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A trial on *Azadirachta indica* as herbal remedy against coccidiosis in poultry

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Abstract

The present study was carried out to assess the anticoccidial and immuno-modulatory effects of indigenous *Azadirachta indica* against experimental coccidiosis in poultry. The ethanolic extract of A. indica at dose rate of 7g/liter of drinking water showed better weight gain ($p < 0.05$) in poultry compared to untreated control group, however, there was non-significant difference in feed conversion ratio. The immunological parameters viz. humoral immune response and cell mediated immune responses were measured by haemagglutination inhibition titre values and delayed type hypersensitivity reaction in terms of skin thickness measurement, respectively. Azadirachta indica treated group maintained a significantly high level of HI titres (1.603 ± 0.06) compared to untreated group (1.405 ± 0.06) and showed the maximum skin thickness (2.51 ± 0.14 mm) at 72 hours post exposure as compared to the untreated (1.405 ± 0.06 mm) suggestive of its immunomodulatory properties. There was a significant ($p < 0.05$) reduction in oocysts per gram of faeces (OPG) in the experimentally infected birds, fed with the ethanolic extract of A. indica at dose rate of 7g/liter of drinking water indicating its therapeutic property against coccidiosis in poultry.

Key words: *Azadirachta indica,* coccidiosis, poultry.

Coccidiosis is a major parasitic disease of poultry affecting mainly the intestinal tract and is caused by the protozoan of the genus *Eimeria*. Coccidiosis causes mortality, malabsorption, inefficient feed utilization, impaired growth rate in broilers and reduced egg production in layers (McDougald and Seibert 1998; Jadhav *et al.* 2011). Coccidiosis is responsible for 6–10% of all broiler mortalities, and the global economic losses occur as a result of reduction in growth rate and feed conversion efficiency. The worldwide intensive use of anticoccidial drugs to prevent coccidiosis, has inevitably led to the development of resistance to almost all anticoccidial drugs as long term exposure to any drug will result in loss of sensitivity.

Allopathic anticoccidial drugs are currently in operation and the most effective means for control of coccidiosis but these have serious limitations, such as their non-availability in some developing countries, risk of misuse leading to drug resistance, environmental pollution and food residues. Therefore, possible alternatives such as the use of plant products that function by mechanisms other than those of chemotherapeutics, with the additional advantage of a natural origin have been recommended (Naidoo *et al*. 2008). *Azadirachta indica A. Juss* (syn. *Melia azadirachta*)/ Neem is well known in Himalayas for more than 2000 years as one of the most versatile medicinal plants having a wide spectrum of biological activity. *Azadirachta indica* has been found to have anticoccidial and immunomodulatory effects (Al-Fifi 2007). Hence, the present study was carried out to assess the anticoccidial and immunomodulatory effects of indigenous *A. indica* against mixed species *Eimeria* sp. infection in poultry.

Materials and Methods

Various poultry farms and slaughter houses in Palampur were screened for the presence of coccidian infection. The positive faecal samples and intestinal contents were then subjected to sporulation as per Venkateshwar Rao *et al*. 2012. Briefly, positive samples were homogenized, mixed with 2.5 % potassium dichromate and sieved through 1 mm sieve to remove the course debris usually present in poultry droppings. The filtrate was kept in wide mouth containers with constant aeration to allow 90 per cent sporulation of oocysts. Oocysts of various *Eimeria* species were examined microscopically for sporulation on a 6 h interval basis until complete sporulation was achieved. The oocysts were concentrated, purified and stored in 2.5% potassium dichromate solution (aq.), stored for future use.

Preparation of extract: Fifty grams (50g) of powdered plant material from *Azadiracta indica* was weighed and mixed with (400ml) absolute ethanol (Bengal chemical limited) for making ethanolic extract and protocol followed as per Chattopadhyay 1998. After getting the dried filtrates, they were lyophilized (lyophilizer- Alpha 1-2 LD Plus, Martin Christ Germany) and stored at 4 °C until use.

Experimental protocol

All the experiments detailed in this study were conducted according to the guidelines of Institutional Animal Ethical Committee. Thirty six (36) day-old broiler chicks were procured. On the day of arrival, the chicks were examined for any abnormality and ill health. They were acclimatized in the new environment for two weeks. Chick wings were banded and they were divided into groups as per Table 1.

