



Effects of Aqueous and Ethanol Root Extracts of *Rauwolfia Vomitori* on Inflammatory Parameters in Complete Freund's Adjuvant-Collagen Type II Induced Arthritic Albino Rat

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Abstract This study was designed to evaluate the effects of *Rauwolfia vomitoria* Oliv. ethanol and aqueous root extracts on adjuvant induced arthritis in albino rats. A total of 135 female albino rats were used for this study. The albino rats were divided into nine groups, each containing 15 animals. Arthritis was induced by intradermal administration of 0.1ml Chicken type II collagen-Complete Freund's adjuvant into the left hind paw of the albino rats. Treatment with the extracts at doses of 400, 600 and 800 mg/kg body weight commenced on day 10 of the 32-day study period. The parameters were analysed using a well known standard methods. The result showed that in the arthritic rats, the levels of interleukin- 6 (IL-6), interleukin-1 β (IL-1 β), erythrocyte sedimentation rate (ESR), rheumatoid factor (RF), C-reactive protein and tumor necrosis factor alpha (TNF- α) were significantly ($P < 0.05$) higher than the control. Treatment of the arthritic rats with the extracts at the specified doses caused a significant reversal ($P < 0.05$) of the effect of the chicken type II collagen –Complete Freund's adjuvant in the rats. The ameliorative effect of the extracts on the arthritic rats was both time and dose dependent. The anti-arthritic potentials of the root extracts was significantly ($P < 0.05$) comparable to that of the standard drug (indomethacine). The results also showed that the potentials of *Rauwolfia vomitoria* ethanol and aqueous root extracts to reverse the effect of the adjuvant on the arthritic rats were significant ($P > 0.05$). The plant extracts contain principles that have the potential to suppress antigen presentation and reverse arthritic conditions developed in adjuvant induced arthritic rats. Hence, this present study provides scientific evidence that *Rauwolfia vomitoria* ethanol and aqueous roots have anti-arthritic potentials.

Keywords *Rauwolfia vomitoria*, weight, paw size, Erythrocyte sedimentation rate, C-reactive protein and Cytokines

Introduction

Rheumatoid arthritis is a known inflammatory condition with an autoimmune component, characterized by bone and cartilage deformations, joint pain, joint oedema, and particularly, the stiffness of the joints of the hands, wrists, and feet [1]. Non-steroidal anti-inflammatory drug (NSAIDs) and disease modifying anti-rheumatic drugs (DMARDs) such as methotrexate, hydroxychloroquine, sulfasalazine and leflunomide are usually the first line treatment of this disease. However, they show common adverse effects such as disorders of the gastro-intestinal tracks, loss of appetite, sore mouth, diarrhea, headaches and hair loss [2 and 3]. Also, the use of biologics such as etanercept, adalimumab, certolizumab etc, which is a new form of treatment for rheumatoid arthritis [4]. They too are not without side effects which include mild skin reactions at the site of injections, infections, nausea, a rise in temperature and headaches [5]. Furthermore, the cost of these drugs are quite high and often beyond the reach of the



common and poor people in the society. Hence many sufferers of arthritis resort to using locally available herbs to manage or treat their ailments. This approach has produced many positive results, and therefore it is becoming the general practice in our society today. *Rauwolfia vomitoria* Afzel. Plant has been successfully used for the treatment of arthritis by the rural dwellers.

Rauwolfia vomitoria is among the common herbs used traditionally in Ebonyi State to manage and/or treat many ailments, especially arthritis. The Yoruba people call it “Asofeyeje”, the Igbos call it “Akanta”, the Hausa tribe call it “Wada”, “Mmoneba” in Efik and “Utoenyim” in Ibibio Languages [6]. It is called “Akan-Brong” in Ivory Coast, “Asaja” in Igbo and Ewe Ifon in Yoruba [6]. *Rauwolfia vomitoria* plant has been proved potent over the years in the management of psychiatric disorders, inflammation, pyretic condition, diabetes and cancer [7]. It is also used therapeutically for the treatment of some ailments such as rheumatoid arthritic condition, diseases of the liver, yellow fever, Jaundice, venereal diseases, guinea worm, asthma, cancer, mental illness and diabetes mellitus [8].

Although this plant has been used over the years locally in the management of arthritis, no scientific base for this has been reported or compared the effects of ethanol and aqueous extracts of the plant. Therefore the study is aimed at determining the effects of aqueous and ethanol root extracts of *Rauwolfia vomitoria* on inflammatory parameters such as Erythrocyte Sedimentation Rate, C-reactive protein and Cytokines such as interleukine 1-B, interleukine-6, tumor necrosis factor, rheumatoid factor in Complete Freund’s Adjuvant-Collagen Type II Induced Arthritic Rats

Materials and Methods

Materials

The Roots of *Rauwolfia vomitoria* were sourced from Ndi-Nwali Village in Izzi Local Government Area of Ebonyi State in South-Eastern Nigeria. The authentication of the plant was carried out by Dr. (Mrs.) Kate Nnamani, a Botanist in the Department of Biological Sciences, Ebonyi State University, Abakaliki. Some of the plant root samples were preserved in the Department of Biological Science herbarium.

A total of one hundred and thirty-five female albino rats were purchased from the Department of Animal Science, University of Nigeria Nsukka, Enugu State, Nigeria and were acclimatized for a period of two weeks at the Animal House of the Department of Biochemistry, Ebonyi State University, Abakaliki. They were kept in cages and were fed on commercial rat feed and were allowed access to clean water. The test rats were weighed daily.

