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Modulatory Effects of *Lawsonia inermis, Waltheria indica, Moringa oleifera* and *Nigella sativa* on Haematology, Blood Pressure and Electrocardiographic Parameters of Wistar Rat Exposed to Acute Pain

Efek Modulator of *Lawsonia inermis, Waltheria indica, Moringa oleifera* dan *Nigella sativa* Terhadap Gambaran Hematologi, Tekanan Darah dan Parameter Elektrokardiografi pada Tikus Wistar yang Diinduksi Nyeri Akut

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ABSTRACT

Background: Pain is an obnoxious stimulus usually triggered by stressors that can affect homeostasis. The inability to sense pain is usually connected to abnormal fluctuations in normal body functions, leading to shortened life expectancy. **Purpose:** This study aimed to assess the effects of *Lawsonia inermis, Waltheria indica, Moringa oleifera* and *Nigella sativa* on hematology, electrocardiography, and blood pressure in Wistar rats experimentally exposed to acute pain. **Methods:** Twenty one 21 Adult female rats were divided into (1-7) of three rats each. Group 1 (negative control), group 2 (positive control), group 3 (*Lawsonia inermis* at 200 mg/kg), group 4 (*Waltheria indica* at 200 mg/kg), group 5 (*Nigella sativa* at 200 mg/kg), group 6 (*Moringa oleifera* at 200 mg/kg), and group 7 (Diclofenac at 10 mg/kg). Rats were dosed for 14 days, after which pain was induced. **Results:** The extracts showed non-significant increases in most hematological parameters, including Packed Cell Volume, Red Blood Cells, and White Blood Cells counts, compared to positive controls. The effects of the extracts on the electrocardiographic parameters were not significant. Although the extract relieved pain, the effect of acetic acid on the heart was not completely ameliorated. The results of this study showed high blood pressure in untreated rats, which could be linked to pain induction. *Moringa oleifera* and *Waltheria indica* exhibited minimal blood pressure-lowering effects. *Waltheria indica* and *Lawsonia inermis* showed transient blood pressure-lowering effects. **Conclusion:** This study concluded that the extract had a modulatory effect on hematology and a lowering effect on blood pressure and electrocardiac parameters in all treatments.

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ABSTRAK

Latar Belakang: Nyeri adalah stimulus menjengkelkan yang biasanya dipicu oleh stresor yang dapat mempengaruhi homeostasis. Ketidakmampuan untuk merasakan rasa sakit biasanya terhubung dengan fluktuasi abnormal dalam fungsi tubuh normal, yang menyebabkan harapan hidup lebih pendek. **Tujuan:** Penelitian ini bertujuan untuk mengkaji pengaruh *Lawsonia inermis, Waltheria indica, Moringa oleifera* dan *Nigella sativa* terhadap gambaran hematologi, elektrokardiografi, dan tekanan darah pada tikus Wistar yang secara eksperimental terpapar nyeri akut. **Metode:** Sebanyak 21 tikus betina dewasa dibagi menjadi 7 kelompok dengan tiga tikus masing-masing kelompok. Kelompok 1 (kontrol negatif), kelompok 2 (kontrol positif), kelompok 3 (*Lawsonia inermis* pada 200 mg / kg), kelompok 4 (*Waltheria indica* pada 200 mg / kg), kelompok 5 (*Nigella sativa* pada 200 mg / kg), kelompok 6 (*Moringa oleifera* pada 200 mg / kg), dan kelompok 7 (Diklofenak pada 10 mg / kg). Tikus diberi dosis selama 14 hari, setelah itu rasa sakit diinduksi. **Hasil:** Ekstrak menunjukkan peningkatan yang tidak signifikan pada sebagian besar parameter hematologi, termasuk jumlah *Packed Cell Volume* (PCV), *Red Blood Cell* (RBC), dan *White Blood Cell* (WBC), bila dibandingkan dengan kontrol positif. Efek ekstrak pada parameter elektrokardiografi menunjukkan hasil yang tidak signifikan. Meskipun ekstrak menghilangkan rasa sakit, efek asam asetat pada jantung tidak sepenuhnya diperbaiki. Hasil penelitian ini menunjukkan tekanan darah tinggi pada tikus yang tidak diobati, dapat dikaitkan dengan induksi nyeri. *Moringa oleifera* dan *Waltheria indica* menunjukkan efek penurun tekanan darah minimal. *Waltheria indica* dan *Lawsonia inermis* menunjukkan efek penurun tekanan darah sementara. **Kesimpulan:** Penelitian ini menyimpulkan bahwa ekstrak memiliki efek modulator pada gambaran hematologi dan efek penurunan pada tekanan darah dan parameter elektrokardiak dalam semua perlakuan.