The doses of different plant extracts were mixed in fresh water daily and given to respective group of birds from 15day of age onwards, before giving infection to birds, and continued till 42 day of experimental trial. Each chick of every group was infected with 20,000 sporulated oocysts of mixed *Eimeria* spp. at 21 day of age and representative birds from each group of experimental trial were sacrificed at 0, 3, 7, 10, 14 and 21 Days Post Infection (DPI).

Body weight and feed conversion ratio:

The Body weight of each bird of every group of the experimental trial was recorded during the time of sacrifice at 0, 3, 7, 10, 14 and 21 Day Post Infection (DPI) and expressed in grams (g). Feed conversion ratio of each bird in every group was calculated and recorded at intervals of one week during the time of sacrifice at 0, 7, 14 and 21 Day Post Infection (DPI).

Immunological parameters

Delayed Type Hypersensitivity Reaction (cell mediated immune response)

Cell-mediated immune response was assessed by delayed type hypersensitivity reaction to DNFB by the method of Phanuphak *et al*. (1974). Six birds from each group were randomly selected and featherless areas of about 10 cm^2 were chosen on left and right lateral side of abdomen for DNFB application. These areas were cleansed with the help of alcohol and 0.25 ml of DNFB (10 mg/ml) in acetone was applied on right side. Acetone (0.25 ml) alone was applied on left side and which served as control. The sensitized birds were challenged two weeks later by applying 0.25 ml of DNFB (1 mg/ml) on right side and 0.25 ml acetone on left side.The skin thickness was measured with the help of vernier caliper at 0, 12, 24, 36 hrs post-

Group	Experimental groups	No. of experimental birds	Dose of plant material	Dose of sporulated coccidian oocyst
1.	Infected non treated(positive control)	6	-	-
2.	*Azadiracta indica*	12	-	20,000
3.	Sulphadimidine	12	7g/liter of water ad libitum	20,000
4.	Non infected non treated(Negative control)	6	0.4% in feed	20,000

challenge. The application area of skin was also examined for erythema, indurations, ulceration and scab formation.

Humoral immune response (haemagglutination inhibition test)

During sacrificing of birds, about 0.5 ml of serum from each bird was taken and heat inactivated to destroy the complement by placing it in water bath at 56°C for 30 minutes. The haemagglutination (HA) test was carried out by the method described by Chauhan and Roy (2000) to determine the HA unit of NCD (Lasota) antigen before performing the HI test.The results were recorded and expressed as $\log_2$ reciprocal of the titre.

Oocyst per gram of faeces

During the trial period, faecal samples were collected from different groups I to IV on 6^{th}, 7^{th}, 8^{th}, 9^{th}, 10^{th}, 14^{th} and 21^{st} day post infection, Oocyst Per Gram was recorded according to modified Stoll's technique (Lee *et al.*1972)

Lesion score

Effects of dietary neem extracts supplementation and standard anticoccidial on pathological parameters were based on gross lesion scoring and on examination of tissue collected during sacrifice of representative birds at 0, 3, 7, 10, 14 and 21 day post infection.

Statistical analysis

Statistical analysis of data was carried out by means of analysis of variance (ANOVA) using Students-Newman-Keuls test (intra-group comparison) and Dunnett's test (inter-group comparison) of Instat software (Graphpad) at 5 % and 1% level of significance.

Results and Discussion

There was a significant decrease in body weight at 21 DPI in the birds infected with mixed coccidian infection (group I) than uninfected control group (group IV) (Table 1). The results of present investigation were in accordance with the observations of Hayat *et al.* (1991); Hashmi *et al.* (1994); Mohsin (1999); McDougald (2003) who reported that coccidiosis result in decrease feed intake and loss in body weight in poultry. The findings of our study revealed that ethanolic extract of *Azadiracta indica* at 7g/liter of drinking water when fed to the infected group showed significantly better weight gains ($p < 0.05$) than the infected untreated control group. These results are in agreement with Tipu *et al.* (2002); Biu *et al*. (2006); Bhattacharyya *et al*. (2013) who have reported that ethanolic neem leaves extract showed a protective effect in broilers against coccidiosis resulting in better weight gain.

