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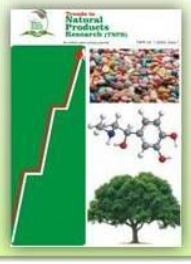

Acute Anti-inflammatory and Haematological Effects of Alkaloidal Leaf Extract of *Combretum dolichopetalum* (Engl & Diels)

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Abstract: Alkaloids are among the common phytochemicals present in the extracts of *Combretum dolichopetalum* that exhibit its diverse effects. This study evaluated the alkaloidal extract of *C. dolichopetalum* for acute antiinflammatory and hematological effects. The phytochemical screening of the extract was done using standard methods. The anti-inflammatory assay was conducted in rats using subplantar injection of the phlogistic agent (egg albumin). The effect of the extract on haematological parameters such as haemoglobin (Hb), Packed Cell Volume *(*PCV), Mean Cell Haemoglobin Concentration (MCHC), White Blood Cell (WBC) and Differential White Blood counts were estimated. Alkaloidal tannates were the major constituents of the extract. The extract significantly ($p < 0.05$) and dose-dependently inhibited the development of acute edema of the rat paw induced by egg abumin. There was a non significant ($p > 0.05$) decrease in some of the haematological parameters however, the white blood cells count was significantly reduced These results indicate that the alkaloidal extract from *Combretum dolichopetalum* leaf presents potent anti-inflammatory effects with minimal effect on haematological indices.

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INTRODUCTION

Inflammation is characterized by redness, swelling, heat, pain, and loss of tissue function, which result from local immune, vascular and inflammatory cell responses to infection or injury (Takeuchi and Akira 2010). Important microcirculatory events that occur during the inflammatory process include vascular permeability changes, leukocyte recruitment and accumulation, and inflammatory mediator release (Chertov *et al*., 2000, Ferrero-Miliani *et al*., 2007). The inflammatory response is the coordinate activation of signaling pathways that regulate inflammatory mediator levels in resident tissue cells and inflammatory cells recruited from the blood (Lawrence, 2009). Although inflammatory response processes depend on the precise nature of the initial stimulus and its location in the body, they all share a common mechanism. Different mediators have been reported to play a key role in the pathogenesis of these inflammatory disorders, including reactive oxygen and nitrogen metabolites, eicosanoids, or cytokines such as tumor necrosis factor- (TNF-) α, interleukin- (IL-) 1β, and IL-6, among many others. Therefore, they are important targets for anti-inflammatory molecules (Guha and Mackman, 2001, Guzit *et al*., 2003). Also, it's a fundamental biological process involving complex pathways that are often induced by the products of bacterial degradation from various microorganisms (lipopeptides, lipopolysaccharides, peptidoglycans, formylmethionyl peptides, flagellin, microbial DNA), fungi (zymosans), viruses (double-stranded RNA), or even the body's own cells upon damage and death (Kulinsky, 2007).

The medicinal use of plants of the family Combretaceae is widely described in the scientific literature. Phytochemical studies carried out in the genus Combretum have demonstrated the occurrence of many classes of constituents, including triterpenes, flavonoids, lignans and non-protein amino acids, among others. The leaves and roots are extensively used in ethno medical practices of many cultures (Barku *et al.*, 2016). In Nigeria, the plant is used in the treatment of burns and gastrointestinal disorders (Asuzu and Njoku, 1992). In Ghana, an infusion of the leaves, roots and stem is used in the management of a condition of "stomach stagger" in cattle (Uzor *et al*., 2014). Hydromethanolic extracts of the leaves possess antidiarrheal properties (Onoja *et al.*, 2015). The leaf of *C. dolichopetalum* was reported to be rich in flavonoids, alkaloids, steroids and good macronutrients. It is recognized that natural-product have great chemical diversity, biochemical specificity, and other molecular properties that make them favourable lead structures (Koehn and Carter 2005, Ekstein and Schachter 2010, Kong, *et al*., 2010, McCloud 2010, Scotti *et al*, 2010, Reis *et al*., 2011,).

