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Original Article

Analgesic effect by using hot Plat and tail flick test in rats models for aqueuos moringa oleifer extract

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ABSTRACT

Introduction Drugs and plants are closely related to each other through the use of traditional medicines that is mainly prepared from plants. Plants are screened for presence of bioactive components responsible for the activity. Various types of plants have been used not only as dietary supplements but also as traditional treatments for many diseases. Moringaoleifera of Moringaceae is a potential source of phytochemical ingredients claimed to have analgesic property. Objective The present study aimed to investigate the analgesic activity of Moringaoleifera extract in albino rats by using taıl flıck and hot plat test. Methods: Leaves of Moringaoleifera were collected April 2022 from Surman (Libya). And then the powder was macerated in 1 L of distilled water for 3 days and evaporated to dryness by rotary evaporator. The crude extract was administered by oral gavaging in doses (50mg/kg_100mg/kg). The study was done by using experimental models, the albino rats were divided into 4 groups, each group consisting of 3 rats. Group I: Control (dH2O given orally at 2 ml/kg); Group II: Standard (100 mg/kg of Panadol orally.); Group III, IV, (MOE 50, 100 mg/kg, respectively), the analgesic effect was evaluated by using taıl flıck and hot plat test, through the reaction time was recorded in various time intervals) 60, 90, and 120 minutes). Results: MOE significantly a high significant increase in latency response (P<0.001) in comparison to control and standrad. Conclusion: These results indicate the ability of MOE to inhibit thermally induced nociceptive processes, which is a characteristic of strong analgesics

Key words: aqueous moringa oleifera extract, antinoceceptive, tail flick test, hot plat test.

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Introduction

Many of the medicines that are currently in use have natural sources, particularly plants. Through the usage of traditional medicines or ethnomedicines, which are mostly made from plants, drugs and plants are closely tied to one another.

Plants of interest are tested for the presence of bioactive components, and the phytochemicals responsible for the bioactivity are extracted, in order to find potential therapeutic candidates. Following the identification of their molecular structures, phytochemicals' original structures may be partially synthesized changed to increase activity or decrease toxicity [1]. In many parts of the world, different kinds of plants have been utilized for many years as both dietary supplements and conventional medicines for a wide range of illnesses [2, 3, 4]. The fact that conventional medications have been used extensively around the world shows the potential of plants as sources of bioactive chemicals, including potential anticancer, antioxidant, antiobesity, and antibacterial agents. These include the extensively farmed Moringaoleifera (Moringa or drumstick tree), a perennial tree with a quick growth rate that was employed by the ancient Romans, Greeks, and Egyptians and has since naturalized from the tropics to the sub-Himalayan areas. Oceania, Latin America, Africa, and tropical Asia (such as India, Pakistan, Bangladesh, and Afghanistan) [5], [6], [7], [8]. since ancient times to treat pain brought on by illness, injury, and surgery In addition to producing basic medications, plants and herbs also contain bioactive chemicals that have the potential to create brand-new drug structures. Due to their historical usage as medicines, various plants and herbs had a role in the creation of the current anaesthetic used to treat pain [9]. M. oleifera has been utilized in Indian traditional medicine for generations as an analgesic and anti-inflammatory. The phytochemical components of leaves, which include alkaloids, glycosides, phenols, saponins, and tannins, provide the mechanism of action for the analgesic effect. The analgesic effect is brought on by cyclooxygenase-2 (cox-2) activity suppression, which prevents prostaglandin synthesis. The extract may possibly have interfered with G-protein-mediated signal transmission, an analgesic mechanism unconnected to the reduction of prostaglandin formation. It could possibly have strengthened the peripheral mechanism by interfering with prostaglandin synthesis in the central nervous system. Non-steroidal anti-inflammatory medicines (NSAIDS), such as aspirin and diclofenac, are known to cause several types of analgesia that have these processes as a contributing factor. Are linked to more negative side effects, opening up the possibility for traditional medicines, which has increased focus on using plant materials as sources of medications for a wide range of human maladies [10]. Therefore, the objective of the current study was to assess the analgesic potential of an aqueous extract of M. oleifera leaves in order to demonstrate this activity utilizing several animal models.

