ORIGINAL ARTICLE

ANALGESIC ACTIVITY OF METHANOL LEAF EXTRACT OF MOMORDICA FOETIDA (SCHUMACH) IN ALBINO RATS

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ABSTRACT

Background

Momordica foetida is been used traditionally in the management of several diseases including pain. However, there is no scientific evidence to validate its efficacy.

Objective

The aim of this study, therefore, is to investigate the analgesic activity of its methanol leaf extract.

Methods

The oral median lethal dose (LD_{50}) in albino rats was determined using Lorke's method. The tail immersion in hot water and formalin-induced tests in albino rats were used to evaluate its analgesic activity at 250, 500, and 1,000 mg/kg, doses and tramadol was employed as the standard analgesic agent.

Results

The LD_{50} value of the plant extract was found to be greater than 5,000 mg/kg. The plant produced a significant (p<0.01) pain latency at the 1,000 mg/kg dose at time 150 minutes as compared to the control group. However, the lower doses (500 and 250 mg/kg) failed to significantly increase the pain latency time. The pain due to formalin was also significantly (p<0.05 and p<0.01) inhibited by the 1,000 mg/kg dose of the extract in both phases of formalin-induced pain. Meanwhile, the lower doses (250 and 500 mg/kg) were only effective in the second phase (p<0.05).

Conclusion

Based on the findings, it was concluded that the present study has demonstrated the analgesic potential of methanol leaf extract of *Momordica foetida* in experimental animals and thus validates the folkloric use of the plant in the management of pain and related conditions. The plant is also safe when taken orally.

Keywords: Analgesic, Formalin, *Momordica foetida*, Pain, Tail-immersion.

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INTRODUCTION

Traditional Medicine (TM) is defined by the World Health Organization (2003), as health practices, approaches, knowledge, and beliefs; including plant, animal, and mineral-based medicines, spiritual therapies, manual techniques, and exercises; applied singularly or in combination to diagnose, treat and prevent illnesses or maintain well-being. TM is the primary health care (PHC) delivery system in the majority of the developing countries of Africa, Latin America, and Asia (1). However, the percentage varies, depending on the country; for instance, 90% of Ethiopians, 70% of Rwandans, and 60% of Ugandans use TM for their PHC (2). The majority of the Ugandans depend largely on herbal medicines for the management of various diseases such as cough, headache, diarrhea, malaria, abdominal pain, measles, flu, mental sicknesses, eye diseases, and dermatitis (1, 2). This is largely due to the affordability, accessibility, and culturally friendly nature of TM.

The three forms of TM practiced in Uganda are herbalism (67%), spiritual counseling (23%), and bone setting (10%) (1). One of the plants used in Ugandan TM is *Momordica feotida. Momordica foetida* (Schumach) is a medicinal plant, which is widely distributed in tropical Africa. It is commonly found in swampy areas in Uganda (3). Its common name is "bitter cucumber" (4), and its vernacular names include *Luiwula* or *Mwishwa* in Luganda (5) and *Omwihura* in Southern Uganda (6). The leaves are rich in primary and secondary metabolites such as proteins, fibers, minerals (iron, calcium, zinc, and magnesium), ascorbic acid, foliate, *β*-carotene, saponin, alkaloids, steroids, phenolics, cardiac glycosides, flavonoids, and tannins (4). Traditionally, the leaves are used to treat cough, stomachache, hypertension, headache, earache peptic ulcers, diabetes mellitus, and intestinal disorders. It is also used as a purgative and to cure snake bites (3, 4, 7).

Pharmacologically, the leaves of *M. foetida* have been reported to possess antidiabetic properties in albino rats (8) antimalarial (9), anti-cholinergic, anti-spasmodic, and anti-oxidant activities (3). However, the leaves of *M. foetida* are frequently reported for their analgesic potentials by traditional healers in different parts of Uganda (2, 6), there is no scientific evidence confirming the analgesic activity of this plant. Therefore, it is prudent to investigate the analgesic activity of the plant scientifically. Thus, the aim of the present study is to investigate the analgesic activity of *M. foetida* leaf extract in albino rats, to validate its folkloric claim or otherwise.

MATERIALS AND METHODS

Plant Collection, Identification, and Extraction

The fresh leaves of *Momordica foetida* were collected by handpicking from a farm in Ishaka in Bushenyi district in October 2021. A herbalist and a botanist identified and authenticated the plant respectively. The leaves were dried at room temperature until a constant weight was obtained and made into powder using mortar and pestle. About 1kg of the powdered plant material was cold extracted using 90% methanol for 2 days with occasional shaking. The mixture was then filtered using Whatman's filter paper No. 1 and the filtrate was concentrated to dryness under reduced pressure to yield a dark brown mass (extract). Subsequently, it was referred to as "Methanol Leaf Extract of *Momordica foetida*" (MLMF). Solutions of MLMF were freshly prepared with distilled water for each study.

