthy adult male Wistar rats weighing between 200g and 250g were completely randomized into fifteen groups (A-O) of five rats each. The test groups were treated by administering the extracts at 100mg/kg, 200mg/kg and 400mg/kg. Arthritis induction was done by intra-dermal injection of 0.1ml of Complete Freund's Adjuvant (10mg/kg of heated-killed *Mycobacterium tuberculosis* in 1ml paraffin oil) into the right hind paw of rats and administration was done for 28days. Arthritis was assessed by measuring the paw thickness with the use of a digital vernier calliper. Body weights and biochemical parameters (serum tumor necrosis-alpha and rheumatoid factors) in the arthritic rats were also measured. Data of study obtained were analyzed using one way analysis of variance (ANOVA), followed by Turkey's post hoc test. From the results of this study, the aqueous leaf and root extracts of *Alafia barteri* caused a significant reduction ($p<0.05$) in paw thickness and increase in body weight of the rats. Serum tumor necrosis factor (TNF)-alpha and rheumatoid factor were decreased in arthritic rats, which is suggestive of its anti-inflammatory effect caused by the root and leaf aqueous and ethanol extracts of *Alafia barteri*.

KEYWORDS: *Alafia barteri,* arthritis, tumor necrosis factor, rheumatoid factor, rat.

INTRODUCTION

Rheumatoid arthritis (RA) is a systemic, autoimmune and a destructive joint disease caused by inflammation in the synovial tissue. It is one of the most common chronic inflammatory disorders and one of the leading causes of disability worldwide (Yoshizaki *et al.*, 1998,Waljee and Chung, 2011, Xin *et al.*, 2019). Its prevalence rate ranks first in the autoimmune connective tissue diseases and the average global incidence is about 0.5–1.0% between the ages of 40–60 years old (Eric and Lawrence, 1996, Xin *et.al.*,2019).

According to Adelowo *et al.*, (2010), rheumatoid arthritis could be a significant medical condition among Nigerians. Its true incidence in Nigeria remains unknown but its crude incidence in an urban city has been estimated to be 12.3% with a female-to-male ratio of 2.4:1 and mean age of 46.9 years (Ali e*t al.*, 2016, Aderemi *et al.*, 2018). Nigerians who suffer RA have also been observed to have high titres of Rheumatoid factor and anti- CCP (anti-cyclic citrullinated peptide) levels (Ishola *et al.*, 2015, Hamid and Aiyelaagbe, 2017, Atilade *et al*., 2018,).

Recent studies have shown the key role played by inflammatory response in the development and progression of RA as the cytokines (IL-1β, IL-6, and TNF-α) have been reported to be involved in the whole process of RA (Xin *et al.*, 2019). A large infiltration of immune cells such as $CD4^+$ T cells, B cells, macrophages and neutrophils into the inflammed joint compartment coupled with significant production of reactive oxygen species (superoxide, hydrogen peroxide and hydroxyl radicals) have also been reported to mediate the progression of joint destruction in RA (Xin *et al.*, 2019, Aderemi *et al.*, 2018, Waljee and Chung, 2011).

Despite the shift towards the use of synthetic medicine and sophisticated drugs, plant-based remedies still play an important role in the world's medicine (Newman *et al*., 2000). Therefore, treating arthritis with plant-derived compounds which are accessible and do not need laborious pharmaceutical synthesis coupled with little or no side effects seems attractive.

Alafia barteri Oliver (Apocynaceae) commonly called Alafia chewing stick and guinea fowl's crest, "agbari-etu

and ibo-agba (yoruba), ota nza(igbo)"(local names) is a vigorous climbing shrub native to the West and Central Africa, stretching from Guinea Bissau to Cameroon, Congo and Nigeria (Irvine, 1961). It is valued for its effectiveness in the traditional medicine system in Nigeria and other African countries (Ishola *et al.,* 2015, Hamid and Aiyelaagbe, 2017, Atilade *et al*., 2018, Adekunle and Okoli, 2002).

