College of Science for Girls Dammam

EXPERIMENTAL STUDY ON EFFECTS OF *NIGELLA SATIVA* SEEDS ON SOME BLOOD COMPONENTS AND COAGULATION IN MALE GOATS

(With 9 Figures)

By

EBTESSAM A.Al- SUHIMI, AMAL A. AKBAR and A.M. HOMEIDA*

*College of Veternary Medicine, King Faisal University, al- Ahsa

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التأثير التجريبي لبذور الحبة السوداء على بعض مكونات الدم والتجلط في ذكور الماعز

ابتسام عبد الله السحيمى ، أمال أسعد أكبر ، عبد القادر موسى حميدة

تبحث هذه الدراسة في تأثير بذور الحبة السوداء على بعض مكونات الدم والتجلط في ذكور الماعز. وقد استخدم عدد من ذكور الماعز السليمة من نوع (*Capra hircus*) ذات عمر ١ – ٢ سنة ، ووزن ٢٦ – ٣٤ كجم. حيث قسمت إلى أربعة مجاميع، المجموعة (١) المجموعة الضابطة واستخدم فيها حيوانات غير معاملة، والمجاميع (٢) و (٣) و(٤) أعطيت معلق مائي بذور الحبة السوداء يومياً ولمدة ١٠ أيام عن طريق الفم، وكانت الجرعات على التوالي ١٠٠ مجم / كجم، ٢٥٠ مجم / كجم ، ١ جم / كجم من وزن الجسم. وقد أخذت عينات الدم من الوريد الودجي لتقدير القياسات المدروسة. وقد أظهرت النتائج أن المعلق المائي لبذور الحبة السوداء لم يدون اختلافات في معدلات الهيموجلوبين، العدد الكلي لكريات الدم الحمراء، العدد الكلي لخلايا الدم البيضاء، النسبة المئوية للخلايا المتعادلة، النسبة المئوية للخلايا اللمفاوية ونسبة الخلايا المتعادلة / الخلايا اللمفاوية في المجاميع المختلفة، كما أشارت إلى أن استخدام ١ جم/كجم لم يبرز أي تأثير سيئ ، ولكن حافظ على القياسات في الحدود الفسيولوجية، على الرغم أن ١ جم والتي تساوي ١٠ أضعاف الجرعة الأولى لم تحدث أي تأثيرات سمية. كما لوحظت تأثيرات جيدة معنوية واضحة للجرعات في زيادة عدد الصفائح الدموية مع تناقص في زمن التجلط بطريقة تعتمد على الجرعة وبعلاقة طردية مع مرور أيام التجربة

SUMMARY

This study was done to investigate effects of *Nigella sativa* seeds on some blood components and coagulation. Selected number of clinically healthy male goats (*Capra hicus*) aged 1 – 2 years,

weighing 26 – 34 kg were divided into 4 groups: 1) control group untreated animals, 2), 3), 4) groups were given black seeds suspension daily for 10 days orally as the following doses respectively 100 mg / kg, 250 mg / kg, 1 g / kg of body weight. Blood samples were taken from jugular vein to determine the studied parameters. The results revealed that *Nigella sativa* seeds aqueous suspension didn't change the values of Hb, total RBCs counts, total WBCs counts, neutrophils per cent (N%) lymphocytes per cent (L%) and neutrophil / lymphocytes ratio (N/L) in different groups. It was noticed that the use of 1 g / kg didn't relieve any bad effects but it maintained the parameters at physiological value although 1 g = 10 times the first dose and don't let any toxic effects. On the other hand, it was noticed significantly the clear effects of the seeds with regard to increased blood platelets count which complained with decreased in coagulation time as dose dependent manner in a positive relation with passage of days.

Key words: *Nigella sativa,* Blood components, Coagulation, Male Goats

INTRODUCTION

The red cells are one of the most important elements in the blood. Bone marrow is the main site of its formation. RBCs is very important because of its capacity to carry O_2 and CO_2. Total red blood cells count ranged 13 – 14 millions / mm^3 blood in goats. The WBCs are called wonderfull cells. Because its amoeboid movement, WBCs differ than RBCs which circulate in circulation while WBCs are out the circulation except those across it to began its activity in the tissues. In goats, total WBCs ranged 8 – 12 (10^3 / mm^3 blood) (Schalm *et al.*, 1975).

