



## **Hematological Values in Sheep Fed a Diet Containing Black Cumin (*Nigella sativa*) Seed Oil**

**M. I. Abd El-Halim<sup>1</sup>, Nabiela M. El-Bagir<sup>1</sup> and M. K. Sabahelkhier<sup>2\*</sup>**

<sup>1</sup>Department of Biochemistry, Faculty of Veterinary Medicine Science, U of K, SUDAN.

<sup>2</sup>Departments of Biochemistry, Molecular Biology, Faculty of Science and Technology, El-Neelain University, SUDAN.

### **Authors' contributions**

*This work was carried out in collaboration between all authors. Author MIAE performed experiments. Author NME supervised experiments and assisted in manuscript preparation. Author MKS conceived the idea, supervised and managed analysis the results and wrote the manuscript. All authors read and approved the final manuscript.*

**Original Research Article**

**Received 6<sup>th</sup> October 2013**  
**Accepted 3<sup>rd</sup> December 2013**  
**Published 9<sup>th</sup> January 2014**

### **ABSTRACT**

The aim of this study was to investigate changes in hematological values after feeding diet mixed with oil pressed from seeds of *Nigella sativa* in sheep. The results indicated that there was no effect on the total Red Blood Cells (RBCs) count due to treatment when compared to the control group, but the treated group showed significant ( $P \leq 0.05$ ) increase in the total RBCs count after six weeks compared to the transient decrease at week two. Hemoglobin concentration showed similar pattern to what was observed in the RBC count in both the treated and control groups. Packed Cells Volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC) weren't affected by feeding *N. sativa* oil to sheep throughout the experiment. However, there were significantly ( $p \leq 0.05$ ) lower values in total mean of White Blood Cells (WBCs), lymphocytes and granulocytes of the treated group compared to the control. The levels of White Blood Cells in the treated group were not time dependent. The Monocytes total mean was similar in both groups but the cells were significantly ( $P \leq 0.05$ ) lower in the *N. sativa* oil treated group compared to the control at week four. The hematological changes could be due to *N. sativa* volatile oils incorporated

\*Corresponding author: Email: [mskhalid53@gmail.com](mailto:mskhalid53@gmail.com);

to in the sheep diet. Conclusion: The results revealed that RBCs, Hb, PCV, MCV, MCH and MCHC for treated group are similar to control group but there are significantly increasing in RBCs and HB in 6<sup>th</sup> week. While WBCs, lymphocytes and granulocytes for treated group are significantly lower than the control group.

**Keywords:** Sheep; *Nigella sativa*; red blood cells; white blood cells and monocytes.

## 1. INTRODUCTION

Black Seed, Black Cumin, Kalunji, Nutmeg Flower, Kalajira and Roman Coriander are all commonly used names for the herbaceous plant (*Nigella sativa*). This plant is widely distributed, belonging to the botanical family *Ranunculaceae*. The genus *Nigella* is native to the Mediterranean region and Western Asia, the name being derived from the Latin word *niger*, meaning black, in reference to the colour of the seeds [1]. The seed has been used as a spice in cooking and as a preservative for cheese products [2]. Raw seeds, seed oil, or seed extract have been used alone or in combination with other ingredients, as a traditional medicine in the treatment of various health conditions, such as eczema, cough, headache, diabetes, asthma, infection and hypertension [1]. Pharmacological activities of seed extracts have been documented and include activities against human and animal diseases and against pests [3]. The crude extract of *N. sativa* has also been shown to cause relaxation of carbachol, histamine and potassium ion induced contractions of isolated guinea-pig trachea [4]. An oral dose of 0.6 ml/kg/day of *N. sativa* extract produced a significant hypotensive effect in spontaneously hypertensive rats comparable to that of 0.5 mg/kg/day of oral nifedipine [5]. In Unani medicine *N. sativa* is promoted for the treatment of oligomenorrhoea, to induce menstruation and to treat infertility [6]. In Unani medicine, It is also used for stomachache and as a digestive, carminative, laxative and anti-jaundice [7]. The antitumor activity study of a methanolic extract of the *N. sativa* seed against Ehrlich Ascites Carcinoma *in vivo* demonstrated that the development of malignancy was completely inhibited at a dose of 2 mg of oil extracted from *N sativa* per mouse per day for 10 days [8]. The isolation of the antimicrobial agent nigellone, of the *N. sativa* volatile oil was achieved by the refrigeration of the oil at 4°C for 3 days, during which time white crystalline needles separated out. The chemical structure of the oil that extracted from *N sativa* was deduced from its physical and chemical properties, as well as its ultraviolet, infrared, proton magnetic resonance and mass spectral data. The volatile oil of *N. sativa* possesses anti-microbial property against gram positive microorganisms [9]. *Nigellone* was very effective in inhibiting histamine released from rat's peritoneal mast cells [10]. The volatile oil of *N. sativa* alone also produced a significant hypoglycemic effect on normal and alloxan-induced diabetic rabbits without changes in insulin levels [11]. *N. sativa* extract prevented the decreases in hemoglobin level and leucocytes count caused by cisplatin in mice [12].

