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Jatropha tanjorensis (Barbados Nut) Lowers Hematological Indices in a Dose-Dependent Fashion

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Abstract **Original Research Article**

The *Jatropha tanjorensis* plant is a vegetable commonly consumed in Southern Nigeria for its purported blood tonic properties. This study aimed to evaluate the hematological effects of the ethanolic leaf extract of *J. tanjorensis* on prepubertal male albino Wistar rats over a 28-day period. The experimental animals were divided into four groups: a control group receiving distilled water, a low-dose group receiving 111.803mg/kg body weight of extract, a medium-dose group receiving 223.606mg/kg body weight of extract, and a high-dose group receiving 335.409mg/kg body weight of extract. The results showed that the extract caused a significant decrease ($P<0.05$) in red blood cell counts, white blood cell counts, hemoglobin concentration, packed cell volume, and neutrophils compared to the control group. The values for lymphocytes were not significantly different ($P>0.05$) in the medium and high dose groups compared to the control group, but a slight increase ($P<0.05$) was observed in the low-dose group. Furthermore, the monocyte count was significantly decreased ($P<0.05$) in the low-dose group compared to the control group, while a significant increase ($P<0.05$) was observed in the medium and high dose groups compared to the low-dose group. Platelet values showed a significant decrease ($P<0.05$) in the medium-dose group compared to the low-dose group, and a significant increase ($P<0.05$) was observed in the high-dose group compared to both the low and medium-dose groups. These findings suggest that prolonged consumption of *J. tanjorensis* may be toxic, as evidenced by negative changes in the hematological indices of the experimental animals.

Keywords: *Jatropha tanjorensis*, hematology, hematological indices, white blood cells, red blood cells.

INTRODUCTION

Throughout history, human societies have relied on the natural environment for their sustenance, and one of the most important applications of plants has been their use in medicine (Jamshidi *et al*., 2018). Indigenous cultures have traditionally utilized various plants and plant-based remedies for their medicinal properties (Aziz *et al*., 2018). In recent times, there has been a global surge in the use of medicinal plants, driven by a growing interest in alternative and complementary medicine. Vyshnavi, (2021) expanded the definition of the term "herb" to include not just the leaves of a plant but also other parts such as fruits, seeds, stems, bark, flowers, stigma, roots, and non-woody plants. Herbs can be utilized in fresh or dried forms, and their medicinal properties can be extracted through techniques like maceration, infusion, decoction, and distillation (Abubakar and Haque, 2020). The World Health Organization (WHO) has identified numerous plant-derived pharmaceutical drugs in current use, many of which have a long history of traditional use as herbal remedies (Hoareau and Silva, 1999). This underscores the relevance and efficacy of medicinal plants in modern medicine. Additionally, the WHO estimates that approximately 88% of the global population uses herbal medicine as their primary healthcare source, highlighting the ongoing importance of plants in human health (WHO, 2022). Plant-based products also play significant roles in the pharmaceutical industry of developed nations, finding applications in the production of pharmaceuticals, cosmetics, and food additives, among other uses (Beyene *et al*., 2016).

Jatropha tanjorensis is commonly referred to as "Physic Nut" or "Barbados Nut" (Oladele *et al*., 2020; Ofor and Nwufo, 2011). Indigenous to tropical regions of Africa, *J. tanjorensis* is used for both medicinal and industrial purposes. In Africa, it is known by various names such as "Hospital too far," "Catholic vegetable," "Iyana-ipaja," and "Lapalapa" (Falodun *et al*., 2013). Traditionally, its leaves are used to treat conditions like anemia, diabetes, and cardiovascular

diseases (Chigozie *et al*., 2018). The plant is commonly grown as a hedge around homes, gardens, and fields due to its resistance to browsing by animals and its long lifespan of over 50 years (Byrappa *et al*., 2018). Several studies have indicated that the seed and leaf extracts of *J. tanjorensis* are used in traditional medicine to treat various ailments. These extracts contain compounds with anti-inflammatory, analgesic, antidiabetic, anticancer, and antimicrobial properties (Falodun *et al*., 2013; Amaechi *et al.*, 2022). Furthermore, the plant is employed in the treatment of wounds, allergies, skin infections, venereal diseases, digestive disorders, and as a laxative (Achika *et al*., 2023; Falodun *et al.*, 2022; Sharma and Singh, 2012).

