E-ISSN: 2618-0618
P-ISSN: 2618-060X

www.agronomyjournals.com
2024; SP-7(1): 09-14
Received: 11-10-2023
Accepted: 15-11-2023

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Hematological effects of *Azadirachta indica* (Neem) extract on New Zealand white male rabbits: A comprehensive study

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DOI: https://doi.org/10.33545/2618060X.2024.v7.i1Sa.211

Abstract
Neem (*Azadirachta indica*) is a versatile tree native to India, with bioactive compounds in its seed, leaf, flower, bark, and roots, offering a wide range of applications. While concerns about neem causing sterility in some bird species have been raised, this study investigates its impact on hematological parameters in New Zealand White male rabbits. The rabbits were divided into control and two treatment groups, receiving varying doses of neem extract. Haematological parameters, including hemoglobin, packed cell volume, total leukocyte count, granulocyte count, lymphocyte count, eosinophil count, total erythrocyte count, and monocyte count, were measured at different time intervals. Results indicated that neem extract did not significantly alter these parameters, suggesting a non-toxic effect. The study provides valuable insights into the hematological impact of neem in rabbits, contributing to our understanding of its safety and potential applications.

Keywords: *Azadirachta indica*, hematological parameters, New Zealand white rabbits

Introduction
Neem (*Azadirachta indica*) is a tree species of the Meliaceae family, which is native to India and has been used for centuries for a wide range of purposes. A large number of bioactive compounds are present in seed, leaf, flower, bark, and roots of neem, which guarantee great versatility in their use (Ogbuewu *et al.*, 2009) [10]. Oliveira (2009) [12] warned of the possibility of neem causing sterility in some species of birds.

Haemoglobin plays an important role in the transport of oxygen, for normal health, production and reproduction (Ganong, 2001) [2]. Hewitt *et al.* (1989) [4], Ozkan *et al.* (2012) [13], Moore *et al.* (2015) [7] and Shousha *et al.* (2017) [14] recorded haemoglobin concentration in normal healthy New Zealand white male rabbits as 10.90-14.50, 8.90-15.50, 14.70-20.80, 10.40-17.40 and 10.45-16.81 g/dl, respectively.

Hewitt *et al.* (1989) [4], Ozkan *et al.* (2012) [13], Moore *et al.* (2015) [7] and Shousha *et al.* (2017) [14] recorded packed cell volume in normal healthy New Zealand white male rabbits which ranged between 26.70-47.20, 41.70-57.00, 33-50 and 38.41-51.50%, respectively. Hewitt *et al.* (1989) [4], Ozkan *et al.* (2012) [13], Moore *et al.* (2015) [7] and Shousha *et al.* (2017) [14] 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. Ozkan *et al.* (2012) [13], Moore *et al.* (2015) [7] observed granulocyte count in normal healthy New Zealand white male rabbits which ranged between 27-73 and 40-61%. Moore *et al.* (2015) [7] and Shousha *et al.* (2017) [14] observed lymphocyte count in normal healthy New Zealand white male rabbits, which ranged between 28-50 and 59.55-73.53%, respectively.

Moore *et al.* (2015) [7] and Shousha *et al.* (2017) [14] recorded eosinophil count in normal healthy New Zealand white male rabbits, which ranged between 0.5-3.5 and 0.40-0.90%. % Moore *et al.* (2015) [7] and Shousha *et al.* (2017) [14] Zealand white male rabbits, which ranged between 4-12 and 0.50-0.90%, respectively.

Ogbuewu *et al.* (2010) [11] recorded non-significant value of red blood cells (RBC), packed cell

volume (PCV) and hemoglobin (Hb) in bucks fed on leaf meal of *Azadirachta indica.* The PCV and Hb values of rabbits on test diets were not significantly (p> 0.05) different from the control group. Ikwuka *et al.* (2020) [5] studied effect of fractionated neem leaf extract on haematological parameters of healthy Wistar rats and concluded that WBC and packed cell volume was not differed between the groups. No significant change in the neutrophil (F=0.81, P=0.47), lymphocyte (F=0.96, P=0.41), monocyte (F=1.86, P=0.20) and eosinophil (F=0.45, P=0.65) numbers (%) was reported.

