Protective Effect of *Chromolena odorata* Aqueous Leaf Extract on Inflammation and Oxidative Damage in Sephadex-Induced Athsmatic Wistar Rats

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Abstract

The aqueous leaf extract of *Chromolena odorata* was investigated for its effects on inflammatory and oxidative stress parameters in sephadex-induced airway inflammation in Wistar rats. Sephadex-treated rats (5 mg/ml) were pre-treated 1 hour earlier with 100 and 200 mg/kg bodyweight of the extract and dexamethasone (2 mg/kg bodyweight) as standard drug. The animals were sacrificed 24 h later. The levels of inflammatory cells and oxidative damage were investigated through bronchoalveolar lavage fluid (BALF) cell count, antioxidant enzyme activity assays and histological analysis of the lung tissue. Sephadex treatment caused a significant increase in total and individual inflammatory cell counts in the rat lungs while increasing the concentrations of MDA and lowering the activities of superoxide dismutase and catalase. These were however reversed in rats pretreated with aqueous leaf extract of *Chromolena odorata* and dexamethasone. These results indicate that the extract may prevent inflammatory cell recruitment and oxidative damage associated with asthma episodes.

Keywords: *Chromolena odorata*, Sephadex, Oxidative stress, Airway inflammation, Antioxidants

1.0 Introduction

The use of medicinal plants for treatment of ailments has been on the increase in recent years. This is due to the little or no side effects attributable to their usage, reasonable cost implication and ease of accessibility [1]. Medicinal plants including *Garcinia kola*, *Alchornea laxiflora*, *Occimum grattissum*, *Cochorous olitorous* possess antifungal, antimalarial, anticancer and anti-inflammatory properties, making them useful in the treatment of various diseases including skin diseases, malaria, airway inflammation and asthma [2,3].

Airway inflammation is one of the early events of an asthma episode [4]. It is characterized by an increased number of inflammatory cells in the lungs resulting in lipid peroxidation caused by generation of free radicals and oxidative stress [5]. These eventually cause damage to the walls of the airways which then lead to histological changes and airway hyperactivity observed in asthmatics [6].

Asthma and airway inflammation are usually treated through the use of established drugs such as corticosteroids and cyclooxygenase inhibitors [7]. These drugs act by inhibiting events following initiation of the inflammatory response and as such, causing severe side effects especially when used in high doses for prolonged periods [8]. This necessitates the need for equally effective alternatives with little or no side effects and which would prevent the early stages of cell recruitment and subsequent oxidative stress induction.

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Chromolena odorata commonly known as Siam weed (English), *ewe akintola (Yoruba)* and *Obuinenawa* (*Igbo*) is a herbaceous perennial that forms dense tangled bushes about 1.5-2.0 m in height with a characteristic aromatic smell. The plant is well-known for the treatment of diseases including malaria, dysentery, toothache, diarrhea, diabetes, skin diseases and fever [2,9,10]. In particular, the plant has been shown to exhibit anti-inflammatory properties [11]. However, there is little or no documented report on its possible effects on airway inflammation parameters. This study therefore aims at investigating the protective role of aqueous leaf extract of *Chromolena odorata* on airways inflammation and oxidative stress in sephadex-treated rats.

2.0 Materials and Methods

2.1 Collection and Authentication of Plant Materials

Fresh leaves of *Chromolena odorata* were collected within the premises of Fountain University, Osogbo, Nigeria in May, 2016. The plant was authenticated at the Herbarium of the Department of Plant Biology, University of Ilorin where a voucher specimen UIH/970 was obtained.

2.2 Preparation of Aqueous Leaf Extract of Chromolena odorata

Fresh leaves of *Chromolena odorata* were thoroughly rinsed with clean water to remove dust and soil particles. The leaves were air dried for two weeks and pulverized using a blender (BL8319 Toyomi, Singapore). The powdered plant sample (50 g) was extracted in 1 L of distilled water for 48 hours. The solution was filtered using Whatman No. 1 filter paper and the resulting filtrate was concentrated using a lyophilizer (DW6-85 Hetodrywinner, Denmark) before being re-constituted into 100 and 200 mg/kg body weight in distilled water for the experiment.

