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Lawsonia inermis possesses a significant analgesic activity compared to *Waltheria indica, Moringa oleifera, Nigella sativa*, and diclofenac in female Wistar rats

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ABSTRACT

Pain is a severe symptom of many diseases, with an increasing percentage of people manifesting various types of pain. Medicinal plants provide analgesic potential with little toxicity. We performed this experiment to compare the analgesic activities of *Lawsonia inermis*, *Waltheria indica*, *Moringa oleifera*, and *Nigella sativa* in Wistar rats using writhing and paw lick responses. We grouped 21 adult female rats into seven groups (n=3), including uninduced and untreated rats (group 1), induced untreated rats (group 2), rats treated by Lawsonia inermis at 200 mg/kg (group 3), rats treated withWaltheria indica at 200 mg/kg (group 4), rats treated with *Nigella sativa* at 200 mg/kg (group 5), rats treated with *Moringa oleifera* at 200 mg/kg (group 6), and rats treated with diclofenac at 10 mg/kg (group 7). We dosed rats for 14 days after inducing the pain. Phytochemical screening showed that methanolic extracts of Lawsonia inermis, Moringa oleifera, and ethanolic extract of *Waltheria indica* contain: Alkaloid, *saponin,* steroid, tannin, flavonoid, phenols, terpene, and glycosides. The rate of weight gain in rats treated with M. oleifera and W. indica was 7%, and with diclofenac was 9% compared to the untreated control. L. inermis and N. sativa possessed a weight gain of 3% and 2%, respectively. All the extracts exhibited analgesic activities by significantly reducing the number of lick and writh in the order of *Lawsonia inermis, Nigella sativa, Moringa oleifera,* and *Waltheria indica.*This study concluded that *Lawsonia inermis* possess significant analgesic activities compared to other plants and the standard drug (diclofenac).

Keywords

Pain, Lawsonia inermis, Nigella sativa, Moringa oleifera, Waltheria indica, Analgesic

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Abbreviations

NSAIDs: Non-steroidal anti-inflammatory drugs
Cox: cyclooxygenase
GCs: Glucocorticoids;
L:Lawsonia
M: Moringa
W: Waltheria
N: Nigella

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Introduction

Scientists describe the pain as an unpleasant sensory experience connected to the potential impairment of tissues [1]. Reports have shown that most pains are usually related to inflammation [2], and inflammation has several mediators like prostaglandins, histamine, serotonin, leukotrienes, and bradykinin that are accountable for inflammatory responses (allergy and hypersensitivity), which results in abnormal production and release of these mediators at an abnormal rate [3]. Analgesic drugs can be produced from natural or synthetic sources. Natural analgesics are mainly derived from *Papaver somniferum* (opium poppy) which is an opioid analgesic (for example, morphine), *Vernonia amygdalina*, and *Zingiber officinale*. These plants can be used for treating acute and chronic pain and are tolerated in geriatrics patients with minimal side effects [4]. However, analgesics like acetaminophen and non-steroidal anti-inflammatories are derived from synthetic sources, predisposing them to toxicities [4, 5]

Reports have shown that medicinal plants have been used as the primary sources of drugs for managing animal and human diseases since ancient times [5]. The World Health Organization (WHO) pronounced that 80% of the world's population depends mainly on medicinal plants for health care delivery and needs, especially those in developing countries [5]. Most plants used for anti-inflammatory purposes also possess analgesic activity. Between 2000 and 2019, about 154 Nigerian medicinal plants have been studied and proven to exhibit analgesic and anti-inflammatory properties [6].

Incessantly untreated pain is one of the most major conditions that can affect the body structure, leading to physical injury and mental disorders [7]. These heart-breaking conditions are the primary cause of body deformities that can lead to death, except treated promptly [8]. Statistical reports have shown that 20% (1 in 5) of adults suffer from pain, and 10% (1 in 10; almost 60 million) of adults are diagnosed with chronic pain yearly [9].

The currently used analgesic drugs include opioids and steroidal and non-steroidal anti-inflammatory agents that can result in severe adverse reactions [10]. Side effects associated with current analgesics (NSAIDs) include gastric ulcers, platelet inhibition, cardiovascular disorder, and organ failure (liver, kidney) [10]. Steroidal anti-inflammatory drugs are related to immunosuppression, muscle weakness, blurry vision, increased weight, and appetite [10]. Side effects of opioids are nausea, vomiting, constipation, drowsiness, dependence, tolerance, and addiction [10]. There is a need for more experimental research to ensure that newer analgesic drugs that possess minimal or no adverse effects are produced from medicinal plants. This study was conducted to evaluate and compare the analgesic effects of four medicinal plants; *Lawsonia inermis* (henna), *Waltheria indica* (sleepy morning), *Moringa oleifera* (Moringa), *Nigella sativa* (black seed) with diclofenac in pain induced Wistar rats.