Methods

Preparation of the plant extracts

The roots of *Rauwolfia vomitoria* were washed under tap water to remove contaminants and were air dried under a shade. They were pulverized using laboratory milling machine and sifted using 0.25 mm sieve. Exactly 800g of the powdered root sample of *Rauwolfia vomitoria* was soaked in 2L each of ethanol and deionized water for 48 hours. They were filtered with a clean white cloth; the process was repeated until nothing could be extracted further. The extracts were pooled together and concentrated using a water bath at 50°C until the solvents were completely removed; the sample were dried and the result was obtained. Extracts obtained were used for various analyses.

Induction of arthritis in albino rats

The induction of arthritis in rats was done by the method described by Pearson, (1956), [9]. Briefly, 0.1 ml of chicken type II collagen-complete Freund’s adjuvant (CFA) was injected into the left hind paw of the rats (The constituents of the CFA are heat killed *Mycobacterium tuberculosis* and sterile paraffin oil (10 mg/ml). A calibrated automatic veneer caliper was used to measure the paw size of all the animal groups twice weekly throughout the duration of the study before and after the administration of the adjuvant. The severity of paw inflammation was measured using a qualitative scoring system. Rats with no visible swelling were scored 0, a score of 1 was given to rats with mild redness and inflammation of individual digits, rats with moderate redness and swelling of the ankle were given a score of 2 while a score of 3 was given to the rats with highly pronounced redness and inflammation of the entire paw including the digits. Those rats that had a score of 3 with elevated level of inflammatory biomarkers when compared to the control were therefore considered to have arthritis and were used for subsequent



experiments. (It was observed that by day 10 after injection of CFA-chicken type II collagen, arthritis had completely set in). The weights of rats were taken daily throughout the study duration.

Treatment of Arthritic rats with plant extracts.

A total of one hundred and thirty-five female rats that weighed between 150- 200 g were employed in this work. The animals were distributed into nine groups with 15 animals in each group. The indomethacin which served as the standard drug (standard control) was made into a solution using normal saline. Group I served as negative control (without induction of arthritis and treatment) and received 5 ml/kg normal saline; Group 2 was induced with arthritis but without treatment. It served as the positive control and received 5 ml/kg normal saline; Group 3 was administered 10 mg/kg indomethacin (standard control), Group 4 - 6 were induced with arthritis and were treated with *Rauwolfia vomitoria* aqueous root extract at doses of 400, 600 and 800 mg/kg body weight, respectively, from day ten after induction till the end of the study; Groups 7 – 9 were induced with arthritis and received *Rauwolfia vomitoria* ethanol root extract at 400, 600 and 800 mg/kg body weight from day ten after induction till the end of the study. Various changes in the body weight and inflammatory index were noted daily.

Preparation of samples for Analysis

Three albino rats from each group (Group 1-9) were sacrificed on days 10, 18, 25 and 32 and blood samples were collected in plain tubes for serum separation. The serum samples were used for the assay of the inflammatory parameters.

Determination of Phytochemical constituents

The phytochemical constituents of *Rauwolfia vomitoria* ethanol and aqueous root extracts were determined were Tannin (10), flavonoids (10), terpenoids (11), steroids (11), alkaloids (11), saponin (11), phenol (11) and carotenoids (12)

Estimation of inflammatory parameters

Inflammatory parameters determined were erythrocyte sedimentation rate (ESR) (13), C-reactive protein (CRP) (14), Rheumatoid factor (RF) (15) and cytokines such as IL-1B (16), IL-6 (16) and TNF (16) were determined

Statistical Analysis

The basic statistics, means, standard deviation and ranges of the measured parameters were estimated using Statistical Analysis System (SAS) windows version 9.0. Data were expressed as means \pm SD of 9 replicates. Values were considered statistically significant at $P < 0.05$.

Results

The results of the percentage yield of ethanol and aqueous root extracts of the plants (Table 1) showed that the percentage yield of ethanol extract of *Rauwolfia vomitoria* was 5% while its aqueous extract percentage yield was 3%. The results of the percentage quantitative phytochemical analysis of *Rauwolfia vomitoria* ethanol and aqueous root extracts are shown in Table 2. The phytochemical results revealed that *Rauwolfia vomitoria* ethanol and aqueous root extracts contain: tannin, alkaloids, terpenoids, steroids, saponin, glycosides, flavonoids, phenols and β -carotenoids in varying amounts and are solvent dependent

Impact of the administration of *Rauwolfia vomitoria* ethanol and aqueous root extracts on the weight of rats is presented in Table 3. The result shows the ameliorator effect of indomethacin, and *Rauwolfia vomitoria* aqueous and ethanol root extracts on the weight of the treated arthritic rats in comparison to the arthritic but untreated group and the normal control. There was a significant ($P < 0.05$) reduction in the weight of arthritic rats. However the administration of *Rauwolfia vomitoria* aqueous and ethanol root extracts on the arthritic rats ameliorated the effect of adjuvant on the weight of the rats while the weight of untreated arthritic rats continued to decrease significantly ($P < 0.05$) until the end of the study period.

The effect of *Rauwolfia vomitoria* ethanol and aqueous root extracts on paw size (inflammation) of rats (Table 4) shows the anti-inflammatory effect of indomethacin, *Rauwolfia vomitoria* ethanol and aqueous root extracts at 400, 600 and 800 mg/kg bd wt respectively, on the changes in paw edema of induced but untreated rats and treated animals. There was increase in paw size and redness developed over a 48 hr period in the feet of experimental



animals injected with chicken type 11 collagen - complete Freund's adjuvant. The administration of *Rauwolfia vomitoria* aqueous and ethanol root extracts at different doses showed a marked reduction in paw size while the paw size of untreated arthritic rats continued to increase.