Alkaloids are a class of amino acid-derived nitrogen-containing organic compounds with low molecular weight, which are mainly contained in various living organisms, such as bacteria, fungi, plants, and animals (Moreira, *et al.*, 2018). In plants, alkaloids are secondary metabolites produced in response to environmental modulations and biotic or abiotic stress (Taha, *et al*., 2009). These properties make alkaloids potential candidate compounds for new drug development. According to their carbon skeletons, alkaloids can usually be classified as indole-, isoquinoline- and pyridine-like alkaloids. Some alkaloids such as isoquinoline, indole and diterpene are known to have good anti-inflammatory activity (Fawole *et al*., 2009). Many alkaloids are used in therapeutics and as pharmacological tools. A wide range of biological effects has been reported for alkaloids, including emetic, anti-cholinergic, antitumor, diuretic, sympatho-mimetic, antiviral, antihypertensive, hypno-analgesic, antidepressant, mio-relaxant, anti-tussigen, antimicrobial and anti-inflammatory activities (Henriques *et al*., 2004, Ezell *et al*., 2010, Aiello *et al*., 2011). In this study the alkaloidal extract of *Combretum dolichopetalum* was investigated for acute inflammatory activity and hematological responses.

MATERIALS AND METHODS

Animals

White albino rats (190-200 g) of either sex bred in the Animal House of Faculty of Biological Sciences and Veterinary Medicine, University of Agriculture, Umudike, Abia State, Nigeria were used for the study. They were acclimatized in the animal house for five days with free access to water and food before the start of the experiment. The rats and mice were fed with standard pellets (Guinea Feeds, Plc, Nigeria). The animals were maintained under standard 12-hour light-dark cycle throughout the duration of the study. All animal experiments were in compliance with the principles for laboratory animal use and care of the National Institute of Health Guide for Care and Use of Laboratory Animals (NIH publication #85-23, revised in 1985).

Collection of Plant Material

The leaves of *Combretum dolichopetalum* were collected between the months of January and February from Orba, Udenu Local Government Area of Enugu State. The botanical identification was confirmed by Mr. A. Ozioko of Bioresources Development and Conservation Programme (BDCP). The voucher specimen was deposited in the BDCP herbarium (BDCP 0094).

Preparation of the Alkaloidal Extract

The alkaloidal extraction method was according to Harborne (1973). The air-dried leaves (750 *g*) of *Combretum dolichopetalum* was weighed into a 1000 ml extraction bottle and 1500 ml of 10 % acetic acid in ethanol was added, covered and allowed to stand for 4 h. This was filtered and the extract was concentrated in a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the preparation was completed. The solution was allowed to settle, and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloidal extract, which was dried and weighed.

Phytochemical Screening

The alkaloidal extract of *C. dolichopetalum* leaves was assessed for the presence of other classes of alkaloids. Phytochemical screening was carried out using both chemical methods and TLC according to the standard procedures (Sofowora 1993, Trease and Evans 1989).

Anti-inflammatory Effects of the Alkaloid Extract

The method of Winter *et al*., (1962) was used. Twenty rats were fasted for 12 h and deprived of water only during the experiment. Deprivation of water was to ensure uniform hydration and to minimise variability in oedematous response. Five treatment groups of five animals each were used. One hour prior to the subplantar injection of the phlogistic agent (egg albumen), group 1 rats were given an equivalent volume of distilled water prior to induction and served as the negative control (without treatment), while the animals in group 2 were administered 10 mg/kg of aspirin (positive control). Groups 3, 4 and 5 were given 100, 200 and 400 mg/kg of the extract (p.o) respectively. The phlogistic agent was injected 1 hr later. Paw volume (oedema) was measured by volume displacement. The inflammation was assessed as the difference between the zero-time volume of the paw and the volume at the various times after the administration of the phlogistic agent. The anti-inflammatory activity was calculated at each time of observation as percent inhibition of oedema in the treated animals in comparison with the vehicle-treated animals. The percent inhibition of oedema (Ahmed *et al*., 1993, Perez, 1996) was calculated using the formula.

$$\% \text{ Inhibition of oedema} = \frac{(V_0 - Vt)}{V_0} \times 100$$

Where Vt is the paw volume (oedema) at corresponding time, and Vo is the paw volume (oedema) of vehicle treated rats at the same time.

