

Analgesic, anti-inflammatory and antipyretic effects of the ethanol extract of *Acalypha wilkesiana* leaves in rats

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Summary: The leaves of *Acalypha wilkesiana* are commonly used for the treatment of pain, fever and ulcer by traditional medical practitioners without any scientific data to evaluate the appropriateness of some of the practices. Therefore, this study was carried out to determine whether the ethanol extract of *Acalypha wilkesiana* has analgesic, anti-inflammatory and antipyretic as well as anti-ulcer effects. The hot plate latency assay and formalin- induced paw licking models were used to evaluate analgesic effects. Animals were divided into groups comprising of five rats each. There were control (administered saline) and reference (administered indomethacin) groups. Also there were three extract groups administered 25, 50 or 100 mg/Kg body weight of extracts. Ulcer was induced using absolute ethanol followed by pylorus ligation in all animals; inflammation was induced using carrageenan while pyrexia was induced by injecting brewer's yeast intramuscularly into the dorsal part of the abdominal cavities of the rats. Different sets of rats were used for the anti-ulcer, anti-inflammatory and antipyretic studies although animal grouping for extract administration were as in analgesic studies. The results show that the extract produced dose-dependent and significant ($p < 0.05$) analgesic and anti-inflammatory activities. The extract also significantly protected against ethanol induced ulcer. Likewise, the extract significantly ($p < 0.05$) reduced the pyretic states of the animals. This study has therefore further provides evidences that may support the ethnomedicinal uses of the ethanolic extracts of *Acalypha wilkesiana* leaves.

Keywords: *Acalypha wilkesiana*, Analgesic, Anti-inflammatory, Antipyretic, Anti-ulcer

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INTRODUCTION

The use of medicinal plants for treatment of ailments are as old as human history and many drugs have been developed either as synthetic or refined components of agents in plants. Despite all the successes achieved using technology, the native people across different geographical terrain especially developing countries still continue to use herbs for the treatment of certain ailments, either as a result of poverty or the efficacy of the herbal products (Jager, 2005; Cordell and Colvard, 2005; Adebayo and Krettli, 2011).

Acalypha wilkesiana (Family Euphorbiaceae) is an ornamental plant that is used commonly for hedging in West Africa and indeed many parts of the world (Jekayinfa *et al*, 1997). The plant is generally referred to as copper leaf and it is a shrub with mostly glossy green or red leaves. The leaves of *A. wilkesiana* have variety of ethnomedicinal uses which includes treatment of gastrointestinal disorders and skin

infections particularly impetigo contagiosa and Tinea versicolour (Akinde and Odeyemi, 1987; Gill, 1992; Jekayinfa *et al*, 1997). It also has anti bacterial, antifungal, immunomodulatory and antimalarial effects (Gill, 1992; Jekayinfa *et al*, 1997; Spelman *et al*, 2006). The leaves of the plant have been reported to be rich in alkaloids, tannins, saponins, anthraquinones, triterpenoids, sesquiterpenoids and polyphenols (Akinde and Odeyemi 1987; Jekayinfa *et al*, 1997). Furthermore, the decoction of the leaves is commonly used for the treatment of pain and ulcer by traditional medical practitioners. Recently, Udobang *et al*, (2010) reported the analgesic and anti-malarial effects of extracts and fractions of *Acalypha wilkesiana*.

The focus of the present work is to investigate the analgesic, anti-inflammatory, antipyretic and anti-ulcer effects of the plant in laboratory animals with a view to establishing the appropriateness of the use of the extract for the treatment of the above listed ailments.

MATERIALS AND METHODS

Preparation of plant extract

Leaves of *A. wilkesiana* were collected at Ilorin and identified at Forestry Research Institute of Nigeria (FRIN) where a voucher specimen (FHI 107114) was deposited. The remaining leaves of the plant were air-dried, reduced to powder and 1kg of the powder was extracted with 5 litres of ethanol to give 65.8g of dark brown solid extract.

Animals

Male albino rats weighing 200±14g were used. All animals were obtained from the Animal house of the Faculty of Basic Medical Sciences, College of Health Sciences, University of Ilorin, Ilorin, Nigeria. They were maintained in standard environmental conditions of temperature, humidity and light dark cycle (12 h light/dark cycle). The Animals also had access to water and standard rodent feeds *ad libitum*.