The decoction of root and leaves of the plant is also taken internally or applied externally to treat toothache and eye infections (Sofidiya *et al.,* 2014) and the fiber from the stem of the plant serves as tying material for roofs. The leaf extracts were found to have antibacterial and antifungal activities (Adekunle and Okoli, 2002, Hamid and Aiyelaagbe *et al*., 2011), anti-plasmodial activity (Lasisi *et al.,* 2016); anti-diabetic activity (Atilade *et al.,* 2018). Its root extract also possessed analgesic activity (Ishola *et al.,* 2015), while the stem extract of the plant has also been reported to show anti-proliferative activity (Hamid *et al*., 2017). Aderemi *et al*.(2018) has also reported its effect on spermatogenesis and steroidogenesis.

This present study is to ascertain the anti-arthritic potential of the aqueous and ethanol leaf and root extracts of *Alafia barteri* in male Wistar rats using the complete Freund's Adjuvant Model.

MATERIALS AND METHODS

Plant Material

The whole plant material of *Alafia barteri* was collected from Abatadu village in Ikire, South West, Nigeria in the month of February, 2018. Botanical identification and authentication was carried out by Dr. A. Kadiri at the Department of Botany, University of Lagos, Lagos, where a voucher specimen was deposited with the herbarium file number: LUH 5517.

Extract Preparations

Fresh leaves were plucked and root barks were air dried for a week and pulverised using the blender. 250g of the powdered leaves and chopped root bark were macerated in 1.5 litres of ethanol for 72h with occasional stirring (ethanol extract). 250g of powdered leaves and root bark were soaked in distilled water for 48h (aqueous extract). Both mixtures were filtered respectively with Whatman No 1 filter paper and concentrated with the oven and rotary evaporator at reduced pressure and at room temperature till a constant weight was obtained.

Reagents Used

Complete Freund's Adjuvant (FCA) from Sigma Chemicals Co., ELISA kits of TNF-α and Rheumatoid Factor(RF) were purchased from Ray Biotech, diclofenac and cerebrex were obtained from a reputable pharmaceutical company, Lagos, Nigeria. All others reagents and chemicals used were of analytical grade.

Experimental Animals

Seventy five (75) healthy adult male Wistar rats weighing between 200g and 250g were obtained from the Laboratory Animal Centre of College of Medicine, University of Lagos, Lagos. The animals were housed in clean plastic cages, had free access to feed and water, and were allowed to acclimatize to the laboratory conditions for 7 days preceding the experiment. The animals were completely randomized into fifteen groups (A-O) of five rats each. Groups A and B represented the non-arthritic and arthritic control, group C was treated with the standard drug, 2mg/kg bwt, celecoxib, groups D-F received 100,200 and 400mg/kg of *Alafia barteri* aqueous leaf extract, groups G-I received 100,200 and 400mg/kg of *Alafia barteri* ethanol leaf extract, groups J-L received 100,200 and 400mg/kg of the aqueous root extract, while groups M-O received 100,200 and 400mg/kg of ethanol root extract. All groups were induced and treated with the extracts, except the normal control group which received distilled water.

Arthritis Induction

Induction was done according to the method of Modal *et al.*,(2016) by intra-dermal injection of 0.1 ml of Complete Freund's Adjuvant (10 mg/kg of heated-killed *Mycobacterium tuberculosis* in 1ml paraffin oil) into the right hind paw of rats after which administration took place for 28 days. Body weights were measured with the weighing balance and arthritis was assessed by measuring the paw thickness over a period of 28 days with the use of a digital vernier calliper. The percentage inhibition of right paw thickness was calculated by the formula: (VC –VT) × 100/VC where, VC represents the paw thickness of control group and VT represents the paw thickness of the test group (Madhavi *et al.*,., 2015, Nishat and Syeda, 2016).

The animals were sacrificed on the 29th day by cervical dislocation under anesthesia and blood samples were collected by ocular puncture into Lithium heparin bottles for biochemical analysis. Centrifugation of blood samples was carried out at 4000rpm for 15min to obtain serum, which was analyzed with the ELISA kits for tumor necrosis factor (TNF)-alpha and rheumatoid factor (RF) respectively, at the Nigerian Institute for Medical Research,Yaba, Lagos, Nigeria.

Statistical Analysis

Data of the study obtained were expressed as mean ± SEM (n = 5). Statistical analysis of the values was performed using one-way ANOVA followed by Turkey's multiple comparison test. GraphPad prism software (USA) version 6.02 was used for this analysis. P value < 0.05 was considered to be statistically significant.

