Future Natural Products
doi: 10.34172/fnp.166 2023;9(2):x-x https://fnp.skums.ac.ir

Original Article

Phytochemical analysis and effect of short-term administration of aqueous seed extract of *Aframomum melegueta* on haematologic indices of female albino rats

Uche Emmanuel Olunkwa[1*], Kizito Munachiso Enyereibenyem Iheanacho[1], Chidi Uzoma Igwe[1], Linus Ahuwaraeze Nwaogu[1]

[1]Department of Biochemistry, Federal University of Technology, P.M.B. 1526, Owerri, Nigeria

*Corresponding Author:
Uche Emmanuel Olunkwa,
Email: uemma.olu@gmail.com

Received: December 13, 2023
Accepted: May 1, 2024
ePublished: Xx xx, 2024

Abstract

Background and aims: *Aframomum melegueta* (Alligator pepper) is a dietary spice widely used for entertainment, religious rites, food flavor enhancer, possessing erythropoietic potentials, and many other medicinal uses. Women are included in eating this widely used spice. This experiment determined the health risk or benefit of short-term administration of aqueous seed extract of *A. melegueta* on haematologic indices of female albino rats.

Methods: Thirty adult female albino rats weighing 160 to 200 g were used for the study. Male rats were introduced into the female rat cages of groups II-V within 12h at the expected estrous phase for mating and withdrawn afterward. Groups I, II, and III were orally administered distilled water only, while groups IV and V received oral doses of 5 µg/kg b.w. Cabergoline and 3000 mg/kg b.w. aqueous seed extract of *A. melegueta* respectively 24 hours postpartum and once daily by oral gavage for three days.

Results: Fourier-transform infrared spectroscopy (FTIR) identified twelve functional groups in the seed extracts, namely –OH, -NH_2, CH, -NH_3^+, -CH_3, -OH, -N=C=O, -C≡N, -C=C=C, -NH, -CH_3 and -1,3,5-trisubstituted benzenes, while gas chromatography with flame ionization detector (GCFID) determined 15 bioactive components namely kaempferol, naringenin, Sapogernin, flavanones, anthocyanin, flavan-3-ol, cyanogenic glycoside, ribalinidine, rutin, catechin, resveratrol, spartein, epicatechin, steroid and phytate. Non-significant alterations in hemoglobin, packed cell volume (PCV), and red blood cell count were observed. There was a significant ($P<0.05$) decrease in WBC during pregnancy, but it was improved postpartum. However, platelet count was significantly ($P<0.05$) reduced after extract administration.

Conclusion: The results indicate no adverse anemic condition elicited during pregnancy and delivery on haematologic parameters, namely red blood cells, hemoglobin, and PCV, with a significant decrease in WBC during pregnancy as well as reduction in platelet counts (thrombocytopenia) of extract treated animals post-partum likely due to the flavonoid, resveratrol as well as rutin and (–)-epicatechin components of *A. melegueta* and thus may increase the risk of bleeding disorders but reflects a positive anti-atherogenic and cardioprotective effect.

Keywords: *Aframomum melegueta*, Cabergoline, Hematology, Platelets, FTIR, GCFID

Introduction

Plants constitute the fundamental ingredient in traditional healing systems, providing the foundation for several major pharmaceutical medications. About 50 000 species of higher plants have been medicinally utilized, thus representing the most extensive use of the natural world in terms of the number of species (1). The ethnobotanical uses of plants are diverse in traditional practice, and the use of plants for medicinal purposes dates back to antiquity (2). Traditional medicine represents an essential aspect of the African culture, with local medicinal systems varying between diverse cultural groups and regions, with various medicinal plant species used in either the form of extracts or decoctions by the indigenes of different regions (3). Medicinal plants have been utilized as a source of medicine to treat illnesses since time immemorial and provide sources of emerging modern medicines and drug compounds. Thus, significant contributions to human health have been made by plant-derived medicines (4). Medicinal plants constitute the most common natural source of human medication, resulting in their broad utilization in alleviating the health care burden worldwide in traditional medicine (5).

Alligator pepper, botanically known as *Aframomum melegueta* (Roscoe), belonging to the family Zingiberaceae (ginger family) of the angiosperms in the Kingdom Plantae is a tropical herbaceous perennial plant of the genus *Aframomum* (6,7). *A. melegueta* is cultivated widely in the tropical region of Africa for its valuable seeds (8). It has trumpet-shaped purple flowers that develop into 5 to 7cm long pods containing hundreds of tiny reddish brown aromatic and pungent seeds widely used spice in several

parts of the world and grows up to a height of 1.5 m (8-10).

Alligator pepper is rich in essential oils that impart peppery pungency and spicy aroma to classic West African soups, which contribute to the pleasant flavor (11). Its antioxidant and antimicrobial properties necessitate the utilization of this spice in traditional medicine (12). Alligator pepper is also used as a spice in West Africa to alleviate stomach aches and diarrhea (13), treatment of hypertension and tuberculosis, as a remedy for snake bites and scorpion stings (11,14). The seeds are also used for culinary purposes due to the seed's pungency (13). *A. melegueta* is an ingredient in pepper soup, a spicy delight in parts of West Africa consumed by pregnant and non-pregnant women. *A. melegueta* possesses many medicinal values, including anti-inflammatory, aphrodisiac, hepatoprotective, antitumor, antidiabetic, antiulcer, anti-venom, antimicrobial, weight loss, erythropoietic potential, and many other medicinal uses have been reported in the literature (15).

