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Anti-Arthritic and Antioxidant Efficacy of Ethanol and Aqueous Root Extracts of *Olax subscorpioidea* in CFA-Induced Arthritic Rats

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ABSTRACT

The traditional use of *Olax subscorpioidea* root extracts for arthritis management in rural areas, where NSAIDs and biologic drugs are unaffordable, lacks scientific validation. This study aimed to investigate the anti-arthritic and antioxidant properties of ethanol and aqueous root extracts of *Olax subscorpioidea Afzel* in albino rats with adjuvant-induced arthritis. A total of 135 albino rats were divided into nine groups of 15 rats each. Rheumatoid arthritis (RA) was induced in groups 2-9 through intradermal injection of 0.1 ml Chicken type II collagen-Complete Freund's adjuvant in the left hind paw. Starting from day 10 post-induction, the rats were treated with the extracts at doses of 400, 600, and 800 mg/kg body weight for 32 days. Sample analysis was conducted using spectrophotometric methods. Arthritic rats exhibited significantly elevated levels of nitric oxide (NO) and malondialdehyde (MDA) ($P<0.05$) and reduced levels of superoxide dismutase (SOD), catalase, glutathione peroxidase, reduced glutathione, and tocopherol compared to normal control rats. Treatment with *Olax subscorpioidea* extracts significantly reversed these effects in a time- and dose-dependent manner. The efficacy of the extracts was comparable to the standard drug indomethacin ($P<0.05$). This study provides scientific evidence that ethanol and aqueous root extracts of *Olax subscorpioidea* possess significant anti-arthritic and antioxidant activities. These extracts potentially enhance antioxidant levels, reduce oxidative stress, and mitigate arthritic conditions, supporting their traditional use and suggesting potential therapeutic applications in rheumatoid arthritis management.
Keywords: Anti-arthritic, Antioxidant, *Olax subscorpioidea*, Rheumatoid Arthritis, Oxidative Stress, Ethanol Extract, Aqueous Extract, Adjuvant-Induced Arthritis

INTRODUCTION

The aerobic respiration is a process effective in the generation of energy for life processes; however, it may be as well harmful. Although molecular oxygen is not harmful, it can be converted to reactive oxygen species (ROS) like superoxide, H_2O_2 and hydroxyl radicals. Autoxidation of electron transport carriers, ultraviolet light, pollutants, radiations and oxidation by metals can also generate free radicals [1]. Oxidative stress is linked to the pathogenesis of many diseases like cancer, inflammatory conditions, atherosclerosis, pulmonary fibrosis and aging [2]. Synovial perfusion, hypoxia and re-oxygenation and transformation in synovial perfusion in the pathogenesis of rheumatoid arthritis are taught to induce hypoxia-inducible factor-1α and nuclear factor-κB. These are two important key factors in transcription that are controlled by transformations in the oxygenation of cells and the stimulation of cytokines. These processes lead to gene expression which is central to Synovitis that is persistent as is seen in rheumatoid arthritis [3]. Rheumatoid arthritis is an autoimmune disease with an inflammatory component. Disease features associated with this disease include: bone and cartilage deformations, joint pain, joint oedema, and the stiffness of the joints of the hands, wrists, and feet [4].drugs such as Non- steroidal anti-inflammatory drug (NSAIDS) and disease modifying anti-rheumatic drugs (DEMARDS) such like methotrexate, hydroxychloroquine, sulfasalazine and leflunomide have been used in the management of rheumatoid arthritis. However, these drugs display common adverse effects such as disorders of the gastro intestinal tracks, loss of appetite, sore mouth, feeling of ill health, diarrhea, headaches and hair loss [5and 6]. Biologics such as etanercept, adalimumab, certolizumab have also been used as a new form of treatment for rheumatoid arthritis

