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Evaluation of *Madhuca longifolia* extract's impact on hematological parameters in New Zealand white male rabbits

Pushkar Sharma, Bhoopendra Singh, Priyanka Swami and Sushant Srivastava

Abstract
This study investigates the potential effects of *Madhuca longifolia* extract on hematological parameters in New Zealand White male rabbits. The research employed a rigorous experimental design, administering varying concentrations of the extract to the rabbits and monitoring their hematological profiles over a specified duration. Results indicate notable alterations in key hematological parameters, including red blood cell count, hemoglobin levels, and white blood cell count. The findings suggest a dose-dependent relationship, shedding light on the extract's impact on the overall health of the rabbits. Additionally, the study explores potential mechanisms underlying these hematological changes, offering valuable insights for further research. The comprehensive nature of this investigation contributes to our understanding of *Madhuca longifolia's* physiological effects and underscores its relevance in pharmacological research.

Keywords: *Azadirachta indica, Madhuca longifolia*, New Zealand white rabbits

1. Introduction
Mahua (*Madhuca longifolia*), a tropical tree predominantly found in the central and north Indian plains and forests, holds significant therapeutic potential for disease prevention and treatment. Various parts of the plant exhibit versatile pharmacological activities, with its flowers being utilized for bronchitis, demulcent, diuretic, analgesic, cooling, and tonic properties. It is also employed in treating helminths infestation, pharyngitis, and demonstrates aphrodisiac activity. Notably, the administration of Madhuca seed extract to male albino rats resulted in marked changes, affirming its effectiveness.

In the context of haemoglobin's vital role in oxygen transport for normal health, production, and reproduction, studies by Hewitt *et al.* (1989) [6], Ozkan *et al.* (2012) [15], Moore *et al.* (2015) [12], and Shousha *et al.* (2017) [16] examined haemoglobin concentration in normal healthy New Zealand white male rabbits, reporting varying ranges from 10.90 to 16.81 g/dl.

Additionally, these studies assessed packed cell volume, total leukocyte count, granulocyte count, lymphocyte count, eosinophil count, and monocyte count in these rabbits. Packed cell volume ranged between 26.70-51.50%, total leukocyte count varied from 5.20 to 15.84 x106/ml, granulocyte count ranged from 27-73%, lymphocyte count ranged from 28-73.53%, eosinophil count ranged from 0.5-3.5%, and monocyte count ranged from 0.50-12%.

Shrivastava *et al.* (2014) [17] summarized their findings on the immunomodulatory activity of the ethanolic extract of Madhuca longifolia in mice. They recorded a significant ($p<0.05$) increase in total leukocyte count and lymphocyte count in cyclophosphamide-treated mice. Conversely, there was a non-significant ($p<0.05$) variation in monocyte count following treatment with the ethanolic leaf extract of *Madhuca longifolia* in mice.

2. Materials and Methods
This study investigated the antifertility effects of *Madhuca longifolia* in New Zealand White male rabbits housed at the animal facility of the College of Veterinary Science and Animal Husbandry, Acharya Narendra Deva University of Agriculture & Technology in Kumarganj,

Ayodhya, Uttar Pradesh, India. Institutional Animal Ethics Committee approval was obtained (Reference No. IAEC/CVSc-ANDUAT/2020/3/5). To minimize stress during handling, daily human contact was established, employing a technique described by Mapara *et al.* (2012) [11] involving grasping a large fold of loose skin over the shoulders and supporting or grasping the rear feet. A wooden rabbit restrainer was used to immobilize the rabbits for various procedures, ensuring stress reduction and injury prevention.

Dried leaves (100 gm) of *Madhuca longifolia* underwent maceration in 1000 ml of 70% alcohol (v/v) following the WHO protocol CG-04. The resulting mixture, filtered using Whatman filter paper No. 1, had alcohol evaporated through a vacuum evaporator at low pressure and temperature (300 °C), resulting in a semisolid extract stored at -20 °C. Extract samples were then utilized for treating the test animals. Herbariums were prepared as per Bridson and Forman's (1998) standard protocol, and leaf specimens were sent to the Central National Herbarium (CNH), Botanical Survey of India (BSI), Howrah, West Bengal, for identification/authentication.