Hematological parameters serve as crucial indicators for assessing the health status of individuals, offering valuable insights into the presence of underlying diseases or conditions (Olafedehan *et al.*, 2010). These parameters encompass various indices, including red blood cell (RBC) count, white blood cell (WBC) count, differential white blood cell count, hemoglobin levels, hematocrit levels, mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration (Etim *et al.*, 2014). Considering the historical use of *J. tanjorensis* in treating diverse ailments and the reported positive effects on hematological indices observed in animal studies, it becomes imperative to conduct further investigations into the potential health benefits of this plant (Ostrander, 2023).

Several studies have explored the impact of *J. tanjorensis* extract on hematological indices. Ndem *et al*. (2019) examined the effects of *J. tanjorensis* leaf extract on hematological indices in Wistar rats, revealing a significant increase in red blood cell count, hemoglobin concentration, and hematocrit following treatment with the extract, suggesting a potential role in treating anemia. Another study by Danborno *et al*., (2019) investigated the influence of *J. tanjorensis* root extract on white blood cells and their components in albino Wistar rats. The results indicated a significant increase in white blood cell count upon treatment with the extract, suggesting a potential immune-boosting effect. However, there are growing concerns regarding the toxicity associated with heavy consumption of herbal plants (Abiare *et al*., 2013; Ugwah-Oguejiofor *et al*., 2019), particularly as Chibuogwu *et al.*, (2021) reported immunosuppression with prolonged use. Moreover, there have been conflicting views on the acute toxicity evaluation of the herb, with some researchers (Idu *et al.*, 2014; Ijioma *et al*., 2014; Chibuogwu *et al*., 2021) finding no signs of toxicity at doses of 5000mg and higher, which would indicate its safety for oral consumption according to Kennedy *et al*., (1986). However, Ndem *et al*., (2019) reported the LD_{50} of the aqueous extract of *J. tanjorensis* leaves as 1161.89 mg/kg.

Thus, the objective of this research is to make a contribution to the expanding body of scientific knowledge concerning the potential health advantages of *J. tanjorensis*. The study intends to examine the prolonged impact of *J. tanjorensis* extract on hematological indices, taking into account the limited investigation conducted on this aspect despite the existence of numerous studies on its short-term effects. Conducting a study to investigate its effects over an extended duration can provide valuable insights into its safety, efficacy, and potential adverse effects, thus establishing it as a natural alternative therapy.

MATERIALS AND METHOD

Plant

The Department of Pharmacognosy and Natural Medicine at the University of Uyo, Uyo, identified fresh leaves of *J. tanjorensis* that were obtained from a garden by Itam market in Uyo, Itam Local Government Area of Akwa Ibom State, Nigeria.

Preparation of Plant Material

The leaves of *J. Tanjorensis* were collected and dried under shade in the laboratory for 14 days. After that, the leaves were cleaned to remove debris and weighed. 500g of leaves were then ground using a grinding machine to increase the surface area. Next, 1.5L of ethanol was measured, placed in a container, and warmed up using a warm water bath. The ground leaves were introduced into the container containing the ethanol in the warm water bath and stirred. The warm water bath was heated up to 50-60 degrees Celsius and kept at this temperature for about 3 hours, stirring occasionally. After 3 hours, the mixture was filtered through a filter paper. Using a rotary evaporator (Resona, Germany), the ethanol was removed from the mixture, leaving behind the extracted compound. The extracted compound was transferred to sterile bottles and refrigerated at $6^{O}C$ until use.

