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Original Article

Evaluation of Aqueous moringa oleifera extract for antinoceceptive effectin ratmodel by using tailflick test

*Asmahan Abukhdir[1] *, Fateh Alsalami2, Fathi Nasser[3], Khaled Aburas[1], Akram Misbah[1], Entesar Knaz[1], Hana Fehelbum[4], Hnan Atwair[4], Wesam Abohallala[6], Mohammed Msaed[6], Saja Oun[6],Amina Bshaena[1]*
1 -Libyan Medical research center, Zawia, Libya
2- Department of Radiology, Faculty of medical technology, Tripoli university, Tripoli, Libya
3 -Department of Intensive Care Unit, Faculty of medical technology, Aljafara University, Aljafara, Libya
4 -Department 0f Histology, Faculty of medicine, Zawia university, Zawia, Libya
5 -Department of laboratory, Faculty of medical Technology, Zawia university, Zawia, Libya
6 -Department of pharmacology,Tripoly Higher Institute of Medical Science,Azzahra Branch, Azzahra, Libya
**Correspondance to asmahan1314ab@gmail.com*

Abstract:

Moringaoleiferais a highly valued plant distributed in many countries of the tropic and subtropics. Moringaoleiferaleaves are a potential source of phytochemical ingredients claimed to have analgesic property. Theanalgesic drugs lacking the side effect as alternative to nonsteroidal anti-inflammatory drugs (NSAIDs) and opiates are in demand by the society. The aim of this study is undertaken to evaluate the analgesic activity of Moringaoleiferausing thermal induced pain by tail flick test. The study was done using experimental models (albino rats). The albino rats were divided into four groups, each group consisting of 3 rats. Group I: (Control dH_2O given orally at 2 ml/kg body weight); Group II:(Standard Panadol given 100 mg/kg orally); Group III, IV, (aqueous extract of Moringaoleifera(AMO) 50, 100 mg/kg, respectively). The AMO were administered orally 1 hour before the experiments. For central analgesic effect was screened using tail flick test.The AMO leaf showed significant ($P < 0.01$) analgesic activity withboth 50,100mg/kg at60 min with a mean rank ranging (3.43±0.29), (4.30±0.23) respectively when comparison with control and standard that with mean rank ranging (2.13±0.08), (2.66±0.08) respectively.The AMO exhibited analgesic activity in tail flick test showing its both central and peripheral analgesic actions. This study confirms the traditional uses of M. oleiferain treatment of diseases, particularly those related to pain and inflammation.

Keywords:aqueousmoringaoleiferaextract, antinoceceptive, tailflicktest

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Introduction

Many of the currently used medicines originate from natural products, especially plants. Drugs and plants are closely related to each other, throuh the use of traditional medicines or ethnomedicines that are mainly prepared from plants. For discovering drug candidates, plants of interest are screened for the presence of bioactive components, phytochemicals responsible for the bioactivity are isolated.Their molecular structures are identified, and then the original structures of phytochemicals may be semi-synthetically modified to enhance the activity or reduce the toxicity [1].

Various types of plants have been used for several centuries worldwide not only as dietary supplements but also as traditional treatments for many diseases [2], [3], [4]. Indeed, the fact that traditional medicines have been widely used worldwide demonstrates the potential of plants as sources of bioactive compounds, including potential antitumor, antioxidant, antiobesity, and antimicrobial molecules. Among these plants, the widely cultivated *Moringaoleifera* (Moringa or drumstick tree), a rapidly growing perennial tree, was used by the ancient Romans, Greeks, and Egyptians, and has been naturalized from the tropics to the sub-Himalayan regions (e.g., India, Pakistan, Bangladesh, and Afghanistan), Oceania, Latin America, Africa and tropical Asia [5], [6], [7], [8].

Moringaoleifera possess highly therapeutic and pharmacological values, so its consumption in regular diet could possibly reduce the risk of degenerative diseases [9]. *Moringaoleifera* is believed to possess numerous medical properties, and is being used for the treatment of ascites, rheumatism [10], venomous bites [11], enhancing cardiac function [12], Inflammation[13], liver disease [14],cancer, hematological, hepatic and renal function [15]. Almost all the parts of this plant: root, bark, gum, leaf, fruit(pods), flowers, seed and seed oil have been used for various ailments in the indigenous medicine of South Asia, including the treatment of inflammation and infectious diseases along with cardiovascular, gastrointestinal, hematological and hepatorenal disorders [16].