All previous researches investigated the biochemical and hematological changes induced by the *N. sativa* in mono gastric animals [13] but no work was done in ruminants. Since *N. sativa* is a promising feed additive due to its known nutritive and protective effects, the main objective of this study is to assess the effect of the *N. sativa* oil on: White blood count (WBC) and deferential, Red blood cells (RBC), Hemoglobin (Hb) concentration, packed cell volume (PCV), Mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC).

## 2. MATERIALS AND METHODS

### 2.1 Materials

#### 2.1.1 Animals

This experiment was conducted in the Department of Biochemistry Faculty of Veterinary Medicine, University of Khartoum (U.K) to investigate the effect of feeding commercial oil of *N. sativa* to sheep. The experimental period was extended for six weeks starting from March 2007. Twelve young male sheep (Hammury × Desert) were used. Their ages range from 3 to 6 months and weights from 13 to 26 kg. All animals were weighted in the (U.K) animal farm and divided into two groups of similar weights. Kept for two weeks as adaptation period and fed commercial fattening diet. Each sheep was kept in a single separate cage. Before getting into cages, the sheep were washed with cypermetherin solution (1 ml in 1 liter), sheep were divided into two groups according to their weights and sample size (n=6 animals). During the adaptation period (which was 2 weeks) all sheep were supplied with concentrate feed that obtained from kenana Company LTD. Table 1. All animals were kept under same conditions that prevented them from experiencing unnecessary pain and discomfort according to guidelines approved by the ethical committee in Faculty of Animal Production, University of Khartoum.

#### 2.1.2 Equipments

Cell counter (Mythic-18, ORPHEE company; Switzerland, Id: 102405-000639) was used for measurement Hematology.

**Table 1. Approximate analysis of kenana ration**

Dry matter	Crude protein	Crude fiber	Crude fat	Ash
87	17.5	11.9	2.3	11.2

Group (A) received 47 gm of oil that extracted from *N. sativa* mixed with one kg of the concentrate (treated group), while the second group (B) received the concentrate feed without *N. sativa* (control group). According to the general recommendation that the total dietary fat should not exceed 6 to 7% of dietary dry matter and those higher levels may adversely affect rumen microbial fermentation [14].

### 2.2 Sample Collection

Blood samples were collected early in the morning per two days interval from each animal from the jugular vein with minimal excitement. 2ml of the blood from each animal were collected in ethylene diamine tetracetate (EDTA) vacuainers and transported to the laboratory for hematological analysis; the samples were analyzed within two hours after collection.

### 2.3 Hematology Method

The hematological estimations were done by using the cell counter (Mythic-18, ORPHEE company; Switzerland, Id: 102405-000639) The method based on the conversion of the hemoglobin by means of drabkin's solution (0.2g potassium cyanide, 0.2 potassium

ferricyanide and 1g sodium bicarbonate per one liter of distilled water) to cyanomethemoglobin concentration was measured in g/dl of blood [15]. The packed cell volume (PCV) was determined by the microhematocrit method [16], Erythrocytes and leucocytes were being determined manually. Whereas, Hb was measured by using the cyanomethemoglobin method. The mean corpuscular hemoglobin concentration (MCHC), the mean cell volume (MCV) and the mean corpuscular hemoglobin were calculated by the formulas:

$$\begin{aligned} \text{MCV (in cubic micros)} &= \text{PCV (\%)} \times 10 \\ \text{MCH (pg)} &= \text{Hb (g/dl)} \times 10 \\ \text{MCHC (\%)} &= \text{Hb (g/dl)} \times 100 \end{aligned}$$

## 2.4 Statistical Analysis

The experiment was designed by the complete randomized design (CRD); factorial arrangement 2×4. Where 2 indicate two groups (A; treated and B; control) and 4 indicate times (0, 2, 4 and 6 weeks). Data generated from the experiment were statistically analyzed by analysis of variance (ANOVA) [15,17]. Then means were separated by Duncan's multiple range tests to determine the significant at 0.05% level of probability [18].