The C-reactive protein result is presented in Table 5. There was significantly ($P < 0.05$) higher levels of C-reactive protein in the arthritic untreated rats than found in normal control rats. This increased significantly ($P < 0.05$) as the exposure period increased with the highest value observed on day 32. However, treatment of the arthritic rats with root extracts of *Rauwolfia vomitoria* aqueous at 800 mg/kg b.w doses, caused a significant ($P < 0.05$) reduction of C-RP levels relative to the levels found in the negative control. The effect was both dose and time-dependent. Nonetheless, the values of C-RP in the treated groups were still significantly ($P < 0.05$) higher than the values found in the control group, but comparable with the values found in the standard control.

The result of the effect of of *Rauwolfia vomitoria* ethanol and aqueous root extracts on Rheumatoid factor (RF) of rats is presented in Table 6. RF count was significantly ($p < 0.05$) higher in the arthritic rat groups. However treatment with *Rauwolfia vomitoria* ethanol and aqueous root extracts at 400, 600 and 800 mg/kg bd wt caused a significant reversal of RF value back to the value found in the normal control when compared to the positive control whose RF value continued to increase until the end of the study. The effect of treatment was time dependent.

Table 7 revealed the effect of *Rauwolfia vomitoria* aqueous and ethanol root extracts on ESR levels in the arthritic rats. The ESR level in arthritic rats was significantly ($P < 0.05$) higher relative to the normal control. However treatment with standard drug, *Rauwolfia vomitoria* aqueous and ethanol root extracts at different doses administered in this study significantly caused a reversal of ESR close to the levels of the normal control groups (negative control) in a dose and time-dependent manner.

The results of cytokine levels are presented in Tables 8-10. The result shows the levels of Serum cytokines measured in the arthritic and non arthritic samples. In comparison to the normal control group, the untreated arthritic animal groups had a significant ($P < 0.05$) higher values of TNF- α , IL-1 β and IL-6 levels. The measured cytokine concentration were observed to have decreased ($P < 0.05$) following the administration of *Rauwolfia vomitoria* ethanol and aqueous extracts to the arthritic rats, the values were comparable to those of the normal control group. The values of TNF- α , IL-1B and IL-6 in the untreated arthritic rats continued to increase significantly ($P < 0.05$) till day 32 when the study was terminated.

Table 1: Percentage Yields of ethanol and aqueous extracts of *Rauwolfia vomitoria* roots

Sample	Yield	
	Aqueous extract	Ethanol extract
<i>Rauwolfia vomitoria</i>	7.3 %	5 %

Table 2: Percentage phytochemical constituent of ethanol and aqueous extracts of *Rauwolfia vomitoria* roots

	Aqueous extract (%)	Ethanol extract (%)
Tannin	4.20	3.93
Terpenoids	0.177	3.24
Steroids	0.017	0.011
Alkaloids	1.52	2.08
Saponin	1.54	0.02
Phenol	3.36	6.28
Flavonoids	2.2	2.78
Carotenoids	0.34	1.24

Table 3: Effect of *Rauwolfia vomitoria* (RV) aqueous and ethanol root extracts on weight of adjuvant-induced arthritic rats

Treatments	Wk1(g)	Wk2(g)	Wk3(g)	wk4(g)
1	180.60 \pm 3.84 ^a	182.00 \pm 6.30 ^b	184.33 \pm 6.15 ^b	191.00 \pm 6.78 ^a
2	168.80 \pm 9.59 ^b	167.87 \pm 4.09 ^d	161.33 \pm 4.72 ⁱ	155.25 \pm 3.59 ^d



3	166.60±10.59 ^{b,c}	176.37±6.16 ^{c,d}	182.50±7.45 ^{b,c}	190.50±5.97 ^a
4	165.50±13.48 ^{b,c}	175.25±6.21 ^{c,d}	181.67±7.06 ^{b,c}	193.75±3.40 ^a
5	159.10±23.07 ^c	166.87±19.30 ^d	174.00±10.03 ^d	180.25±11.70 ^e
6	165.30±9.96 ^{b,c}	173.37±9.41 ^{c,d}	177.67±12.46 ^d	184.00±6.95 ^b
7	168.60±13.64 ^b	180.37±10.64 ^{b,c}	182.00±9.06 ^{b,c}	184.25±9.50 ^b
8	166.60±13.84 ^{b,c}	170.25±9.48 ^d	175.50±10.56 ^d	181.75±13.05 ^{b,c}
9	166.10±7.26 ^{b,c}	186.87±2.70 ^a	191.17±3.19 ^a	192.75±2.50 ^a

Difference in weight of adjuvant induced arthritic rats treated with *Rauwolfia vomitoria* ethanol and aqueous root extract. RV= *Rauwolfia vomitoria*. 1= Negative control, 2= positive control, 3= Standard control, 4= 400 mg/ kg RV aqueous extract, 5= 600 mg/kg RV aqueous extract, 6= 800 mg/kg RV aqueous extract, 7= 400 mg/kg RV ethanol extract, 8= 600 mg/kg RV ethanol extract, 9= 800 mg/kg RV ethanol extract. * Means with the same letter are not significantly different.