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Kata kunci: Elektrokardiogram; Hematologi; Tanaman Obat; Tekanan Darah; Tikus Wistar

INTRODUCTION

Pain is an obnoxious stimulus usually triggered by stressors that can affect homeostasis, and the inability to sense pain is usually associated with shortened life expectancy (Swift, 2018). Internal medicine prioritizes the association between pain and changes in vital signs associated with hypertension and tachycardia (Dayoub and Jena, 2015). Reports have shown that acute pain is physiologically linked to stress responses involving increases in blood pressure, heart rate, pupil diameter, and plasma cortisol level (Rathmel and Fields, 2012). A Previous study examining the physiological response to pain in a small group of healthy patients found an increase in heart rate when individuals are exposed to noxious stimuli (Hilgard *et al.,* 1974). The degree and interval of the stimulus in these spinal reflexes causes activation of the sympathetic nervous system, which increases peripheral resistance, heart rate, and stroke volume (Silvani *et al.,* 2016).

Medicinal plants are the primary source of drugs for the management of animal and human diseases (Aremu *et al.,* 2022). The World Health Organization (WHO) estimates that approximately 80% of the world's population, particularly in developing countries, relies mainly on herbal plants for health care needs, primarily using the active principles in plant extracts (Aiyelero *et al.,* 2009). These plants have minimal side effects and have been used to treat various diseases since ancient times (Aiyelero *et al.,* 2009; Aremu *et al.,* 2022). Kang-Hua Chen *et al.,* (2012) reported the use of *Moringa oleifera* in the treatment of hypertension by lowering the pulmonary arterial blood pressure in monocrotaline-induced hypertensive rats. It has also been reported that the seed of *Moringa oleifera* shows heart-protective function in spontaneously hypertensive rats by raising diastolic cardiac function and lowering the nocturnal heart rate without altering the rat's blood pressure (Randriamboavonjy *et al.,* 2016) the modulatory effect of medicinal plants on hematology has been reported as shown by *Waltheria indica* increasing the Packed Cell Volume, Red Blood Cells, Hemoglobin Count, and Mean Corpuscular Hemoglobin in rats fed an iron-deficient meal (Oladijin and Yakubu, 2005). *Nigella sativa* oil has been reported to increase hematocrit levels but reduce Mean Corpuscular Hemoglobin Concentration (MCHC) and Mean Corpuscular Volume (MCV) concentration (Kökdil *et al.,* 2006) whereas Lawsonia inermis has no significant effect on Packed Volume Cell (PVC), Red Blood Cells (RBC), and Hemoglobin concentrations, as reported by (Aremu *et al.,* 2022).

Reports have shown that reduced hemoglobin levels, which are usually characterized by anemia, have been associated with fibromyalgia and chronic fatigue syndrome, both of which are frequently accompanied by chronic pain (Yao *et al.,* 2021). Additionally, by affecting neuronal activity and tissue viability, insufficient oxygen transport to tissues as a result of low hemoglobin levels might worsen pain perception (Premont *et al.,* 2020). Full blood counts and haematological parameters are useful, inexpensive, and widely accessible paraphernalia for the management and prognosis of patients with coronary heart disease, heart failure, hypertension, arrhythmias, and stroke (Mozos, 2015). The present study aimed to assess the comparative and modulatory effects of *Lawsonia inermis, Waltheria indica, Moringa oleifera* and *Nigella sativa* on the hematology, electrocardiography, and blood pressure of Wistar rats experimentally exposed to acute pain.

MATERIAL and METHODS

Plant Collection, Preparation and Authentication

The leaves of *Lawsonia inermis* and *Moringa oleifera* and the stem bark of *Waltheria indica* were harvested from different areas in Kwara State. The parts of these plants were used for this study because of their reported medicinal activities (Azeez *et al.,* 2017; Aremu *et al.,* 2022) 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 the stem-bark of *Waltheria indica* were washed, dried, and crumpled into powder. Powdered particles (500g) were soaked in 99% methanol (ratio 1:3, w/v), and *Waltheria indica* was infused into 98% ethanol at room temperature for 48 h. This procedure was repeated twice for the second and third extraction processes. The filtrates were sieved through filter paper (Whatman No.1) and vaporized using a rotary evaporator. The dry residue was weighed and preserved in a refrigerator at 4^{0}C until used.

