Comparative Anthelmintic Efficacy of *Caesalpinia Crista*, *Nigella Sativa* and Oxfendazole in Broilers with Experimentally Induced *Ascaridia Galli* Infection

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Abstract The study was conducted to explore the effect of two herbal drugs, *Caesalpinia crista* and *Nigella sativa* in comparison with Oxfendazole in broilers experimentally induced with *Ascaridia galli* infection. A total of 130 day old broiler chicks were divided into five groups i.e. A, B, C, D and E each group having 26 birds. Group E was kept as negative control, while all other groups were induced with experimental *Ascaridia galli* infection on 15th day. Group A was kept as positive control and groups B and C were treated with *Caesalpinia crista* and *Nigella sativa* as methanolic extract orally once @50mg/kg body weight and group D was medicated with Oxfendazole @10mg/kg body weight orally once on 25th day post infection. On 25th day post infection before treatment 4 birds from each group were slaughtered, and worm count was done. The efficacy was confirmed by counting the worm, slaughtering all the bird on 30th day post infection. The mean worm count was significantly different ($p < 0.05$) between the treated and non treated groups. The efficacy of the plant extracts were less as compare to Oxfendazole. The efficacy of *Caesalpinia crista* and *Nigella sativa* was 70.83% and 57.5% respectively while the efficacy of Oxfendazole was 90.83%. In vitro treatment at $37 \pm 1^\circ\text{C}$ revealed that a serial dose of the plant extract caused dose-dependent vermifugal effect on the worm (significant efficacy at $P < 0.05$), except at the lowest dose (i.e. 5 mg/mL). The efficacy of drugs was calculated on the basis of reduction in total worm count after treatment.

Keywords Anthelmintic, Broiler and Methanol Extract

1. Introduction

The increasing demand on poultry products from backyard and commercial poultry systems is restricted by the heavy burden of different poultry parasites which are emerging in such production systems (Fossum et al.[17]). There are emerging several parasitic infections, like

Ascaridia, *Heterakis*, *Subulura* and *Strongyloides*. *Ascaridia galli* is the most prevalent helminth species in different poultry production systems (Permin et al.[34]; Abdelqader et al.[2]; Kaufmann et al.[27]). Heavily infected chickens may damage the integrity of intestinal mucosa and nutrient utilization can be hampered which decrease weight gain (Kilpinen et al.[29]; Das, et al.[14]). Economic losses are usually associated with *Ascaridia galli* infection because of treatment cost and impair performance (Ruff.[36]; Martin-Pacho et al.[32]). The chief economic importance lies in the ability of *A. galli* to act as a vector for transmission of other infectious organisms like *Salmonella enterica* and *Escherichia coli* (Chadfield et al.[13]; Permin et al.[35]).

Despite remarkable achievements in the discovery and improvement of pharmaceutical anthelmintics, diseases due to nematode infections continue to be the greatest constraint in sustainable livestock production worldwide, primarily due to rapid evolution of drug resistance in these parasites to all classes of anthelmintics (Stear et al.[42]). *A. galli* is a roundworm parasitizing the small intestine of birds, and is by far the most prevalent of all helminths infecting poultry (Permin et al.[34]). *A. galli* infections continue to be the most debilitating factor impeding poultry productivity resulting in retarded growth, weight loss, diarrhea, poor absorption of nutrients, death and even the spread of fatal bacterial infections (Gauly et al.[19]).

The development of drug resistant helminths against chemotherapeutical products and the associated risks of chemical residues in poultry products have drawn the attention to alternative approaches. Different approaches were employed to sustainable control of *A. galli*, for example, utilization of genetic resistance (Gauly et al.[18]; Abdelqader et al.[1]; Kaufmann et al.[26]), nutrition of host animal (Das, et al.[14]), biological control (Braga et al.[11]), and the use of plants with anti-parasitic properties (Siamba et al.[40]; Kholhring et al.[28]). For centuries, medicinal plants have been used to combat parasitism, and in many parts of the world. The nicker nut *Caesalpinia crista* L. (*Caesalpiniaceae*), a perennial climbing shrub, which is

