

Clinicopathological effects of *Nigella sativa* (kalonji) seeds in *Escherichia coli* infected *Psittaculakrameri*.

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Abstract

The clinicopathological effects of *E. coli* infected *Psittaculakrameri* (ring necked parrots) supplemented with *N. sativa* seeds in feed were evaluated. Faecal samples from "psittacine" species parrots were collected and *E. coli* was isolated for cultural, morphological, biochemical and in-vitro studies. A total of 27 parrots were selected and divided into 03 groups (A= control, B= no treatment and C= treatment+ infection) having 09 parrots in each and were fed with *N. Sativa* seeds @100mg/kg from 3rd day. The parrots of group B and C were challenged with 0.1ml pathogenic *E. coli* @10⁶CFU/ml/bird subcutaneously on day 9th. Clinical signs, mortality and morbidity rates were observed during the trial. For haematology, histopathology and CRP in sera, three birds were slaughtered weekly from each group. Significant decreased in feed consumption, haematological values like red blood cells, haemoglobin and pack cell volume while significant increase in clinical signs, morbidity, mortality, liver, heart, kidneys weights and white blood cell were recorded in group B. C-Reactive protein values was significantly decreased in group C as compared to group B while degenerative changes were seen in liver, heart and kidneys of group B.

Introduction

Parrots are among the most distinguishable and widely distributed of all the birds, referring to more than 350 species of the order Psittaciformes occupying major parts of the tropics (Snyder and McGowan, 2000). Several species of parrots are kept as pets because of their friendly and loving nature, ability to emulate human voices, phenomenal feather coloration and intellect (Gill, 1997). The Rose-Ringed Parakeet (*Psittaculakrameri*) belongs to the family Psittacidae and it is native to Africa and India. Its size is 38-42 cm as it is a medium sized green bird and its tail accounts 25 cm which is more than half of its length immature birds are difficult to differentiate from females as these juveniles begin developing of red ring when they are three years old. Four sub-species of *Psittaculakrameri* are currently recognized as *P. k. krameri*, *P. k. parvirostris*, *P. k. borealis* and *P. k. manillensis* (Butler, 2003). They can take a large variety of feed but usually prefer fruits, vegetables, seeds, cereal grains and oil seed crops (Khan and Ahmad, 1983). *E. coli* is classified into its pathotypes by the help of genes which are responsible for their expression of virulence factors. Avian pathogenic *E. coli*, Enteropathogenic *E. coli* and Uropathogenic *E. coli* are the most commonly described pathotypes of *E. coli*. APEC is famous for providing massive economic losses to the poultry sector resulting in septicemic and respiratory diseases, while EPEC causes diarrhoea in infants (Nataro and Kaper, 1998). UPEC is responsible for urinary diseases in humans (Johnson et al., 2000). A large number of medicinal plants and their constituents are known for their therapeutic properties. *Nigella sativa* (*N. sativa*) is a medicinal plant, belongs to family Ranunculaceae. The seeds of *N. sativa* are the source of active ingredients of this plant and these are being employed for thousand years. The seeds of *N. sativa* and their oil have been widely used in the treatment of various disorders throughout the world for centuries and it is an important drug in traditional medicine system. The seeds of *N. sativa* contain pharmacologically active substances like thymoquinone (30-48%), dithymoquinone (7-15%), thymohydroquinone (1-4%), carvacol (6-12%) and thymol. It also contains large amount of fixed oils, proteins, alkaloids, essential oil, mineral elements, carbohydrates, proteins and fats. Thymoquinone is the most active component largely considered for therapeutic ability of *N. Sativa* (Ghosheh et al., 1999). This plant has been suggested to possess many properties like antimicrobial, antioxidant, anti-inflammatory, antihistaminic, antitumor, antidiabetic, spasmolytic, anticancerous, bronchodilator, hepato-protective, renal protective, gastro-protective, immunomodulatory etc. The photochemical analysis of *N. sativa* shows that the plant is safe to use and possess harmless effects (Ali and Blunden, 2003).

Ethical Approval

The study was approved by University ethical Committee to ensure that no harm is provided to the animals.