## 3. RESULTS AND DISCUSSION

### 3.1 Results

#### 3.1.1 Red Blood Cells (RBCs)

Fig. 1 showed that Maximum value of RBCs ( $8.09 \times 10^6/\mu\text{L}$ ) was observed in 6<sup>th</sup> week, while minimum value of RBCs ( $7.19 \times 10^6/\mu\text{L}$ ) was observed in 2<sup>nd</sup> week comparing with the control group. It is indicated that the *N. sativa* oil has no effect on the total mean (RBCs) count, since there was no significant difference between the treated group (A) and the control (B). This result agrees with previous findings [6] but the numerical decreased of the RBCs values observed in both groups in week two. These variations could be due to differences in doses that received by sheep. However in the present study the decrease seen in RBCs count after two weeks in the treated group, increased in week four and reached significantly ( $P \leq 0.05$ ) higher level in week six compared to week two, this results are in agreement with [19].

#### 3.1.2 Hemoglobin (Hb)

Fig. 2 showed that Hb value was ranged from 8.65 g/L in 2<sup>nd</sup> week to 10.57 g/L in 6<sup>th</sup> week for treated group, while Hb value was ranged from 8.5 g/L (2<sup>nd</sup> week) to 10.95 g/L (6<sup>th</sup> week) for control group. It is clearly illustrated that the *N. sativa* oil has no effect on Hb in sheep, whereas *N. sativa* oil in rats has been observed to increase the hematocrit and hemoglobin levels by 6.4 and 17.4%, respectively [5]. The effect of feeding different doses of the seeds of *N. sativa* 2 and 10% w/w for considerably long periods in male and female rabbits showed an increase in Hb levels in all groups [19]. In the present study a similar pattern to what was observed in the RBCs count has been found in the level of Hb, but at week six the values were significantly ( $P \leq 0.05$ ) higher than in time zero in treated groups and the control one.

RBCs X 10<sup>6</sup>/μ

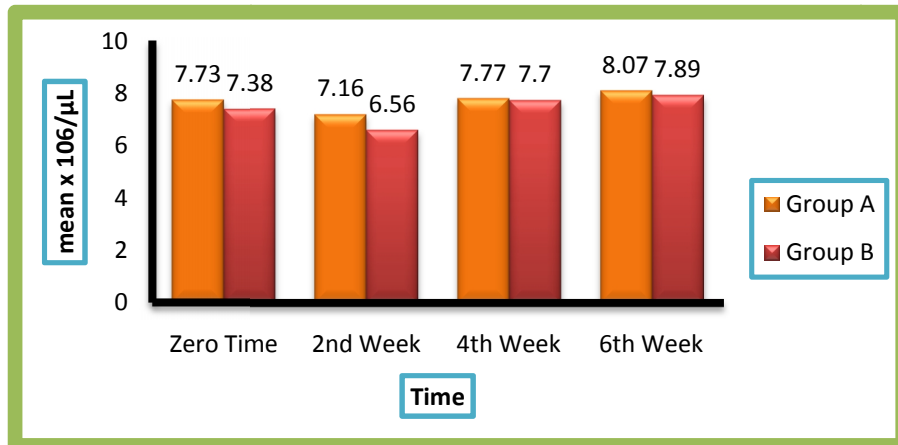


Fig. 1. Effect of feeding *N. sativa* oil on red blood cells in sheep Group A: treated with *N. sativa* oil; Group B: control

Hb (g/dl)

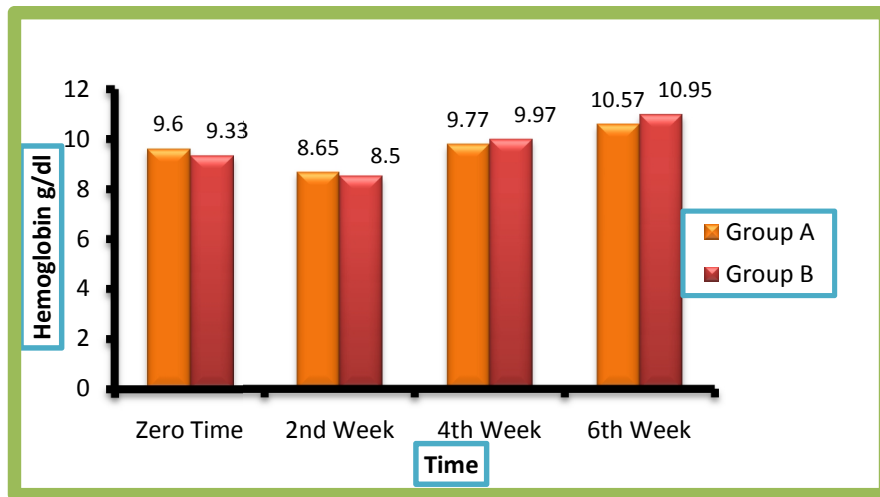


Fig. 2. Effect of feeding *N. sativa* oil on hemoglobin in sheep Group A: treated with *N. sativa* oil; Group B: control

