Effect of Hydro-Methanolic Extract of *Acalypha Wilkesiana* Leaves on Pyretic, Inflammatory and pain-induced scenarios In WistarRats

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ABSTRACT - Locally, the shrub plant, *Acalypha wilkesiana* is regularly used in the treatment of a range of ailments like diabetes, arthritis, fever and others. this study was carried out to evaluate the effect of hydro-methanolic extract of *Acalypha wilkesiana* leaves on pyrexia, pain and inflammation in wistar rats using a non-invasive method. The experimental animals were procured and separated into five (5) groups made up of 5 rats each. The control group (group 1) was given distilled water as placebo and reference group (given aspirin or piroxicam as the case may be) (group 5). Also there were three extract groups administered 40, 80 or 160 mg/Kg body weight of extracts. inflammation was induced using formalin while pyrexia was induced by injecting Baker's yeast intramuscularly into the dorsal part of the abdominal cavities of the rats. Different sets of rats were used for the anti-inflammatory and antipyretic studies although animal grouping for the extract administration were as in analgesic studies. The result demonstrated that the extract elicited significant ($p<0.05$) anti-inflammatory and pain relieving potentials; as can be seen in the extract treated groups with shorter periods of limb licking responses in experimental-inflammatory conditions. Similarly, the extract remarkably ($p<0.05$) depressed the pyretic conditions of the experimental animals after about 30 minutes of the treatments. In conclusion, this investigation has shown that the hydro-methanolic extract of *Acalypha wilkesiana* leaves possess anti-inflammation, analgesic and anti-pyretic potentials. In turn, these findings justifies the ethnomedicinal uses of *A. wilkesiana* for quite a range of ailments.

Keywords:*Acalypha wilkesiana,* pyrexia, inflammation, pain, pieoxicam.

Introduction

Therapeutic plants have been utilized in healthcare right from antiquity. Studies from all over the world have confirmed their viability and a portion of the discoveries have prompted the generation of plant-based medicinal agents. The worldwide market estimation of therapeutic plant items is above $100 billion per year. Due to increasing morbidity and the elevated burden of side effects of orthodox drugs, most researches are focused on investigating possible therapeutic plants in handling the diseases of public health importance, with specific interest on the current key ways to achieve prevention of diseases (Sofowora*et al.,* 2013).

Medicinal plants are frequently utilized in treating several disorders/illnesses by trado-medical practitioners. These herbs and their products have active components, aerial or root parts of the plants, or other materials or mixtures thereof whether in natural forms or as plant products. Presently, so many plants are used in the management of a wide range of diseases by traditional health care practitioners (Sofowora*et al.,* 2013).

Herbs, fruits and vegetables are known common wellsprings of various bioactive constituents and these attributes are often because of the availability of abundant phyto-constituents in them. For example, biologically beneficial component like flavonoids, nutrients, dietary fiber, phenolic constituents and carotenoids etc, are found in them (Akuodor et al., 2012; Pennington and Fisher, 2010).*Acalyphawilkesiana* is one of such plants with these attributes (WHO, 1991; Owoyele et al., 2011). *A. wilkesiana* (Family-Euphorbiaceae) is a beautification plant that is utilized frequently for supporting in West Africa and in fact numerous parts of the world. The plant is also popularly called copper leaf; most part of it is reflexive green or red leaves. The leaves of A. wilkesiana have assortment of ethnomedicinal relevance like treatment of digestive tract issues and skin diseases (Owoyele et al., 2011). Some scholars have described it to possess antifungal, anti-bacterial, immune-modulatory and antimalarial potentials (Spelman et al, 2006).

Materials and Methods

Research Design

The present study was a laboratory experimental-based study that was carried out in three different phases. Sixty five (65) male wistar rats were used throughout this experimental study and were separated into thirteen (13) groups as follows:

1. antipyretic study 5 groups (25 rats)
2. analgesic study 5 groups (25 rats)
3. anti-inflammatory study 3 groups (15 rats)

The animals weighed between 180g and 230g were all treated according to National Institute of Health (NIH) guidelines for care and use of experimental laboratory animals (NRC, 2017).

