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## COMPARATIVE EFFICACY OF ALCOHOLIC EXTRACTS OF BLACK PEPPERS (*Piper nigrum*) AND CHUTRA LEAVES (*Urtica dioica*) WITH ESB3 AGAINST COCCIDIOSIS IN CHICKENS

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### ABSTRACT

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The present study is undertaken to compare the efficacy of alcoholic extracts of Black Peppers (*Piper nigrum*) and Chutra leaves (*Urtica dioica*) with a patent drug Esb3 against coccidiosis in chicken. 16 Fayoumi breed chickens were collected from a local farm and divided into four groups; A, B, C and D, each consisting of four chickens, Group A (control), Group B (alcoholic extracts of Black Peppers @ 9ml/kg bd wt.), Group C (alcoholic extracts of Chutra leaves @ 9ml/kg bd wt.) and Group D (Esb3 @ 1 ml/ liter drinking water). All the treated chickens were kept under close observation for 18 days and data was collected at 3 days interval. In group B, two chickens died within 4-7 days of treatment and in group C one chicken died on 5<sup>th</sup> day of medication. All the chickens of control group died within 5-7 days of medication. Oocyst was counted for per gram of feces in all groups. Biochemical parameters like SGPT and haematological parameters like Total erythrocyte count (TEC), Haemoglobin count (Hb), Packed cell volume (PCV), Erythrocyte sedimentation rate (ESR) were determined. There were significant decrease in oocyst count in group B and C in compared to control and very few oocysts were present in faeces of 6 days onward of medication. All the chickens were survived in group D and oocyst started to disappear in the faeces from 3<sup>rd</sup> day onward of medication. Our study suggests Both Black Peppers and Chutra are effective against coccidiosis in chicken and Chutra is more effective than Black peppers.

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## INTRUDUCTION

Coccidiosis is the most important protozoan disease affecting the poultry industry worldwide. Control of poultry coccidiosis is presently based on managerial skills and the use of prophylactic coccidiostatic drugs (Tewari and Maharana, 2011). It is a ubiquitous intestinal protozoan infection of poultry seriously impairing the growth and feed utilization of infected animals. Conventional disease control strategies rely heavily on chemoprophylaxis, which is a tremendous cost to the industry. The economic significance of coccidiosis is attributed to decreased production (higher feed conversion, growth depression and increased mortality) and the costs involved in treatment and prevention. Worldwide, the annual costs inflicted by coccidiosis to commercial poultry have been estimated at 2 billion euro, stressing the urgent need for more efficient strategies to control this parasite (Peek and Landman, 2011). Numerous anticoccidial drugs have been introduced since 1948, when sulphaquinoxaline and nitrofurazone were first approved by the American Food and Drug Administration (Conway DP, 2007). Existing vaccines consist of live virulent or attenuated *Eimeria* strains with limited scope of protection against an ever-evolving and widespread pathogen. The continual emergence of drug-resistant strains of *Eimeria*, coupled with the increasing regulations and bans on the use of anticoccidial drugs in commercial poultry production, urges the need for novel approaches and alternative control strategies (Dalloul and Lillehoj, 2005).

The ecological factors prevailing in Bangladesh are highly conducive for the survival, multiplication and perpetuation of poultry parasites of which coccidiosis is encountered in chicken. Mortality in young birds which varies from 25-90% is predominant factor of economic loss. Most of the farmers are very poor and cannot afford to buy modern drugs for the treatment various diseases due to unavailability and higher price of the drugs. If the farmers can use the traditional medicinal plants for the treatment of various animal and poultry diseases it will be very much helpful for them and for the overall improvement of livestock. In Bangladesh very few works have been done to explore the possibilities of utilizing the indigenous plants in poultry coccidiosis. Black peppers have been shown as a growth promoter in poultry (Abou-Elkhair, Ahmed, and Selim, 2014) and sulfachlorpyrisine-Na (Esb3) was active against coccidiosis (Penev and Lozanov, 1983). However, resistance has been reported for Esb3 in poultry (Harfoush et al., 2010). No data yet available about the efficacy of Black Peppers and Chutra leaves in coccidiosis. Therefore, the aim of this study was to evaluate the efficacy of plant extracts (Black Peppers and Churta leaves) compared to ESB3 drug in poultry coccidiosis.

## MATERIALS AND METHODS

The experiment was conducted in the Department of Pharmacology and Department of Physiology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh.

