Qualitative phytochemical screening, anti-inflammatory and haematological effects of alkaloid extract of *Combretum dolichopetalum* leaves

Abstract

Inflammation has been implicated in a number of wounds and disease conditions, spurring the search for compounds with anti-inflammatory properties. The study investigated the anti-inflammatory potentials and effect of alkaloid extract of *Combretum dolichopetalum* on haematological parameters of Wistar albino rats. Alkaloids in the leaves were extracted using 10% acetic acid as solvent. The anti-inflammatory potential was investigated using egg-albumin paw edema model, while the haematological effects of the extract was observed on plasma. Phytochemical screening showed the presence of alkaloids, saponins and tannins I moderate concentration. Alkaloid specific test showed the presence of six classes of alkaloids. Result for anti-inflammatory potentials showed a significant *($p<0.05$)* reduction in paw size of extract treated group compared to negative control. the extract also stabilized haematological parameters compared to negative control. in conclusion, the study confirms the use of the alkaloid extract of *Combretum dolichopetalum* leaves in pain management and inflammatory conditions.

Keywords: Alkaloids, *Combretum dolichopetalum*, phytocompounds, inflammation

INTRODUCTION

The plant *C. dolichopetalum* is known to be a tropical plant that is well pronounce in Nigeria. It belongs to the class Combretaceae. The medicinal and therapeutic role of the plant has been well established in literature (Asuzu *et al.,* 1998; Gugu *et al.,* 2019). The root extract has been reported to be anti-ulcerative due to large amount of tannins and saponins (Asuzu and Onu, 1990). Gugu *et al.,* (2019) reported the use of *C. dolichopetalum* in the management of some tropical disease that are caused by parasites and bacteria.

Inflammation is associated with many infections and injuries. it is a part of the system response to harmful stimuli. Combating inflammation is critical to treating many disease conditions. Most anti-inflammatory drugs target the production of prostaglandins *via* inhibiting the activity of cyclooxygenase (Onoja *et al.,* 2017).

Studies by Asuzu *et al.,* (1988) reported the anti-inflammatory potentials of root extract of *C. dolichopetalum* and propose a mechanism of action to be through shutting down cox-2 activity. however, little is known about the anti-inflammatory potentials of extract of other parts of the plant such as leaves, stems, flowers etc. The main purpose of the study was to investigate the anti-inflammatory and haematological effects of alkaloid extract of Combretum *dolichopetalum* leaves.

MATERIALS AND METHOD

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).

Plate 1: leaves of *Combretum dolichopetalum*

Alkaloid extract preparation

The alkaloid extract was prepared as described by Harborne (1973). Briefly, air-dried leaves (750 *g*) of *Combretum dolichopetalum* was weighed into a 2500 *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 complete. The whole solution was allowed to settle and the precipitate was

collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid extract, which was dried and weighed.

Phytochemical analyses

Qualitative phytochemical screening and test for specific alkaloids (quinoline alkaloids, tropane alkaloids, purine alkaloids, isoquinoline alkaloids, indole alkaloids, alkaloidal tannates, were performed as described by Harborne, 1984; Sofowora, 2008).

Animals

Twenty-five albino rats (90- 120 *g*) bred in the animal house of Faculty of Biological Sciences and Veterinary Medicine were used for the study. They were acclimatised in the animal house for two weeks with free access to water and feed before the start of the experiment. The animals 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. Before commencement of study, ethical approval was sought from the Ethical Committee, Department of Biochemistry, Faculty of Biological Sciences, University of Nigeria, Nsukka, Enugu State, Nigeria.

Experimental design and biochemical analysis

Rats in group A were administered distilled water and inflammation was induced (n=5) (negative control)
Rats in group B were administered 100mg/kg aspirin and inflammation was induced (n=5) (positive control)
Rats in group C were administered 100mg/kg ALKE and inflammation was induced (n=5)
Rats in group D were administered 200mg/kg ALKE and inflammation was induced (n=5)
Rats in group E were administered 400mg/kg ALKE and inflammation was induced (n=5)

The animals were fasted for 12 hours and deprived of water only during the experiment. Deprivation of water was to ensure uniform hydration and to minimise variability in edematous response. The animals were treated with the different test compounds as indicated above orally, and one hour after, the sub plantar injection of the phlogistic agent (egg albumen 0.05ml) was performed. At the end of experiment, blood samples were collected from the retro-orbital venous plexus of the eye into EDTA bottle. Blood samples were spun at 3000rpm for 10minutes to obatin plasma used for haematology analysis. Anti-inflammatory effect of the alkaloid extract was performed as described by Winter *et al.,* (1962), the percentage inhibition of edema (Ahmed *et al.,* 1993; Perez 1996) was calculated using the formula.