Hematological Effects of the Alkaloidal Extract

Haemoglobin Estimation (Methemoglobin Method)

The method was as described by Baker *et al*., (1998). The modified Drabkin's solution was used to spectrophotometrically measure the conversion of haemoglobin to cyanomethemoglobin. The whole blood of the rat (0.02 *ml*) was diluted to 4 ml with modified Drabkin's solution. This was allowed to stand for 10 minutes and the optical density was read at 540 nm against a Drabkin's solution (as a blank). Similarly, 57.2 mg/100 ml of the standard cyanomethemoglobin was treated as the sample. The final haemoglobin result is calculated as follows:

$$\text{Hb. Conc.} = \frac{T \times C \times D}{A \times 1000} \text{ g/100}$$

A, the standard absorbance
C,the cyanomethaemoglobin standard concentration (57.2 mg/100 ml); and D, the dilution factor; 1000 converts from mg/100 ml to g/dl.

The Packed Cell Volume (PCV)

The standard microhematocrit method was used in determining the PCV as described in Baker *et al*., (1998). The PCV or the haematocrit measures the relative mass of erythrocytes present in a whole blood. The blood was collected in sample containing K_2EDTA. The sealed haematocrit tube is placed and centrifuged in at 12000 *g* for 10 minutes. The PCV is subsequently determined by measuring the height of the erythrocyte column and expressing this as fraction of the total blood volume. That is:

$$\text{PCV} = \frac{\text{Height of the packed cell column}}{\text{Height of whole blood column}}$$

The Mean Cell Hemoglobin Concentration (MCHC)

The MCHC refers to the amount of haemoglobin in 100 ml of packed red cells, as opposed to the amount of haemoglobin present in whole blood. It is calculated from the haemoglobin and PCV as follows:

$$MCHC = Hb / PCV$$

Effect of the Alkaloidal Extract on the Total and Differential White Blood Cell Count

The total white blood cell count was estimated according to the method of Baker *et al*, (1998) using the improved Neubauer counting chamber.

Statistical Analysis

Results were expressed as mean ± Standard Error of Mean (SEM) using SPSS version 12.00. Data were analysed using one way analysis of variance (ANOVA) and LSD post Hoc test. Difference between mean values of the treated groups were compared to the control, ands regarded as significant at $p < 0.05$.

RESULTS

Phytochemical Analyses

The alkaloidal extract contained other constituents in small amounts but had more of the alkaloidal tannates (Tables 1 and 2).

Effect of the Alkaloidal Extracts on Acute Inflammation

Oral administration of the extract significantly ($p < 0.05$) suppressed the development of acute edema of the rat paw induced by egg abumin at the 3 doses tested. The extract evoked a dose-related effect with the higher dose (200 and 400 mg/kg) exhibiting the highest inhibition which was comparable to Asprin (100 mg/kg) (Table 3).

Effects of the Alkaloid Extracts on the Hb, PCV and MCHC during Inflammation.

The haemoglobin, PCV and MCHC indicated a non significant ($p > 0.05$) decrease after induction of the inflammation. The PCV showed a dose dependent increase with the ALKE 400 mg/kg giving a better stability. There was a dose-dependent decrease in MCHC level (Table 4).

Effects of the Alkaloid Extracts on Total White blood cells and its differentials .

The total white blood cell was non significantly increased ($p > 0.05$) in all the treated groups (Table 5). There was an overall increase ($p > 0.05$) in the eosinophils, lymphocytes and neutrophils in all the treated groups (Table 5).

Table 1. The Phytochemical Constituents of the Alkaloidal Extract.