The Feed Conversion Ratio (FCR) was significantly higher FCR (2.03 ± 0.08) at 21 DPI (Table 2) in birds of group I (infected untreated) as compared to group IV (uninfected untreated-1.80 ± 0.09). This can be attributed to the fact that coccidiosis causes a decrease in feed intake in poultry (Jithendran 2001; Chandrakesan *et al*. 2009). In the present study there was no significant difference in FCR in *A. indica* group (1.97 ± 0.06) while sulfadimidine treated group showed significantly lower FCR (1.78 ± 0.04) as compared to the control (Group I). Similarly, Hady and Zaki (2012) reported no significant difference in body weight gain and feed conversion ratio in the birds fed with neem. However, Chakeravarty and Prasad (1991) reported better feed conversion ratio in broilers fed with neem extract in water than control group. Contrary to our findings, Al-Fifi (2007) reported that addition of 7g of neem leaves extract per liter of drinking water to the broilers significantly improved feed conversion ratio.

The immunological parameters viz. humoral immune response and cell mediated immune responses were measured by haemagglutination inhibition titre values and delayed type hypersensitivity reaction in terms of skin thickness measurement, respectively (Table 3). At 21 DPI, *A. indica* treated group maintained a significantly high level of HI titres (1.603 ± 0.06) compared to untreated group I(1.405 ± 0.06). But the highest levels were exhibited by group III (1.605 ± 0.06). This suggested that the birds fed with neem extracts exhibited fairly good cell mediated immune response which is suggestive of its immunomodulatory properties. A significant ($p<0.05$) increase in the skin thickness was recorded at 12, 24, 36, 48 and 72 hrs post infection in the treatment groups as compared to control groups. Group II (*A. indica* 2.51 ± 0.14 mm) showed the maximum skin thickness at 72 hours post exposure as compared to group III (2.30 ± 0.07 mm) and group I (control - 2.03 ± 0.06 mm) indicative of enhanced humoral antibody response. Similarly, enhanced humoral response against New castle disease was seen in broilers fed with neem leaves (Sarang and Durrani 2005; Garba *et al.* 2013). Ray *et al*. (1996) studied cell mediated immune responses in mice fed with

Table 1. The effect of dietary supplementation of *Azadirachta indica* (ethanolic extract) on body weight in broilers with experimental coccidiosis

Treatments	Dose	Body weight (g) in Broilers infected with experimental coccidiosis (Mean ± S.E.)					
		0	3	7	10	14	21
		Days post infection with sporulated oocysts					
Group I (Infected, Non Treated Control)	-	310 ± 3.74^{a}	330 ± 1.62^{a}	360 ± 6.54^{a*}	420 ± 4.21^{a*}	500 ± 9.80^{a*}	1140 ± 42.08^{a*}
Group II (Neem ethanolic extract)	7g/l of water	303.3 ± 4.21^{a}	320 ± 6.14^{a}	420 ± 4.94^{b*}	460 ± 13.33^{b*}	550 ± 12.75^{bc*}	1211 ± 31.45^{b*}
Group III (Sulphadimidine)	0.4% in feed	280 ± 3.09^{b}	300 ± 2.10^{b}	310 ± 4.28^{c}	340 ± 14.06^{b}	450 ± 12.91^{b*}	1424 ± 35.70^{bc*}
Group IV (Non infected, Non treated control)	-	320 ± 3.01^{c}	360 ± 4.21^{c}	420 ± 2.22^{b*}	500 ± 3.15^{bc*}	700 ± 8.47^{d*}	1510 ± 25.07^{bc*}

Means with same superscripts in between groups does not differ significantly at 5% level; In intra-group comparison, values with (*) significantly varies ($p<0.05$) with base values at 0DPI

Table 2. The effect of dietary supplementation of *Azadirachta indica* (ethanolic extract) on feed conversion ratio (FCR) in broilers with experimental coccidiosis

