

Methanolic Extract of *Acalypha Wilkesiana* Inhibited Thermally but not Chemically Induced Pain in Diabetic Rats

*Owoyele B.V., Imam-Fulani A.O., Joseph L.A., Arikawe O.O. and Olayaki L.A.

Department of Physiology,
University of Ilorin, Ilorin, Nigeria.

Corresponding Author: deleyele@yahoo.com, +2348032146355

Abstract

The methanolic extract of *Acalypha wilkesiana* leaves was investigated for its effect on pain in diabetic Wistar rats using hot plate test and formalin paw licking. The rats were administered low dose extract of 100mg/kg(bw) and high dose extract of 200mg/kg b.w while the control group and diabetic control group received 10mg/kg(bw) normal saline. For the hot plate test, the mean latency in diabetic rat was significantly increased ($p < 0.05$) by the two doses administered compared to the control. However, in the formalin induced paw licking there was no significant difference seen across the groups. Thus, the study therefore suggests that *Acalypha wilkesiana* has analgesic property especially for thermally induced pain in diabetic rats.

Keywords: Analgesic, anti-inflammatory, nociceptive pain, neuropathic pain, diabetes, *Acalypha wilkesiana*, phytochemicals.

Introduction

Pain is a major symptom in many medical conditions, and can significantly interfere with a person's quality of life and general body functioning (1). Most pain resolves promptly once the painful stimulus is removed and the body has healed but sometimes, pain persists despite removal of the stimulus and apparent healing of the body; and sometimes pain arises in the absence of any detectable stimulus, damage to tissues or in a disease state (2).

Stimulation of nociceptors due to thermal, chemical or mechanical event that has the potential to damage body tissue may cause nociceptive pain (3), while damage or disease affecting the central portions of the nervous system involved in bodily feelings causes neuropathic pain (4).

Diabetic neuropathy affects approximately 131 million people globally as of 2010 (5). Diabetes is the leading known cause of neuropathy in developed countries, and neuropathy is the most common complication and greatest source of morbidity and mortality in diabetes patients. It is estimated that the prevalence of neuropathy in diabetes patients is approximately 20%. Diabetic neuropathy is implicated in 50–75% of non-traumatic amputations (5).

Diabetic peripheral neuropathy is the most likely diagnosis for someone with diabetes who has pain in a leg or foot, although it may also be caused by vitamin B₁₂ deficiency or osteoarthritis (6).

Despite advances in the understanding of the metabolic causes of neuropathy, treatments aimed at interrupting these pathological processes have been limited. Thus, with the exception of tight glucose control, treatments are for reducing pain and other symptoms (7).

Options for pain control include tricyclic antidepressants (TCAs), serotonin reuptake inhibitors (SSRIs) and antiepileptic drugs (AEDs). A systematic review concluded that "tricyclic antidepressants and traditional anticonvulsants are better for short term pain relief than newer generation anticonvulsants (7).

Acalypha wilkesiana is a medicinal plant and has been known from the time past traditionally to cure fever when taken boiled. It has been reported to have antimicrobial property (8) and the leaves of *Acalypha wilkesiana* are used in Southern Nigeria for the management of hypertension and diabetes mellitus (9). The expressed juice or boiled decoction is used for the treatment of gastrointestinal infection and fungal skin infections such as pityriasis versicolor, impetigo contagiosa, candida intertrigo, tinea corporis and tinea pedis (10).

It also has antibacterial, antifungal, immunomodulatory and antimalarial effects (11, 12, 13). The leaves of the plant have been reported to be rich

in alkaloids, tannins, saponins, anthraquinones, triterpenoids, sesquiterpenoids and polyphenols (14, 12).

A previous study has shown that *Acalypha wilkesiana* has anti-inflammatory and anti-pyretic activities (15). Specifically this study was carried out on normal animals. The extract produced significant analgesic effects in the two models of pain employed. This gave an indication that the extract has the capacity to inhibit strong thermally induced pain and inflammatory type of pain which was represented by its action on the late phases of the formalin pain model (16) respectively.

The focus of the present work is to investigate further, the analgesic effect of *A. wilkesiana* leaves' extract in diabetic animals.

