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Amelioration of Extra-articular Effects Associated with Rheumatoid Arthritis Using Anti-arthritic Herbal Formulations

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Authors' contributions

This work was carried out in collaboration among all authors. Authors KNEA and EON designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors ONB and EASB managed the analyses of the study. Author BHO managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This study investigated the ability of some herbal formulations to ameliorate extra-articular effects of some herbal formulations used in the management of rheumatoid arthritis in Nigeria.
Methodology: Forty-nine (49) female albino Wistar rats were used for this study. They were divided into seven groups: A, B, C, D, E, F and G of seven rats each, with Group A serving as negative control while Group B was a positive control. Groups B, C, D, E, F and G were induced with rheumatoid arthritis by injecting 0.1 ml of Complete Freund's Adjuvant into the right hind paw of each rat. The rats were treated with the standard drug and herbal formulations 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 herbal drug, Arthropower), Group F (treated with

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combination therapy of Jointeez and Celebrex) and Group G (treated with combination therapy of Arthropower and Celebrex). At the end of the 28-day treatment period, the rats were anaesthetized with chloroform and sacrificed through puncture of the jugular vein. Five millilitres (5 ml) of blood samples were put into plain bottles for the analysis of biochemical parameters and 3 ml into K3EDTA bottles for haematological analysis. The lipid parameters were analysed using Mindray autoanalyzer while haematological parameters were determined using Sysmex haematology auto analyzer.

Results: Total cholesterol ($p<0.001$), HDL ($p=0.005$) and LDL ($p=0.004$) were significantly reduced in the treated rats compared to the positive control group. Conversely, Packed Cell Volume ($p<0.001$) and Haemoglobin levels ($p<0.001$) were significantly reduced in the positive control rats compared to the treated rats. However, Total WBC count was significantly higher in the positive control rats than in the treated rats ($p=0.001$). The combination therapies used in this study did not offer a significantly different therapeutic advantage over the monotherapies used. The herbal formulations gave therapeutic effects on the extra-articular effects similar to that obtained from the orthodox drug used in this study.

Conclusion: The herbal formulations can be used as alternative regimens for rheumatoid arthritis. It is recommended that herbal formulations be considered for integration into our healthcare system for the management of rheumatoid arthritis.

Keywords: Anti-arthritic; herbal formulation; complete Freund's adjuvant; rheumatoid arthritis; Nigeria.

1. INTRODUCTION

Rheumatoid arthritis is a chronic inflammatory disease that affects the joints, extra-articular tissues and organs of the body [1]. It has been reported to be the most frequent type of autoimmune arthritis [2], and is characterized by polyarthritis of the peripheral system which occurs in a symmetrical pattern; there is joint deformation due to inflammation of the synovial membrane of the joint [3]. Rheumatoid arthritis affects between 0.5 to 1% of the general population[4]. The pathogenesis of rheumatoid arthritis is a complex mechanism, which is initiated with the activation of innate immunity. The cells of the innate immunity including dendritic cells, macrophages, present the arthritogenic antigens to the cells of the adaptive immunity, especially T-cells1. The T-cells produce inflammatory cytokines like interleukins and interferons. Another group of cells of the adaptive immune system, the B-cells, produce antibodies such as anti-cyclic citrullinated protein (anti-CCP) antibodies and rheumatoid factor (RF). At the affected joint, there is the activation of the synoviocytes and these cells produce cytokines, matrix metalloproteinases and prostaglandins. Angiogenesis and osteoclastogenesis also occur in the joint. The resulting inflammatory responses cause damage to the cartilage of the joint [1].

Rheumatoid arthritis, although principally affecting the articular cartilage, also has extra-articular effects, generally referred to as extra-articular manifestations [5]. These include cardiovascular diseases and anaemia. Atherosclerosis, a low-grade inflammation (that is, inflammation characterized by high levels of cytokines and acute-phase reactants in circulation), is the underlying pathological process that leads to cardiovascular disease in rheumatoid arthritis. This inflammation contributes to the destabilization and rupture of the atherosclerotic plaques, leading to instability in the plaques which is responsible for the cardiovascular events that result from it [6]. The involvement of the immune system is very central to the development of heart disease in rheumatoid arthritis. The levels of acute-phase reactants increase, which increases the risk of heart disease [7]. It has been reported that there is an increase in the levels of C-reactive protein which is associated with both incident and progressive carotid plaque [8]. Individuals with rheumatoid arthritis are more likely to have heart failure due to diastolic dysfunction that may result from systemic inflammation [7].

