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Impact of *Justicia carnea* on haemostatic parameters of albino rats

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Abstract

This study evaluates the impact of ethanolic leaf extract of *Justicia carnea* on hemostatic parameters in albino Wistar rats, focusing on platelet count, prothrombin time (PT), and activated partial thromboplastin time (APTT). *Justicia carnea*, a medicinal plant widely used in West Africa for blood-related disorders, was investigated for its coagulation-modulating effects. Thirty rats were divided into four groups: a control group receiving distilled water and three treatment groups receiving low (0.3 ml), medium (1.75 ml), and high (3.5 ml) doses of the extract daily for 14 days. Blood samples were analyzed for PT, APTT, and platelet count, with comparisons made across doses and genders. Results showed a dose-dependent increase in platelet count, with the high-dose group reaching 742.75 ± 12.23 $\times 10^9$/L compared to the control's 582.43 ± 7.93 $\times 10^9$/L ($p = 0.000$, Table 3). PT was significantly prolonged, increasing from 15.14 ± 0.90 seconds (control) to 24.13 ± 1.13 seconds (high-dose, $p = 0.000$). APTT also extended from 33.71 ± 1.89 seconds (control) to 47.50 ± 1.20 seconds (high-dose, $p = 0.000$). Female rats exhibited more pronounced effects, with platelet counts up to 750.60 ± 6.58 $\times 10^9$/L and PT at 24.40 ± 1.14 seconds ($p = 00.000$, Table 8), suggesting sex-specific responses possibly due to hormonal influences. These findings, consistent with prior studies, indicate thrombopoietic and anticoagulant properties, likely driven by flavonoids and phenolics. The dual action of *Justicia carnea* suggests therapeutic potential for conditions like thrombocytopenia or hypercoagulability, but its anticoagulant effects warrant caution to avoid bleeding risks. Further studies are needed to identify active compounds, assess long-term safety, and validate clinical applications.

Keywords: Hemostasis; *Justicia carnea*; Hemostatic Parameters; Albino Rats; And Medicinal Plant

1. Introduction

Hemostasis is a vital physiological process that ensures the integrity of the circulatory system by stopping bleeding from injured blood vessels, involving a complex interaction between the vascular endothelium, platelets, and coagulation factors. Any impairment in this process can lead to excessive bleeding (haemorrhage) or unwanted clotting (thrombosis), both of which can be life-threatening (Hoffbrand *et al.*, 2019). Monitoring and maintaining haemostatic balance are, therefore, critical in medical practice, particularly in conditions that may compromise coagulation such as liver diseases, malignancies, and haematological disorders.

Coagulation is a complex physiologic process by which blood forms clots. It is an important part of hemostasis (the cessation of blood loss from a damaged vessel), wherein a damaged blood vessel wall is covered by a platelet and fibrin-containing clot to stop bleeding and begin repair of the damaged vessel (Shapiro, 2003). Disorders of coagulation can lead to an increased risk of bleeding (hemorrhage) or obstructive clotting (thrombosis). Coagulation involves both a cellular (platelet) and a protein (coagulation factor) component. The system in humans has been the most extensively researched and is therefore the best understood (Norris, 2003).

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Coagulation begins almost instantly after an injury to the blood vessel has damaged the endothelium (lining of the vessel) (Cotran *et al*., 2005) Exposure of the blood to proteins such as tissue factor initiates changes to blood platelets and the plasma protein fibrinogen, a clotting factor, platelets immediately form a plug at the site of injury, this is called primary hemostasis. The secondary hemostasis occurs simultaneously: proteins in the blood plasma, called coagulation factors respond in a complex cascade to form fibrin strands, which strengthen the platelet plug (Furie and Furie, 2005).

Natural products and medicinal plants have long been explored for their pharmacological effects, including their potential to influence haemostatic functions. One such plant is *Justicia carnea*, commonly known as the blood of Jesus plant or purple-leaved Justicia. It belongs to the family Acanthaceae and is widely distributed in tropical regions, particularly in West Africa. It is traditionally used in herbal medicine for treating anaemia, inflammation, and boosting general vitality (Ezeugwu *et al.,* 2020). Anecdotal and preliminary scientific evidence suggests that *Justicia carnea* possesses heamatopoietic and antioxidant properties, making it a candidate of interest in studies related to blood health and coagulation.