Materials and Methods

The present study was undertaken to find out the antifertility effect of *Azadirachta indica* in New Zealand White male rabbits. The rabbits kept at the animal house of College of Veterinary Science and Animal Husbandry, Acharya Narendra Deva University of Agriculture & Technology, Kumarganj, Ayodhya, Uttar Pradesh (India) were used for the study. The proposed experimental work was approved by Institutional Animal Ethics Committee (Reference No. IAEC/CVSc-ANDUAT/2020/3/5)

Daily human contact was helpful to reduce stress during handling. Animals were handled by grasping a large fold of loose skin over the shoulders with one hand and either supporting or grasping the rear feet with the other hand **(**Mapara *et al.*, 2012) [6]. To reduce stress and prevent injury, a wooden rabbit restrainer was used to immobilize the rabbits for blood collection, tissue biopsy, administration of drugs, etc.

The powder of dried leaves (100 gm) was mixed with 1000 ml of 70% alcohol (v/v) separately and left to macerate at room temperature for 20 hours (WHO protocol CG-04). The mixture was stirred at an interval of 6 hours. Then, the mixture was filtered using Whatman filter paper No. 1. After filtration, alcohol was evaporated from the extract. A vacuum evaporator under low pressure and temperature @ 30^0 C was used to obtain a semisolid mass (extract). Finally, the extract was stored in deep freezer at -20 °C. The samples of extracts were used to treat test animals.

The herbariums were made as per the standard protocol described by Bridson and Forman (1998). The specimens of leaves were sent to the Central National Herbarium (CNH), Botanical Survey of India (BSI), Howrah, West Bengal for identification/ authentication.

The animals included in the present study were randomly divided into five groups (six male rabbits/group) as Group A (Control), Group B (*A. indica* @ 100 mg/kg b. wt./animal/day), Group C (*A. indica* @ 200 mg/kg b. wt./animal/day). Before feeding, the extract was dissolved in 1 ml distilled water. The suspension of extract was administered orally with the help of 2 ml syringe.

Results and Discussion

Haemoglobin (gm/dl)

The mean haemoglobin concentration in different groups (A to C) of rabbits before (0 day) and after treatment (day 15, 30, 60 and 120) are presented in the Table1. Prior to treatment (day 0), the mean haemoglobin concentration of group A, B and C were 10.53±0.20, 11.19±0.27, and 11.50±0.47 gm/dl, respectively. After treatment (day 60), the corresponding values were 11.28±0.18 and 11.65±0.20 gm/dl, respectively.

Prior to treatment (0 day), the mean haemoglobin concentration of group A, B and C varied non-significantly. In all the treatment and control groups, the mean haemoglobin concentration differed non-significantly at different time intervals (on day 0, 15, 30, 60 and 120). In the present study, the mean haemoglobin concentration varied non-significantly among the treatment and control groups at different time intervals.

Haemoglobin plays an important role in the transport of oxygen, for normal health, production and reproduction (Ganong, 2001) [2]. Hewitt *et al.* (1989) [4], Ozkan *et al.* (2012) [13], Moore *et al.* (2015) [7] and Shousha *et al.* (2017) [14] recorded haemoglobin concentration in normal healthy New Zealand white male rabbits as 10.90-14.50, 8.90-15.50, 14.70-20.80, 10.40-17.40 and 10.45-16.81 g/dl, respectively. In the present study, the haemoglobin concentration was in the same range (10.00-12.80 g/dl) as reported by Hewitt *et al.* (1989) [4] and Moore *et al.* (2015) [7]. However, Ozkon *et al.* (2012) [13] and Shousha *et al.* (2017) [14] observed higher concentration and Odetola *et al.* (2012) [8] reported lower concentration (8.48 g/dl) of haemoglobin than the present study. Variation in the haemoglobin concentration of rabbits in the different studies might be due to difference in feeding and environmental conditions (Abdel-Azeem *et al.*, 2010) [1].

In all the treatment groups, haemoglobin concentration did not alter post-treatment indicating non-toxic effect of *Azadirachta indica* in New Zealand White male rabbits. Our findings are similar to the findings of Gowda *et al.* (1996) [3] and Ogbuewu *et al.* (2010) [11], who also reported non-significant variation in the concentration of haemoglobin in New Zealand White rabbits following treatment with leaf meal of *Azadirachta indica*.

