ANTIPLATELET ACTIVITY OF *MORINGA OLEIFERA* SEED AND LEAF EXTRACTS IN NEW ZEALAND RABBITS

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ABSTRACT

Background: Many people rely on herbal medicine globally, especially rural dwellers in Sub-Saharan Africa. In a quest to search for African traditional medicine to alleviate the possible impact of transfusion-induced thrombocytosis among transfusion-dependent patients. Thus, it employs *Moringa oleifera* for antiplatelet activity; since it has been used traditionally to treat various illnesses.

Aim: The study aimed to determine the antiplatelet activity of *Moringa oleifera* seed and leaf extracts in New Zealand Rabbits.

Materials and Methods: The lethal dose of 50% of the total population (LD_{50}) was examined. Bleeding Time and Clotting Time were carried out at an ambient temperature, and Prothrombin Time, and Activated Partial Thromboplastin Time were estimated using a coagulometer. Platelet count using a haematology analyser. Biochemical parameters were analysed with the aid colorimetric method. Standard statistical software (Graphpad prism version 8.0.2) was used to evaluate the data.

Results: The results revealed low platelet count, as platelet counts at different grades of intervention 125mg/kg, 250mg/kg, and 500mg/kg body weight in rabbits treated with *Moringa oleifera* seed extracts; 194.0±0.0*(*P*=0.001), 230.0±0.0*(*P*=0.001) and 225.0±0.0*(*P*=0.001) were significantly below the control group 374.0±55.3; Whereas, for rabbits treated with *Moringa oleifera* leaf extract the counts were lower at 250mg/kg and 500mg/kg; 190.3±19.1*(*p=0.001*) and 158.0±0.0*(*P* =0.001) compared to the same control group. Also Clotting time for the same extract at 125mg/kg and 250mg/kg; 642.0±95.5*(*P*=0.002) and 570.0±17.3*(*P*=0.034) were significantly higher compared to the control group 383.7±132.3.

Conclusion: *Moringa oleifera* is used widely not only in Africa for nutritional values and to cure many ailments. In this study, it was observed that the plant is safe and may be used as an antiplatelet agent.

Keywords: New Zealand Rabbits, Antiplatelet, *Moringa oleifera*, seed and leaf extracts

INTRODUCTION

Moringa oleifera is known and named by three major tribes across Nigeria as Zogale (Hausa); Ewe-Igbale, Ewe-Iba, IgiIgbale (Yoruba); (Stevens *et al*., 2015) Agbaje, Npataka, Zele, Okwe-Olu, Okwe-Oyibo, Odudu-Oyibo (Ibo). *Moringa oleifera* (*Moringa pterygosperma*) is the most frequently grown species in the genus Moringa, according to Fuglie (2001).

Because of the appearance of long, slender, triangular seed pods, (Mishra *et al*., 2012; Alakali *et al*., 2015) and noted that this tree is also known as the drumstick tree. The tree is thin, with drooping limbs that reach a height of around 10 meters. It is frequently pruned down to 1-2 m in cultivation and allowed to re-grow so that the pods and leaves are always within reach (Fuglie, 2001). Iron, potassium, calcium, zinc, magnesium, and essential vitamins are all found in the *Moringa oleifera* tree, which generates four times the amount of vitamin A found in carrots (Alakali *et al*., 2015). *M. oleifera* contains beta-carotene, which is a precursor to retinol.

Phytotherapy: Malnutrition has been treated with moringa plants, particularly in newborns and nursing mothers. According to Kasolo (2010), three non-governmental groups have endorsed Moringa as "natural nutrition for the tropics." Leaves can be consumed raw, roasted, or stored as a dry powder for months without losing nutritious content, according to reports. Moringa is particularly promising as a food source in the tropics since it is in full leaf at the end of the dry season when other foods are limited.

Several studies on Moringa's nutritional properties have now been published in both scientific and popular sources. Moringa leaves are believed to provide "more Vitamin A than carrots, more calcium than milk, more iron than spinach, more Vitamin C than oranges, and more potassium than bananas," and its protein quality is comparable to (Kasolo, 2010)milk and eggs. Moringa is credited with countless life-saving nutritional rescues in Senegal and throughout West Africa, according to oral histories. In fact, Moringa's nutritional characteristics have become so well-known that there appears to be little dispute about the significant health benefits that can be obtained by consuming Moringa leaf powder in situations where famine is a genuine possibility. Nonetheless, the results of well-controlled and well-documented clinical trials would definitely be beneficial (Kasolo, 2010). The aim of the study was an assessment of the possible antiplatelet values and toxicological study of *Moringa oleifera* seed and leaf extracts in New Zealand rabbits.

