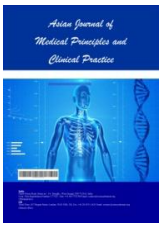

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Toxicological Evaluation and Anti-inflammatory Effects of the Anti-arthritic Herbal Formulations, Jointeez and Arthropower in Albino Wistar Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Author KNEA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors HB, ONB and EON 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 toxicological and anti-inflammatory effects of some anti-arthritic herbal formulations.
Methodology: Forty-nine (49) female albino wistar rats grouped into seven groups of seven rats per group, were used for this study. Group A was the negative control while Group B was the positive control groups respectively. The rats in group B, C, D, E, F and G were induced with rheumatoid arthritis by injecting 0.1 ml of Complete Freund's Adjuvant into their right hind paw. They were treated with the standard drug and herbal formulations respectively for 28 days as follows: Group C (treated with the standard orthodox drug, Celebrex), Group D (treated with the herbal drug, Jointeez), Group E (treated with a herbal drug, Arthropower), Group F (treated with a combination therapy of Jointeez and Celebrex) and Group G (treated with a combination therapy of Arthropower and Celebrex). The treatment was for 28 days, once daily. On the 29th day the rats

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were anaesthesized with chloroform and sacrificed through puncture of the jugular vein. Five milliliters (5 ml) of blood samples were put into plain bottles for the analysis of biochemical parameters and 3 ml into K_3EDTA bottles for haematological analysis. The biochemical parameters were analysed using ELISA technique while haematological parameters were determined using Sysmex haematology autoanalyzer.

Results: The AST ($p=0.006$), ALT ($p<0.001$) and ALP ($p=0.001$) were significantly reduced in the treated rats compared to the arthritic control group. TNF-α ($p=0.001$), IL-6 ($p<0.001$), CRP ($p=0.001$) and WBC ($p=0.001$) were significantly reduced in the treated rats compared to the positive control group. Conversely, Haemoglobin ($p<0.001$), Packed Cell Volume ($p<0.001$) levels were significantly reduced in the positive control rats compared to the treated rats. The combination therapies used in this study did not offer 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 serve as safe regimens for rheumatoid arthritis in our population. 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; anti-inflammatory; rheumatoid arthritis; toxicological.

1. INTRODUCTION

Arthritis disease is a group of over 100 diseases that affect the joints in the body and their surrounding tissues [1]. Essentially, arthritis is an inflammatory disease. Inflammation is a complex homeostatic process which, among other things, involves the migration of leucocytes from the vascular system into the tissues [2].

Rheumatoid arthritis is a chronic inflammatory disease that affects the joints as well as extra-articular tissues and organs of the body [3]. Rheumatoid arthritis is the most frequent type of autoimmune arthritis [4]. Its characteristic features include polyarthritis of the peripheral system which occurs in a symmetrical pattern. There is also joints deformation owing to the characteristic damage to the synovial membrane [5].

It has proven difficult to identify effective therapies for rheumatoid arthritis, mainly due to the complex etiology of the disease [6]. Currently, the drugs that are used for the treatment of rheumatoid arthritis have been reported to have adverse side effects [7]. There is a growing interest in the use of herbal formulations for the treatment and management of human diseases including rheumatoid arthritis, because the formulations are credited with medicinal efficacies [8] and have little or no adverse effects as the orthodox drugs [9]. 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 [10]. Despite the increasing use of alternative therapies for the diseases, scientific evidence for the validation of the effectiveness and safety of these therapies has been scarce [11]. The promises and hopes of the research findings so far as regards the use of herbal formulations for the treatment of rheumatoid arthritis notwithstanding, issues of safety and adverse reactions are still of great concern, especially as more people in developing as well as developed countries take more interests in the use of herbal therapies.

2. MATERIALS AND METHODS

2.1 Experimental Animals

Forty-nine (49) female Albino Wistar rats, weighing 150-200 g were used for this study.

They were housed in a cage and allowed to acclimatize for two weeks and exposed to a daily 12-hourly light and dark cycle. They had unhindered access to standard feed and clean water *ad libitum.*

2.2 Experimental Drugs

The standard drug used for this study was Celebrex (Celecoxib), manufactured by Pfizer Pharmaceuticals, Puerto Rico. The two herbal formulations that were used for this study were Jointeez (product of Kedi Healthcare Industries Limited, Nigeria) and Arthropower (product of New Green World 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, according to the Paget and Barnes (1964) conversion table [12].

