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Serum Protein Levels and Hematological Values in Rats Fed a Diet Containing Black Cumin Seed

[1]Nabiela M. El Bagir, [1]Rania T.H. El Amin, [2]Ahmed Alhaidary,
[2]Hasab E. Mohamed and [2]Anton C. Beynen
[1]Department of Biochemistry, Faculty of Veterinary Medicine,
University of Khartoum, Khartoum North, Sudan
[2]Department of Animal Production, College of Food and Agricultural Sciences,
King Saud University, Riyadh, Kingdom of Saudi Arabia

Abstract: In this study with rats, the influence of a dietary black cumin seed on both serum proteins and blood cell counts was determined. Rats were fed a diet either without or with 15% black cumin seed for 9 weeks. At the end of the experiment, body weights were similar for the two groups of rats. The diet containing black cumin seed slightly but significantly, lowered the concentration of plasma total proteinsbut did not affect the concentrations of albumin, globulins and hemoglobin. The numbers of white and red blood cells were not affected by black cumin seed in the diet and so was the the composition of white blood cells in terms of neutrophils, lymphocytes and monocytes. After comparing the results of this study with those of studies found in the literature data, it is concluded that black cumin seed has clear effects on serum proteins and blood cells only in rats that are exposed to toxic agents.

Key words: Rats, serum proteins, hematological values, black cumin, lymphocytes, monocytes

INTRODUCTION

Black cumin seed is derived from *Nigella sativa,* a plant of the Ranunculacae family. The seed or its oil fraction is used to treat a wide variety of human diseases such as asthma, inflammatory diseases, diarrhea and hyperlipidemia (Ali and Blunden, 2003). Controlled research with patients is necessary to prove or disprove the assumed therapeutic effects of black cumin seed. Research with laboratory animals is important to unravel the mechanism of action of black cumin seed and to identify its effects on metabolism.

It has shown in rabbits that the inclusion of either 10 or 15% black cumin seed in the feed did not affect serum concentrations of total proteins, albumin and globulins but a level of 20% caused a lowering effect (El Bagir *et al*., 2010). For rats which are used in studies on black cumin seed more commonly than rabbits, the literature data are inconclusive.

The administration of black cumin seeds or oil caused an increase in serum albumin (Al-Jishi and Abuo Hozaifa, 2003; Kocyigit *et al*., 2009) or produced a decrease in serum albumin (El-Daly, 1998; Mohamed and Awad, 2008) and total protein concentrations (El-Daly, 1998; Ekanem and Yusuf, 2008) or had no influence (Abdel-Wahhab and Aly, 2005).

The outcomes of the various rat studies also show inconsistency with regard to hematological values. The administration of black cumin seeds or oil has been found to increase the number of white blood cells (Mohamed and Awad, 2008), to decrease the counts (Zaoui *et al*., 2002a, b) or to have no effect (Abdel-Wahhab and Aly, 2005; Ekanem and Yusuf, 2008; Kocyigit *et al*., 2009; Al-Attar and Al-Taisan, 2010). As to the influence of black cumin seed on the number of red blood cells there are studies that show an increase (Mohamed and Awad, 2008) or no effect (Abdel-Wahhab and Aly, 2005; Ekanem and Yusuf, 2008; Kocyigit *et al*., 2009; Al-Attar and Al-Taisan, 2010).

The literature data mentioned above represent conflicting results on the effect of black cumin on serum proteins and blood cell counts both within and between rat studies. This prompted us to undertake the present study with rats, describing the influence of a high level of dietary black-cumin seed on both serum proteins and blood cell counts.

Corresponding Author: Nabiela M. El Bagir, Department of Biochemistry, Faculty of Veterinary Medicine, University of Khartoum, Khartoum North, Sudan

MATERIALS AND METHODS

Animals and diets: Wistar rats with average body weight of 109 g were used. The rats were housed in cages with wire-mesh bottom. The cages were placed in a room with adequate ventilation and windows transmitting day light. The control diet consisted of sorghum flour enriched with 1% sodium chloride. The experimental diet was a mixture of the control diet and black cumin seed in a 85:15 (w/w) ratio. The two diets were mixed with water and from the dough small balls were formed that were dried subsequently. Table 1 shows the composition of the diets. There were five rats per dietary group. The diets were fed in a restricted amount of 6 g/rat/day. The rats had free access to water. The experimental period lasted 9 weeks.

