

RESEARCH ARTICLE

INVESTIGATING THE EFFECT OF ETHANOLIC EXTRACT OF *MORINGA OLEIFERA* SEED ON BLEEDING TIME AND WHOLE BLOOD CLOTTING TIME OF WISTAR ALBINO RATS

Changjul L. Singnap^{1*}, Ahmed M. Sabo², Oto-Obong V. Idah², Bitrus N. Lekshak¹, Thomas P. Yakubu³, Moses D. Lugos⁴.

¹Department of Haematology and Blood Transfusion, University of Jos, Nigeria

²Department of Human Physiology, University of Jos, Nigeria

³Department of Pharmacognosy University of Jos, Nigeria

⁴Department of Medical Laboratory Science, Faculty of Health Sciences & Technology, University of Jos, Nigeria

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ABSTRACT: Background: It is estimated that over 80% of people still depend mainly on the traditional use of parts of plants and herbs to treat ailments. The medicinal properties of these plants and herbs are linked to the presence of a variety of phytochemicals and their elemental composition. **Justification:** Every year, 1 in 4 people die of conditions related to thrombosis, with many never knowing their risk for the condition. Prevention of intravascular thrombosis, however, has a narrow therapeutic window, bleeding risk, the incidence of resistance, and unwanted drug interactions, hence the need for anti-thrombotic drugs that deliver more effective prevention of intravascular thrombosis. **Aim:** The research sought to investigate the effect of *Moringaoleifera* ethanolic seed extract on Bleeding Time (BT) and Clotting Time (CT), in Wistar albino rats. **Materials and Methods:** Forty (40)Wistar rats were weighed and randomly divided into two groups, group I=15 rats for BT, group II=15 rats for CT and 10 rats for control tests. After the administration of ethanolic extract of *M. oleifera* seed at the dose of 100mg/kg, 200mg/kg and 400mg/kg (administered to a group of 5 rats per dose) for 28 days, BT and CT were determined using Tail transection and Lee & White method respectively. The data were analysed using GraphPadPrism (7.03). **Result:** There was a statistically significant increase at $P < 0.0001$ of BT and CT compared to the control (administered with only distilled water). **Conclusion:** The prolonged BT and CT indicate that the seed extract of *M. oleifera* could pose an antagonising effect on both the primary (platelets) and secondary haemostatic activities, a property that can be explored in the management of thrombotic diseases.

KEYWORDS: *Moringaoleifera*, Bleeding Time, Clotting Time, Thrombosis.

Corresponding Author:

Changjul L. Singnap,
Department of Haematology and Blood Transfusion, University of Jos,
Nigeria



INTRODUCTION:

For thousands of years, plants have been a vital source of medicine. Even today, the World Health Organization (WHO) has reported that up to 80% of people still depend essentially on traditional medications such as the use of herbs to treat ailments.^{1,2} Diverse plants phytochemicals and their elemental composition have been shown to possess medicinal values.^{3,4}

A growing concern in the development and evaluation of natural antioxidants from medicinal plants mainly in the field of preventive health care and the food industry is reported. Among those herbs, one promising species is *Moringa oleifera* Lam (*Moringa* or drumstick tree), which is native to the sub-Himalayan regions of Northwest India.⁴ *Moringa oleifera* (*Moringaceae*) is a well-known plant in North Eastern, Nigeria.⁵ Lam seed; is a highly valued plant, with an impressive range of medicinal uses and high nutritional value.⁶ A good number of traditional medicine references attest to its therapeutic potential, and scientific validation of these plants is developing to support at least some of the claims.⁷ Historically, ancient Romans, Greeks and Egyptians, as well as Asian communities, have been using the plant for various medicinal and nutritional purposes.⁸

The plant is used locally for various medicinal purposes by traditionalists and herbalists in Maiduguri, North Eastern Nigeria.⁵

M. oleifera roots and seeds are prescribed for the treatment of snake bites, and scorpion stings and the plant contains a mixture of several hydrolytic enzymes, in which proteases are the key enzymes responsible for the observed pharmacological actions.^{9,10} Ajibade et al., 2011 also reported that the seed could reduce the number of platelets, hence the need to look into its effect on haemostatic activities in detail.^{11,12} Platelets, also called

