Original Research Article

ANTI-ARTHRITIC ACTIVITY OF A HERBAL FORMULATION (JOINTEEZ) USED IN NIGERIA

ABSTRACT

Aim: This study evaluated the anti-arthritic activity of a herbal formulation used in the management of rheumatoid arthritis in Nigeria.

Design: Thirty-five (35) albino wistar rats were used. They were divided into seven groups of seven rats each, with Group A serving as negative control while Group B was the positive control. Groups B, C, D and E were induced with rheumatoid arthritis by injecting 0.1ml of Complete Freund's Adjuvant into the right hind paw of each rat. The rats were treated with the standard drug and herbal formulation respectively for 28 days as follows: Group C (treated with a standard drug, Celebrex), Group D (treated with the herbal drug, Jointeez), Group E (treated with a combination therapy of Jointeez and Celebrex). At the end of the 28-day treatment period, the rats were anaesthesized with chloroform and sacrificed through puncture of the jugular vein. Five millilitres (5ml) of blood samples were put into plain bottles for the analysis of biochemical parameters.

Place and duration of study: This study was conducted in the Department of Medical Laboratory Science, Rivers State University, from September to December, 2018.

Methodology: The inflammatory markers, TNF-α, IL-6 and C-reactive protein, were analysed using ELISA technique.

Results: The levels of TNF-α (p<0.001), IL-6 (p =0.01) and C-reactive protein (p <0.001) were significantly reduced in the treated rats compared to the positive control group. There were significant reduction in the paw diameters of the treated rats (p <0.001). The combination therapy used in this study did not offer significantly different therapeutic advantage over the monotherapies used in this study. The herbal formulation used in this study offered similar therapeutic activities as the orthodox drug used in this study.

Conclusion: The herbal formulations can be used as safe therapies for the management of rheumatoid arthritis in our population. It is recommended that herbal formulations be integrated into our healthcare system in the management of rheumatoid arthritis.

KEYWORDS: Anti-arthritic, herbal formulation, Complete Freund's Adjuvant, Rheumatoid Arthritis, Nigeria, Jointeez

INTRODUCTION

Rheumatoid arthritis is a chronic, autoimmune disease that affects the joints and also has extra-articular as well as systemic manifestations (Choy, 2012). Rheumatoid arthritis causes severe pain, swelling, early morning stiffness of the joint, and often there may be loss of function (Okoronou *et al.*, 2016).

The aetiology of rheumatoid arthritis is not known, but some factors have been reported to be the likely causative or predisposing factors. These include genetic, environmental and hormonal factors (Gibofskey, 2012). The Genome Wide Association Studies (GWAS) have enabled researchers to identify the genetic risk factors for many human diseases including rheumatoid arthritis. The greatest risk of the disease lies within the HLA (Human Leukocyte Antigen) region which codes for HLA – DRB1 *04 molecule (Yarwood *et al.,* 2016). HLA – DRB1 * 01 and HLA – DRB1 *04 have been associated with the susceptibility of individuals to rheumatoid arthritis (Kurko *et al.,* 2013). Similarly, non-HLA genes have been associated with rheumatoid arthritis. Single nucleotide polymorphisms in PTPN22, IL23R, TRAF1, CTLA4 and others have been linked with the pathogenesis of rheumatoid arthritis (de Vries, 2011).

Environmental risk factors are also known to predispose to rheumatoid arthritis. The strongest known of these environmental factors is smoking. This risk is higher in predisposed individuals who are anti-citrullinated peptide antibody (ACPA) – positive or rheumatoid factor – positive (Liao *et al.,* 2009). This gene-environment interaction further increases the risk by the number of shared epitopes. The shared epitope refers to a sequence of amino acids on the HLA – DRB1 allele (Liao *et al.,* 2009). It has been reported that smoking accounts for about 20 – 30% of environmental risks of rheumatoid arthritis (Klareskog *et al.,* 2010). Another environmental risk factor is exposure to silica or industrial dust (Liao *et al.,* 2009).

There is a growing popularity of CAM among the general population. In many developed countries, about 70 – 80% of the population use CAM (WHO, 2008). In spite of this, phytotherapeutics, or the use of herbs for medicines has not been accepted into mainstream

healthcare delivery, probably due to lack of knowledge by orthodox practitioner (Carmona & Pereira, 2013).