RESULTS

The ethanol leaf extract had the highest yield compared to the aqueous leaf and root extracts after extraction as presented in Table 1. On induction with Complete

Freund's Adjuvant, a loss in body weight was observed in all rats, which was restored on administration of the aqueous leaf and root extracts of *Alafia barteri* (Table 2). The standard drug celecoxib was seen to cause an increase though not significant (p> 0.05).

Table 3 shows the effect of the extracts on paw diameter during the period of administration. The Complete Freund's Adjuvant administration in the arthritic induced rats resulted a significant (P< 0.05) increase (swelling) in the paw of rats as seen on day 7.Treatment with the leaf and roof extracts however caused a significant reduction in the inflamed paws on days 21 and 28, with the ethanol leaf extract having the highest percentage inhibition (51.14%).

A rise in serum TNF alpha levels was also observed on induction, however, treatment with the extracts had no significant reduction in their levels.(Figures 2 and 3). Rheumatoid factor levels increased on arthritis induction as seen in the arthritic control rats(Fig. 3), while both the standard drug, celecoxib and *Alafia barteri* extracts respectively caused a significant reduction in its serum levels of TNF and RF.

Table 1: Percentage yield of leaf and root aqueous and ethanol extracts of *Alafia barteri.*

Aqueous leaf	7.48 ± 0.46%
Ethanol leaf	11.06 ±.57%
Aqueous root	7.34 ± 0.73%
Ethanol root	10.53 ± 0.65%

Table 2: Body Weights of rats administered with aqueous and ethanol leaf and root extracts of *Alafia barteri* for 28 days

Treatment Group	Dose	Day 1	Day 14	Day 28
A(NAC)	Distilled Water	109.80±4.13	119.50±5.81	141.80±7.00*
B(AC)	Distilled Water+1ml CFA	103.00±5.15	104.00±5.37	103.50±6.46
C	2mg/kg celecoxib +0.1ml CFA	95.80±4.97	111.20±6.79	120.60±7.52
D	100mg/kg aqueous leaf extract +0.1ml CFA	108.00±6.05	120.20±6.38	135.00±8.17*****
E	200mg/kg aqueous leaf extract +0.1ml CFA	111.60±4.68	126.20±2.01*	135.80±3.34*
F	400mg/kg aqueous leaf extract +0.1ml CFA	103.80±6.46	120.00±5.89	132.80±5.81*
G	100mg/kg ethanol leaf extract +0.1ml CFA	102.60±5.32	113.20±4.43	124.20±3.80*
H	200mg/kg ethanol leaf extract +0.1ml CFA	103.00±5.55	119.50±3.12	128.30±3.68*
I	400mg/kg ethanol leaf extract +0.1ml CFA	100.50±2.22	112.30±4.63	125.30±5.51*
aqueous and ethanol root extract of *Alafia barteri* for 28 days				
J	100mg/kg aqueous root extract +0.1ml CFA	106.00±4.59	118.20±5.21	125.20±6.21*
K	200mg/kg aqueous root extract +0.1ml CFA	101.40±3.61	113.40±4.25	124.00±4.30
L	400mg/kg aqueous root extract +0.1ml CFA	108.80±3.09	121.00±7.47	134.00±9.76*
M	100mg/kg ethanol root extract +0.1ml CFA	112.80±7.86	122.00±8.12	133.75±9.00*
N	200mg/kg ethanol root extract +0.1ml CFA			
O	400mg/kg ethanol root extract +0.1ml CFA			

Results represent Mean ± SEM: Values that carry superscript (*) are significantly different from the arthritic control (P<0.05)

Table 3: Effect of aqueous and ethanol leaf and root extracts of *Alafia barteri* on paw diameter of Wistar rats.