### 3.1.3 PCV (%)

Fig. 3 showed that PCV% for treated group in zero, 2, 4 and 6<sup>th</sup> week was 20.53, 19.4, 22.23 and 22.23%, respectively. While PCV% for control group in zero, 2, 4 and 6<sup>th</sup> week was 21.25, 17.98, 22.17 and 21.25%, respectively. This results were indicated that addition of *N. sativa* oil resulted in similar values in treated and control groups which observed no significant effect of the oil on PCV value in sheep. This comes in line with the study [20] that found the treatment with *N. sativa* oil for two months to the anemic patients showed that the

Hb concentration has increased by 10% for the initial level. PCV and MCV also have increased, but not significantly. This result also agrees [6] but contradicted.

PCV %

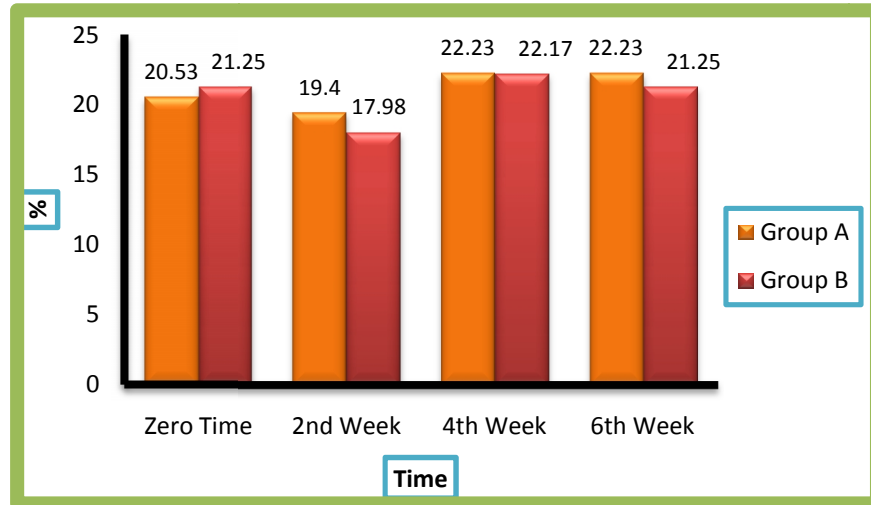


Fig. 3. Effect of feeding *N. sativa* oil on PCV in sheep Group A: treated with *N. sativa* oil; Group B: control

### 3.1.4 MCV ( $10^3 \mu\text{L}$ )

Fig. 4 indicated that MCV value for treated group was ranged from  $28.65 \times 10^3 \mu\text{L}$  in zero week) to  $34.73 \times 10^3 \mu\text{L}$  in 6<sup>th</sup> week, while MCV value for control group was ranged from  $28.81 \times 10^3 \mu\text{L}$  in zero week to  $26.95 \times 10^3 \mu\text{L}$  in 6<sup>th</sup> week. These results were illustrated that the *N. sativa* oil treated group was similar to control group which indicates no effect of the oil on MCV value in sheep. The pattern observed in MCH, PCV and MCV was similar to what was observed in RBC count. This agrees with the finding [20] which showed MCV value between  $96 \mu\text{m}^3$  and  $85 \mu\text{m}^3$ . This indicates that most of them were in normocytic condition. After 2 months treatment the MCV increased to the average value of 109.8, which indicate the lack of iron.

### 3.1.5 MCH (Pg)

Fig. 5 indicated that low value of MCH (12.48 Pg) was observed in zero week while high value of MCH (13.18 Pg) was obtained in 6<sup>th</sup> week for treated group compared with control, the low value of MCH (13.48 Pg) was clearly observed in zero week while high value of MCH (14.43 Pg) was indicated in 6<sup>th</sup> week. These findings were indicated that the *N. sativa* oil treated group was similar to control group which indicates no effect of the oil on MCH value in sheep. The pattern observed in MCH was similar to what was observed in RBCs and changes were also not significant. This contradicted with previous findings [20].