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

Where Vt is the volume of edema at corresponding time, and Vo is the volume of edema of vehicle treated rats at the same time.

The Haematological effects of the alkaloid extract were analysed using blood. Haemoglobin estimation and pack cell volume (PCV) were performed as described by Baker and Silverton (1985). The mean cell haemoglobin concentration (MCHC) was calculated from

haemoglobin and PCV value. The total white blood cell count was performed as described by Baker and Silverton (1985) using the improved Neubauer counting chamber.

Statistical analysis

Results were expressed as mean ± Standard Error of Mean (SEM) using SPSS version 22. Data obtained 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 controls regarded significant at $p<0.05$.

RESULTS

Table 1: The phytochemical constituents of the extract.

Phytoconstituents	Alkaloid Extract (ALKE)
Alkaloids	++
Terpenoids	+
Steroids	+
Glycosides	+
Resins	+
Saponins	++
Tannins	++
Flavonoids	-

- implies absent; + implies present in small amount; ++ implies present in moderately high concentration

Table 2: Specific alkaloids in the alkaloid extract The phytochemical constituents of the extract.

Phytoconstituents	Alkaloid Extract (ALKE)
Quinoline alkaloids	++
Tropane alkaloids	+
Purine alkaloids	+
Isoquinoline alkaloids	+
Indole alkaloids	+
Alkaloidal tannates	++

- implies absent; + implies present in small amount; ++ implies present in moderately high concentration

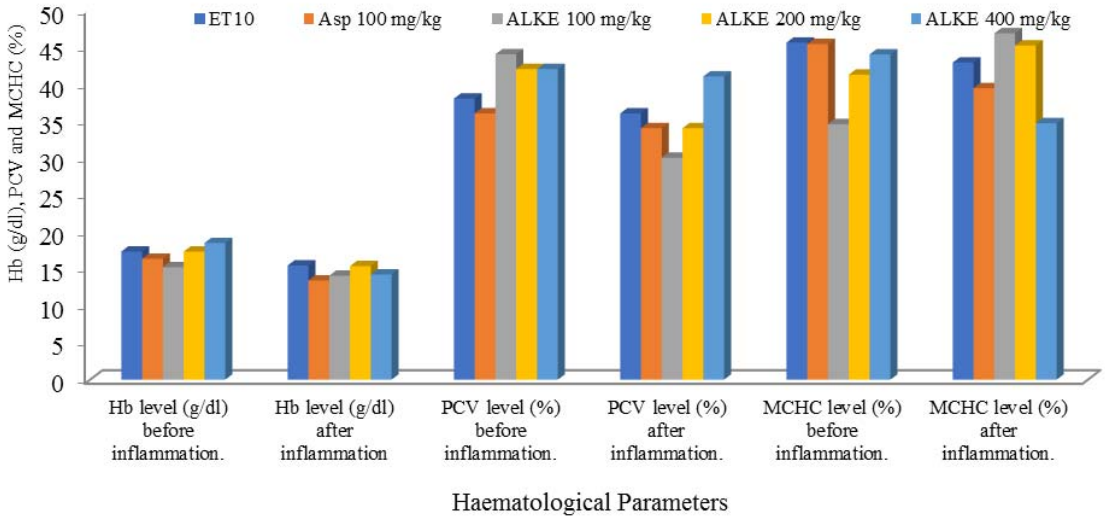
Table 3: Effect of extract on egg albumin-induced acute edema of the rat

Treatment	**Mean increase in paw volume (ml) + SEM (% Inhibition of edema)**				
Group/Time	1h	2h	3h	4h	24h
DW 5mg/ml	0.41±0.02 (-)	0.43±0.02 (-)	0.43±0.01 (-)	0.45±0.03 (-)	0.45±0.04 (-)
ASP 100mg/kg	0.25±0.03*,y (39.02%)	0.27±0.03*,y (37.21%)	0.29±0.07*,y (32.56%)	0.30±0.05*,y (33.33%)	0.31±0.02*,y (311.11%)
ALKE 100mg/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*,y (24.39%)	0.32±0.00*,y (25.584%)	0.32±0.01* (25.584%)	0.27±0.01* (26.67%)	0.25±0.03* (40.00%)

*significant at $P < 0.05$ compared to control, ʸsignificant at $P < 0.05$ compared to test groups. ALKE =Alkaloid extract; Values in bracket represent percent inhibition of edema calculated relative to the control. (DW- Distilled water).