Materials And Methods

Bacterial isolation

A total of 12 faecal samples were taken from Jhang Bazar, Faisalabad 38000, Punjab, Pakistan derby and Ghulam Muhammadabad Faisalabad 38000, Punjab, Pakistan derby. Using standard measures Bacteria needed for the research was obtained from the faecal sample of *Psittacine* family in a series of steps i.e. *Escherichia coli* (*E. coli*). Instantaneously, after the collection of faecal swab, these swabs were inoculated in a liquid medium named as nutrient broth and kept for 24 hours at 37°C for enrichment of these samples. Following the enrichment of the faecal swabs in the nutrient broth, swabs were streaked on the nutrient agar. Swabs were further purified on the surface of MacConkey Agar to obtain pink coloured colonies of *E. coli*.

Identification and biochemical characterization of *E. coli*

Probable identification of *E. coli* was performed using gram's staining and biochemical tests. Slide was observed under the microscope to observe the colonies of *Escherichia coli* at 40X. Citrate test, Indole test, Lactose fermentation and Methyl red test were applied for *E. coli* confirmation. Determination of pathogenicity by Congo red dye by method described by (Ali and Blunden, 2003).

Management and procurement of Parrots

A total of 27 parrots were purchased from local derby of district Faisalabad approximately of same age and they were kept in cages under proper standard managerial conditions. Parrots were divided into 3 different groups A, B and C from 3rd day of experiment and were inoculated with 0.1ml 10⁶ CFU of Avian Pathogenic *E. coli* (APEC) subcutaneously to group B and C at day 9 of experiment and group A and B were kept separate to avoid contamination.

Treatments of different groups

Parrots were divided into 3 groups from day 3rd day of the experiment group A was kept as control positive group in which parrots were not given any infection and treatment group B was kept as control negative in which parrots were infected with *E. coli* and not received and treatment and Group C was

supplemented with *N. sativa* at 100mg/kg in feeds. Parrots of group B and C were challenged with pathogenic *E. coli* at 9th day of experiment. About 3 birds were slaughtered at weekly basis and blood was collected from the jugular vein from which serum was separated for determination of CRP and Liver, Heart and Kidneys were collected and weighed and organs showing any abnormality were preserved in neutral buffered formalin for histopathology (Bancroft and Gamble, 2008). (Table 1)

Hematological parameters such as RBC, WBC, Hb and PCV were studied following (Benjamin, 1978). While absolute and relative weight of internal organs (liver, heart and kidney) were performed and concentration of C-reactive protein was measured according to (Singer et al., 1957). Histopathology was performed following technique described by Bancroft & Gamble (2008). Data thus obtained was analysed statistically using two-way ANOVA and DMR tests.

Results And Discussions

Cultural properties of *E. coli*

Test for presence of *E. coli* were performed by culturing on various growth media it showed white, greyish and opaque colonies on nutrient agar while on MacConkey agar it showed dark pink coloured colonies which were indicative for *E. coli* in samples

Morphological characteristics

Microscopic examination of Gram's stained smear of these bacteria revealed Gram negative rod shape with peritrichous flagella.

Biochemical Tests

Standard biochemical tests were performed to check the presence of *E. coli* where observation of red coloured ring in Indole positive test was noticed for *E. coli* while shift of red colour of methyl red to yellow was observed indicating presence of *E. coli*. Green color during Simmons citrate negative test and immediate bubble formation (within 45 Seconds) in catalase positive test

In-vitro pathogenicity test of *E. coli*

In-vitro pathogenicity testing of *E. coli* was performed by Congo red dye binding test. Appearance of red colour colonies indicated the presence of pathogenic *E. coli*.

Morbidity and Mortality Rate

Marked variation in days of post infection. Morbidity percentage was 75% and mortality was 50% in group B. The parrots of group C were challenged with *E. coli* and supplemented with *N. sativa* seeds in feed. Decreased morbidity percentage was seen in this group as compared to group B. Morbidity percentage was 37.5% and mortality percentage was 25% in group C.

