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Actions of Aqueous and Ethanol Root Extracts of *Rauwolfia Vomitoria* on Tocopherol, Adenine Deaminase and Antioxidant Indices in Complete Freund's Adjuvant-Chicken Type II Collagen Induced Arthritic Rats

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Abstract

The study examined the impact of *Rauwolfia vomitoria* Oliv. ethanol and aqueous root extracts on tocopherol, adenine deaminase, and antioxidant parameters in arthritic albino rats. A complete set of 135 albino rats were used in this investigation. Nine groups, each including fifteen rats, were formed from the rats. The albino rats' left hind foot was intradermally administered with 0.1 millilitres of Chicken type 11 collagen-Complete Freund's adjuvant to trigger arthritis. On the 10th day of arthritis induction, administration with the extract at dosages of 400, 600, and 800 mg/ kg body weight began. The 32-day research was conducted, and the spectrophotometric technique was used to obtain the results. Our findings demonstrated that the RA rats had considerably higher ($P<0.05$) concentrations of malondialdehyde (MDA) and nitric oxide (NO) than the normal control rats, but considerably decreased ($P<0.05$) levels of glutathione peroxidase, catalase, superoxide dismutase (SOD), reduced glutathione, and tocopherol. Nevertheless, treatment of RA rats with the extracts caused a reversal of the observed effects. The extracts' ability to relieve the arthritic rats' symptoms depended on both time and dosage. The conventional medicine (indomethacine) and the root extracts have considerably ($P<0.05$) equivalent anti-arthritic abilities. The findings demonstrated that the ethanol and aqueous root extracts of Rauwofia vomitoria possess components that may have improved the arthritic rats' antioxidant levels, reduced oxidative stress, and reversed the arthritic conditions brought on by adjuvant-induced arthritic rats. Therefore, the current study offers proof from science that the ethanol and aqueous root extracts of *Rauwolfia vomitoria* include compounds that may be used to treat arthritis.

Keywords: *Rauwolfia vomitoria*; Tocopherol; Adenine deaminase; Antioxidant potentials; Nitric oxide and Arthritis

Introduction

Rheumatoid arthritis (RA) is an autoimmune disorder with an inflammatory component. Deformations of the bones and cartilage, joint discomfort, oedema, and stiffness in the joint of fingers, wrist, as well as feet are among the symptoms linked to this illness (Guo et al.). Treatment of RA requires medications such as leflunomide, hydroxychloroquine, methotrexate, and sulfasalazine have been utilized (Ben Mrid et al.). The latter category includes disease-modifying anti-rheumatic medicines (DEMARDS), however, these medications also have common side effects, such gastrointestinal tract issues, appetite loss, mouth soreness, feeling unwell, diarrhoea, migraines, and hair loss (Ben Mrid et al.; Bullock et al.). Novel treatments for rheumatoid arthritis have also been used, including biologics like certolizumab, adalimumab, and etanercept (Bullock et al.). They also have deleterious effects, like minor skin responses where injections are made, infections, nausea, fever, and headaches (Bullock et al.). Due to the difficulty low-income people have affording these medications, a lot of RA sufferers turn to employing plant herbs that grow nearby to control or treat their conditions. Herbal medicine has shown great promise in treating conditions such as rheumatoid arthritis, and as a result, it is now widely accepted in modern culture. Rural residents have had success treating their arthritis with the *Rauwolfia vomitoria* plan.

The respiration process may be harmful since it uses oxygen molecules to form reactive oxygen species, even though it is necessary for the production of energy in living cells. Oxidative stress is brought on by UV light, pollution, radiation, and metal oxidation which have been traced to a number of illnesses, including inflammatory disorders, cancer, atherosclerosis, pulmonary fibrosis, and ageing (Jomova et al.). Key transcription factors regulated by cell oxygenation and cytokine stimulation, and nuclear factor-κB are induced in the pathogenesis of RA by synovial perfusion, hypoxia, and re-oxygenation (Quiñonez-Flores et al.). These mechanisms result in gene expression, which is essential for rheumatoid arthritis-related chronic synovitis.