Animals

Albino rats of either sex (150-180g) were used for the experiments. The albino rats were obtained from the Departmental Animal House Facility of Pharmacology and Toxicology, Kampala International University, Western Campus, Ishaka-Bushenyi. The animals were housed in plastic cages; under standard environmental conditions and were fed with standard rats' feeds and water *ad libitum.* The experiments were carried out in accordance with the criteria outlined in the Guide for the Care and Use of Laboratory Animals by the National Institutes of Health (Publication No. 80-23, revised 1996).

Acute Toxicity Studies

The oral acute toxicity studies of MLMF in rats were conducted according to the previously described bi-phasic method (10). In the first phase, 3 groups of rats (n=3) were administered MLMF 10, 100, and 1,000 mg/kg. The rats were physically observed for signs of toxicity and death for the first 4 hours and intermittently for 24 hours. In the second phase, three rats were administered MLMF 1600, 2900, and 5,000 mg/kg and were also physically observed for signs of toxicity and death for the first 4 hours and intermittently for 24 hours. The median lethal dose (LD_{50}) value was determined by calculating the geometric mean of the lowest dose that caused death and the highest dose for which the animal survived.

Animal Grouping and Dosing

Rats of either sex were randomly divided into 5 groups of 5 rats each. Group-I was assigned as negative control and received distilled water (1 ml/kg). Group-V served as positive control and was treated with standard drugs, tramadol (12.5mg/kg). Groups II-IV were administered graded doses of MLMF (250, 500, and 1,000 mg/kg, respectively) for both tail-flick test and formalin-induced pain test. All administration was performed orally, with the exception of group V, which was administered intraperitoneally.

Tail Immersion Test (Hot Water)

To examine the central analgesic activity of MLMF, the tail-flick hot water immersion test, which measured brief threshold-level pain, was used. The lower 5cm portion of the tail of each rat was marked. This part of the tail is immersed in a water bath set at 55 °C. Within a few seconds, the rat reacts by withdrawing the tail. A stopwatch was used to record the reaction time. After each determination, the tail is carefully dried. The reaction time is determined before drug administration and 60, 90, 120, and 150 minutes after the administration. The prolongation of the latency times was taken as an analgesic response (11).

Formalin-induced pain in rats' tests

A standard method previously described was adopted for the formalin-induced pain tests in rats (12). Sixty minutes after oral administration (30 minutes after intraperitoneal), 0.1 ml of freshly prepared 2.5% formalin in saline was injected subcutaneously into the left hind paw of each rat. The rats were then placed, individually, in an observation chamber and monitored. The cumulative time of paw licking during 0–5 min (first phase) and 15–30 min (second phase) after formalin treatment were observed and recorded. A reduction in paw licking time was taken as an analgesic response.

Statistical analysis

All values were expressed as Mean ± Standard Error of the Mean (SEM). The data were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's Post hoc test for Multiple Comparison using the Graph Pad Prism (statistical) software (version 8). The differences between means were considered significant when $p<0.05$.

RESULTS

Acute toxicity test (LD_{50}): In the acute toxicity test, the oral median lethal dose of MLMF was estimated to be greater than 5,000 mg/kg in albino rats (Table 1). The physical signs of toxicity such as diarrhea, coma, sleep, tremors, convulsions, and lethargy were not seen in the rats even as the doses increased. No death was also observed in the first 4 hours and throughout the period of the experiment.

Tail immersion hot water test: MLMF produced a dose-dependent analgesic effect in the tail immersion hot water test in albino rats by increasing the pain latency of the treated rats. MLMF (1,000 mg/kg) at 150 minutes produced a significant ($p<0.01$) pain latency when compared with the control. Its analgesic effect is comparable to that of the standard drug, tramadol. The lower doses (250 and 500 mg/kg) of MLMF increased pain latency but not to a significant level (Figure 1).

Formalin-induced pain test in rats: MLMF (1,000 mg/kg) significantly ($p<0.05$ and $p<0.01$) inhibited both phases of the formalin-induced pain in rats. The lower doses (250 and 500 mg) were not significantly effective in the first phase but were able to significantly ($p<0.05$) reduce the paw licking time in the second phase (Figure 2).