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 tested doses. The effect was dose depended, with the highest does exhibiting the highest level of inhibition, comparable to standard.

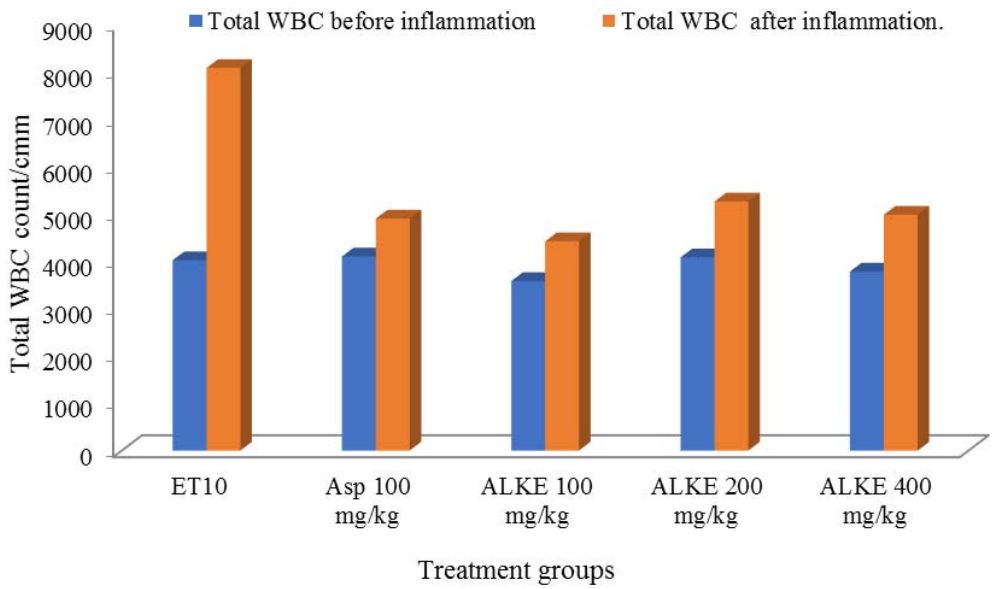
Figure 1: Effects of alkaloid extract on the Hb, PCV and MCHC.



Haemoglobin, PCV and MCHC values showed a non-significant ($p>0.05$) decrease in test groups before induction of inflammation compared to controls (negative and positive). The

haemoglobin were better stabilised with the extracts compared to the negative control. The PCV showed a dose dependent increase with the ALKE 400 *mg/kg* indicating a better stability than all the treated group. The MCHC however indicated a reverse in dose dependent response in the overall effect.

Figure 2: Effects of Alkaloid extract on the total White Blood cell counts



The total white blood cell showed a significant increase ($p<0.05$) in all the negative control compared to other groups. This significant increase indicates the extract marked effect in reducing the elevated levels of blood white cells. This trend is also similar with the monocytes as indicated in the white cell differentials. There was insignificant increase in the eoinophils after the inflammation. However there were an overall increase in the lymphocytes and the neutrophils in all the treated groups after the inflammation compared to the their overall level before the inflammation.