Treatment Group	Dose	Day 0	Day 7	Day 14	Day 21	Day 28
A(NAC)	Distilled Water	2.04±0.004	2.04±0.004	2.04±0.004	2.04±0.004	2.04±0.004
B(AC)	Distilled Water+1ml CFA	2.04±0.007	4.25±0.31	5.27±0.19	5.50±0.15	5.69±0.19
C	2mg/kg celecoxib +0.1ml CFA	2.04±0.007	4.74±0.33	4.60±0.21 12.71%	4.17±0.19 24.18%	3.94±0.17**** 30.76%
D	100mg/kg aqueous leaf extract +0.1ml CFA	2.02±0.006	5.46±0.24	4.99±0.29 5.31%	4.19±0.29 23.82%	3.76±0.27**** 33.92%
E	200mg/kg aqueous leaf extract +0.1ml CFA	2.04±0.007	4.76±0.11	4.11±0.08* 22.01%	3.59±0.14**** 34.72%	3.05±0.10**** 46.39%
F	400mg/kg aqueous leaf extract +0.1ml CFA	2.04±0.01	4.78±0.20	4.17±0.19* 20.80%	3.59±0.11**** 34.72%	2.91±0.15**** 48.86%
G	100mg/kg ethanol leaf extract +0.1ml CFA	2.04±0.007	4.56±0.14	3.74±0.22* 29.03%	4.09±0.16 25.64%	3.66±0.23**** 25.68%
H	200mg/kg ethanol leaf extract +0.1ml CFA	2.03±0.009	4.74±0.33	4.47±0.29 15.18%	3.91±0.23**** 28.91%	3.24±0.20**** 43.06%
I	400mg/kg ethanol leaf	2.03±0.007	4.61±0.25	3.63±0.13*	3.19±0.09****	2.78±0.17****

	extract +0.1ml CFA			31.12%	42.00%	51.14%
J	100mg/kg aqueous root extract +0.1ml CFA	2.03±0.009	5.74±0.19*	5.14±0.29 2.47%	4.59±0.2 16.565%	4.09±0.29**** 28.12%
K	200mg/kg aqueous root extract +0.1ml CFA	2.04±0.007	5.04±0.24	4.92±0.25 6.64%	4.56±0.34 17.09%	3.94±0.39**** 30.76%
L	400mg/kg aqueous root extract +0.1ml CFA	2.03±0.007	4.93±0.22	4.65±0.21 11.76%	3.93±0.15 28.55%	3.31±0.16**** 41.83%
M	100mg/kg ethanol root extract +0.1ml CFA	2.03±0.008	4.59±0.09	4.13±0.11** 21.63%	3.54±0.15**** 35.64%	3.22±0.12**** 43.41%
N	200mg/kg ethanol root extract +0.1ml CFA	2.02±0.005	5.21±0.33	4.06±0.33** 22.96%	3.56±0.34**** 35.27%	2.92±0.39**** 48.68%
O	400mg/kg ethanol root extract +0.1ml CFA	2.03±0.007	4.54±0.29	3.86±0.39** 26.76%	3.40±0.37**** 38.18%	2.98±0.39**** 47.63%

Results represent Mean ± SEM: Values that carry superscript (*) are significantly different from the arthritic control (P<0.05)