MATERIALS AND METHODS

Plant Collection and Identification: *Moringa oleifera* leaf and seed were purchased at the "Sokoto Central market" in Sokoto State, Nigeria. The plant's identity and legitimacy were confirmed by taxonomist Namadi Sunusi of the Herbarium Unit, Department of Botany, Faculty of Life Sciences, Ahmadu Bello University, Zaria, Nigeria, who compared the plant's morphological features to those found in the literature. Voucher Number: ABU0517 for *Moringa oleifera* was allocated to the voucher specimen and stored in the same department's herbarium. All procedures are performed in compliance with relevant laws and guidelines of the Animal Use and Care Committee of Ahmadu Bello University (ABUCAUC), Zaria.

Preparation and Extraction: *Moringa oleifera*, leaf, and seed were gathered and air-dried for six weeks. It was then manually ground with a mortar and pestle. The extraction was carried out using a soxhlet device. The obtained crude extract was concentrated in an oven at 48°C. The dried crude extract was maintained in sterile plastic bottles in a refrigerator at a low temperature (4°C) at the Department of Pharmacology, Faculty of Pharmaceutical Sciences, ABU, Zaria, until it was needed.

The residual extract was then reconstituted, taking into account the rabbits' average weight, the length of extract administration, and the needed dose volume. To acquire different extract quantities per kilogram of body weight, the crude extracts were diluted in cold water. It was then kept refrigerated at four degrees Celsius until usage, and the rabbits were given the appropriate dose orally.

Experimental Animals: A total of 42 rabbits were purchased from the Samaru area of Zaria and transported to the Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, ABU, Zaria, where they were housed. The temperature in the experimental animal room was 22°C (±3°C), and the relative humidity was 50-60%. The animal cages were cleaned every day and regularly. The lighting was artificial, with a photoperiod of 12 hours of light followed by 12 hours of darkness. Traditional animal diets were used for feeding and an unrestricted supply of drinking water. The animals were kept in cages with only one animal per cage (Organization for Economic Co-operation and Development guidelines, 2008a).

Experimental design: The study was a randomized placebo trial in which animals were purchased continually. The extracts were given to the rabbits in each group individually through intragastric gavage with an oral cannula (an oral feeding tube).

Assessment of the lethal dose for 50% of the population, consisting of 10 rabbits divided into two groups at random. Each group is made up of five rabbits. In the antiplatelet value study, 42 rabbits including nine (9) survived rabbits during the lethal dose study were randomly divided into 7 groups, each with six rabbits.

Lethal Dose of 50% of the Total population (LD_{50}): Ten (10) rabbits were randomly divided into two groups, and each group consisting of 5 rabbits as follows:

Group I- A total of five rabbits and were given *Moringa oleifera* seed extract 5000mg/day/kg body weight dosage.

Group II- A total five rabbits and were given *Moringa oleifera* leaf extract 5000mg/day/kg body weight dosage.

An overnight starved rabbit was given a single bolus dose of 5000 mg/kg body weight orally via an oral feeding tube and monitored for symptoms of toxicity or mortality for the first 30 minutes after administration and frequently for the next 24 hours, with specific attention paid to the first 4 hours. Food and water were not allowed for the first four hours after the administration. After 48 hours of no mortality, it was terminated and observed for 14 days for *Moringa oleifera* leaf, and seed extract, respectively (Organization for Economic Co-operation and Development guidelines, 2008b).

Secondly, seven groups were formed, each with six rabbits. The animals were divided into the following groups at random:

Group I – control: It consists of six rabbits.

Group II- A total of six rabbits and were given *Moringa oleifera* seed extract 500/day/kg body weight dosage.

Group III- A total of six rabbits and were given *Moringa oleifera* seed extract 250/day/kg body weight dosage.

Group IV- A total of six rabbits and were given *Moringa oleifera* seed extract 125/day/kg body weight dosage.

