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EVALUATION OF ANALGESIC ACTIVITY OF HYDROALCOHOLIC EXTRACT OF *LUDWIGIA OCTOVALVIS* (JACQ.) P.H.RAVEN (ONAGRACEAE) LEAVES IN MICE

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ABSTRACT

This study was done to investigate the analgesic activity of *Ludwigia octovalvis* leaves'extract in Swiss-Albino mice. Peripheral action was assessed by acetic acid-induced writhing method and heat tail-flick test for its central action. Extract was administered orally at doses 100, 200 and 400 mg/kg. In the acetic acid-induced writhing test, the extract in doses of 200 and 400 mg/kg showed 64.63% and 91.67% ($p < 0.05$) inhibition of writhing, respectively. In heat tail-flick method the extract produced 47.5% and 66.27% elongation of tail flicking time 60 minutes after oral doses of 200 and 400 mg/kg body weight respectively. These results demonstrate the analgesic activity of this plant.

KEYWORDS: *Ludwigia octovalvis*, analgesic, mice.

INTRODUCTION

Ludwigia octovalvis is a well-known traditional medicine remedy in Madagascar. It is used to treat different diseases and as pain killer. Pain is a signal which makes us aware that something is wrong to protect the body's integrity. It can be caused by injuries, diseases, medication, functional pain syndromes….. Stimulus nociceptive causes damage on tissues which provokes the release of endogenous pain modulating agents such as prostaglandins, leukotrienes, histamine and kinins. Prostaglandins (PG) activate nociceptor of sensory neurons to produce pain, they also potentiate the aglycogenic power of the pain producing substances and sensitize afferent nerve endings to those pain agents, thereby increasing the intensity and duration of pain.[1,2]

Opioid-containing cells release opioid peptides that activate opioid receptors to induce antinociception. Endogenous opioid peptides, including β-endorphins (β-END), enkephalins (ENK), and dynorphins (DYN), have been implicated in peripheral analgesia during the early stages of inflammation, whereas antinociception is mediated by β-END in the late stages of inflammation.[3,4,5,6]

Even though *L. octovalvis* is largely used for its antinociceptive and anti-inflammatory actions by traditional healers in different parts of Madagascar, no study has investigated its effectiveness in relieving pain. Therefore, the aim of this study was intended to investigate the effect of *L. octovalvis* as an analgesic using experimental models that employed chemical and thermal-induced nociception. Acetic acid-induced writhing test was employed to assess the peripheral analgesic activity, while its central mechanism in producing analgesia was assessed using tail-flick tests in mice.[7]

MATERIALS AND METHODS

Experimental animals

Swiss mice weighing 25-30 g, 3-4 months old of either sex were selected for the experiments. Animals were obtained from the animal house of the Pharmacology Dept., Faculty of Sciences, University of Antananarivo, Madagascar. Animal studies were performed in accordance with the guideline of the Faculty of Sciences Animal Ethics Committee, and the study was approved under (Reg. no.). The animals were housed in light and darkness (12:12hr) alternation and at 22±2°C temperature. They were fed with standard laboratory diet and had water *ad libitum*.

Plant materials

The leaves of *Ludwigia octovalvis* were collected in March from Ambositra, Fianarantsoa (Madagascar), and were authenticated at the Botany Department of "Parc Botanique et Zoologique de Tsimbazaza" Antananarivo. The collected plant materials were cleaned, air dried in shade at room temperature. The dried leaves were ground and macerated in a mixture of ethanol-water (60:40) at room temperature for 3 days. The macerate was filtered, and the filtrate was evaporated to dryness using a rotary evaporator. The extract collected was stored in refrigerator at 4-8˚C for further use in the experiment. The hydro alcoholic extract was subjected to qualitative phytochemical analysis using Fong *et al*. methods.[8]

Methods for peripheral analgesic activity

Peripheral analgesic activity of *Ludwigia octovalvis* extract was tested with acetic acid induced writhing response. Fastened animals, 12 hours prior the test, were used. They were divided into 4 groups: the animals of the first group were given distilled water and served as control, while the animals of the 3 groups were respectively given 100, 200 and 400 mg/kg of the extract by oral route.

Thirty minutes after administration of drugs, acetic acid (0.6%) was injected intra-peritoneally in a volume of 5 ml/kg body weight. The number of writhing responses were counted and recorded for 20 minutes in each group and percentage protection was noted.[9]

Methods for central analgesic activity

Tail flick method was used to assess central analgesic activity of *Ludwigia octovalvis* extract. Experimental animals were fastened for 12 hours prior to tests and divided into four groups with six animals in each group. The first group served as control and was given distilled water, and the rest were respectively given 100, 200 and 400 mg/kg of the extract by oral route.