distributed worldwide in tropical and subtropical regions such as southern and western Africa and the Indo-Pakistan subcontinent, and is native in tropical Asia. The leaves have an antifilarial effect (Datt'e et al.[15]) and the seeds are also considered to be effective for the treatment of helminths of sheep (Akhtar and Aslam.[6]). *Caesalpinia crista* seed powder at the dose rate of 50 mg/kg body weight, its equivalent methanolic extract and piperazine at dose rate of 200 mg/kg body weight have similar effectiveness in treating the Ascarid infection of poultry (Javed et al. [25]). *Nigella sativa* is an annual herb of the Ranunculaceae family, which grows in countries bordering the Mediterranean Sea, Pakistan and India. This widely distributed plant is native to Arab countries and other parts of the Mediterranean region. *Nigella sativa* have shown efficacy against ovine and caprine helminthiasis in the form of reduction of faecal egg counts without any side effect (Akhtar.[5]). The extracts of *N. sativa* seeds have been used by patients to retard the carcinogenic process (Hassan and El-Dakhkhny.[22]; Worthen et al.[44]), treat abdominal pain, diarrhoea, flatulence and polio (Enomoto et al.[16]), exert antiinflammatory (Chakravarty.[12]; Houghton et al.[24]) and antioxidant effects (Mansour et al.[31]). Besides, the essential oil was shown to have antinematodal (Akhtar and Riffat.[7]), antischistosomal (Mahmoud et al.[30]), antimicrobial (Hanafy and Hatem.[21]; Aboul-Ela et al.[3]) and antiviral (Salem and Hossain.[37]) effects.

The present study encourages the possible use of indigenous plants for the treatment of *Ascaridia galli* infection in poultry, because these indigenous drugs are cheaper and easily available.

2. Materials and Methods

Feeding, management and grouping of birds

A total of one hundred and thirty day-old broiler chicks were procured from a local hatchery. The birds were reared under standard environmental condition for 45 days. The birds were fed with a local poultry feed named Big Bird feed. The birds were divided randomly into five groups namely A, B, C, D and E. Each group had 26 birds. Group A and E were positive and negative controls while group B, C and D were treated with *Caesalpinia crista*, *Nigella sativa* and Oxfendazole respectively.

Source of Infection

Infected intestinal contents were collected from the poultry meat sale shops, poultry farms (layer farms) and hotels where birds were slaughtered. These intestines were processed in the laboratory of the Department of Clinical Medicine and Surgery, University of Veterinary and Animal Sciences, Lahore, Pakistan. On 15th day of experiment, four groups were infected orally with *Ascaridia galli* while group E was kept as control. On 25th day post-infection before treatment, four birds from each group including control were

randomly selected and slaughtered. The intestines were examined by setting and decanting method. The worms obtained from the birds of each group (except non-infected non-medicated) were counted (Ashraf.[10]). After the confirmation of development of the infection on the 25th day post infection, all the birds of the group B and C were treated orally with *Caesalpinia crista* and *Nigella sativa* at the dose rate of 50 mg/kg each. Group D was fed Oxfendazole at the rate of 10 mg/kg body weight in drinking water. At 30th day post-infection, all birds were slaughtered and above mentioned procedure were adapted to count worms.

Collection of Worms

Mature worms were collected from the intestines of naturally infected chicken. Adult female were identified on the basis of size and absence of spicules (Souslby.[41]).

Collection of Experimental Ova

Ova of the worms were obtained by splitting open the female worms to liberate eggs. Uteri were chopped with scissors and water was added to make the suspension. The suspension was poured through wire mesh screen and material on the screen was discarded. The suspension was again poured through a wire mesh screen. The eggs were resuspended in a 2% formalin solution to prevent contamination as described by Permin et al.[33].

Preparation and Administration of Herbal Drugs

Seeds of *Caesalpinia crista* and *Nigella sativa* were purchased from local herbal market of Lahore then these seeds were dried in a hot air oven at 50°C. The dried seeds were macerated to fine powder and then refluxed with methanol (100 g/L) for 8h at 60°C. The solution obtained was filtered through Whatman filter paper (No. 1) and the solution was evaporated to complete dryness at 50°C. The methanol extract was obtained as a deep brown powdered material, which was then refrigerated at 4°C until further in vitro use. 1 h prior to *in vitro* assay, a stock concentration of the extract, 80 mg/mL, was prepared by dissolving in 0.9% neutral phosphate-buffered saline (PBS, pH~ 7.1) supplemented with 1% dimethylsulfoxide (DMSO). Varying doses, viz. 5, 10, 20 and 40 mg/L, were then prepared by serial dilution of the stock solution using PBS with DMSO. The methanolic extract of *Caesalpinia crista* and *Nigella sativa* powder was prepared using a Soxhlet apparatus (Javed.[25]) for *in vivo* use. The evaporated extract representing 50 mg/kg body weight of powder was administered orally to chicks individually (Akhter.[5]).

Synthetic Drug "Oxfendazole" (Systamex ICI Pakistan Ltd.)

Oxfendazole was medicated in the form of drench given per oral solution at the dose rate of 10 mg/kg body weight (Georgi et al.[20]). The reductions in the worms count and body weight gain were recorded for each group.