Preparation of plant extract

Leaves of *A. wilkesiana* were collected at University of Port Harcourt premises and voucher sample was deposited at the University of Port Harcourt Herbarium for identification and voucher number designation (UPH/V/1382).The leaves were air-dried and grinded into powder form out of which 1kg weighed and dissolved into 5 litres of hydromethanol in a ratio of 20:80 (that is distilled water and analytical grade methanol respectively). The solution was soaked and macerated continually for about 72 hour interval. Thereafter, the mixture was filtered through a Whatmann number 1 filter paper. The resulting filtrate was further concentrated using a rotary evaporator in vacuum at 40^0C (Nworah*et al.,* 2012). This produced about 65g of dark brown semi-solid extract which was later freeze drieduntil the time of use.

Preparations and Handling of the Experimental Animals

Sixty five newly bred male wistar rats weighing between 180 and 230g were obtained from the animal house of the Faculty of Basic Medical Sciences in the University of Port Harcourt and were used for the study. They were acclimatized for fourteen days before the commencement of experimentations. The animals were housed in well constructed wire-gauzed cages and were maintained under the natural day night cycles, under a humidity/temperature of 23-25^0C. The animals were allowed access to normal finisher mash feed and water *ad libitum* (Owoyele et al., 2011).

Different Experimental Animal Groupsfor the Study

A. Antipyretic Study (used 25 rats, i.e. 5 rats per group)

Group 1(Control group): normal rats with no treatment

Group 2(Treatment group 1):pyrexia-induced rats that received 40mg/kg body weight (b.w) of hydro-methanolic extract of *Acalypha wilkesiana* leaves (HEAWL)

Group 3 (Treatment group 2):pyrexia-induced rats that received 80mg/kg- b.w of HEAWL

Group 4 (Treatment group 3):pyrexia-induced rats that received 160mg/kg- b.w of HEAWL

Group 5 (Treatment group 4):pyrexia-inducedrats that received0.9mg/ml of Aspirin

B. Analgesic Study Group (25 rats)

Group 1(Control group): normal rats with no treatment and placed on the infrared spot of the tail flick instrument

Group 2(Treatment group 1): normal rats that received 40mg/kg b.w of HEAWL and placed on the infrared spot of the tail flick instrument

Group 3 (Treatment group 2): normalrats that received 80mg/kg- b.w of HEAWL and placed on the infrared spot of the tail flick instrument

Group 4 (Treatment group 3): normal rats that received 160mg/kg- b.w of HEAWL and placed on the infrared spot of the tail flick instrument

Group 5 (Treatment group 4): normal rats that received 0.04mg/ml of Piroxicam and placed on the infrared spot of the tail flick instrument

C. *Anti-inflammatory Study Group (15 rats)*

Group 1(Control group): normal rats infused with normal saline (0.9% sodium chloride)

Group 2(Treatment group 1): Inflammation-induced rats that received 40mg/kg body weight (b.w) of hydro-methanolic extract of *Acalypha wilkesiana* leaves (HEAWL)

Group 3 (Treatment group 2): Inflammation-induced rats that received 80mg/kg- b.w of HEAWL

Group 4 (Treatment group 3): Inflammation-induced rats that received 160mg/kg- b.w of HEAWL

Group 5 (Treatment group 4): Inflammation-induced rats that received0.04mg/ml of Piroxicam

Experimental Protocol

1) **Analgesic Study:**

The tail flick test is a test of the pain response in animals, similar to the hot plate test. It is used in basic pain research and to measure the effectiveness of analgesics, by observing the reaction to heat. It was first described by D'Amour and Smith in 1941 (D'Amour and Smith, 1941). As a principle, an intense light beam was focused on the animal's tail and a timer started reading. When the animal flicked its tail, the timer stoped and the recorded time (latency) which represent the measure of the pain threshold (Tzschentke *et al.*, 2007). The essence of using the tail flick machine was to investigate the effectiveness of extract/drug being used. The tail flick test to measure the extent to which the extract/drug being tested has reduced the amount of pain felt by the study rats (Doebel and Gagneux, 2012).