Animal grouping

In the analgesic, anti-inflammatory and antipyretic studies, animals were divided into five groups each comprising of five animals. Groups A & E received saline (control) and 5mg/kg indomethacin (positive control) respectively while groups B, C & D were administered 25, 50 and 100 mg/kg of body weight of the extract respectively. In the ulcer experiment, the control received saline while the positive control received cimetidine (11.5mg/kg). The remaining three groups received 400, 800 or 1200mg/kg of body weight of the extract. Saline, extract and drug administration were through the oral route.

Experimental Design

Analgesic Studies: The hotplate and formalin models were used for these studies. In the hot plate test the animals were placed individually on a hotplate (maintained at 55°C) 30 min after the extract, indomethacin or saline had been administered to them orally. The time taken for each rat to respond to the thermal stimulus by jumping off the hotplate was recorded. In the formalin test, animals were injected 0.1ml of 3% formalin on the left hind paw one hour after extracts, saline or indomethacin administration. The times spent in licking the injected paws in the first phase (0-5min) and in the second phase (20-30 min) were recorded.

Anti-inflammatory study: Carrageenan was used to induce oedema in this study. Animals were injected 0.1ml of 1% carrageenan on the right foot of each rat one hour after the oral administration of the extract, saline or indomethacin. Measurement of paw size was carried out as described previously (Owoyele *et al.*, 2009) by wrapping a piece of cotton thread round

the paw and measuring the circumference with a metre rule. Paw sizes were measured immediately before and 1-5hrs after carrageenan injection. Oedema inhibitory activity was calculated according to the following formula.

$$\% \text{ inhibition} = \frac{(C_t - C_o)_{\text{Control}} - (C_t - C_o)_{\text{treated}}}{(C_t - C_o)_{\text{Control}}} \times 100$$

Where C_t = paw circumference at time t, C_o = paw circumference before carrageenan injection and $C_t - C_o$ = Oedema

Antipyretic studies: Brewer's yeast (20 w/v) were injected (10ml/Kg) into the groin area of animals after 12 hours of fasting and the animals were subsequently administered the extract, saline or indomethacin orally after 18 hours. The rectal temperatures of the animals were monitored using a digital thermometer.

Anti-ulcer studies: ulcer was induced in 12 hour pre-fasted animals by orally administering 5 ml/Kg of absolute ethanol 1 hour after extract, saline or cimetidine administration and the pylorus of each animal was immediately ligated. Six hours later, the abdominal cavities of the animals were dissected and the gastric mucosae were observed for ulcers. Ulcers were scored using arbitrary scales of Singh *et al.* (1997). 0 = no lesion, 0.5 = hyperaemia, 1 = one or two slight lesions, 3 = very severe lesions, 4 = mucosa full of lesions. Ulcer index was calculated as mean ulcer scores (Tan *et al.*, 1996). Gastric contents of the stomach of each animal was emptied into a test tube and centrifuged at 3000 g for 5 minutes. The clear supernatant was titrated against 0.01mol/L solution of sodium hydroxide (NaOH) to pH7.0. The acid content of the stomach was calculated according to the method of Shay *et al.* (1954) and expressed as MEq/L.

Phytochemical analysis: The phytochemical analyses were carried out using the method of Trease and Evans, (1989).

Statistical analysis

Values were recorded as Mean ± standard error of the mean (SEM). Data were subjected to analysis of variance (ANOVA) followed by Waller-Duncan *post hoc* test using SPSS v16 statistical package. $p < 0.05$ was considered statistically significant.

RESULTS

Analgesic studies: Table 1. shows the results of the hot plate latency test, in which the extract of *A. Wilkesiana* (at the doses of 25, 50 and 100mg/Kg) significantly ($p < 0.05$) increased

(analgesia) the time spent by animals on the hot plate after 30, 60 and 90 min of its administration compared with the control. This shows that the extract can inhibit thermal pain.

In Table 2, the results of the formalin test shows that the time spent in licking the injected paw was significantly ($p < 0.05$) reduced (analgesia) both in the early and late phases of the test compared with the control.

Anti-inflammatory study: Table 3 shows the results of the carrageenan oedema test. The extract as well as indomethacin produced significant ($p < 0.05$) reduction in paw oedema after 3 and 5 hours of carrageenan injection compared with control group.

Antipyretic studies: the extract of *A. Wilkesiana* significantly ($p < 0.05$) reduced the elevated rectal

Table 1.