It is known that many human diseases have been treated using herbal remedies all through human history (Balasundaram *et al*.,2011). Thus, it is possible to discover new, effective and more affordable drugs for the treatment of human diseases (Kalita *et al*., 2012). Herbal formulations are being used for improving health and for the treatment or prevention of human diseases (Vieira & Huang, 2012). This widespread acceptance and use can be attributed to the notion that herbal medicines are generally safe and non-toxic (Calitz *et al*., 2015). This is more so as it has been reported that about 80% of hospital admissions in the United States of America alone are due to the toxicity of synthetic drugs (Philomena, 2011).

The renewed and growing interest of the world population for use of alternative medicines is predicated on several factors. Some of these factors include high cost and side effects of orthodox drugs amongst other factors (Ekor, 2013). In the case of rheumatoid arthritis, the drugs used for its treatment have been reported to cause a number of safety and efficacy problems. Some of the side effects of conventional anti-arthritic drugs include stomatitis, myelosuppression (common with DMARDs like methotrexate), GIT problems, renal problems, haematological abnormalities (common with NSAIDs) (Wadekar *et al*., 2015). The effort to search for affordable and safer alternatives for these conventional drugs is the major driving force for the increased interests in the use of herbal formulations (Patel *et al*., 2013).

MATERIALS AND METHODS

Experimental Animals

Thirty-five (35) female Albino Wistar rats, weighing 150-200g were used for this study.

The rats were housed in compartmentalized cage and allowed to acclimatize for two weeks, in a daily 12-hourly light and dark cycle. They were allowed access to standard feed and water *ad libitum*.

Experimental Drugs

The standard drug used for this study was Celebrex (Celecoxib), a product of Pfizer Pharmaceuticals, Puerto Rico. The used herbal formulation used for this study was Jointeez (product of Kedi Healthcare Industries Limited, Nigeria).

Determination of Therapeutic Doses

The rat doses of the herbal formulations and orthodox drugs were extrapolated from the human therapeutic doses based on body surface area ratio, using the Paget and Barnes (1964) conversion table.

The daily dose of both the standard drug and the herbal formulations were determined based on OECD's Guidelines (OECD, 2001).

Acute Toxicity Testing of the Herbal Drugs

This was done using the Fixed Dose Procedure (OECD, 2001).

Three rats were put in a cage, fasted overnight, and then given 2000mg/kg of Jointeez. They were observed for three days for signs of toxicity of the drugs.

Experimental Design

Thirty-five (35) rats were put into seven (7) groups of seven (7) rats each as follows:

a) Group A was not induced, and served as negative control group.
b) Group B was induced with rheumatoid arthritis using Complete Freund's Adjuvant, and given distilled water. This was the positive control group.
c) Group C was induced with rheumatoid arthritis using Complete Freund's Adjuvant, and treated with 36mg/kg body weight of the standard drug, Celecoxib (commonly known as Celebrex).
d) Group D was induced with rheumatoid arthritis using Complete Freund's Adjuvant, and treated with 126mg/kg body weight of Jointeez
e) Group F was induced with rheumatoid arthritis using Complete Freund's Adjuvant and treated with a combination therapy of Jointeez and Celebrex at therapeutic doses

Induction of Rheumatoid Arthritis

Rheumatoid arthritis was induced in the rats in groups B, C, D, and E, using 0.1ml (100µl) of Complete Freund's Adjuvant (CFA). This induction was done using the method of Foyet *et al.*, (2015). Briefly, each rat was given 0.1ml of the adjuvant in the subplantar region of the right foot and observed for 14 days before commencement of therapy.

The paw diameter of the induced rats was measured using Vernier Calipers before the induction, and once every week during the period of the study. The dorsoventral area of the paw was measured according to the method of Hussein *et al*.,(2012).

Treatment

The rats that were induced with rheumatoid arthritis were treated for four (4) weeks after induction of the arthritis. The treatment, using the herbal formulations and the standard drugs, was given by oral gavage once daily for four weeks.