When tissue necrosis factor-alpha (TNF-α) binds to any of its receptors (tissue necrosis factor receptor 1, TNFR1 and tissue necrosis factor receptor 2, TNFR2), nuclear factor kappa B (NFkB) is activated [9], as well as p38 mitogen-activated protein kinase (p38 MAPK). Consequently, there is transcription of genes for pro-inflammatory molecules like interleukins (IL-1β and IL-8), intercellular adhesion molecules

(ICAM), vascular cell adhesion molecule (VCAM), in several immune cells [10]. TNF-α and IL-6 induce the upregulation of scavenger receptors in T-helper-1 cells and macrophages. They also lead to the accumulation of oxidized LDL in these immune cells thereby transforming them into foam cells [11]. There is also an alteration in the structure and function of HDL in rheumatoid arthritis. HDL usually exerts its anti-atherogenic functions by promoting the efflux of cholesterol from the arterial walls, and by protecting LDL against oxidation [12].

Rheumatoid arthritis causes endothelial dysfunction and leads to atherosclerosis [12,13]. The formation and accumulation of atherosclerotic plaques lead to coronary heart disease (CHD) also known as a coronary arterial disease (CAD), which is the narrowing of the blood vessels around the heart. This narrowing reduces the amount of oxygen and other nutrients that reach the heart. CHD leads to myocardial infarction, unstable angina and heart failure [14]. It has been reported that the leading cause of death among individuals with rheumatoid arthritis is myocardial infarction [15]. Congestive heart failure is another cardiovascular manifestation in rheumatoid arthritis but it is reported to be less clinically evident [16]. Valvular heart disease, another manifestation, results from the formation of rheumatoid nodules in the valves (Mitral or aortic) of the heart [5].

Anaemia is the second most common systemic manifestation of rheumatoid arthritis after cardiovascular diseases [1] and it is said to occur very early in the rheumatoid arthritis disease [17]. Inflammatory cytokines, especially IL-6, can induce hepcidin during inflammatory conditions, which can consequently reduce serum iron levels. Also, it has been reported that plasma levels of haemoglobin are inversely related to the levels of IL-6 [18]. In the human population, hepcidin levels have been reported to be significantly high in rheumatoid arthritis subjects than in the healthy subjects [19]. Three principal mechanisms contribute to anaemia in inflammation and these mechanisms are immune-driven [20]. The mechanisms are reduction of the lifespan of red blood cells, impairment in the proliferation of progenitor cells and increased uptake and retention of iron in the reticuloendothelial system [20].

The treatment for rheumatoid arthritis has tremendously changed over the past two decades. Non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids like glucocorticoids and disease-modifying antirheumatic drugs (DMARDs) have been used over the years for the treatment of the disease. While NSAIDs and corticosteroids have been used for treating acute inflammations in rheumatoid arthritis, DMARDs have been used for maintenance therapy [21]. It has been reported that these anti-arthritic drugs have adverse effects. NSAIDs can cause an ulcer, haemorrhage, haematological, pulmonary and dermatologic abnormalities. DMARDs have been reported to cause stomatitis, hepatotoxicity, myelosuppression and even potential pulmonary toxicity [22]. Due to these side effects of NSAIDs and DMARDs, there is a growing interest in the search for alternative therapies for rheumatoid arthritis [22]. Some researchers have reported case studies of rheumatoid arthritis that were successfully treated with some alternative medicines [23]. The interest in alternative medicines has grown because they are considered efficacious and safer than orthodox medicines [24]. There is no cure yet for rheumatoid arthritis [25] (Curtis & Singh, 2011) and to the best of our knowledge studies on the amelioration of the extra-articular manifestations of rheumatoid arthritis using herbal formulations are scanty. The present study was, therefore, designed to investigate the amelioration of extra-articular effects associated with rheumatoid arthritis in individuals using anti-arthritic herbal formulations.

2. MATERIALS AND METHODS

2.1 Experimental Animals

Forty-nine (49) female Albino Wistar rats, weighing 150-200 g were used for this study. The rats were housed in a compartmentalized cage and allowed to acclimatize for two weeks, in daily 12-hourly light and dark cycle, and had unhindered access to standard feed and water ad libitum.

2.2 Experimental Drugs

The standard drug used for this study was Celebrex (Celecoxib), a product of Pfizer Pharmaceuticals, Puerto Rico. Two herbal formulations used for this study were Jointeez (product of Kedi Healthcare Industries Limited, Nigeria) and Arthropower (product of New GreenWorld Inc., Michigan, USA).