The extract of Moringaoleifera has medicinal properties including ,antioxidant[17]andanticarcinogenesis[18], anti-inflammatory, antispasmodic [19]and diuretic [20]antiulcer,antibacterial, and antifungal [21], and Analgesic activity [22].

The present study is undertaken to evaluate the analgesic activity of Moringaoleiferausing thermal induced pain by tail flick test.

Materials And Methods

Collection of plant material:M. oleifera leaves were collected inMay 2021 fromSwani Benadem (Libya), and identified by Department of Botany, Aljafara university. The leaves shade dried under appropiate condition and the dried leaves ground to powder, after that 100 gramms of M.oleiferapowder was weighted accurately.The powder mixed with 1 L of distilled water (1:10 ;w/v) andthe macerated was lefted for 3 days with continuous shaking. After completion of maceration the dark green

solution was filtred through filter paper according to study by Yadu ND, Shankhajit D, Ajoy KG [19]with slight modifications. The filtrated solution was transferred to Buchner funnel fitted with Whatman No.1 filter paper where the filtration using by vacuum units.

The homogeneous solution was kept in freezer for over nightat -20 °C in tightly sealed jars after that were used in the feeding experiments.The yield was calculated according to the doses and diluted with distilled water (dH_2O) to use as experimental doses.

Experimental Rats (model): 12 male albino rats*(swiss rat)*weighing between (120-180 gm) were obtained fromlocal animal house inLibyan Medical Research Center, Alzawia, Libya. They were housed in standard environmental condition. The animals were kept in a room temperature varying between 20 ± 25c° and 12/12h light dark cycle and adapted to the condition for 10 days in the laboratory before examination .

Experimental design: This study included one experiment with four groups which each group according to doses, namely (control, standrad, sample 50, sample 100) with 3 rats in each group were distributed randomly.

a) Control group:

They were on basal diet (commercial rat food) with fed 0.5 ml of dH_2Oorally only.

b)Standradgroup:

The rats were treated orally with 100 mg of thepanadoldose (500 mg/kg) was prepared by weighed one tablet of panadol and gruond in mortarand then dissolved in 20 ml of distilled waterto increase solubility used vortex mixer. Finally calculated doses per weight that fed 0.6 ml of solution to each rat orally by using (metal PTFE oral feeding needle).

c) Sample 50mg group:

The sample group rats were treated with 50 mg/kg of AMO extractorally administration by using metal PTFEoral feeding needle.

d) Sample 100mg group:

The rats were treated with 100 mg/kg of AMO extract orally administration by using metal PTFE oral feeding needlewith weighing each rat befor treated.

Antinociceptive assays

Tail flick test: Radiant heat was applied over the tail on a single spot over the proximal one third with the help of analgesiometer by infra red radiation source (IR). The time taken by the animal to withdraw (flick) the tail was taken as the reaction time. Before administration of the test compound or the standard drug, the normal reaction time was recorded. Animals were subjected to a preliminary screening and rats showing tail flick response in 2-3 seconds were selected. The animals were submitted to the same testing procedure after 60, 90 and eventually 120 minutes after administration of the drug and test compound. For each individual animal, the reaction time was noted. Panadol (500 mg/Kg. orally.) was given as reference standard.

Statıstıcal Analysıs

Data in this study were analysed using Graph pad prism 5.01 soft ware (Graph pad soft ware Inc). One –way ANOVA of variance with Bonferroni post-boc testing (with correction for mltiple test) was performed . results were viewed as statistically significant with (p value □ 0.05)

Results

The experimental data were expressed as Mean ± SEM (standard error of mean).

All results of latency rseponse (analgesic activity) (sec) were given in Table (1). The rats were orally supplemented with MOA extract at various doses (50mg kg-1 and 100mg kg-1) showed significant (P< 0.001) difference in results when compared to standrad at 60min after treated with aqueous M.oleifera extractby using tail flick test.