Materıals And Methods

Collection of plant and extract preparation:

In April 2022, leaves of M. oleifera were gathered in Surman (Libya), and the Libyan Medical of Research Center identified the leaves. The leaves shade dried under appropiate condition. hence, including it in a regular diet may lower the likelihood of developing degenerative disorders [11]. 100 grams of powdered leaves from the shade were combined with

1 L of distilled water and macerated for 3 days with continuous shaking. the dark green solution was filtered via filter paper once the maceration process was complete [12]. Transferring the filtrated solution to a Buchner funnel furnished with Whatman No. 1 filter paper allowed vacuum machines to do the filtration. The homogenous solution was placed in firmly sealed jars and frozen over night at -25 °C before being utilized in the feeding tests. With distilled water (dH2O) diluted to use as experimental doses, the yield was determined in accordance with the dosages.

Experimental animal (model):

12 male albino rats, weighing between 120 and 180 g for each test , were obtained from the National Medical Research Center in Alzawia, Libya. They were maintained at regular laboratory temperatures of 20 to 25 C in alternately dark and light situations. There was unlimited access to food and drink. Prior to testing, the animal spent 10 days in the lab before examination

Animal Grouping:

The rats were placed into four groups of three randomly for each test, with group 1 serving as a control group that simply received the vehicle (distilled water). Group 2 received a standard dose of 100 mg/kg) of panadol (paracetamol 500mg) orally. the two sample or treatment groups (3 and 4) that received oral doses of M. oleifera extract of 50 and 100 mg/kg, respectively.

Procedure used for testing analgesic activity
Eddy's Hot plate method:

The hot plate technique of Eddy and Leimbach Was used to evaluate the analgesic efficacy of M. oleifera[13]. The temperature was kept at 55 ± 0.2 c 0. It's hot enough to be uncomfortable without harming tissue. In order to show that they were in agony, animals licked and jump. Following are the suspensions that were administered to these rats: group under control received distilled water. M. oleifera extract was given to the test groups at doses of 50 mg/kg and 100 mg/kg. Panadol 100 mg/kg was administered orally to the standard group. Following the administration of the medicine and test substance for 60, 90, and eventually 120 minutes, the animals underwent the same testing process. The time taken by the animal to lick the fore or hind paw or jump out of the plate was taken as the latency time.

Tail flick test:
Using an analgesiometer and an infrared (IR) radiation source, radiant heat was delivered to the tail at a single location over the proximal third. 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 (60, 90, and eventually 120 minutes) . Rats that responded to a tail flick within two to three seconds were chosen after an initial screening of the animals.

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 presented as Mean ± SEM (standard error of mean).

Rats are given the sensation of pain by the application of heat. The hot plate method's results are presented in [Table.1] and tailflick test results are presented in [Table2].

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

Group / Dose (mg/kg)	Duration of latency of jumping response interval		n (sec) at various time
	x60min	90min	120min
Control	8.43±0.27	8.83±0.17	8.66±0.14
standrad	10.47±0.38	13.70±2.04	11.53±0.63
sample (50mg/kg)	13.23±0.61**	12.00±3.29**	12.23±1.21
sample(100mg/kg)	19.00±0.52***	18.40±0.15**	12.67±0.43

comparison with control group (one way ANOVA
)