2.3 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 [26]. The daily dose of both the standard drug and the herbal formulations were determined based on OECD's Guidelines [27].

2.4 Acute Toxicity Testing of the Herbal Drugs

This was done using the Fixed-Dose Procedure [27]. Six rats were put in two cages, fasted overnight, and then given 2000 mg/kg each of Jointeez and Arthropower. They were observed for three days for signs of toxicity of the drugs.

2.5 Experimental Design

Forty-nine (49) rats were put into seven (7) groups of seven (7) rats each as follows:

Group A was not induced and served as a negative control group.

Group B was induced with rheumatoid arthritis using Complete Freund's Adjuvant and given distilled water. This was the positive control group.

Group C was induced with rheumatoid arthritis using Complete Freund's Adjuvant, and treated with 36 mg/kg bodyweight of the standard drug, Celecoxib (commonly known as Celebrex).

Group D was induced with rheumatoid arthritis using Complete Freund's Adjuvant and treated with 126 mg/kg bodyweight of Jointeez.

Group E was induced with rheumatoid arthritis using Complete Freund's Adjuvant and treated with 180 mg/kg bodyweight of Arthropower.

Group F was induced with rheumatoid arthritis using Complete Freund's Adjuvant and treated with combination therapy of Jointeez and Celebrex at therapeutic doses.

Group G was induced with rheumatoid arthritis using Complete Freund's Adjuvant and treated with combination therapy of Arthropower and Celebrex at therapeutic doses.

2.6 Induction of Rheumatoid Arthritis

Rheumatoid arthritis was induced in the rats in groups B, C, D, E, F and G, using 0.1 ml (100µl) of Complete Freund's Adjuvant (CFA). This induction was done using the method of Foyet et al. [28]. Briefly, each rat was given 0.1 ml 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. [29].

2.7 Treatment

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

2.8 Morphological Assessment

The morphological assessment (arthritis score) was done using the method of Vijayalaxmi et al. [30]. 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 the study.

2.9 Sample Collection

The rats were sacrificed after an overnight fast. They were anaesthetized using chloroform. Blood samples were collected by puncture of the jugular vein and put into plain bottles and K3EDTA bottles for lipid profile and analysis of haematological parameters respectively.

2.10 Laboratory Analysis

Lipid parameters were assayed using Mindray biochemistry autoanalyzer (Model BS120, Shenzhen, China), while haematological analysis was done using Sysmex KX-21n auto-analyser, Kobe, Japan.

2.11 Disclaimer

This study was carried out by the Guidelines of the Organization for Economic Cooperation and Development [27].

2.12 Data Analysis

Data from this study were analyzed using SPSS version 23. The analytical tool used was ANOVA followed by Tukey multiple comparisons. P-values less than 0.05 were considered statistically significant in this study.

3. RESULTS

3.1 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 2000 mg/kg body weight.

3.2 Mean ± SD of Lipid Parameters

The mean ± SD of total cholesterol (TC), high-density lipoprotein (HDL-C) and low-density lipoprotein (LDL-C) levels in the positive control (PC) group were significantly lower ($p<0.05$) compared to the treated groups, while there was no significant difference ($p>0.05$) in the triglycerides (TG) levels. There were no significant differences ($p>0.05$) in means of the parameters between the negative control (NC) and the treated groups.

3.3 Mean ± SD of Haematological Parameters of the Positive Control, Negative Control and Treated Groups of the Experimental Animals

The packed cell volume (PCV), and haemoglobin (Hb) for the positive control (PC) group were significantly lower ($p< 0.05$) than those of the treated groups, while the white blood cell count (WBC) was significantly higher ($p<0.05$) than those of the treated groups. There were no

Table 1. Mean ± SD of lipid parameters of the positive control, negative control and treated groups of the experimental animals

	TC (mmol/L)	HDL-C(mmol/L)	TG (mmol/L)	LDL- C (mmol/L)
Group A (NC)	2.23±0.11a	1.41±0.09a	1.17±0.13	0.38±0.08a
Group B (PC)	2.00±0.32b	1.17±0.29b	1.18±0.25	0.28±0.15b
Group C(CB)	2.31±0.37a	1.40±0.04a	1.04±0.08	0.38±0.11a
Group D(JZ)	2.32±0.22a	1.41±0.05a	1.03±0.15	0.34±0.21a
Group E (AP)	2.21±0.15a	1.41±0.11a	1.01±0.08	0.33±0.22a
Group F (JZ + CB)	2.30±0.17a	1.40±0.05a	1.13±0.20	0.35±0.14a
Group G (AP + CB)	2.33±0.14a	1.48±0.13a	1.13±0.20	0.34±0.11a
p-value	<0.001	0.005	0.27	0.004
F-value	6.465	3.652	1.578	3.876