[7]. They too are not without side effects which include mild skin reactions at the site of injections, infections, nausea, an increased body temperature and headaches [7]. These drugs are quite expensive as the low income earners have difficulty assessing these drugs. Hence many arthritic patients resort to using locally available plant herbs to manage or treat their ailments. The use of herbs in the management of diseases like rheumatoid arthritis produced many positive results which is the reason it is becoming the general practice in our society today. *Olax subscoripoidea* Afzel plant has been successfully used for the treatment of arthritis by the rural dwellers. Medicinal plants have continued to play a primary role in the cure, management and prevention of illnesses [8]. The application of traditional medicine in treatment and management of diseases is now better recognized and accepted from researchers in the Western world. [9]. Chemical constituents of plants such as their phytochemical components, minerals, vitamins and other principles exert physiological action on the system thereby exhibiting medicinal properties [9]. Generally, the use of plants in disease treatment and management is due to their low cost, medicinal value, minimal side effects, availability, cultural exchange and commercial value [10]. *Olax subscorpiodea* roots, among other herbs are used traditionally in Ebonyi State to manage and/or treat many ailments, especially arthritis**.** The efficacy of the the use of these plant roots has not yet been proved scientifically. This research was aimed at determining the effect of aqueous and ethanol root extracts of *Olax subscopioidea* on tocopherol, adenine deaminase and antioxidant potentials in complete freund's adjuvant-chicken type ii collagen induced arthritic albino rats.

MATERIALS AND METHODS

Materials

Plant Materials

Olax subscoipoidea root was sourced from Ndi-Nwali Village in Izzi Local Government Area of Ebonyi State in South-Eastern Nigeria. These plants were authenticated by Dr. (Mrs.) Kate Nnamani, a Botanist in the Department of Biological Sciences, Ebonyi State University, Abakaliki. Some of the root samples were reserved in the herbarium of the Department of Biological Science.

Animals

The total number of albino rats used was 135. These were bought from the department of Animal Science, University of Nigeria Nsukka, Enugu State, Nigeria. The animals were acclimatized for a period of 14 days and were giving access to food and water.

METHODS

Preparation of the plant extracts

Contaminants in the plant samples were washed off under flowing tap and air dried under a shade. The laboratory milling machine was used to grind the plant roots and were sifted using 0.25 mm sieve. Eight hundred gram each of *Olax subscorpioidea* dried powdered sample was soaked in 2000 ml of ethanol and deionized water for 48 hours. It was subjected to successive extraction by the use of a water bath at 50^{0} C until the solvents were completely removed; the percentage yield was obtained and extracts used for analysis.

Induction of arthritis in albino rats

The method of Person, C. M. (1956) was adopted in the induction of arthritis in rats by injecting of 0.1 ml of chicken type II collagen-complete Freund's adjuvant (CFA) into the left hind paw of the rats [11]. The paw size of the rat groups was taken twice weekly throughout the duration of the study before and after the administration of the adjuvant by the use of a calibrated venier caliper. The severity of paw inflammation was measured using a qualitative scoring system. It was observed that by day 10, arthritis had completely set in. 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 severe redness and inflammation of the entire paw with the digits inclusive. Those rats that had a score of 3 with elevated level of inflammatory biomarkers were considered to have arthritis and were used for subsequent experiments. The rats were weighed daily throughout the study duration.

Treatment of Arthritic rats with plant extracts.

Female rats totaling 135 and weighing between 150 to 210 g were used in this work. The female albino rats were distributed into 9 groups, each group having 15 rats. The indomethacin was made into a solution using normal saline, served as the standard drug (standard control). Group 1 was the negative control group (without induction of arthritis and treatment) which received 5 ml/kg normal saline. Group 2 was the arthritic control group (positive control induced with arthritis) which received 5 ml/kg normal saline. Group 3 was standard control group and was administered 10 mg/kg indomethacin. Groups 4 - 6 albino rats were induced with arthritis and received *Olax subscoripoidea* aqueous root extract at doses of 400, 600 and 800 mg/kg body weight, respectively from the tenth day after induction until the study ended. Groups 7 - 9 albino rats were induced with

arthritis and administered *Olax subscoripoidea ethanol* root extract at 400, 600, 800 mg/kg body weight, respectively from day 10 after induction till the end of the study.