Each value represented in Mean ± SEM, *p < 0.05, **p < 0.01, ***p < 0.001 in

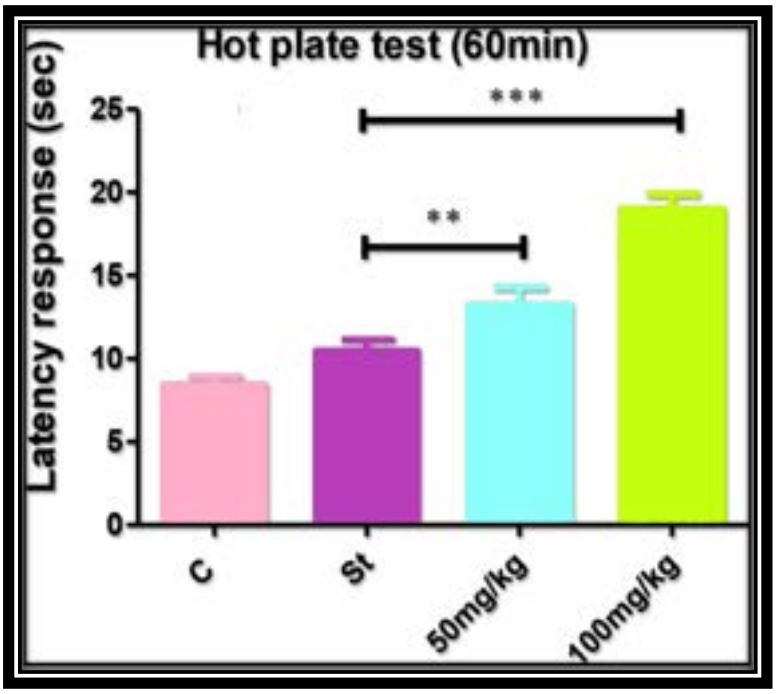
Table 2.Analgesic activity: Effect of aqueous extract of M.oleifera leaves on thermal stimuli Pduced pain in rats by using tail flick test.

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**	pp2.30±0.40

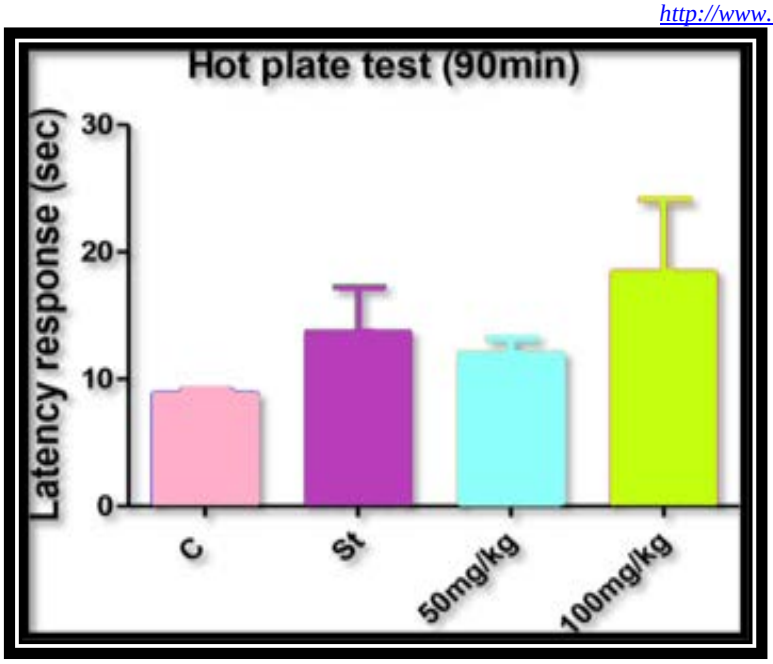
Each value represented in Mean ± SEM, *p < 0.05, **p < 0.01, ***p < 0.001 in comparison with control group (one way ANOVA).