Most medicinal plants do not harm hematological and serum biochemical parameters. Hematological profiles, both in humans and animals, are an important index for an individual's physiological state. Pregnancy and lactation are physiological statuses considered to modify metabolism in animals (16,17). The pregnant woman experiences physiological changes to support fetal growth and development (18). The end of gestation and the beginning of lactation are the two critical periods where most changes in serum metabolic profile happen (19).

Health status can be determined by measuring blood hematology (20,21). Pregnancy is associated with many changes in physiologic hematological parameters, which can be considered pathological (22,23). Changes in hematology occur due to the increased energy demand by fetal development, lactogenesis, endocrine, metabolic changes, and body preparation for parturition and lactation (24). Bleeding and very stressful conditions may worsen during birth; thus, maintaining normal hemostatic change in blood is important during pregnancy (21,25). Postpartum hemorrhage occurs in up to 18 percent of births and is the most common maternal morbidity in developed countries (26). Anaemia is considered a risk factor for an unfavorable outcome of pregnancy as more than half of the cases of anemia, especially among pregnant women. The most frequent cause of postpartum anemia is iron deficiency, occurring in the third trimester of pregnancy, and loss of blood during delivery (27). Uterine atony is responsible for most cases and can be managed with uterine massage in conjunction with oxytocin, prostaglandins, and ergot alkaloids (26).

Hematological parameters are good indicators of physiological health status whose evaluation is essential in assessing the animal's response to various physiological stressful conditions such as pregnancy, parturition, and lactation (19,28). Additional medications or blood transfusion may be required for postpartum hemorrhage to stop bleeding.

This study investigates the health risks or benefits of short-term administration of aqueous seed extract of *A. melegueta* on haematologic indices of female albino rats.

Materials and Methods

Plants Collection and Preparation

The fruits of *A. melegueta* were purchased from Relief Market, Owerri, Imo State, Nigeria. The plants were authenticated by Mr. Francis Iwueze, a botanist at the Department of Forestry and Wildlife, Federal University of Technology, Owerri (FUTO), and compared with existing collections deposited at the herbarium of the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Umudike, with voucher specimen catalog no. MOUAU/CVM/VPP/2013/03. The fruits were air-dried at room temperature for two weeks. The outer coats of the seeds were removed and dried in an oven at 50 °C for 4 hours. The dried seeds were ground to powder form with an electric blender and preserved in a labeled air-tight container until required for extraction.

Extraction of Aframomum melegueta seeds

Five hundred milligrams of ground seed were soaked in 500 mL of distilled water. The solution was allowed to stand for 48 hours with intermittent mixing and later filtered with Whatman No. 1 filter paper. The filtrate was then evaporated in a water bath to concentrate the extract. The concentrate was dispensed into an airtight sterile container and refrigerated at 4 °C until required.

Percentage yield

The extract obtained was weighed using an electronic weighing balance, and the percentage yield was calculated as follows:

$$\text{Percentage yield} = \frac{Weight\ of\ extract}{Weight\ of\ starting\ material} \times 100$$

Phytochemical Analysis of Aframomum melegueta

The phytochemicals and functional units present in the aqueous seed extracts were analyzed using gas chromatography with flame ionization detection (GCFID) and Fourier-transform infrared spectroscopy (FTIR), respectively.

Fourier-transform infrared spectroscopy

This was determined by weighing 0.5 g of the sample, which was mixed with 0.5 g of potassium bromide. Afterward, 1 mL of Nujol solvent was introduced using a syringe to form a paste, which was then placed into the instrument's sample mold and scanned at a wavelength of 600-4000 nm to obtain the sample's spectra wavelength.

Gas chromatography with flame ionization detector

This was determined by weighing 0.2 g of the sample into a test tube to which 15 mL of ethanol was added and placed in a water bath to react for 60 minutes at 60 °C. After the reaction time, the product was transferred to a

separatory funnel and separately washed with 20 mL of ethanol, then 10 mL of cold water, 10 mL of hot water, and 3 mL of hexane. The extract was then washed thrice with 10 mL ethanol aqueous solution of 10% v/v and dried using anhydrous sodium sulfate, after which the solvent was evaporated. The sample was then solubilized in 1000 µL petroleum ether, out of which 200 µL of it was subsequently transferred to a vial for the analysis. A BUCK M910 gas chromatography equipped with an HP-5MS column (30 m in length × 250 µm in diameter × 0.25 µm in thickness of film) was used to perform the analysis of phytochemicals. Spectroscopic detection utilized a high-energy electron (70 eV) ionization system. Pure helium gas (99.995%) was used as carrier gas at 1 mL/min flow rate—one microliter of 1% of the diluted extract prepared with respective solvents and injected in splitless mode. The relative quantity of the chemical compounds in each extract based on the peak area produced in the chromatogram was expressed as a percentage.

