

ANTICOCCIDIAL SCREENING OF *Azadirachta indica* (NEEM) IN BROILERS

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Summary

Anticoccidial screening of *Azadirachta indica*, fruit (Neem) was carried out in broiler chickens naturally infected with a mixed *Eimeria* infection @ 50,000 per bird. Powdered *Azadirachta indica* fruits were administered orally in doses of 10, 15 and 20 mg/Kg body weight and their water and methanol extracts in amounts equivalent to 20mg/Kg of the powder were also administered. As a control drug Amprol plus (R) was administered orally. Faecal oocyst per gram (OPG) counts were made by the Mc Master Egg Counting technique. *Azadirachta indica* powder as well as its extracts in methanol and water significantly ($P < 0.05$) reduced the OPG counts at the dosage levels of 10, 15 and 20 mg/Kg. The percentage reduction in OPG counts on the 3rd, 10th and 15th days after administration of 10mg/Kg body weight of drug were 8.2 ± 5.5 , 13 ± 8.0 and 22 ± 4.1 , respectively. Treatment with 15 mg/Kg body weight of the *Azadirachta indica* powder produced 18 ± 5 , 29 ± 8 and 33 ± 11 percent reductions on days 3rd, 10th and 15th, respectively. The respectively OPG reductions in the group treated with 20mg/Kg were 22 ± 8 , 70 ± 14 and 85 ± 20 percent. The OPG count reductions at this dose were non significantly different from that of control drug on day 15th. The aqueous extract equivalent to 20mg/Kg body weight of the powder produced percentage reductions of 24 ± 6 , 61 ± 11 and 68 ± 16 on 3rd, 10th and 15th days post treatment, respectively. The OPG reduction produced by the methanol extract of *Azadirachta indica* were 12 ± 6 , 58 ± 13 and 78 ± 14 percent on days 3rd, 10th and 15th, respectively, showing a non significant ($P > 0.05$) difference in the OPG count reduction from the control drug on the 15th day. The data suggest that single oral administration of 20mg/Kg of *Azadirachta indica* fruits and their extracts in methanol and water in equivalent amounts are effective in controlling the *Eimeria* infection in chickens. However, since at higher doses mortality was observed they should not be used alone for therapeutic purposes in the poultry birds but may be recommended perhaps in smaller amounts along with other herbal ingredients.

Introduction

Coccidiosis is one of the most detrimental and lethal managemental disease of poultry (1). It causes high mortality in affected flocks. Many anticoccidial drugs have been developed and introduced in the poultry industry all over the world. Since Levine (2) discovered sulfanilamide would cure coccidiosis in chickens, various anticoccidial feed additives, predominantly polyether ionophorous antibiotics, have been developed and used (3). While effective for avian coccidiosis, the continuous use and misuse of anticoccidial drugs have led to the emergence of drug-resistant strains (4,5).

To prevent the emergence of drug resistant strains, new drugs have been developed and administered on a rotational basis with existing drugs. However, this has resulted in the increased cost of poultry products. Furthermore, drug- or antibiotic-residue in the poultry product is potentially annoyance to consumer. Therefore, it is sought that the regulations for anticoccidial drugs should be strengthened gradually. Halofuginone was derived from an extract of the *Dichroa febrifuga*. The original extract, febrifugine, was known for antimalarial and anticoccidial activity, but was never marketed because of a very narrow safety margin at the dose of 3 ppm. Other extracts of herbs were known to be effective against parasites, such as malaria, amoeba, trichomonad, arthropods and helminths (6-15, 3,16, 17-19). *Azadirachta indica* (Neem) has been used since centuries in the folk medicine to treat various parasitic infections of man and animals (20). Therefore, in order to evaluate the anticoccidial activity of *Azadirachta indica* (Neem) fruit, their powder and the extracts in water and methanol were administered orally in graded doses to the chickens.

Keywords: Coccidiosis, *Azadirachta indica*

Materials and methods

Chicken and feed composition: Eighty four, 1-day-old broiler chicks were purchased from a local hatchery. The chicks were reared in groups of 12 per cage. In order to prevent outbreak of newcastle disease (ND), they were vaccinated at 2 weeks of age with ND oil-emulsion vaccine. Experimental feed manufactured for starters without anticoccidial feed additives was obtained from Nashat Feed Mills Pvt. Ltd. Pakistan. Its composition followed the commercial chicken production manual (21). Feed and water were offered ad libitum.

Parasite and dose: To obtain the mixed *Eimeria* species, guts were collected from different commercial poultry shops in and around Faisalabad, Pakistan. The oocysts were preserved in 2.5% potassium dichromate solution to induce sporulation and kept in a refrigerator (2–5⁰C) until use. Each bird was challenged with 50,000 oocysts/chicken of mixed *Eimeria* species.

Experimental groups: There were 7 experimental groups and each was having 12 chicks. Different groups of chicks were assigned to various rations with different supplementations from day 25 till end of experiment and infection was given at day 18th.

Group 1: Untreated control

Group 2: Treated control (Amprol plus 2g/Kg of feed)

Group 3: *Azadirachta indica* (10mg/Kg, B.Wt.)

Group 4: *Azadirachta indica* (15mg/Kg, B.Wt.)

Group 5: *Azadirachta indica* (20mg/Kg, B.Wt.)

Group 6: Water extracts of *Azadirachta indica* (20mg/Kg, B.Wt.)

Group 7: Metanol extracts of *Azadirachta indica* (20mg/Kg, B.Wt.)