Firstly, In hot plate method's at 60 minutes after the administration of a 100 mg/kg dosage of M. oleifera extract demonstrated a high significant increase in latency response (P***<0.001) (19.00±0.52) compared to control and standard. Similar

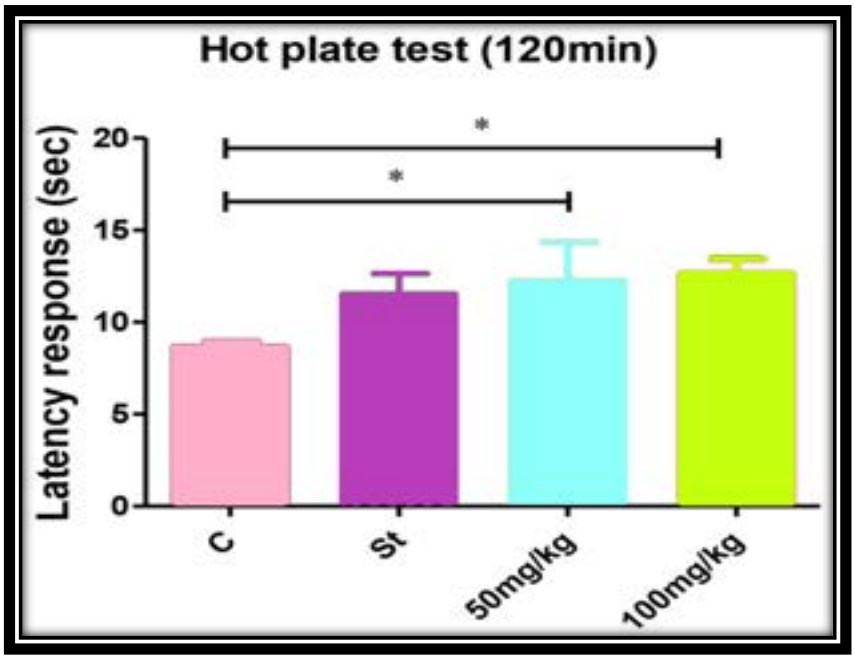
results were observed 90 minutes later. When compared to control and standard, the dosages of 50 mg/kg slightly extended the delay of the hot plate response (*p<0.05) at 60 minutes (3.43±0.29). While the groups that obtained extract at doses of 50 mg/kg and 100 mg/kg exhibited decreasing response after 90 minutes. The group that received 50 mg of the M. oleifera leaves aqueous extract 120 minutes after treatment (1.60±0.25) at the conclusion of the experiment showed the lowest analgesic activity values.



Graphic.1. Analgesic activity (latency response) of aqueous extract of M.oleiferaleaves at 60min after treated.
c= control group. st=standrad group were fed panadol. 50mg/kg, 100mg/kg=sample group were fed aqueous extract of M.oleiferaleaves.
tailflick test p



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



Graphic.3. Analgesic activity (latency response) of aqueous extract of M.oleiferaleaves at 120min after treated.

Secondly, tailflick method's at 60 minutes after being treated with aqueous M. oleifera extract, the rats that was given oral supplements of MOA extract at different

concentrations (50 mg/kg and 100 mg/kg) had significantly different outcomes from controls (P< 0.001). Rats are made to feel pain by the application of radiant heat. At 60 minutes after drug administration, aqueous extract M. oleifera dosage of 100mg/kg shown a substantial increase in tail flick response latency compared to control and standard. After 90 minutes, a similar response was seen. When compared to control and standard, the dosage of 100 mg/kg increased the latency of the tail flick response at the 60-minute (**p=0.0017) (4.30±0.23). Compared to control and standard, the dosage of 50mg/kg slightly increased the latency response (*p<0.05) (3.43±0.29) at 60 minutes. Whereas, at 90 minutes, the groups who received extract at 50 mg/kg and 100 mg/kg exhibited a decreasing response. The group that received 50 mg of an aqueous extract of M. oleifera leaves was found to have the lowest analgesic activity (1.60±0.25) at 120 minutes following treatment.