Table 4: Effect of *Rauwolfia vomitoria* (RV) aqueous and ethanol root extracts on paw size (inflammation) of adjuvant-induced arthritic rats treated

Treatments	Before (mm)	week 1(mm)	week 2(mm)	week 3(mm)	week 4 (mm)
1	2.73±0.02 ^a	2.73±0.20 ^e	2.73±0.02 ^g	2.73±0.01 ^e	2.73±0.01 ^{h,g}
2	2.72±0.01 ^a	5.37±0.08 ^a	6.52±0.23 ^a	7.31±0.07 ^a	7.79±0.04 ^a
3	2.70±0.04 ^a	4.53±0.51 ^{c,d}	3.76±0.69 ^f	2.79±0.23 ^e	2.68±0.12 ^h
4	2.69±0.16 ^a	4.79±0.35 ^{b,c}	4.49±0.27 ^{b,a}	3.84±0.04 ^{c,b,d}	2.94±0.05 ^{f,h,e,g}
5	2.68±0.07 ^a	4.97±0.47 ^b	4.44±0.33 ^{c,e,d}	3.68±0.17 ^{c,b,d}	2.94±0.06 ^{f,h,e,g}
6	2.72±0.07 ^a	4.67±0.20 ^{b,c}	4.42±0.17 ^{c,e,d}	3.47±0.16 ^d	3.02±0.12 ^{f,c,e,d,g}
7	2.69±0.08 ^a	4.73±0.34 ^{b,c}	4.59±0.35 ^{c,b,d}	4.09±0.12 ^{c,b}	3.35±0.05 ^{c,b}
8	2.73±0.08 ^a	4.35±0.66 ^d	4.12±0.26 ^{e,f}	3.98±0.19 ^{c,b}	3.24±0.06 ^{c,e,b,d}
9	2.74±0.04 ^a	4.75±0.47 ^{b,c}	4.61±0.45 ^b	3.65±0.21 ^{c,d}	2.83±0.05 ^{h,g}

Paw size (inflammation) of adjuvant induced arthritic rats treated with *Rauwolfia vomitoria* ethanol and aqueous root extracts. RV= *Rauwolfia vomitoria*. 1= Negative control, 2= positive control, 3= Standard control, 4= 400 mg/ kg RV aqueous extract, 5= 600 mg/kgRV aqueous extract, 6= 800 mg/kgRV aqueous extract, 7= 400 mg/kg RV ethanol extract, 8= 600 mg/kg RV ethanol extract, 9= 800 mg/kg RV ethanol extract,. * Means with the same letter are not significantly different.

Table 5: Effect of *Rauwolfia vomitoria* (RV) aqueous and ethanol root extracts on C-RP level of adjuvant induced arthritic rats

Treatment	DAY10(mg/dl)	DAY18 (mg/dl)	DAY25 (mg/dl)	DAY32 (mg/dl)
1	4.22±0.15 ^f	4.35±0.86 ^f	4.96±0.01 ^h	4.36±0.01 ^f
2	10.34±0.91 ^a	12.61±0.35 ^a	17.51±0.17 ^a	21.42±1.49 ^a
3	7.52±0.012 ^e	7.41±0.78 ^{g,f}	5.36±0.04 ^g	4.45±0.04 ^f
4	8.38±0.07 ^{c,b,d}	8.30±0.11 ^{c,b}	6.87±0.69 ^{c,d}	6.15±1.10 ^{c,b,d}
5	8.39±0.04 ^{c,b,d}	8.31±0.08 ^{c,b}	6.42±0.00 ^{f,e}	6.28±0.39 ^{b,c}
6	8.27±0.06 ^{c,b,d}	7.93±0.16 ^{c,e,b,d}	6.27±0.04 ^f	6.20±0.11 ^{c,b,d}
7	8.55±0.25 ^{c,b}	8.29±0.13 ^{c,b,d}	7.35±0.04 ^b	6.39±0.12 ^{c,b}
8	8.26±0.06 ^{c,b,d}	7.26±0.02 ^{g,h}	6.71±0.40 ^{c,e,d}	6.43±0.00 ^{c,b}
9	8.23±0.03 ^{c,b,d}	7.20±0.01 ^{g,h}	6.88±0.24 ^{c,d}	5.84±0.05 ^{c,e,d}

C-reactive protein levels of adjuvant induced arthritic rats treated with *Rauwolfia vomitoria* ethanol and aqueous root extracts. RV= *Rauwolfia vomitoria*. 1= Negative control, 2= positive control, 3= Standard control, 4= 400 mg/ kg RV aqueous extract, 5= 600 mg/kgRV aqueous extract, 6= 800 mg/kgRV aqueous extract, 7= 400 mg/kg RV ethanol extract, 8= 600 mg/kg RV ethanol extract, 9= 800 mg/kg RV ethanol extract, * Means with the same letter are not significantly different.



Table 6: Effect of *Rauwolfia vomitoria* (RV) aqueous and ethanol root extracts on rheumatoid factor level of adjuvant induced arthritic rats

Treatments	DAY10 (IU/ml)	DAY18 (IU/ml)	DAY25 (IU/ml)	DAY32 (IU/ml)
1	26.42±0.99 ^f	25.06±0.46 ^d	27.31±0.46 ^f	27.96±0.05 ^{b,c}
2	52.64±0.35 ^a	58.41±0.64 ^a	62.23±2.62 ^a	71.28±1.01 ^a
3	38.99±0.88 ^e	32.61±1.80 ^c	29.48±1.29 ^e	27.64±1.41 ^{b,c}
4	47.04±0.94 ^b	42.87±0.72 ^b	36.44±0.78 ^b	30.11±0.00 ^b
5	46.69±0.57 ^b	40.41±0.11 ^b	34.88±1.21 ^c	29.64±0.00 ^b
6	44.77±0.87 ^c	40.46±0.24 ^b	34.74±0.64 ^c	27.66±0.04 ^{b,c}
7	41.03±0.21 ^{d,c}	40.15±0.66 ^b	33.70±0.09 ^{c,d}	27.87±0.06 ^{b,c}
8	43.32±0.57 ^c	40.26±2.30 ^b	35.55±0.23 ^c	27.89±0.01 ^{b,c}
9	42.81±0.08 ^c	41.36±0.66 ^b	33.19±0.27 ^{c,d}	27.32±0.59 ^{b,c}