### Experimental Chickens

Total 16 chickens of Fayoumi breed were collected from a local farm. The chicks were 3-4 weeks of age and weighing 250-300 gm. The experimental poultry shed was properly brushed with broom and then washed by forced water using a hosepipe. The room was disinfected with bleaching powder and it was left for 7 days. After the interval, the shed was again disinfected with Virkons (Antes International Limited, England). Additionally the poultry shed was also fumigated by formalin and potassium permanganate for a period of 24 hours for disinfection.

### Collection of Coccidia Affected Birds and Isolation of Oocyst and Infection

Four chickens suffering from coccidiosis were collected from a private farm. The ceca of coccidian infected chickens were separated and opened with scissors and forceps and the fecal contents were taken out in a petri dish containing water. The content was then stirred for 20 minutes and filtered. 50 ml of filtrate was taken in a glass jar. A drop of filtrate was examined under the microscope to observe oocyst. In 50 ml of filtrate 1 gm of potassium dichromate was added and mixed thoroughly and was kept for 24 hours for sporulation. After 24 hours, a drop of filtrate was examined under the microscope to observe sporulated oocyst. The oocysts were counted by McMaster Egg counting method.

All experimental birds were divided into four groups and were kept in four different cages. Oocyst containing filtrate was fed to all birds of the groups at the dose rate of 20 ml / bird. All the birds become infected within 3-5 days. The infection of the bird was confirmed by observing the visible symptoms and also by faecal egg count per gm by McMaster Egg counting method.

#### **Alcoholic Extracts of Black Peppers and Chutra**

The leaves of Chutra and whole of black Peppers were collected in fresh condition. They were washed thoroughly with fresh water. These were cut into small pieces, sundried and grinded in a grinding machine. The powdered sample was measured with a balance. 100 gm of each sample was taken and preserved in air tight bottle separately. Alcoholic extracts of Black Peppers and Chutra were obtained by Soxhlet method (250 ml of alcohol was used for 100 gm of powdered sample).

#### **Determination of Biochemical and Hematological Parameters**

Biochemical parameters like serum glucose and SGPT (serum glutamic pyruvic transaminase) were determined by using autoanalyser (Model No. Reflotron M-06). Haematological parameters like Total Erythrocyte Count (TEC), Haemoglobin count (Hb), Packed Cell Volume (PCV), Erythrocyte Sedimentation Rate (ESR) was determined. The parameters were determined as per method cited by (Coffin, 1955).

#### **Statistical Analysis**

The data was analysed statistically between normal and treated values by student's t test and a p value of  $\leq 0.5$  was considered significant.

## **RESULT AND DISCUSSION**

#### **Effect of Black Peppers on Oocyst Count**

The data obtained from oocyst count of per gm of feces showed Black Peppers reduced the oocyst count significantly as compared to control. Data are presented in Table 1. Data was collected up to 12 days during the treatment period and up to 6 days post treatment period at 3 days interval. All the chickens of control group died within 4-8 days of medication. Two chicks of group B died within 4-7 days of medication. Rest two chicks became apparently normal within 7-10 days of treatment. At day 0, oocyst count of per gm of feces in control was  $5272.50 \pm 31.98$  compared to  $5212.40 \pm 94.20$  in Black peppers treated group (group B). Oocyst count started to fall 3 days after treatment in group B. However, in control, oocyst count never reduced. All the chickens in group A (control) died after 9 days of treatment. Therefore, the data obtained could be compared with the control up to 9 days during treatment period. On day 9, average oocyst count in group B was 580 compared to 6127 in control group. This observation is statistically significant. While the efficacy of Black Peppers compared with standard Esb3 treated group (group D), the pattern of reducing oocyst count is identical in both group B and D. In both groups, oocyst count started to fall 3 days after treatments. At the end of treatment, oocyst count in group B was 130 compared to  $52 \pm 8.62$  in group D. Observations in post treatment period at day 6 showed oocyst count in group B was 37 while in group D was  $14 \pm 5.32$ . These data suggests alcoholic extract of Black Peppers is almost as effective as Esb3 in coccidiosis.

