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Anti-inflammatory, Anti-Nociceptive and antipyretic activity of *Acalypha wilkesiana* hydro-methanolic leave extract: A non-invasive investigation

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Abstract
Acalypha wilkesiana is an evergreen shrub usually planted around homes for horticultural purposes. This study investigated the potential ameliorating effect of *A. wilkesiana* leaf extract in inflammatory, pyretic and pain-induced conditions. Sixty five (65) male wistar rats with average weight of 205 grams were sampled randomly in three separate treatment protocols. First treatment include 5 groups *a* to *e* administered Normal saline, aspirin (0.9mg/ml), 40mg/kg-AW, 80 mg/kg-AW, 160 mg/kg-AW respectively; second and third treatments *a* to *e* were; Normal saline, Piroxicam, 40mg/kg-AW, 80 mg/kg-AW, 160 mg/kg-AW respectively. The extract caused significant changes ($P<0.05$) similar to aspirin and piroxicam on pyrexia and inflammation, but showed no tendency of ameliorating pain. From this investigation, *A. wilkesiana* leaf extract has significant anti-inflammatory and anti-pyretic phytotherapeutic tendency, and this effect may be dependent on the dose of treatment.

Keywords: *a. wilkesiana*; phytotherapy; aspirin; piroxicam; extract

Introduction
Medicinal plants have achieved great popularity in the treatment of various diseases for many centuries. Medicinal plants are plants used for therapeutic purposes [1]. The discovery of these useful plants was as a result of man's inquisitive and inventive nature as well as necessity to feed [2]. The active principles of medicinal plants are to be able to alleviate illnesses based on their physiological and pharmacological actions and uses [1, 2]. These medicines could be any component or part of a plant like fruits, stems and leaves which are known common wellsprings of various bioactive constituents and these attributes are often because of the availability of abundant phyto-active constituents in them [3]. Medicinal plants are classified as; neuroprotective [4], anti-inflammatory [5], anti-nociceptive [6], antipyretic [7], anti-allergic [8], aromatherapeutic [9], anti-diabetic [10], cytoprotective [11], antioxidants [12] as well as antibiotics [13]. A medicinal plant may have multidimensional effects falling into more than one of the classes already mentioned. The relevance of home grown medicinal plants is of worldwide significance because of their practically reduced side-effects when used in treating bodily disorders. Plant based medications have now been considered as reliable and alternative treatment for different infections and ailments. *Acalypha wilkesiana*, commonly called Irish petticoat [1], is native to the south pacific islands [2]. It is a plant of great ornamental value due to its showily colored foliage and is widely cultivated in the tropical and subtropical countries [1, 13]. *A. wilkesiana* is an evergreen shrub usually plantedaround homes for horticultural purposes. The plant may grow up to 3meters high with erect stems and many branches. The genus "*Acalypha*" comprises about 570 species [2]. *A. wilkesiana* belongs to the family *Euphorbiaceae* [14] and grows as an annual bedding plant [3]. This plant has been reported to have antibacterial and antifungal properties as the expressed juice or boiled decoction is locally used within Nigeria and some other parts of West Africa for the treatment of malaria, dermatological and gastrointestinal infections [13, 14]. This study investigated the potential medicinal effect of *A. wilkesiana* leaf extract in inflammatory, pyretic and nociceptive conditions.

Materials and methods
Plant samples
Leaves of *A. wilkesiana* were collected within the Matrices University of Port Harcourt Horticultural Garden. The leaves were authenticated by a plant scientist in same institution.

Extract preparation
Hydromethanolic solvent 2:8 (*v/v*) was used following standard protocols previously described [12].

Study design
Sixty five male wistar rats with average weight of 205 grams were procured from Experimental Animals Unit of Faculty of Basic Medical Sciences, University of Port Harcourt. They were acclimatized for fourteen days before the commencement of treatments.

Table 1: Treatment protocols

Study groups			
Antipyretic		**Analgesic**	**Antin-flammatory**
a	Normal saline	Normal saline	Normal saline
b	Aspirin (0.9mg/ml)	Piroxicam	Piroxicam
c	40mg/kg-AW	40mg/kg-AW	40mg/kg-AW
d	80 mg/kg-AW	80 mg/kg-AW	80 mg/kg-AW
e	160 mg/kg-AW	160 mg/kg-AW	160 mg/kg-AW
	N=5	*N*=5	*N*=3

Key: *AW*= *Acalypha wilkesiana*, *N*=Number of samples in each group.

Experimental Protocols

Analgesic Study

The tail flick model was adopted for the current study. In the tail flick instrument each animal was positioned on an infrared spot (maintained at about 50°C) 35 minutes after treatment with the extract, piroxicam or distilled water had been administered to them orally. The time taken for each rat to respond to the thermal stimulus by retrieving its tail from the infrared spot of the tail flick machine was then recorded.