“thrombocytes”, are a component of blood whose function (along with the coagulation factors) is to stop bleeding by clumping and clogging blood vessel injuries. Platelets release a variety of substances that promote blood clotting, which causes haemostasis. The normal blood platelets count is between $150 \times 10^9/L$ and $450 \times 10^9/L$ (150,000 – 450,000/ mm^3). Platelets have no cell nucleus: they are produced by budding-off of the cytoplasm of megakaryocytes.¹³ Formation of this platelets plug (primary haemostasis) is associated with activation of the coagulation cascade; which results in fibrin formation and crosslinking (secondary haemostasis). They also play an important role in the conversion of prothrombin to thrombin because much of the prothrombin first attaches to prothrombin receptors on the platelets already bound to the damaged tissue.¹⁴ Intravascular thromboses such as deep vein thrombosis (DVT), a major cause of myocardial infarction, stroke and pulmonary embolism, can be caused by abnormal platelet activation, augmented clotting, vascular dysfunction and shear stress due to interrupted blood flow and atherosclerosis.^{15,16} This research was therefore designed to investigate the effect of *Moringa oleifera* seed extract on bleeding time (BT) and clotting time (CT).

MATERIALS AND METHODS:

Seed Collection and Authentication

Dried seeds of *Moringa oleifera* were obtained from Lamingo Jos-North local government Area of Plateau State Nigeria.

Preparation of Seed Extract

Good quality seeds were selected for this study. The coat and wings of the seeds were removed and the kernel collected. The kernel was dried for 5 days at room temperature (25-38°C) in the Department of Pharmacognosy. Then we produced a fine powder of the seeds by using

coffee mill attachment of Moulinex domestic food blender (Moulinex Genuine Blender 1.25 Liter, 400 Watt and White – LM2421EG made in France). The seeds were weighed before and after blending. The quantity after blending was soaked in 4 litres of 70% ethanol at room temperature for 3 days and filtered; the bulked filtrate was evaporated using hot air oven at 41°C for 24 hours. Until needed, the powder produced was stored at room temperature. A fresh solution of the seed extract was prepared when needed. The test solution was prepared by dissolving the fine powder of *Moringa oleifera* seed in distilled water in the ratio of 10g to 100ml (to give 100mg/ml concentration) and stirred for two minutes before intubation.¹⁷

Experimental Animals

A total of forty (40) male and female Wistar rats weighing between 150g to 250g were used for the research. The rats were kept in a plastic cage at room temperature with twelve hours a night/dark cycle. They had access to their feed and a hygienic environment maintained to prevent infection. The rats were weighed using an electric beam balance at the commencement of the experiments and weekly throughout the duration of the experiment.

Experimental design

A total of 40 Wistar rats were used for this study to determine the BT and CT; 15 rats were used per test by grouping them into three (5 rats/each dose) 100 mg/kg (low dose), 200 mg/kg (medium dose) and 400 mg/kg (high dose) of the seed extract in that order. We used 5 rats to control for BT and 5 rats were used to control for the CT. The oro-gastric method of intubation was adapted for the administration of seed extract for experimental rats while distilled water was administered to control groups, these were done in the morning hours before feeding the animals.

The rats were intubated daily throughout the period of the experiment (for 28 days).

Sample Collection

Animals were euthanized 24 hours after the last doses administered to them. The rats were weighed and then anaesthetized by placing them in a closed jar containing cotton wool sucked with chloroform and euthanized by cervical dislocation and collected from jugular vein into sodium-citrate sample bottle (at the ratio of 4.5 to 0.5) and the content properly mixed by gentle rolling of the bottle for haematological analysis. The blood parameters analyzed included Bleeding Time (Duke's method) and Clotting Time (Lee and White method).

Laboratory Methods

Bleeding Time (Tail transection; a modified Duke's method)

After 24 hours of the final oral administration of *Moringa oleifera* seed extract, the bleeding time was measured in rats by modified Duke's method as described briefly; the tail of the anaesthetized rat was cut 1 cm from the end using a sharp pair of surgical scissors and then the tail lesion blotted with a clean Whatman filter paper every 15 seconds. The interval, from the time the tail incision was made until the time blood was no longer apparently transferred to the filter paper, was recorded as the bleeding time in seconds.¹⁵

Whole blood clotting time (Lee White method)

Three test tubes were placed in a 37°C water bath and 3ml of blood sample was collected from the rats by the jugular method. As soon as blood appeared in the syringe, the stop watch was started. To each of the pre-warmed glass test tubes, 1ml of the blood was added. After the initial 3 minutes of incubation, the three tubes were simultaneously tilted to observe for clot formation at 30 seconds intervals. This procedure was repeated until the blood was clotted. The time taken for the first blood clot

to appear was recorded in seconds. Subsequently, the process of tilting was repeated for the remaining 2 tubes until clotting was observed. The average value of clotting time of the three tubes was reported as clotting time of the rat in seconds.