Effect of the ethanolic extract of *Acalypha wilkesiana* leaves on hot plate test in rats

Group	Dose (mg/kg) Orally	Reaction time ^a (sec)		
		30 (min)	60 (min)	90 (min)
Control (saline)	-	3.81 ± 0.19	3.41 ± 0.16	2.66 ± 0.20
<i>A. wilkesiana</i>	25	5.07 ± 0.04*	3.82 ± 0.06*	2.98 ± 0.02
<i>A. wilkesiana</i>	50	5.85 ± 0.04*	4.62 ± 0.04*	4.00 ± 0.04*
<i>A. wilkesiana</i>	100	6.07 ± 0.05*	4.98 ± 0.05*	4.20 ± 0.25*
Indomethacin	5	4.97 ± 0.07*	4.21 ± 0.06*	3.15 ± 0.04*

^aData are mean ± S.E.M. for 5 rats, * $p < 0.05$ compared with control, ANOVA.

Table 2.

Effect of the ethanolic extract of *Acalypha wilkesiana* leaves on formalin-induced paw licking in rats

Group	Dose (mg/kg) orally	Licking time ^a (sec)	
		Early phase	Late phase
Control (saline)	-	92.51 ± 5.79	73.0 ± 3.66
<i>A. wilkesiana</i>	25	67.34 ± 5.21*	53.99 ± 4.61*
<i>A. wilkesiana</i>	50	52.42 ± 4.85*	35.92 ± 2.94*
<i>A. wilkesiana</i>	100	47.47 ± 3.64*	25.96 ± 2.24*
Indomethacin	5	68.22 ± 3.68*	44.16 ± 4.62*

^aData are mean ± S.E.M. for 5 rats, * $p < 0.05$ compared with control, ANOVA.

Table 3.

Effects of the aqueous extract of *Acalypha wilkesiana* leaves on Carrageenan-induced paw oedema in rats.

Groups	Dose (mg/kg) Orally	Increase in paw size ^a (mm)		Inhibition (%)	
		3 h	5 h	3 h	5h
Control (saline)	-	7.80 ± 0.49	5.20 ± 0.37	-	-
<i>A. wilkesiana</i>	25	5.20 ± 0.8*	3.00 ± 0.71*	33.3	42.3
<i>A. wilkesiana</i>	50	4.80 ± 0.37*	3.20 ± 0.58*	38.5	38.5
<i>A. wilkesiana</i>	100	4.40 ± 0.93*	3.00 ± 0.51*	43.6	42.3
Indomethacin	5	4.40 ± 0.68*	2.00 ± 0.45*	43.6	61.5

^aData are mean ± S.E.M. for 5 rats, * $p < 0.05$ compared with control, ANOVA.

Table 4.

Effects of ethanolic extract of *Acalypha wilkesiana* leaves on gastric ulceration and acidity in wistar rats^a

Treatment	Dose (mg/Kg)	Ulcer index	Ulcerated surface area (mm ²)	Inhibition (%)	Volume of gastric juice (ml)	Gastric acidity (mEq/L)
Control (saline)	-	3.8 ± 0.2	12.6 ± 1.6	0	3.5 ± 0.11	141 ± 3.1
Cimetidine	11.5	0.3 ± 0.1*	3.1 ± 1.1*	75.4	3.0 ± 0.1*	71.4 ± 1.7*
Extract	400	0.4 ± 0.2*	2.7 ± 1.0*	79.6	2.7 ± 0.2*	64.6 ± 2.5*
Extract	800	0.2 ± 0.1*	2.0 ± 1.1*	84.1	1.8 ± 0.2*	51.4 ± 2.5*
Extract	1200	0.1 ± 0.1*	1.6 ± 1.0*	87.3	1.2 ± 0.2*	42.4 ± 2.3*

^aData are mean ± S.E.M. for 5 rats, * $p < 0.05$ compared with control, ANOVA.