Preliminary acute toxicity and lethality LD_{50} test

Acute toxicity

Eighteen albino mice weighing 25 g to 30 g were purchased from the Animal Breeding Unit, Zoological Garden, University of Nigeria Nsukka, Enugu state. The animals were kept in a well–ventilated room at a temperature of 25 ± 2 °C and 55%–65% relative humidity with a diurnal 12 hours light cycle in stainless–steel cages. The mice had access to water and pelletized standard finisher mesh (Vital finisher, United Africa Company Nigeria Plc., Jos, Nigeria) *ad libitum*. A period of 1 week was allowed to acclimate the mice to the environmental conditions.

LD_{50} determination

The index of acute toxicity was the LD_{50}, which is the dose of a substance capable of producing death in 50% of the population of animals exposed to the substance. Lorke's method (29) was used. This method has two different phases.

Phase I

Three groups of three mice each were utilized. The extract was administered at different concentrations of 10, 100, and 1000 mg/kg body weight to group I, II, and III mice, respectively, that formed the LD_{50} phase one group. The extract was administered orally, and the animals were monitored for 24 hours for signs of abnormal reactions or death.

Phase II

Phase two consisted of three groups of mice administered with the crude extract at concentrations of 1600, 2900, and 5000 mg/kg body weight in groups 1, 2, and 3 mice, respectively. The animals were observed and recorded for 24 hours. The extract's lethal dose (LD_{50}) was estimated by calculating the geometric mean of the maximum dose with 0 % mortality and the minimum dose with mortality (29).

$$LD_{50} = \sqrt{\frac{Maximum\ dose\ with\ 0\%\ mortality \times 100}{Minimum\ dose\ with\ mortality}}$$

Standard drug preparation

Cabergoline (Dostinex® 0.5 mg, Pfizer Limited) was purchased at Orchad Pharmacy, Owerri. Each 0.5mg tablet was dissolved in 500mL distilled water, reconstituted, and administered as 5 µ/kg body weight of the animals.

Laboratory animals

Laboratory animals comprised 30 females and 15 male adult albino rats (*Rattus norvegicus*) weighing 180 g and 200 g, aged about 5 weeks. Animals were procured from the Department of Veterinary Medicine, University of Nigeria, Nsukka, and housed in cages under standard environmental conditions of temperature (30 ± 1 °C), humidity (60 ± 0.2%), and a 12 hours light/dark cycle in the Animal House Unit of Department of Biochemistry, Federal University of Technology, Owerri, Imo State, Nigeria, for a 14-day period to acclimatize. The animals were fed a standard rodent diet (Growers Mash, Vital Feeds Ltd) and water *ad libitum*. Ethical approval was obtained for the study protocol from the University Ethical Committee, and Principles of Laboratory Animal Care (30) was adopted for all the experimental procedures involving the use and handling of laboratory animals.

Study design

The female rats were divided randomly into five groups of 6 rats each. The weight differences within and between groups did not exceed ± 20% of the average weight of the total rats. The animals were grouped and treated as detailed in Table 1. The estrous cycles of the female albino rats were monitored. Then, three male rats were introduced into each of the female rat's cages of groups III-V within 12 hours at the expected estrous phase and left in the cages for three days to allow mating to occur and later withdrawn. Subsequently, 14 days after that, group II female rats were also mated, while group I female rats were not mated at all. The day sperm was observed in

Table 1. Animal treatment groups

Group	Treatment
Group 1 (Non-pregnant unmated control)	Orally administered distilled water only
Group II (Pregnant control)	Orally administered distilled water only
Group III (Post-partum negative control)	Orally administered distilled water only
Group IV (Post-partum positive control)	Received a single oral dose of 5 µg/kg b.w. carbagoline (positive control drug) for three days
Group V (Post-partum Test)	Received a single oral dose of 3,000 mg/kg b.w. *Aframomum melegueta* for three days

the vaginal smear of the female rats or the presence of a cornified plug on the floor of the cages was taken as day 1 of pregnancy (10). The post-partum rats were not allowed to suckle their pups by having their pups removed within 12 hours of birth.

Administration of the drug/extract commenced 24 hours postpartum once daily by oral gavage for a total of 3 days. The chosen dosage of aqueous plant extracts used was based on the results of preliminary acute toxicity studies on the median lethal dose (LD_{50}).

Sample collection

All the animals were weighed on the first and last days of treatment. After treatment, they fasted overnight, were anesthetized with diethyl ether, and were sacrificed. Then, 5 mL of whole blood was collected through cardiac puncture and gently dispensed into well-labeled bottles of ethylene diamine tetra acetic acid (K_3EDTA), which was subsequently utilized for hematological parameter analysis.

Hematological analyses

The cyan-methaemoglobin method was used to determine the hemoglobin concentration of the anticoagulated blood. The packed cell volume (PCV) was determined using the hematocrit method. In contrast, white and red blood cells were counted with the Romanowsky stains/ May-Grunwald-Giemsa stain technique (31,32).