#### **Effect of Chutra on Oocyst Count**

Oocyst count in group C (Chutra treated group) also showed significant reduction compared to control (group A) and Esb3 treated group (group D). Three chicks of group B and two chicks of group C died within 4-8 days of medication. Rest one and two chicks of group B and C respectively cured. It was observed that, 3 days after treatment, oocyst count started to fall unlike control group. On day 0, oocyst count in control group, Chutra treated group and Esb3 treated groups were  $5272.50 \pm 31.98$ ,  $5265.00 \pm 57.81$  and  $5285 \pm 95.96$  respectively. However, on day 3 during treatment, oocyst count reduced 54% in Chutra treated group and 62% in Esb3 treated group. On day 9, oocyst count in Chutra treated group and Esb3 treated group was 580 and  $210 \pm 11.09$  respectively compared to 6127 in control group. The number of oocyst count 6 days after post treatment was 37 in group B compared to  $14 \pm 5.32$  in group D (Table 1).

**Table 1.** Comparative efficacy of alcoholic extracts of Black Peppers, Chutra and Esb3 on oocyst count (per gm of feces) against experimentally induced coccidiosis in chickens

Group	Oocyst count during treatment and post treatment period						
	Treatment period					Post treatment period	
	Day 0	Day3	Day 6	Day 9	Day 12	Day 3	Day6
A	5272.50±31.98	5442.40±55.88	5945b	6127c	-	-	-
B	5212.40±94.20	3882.50±69.39	1652.30±36.83	580b*	130b	75b	37b
C	5265.00±57.81	2422.50±39.66	1575 ± 22.55a	450±29.26*a	125±17.32a	70±14.56a	32±12.20
D	5285±95.96	2032.5±87.97	615±124.09	210±11.09	52±8.62	24±7.39	14±5.32

The values above are mean ± SE of 4 chicks unless otherwise stated. a= values are mean ± SE of 3 chicks, b= values are mean of 2 chicks, c= value of 1 chick, \* Significant decrease ( $p<0.01$ ), - Death of all chickens.

#### Effect of Black Peppers, Chutra Leaves and Esb3 on Biochemical Parameters

SGPT level was calculated up to day 12 during treatment and up to day 6 post treatment. The data are presented in Table 2. On day 0, SGPT level was recorded in group A, B, C and D as 10.55±0.28, 9.425±0.30, 7.595±0.25 and 8.695±0.45 respectively. However, changes of SGPT level in all treatment groups were changes slightly and in significant in comparison to control group. However, the SGPT level was slightly increased from the initial value in group B, C and D and decreased in control group.

**Table 2.** Effect of alcoholic extracts of leaves of whole of Black Peppers, Chutra and patent drug ESB3 on SGPT (U/L) in chickens

Group	SGPT level on different days (U/L) of treatment and post treatment period						
	Treatment period					Post treatment period	
	Day 0	Day3	Day 6	Day 9	Day 12	Day 3	Day6
A	10.55±0.28	10.625±0.28	10.50b	10.5c	-	-	-
B	9.425±0.30	9.375±0.29	9.40a±0.46	9.60b±	9.50b0.70b	9.572b±0.715b	9.59b±0.15b
C	7.595±0.25	7.875±0.23	8.475±0.225a	8.40a±0.21a	8.23a±0.09a	8.40a±0.13a	8.42a±0.16a
D	8.695±0.45	8.65±0.39	8.725±0.36	8.725±0.49	8.825±0.30	8.765±0.36	8.83±0.39

The values above are mean ± SE of 4 chicks unless otherwise stated. a= values are mean ± SE of 3 chicks. b= values are mean of 2 chicks, c= value of 1 chick, \*Significant decrease ( $p<0.01$ ). -Death of all chickens

#### Effect of Black Peppers, Chutra Leaves and Esb3 on Blood Glucose Level

Blood glucose (U/L) level was calculated up to day 12 during treatment and up to day 6 post treatment in control group as well as treated groups. The data are presented in Table 3. No significant difference was observed in treatment group in comparison to control.

**Table 3.** Effect of alcoholic extracts of Black Peppers, Chutra and patent drug Esb3 on blood glucose (U/L) in chicken

Group	Glucose level on different days (U/L) of treatment and post treatment period						
	Treatment period					Post treatment period	
	Day 0	Day3	Day 6	Day 9	Day 12	Day 3	Day6
A	84.55±0.34	84.65±0.36	84.85b	85.4c	-	-	-
B	84.325±0.70	84.4±0.89	84.1a±0.89	84.95b	85.1b	84.94b	85.24b
C	84.65 ± 0.31	84.825 ± 0.34	84.825 ± 0.36a	84.13 ± 0.12a	84.23a ± 0.12a	84.72 ± 0.23a	84.85a ± 0.24a
D	83.375±0.84	83.475±0.81	83.70±0.72	83.675±0.70	83.925±0.76	83.85±0.65	84.12±0.73

The values above are mean ± SE of 4 chicks unless otherwise stated. a= values are mean ± SE of 3 chicks, b= values are mean of 2 chicks, c= value of 1 chick, \* Significant decrease ( $p < 0.01$ ), -Death of all chickens

#### Effect of Black Peppers, Chutra Leaves and Esb3 on Blood Parameters (TEC) Level in Chicken

Blood parameters (TEC) were observed in control and treatment groups. The data are presented in Table 4. No significant difference was observed in treatment group in comparison to control.