Results

Phytochemical analysis

The tables below showed the various phytoconstituents present in the methanolic leave extract of Lawsonia inermis Linn, (Table 1), Moringa oleifera (Table 2), and ethanolic root extract of Waltheria indica Table 3.

Table 1.
Phytochemical constituent of *Lawsonia* inermis Linn. leaves

Test	Crude methanol extract
Saponins	++
Tannins	++
Cardiac glycoside[s]	++
Flavonoids	++
Steroids	+
Alkaloids	+
Anthraquinones	+
Terpenoids	+

Interpretations -: Absent, +: Present, ++: Abundantly present

Table 2.
Phytochemical constituent of *Moringa oleifera* Linn. leaves

Test	Crude methanol extract
Tannins	+
Alkaloid	+
Saponin	+
Flavonoid	+
Glycosides	+
Phenol	+
Carbohydrate	+
Terpenoids	+

Interpretations -: Absent, +: Present, ++: Abundantly present

Table 3.
Phytochemical constituent of *W. indica* Linn. roots

Test	Crude ethanol extract
Alkaloids	++
Saponins	+
Tannins	+
Cardiac glycosides	++
Flavonoids	++
Steroids	+
Glycosides	+
Anthraquinones	+
Terpenes	+
oxalates	+
Trypsin	+

Interpretations -: Absent, +: Present, ++: Abundantly present

Weight gain

The changes in the weight of rats treated with Lawsonia inermis, Waltheria indica, Moringa oleifera, Nigella sativa, and diclofenac are listed in Table 4.

After seven days of treatment, the percentage of weight gain in all other treated rats W. indica showed a significant increase (6%.) compared to other treatment groups. The percentage of weight gain in all other treated rats was non-significant except for groups treated with N. sativa that showed considerably low weight gain (0.4%) when compared to all other treatment groups and the two controls. After 14 days of treatment, diclofenac-treated rats showed a significantly increased weight (9%) compared to all other treatment groups and the two control. W. indica and M oleifera treated groups showed moderate weight gain (7%) compared to L. inermis (4%) and N. sativa (1.8%), as shown in Table 4.4.

The results of the analgesic activities of the extracts of Lawsonia inermis, Waltheria indica, Moringa oleifera, and Nigella sativa

The analgesic effect of Lawsonia inermis, Waltheria indica, Moringa oleifera, and Nigella sativa extracts are present in Tables 5 and 6.

Formalin test

Table 5 shows the results of the analgesic effect of the extracts using the formalin-induced paw lick response. The lower the lick, the higher the analgesic potency. L. inermis has the lowest lick response (11.7 ± 5.13) compared to W. indica (37.3 ± 8.62), N. sativa (17.7 ± 6.51), M. oleifera (26.3 ± 3.51), diclofenac (29.0 ± 7.94) and the control (44.7 ± 5.69). All the extract treatment groups had considerable analgesic activities compared to diclofenac.

Percentage inhibition of induced pain

The percentage inhibition is indicated in Figure 1. L. inermis-treated rats had higher analgesic potency than other treatment groups and the control. Rats treated with N. sativa also showed a considerable analgesic effect compared to the control group. The M. oleifera dosed group showed similar analgesic potency with the group treated with the conventional drug (diclofenac) compared to the control.

Acetic acid test

Table 4.6 shows the results of the analgesic properties of the extracts using the acetic acid-induced writhing response. The lower the writh, the higher the analgesic effect. L. inermis showed the highest analgesic activities compared to other extracts and the standard drug

Percentage inhibition

The percentage inhibition is indicated in

Table 4.
Weight gain of rats treated with *Lawsonia inermis*, *Waltheria indica, Moringa oleifera, Nigella sativa* and *diclofenac*