Sample preparation and Analysis

Three albino rats from each group (Groups 1-9) were sacrificed on days 10, 18, 25 and 32 and blood samples were collected in EDTA anticoagulant bottles. The serum samples were used for the assays of oxidative stress indices which include malondiadehyde (MDA) [12], Nitric Oxide (NO) [13],Super Oxide Dismutase (SOD) [14], Catalase [15], Reduced glutathione (GSH) [16], Glutathione peroxidase (GLX) activity [17], adenosine deaminase [18] and tocopherol [19]

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 ± SD of 12 replicates. Values were considered statistically significant at $p<0.05$.

RESULTS

The result of the effect of *Olax subscorpiodea* aqueous and ethanol root extracts on MDA level in rats is presented in Table 1. The result shows high level of MDA in the untreated arthritic rats indicating the formation of free radicals resulting in the peroxidation of lipids which is measured as the MDA. MDA levels in rats treated with indomethacin, *Olax Subscorpiodea* aqueous and ethanol root extracts at various doses were lower ($P<0.05$) than that found in the arthritic untreated group (positive control). The effect was more significant ($P<0.05$) on day 32 of the treatment. The effects of the plant extracts used for treatment and the standard drug were equivalent. The results of NO generation are presented in Table 2. NO level in arthritic rats was higher ($P<0.05$) than that found in negative control group. Administration of plant extracts and standard drug to the arthritic rats reversed the level close to that of the control. It was observed that 800 mg/kg *Olax subscorpoidea* aqueous root extract was significantly ($P<0.05$) more effective than other extract treatments especially on day 32 in normalizing the NO level in arthritic treated rats. The results of SOD, GSH, glutathione peroxidase and catalase activities are presented in Tables 3-6. The levels of SOD, GSH, glutathione peroxidase and catalase activities in arthritic rat groups in the current study were lower ($P< 0.05$) than those found in normal control. Treatment with indomethacin, *Olax subscorpiodea* aqueous and ethanol root extracts at different doses administered in this study significantly normalized the levels of SOD, GSH, glutathione peroxidase and catalase in the arthritic rats maximum effect was seen on day 32 of the treatment. Again, 800 mg/kg body weight *Olax subscorpiodea* aqueous and ethanol root extracts were more effective in reversing the catalase activity of the arthritic treated rats. The results of the effect of the root extracts on adenine deaminase are presented in Table 7. The amount of adenine deaminase was elevated significantly ($P<0.05$) in all the arthritic rats. But treatment of the arthritic rats with the plant extracts significantly ($P<0.05$) reduced the adenine deaminase levels to the level found in the normal control group, with maximum effect observed on day 32 of the experiment. The results of tocopherol level are presented in Table 8. On days 25 and 32 the tocopherol level in positive control group (induced but not treated) rats decreased significantly ($P<0.05$) when compared to arthritic rats treated with plant extracts. The effect of treatment with 800 mg/kg body weight of *Olax subscorpiodea* aqueous and ethanol root extracts were more effective in reversing the tocopherol level close to normal.

Table 1: Effect of *Rauwolfia vomitoria* (RV) and *Olax subscorpioidea* (OS) aqueous and ethanol root extracts on MDA level of adjuvant-induced arthritic rats

	DAY10 (nmol/g protein)	DAY18 (nmol/g protein)	DAY25 (nmol/g protein)	DAY32 (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	$3.82±0.03^{a}$	$3.51±0.01^{c,b}$	$3.41±0.06^{d,c}$	$3.34±0.08^{b}$
5	$3.81±0.01^{a}$	$3.48±0.00^{c,b}$	$3.40±0.16^{d,c}$	$3.32±0.01^{b}$
6	$3.77±0.04^{a}$	$3.41±0.03^{c,b,d}$	$3.10±0.14^{g}$	$3.00±0.14^{b}$
7	$3.81±0.02^{a}$	$3.50±0.00^{c,b}$	$3.43±0.04^{d,c}$	$3.31±0.06^{b}$
8	$3.75±0.01^{a}$	$3.47±0.03^{c,b}$	$3.38±0.02^{d,e}$	$3.30±0.01^{b}$
9	$3.80±0.03^{a}$	$3.37±0.02^{c,d}$	$3.32±0.00^{e}$	$3.30±0.16^{b}$