Anti-inflammatory study

In the evaluation for the anti-inflammatory potential in the present study, the study model were infused with 0.1ml of three percent (3%) formalin on the right hind paw 45 minutes after treatments with either of the extracts, normal saline or piroxicam. The times it took the rat to lick the injected paws in the initial phase (0-5min) and the second phase (30-35 min) and in the third phase (65-70 min) were recorded.

Antipyretic studies

Precisely 1ml/100g body weight of 50% dried baker's yeast suspended in normal saline were injected into the groin area of animals following a 12 hours of fasting and the animals were subsequently administered the extract, normal saline or aspirin orally as the case may be after 18 hours. Thereafter, the rectal temperatures of the animals were watched using a digital thermometer.

Statistical Analysis

Quantitative data obtained from the study were subjected to analysis of variance (ANOVA) followed by least significant (LSD) *Post hoc* test using SPSS v20.0. The values were presented as mean ±standard error of mean (SEM) and Percentage relative changes (%c). P<0.05 was considered statistically significant.

Results

Table 2: Time-dependent Anti-inflammatory effects of *Acalypha wilkesiana*

Groups		Time (minutes)						
		0-5	**%I**	**%AI**	**30-35**	**%I**	**%AI**	**65-70**
a	Control	3.00±0.00	0	-94.3	0.00±0.00	-	-100	0.00±0.00
b	Piroxicam	52.67±0.88 [a]	1656	0	29.00±1.15 [a]	-	0	1.60±0.84 [a]
c	40mg/kg-AW	78.00±0.18 [a, b]	2500	48.1	25.00±0.58 [a, b]	-	-13.8	0.00±0.00 [b]
d	80 mg/kg-AW	45.00±0.58 [a, b]	1400	-14.6	15.00±0.57 [a, b]	-	-48.3	0.00±0.00 [b]
e	160 mg/kg-AW	35.00±0.58 [a, b]	1066	-33.5	8.00±0.58 [a, b]	-	-72.4	0.00±0.00 [b]
N=5								

Values are expressed as Mean ± SEM; n=5; [a] Significant at P<0.05 when compared with control, *a*. [b] Significant at P<0.05 when compared with Group *b*. %I=Percentage inflammatory, %AI =percentage anti-inflammatory, AW= *Acalypha wilkesiana*

Table 2: Time-dependent Antipyretic activity of *Acalypha wilkesiana*

Groups		Rectal Temperatures (^{0}C)					
		Before	**After**				
			30min	**60min**	**90min**	**120min**	**150min**
a	Control	36.64±0.19	36.56±0.17	36.61±0.20	36.70±0.03	36.56±0.17	36.56±0.17
	%AP	0	0	0	0	0	0
b	Aspirin 0.9mg/ml	38.00±0.07 [a]	37.40±0.04[a]	36.80±0.03	36.70±0.05	36.50±0.05	36.30±0.04
	%AP	3.71	2.50	0.52	0	-0.16	-0.71
c	40mg/kg-AW	38.10±0.13 [a]	37.28±0.37	37.10±0.06	37.00±0.07	36.80±0.18	36.60±0.07
	%AP	3.98	2.14	1.01	0.82	0.66	0.11
d	80 mg/kg-AW	37.80±0.50 [a]	37.10±0.40	36.98±0.05	36.80±0.07	36.70±0.07	36.50±0.04
	%AP	3.17	2.02	0.52	0.27	0.38	-0.16
e	160 mg/kg-AW	38.20±0.08 [a]	37.30±0.07	36.80±0.05	36.90±0.20	36.50±0.07	36.30±0.03
	%AP	4.25	2.02	0.52	0.54	-0.16	-0.71
N=5							

Values are expressed as Mean ± SEM; *N*=5; [a] Significant at P<0.05 when compared with the control, *a*. [b] Significant at P<0.05 when compared with Group *b*. %AP =Percentage antipyrexia, AW= *Acalypha wilkesiana*

Table 3: Analgesic effects of *Acalypha wilkesiana* using tail flick apparatus

		Reaction Time (sec)	**%N**	**%AN**
a	Control	5.45±0.01	0	-6.03
b	Piroxicam	5.80±0.19 [a]	6.42	0
c	40mg/kg-AW	7.40±0.05 [a, b]	35.8	27.6
d	80 mg/kg-AW	7.70±0.08 [a, b]	41.3	32.8
e	160 mg/kg-AW	9.48±0.60 [a, b]	73.9	63.4
N=3				