Phytochemical analyses of *Justicia carnea* have revealed the presence of bioactive compounds such as flavonoids, saponins, alkaloids, and tannins (Akinmoladun *et al.,* 2015). These phytochemicals have been shown to exert various physiological effects, including antioxidant, anti-inflammatory, and haemostatic-modulating activities. For instance, flavonoids are known to stabilize blood vessels and inhibit platelet aggregation, while tannins can enhance blood clotting by promoting vasoconstriction and reducing capillary permeability (Omoregie and Osagie, 2011). These properties suggest that *Justicia carnea* may have a significant impact on haemostatic parameters such as bleeding time, clotting time, platelet count, and prothrombin time.

The use of animal models, particularly albino Wistar rats, provides an ethical and scientifically valid method to investigate the biological effects of medicinal plants. These models allow researchers to evaluate the influence of interventions like *Justicia carnea* on specific physiological and biochemical parameters under controlled conditions. The importance of understanding the influence of natural plant extracts on haemostasis has gained momentum in light of the growing interest in complementary and alternative medicine, especially in regions where access to conventional drugs is limited.

Despite the extensive traditional use of *Justicia carnea*, there is limited scientific evidence regarding its impact on coagulation and haemostatic function. Investigating this relationship is crucial to validate traditional claims and explore potential therapeutic or adverse effects. The haemostatic system is particularly sensitive to external agents, and any substance that affects blood clotting could have implications for conditions such as thrombosis, haemophilia, and other clotting disorders (Cheesbrough, 2014). Thus, this study seeks to scientifically assess the effects of *Justicia carnea* extract on the haemostatic parameters of albino Wistar rats, providing a basis for further pharmacological investigations and potential clinical applications.

Understanding the role of herbal extracts in modifying blood parameters also contributes to the safety evaluation of these plants when used in folk medicine. While herbal medicines are often considered safe due to their natural origin, they may contain bioactive compounds capable of interfering with physiological systems. This highlights the need for rigorous scientific research to determine their efficacy, safety, and possible contraindications. Hence, this study is poised to contribute to the growing body of knowledge regarding the pharmacodynamic effects of medicinal plants on the hemostatic system.

Despite the widespread traditional use of *Justicia carnea* for blood-related ailments, scientific evidence on its actual impact on hemostatic processes is lacking. This knowledge gap poses a potential risk, especially when such herbs are consumed without regulation or understanding of their biological effects. Hemostatic imbalances can lead to serious health complications such as thrombosis, stroke, or hemorrhagic conditions, which necessitates the evaluation of substances that may influence these pathways. Moreover, the increasing reliance on herbal remedies in regions with limited access to orthodox medicine emphasizes the urgency to scientifically validate the safety and efficacy of these plants. Without proper research, the continued use of *Justicia carnea* might result in unintended consequences, especially in individuals with underlying coagulation disorders or those on anticoagulant therapy. The growing global interest in plant-based therapeutics and the urgent need to scientifically evaluate traditional medicines. The investigation into the hemostatic effects of *Justicia carnea* will not only validate or refute traditional claims but may also uncover novel applications or warnings associated with its use. As clotting abnormalities remain a significant cause of morbidity and mortality, identifying natural agents that can positively or negatively affect coagulation is of medical and pharmacological importance.

Furthermore, this research will provide baseline data for future studies and support evidence-based integration of herbal medicine into clinical practice. It may also contribute to the development of new anticoagulant or procoagulant agents derived from natural sources, promoting safer health practices and drug discovery.

2. Materials and Methods

2.1. Study Area

The study was carried out in the Animal House and Medical Laboratory Science Department of Madonna University, Elele, Rivers State, Nigeria. The university has a well-equipped biomedical laboratory suitable for biochemical and Hematological research. The area is located in the tropical rainforest belt, with environmental conditions conducive for laboratory animal care and research work.