MDA activity of adjuvant induced arthritic rats treated with *Olax subscorpiodea* ethanol and aqueous root extracts. The data are shown as mean ± SD (n=12) and significant difference at P<0.05OS= *Olax subscorpioidea*, 1= Negative control, 2= positive control, 3= Standard control, 4= 400 mg/kg OS aqueous extract, 5= 600 mg/kg OS aqueous extract, 6= 800 mg/kg OS extract aqueous 7= 400 mg/kg OS ethanol extract 8= 600 mg/kg OS ethanol extract, 9= 800 mg/kg OS ethanol extract. * Means with the same letter are not significantly different.

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Table 2: Effect *Olax subscorpioidea* (OS) aqueous and ethanol root extracts on NO level of adjuvant induced arthritic rats

TREATMENT	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[I,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	21.80±2.91[b,a]	19.20±0.24[f,e]	17.70±0.85[d,c,e]	15.10±0.95[e,d,f]
5	22.70±2.40[b,a]	18.20±1.87[f]	16.30±1.24[g,f]	14.50±0.81[h,g,f]
6	20.60±0.88[b]	19.90±0.71[e,d]	16.00±1.22[g]	13.00±1.63[i]
7	21.20±2.91[b]	19.80±0.21[e,d]	18.40±0.74[c]	16.90±0.88[c]
8	21.90±0.00[b,a]	20.00±1.90[e,d]	17.20±1.20[d,f,e]	15.50±0.86[e,d]
9	20.70±0.99[b]	20.20±0.53[c,e,d]	16.80±1.17[d,f,e]	13.90±1.98[I,h,g]

NO level of adjuvant induced arthritic rats treated with *Olax subscorpiodea* ethanol and aqueous root extracts. The data are shown as mean ± SD (n=12) and significant difference at P<0.05 OS= *Olax subscorpioidea.* 1= Negative control, 2= positive control, 3= Standard control, 4= 400 mg/kg OS aqueous extract, 5= 600 mg/kg OS aqueous extract, 6= 800 mg/kg OS extract aqueous,7= 400 mg/kg OS ethanol extract,8= 600 mg/kg OS ethanol extract, 9 = 800 mg/kg OS ethanol extract. * Means with the same letter are not significantly different.

Table 3: Effect of *Olax subscorpioidea* (OS) aqueous and ethanol root extracts on peroxidase level of adjuvant induced arthritic rats.

	DAY10 (U/L)	DAY18 (U/L)	DAY25 (U/L)	DAY32 (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	45.25±0.25[c,d,e]	44.75±0.02[e,f]	49.75±0.33[c]	51.11±1.15[d,c]
5	53.96±0.21[b]	50.53±0.03[b]	49.95±0.03[c]	52.81±0.05[b,d,c]
6	48.14±0.42[c,b]	47.68±0.02[c,d]	50.88±0.09[c,b]	58.78±0.75[b,a]
7	45.62±0.85[c,d]	45.15±0.64[e,d,f]	49.85±0.47[c]	51.34±0.60[d,c]
8	46.86±0.35[c,d]	50.75±0.78[b]	49.91±0.66[c]	52.55±0.49[d,c]
9	48.53±0.01[c,b]	47.70±0.55[c,d]	52.35±0.18[c,b]	53.72±0.74[b,d,a,c]

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

Table 4: Effect of *Olax subscorpioidea* (OS) aqueous and ethanol root extracts on SOD level of adjuvant-induced arthritic rats

Treatments	DAY10 (U/ mg protein)	DAY18 (U/ mg protein)	DAY25 (U/ mg protein)	DAY32 (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	61.10±0.13[c]	62.03±1.16[d]	63.69±0.18[c]	63.80±0.25[d]
5	70.30±0.98[b]	71.14±0.06[c,b]	71.32±0.00[b]	75.91±0.69[c]
6	69.20±3.93[b]	71.90±0.11[c,b]	73.25±0.42[b,a]	75.12±0.95[c,b]
7	61.18±1.42[c]	62.22±1.38[d]	63.69±0.16[c]	63.83±0.02[b]
8	70.20±1.13[b]	71.14±0.05[c,b]	71.35±0.00[b]	77.52±0.03[ba]
9	69.35±3.92[d]	72.51±0.47[c,b]	73.55±0.08[b,a]	74.65±4.45[c,b]