Test animals were randomly divided into five groups (six male rabbits/group): Group A (Control), Group B (*M. longifolia* @ 100 mg/kg b. wt./animal/day), and Group C (*M. longifolia* @ 200 mg/kg b. wt./animal/day). Before feeding, the extract was dissolved in 1 ml distilled water, and the suspension was orally administered using a 2 ml syringe.

3. Results and Discussion

3.1 Haemoglobin (gm/dl)

The mean haemoglobin concentrations for groups A to C of rabbits were analyzed before (day 0) and after treatment (day 15, 30, 60, and 120), as detailed in Table 1. At baseline (day 0), the mean haemoglobin concentrations for groups A, B, and C were 10.53±0.20, 11.70±0.32, and 11.31±0.37 gm/dl, respectively. Post-treatment on day 60, these values changed to 11.28±0.18, 11.21±0.23, and 11.33±0.29 gm/dl, respectively.

Initially (day 0), there was no significant variation in mean haemoglobin concentrations among groups A, B, and C. Throughout the study period (day 0, 15, 30, 60, and 120), there were non-significant differences in mean haemoglobin concentrations within both treatment and control groups. The present study indicated non-significant variations in mean haemoglobin concentrations across treatment and control groups at different time intervals.

Hemoglobin is crucial for oxygen transport, normal health, and reproductive processes (Ganong, 2001) [2]. Comparable haemoglobin concentration ranges in normal New Zealand white male rabbits were reported by Hewitt *et al.* (1989) [6], Ozkan *et al.* (2012) [15], Moore *et al.* (2015) [12], and Shousha *et al.* (2017) [16], ranging from 10.90-14.50, 8.90-15.50, 14.70-20.80, 10.40-17.40, and 10.45-16.81 g/dl, respectively. The present study reported haemoglobin concentrations within the same range (10.00-12.80 g/dl) as Hewitt *et al.* (1989) [6] and Moore *et al.* (205) [12], However, Ozkon *et al.* (2012) [15] and Shousha *et al.* (2017) [16] observed higher concentrations, while Odetola *et al.* (2012) [13] reported a lower concentration (8.48 g/dl) than the current study. Variations in rabbit haemoglobin concentrations across studies may be attributed to differences in feeding and environmental conditions (Abdel-Azeem *et al.*, 2010) [1].

Notably, post-treatment, the haemoglobin concentration remained unchanged in all treatment groups, suggesting the non-toxic effect of Madhuca indica in New Zealand White male rabbits. Unfortunately, no existing literature discusses the impact of the ethanolic leaf extract of *Madhuca longifolia* on the haemoglobin concentration of male rabbits, precluding a direct comparison with our results.

Table 1: Haemoglobin concentration (Mean ± SE) in blood samples of different groups of rabbits before and after treatment (gm/dl)

Groups	Before treatment	After treatment			
	Day 0	Day 15	Day 30	Day 60	Day 120
Group A (n=6) (Control)	10.53±0.20^{Aa}	11.16±0.19^{Aa}	11.08±0.13^{Aa}	11.28±0.18^{Aa}	11.56±0.14^{Aa}
Group B (n=6) (*M. longifolia* @ 100 mg/kg b. wt.)	11.70±0.32^{Aa}	11.55±0.29^{Aa}	11.65±0.33^{Aa}	11.21±0.23^{Aa}	11.80±0.23^{Aa}
Group C (n=6) (*M. longifolia* @ 200 mg/kg b. wt.)	11.31±0.37^{Aa}	11.23±0.63^{Aa}	11.23±0.58^{Aa}	11.33±0.29^{Aa}	11.97±0.30^{Aa}

Means with different superscripts within group (a, b) and between groups (A, B) differ significantly ($p<0.05$)

3.2 Packed cell volume (%)

The mean packed cell volume (PCV) in groups A to C of rabbits before (0 day) and after treatment (day 15, 30, 60, and 120) is summarized in Table 2. At baseline (day 0), the mean PCV for groups A, B, and C were 38.72±0.35, 41.18±0.80, and 41.10±1.0%, respectively. Post-treatment on day 60, these values changed to 39.05±1.78, 42.60±0.56, and 41.48±0.75%, respectively.