2.2. Collection and Identification of the Plant

Fresh leaves of *Justicia carnea* were collected from a local farm in Elele, Rivers State, Nigeria, and authenticated by a plant taxonomist in the department of pharmacy, Madonna University Elele.

2.3. Study population

The study population consisted of thirty (30) albino rats that was further subdivided into four (4) groups used in this research.

2.4. Research design

This study adopted an experimental design involving the administration of aqueous extract of *Justicia carnea* leaves to albino Wistar rats. The animals were randomly assigned into groups, with a control group receiving no treatment only distilled water and feed while the treatment groups received varying doses of the extract. The primary aim was to observe changes in haemostatic parameters specifically Prothrombin Time (PT), Activated Partial Thromboplastin Time (APTT), and Platelet Count following extract administration over a defined period. This design allowed for the evaluation of cause-effect relationships between the extract and coagulation status in the animal model.

2.5. Inclusion criteria

- The albino rats had acclimatized to the new environment for seven (7) days
- Albino rats used, weighed from 150-200g
- The rats used were healthy and free from infection

2.6. Exclusion criteria

- Albino rats showing signs of infection were not used
- Albino rats that were below 130g were not used
- the rats were not stored in a dirty environment

2.7. Preparation of Plant Extract

Fresh leaves of *Justicia carnea* were collected from a pharmaceutical garden in Madonna University, Elele. The leaves were washed thoroughly with clean water to remove dirt and debris, then shade-dried at room temperature for 10 days. Once fully dried, the leaves were pulverized into a fine powder using an electric blender. Approximately 5.5 g of the powdered leaves were macerated in 1000 ml of 95% ethanol for 48 hours with intermittent shaking. After extraction, the mixture was filtered first through muslin cloth and then through Whatman No. 1 filter paper. The resulting filtrate was concentrated using a rotary evaporator and then a water bath at 40–50°C to obtain a dark green ethanolic extract. The semi-solid extract was transferred to an airtight container and stored at 4°C in a refrigerator until further use.

Experimental design / Experimental: A total of thirty (30) healthy albino Wistar rats which was subdivided into four (4) groups consisting of seven (7) control, and the treatment groups were eight (8) for low dose (0.3), (8) for moderate dose (1.75) and another (8) for high dose (3.5). Rats weighing between 150–200 g, were used for this study. The animals were procured from a reputable animal breeder and housed in well-ventilated standard plastic cages. The rats were allowed to acclimatize for two weeks under controlled temperature and humidity, with a 12-hour light/dark cycle. They were fed with a standard pellet diet and provided water and libitum. Animal handling and experimentation were carried out in accordance with institutional ethical guidelines for animal research.

Grouping and Administration of Treatment

- The 30 rats were further subdivided into four groups:
- Group A (Control): Received distilled water and feed.
- Group B (Low Dose): Received 0.3 ml of *Justicia carnea* extract.
- Group C (Medium Dose): Received 1.75mls of extract.
- Group D (High Dose): Received 3.5mls of extract.

The extract was administered orally using an orogastric tube once daily for 14 consecutive days. On the 15th day, the animals were sacrificed under light chloroform anesthesia, and blood was collected via cardiac puncture into tri-sodium citrate anticoagulated tubes for coagulation analysis.

2.8. Collection of Blood Samples

At the end of the experiment, four milliliters (4 mL) of blood were collected from each rat via venipuncture into EDTA containers and labeled. The samples were taken to the laboratory for analysis.

Determination of Hemostatic Parameters: The following hemostatic parameters were evaluated using the standard method as describe by the recent laboratory animal studies adopted by Tynngard *et al.*, (2015):

Determination Prothrombin Time (PT): The automated photo-optical clot detection method as described by Favaloro, (2025) was used in determining the prothrombin time. In this method, four (4) ml of Blood was collected in citrate tubes and was centrifuged at 1500 rpm for 15 minutes to obtain platelet-poor plasma and unto a clean test tube, 0.2 ml of thromboplastin reagent containing calcium was added and incubated in the water bath at 37ºC for 2 minutes, after 2 minutes, 0.1 ml of plasma was dispensed into the same tube in the water bath. A stopwatch was started immediately, and the clotting time was recorded in seconds as prothrombin time (PT). Reference Range: 11- 16 seconds