Statistical analysis

The obtained data from the analyses were presented as mean ± standard deviation and then statistically analyzed by one-way analysis of variance (ANOVA) and Tukey post hoc test aided with GraphPad Prism version 5.3 (GraphPad, USA). Values with $P \leq 0.05$ were taken to be statistically significant.

Results

The results show no significant ($P < 0.05$) difference in hemoglobin concentration (g/dL) concentration of the non-pregnant control (16.68 ± 1.63), pregnant CTRL (16.00 ± 1.89), post-partum CTRL (17.12 ± 1.01), post-partum + STD drug (14.96 ± 2.12) and post-partum + extract (15.68 ± 1.71) groups.

The PCV (%) of the non-pregnant control (50.04 ± 4.89), pregnant CTRL (48.00 ± 2.62), post-partum CTRL (51.36 ± 3.03), post-partum + STD drug (44.88 ± 3.84) and post-partum + extract (47.04 ± 5.12) groups were not significantly ($P < 0.05$) different from each other.

Total white blood cell (WBC) count ($\times 10^9$ cells/L) of the non-pregnant control (4.88 ± 0.48), post-partum CTRL (4.54 ± 0.50) and post-partum + extract (4.64 ± 0.37) groups were not significantly ($P < 0.05$) different from each other. However, total WBC count ($\times 10^9$ cells/L) of post-partum + STD drug (5.92 ± 0.08) was significantly ($P < 0.05$) elevated when compared to the non-pregnant control (4.88 ± 0.48), Pregnant CTRL (3.50 ± 0.54), post-partum CTRL (4.54 ± 0.50) and post-partum + extract (4.64 ± 0.37) groups.

Total red blood cell count ($\times 10^9$ cells/L) of the non-pregnant control (5.52 ± 0.67), pregnant CTRL (5.80 ± 0.53), post-partum CTRL (5.58 ± 0.80), post-partum + STD drug (5.76 ± 0.59) and post-partum + extract (6.12 ± 0.52) groups were not significantly ($P < 0.05$) different from each other.

The results from the platelet count ($\times 10^9$ cells/L) of non-pregnant control (240.2 ± 7.16), pregnant CTRL (244.50 ± 15.35) and post-partum CTRL (219.60 ± 10.57) groups were not significantly ($P < 0.05$) different from each other. However, were significantly ($P < 0.05$) decreased when compared to the post-partum + STD drug (273.60 ± 28.99) group but showed significant ($P < 0.05$) elevation when compared to the post-partum + extract (182.60 ± 19.19) group.

Discussion

Fourier-transform infrared spectroscopy identified important functional groups (Table 2) of organic compounds ranging from complex –OH contained in alcohols and phenols, $-NH_2$ of aromatic amines, primary amines and amides, ≡CH found in acetylenes, $-NH_3^+$ in amino acids, $-CH_3$ linked to O or N, -OH in phosphorus oxyacids, -N = C = O of isocyanates, -C≡N in thiocyanates, -C = C = C of alkenes, -NH in primary amides, $-CH_3$ in aliphatic compounds, and -1,3,5-trisubstituted benzenes.

According to retention time, the identified bioactive components comprised kaempferol, naringenin, sapogernin , flavanones, anthocyanins, flavan-3-ol, cyanogenic glycosides, ribalinidine, rutin, catechin, resveratrol, spartein, epicatechin, steroids, and phytates (Table 3).

The index of acute toxicity using LD_{50}, which is the dose of a substance capable of producing death in 50% of the animals exposed to the aqueous seed extract, showed that no lethality was recorded in the animals after administration of extracts up to 5000 mg/kg body weight (Table 4).

Table 2. FTIR determined functional groups of aqueous seed extract of *Aframomum melegueta*

Peak value	Bonds	Group and class
3696.513	OH stretch	-OH of alcohols and Phenols
3382.721	NH stretch	$-NH_2$ found in aromatic amines, primary amines and amides
3275.926	≡CH-H stretch	≡CH in acetylenes
3170.204	$-NH_3^+$anti symmetric stretch	$-NH_3^+$in amino acids
2772.403	CH stretch modes	$-CH_3$ linked to O or N
2617.028	Associated OH stretching	-OH in phosphorus oxyacids
2258.953	N = C = O anti symmetric stretch	-N = C = O in isocyanates
2159.471	C≡N stretch	-C≡N found in thiocyanates
1968.142	C = C = C stretch	-C = C = C in allenes
1629.664	NH deformation (amide ii band)	-NH in primary amides
1446.7	$-CH_3$ anti symmetric deformation	$-CH_3$ found in aliphatic compounds
1370.012	CH_3 symmetric deformation	$-CH_3$ in aliphatic compounds
836.568	CH out-of-plane deformation	-1,3,5-trisubst benzenes

There were no observed significant changes in the blood parameters (Hb, PCV, and RBC counts) across the treatment groups, unlike previous studies by Muthuramalingam et al (19), who reported elevated levels of Hb, PCV, and RBC throughout pregnancy. The absence of significant changes in these functional properties shows no anemic condition was elicited across the groups (33). Decreased hemoglobin and hematocrit levels, indicative of anemia, are associated with hemolysis from antigen-antibody response (34). Hemoglobin is a natural constituent of RBCs and biochemically adapted to carry oxygen in the lungs and deposit it in tissues for oxidative metabolism; it has been characterized to also play a significant role in physiological carbon dioxide removal and acid-base balance; increased production of hemoglobin is an advantage to an organism (35).