3.8 Morphological Assessment

The morphological assessment (arthritis score) was done using the method of Vijayalaxmi *et al*., (2015). Briefly, scoring for morphological assessment was done as follows:

Normal paw = 0, mild swelling and erythema of digits = 1, moderate swelling and erythema of digits = 2, severe swelling and erythema = 3, gross deformity and inability to use limbs = 4. The maximum score for both paws is 8

The morphological assessment was done once weekly for the duration of study.

Sample Collection

The rats were sacrificed after an overnight fast. They were anaesthesized using chloroform. Blood samples were collected by puncture of the jugular vein and put into plain bottles for the analysis of TNF-α, IL-6 and C- reactive protein.

Laboratory Analysis

TNF-α, IL-6 and C-reactive protein were assayed using ELISA technique

Data Analysis

Data from this study were analyzed using SPSS version 23. P-values less than 0.05 were considered statistically significant in this study.

RESULTS

4.1 Acute Toxicity Study

The result of the acute toxicity study of the herbal drug shows that there was no mortality or any sign of toxicity observed after three days of administration of the herbal formulation. The herbal formulation was therefore considered safe and non-toxic up to 2000mg/kg body weight.

4.2 Biochemical Parameters

The results of the biochemical parameters are as shown in the table below:

Table 1: Mean ± SD of Biochemical Parameters

Groups	TNF-α (pg/ml)	CRP (ng/ml)	IL-6 (pg/ml)
Group A (NC)	13.96±2.58[a]	217.73±8.08[a]	7.32±0.30[a]
Group B (PC)	20.15±0.92[b]	251.72±15.34[b]	11.31±2.74[b]
Group C (CB)	15.67±2.49[a]	214.35±25.36[a]	6.75±1.32[a]
Group D (JZ)	16.61 ±0.72[a]	216.62±17.39[a]	7.15±1.66[a]
Group E (CB + JZ)	15.18±3.21[a]	213.44±9.34[a]	6.80±0.98[a]
p -value	< .001	< .001	0.010
F-value	7.840	6.956	4.124

ANOVA, followed by Tukey's multiple comparison.

a = significantly different compared with positive control (p <0.05)

b = significantly different compared with negative control (p <0.05)

4.3 Paw Volume of Rats

The changes in the paw diameters are as shown in the table below:

Table 2: Mean ± SD of Paw Volume of Rats

	Group A	Group B	Group C	Group D	Group E
Week 1	0.36±0.05	0.60±0.07	0.72±0.05	0.70±0.06	0.67±0.08
Week 2	0.36±0.05[a]	0.61±0.07[a]	0.69±0.07[b]	0.65±0.10[b]	0.64± 0.05[a]
Week 3	0.34±0.05[a]	0.61±0.08[a]	0.54±0.10[b]	0.59±0.07[b]	0.47±0.06[b]
Week 4	0.36±0.05[a]	0.61±0.10[a]	0.44±0.08[b]	0.46±0.06[b]	0.43±0.05[b]
p -value	0.79	0.42	< .001	< .001	< .001
F-value	15.322	11.514	4.312	2.388	8.037

ANOVA, followed by Dunnet's multiple comparison test against week 1.

a= No significant difference at $p < 0.05$.