Experimental Design

A total of 21 matured female Wistar rats (8 weeks old and non-gravid) 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 follows: **Group 1:** (Negative control) was untreated and uninduced. **Group 2:** (Positive control) untreated induced. **Group 3:** Lawsonia inermis extract administered orally at 200 mg/kg daily for 14 days. **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 200 mg/kg daily for 14 days. **Group 6:** *Moringa oleifera* extract administered at 200 mg/kg daily for 14 days. **Group 7:** Diclofenac was orally administered at a dose of 10 mg/kg daily for 14 days.

Pain Induction Using Acetic Acid

Pain was induced using the writhing test. The rats were treated with various extracts for 14 days and injected intraperitoneally with 0.6% acetic acid at 10 ml/kg to induce acute pain. Writhing activity characterized by hindlimb extension, abdominal muscle contraction, and arching of the back has been observed in animals.

Electrocardiograph and Blood Pressure Measurement

Electrocardiography was performed to assess cardiac function using an EDAN®-VE 1010 digital veterinary electrocardiographic machine, as described by (Azeez *et al.,* 2017). Parameters such as HR (Heart Rate), P (atrial contraction), PR (delay at Atrioventricular bundle), QRS (ventricular contraction), QT (time from ventricular contraction to ventricular relaxation), and QTc (QT corrected) were measured. The blood pressure of each rat was recorded. Electrocardiogram (ECG) and blood pressure were monitored on day 14.

Blood Collection and Analysis

At the end of the experiment, the rats were humanely euthanized (rats were anesthetized with light chloroform followed by cervical dislocation). Blood samples were collected via the intraocular route for hematology. The method adopted by Aremu *et al.,* (2017) was used to insert a heparinized capillary tube into the ocular vein, and blood was drawn from the median canthus of the eye. 1-2 ml of blood were drawn and placed in EDTA sample containers.

Total Blood Count (TBC), Red Blood Cell (RBC) count, White Blood Cell (WBC) count, Packed Cell Volume (PCV), hemoglobin (HB), platelet (PLT), and differential count, including Mean Corpuscular Volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), were the haematological parameters analyzed using an Auto Hematology Analyzer (Biogen GmbH).

Statistical Analysis

Values are expressed as means ± standard deviation (mean ± SD). The differences within the groups were compared using Dunnett's post hoc ANOVA with GraphPad Prism statistical package (San Diego, California, U.S.A; www.Graphpad.Com).

RESULTS

Haematology

Table 1 shows the effect of Rats treated with *Lawsonia inermis, Waltheria indica, Moringa oleifera,* and *Nigella sativa* on blood parameters after 14 days of treatment following pain induction. There were no significant ($p>0.05$) changes in any of the haematological parameters (PCV, RBC, HB, and WBC) when compared to the two controls.

Electrocardiograph (ECG)

The ECG results of untreated rats showed a tall P, irregular RR interval, and widened T, which was higher than half of R, whereas rats treated with Lawsonia inermis presented a very short R and irregular RR interval. Waltheria indica showed tall P waves, and inversion of the P waves was observed in *Nigella sativa.* Moringa oleifera showed widening of P, PR, and T waves, while diclofenac showed very distressed R and Irregular RR intervals when compared to the negative control, which showed normal PQRS and T wave patterns (Figure I-VII). Table 2 shows the effects of *Lawsonia inermis, Waltheria indica, Moringa oleifera,* and *Nigella sativa* on electrocardiographic parameters when administered orally for 14 days following the induction of pain. No significant ($p>0.05$) changes were observed in any of the treated groups when compared to the two controls.

Blood Pressure

Table 3 shows the effects of *Lawsonia inermis, Waltheria indica, Moringa oleifera,* and *Nigella sativa* on blood pressure. There were no significant changes compared to the control group.