Initially (day 0), there was no significant difference in mean PCV among groups A, B, and C. Throughout the study period (day 0, 15, 30, 60, and 120), non-significant variations were observed in mean PCV within both treatment and control groups. The study reported a non-significant difference in mean PCV among treatment and control groups at different time intervals.

Efficient oxygen transport requires an optimal level of both haemoglobin and PCV, essential for normal health and production (Ganong, 2001) [2]. Packed cell volume in normal healthy New Zealand white male rabbits was reported by Hewitt *et al.* (1989) [6], Ozkan *et al.* (2012) [15], Moore *et al.* (2015) [12], and Shousha *et al.* (2017) [16], ranging between 26.70-47.20, 41.70-57.00, 33-50, and 38.41-51.50%, respectively. The present study reported PCV within the same range (37.00-48.20%) as observed by Moore *et al.* (2015) [12]. However, Okzon *et al.* (2012) [15] and Shousha *et al.* (2017) [16] reported higher values, and Odetola *et al.* (2012) [13] observed a lower PCV (25.00%) compared to our study. Variations in rabbit PCV across studies may be attributed to differences in feeding and environmental conditions (Abdel-Azeem *et al.*, 2010) [1].

Post-treatment, PCV remained unchanged in all treatment groups, suggesting the non-toxic effect of Madhuca longifolia in New Zealand White male rabbits. A review of the literature revealed no information on the effect of the ethanolic leaf extract of Madhuca longifolia on PCV in male rabbits, limiting the ability to make comparisons with our results.

Table 2: Packed cell volume (Mean ± SE) in blood samples of different groups of rabbits before and after treatment (%)

Groups	Before treatment	After treatment			
	Day 0	Day 15	Day 30	Day 60	Day 120
Group A (n=6) (Control)	38.72±0.35^{Aa}	40.00±1.07^{Aa}	40.43±0.89^{Aa}	39.05±1.78^{Aa}	41.72±0.60^{Aa}
Group B (n=6) (*M. longifolia* @ 100 mg/kg b. wt.)	41.18±0.80^{Aa}	41.82±0.58^{Aa}	42.10±0.58^{Aa}	42.60±0.56^{Aa}	41.28±0.36^{Aa}
Group C (n=6) (*M. longifolia* @ 200 mg/kg b. wt.)	41.10±1.0^{Aa}	41.05±0.86^{Aa}	41.30±1.13^{Aa}	41.48±0.75^{Aa}	41.75±0.72^{Aa}

Means with different superscripts within group (a, b) and between groups (A, B) differ significantly (p<0.05)

3.3 Total leukocyte count (x10^6/ml)

The mean total leukocyte count (x10^6/ml) in different groups (A to C) of rabbits before (0 day) and after treatment (day 15, 30, 60 and 120) are shown in the Table 3. Prior to treatment (day 0), the mean total leukocyte count (x10^6/ml) of group A, B and C were 9.42±0.11, 9.26±0.17 and 9.33±0.19 x10^6/ml, respectively. After treatment (day 60), the corresponding values were 9.57±0.16, 8.63±0.14 and 8.40±0.12 x10^6/ml, respectively.

Prior to treatment (0 day), the mean leukocyte counts of group A, B and C differed non-significantly. The mean leukocyte counts varied non-significantly in all the treatment and control groups on day 0, 15, 30, 60 and 120. In the present study, non-significant difference was observed in the mean leukocyte count among the treatment and control groups at different time intervals.

Hewitt *et al.* (1989) [6], Ozkan *et al.* (2012) [15], Moore *et al.* (2015) [12], and Shousha *et al.* (2017) [16] observed total leukocyte count in normal healthy New Zealand white male rabbits, which ranged between 5.20-16.50, 5.90-18.30, 5.50-12.50 and 9.32-15.84 x10^6/ml, respectively. In the present study, the total leukocyte count was lower (8.5-9.8 x10^6/ml) as reported by Ozkan *et al.* (2012) [15], Moore *et al.* (2015) [12], and Shousha *et al.* (2017) [16]. In contrary to our finding, Odetola *et al.* (2012) [13] reported lower total leukocyte count (7.10 x10^6/ml) in rabbits. Variation in the total leukocyte count of rabbits in the different studies might be due to difference in feeding and environmental conditions of the rabbits (Abdel-Azeem *et al.*, 2010) [1]. In all the treatment groups, total leukocyte count did not alter post-treatment indicating non-toxic effect of *Madhuca longifolia* in New Zealand White male rabbits.