Herbal remedies have been widely recognized and embraced by researchers for their effective treatment, control, and prevention of diseases (Liheluka et al.). Plant-based chemicals, including their phytochemical components, minerals, vitamins, and other elements, influence the body physiologically and have essential therapeutic effects (Liheluka et al.). In broad terms, plants are used to treat and control diseases because of their low cost, therapeutic efficacy, minimal negative side effects, accessibility, potential for cross-cultural interaction, and economic significance (Isola). *Rauwolfia vomitoria* roots, native to Ebonyi State in Nigeria, have been historically used to treat various illnesses, including arthritic conditions, but experimental evidence on their effectiveness remains inconclusive. This led to the aim of this study: to ascertain the impact of *Rauwolfia vomitoria* aqueous and ethanolic root extractson antioxidant potentials, tocopherol, and adenine deaminase levels in complete freund's adjuvant-chicken type II collagen induced arthritic rats.

Materials and Methods

Materials

Plant Materials

Rauwolfia vomitoria (RV) root was acquired from Ndinwali axis in Izzi of Ebonyi State in Nigeria. Professor Kate Nnamani, a botanist at Ebonyi State University in Abakaliki's Department of Applied Biological Sciences, verified the authenticity of the plant. A portion of the root samples were kept at the Department of Applied Biological Science's herbarium.

Animals

The total number of 135 female albino rats were used. Animals were bought at the University of Nigeria's Department of Animal Science in Nsukka, Enugu State, Nigeria. The animals were allowed unrestricted access to food and water during their 14-day acclimatization phase.

Methods

Plant extract preparations

Contaminants in the plant samples were washed off under flowing tap and dried by air under a shade. The laboratory milling machine was used to grind the plant roots and were filtered through 0.25 mm mesh. Eight hundred grams of RV dried powdered root samples were soaked in 2000 ml of alcohol and distilled water respectively for 48 hours. They were subjected to successive extraction by the use of a water bath at 500 C until the solvents were completely removed; the percentage yield was obtained and extracts used for analysis.

Arthritis initiation in albino rats

Rats were induced arthritis using the technique outlined by Aloke et al. (2021) by injecting 0.1 ml of chicken type II collagen-complete Freund's adjuvant (CFA) into the rats' left hind paw. The paw circumference of the rat groups was measured twice weekly before and after adjuvant administration, and paw inflammation severity was assessed using a qualitative scoring system. By day 10, arthritis had fully developed. Rats with no visible oedema scored 0, mild redness and individual digit inflammation was scored 1, moderate redness and ankle swelling was scored 2, and severe redness and paw inflammation was scored 3. Rats scoring three were deemed to have arthritis and were employed in the experiments that followed.

Treatment of Rheumatoid Arthritic Rats with Root Extracts

In all, 135 female rats weighing 150-210 g were used in this study. Nine groups of 15 female albino rats each were formed from the distribution of the rats. Normal saline was used to make a solution of the standard drug, indomethacin (standard control). Group I: 5 ml/kg of normal saline was given daily. Group 2: (5 ml/kg normal saline daily): this group (also known as the positive control or arthritis control) was induced with arthritis. Group 3 (the standard control) received an indomethacine dose of 10 mg/kg after being provoked with arthritis. From the tenth day following induction to the end of the trial, groups 4, 5, and 6 were induced with arthritis and administered dosages of 400, 600, and 800 mg/kg body weight, respectively, of RV aqueous root extract. From the tenth day following induction to the last day of the experiment, groups 7, 8, and 9 were given RV ethanol root extract at doses of 400, 600, and 800 mg/kg body weight.

Processing and Analysis of Blood samples

At days 10, 18, 25, and 32, three albino rats from every group (Groups 1-9) were sacrificed and blood samples were taken in bottles bearing EDTA anticoagulant. The serum samples were used for the assays of oxidative stress indices which include malondiadehyde (MDA) (Buege and Aust), Nitric Oxide (NO) (Choy and Panayi), Super Oxide Dismutase (SOD) (McCord and Fridovich), Catalase (Sinha), Reduced glutathione (GSH) (Ellman), Glutathione peroxidase (GLX) activity (Rotruck et al.), adenosine deaminase (Whittingham)and tocopherol (Desai).

Statistical Analysis

The basic statistics, averages, standard deviation, and ranges of the observed data were estimated using the Statistical Analysis System (SAS) windows version 9.0. Means ± SD of 12 replicates were used to express the data. When a value was $p<0.05$, it was deemed statistically significant.