The study was done in replicate. ANOVA, followed by Tukey's multiple comparison test. A = significantly different compared to positive control at p<0.05, b = significantly different compared to negative control at p<0.05, NC-Negative control, PC-positive control, CB-Celebrex, JZ- Jointeez, AP-Arthropower

Table 2. Mean ± SD of haematological parameters of the positive control, negative control and treated groups of the experimental animals

	PCV(%)	Hb(g/dl)	WBC(x109/µl)
Group A (NC)	37.74±0.99a	13.53±0.19a	6.80±0.98a
Group B (PC)	23.64±1.35b	10.03±0.69b	9.54±0.50b
Group C(CB)	34.29±2.29ab	12.41±0.95ab	6.50±1.49a
Group D(JZ)	35.74±1.14a	12.66±0.60a	6.99±1.52a
Group E (AP)	34.90±1.41ab	12.47±0.31ab	7.04±1.75a
Group F (JZ + CB)	35.69±1.34a	12.44±0.22ab	6.00±1.04a
Group G (AP + CB)	34.74±1.56ab	12.29±0.59ab	6.97±1.67a
p-value	<0.001	< 0.001	0.001
F-value	67.571	24.659	4.942

The study was done in replicate. ANOVA, followed by Tukey's multiple comparison test. a= significantly different compared to positive control at p<0.05, b =significantly different compared to negative control at p<0.05, NC-Negative control, PC-positive control, CB-Celebrex, JZ- Jointeez, AP-Arthropower

significant differences (p>0.05) in CD4 and WBC counts between the negative control group (NC) and the treated groups, but the Hb and PCV of the negative control (NC) group were significantly higher than those of the treated groups.

3.4 Morphological Scores

Fig. 1 shows the morphological score of the rats measured weekly during the study. There was a reduction in the morphological score of the rats in the treated groups as the treatment progressed weekly, while that of the arthritic control group (positive group) did not show a reduction.

4. DISCUSSION

Data from this study has shown that the arthritic control group had significantly lower levels of total cholesterol, HDL-C and LDL-C than the rats that were treated with herbal formulations. However, there was no significant difference in the levels of triglycerides. There is an alteration in lipid metabolism in rheumatoid arthritis patients [31] and this predisposes them to increased risk of cardiovascular disease even with low levels of cholesterol [32]. The low level of lipid parameters is probably due to the lowering effects of inflammation on circulating lipids [33]. Our findings agree with some other research findings [34,35]. Cardiovascular disease is among the extra-articular manifestations of rheumatoid arthritis [36]. The mechanism of CVD in rheumatoid arthritis in the presence of low levels of lipid parameters is largely not understood [32]. However, this may be attributed to the low levels of HDL and raised triglyceride levels as seen in our data. The significant increase in the levels of the lipid parameters in the rats that were treated with the herbal formulations does not translate into increased risk of cardiovascular disease since rheumatoid arthritis can predispose to cardiovascular disease even at low lipid levels [35]. The herbal formulations improved the lipid levels probably by controlling inflammation. This is possible because natural products have been reported to have the ability to control inflammation [37]. Other researchers have reported that rheumatoid arthritis subjects had low lipid levels and that these lipid levels are increased in those who are on anti-TNF therapy but not receiving lipid-lowering therapy [38].

This effect of the herbal formulations was comparable to that obtained from the orthodox drug used for this study. This finding agrees with the work of Akramas et al. [39] who reported similar findings using EM1201, a polyherbal formulation and diclofenac, an orthodox drug. Natural substances exert their medicinal effects in ways similar to allopathic medicines; they inhibit the pathways of inflammation [40].