Animals and Methods

Preparation of plant extract

Acalypha wilkesiana leaves were collected at Pipe-line area, Ilorin, Nigeria and identified at the Forestry Research Institute of Nigeria (FRIN, Ibadan) where a voucher specimen (FHI 107114) was deposited. The leaves of the plant were air-dried, and the cold extraction method was applied using pure methanol. The pure methanol was poured into container containing 1588.25g air-dried *A. Wilkesiana* leaves, and it was allowed to stay for 72hrs. The mixture was decanted and filtered; the filtrate was concentrated in a distiller to get the crude extract and was stored at room temperature.

Animals

Forty male albino rats weighing averagely 138 ± 5.61 were used. They were kept at the animal house of Faculty of Basic Medical Sciences, University of Ilorin, Ilorin, Nigeria. The rats were housed in temperature- controlled rooms with a constant light-dark cycle (12:12 h) with free access to water and food. The protocols comply with the guidelines for the Care and Use of Laboratory Animals, published by University of Ilorin, Ilorin, Nigeria.

Experimental

Induction of diabetics: Alloxan was administered intraperitoneally as a single dose (150 mg/kg BW) to induce diabetes in rats. Diabetes was

confirmed in the rats by measurement of the blood glucose which was elevated to $\geq 250\text{mg/dl}$.

Analgesic study using hotplate model: This was carried out by the method used previously (17). The rats were placed on hot plate maintained at $55^{\circ}\text{C} \pm 1^{\circ}\text{C}$ with a standard restrainer. The time taken for the rat to jump off the plate was recorded as the latency in seconds. The rats used for this study were fasted 12 hours before the test and subsequently divided into four groups ($n=5$); control (administered 10mg/kg normal saline), diabetic control (administered 10mg/kg normal saline), diabetic administered low dose extract (100mg/kg) and diabetic administered high dose extract (200mg/kg). The rats were placed on the hotplate after 30mins, 60mins and 90mins after saline or extract has been administered orally. Time spent by each animal on the hotplate was recorded

Analgesic study using formalin model: This was carried out by method previously described by Hunskaar et al., (18). The animals were grouped into four ($n=5$); control (administered 10mg/kg normal saline), diabetic control (administered 10mg/kg normal saline), diabetic administered low dose extract (100mg/kg) and diabetic administered high dose extract (200mg/kg). The animals were injected with 0.1ml of 4% formalin on the left hind paw one hour after saline or extract administration. The time spent in licking the injected paw was recorded in the early phase (0-5mins) and the late phase (15-30mins).

Statistical analysis

In the studies, values are expressed as mean $\pm$ standard error of mean (SEM). Statistical significance was determined using the ANOVA followed by Duncan post hoc test and values were accepted as significant when $p < 0.05$.

Results

Analgesic study using hotplate model: Fig.1 shows the result of the hot plat latency test, in which the extract of *A. wilkesiana* (100 and 200mg/kg) significantly ($p < 0.05$) increase the time spent by the animals on the hot plate and it is dose and time dependent. This shows that the extract has analgesic effect on thermal stimuli. It also indicated the mean latency, across all groups at different time interval. This illustration indicates how the activity of the extract wears off over time.

Analgesic study using formalin model: Figure 2 shows the result of the formalin paw licking test in which the extract of *A. wilkesiana* (100 and 200mg/kg) shows no significant difference for both the early and late phase paw licking across the groups. Thus this shows that the extract may have no effect on chemical stimuli.

Discussion and Conclusion

The present study investigated the analgesic effect of *Acalypha wilkesiana* leaf extract in diabetic rats. The study was based on the desire to investigate if the plant extract can also be effective at ameliorating nociceptive activities in animals rendered diabetic as it did in a previous study (15) in non-diabetic rats. The results showed that the extract produced significant analgesic effects in the hot plate latency test and no significant difference is seen in the formalin paw-licking model. These studies have indicated the possible analgesic effect of *A. wilkesiana* to thermal stimuli in diabetic rats which could be dose and time dependent.

In the analgesic study conducted by Prado et al., (19), 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. Whereas, in a study conducted by Tjølsten (16), the extract demonstrated significant inhibitory activity against neurogenic and inflammatory types of pain as indicated by its action on the early and late phases of the formalin paw-licking model. The observation on the analgesic effects of the leaf extract agrees with the report of Udobang et al., (20) which showed that *Acalypha wilkesiana* leaves at the dose range of 220-659mg/Kg have analgesic effects in chemically and heat induced pain in mice. In the previous study, (15), the analgesic study of the extract of *Acalypha wilkesiana* produced significant analgesic effects in the two models of pain employed; however our study went further to demonstrate the possible effect of this extract on pain in diabetic rats and we observed an analgesic effect in the hot plate latency test.