Determination of Activated Partial Thromboplastin Time (APTT): Activated partial thromboplastin time was determined using the photo-optical coagulometric method as described by Shimonishi et al., (2025). In this method, 0.2 ml of kaolin platelet substitute was aspirated and dispensed into a test tube, 0.1 ml of the platelet poor plasma was added into the same tube and was incubated at 370 C in the water bath for 2 minutes, after which 0.1 ml of calcium chloride was added and the stopwatch was started Immediately, the time taken for clot formation was recorded in seconds. Reference Range: 30-50 seconds.

2.9. Determination of Platelet Count

The fluorescence flow cytometric method as described by Tantanate *et al.*, (2017) was used in determining the platelet count. In this method, the blood was diluted 1:20 with ammonium oxalate diluent to 0.38ml of ammonium oxalate and 0.20ml of whole blood into a clean tube, mixed and was allowed to stand for 5 minutes to lyse red cells. The Neubauer chamber was charged with the diluted sample and left in a moist chamber for 20 minutes. Platelets in the central large square (comprising 25 medium squares) were counted using x10 objective lens to focus and x40 objective lens to view

Platelet count is done to determine the number of circulating thrombocytes in the blood. Ammonium oxalate lyses the red blood cells while preserving platelets, which are then counted using a hemocytometer and is expressed in x10^9. Abnormal platelet counts indicate disturbances in hemostasis. Reference Range: 400 – 150 x10^9/l

2.10. Statistical Analysis

Data obtained from the experiment were expressed as mean ± standard deviation. The statistical analysis was performed using SPSS version 25.0. One-way Analysis of Variance (ANOVA) was used to compare means among the groups, and significance was considered at $p< 0.05$.

3. Results

Table 1 presents a comparative analysis of platelet count, prothrombin time (PT), and activated partial thromboplastin time (APTT) between non-treated (control) rats and those administered a low dose of ethanolic extract of *Justicia carnea*. The results indicate a statistically significant increase in Platelet count among the treated group (612.29 ± 8.90 $\times 10^9$/L) relative to the control group (582.43 ± 7.93 $\times 10^9$/L), with a P-value of 0.000. Furthermore, a marked prolongation of Prothrombin time was observed in the treated rats (18.86 ± 1.35 seconds) compared to the control (15.14 ± 0.90 seconds), also statistically significant (P = 0.000). Similarly, activated partial thromboplastin time was

significantly elevated in the treated group (38.57 ± 1.27 seconds) versus the control (33.71 ± 1.89 seconds), with a P-value of 0.000.

Table 2: displays the comparative effects of a medium dose of ethanolic extract of *Justicia carnea* on platelet count, prothrombin time (PT), and activated partial thromboplastin time (APTT) in rats, relative to non-treated controls. Administration of the medium dose resulted in a significant elevation in platelet count, with treated rats showing a mean value of 699.50 ± 13.31 $\times 10^9$/L compared to 582.43 ± 7.93 $\times 10^9$/L in the control group (P = 0.000). In addition, prothrombin time was markedly prolonged in the treated group (21.25 ± 0.89 seconds) relative to the control (15.14 ± 0.90 seconds), with statistical significance (P =0.000). Similarly, activated partial thromboplastin time was significantly increased in the treated rats (42.50 ± 1.20 seconds) when compared to the control group (33.71 ± 1.89 seconds), also with a P-value of 0.000.

Table 3 provides a comparative analysis of platelet count, prothrombin time (PT), and activated partial thromboplastin time (APTT) between non-treated rats and those administered a high dose of ethanolic extract of *Justicia carnea*. A significant increase in platelet count was observed in the high-dose treatment group (742.75 ± 12.23 $\times 10^9$/L) compared to the control group (582.43 ± 7.93 $\times 10^9$/L), with a P-value of 0.000. In addition, prothrombin time was substantially prolonged in the treated rats (24.13 ± 1.13 seconds) relative to controls (15.14 ± 0.90 seconds), with statistical significance (P =0.000). Similarly, activated partial thromboplastin time was markedly increased in the high-dose group (47.50 ± 1.20 seconds) when compared with the control group (33.71 ± 1.89 seconds), also with a P-value of 0.000.