Values are expressed as Mean ± SEM; *N*=3; [a] Significant at P<0.05 when compared with the control, ***a.*** [b] Significant at P<0.05 when compared with Group ***b.*** %N= Percentage nociceptive, %AN =Percentage antinociceptive, AW= *Acalypha wilkesiana*

Anti-inflammatory effect of *A. wilkesiana*

After the first 5 minutes of injection of formalin in the right hind paw, the positive control group *b* showed predominant anti-

inflammatory activity by exhibiting lowest frequency and duration of right hind paw licks. However, after 35 minutes there was a significant reduction in the behavior of interest in groups' c to d compared to group b. The Anti-inflammatory effects of *A. wilkesiana* can be said to be dose and time-dependent.

Antipyretic activity of *A. wilkesiana*

At all doses, *A. wilkesiana* showed similar antipyretic activity to aspirin. A slight decrease in rectal temperature was noticed in all test groups but better similarity exists between Aspirin 0.9mg/ml treated groups and *A. wilkesiana* at 160mg/kg.

Analgesic activity of *A. wilkesiana*

Analgesic response in *A. wilkesiana* was significantly lower than piroxicam-treated groups. When treated with *A. wilkesiana* at doses 40, 80 and 160 mg/kg, a progressively higher reaction time was recorded compared to control.

Discussion

Relatively few studies have investigated the phytomedicinal value of *A. wilkesiana* leaves [15, 16]. In the present study, the anti-inflammatory, anti-nociceptive and antipyretic activities of *A. wilkesiana* hydro-methanolic leave extract were revealed using a non-invasive technique. There were inflammatory, nociceptive and pyretic responses induced by formalin, infrared stimulation and baker's yeast respectively, this was in consonance with a study by Wali, *et al*. 2018 [17]. Flavonoids, alkaloids, tannins, glycosides, steroids and terpenoids are some bioactive agents in *A. wilkesiana* leaves [17, 18]. These agents have been implicated in several phytotherapeutic manifestations [17]. Inflammation is the body's response to injury [19]. The signs and symptoms of inflammation can be uncomfortable but may be regarded as reactions that allow the body to heal itself. Chronic inflammation can eventually cause several diseases and conditions, including some cancers, rheumatoid and Alzheimers disease [20]. Evidence points that anti-inflammatory drugs like the Nonsteroidal anti-inflammatory drugs (NSAIDs) produce their therapeutic activities through inhibition of cyclooxygenase (COX) [5], the enzyme that makes prostaglandins (PGs). Elevation in body temperature occurs when the concentration of prostaglandin E_2 (PGE_2) increases within parts of the brain [19]. Such an elevation contributes to a considerable alteration in the firing rate of neurons that control the thermoregulatory process in the hypothalamus [12]. Evidence points to the fact that antipyretic drugs exert their action by inhibiting the enzymatic activity of cyclooxygenase and consequently reducing the levels of PGE_2 within the hypothalamic region [12, 19]. Flavonoids have been shown to exert anti-inflammatory and antipyretic effect by suppressing TNF-α [19], an inflammatory mediator. Flavonoids and tannins are known to inhibit prostaglandin synthesis as reported earlier [20]. It is therefore suggested that the flavonoids and tannins present in *A. wilkesiana* extract played a role in the observed antipyretic effect. Alkaloids have also been reported to inhibit the synthesis of prostaglandin E_2, which could eventually reduce elevations of body temperature. This phytochemical is also suggested to have played a part in the observed antipyretic effect. The extract exhibited antipyretic activity in rats made pyrexic by brewer's yeast injection. This study corresponds with the study of Zakaria, *et al.* 2007 [21] in which compounds like flavonoids and saponins were suggested to act synergistically to exert observed pharmacological activity. The mechanism of nociception requires B_2 receptor activation which is expressed constitutional in neurons and facilitates PGE_2 synthesis [13]. The extract at treatment doses of this study showed no antinociceptive effect compared to normal saline treated control group. Probably, the dose administered and phytoconstituents present in these doses were lower than what was required to suppress facilitation of pain signals in rat models. Besides, pain transmission may require several molecular mechanisms out of which the anti-nociceptive tendency of the extract may influence one or only a few. The result of the present study indicates that hydromethanolic extract of *Acalypha wilkesiana* possesses significant anti-inflammatory, and antipyretic activity. Its anti-nociceptive effect may be clearly appreciated at a higher dose.

Conclusion

The outcome of this study suggests that *A. wilkesiana* leave hydromethanolic extract possesses significant anti-inflammatory and antipyretic activity, but showed no anti-nociceptive tendency. Its therapeutic potential may be dose-dependent and determined by its phytomedicinal constituents.

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