Rheumatoid factor levels of adjuvant induced arthritic rats treated with *Rauwolfia vomitoria* ethanol and aqueous root extracts. RV= *Rauwolfia vomitoria* 1= Negative control, 2= positive control, 3= Standard control, 4= 400 mg/kg RV aqueous extract, 5= 600 mg/kgRV aqueous extract, 6= 800 mg/kg RV aqueous extract, 7= 400 mg/kg RV ethanol extract, 8= 600 mg/kg RV ethanol extract, 9= 800 mg/kg RV ethanol extract, * Means with the same letter are not significantly different.

Table 7: Effect of *Rauwolfia vomitoria* (RV) aqueous and ethanol root extracts on ESR level of adjuvant

Treatments	DAY 10 (mm/hr)	DAY 18 (mm/hr)	DAY 25 (mm/hr)	DAY 32(mm/hr)
1	3.68±0.07 ^g	3.53±0.01 ^f	3.42±0.15 ^g	3.35±0.24 ^h
2	7.57±0.21 ^{b,a,c}	8.11±1.02 ^a	10.39±0.02 ^a	11.82±0.15 ^a
3	6.58±0.45 ^{*f}	5.15±0.05 ^e	4.86±0.18 ^f	3.81±0.01 ^g
4	7.39±0.01 ^{b,a,c}	6.86±0.11 ^b	5.99±0.36 ^c	5.61±0.01 ^b
5	7.46±0.06 ^{b,a,c}	6.50±0.16 ^b	5.57±0.06 ^d	4.36±0.09 ^{d,c}
6	7.48±0.08 ^{b,a,c}	5.265±0.11 ^b	5.22±0.03 ^e	4.01±0.13 ^f
10	7.39±0.71 ^{b,a,c}	6.85±0.12 ^b	5.99±0.35 ^c	5.60±0.00 ^b
11	7.31±0.13 ^{b,d,c}	6.57±0.05 ^b	5.30±0.07 ^e	4.27±0.08 ^{d,e}
12	7.37±0.08 ^{b,c}	5.21±0.04 ^e	5.21±0.03 ^e	4.02±0.13 ^f

Erythrocyte sedimentation rate levels of adjuvant induced arthritic rats treated with *Rauwolfia vomitoria* ethanol and aqueous root extracts. RV= *Rauwolfia vomitoria*. 1= Negative control, 2= positive control, 3= Standard control, 4= 400 mg/kg RV aqueous extract, 5= 600 mg/kgRV aqueous extract, 6= 800 mg/kgRV aqueous extract, 7= 400 mg/kg RV ethanol extract, 8= 600 mg/kg RV ethanol extract, 9= 800 mg/kg RV ethanol extract, * Means with the same letter are not significantly different.

Tables 8: Effect of *Rauwolfia vomitoria* aqueous and ethanol root extracts on TNF- α level of adjuvant induced arthritic rats

Treatments	DAY 10(pg/ml)	DAY 18(pg/ml)	DAY 25(pg/ml)	DAY 32(pg/ml)
1	198±10.73 ^b	200±12.02 ^k	197±13.03 ⁱ	198±11.01 ^g
2	480±18.08 ^a	760±16.71 ^a	800±17.15 ^a	820±15.89 ^a
3	470±11.11 ^a	350±10.80 ^j	320±9.24 ^e	205±8.01 ^{e,f}
4	475±10.05 ^a	450±11.16 ^{c,b}	360±11.06 ^b	220±10.10 ^{c,b}
5	476±12.34 ^a	420±9.06 ^{f,e}	350±10.03 ^c	210±9.26 ^{e,d}
6	474±16.16 ^a	400±10.20 ^h	330±9.19 ^d	205±7.02 ^{e,f}
7	477±13.48 ^a	452±6.21 ^b	358±7.06 ^b	222±3.40 ^b
8	474±23.07 ^a	425±19.30 ^e	348±10.03 ^c	215±11.70 ^{c,d}
9	470±9.96 ^a	385±9.41 ⁱ	330±12.46 ^d	210±6.95 ^{e,d}

TNF- α level of adjuvant induced arthritic rats treated with *Rauwolfia vomitoria* aqueous and ethanol root extracts. The data are shown as mean \pm SD (n=12) and significant difference at P<0.05. RV= *Rauwolfia vomitoria*. 1=



Negative control, 2= positive control, 3= Standard control, 4= 400 mg/ kg RV aqueous extract, 5= 600 mg/kgRV aqueous extract, 6= 800 mg/kgRV aqueous extract, 7= 400 mg/kg RV ethanol extract,8= 600 mg/kg RV ethanol extract, 9= 800 mg/kg RV ethanol extract, * Means with the same letter are not significantly different.