MCV  $10^3/\mu\text{L}$

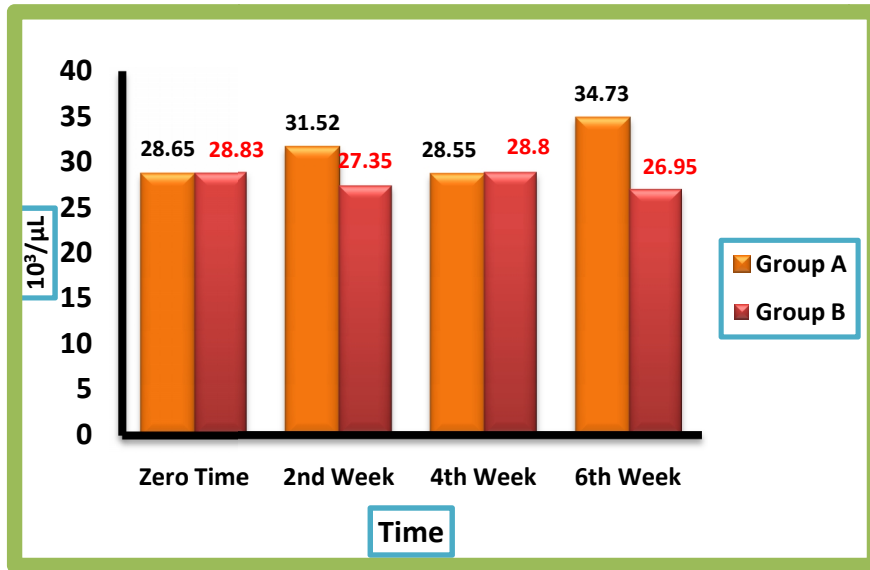


Fig. 4. Effect of feeding *N. sativa* oil on MCV in sheep Group A: treated with *N. sativa* oil; Group B: control

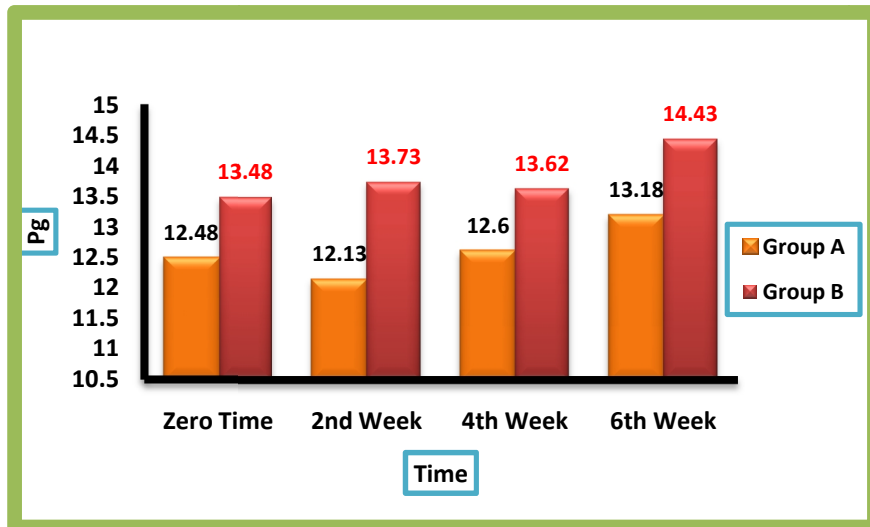


Fig. 5. Effect of feeding *N. sativa* oil on MCH in sheep Group A: treated with *N. sativa* oil; Group B: control

### 3.1.6 MCHC (g/dl)

Fig. 6 showed that the MCHC values for treated group in zero, 2,4 and 6<sup>th</sup> week were 44.1, 45.1,44.37 and 48.05 g/dl respectively, while The MCHC values of control group in zero,2,4 and 6<sup>th</sup> week were 47.12,50.2,47.35 and 53.58 g/dl, respectively. These findings were

illustrated that the *N. sativa* oil treated group was similar to control group. The *N. sativa* oil treated group was similar to control group. This was indicated no effect of the oil on MCHC value in sheep. The MCHC values are dependent upon RBC, Hb and PCV values. The result comes on line with the finding of [20]. The MCHC was always below the normal level and change was not significant.