The daily dose of both the standard drug and the herbal formulations were determined using the OECD's Guidelines (OECD, 2001) [13].

2.4 Experimental Design

Forty-nine (49) 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 36 mg/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 126 mg/kg body weight of Jointeez.
e) Group E was induced with rheumatoid arthritis using Complete Freund's Adjuvant and treated with 180 mg/kg body weight of Arthropower.
f) 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.
g) Group G was induced with rheumatoid arthritis using Complete Freund's Adjuvant and treated with a combination therapy of Arthropower and Celebrex at therapeutic doses.

2.5 Induction of Rheumatoid Arthritis

The rats in groups B, C, D, and E, were induced with rheumatoid arthritis using 0.1 ml (100 µl) of Complete Freund's Adjuvant (CFA), according to the method of Foyet et al. [14]. Briefly, each rat was given 0.1 ml of the CFA in the sub-plantar region of the right foot and observed for 14 days before therapy was commenced.

The paw diameter of the induced rats was measured using Vernier Calipers 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. [15].

2.6 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.

2.7 Morphological Assessment

The morphological assessment (arthritis score) was done using the method of Vijayalaxmi et al. [16]. 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.

2.8 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 and K_3EDTA bottles for biochemical and haematological analyses respectively.

2.9 Laboratory Analysis

Lipid parameters were assayed using Mindray biochemistry autoanalyzer (Model BS120, china) while haematological parameters were determined using Sysmex KX-21n auto-analyser, Japan. CD4 count was performed using Fluorescent Activated Cell Sorter Count (FACSCount) automation.

2.10 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.

3. RESULTS

The arthritic control group had significantly higher activities of the hepatic parameters compared to the treated and control groups.

The arthritic control group had significantly higher levels of tumour necrosis factor alpha (TNF-α), interleukin-6 (IL-6) and C-reactive protein (CRP) compared to the treated and control groups.

The arthritic control group had significantly lower packed cell volume (PCV), haemoglobin (Hb) but significantly higher white blood cell (WBC) count.

Table 1. Mean ± SD of hepatic parameters

	AST(IU/L)	ALT(IU/L)	ALP (IU/L)
Group A (NC)	80.34±13.17[a]	12.03±0.98[a]	113.36±7.09[a]
Group B (PC)	105.65±3.28[b]	17.29±1.20[b]	133.61±5.43[b]
Group C (CB)	86.36±12.30[a]	13.44±0.46[a]	125.61±3.80[a]
Group D (JZ)	83.72±1.89[a]	14.53±1.76[a]	117.21±14.85[a]
Group E (AP)	85.14±9.33[a]	14.15±2.14[a]	123.57±2.94[a]
Group F (JZ + CB)	87.08±11.93[a]	13.38±2.23[a]	121.84±6.59[a]
Group G (AP + CB)	88.71±11.14[a]	14.97±0.54[a]	120.59±5.60[a]
P-value	0.006	<0.001	0.001
F-value	3.592	8.506	5.117

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 inflammatory parameters

	TNF-α(pg/ml)	1L-6(pg/ml)	CRP(ng/ml)
Group A (NC)	13.96±2.58[a]	7.32±0.30[a]	217.73±8.08[a]
Group B (PC)	20.15±0.92[b]	11.31±2.74[b]	251.72±15.34[b]
Group C (CB)	15.67±2.49[a]	6.75±1.32[a]	214.35±25.36[a]
Group D (JZ)	16.61±0.72[a]	7.15±1.66[a]	216.62±17.37[a]
Group E (AP)	15.05±3.37[a]	6.76±0.73[a]	216.54±14.24[a]
Group F (JZ + CB)	15.18±3.22[a]	6.80±0.98[a]	213.44±9.33[a]
Group G (AP + CB)	15.99±2.13[a]	7.34±0.76[a]	215.33±18.69[a]
P-value	0.001	<0.001	0.001
F-value	4.714	9.321	4.882

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 3. Mean ± SD of haematological parameters

