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EFFECT OF AN ANTI-ARTHRITIC HERBAL MEDICINE ON INFLAMMATORY MARKERS IN ALBINO WISTAR RATS

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ABSTRACT
The anti-arthritic activity of a herbal drug was evaluated in this study. Thirty-five (35) albino wistar rats were used in this study. They were divided into seven groups, A, B, C, D, E, with seven rats each group. Group A served as negative control while Group B was the positive control. Rats in Groups B, C, D and E were induced with rheumatoid arthritis by injecting 0.1ml of Complete Freund's Adjuvant into their right hind paw. They were treated with the standard drug and herbal drug respectively for a period of 28 days as follows: Group C was treated with a standard drug, Celebrex, Group D was treated with the herbal drug, Arthropower, and Group E was treated with a combination therapy of Arthropower and Celebrex). The negative control and positive control groups were not given any treatment. At the end of the 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. The ELISA technique was used to analyze the inflammatory markers, TNF-α, IL-6 and C-reactive protein. The levels of TNF-α ($p<0.001$), IL-6 ($p=0.04$) 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 herbal formulation used in this study offered similar therapeutic activities as the orthodox drug used. The combination therapy gave results that were comparable to the monotherapies used in this study. Thus, the herbal drug can serve as a safe anti-arthritic therapy. It is recommended that herbal drug be considered for integration into our management regimen for rheumatoid arthritis.

KEYWORDS: Anti-arthritic, herbal drug, Complete Freund's Adjuvant, Rheumatoid Arthritis, Nigeria, Arthropower.

INTRODUCTION
Rheumatoid arthritis is a chronic inflammatory disease that affects the joints, extra-articular tissues and organs of the body (Choy, 2012). It is reportedly the most frequent type of autoimmune arthritis (Koop *et al*., 2015).

The pathogenesis of rheumatoid arthritis is complex, and there is involvement of a variety of cells like T-cells, B-cells, dendritis cells, chondrocytes, macrophages and other cells (Anic & Mayer, 2014). In other words, both the innate and the adaptive immune systems are involved in the pathogenesis of rheumatoid arthritis.

The earliest biological event that occurs in rheumatoid arthritis is the activation of the innate immunity. Exogenous material and autologous antigens activate the dendritic cells (Smolen *et al*., 2007). Dendritic cells, macrophages and activated B-cells, which serve as antigen-presenting cells (APCs) present antigens associated with arthritis to the T-cells. At the same time, CD4 cells, which secrete inflammatory substances like interleukins and interferon-gamma, move into the synovial membrane (Choy, 2012).

Within the synovium of the joint, increased activation of synoviocytes occurs, with production of pro-inflammatory cytokines, prostaglandins and metalloproteinases (MMPs). The synoviocytes participate in cartilage and bone destruction by two ways – secretion of MMPs into the synovial fluid and also by direct invasion (Smolen *et al*., 2007). The cytokines also activate chondrocytes which release more MMPs into the cartilage, leading to more destruction (Smolen *et al*., 2007).

These physiological activities lead to joint destruction. There are mobility problems, systemic and extra-articular complications and socio-economic inabilities as a result of this destruction.

The conventional therapies for rheumatoid arthritis have been reported to have some serious side effects, like ulcer, haemorrhage, haematological, pulmonary and dermatologic abnormalities. Other effects include stomatitis, hepatotoxicity, myelosuppression and even potential pulmonary toxicity (Wadekar *et al*., 2015). Given these concerns, a variety of herbal products, minerals or metals, reportedly without these side effects, have been produced and are being used in an effort to cure or control arthritis (Joseph *et al*., 2012).

This study was concerned with evaluating the anti-arthritic activity of a herbal medicine used in the management of rheumatoid arthritis in Nigeria.

MATERIALS AND METHODS

The Animals

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

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

The Drugs

The orthodox drug used for this study was Celebrex (Celecoxib), a product of Pfizer Pharmaceuticals, Puerto Rico, while the herbal formulation was Arthropower (product of Green World Inc., USA).

Determination of the Therapeutic Doses of Drugs

The doses of both the herbal formulation and orthodox drugs given to the rats were extrapolated from the human therapeutic doses using the Paget and Barnes (1964) conversion table.

The daily dose of both drugs were determined according to OECD's Guidelines (OECD, 2001).

Acute Toxicity Testing

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 Arthropower. 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 180mg/kg body weight of Arthropower
e) Group F was induced with rheumatoid arthritis using Complete Freund's Adjuvant and treated with a combination therapy of Arthropower 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 was given by oral gavage once daily for four weeks.

Arthritis Score

The 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 assessment was done once every week throughout the duration of study.

Sample Collection

The rats were sacrificed after an overnight fast, anaesthesized using chloroform and sacrificed by means of jugular vein puncture. Blood samples were collected 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 rats did not show any sign of toxicity or mortality three days after the administration of the herbal

formulation. The herbal drug was therefore considered safe and non-toxic up to 2000mg/kg body weight.