Tables 9: Effect of *Rauwolfia vomitoria* (RV) aqueous and ethanol root extracts on interleukin-6 level of adjuvant-induced arthritic rats

Treatments	DAY 10 (pg/ml)	DAY 18 (pg/ml)	DAY 25 (pg/ml)	DAY 32 (pg/ml)
1	180±3.84 ^b	188±6.30 ⁱ	185±6.15 ^k	187±6.78 ^h
2	420±9.59 ^b	657±4.09 ^a	789±4.72 ^a	811±3.59 ^a
3	410±10.59 ^a	325±6.16 ^h	315±7.458 ^j	205±5.97 ^g
4	412±13.64 ^a	385±10.64 ^b	370±9.06 ^b	240±9.50 ^b
5	409±13.84 ^a	370±9.48 ^c	352±10.56 ^d	230±13.05 ^{c,d}
6	413±7.26 ^a	357±2.70 ^e	340±3.19 ^{e,f}	225±2.50 ^{e,d}
7	418±15.64 ^a	375±14.20 ^c	363±13.75 ^c	238±10.15 ^b
8	407±16.67 ^a	364±12.10 ^d	346±14.85 ^{e,d}	235±9.93 ^{c,b}
9	410±12.25 ^a	340±11.88 ^f	335±10.61 ^{g,f}	224±9.81 ^e

Tables 10: Effect of *Rauwolfia vomitoria* aqueous and ethanol root extracts on IL-1 β level of adjuvant-induced arthritic rats

Treatments	DAY 10 (pg/ml)	DAY 18 (pg/ml)	DAY 25 (pg/ml)	DAY 32 (pg/ml)
1	196±10.71 ^e	194±11.34 ^f	190±1.70 ^h	194±0.17 ^{c,f}
2	258±13.79 ^{b,a}	470±10.00 ^a	590±0.21 ^a	650±0.76 ^a
3	242±7.47 ^{d,c}	220±13.61 ^{c,e,d}	212±4.27 ^{f,e,d}	198±1.22 ^{e,d}
4	265±9.18 ^a	230±8.10 ^b	220±1.24 ^{c,b}	210±0.35 ^b
5	254±11.71 ^{b,d,a,c}	225±12.89 ^{c,b,d}	220±1.13 ^{c,b}	207±0.40 ^{c,b}
6	240±12.85 ^d	220±14.09 ^{c,e,d}	215±2.47 ^{c,e,d}	198±3.60 ^{e,d}
7	258±7.64 ^a	225±10.64 ^{c,e,d}	218±9.06 ^{c,b}	207±9.50 ^{c,b}
8	258±6.84 ^{b,a}	220±9.48 ^{c,e,d}	221±6.55 ^b	203±7.05 ^{c,d}
9	241±7.26 ^{d,c}	218±2.70 ^{e,d}	212±3.19 ^{f,e,d}	190±2.50 ^f

IL-1 β level of adjuvant induced arthritic rats treated with *Rauwolfia vomitoria* ethanol and aqueous root extracts. The data are shown as mean \pm SD (n=12) and significant difference at P<0.05., RV= *Rauwolfia vomitoria*. 1= Negative control, 2= positive control, 3= Standard control, 4= 400 mg/ kg RV aqueous extract, 5= 600 mg/kg RV aqueous extract, 6= 800 mg/kg RV aqueous extract,7= 400 mg/kg RV ethanol extract,8= 600 mg/kg RV ethanol extract, 9= 800 mg/kg RV ethanol extract,. * Means with the same letter are not significantly different

Discussion

Rheumatoid arthritis (RA) is an autoimmune disease in which the body's immune systems that fight foreign substances such as bacteria and viruses mistakenly attack the human joints. This leads to inflammation of the synovium, causing pain and swelling in the joints [17]. At present, NSAIDs as well as DMARDs are employed in the management of rheumatoid arthritic condition to lower the associated pain and inflammation and also to decrease the continuity of the disease. DMARDs act by suppressing the immunological processes linked to the progression of this disease [18]. Due to unwelcomed side effects of NSAIDs and DEMARDS and the cost of the drugs, there is an increased demand and preference for alternative medicines [19]. Among the plants that have been used by rural dwellers for the management of arthritis with a claim of effectiveness is *Rauwolfia vomitoria*. In an attempt to model human arthritis, a systemic injection of chicken type II collagen -CFA resulted in polyarthritis and systemic disease that mimicks changes as is seen in human rheumatoid arthritis [19]. Some Inflammatory biomarkers were measured to ascertain the effectiveness of these plants.



The presence of the phytochemicals in *Rauwolfia vomitoria* are without doubt responsible for the medicinal use of the plant in the management of arthritis. For example, several studies have shown that alkaloids decrease lymphocyte proliferation triggered by antigen and mitogen, the cytotoxicity of natural-Killer cell, the synthesis of histamine by mast cells, the release of interleukin-1 by human monocytes and the platelet activating factor (PAF) action on platelets [20]. Alkaloids such as tetrandine and its analogue, berbamine have been shown to stop prostaglandin and leukotriene release by monocytes and neutrophils in humans. The inhibitory effect of berbarine on inflammation has shown that alkaloids may exert an important activity in chronic inflammation [20]. However, in this study, alkaloids profiling was not carried out, and it is not possible to pin the observed anti-arthritic effects of the plant extract to any of the alkaloids mentioned above. In addition, tannins are known to have free radical scavenging ability and hence could ameliorate inflammatory responses in animals [21, 22]. Several other promising studies have connected the anti-oxidant (radical scavenging) activity of tannins with the inflammatory system as many of those studies have helped to describe the molecular action of the active component found in tannin. Poly-[1-6]- β -D-glucopyranosyl-[1-3]- β -D-glucopyranose (PGG-glucan) for example, along with five galloyl ester groups, exert a strong anti-tumor action, via a decreased iNOS expression and has shown to inhibit the production of prostaglandin E2 (PGE2) [23]. This helps explain the possible ameliorative effects these plant extracts on the arthritic rats.