Figure 3: Effects of Alkaloid extract on the lymphocytes and neutrophil counts

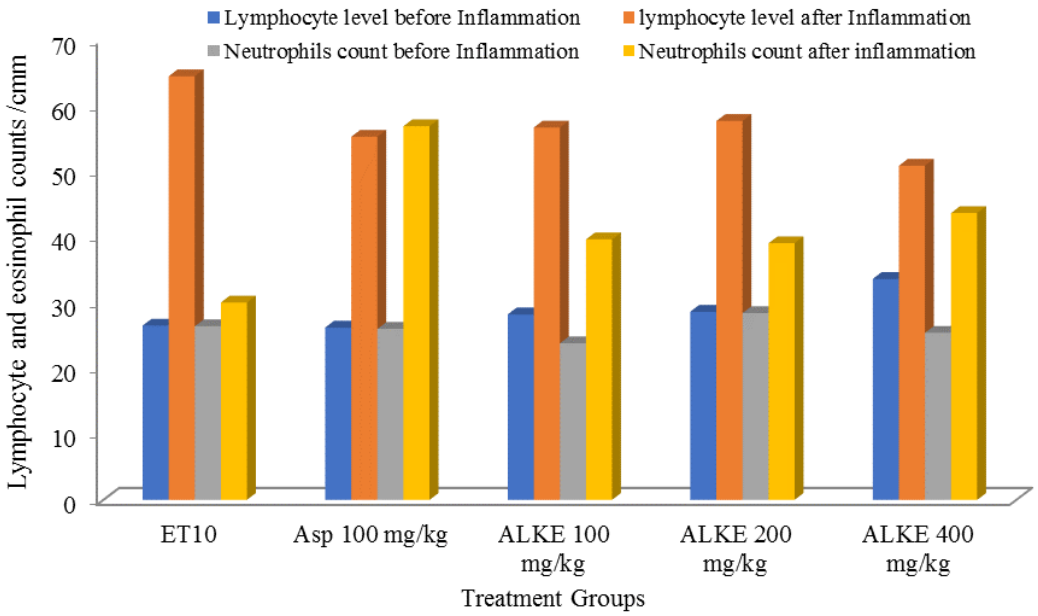
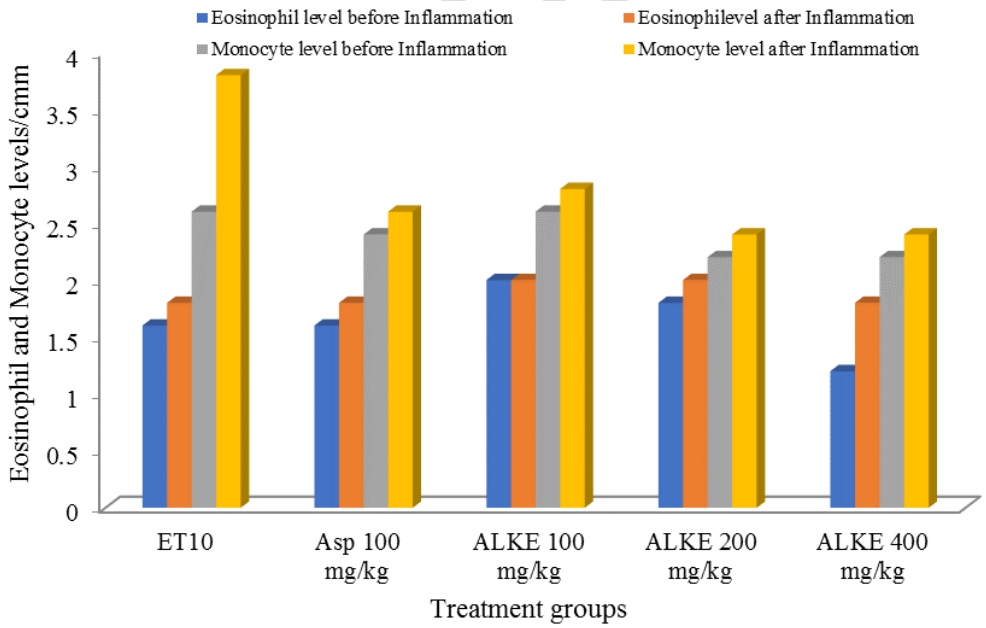


Figure 4: Effects of Alkaloid extract on the monocytes and eosinophil counts



Discussion

Alkaloids are considered one of the largest classes of secondary metabolites, with therapeutic potentials present in all plant species (Husein and El-Anssary, 2018). The phytochemical analysis from this study showed that the extract was rich in tannins, saponins and alkaloids, while flavonoids were absent. This agrees with the findings of Gugu *et al.* (2019) who reported high concentration of tannins and saponins in extract of *Combretum dolichopetalum* leaves extract. The specific alkaloid test showed the presence of quinoline alkaloids, tropane alkaloids, purine alkaloids, isoquinoline alkaloids, indole alkaloids and alkaloidal tannates. Several studies have reported anti-diarrhoeal (Yasmeen *et al*., 2010), antibacterial (Karou *et al,* 2005), anti-inflammation (Bribi *et al.,* 2015) and anti-infection (Vieira, 2010) activities of alkaloids.