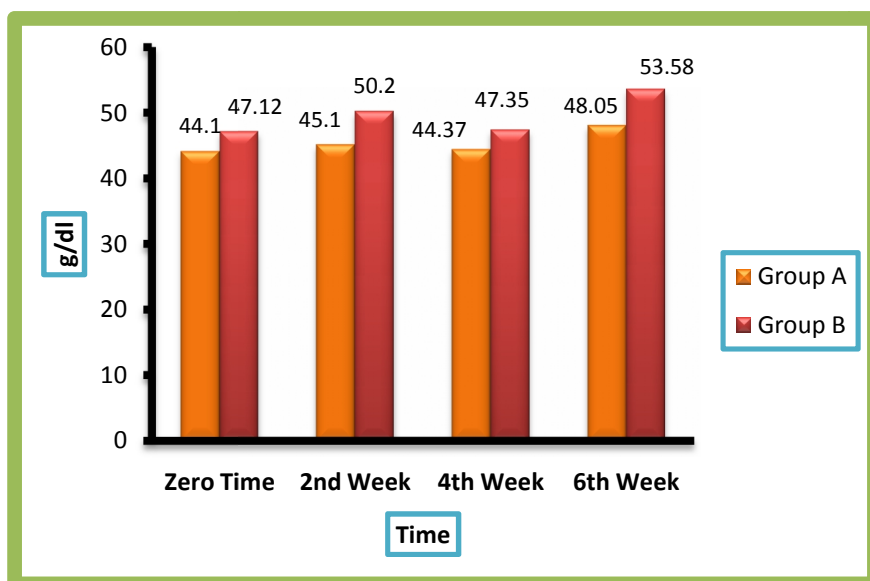


Fig. 6. Effect of feeding *N. sativa* oil on MCH in sheep Group A: treated with *N. sativa* oil; Group B: control

### 3.2 White Blood Cells WBCs ( $10^3 \times \mu\text{L}$ )

Fig. 7 showed that the white blood cells (WBCs) in zero, 2, 4 and 6<sup>th</sup> week for treated group were 6.92, 35.8, 18.57 and 27.85  $10^3 \times \mu\text{L}$  respectively. While WBCs in zero, 2, 4 and 6<sup>th</sup> week for control group were 10.35, 49.2, 29.9 and 47.08  $10^3 \times \mu\text{L}$  respectively. The WBCs are soldiers of the body and their high counts may be due to increase or development of the immune systems of the animals at the early stage of life which they may not obtain from the colostrums of the dam [18]. These findings are indicated that the general effect of *N. sativa* oil on white blood cells in sheep produced significantly ( $P \leq 0.05$ ) lower value as total mean of WBCs of group A compared to the control. This result agree with [5] and disagree with [6]. In the present study during the experimental period, week two showed increased WBCs values in both groups compared to other weeks, other increase was also observed in week six but it was not significant in the treated group compared to week four. This may be due to the stress during sample collection, which could be attributed to physiological phenomenon i.e. excitement or strenuous exercise during handling [20].

#### 3.2.1 Lymphocytes ( $10^3 \times \mu\text{L}$ )

Fig. 8 indicated that the Lymphocytes for treated group was ranged 4.75  $10^3 \times \mu\text{L}$  in zero week to 26.75  $10^3 \times \mu\text{L}$  in 6<sup>th</sup> week but it was ranged from 7.39  $10^3 \times \mu\text{L}$  in zero week to 41.05  $10^3 \times \mu\text{L}$  in 6<sup>th</sup> week for control group. These results were indicated that the *N. sativa* oil affected lymphocytes of sheep in the present work by resulting in significant ( $P \leq 0.05$ ) reduction of



total mean value of lymphocytes in the *N. sativa* oil treated group compared to the control group. This result disagrees with previous finding [6]. The lymphocytes were elevated in week two in both groups compared to other weeks, this could also be due to the excitement and stress during sampling. The lymphocytes constituted majority of the WBCs counts and the cells increase with age in early life of animals, this is favored as stated in the finding [20] and it might be attributed to stress and immune response to the environment [20].

### 3.2.2 Monocytes ( $10^3 \times \mu\text{L}$ )

Fig. 9 indicated that the higher value of monocytes for treated group ( $10.4310^3 \times \mu\text{L}$ ) was observed in 2<sup>nd</sup> week and lower value of ( $0.7310^3 \times \mu\text{L}$ ) monocytes was observed in 6<sup>th</sup> week compared with control group, high value of monocytes ( $3.7510^3 \times \mu\text{L}$ ) was noted in 6<sup>th</sup> week and low value of monocytes ( $1.2510^3 \times \mu\text{L}$ ) was noted in 2<sup>th</sup> week. These findings indicate that total mean of monocytes count of the *N. sativa* oil for treated group was not significantly different compared to the control but in week four the treated group showed significantly ( $P \leq 0.05$ ) lower level compared to the control group F. This result contradicted with previous finding [6] but come in line with other work [19]. However a significant ( $P \leq 0.05$ ) increase was observed in group A at week two but followed by significant ( $P \leq 0.05$ ) decrease in week four which decrease further in week six this effect was not observed in the control group.