Group	Day 0	Day 7	Day 14
Negative control	166.7 ± 20.11	172.7 ± 21.78(3.6%)	179.7 ± 21.94 (4%)
Positive control	136.3 ± 11.15	141.3 ± 10.12(3.67%)	147.7 ± 18.23 (5%)
L. inermis	138.3 ± 24.13	144.3 ± 25.58(4.34%)	149.7 ± 24.34 (4%)
W. indica	117.7 ± 2.076	124.7 ± 1.528(6%)	133.7 ± 2.518 (7%)
N. sativa	178.3 ± 3.507	179.0 ± 9.167(0.4%)	182.3 ± 12.58 (1.8%)
M. oleifera	197.0 ± 11.27	202.0 ± 11.14(2.54%)	216.0 ± 15.62 (7%)
Diclofenac	118.3 ± 1.528	122.7 ± 7.369(3.72%)	134.3 ± 13.80(9%)

Table 5.
Analgesic effect of the extracts and diclofenac on response to pain using the formalin test

Group	No of licks/10mins	Mean ± SD
Positive control	43	44.7 ± 5.69
	40	
	51	
L. inermis	13	11.7 ± 5.13***
	6	
	16	
W. indica	28	37.3 ± 8.62
	39	
	45	
N. sativa	18	17.7 ± 6.51**
	11	
	24	
M. oleifera	30	26.3 ± 3.51*
	26	
	23	
Diclofenac	26	29.0 ± 7.94*
	38	

Results are shown as Mean ± SD: n=5
*Significant *$p \leq 0.05$ **$p \leq 0.01$ ***$p \leq 0.001$

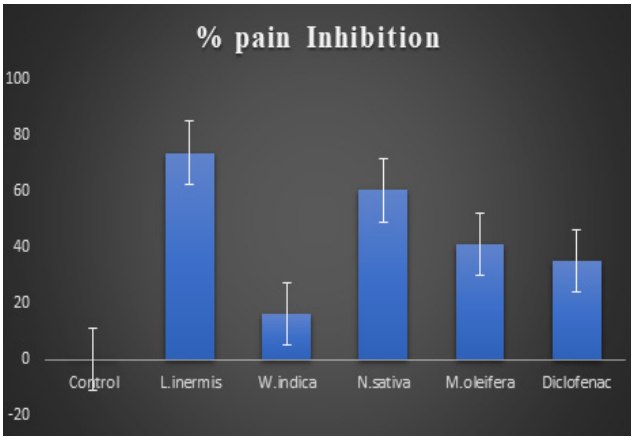

Figure 1.
Percentage inhibition of formalin induce paw lick pain treated with various extracts of *L. inermis, W. indica, N. sativa, M. oleifera,* and *diclofenac*

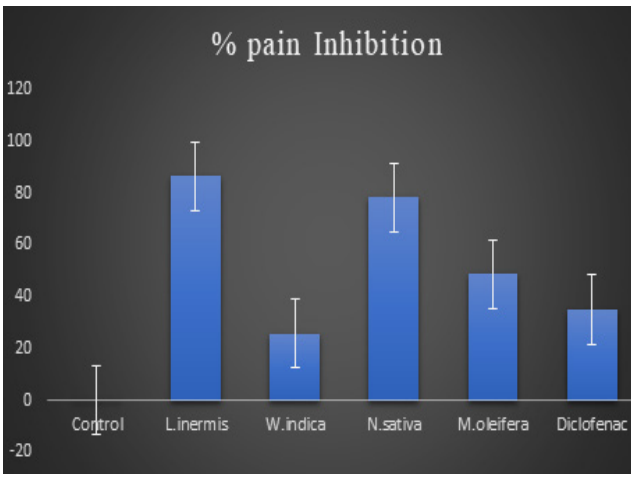

Figure 2.
Percentage inhibition of acetic acid writhing test (pain) treated with various extracts of *L. inermis, W. indica, N. sativa, M. oleifera,* and *diclofenac*

Figure 2. L. inermis group had significantly higher analgesic potency than other treatment groups and the control. Rats treated with N. sativa also showed a considerable analgesic effect compared to the control group. The M. oleifera dosed group showed similar analgesic potency with the group treated with the conventional drug (diclofenac) compared with the control.

Discussion

Bioactive phytoconstituents usually found in medicinal plants include alkaloids, tannins, flavonoids, antioxidants, carotenoids, and phenolic compounds. Phytochemical screening of the extracts used in this study revealed the presence of alkaloids, saponin, steroids, tannin, flavonoids, phenol, terpene, and glycosides. The result obtained from this study conforms with the previous work of Aremu et al. [12] Aremu et al. [16], and Basiru et al., [17] who reported similar phytoconstituents in methanolic extract of Lawsonia inermis Linn leaves, Moringa oleifera Linn. Leaves and ethanolic extract of Waltheria indica Linn. root, respectively.