Table 4 presents a comparison of platelet count, prothrombin time (PT), and activated partial thromboplastin time (APTT) between non-treated female rats and female rats treated with a low dose of ethanolic extract of *Justicia carnea.* Treatment with the low dose of the extract led to a significant increase in platelet count, with the treated group recording 618.67 ± 6.03 $\times 10^9$/L compared to 584.50 ± 10.41 $\times 10^9$/L in the control group (P =0 .004). Prothrombin time was also significantly prolonged in treated female rats (19.00 ± 1.00 seconds) relative to controls (14.75 ± 0.96 seconds), with a P-value of 0.002. Additionally, a significant increase in activated partial thromboplastin time was observed in the treated group (38.33 ± 1.15 seconds) compared to the control group (33.25 ± 2.22 seconds), with a P-value of 0.016.

Table 5 compares platelet count, prothrombin time (PT), and activated partial thromboplastin time (APTT) between non-treated male rats and male rats administered a low dose of ethanolic extract of *Justicia carnea*. Male rats treated with the extract exhibited a significant increase in platelet count (607.50 ± 7.94 $\times 10^9$/L) relative to the control group (579.67 ± 2.52 $\times 10^9$/L), with a P-value of 0.002. Prothrombin time was significantly prolonged in the treated group (18.75 ± 1.71 seconds) compared to the control group (15.67 ± 0.58 seconds), with a P-value of 0.032. Similarly, activated partial thromboplastin time was significantly elevated in treated rats (38.75 ± 1.50 seconds) versus the controls (34.33 ± 1.53 seconds), with a P-value of 0.012.

Table 6 presents the comparative analysis of platelet count, prothrombin time (PT), and activated partial thromboplastin time (APTT) between non-treated female rats and female rats treated with a medium dose of ethanolic extract of *Justicia carnea.* Administration of the medium dose resulted in a highly significant increase in platelet count in the treated group (710.75 ± 6.18 $\times 10^9$/L) compared to the control group (584.50 ± 10.41 $\times 10^9$/L), with a P-value of 0.000. Similarly, prothrombin time was markedly prolonged in the treated female rats (20.75 ± 0.96 seconds) relative to controls (14.75 ± 0.96 seconds), with a P-value of0.000. Activated partial thromboplastin time was also significantly elevated in the treated group (42.00 ± 1.15 seconds) when compared to the control group (33.25 ± 2.22 seconds), with a P-value of 0.000.

Table 7 compares platelet count, prothrombin time (PT), and activated partial thromboplastin time (APTT) between non-treated male rats and male rats treated with a medium dose of ethanolic extract of *Justicia carnea*. A significant increase in platelet count was observed in the treated group (688.25 ± 6.13 $\times 10^9$/L) compared to the control group (579.67 ± 2.52 $\times 10^9$/L), with a P-value of 0.000. Prothrombin time was markedly prolonged in treated male rats (21.75 ± 0.50 seconds) relative to the controls (15.67 ± 0.58 seconds), with statistical significance (P = 0.000). In addition, activated partial thromboplastin time was significantly elevated in the treated group (43.00 ± 1.15 seconds) compared to the control group (34.33 ± 1.53 seconds), also with a P-value of 0.000.

Table 8 presents the comparative analysis of platelet count, prothrombin time (PT), and activated partial thromboplastin time (APTT) between non-treated female rats and female rats treated with a high dose of ethanolic extract of *Justicia carnea.* Administration of the medium dose resulted in a highly significant increase in platelet count in the treated group (750.60± 6.58$\times 10^9$/L) compared to the control group (584.50 ± 10.41 $\times 10^9$/L), with a P-value of 0.000. Similarly, prothrombin time was markedly prolonged in the treated female rats (24.40 ± 1.14seconds) relative to controls (14.75 ± 0.96 seconds), with a P-value of 0.000. Activated partial thromboplastin time was also significantly

elevated in the treated group (47.80 ± 0.84 seconds) when compared to the control group (33.25 ± 2.22 seconds), with a P-value of 0.000.