2.3 Induction of Airway Inflammation using Sephadex

Sephadex suspension (5 mg/ml) was prepared following the method described by Belvisi *et al*. [4]. Sephadex (G-200, 0.5 g) was dissolved in 100 mL of normal saline for 72 hours. The suspension (0.5 mL) was administered intravenously to the animals via the tail vein.

2.4 Pre-treatment with Extract and Dexamethasone

Pre-treatment was done following the method of De Bie *et al*. [12]. The extract (2 ml) at 100 and 200 mg/kg body weight were administered orally to the animals. Dexamethasone (2 ml, 2 mg/kg body weight) in normal saline was administered intraperitoneally to the animals.

2.5 Experimental Design

Twenty-five male Wistar rats with a mean weight of 200 ± 10 g were obtained from the Animal House of the Department of Biochemistry, University of Ilorin, Nigeria. They were acclimatized in clean ventilated cages for three weeks and provided with feed and water *ad libitum*. The animals were divided into 5 treatment groups as follows:

Group 1 (normal control): received distilled water only
Group 2: received sephadex (5 mg/ml)
Group 3: received dexamethasone pre-treatment (2 mg/kg body weight) 1h before sephadex injection
Group 4: pre-treated with 100 mg/kg body weight of *Chromolena odorata* extract 1 h before sephadex injection
Group 5: pre-treated with 200 mg/kg body weight of *Chromolena odorata* extract 1 h before sephadex injection

Twenty four hours after sephadex administration, the animals were humanely sacrificed under ether anaesthesia. The rats were carefully dissected using sharp sterile blade after which the lung was excised and blotted with clean laboratory tissue paper.

2.6 Bronchoalveolar Lavage and Cell Counts

Bronchoalveolar lavage was performed following the method of Evaldsson *et al*. [8]. Phosphate buffered saline (5 mL, pH 7.4) was used to flush the lungs and the lavage fluid samples were collected in test tubes. The lavage fluid was centrifuged at 800 g for 10 min at 4^0C. Thereafter, the cell pellets were re-suspended in 1 mL of Hanks' balanced salt solution. The cell suspension was then added to Turk's reagent, and the cells were counted under the light microscope in a Burker-Turk chamber (Erma Optical Works, Tokyo, Japan). Differential cell counts were made from cytospin preparations (Cytospin 3; Shandon Scientific, Cheshire, UK) stained using a Diff-Quik staining kit. Cells were identified as monocytes, neutrophils, eosinophils and lymphocytes by standard morphologic techniques.

2.7 Preparation of Lung Homogenate

Known weight of lung tissue was washed in ice-cold 1.15% KCl and homogenized in Tris-buffer (50 mM, pH 7.4) using a Teflon homogenizer. The homogenate was centrifuged at 9000 rpm for 20 minutes at 4°C to remove debris. The supernatant was then taken and used for the various biochemical assays.

2.8 Assay for Lipid Peroxidation

Lipid peroxidation was determined by adapting the method of Uchiyama and Mihara [13]. To 0.5 ml of supernatant, 0.2 ml of 8.1% sodium dodecyl sulphate salt (SDS), 1.5 ml of 1% phosphoric acid, 0.2 ml of distilled water and 1.0 ml of 0.6% 2-thiobarbituric acid were added. The mixture was heated in a boiling water bath for 45 minutes, cooled in an ice bath, followed by an addition of 4.0 ml of n-butanol to extract the cold thiobarbituric acid reactants. The absorbance of the n-butanol layer was read at 532 nm after centrifugation at 1,000 rpm for five minutes. Lipid peroxidation was expressed in terms of malondialdehyde (MDA) content.

2.9 Assay of Superoxide Dismutase (SOD) Activity

SOD activity was assayed according to the method of Kakkar *et al*. [14]. To 0.l ml supernatant, 1 ml of sodium carbonate (1.06 g in 100 ml water), 0.4 ml of 24 mM NBT and 0.2 ml of EDTA (37 mg in 100 ml water) was added and zero minute reading was taken at 560 nm. Reaction was initiated by addition of 0.4 ml of 1 mM Hydroxylamine hydrochloride, incubated at 25°C for 5 minutes and the reduction of NBT was measured at 560 nm and expressed as Units/g of wet tissue.