b= significantly different at $p < 0.05$

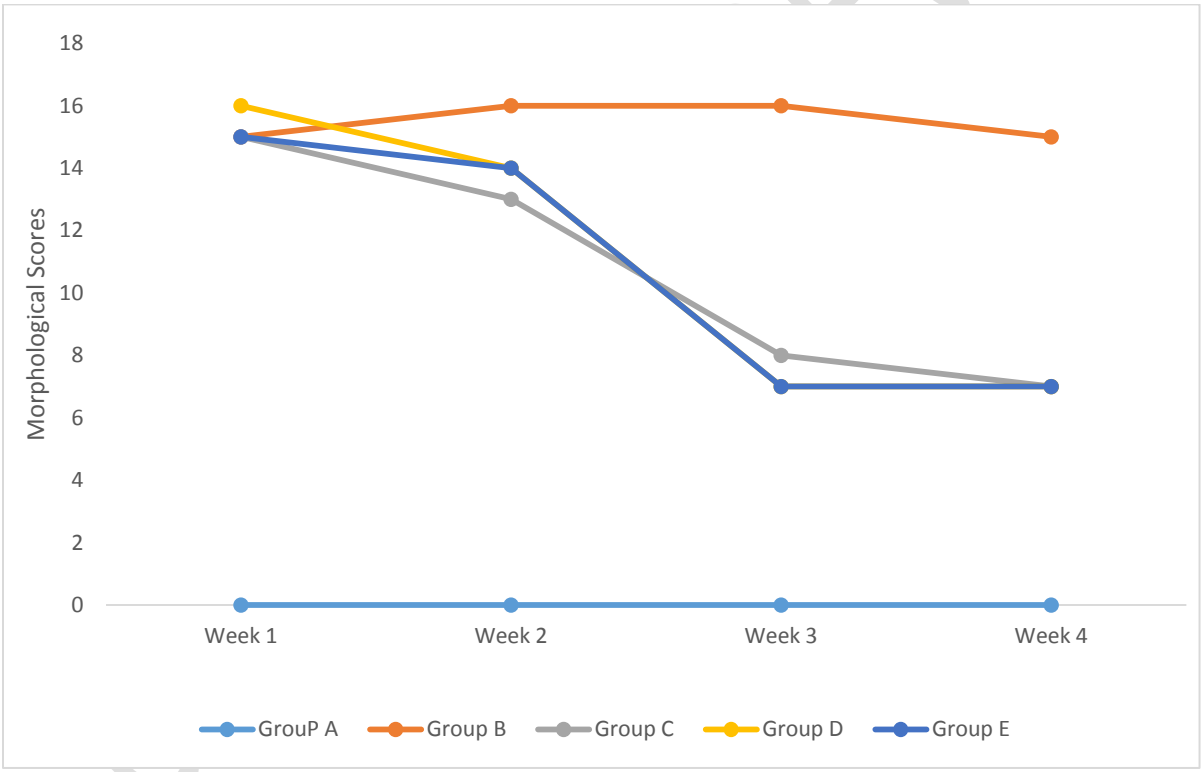
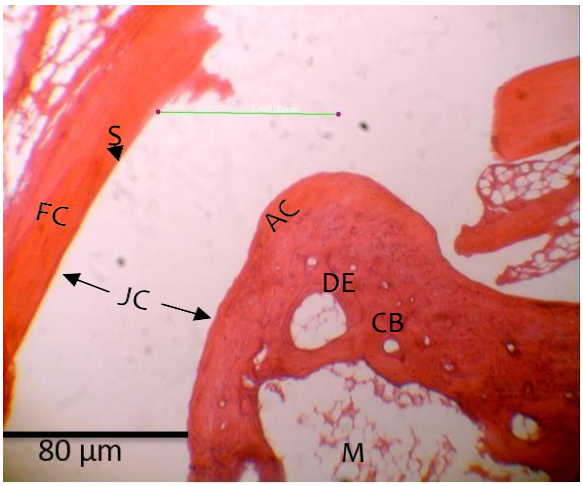
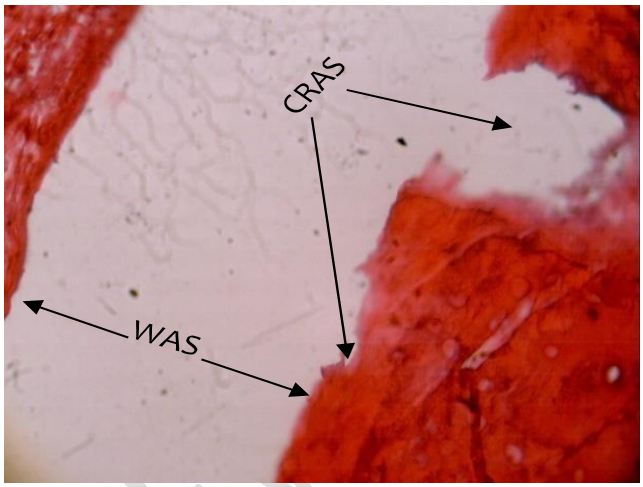