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) (*A. indica* @ 100 mg/kg b. wt.)	11.19±0.27^{Aa}	11.38±0.23^{Aa}	11.51±0.21^{Aa}	11.65±0.20^{Aa}	11.68±0.30^{Aa}
Group C (n=6) (*A. indica* @ 200 mg/kg b. wt.)	11.50±0.47^{Aa}	11.71±0.40^{Aa}	11.77±0.33^{Aa}	12.08±0.32^{Aa}	12.31±0.30^{Aa}

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

Packed Cell Volume (%)

The mean packed cell volume in different groups (A to C) of rabbits before (0 day) and after treatment (day 15, 30, 60 and 120) are depicted in the Table 2. Prior to treatment (day 0), the mean packed cell volume of group A, B and C were 38.72±0.35, 40.43±1.11 and 42.40±1.76%, respectively. After treatment (day 60), the corresponding values were 39.05±1.78, 42.63±1.40 and 41.60±0.95, respectively.

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

An optimum level of haemoglobin and packed cell volume is required for efficient transport of oxygen, as they are essential for normal health (Ganong, 2001) [2]. Hewitt *et al.* (1989) [4], Ozkan *et al.* (2012) [13], Moore *et al.* (2015) [7] and Shousha *et al.* (2017) [14] recorded packed cell volume in normal healthy New

Zealand white male rabbits, which ranged between 26.70-47.20, 41.70-57.00, 33-50 and 38.41-51.50%, respectively. In the present study, the packed cell volume was in the same range (37.00-48.20%) as reported by Moore *et al.* (2015) [7]. However, Okzon *et al.* (2012) [13] and Shousha *et al.* (2017) [14] reported higher and Odetola *et al.* (2012) [8] observed lower PCV (25.00%) in comparison to our study. Variation in the PCV of rabbits in the different studies might be due to difference in feeding and environmental conditions (Abdel-Azeem *et al.*, 2010) [1].

In all the treatment groups, PCV did not differed after treatment indicating non-toxic effect of *Azadirachta indica* in New Zealand White male rabbits. Our findings are in agreement with the findings of Ogbuewu *et al.* (2010) [11], who also reported non-significant variation in the values of PCV following treatment with leaf meal of *Azadirachta indica* in female rabbits. Non-significant variation in the mean values of PCV was also recorded by Ikwuka *et al.* (2020) [5] after treatment with fractionated neem leaf extract in wistar rats.

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) (*A. indica* @ 100 mg/kg b. wt.)	40.43 ± 1.11^{Aa}	41.20 ± 0.92^{Aa}	42.03 ± 0.90^{Aa}	42.63 ± 1.40^{Aa}	41.96 ± 1.40^{Aa}
Group C (n=6) (*A. indica* @ 200 mg/kg b. wt.)	42.40 ± 1.76^{Aa}	42.10 ± 2.01^{Aa}	42.15 ± 1.54^{Aa}	41.60 ± 0.95^{Aa}	41.80 ± 1.48^{Aa}

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

Leukocyte Count ($\times10^6$/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, 9.13±0.14, and 9.20±0.26 $x10^6$/ml, respectively. After treatment (day 60), the corresponding values were 9.57±0.16, 9.18±0.08 and 8.86±0.15 $x10^6$/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) [4], Ozkan *et al.* (2012) [13], Moore *et al.* (2015) [7] and Shousha *et al.* (2017) [14] 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 Okzon *et al.* (2012) [13], Moore *et al.* (2015) [7] and Shousha *et al.* (2017) [14]. In contrary to our finding, Odetola *et al.* (2012) [8] 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].

Table 3: Total leukocyte count (Mean ± SE) in blood samples of different groups of rabbits before and after treatment ($\times10^6$/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) (*A. indica* @ 100 mg/kg b. wt.)	9.13 ± 0.14^{Aa}	9.18 ± 0.1^{Aa}	9.22 ± 0.08^{Aa}	9.18 ± 0.08^{Aa}	9.12 ± 0.11^{Aa}
Group C (n=6) (*A. indica* @ 200 mg/kg b. wt.)	9.20 ± 0.26^{Aa}	9.13 ± 0.16^{Aa}	8.83 ± 0.17^{Aa}	8.68 ± 0.15^{Aa}	9.11 ± 0.11^{Aa}

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

In all the treatment groups, total leukocyte count did not altered post-treatment indicating non-toxic effect of *Azadirachta indica* in New Zealand White male rabbits. No citation is available regarding effect of ethanolic extract of neem *Azadirachta indica* (Neem) on total leukocyte count in New Zealand White male rabbits, So, our results could not be compared.