2.10 Assay of Catalase (CAT) Activity

The activity of CAT was determined following the method of Luck [15]. Hydrogen peroxide–phosphate buffer (3 ml, 0.067 M, pH 7.0) was taken followed by the addition of an aliquot of 40 µl of the supernatant and mixed thoroughly. The time required for a decrease in absorbance by 0.05 units was recorded at 240 nm using a spectrophotometer. The enzyme solution containing hydrogen peroxide–free phosphate buffer served as control. One enzyme unit was calculated as the amount of enzyme required to decrease the absorbance at 240 nm by 0.05 units.

2.11 Histological Analysis

Formalin-fixed lung samples were cut into about 2 µm sections. The sections were first flamed on a burner, placed in xylene to remove the formalin and then passed through decreasing concentration of alcohol baths and water (100, 90, 80 and 70%).Thereafter, sections were stained with haematoxylin for 3 minutes, washed under running tap water until they turned blue and placed in acid alcohol (1% HCl in 70% alcohol) for 5 minutes. Washing under running tap water was repeated until they turned blue again. Finally, sections were stained using 1% eosin Y for 10 minutes, washed under tap water, placed indecreasing concentration of alcohol baths and water (100, 90, 80 and 70%) and mounted on microscope slides for viewing.

2.12 Statistical Analysis

Values reported were expressed as mean ± SEM, using one way analysis of variance (ANOVA) followed by Tukey's test. The values were considered to be significant when $p< 0.05$.

3.0 Results

3.1 Effect of Chromolena odorata Aqueous Leaf Extract on BALF Cell Count

The total number of cells in the BALF was found to be significantly increased in rats treated with sephadex only when compared with the control. Individual cell counts including eosinophils, neutrophils and lymphocytes were also significantly increased by 22%, 112%, 45% respectively when compared with the control except for monocytes which were reduced. However, the reverse was the case in the animals pre-treated with the extract, having significantly reduced total cell count as well as individual cell counts when compared with the animals treated with sephadex alone (Table 1).

3.2 Effect of Chromolena odorata Aqueous Leaf Extract on Lipid Peroxidation

MDA concentration was significantly increased in the animals treated with sephadex alone when compared with the control. A significant reduction was however observed in the extract-treated groups when compared with the animals treated with sephadex alone (Table 2).

3.3 Effect of Chromolena odorata Aqueous Leaf Extract on SOD and CAT Activities

A significant reduction in the activities of SOD and CAT was observed in the rats treated with sephadex when compared with the control. The activities of these enzymes were however significantly increased in animals pre-treated with the extract, when compared with the animals treated with sephadex alone (Table 2).

3.4 Effect of Chromolena odorata Aqueous Leaf Extract on Histopathology of Lungs

Histological study on the lung tissues of animals in the control group revealed the alveolar sac and bronchioles with normal epithelium. Sephadex treatment caused accumulation of inflammatory cells around the inter-alveolar septum. This was however prevented by pre-treatment with the extract (200 mg/kg body weight) as inflammatory cell accumulation was less and scattered (Figure 1).

Table 1: Effects of Aqueous Leaf Extract of *Chromolena odorata* and Dexamethasone on Bronchoalveolar Lavage (BAL) Cell Counts in Sephadex-Treated Rats

	Eosinophil count (units/cmm) ($x10^2$)	**Neutrophil count (units/cmm) ($x10^2$)**	**Lymphocyte count (units/cmm) ($x10^2$)**	**Monocyte count (units/cmm) ($x10^2$)**	**Total cell count (units/cmm) ($x10^2$)**
Control	4.08 ± 0.65^a	17.63 ± 5.69^a	32.53 ± 6.21^a	3.61 ± 1.09^a	61.80 ± 12.65^a
Sephadex only (5 mg/ml)	5.01 ± 0.61^b	37.41 ± 3.02^b	47.26 ± 6.81^b	3.55 ± 1.15^a	10.980 ± 11.71^b
dexamethasone (2 mg/kg b.w.) + Sephadex	4.21 ± 0.66^c	20.00 ± 4.12^c	31.06 ± 7.95^a	3.00 ± 1.81^a	65.80 ± 14.14^c
100 mg/kg b.w. extract + Sephadex	4.23 ± 0.91^c	27.01 ± 3.28^d	40.57 ± 6.92^c	2.90 ± 1.76^b	70.60 ± 19.22^d
200 mg/kg b.w. Extract + Sephadex	4.00 ± 0.66^a	20.09 ± 3.73^c	33.83 ± 7.12^a	2.87 ± 1.63^b	59.75 ± 10.43^d

Results are expressed as cell numbers in BALF. Each value represents mean ± SEM of five replicates. Different superscripts represent significant difference. The values were considered to be significantly different when $p < 0.05$.