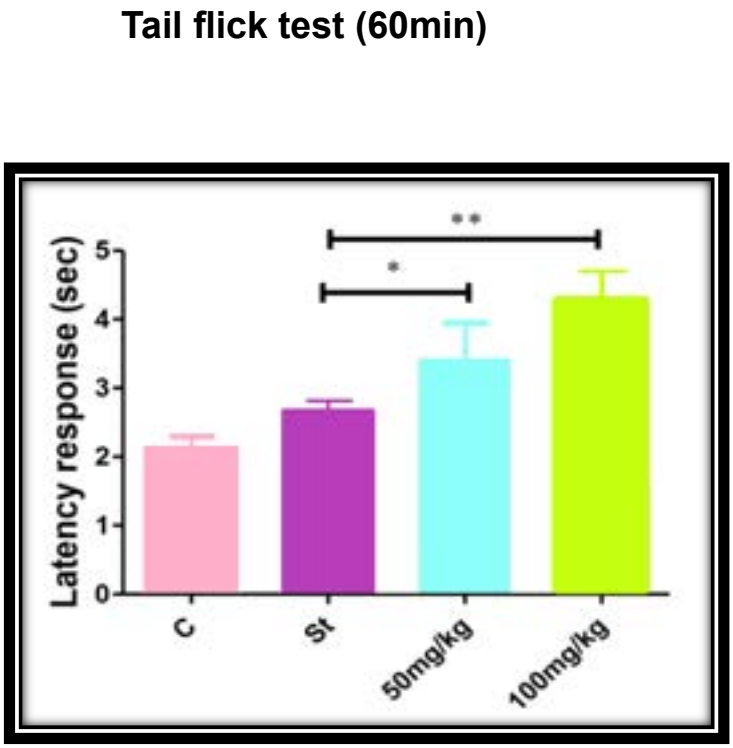


Figure 4. Analgesic activity (latency response) of aqueous extract of M.oleiferaleaves at60min after treated. c = control group. st=standrad group were fed panadol. 50mg/kg, 100mg/kg=sample group were fed aqueous extract of M.oleiferaleaves

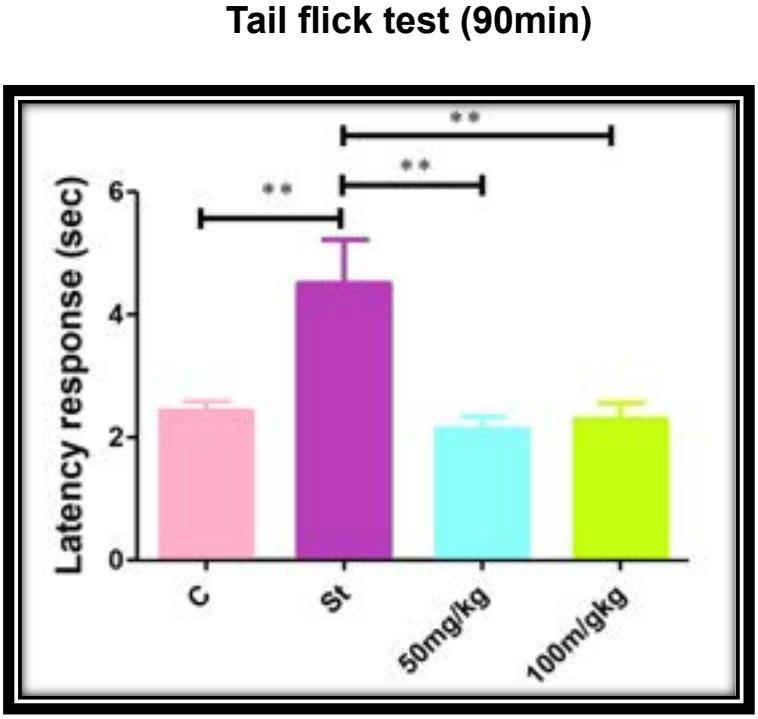


Figure 5.Analgesic activity (latency response) of *aqueous extract of M.oleifera*leaves at 90min after treated.