Efficacy Parameter: The post treatment oocysts per gram counts of the droppings were determined by McMaster technique (22) on the 3rd, 10th, and 15th days after oral administration of 10, 15 and 20mg/Kg of *Azadirachta indica* fruit powder and its extracts in water and methanol equivalent to 20mg/Kg body weight of *A. indica* fruit.

Statistical analysis: The statistical analysis of the data was performed after the methods described by (23).

Results and Discussion

Table 1 shows that the oocysts per gram (OPG) counts in the droppings of untreated chickens were not significantly different (P>0.05) at all the time intervals checked.

It also showed that the administration of the single oral dose of 10mg/Kg body weight of *Azadirachta indica* fruit powder has not produced any significant reduction of the OPG counts. The pre-treatment OPG count (Means \pm SEM) was 14206 ± 800 and the values on the 3rd, 10th and 15th day post treatment were 13800 ± 1770 , 12500 ± 600 and 11200 ± 450 , respectively.

Table 1: Mean \pm SEM oocysts count per gram of faeces (OPG) and their %age reduction in chickens before and after single oral treatment with Powdered *Azadirachta indica* fruit, their aqueous and methanol extracts and Amprol Plus (R)

Treated (Oral dose)	Pretreatment OPG counts Means \pm SEM	Post treatment faecal OPG counts (Means \pm SEM)			Post treatment %age reductions of OPG counts (Means \pm SEM)		
		3 rd day	10 th day	15 th day	3 rd	10 th	15 th
Untreated control	13900 ± 720	13500 ± 780	14200 ± 820	15100 ± 980	–	–	–
Amprol plus (2g/5Kg of feed)	12500 ± 630	$1000 \pm 80^{**}$	$230 \pm 91^{**}$	$130 \pm 51^{**}$	91.8 ± 16	96 ± 19	99 ± 16
<i>A.indica</i> (10mg/Kg, B.Wt.)	14200 ± 800	13800 ± 1770	12500 ± 600	11200 ± 450	8.2 ± 5.5	13 ± 8	22 ± 4.1
<i>A.indica</i> (15mg/Kg, B.Wt.)	17900 ± 2750	14600 ± 2620	13100 ± 1370	10200 ± 1120	18 ± 5	29 ± 8	33 ± 11
<i>A.indica</i> (20mg/Kg, B.Wt.)	18900 ± 1780	15100 ± 1400	$7950 \pm 1250^*$	$6950 \pm 880^*$	22 ± 8	70 ± 14	85 ± 20
Water extract (20mg/Kg, B.Wt.)	17100 ± 1170	$13100 \pm 1010^*$	$7060 \pm 900^{**}$	$6760 \pm 630^{**}$	24 ± 6	61 ± 11	68 ± 16
Methanol extract (20mg/Kg, B.Wt.)	15300 ± 1270	13300 ± 480	$9070 \pm 460^{**}$	$6540 \pm 340^{**}$	12 ± 6	58 ± 13	78 ± 14

* = Significantly (P<0.05) less than the pretreatment value. ** = Highly significantly (P<0.001) less than the pretreatment value.

In chicks treated with 20mg/Kg body weight of crude powder, OPG count prior to treatment was 18900 ± 1780 which was reduced to 15100 ± 1400 , 7950 ± 1250 and 6950 ± 880 , showing a reduction of 67% on the last day. This value was significantly different on days 10th and 15th ($P < 0.05$) from the pre-treatment OPG value but the mortality rate was about 67% in this group. Thus next higher doses of this group were not tried. Water extract equivalent to 20 mg/Kg body weight was tried in another group. Pre-treatment OPG count had a mean \pm SEM of 17100 ± 1170 which was reduced to 13100 ± 1010 , 7060 ± 630 on days 3rd, 10th and 15th respectively. These OPG counts are significantly ($P < 0.05$) lower than that of pre-treatment values on the 3rd day and highly significantly lower ($P < 0.001$) on days 10th and 15th. These values showed 60% reduction in oocyst counts and the mortality in this group was 33 percent. In the group treated with methanol extract of *Azadirachta indica* equivalent to 20mg/Kg, the pre-treatment count was 15300 ± 1270 which was reduced to 13300 ± 480 , 9070 ± 460 , 6540 on days 3rd, 10th and 15th respectively, showing a reduction of 41 percent on 10th day and a reduction of 57 percent on 15th day, while the mortality rate in this group was again 33 percent

The results of group treated with dry powder of *Azadirachta indica* showed maximum reduction of 67 percent while the water extract showed a maximum reduction of 60 percent and methanol extract was least potent showing 57 percent reduction of oocyst counts. All these values were not comparable with the control drug Amprol Plus (R) (2g/Kg of feed) which had a pre-treatment count of 12500 ± 639 and caused a significant decrease in OPG counts even on the 3rd day. The post treatment counts on the 3rd, 10th and 15th days were 1000 ± 80 , 230 ± 91 and 130 ± 51 , showing 99 percent reduction in the OPG counts. These reductions were comparatively higher than the *Azadirachta indica* treated groups where the maximum reduction was about 67 percent.

It was observed during these studies that all the chicks infected with *Eimeria* species after about 5 days of infection started passing loose droppings and suffered from watery diarrhea. This would have caused an electrolyte imbalance as already reported by (24). The diseased birds showed slow reflexes, ataxia and petechial haemorrhages in the G.I. tract. The caecum was found filled with large amount of clotted blood. Therefore, it is conceivable that the mortality observed in the *Azadirachta indica* treated groups was due to its low efficacy but not perhaps due to its direct toxic effect. However, further investigations are needed to clarify this point. It is suggested that *Azadirachta indica* should be used to control coccidiosis in birds along with other herbal ingredients as employed in some propriety remedies.

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