Results and Discussion

Results

Table 1 shows the impact of ethanol and aqueous root extracts of RV on the MDA level in rats. The results indicate that the untreated RA rats had significant levels of MDA, which is a measure of the peroxidation of lipids caused by the generation of free radicals. MDA levels were observed to be lower ($P<0.05$) in rats treated with indomethacin, RV aqueous extract, and ethanol root extracts at different

dosages than in the group of rats without treatment for arthritis (positive control). On day 32 of the therapy, the impact was statistically significant (P<0.05). The regular medication and the plant extracts used for therapy had similar results.

Table 2 displays the NO generation outcomes. Rats with arthritis had an elevated NO level (P<0.05) than the negative control group. When standard drugs and plant extracts were given to the arthritic rats, the level was nearly restored to that of the control group. In rats treated with arthritis, it was shown that 800 mg/kg of aqueous root extract from RV was notably (P<0.05) more effective than ethanol extract treatments, particularly on day 32 when it came to returning the NO level to normal.

Tables 3-6 provide the results of the activities of glutathione peroxidase, SOD, GSH, and catalase. In the present study, the arthritic rat groups had a decreased level (P<0.05) of SOD, GSH, glutathione peroxidase, and catalase activities than the normal control group. The study found that administering varying dosages of indomethacin and RV aqueous and ethanol root-extract considerably normalized the levels of SOD, GSH, glutathione peroxidase, and catalase in arthritic rats in a time-dependent manner. Day 32 of the therapy was when the most significant effect was observed. The GSH activity of the rats treated with arthritis was shown to be reversed more effectively (P<0.05) by 600 mg/kg body weight *Rauwolfia vomitoria* aqueous root extract than by the standard drug.

Table 7 presents the findings of the root extracts' impact on adenine deaminase. All of the arthritic rats had notably higher levels of adenine deaminase (P<0.05). However, the use of plant extracts on the arthritic rats resulted in a substantial (P<0.05) decrease in adenine deaminase levels, attaining that of the normal control group by day 32 of the experiment.

Table 8 presents the tocopherol level result. When compared to arthritic rats treated with plant extracts, the tocopherol level in the positive control group (induced but not treated) animals decreased considerably (P<0.05) on days 25 and 32. reversing the tocopherol level to near normal was more effective when 800 mg/kg body weight of RV aqueous aqueous and ethanol root extracts were administered as a treatment.

Tables

Groups	***DAY 10 (nmol/g protein)***	***DAY 18 (nmol/g protein)***	***DAY 25 (nmol/g protein)***	***DAY 32 (nmol/g protein)***
1	3.21 ± 0.17^{b}	3.20 ± 0.08^{d}	3.19 ± 0.01^{f}	3.32 ± 0.23^{b}
2	3.85 ± 0.05^{a}	4.03 ± 0.03^{a}	5.30 ± 0.01^{a}	6.46 ± 0.08^{a}
3	3.74 ± 0.03^{a}	3.65 ± 0.12^{b}	3.61 ± 0.03^{b}	3.43 ± 0.18^{b}
4	3.77 ± 0.44^{a}	$3.57\pm0.37^{c,b}$	$3.44\pm0.01^{d,c}$	3.34 ± 0.76^{b}
5	3.78 ± 0.77^{a}	$3.54\pm0.50^{c,b}$	$3.43\pm0.08^{d,c}$	3.31 ± 0.84^{b}
6	$3.72\pm0.83^{b,a}$	$3.52\pm0.18^{c,b}$	$3.38\pm0.10^{d,e}$	3.35 ± 0.89^{b}
7	3.75 ± 0.01^{a}	$3.55\pm0.02^{c,b}$	3.49 ± 0.08^{c}	3.32 ± 0.18^{b}
8	$3.73\pm0.01^{b,a}$	$3.51\pm0.08^{c,bc,b}$	$3.41\pm0.05^{d,c}$	3.33 ± 0.00^{b}
9	3.74 ± 0.04^{a}	$3.49\pm0.07^{c,b}$	$3.37\pm0.06^{d,e}$	3.32 ± 0.01^{b}

Table 1: Impact of ethanol and aqueous root-extracts of RV on the concentration of MDA in rats given an adjuvant-induced arthritis.