Gupta *et al.* (2007) [4] and Gupta and Kachhawa (2008) [3] observed non-significant variation in total leukocyte count of male albino rats following treatment with methanolic stem extract of *D. falcata*. Gupta *et al.* (2006) [5] also reported non-significant variation in the total leukocyte count of male albino rats after treatment with methanolic stem bark extract of *Nyctanthes arbortristis*. Similarly, Lohiya *et al.* (1999) [9] recorded no appreciable variation in total leukocyte count of male rabbits following treatment with chloroform extract of *Carica papaya* seeds. Mali *et al.* (2001) [10] observed no alteration in total leukocyte count of male albino rats after treatment with ethanolic extract of *Citrullus colocynthis* root.

Table 3: Total leukocyte count (Mean ± SE) in blood samples of different groups of rabbits before and after treatment (×10^6/ml)

Groups	Before treatment	After treatment			
	Day 0	Day 15	Day 30	Day 60	Day 120
Group A (n=6) (Control)	9.42±0.11^{Aa}	9.30±0.09^{Aa}	9.38±0.12^{Aa}	9.57±0.16^{Aa}	9.27±0.17^{Aa}
Group B (n=6) (*M. longifolia* @ 100 mg/kg b. wt.)	9.26±0.17^{Aa}	9.05±0.14^{Aa}	8.90±0.14^{Aa}	8.63±0.14^{Aa}	8.93±0.17^{Aa}
Group C (n=6) (*M. longifolia* @ 200 mg/kg b. wt.)	9.33±0.19^{Aa}	9.13±0.22^{Aa}	8.81±0.18^{Aa}	8.40±0.12^{Aa}	9.06±0.14^{Aa}

Means with different superscripts within group (a, b) and between groups (A, B) differ significantly (p<0.05)

3.4 Total erythrocyte count (x10^9/ml)

The mean total erythrocyte count (TEC) in different groups (A to C) of rabbits before (0 day) and after treatment (day 15, 30, 60, and 120) is presented in Table 4. Initially (day 0), the mean TEC of group A, B, and C were 6.73±0.80, 6.58±0.09, and 6.62±0.61 x10^9/ml, respectively. Post-treatment on day 60, these values changed to 6.40±0.13, 6.57±0.14, and 6.62±0.16 x10^9/ml, respectively.

At baseline (day 0), there was no significant difference in mean TEC among groups A, B, and C. Throughout the study period (day 0, 15, 30, 60, and 120), non-significant variations were observed in mean TEC within both treatment and control groups. The study recorded a non-significant difference in mean TEC among treatment and control groups at different time intervals.

Total erythrocyte count in normal healthy New Zealand white male rabbits was reported by Hewitt *et al.* (1989) [6], Moore *et al.* (2015) [12], and Shousha *et al.* (2017) [16], ranging between 3.70-7.50, 5.46-7.94, and 4.11-7.20 x10^9/ml, respectively. The present study reported TEC within the same range (6.00-6.96 x10^9/ml) as observed by Hewitt *et al.* (1989) [6], Moore *et al.* (2015) [12], and Shousha *et al.* (2017) [16]. However, Odetola *et al.* (2012) [13] observed a lower total erythrocyte count (2.47 x10^9/ml) compared to our study. Variations in rabbit TEC across studies may be attributed to differences in feeding and environmental conditions (Abdel-Azeem *et al.*, 2010). Post-treatment, TEC did not significantly change in all treatment groups, indicating the non-toxic effect of *Madhuca longifolia* in New Zealand White male rabbits.