Furthermore, it is possible that the anti-arthritic effect displayed by our plant extracts could be due to the presence of flavonoids in these plants. Flavonoids have been known to display anti-inflammatory potential [24]. It has been suggested that the inhibition of inflammation causing enzymes such as cyclooxygenase-2, lipoxygenase and inducible NO synthase, nuclear transcription factor-kappaB (NF-kB) and activating protein-1 (AP-1) and activation of phase II antioxidant detoxifying enzymes in rat are possible molecular mechanisms involved in the anti-inflammatory activities of flavonoids [25]. It has been ascertained that several flavonoids are able to suppress the release of different pro-inflammatory cytokines/chemokines like TNF α , IL-1 β , IL-6, IL-8 and monocyte-chemo attractant protein-1, in different cell types such as RAW macrophages, Jurkat T-cells and peripheral blood mononuclear cells [26]. Therefore the anti-arthritic effects of these plant extracts may be associated with their saponin and carotenoid content [20].

The results presented in Table 3 showed that rheumatoid arthritis was induced in rats within a period of 10 days by administering chicken type 11 collagen-complete Freund's adjuvant. Induction of arthritis condition caused significant ($P < 0.05$) weight loss in rats. Weight loss has been reported to be a marker for an upsurge in the synthesis of TNF- α and interleukin-1 which are pro-inflammatory cytokines [27]. It has also been reported that reduction in body weight in arthritic rats could be caused by muscle wasting in experimental arthritis which manifests as a result of heightened breakdown of protein by the ubiquitin-proteasome proteolytic pathway [27]. Also, our result agrees with a research done by Thiagarajan *et al.* (2015) [28] who noted that signs such as weight loss, slight fever, malaise, and fatigue, loss of appetite and morning stiffness are common systematic symptoms seen in people with active rheumatoid arthritis. In CFA induced arthritis in rats, there is a rapid increase in leptin level just within twenty-four hours of injecting of CFA in rats leading to anorexia and loss of weight [19]. However, our results showed ameliorator effect of indomethacin and *Rauwolfia vomitoria* aqueous and ethanol root extracts on the weight of the rats administered extracts when compared to the arthritic but untreated group (positive control) and the normal control (negative control). The ameliorative effect of these extracts may have been due to the presence of phytochemical constituents like alkaloids, flavonoids and terpenoids that suppress antigen presentation, cytokine and prostaglandin release [20, 26]. Our result also agrees with the report of Ezejindu *et al.*, [29] on the hepatoprotective effects of *Rauwolfia vomitoria* extract on the liver of adult wistar rats. He stated that it did not cause weight loss to the experimental rats rather enhanced weight gain and growth, hence may boost the tolerance capacity against toxic compounds due to the presence of antioxidants in the plant extract.

Effect of adjuvant-induced arthritis on Paw size rats (Table 4) shows the anti-inflammatory effect of indomethacin, *Rauwolfia vomitoria* ethanol and aqueous root extracts at 400, 600 and 800 mg/kg bd wt respectively, on the changes in paw edema of induced untreated rats and treated animals. There was increase in paw size and redness developed over a 48 hr period in the feet of experimental animals injected with chicken type 11 collagen - complete



Freund's adjuvant. The *Rauwolfia vomitoria* aqueous and ethanol root extracts at different doses showed a marked reduction in paw size. Increase in paw size is an index applied in the measurement of the anti-arthritic activity of different drugs administered in the management of inflammatory conduction [20]. It was employed in this study to access the activity of *Rauwolfia vomitoria*. Our result shows that there is a link between the level of inflammation and body weight loss. Paw size is a physical sign of inflammation in early as well as the chronic phase of the disease and are used as indicators of inflammation in animal studies [28]. The characteristics of arthritis progression include an increase in paw foot pad and tibio tarsal joint diameters after 8 days, which can be due to the delayed immunological upsurge in the disease [30]. The process of inflammation reaction increased over time with arthritis associated swelling appearing and remaining constant by the end of 1 week. T-cell proliferation especially the Th-1 helper cells is an important mechanism of adjuvant diseases [31]. In addition, influx of inflammatory cells in rats causes the development of a chronic swelling in many joints, loss of joint cartilage, destruction of bone and remodeling in adjuvant induced arthritis model. The loss of joint integrity and function in the affected animals is the result of these inflammatory changes. The increase in swelling of the hind paw decreased significantly ($P < 0.05$) in the days that followed after arthritis induction when compared to the induced but not treated group (negative control). It can therefore be said that the likely mechanism for the reduction in paw size may be as a result of indomethacin and *Rauwolfia vomitoria* aqueous and ethanol root extracts treatment suppressive effect which may be linked to the action of the phytochemical components present in the extracts. Some alkaloids suppress antigen presentation and immune cells release while terpenoids regulate important cell to cell information transfer pathways that play a role in the course of inflammation for example, nuclear transcription factor-kappaB (NF-kappaB) [32].

The C-reactive protein result is presented in Table 4. The C-reactive protein (C-RP) level in serum samples were found to be significantly ($P < 0.05$) higher in all arthritic rats than the normal control rat group. The rate of disease progression determines the CRP levels. In response to inflammatory condition, IL-6 action triggers the release of CRP by the liver and also enhances the presentation of antigens by inflammatory cells like the monocyte. The rise in serum C-RP levels was sustained on days 18, 25 and 32 in the positive control; however treatment with indomethacin, and *Rauwolfia vomitoria* aqueous and ethanol root extracts at 400, 600 and 800 mg/ kg bd wt caused a significant ($P < 0.05$) reversal of the C-reactive proteins to levels close to that found in the normal control rats. This effect could be attributed to the presence of active principles found in *Rauwolfia vomitoria* including flavonoides and phenols. These phytochemicals are known for their anti-inflammatory and anti-oxidant properties. They prevent disease progression by inhibiting synthesis of inflammatory mediators in rats. Our result is in agreement with Patel, S. S. and Shah, P. V (2013), [29], who reported of anti-inflammatory and immunosuppressive effect of their herbal drug, whose effects was linked to the action of active principles (antioxidants) such as flavonoids and phenols which are also found in our extracts.