The platelets are small components (250000 – 500000 plate / mm^3 blood in human), and affected by number of physiological and pathological factors. These platelets are formed during embriogenic phase in liver, spleen, bone marrow. In mammals, the bone marrow is the main source of it. It has ability to phagocytosis. It has important role in coagulation process by agglutination of RBCs surface beside capillaries pores and block it, or by serotinin release which has a vasoconstriction effect. It can accelerate coagulation by thromboplastin enzyme production, the main of this process and it has a role in clot shrinking by retractozyme release (عداي وحنا ١٩٨٧، محي الدين يوسف ١٩٨٧، سلامة ١٩٩٢، الشاعر وآخرون ١٩٩٣، خليفة وآخرون

١٩٩٥ وزايد ومبارك ١٩٩٥، Macsween and Whaley, 1992; Clancy and McVicar, 1995; Ganong, 1995; Marieb, 1997 and Shier *et al.*, 1999).

There are no more studies on the effect of herbal treatment on goat blood thrombocytes. However, some researchers found that black seeds extract has a role in fibrinolysis inhibition in blood and it possess a function in blood coagulation and fibrinolysis in vitro (Ghoneim *et al.*, 1982). Nair *et al.* (1991) found that a given extract of *Crous sativa* stigmas to mice, partially prevented the decrease in body weight, hemoglobin level and leucocyte count, in a dose of 2 mg / kg of cisplastin i.p. for 5 days. Also, prolonged the life span of cisplastin-treated mice almost threefold. An extract of *Nigella sativa* seed only tended to protect from cisplastin-induced falls in hemoglobin levels and leucocyte counts. While, Sallal and Al-Kofahi (1996) found that cinnamonum zeylanicum, *Nigella sativa* and *Quercus aegilops* enhanced red cell haemolysis rather than inhibiting the venom activities.

The aim of this work was to study the effect of administration of aqueous suspension of *Nigella sativa* seeds on some blood cellular components, Hb and coagulation in male goats.

MATERIAL and METHODS

Animals, sixteen clinically healthy male goats (*Capra hicus*) aged 1–2 years and weighed 26-34 kg, were tested experimentally, kept under a normal management system regarding temperature, humidity, food, water ad libitum and vaccination system.

Animal were divided into four equal groups:

1- Control group given water without black seeds.
2- Group 2, given the black seeds in a dose of 100 mg / kg of body weight, daily for 10 successive days.
3- Group 3, given the black seeds in a dose of 250 mg / kg of body weight, daily for 10 successive days.
4- Group 4, given the black seeds in a dose of 1 g / kg of body weight, daily for 10 successive days.

Materials:

The dose were given orally as aqueous suspension, after seeds weight for every animal alone, directly put in 100 ml volumetric flask to prevent a loss of active principals, the volume was completed to 100 ml with distilled water.

Blood sampling:

Blood sampling were collected daily from animals at morning before morning ration (fasting) by jugular vein puncture in ready treated tubes with 1.8 mg heparin per ml of blood.

Determinations:

1- Haemoglobin concentration, RBCs, WBCs counts, differential leucocytic count (Neutrophils, lymphocytes and N/L) and blood platelets were estimated using Clay Adams-logic-800 Haematology Analyzer, Clay Adams, Presipany, NJ. USA.

2-For PCV determination we used MSE microhaematocrit centrifuge, type 346 (MSE Scientific Instruments), (Manor Royal Crawley, Sussex-RH 102 qq, England).

3- Coagulation tine was calculated according to Schalm (1965). Blood samples were collected from ear by sterilized syringe then the blood flowed into capillary tube (15 cm x 1.5 mm) with horzind shape. After every 30 seconds part of tube was broken until fibrin filaments appearance, then record the time.

Statistical methods:

Recorded data were analyzed statistically using Snedecor and Cochran (1980) methods for Mean, ANOVA, LSD and F-degree.

RESULTS

1- **Fig (1);** shows no significant differences in the concentration of Hb between treated groups and control one.

2- **Fig (2);** RBCs count behave no significant differences.

3- **Fig (3);** shows significant decrease in PCV% between control groups allover the days of experiment when compared with the basal level (control level of the first day). In general, the significant differences between the experimental and control groups were observed.

4- **Fig (4);** total white cells count (WBCs), Fig (5); Neutrophil % (N%), Fig (6); Lymphocyte% (L%) and N/L ratio revealed no significant differences between treated and control groups, except at the last three days of experiment where there was clear significant increase in the total L% and N/L ratio of treated groups in comparison to control one.

5- **Fig (8);** platelets count increased in a dose dependent manner in black seeds treated animals if compared to control one.

6- **Fig (9);** coagulation time, in groups treated with *Nigella sative* seeds there were significant dose-dependent decrease in coagulation time compared to control group.

