ANALGESIC PROPERTIES OF AQUEOUS LEAF EXTRACT OF *Alchornea cordifolia* (Christmas bush) ON WISTAR RATS

ABSTRACT

Analgesic effects of aqueous leaf extract of *Alchornea cordifolia* on wistar rats were investigated. Twenty wistar rats of both genders weighing between 110-178g were utilized. Acetic acid was used for induction of pain. The rats were grouped into five groups of 4 per group in each study. Groups 3, 4 and 5 received 400, 800mg/kg b.w of aqueous extract and reference drug respectively after induction. Groups 1 and 2 served as normal and negative controls. Rats were sacrificed and blood samples collected for hematological and biochemical analyses. Phytochemical screening of the plant revealed the presence of alkaloid, phenolic compounds, Tannins and Quinine. The result showed significant increase ($p \leq 0.05$) in paw size, and number of writhing in group 2 and non-significant reduction ($p \geq 0.05$) in group 4 were seen when contrasted to group 1 and 2 respectively. Highest pain inhibition (16%) was seen in group 4. Significant ($p \leq 0.05$) increase in C-reactive protein and nitric oxide concentrations were observed in groups 3 and 4 when contrasted to group 2 in the study. Non-significant differences in all hematological parameters in all treated groups were observed when compared to group 2. Aqueous leaf extract of *Alchornae cordifolia* displayed an analgesic effect.

1. INTRODUCTION

Disease conditions leading to inflammation, pain and pyrexia are major healthcare problem worldwide. Incidences of pain and inflammation-related disorders have been on the increase worldwide. Although, several synthetic agents are available in treatment of these disorders, long-term usage has been reported by Qandil [1] to potentially lead to the undesired effect.

Plants have a reputation as remedies in ethnomedicine for the management of pain (Sofidiya *et al.,* 2014). Extracts from plant reportedly possess vast secondary metabolites with myriad biological activities (Chung *et al.,* 2010). Plants are an interesting focal point for novel analgesic compounds. This necessitates the need for this study which was designed to evaluate the analgesic properties of aqueous leaf extract of *Alchornea cordifolia* on wistar rats. Synthetic analgesics drugs usage have been gaining popularity in the last few decades due to the physical, mental and exhaustive nature of work and occupation of many people especially in impoverished countries. Studies have established that prolonged usage of over the counter and prescription analgesic and anti-inflammatory medication are linked to side effects which ranges from mild (such as irritation and nausea) to severe (such as Renal Failure, Respiratory Distress, Hepatotoxicity, Gastric and Duodenal ulcers, addiction amongst others) adverse effect (Marret *et al.,* 2005). Some of these effects may be temporal or permanent. Therefore, finding un-toxic and efficient drug to lower or manage pain remains a burden. Few years past, phytochemicals were the main source for evaluating analgesic properties of plants, some (phytoconstituents) of which are currently looked at as components (bioactive) for analgesic,actions (Kalpesh *et al.,* 2019).

Alchornea cordifolia commonly known as Christmas bush is a vertical shrub or a perennial small tree with a lightly granulated bark, greyish and woody stem and a simple, alternate heart shaped based leaves (Olaleye *et al.,* 2006). Reported as an evergreen small tree from the *Euphobiaceae* family, Boniface *et al.* (2016) opined that the plant parts are ethnobotanically used in management of myriad ailments.

Its medicinal properties have been reported by Boniface *et al.* (2016) to include anti-diabetic, spasmolytic, anti-bacterial and anti-microbial. Over the years, more revelations on the medicinal potentials of *Alchornea cordifolia* including the Anti-*Helicobacter Pylori* induced-gastric ulcers (Adeyemi *et al.,* 2008). Report by Agrawal *et al.* (2003) suggest that *Alchornea*

cordifolia leaf alongside leaves from *Boerhavia Diffusa* plants possess Antifungal property although the level of this property is yet to be fully understood. The leaves, bark and roots have been reportedly used in ethnomedicine by the Urhobo people of Nigeria in curing conjunctivitis, amoebic dysenteries and venereal diseases (Boniface *et al.*, 2016). Hence, given the myriad spectrum of ethnobotanical applications and reported multiple pharmacological properties attributed to *Alchornea* cordifolia as a medicinally important plant, this research was designed to further investigate the biological activities of this specie by evaluating anti-inflammatory, anti-pyretic and analgesic properties of aqueous leaf extract on wistar rats.

2. MATERIALS AND METHODS

Study design

The Wistar rats were purchased from the Department of Pharmacy, Faculty of Pharmaceutical Science, University of Port Harcourt, Rivers state State, Nigeria. They were housed in different cages by groups with renewable bedding, and were fed with standard rat feed and clean water, allowing them to acclimatize for fourteen days under normal temperature, humidity and light-dark cycle. After acclimatization, the animals were weighed and divided into 5 groups for analgesic study.