Chemical methods: Blood samples were taken at the beginning of the experiment and then weekly. Samples were taken by orbital puncture with a capillary glass tube while the rats were under light halothane anesthesia. For each rat, blood was collected into two glass tubes with EDTA as anti-coagulant. Whole blood was immediately used for hematology tests. Plasma was collected by low speed centrifugation and kept frozen at +4°C until protein analyses.

Total protein in plasma was analyzed colorimetrically using copper sulphate as reagent. Plasma albumin was determined with the use of bromocrysol green. Total globulins were calculated as the difference between total protein and albumin. Hemoglobin was measured by matching the brown hematein solution against a glass standard in a comparator block.

The numbers of white and red blood cells were based on the known dilution factor of the blood samples using fluids and counting pipettes. Individual white blood cells were counted in a hemocytometer.

Table 1: Ingredient and calculated macronutrient composition of the experimental diets

	Dietary black cumin	
Diets	0%	15%
Ingredients (g 1000 g)		
Sorghum	990.0	841.5
Sodium chloride	10.0	8.5
Black cumin	-	150.0
Macronutrients (g/100 g)		
Crude protein	9.2	11.0
Crude fat	2.9	7.8
Crude fiber	2.4	2.8
Ash	2.5	2.7
Moisture	12.7	11.6
Carbohydrates (NFE)	70.3	64.1

The composition of sorghum and black cumin seed, respectively was taken to be as follows (g/100 g product): crude protein, 9.3, 21.3; crude fat, 2.9, 35.5; crude fiber, 2.4, 5.5; ash, 1.5, 3.8; moisture, 12.8, 5.5

Data analysis: The data are presented as group means and SD at the beginning of the experiment (Initial) and as within-group averages for the 9 blood samples taken during the experiment (Treatment). Statistically significant differences between group means for the initial and treatment values were identified with the use of Student's t-test. The level of statistical significance was pre-set at $p<0.05$.

RESULTS AND DISCUSSION

Table 2 shows that the control rats were heavier than the test rats at the beginning of the experiment. After the end of the experiment, body weights were similar for the two groups of rats.

The diet containing black cumin seed slightly but significantly, lowered the plasma concentration of total proteins (Table 3). There was no significant diet effect on plasma albumin and globulins. The plasma concentration of hemoglobin was unaffected by the dietary treatment.

In the course of the experiment, the number of white red blood cells fell by on average 10.2% and that of red blood cells by 45.5% (Table 4). The numbers of white and red blood cells were not affected by black cumin seed in the diet. The composition of white blood cells in terms of neutrophils, lymphocytes and monocytes was not influenced by dietary treatment.

The experimental diet was formulated by the addition of black cumin seeds to the sorghum-base diet. As a result, the experimental diet contained more protein and more fat (Table 1). Because the control and test rats were given an identical weight of feed, the latter received

Table 2: Body weights of the rats fed the experimental diets

	Dietary black cumin	
Body weight (g)	0%	15%
Initial	120±20.5	98±7.2*
Week 4	123±19.6	100±8.1*
Week 9	103±28.5	102±10.0

*Significant difference versus control group

Table 3: Response of plasma proteins to dietary black cumin in rats

	Dietary black cumin	
Parameters	0%	15%
Total protein (g/100 mL)		
Initial	7.96±0.30	7.40±0.49
Treatment	9.34±0.15	8.84±0.16*
Albumin (g/100 mL)		
Initial	4.56±0.17	4.02±0.18
Treatment	5.11±0.15	4.91±0.16
Total globulins (g/100 mL)		
Initial	3.40±0.30	3.38±0.61
Treatment	4.23±0.21	3.93±0.24
Hemoglobin (g/100 mL)		
Initial	73.80±1.26	77.60±0.76
Treatment	69.20±2.24	69.70±2.85

* Significant difference versus control group

Table 4: Response of blood cell spectrum to dietary black cumin in rats

	Dietary black cumin	
Parameters	0%	15%
White blood cells (number $\times 10^3$ mL^{-1})		
Initial	7.48±1.06	8.60±1.31
Treatment	7.05±0.97	7.34±0.89
Red blood cells (number $\times 10^6$ mL^{-1})		
Initial	6.23±0.54	5.84±0.69
Treatment	3.31±0.45	3.26±0.41
Neutrophils (percentage of white blood cells)		
Initial	42.0±1.150	43.80±1.21
Treatment	42.6±2.320	43.20±1.72
Lymphocytes (percentage of white blood cells)		
Initial	57.6±1.140	55.40±1.22
Treatment	57.4±2.440	56.70±1.91
Monocytes (percentage of white blood cells)		
Initial	2.0±0.00	1.30±0.30
Treatment	1.2±0.20	1.00±0.20