4.2 Mean ± SD of Inflammatory 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 (AP)	15.05 ±3.39[a]	216.54±14.24[a]	6.76±0.73[a]
Group E (CB + AP)	15.99±2.13[a]	215.33±18.69[a]	7.34±0.76[a]
p-value	<0.001	0.001	0.04
F-value	6.575	6.012	2.462

ANOVA, followed by Tukey's multiple comparison.
a = significantly different compared with positive control ($p<0.05$)
b = significantly different compared with negative control ($p<0.05$)
Key: NC- Normal control, PC- Positive control, CB- Celebrex, AP- Arthropower

4.2 Mean ± SD of Arthritis Score

	Group A	Group B	Group C	Group D	Group E
Week 1	0.36±0.05	0.60±0.07	0.72±0.05	0.64±0.05	0.74±0.05
Week 2	0.36±0.05[a]	0.61±0.07[a]	0.69±0.07[b]	0.64±0.05[b]	0.73± 0.05[a]
Week 3	0.34±0.05[a]	0.61±0.08[a]	0.54±0.10[b]	0.48±0.04[b]	0.50±0.10[b]
Week 4	0.36±0.05[a]	0.61±0.10[a]	0.44±0.08[b]	0.46±0.05[b]	0.46 ±0.05[b]
p-value	0.79	0.42	<0.001	<0.001	<0.001
F-value	15.322	11.514	4.312	4.883	5.611

ANOVA, followed by Dunnet's multiple comparison test against week 1.
a= No significant difference at $p<0.05$.
b= significantly different at $p<0.05$

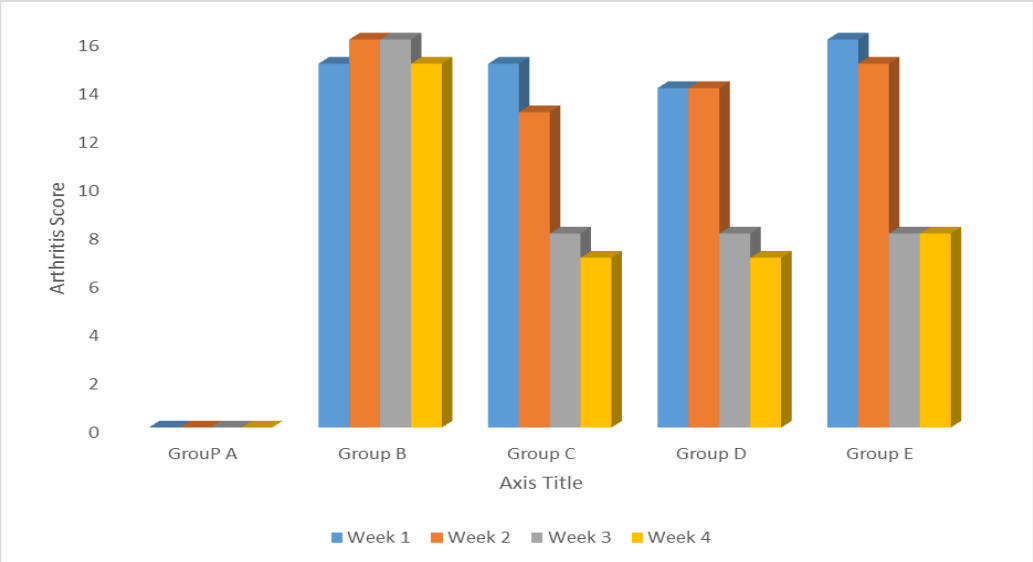

Fig. 1: Arthritis Score of the Rats.

DISCUSSION

The results of this study shows that the herbal drug used for this study did not cause toxicity in the rats up to 2000mg/kg body weight of rats. Sundera *et al.*, (2013) have reported a similar finding using another anti-arthritic herbal drug known as Dazzle. Herbal substances are not always toxic at recommended doses. Nwachuku and Elekima (2018) have reported that Action Bitters, a commonly consumed herbal mixture, did not cause obvious derangements in biochemical parameters in rats at 0.68ml/kg body weight of rats.

The results also show that there were significant reduction in the levels of inflammatory parameters in the treated rats. TNF-α, C-reactive protein and IL-6 significantly reduced in the rats there were treated with the drugs. This is probably because herbal substances have the ability to inhibit the synthesis of inflammatory molecules (Maladi *et al.*, 2018). Patel &

Pundarikakshudu (2016) and Appusamy *et al*., (2018) have reported a similar findings using different herbal formulations. The anti-inflammatory effect of the herbal drug was comparable to that of the orthodox drug used.

There were also significant reduction in the paw diameters of the treated rats at the end of the treatment period. The pathology of rheumatoid arthritis involves the infiltration of the paw by immune cells with attendant production of inflammatory molecules. The reduction in the paw diameters of the treated rats may be due to the ability of the natural drugs to prevent the infiltration of the paw by the immune cells (Nargarkar & Jagtap, 2017). Herbal drugs have also been reported to have the ability to inhibit the formation of pannus and bone erosion that occur in rheumatoid arthritis (Bhatt & Maithani, 2017).

Our findings here also show that the anti-arthritic effect of the herbal drug was comparable to that of the orthodox drug. Also, a combination therapy of the herbal drug and the orthodox drug did not offer a significantly beneficial effect over monotherapies used.

Herbal therapies can be incorporated into the treatment regimen for rheumatoid arthritis. This is because the orthodox drug have been reported to have adverse side effects (Wadekar *et al*., 2015).

CONFLICT OF INTEREST: There was no conflict of interest.

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