Statistical Analysis

Data collected were expressed as mean ± S.D and analysed using the SPSS software program (Graph PadPrism, 7.03). One Way analysis of variance (ANOVA) was used to determine the level of significance of confidence at 95%, (p-value < 0.05 was considered significant).¹⁸

RESULTS:

A total of 40 rats were used for the experiment to determine the BT and CT; 5 rats/each dose of: 100 mg/kg (low dose), 200 mg/kg (medium dose) and 400 mg/kg (high dose) of the seed extract in that order. We used 5 rats to control for BT, and 5 rats were used to control for the CT. The result showed that the mean value of BT and CT in control groups fall within the normal range of 60-420s and 240-540s respectively. At 100mg/ml of the extract, the mean value of BT and CT were 1059.60s and 253.30 seconds respectively, at 200mg/ml BT= 1773.00s, while CT= 346.40s, and at 400mg/ml, BT= 1917.20s, while CT= 392.00s (Table 1). The effect of ethanolic extract of *M. oleifera* seed as presented in figures 1 & 2 was seen to be accompanied by significant increase in both BT and CT with increasing concentration of the dosage of extract in the order of 100mg/ml, 200mg/ml, and 400mg/ml (P<0.0001).

Table 1: Mean values of Bleeding Time (BT) & Clotting Time (CT) of experimental rats fed on daily doses of 100mg/ml, 200mg/ml, and 400mg/ml of ethanolic extract of *m. oleifera* seed and a control group on distilled water.

Dose of <i>m. oleifera</i> extract	No. of Rats	Mean BT (Secs)	Mean CT (Secs)
Control	5	173.20 ± 25.19	240.60 ± 12.60
100mg/ml	5	1059.60 ± 52.05	253.30 ± 9.32
200mg/ml	5	1773.00 ± 64.43	346.40 ± 9.43
400mg/ml	5	1917.20 ± 47.32	392.00 ± 9.62

Footnote: The mean and standard deviation of bleeding time and whole blood clotting time of experimental and control rats are reported in seconds. While the total is the average values for the total means of BT and CT.

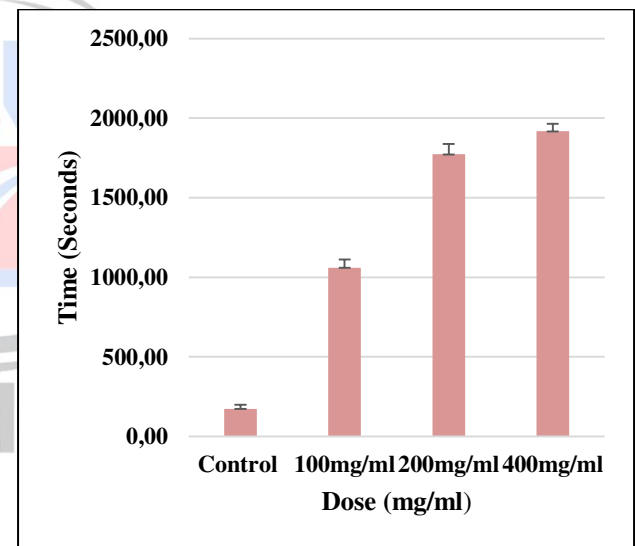


Figure 1: Bar charts showing the dose effect of ethanolic extract of *M. oleifera* seed on Bleeding Time of experimental and control rats.

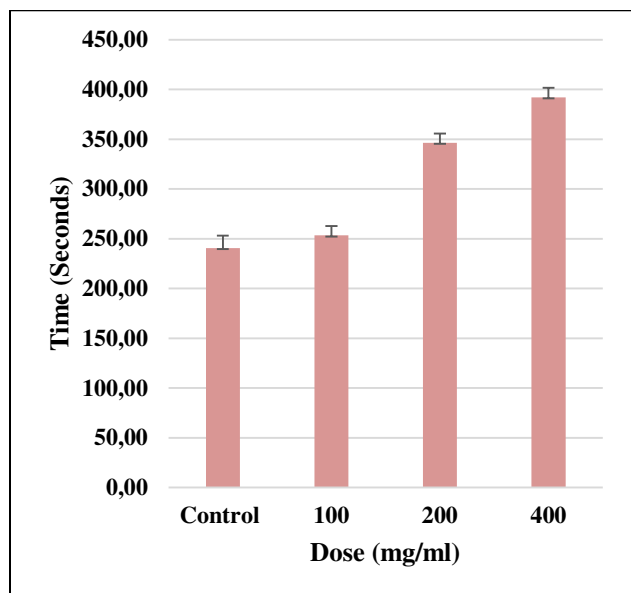


Figure 2: Bar charts showing the dose effect of ethanolic extract of *M. oleifera* seed on whole blood Clotting Time of experimental and control rats

DISCUSSION:

In this work, we investigated the effect of *Moringa oleifera* on haemostatic profile particularly BT and whole blood CT in vivo using Wistar albino rats.