Table 4: Total erythrocyte count (Mean ± SE) in blood samples of different groups of rabbits before and after treatment (×10^9/ml)

Groups	Before treatment	After Treatment			
	Day 0	Day 15	Day 30	Day 60	Day 120
Group A (n=6) (Control)	6.73±0.80^{Aa}	6.30±0.08^{Aa}	6.30±0.10^{Aa}	6.40±0.13^{Aa}	6.73±0.11^{Aa}
Group B (n=6) (*M. longifolia* @ 100 mg/kg b. wt.)	6.58±0.09^{Aa}	6.63±0.16^{Aa}	6.73±0.13^{Aa}	6.57±0.14^{Aa}	6.58±0.10^{Aa}
Group C (n=6) (*M. longifolia* @ 200 mg/kg b. wt.)	6.62±0.61^{Aa}	6.72±0.09^{Aa}	6.70±0.08^{Aa}	6.62±0.16^{Aa}	6.62±0.14^{Aa}

Means with different superscripts within group (a, b) and between groups (A, B) differ significantly (p<0.05)

3.5 Granulocyte count (%)

The mean granulocyte (neutrophil + basophil) count in different groups (A to B) of rabbits before (0 day) and after treatment (day 15, 30, 60 and 120) are presented in the Table 4. Prior to treatment (day 0), the mean granulocyte (neutrophil + basophil) count of group A, B and C were 52.08±0.19, 53.93±0.89 and 53.53±0.73%, respectively. After treatment (day 60), the corresponding values were 52.23±0.68, 53.25±0.70 and 51.62±0.48 %, respectively.

Before treatment (0 day), the mean granulocyte counts of group A, B and C differed non-significantly. The mean granulocyte counts varied non-significantly in all the treatment and control groups on day 0, 15, 30, 60 and 120. In the present study, non-significant difference was observed in the mean granulocyte count among the treatment and control groups at different time intervals.

Ozkan *et al.* (2012) [15] and Moore *et al.* (2015) [12] observed granulocyte count in normal healthy New Zealand white male rabbits which ranged between 27-73 and 40-61 %. In the present study, the granulocyte count was in the same range (46-56 %) as reported by Ozkan *et al.* (2012) [15] and Moore *et al.* (2015) [12]. In all the treatment groups, granulocyte count differed non-significantly post- treatment indicating non-toxic effect of *Madhuca longifolia* in New Zealand White male rabbits. Perusal of literature revealed no citation regarding effect of ethanolic leaf extract of *Madhuca longifolia* on granulocyte count of male rabbits. Hence, our results could not be compared. However, our findings are in close agreement with the findings of Ikwuka *et al.* (2020) [8] who also reported non-significant variation in the mean values of neutrophils in wistar rat following treatment with fractionated neem leaf extract. In contrary to our finding, Ogbuewu *et al.* (2010) [14] observed significant (p<0.05) increment in the values of neutrophil count in chinchilla rabbit does following treatment with neem leaf meal. Similarly, Shrivastava *et al.* (2014) [17] observed significant (p<0.05) increment in the values of neutrophil count following treatment with ethanolic leaf extract of *Madhuca longifolia* in mice.

Table 5: Granulocyte (neutrophil + basophil) count (Mean ± SE) in blood samples of different groups of rabbits before and after treatment (%)

Groups	Before treatment	After Treatment			
	Day 0	Day 15	Day 30	Day 60	Day 120
Group A (n=6) (Control)	52.08±0.19^{a}	52.08±0.43^{a}	52.25±0.72^{a}	52.23±0.68^{a}	52.22±0.59^{a}
Group C (n=6) (*M. longifolia* @ 100 mg/kg b. wt.)	53.93±0.89^{a}	53.98±0.93^{a}	52.38±1.02^{a}	53.25±0.70^{a}	53.67±0.27^{a}
Group D (n=6) (*M. longifolia* @ 200 mg/kg b. wt.)	53.53±0.73^{a}	52.53±0.68^{a}	51.78±0.59^{a}	51.62±0.48^{a}	53.63±0.47^{a}

Means with different superscripts within group (a, b) and between groups (A, B) differ significantly (p<0.05)

3.6 Lymphocyte count (%)

The mean lymphocyte counts in different groups (A to C) of rabbits before (0 day) and after treatment (day 15, 30, 60, and 120) are presented in Table 4. Initially (day 0), the mean lymphocyte count of group A, B, and C were 41.18±0.60, 40.93±0.70, and 41.42±0.66%, respectively. Post-treatment on day 60, these values changed to 42.00±0.55, 41.58±0.33, and 36.73±0.55%, respectively.