Animals

A group of forty prepubertal albino Wistar male rats, weighing between 59-99g, were obtained from the Department of Pharmacology and Toxicology at the University of Uyo, Uyo and kept in the Pharmacology Laboratory throughout the duration of the experiment. The rats were placed in wooden cages with 10 rats in each of the four cages. Before starting the experiment, the animals were kept at room temperature and fasted for 12 hours. The animals were exposed to feed ad libitum and the experiment carried out in accordance with the NIH guidelines for the care and use of laboratory animals, as described by Albus, (2012). The study was performed at the Pharmacology Laboratory of the Department of Pharmacology and

Toxicology located at the University of Uyo, Uyo, Akwa Ibom State, Nigeria.

Acute Toxicity Study (Ld50)

Twenty-four (24) male mice weighing 25-36g were divided into 8 groups of 3 mice per group. Using Lorke's method (1983), they were assigned graded doses of *J. tanjorensis* intraperitoneally in the following order: 750, 1000, 1250, 1500, 2000, 2250, 3000, and 5000 mg/kg body weight. The mice were kept in aluminum cages and allowed free access to feed and water. Observations were made for toxicity signs and the number of deaths at the end of 24 hours. The LD_{50} was determined as the geometric mean of the maximum dose producing 0% mortality and the minimum dose producing 100% mortality. The LD_{50} calculation was done in two phases. In the first phase, the mice were fasted for at least 18 hours and given a high dose of *J. tanjorensis*, 3000 mg/kg and 5000 mg/kg, and observed for toxicity signs and mortality for 24 hours. Based on the results of phase 1, phase 2 was carried out where the mice were again fasted for at least 18 hours and given lower doses of 750, 1000, 1250, 1500, 2000, and 2500 mg/kg and observed for toxicity signs and mortality for 24 hours. After 24 hours, the doses of 750 mg/kg and 1000 mg/kg showed 0% mortality while the doses of 1250, 1500, 2000, and 2500 mg/kg resulted in 100% mortality.

Using the Lorke's method, the LD_{50} was calculated as follows:

$LD_{50} = \sqrt{AB}$

Where A is the maximum dose that produces 0% mortality and B is the minimum dose that produces 100% mortality.

$LD_{50} = \sqrt{(1000 \times 1250)}$
$= 1118.03$ mg/kg

The working doses were 10%, 20%, and 30% of 1118.03 mg/kg.

Experimental Design

Forty (40) prepubertal male albino Wistar rats were used in this study. They were randomly distributed into four (4) groups of 10 rats each. The rats were fed graded doses of ethanol extract of *J. tanjorensis* through orogastric gavage method for 28 days. The groups and doses administered are summarized below:

Group 1: Control group without *J. tanjorensis* extract administration. Rats were given feed ad libitum and distilled water.

Group *2:* Low dose test group. Rats were treated with 111.8 mg/kg body weight of ethanol extract of *Ja. tanjorensis*. Serial dilution was performed to reduce the concentration of the stock solution due to the low body weight of test group animals.

Group 3: Medium dose test group. Rats were treated with 223.6 mg/kg body weight of ethanol extract of *J. tanjorensis*.

Group 4: High dose test group. Rats were treated with 335.4 mg/kg body weight of ethanol extract of *J. tanjorensis*.

Doses for the experimental groups were arrived as thus

Group 2 (low dose) 10% of LD_{50}: (10/100) * (1118.03) mg/kg = 111.8mg/kg

Group 3 (Medium dose) 20% of LD_{50}: (20/100) * (1118.03) mg/kg = 223.6mg/kg

Group 4 (High dose) 30% of LD_{50}: (30/100) * (1118.03) mg/kg = 335.4mg/kg

Collection of Blood Samples

On the 28th day, administration of *J. tanjorensis* extract was stopped, and the rats were fasted for 24 hours. Afterward, five animals were selected from each group and euthanized intraperitoneally with 1.5ml ketamine according to procedures acceptable to the Faculty of Basic Medical Sciences Animal Ethical Committee, University of Uyo, Nigeria. Then, the animals were opened up peritoneally using surgical scissors to expose the heart. Blood was collected via cardiac puncture using a 5ml syringe, and 2ml of the blood was placed into an EDTA bottle to prevent blood coagulation. The collected blood was then taken to the laboratory and analyzed using the Zybio hematology analyser.