Total erythrocyte count ($x10^9$/ml)

The mean total erythrocyte count ($x10^9$/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 4. Prior to treatment (day 0), the mean total erythrocyte count ($x10^9$/ml) of group A, B and C were 6.73±0.80, 6.40±0.16 and 6.52±0.12 $x10^9$/ml, respectively. After treatment (day 60), the corresponding values were 6.40±0.13 and 6.49±0.14 $x10^9$/ml, respectively.

Prior to treatment (0 day), the mean total erythrocyte count (TEC) of group A, B and C varied non-significantly. The mean total erythrocyte count altered 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 recorded in the mean total erythrocyte count among the treatment and control groups at different time intervals.

Hewitt *et al.* (1989) [4], Moore *et al.* (2015) [7] and Shousha *et al.* (2017) [14] recorded total erythrocyte count in normal healthy New Zealand white male rabbits, which ranged between 3.70-7.50, 5.46-7.94 and 4.11-7.20 $x10^9$/ml. In the present study, the TEC was in the same range (6.00-6.96$x10^9$/ml) as reported by Hewitt *et al.* (1989) [4], Moore *et al.* (2015) [7] and Shousha *et al.* (2017) [14] recorded total. However, Odetola *et al.* (2012) [8] observed lower total erythrocyte count (2.47$x10^9$/ml) in comparison to our study. Variation in the TEC 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, TEC did not alter significantly post-treatment indicating non-toxic effect of *Azadirachta indica* 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) (*A. indica* @ 100 mg/kg b. wt.)	6.40±0.16^{Aa}	6.50±0.09^{Aa}	6.48±0.13^{Aa}	6.49±0.14^{Aa}	6.40±0.17^{Aa}
Group C (n=6) (*A. indica* @ 200 mg/kg b. wt.)	6.52±0.12^{Aa}	6.62±0.15^{Aa}	6.64±0.14^{Aa}	6.74±0.06^{Aa}	6.52±0.10^{Aa}

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

Granulocyte count (%)

The mean granulocyte (neutrophil + basophil) count in different groups (A to C) of rabbits before (0 day) and after treatment (day 15, 30, 60 and 120) are presented in the Table 5. Prior to treatment (day 0), the mean granulocyte (neutrophil + basophil) count of group A, B and C were 52.08±0.19, 52.87±0.55 and 51.65±1.21%, respectively. After treatment (day 60), the corresponding values were 52.23±0.68, 51.35±0.40 and 51.92±0.20%, 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) [13] and Moore *et al.* (2015) [7] 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) [13] and Moore *et al.* (2015) [7]

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 B (n=6) (*A. indica* @ 100 mg/kg b. wt.)	52.87±0.55^{a}	52.62±0.70^{a}	50.27±0.75^{a}	51.35±0.40^{a}	52.45±0.38^{a}
Group C (n=6) (*A. indica* @ 200 mg/kg b. wt.)	51.65±1.21^{a}	52.28±0.41^{a}	52.90±0.64^{a}	51.92±0.20^{a}	52.35±0.68^{a}

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

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 depicted in the Table 6. Prior to treatment (day 0), the mean lymphocyte count of group A, B and C were 41.18±0.60, 41.62±0.24 and 42.78±0.93%, respectively. After treatment (day 60), the corresponding values were 42.00±0.55, 45.31±0.22 and 36.87±0.49%, respectively.

Prior to treatment (0 day), the mean lymphocyte counts of group A, B and C altered non-significantly. The mean lymphocyte counts differed 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 recorded in the mean lymphocyte count among the treatment and control groups at different time intervals.

Moore *et al.* (2015) [7] and Shousha *et al.* (2017) [14] observed lymphocyte count in normal healthy New Zealand white male rabbits, which ranged between 28-50 and 59.55-73.53%. In the present study, the lymphocyte count was in the same range (39-47%) as reported by Moore *et al.* (2015). However, Odetola *et al.* (2012) [8] and Shousha *et al.* (2017) [14] observed higher count of lymphocyte than the present study. Variation in the lymphocyte 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, lymphocyte count varied non-significantly post- treatment indicating non-toxic effect of *Azadirachta indica* in New Zealand White male rabbits.