There was observed significant ($P<0.05$) reduction in WBC count of the pregnant control animals compared with the non-pregnant rats, probably due to pregnancy-related stress and mobilization of WBC to the fetus. However, there was a significant ($P<0.05$) improvement in the WBC count of the rats after delivery. On the contrary, Muthuramalingam et al (19) reported higher WBC during late pregnancy and late lactation. The increase in WBC could be due to the defense mechanism against the entrance of foreign material into the body system of the rats (35). White blood cells form part of the immune system in animals and show a significantly ($P<0.05$) adverse xenobiotic response to carbagoline standard drug compared to the extract-treated animals due to the stimulation of the production of plasma-cell derivatives of B-cells.

The results showed no significant increase ($P<0.05$) in platelet count between the non-pregnant and pregnant control but a slight non-significant decrease after delivery. Platelet is one of the critical elements of human blood. Normal pregnancy is characterized by an increase in platelet aggregation and a decrease in the number of circulating platelets with gestation. Increased consumption of platelets in the uteroplacental circulation has been suggested to explain the reduction in the number of circulating platelets (36). However, there was a non-significant increase ($P<0.05$) in the post-partum + STD drug animals' platelet count, which is likely due to the drug's adverse xenobiotic effect and cellular injury elicited by the standard drug. However, there was a significant ($P<0.05$) reduction (thrombocytopenia) in the post-partum + extract group when compared to the Post-partum control animals, which may be attributed to a decrease in the number of circulating platelets post-partum likely due to the flavonoid, resveratrol contained in *A. melegueta* which is reported to dilate blood vessels, hence reducing blood clotting, and thus may increase the risk of bleeding disorders (37), as well as the reported antiplatelet activities of the rutin component of the plant extract (38). Additionally, the plant extract (–)-epicatechin component is reported to be the only catechin stereoisomer capable of inducing vasodilation of the femoral artery upon direct infusion into the bloodstream (39). However, this reflects a positive anti-atherogenic and cardioprotective effect effect (Table 5).

Table 3. GCFID determined phytochemical constituents of aqueous seed extracts of *Aframomum melegueta*

Component	Concentration (µg/mL)
Kaempferol	15.68
Naringenin	1.06
Sapogernin	23.10
Flavanones	18.41
Anthocyanins	13.45
Flavan-3-ol	11.09
Cyanogenic glycosides	12.64
Ribalinidine	10.13
Rutin	11.75
Catechin	10.57
Resveratrol	6.73
Spartein	1.92
Epicatechin	1.23
Steroids	1.93
Phytates	6.73

Table 4. LD_{50} acute toxicity test result of albino mice administered aqueous seed extract of *Aframomum melegueta*

Groups	No. of mice	Mortality
Phase I		
I 10 mg/kg body weight	3	0
II 100 mg/kg body weight	3	0
III 1000 mg/kg body weight	3	0
Phase II		
IV 1600 mg/kg body weight	3	0
V 2900 mg/kg body weight	3	0
VI 5000 mg/kg body weight	3	0

Acute toxicity test using LD_{50} determination revealed no death was recorded among the animals up to 5000mg extract/kg body weight administration.

Table 5. Haematologic indices of animals treated with aqueous seed extracts of *Aframomum melegueta*

Parameter	Non-pregnant CTRL	Pregnant CTRL	Post-partum CTRL	Post-partum + STD Drug	Post-partum + Extract
Haemoglobin	16.68 ± 1.63^{a}	16.00 ± 1.89^{a}	17.12 ± 1.01^{a}	14.96 ± 2.12^{a}	15.68 ± 1.71^{a}
PCV	50.04 ± 4.89^{a}	48.00 ± 2.62^{a}	51.36 ± 3.03^{a}	44.88 ± 3.84^{a}	47.04 ± 5.12^{a}
WBC ($\times 10^{9}$/L)	4.88 ± 0.48^{a}	3.50 ± 0.54^{b}	4.54 ± 0.50^{a}	5.92 ± 0.08^{c}	4.64 ± 0.37^{a}
RBC	5.52 ± 0.67^{a}	5.80 ± 0.53^{a}	5.58 ± 0.80^{a}	5.76 ± 0.59^{a}	6.12 ± 0.52^{a}
PLT	240.2 ± 7.16^{ab}	244.50 ± 15.35^{ab}	219.60 ± 10.57^{a}	273.60 ± 28.99^{b}	182.60 ± 19.19^{c}

Values are mean ± standard deviation. The values having different alphabet letters per row are statistically significant ($P<0.05$).