Rats with adjuvant-induced arthritis that were given ethanol and aqueous root extracts of RV had elevated MDA levels. The overall mean ± SD (n = 9) as well as the significant difference at P<0.05 are displayed for the data. RV stands for *Rauwolfia vomitoria*. 1 is standard control, 2 is positive control, and 3 is negative control. The means with the same letter do not differ significantly: 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, and 9 = 800 mg/kg RV ethanol extract.

Groups	DAY 10 (nmol/ml)	DAY 18 (nmol/ml)	DAY 25 (nmol/ml)	DAY 32 (nmol/ml)
1	13.30±0.08^{c}	13.40±0.28^{g}	13.60±0.02^{h}	13.80±0.84^{l,h}
2	23.80±2.04^{a}	25.70±0.11^{a}	32.30±0.47^{a}	45.60±0.76^{a}
3	20.60±0.76^{b}	19.70±1.64^{e,d}	16.80±0.00^{g,f,e}	14.50±0.62^{h,g,f}
4	21.90±1.36^{ba}	20.80±0.03^{c,b,d}	18.20±1.83^{d,c}	16.89±0.16^{c}
5	21.10±0.00^{b}	20.60±0.57^{c,b,d}	17.80±0.35^{d,c,e}	15.80±0.05^{d}
6	20.90±0.25^{b}	18.40±1.03^{f}	16.80±0.72^{g,f,e}	14.70±0.16^{e,h,g,f}
7	21.80±1.56^{b,a}	21.20±0.09^{c,b}	19.60±1.77^{b}	18.70±0.07^{b}
8	21.90±0.01^{b,a}	21.40±0.06^{b}	19.60±0.04^{b}	17.40±0.08^{c}
9	20.70±0.01^{b}	19.80±0.38^{e,d}	17.10±0.23^{f,e}	14.80±0.08^{e,g,f}

Table 2: Impact of root-extracts from RV on the level of NO in rats with adjuvant-induced arthritis.

Adjuvant-induced arthritic rats treated with ethanol and aqueous root extracts from RV showed Nitric oxide level of RA. The mean ± SD (n = 9) and significant difference at P<0.05 are displayed for the data. RV stands for *Rauwolfia vomitoria*. 1 is standard control, 2 is positive control, and 3 is negative control. The following are the values of the RV aqueous extract: 4 = 400 mg/kg, 5 = 600 mg/kg, 6 = 800 mg/kg, 7 = 400 mg/kg, 8 = 600 mg/kg, and 9 = 800 mg/kg. The means that have the same letter do not differ much.

Groups	DAY 10 (U/L)	DAY 18 (U/L)	DAY 25 (U/L)	DAY 32 (U/L)
1	66.00±1.41^{a}	65.15±0.02^{a}	65.24±0.59^{a}	65.00±1.51^{a}
2	41.10±0.71^{f,d,e}	41.26±0.02^{g}	36.29±1.70^{f}	36.05±0.17^{e}
3	45.35±13.79^{c,d,e}	43.10±0..00^{e,g,f}	53.96±0.21^{b}	54.38±0.76^{b,a,c}
4	37.95±0.64^{f}	42.77±0.00^{g,f}	43.45±0.75^{e}	47.62±1.15^{d}
5	43.94±1.67^{c,f,d,e}	45.49±0.01^{e,d}	48.49±0.85^{c,d}	51.54±0.93^{d,c}
6	45.17±0.258^{c,d,e}	49.47±0.02^{c,b}	50.55±0.61^{c,b}	53.42±0.81^{b,d,a,c}
7	38.97±0.47^{f,e}	43.15±0.61^{e,g,f}	44.62±0.27^{e,d}	48.67±1.22^{d,c}
8	44.68±1.18^{c,d,e}	45.66±0.10^{e,d}	48.98±0.24^{c}	52.35±0.35^{d,c}
9	45.50±0.71^{c,d}	49.53±0.89^{c,b}	50.80±0.13^{c,b}	53.89±0.40^{b,a,c}

Table 3: The impact of root extracts (ethanol and aqueous) of RV on the peroxidase activity in rats with adjuvant-induced arthritis.