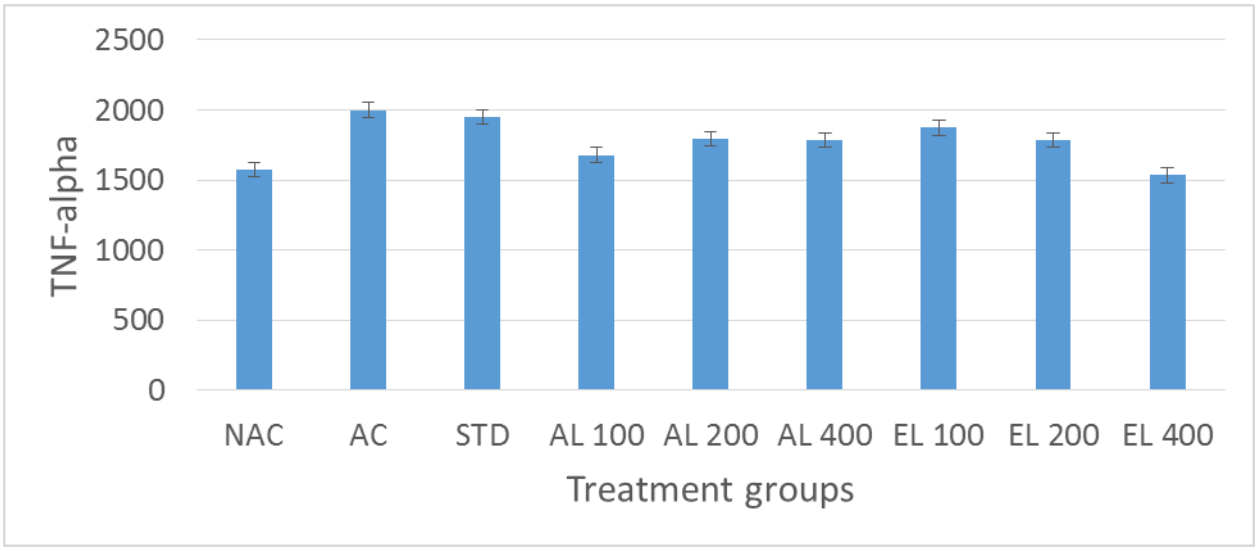

Figure 1: Serum TNF-alpha of arthritic rats administered aqueous and ethanol leaf extracts of *Alafia barteri* (Values are expressed as mean ± SEM, p< 0.05).

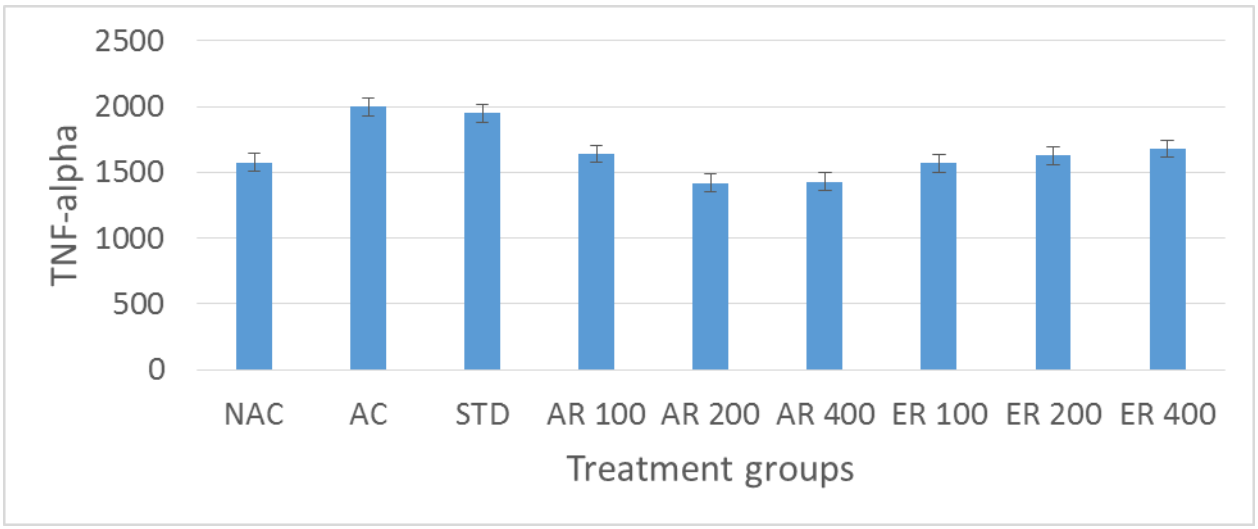

Figure 2: Serum TNF-alpha of arthritic rats administered aqueous and ethanol root extracts of *Alafia barteri* (Values are expressed as mean ± SEM, p< 0.05).