The observed analgesic effect could be due to the presence of some active substances in the plant extract. The phytochemical analysis of the plant extract shows that it has active ingredients such as terpenoids, carbohydrate and glucose which are also components of analgesic drugs.

The study also suggests that the extract could be analgesic to thermal stimuli by reducing thermal pain in diabetic animals but not effective in inducing analgesia on chemically (formalin) induced pain in diabetic animals. Studies of the effect of this extract on the haematological parameters in diabetic rats as reported by Ikewuchi et al., (21) showed that the extract was hypoglycemic, positively affected the hemopoietic system, integrity and function (dose dependently) of the liver and kidney of the diabetic rats; it improved the lipid profile and had no deleterious effect on red cell morphology. It also protected against oxidative stress in ocular tissues. Their study also revealed the presence of pharmacologically active compounds in the leaf extract (2).

It was also reported by Iniaghe et al., (22) that aqueous leaf extract of *A. wilkesiana* administration did not alter the behavioral, neurological and autonomic profiles of rats. No mortality or morbidity was observed in the rats at 10,000mg/kg body weight of the extract. This is an indication that the extract may have been well tolerated by the rats (22). All of these highlights the protective potential of the leaves of *Acalypha wilkesiana* and support its use in traditional health practices for the management of diabetes mellitus.

In conclusion *Acalypha wilkesiana* can inhibit thermally induced pain in animals with diabetes. Further studies should be carried out to evaluate the active agent of *A. wilkesiana* on its analgesic property as well as its mechanism of action. Also studies on the toxicity of some content of this extract should not be overlooked if clinical application is to be achieved.

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Illustrations

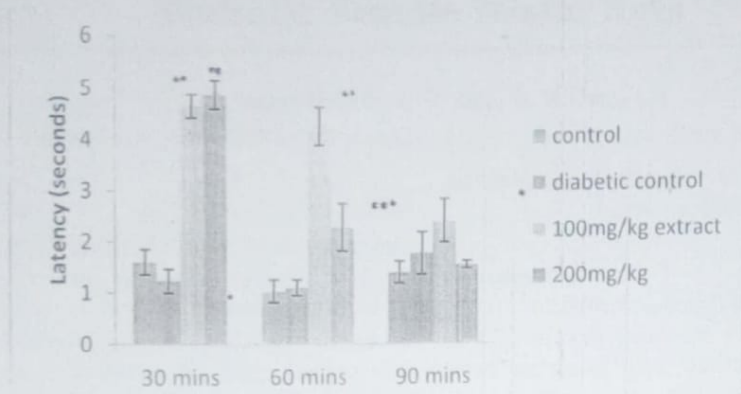


Figure 1. chart showing latency (s) across groups at different time interval. *P < 0.05 compared to control; **P < 0.05 compared to control and diabetic control; ***P < 0.05 compared to control, diabetic control and 100mg/kg ext. Centrepont Journal Volume 22, No. 1 (2016)

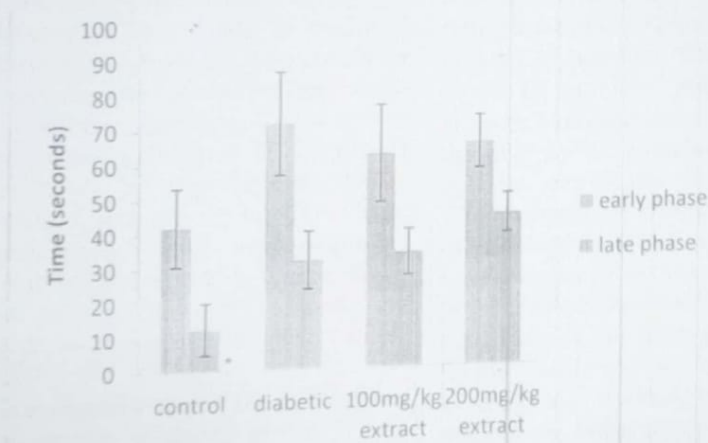


Figure 2. Effect of the ethanolic extract on Analgesic study using formalin paw-licking model