### 3.2.3 Granulocytes ( $10^3 \times \mu\text{L}$ )

Fig. 10 showed that granulocytes for treated group in zero, 2, 4 and 6<sup>th</sup> week were 1.6, 2.47, 1.17 and  $3.34 \times 10^3 \times \mu\text{L}$ , respectively. While for control group the granulocytes in zero, 2, 4 and 6<sup>th</sup> week were 1.6, 4.77, 1.83 and  $5.13 \times 10^3 \times \mu\text{L}$ , respectively. The total mean value of the granulocyte count in the *N. sativa* oil treated group was significantly ( $P \leq 0.05$ ) lower compared to the control group. This result comes on line with previous study [5] but it is contradicted other work [21]. The pattern followed of the granulocytes count was similar to the behavior of the WBCs and contributed in the total count result.

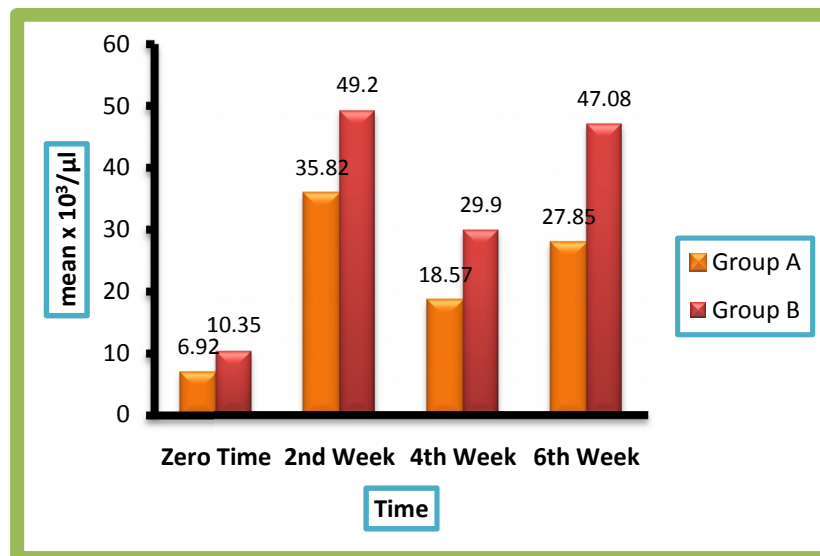


Fig. 7. Effect of feeding *N. sativa* oil on white blood cells in sheep Group A: treated with *N. sativa* oil; Group B: control

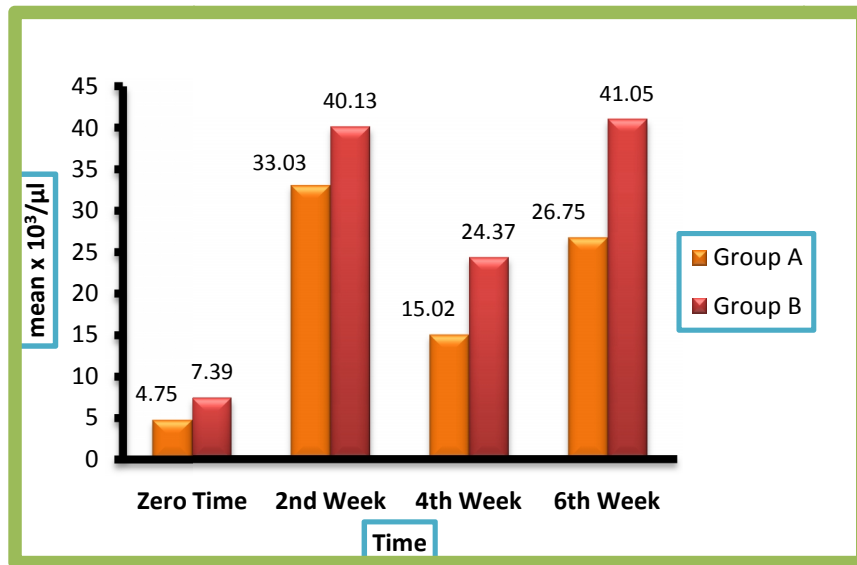


Fig. 8. Effect of feeding *N. sativa* oil on Lymphocytes in sheep Group A: treated with *N. sativa* oil; Group B: control