Pain induced by application of radiant heat in rats. *Aqueous extract M.oleifera*in dose of 100mg/kg showed significant increase in latency of tail flick response compared to control and standrad at 60 minutes onwards following administration of drug. Similar result was seen after 90 minutes. The dose of 100 mg/kg highly increased the latency of tail flick response (**p= 0.0017) (4.30±0.23)at 60min after treated compared to control and standrad. While the dose of 50mg/kg mildly increase the latency response (*p < 0.05) (3.43±0.29) at 60min compared to control and standrad (Graphic 4.1).Whereas showed deccreasing the response at 90min in the groups were fed both 50mg/kg and 100mg/kg of extract (Graphic 2). The lowest values of analgesic activity was noticed in the group that receiving 50 mg of *aqueous extract of M.oleifera leaves* at 120min after treated (1.60±0.25) at the end of experiment (Graphic 3).

Table 1.*Analgesic activity: Effect of aqueous extract of M.oleifera leaves on thermal stimuli induced pain in rats by using tail flick test.*

Group / Dose (mg/kg)	Duration of latency of withdraw (flick) response in (sec) at various time interval		
	60min	**90min**	**120min**
Control	2.13±0.08	2.43±0.08	2.23±0.08
Standrad	2.66±0.08	4.50±0.41	2.70±0.70
sample (50mg/kg)	3.43±0.29*	2.13±0.12**	1.60±0.25
sample(100mg/kg)	4.30±0.23**	2.30±0.15**	2.30±0.40

Each value represented in Mean ± SEM, *p < 0.05, **p < 0.01, ***p < 0.001 in comparison with control group (one way ANOVA).

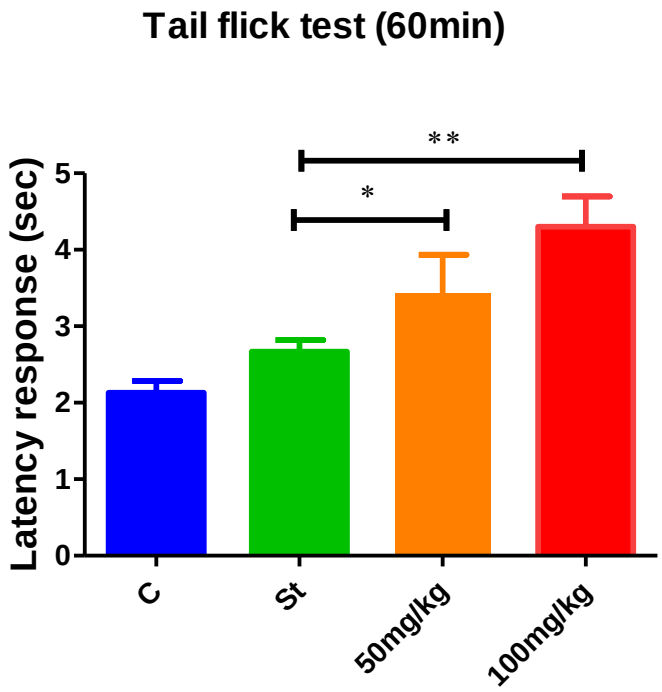

Figure 1. Analgesic activity (latency response) of *aqueous extract of M.oleifera*leaves at60min after treated.
c = control group. st=standrad group were fed panadol. 50mg/kg, 100mg/kg=sample group were fed *aqueous extract of M.oleifera*leaves