DISCUSSION

The results of control group showed that Hb values revealed no change and there is no significant differences in RBCs, WBCs, N%, L%, N/L, blood platelets, while significant decrease in some values of PCV and coagulation time was detected in treated animals in comparison to the control one. Hb values ranged 10.45 ± 0.06 – 11.10 ± 0.04 g/dl, PCV 32.50 ± 0.29 – 34.75 ± 0.75%, RBCs 14.03 ± 0.03 – 14.95 ± 0.14 x 10^{12} / L, WBCs 10.33 ± 0.19 – 11.20 ± 0.04 x 10^{9} / L, N% 69.93 ± 0.42 – 71.38%, L% 20.00 ± 0.00 – 21.53 ± 0.88%, N/L 3.23 ± 0.05 – 3.53 ± 0.03.

Ashmawy (2000) found that Hb concentration reaches 8.1 ± 0.1 g / 100 ml and 30.8 ± 0.3% for PCV in Ossimi ewes.

Our results are closely similar to (١٩٨٧) محي الدين يوسف also (١٩٩٥) زايد و مبارك who recorded that mean Hb value was 11 g / 100 ml while they found that PCV% was 32% in goats and the count of RBCs in the same animals was 13 – 14 x 10^{6} / mm^{3} and 8000 – 12000 for WBCs, while N% and L% were ranged between 35 – 40% and 50 – 55% respectively.

There was noted that the percentage of L% lesser than N% at $P<0.05$ (present mentioned data). These results may be explained by (١٩٩٥) زايد ومبارك who showed that L% constituted the most ratio of white blood cells in ruminants (75%) but this ratio don't appear in blood but mostly was found in lymphatic tissues.

Our result recorded that coagulation time ranged between 4.08 ± 0.03 to 4.45 ± 0.18 minutes, meanwhile (١٩٩٥) زايد و مبارك found that prothrombin time was 9 – 12 seconds, in domestic animals and 20 – 25 seconds in cows, goats which is different with the present results.

Animals treated with different doses of *Nigella sativa* seeds didn't show significant differences in Hb concentration and RBCs count along the days of treatment when compared with the control groups or the basal level (first day of experiment). Although PCV values were stable in treated groups there are only transient decrease or increase of those control groups at $P<0.05$ which returned to be insignificant at the end of experimental period.

Black seeds were able to increase total WBCs count, neutrophils and N/L ratio compared to control.

These results give good conclusion that the *Nigella sativa* seeds extract has no deleterious effect on studied blood components but it remains at physiological values and levels.

These results were in agreement with Nair *et al.* (1991) and El-Daly (1998) who found that with vitamin E, Saffaron and *Nigella sativa* aqueous suspension able to decrease coagulation time in the same time of platelets increase.

These results are very significant and clear in most days of experimental for almost treated groups. Previous studies proved that coagulation time decreased with an increase of platelets and when the platelets decrease, coagulation time would delayed (١٩٩٢) سلامة.

It is noticed that the decrease in coagulation time, that was accompanied with platelets increase was indirectly related to length of experimental days and dose dependent manner.

This result coincided with Ghoniem *et al.* (1982) who found that there was a ppositive role of *Nigella sativa* seeds on coagulation time by inhibition of fibrinolysis in blood. The authors explained this phenomena which could be caused by fats found in the extract which play a role in bleeding stop mechanism.

In relative to the dose, our results didn't agree with El-Tahrir *et al.* (1993) who found that small doses of *Nigella sativa* has effects better than larger doses of volatile oil of the black seeds on the cardiovascular depressant effect.

This study suggested that: the seeds has a positive significant role in coagulation process and haemostatis, their result perhaps useful for haemophilia.

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Fig (1) : Effects of *Nigella sativa* seeds on Haemoglobin level (Hb) in male goats