All the rabbits received the respective intervention for 28 days. On the 29th day, blood was collected from the marginal ear vein for haemostatic study.

Then an assessment of the haemostatic values of the extract by carrying out the following investigations:

Bleeding Time, Clotting Time, Prothrombin Time (PT) Test, Activated Partial Thromboplastin Time (APTT) Test, and Platelet Count

Blood Processing: Two hundred and fifty (250µl) of 3.8 percent Tri-Sodium Citrate was added to a plain graduated container, 2.5 mL of blood samples were added, platelets were counted, centrifuged for 15 minutes, and platelet-poor plasma was carefully collected into sample vials and stored frozen at -20°C until analysis.

Bleeding Time Measurement: One of the rabbits' forelegs was shaved, and three insertions with a lancet were made, with the bleeding time measured from the start of the bleeding to the end.

When wiping the blood with Whatman's filter paper, visible veins and pierced areas were avoided. I made spots on blotting paper every 15 seconds till the bleeding stopped and recorded the bleeding time accordingly, or I took the time between the appearance of blood and the cessation of bleeding as the bleeding time was expressed in seconds (Shrivastava and Das, 1987; Dacie *et al*., 1995).

Clotting Time: Two milliliters of blood were extracted and placed in a small glass tube 10x75mm after one of the rabbits' ears was cleaned with xylene, and the veins appeared to be very visible. The measurement time begins when blood enters the needle and ends when the blood clots. After that, tilted gently and checked for agglutination every 30 seconds.

Platelets Count: The rabbit's platelet count was determined by placing blood samples in anticoagulant tubes and counting them with Mindray BC-3600 automated platelet count equipment.

Pioway CL-2000B coagulometer Semi-Automatic Coagulation Analyser: Prothrombin Time (PT) and Activated Partial Thromboplastin Time (APTT) assays were all performed using a coagulometer.

Statistical Analysis: Standard statistical software (Graphpad prism version 8.0.2) was used to evaluate the data. In all statistical comparisons, a p-value of <0.05 was considered significant. In the figures and tables, the results were expressed as mean±SEM. One-way analysis of variance (ANOVA) was used to compare groups.

RESULTS

Table 1 show the LD_{50} of *Moringa oleifera* seed and leaf methanolic extracts, respectively was greater than 5000mg since three rabbits survived per each extract.

Table 1: Lethal Dose of fifty percent of the total population (LD_{50}) for rabbits treated with Moringa *oleifera* seed and leaf methanolic extracts

Plants	Extracts	Groups	No. of Rabbits	No. Survived	No. Death
Moringa oleifera	Seed	I	5	5	0
	Leaf	II	5	4	1

Legend: Data shows lethal dose at 50% of the population according to Organization for Economic Cooperation and Development (OECD) guideline for acute toxicity study, 5000mg/kg body weight was administered and observed after the first 24h, and after 14 days.

Table 2: Haemostatic Parameters for rabbits treated with M*oringa oleifera* seed and leaf methanolic extract

Extracts	Dosage (n=6)	Bleeding time (sec)	Clotting time (sec)	Platelet count ($x10^9$/l)	PT (sec)	APTT (sec)
	Control	30.0±0.3	383.7±132.3	374.0±55.3	6.4±0.1	22.5±2.9
Seed	125mg/kg	30.0±0.0	375.0±77.9	194.0±0.0[a]	7.5±0.0	14.4±3.8
	250mg/kg	30.0±0.0	311.0±0.0	230.0±0.0[b]	7.4±0.0	11.4±6.8
	500mg/kg	30.0±0.0	410.0±0.0	225.0±0.0[b]	6.9±0.0	12.5±2.5
Leaf	125mg/kg	30.0±0.0	642.0±95.5[a]	218.7±56.3	5.7±0.1	25.2±0.6
	250mg/kg	40.0±10.0	570.0±17.3[b]	190.3±19.1[b]	11.9±2.9	15.6±2.8
	500mg/kg	30.0±0.0	352.7±99.6	158.0±0.0[a]	11.4±0.0	15.2±0.0

Legend: The data were expressed as mean±SEM; haemostatic parameters for the control group and three different grades of intervention in ascending order, after 28 days of treatment with *Moringa oleifera* seed and leaf methanolic extracts respectively, n, number of rabbits per group. Different letters indicate significant differences ($P<0.05$) when compared with the control, one-way ANOVA followed by Dunnett's post *hoc* test.