Statistical Analysis

The data was presented by mean and standard error of means. Increase or decrease in worm count was presented by percentage. The data collected from this experiment were statistically analyzed by applying analysis of variance test and significance difference was determined using Least Significant Difference (Steel and Torrie.[43]).

3. Results and Discussion

Ascaridia galli in the control experiment survived for 97.73 ± 0.90 h in a medium composed only of PBS with 1% DMSO. The nematodes thrived relentlessly, but once their movement ceased, death ensued abruptly. Figure-1 Presents the response in physical activity of the nematodes after treatment with a broad-spectrum antiparasitic drug, Oxfendazole, and the extracts of *Caesalpinia crista*, *Nigella sativa* at the doses of 5, 10, 20, 40 and 80 mg/mL, respectively. Oxfendazole was found to be a highly effective nematocide exerting profound dose-dependent activity at all doses tested. The plant extract also indicated concentration-dependent efficacy on the nematode. However, at the lowest concentration, i.e. 0.5 mg/mL, the nematodes did not show any significant mortality with respect to the control, although average death was observed earlier than those in control group. Figure 1 shows the comparative efficacy of the drug and the plant extract, depicting dose-dependent activity for both cases.

In our vivo experiment, *Caesalpinia crista* seed powder was fed to the birds on 25th day post-infection. At 30th day post-infection, the anthelmintic efficacy of *Caesalpinia crista* was found to be 57.05%. Shaker et al.[39] reported that *Caesalpinia crista* at the dose rate of 0.4g/kg body weight reduced worm count by 96.07% in turkey pullets against intestinal nematodes. Similarly, Akhtar.[8] showed that

anthelmintic efficacy of *Caesalpinia crista* at the dose rate of 50 mg/kg body weight against *Ascaridia galli*. The findings of our study are in contrast to the results of Javed et al. [25] and Akhtar. [5]. The differences in the results may be due to difference in experimental procedures, dose rate, and infectivity. The drug efficacy of *Nigella sativa* was found to be 56.59%. Akhter et al.[5] reported that *Nigella sativa* reduced ovine and caprine helmenthiasis without any side effects. Similarly, Aboul Ela and Al-Homidan et al.[4] reported that *Nigella sativa* reduced fecal count by 88% and 86% respectively.

The body weight of different experimental groups A, B,C,D & E were recorded and results shown that there was no significant difference in weight gain amongst treated groups on 15th & 30th day post infection, while there is significant difference in weight gain of infected non medicated group and medicated groups (Table-2). These body weights completely correlates with the results of Shaker et al 2011 where he reported that alendazole and caesalpinia crista treated groups had no significant body weights difference at 28th and 42 days of age in turkey poultts infected with heterakis gallinarium, body weights at 28th and 42 days of age were 947.33 ± 8.09 , 951.27 ± 8.45 and 2235.33 ± 7.41 , 2292.34 ± 7.79 respectively. body weight was significantly different in infected non medicated group and body weights were 757.47 ± 2.82 and 1392.00 ± 15.19 at 28th and 42 days of age. ABZ and ACEA treatments exerted beneficial effects by significantly improving body weight gain and feed conversion ratio values compared to the positive control group. Moreover, ACEA significantly ($P \leq 0.05$) enhanced growth performance of turkey poultts than that of ABZ. Comparable to our results, A. herb-alba improved the growth performance of chickens infested with *A. galli* (Seddiek et al.[38])

Table 1. Mean worm count after infection and before treatment on 25th day and 30th day post

Groups	Post infection (25 th day)	Post treatment (30 th day)	Percent decrease or increase
A	23±8	33±8	30.29 (Increase)
B	14±7	6±3	57.05 (Decrease)
C	32±9	14±6	56.59 (Decrease)
D	45±8	6±4	87.50 (Decrease)
E	0±0.00	0±0.00	0.00 (Decrease)

A: Infected nonmedicated B: *Caesalpinia crista* C: *Nigella sativa*
D: oxfendazole E: Non infected non medicated

Table 2. weight gain in broilers after infection and treatment

Groups	Post infection (15 th day)	Post treatment (30 th day)
A	795 ± 15.83^d	1720 ± 22.5^c
B	975 ± 16.83^b	1889 ± 11.9^b
C	945 ± 14.83^c	1846 ± 21.15^b
D	982 ± 12.83^b	1897 ± 11.94^b
E	1060 ± 18.83^a	1960 ± 12.96^a

^{a-d}Mean values having similar alaphabets differ significantly ($P < 0.05$)

A: Infected nonmedicated B: *Caesalpinia crista* C: *Nigella sativa*
D: oxfendazole E: Non infected non medicated

Figure 1. Comparison of the Efficacy of Oxfendazole and the Extract of *Caesalpinia Crista* and *Nigella Sativa* on the Survival of *a. Galli* *In Vitro*

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