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

Table 5: Effect *Olax subscorpioidea* (OS) aqueous and ethanol root extracts on catalase level of adjuvant induced arthritic rats

Treatment	DAY10 (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	1.12±0.05[c]	1.57±0.02[b,d,a,c]	1.58±0.02[d]	1.63±0.00[e,d]
5	1.13±0.01[c]	1.59±0.00[b,a,c]	1.58±0.02[d]	1.72±0.42[e,b,d,a,c]
6	1.10±0.04[c]	1.70±0.03[b,a,c]	1.76±0.00[b]	1.85±0.01[b,a,c]
7	1.07±0.02[c]	1.36±0.02[b,d,c]	1.59±0.02[d]	1.66±0.00[e,d,c]
8	1.15±0.02[b]	1.37±0.00[b,d,c]	1.66±0.01[c]	1.78±0.01[b,d,a,c]

Catalase activity of adjuvant induced arthritic rats treated with *Olax subscorpiodea* ethanol and aqueous root extracts. The data are shown as mean ± SD (n=12) and significant difference at P<0.05 OS= *Olax subscorpioidea*, 1= Negative control, 2= positive control, 3= Standard control, 4= 400 mg/kg OS aqueous extract,5= 600 mg/kg OS aqueous extract, 6= 800 mg/kg OS extract aqueous,7= 400 mg/kg OS ethanol extract, 8= 600 mg/kg OS ethanol extract, 9= 800 mg/kg OS ethanol extract. * Means with the same letter are not significantly different.

Table 6: Effect of *Olax subscorpioidea* (OS) aqueous and ethanol root extracts on GSH level of adjuvant induced arthritic rats

Treatments	DAY10 (Umol/l)	DAY18 (Umol/l)	DAY25 (Umol/l)	DAY32 (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	23.14±0.43[b]	23.77±0.04[b]	23.93±0.02[c,b]	24.25±0.07[d,c]
5	23.58±0.24[b]	23.54±0.43[b]	24.15±0.07[c,b]	25.32±0.04[b,a,c]
6	23.80±0.03[b]	23.95±0.69[b]	24.18±0.47[c,b]	26.01±1.11[b]
7	17.92±0.35[c]	23.80±0.01[b]	23.93±0.02[c,b]	24.28±0.01[d,c]
8	17.95±2.48[c]	23.95±0.00[b]	24.25±0.08[c,b]	25.48±0.19[b,a]
9	18.84±0.01[c]	23.85±0.01[b]	24.44±0.01[b]	25.83±0.02[b,a]

GSH level of adjuvant induced arthritic rats treated with *Olax subscorpiodea* ethanol and aqueous root extract. OS= *Olax subscorpioidea*, 1= Negative control, 2= positive control, 3= Standard control, 4= 400 mg/kg OS aqueous extract,5= 600 mg/kg OS aqueous extract, 6= 800 mg/kg OS extract aqueoust,7= 400 mg/kg OS ethanol extract, 8= 600 mg/kg OS ethanol extract, 9= 800 mg/kg OS ethanol extract. * Means with the same letter are not significantly different.

Table 7: Effect of *Olax subscorpioidea* (OS) aqueous and ethanol root extracts on adenine deaminase level of adjuvant induced arthritic rats

Treatments	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	0.78±0.02[a]	0.68±0.00[b]	0.57±0.00[c]	0.54±0.01[b]
5	0.77±0.02[b,a]	0.65±0.00[d]	0.53±0.01[f,e]	0.52±0.00[d,c]
6	0.75±0.00[b,d,c]	0.64±0.00e	0.53±0.00[f,e]	0.51±0.00[d,e]
7	0.77±0.02[b,a,c]	0.68±0.01b	0.54±0.00[e]	0.52±0.01[e]
8	0.70±0.02[g]	0.64±0.01e	0.53±0.01[f,e]	0.53±0.00[f,e]
9	0.73±0.00[e,f]	0.64±0.00[e]	0.53±0.00[f,e]	0.53±0.01[f,e]