DISCUSSION

Reports have shown a complicated and multifaceted phenomenon linking haematological parameters and acute pain (Orsolini *et al.,* 2020). The results obtained in this study showed that the extracts increased the values of RBC, PCV,

Table 1.
Effect of *Lawsonia inermis, Waltheria indica, Moringa oleifera*, and *Nigella sativa* on Haematological Parameters

Parameters	Negative Control	Positive Control	*L. inermis*	*W. indica*	*N. sativa*	*M. oleifera*	Diclofenac
RBCX 10^{12}/L	6.0±1.60	5.3±1.70	6.7±0.57	6.9±0.44	6.6±0.68	6.5±0.56	6.7±0.86
HGB (g/dl)	11±2.60	12±3.00	13±1.50	13±0.48	11±0.69	11±0.64	13±1.90
PCV (%)	36±7.50	38±8.40	40±2.60	41±2.1	34±3.80	34±3.50	40±4.00
MCV (fl)	61±3.80	61±4.20	59±1.20	59±0.95	61±0.98	62±0.00	60±1.70
MCH (pg)	19±1.90	20±0.91	19±1.10	20±0.59	19±1.10	19±0.87	19±0.70
MCHC (g/dl)	31±1.00	32±0.76	32±2.20	33±0.61	31±1.50	31±1.40	32±1.70
WBC X 10^{9} /L	5.6±1.50	8.6±2.30	7.7±3.00	7.7±3.00	6.5±1.00	8.1±3.10	7.5±2.30
NEUT (%)	45±3.10	38±10.00	38±3.00	41±5.00	35±6.10	37±4.50	45±6.10
LYM (%)	52±3.10	60±11.00	57±1.00	55±5.70	61±6.00	61±3.80	57±4.40
MON (%)	2.0±1.00	1.7±0.58	3.0±1.00	2.3±1.20	2.7±1.20	1.0±1.00	1.7±0.58

Note: All values are expressed as Mean ± SD

Picture 1. A. Negative control (uninduced) showing a normal PQRS and T waves pattern. **B.** Positive control (induced but not treated) showing very tall P, irregular RR interval, and widened T which is higher than half of R suggestive of myocardial infarction. **C.** *Lawsonia inermis* 200 mg/kg indicating atrial and ventricular fibrillation, very short R, and irregular RR interval. **D.** *Waltheria indica* 200 mg/kg showing tall P waves which is indicative of inconsistent SA stimulation. **E.** *Nigella sativa* 200 mg/kg showing Inversion of the P waves. **F.** *Moringa oleifera* 200 mg/kg indicating ectopic stimulation and widening of P, PR, and T waves. **G.** Diclofenac 10 mg/kg showing atrial fibrillation and ventricular fibrillation, very distressed R and Irregular RR interval.

Table 2.
Effect of *Lawsonia inermis, Waltheria indica, Moringa oleifera*, and *Nigella sativa* on the Electrocardiographic indices of pain induced Wistar rats

Parameters	Negative Control	Positive Control	*L. inermis*	*W. indica*	*N. sativa*	*M. oleifera*	Diclofenac
HR (bpm)	234.5±26	255.3±8.73	199.3±51.6	239.4±38.6	260.3±21.9	246±10	219.3±25.0
P (ms)	88.7±49.2	59.3±25.11	96±111.20	81±53.2	63±59.0	115±7.0	105.8±28.1
PR (ms)	94.1±47.4	78±30.5	107.7±122.2	128.1±65.6	86.3±63.1	130.7±4.51	114±27.30
QRS (ms)	24.3±4.51	16.0±1.00	18.7±3.79	18.7±4.16	17.7±1.15	22.0±5.00	18.3±3.06
QT (ms)	152±65.7	149±46.2	146±30.0	112±12.5	110±14.0	154±11.0	150±41.30
QTc (ms)	302±109	306±91.6	259±22.1	248±19.1	229±37.4	311±19.6	255±41.20

Note: All values are expressed as Mean ± SD

Table 3.
Effect of *Lawsonia inermis, Waltheria indica, Moringa oleifera*, and *Nigella sativa* on the Electrocardiographic indices of pain induced Wistar rats

Parameters	Negative Control	Positive Control	*L. inermis*	*W. indica*	*N. sativa*	*M. oleifera*	Diclofenac
Blood pressure	147±20	161±10	134±23	128±22	147±13	123±26	144±25