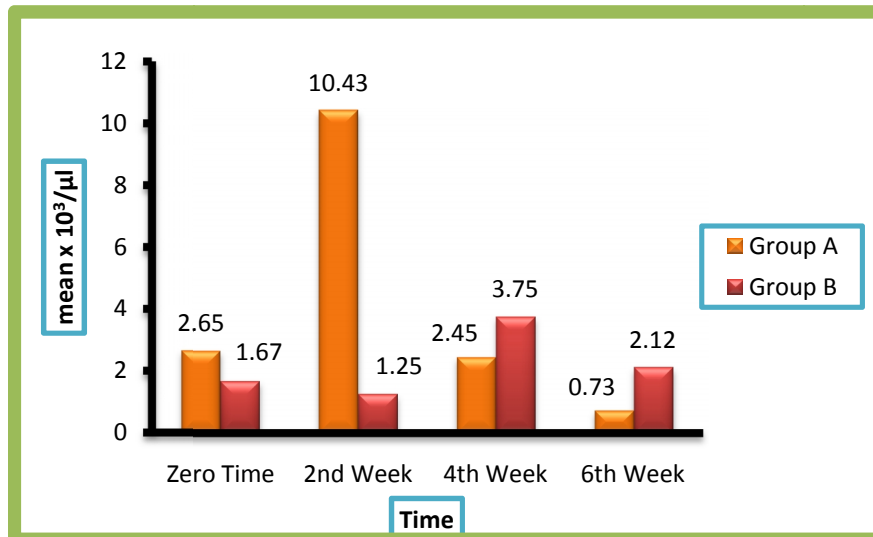


Fig. 9. Effect of feeding *N. sativa* oil on Monocytes in sheep Group A: treated with *N. sativa* oil; Group B: control

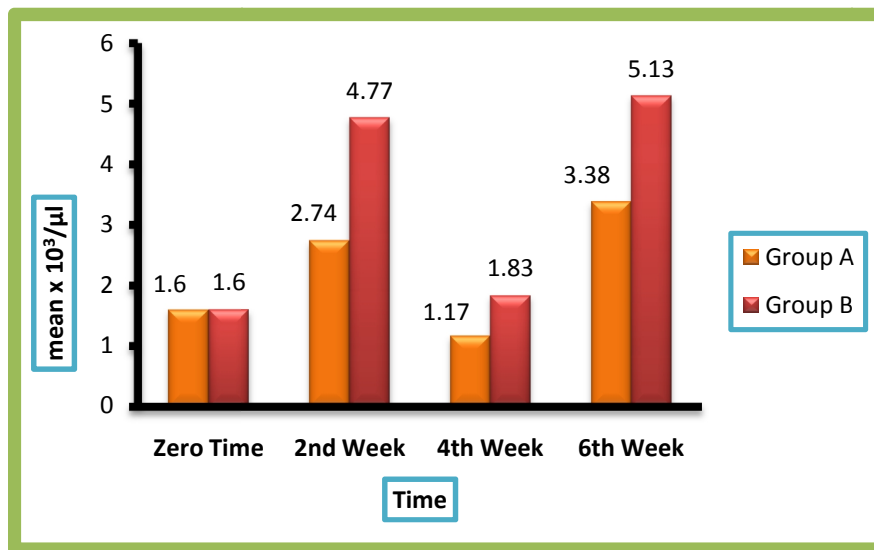


Fig. 10. Effect of feeding *N. sativa* oil on Granulocytes in sheep Group A: treated with *N. sativa* oil; Group B: control

### 3.3 Discussion

Findings in the present study showed that Red Blood Cells, Hemoglobin, PCV, MCV, MCH, and MCHC total mean of the *N. sativa* oil treated group is similar to control group, only Red Blood Cells (RBCs) count of the *N. sativa* oil treated group showed significant ( $P \leq 0.05$ ) increase at week six, whereas no significant change was observed in the control group. Hemoglobin concentration also showed similar pattern to what was observed in the RBC count in both treated and control groups. PCV, MCV, MCH and MCHC weren't affected by feeding *N. sativa* oil to sheep throughout the experiment. The effect of *N. sativa* oil on white blood cells in sheep produced significantly lower values, in the total count of the (WBCs), the lymphocytes and granulocytes of the treated group compared to the control and the count of these cells presented the same pattern in the two groups, that the levels were significantly ( $P \leq 0.05$ ) increased, then significantly ( $P \leq 0.05$ ) decreased and significantly ( $P \leq 0.05$ ) increased by the end of the experimental period. The Monocytes total means was similar in both groups, but the cells were significantly ( $P \leq 0.05$ ) lower in the *N. sativa* oil treated group compared to the control group at week four.

### 4. CONCLUSION

Findings in the present work showed some changes in the blood cell related to the use of the *Nigella sativa* oil and the changes were more pronounced in the white cells, the different effect of this treatment in ruminants was expected, if compared to findings in mono gastric animals, due to the effect of the rumen atmosphere on the digestion of fats. The blood cells changes in sheep here may be mainly due to the effects of *N. sativa* volatile oils.

### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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