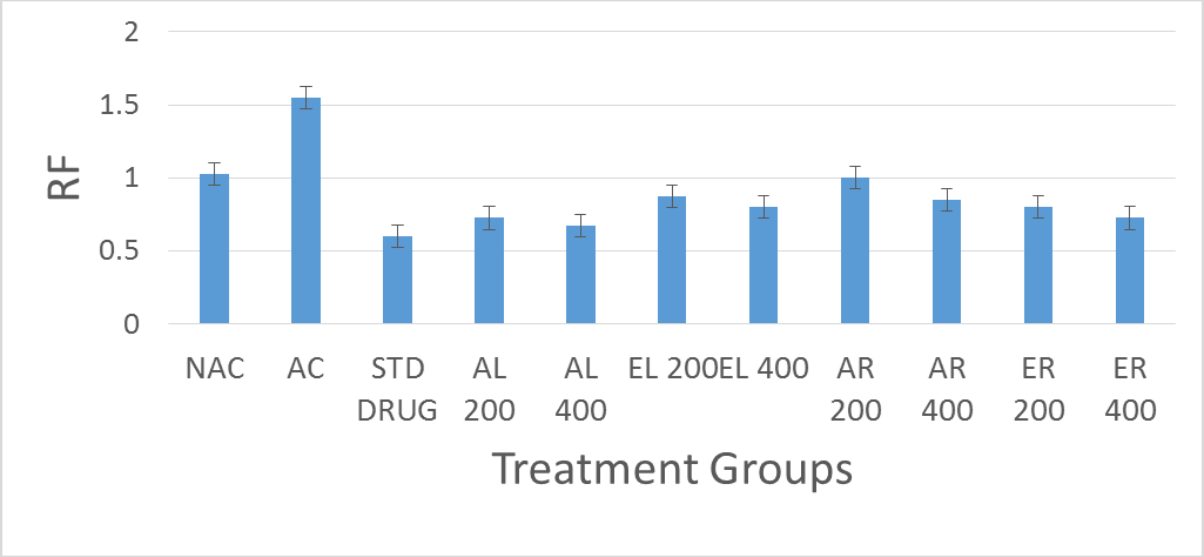

Figure 3: Serum Rheumatoid factor of arthritic rats administered aqueous and ethanol leaf extracts of *Alafia barteri* (Values are expressed as mean ± SEM, p< 0.05).

DISCUSSION

In the anti-arthritic study, the activity of *A. barteri* leaf and root extracts was evaluated against CFA-induced arthritis. Freund's Complete Adjuvant consists of inactivated and dried *Mycobacterium*, which effectively stimulates cell mediated immunity and ultimately leads the immunoglobulin production. CFA-induced arthritis remains one of the oldest experimental animal models used to mimic human rheumatoid arthritis and it is mostly used in anti-arthritic studies (Roubenoff *et al*., 1997). Celecoxib, a non-steroidal anti-inflammatory drug was used as the standard drug for comparison due to its frequent use in the treatment of arthritis (Kore *et al*., 2013, Rall and Roubenoff, 2004, Madhavi *et al*., 2015).

It is well documented in literature that weight loss and cachexia (body wasting due to chronic illness) cachexia which leads to the decreased physical activity, muscle strength and decreased daily performance are common features in rheumatoid arthritis. These effects have been found to be as a result of raised cytokines levels which affects metabolic rate and protein breakdown. (Eric and Lawrenece, 1996, Yoshizaki *et al.*, 1998, Erukainure *et al.*, 2015, 2020, Igietseme *et al*., 2014, Ukwade *et al.*, 2014).

A significant finding of the present study is the effect of AB treatment on body weight changes of arthritis induced rats. The arthritic control rats were observed to have reduced weight gain pattern over the treatment period when compared with the standard drug and extract treated arthritic rats which is similar to that previously reported by Roubenoff *et al*., 1997). The body weight gain on the plant extract administration may be due to its ability to increase or restore the absorption of nutrients showing effective management of rheumatoid cachexia.

Prostaglandins are generated in primary inflammatory phase of arthritis and auto antibodies are generated in secondary immunological state. Release of various inflammatory mediators including cytokines (IL-1B and TNF-α), interferons and prostaglandins are responsible for the initiation of pain followed by limb and joint swelling, bone deformation and joint disability (Eric and Lawrenece, 1996).

The results of this study (Table 3) showed a significant increase in paw diameter on induction of arthritis with CFA which reflected the status of arthritis. Arthritis induced rats were treated with AB extracts and a significant reduction in paw diameter on the 21st and 28th day was observed. This may be as a result of decrease in the production of inflammatory mediators as also seen in igure 1 showing anti-inflammatory activity of AB extract.

CONCLUSION

Data of the present study indicate that anti-arthritic potential of aqueous and ethanol leaf and root extracts of *Alafia barteri* which was seen in its ability to reduce paw inflammation and serum TNF levels. Further studies on the isolation of the active ingredients of the extracts responsible for the anti-arthritic effect would be required in the future.

ACKNOWLEDGEMENT

The authors are grateful for the laboratory and technical assistance received from the technologists, Mr.S.O. Adenekan, Mr.P.Okoh and others, of the Department of Biochemistry, College of Medicine, University of Lagos, Nigeria. We are indebted to the laboratory assistance received from the technical staff and students of the Biochemistry Unit, Mountain Top University, Ibafo, Ogun State, Nigeria.