The significant ($p<0.05$) effect of dose-dependent reduction of the paw size caused by the phlogistic agent indicates the extract anti-inflammatory potentials against inflammatory events, and this was corroborated by results of the haematological assay performed. Earlier mentioned, Bribi et al. (2015) reported the anti-inflammatory properties of alkaloids, specifically the quinoline, indole and isoquinoline alkaloids, and these compounds were present in the extract used for this study. The alkaloids in the extract may have inhibited the activity of cyclooxygenase (COX), an enzyme involved in the synthesis of prostaglandins a key inflammatory mediator (Alam *et al.,* 2009; Bribi *et al.,* 2015), and this is similar to the mechanism of action of aspirin which involves the irreversible blockage of COX activity by acetylation of a specific serine hydroxyl group, thus inhibiting the production of prostaglandins used in this study (Balakumar *et al.,* 2010).

It is well established that egg-albumin induces inflammation via stimulation of inflammatory mediators and enhancers such as cytokine, histamine, prostaglandins, polymorphonuclear leucocytes and thromboxane in the tissue and increased vascular permeability (Silva *et al.,* 2014). In this study, the extract could have inhibited inflammation via the inhibition of production of inflammatory factors and this has earlier been reported by Onoja *et al.,* 2017, as a potential mechanism of action. The standard anti-inflammatory drug used in this study is believed to carry out the irreversible blockage of cyclooxygenase (COX) activity thereby shutting down the synthesis of prostaglandins (Onoja *et al.,* 2017). Although pathological circumstances can alter surface-volume ratio of the cell via membrane surface loss or gain in volume, the physical integrity of the cell membrane of the extract treated groups may have been enhanced by the specific and/or interacting bioactive agent of the extract (through a direct protective interaction with the membrane proteins) to hinder cell lysis including that caused by products such as those of the complement system involved in the inflammatory response cascade and hypotonic solutions that cause the cell to swell and rupture (Rowman, 1996).

Haematological parameters are widely used to access stress associated with certain factors such as environmental, nutritional and/or pathological factors (Adeyemo, 2007). This study indicates significant ($p<0.05$) increase in neutrophils and lymphocytes after the inflammation event among all the analysed parameters. Neutrophils play an important role in the mediation of inflammation. These cells are useful in combating pathogens (enzymatic killing mechanisms), and are gaining more significance in terms of their role at various stages of inflammation. In addition to the recent findings on the generation of lipid mediators of

inflammation, persistent activity of neutrophils also contributes to the tissue destruction. Through this mechanism, the protective role of these cells can turn into a deleterious action targeting it host (Kantarci *et al.,* 2003). They can also give rise to neutrophil-dependent vascular injury and contribute to increased vascular permeability, edema, and further release of chemoattractant, with a net pro-inflammatory effect (Pouliot *et al.*, 2000). Lymphocyte subsets (eg monocyte derived macrophages) are known to be controlling immune responsiveness in the gut and their mechanisms of control, which involve maintenance of intestinal barrier function and suppression of chronic inflammation. They also play a nonredundant role in the maintenance of intestinal homeostasis through IL-10- and TGF-β-dependent mechanisms. Their activity is complemented by other T and B lymphocytes. Because breakdown in immune regulatory networks in the intestine leads to chronic inflammatory diseases of the gut, regulatory lymphocytes are an attractive target for therapies of intestinal inflammation (Izcue *et al,* 2009). Although an infection caused parasite with large intestine inflammation manifested by permanent bloody mucoid diarrhoea that are probably responsible for the observed haematological changes in the animals (The extract insignificant decrease in effect on haemoglobin, packed cell volume and mean corpuscular haemoglobin concentration indicates the comparable efficacy with aspirin in limiting acute inflammation. The decrease could be associated with partially anaemic condition with dehydration. The occurring changes in total WBC counts and the percentages of WBC classes during the period of the study on the negative control could be explained by the suppression of the mechanisms of systemic non-specific defence against the inflammation event.

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