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 had been administered to them orally and piroxicam intramuscularly. 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.

2) **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 45minutes after treatments with either of the extracts, normal saline or piroxicam.

a. The times it took the rat to lick the injected paws in the initial phase (0-5min) and

b. the second phase (30-35 min) and in the third phase (65-70 min) were recorde.

3) **Antipyretic studies**

Precisely 1ml/100g body weight of 50% dried baker's yeast suspended in normalsaline 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 (Nwoke*et al.,* 2016).

3.4 Statistical Analysis

Quantitative data emanating from the study were statistically analysed using SPSS (statistical package for social science). Values were reported as Mean ± standard error of mean (SEM) while one way ANOVA was used to test for differences between treatment groups. Values were accepted as significant at p-values of less than 0.05, that is, at 95% confidence level ($p<0.05$).

Results

Presentation of Data

This section presents the results and the interpretation and discussion of findings of the current research.

Results of analgesic; anti-inflammatory and antipyretic effects of the hydro-methanolic leaf extract of *A. wilkesiana.*

Table 1:Anti-inflammatory effects of hydro-methanolic extract of *Acalypha wilkesiana* leaves(HEAWL) in wistar rats

S/no.	GROUP	Post-Treatment Limb Licking Response		
		0-5minutes	30-35minutes	65-70minutes
1.	Control	3.00±0.00	0.00±0.00	0.00±0.00
2.	40mg/kg-HEAWL	78.00±0.18[a, b]	25.00±0.58[a, b]	0.00±0.00[b]
3.	80 mg/kg-HEAWL	45.00±0.58[a, b]	15.00±0.57[a, b]	0.00±0.00[b]
4.	160 mg/kg-HEAWL	35.00±0.58[a, b]	8.00±0.58[a, b]	0.00±0.00[b]
5.	Piroxicam (0.04mg/ml)	52.67±0.88[a]	29.00±1.15[a]	1.60±0.84[a]

Values are expressed as Mean ± SEM; n=3;[a]Significant at $P<0.05$ when compared with the control**.**

[b]Significant at $P<0.05$ when compared with the Group 5**.**

Table 2:Antipyretic effects of hydro-methanolic extract of *Acalypha wilkesiana* leave (HEAWL) in male wistar rats

S/No.	GROUP	Pre-Treatment Rectal Temperature (^{0}C) (After inducing Pyrexia)	Post-Treatment Rectal Temperature (^{0}C)				
			After 30min	After 60min	After 90min	After 120min	After 150min
1.	**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
2.	**40mg/kg- HEAWL**	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
3.	**80 mg/kg- HEAWL**	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
4.	**160 mg/kg- HEAWL**	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
5.	**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

Values are expressed as Mean ± SEM; n=5;[a]Significant at P<0.05 when compared with the control**.**

[b]Significant at P<0.05 when compared with the Group 5**.**

AW= *Acalypha wilkesiana*

Table 3:Analgesic effects of hydro-methanolic extract of *Acalypha wilkesiana*leaves (HEAWL)in male wistar rats using tail flick apparatus

S/no.	GROUP	Reaction Time (sec)
1.	**Control**	5.45±0.01
2.	**40mg/kg- HEAWL**	7.40±0.05[a]
3.	**80 mg/kg- HEAWL**	7.70±0.08 [a]
4.	**160 mg/kg- HEAWL**	9.48±0.60 [a]
5.	**Piroxicam (0.04mg/ml)**	5.80±0.19 [a]

Values are expressed as Mean ± SEM; n=5;[a]Significant at P<0.05 when compared with the control**.**