Treatments	Dose	Feed Conversion Ratio in Broilers infected with experimental coccidiosis (Mean ± S.E.)			
		0	7	14	21
		Days Post Infection with sporulated oocysts			
Group I (Infected, Non Treated Control)	-	3.06 ± 0.09^{a}	3.16 ± 0.06^{a}	2.93 ± 0.08^{a*}	2.03 ± 0.08^{a*}
Group II (Neem ethanolic extract)	7g/l of water	3.06 ± 0.16^{a}	2.63 ± 0.03^{b*}	2.70 ± 0.06^{a*}	1.97 ± 0.06^{a*}
Group III (Sulphadimidine)	0.4% in feed	3.21 ± 0.01^{b}	3.13 ± 0.05^{a}	2.96 ± 0.87^{a*}	1.78 ± 0.04^{b*}
Group IV (Non infected, Non treated control)	-	2.96 ± 0.16^{a}	2.61 ±0.07^{b*}	2.31 ± 0.05^{b*}	1.80 ± 0.09^{b*}

Means with same superscripts in between groups do not differ significantly at 5% level; In intra-group comparison, values with (*) significantly varies ($p<0.05$) with base values at 0DPI

Table 3. The effect of dietary supplementation of *Azadirachta indica* (ethanolic extract) on haemagglutination Inhibition Titre (HI) (log_2 values) in broilers with experimental coccidiosis

Treatments	Dose	Haemagglutination Inhibition Titre (log_2 values) in broilers infected with experimental coccidiosis(Mean values ± S.E.)					
		0	3	7	10	14	21
		Days Post Infection with sporulated oocysts					
Group I (Infected, Non Treated Control)	-	1.906 ± 0.06[a]	1.605 ± 0.06[a*]	1.405 ± 0.06[a*]	1.054 ± 0.07[a*]	1.455 ± 0.09[a*]	1.405 ± 0.06[a*]
Group II (Neem ethanolic extract)	7g/l of water	2.256 ± 0.07[b]	1.605 ± 0.06[a*]	1.806 ± 0.00[b*]	1.655 ± 0.07[b*]	1.706 ± 0.06[b*]	1.550 ± 0.05[b*]
Group III (Sulphadimidine)	0.4% in feed	2.508 ± 0.10[b]	1.806 ± 0.07[a*]	2.056 ± 0.09[c*]	2.006 ± 0.15[c*]	1.756 ± 0.09[b*]	1.605 ± 0.06[c*]
Group IV (Non infected, Non treated control)	-	2.107 ± 0.02[b]	2.006 ± 0.03[b]	1.806 ± 0.00[b*]	1.806 ± 0.05[b*]	1.806± 0.02[b*]	1.505 ± 0.04[b*]

Means with same superscripts in between groups do not differ significantly at 5% level; In intra-group comparison, values with (*) significantly varies ($p<0.05$) with base values at 0DPI

Table 4. The effect of dietary supplementation of *Azadirachta indica* (ethanolic extract) on delayed type hypersensitivity (skin thickness in mm) in broilers with experimental coccidiosis

Treatments	Dose	Delayed Type Hypersensitivity (Skin Thickness in mm) in Broilers with experimental coccidiosis (Mean values ± S.E.)					
		0	12	24	36	48	72
		Hours Post Sensitization with DNFB dye					
Group I (Infected, Non Treated Control)	-	2.05 ± 0.07[a]	2.91 ± 0.10[a*]	3.33 ± 0.06[a*]	3.15 ± 0.04[a*]	3.11 ± 0.04[a*]	2.03 ± 0.06[a]
Group II (Neem ethanolic extract)	7g/l of water	1.90 ± 0.10[a]	2.88 ± 0.21[a*]	3.16 ± 0.14[a*]	3.45 ± 0.11[a*]	3.16 ± 0.13[a*]	2.51 ± 0.14[b*]
Group III (Sulphadimidine)	0.4% in feed	2.03 ± 0.14[a]	3.26 ± 0.13[b*]	3.47 ± 0.15[a*]	3.70 ± 0.16[b*]	3.45 ± 0.15[a*]	2.30 ± 0.07[a]
Group IV (Non infected, Non treated control)	-	1.77 ± 0.07[a]	1.79 ± 0.09[c]	1.83 ± 0.13[d]	1.85 ± 0.09[c]	1.75 ± 0.06[b]	1.71 ± 0.10[c]