Rats with induced arthritis treated with RV ethanol and aqueous root extracts showed increased peroxidase activity. The information is displayed as mean ± SD (n = 9) with a significant difference at P<0.05. As in, *Rauwolfia vomitoria* = RV. 1, 2, and 3 stand for negative, positive, and standard control, respectively. 400 mg/kg RV aqueous extract, 600 mg/kg RV aqueous extract, 800 mg/kg RV aqueous extract, 400 mg/kg RV ethanol extract, 600 mg/kg RV ethanol extract, and 800 mg/kg RV ethanol extract are the values obtained from the preceding equations. There is no discernible difference between means with the same letter.

Treatments	*DAY 10 (U/ mg protein)*	*DAY 18 (U/ mg protein)*	*DAY 25 (U/ mg protein)*	*DAY 32 (U/ mg protein)*
1	78.91±5.02^{a}	78.96±6.36^{a}	78.88±5.07^{a}	78.67±2.20^{a}
2	40.15±2.04^{e}	42.25±0.78^{f}	33.73±0.58^{e}	31.88±0.96^{e}
3	74.25±2.57^{b,a}	75.15±2.42^{b,a}	77.72±0.28^{a}	77.75±0.15^{b,a}
4	51.02±5.13^{d}	57.41±2.81^{e}	60.50±0.42^{d,c}	60.81±0.01^{d}
5	68.42±0.01^{b}	69.85±0.49^{d}	72.99±1.20^{b,a}	75.54±0.62^{c,b,a}
6	73.73±0.96^{b,a}	73.36±0.65^{c,b}	73.77±0.6^{b,a}	74.17±1.10^{c}
7	51.96±5.20^{d}	60.06±1.36^{e,d}	60.81±7.90^{d,c}	61.50±0.03^{d}
8	71.83±1.70^{b}	72.73±1.59^{c,b}	74.83±0.07^{b,a}	75.10±0.17^{c,b}
9	74.61±1.40^{b,a}	74.34±0.76^{c,b}	75.04±0.11^{b,a}	75.40±0.14^{c,b}

Table 4: Impact of root extracts from Rauwolfia vomitoria (RV) on the SOD activity in rats given an adjuvant-induced arthritis.

Rats with adjuvant-induced arthritis that were given ethanol and aqueous root extracts from RV revealed super oxide dismutase activities. The mean ± SD (n =9) and significant difference at P<0.05 are displayed for the data. RV stands for *Rauwolfia vomitoria*. 1 is standard control, 2 is positive control, and 3 is negative control. The following are the values of the RV aqueous extract: 4 = 400 mg/kg, 5 = 600 mg/kg, 6 = 800 mg/kg, 7 = 400 mg/kg, 8 = 600 mg/kg, and 9 = 800 mg/kg. The means that have the same letter do not differ substantially.

Groups	*DAY 10 (U/ mg protein)*	*DAY 18 (U/ mg protein)*	*DAY 25 (U/ mg protein)*	*DAY 32 (U/ mg protein)*
1	1.94±0.03^{a}	1.84±0.01^{a}	1.87±0.09^{a}	1.91±0.07^{a}
2	1.07±0.05^{d}	0.88±0.01^{e}	0.57±0.00^{g}	0.42±0.01^{f}
3	1.18±0.04^{cb}	1.31±0.05^{b,d}	1.79±0.01^{b}	1.88±0.00^{ba}
4	1.09±0.01^{b}	1.20±0.00^{d,e}	1.46±0.00^{e}	1.58±0.01^{e,d}
5	1.11±0.02^{c}	1.32±0.01^{b,d,c}	1.49±0.01^{e}	1.68±0.20^{e,b,d,c}
6	1.09±0.01^{c}	1.50±0.04^{b,d,a,c}	1.63±0.01^{d,c}	1.72±0.01^{e,b,d,a,c}
7	1.10±0.04^{c}	1.70±0.03^{b,a,c}	1.76±0.00^{b}	1.85±0.01^{b,a,c}
8	1.17±0.02^{b}	1.21±0.01^{d,e}	1.37±0.01^{f}	1.51±0.04^{e}
9	1.07±0.01^{c}	1.27±0.01^{d}	1.47±0.01^{e}	1.62±0.03^{e,d}

Table 5: Aqueous and ethanol root extracts of RV and their impact on the catalase activities in adjuvant-induced arthritic rats.