Table 1: Experimental design for analgesic study

Groups	Description	Dose
Group 1	Normal control	No induction

Group 2	Negative control	1% acetic acid
Group 3	*Alchornea cordifolia* aqueous extract	1% acetic acid + 400mg/kg
Group 4	*Alchornea cordifolia* aqueous extract	1% acetic acid + 800mg/kg
Group 5	Reference drug	1% acetic acid + 50mg diclofenac (It is mandatory to change the drug to aspirin as the researcher has used aspirin in the study.)

Plant Collection and Identification

The fresh leaf of Alchornea Cerdifolia *cordifolia* was obtained from Ekrejeta in Abraka in Ethiop East Local Government area of Delta State, Nigeria. After collection, the plant was sent to the Department of Plant Science and Biotechnology, University of Port Harcourt, where it was properly identified.

Extract Preparation

The leaves were properly washed in running tap H_2O water and allowed to air dry, and blend into powder form. 25g of the powdered powder was macerated in 100ml of deionized water for 24 hours under mechanical agitation at room temperature. The suspension was filtered using whatman filter paper and dried in a water-bath at approximately 55 ^oC. Crude extract gotten was store at 4 ^oC.

Haematological analysis

Total WBC count

Method: Improved Neubauer ruled chamber

Principle: Whole blood is diluted 1 in 20 in an acid reagent which haemolyzes the red cells leaving the white cells to be counted.

Reagents:

i. Turk's solution.

Procedure: White blood cell count was done manually with the use of counting chambers. 0.38ml of Turk's solution was aliqouted aliquoted into a plain bottle, 20ul of whole blood collected with an EDTA bottle, was pipetted to each bottles bottle, it was mixed gently for sedimenting. The counting chamber was covered with a cover slide and the edges were sealed of distilled water. The solution in the plain bottle was then charged into the counting chamber at an angle of 45°, it was mounted on the microscope and focused using X40 magnification, the cells were accurately counted and the value was then multiplied by 50 and divided by 1000 to get the actual white blood cell count. This was done for all the samples

White blood cell differential count

Method: Leishman stain (Leishman, 1901)

Principle: Methanolic mixture-based of Methylene blue and eosin. The methanolic stock solution is undisturbed serving the intention of directly fixing the smear thereby eliminating a prefixing step.

Reagents:

i. Leishman's stain

Procedure: A thin film was made on a microscope slide for each sample it was allowed to air dry. It was stained with Leishman's stain for 2-3 minutes, after which, it was diluted with distilled water for another 2 minutes then allowed to air dry. A drop of oil immersion introduced and mounted on the microscope using X100 magnification to count the different differential cells (neutrophils, lymphocytes, monocytes, eosinophils and basophils). The cells were noted and documented. This was replicated for all collected samples.

Data Analysis

Laboratory data were analyzed with statistical packages for Social sciences (SPSS version 20). Values were reported as mean plus-minus standard error of mean employing analysis of variance (ANOVA) and least significant difference (LSD) for multiple comparison.

3. RESULT AND DISCUSSION

Table 2. Phytochemical Analysis of Alcharnia carclifolia Alchornea cordifolia (urbobo)

Alkaloid	**positive**
Phenolic_compound	**positive**
Tannins	**positive**
Flavonoid	**negative**
Saponnin	**positive**
Quinine	**Positive**
Coumarin	**negative**
Protein	**negative**
Cardiac glycoside	**negative**
Steroid	**negative**

Table 3: **Effect of oral administration of aqueous leave extract of *Alchornea cordifolia* on acetic acid induced writhing in wistar rats**

Group	Treatment	Average number of Writhing	Percentage Inhibition (%)
1	Control (0.5ml distilled water)	0.00±0.00[a]	-
2	Control (-ve) 1% acetic acid	4.50±1.55[a]	-
3	1% acetic acid + 400mg/kg *Alchornea cordifolia*	4.25±0.48	5.56
4	1% acetic acid + 800mg/kg *Alchornea cordifolia*	3.75±0.48	16.67
5	1% acetic acid + 300mg/kg Aspirin	4.25±0.48	5.56

Values are reported as mean ± standard error of mean (M±SEM) (n =4). Values with similar superscript letters indicate statistical statistically significant differences ($p \leq 0.05$) down the column while those without superscripts show non-significant differences ($p \geq 0.05$) down the column when compared with the control and between groups.