M. oleifera was described as a plant with many medicinal values. The different parts of the plant such as the leaves, seeds, fruits, flowers and bark act as cardiac and circulatory stimulants and antihypertensive among others.⁷ Also, the coagulant property of the seed has been shown to be useful in water purification and treatment processes.¹⁹

We, therefore, sought to investigate the effect of the *Moringa oleifera* seed extract on blood coagulation in rats.

The study showed that *Moringa oleifera* seed extract might be responsible for the prolonged bleeding and whole blood clotting time in Wistar albino rats as reported in the results section. The average BT of the control (173.20 ± 25 seconds) falls within the normal range of between 120 – 420 seconds (2-7 minutes).

However, at 100mg/kg concentration of the seed extract, a significant increase in the average BT of 1060.0 ± 52.05 seconds (17minutes ± 52.05 s) was observed ($p \leq 0.0001$). The result further showed a dose-dependent increase with the highest at 1917.20 ± 47.32 seconds (about 33 minutes) at the dose of 400mg/kg. Also, the CT of the control group at 240.60 ± 12.06 seconds (4.10 minute) falls within the normal range of 240 – 540 seconds (4-9 minutes). However, there was no significant difference in the CT at 200mg/ml concentration (346.40 ± 9.43 seconds) of *M. oleifera* seed extract compared to mean value at 100mg/ml (253.80 ± 9.32 seconds). A statistically significant increase in the mean CT value of 392.00 ± 9.62 seconds was observed compared to CT mean value of 253.80 ± 9.32 seconds at 100mg/ml ($P \leq 0.005$). Data revealed that a higher dose of *M. oleifera* extract seed may prolong both bleeding and whole blood clotting time.

The delay in BT and CT may be due to the seed's ability to cause platelets aggregation hence reducing the number of platelets receptor sites needed for adhesion (the initial stage of bleeding arrest). Our work supported the findings of Luz et al., which reported that a coagulant isolated from *M. oleifera* seed called Lectin significantly prolongs activated partial thromboplastin time (aPTT) and prothrombin time (PT).²⁰ The research of Luz et al., 2017 was done in vitro using human blood, but we investigated whole blood CT in vivo using rats.

Analysis of variance was performed to compare the mean of extract doses and concentrations which reveals significantly ($p \leq 0.05$) that all the doses (100mg/ml, 200mg/ml, and 400mg/ml) resulted in increased coagulation (haemostatic) time in rats. The higher the dose the longer it takes for clotting to take place. This means that hypercoagulability (one of the causes of thrombotic events) can be delayed with the

right doses of *Moringa oleifera* seed extract. This findings could be the reason why *M. oleifera* seeds are used as anti-scorpion, anti-snake bites.¹⁰

CONCLUSION:

The results of this study showed that *M. oleifera* seed could delay haemostatic activity for as long as the administered dose lasts, usually within twenty-four hours (24hrs.). Since *M. oleifera* works similarly to heparin and aspirin; it may be a suitable replacement or another alternative of blood thinners. The seed extract of *M. oleifera* has the advantage of being a nutritional supplement meaning it has far less side effect if any.

RECOMMENDATIONS:

1) It is necessary to look into this wonderful gift (*M. oleifera*) to mankind with the aim of developing the seeds as a nutritional supplement or even as a drug which could be recommended in the management of thrombotic events such as deep vein thrombosis (DVT) or atherosclerosis in order to minimize ischaemic heart attack or even stroke since normal haemostasis cannot be possible without the activation of platelets.

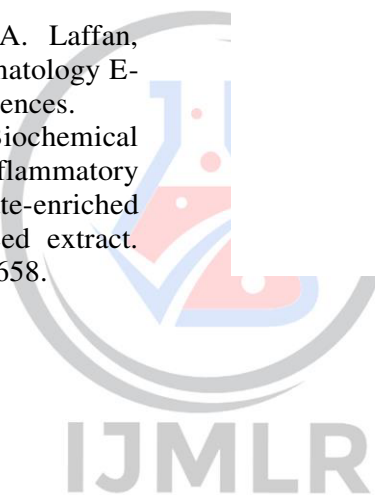
2) Further research on the effect of *M. oleifera* seed tract on other haemostatic profiles such as PTT, PT, clotting factors & co-factors, vWf, and platelets satellitism may be able to reveal more on the efficacy of this plant seed in arresting thrombotic activities.

3) When used as nutritive supplement at the right dose, it can help in the prevention of unnecessary activation of platelets leading to thrombosis; this is because the extract of *M. oleifera* acts efficiently at both the primary and secondary stages of haemostasis.

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