Adjuvant-induced arthritic rats given ethanol and aqueous root extracts from RV demonstrated increased catalase activity. RV is *Rauwolfia vomitoria*, mean ± SD (n = 9), and significant difference at P<0.05 are displayed for the data. 1 is standard control, 2 is positive control, and 3 is negative control. There are four different amounts of RV aqueous extract: 400 mg/kg, 600 mg/kg, and 800 mg/kg. There are also twelve different amounts of RV ethanol extract: 10 mg/kg, 600 mg/kg, and 800 mg/kg. The means that have the same letter do not differ substantially.

Groups	DAY 10 (Umol/l)	DAY 18 (Umol/l)	DAY 25 (Umol/l)	DAY 32 (Umol/l)
1	26.44±2.37^{a}	26.82±0.59^{a}	26.75±0.02^{a}	25.98±0.08^{b}
2	22.88±0.71^{b}	19.18±0.62^{f}	16.35±0.18^{f}	10.17±1.43^{f}
3	18.56±0.86^{c}	21.98±0.39^{d,c,e}	22.03±0.41^{e}	22.38±0.76^{e}
4	18.38±0.66^{c}	18.52±0.44^{g}	22.82±0.02^{d}	25.92±1.40^{b,a}
5	17.21±2.33^{c}	21.73±0.58^{e}	23.87±0.18^{c}	29.92±0.39^{a}
6	18.24±1.54^{c}	22.18±0.57^{d,c}	23.94±0.16^{c,b}	25.03±0.95^{b,a,c}
9	23.80±0.03^{b}	23.95±0.69^{b}	24.18±0.47^{c,b}	26.01±1.11^{b}
7	18.38±0.69^{c}	18.53±0.42^{g}	22.83±0.00^{d}	25.91±1.42^{b,a}
8	17.21±2.34^{c}	21.78±0.49^{d,e}	22.89±1.22^{d}	24.87±1.60^{b,c}
9	18.24±1.53^{c}	22.22±0.59^{c}	23.94±0.14^{c,b}	23.23±1.61^{e,d}

Table 6: Impact of ethanol and aqueous root-extracts from RV on the GSH concentration in rats given an adjuvant-induced arthritis.

Adjuvant-induced arthritic rats given ethanol and aqueous root extracts from RV demonstrated increased GSH activity. The mean ± SD (n = 9) and significant difference at P<0.05 are displayed for the data. RV stands for *Rauwolfia vomitoria*. 1 is standard control, 2 is positive control, and 3 is negative control. The means with the same letter do not differ significantly: 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, and 9 = 800 mg/kg RV ethanol extract.

Groups	DAY 10 (mg/dl)	DAY 18 (mg/dl)	DAY 25 (mg/dl)	DAY 32 (mg/dl)
1	0.52±0.00^{h}	0.52±0.01^{i}	0.52±0.01^{g}	0.52±0.00^{d,e}
2	0.79±0.00^{a}	0.78±0.01^{a}	0.85±0.01^{a}	0.89±0.01^{a}
3	0.75±0.01^{e,d,c}	0.63±0.00^{h}	0.52±0.01^{g}	0.50±0.01^{g}
4	0.755±0.01^{b,d,c}	0.64±0.00^{f}	0.58±0.00^{b}	0.53±0.00^{c}
5	0.75±0.01*^{,d,c}	0.63±0.00^{h}	0.55±0.00^{d}	0.52±0.01^{d,c}
6	0.74±0.01^{e,d}	0.63±0.01^{g}	0.53±0.01^{f,e}	0.51±0.01^{f,e}
7	0.72±0.02^{g,f}	0.63±0.00f	0.53±0.00^{f,g}	0.52±0.00^{d,c}
8	0.74±0.01^{e,d,c}	0.63±0.00h	0.52±0.00^{g}	0.52±0.00^{d,c}
9	0.74±0.01^{e,d}	0.65±0.00c	0.52±0.00^{g}	0.51±0.01^{f,e}

Table 7: Impact of ethanol and aqueous root-extract of RV on the adenine deaminase level in rats given an adjuvant-induced arthritic stimulus.