The activation of B-cells through toll-like receptors and other genetic predisposition factors lead to rheumatoid factor generation in arthritis. Rheumatoid factor (RF) is an antibody directed to determinants in the Fc portion of immunoglobulin G molecule. It serves as a marker of rheumatoid arthritis. There is an increase in RF in adjuvant diseases study of rheumatoid arthritis [30]. In this study, the arthritic rats had significantly ($P < 0.05$) elevated amount of rheumatoid factor compared to negative control (normal control) group. However, treatment with varied doses of *Rauwolfia vomitoria* extracts and indomethacin reduced ($P < 0.05$) the amount of RF and decreased auto-immune stimulation in the arthritic treated rats (Table 5). The effect of both the extract was time and dose dependent. On day 32, the plant extracts were able to significantly ($P < 0.05$) reversed the RF level close to normal while the level in the untreated arthritic rats were still significantly higher. Our result showed that the anti-inflammatory effects of *Rauwolfia vomitoria* could as a result of the inhibition of B-cell action. Our result corresponds with that of Senthamil and Viji (2014), [31] who reported on the anti-arthritic potential of their plant extracts; *Cardiosperm halicacabum* (L) and *Cissus vitiginia* on Freund's complete adjuvant induced arthritis. They reported that induction of arthritis caused an upsurge in RF levels which reversed on treatment with their plant extracts. They attributed this to the action of flavonoids, tannins, saponins and other phytochemical constituents present in their plants.

The result of Erythrocyte sedimentation rate (ESR) shows the suspension stability of red blood cells in plasma, in relation to red blood cell count, size and to the relative concentration of plasma proteins with emphasis on fibrinogen



and the α and β globulins. ESR is an indicator of the chronic state of inflammation in a disease condition [32] There was significantly ($P < 0.05$) higher ESR level in the arthritic groups. Certain factors such as increase in tissue damage, inflammation and infection that has become chronic probably played important roles in erythrocyte aggregation. It has been reported that rheumatoid factor, along with raised CRP level are involved in the induction of enhanced aggregation of red blood cells in rheumatoid arthritic patients [32]. Ramesh *et al.*, (2015), [33], reported an initial upsurge in the level of ESR in adjuvant induced arthritis which was reversed on treatment with their herbal formulation that exerted an anti-arthritic activity by significantly halting the pathological pathway of disease onset in complete Freund's adjuvant induced arthritis in the rats. This effect was also observed on administration of our plant extracts.

Treatment of the arthritic rats with the plant extracts however caused time-dependent reduction in ESR level (Table 6), with effect being more pronounced on day 32 of the study. Treatment with 600 and 800 mg/kg b.w ethanol extracts showed a greater effects ($P < 0.05$) than aqueous plant extract dose treated groups. A heightened disease processes is indicated by increases in ESR. In this study, the decrease in ESR denotes the anti-arthritic activity of the plant extracts and the standard drug.

The results of cytokine levels are presented in Tables 7-9. The result shows the levels of serum cytokines measured in the arthritic and non arthritic samples. In comparison to the normal control group, the arthritic rat groups demonstrated a significantly ($P < 0.05$) higher in TNF- α , IL-1 β and IL-6 levels in contrast to the normal control group. TNF- α increases proliferation and cytokine (IL-1 β and IL-6) productions in infiltrating cells. IL-6 was also elevated in rheumatoid arthritic rats plays a vital function in inflammation of RA by activating the synthesis of acute phase proteins like C-reactive protein, fibrinogen and serum amyloid. The elevated level of IL-6 is correlated with clinical variables like duration of morning joint stiffness and number of joints affected and laboratory variables such as erythrocyte sedimentation rate, C-reactive protein and rheumatoid factor titer. It switches on the CRP- gene expression leading to production of CRP [34].

Cytokine concentration decreased ($P < 0.05$) following the administration of plant extracts to arthritic rats to a level comparable to the normal control group. Arthritic untreated rats continued to increase significantly ($P < 0.05$) till day 32 when the study was terminated. Our result is in agreement with the findings of Hend *et al.*, (2014) [35]. They reported a significant increase in the levels of IL-1 β , IL-6 and TNF- α on induction of arthritis using adjuvant on mice. On treatment with their lemon fruit peel and lemon leaf extract, the cytokine level decreased significantly. Gonzalez-Gay *et al.*, (2005), [36], also observed that rheumatoid arthritis is caused by a number of inflammatory molecules released by macrophages and fibroblasts. Such molecules include IL-1 β , IL-6 and TNF- α , prostaglandins and reactive oxygen species [36]. Hend *et al.*, (2014), [35], also reported that attributes of arthritic joints is the persistence of pro inflammatory cytokines produced by the inflamed synovium as well as by chondrocytes in the arthritic joints.

In conclusion, the study has provided evidence of the anti-arthritic potentials of root extracts (ethanol and aqueous) of *Rauwolfia vomitoria* by reducing the arthritic markers such as C-RP, ESR, RF and cytokine levels (TNF- α , IL-1 β and IL-6) in adjuvant induced arthritic rats. The effect was time and concentration dependent. The observed effects are attributed to the presence of some active principles of *Rauwolfia vomitoria* such as flavonoides and phenols which have anti-inflammatory and anti-oxidant properties.

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