After 30 minutes, animals were individually put in animal holder which allows the tip of their tail to be out.[9] The animal holder was put above hot water bath maintained at 50 °C, and the distal part of the mice's tail was immerged in the hot water. The reaction time until animal flicked the tail from the hot water was measured with a timer. The cut off reaction time was fixed at 15 seconds to avoid tissue damage. Experiment was performed at 15, 30, 60, 90 and 120 minutes after administration of the drugs.

Statistical analysis

Statistical analysis was done using one-way ANOVA followed by Student 't' test. Significance level of $p<0.05$ was considered as significant.

RESULTS

Peripheral analgesic activity of *Ludwigia octovalvis*

Writhing reaction occurs 5 minutes after acetic acid injection intra peritoneally. As shown in Figure 1, *Ludwigia octovalvis* reduces the writhing reaction compared to the control group. During the 20 minutes observation time, the animals in control group manifested 84,83 ± 3,29 writhing, versus 37.57 ± 1.54, 30 ± 1.13 and 7.06 ± 0.82 in animals treated with *Ludwigia octovalvis* hydro alcoholic extract at doses 100, 200 and 400 mg/kg respectively ($p<0.05$), which corresponds to 55.71, 64.63 and 91.67 % of inhibition. These results indicate that this extract has an antinociceptive effect on acetic acid-induced writhing response.

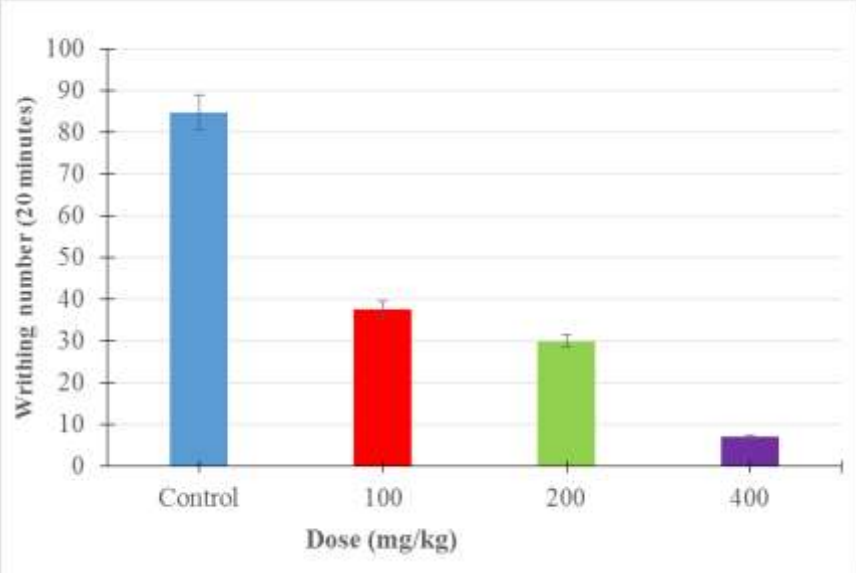

Figure 1: **Acetic acid (0.6%) induced writhing number in control group and mice treated with *L. octovalvis* extract ( ), administered orally at doses 100 ( ), 200 ( ) and 400 mg/kg ( ), in 20 minutes observation ($\bar{x} \pm \bar{\sigma}$; n = 5; p < 0.05).**

Central analgesic activity of *Ludwigia octovalvis*

In response to the pain induced by hot water, animals withdraw their tails. According to the results obtained during the 120 minutes observation, *Ludwigia octovalvis* extract increases the animal's reaction time, the latency time is maximal at 45 minutes after oral administration of the extract. The animals of control group withdraw their tail from the hot water bath after 1.85 ± 0.06 seconds and this latency time remains stable during the observation time. While it is respectively equal to 2.83 ± 0.05, 3.47 ± 0.37 and 4.11 ± 0.83 seconds after 45 minutes of oral administration of the extract at doses 100, 200 and 400 mg/kg respectively ($p<0.05$) (Figure 2), which corresponds to a decrease of 28.73, 47.5 and 66.27 % of pain. These results demonstrate the central analgesic activity of *Ludwigia octovalvis*.