Adjuvant-induced arthritic rats given ethanol and water root extracts from RV demonstrated increased adenine deaminase activity. The mean ± SD (n = 9) and significant difference at P<0.05 are displayed for the data. RV stands for *Rauwolfia vomitoria*. 1 is standard control, 2 is positive control, and 3 is negative control. The means with the same letter do not differ significantly: 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, and 9 = 800 mg/kg RV ethanol extract.

Treatments	DAY 10 (mg/dl)	DAY 18 (mg/dl)	DAY 25 (mg/100g)	DAY 32 (mg/100g)
1	0.93±0.04^{a}	0.95±0.06^{a}	0.94±0.07^{a}	0.98±0.03^{a}
2	0.78±0.05^{f,c,e,d}	0.68±0.10^{d}	0.61±0.01^{c}	0.55±0.09^{e}
3	0.86±0.01^{b}	0.90±0.08^{b,a}	0.93±0.00^{a}	0.96±0.01^{b,a,c}
4	0.77±0.00^{f,e}	0.82±0.00^{c}	0.84±0.00^{b}	0.87±0.10^{d}
5	0.77±0.08^{f,e,d}	0.81±0.01^{c}	0.85±0.00^{b}	0.89±0.01^{d}
6	0.81±0.01^{f,c,e,b,d}	0.82±0.09^{c}	0.84±0.06^{b}	0.88±0.06^{d}
7	0.75±0.01^{f}	0.83±0.00^{c}	0.84±0.00^{b}	0.86±0.08^{d}
8	0.77±0.07^{f,e}	0.81±0.09^{c}	0.87±0.00^{b}	0.88±0.01^{d}
9	0.81±0.01^{f,c,e,b,d}	0.82±0.10^{c}	0.86±0.06^{b}	0.88±0.07^{d}

Table 8: The impact of ethanol and aqueous root-extract of RV on the tocopherol levels in rats with adjuvant-induced arthritis.

Rats with adjuvant-induced arthritis that were given ethanol and aqueous root extracts from RV had a high tocopherol concentration. The mean ± SD (n = 9) and significant difference at P<0.05 are displayed for the data. RV stands for *Rauwolfia vomitoria*. 1 is standard control, 2 is positive control, and 3 is negative control. The means with the same letter do not differ significantly: 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, and 9 = 800 mg/kg RV ethanol extract.

Discussion

Rats group that was induced RA without treatment had elevated level of MDA, according to the findings of this study (Table 1). Likewise, the RA rats had extremely elevated nitric oxide (NO) levels. In the groups comprising those rats with untreated RA, MDA levels steadily increased until day 32nd day. But, treatment indomethacin (standard RA drug) and the *Rauwolfia vomitoria* root-extract in ethanol and aqueous solutions decreased (P<0.05) the MDA level. The effects root-extract on the MDA level were relative to those that received standard drug. Although, nitric oxide is a crucial chemical mediator produced by nerve cells, macrophages, and endothelial cells; they are involved in the regulation of several physiological processes, the body suffer when it is released uncontrollably into the cells (Andrabi et al.). The arthritic rats' NO levels were considerably (P<0.05) lowered (P<0.05) when indomethacin, as well as the aqueous and ethanol root-extract of *RV*, were administered. Numerous medical illnesses are primarily caused by oxidative stress and the damage it causes(Kondo et al.). The adjuvant used to induce RA in this study was responsible for increasing the amount of reactive oxygen species released but, when the root extracts were administered to the treatment group rats, the effect was reversed because, the root extracts contain bioactive compounds which break down peroxides, singlet and triplet oxygen by absorbing and neutralizing free radicals like MDA and NO(Andrabi et al.; Kondo et al.).

Tables 3-6 show the outcome of antioxidant (GSH, SOD, catalase, and glutathione peroxidase) concentrations. Cells and tissue structures are shielded from oxidative damage and free radicals by antioxidants like GSH, SOD, catalase, and glutathione peroxidase. They also help to regulate immunological function and detoxify xenobiotics, peroxides, and free radicals. In this study, the arthritic rat groups had lower levels (P<0.05) of SOD, GSH, and catalase activities than the normal control group which align with the study of (Ogbu et al.). Administration of indomethacin and RV aqueous and ethanol root-extract at varying dosages to the arthritic rats normalised (P<0.05) the activities of SOD, GSH, and catalase. The highest activities of these enzymes were observed on day 32 of the treatment. The standard drug was apparently less efficacious (P<0.05) than 600 mg/kg body weight RV aqueous root extract in restoring GSH activity.