Phytoconstituents	Alkaloid Extract
Alkaloids	++
Terpenoids	+
Steroids	+
Acidity	-
Glycosides	+
Resins	+
Carbohydrates	+
Saponins	++
Tannins	++
Flavonoids	-
Fats and oil	+
Reducing sugar	-
Protein	-
Vitamin E	++
Vitamin A	+

- Absent, + small amount, ++ moderately high, +++ very high

Table 2. Specific Alkaloid in the Alkaoidal Extract

Phytoconstituents	Alkaloidal extract
Quinoline Alkaloids	-
Tropane Alkaloids	-
Purine Alkaloids	-
Isoquinoline Alkaloids	-
Indole Alkaloids	-
Alkaloidal Tannates	+++

- Absent, + small amount, ++ moderately high, +++ very high

Table 3: Effect of Extract on Egg Albumin-induced Acute Oedema in Rat

Treatment Group/Time	**Edema (ml) (% Inhibition of edema)**				
	1h	2h	3h	4h	24 hr
DW (10 ml/g)	0.41±0.02	0.43±0.02	0.43±0.01	0.45±0.03	0.45±0.04
AsA 100 mg/kg	0.25±0.03* (39.02%)	0.27±0.03* (37.21%)	0.29±0.07* (32.56%)	0.30±0.05* (33.33%)	0.31±0.02* (31.11%)
ALKE 100 mg/kg	0.35±0.02* (14.63%)	0.36±0.03* (16.28%)	0.38±0.06* (11.63%)	0.40±0.04* (11.11%)	0.35±0.05 (22.22 %)
ALKE 200 mg/kg	0.32±0.01 (21.95%)	0.34±0.01 (20.93%)	0.35±0.02 (18.60%)	0.33±0.03 (26.67%)	0.32±0.01 (28.89 %)
ALKE 400 mg/kg	0.31±0.01 (24.39%)	0.32±0.00* (25.584%)	0.32±0.01* (25.584%)	0.27±0.01* (26.67%)	0.25±0.03* (40.00%)

* $p < 0.05$ compared to control, (n = 5); DW=Distilled water, ALKE =Alkaloid extract; Values in parenthesis represent percent inhibition of edema

Table 4: Effects of the ALKE on some Haematological Parameters before and after Inflammation

Treatment Group.	Hb level (g/dl) before inflammation.	Hb level (g/dl) after inflammation	PCV level (%) before inflammation.	PCV level (%) after inflammation.	MCHC level (%) before inflammation.	MCHC level (%) after inflammation.
DW (10 ml/g)	17.32±0.97	15.44±1.05	38.00±4.01	36.00±4.05	45.58±4.03	42.88±3.98
Asp 100 mg/kg	16.34±1.01	13.40±0.89	36.00±5.22	34.00±5.12	45.39±5.21	39.41±4.93
ALKE 100 mg/kg	15.20±1.01	14.05±1.12	44.00±4.41	30.00±3.23	34.55±4.01	46.83±3.56
ALKE 200 mg/kg	17.32±1.21	15.36±0.98	42.00±3.67	34.00±4.01	41.24±3.45	45.18±3.87
ALKE 400 mg/kg	18.47±1.12	14.22±1.07	42.00±5.61	41.00±3.63	43.98±4.01	34.68±4.82

Values of percentage and/or concentration are shown as Mean ± SEM (n = 5).

Table 5: Effect of the ALKE on total White Blood Cell and its differentials (%/cmm*)* before and after Inflammation

Treatment Group	Eosinophil level before Inflammation	Eosinophil level after Inflammation	Monocyte level before Inflammation	Monocyte level after Inflammation	Lymphocyte level before Inflammation	Lymphocyte level after Inflammation	Neutrophils count before Inflammation	Neutrophils count after inflammation	Total WBC before inflammation	Total WBC after inflammation.
ET_{10}	1.60±0.40	1.80±0.20	2.60±0.25	3.80±0.37*	26.50±2.32	64.40±4.79*	26.40±2.14	30.00±2.77	4020.00±386.52	8080.00±658.33*
Asp 100 mg/kg	1.60±0.25	1.80±0.37	2.40±0.25	2.60±0.25	26.20±2.08	55.20±6.22	26.00±2.72	56.80±8.22*	4100.00±404.97	4900.00±632.46
ALKE 100 mg/kg	2.00±0.00	2.00±0.00	2.60±0.25	2.80±0.37	28.20±2.58	56.60±4.31*	23.80±2.52	39.60±2.04*	3580.00±231.08	4420.00±558.93
ALKE 200 mg/kg	1.80±0.20	2.00±0.32	2.20±0.20	2.40±0.25	28.60±3.12	57.60±5.03*	28.40±2.48	39.00±2.63*	4080.00±404.23	5260.00±847.70
ALKE 400 mg/kg	1.20±0.20	1.80±0.20	2.20±0.20	2.40±0.25	33.60±2.98	50.80±2.35*	25.40±2.74	43.60±2.98*	3780.00±307.25	4980.00±959.37