DISCUSSION

A total of forty-two rabbits (42) were used in this Randomized Control Trial, which was divided into seven (7) groups with six rabbits in each group. The continued use of medicinal plants around the world, particularly in underdeveloped countries, is partly because nature is the best cure for many of man's health problems. According to the World Health Organization (WHO), 80 percent of the world's population relies on herbal medicine for their health requirements (Malviya *et al*., 2011), with a higher proportion of dependence among rural inhabitants in African countries. Previous studies have shown that modern medicine is more expensive and ineffective than phytotherapy. Because of a lack of political will and a lackadaisical attitude of authorities concerned with good healthcare delivery to the poor and (Anthony *et al*., 2020) vulnerable population, medicinal plants have continued to play a significant role in the healthcare systems of the majority of the world's population.

The leaves of *Moringa oleifera* have anti-hyperglycaemic, anti-inflammatory, and hemopoietic effects (Fidelis and Ifaenyi, 2016). It also boosted humoral and cellular defences (Fidelis and Ifaenyi, 2016). It is used in the manufacturing of cow and buffalo ghee in rural areas to improve the flavour, taste, and shelf life of the product. Moringa oil contains a high amount of oleic acid (72%) and is used in cosmetic goods to improve the health and strength of hair and skin (Chelliah *et al*., 2017). *Moringa oleifera* Lam. leaves, in particular, have some distinct nutritional and medicinal potential (Fidelis and Ifaenyi, 2016). The plant is used to cure anaemia, heart illness, high blood pressure, diabetes, and even fatal cases of meningitis (Sunday *et al*., 2015)

Ten (10) rabbits were divided into two groups, each with five rabbits. The rabbits were given methanolic extracts of *Moringa oleifera* seed and leaf, and showed no signs of toxicity after four hours, twenty-four hours, and fourteen days, respectively. This is in accordance with the guidelines for acute toxicity studies established by the Organization for Economic Cooperation and Development (OECD, 2008b). For rabbits, the median oral lethal dosage was reported to be greater than 5000mg/kg.

Alamgeer *et al.* (2018) reported a statistically significant increase in bleeding time among rabbits treated with *Mentha longifolia* when compared to control, Aziakpono *et al.* (2021) reported a significant decrease in bleeding time among rats treated with hydrocortisone when compared to control ($P< 0.05$). This disagreed with my findings that no significant relation in all the groups of rabbits treated with *Moringa oleifera* seed and leaves methanolic extracts respectively, compared with the control as in Table 2. Bleeding time statistically significantly increased in rabbits treated with *Moringa oleifera* leaf at a concentration of 250mg/kg ($P = 0.012$) compared with *Moringa oleifera* seed Table 2. Singnap *et al* (2019) reported prolonged bleeding time results in Wistar albino rats treated with *Moringa oleifera* seed extract, it is in contrast with the findings in this research the increase in bleeding time was significant in rabbits treated with *Moringa oleifera* leaf compared with seed extract, maybe because their investigation was limited to only seed and also species variation. Moreover, Caballero and Cachuela (2017) established that *Moringa oleifera* leaf extract was associated with significantly shorter bleeding time than saline control in adult male New Zealand white rabbits with ages ranging from 16 – 20 weeks; as an experimental epistaxis model. Thus, may be worth investigating further as a haemostatic agent for epistaxis, it disagreed with this study may be due to the different sites of investigation; one-centimeter long, full-thickness mucosal wounds in the junction of the nasal floor and anterior part of the septum were treated randomly with tropical *M. oleifera* extract.

The duration of bleeding time was recorded.

Aziakpono *et al.* (2021) reported that clotting time was reduced in rats treated with hydrocortisone, it disagrees with the findings in this study as clotting time in rabbits treated with *Moringa oleifera* seed and leaf extract, respectively, shows a statistically significant relationship between rabbits treated with MOL increased at 125mg/kg (P = 0.005); compared with a control group; Table 2. This may be due to the difference in animal species as well as the extract administered. Alamgeer *et al.* (2018) reported significant and dose-dependent (2.5, 5 and 10%) increases in blood clotting time with maximum effect at 10% extract solution in rabbits treated with aqueous methanolic extract of *Mentha longifolia*, it concurred with my finding even though different extract was used for a reverse purpose on same animal species.The vascular/platelet phase and the coagulation phase have been separated in haemostasis.