Catalase activity was notably (P<0.05) boosted by RV root-extract, indicating increased hydrogen peroxide breakdown to water and oxides. The oxygen radical (O2-) steady state level is decreased by superoxide dismutase. In order to shield cells and tissues from superoxide radicals and other peroxides such lipid peroxides, superoxide dismutase enzyme acts as a catalyst for the dismutation of superoxide radicals into peroxides and molecular oxygen (Zheng et al.). It is possible the decreased level of superoxide dismutase

enzyme activity was caused by increased superoxide anion generated after induction of RA (Zheng et al.). However, on administration of ethanol and aqueous root extracts of RV there was a significant ($P<0.05$) increase in the activity of glutathione peroxidase, which reduces peroxides to water and concurrently oxidises glutathione (GSH) to glutathione disulphide (GSSG), which is an antioxidant (Bisong et al.). The presence of certain antioxidant molecules in the root extracts of RV may have contributed to its anti-oxidative and radical scavenging properties by increasing the activity of enzymes such as glutathione peroxidase, catalase, and superoxide dismutase (SOD) as shown in Tables 3-6.

The level of GSH was observed to be increased in the RA induced rat groups receiving RV root-extract. Functionally, GSH is a cofactor for detoxifying enzymes such as GPx, which help to regenerate oxidized versions of antioxidant vitamins C and E while preventing lipid oxidative damage by lowering lipid peroxides(Lushchak). Our findings in this study demonstrate that an imbalance between oxidants and the antioxidant system was caused by type II collagen-induced CFA in RA. The administration of root extracts of RV reversed this imbalance to a nearly normal level, presumably because the root extract contains vital antioxidants. According to (Hussen and Endalew), the treatment of RV aqueous root-extract restored the considerable suppression of serum DPPH radical scavenging activity and the decrease in total antioxidant power by CCl4 and the results of our study are consistent with their findings.

All of the arthritic rats had increased levels of adenine deaminase ($P<0.05$) (Table 7). The enzyme known as adenosine deaminase (ADA) releases ammonia during the conversion of adenosine to deoxyadenosine and inosine to deoxyinosine. Adenosine deaminase levels in the blood are elevated, which suggests that cellular immunity is being stimulated. This has been noted in cases of rheumatoid arthritis, lymphoblastic leukaemia, acute hepatitis, and infection with the human immune deficiency virus. According to reports, the monocyte/macrophage cell system is one of the main producers of the common type of adenosine deaminase in blood (Haskó et al.; Tiwari-Heckler et al.). On day 32, the treatment with the root extracts had the most significant effect, lowering the adenine deaminase levels to those of the normal control group by a significant ($P<0.05$) margin.

Similarly, the tocopherol level in the arthritic rats in this study was notably ($P<0.05$) lower than the values observed in the normal control group (Table 8). Arthritis symptoms reported to have been successfully managed with the use of vitamin E in conjunction with other medications(Chin and Ima-Nirwana). Administering aqueous and ethanol root-extract of RV to the arthritic rats has shown to be equally beneficial as conventional indomethacin therapy, particularly when administered at a dose of 800 mg/kg body weight. Furthermore, reduced serum levels of α tocopherol, β carotene, and selenium have been documented by (Karlson et al.), before the diagnosis of rheumatoid arthritis, and low antioxidant values may be a risk factor or a marker for the illness (Costenbader et al.; Quiñonez-Flores et al.).

Conclusion

In conclusion, it is possible that the phytochemicals reported in our earlier study of RV ethanol and aqueous root extracts boosted the antioxidant level of the arthritic rats, reduced oxidative stress markers, and reversed the arthritic conditions that the adjuvant-induced arthritic rats had established. Therefore, the current study offers scientific proof that the ethanol and aqueous root-extract of RV could be used to treat RA.

Conflict of interest

The author has declared no conflict of interests.

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