Table 2: Effect of Aqueous Leaf Extract of *Chromolena odorata* and Dexamethasone on Lipid Peroxidation and Antioxidant Enzymes in the Lungs of Sephadex-Treated Rats

Groups	MDA Concentration (µmols g^{-1} fw)	Superoxide Dismutase Activity (U/L)	Catalase Activity (U/L)
Control	0.34 ± 0.02^{a}	34.05 ± 0.51^{a}	60.65 ± 3.13^{a}
Sephadex only (5 mg/ml)	0.85 ± 0.04^{b}	10.77 ± 0.72^{b}	23.07 ± 2.12^{b}
Dexamethasone (2 mg/kg b.w.) + Sephadex	0.54 ± 0.04^{a}	30.58 ± 2.92^{a}	37.64 ± 3.59^{c}
100 mg/kg b.w. Extract + Sephadex	0.58 ± 0.04^{b}	18.84 ± 2.89^{c}	38.91 ± 2.95^{c}
200 mg/kg b.w. Extract + Sephadex	0.40 ± 0.09^{a}	23.33 ± 1.29^{c}	40.86 ± 3.26^{c}

Each value represents the mean ± SEM of five animals. Different superscripts represent significant difference. The values were considered to be significantly different when $p < 0.05$.

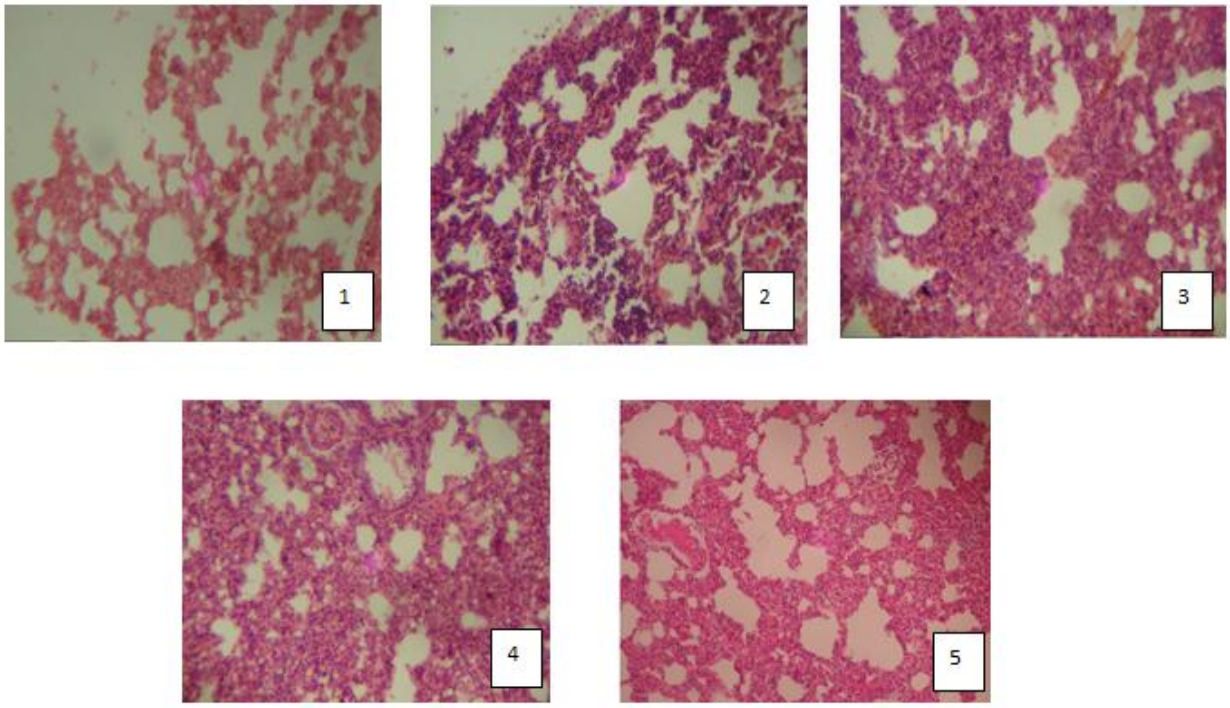

Figure 1: Haematoxylin-Stained Lung Sections from Rats Treated with Aqueous Leaf Extract of *Chromolena odorata*