REFERENCES

1. Adekunle AA and Okoli SO. Antifungal activity of the crude extracts of *Alafia barteri Oliv. (Apocynaceae*) and *Chasmanthera dependens* (Hochst). Menispermaceae. Hamdard, 2002; 45: 52-56.
2. Adelowo OO[1], Ojo O, Oduenyi I, Okwara CC. Rheumatoid arthritis among Nigerians: the first 200patients from a rheumatology clinic. Journal of Clinical Rheumatology, 2010; 29(6): 593-597. doi: 10.1007/s10067-009-1355-0.
3. Aderemi, A., Ogunlade, B., & Omotoso, O. Histomorphological responses to aqueous crude leaf extract of *Alafia barteri* on prefrontal cortex, heart, kidney, liver and testis of adult male Sprague-Dawley rats. European Journal of Biological Research, 2018; 8(2): 105-111.
4. Ali AA, El-Zaitony AS, Al-Haleem ENA. Evaluation of therapeutic efficacy of Vinpocetine in adjuvant induced arthritis model in rats. J Pain Management Med, 2016; 2: 115.
5. Atilade AO, Ogunniyi, Victoria O, Joseph DB, Segun EA, *et al*. Aqueous leaf extract of *Alafia Barteri* maintained renal integrity in diabetic Wistar rats. J . Physiol. Stud. 2018, 1: 001-008.
6. Eric GB, Lawrence JL. Rheumatoid arthritis and its therapy: The textbook of therapeutics drug and disease management, Baltimore: Williams and Wilkins company, 1996; 579–595.
7. Erukainure, O, Ebuehi, O.A.T., Choudhary, I., N Ashraf, A Navqi, A Muhammad, Zaruwa, M., & Elemo G.N. Dietary Fatty Acids from a local Nigerian Spice (*Clerodendrum volubile*) Suppresses Tumor Cell Migration and Invasion, Attenuates Oxidative Stress, and Regulates Cell Cycle Progression in Human Neuronal Glioblastoma Cells. Annals of Nutrition and Metabolism, 2015; 67: 454-455.
8. Erukainure, O.L., Salau, V.F., Alabi, O.O., Ebuehi, O.A.T., Koorbanally, NA and Shahidul, Md. Casein micelles from bovine milk exerts neuroprotection by stalling metabolic complication linked to oxidative brain injury. Metabolic Brain Disease, 2020; 35(5): 1417-1428, doi. org/ 10.1007/s 11011-020-00621-3.
9. Hamid AA and Aiyelaagbe OO. Pharmacological screening of *Alafia barteri* leaves for its antibacterial and antifungal properties against pathogenic bacteria and fungi. Int J Pharm Sci Health Care, 2011; 1: 93-104.
10. Hamid, A.A., Aiyelaagbe, O.O., Kaneez, F. *et al.* Isolation, characterization and antiproliferative evaluation of constituents from stem extracts of *Alafia barteri Oliv. Hook. F. . Med. Chem. Res*, 2017; 26: 3407–3416. https://doi.org/10.1007/s00044-017-2033-4s
11. Igietseme, JU, Ukwade C, Ouburg S, Land JA, Omosun Y, Joseph K, Partin J, He Q, Eko FO, Ellerson D, Bandea C, Morre SA, Zhong G, Ebuehi, O.A.T. and Black CM. Profile of Anti-chlamydial immune responses in complicated (infertile) and Non-complicated (fertile) genital infections. J. Clinical Immunology & Immunotherapy, 2014; 2: 1-5.
12. Irvine V.R. Woody plants of Ghana with special reference to their uses: London Oxford University press, 1961; 110—145.
13. Ishola IO, Oreagba IA, OkekeOgochukwu N, Olayemi SO. Analgesic and anti-inflammatory actions of *Alafia barteri:* Involvement of monoaminergic, nitrergic and opioidergic pathway. *Nig. Qt. J. Hosp. Med*, 2015; 25(2): 118-123.
14. Kore K. J., Shete R.V., Desai N. V. Anti- arthritic activity of hydroalcoholic extract of *Lawsonia Innermis*. Int. J. Drug Dev. & Res, 2013; 4: 217-224.
15. Lasisi AA, Olayiwola MA, Balogun SA, Akinloye OA, Ojo DA. Phytochemical composition, cytotoxicity and *in vitro* antiplasmodial activity of fractions from *Alafia barteri* Oliver (Hook F. Icon) - Apocynaceae. J. Saudi Chem Soc, 2016; 20: 2-6.
16. Madhavi G. Patel and Kilambi Pundarikakshudu. Anti-arthritic activity of a classical Ayurvedic formulation *Vatari Guggulu* in rats. J. Tradit. Complement. Med, 2016; 6(4): 389–394. doi: 10.1016/j.jtcme.2015.08.007.
17. Mondal P, Das S, Mahato K, Borah S, Junejo JA and Zaman K. 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. Int. J. Pharm. Sci. Res., 2016; 7(9): 3675-88. doi: 10.13040/IJPSR.0975-8232.7(9).3675-88.
18. Newman DJ, Cragg GM, Snader KM. The influence of natural products on drug discovery. Nat Prod Rep, 2000; 17(3): 215-34.
19. Nishat Fatima, Syeda Jabeen Fatima. Pharmacological screening for anti-arthritic activity of *Moringa Oleifera.* Asian Journal of Pharmaceutical and Clinical Research, 2016; 9(3): 106-111.
20. Rall LC, Roubenoff R. Rheumatoid cachexia: metabolic abnormalities, mechanisms and interventions. *Rheumatology (Oxford)*, 2004; 43(10): 1219-1223. doi:10.1093/rheumatology/keh321.
21. Roubenoff R, Freeman LM, Smith DE, Abad LW, Dinarello CA, Kehayias JJ. Adjuvant arthritis as a model of inflammatory cachexia. Rheumatism, 1997; 40: 534–539.
22. Sofidiya M. and Essien E. Imeh and C. Ezeani and F. Aigbe and A. Akindele: Antinociceptive and anti-inflammatory activities of ethanolic extract of *Alafia barteri*: Revista Brasileira De Farmacognosia-brazilian Journal of Pharmacognosy, 2014; 24: 348-354, https://doi.org/10.1016/j.bjp.2014.07.013.
23. Ukwade, C.E., Ebuehi, O.A.T., Igietseme, JU, Ouburg, S, Land, JA, Omosun, J, Joseph, K, Partin, J, He, Q, Eko, FO, Ellerson, D, Bandea, C, Morre, SA, Zhong, G. and Black, CM. Immunological Profiles of Mice Protected from Chlamydia-induced