*Significant at $p < 0.05$ compared to control (ANOVA). Values of percentage and/or concentration are shown as Mean ± SEM (n = 5).

DISCUSSIONS

Acute inflammatory reactions are physiological characteristics of vascularized tissues and increased vascular permeability associated with it. It is known to cause exudation of fluid rich in plasma protein including immunoglobulin, coagulation factors and cells into the injured tissue with subsequent oedema at the site (Cotran *et al*., 1999). Studies have reported that alkaloids are effective in treating intestinal inflammatory disorders (Lv *et al.*, 2015, Chen *et al*., 2017a, 2017b, Fu *et al*., 2017, Zhang *et al*., 2018)

Alkaloids are known as one of the largest classes of secondary metabolites with basic properties that make them particularly pharmacologically active. Several studies have reported the antidiarrheal (Yasmeen *et al*., 2010), antibacterial (Karou *et al*., 2006), anti-inflammatory and anti-infective (Vieira, 2010) activities of alkaloids. The effect of the extract on the phlogistics agent-induced acute edema indicates its efficacy in the initiated, enhanced, or coordinated inflammatory events by the actions of various chemical mediators. Alkaloids have been shown to improve DSS-induced colitis by reducing the disease activity index, alleviating inflammatory cell infiltration, inhibiting MPO activity and cytokines (TNF-α, IFN-γ, IL-1β, IL-6, and IL-4) production, upregulating the expression of tight junction (TJ) proteins (zonula occludens-1, zonula occludens-2, claudin-1, and occludin) and mRNA expression of mucins, and decreasing the Bax/Bcl-2 ratio (Yu *et al*., 2018).

Although pathological conditions can alter surface-volume ratio of the cell through loss of membrane surface or gain in volume, the physical integrity of the treated cell membranes may have been enhanced by the specific and/or interacting bioactive agent of the extract to hinder cell lysis including that caused by products such as those of the complement system involved in the inflammatory response cascade that cause the cell to swell and rupture (Rowman, 1996).

Important functions of certain WBCs include, but not limited to, destruction of virus-infected cells, directing the immune response through cytokine secretion, secretion of antibodies for phagocytosis detection (lymphocytes), destruction of pathogens by phagocytosis (neutrophils), and transformation into macrophages (monocytes) (Pritchett and Reddy, 2015). It is important to note that elevations in WBCs can represent a normal response to infection and wound healing (Labrecque and Cermakian, 2015). This study indicates significant increase in neutrophils and lymphocytes after the inflammation. Neutrophils play an important role in the mediation of inflammation. These cells, previously regarded as solely generators of enzymes and thus contributors of enzymatic killing mechanisms against pathogens, are gaining more significance in terms of their role at various stages of inflammation. The non-significant effect of the extract on haemoglobin, packed cell volume and mean corpuscular haemoglobin concentration indicates its efficacy in limiting acute inflammation. WBCs have been considered a reliable cellular biomarker of inflammation (Kounis *et al*., 2015). Chronic elevation of WBCs has been linked to several chronic conditions including Type 2 diabetes, coronary artery disease, stroke, and leukemia (Costas *et al*., 2016; Libby *et al.*, 2016).

In conclusion, the present study suggests that the alkaloidal extract from *Combretum dolichopetalum* is endowed with anti-inflammatory effects and can be useful in managing inflammatory conditions.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests.

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