Statistical Analysis

Data are expressed as mean ± Standard Error of Mean (SEM). Differences between mean values were evaluated by analysis of variance (ANOVA), followed by Tukey's post-hoc test for pairwise comparisons. Values of $P<0.05$ were considered statistically significant. Graph Pad Prism 7.0 software (Graph Pad Inc., USA) was used for the statistical analysis.

RESULTS

Effect of *J. tanjorensis* (Hospital too far) leaf Extract on White blood cell count

White blood cell count was significantly decreased ($p<0.05$) in the rats given low, medium and high doses of *J. tanjorensis* leaf extract when compared with the control. White blood cell count was significantly decreased ($p<0.05$) in the rats given medium and high doses of *J. tanjorensis* leaf extract when compared with the low dose *J. tanjorensis* leaf extract treated groups respectively (Figure 1).

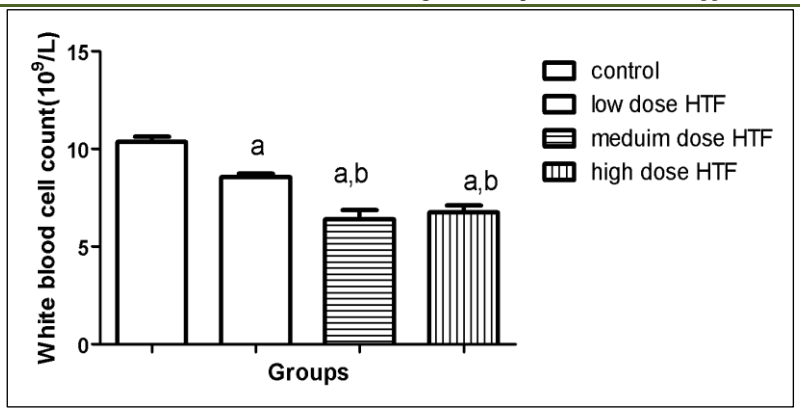

Figure 1: Effect of *J. tanjorensis* leaf extract on white blood cell concentration of male Wistar Rats. Columns represent mean ± SEM. n= 5; {HTF: Hospital too far*: J. tanjorensis leaf extract*}; a = p<0.05 when compared with control; b = p<0.05 when compared with low dose treated group.

Effect of *J. tanjorensis* (Hospital too far) leaf Extract on hemoglobin concentration

Hemoglobin concentration was significantly decreased (p<0.05) in the rats given low, medium and high doses of *J. tanjorensis* leaf extract when compared with the control. Hemoglobin concentration was significantly decreased (p<0.05) in the rats given a high dose of *J. tanjorensis* leaf extract when compared with the low dose *J. tanjorensis* leaf extract treated group (Figure 2).

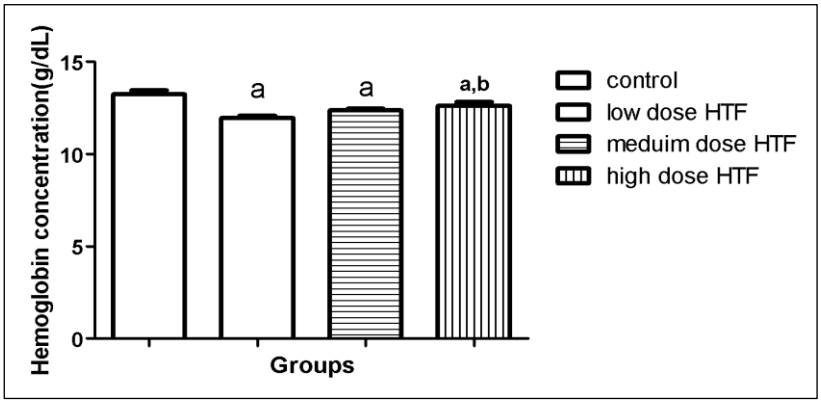

Figure 2: Effect of *J. tanjorensis* leaf extract on hemoglobin concentration of male Wistar Rats. Columns represent mean ± SEM. qn= 5; {HTF: Hospital too far*: J. tanjorensis leaf extract*}

Effect of *J. tanjorensis* (Hospital too far) leaf Extract on packed cell volume

Packed cell volume was significantly decreased (p<0.05) in the rats given low, medium and high doses of *J. tanjorensis* leaf extract when compared with the control (Figure 3).