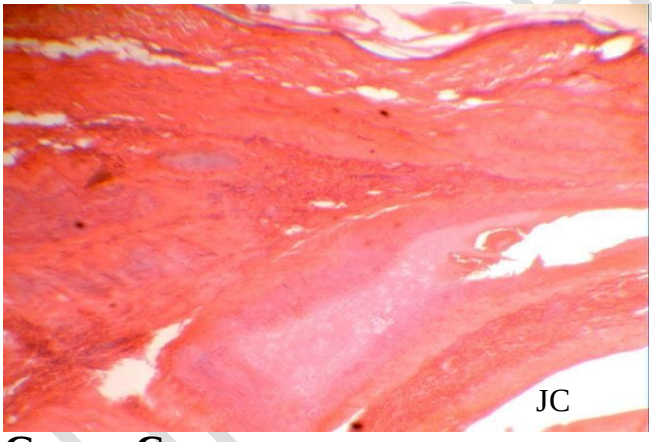
Fig. 4.1: Morphological Scores of the Rats according to Weeks

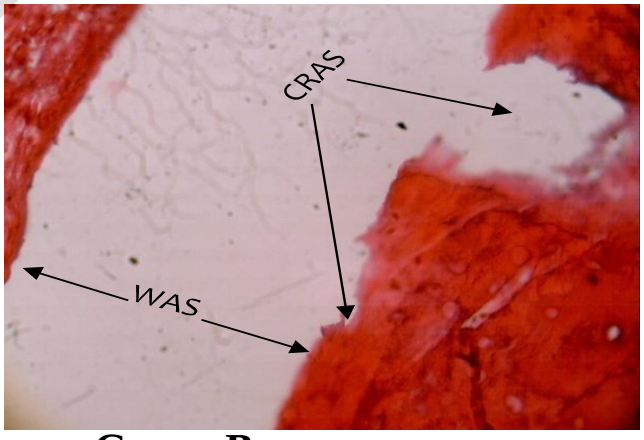




Group A **Group B**

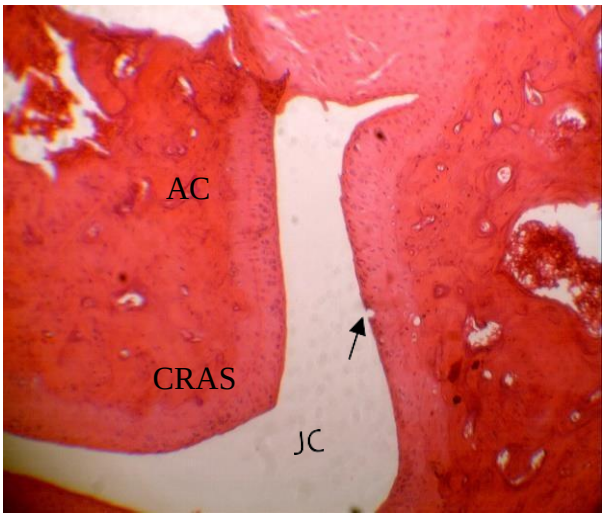
Fig. 4.2: Histology of Femororbital Joints of Group A and Group B Rats (x400)