Most medicinal plants contain alkaloids, saponins, steroids, tannins, flavonoids, phenols, terpenes, and glycosides [17]. These compounds have been reported for their analgesic and anti-inflammatory potential. Alkaloids specifically can be found in the papaver somniferum (poppy plant), a plant that produces opium which is a source of many narcotic analgesics [18], while steroids are essential anti-inflammatory agents [19]. Tannins (tannic acid) are polyphenols that are soluble in water and are found in several plants with marked antioxidant activities [20]. Flavonoids are secondary metabolites that possess multiple functions such as analgesic and antioxidant activities [21]. Phenols are abundantly present in plants and their metabolites possess many physiological functions. Terpenoids are the largest naturally occurring

plant compounds that form the primary component of their essential oil. Glycosides are organic substances made of sugars with alcohol and phenol with major effects on the heart either positively (cardiotonic) or negatively (toxicity) [22].

The result from this study showed that the percentage weight of rats treated with diclofenac had 9% weight gain when compared with W. indica (7%), M. oleifera (7%), N. sativa (1.4%), and the two controls. This result agrees with Allo et al. [23] who reported that moringa improves weight gain. This result also contradicts the outcome of Waterman et al., [24] who reported that isothiocyanate-rich M. oleifera extract reduces weight.

Paw lick and writhing tests are standard methods used to induce experimental pain by injecting irritant substances such as formalin and acetic acid in rats. Analgesic potentials of the test compound are predominantly evaluated through the decreased frequency of paw licking and stomach writhing, respectively [25]. The result of the acetic acid-induced writhing and the formalin paw lick tests obtained from this study disclosed that all the extracts exhibit significant analgesic potentials. This observation is due to the reduction in the number of writhes and paw lick responses as compared to the untreated control rats. L. inermis exhibited the highest analgesic effect, followed by N. sativa oil. This result agrees with Ghannadi et al. [25]) who reported that ethanolic extract of L. inermis Linn. leaf and black seed oil possess a dose-dependent analgesic, anti-inflammatory, and antipyretic effect, respectively. M. oleifera, on the other hand, showed moderate analgesia when compared to both L. inermis and N. sativa. This result conforms with the report of Bhattacharya et al., [26] and Adedapo et al., [15] who ascertained that various extracts of M. oleifera exhibit dose-dependent analgesia when compared to indomethacin. W. indica had the least analgesic effect compared to all other extracts. Nirmala and Sridevi, [27] and Termer et al. [28] attributed the analgesic potency of W. indica Linn. roots to the presence of tiliroside, epicatechin, and quercetin which are flavonoid derivatives as seen from the phytoconstituents reported above.

It was noted that M. oleifera and W. indica showed increased weight and minimal analgesic activities, while L. inermis and N. sativa showed marked analgesic effects with minimal weight changes. Reports have shown that many drugs cause weight gain, which can lead to overweight and obesity [29]. Most conventional analgesic drugs usually enhance body fat redistribution by increasing central adiposity, resulting in resistance to insulin, dyslipidemia, metabolic syndrome, and risk for the non-insulin-dependent type of diabetes (type II) [30]. Thus, from the result above, L. inermis and N. sativa will not cause abnormal weight changes even when used for the long term.

Conclusions

All the evaluated extracts contained phytochemicals with known antioxidant properties. All the extracts exhibited analgesic activities by significantly reducing the number of lick and writh in the order of Lawsonia inermis, Nigella sativa, Moringa oleifera, and Waltheria indica. Lawsonia inermis possesses significant analgesic activities compared to other plants and the standard drug (diclofenac). Lawsonia inermis is effective in the treatment of pain, and its effect on various types of pain could be investigated further by researchers.

Materials & Methods

Drug

Diclofenac sodium was acquired from a reputable pharmaceutical company in Ilorin, Kwara State, Nigeria.

Plant collection, preparation, and authentication

The leaves of Lawsonia inermis, Moringa oleifera, and stem bark of Waltheria indica were harvested from different areas in Kwara State The plants were authenticated at the Herbarium, Department of Botanical Sciences, University of Ilorin, Ilorin with voucher specimen no.134289 which was deposited for reference.

Nigella sativa oil (Hemani 125 mL) was obtained from a reputable store in Ilorin, Kwara State, Nigeria.