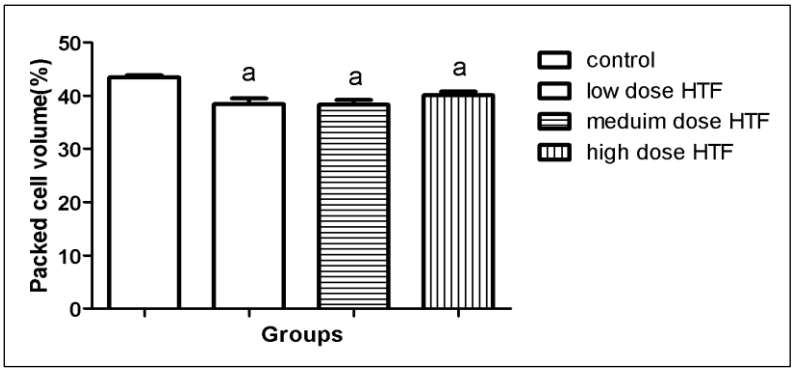

Figure 3: Effect of *J. tanjorensis* leaf extract on packed cell volume of male Wistar Rats. Columns represent mean ± SEM. n= 5; {HTF: Hospital too far*: J. tanjorensis leaf extract*}

Effect of *J. tanjorensis* (Hospital too far) leaf Extract on red blood cell count

Red blood cell count was significantly decreased ($p<0.05$) in the rats given low, medium and high doses of *J. tanjorensis* leaf extract when compared with the control (Figure 4).

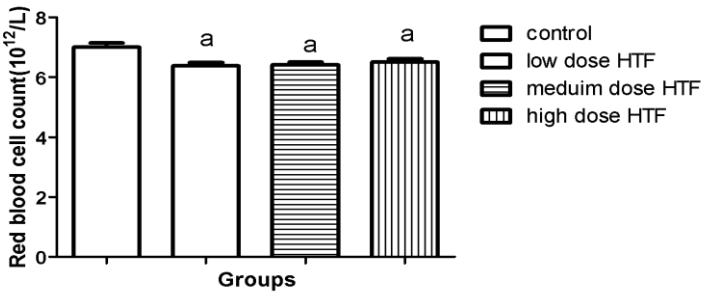

Figure 4: Effect of *J. tanjorensis* leaf extract on red blood cell count of male Wistar Rats. Columns represent mean ± SEM. n= 5; {HTF: Hospital too far*: J. tanjorensis leaf extract*}

Effect of *J. tanjorensis* (Hospital too far) leaf Extract on platelet count

Platelets count was significantly decreased ($p<0.05$) in the rats given medium dose of *J. tanjorensis* leaf extract when compared with the control and low dose *J. tanjorensis* leaf treated groups respectively Platelets count was significantly increased ($p<0.05$) in the rats given high dose of *J. tanjorensis* leaf extract when compared with the medium dose *J. tanjorensis* leaf extract treated groups respectively (Figure 5).

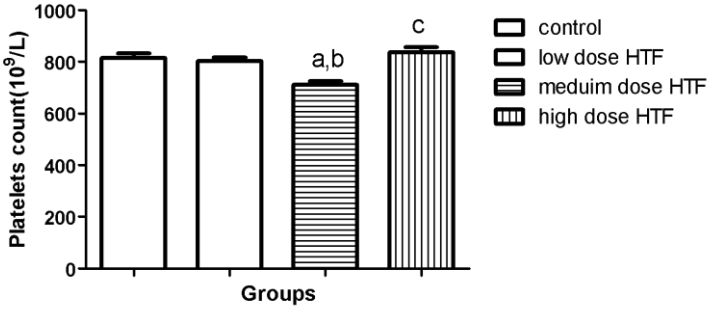

Figure 5: Effect of *J. tanjorensis* leaf extract on platelets count of male Wistar Rats. Columns represent mean ± SEM. n= 5; {HTF: Hospital too far*: J. tanjorensis leaf extract*}; c = p<0.05 when compared with medium dose treated group

Effect of *J. tanjorensis* (Hospital too far) leaf Extract on neutrophil count

Neutrophil count was significantly decreased $p<0.05$) in the rats given low, medium and high doses of *J. tanjorensis* leaf extract when compared with the control (Figure 6).