Table 9 compares platelet count, prothrombin time (PT), and activated partial thromboplastin time (APTT) between non-treated male rats and male rats treated with a high dose of ethanolic extract of Justicia carnae. A significant increase in platelet count was observed in the treated group (729.67 ± 5.13×10^9/L) compared to the control group (579.67 ± 2.52 ×10^9/L), with a P-value of 0.000. Prothrombin time was markedly prolonged in treated male rats (23.67± 0.50 seconds) relative to the controls (15.67 ± 0.58 seconds), with statistical significance (P = 0.000). In addition, activated partial thromboplastin time was significantly elevated in the treated group (47.00± 1.15 seconds) compared to the control group (34.33 ± 1.53 seconds), also with a P-value of 0.000.

Table 1 Comparison of Platelet count, Prothrombin time and Activated Partial Thromboplastin time between non-treated rats and rats treated with a low dose of ethanolic extract of Justicia crane

S/N	Parameters	Control	Test	P-Value
1	Platelet count (×10^9/L)	582.43 ± 7.93	612.29 ± 8.90	0.000
2	PT (secs)	15.14 ± 0.90	18.86 ± 1.35	0.000
3	APTT (secs)	33.71 ± 1.89	38.57 ± 1.27	0.000

Key: Results are expressed as Mean + Standard Deviation.PT = Prothrombin Time, APTT = Activated Partial Thromboplastin Time. P<0.05 is statistically significant.

Table 2 Comparison of Platelet count, Prothrombin time and Activated Partial Thromboplastin time between non-treated rats and rats treated with a medium dose of ethanolic extract of *Justicia carnea*

S/N	Parameters	Control	Test	P-Value
1	Platelet count	582.43 ± 7.93	699.50 ± 13.31	0.000
2	PT	15.14 ± 0.90	21.25 ± 0.89	0.000
3	APTT	33.71 ± 1.89	42.50 ± 1.20	0.000

Key: Results are expressed as Mean + Standard Deviation.PT = Prothrombin Time, APTT = Activated Partial Thromboplastin Time. P<0.05 is statistically significant.

Table 3 Comparison of Platelet count, Prothrombin time and Activated Partial Thromboplastin time between non-treated rats and rats treated with a high dose ethanolic of extract of Justicia carnae

S/N	Parameters	Control	Test	P-Value
1	Platelet count	582.43 ± 7.93	742.75 ± 12.23	0.000
2	PT	15.14 ± 0.90	24.125 ± 1.13	0.000
3	APTT	33.71 ± 1.89	47.50 ± 1.20	0.000

Key: Results are expressed as Mean + Standard Deviation.PT = Prothrombin Time, APTT = Activated Partial Thromboplastin Time. P<0.05 is statistically significant.

Table 4 Comparison of Platelet count, Prothrombin time and Activated Partial Thromboplastin time between femalerats not treated and female rats treated with a low dose of ethanolic extract of Justicia carnae

S/N	Parameters	Control	Test	P-Value
1	Platelet count	584.50 ± 10.41	618.67 ± 6.03	0.004
2	PT	14.75 ± 0.96	19.00 ± 1.00	0.002
3	APTT	33.25 ± 2.22	38.33 ± 1.15	0.016

Key: Results are expressed as Mean + Standard Deviation.PT = Prothrombin Time, APTT = Activated Partial Thromboplastin Time. P<0.05 is statistically significant.

Table 5 Comparison of Platelet count, Prothrombin time and Activated Partial /Thromboplastin time between male rats not treated and male rats treated with a low dose of ethanolic extract of Justicia carnae

S/N	Parameters	Control	Test	P-Value
1	Platelet count	579.67 ± 2.52	607.50 ± 7.94	0.002
2	PT	15.67 ± 0.58	18.75 ± 1.71	0.032
3	APTT	34.33 ± 1.53	38.75 ± 1.50	0.012

Key: Results are expressed as Mean + Standard Deviation.PT = Prothrombin Time, APTT = Activated Partial Thromboplastin Time. P<0.05 is statistically significant.