Clotting Time for *Moringa oleifera* plant extracts, the result shows a statistically significant increase in rabbits treated with MOL at 125mg/kg (P = 0.005), 250mg/kg (P = 0.007) compared with MOS, Table 2. Singnap *et al.* (2019) reported prolonged results in Wistar rats treated with *Moringa oleifera* seed extract, it is in line with the findings in this research despite the difference in the species used and the increase in the clotting time was more pronounced in *Moringa oleifera* leaf compared with seed extract. It has been reported that moving to a colder climate (Pichotka and Reichel, 1950) slows down the clotting process.

Alamgeer *et al.* (2018) reported the effect of *Mentha longifolia* on in vitro prothrombin time, that different concentrations of plant extract (2.5, 5 and 10%) exhibited considerable (P<0.001) increase in prothrombin time, in the same vein; in the current study prothrombin time statistically increased in rabbits treated with MOL at 250mg/kg, and 500mg/kg (P = 0.001); respectively, compared with control, Table 2. Although the species varies and no intervention was administered in his study, Mohammed (2019) reported a mean PT value of 7.35±0.91seconds in his study on the effect of breed, sex, and age on coagulation parameters of healthy police dogs in Sudan, which was slightly lower than the findings in this study.

Prothrombin Time comparison of *Moringa oleifera* seed and leaf extracts; the result shows a statistically significant increase in rabbits treated with MOL at 250mg/kg, and 500mg/kg (P = 0.001), compared with MOS respectively, Table 2. Zimmerman *et al* (1971) reported no statistically significant difference for the prothrombin time test in C_6-deficient rabbits at 12.4sec compared to controls at 12.2sec. This disagreed with the findings in this study as mentioned above, which may be due to the difference in the intervention administered. Activated Partial Thromboplastin Time shows a statistically significant decrease in rabbits treated with MOS at 250mg/kg (P = 0.004) and 500mg/kg (P = 0.011); an increase in MOL at 125mg/kg (P = 0.004) compared with control group respectively, Table 2. This corroborates with the study of Bhatnagar and colleagues (2013) Activated Partial Thromboplastin Time was shorter in the *Moringa oleifera* seed biopolymer solution respectively compared with the control. In rabbits treated with aqueous methanolic extract of *Mentha longifolia*, Alamgeer and colleagues (2018) found that varying concentrations of plant extract (2.5, 5, and 10%) caused a significant increase in activated partial thromboplastin time in a concentration-dependent way, this could be the due difference in the plant's extracts used.

Platelet Count statistically significantly decreased in rabbits treated with both *Moringa oleifera* seed and leaf at 125mg/kg, 250mg/kg, and 500mg/kg (P = 0.001); compared with the control group, respectively (Table 2). However, the reduction in platelet count was directly proportional to the increase in extract concentration for rabbits treated with *Moringa oleifera* seed extract; whereas slight in consistent for rabbits treated with *Moringa oleifera* leaf extract was observed. Kipyegon (2020) found no statistically significant difference in platelet count in Sprague-Dawley rats treated with *Zingiber officinale* Roscoe (ginger), however, the treatment group has a lower count than the control group; but different animal species and plant extracts were used.

CONCLUSION

According to some writers, *Moringa oleifera*, often known as the Drumstick tree, is regarded as a versatile tree due to its contributions to nutrition, anemia treatment, and other fields. The study was a randomized placebo experiment. The lethal dose was determined for 50% of the population, which consisted of 10 rabbits randomly divided into two groups. There were five rabbits in each group. Forty-two (42) rabbits were randomly separated into 7 groups, each with six rabbits, for a study on antiplatelet activities. Slight prolongation of extrinsic as well as inconsistently reduced intrinsic effects between the treated and control group made it cumbersome to draw a substantive inference. However, uniform reduction in the platelet count between the treated and control group suggested that rabbits treated with *Moringa oleifera* seed and leaf extracts possessed a potential antiplatelet property.

RECOMMENDATION

Further study to identify the active compound in *Moringa oleifera* seed and leaf with the antiplatelet activity was recommended.

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