DISCUSSION

The present study aimed at evaluating the analgesic potentials of methanol leaf extract of *Momordica foetida* (MLMF) in tail immersion (in hot water), and formalin-induced pain tests in rats. *M. foetida* is widely used in the Ugandan traditional medicine system for the management of pain and related conditions; unfortunately, there is no scientific evidence confirming its analgesic potentials, thus, this study is prudent. The tail immersion in hot water test is a standard method used for the discovery of new analgesic agents. The lower end of the tail of the rat (5cm) is immersed in hot water set at 55 °C. The rat reacts by withdrawing the tail within a few seconds. The prolongation of the reaction time of the tail-withdrawal reflex is evidence of analgesia (13). This test has been developed to be selective for central-acting analgesic agents (opioid analgesics). Opioids analgesics, such as morphine and tramadol, are capable of prolonging the reaction time of the tail-withdrawal reflex. Non-opioid analgesics, such as the non-steroidal anti-inflammatory drugs (NSAIDs) are non-active in this test. MLMF in a dose-dependent manner significantly prolonged the reaction time of the tail-withdrawal reflex in the test rats. However, this effect is observed more in the highest dose (1,000 mg/kg) at 150 minutes. This suggests that MLMF might be eliciting its analgesic activity through the central analgesic mechanism.

The formalin-induced pain test in rats is a chemical-induced test used to test for central and peripheral acting analgesics (13). The nociceptive stimulus and response, in formalin-induced pain, are persistent rather than transient (14). This method enables the evaluation of analgesic activity towards moderate, continuous pain generated by the injured tissue. The formalin test produces a biphasic nociceptive response. The first (or early) phase response starts immediately after formalin injection and lasts for about 5 minutes. The early phase response has been attributed to direct chemical stimulation of nociceptors (acute or neuropathic pain) (15). The second (or late) phase response lasts 15 to 30 minutes after injecting formalin (16). The second phase response has been attributed to the involvement of inflammatory mediators (inflammatory pain) (15). Central acting (or opioid-like) analgesics are effective in attenuating both phases of formalin-induced pain. Whereas, NSAIDs are ineffective in attenuating the first phase response, but are effective in attenuating the second phase response (14). Nociceptive behavior in the formalin-induced pain test can be quantified in several ways; the scaling method, counting the number of flinches in the formalin-injected paw, and counting the time spent licking the injected paw. However, the most commonly used method of quantifying formalin-induced pain is the time spent licking the paw (17).

MLMF at the highest dose (1,000 mg/kg) was able to significantly reduce the paw licking time in both phases of the formalin-induced pain test. However, the lower doses (250 and 500 mg/kg) only significantly reduced the paw licking time in the second phase. The analgesic activity of the MLMF in the two phases of formalin-induced pain suggests that the plant might relieve pain by both central and peripheral mechanisms. The oral administration of MLMF up to 5,000 mg/kg in rats caused no death or any physical sign of toxicity. These suggest that MLMF may be relatively safe when administered orally (10).

CONCLUSION

Because of these results, it is suggested that methanol leaf extract of *Momordica foetida* (Schumach) possesses analgesic activities that may be mediated through the central and peripheral pain pathways and this therefore validates and supports its folkloric use in pain management.

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Table 1: Oral Acute Toxicity Studies of Methanol Leaf Extract of *Momordica foetida* (MLMF) in Albino Rats

Treatment	Phase 1 (n=3)		Phase 2 (n=1)		LD_{50}
	Dose (mg/kg)	Mortality	Dose (Mg/kg)	Mortality	
MLMF	10	0/3	1,200	0/1	>5,000 mg/kg
	100	0/3	2,900	0/1	
	1,000	0/3	5,000	0/1	