	PCV(%)	Hb(g/dl)	WBC(x10^9/µl)
Group A (NC)	37.74±0.99[a]	13.53±0.19[a]	6.80±0.98[a]
Group B (PC)	23.64±1.35[b]	10.03±0.69[b]	9.54±0.50[b]
Group C (CB)	34.29±2.29[ab]	12.41±0.95[ab]	6.50±1.49[a]
Group D (JZ)	35.74±1.14[a]	12.66±0.60[a]	6.99±1.52[a]
Group E (AP)	34.90±1.41[ab]	12.47±0.31[ab]	7.04±1.75[a]
Group F (JZ + CB)	35.69±1.34[a]	12.44±0.22[ab]	6.00±1.04[a]
Group G (AP + CB)	34.74±1.56[ab]	12.29±0.59[ab]	6.97±1.67[a]
P-value	< 0.001	<0.001	0.001
F-value	67.571	24.659	4.942

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

4. DISCUSSION

This study evaluated the effect of the herbal formulations on the activities of the liver enzymes. The arthritic control group had significantly higher activities of AST, ALT and ALP than the treated groups. The inflammation in the liver leads to an increase in the activities of the liver enzymes. This is indicative of hepatic dysfunction that are features of adjuvant-induced arthritis [16]. AST and ALT have been reported to play important roles in the synthesis of active chemical mediators of inflammation in rheumatoid arthritis [17]. The increase in ALP activity may also be due to associated increase in either its liver or bone isoforms or both in inflammation [17], which may imply bone erosion as seen in rheumatoid arthritis [18].

The assay of these enzymes has been seen as a simple method of evaluating the anti-arthritic activity of any target drug [17]. The levels of these enzymes were significantly reduced in the rats that were treated with the herbal formulations. Thus, the herbal formulations used in this study were able to reverse the liver impairments that are associated with adjuvant-induced rheumatoid arthritis. This is probably due to the anti-inflammatory potentials of these herbal formulations, which brings about the reduction in the release of chemical mediators of inflammation [19]. These results were comparable to that of the orthodox drug used for this study.

The reduction in the activities of these enzymes also indicate that, at therapeutic doses, these herbal formulations were not toxic to the liver and therefore do not pose any threat to the integrity of the liver.

The data from this study showed that the levels of the inflammatory markers were significantly higher in the arthritic control group compared to the other groups. Acute phase reactants are usually produced during inflammation [20] such as rheumatoid arthritis. 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 [21].

The significantly reduced levels of the markers in groups treated with the herbal formulations, compared to the arthritic control group, is probably due to the inhibitory effects of the herbal formulations on the production of inflammatory markers [22]. The anti-inflammatory effects of the herbal formulations were comparable with that observed with the orthodox drug. Similar findings have been reported by Appusamy et al. [23], using Balaguluchyai Kwatham tablet, a polyherbal formulation and leflunomide, an orthodox drug. Other studies have also reported a similar finding using Vatari Guggulu and Indomethacin [24]. The herbal drug has been reported to have anti-arthritic effects, in which the levels of the inflammatory markers were reduced significantly in rats that were treated with the herbal drugs [25].

There were significant increases in the PCV, Hb and CD4 counts 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 [26]. The anaemia, which may be may be of chronic disease (ACD) and iron deficiency anaemia (IDA) or both, may be due to the abnormal metabolism of hepcidin, as the hormone that regulates iron metabolism [27]. Hepcidin levels are increased during inflammation [28] as seen in patients who suffer from rheumatoid arthritis. Similarly, ACD may arise due to altered iron metabolism as an effect of hepcidin, shortened lifespan of the reticulocytes and diminished response to erythropoietin by red blood cell precursors [29]. The herbal formulations improved the haematological parameters thereby, reversing the observed anaemia. This is probably because herbal formulations can improve intestinal absorption of nutrients by altering the microbiota of the GIT [30] and reducing inflammation [31].

Total White blood cell count was, however, higher in the arthritic control group than in the other groups. This high WBC count has been reported by Neeraji et al. [32]. The increased WBC may be due to the activation of the immune system which occurs in rheumatoid arthritis [33]. There is release of interleukins which causes an increase in the synthesis of colony stimulating factors for both macrophages and granulocytes [23].

The herbal formulations restored the WBC in the treated rats to normal levels. This is probably because bioactive components in natural products have the ability to control molecular mediators of inflammation thereby inhibiting

effector molecules such as the proinflammatory cytokines [34], thereby inhibiting the activation of the immune system.

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 other researchers [22,35,23].

5. CONCLUSION

It is concluded that these herbal formulations have the potential to reduce the inflammation associated with rheumatoid arthritis. The results from this study show that they are not toxic at therapeutic doses.

CONSENT

It is not applicable.

ETHICAL APPROVAL

This study was carried out in accordance with the Guidelines of the Organization for Economic Cooperation and Development [13].

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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