Table 6 Comparison of Platelet count, Prothrombin time and Activated Partial Thromboplastin time between female rats not treated and female rats treated with a medium dose of ethanolic extract of *Justicia carnea*

S/N	Parameters	Control	Test	P-Value
1	Platelet count	584.50 ± 10.41	710.75 ± 6.18	0.000
2	PT	14.75 ± 0.96	20.75 ± 0.96	0.000
3	APTT	33.25 ± 2.22	42.00 ± 1.15	0.000

Key: Results are expressed as Mean + Standard Deviation.PT = Prothrombin Time, APTT = Activated Partial Thromboplastin Time. P<0.05 is statistically significant.

Table 7 Comparison of Platelet count, Prothrombin time and Activated Partial Thromboplastin time between male rats not treated and male rats treated with a medium dose of ethanolic extract of *Justicia carnea*

S/N	Parameters	Control	Test	P-Value
1	Platelet count	579.67 ± 2.52	688.25 ± 6.13	0.000
2	PT	15.67 ± 0.58	21.75 ± 0.50	0.000
3	APTT	34.33 ± 1.53	43.00 ± 1.15	0.000

Key: Results are expressed as Mean + Standard 'Deviation.PT = Prothrombin Time, APTT = Activated Partial Thromboplastin Time. P<0.05 is statistically significant.

Table 8 Comparison of Platelet count, Prothrombin time and Activated Partial Thromboplastin time between female rats not treated and female rats treated with a high dose of ethanolic extract of *Justicia carnea*

S/N	Parameters	Control	Test	P-Value
1	Platelet count	584.50 ± 10.41	750.60 ± 6.58	0.000
2	PT	14.75 ± 0.96	24.40 ± 1.14	0.000
3	APTT	33.25 ± 2.22	47.80 ± 0.84	0.000

Key: Results are expressed as Mean + Standard Deviation.PT = Prothrombin Time, APTT = Activated Partial Thromboplastin Time. P<0.05 is statistically significant.

Table 9 Comparison of Platelet count, Prothrombin time and Activated Partial Thromboplastin time between male rats not treated and male rats treated with a high dose of ethanolic extract of *Justicia carnea*

S/N	Parameters	Control	Test	P-Value
1	Platelet count	579.67 ± 2.52	729.67 ± 5.13	0.000
2	PT	15.67 ± 0.58	23.67 ± 1.15	0.000
3	APTT	34.33 ± 1.53	47.00 ± 1.73	0.001

Key: Results are expressed as Mean + Standard Deviation.PT = Prothrombin Time, APTT = Activated Partial Thromboplastin Time. P<0.05 is statistically significant.

4. Discussion

This study assessed the hematological and coagulative effects of ethanolic extract of *Justicia carnea* on male and female Wistar rats, with specific focus on platelet count, prothrombin time (PT), and activated partial thromboplastin time (APTT) across different doses. The findings consistently revealed dose-dependent increases in platelet count and significant prolongation of PT and APTT across both sexes. These outcomes provide insightful parallels and contrasts with earlier research and help elucidate the underlying mechanisms of *Justicia carnea's* action. Interestingly, across all treatment groups, platelet count significantly increased relative to the control, with the highest elevation observed in rats administered a high dose (e.g., 742.75 ± 12.23 $\times 10^9$/L in Table 3 vs. 582.43 ± 7.93 $\times 10^9$/L control). This outcome aligns with the findings of Olaiya et al. (2020), who reported enhanced thrombopoiesis in sodium nitrite-induced anaemic rats following *Justicia carnea* extract administration. Their interpretation, attributing the effect to bioactive flavonoids and phenolics promoting megakaryopoiesis, likely holds relevance here. Similarly, Ugwu et al. (2019) observed a comparable rise in platelet counts, supporting the extract's potential to boost hematopoietic parameters. The stronger effect observed in high-dose groups and among females may be due to estrogen-enhanced hematopoietic sensitivity or sex-specific pharmacokinetics. Contrastingly, Okonkwo et al. (2021) noted a dose-dependent platelet increase but cautioned about possible toxicity at higher doses, which this current study did not observe within the test range. Therefore, *Justicia carnea* demonstrates a reproducible thrombopoietic effect, though dose monitoring remains essential.