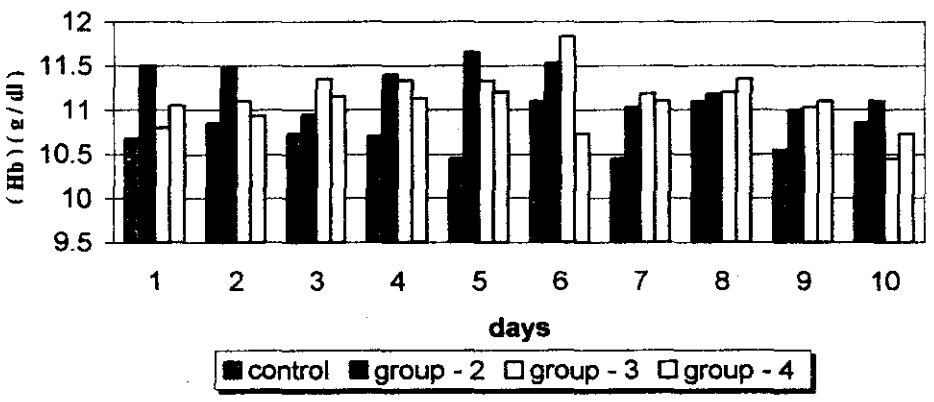

Fig (2) : Effects of *Nigella sativa* seeds on (RBCs) counts in male goats

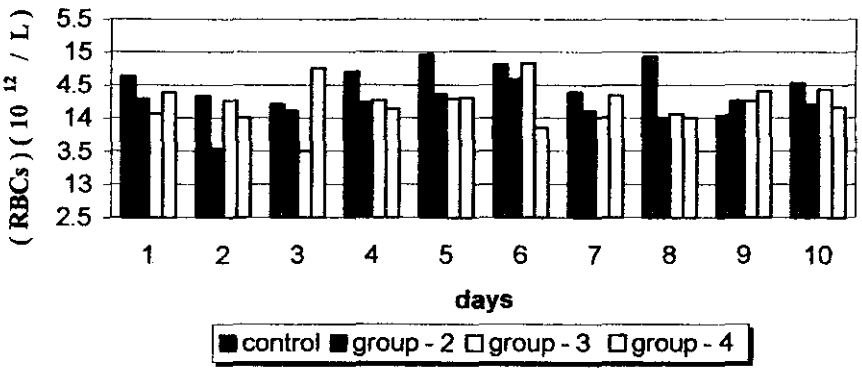

Fig (3) : Effects of *Nigella sativa* seeds on (PCV) % in male goats

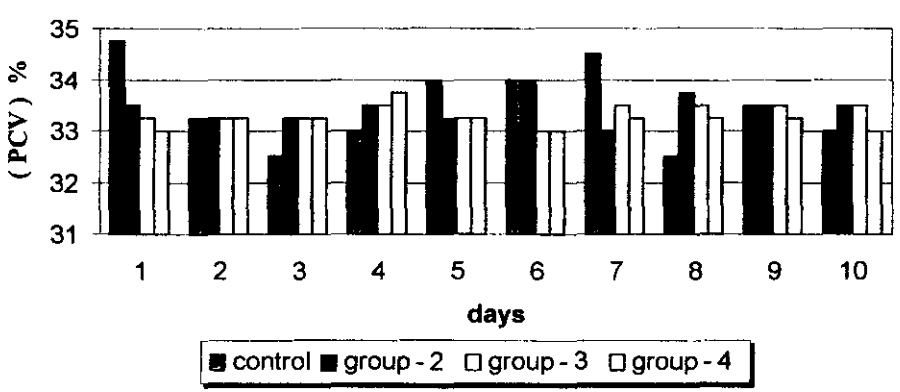

Fig (4) : Effects of *Nigella sativa* on (WBCs) counts in male goats

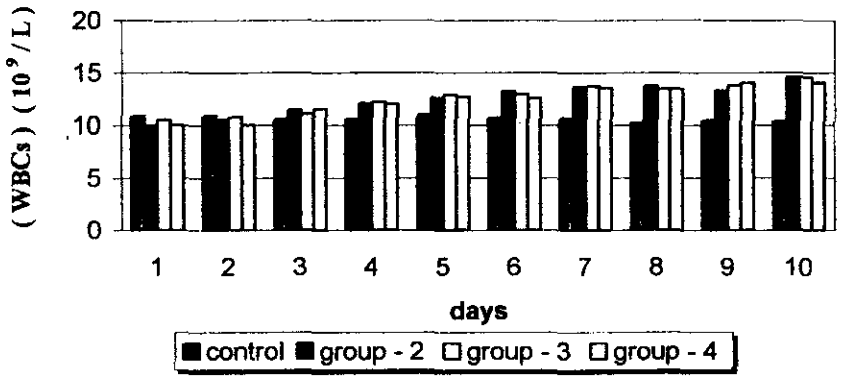

Fig (5) : Effects of *Nigella sativa* seeds on neutrophils (N) % in male goats