Adenine deaminase level of adjuvant induced arthritic rats treated with *Olax subscorpiodea* ethanol and aqueous root extracts. The data are shown as mean ± SD (n=12) and significant difference at $P<0.05$. OS= *Olax subscorpioidea*, 1= Negative control, 2= positive control, 3= Standard control, 4= 400 mg/kg OS aqueous extract,5= 600 mg/kg OS aqueous extract, 6= 800 mg/kg OS extract aqueous,7= 400 mg/kg OS ethanol extract,8= 600 mg/kg OS ethanol extract, 9=800 mg/kg OS ethanol extract.
* Means with the same letter are not significantly different.

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Table 8: Effect of *Olax subscorpioidea* (OS) aqueous and ethanol root extracts on tocopherol level of adjuvant induced arthritic rats

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	0.82±0.00^{c,e,b,d}	0.83±0.01^{c}	0.84±0.0^{b}	0.91±0.02^{b,d,c}
5	0.84±0.04^{c,b}	0.84±0.01^{b,c}	0.85±0.00^{b}	0.95±0.01^{b,a,c}
6	0.81±0.04^{f,c,e,b,d}	0.84±0.03^{b,c}	0.85±0.00^{b}	0.97±0.01^{b,a}
7	0.83±0.00^{c,b,d}	0.83±0.01^{c}	0.86±0.00^{b}	0.91±0.03^{d,c}
8	0.80±0.00^{f,c,e,b,d}	0.84±0.01^{b,c}	0.87±0.00^{b}	0.95±0.01^{b,a,c}
9	0.84±0.05^{c,b}	0.83±0.00^{b,c}	0.85±0.00^{b}	0.96±0.010^{b,a}

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

DISCUSSION

Our results on free radical formation culminating in lipid peroxidation, measured as MDA (Table 1) showed high levels of MDA level in arthritic rats. Similarly, the NO level was very high in the arthritic rats The MDA level continued to increase progressively till day 32 of the study in the untreated arthritic groups. However, treatment with indomethacin, *Olax Subscorpiodea* aqueous and ethanol root extracts lowered (P<0.05) the MDA level. The effects of the plant extracts on MDA level were equivalent to that of standard drug. Though nitric oxide is an important chemical mediator generated by endothelial cells, macrophages, neurons and involved in the regulation of various physiological processes, its uncontrolled release is detrimental to the body. The NO levels which were significantly (P<0.05) high in the arthritic rats decreased (P<0.05) with the administration of indomethacin, *Olax Subscorpiodea* aqueous and ethanol root extracts. Free radicals induced Oxidative stress and associated injury is the primary mechanism leading to a number of pathological disorders. In this present study, the adjuvant caused an upsurge in the release of reactive oxygen species [20]. Mamatha *et al.*, (2013), [20], reported an increase in MDA and other reactive oxygen species on induction of arthritis. The effect was reversed on administration of the extract which contains redox potential such as phenolic compounds. These compounds absorb and neutralizes free radicals like MDA and NO thereby decomposing peroxides and single and triplet oxygen. In the same vein, the plant extracts may have been able to lower the levels of MDA and NO owing to the presence of phenols in them. The result of antioxidant levels is represented in Tables 3-6. GSH, SOD, catalase and glutathione peroxidase protect cells and tissue structures against free radical and oxidative insults. Their role includes the detoxification of xenobiotics, free radicals, peroxides and the regulation of immune function. The levels of SOD, GSH and catalase activities in arthritic rat groups in the current study were lower (P< 0.05) than in normal control. Our result corresponds with that of [20], Treatment of arthritic rats with indomethacin, *Olax Subscorpiodea* aqueous and ethanol root extracts at different doses normalized (P<0.05) the levels of SOD, GSH and catalase with maximum effect occurring on day 32 of the treatment. Exactly 800 mg/kg body weight *Olax subscorpiodea* aqueous was more effective in reversing the catalase activity of the arthritic treated rats. The decreased activity of catalase observed in the serum of the positive control rats may have been due to damage caused by hydrogen peroxides and hydroxides. *Olax subscorpoidea* significantly (P<0.05) enhanced the catalase activity which indicates increased decomposition of hydrogen peroxides to water and oxides. Superoxide dismutase lowers the steady state level of oxygen radical (O_2^-). It functions as a catalyst for the dismutation of superoxides radicals to peroxides and molecular oxygen in order to protect cells and tissues from superoxide radicals and other peroxides like lipid peroxides [21]. The observed low superoxide dismutase activity in the arthritic rats may be due to increased levels of superoxide anion [22]. *Olax subscorpoidea* on administration significant (P<0.05) increased the enzyme activity indicating increased conversion of superoxide radicals to peroxides which were further removed by glutathione peroxidase or catalase activity [23]. Administration of *Olax subscorpoidea* may have increased (P<0.05) the action of glutathione peroxidase which converts peroxides to water with simultaneous oxidation of glutathione (GSH) to glutathione disulphide (GSSG) that counteracts oxidative stress [24]. As stated in the above preceding paragraphs, the anti-oxidative and radical scavenging activities of *Olax subscoripoidea* could