[b]Significant at P<0.05 when compared with the Group 5**.**

AW= *Acalypha wilkesiana*

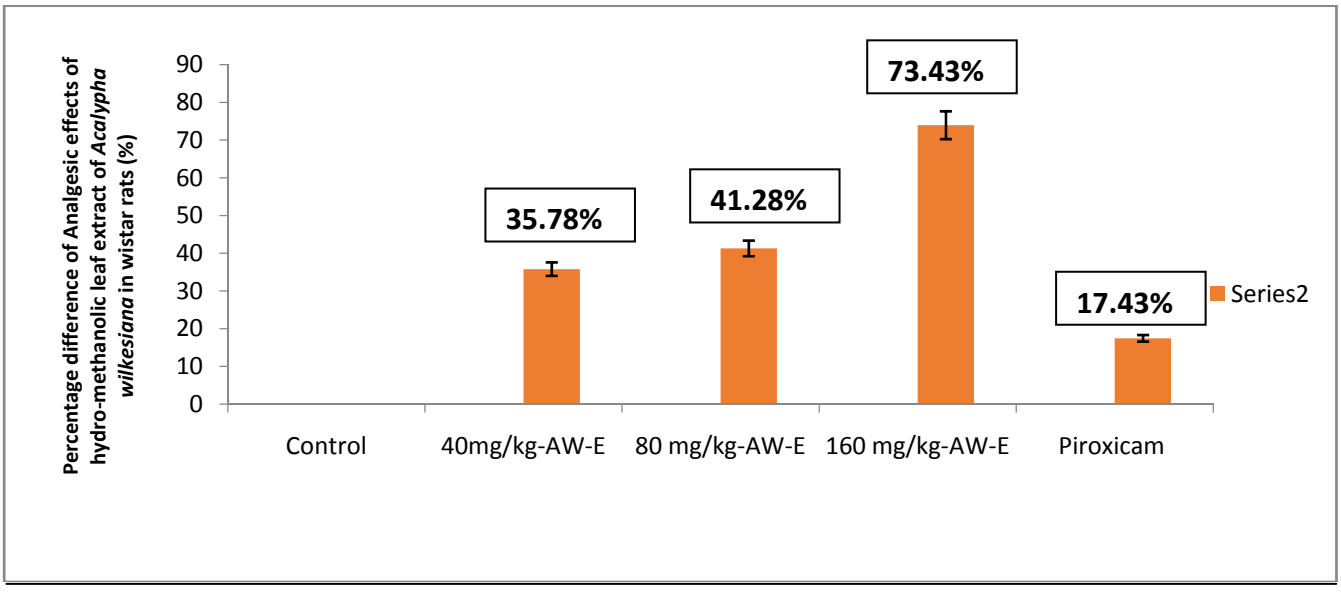

Figure 1: Percentage difference of Analgesic effects of hydro-methanolic leaf extract of *Acalypha wilkesiana* in wistar rats

AW= *Acalypha wilkesiana*

Discussion of Findings

Locally available medicinal plants are broadly utilized in customary therapeutic medicines for the management of different sicknesses (WHO, 1991). An extraordinary number of plants are right now utilized in the administration of a wide scope of ailments by conventional medicinal services professionals. *Acalypha wilkesiana* (*A. wilkesiana*) is one of such (Ikewuchi, 2013). Madziga *et al.,* (2010) in his report submitted that *A. wilkesiana* extractcontains pharmacologically valuable components, thus the reason behind its numerous medicinal applications.The anti-inflammatory study in this research demonstrates that, the hydro-methanolic extract of the *Acalypha wilkesiana* leaves (HEAWL) caused an significant inhibition of paw inflammation initiated by 3% formalin. This shows the plant have the capacity to depress severe inflammation which the model represents and this finding is consistent with the earlier reports of Oladunmoye, (2006) and Owoyele *et al.,*(2011). This assessment has helped to reveal that the extract can be a good local oral agent for treating inflammation. In this manner, the extract of *A. Wilkesiana*can be said to be active inflammation ameliorating agent.