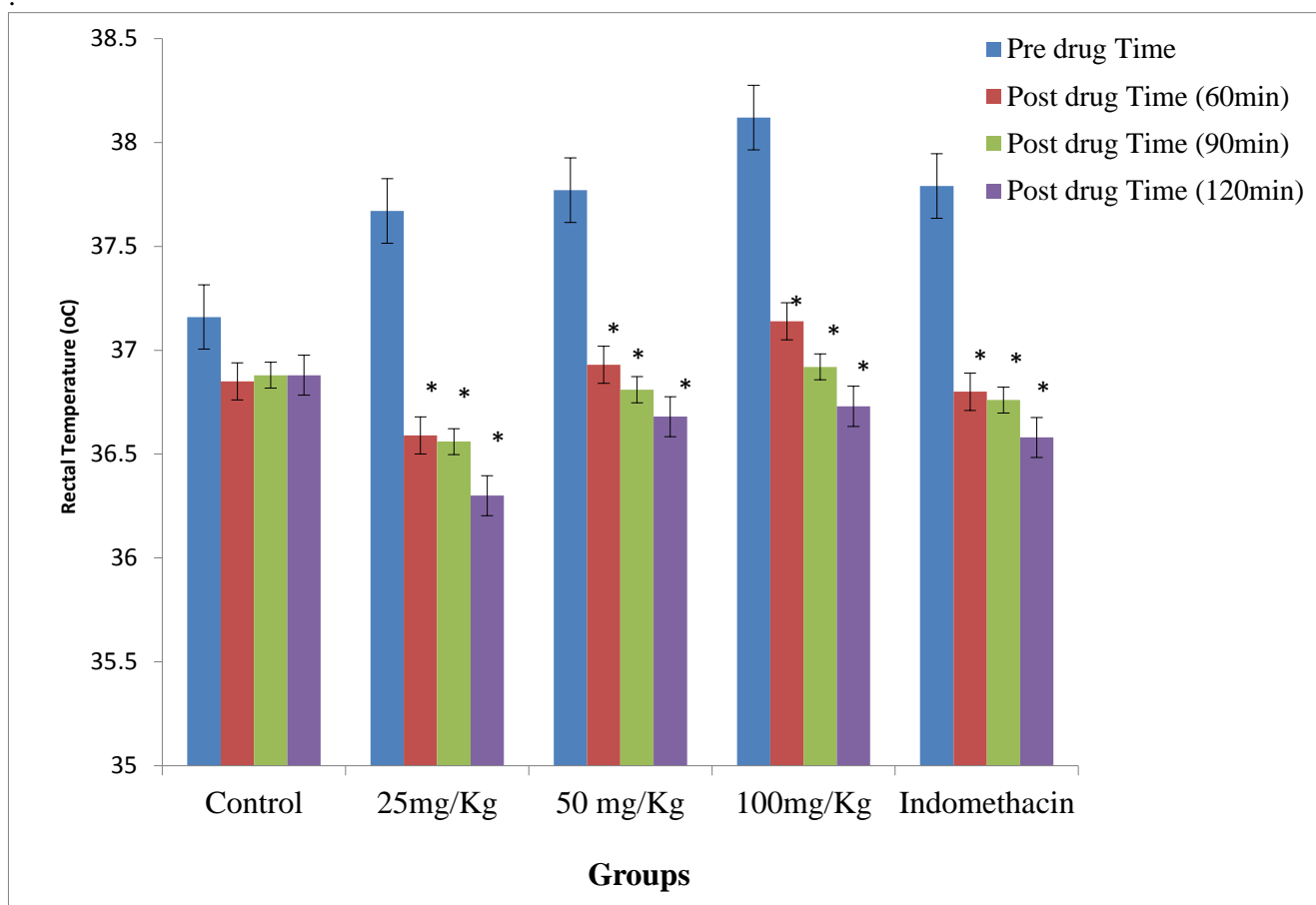


Figure 1:

Effect of ethanol extract of *Acalypha wilkesiana* leaves on Brewer’s yeast induced pyrexia in rats. 25mg, 50mg &100mg are doses of extract. ^aData are mean \pm S.E.M. for 5 rats, * $p < 0.05$ compared with control, ANOVA.

temperature induced by Brewer’s yeast injection after 60 and 90 minutes of extract administration compared with the pre-drug (extract) temperature (Figure 1).

Anti-ulcer studies: Ethanolic extract of *Acalypha wilkesiana* produced significant ($P < 0.05$) reduction in gastric ulceration from 3.8 ± 0.18 (control) to 0.10 ± 0.09 (1200 mg/Kg). Likewise the gastric acidity was significantly reduced from 141.0 ± 3.14 to 42.4 ± 2.25 . Volume of gastric juice and percentage ulcerated surface were also significantly reduced. The results are shown in Table 4.