Means with same superscripts in between groups do not differ significantly at 5% level; In intra-group comparison, values with (*) significantly varies ($p<0.05$) with base values at 0hour post application.

neem meal (100 mg neem leaf extract/kg diet) and showed higher skin thickness than control group. Similarly, Zahid *et al*. (2013) investigated in broilers that the groups fed with neem leaf meal showed higher mean antibody titer values against New Castle disease virus as compared to the negative control group. They also compared two commercially available immunostimulants Livol (herbal neem supplement) and Immunotone (selenium and vitamin E) on growth performance and humoral response in chicken against IBD through indirect haemagglutination (IHA) test and showed higher HI titre values in Livol as compared to Immunotone.

The effects *A. indica* on oocyst per gram (OPG) counts has been depicted in Table 5. The OPG count of birds of all the groups was done at day 6, 7, 8, 9, 10, 14 and 21day post infection, which revealed that group II showed comparable and significantly high efficacy similar to group III. The anticoccidial effect of neem might be ascribed to some bioactive chemicals such as *azadirachtin* which has a significant efficacy on viruses, fungal pathogens and protozoan parasites such as coccidian species (Biu *et al*. 2006). A significant decrease in oocyst count in birds was observed in neem fed group as compared to positive control by Tipu *et al.* (2006). Similarly, Hady and Zaki (2012) mentioned that neem at 10% level in diet of broilers infected with *Eimeria tenella* produced significantly lower OPG as compared to control, which is in agreement with our study. The neem fed group showed a gross lesion score of mostly 0 and 1in the anterior, middle, posterior intestine as well as in caeca on various days except on 14 day post infection, where a score of 2 was observed. This is in agreement with the findings of Tipu *et al*. (2002) whoreported that the group fed with neem showed significantly lower gross lesion scores than the infected group (Table6 &Fig.1). Thus, *Azadirachta indica* was found

Table 5. The effect of dietary supplementation of *Azadirachta indica* (ethanolic extract) on on oocyst per gram of faeces in broilers with experimental coccidiosis

Treatments	Dose	Parasitological parameters (oocyst per gram of faeces) in broilers infected with experimental coccidiosis (Mean ± S.E.)						
		6	7	8	9	10	14	21
		Days Post Infection with sporulated oocysts						
Group I (Infected, Non Treated Control)	-	5033.33 ± 133.33^{a}	11000 ± 365.15^{a*}	14833.33 ± 477.26^{a*}	17333.33 ± 333.33^{a*}	11500 ± 428.17^{a*}	7666.66 ± 421.64^{a*}	5666.66 ± 210.82^{a}
Group II (Neem ethanolic extract)	7g/l of water	4700 ± 230.94^{a}	6333.3 ± 130.81^{b*}	6000 ± 139.04^{b*}	5633.3 ± 231.90^{b*}	5000 ± 139.04^{b}	1483.33 ± 144.72^{b*}	200 ± 36.51^{b*}
Group III(Sulphadimidine)	0.4% in feed	4500 ± 150.55^{a}	4866.66 ± 142.98^{c}	4316.66 ± 155.81^{c}	3250 ± 99.16^{c*}	1833.33 ± 84.32^{d***}	983.33 ± 110.81^{b*}	116.66 ± 16.67^{b*}
Group IV (Non infected, Non treated control)	-	0.0 ± 0.00^{c}	0.0 ± 0.00^{c}	0.0 ± 0.00^{d}	0.0 ± 0.00^{e}	0.0 ± 0.00^{f}	0.0 ± 0.00^{d}	0.0 ± 0.00^{d}

Means with same superscripts in between groups do not differ significantly at 5% level; In intra-group comparison, values with (*) significantly varies ($p<0.05$) with base values at 6 DPI

to be highly effective against mixed species *Eimeria* infection and exhibited very good immunomodulatory properties. Therefore, this herb presents itself as an economically viable, safe and efficient alternative to chemical anticoccidials and can be utilized as cost effective and safe herbal remedy for coccidiosis in poultry. Further research may be embarked to determine the toxicity level, in-depth study of mechanism of action, determination of lethal dose, therapeutic level and economic viability of this plant against coccidiosis in poultry.

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