In this study, prothrombin time was significantly prolonged in all treated groups compared to controls, with values increasing from 15.14 ± 0.90 seconds in the control to as high as 24.13 ± 1.13 seconds in the high-dose group. This finding is in agreement with the work of Okonkwo et al. (2021), who demonstrated that *Justicia carnea* extract has anticoagulant-like effects by prolonging 6PT. The authors hypothesized that certain phytoconstituents in the plant may interfere with vitamin K-dependent clotting factors (such as factor II or X), possibly mimicking warfarin-like activity. Our findings reinforce this assertion and further reveal a dose-dependent response, especially pronounced in females. Notably, this study also adds a sex-differentiated dimension, which prior studies lacked. However, in the study by Olaiya et al. (2020), PT values were not evaluated, limiting comparison. The marked prolongation in PT raises a therapeutic interest in the plant's potential for conditions where reduced coagulability is beneficial, though clinical translation requires cautious consideration due to potential bleeding risks.

Consistent with the PT findings, APTT was significantly prolonged in all treatment groups, with values rising from 33.71 ± 1.89 seconds in the control to 47.50 ± 1.20 seconds in the high-dose group. This suggests that *Justicia carnea* not only affects the extrinsic pathway (reflected in PT) but also significantly influences the intrinsic and common coagulation cascades. These findings mirror those reported by Okonkwo et al. (2021), who documented APTT prolongation with J. carnea extract. Their study suggested that certain polyphenols may interfere with factors VIII, IX, XI, or XII, or even the activity of thrombin. This adds a mechanistic rationale for the observed anticoagulant properties. Ugwu et al. (2019) did not report APTT data, creating a literature gap that this current work helps fill. Interestingly, the effect was again more marked in female rats, which may be explained by sex-dependent metabolic processing or hormonal influences on coagulation factor expression. These findings suggest a dual anticoagulant mechanism of the extract that may mimic low-intensity heparin-like activity. This study revealed that female rats generally showed more pronounced hematologic changes than their male counterparts at equivalent doses. For instance, female rats treated with a high dose exhibited a platelet count of 750.60 ± 6.58 $\times 10^9$/L compared to 729.67 ± 5.13 $\times 10^9$/L in males. Similarly, both PT and APTT were slightly higher in females. This sex difference could be attributed to hormonal modulation of hematopoietic and coagulation pathways. Estrogens are known to influence thrombopoiesis and coagulation protein synthesis, potentially enhancing the effects of bioactive compounds (Yang et al., 2018). This finding underscores the importance of considering sex as a biological variable in preclinical studies and herbal pharmacology research.

The data in this study clearly support a dose-dependent relationship. Higher doses consistently resulted in increased platelet counts and prolonged coagulation times, indicating a balance between thrombopoietic and anticoagulant effects. This dual action could have therapeutic potential in specific hematologic disorders, such as thrombocytopenia with hypercoagulability, but may also raise caution about hemorrhagic risks if administered in high doses without monitoring. These results support the conclusions by Okonkwo et al. (2021), while also expanding the scope by including gender-based analysis.

5. Conclusion

This study demonstrated that ethanolic extract of *Justicia carnea* significantly elevates platelet count and prolongs PT and APTT in a dose-dependent manner in both male and female rats. The hematologic effects were more pronounced

at higher doses and in female rats, indicating both potency and gender influence. These findings corroborated with earlier studies on the plant's thrombopoietic and anticoagulant properties and add new dimensions regarding sex-specific and dose-specific responses. However, while the extract holds therapeutic promise, especially in enhancing platelet production and modifying coagulation, cautious dose titration is advised to prevent adverse bleeding tendencies.

Compliance with ethical standards

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Disclosure of conflict of interest

Authors have declared that no competing interests exist.

Statement of ethical approval

Ethical approval for this study was obtained from the Ethical Committee on Animal Use and Care of Madonna University, Elele. All animal handling and procedures were conducted in accordance with institutional and international guidelines on animal research and welfare.

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