Haematological parameters in *E. coli* infected *Psittaculakrameri* supplemented with *N. sativa* seeds in feed.

In this trial, total erythrocyte count was significantly decreased in parrots of group B as compared to that of group A and C. Total erythrocyte count of parrots of group A and C were non-significantly different from each other as given in table (2).

Total leukocyte count was significantly increased in parrots of group B as compared to that of group A and C. Total leukocyte count of parrots of group A and C were non-significantly different from each other as given in table (2).

Hemoglobin (Hb) concentration (g/dl)

Hemoglobin concentration was significantly decreased in parrots of group B and there was non-significant difference among values of hemoglobin of parrots of group A and C (Table 2).

Packed cell volume percentage (%)

Packed cell volume also decreased significantly in parrots of group B as compared to that group A and C. Packed cell volume of parrots of group A and C were non-significantly different from each other.

Absolute and relative weight of visceral organism *E. coli* infected *Psittaculakrameri* supplemented with *N. sativa* seeds in feed.

Absolute and Relative weight

After *E. coli* infection, there was a significant increase in absolute weights of liver, heart and kidneys of parrots in group B as compared to that of group A and C in this trial. The absolute weights of organs of group C parrots were also significantly higher than group A parrots.

After *E. coli* infection, there was a significant increase in relative weights of liver, heart and kidneys of parrots in group B as compared to that of group A and C in this trial. The relative weights of organs of group C parrots were also slightly higher than group A parrots.

C - reactive protein (CRP)

In this experimental trial, CRP concentration was significantly increased in parrots of group B and C as compared to that of group A. The CRP concentration in parrots of group C was significantly decreased as compared to that of group B.

Histopathology

In *E. coli* infected group of parrots, hydropic degeneration of hepatocytes with karyolysis of nuclei was observed. At some places, blood vessels were congested. Severe sloughing of cuboidal epithelium with necrotic changes in convoluted tubules and atrophied Bowman's capsule were seen in *E. coli* infected group of parrots. Accumulation of inflammatory cells and loss of striations were seen in *E. coli* infected group of parrots (Figure 1).

Conclusion

In the current study, it was concluded that *N. sativa* seeds supplementation @ 100 mg/kg in feed had antibacterial action against *E. coli* infection in parrots as it reduced morbidity and mortality rate. The decrease in CRP concentration also showed antibacterial activity of *N. sativa* seeds. It has no deleterious effects on hematological parameters and physiology of major body organs (liver, heart and kidney) of parrots.

Declarations

Authors' Contribution

MW, SN and MA designed the idea, performed experiments and wrote the article. FUR and MSI did analysis and reviewed the article FAK, MKK and MS collected and processed the samples.

Novelty Statement

To the author's knowledge limited research work has been done on *Nigella sativa* (kalonji) seeds in *Escherichia coli* infected *Psittaculakrameri*, so it is a new era to find the effect of *Nigella sativa* (kalonji) seeds in *Escherichia coli* infected *Psittaculakrameri* to reduce the dose, cost and side effects of the drugs.

Data availability statement

The Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

Statement of conflict of interest

The authors have declared no conflict of interest.

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Tables

Table 1. Experimental trail showing different treatment groups

Groups	Total No. of exp. birds	Given Treatments
A	9.0	Control Negative (No given treatment + No Vaccination)
B	9.0	Control Positive (Infection with pathogenic strain of <i>E. coli</i> + Vaccination performed)
C	9.0	<i>N. sativa</i> seeds fed at a ratio of 100 mg in their feed + Infection given with pathogenic <i>E. coli</i> strain + Vaccination performed)

Table 2: showing Variation in Different haematological parameters in various groups

Days post infection	A			B			C		
	RBC Count	WBC Count	Hb Concentration	RBC Count	WBC Count	Hb Concentration	RBC Count	WBC Count	Hb Concentration
7	2.9±0.29a	19.75±1.13b	11.90±1.33a	1.63±0.40c	36.83±3.00a	9.16±0.75c	2.59±0.25a	16.04±2.30b	11.05±0.85a
14	2.87±0.29a	22.50±2.15c	12.10±1.05a	1.58±0.30b	41.66±9.14a	8.76±0.69b	2.76±0.35b	16.30±1.28c	10.64±0.64a
21	2.83±0.17a	20.80±2.58c	11.70±1.69a	1.57±0.33c	36.08±4.08a	8.00±0.72c	2.59±0.38a	14.16±1.16c	10.81±0.18a

A = Control negative (no treatment, no infection), B = Control positive (*E. coli* infection 10⁶CFU/ml), C = Infection + Treatment (*N. sativa*@100mg/kg in feed).