Tail flick test (120min)

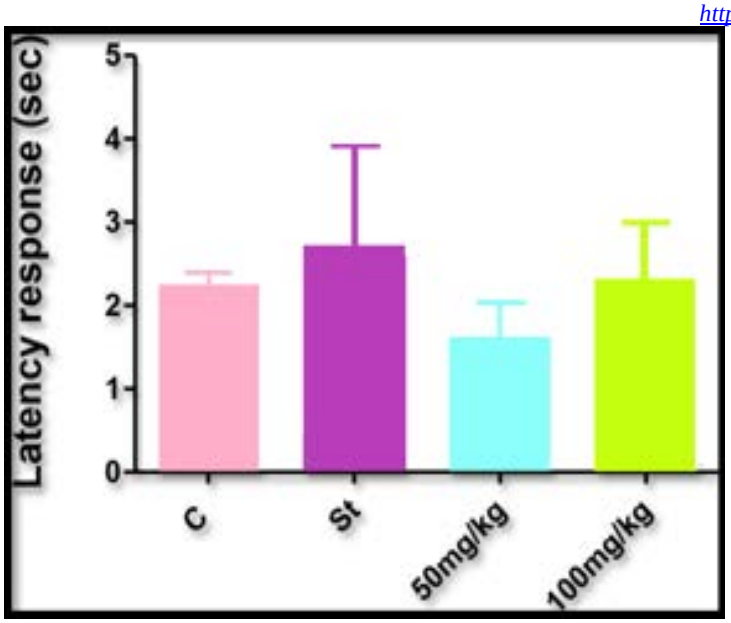


Figure 6. Analgesic activity (latency response) of *aqueous extract of M.oleifera*leaves at 120min after treated.

DISCUSSION

Analgesics are drugs that act on peripheral or central nervous system to selectively relieve pain without significantly altering consciousness [14]. Centrally acting analgesics act by raising the threshold for pain and also altering the physiological response to pain. On the other hand, peripherally acting analgesics act by inhibiting the generation of impulses at chemoreceptor site of pain [15]. The animal models employed for screening of analgesic activity in this study are pain-state models using thermal stimuli which include tail-flick and hot plate methods. Both methods are useful in illustrating centrally mediated antinociceptive responses which focus generally on changes above the spinal cord level [16]. While the tail-flick method mediates a spinal reflex to a nociceptive stimulus, hot plate method involves higher brain functions and is regarded a supra spinally organized response [17].

Tail-flick and hot plate are two of the several methods available for evaluating central analgesic activity [18]. Although both methods employed thermal stimuli, the tail-flick response indicates spinally mediated reflex while the paw-licking hot plate response is due to complex supraspinally integrated behaviour [17].

In tail flick, 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 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 [19] .More over

study by Sulaiman ın 2008 observed that the highest latency activity was at high dose (100 mg kg-) [20] . 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[21] .

In the method of pain induction by application of radiant heat on hot plate, M.oleifera extract at the dose of 100mg. /kg showed highly significant increase in latency of which is comparable to that of panadol at the dose of 100 mg/kg of body weight [Tables 1].In a similar study by Manaheji et al; found significant reductions in both thermal hyperalgesia and mechanical allodynia in adult Male Wistar rats with CFA-induced arthritis compared to indomethacin 5mg/Kg [22]. Another study by Nitin G et al; found that the seeds of Moringa oleirera Lam. possess marked analgesic activity and is equipotent to standard drug (Panadol) [23].On contrast higher latency response values have been shown by Manoj in 2011 on albino mice treated with ethanolic M.oleifera extract at 400 mg/kg from 15 min to 90 min [24] . From this study, it can be concluded that the seeds of MoringaoleireraLam. possess marked analgesic activity [Graphic. 1and 2] .The present study establishes the use of Moringaoleifera.leaves as regular analgesic.

CONCLUSION

The present study results summarizes that, the analgesic effect of AMO is exhibited in a dose-dependent manner and which may be due to the presence of various bioactive constituents in the extract. However, the study is needed to isolate the active constituents responsible for the observed effect. These findings further support the ethnomedical claim of the use of the plant in the management of painful and inflammatory conditions. Further, the ability of MOE to inhibit thermally induced nociceptive processes also demonstrated its potential to influence the peripheral and central antinociceptive mechanisms, which is a characteristic of strong analgesics.

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