Platelets are essential in thrombogenesis (36). Platelets, also called thrombocytes, are the smallest human blood cells that play a crucial role in blood clotting. When released by the bone marrow, the quantity of these specialized cells in the blood is generally reported on a complete blood count (40). Platelets play an important role in atherogenesis and the progression of atherosclerotic lesions (36). The interaction of platelets with the blood vessel walls and their subsequent contribution to atheroma formation and thrombosis is essential in the etiology and pathogenesis of peripheral, coronary, cerebrovascular, and other vascular diseases (36,41). However, pregnancy is generally a "pro-clotting" state, and this is quite useful as clotting helps protect women from excessive bleeding at delivery as platelets combine with other factors in the blood to control bleeding and help plug holes in the walls of blood vessels (40). Inappropriate platelet activation is common in atherosclerosis, and many of its risk factors, such as smoking and diabetes, play a prime role in increasing the heart disease burden of society. There is still no generally accepted ideal measure of platelet activation that would indicate a state of 'high risk' (36). Platelets can form clots in blood vessels in areas that have plaque buildup, leading to an increased risk for heart attacks and strokes. Thrombocytopenia occurs in maternal complications such as excessive bleeding, premature delivery, or inability to get an epidural (40). Platelet activation markers could be a helpful guide in distinguishing different subgroups of patients, following the observation that stroke patients with carotid artery disease exhibit significantly more platelet activation than those strokes with a cardio-embolic etiology (36,42). It is normal for platelet count to dip by a few thousand during pregnancy due in part to hemodilution: the body makes more plasma during pregnancy, so the total number of platelets per blood volume will be lower (40).

Conclusion

FTIR spectroscopy identified critical functional groups of organic compounds ranging from complex –OH, $-NH_2$, primary amines and amides, CH, $-NH_3^+$, $-CH_3$, -OH, -N=C=O, -C≡N, -C=C=C, -NH, $-CH_3$, and -1,3,5-trisubstituted benzenes. According to retention time, the identified bioactive components ranged from 1.06 µg/mL to 23.10 µg/mL. The highest concentration of bioactive components was sapogernin, 23.10 µg/mL, while the lowest concentration of 1.06 µg/mL was for naringenin. There were no deaths following acute toxicity tests after administration of extracts up to 5000 mg/kg body weight.

No adverse anemic condition was observed during pregnancy and delivery on haematologic parameters, namely hemoglobin, PCV, and red blood cell count. However, a significant decrease in WBC count was observed during pregnancy. However, the WBC count improved significantly ($P < 0.05$) after delivery. There was observed significant reduction (thrombocytopenia) in the aqueous extract-treated animals attributable to a possible decrease in the number of circulating platelets post-partum, likely due to the flavonoid, resveratrol contained in *A. melegueta*, which is reported to dilate blood vessels, hence reducing blood clotting, and thus may increase the risk of bleeding disorders (37), as well as antiplatelet activities of the rutin and (–)-epicatechin components of the plant extract (38,39). However, this reflects a positive anti-atherogenic and cardioprotective effect.

Authors' Contribution

Conceptualization: Uche Emmanuel Olunkwa.
Data curation: Uche Emmanuel Olunkwa.
Formal analysis: Chidi Uzoma Igwe.
Funding acquisition: Uche Emmanuel Olunkwa.
Investigation: Uche Emmanuel Olunkwa.
Methodology: Uche Emmanuel Olunkwa.
Project administration: Uche Emmanuel Olunkwa.
Resources: Uche Emmanuel Olunkwa.
Software: Chidi Uzoma Igwe.
Supervision: Kizito Munachiso Enyereibenyem Iheanacho, Chidi Uzoma Igwe and Linus Ahuwaraeze Nwaogu.
Validation: Kizito Munachiso Enyereibenyem Iheanacho, Chidi Uzoma Igwe and Linus Ahuwaraeze Nwaogu.
Visualization: Chidi Uzoma Igwe.
Writing–original draft: Uche Emmanuel Olunkwa.
Writing–review & editing: Uche Emmanuel Olunkwa, Kizito Munachiso Enyereibenyem Iheanacho, Chidi Uzoma Igwe, Linus Ahuwaraeze Nwaogu.

Competing Interests

None declared.

Ethical Approval

Ethical approval was obtained for the study protocol from the University Ethical Committee, and Principles of Laboratory Animal Care was adopted for all the experimental procedures involving the use and handling of laboratory animals.

Funding

None.

References

1. Mohammed A, Ngwen AL, Umaru IJ, Ahmed MU, Boyi NR. Antimicrobial property of aqueous and petroleum ether leaf extracts of *Jatropha curcas*. International Journal of Science Inventions Today. 2013;2(6):549-55.
2. Usman MM, Sule MS. Effect of oral administration of aqueous leaves extracts of *Euphobia lateriflora* (Schum and Thonn) on liver and kidney function in rats. IOSR J Environ Sci Toxicol Food Technol. 2014;8(2):64-7. doi: 10.9790/2402-08236467.
3. Augustine A, Francis EU, Ivy AU. Biochemical assessment of the effect of aqueous leaf extract of *Euphorbia heterophylla* Linn on hepatocytes of rats. IOSR J Environ Sci Toxicol Food Technol. 2013;3(5):37-41.
4. Prasad DR, Izam A, Khan MM. *Jatropha curcas*: plant of medical benefits. J Med Plants Res. 2012;6(14):2691-9. doi: 10.5897/jmpr10.977.
5. Akpanabiatu MI, Ekpo ND, Ufot UF, Udoh NM, Akpan EJ, Etuk EU. Acute toxicity, biochemical and haematological study of *Aframomum melegueta* seed oil in male Wistar albino rats. Journal of Ethnopharmacology. 2013;150(2):590-4. doi: 10.1016/j.jep.2013.09.006.
6. Okoli CO, Akah PA, Nwafor SV, Ihemelandu UU, Amadife C. Anti-inflammatory activity of seed extracts of *Aframomum melegueta*. J Herbs Spices Med Plants. 2007;13(1):11-21. doi: 10.1300/J044v13n01_02.