Table 4: Effect of aqueous leave extract of *Alchornea cordifolia* on interleukin-6, C-reactive protein and nitric oxide concentration of acetic acid induced-writhing in wistar rats

Group	Treatment	Interleukin-6 (pg/ml)	C-reactive protein (mg/l)	Nitric Oxide (mg/ml)
1	Control (0.5ml distilled water)	249.75±59.79	0.06±0.00	0.09±0.00
2	Control (-ve) 1% acetic acid	180.05±8.80[a]	0.50±0.25	0.57±0.27
3	1% acetic acid + 400mg/kg *Alchornea cordifolia*	109.03±20.74	0.42±0.13	0.11±0.04[a,b]
4	1% acetic acid + 800mg/kg *Alchornea cordifolia*	80.48±17.80[a]	0.74±0.43	0.77±0.31[a]
5	1% acetic acid + 300mg Aspirin	119.90±0.00	0.05±0.00	0.84±0.00[b]

Values are reported as mean ± standard error of mean (M±SEM) (n =4). Values with similar superscript letters indicate statistical significant differences ($p \leq 0.05$) down the column while those without superscripts show non-significant differences ($p \geq 0.05$) down the column when compared with the control and between groups.

Table 5: Effect of aqueous leave extract of *Alchornea cordifolia* on some haematological parameters of acetic acid induced-writhing in wistar rats

Group	Treatment	ESR (mm/hour)	WBC (X 10^9/L)	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)	Basophils (%)

1	Control (0.5ml distilled water)	0.00±0.00 value required	9.00±0.24	40.00±1.22	48.50±0.61	5.00±0.41[a]	1.50±0.20[a]	0.00±0.00
2	Control (-ve) 1% acetic acid	11.50±1.43[a]	13.15±0.27	39.00±0.41	40.00±2.04[a]	16.50±3.06[a]	4.50±0.61[a]	0.00±0.00[a]
3	1% acetic acid + 400mg/kg *Alchornea cordifolia*	30.00±11.02[a,b]	21.60±7.60	32.75±2.17[a]	51.50±3.40[a,b]	12.25±2.56	2.50±0.65	0.75±0.25[b]
4	1% acetic acid + 800mg/kg *Alchornea cordifolia*	28.00±7.48[b]	12.70±2.78	40.25±4.48	43.75±3.94	10.00±1.41[a]	4.75±1.18	1.75±0.63[a,b,c]
5	1% acetic acid + Aspirin	9.33±1.89[b]	11.80±1.53	46.00±2.27[a]	42.00±3.56[b]	7.67±1.18[a]	3.67±1.03	0.50±0.29[c]

Values are reported as mean ± standard error of mean (M±SEM) (n =4). Values with similar superscript letters indicate statistical significant differences ($p \leq 0.05$) down the column while those without superscripts show non-significant differences ($p \geq 0.05$) down the column when compared with the control and between groups

4. Discussion of findings

Writhings generated by parenteral administration of acetic acid in rodents is due to profound pain of endogenous nature which recurs over a time period (Shivaji, 2012). Investigation on the analgesic properties of *Alchornea cordifolia* (Table 3- 4) showed a non-significant decrease ($p \geq 0.05$) in the number writhing in the 400 and 800mg/kg *Alchornea cordifolia* treated groups, implying ability of *Alchornea cordifolia* to have potential analgesic properties. This finding agrees with Nsonde *et al.* (2020) who reported a significant analgesic effect with 400 and 800mg/kg of *Alchornea cordifolia* leaves extract. The analgesic effects of *Alchornea cordifolia* could be attributed to alkaloids and terpenes reportedly found in the leaf.

Aspirin is an analgesic used in management of pain. The study found that *Alchornea cordifolia* extract at 800mg/kg treated group showed non-significant reduction ($p \geq 0.05$) in writhing display compared with aspirin treated group, implying extract ability to block signals transmitted to brain and spinal cord in reaction to pain due to irritation, which causes release of mediators such as prostaglandins (Shivaji, 2012) thereby elevating to nociceptors sensitivity.

Furthermore, the analgesic investigations (Table 5) shows greater percentage inhibition at 800mg/kg against 400mg/kg *Alchornea cordifolia* and aspirin treated groups, which is an indication of dose-dependent analgesic effect in conformity with Nsonde *et al.* (2020) findings.

The study also observed decreased decrease in interleukin-6, C-reactive protein and NO in the acetic acid-induced animals after dosing with *Alchornea cordifolia* aqueous leaf extract at 400 and 800mg/kg b.w. The ESR, WBC, lymphocytes levels were significantly increased (p

≤ 0.05). However, neutrophils monocytes and eosinophils levels revealed non-significant decrease ($p \geq 0.05$).

Conclusion

This research findings indicates indicate that *Alchornea cordifolia* at 400 and 800mg/kg showed potential analgesic properties.

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