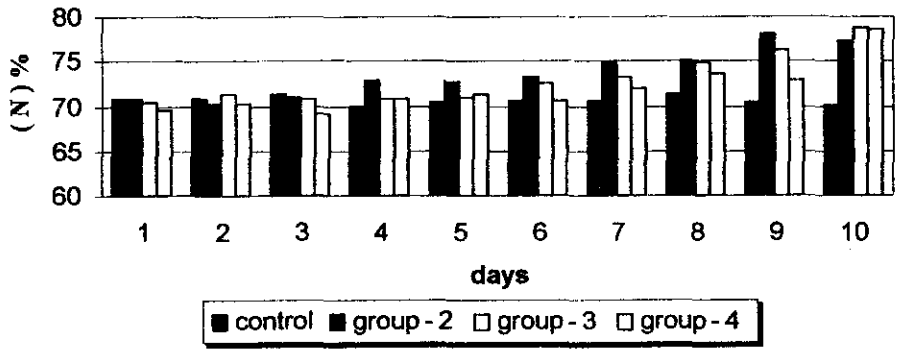

Fig (6) : Effects of *Nigella sativa* seeds on lymphocytes (L) in male goats

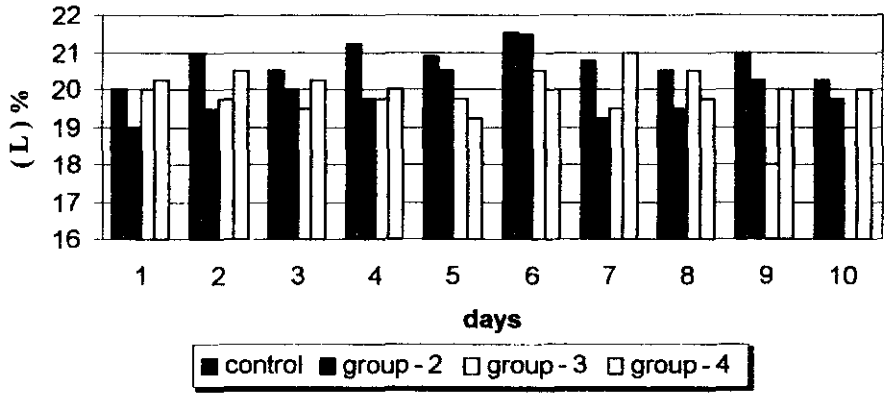

Fig (7) : Effects of *Nigella sativa* seeds on neutrophisl / lymphocytes (N / L) in male goats

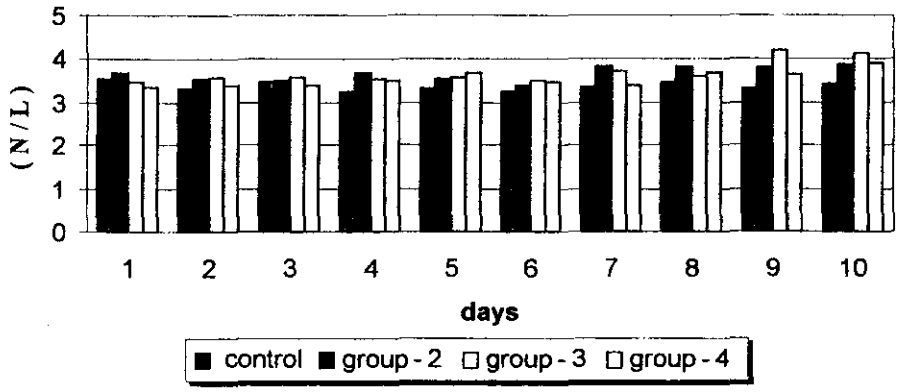

Fig (8) : Effects of *Nigella sativa* seeds on platelets count in male goats

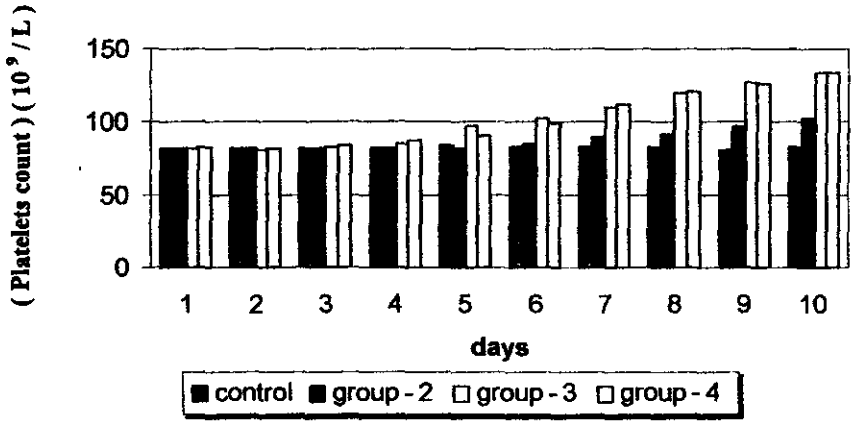

Fig (9) : Effects of *Nigella sativa* seeds on coagulation time in male goats