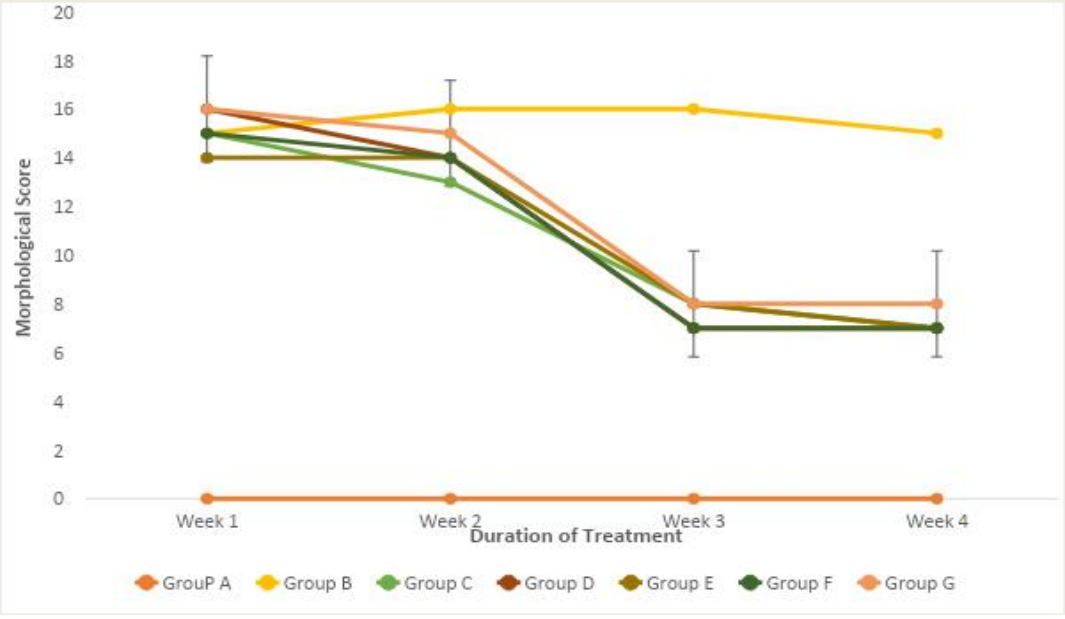

Fig. 1. Morphological score of rats according to groups

From this study, there were significant increases in the PCV and Hb of the rats that were treated with the herbal formulations, compared to the arthritic group which exhibited significantly low levels of these parameters. The low levels of these haematological parameters in the arthritic control group indicate anaemia which has been associated with rheumatoid arthritis [41]. It has been reported that both anaemias of chronic disease (ACD) and iron deficiency anaemia (IDA) occurs in rheumatoid arthritis [42]. The anaemia may be due to the abnormal metabolism of hepcidin, the hormone that regulates iron metabolism [43]. Hepcidin levels are increased during inflammation44. Thus, hepcidin is induced by inflammatory factors like tumour necrosis factor and interleukin-6, and this can bring about functional iron deficiency thereby reducing iron absorption in the intestine. Similarly, ACD may arise due to altered iron metabolism due to the effect of hepcidin, shortened lifespan of the reticulocytes and diminished response to erythropoietin by red blood cell precurssors [42]. Anaemia in rheumatoid arthritis could also be an indicator of the deficient anti-inflammatory capacity of erythropoietin, since erythropoietin can impair the formation of pro-inflammatory molecules like interleukin-6 and Tumour Necrosis Factor-alpha, through the inhibition of nuclear factor-kappa B-dependent production of cytokines [45]. The herbal formulations improved the haematological parameters thereby reversing the observed anaemia. This is probably because herbal formulations can improve the intestinal absorption of nutrients by altering the microbiota of the GIT [46] and reducing inflammation [47].

Total White blood cell count was, however, higher in the arthritic control group than in the other groups. This high WBC count has earlier been reported by other study [48]. The increased WBC may be due to the activation of the immune system which occurs in rheumatoid arthritis [49]. There is a release of interleukins which causes an increase in the synthesis of colony-stimulating factors for both macrophages and granulocytes [50,51,52]. The herbal formulations restored the WBC in the treated rats to normal levels. This is probably because bioactive components in natural products can control molecular mediators of inflammation thereby inhibiting effector molecules such as the proinflammatory cytokines [37]. The inhibition of the activation of the immune system may be due to the ability of herbal drugs to reduce inflammatory markers [53]. The observed attenuation of inflammation can also explain the weekly reduction in the morphological scores of the rats as our data have shown.

In this study, the herbal formulations restored the haematological parameters in the treated groups to levels that were non-significantly different from those in the negative control group. These findings agree with the work of [50,54,55].

The herbal formulations used for this study attenuated the extra-articular effects in the treated rats. The formulations have been reported to offer similar effect as some orthodox drugs [53].

5. CONCLUSION

The herbal formulations used for this study can be used as alternative regimens for rheumatoid arthritis. It is recommended that herbal formulations be considered for integration into our healthcare system for the management of rheumatoid arthritis.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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