Phytochemical analysis: The preliminary phytochemical screening of the leaves of *A. wilkesiana* revealed the presence of saponins, reducing sugars, tannins, alkaloids, anthraquinones and triterpenoids

DISCUSSION

The present study investigated the analgesic, anti-inflammatory, antipyretic and anti-ulcer effects of an ethanolic extract of *Acalypha wilkesiana* leaves in rats. The study was based on ethnomedicinal

practices by some local medical practitioners which involves the use of the plant for treatment of some of the ailment modelled by the study. In the analgesic study, the extract produced significant analgesic effects in the two models of pain employed. This indicates that the extract has the capacity to inhibit strong and centrally mediated types of pain represented by the Hot plate test (Prado *et al*, 1990). Likewise, the extract demonstrated significant inhibitory activity against neurogenic and inflammatory types of pain which was represented by its action on the early and late phases of the formalin pain model (Tjølsen, 1992) respectively. The observation on the analgesic effects of the leaves extract agrees with the report of Udobang *et al*, (2010) which showed that *Acalypha wilkesiana* leaves at the dose range of 220-659mg/Kg has analgesic effects in chemically and heat induced pain in mice. However our study employed lower dosages (25-100 mg/kg) and the study was carried out in rats. In the anti-inflammatory study, the leaves extract of the plant produced significant inhibition of paw inflammation induced by carrageenan. This indicates

that the extract can reduce acute inflammation which the model represents (Olajide *et al*, 2003; Owoyele *et al*, 2009). In the carrageenan model, chemical mediators of inflammation are involved and these include histamine, prostaglandins and nitric oxide (Kayyal *et al*, 1993; Tonks *et al*, 2003). Thus the extract might be inhibiting one or a combination of these inflammatory mediators. Another significance of this test is that it helps in the detection of orally active anti-inflammatory agents (Ismail *et al*, 1997; Owoyele *et al*, 2005). Therefore, the extract of *A. Wilkesiana* is an orally active anti-inflammatory extract.

The antipyretic study showed that the extract of *A. wilkesiana* produced significant inhibition of brewer's yeast induced pyrexia. Brewer's yeast is commonly used to induce pyrexia because the yeast serves as a foreign organism within the biological system of the animals and this has pyrogenic effect which ultimately increases the temperature of the body (Adesokan *et al*, 2008). Therefore, the results of this study indicate that the extract has the potential to inhibit the elevated body temperature induced by pyrogens within biological system.

Results obtained from the anti-ulcer study show that the extract of *A. wilkesiana* has the ability to inhibit ulcers induced by oral administration of ethanol. Two experimental methods of generating gastric ulcer were combined in the study (Parmar and Parma 1998; Maity *et al*, 2003) i.e. ethanol and pylorus ligation. Both methods have been proven to be effective models for inducing gastric mucosal damage (Shah *et al*, 1997; Maity *et al*, 2003; Odetola *et al*, 2006). Therefore the result indicates that the extract at the doses administered is acting as a powerful anti-ulcer agent. This is probably the reason why some local medical practitioners and some individual drink the fresh juice of the leaves of the plant for the treatment of ulcer.

The list of constituents obtained from our phytochemical analysis of the leaves of *A. wilkesiana* agreed with those that were previously reported (Akinde and Odeyemi, 1987; Gill, 1992) and this showed that the leaves contain substances such as alkaloids, tannins, saponins, anthraquinones and triterpenoids. Therefore, the activity of the extract might be due to the presence of some of these active agents which have been linked with analgesic, anti-inflammatory and anti ulcer effects in other studies (Backhouse *et al.*, 1994; Parmar and Parma 1998; Maity *et al*, 2003; Nguielefack *et al*, 2005; Graziani *et al*, 2005). Furthermore, the anti-ulcer effect of *A. wilkesiana* may also in fact be due to the anti secretory effect since the extract dose dependently inhibited the gastric juice output as well as acidity.

In conclusion, this study has established the analgesic, anti-inflammatory, antipyretic and anti-ulcer effect of *A. wilkesiana* in laboratory animal and thus justifies the local uses of the plant for the treatment of these conditions in humans. Further studies will attempt to look into the identification, purification and characterization of specific phytochemical agents that are responsible for the observed biological effects.

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