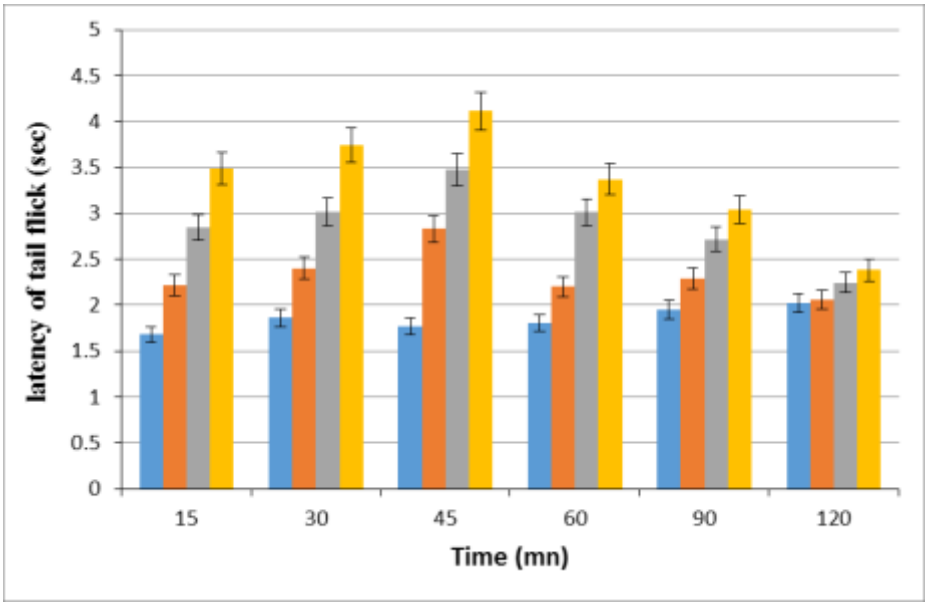

Figure 2: Latency time of tail removal from hot water at 50°C of control group ( ) and mice treated with *L. octovalvis*, administered orally at doses 100 ( ), 200 ( ) and 400 mg/kg ( ) ($\bar{x} \pm \bar{\sigma}$; n = 5; p < 0,05).

DISCUSSION

Drugs commonly used for pain management are classified as non-opioids and opioids drugs. Non-steroidal and steroidal anti-inflammatory; antidepressant, antiepileptic and local anaesthetics drugs are part of the first group. They are prescribed to relieve light to mild pain.[10,11] While opioids drugs such as morphine take care of strong pain.[12]

Acetic acid-induced writhing test is performed to assess the analgesic effects of a drug on peripheral pain. Reportedly, acetic acid injection into the peritoneal cavity induces pain by releasing pain mediators (such as prostaglandins, kinins, histamine, etc.) which activate local peritoneal receptors leading to a writhing response in animals.[13] Increasing PG levels within the peritoneal cavity enhances inflammatory pain by increasing capillary permeability and activating primary afferent nociceptors.[9] In this study, *Ludwigia octovalvis* showed a significant and dose-dependent inhibition of writhes in mice. This suggests that the extract might have a peripheral analgesic action.

The possible mechanism by which the extract produced peripheral analgesia in this model might be associated with inhibiting the synthesis and release of various endogenous inflammatory mediators and suppression of sensitivity of peripheral nociceptors in the peritoneal free nerve endings for chemical-induced pain. These proposed mechanisms are in line with the principles that stated, any agent that decreases the number of writhing will demonstrate analgesia by inhibiting the synthesis and release of PGs, and the peripheral pain transmission.[14]

The decrease of writhing observed in this work suggests peripheral anti nociception of this plant, probably by inhibiting cyclooxygenase which inhibits PGs synthesis. This is in accordance with previous reports indicating that this test is sensitive to non-steroidal anti-inflammatory drugs (NSAIDs).[15]

Aiming to study the spinal antinociceptive action, we performed the tail flick test. It is an extensively used test of nociception in rats and mice, first described in 1941 by D'Amour and Smith.[9] The noxious stimulus is usually radiant heat on the tail or tail immersion in hot water, and the response is a flick of the tail. This reflexive response is an indicator of pain sensitivity which is reduced by central analgesics.[16,17]

In this test, pain is not measured directly but instead, the spinal nociceptive reflex was observed. The tail–flick latency is a measure of the nociceptive sensitivity of the animal and is prolonged by central analgesics.[18]

Tail flick test is considered selective for opioid-like analgesic compounds. Those centrally acting analgesic drugs alleviate pain threshold of animals to heat and pressure, expressed by the latency increase. The results obtained in this test indicate a significant, dose and time related analgesic activity of the extract in tail flick assays. Prolongation of reaction time in tail immersion test confirms that *L. octovalvis* extract possesses central analgesic action. This finding is in accordance with what was reported by Owolabi and collaborators on *Parkia filicoidea* (Fabaceae) extract and also on what was reported on *Alpinia nigra* extract.[19,20]

CONCLUSION

From the results of the present study taken together, one can conclude that *Ludwigia octovalvis* (Onagraceae) extract possesses analgesic activity, which may be mediated via peripheral and central mechanisms; it might act partly through an opioid and prostaglandins mediated mechanism. This provides evidence for its use locally in human medicine to relief pain in the treatment of ailments accompanied with pain. Further studies are needed in order to know the mechanism behind the observed antinociceptive action, in view of the need for new, safe and effective therapies. The antinociceptive action demonstrated in the present study supports, at least in part, the ethno-medical uses of this plant. It represents a promising source of herbal medicine for the treatment of pathologies for which no efficacious treatment exists, such as chronic pain.

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