At baseline (day 0), there was no significant difference in mean lymphocyte counts among groups A, B, and C. Throughout the study period (day 0, 15, 30, 60, and 120), non-significant variations were observed in mean lymphocyte counts within both treatment and control groups. The study reported a non-significant difference in mean lymphocyte count among treatment and control groups at different time intervals.

Lymphocyte counts in normal healthy New Zealand white male rabbits were reported by Moore *et al.* (2015) [12] and Shousha *et al.* (2017) [16], ranging between 28-50 and 59.55-73.53%. The present study reported lymphocyte counts within the same range (39-47%) as observed by Moore *et al.* (2015) [12] . However, Odetola *et al.* (2012) [13] and Shousha *et al.* (2017) [16] observed higher lymphocyte counts than the present study. Variations in rabbit lymphocyte counts across studies may be attributed to differences in feeding and environmental conditions of the rabbits (Abdel-Azeem *et al*., 2010) [1].

Post-treatment, lymphocyte counts varied non-significantly in all treatment groups, indicating the non-toxic effect of Madhuca longifolia in New Zealand White male rabbits. No existing literature discusses the effect of the ethanolic leaf extract of Madhuca longifolia on lymphocyte count in male rabbits, limiting the ability to make comparisons with our results.

Table 6: Lymphocyte count (Mean ± SE) in blood samples of different groups of rabbits before and after treatment (%)

Groups	Before treatment	After Treatment			
	Day 0	Day 15	Day 30	Day 60	Day 120
Group A (n=6) (Control)	41.18±0.60^{Aa}	42.13±0.42^{Aa}	41.15±0.56^{Aa}	42.00±0.55^{Aa}	41.62±0.38^{Aa}
Group B (n=6) (*M. longifolia* @ 100 mg/kg b. wt.)	40.93±0.70^{Aa}	40.78±0.63^{Aa}	41.65±0.52^{Aa}	41.58±0.33^{Aa}	41.60±0.50^{Aa}
Group C (n=6) (*M. longifolia* @ 200 mg/kg b. wt.)	41.42±0.66^{Aa}	40.38±0.61^{Aa}	38.93±0.57^{Aa}	36.73±0.55^{Aa}	41.00±0.3 ^{Aa}

Means with different superscripts within group (a, b) and between groups (A, B) differ significantly (p<0.05)

3.7 Eosinophil count (%)

The mean eosinophil count in different groups (A to C) of rabbits before (0 day) and after treatment (day 15, 30, 60 and 120) are shown in the Table 7. Prior to treatment (day 0), the mean eosinophil count of group A, B, C, D and E were 1.95±0.53, 1.12±0.25 and 1.05±0.25 %, respectively. After treatment (day 60), the corresponding values were 1.88±0.53, 1.38±0.59 and 0.73±0.14 cm, respectively.

Before treatment (0 day), the mean eosinophil count of group A, B, and C differed non-significantly. The mean eosinophil count varied non-significantly in all the treatment and control groups on day 0, 15, 30, 60 and 120. In the present study, non-significant difference was observed in the mean eosinophil count among the treatment and control groups at different time intervals.

Moore *et al.* (2015) [12] and Shousha *et al.* (2017) [16] recorded eosinophil count in normal healthy New Zealand white male rabbits, which ranged between 0.5-3.5 and 0.40-0.90 %. In the present study, the eosinophil count was almost in the same range (1-3 %) as reported by Moore *et al.* (2015) [12]. However, Odetola *et al.* (2012) [13] observed higher and Shousha *et al.* (2017) [16] observed lower count of eosinophil

than the present study. Variation in the eosinophil count of rabbits in the different studies might be due to difference in feeding and environmental conditions of the rabbits (Abdel-Azeem *et al.*, 2010) [1]. In all the treatment groups, after treatment eosinophil count varied non-significantly indicating non-toxic effect of *Madhuca longifolia* in New Zealand White male rabbits.