Group 1 (Distilled water only)
Group 2 (Sephadex only)
Group 3 (Sephadex + Dexamethasone 2 mg/kg bodyweight)
Group 4 (Sephadex + 100 mg/kg body weight extract)
Group 5 (Sephadex + 200 mg/kg body weight extract)

4.0 Discussion

A major area of scientific research in recent years is the discovery of pharmacological agents from medicinal plants. Results from this study have shown that aqueous leaf extract of *Chromolena odorata* has preventive roles in inflammatory cell recruitment and free radical generation during an airway inflammation episode.

Inflammatory cells, especially the neutrophils are recruited in large amounts to sites of inflammation and are therefore used as an index of inflammation onset [16]. This may have accounted for the significant increase in total and individual cell counts in the lungs of rats treated with sephadex only, especially the neutrophils. This is an indication of inflammation caused by sephadex treatment and conforms to earlier studies that reported a direct link which neutrophils have with airway inflammation [16,17]. Glucocorticoids including dexamethasone inhibit the action of NF-κB-responsive elements required for the induction of many genes involved in inflammation process [18]. This may be responsible for the significant reduction in inflammatory cell counts in the dexamethasone pre-treated rats and conforms to earlier studies that reported inhibitory effects of dexamethasone on inflammatory cell recruitment [18,19]. Plant polyphenols also inhibit airway inflammation processes including production of inflammatory mediatorsand neutrophil function [5]. This may have accounted for the significant inhibition of neutrophil recruitment exhibited by the aqueous leaf extract of *Chromolena odorata.* Aqueous leaf extract of *Chromolena odorata* contains polyphenols among other important secondary metabolites and thus may be a potential inhibitor of neutrophil recruitment [20].

Neutrophils secrete elastase, an enzyme that causes degeneration of lung elastin, thereby leading to generation of free radicals which could cause lipid peroxidation [21]. Therefore, the significant increase in MDA concentration in the rats treated with sephadex only is an indication of membrane damage resulting from sephadex treatment. This is in line with earlier studies that reported an increase in MDA content of rat lungs treated with sephadex [22]. The reduced MDA levels observed in the rats pre-treated with dexamethasone is an indication of the antioxidative effect of the glucocorticoid. Dexamethasone may reduce the spontaneous production of oxygen radicals by neutrophils through blocking of early events in the inflammation cascade, thus preventing tissue damage caused by oxidative burst during acute inflammation [18]. Plant secondary metabolites including the polyphenols have the ability to direct enzymatic breakdown of free radicals through endogenous antioxidants thereby inhibiting the actions of the inflammatory cells [23]. Thus, the significant reduction in MDA content in the extract-treated group when compared with the untreated animals indicates the presence of antioxidant polyphenols in the extract and conforms to an earlier study that reported a significant reduction in MDA content of sephadex challenged rats pre-treated with aqueous extract of *Ginkgo biloba* [24]. Increase in MDA concentration in tissues, thus serves as an indicator of oxidative stress condition and is usually accompanied by alteration in antioxidant enzyme activities [5].

The significant reduction in the antioxidant enzyme activities in the sephadex-induced rats when compared with the normal group may be attributed to high amounts of free radicals which were generated in the lungs [23]. Excess free radicals may overwhelm the antioxidant enzymes leading to reduction in their activities [5]. These observations are in line with previous studies that reported alterations in the activities of antioxidant enzymes in the lungs of rats challenged with sephadex [22]. Glucocorticoids may donate amino acids such as cysteine to intracellular antioxidants [25]. This may have also contributed to the significant increase in antioxidant enzyme activities in the dexamethasone pre-treated rats when compared with the sephadex treated rats. Plant secondary metabolites with antioxidant properties may prevent the production of free radicals or even stimulate the synthesis of antioxidant enzymes thus resulting in the production of more antioxidant enzymes as seen in the group pre-treated with the extract [5].

In conclusion, the present study clearly showed that aqueous extract of *Chromolena odorata* prevented recruitment of harmful cells and oxidative damage that may result from airway inflammation, an indication that the extract contains substances which may be useful in the management of acute asthma.

5.0 References

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