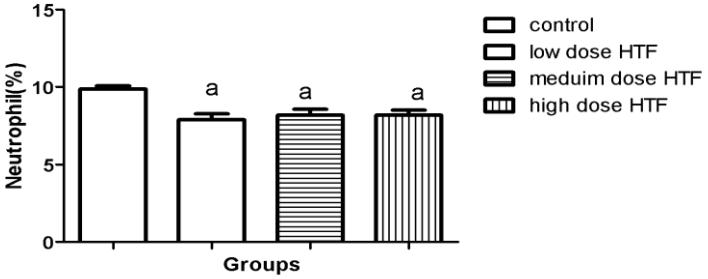

Figure 6: Effect of *J. tanjorensis* leaf extract on neutrophil count of male Wistar Rats. Columns represent mean ± SEM. n= 5; {HTF: Hospital too far*: J. tanjorensis leaf extract*; a = p<0.05 when compared with control

Effect of *J. tanjorensis* (Hospital too far) leaf Extract on lymphocyte count

There was no statistical difference in the lymphocyte count in the low, medium and high dose of *J. tanjorensis* leaf extract treated rats when compared with the control group (Figure 7).

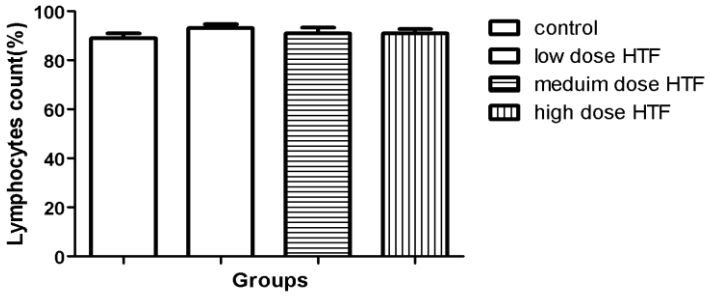

Figure 7: Effect of *J. tanjorensis* leaf extract on lymphocyte count of male Wistar Rats. Columns represent mean ± SEM. n= 5; {HTF: Hospital too far*: J. tanjorensis leaf extract*}

Effect of *J. tanjorensis* (Hospital too far) leaf Extract on monocytes count

Monocytes count was significantly decreased ($p<0.05$) in the rats given low dose of *J. tanjorensis* leaf extract when compared with the control. Monocytes count was significantly increased($p<0.05$) in the rats given medium and high doses of *J. tanjorensis* leaf extract when compared with the low dose *J. tanjorensis* leaf extract treated groups respectively (Figure 8).

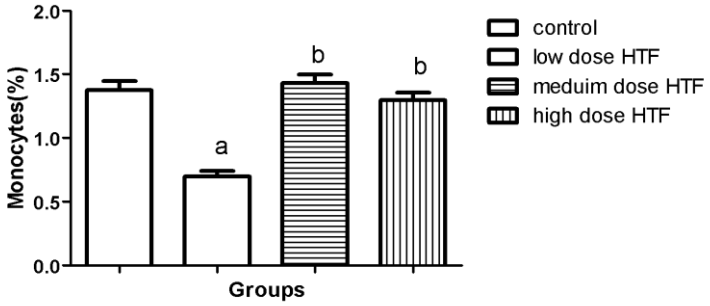

Figure 8: Effect of *J. tanjorensis* leaf extract on monocyte count of male Wistar Rats. Columns represent mean ± SEM. n= 5; {HTF: Hospital too far*: J. tanjorensis leaf extract*}; a = p<0.05 when compared with control; b = p<0.05 when compared with low dose treated group.