7. Ukeh DA, Birkett MA, Pickett JA, Bowman AS, Luntz AJ. Repellent activity of alligator pepper, *Aframomum melegueta*, and ginger, *Zingiber officinale*, against the maize weevil, *Sitophilus zeamais*. Phytochemistry. 2009;70(6):751-8. doi: 10.1016/j.phytochem.2009.03.012.
8. Onoja SO, Omeh YN, Ezeja MI, Chukwu MN. Evaluation of the in vitro and in vivo antioxidant potentials of *Aframomum melegueta* methanolic seed extract. J Trop Med. 2014;2014:159343. doi: 10.1155/2014/159343.
9. Tropilab Inc. *Aframomum melegueta* - Allligator Pepper. 2008. Available from: https://tropilab.com/nengrekondrepepre.html.
10. Inegbenebor U, Ebomoyi M. Effect of saline extract of alligator pepper (Zingiberaceae *Aframomum melegueta*) on serum progesterone in pregnant Sprague-Dawley rats. Int J Herbs Pharmacol Res. 2014;3(3):46-51.
11. Ajaiyeoba EO, Ekundayo O. Essential oil constituents of *Aframomum melegueta* (Roscoe) K. Schum. seeds (alligator pepper) from Nigeria. Flavour Fragr J. 1999;14(2):109-11. doi: 10.1002/(sici)1099-1026(199903/04)14:2<109::aid-ffj775>3.0.co;2-m.
12. Okibe GO, Ajibola VO, Agbaji EB. Evaluation of proximate composition and the stability of alligator pepper (*Aframomum melegueta*) and Ethiopian pepper (*Xylopia aethiopica*) extracts. N Y Sci J. 2020;13(8):45-54. doi: 10.7537/marsnys130820.04.
13. Ilic N, Schmidt BM, Poulev A, Raskin I. Toxicological evaluation of grains of paradise (*Aframomum melegueta*) [Roscoe] K. Schum. J Ethnopharmacol. 2010;127(2):352-6. doi: 10.1016/j.jep.2009.10.031.
14. Oyedele AO, Gbolade AA, Sosan MB, Adewoyin FB, Soyelu OL, Orafidiya OO. Formulation of an effective mosquito-repellent topical product from lemongrass oil. Phytomedicine. 2002;9(3):259-62. doi: 10.1078/0944-7113-00120.
15. Okaiyeto K, Adeoye RI, Oguntibeju OO. Some common West African spices with antidiabetic potential: a review. J King Saud Univ Sci. 2021;33(6):101548. doi: 10.1016/j.jksus.2021.101548.
16. Krajničáková MÁ, Kováč GA, Kostecký MA, Valocký IG, Maraček IM, Šutiaková IR, et al. Selected clinico-biochemical parameters in the puerperal period of goats. Bull Vet Inst Pulawy. 2003;47:177-82.
17. Iriadam M. Variation in certain hematological and biochemical parameters during the peri-partum period in Kilis does. Small Rumin Res. 2007;73(1-3):54-7. doi: 10.1016/j.smallrumres.2006.11.001.
18. Bacq Y. The liver in normal pregnancy. In: Madame Curie Bioscience Database. Austin, TX: Landes Bioscience; 2013. Available from: https://www.ncbi.nlm.nih.gov/books/NBK6005/.
19. Muthuramalingam T, Sundaram SM, Gnanaraj PT, Sivakumar T, Vairamuthu S, Rangasamy S, et al. Comparative body condition and haemato-biochemical profile of Tellicherry does during pregnancy, lactation and dry period. Indian J Anim Res. 2022;56(3):298-304. doi: 10.18805/ijar.b-4459.
20. Etim NN, Enyenihi GE, Akpabio U, Offiong EE. Effects of nutrition on haematology of rabbits: a review. Eur Sci J. 2014;10(3):413-24.
21. Nurlatifah A, Khotijah L, Arifiantini RI, Maidin MS, Astuti DA. Change in hematology prepartum and postpartum of Garut ewe fed with flushing diet contain lemuru oil. IOP Conf Ser Earth Environ Sci. 2022;1020(1):012005. doi: 10.1088/1755-1315/1020/1/012005.
22. Lurie S, Mamet Y. Red blood cell survival and kinetics during pregnancy. Eur J Obstet Gynecol Reprod Biol. 2000;93(2):185-92. doi: 10.1016/s0301-2115(00)00290-6.
23. Chandra S, Tripathi AK, Mishra S, Amzarul M, Vaish AK. Physiological changes in hematological parameters during pregnancy. Indian J Hematol Blood Transfus. 2012;28(3):144-6. doi: 10.1007/s12288-012-0175-6.