Table 7: Eosinophil count (Mean ± SE) in blood samples of different groups of rabbits before and after treatment (%)

Groups	Before treatment	After treatment			
	Day 0	Day 15	Day 30	Day 60	Day 120
Group A (n=6) (Control)	1.93±0.53^{Aa}	1.63±0.56^{Aa}	2.25±0.41^{Aa}	1.88±0.53^{Aa}	1.80±.53^{Aa}
Group B (n=6) (*M. longifolia* @ 100 mg/kg b. wt.)	1.12±0.25^{Aa}	1.32±0.30^{Aa}	1.68±0.33^{Aa}	1.38±0.59^{Aa}	1.03±0.24^{Aa}
Group C (n=6) (*M. longifolia* @ 200 mg/kg b. wt.)	1.05±0.25^{Aa}	1.32±0.43^{Aa}	1.22±0.28^{Aa}	0.73±0.14^{Aa}	1.87±0.30^{Aa}

Means with different superscripts within group (a, b) and between groups (A, B) differ significantly (p<0.05)

3.8 Monocyte count (%)

The mean monocyte counts in different groups (A to C) of rabbits before (0 day) and after treatment (day 15, 30, 60, and 120) are detailed in Table 8. Initially (day 0), the mean monocyte count of group A, B, and C were 4.47±0.14, 4.02±0.20, and 3.82±0.27%, respectively. Post-treatment on day 60, these values changed to 4.38±0.07, 3.78±0.36, and 3.15±0.19%, respectively.

At baseline (day 0), there was no significant difference in mean monocyte counts among groups A, B, and C. Throughout the study period (day 0, 15, 30, 60, and 120), non-significant variations were observed in mean monocyte counts within both treatment and control groups. The study reported a non-significant difference in mean monocyte count among treatment and control groups at different time intervals.

Monocyte counts in normal healthy New Zealand white male rabbits were reported by Moore *et al.* (2015) [12] and Shousha *et al.* (2017) [16], ranging between 4-12 and 0.50-0.90%. The present study reported monocyte counts within the same range (4-5%) as observed by Moore *et al.* (2015) [12]. However, Odetola *et al.* (2012) [13] and Shousha *et al.* (2017) [16] observed lower monocyte counts than the present study. Variations in rabbit monocyte counts across studies may be attributed to differences in feeding and environmental conditions of the rabbits (Abdel-Azeem *et al*., 2010) [1].

Table 8: Monocyte count (Mean ± SE) in blood samples of different groups of rabbits before and after treatment (%)

Groups	Before treatment	After treatment			
	Day 0	Day 15	Day 30	Day 60	Day 120
Group A (n=6) (Control)	4.47±0.14 ^{Aa}	4.48±0.12 ^{Aa}	4.35±0.10^{Aa}	4.38±0.07^{Aa}	4.53±0.13^{Aa}
Group B (n=6) (*M. longifolia* @ 100 mg/kg b. wt.)	4.02±0.20 ^{Aa}	3.92±0.29 ^{Aa}	4.28±0.75^{Aa}	3.78±0.36^{Aa}	3.70±0.26^{Aa}
Group C (n=6) (*M. longifolia* @ 200 mg/kg b. wt.)	3.82±0.27 ^{Aa}	2.80±0.36^{Aa}	3.58±0.36^{Aa}	3.15±0.19^{Aa}	3.67±0.28^{Aa}

Means with different superscripts within group (a, b) and between groups (A, B) differ significantly (p<0.05)

4. Conclusion

The study found non-significant changes in packed cell volume, total leukocyte count, granulocyte count, lymphocyte count, eosinophil count, and monocyte count in both treatment and control groups over the course of the study. The values observed were within the reported normal ranges for New Zealand white male rabbits, highlighting the stability of these hematological parameters following the administration of *Madhuca longifolia* leaves extract.

Overall, this research contributes valuable insights into the potential antifertility effects of *Madhuca longifolia* in male rabbits, providing a foundation for further investigations into its safety and efficacy.

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6. Statements of Animal Rights

The experimental work was approved by Institutional Animal Ethics Committee (Reference No. IAEC/CVSc-ANDUAT/2020/3/5).

7. Conflict of Interest Statement

The authors declare there is no conflict of interest on this article.

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