DISCUSSION

In this study, the effect of *J. tanjorensis* leaf extract on hematological indices was investigated. Omoregie and Osagie, (2007) had previously identified bioactive compounds in the plant responsible for its medicinal properties. The study found that the white blood cell (WBC) count and neutrophil count decreased in all groups receiving low, medium, and high doses of the extract compared to the control group. Notably, the WBC count was significantly lower in the medium and high dose groups than in the low dose group, suggesting a dose-dependent response to the extract. These findings support the claims of (Asuk, 2017; Chibuogwuo *et al*., 2021) regarding *J. tanjorensis* potential for immunosuppression, as well as the observations of (Ijioma *et al*., 2014; Ndem *et al*., 2019) regarding its ability to reduce WBC count. Asuk *et al*., (2013) proposed that the observed immunosuppression could be attributed to a non-agitated system due to the absence of foreign bodies. It should be noted that (Asuk, 2017) and Chibuogwuo *et al*., (2021) utilized similar methodologies, which could explain the similarity in their results. However, studies by (Omoregie and Osagie, 2007; Danborno *et al*., 2021) contradict these findings as they reported an increase in WBC count.

Regarding the red blood cell count, packed cell volume, and hemoglobin concentration, all three parameters were significantly decreased in the low, medium, and high dose groups compared to the control group. These results do not align with the claims of (Ndem *et al*., 2019; Danborno *et al*., 2019; Chibuogwuo *et al*., 2021) regarding the anti-anemic properties of *J.*

tanjorensis, which have reported a hematopoietic activity when administering the extract. This study hypothesized that prolonged exposure to the J. tanjorensis extract may lead to hemolysis, based on observations from Danborno *et al*., (2019) study, where a decrease in the red blood cell count was observed at a high dose of 750 mg/kg of the plant extract. Therefore, this study further hypothesized that prolonged exposure to the plant extract gradually accumulates its bioactive compounds in the body, increasing the concentration of the drug's constituents to levels that produce effects similar to those seen with a high dose.

The results of the monocyte count showed a significant decrease in the low dose group, while a significant increase was seen in the medium and high dose groups. Monocytes are important for regulating cellular homeostasis, especially during inflammation and infection (Yáñez *et al*., 2017). The increase in the medium and high dose groups may be linked to the suppressed immune system, as a suppressed immune system can exhibit an exaggerated inflammatory response as compensation, leading to an increased release of monocytes from the bone marrow. Regarding the platelet count, a significant decrease was observed in the group given the medium dose of the extract compared to the low dose and control groups.

However, the high dose of the extract significantly increased platelet count. Platelets play a crucial role in cellular hemostasis and thrombosis (Mc Ewen, 2015). The fluctuation in platelet count may be due to alterations in the immune system at different dose administrations, supporting the claims of Mc Ewen, (2015) that some herbal medicines can alter platelet function and coagulation. The increase observed in the high dose group aligns with the findings of (Ndem *et al*., 2019; Danborno *et al*., 2019). Lastly, the results obtained for lymphocyte count did not show a significant difference among the low, medium, and high dose groups. Thus, the plant extract did not cause any significant changes, as also reported by (Igbinaduwa *et al*., 2011).

CONCLUSION

J. tanjorensis harbors bioactive compounds with potential medicinal applications. However, the study reveals that prolonged consumption or exposure to the plant material may result in toxicity, as indicated by adverse alterations in the hematological indices of the experimental animals. These findings align with prior research by Chibuogwu *et al.*, 2021; Ndem *et al*., 2019; Idu *et al.*, 2014), which also identified immune suppression as a potential toxic effect associated with the plant. Given the crucial role of white blood cells in defending the body against infections and diseases, a decrease in their count can compromise immune response and heighten susceptibility to infections. Therefore, while the plant exhibits potential for enhancing blood parameters, caution should be taken against its prolonged usage. Standardization of the plant material is a critical step in ensuring its safety and effectiveness for therapeutic purposes. This study advocates for further investigations to elucidate the plant's underlying mechanism of action.

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