Extraction of plants material

The leaf samples of Lawsonia inermis, Moringa oleifera, and stem bark of Waltheria indica were washed, dried, and macerated into powdery form. 500 g of different leaves (L. inermis and M. oleifera) were soaked in 99% methanol (ratio 1:3, w/v), while Waltheria indica was soaked into 98% ethanol at room temperature, for 48 hours respectively. The procedures were repeated twice for the second and third extraction processes. The filtrates were sieved through filter paper (Whatman size no.1) and vaporized using a rotary evaporator. The dry residues were weighed and preserved in a refrigerator at 4oC until used.

Ethical approval

Ethical approval was obtained from the Ethical Committee on Animal Use and Care, Faculty of Veterinary Medicine, University of Ilorin, Nigeria with the approval code number UREC/FVM/15/32TA037.

Phytochemical analysis

Phytochemical screening of the leave samples of Lawsonia inermis, Moringa oleifera, and stem bark of Waltheria indica was conducted to identify the presence of various phytoconstituents following standard analytical procedures [11].

Experimental animals

Adult female rats (120 g) were obtained from the laboratory animal unit, Department of Biochemistry, University of Ilorin. They were housed in hygienic cages in a fly-proof animal house adhering to the internationally accepted standards for laboratory animal use and care laid down by the Canadian Council on Animal Care, (CCAC). The rats were fed with standard pelletized feed (Chikun feeds) and pro-

vided with clean water ad libitum. The rats were allowed to acclimatize for two weeks before the commencement of the experiment. This is in accordance with the guidelines for laboratory animal care and use (Institute for laboratory animal research).

Experimental design

A total of 21 female Wistar rats were divided into seven groups, each consisting of three rats, and the extracts were administered through the oral route using a cannula. Each group was treated as:
Group 1: (Negative control) was untreated and was uninduced.
Group 2: (Positive control) induced and untreated.
Group 3: Treated with Lawsonia inermis extract at 200 mg/kg daily for 14 days orally
Group 4: Waltheria indica extract was administered orally at 200 mg/kg daily for 14 days
Group 5: Nigella sativa oil was administered orally at 100 mg/kg daily for 14 days
Group 6: Moringa oleifera extract was administered at 200 mg/kg daily for 14 days.
Group 7: treated with diclofenac 10 mg/kg daily for 14 days orally.

Weight measurement

The weight was assessed using an automated electronic scale (Sensor Disc Technology, London) from day one and later on a weekly basis. In doing this, a round plastic bowl was positioned on the scale and tared to zero after which a rat was placed inside the bowl and consequently weighed as described by [12].

Assessment of analgesic activities

Two models were used to induce pain in this experiment (the paw-licking test and the acetic-acid-induced writhing test).

Formalin paw lick test

The formalin test was performed as described by (Hunskaar and Hole, 1987). 20 µL of 2.5% formalin were injected into the sub-plantar space of the right hind paw of the mice and immediately kept in a cage where they can be easily monitored. The frequency of lick and licking time of the injected paw was documented for 10 mins [13]. The percentage inhibition of the paw lick response was estimated using the formula;

$$\% \text{ Inhibition} = D0 - Dt / D0 \times 100$$

D0 is the average paw lick response of the control group
Dt is the average paw lick response of the drug-treated groups

Acetic acid-induced writhing test

The writhing test was carried out as first demonstrated by Sigmund et al. The rats were treated with various extracts an hour before the injection of 0.6% acetic acid at 10 mL/kg intraperitoneally. Writhing activity characterized by hindlimb extension, abdominal muscle contraction, and arching of the back was observed in the animals [14]. The number of writhes i.e. abdominal constrictions were recorded for 5mins following the injection of acetic acid [15]. The percentage inhibition of the writhing response was estimated using the formula:

$$\% \text{ Inhibition} = D0 - Dt / D0 \times 100$$

Do is the average writhing response of the control group
Dt is the average writhing response of the drug-treated groups

Data analysis

The values were expressed as mean ± standard deviation (Mean ± SD). The differences within the groups were compared with the Dunnett post hoc method of ANOVA with GraphPad Prism statistical package, San Diego, California, U.S.A (www.Graphpad.Com).

Authors' Contributions

Aremu A and Idris J. F planned the experiments. Akorede G. J, Aremu A and Idris G. J carried out the experimental dosing. Olatunji A. O and Basiru A carried out analgesic test. Aremu A. Idris J. F and Ahmed O. A. contributed to the interpretation of the results. Idris J. F took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

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Competing Interests

The authors declare that there is no conflict of interest.

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