MLMF: Methanol leaf extract of *Momordica foetida*

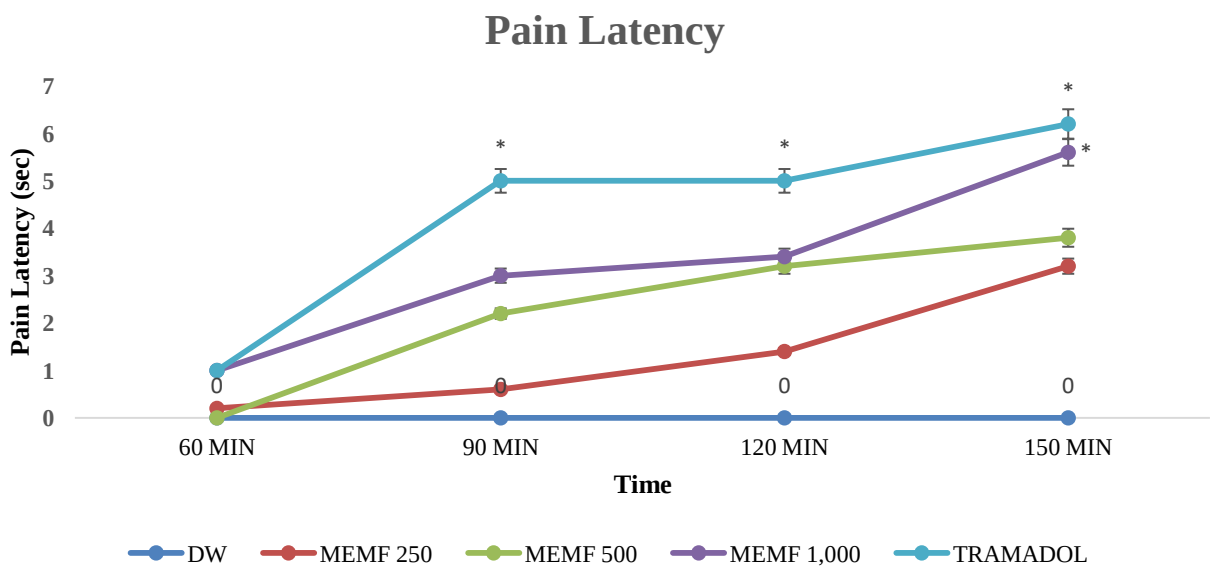

Figure 1: Effect of methanol leaf fraction of *Momordica foetida* (MLMF) on tail immersion Test in rats. Values presented as Mean ± SEM, * *p*<0.01, versus control. Repeated measures ANOVA followed by Dunnett's Post hoc test.

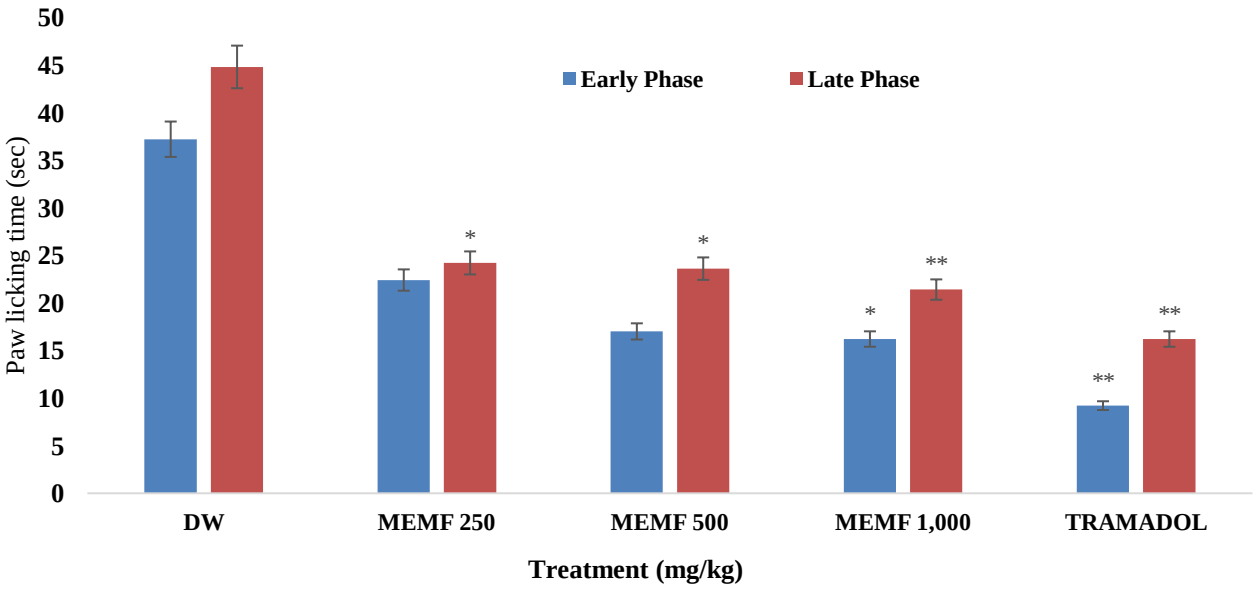

Figure 2: Effect of methanol leaf fraction of *Momordica foetida* (MLMF) on formalin-induced pain in rats. Values presented as Mean ± SEM, *=p<0.05, **=p<0.01, versus control. Repeated measures ANOVA followed by Dunnett's Post hoc test.