24. Greguła-Kania M, Kosior-Korzecka U, Patkowski K, Juszczuk-Kubiak E, Plewik M, Gruszecki TM. Acute-phase proteins, cortisol and haematological parameters in ewes during the periparturient period. Reprod Domest Anim. 2020;55(3):393-400. doi: 10.1111/rda.13628.
25. Franchini M. Haemostasis and pregnancy. Thromb Haemost. 2006;95(3):401-13. doi: 10.1160/th05-11-0753.
26. Anderson JM, Etches D. Prevention and management of postpartum hemorrhage. Am Fam Physician. 2007;75(6):875-82.
27. Wojtyła C, Biliński P, Paprzycki P, Warzocha K. Haematological parameters in postpartum women and their babies in Poland - comparison of urban and rural areas. Ann Agric Environ Med. 2011;18(2):380-5.
28. Manat TD, Chaudhary SS, Singh VK, Patel SB, Puri G. Hematobiochemical profile in Surti goats during post-partum period. Vet World. 2016;9(1):19-24. doi: 10.14202/vetworld.2016.19-24.
29. Lorke D. A new approach to practical acute toxicity testing. Arch Toxicol. 1983;54(4):275-87. doi: 10.1007/bf01234480.
30. DHHS. Guide for the Care and Use of Laboratory Animals. Washington, DC, USA: Institute of Laboratory Animal Resources Commission on Life Sciences, National Research Council, National Academy Press; 1985.
31. Lewis SM, Bain BJ, Bates I. Dacie and Lewis Practical Haematology. 9th ed. New York: Churchill Livingstone; 2001.
32. Central Board of Health (CBOH). Haematology Standard Operating Procedures for Hospital Laboratories Level III. Lusaka, Zambia: Mipal Printers; 2003.
33. Igwe CU, Nwaogu LA, Olunkwa EU, Otaba M, Onwuliri VA. Atherogenic and haematologic indices of paracetamol-overdosed albino rats treated with aqueous leaf extracts of *Euphorbia heterophylla* and *Jatropha curcas*. J Clin Toxicol. 2017;7(2):344. doi: 10.4172/2161-0495.1000344.
34. Igbinosa OO, Oviasogie EF, Igbinosa EO, Igene O, Igbinosa IH, Idemudia OG. Effects of biochemical alteration in animal model after short-term exposure of *Jatropha curcas* (Linn) leaf extract. ScientificWorldJournal. 2013;2013:798096. doi: 10.1155/2013/798096.
35. Omoboyowa DA, Aja AO, Eluu F, Ngobidi KC. Effects of methanol seed extract of *Aframomum melegueta* (alligator pepper) on Wistar rats with 2,4-dinitrophenylhydrazine-induced hemolytic anemia. Recent Advances in Biology and Medicine. 2017;3:11-7. doi: 10.18639/rabm.2017.03.443648.
36. Juan P, Stefano G, Antonella S, Albana C. Platelets in pregnancy. J Prenat Med. 2011;5(4):90-2.
37. WebMD. 2005. Available from: https://www.webmd.com/vitamins/ai/ingredientmono-307/resveratrol.
38. Novakovic A, Bukarica LG, Kanjuh V, Heinle H. Potassium channels-mediated vasorelaxation of rat aorta induced by resveratrol. Basic Clin Pharmacol Toxicol. 2006;99(5):360-4. doi: 10.1111/j.1742-7843.2006.pto_531.x.
39. Schwarz NA, Blahnik ZJ, Prahadeeswaran S, McKinley-Barnard SK, Holden SL, Waldhelm A. (-)-Epicatechin supplementation inhibits aerobic adaptations to cycling exercise in humans. Front Nutr. 2018;5:132. doi: 10.3389/fnut.2018.00132.
40. Horsager-Boehrer R. How Can Having Low Platelets Affect My Pregnancy and Birth Plan? UT Southwestern Medical Center; 2021. Available from: https://utswmed.org/medblog/low-platelets-pregnancy/.
41. White JG. Platelets and atherosclerosis. Eur J Clin Invest. 1994;24 Suppl 1:25-9. doi: 10.1111/j.1365-2362.1994.tb02422.x.
42. Nurden P, Bihour C, Smith M, Raymond JM, Nurden AT. Platelet activation and thrombosis: studies in a patient with essential thrombocythemia. Am J Hematol. 1996;51(1):79-84. doi: 10.1002/(sici)1096-8652(199601)51:1<79::aid-ajh13>3.0.co;2-6.

Cite this article as: Olunkwa UE, Iheanacho KME, Igwe CU, Nwaogu LA. Phytochemical analysis and effect of short-term administration of aqueous seed extract of *Aframomum melegueta* on haematologic indices of female albino rats. Future Nat Prod. 2023;9(2):x–x. doi: 10.34172/fnp.166.