Note: All values are expressed as Mean ± SD

WBC, and other haematological parameters. This result agrees with (Günaydın and Günaydın, 2022) who reported that RBC distribution was significantly higher in patients exposed to subacute/chronic pain than in those exposed to acute pain. This result contradicts (Abdelgadir *et al.*, 2010). reported a significant decrease in blood parameters in rats treated with an aqueous extract of *Lawsonia inermis*. This difference could be due to variations in the doses administered and intensity of pain. This result also disagrees with (Ajugwo *et al.*, 2017) who reported a significant increase in RBC, PCV, Hb, and WBC counts in rats treated with *Moringa oleifera*. This result agrees with (Basiru and Olayemi, 2014) who reported no significant changes in the haematological parameters of rats treated with 200 mg/kg of *Waltheria indica*. Reports have shown that *Nigella sativa* seed oil increases the total number of RBCs, hemoglobin levels, and hematocrit percentage (Fadeifard *et al.*, 2018). The results obtained from this study followed the reports of (Liang *et al.*, 2021) who showed that *Nigella sativa* has a potential stimulatory effect on erythropoiesis. These findings also corroborate the reports that *Nigella sativa* enhances WBC function by increasing its phagocytosis and natural killer cell activities (Majdalawieh and Fayyad, 2015).

Electrocardiography (ECG) is useful for indicating the perception of pain among all the physiological signals captured by wearable devices. The ECG results from this study showed irregularities in heart function in the form of arrhythmias and myocardial infarction. This could be associated with the presence of cardiac glycosides in the extracts, which have positive ionotropic effects and predispose to cardiotoxicity (Biobaku *et al.*, 2021). The effects of the extracts on the electrocardiographic parameters were not significant. However, it could be deduced that acetic acid has strong negative effects on cardiovascular function (Bao *et al.*, 2017; Kalishwaralal *et al.*, 2018) because the electrical conduction systems of the heart were affected in all treated and untreated rats. Although the extract relieved pain, the effect of acetic acid on the heart was not ameliorated. Reports have shown that pain activates the sympathetic nervous system, resulting in increased blood pressure, leading to increased stimulation of baroreceptors that consecutively activate the descending inhibitory pathways of pain, thereby restoring initial homeostasis. The relationship between pain and hypertension is potentially of great pathophysiological and clinical interest but is poorly understood (Saccò *et al.*, 2013). The results of this study showed high blood pressure in untreated rats, which may be linked to acetic acid-induced pain. *Moringa oleifera* and *Waltheria indica* exhibited minimal blood pressure-lowering effects.

Medicinal plants offer a diverse range of mechanisms through which they can effectively lower blood pressure via vasodilation, diuretic effects, ACE inhibition, and antioxidant and calcium blockade (Verma *et al.*, 2021). *Moringa oleifera* and *LLawsonia inermis* leaves have been reported to inhibit angiotensin converting enzyme (ACE), and this effect is attributable to various phytochemical compounds present in the extract (Mounika *et al.*, 2022). This outcome conforms to the report of (Aekthammarat *et al.*, 2019) stating that *M. oleifera* possesses traditional antihypertensive activities by alleviating vascular dysfunction and oxidative stress (Aekthammarat *et al.*, 2019).

Reports have shown that supplementation of *N. sativa* in hypertensive patients significantly reduced both systolic and diastolic blood pressure compared with placebo (Rashidmayvan *et al.*, 2022). The results of this study also showed a decrease in blood pressure, which could be attributed to the active compounds found in *N. sativa*. Moreover, its antioxidant and anti-inflammatory properties may contribute significantly to its potential benefits in lowering blood pressure (Maideen, 2020). However, reports on the antihypertensive effect of W. indica and L. inermis are still scarce, even though there is a transient blood pressure-lowering effect, as seen in this study.

CONCLUSION

All extracts showed non-significant increases in PCV, RBC, WBC, and other haematological parameters in Wistar rats exposed to acute pain when compared to the positive control (pain without treatment). The extracts showed significant modulatory effects on electrocardiographic parameters and blood pressure when compared to untreated rats (positive control).

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CONFLICT of INTEREST

The author declares no conflict of interest.

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ETHICAL APPROVAL

The Faculty of Veterinary Medicine at the University of Ilorin in Nigeria approved this study under the following approval code: UREC/FVM/15/32TA037

AUTHORS' CONTRIBUTIONS

A. A. and I.J. F planned the experiments. A.G. J, A. A., and I.G. J carried out the experimental dosing. B. A and A.O. M performed ECG and blood pressure tests. A. A., I.J. F, and A.O. M contributed to the interpretation of the results. A. A and I.J. F took the lead in writing the manuscript.

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