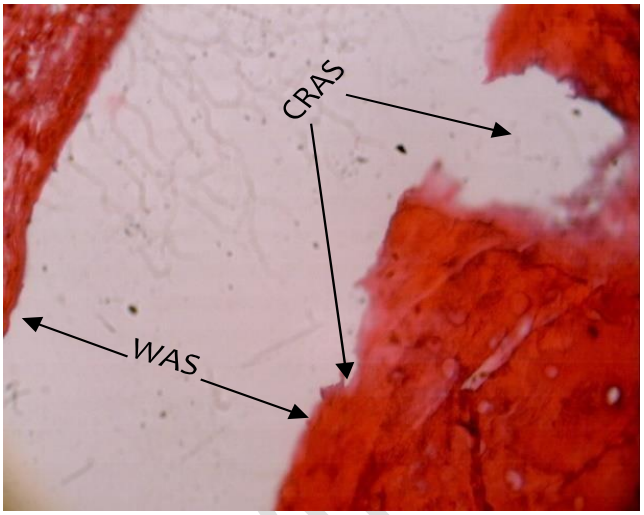




Group C **Group B**

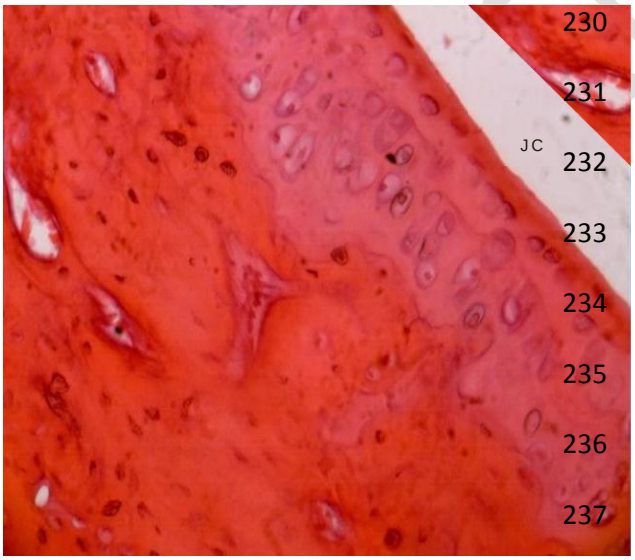
Fig. 4.3: Histology of Femororbital Joints of Group C and Group B Rats (x400)

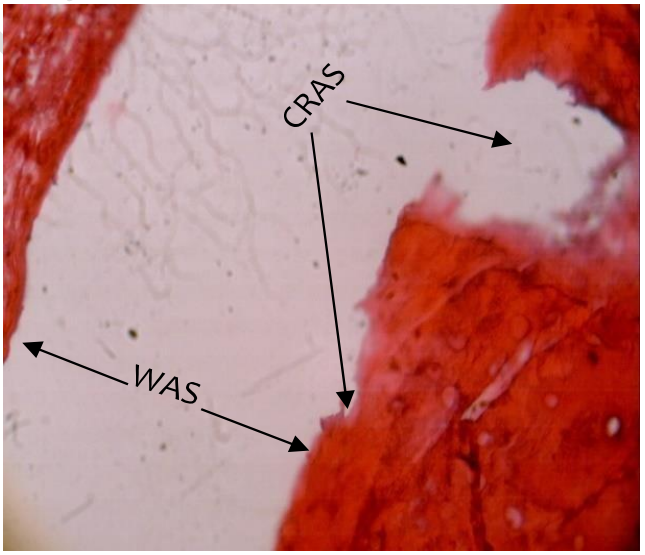




Group D **Group B**

Fig. 4.4: Histology of Femororbital Joints of Group D and Group B Rats (x400)





Group E **Group B**

Fig. 4.5: Histology of Femororbital Joints of Group E and Group B Rats (x400)

Discussion

This study evaluated the anti-arthritic activity of a herbal formulation used in the treatment of rheumatoid arthritis in Nigeria. The result of the acute toxicity study on the herbal formulation indicates that it is safe and non-toxic at therapeutic doses. This result is consistent with the work of Sundera *et al*., (2013), who evaluated the anti-inflammatory activity of Dazzle Capsule, another polyherbal formulation used in India.

There were significant reduction in the paw diameters of the rats treated with the herbal formulations. In rheumatoid arthritis, there is infiltration of the paw tissues by immune cells, chiefly neutrophils and macrophages. The reduction in the paw diameters may be due to inhibition of the infiltration by the herbal formulations (Nargarkar & Jagtap, 2017) as well as inhibition of pannus formation and bone erosion (Bhatt & Maithani, 2017). This effect was comparable to that observed in the rats treated with the orthodox drug, Celebrex.

The levels of the inflammatory markers were significantly reduced in the groups treated with the herbal formulations, compared to the arthritic control group. This finding is probably due to the inhibitory effects of the herbal formulations on the production of inflammatory markers (Maladi *et al*., 2018). The anti-inflammatory effects of the herbal formulations were comparable with that observed with the orthodox drug.

Acute phase reactants such as C-reactive proteins are usually produced during inflammation such as in rheumatoid arthritis (Jain *et al*., 2011). Also, immune cells, which are usually attracted to the inflamed synovium, produce TNF-α, IL-6 and other pro-inflammatory cytokines, and these contribute greatly to the pathology of rheumatoid arthritis (Alghashan & Rasheed, 2014).

The combination therapy did not significantly reduce the parameters compared to the results obtained using the herbal drug alone or the orthodox drug alone.

Anti-arthritic herbal formulations can be used as effective therapeutic alternative for the management of rheumatoid arthritis. It may be necessary to consider them for integration into the regular health system.

CONFLICT OF INTEREST: There was no conflict of interest.

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