Pyrexia or fever has been submitted by scholars in biomedical discipline to be helpful in the battle against diseases; as it causes the immune system to expand versatility of leucocytes and its phagocytic action, depress endotoxin impacts, elevate multiplication of T-cells and upgrade capacity of interferons movement so as to kill the disease causative agent or detoxify their effects (Lewis *et al.,* 2007). To the other far extreme, pyrexia may be dangerous as it could prompt derangement in the function of the biological system and in most occasions lethal particularly in kids. For this situation, treatment ends up unavoidable (Nwoke et al., 2016).

This study has shown that the hydromethanolic leaf extract of *A. wilkesiena*significantly ($p<0.05$) reduced the body temperature of the models. The result has found that the extract is able to effectively compete with orthodox medicines in body temperature guideline. This impact might be because of the reported presence of the phytochemical "bioflavanoid" which is known to discourage the cyclooxygenase response, which thus limit PG-E2 biosynthesis and henceforth decline the rodent body temperature (Nwoke et al., 2016). This revelation is in line with the result of a similar study on the ethanolic concentrate of the bark of mango plant completed by Nwoke *et al.,* (2016).

Pain is a multifaceted and subjective feeling that often serves physiological cautioning signals from wounds or other underlying ailments. In spite of the fact that, diagnosing and treating the causative factor of pain is substantially more essential; be that as it may, due to discomforting background, a quick relief from discomfort is wanted (Younger et al., 2009).

In the screening for analgesic potentials of the extract, it appreciably elicited pain relieving ability which reveal that the extract may possess the capacity to inhibit strong and higher centers mediated kinds of pain as represented by the tail flick machine study (Younger et al., 2009).The result on the pain relieving impacts of the extract is in line with the findings of Younger et al, (2009) which clarified that the plant at doses between 220 mg/Kg and 659mg/Kg has pain relieving potentials in synthetically and heat actuated pain in mice. Nevertheless, this investigation utilized lower doses (40, 80 and 160 mg/kg) of the same plant and this study was carried out in rat-model.

In other words, the outcome of the present study on anti-inflammatory effects of the extract has shown that, the extract may possess considerable anti-inflammatory potentials as it effected inhibition of paw inflammation induced by 3% formalin. In the 3% formalin experimental animal model, chemical mediators of inflammation are responsible and these comprise of prostaglandins, histamine, amongst others. In other words, the extract could have inhibited the activities of any of these inflammatory mediators.

The present study also discovered that the hydro-methanolic leaf extract of *Acalypha wilkesiena* remarkably depressed the elevated core body temperature of the rats in the test groups and this was in a rising dose-dependent manner. Due to the undesirable outcome severe fever, one immediate is to put it under control. From the outcome of this study, the hydromethanolic leaf extract of *Acalypha wilkesiena*may be a useful analgesic agent.

The present study has also revealed that the extract may act faster than some synthetic anti-inflammatory agent like aspirin at 200mg/kg in reducing the core body temperature in experimentally induced pyretic rats.

The outcome of the evaluation of the analgesic effects of the hydromethanolic extract of *Acalypha wilkesiena*leaf in this study revealed the extract elicited a pain relieving effect. This is an indication that the extract may possess analgesic properties. Of course, as can be seen in the data section, the extract treated animals a longer reaction time on the tail-flick apparatus.

Conclusion

This study has revealed the analgesic, anti-inflammatory and antipyretic effect of *A. wilkesiana* in laboratory using dry baker's yeast induced pyretic model; tail flick for analgesic model and formalin induced rat paw inflammation models.

Interestingly as can be seen in this study, the extract treated groups showed an increasing dose-dependent anti-inflammation potentials. The outcome of the anti-pyrexia study revealed that all doses of the extract like the standard drug , aspirin, rectal temperature reduction efficacy. Finally, this study found a dose-dependent significant delay in the analgesic study.

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