more dietary energy than did the control rats. The diet with black cumin contained more fat and thus had a higher energy density than the control diet. The control diet contained 9.2% protein which may have supported maintenance (Bricker and Mitchell, 1947). During the course of the experiment, the control rats lost weight, whereas the test rats gained weight. The numbers of white and red blood cells fell in the two dietary groups. In both the control and test rats, plasma total protein concentrations were higher at the end of the experiment than at the beginning. The observations as to the changes in body weight, blood cell counts and plasma protein are difficult to reconcile. In any event, it cannot be excluded that the outcome of the present study was influenced by the differences in nutrient composition between the control and test diet and by opposite weight changes in the control and test rats.

At the end of the experiment, plasma total protein concentrations were on average 5.4% lower in the rats fed black cumin seed. This lowering was statistically significant. No effect of black cumin seed was seen with regard to plasma albumin and globulin concentrations. The observed decrease in plasma total protein agrees with earlier observations in rats. El-Daly (1998) administered an ethanol extract of black cumin seed intraperitoneally at a level of 50 mg kg^{-1} body weight for 5 alternate days. The treatment lowered serum total protein concentration by about 7%. Ekanem and Yusuf (2008) found that the daily intraperitoneal administration of black cumin seed oil (3 mg kg^{-1} body weight) for 8 days diminished serum total protein by 40%.

Feeding the diet with 15% black cumin seed to the rats did not influence the counts of white and red blood cells. This observation corroborates the outcome of various previous rat studies with different experimental designs. Adbel-Wahhab and Aly (2005) administered *Nigella sativa* oil orally at a level of 5 mg kg^{-1} body weight for 30 consecutive days. Ekanem and Yusuf (2008) applied daily intraperitoneal administration of black cumin seed oil at a level of 3 mg kg^{-1} body weight for 8 days. Kocyigit *et al.* (2009) fed diets containing powdered black cumin seed up to amounts equivalent to 600 mg seed kg^{-1} body weight. Al-Attar and Al-Taisan (2010) prepared an ethanol extract from black cumin seed and used oral supplementation at a daily dose of 300 mg kg^{-1} body weight for 6 weeks. In the four published studies the treatment with black cumin left the numbers of white and red blood cells unchanged. In this study, the rats fed the diet with 15% black cumin seed had daily intake of whole seed and oil of about 9000 and 3195 mg kg^{-1} body weight. The dose in the present study was much higher than that in the four studies showing that black cumin did not influence the numbers of white and red blood cells. This reinforces the lack of effect in this study.

Various rat studies have addressed the question whether the administration of black cumin seed or oil was able to antagonize a specific toxic influence. *Nigella sativa* oil has been reported to counteract the decrease in white and red blood cells as induced by *Trypanosoma brucei* infection (Ekanem and Yusuf, 2008). The aflatoxine-mediated lowering of serum albumin and white and red blood cells was partly restored by black cumin oil (Abdel-Wahhab and Aly, 2005). Black cumin seed also protected against the cisplatin-induced lowering of serum albumin and number of white blood cells (El-Daly, 1998). The effects of diazinon on white and red blood cells were antagonized by an extract of black cumin seed (Al-Attar and Al-Taisan, 2010). Likewise, black cumin seed extracts were able to counteract the decrease in white and red blood cells as induced by exposure to carbon tetrachloride (Meral and Kanter, 2003; Abuelgasim *et al.*, 2008), cadmium chloride (Demir *et al.*, 2006) or aluminum chloride (Mohamed and Awad, 2008).

In the rats fed black cumin seed, blood hemoglobin levels were not changed. In contrast, Zaoui *et al.* (2002b) showed in rats that a daily oral dose of 2 mL *Nigella sativa* oil/kg body weight for 12 weeks significantly raised hemoglobin concentrations. Black cumin seed extracts have also been shown to counteract the decrease in hemoglobin as induced by treatment with either cisplatin (El-Daly, 1998) or carbon tetra chloride (Meral and Kanter, 2003).

CONCLUSION

This study with rats fed a diet containing whole black cumin seed contributes to the data published elsewhere. It was found that black cumin seed slightly lowered serum total proteins and did not affect the numbers of white and red blood cells. In the light of the literature data

mentioned above, it would appear that black cumin seed has clear effects on serum proteins and blood cells only in rats that are exposed to toxic agents.

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