Infertility by Anti-caspase Treatment. British J. Medicine & Medical Research, 2014; 15(3): 1-9.
24. Waljee JF, Chung KC.: Outcomes Research In Rheumatoid Arthritis. Hand Clin, 2011; 27(1): 115-26. Doi: 10.1016/J.Hcl.2010.10.005.
25. Xin Cui, Ruijing Wang, Peimin Bian, Qingke Wu, Vidya Devanatha Desikan Seshadri and Lun Liu. Evaluation of anti-arthritic activity of Nimbolide against Freund's Adjuvant Induced Arthritis in rats. Artificial Cells, Nanomedicine and Biotechnology, 2019; 47(1): 3391-3398, Doi: 10.1080/21691401.2019.1649269.
26. Yoshizaki K, Nishimoto N, Mihara M *et al*,: Therapy of rheumatoid arthritis by blocking IL-6 signal transduction with a humanized antiIL-6 receptor antibody. Springer Semin Immunopathol, 1998; 20: 247-259, https://doi.org/10.1007/BF00832010.
27. Igietseme, JU, Ukwade C, Ouburg S, Land JA, Omosun Y, Joseph K, Partin J, He Q, Eko FO, Ellerson D, Bandea C, Morre SA, Zhong G, Ebuehi, O.A.T. and Black CM., Profile of Anti-chlamydial immune responses in complicated (infertile) and Non-complicated (fertile) genital infections. J. Clinical Immunology & Immunotherapy, 2014; 2: 1-5.
28. Ukwade, C.E., Ebuehi, O.A.T., Igietseme, JU, Ouburg, S, Land, JA, Omosun, J, Joseph, K, Partin, J, He, Q, Eko, FO, Ellerson, D, Bandea, C, Morre, SA, Zhong, G. and Black, CM. Immunological Profiles of Mice Protected from Chlamydia-induced Infertility by Anti-caspase Treatment. British J. Medicine & Medical Research, 2014; 15 (3): 1-9.