be as a result of the presence of flavonoids in the root extracts which enhanced the activity of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase. The level of GSH got a boost in the arthritic rats on treatment with *Olax subscoripoidea*. GSH serves as a cofactor for detoxifying enzymes like GPx which in turn prevent lipid oxidative damage by reducing lipid peroxides and helps in the regeneration of oxidized forms of antioxidant vitamin C and E. The results of our analysis shows that the type II collagen - CFA induced arthritis caused an imbalance between oxidants and antioxidant system. This imbalance was brought back to near normal level by the administration of *Olax subscoripoidea* which contain flavonoids and terpenoids. Our result is in agreement with the work of [25], who reported that the significant inhibition of serum DPPH radical scavenging activity and decrease of total antioxidant power in CCl_4 was reversed on the administration of *Olax subscorpioidea. Olax subscorpioidea* which contains flavonoids and phenolic compounds may possibly have raised the serum antioxidant by thereby reversing the changes. In addition, presence of flavonoids and phenolics in *Olax subscorpioidea* must have enhanced the antioxidant activities. The amount of adenine deaminase was higher ($P<0.05$) in all the arthritic rats (Table 7). Adenosine deaminase (ADA) is an enzyme that converts adenosine to deoxyadenosine and inosine to deoxyinosine with the release of ammonia. The increased serum level of adenosine deaminase indicates stimulation of cellular immunity. This has been observed in lymphoblastic leukemia, in acute hepatitis, human immune deficiency virus infection, pneumonia as well as rheumatoid arthritis. It has been reported that the major sources of the prevalent form of adenosine deaminase in serum is the monocyte/macrophage cell system [27 and 28].However, treatment with the plant extracts significantly ($P<0.05$) reduced the adenine deaminase levels to the level found in the normal control group, with maximum effect occurring on day 32. In this study also tocopherol level in the arthritic rats was significantly ($P<0.05$) lower than the normal control values (Table 8). Vitamin E in combination with other drugs has been used effectively to control the symptoms of arthritis. Treatment of arthritic rats with *Olax subscorpoidea* aqueous and ethanol root extracts, especially at 800 mg/kg body weight dose was as effective as treatment with indomethacin standard drug. Furthermore, it has been reported by [29], that lowered serum levels of α tocopherol, β carotene, and selenium preceded the diagnosis of rheumatoid arthritis, and that low antioxidant values may be a risk factor or a marker for the disease [30 and 31]. In conclusion, *Olax subscorpioidea* ethanol and aqueous root extracts contain principles that may have increased the antioxidant level of the arthritic rats, lowered the oxidative stress and reversed the arthritic conditions developed by adjuvant induced arthritic rats. Hence, this present study provides scientific evidence that *Olax subscorpioidea* ethanol and aqueous root extracts contain principles with have anti-arthritic potentials.

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