Figures

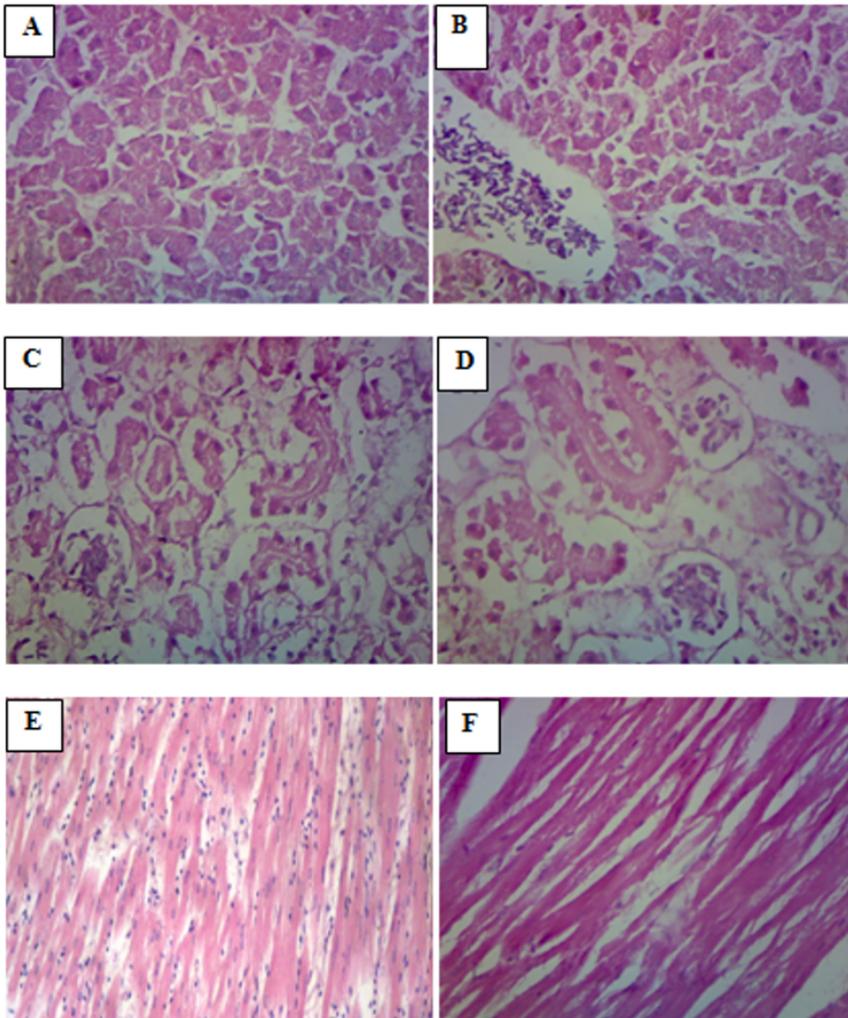


Figure 1

(A) Photomicrograph of liver showing degenerative changes in *E. coli* infected parrots. (B) Photomicrograph of liver showing hydropic degeneration and congestion in *E. coli* infected parrots. (C) Photomicrograph of kidney showing sloughed epithelium and necrotic changes in *E. coli* infected parrots. (D) Photomicrograph of kidney showing atrophied Bowman's capsule in *E. coli* infected parrots. (E) Photomicrograph of heart showing accumulation of inflammatory cells. (F) Photomicrograph of heart showing loss of striations in *E. coli* infected parrots.