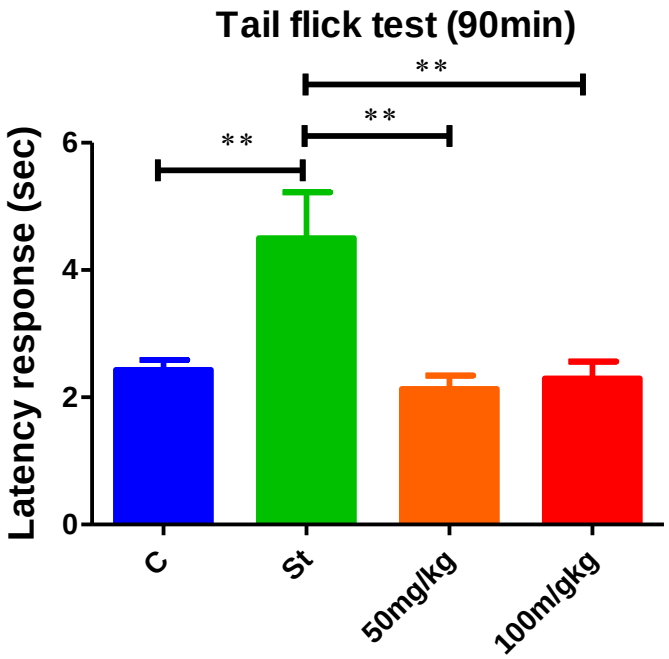

Figure 2.Analgesic activity (latency response) of *aqueous extract of M.oleifera*leaves at 90min after treated.

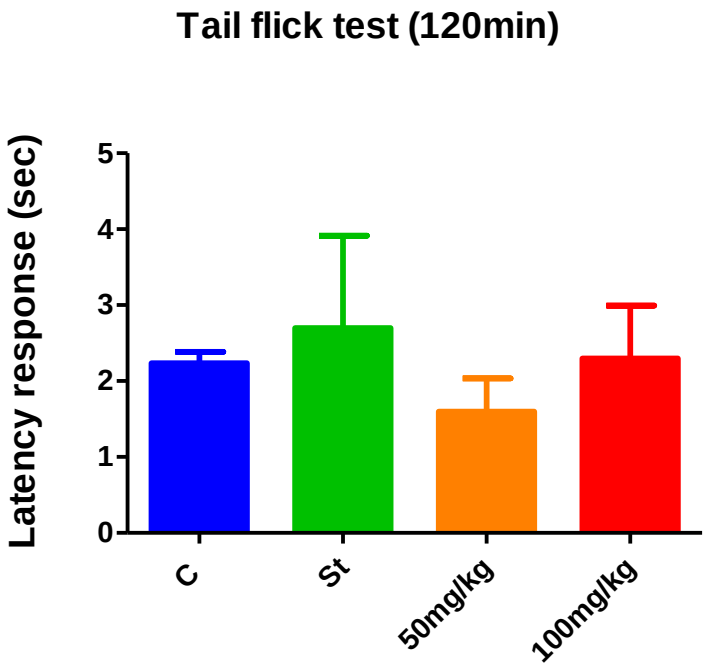

Figure 1 -3. Analgesic activity (latency response) of *aqueous extract of M.oleifera*leaves at 120min after treated.

Discussion

The bioactive compounds that in herbs facilitate in treatment and prevention of various diseases, increase the natural resistance of the body to infection and promote health [23], [24].This study demonstrated the potential antinociceptive effect of the AMO. Tail flick and hot plate tests are the most common tests of central nociceptive activity that are based on a phasic stimulus of high intensity [25]. Pain induced by thermal stimulus of the tail flick is specific for centrally mediated activity[26]. AMO showed central antinociceptive activity by increasing the latency to discomfort in the tail flick test, are suggested to act like centrally mediated drugs [22] by activating the periaqueductal gray matter (PAG) to release endogenous peptides (i.e., endorphin or enkephalin). These endogenous peptides descend the spinal cord and function as inhibitors of the pain impulse transmission at the synapse in the dorsal horn [27] . The ability of the extract to prolong the reaction latency to pain thermally induced in rats by the tail flick test further suggests central nociceptive activity. In the present study according to the results get from the tail flick test that were significant increase ($P<0.01$) at the beginning of the experiment (60min) according to control and standrad. In the study that reported by Veena in 2012 showed increase in latency response by chemical (i.e., acetic acid induced writhing and formalin tests) in albino mice[28]. The results were observed decrease at 90 min and remaining this activity until the end of experiment at 120 min. This result is attributed to pharmacokinic of M.oleifera by different processes. The AMO take 1h

to reach maximum concentration and give high value of latency response which observed decrease the latency response at 60 min and 90 min this mean the extract was excreted from the body.[21].More over study by Sulaiman ın 2008 observed that the highest latency activity was at high dose (100 mg kg-) [29]. On contrast previous study showed higher latency response values on albino mice that treated with *ethanolic M.oleifera* extract at 400 mg/kg from 15 min to 90 min[30].

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