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) (*A. indica* @ 100 mg/kg b. wt.)	41.62±0.24^{Aa}	42.68±0.17^{Aa}	44.00±0.22^{Aa}	45.31±0.22^{Aa}	41.75±0.16^{Aa}
Group C (n=6) (*A. indica* @ 200 mg/kg b. wt.)	42.78±0.93^{Aa}	40.83±0.51^{Aa}	39.67±0.49^{Aa}	36.87±0.49^{Aa}	40.17±0.51^{Aa}

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

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 and C were 1.95±0.53, 1.20±0.20 and 1.12±0.38%, respectively. After treatment (day 60), the corresponding values were 1.88±0.53, 1.47±0.25 and 1.25±0.28 cm, respectively.

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) (*A. indica* @ 100 mg/kg b. wt.)	1.20±0.20^{Aa}	1.15±0.14^{Aa}	1.53±0.26^{Aa}	1.47±0.25^{Aa}	1.80±0.37^{Aa}
Group C (n=6) (*A. indica* @ 200 mg/kg b. wt.)	1.12±0.38^{Aa}	1.53±0.35^{Aa}	1.83±0.29^{Aa}	1.25±0.28^{Aa}	1.55±0.46^{Aa}

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

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 given in the Table 8. Prior to treatment (day 0), the mean monocyte count of group A, B and C were 4.47±0.14, 4.32±0.30 and 4.00±0.22, respectively. After treatment (day 60), the corresponding values were 4.38±0.07, 3.87±0.34 and 3.95±0.30, respectively.

Prior to treatment (0 day), the mean monocyte count of group A, B and C varied non-significantly. The mean monocyte count altered non-significantly in all 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) [7] and Shousha *et al.* (2017) [14] 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) [7]. However, Odetola *et al.* (2012) [8] observed higher and Shousha *et al.* (2017) [14] 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]. The treatment and control groups on day 0, 15, 30, 60 and 120. In the present study, non-significant difference was recorded in the mean monocyte count among the treatment and control groups at different time intervals.

Moore *et al.* (2015) [7] and Shousha *et al.* (2017) [14] reported monocyte count in normal healthy New Zealand white male rabbits, which ranged between 4-12 and 0.50-0.90%. In the present study, the monocyte count was in the same range (4-5%) as reported by Moore *et al.* (2015) [7]. However, Odetola *et al.* (2012) [8] and Shousha *et al.* (2017) [14] observed lower count of monocyte than the present study. Variation in the monocyte 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, monocyte count differed non-significantly post- treatment indicating non-toxic effect of *Azadirachta indica* in New Zealand White male rabbits. Perusal of literature revealed no citation regarding effect of ethanolic leaf extract of *Azadirachta indica* on monocyte count of male rabbits.

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) (*A. indica* @ 100 mg/kg b. wt.)	4.32±0.30^{Aa}	3.62±0.28^{Aa}	4.20±0.26^{Aa}	3.87±0.34^{Aa}	4.00±0.20^{Aa}
Group C (n=6) (*A. indica* @ 200 mg/kg b. wt.)	4.00±0.22^{Aa}	3.87±0.29^{Aa}	3.77±0.25^{Aa}	3.95±0.30^{Aa}	4.05±0.35^{Aa}

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

Conclusion

The comprehensive analysis of various hematological parameters, including haemoglobin concentration, packed cell volume, leukocyte count, total erythrocyte count, granulocyte count, lymphocyte count, eosinophil count, and monocyte count, across different time intervals in both treatment and control groups revealed non-significant differences. The consistent non-significant variations in these parameters indicate that the interventions applied in the study did not exert a statistically significant impact on the hematological profile. This suggests a stable and comparable baseline across the groups and showed oral feeding of ethanolic leaf extract of *Azadirachta indica* at both dose rates was found to be nontoxic to male rabbits.

Acknowledgment

The author would like to express gratitude to the Dean, College of Veterinary Science and Animal Husbandry, A.N.D.U.A. & T., Kumarganj, Ayodhya (Uttar Pradesh) for providing funds and necessary support for the research.

Statements of Animal Rights

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

Conflict of Interest Statement

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

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