**Table 4.** Effect of alcoholic extracts of leaves of Black Peppers, Chutra leaves and patent drug Esb3 on blood parameter (TEC) in chicken

Group	TEC level on different days of treatment and post treatment period in chicken						
	Treatment period					Post treatment period	
	Day 0	Day3	Day 6	Day 9	Day 12	Day 3	Day6
A	1.425 ±	1.45a ± 0.17	1.46b	1.52c	-	-	-
B	1.85 ± 0.11	1.93 ± 0.062	2.25a ± 0.085	2.84b	3.16b	3.24b	3.18b
C	2.14 ± 0.04	2.35 ± 0.028	2.84 ± 0.066a	2.95 ± 0.1a	2.94 ± 0.082a	2.95 ± 0.067a	2.96a ± 0.058a
D	1.96 ± 0.07	2.14±0.062	2.47±0.086	2.47±0.085	2.63±0.11	3.15±0.073	3.23±0.09

The values above are mean ± SE of 4 chicks unless otherwise stated. a= values are mean ± SE of 3 chicks, b= values are mean of 2 chicks, c= value of 1 chick, \* significant decrease ( $p < 0.01$ ), Death of all chicks.

#### Effect of Black Peppers, Chutra Leaves and Esb3 on Blood Parameters (Hb) Level in Chicken

Blood parameter (Hb) was observed in control and treatment groups. The data are presented in Table 5. Hemoglobin level was significantly increased in Black Peppers treated group (from  $7.32 \pm 0.19$  on day 0 of treatment period to  $11.29$  on day 6 post treatment period) compared to control (from  $6.43 \pm 0.13$  on day 0 to  $6.86$  on day 9 before they died). The level of hemoglobin was also significantly increased (from  $6.85 \pm 0.13$  on day 0 to  $9.55 \pm 0.53$  on day 6 post treatment) in comparison to control. However, the Hb level obtained in group B is higher and group C is lower than Esb3 treated group (group D).

**Table 5.** Effect of alcoholic extracts of Black Peppers, Chutra leaves and patent drug ESB3 on blood parameter (Hb) in chicken

Group	Haemoglobin level on different days (gm%) of treatment and post treatment period						
	Treatment period					Post treatment period	
	Day 0	Day3	Day 6	Day 9	Day 12	Day 3	Day6
A	6.43 ± 0.13	6.40 ± 0.12	6.40b	6.86c			
B	7.32 ± 0.19	7.59 ± 0.37	8.20a ± 0.45	9.13b*	10.50b	10.80b	11.29b*
C	6.85 ± 0.13	7.30 ± 0.63	7.85a ± 0.23a	7.86a ± 0.13*a	8.35a ± 0.57a	8.73a ± 0.13a	9.55a ± 0.53a
D	7.57 ± 0.17	8.13 ± 0.63	8.85 ± 0.23	9.29 ± 0.53	9.78 ± 0.13	10.16 ± 0.62	10.57 ± 0.13

The values above are mean ± SE of 4 chicks unless otherwise stated. a= values are mean ± SE of 3 chicks, b= values are mean of 2 chicks, c= value of 1 chick, \* significant decrease ( $p < 0.01$ ), Death of all chicks

#### Effect of Black Peppers, Chutra leaves and Esb3 on PCV (%) in chicken

PCV (%) level was calculated up to day 12 during treatment and up to day 6 post treatment. The data are presented in Table 6. On day 0, SGPT level was recorded in group A, B, C and D as 28.53 ± 0.13, 29.56 ± 0.32, 28.36 ± 0.36 and 27.56 ± 0.25 respectively. However, PCV (%) in group B was significantly increased (31.82 on day 6 post treatment) in comparison to control and Esb3 treated group (30.03 ± 0.73). PCV (%) in group C was also increased but significant in comparison to control and insignificant in comparison to Esb3 treated group.

**Table 6.** Effect of alcoholic extracts of Black Peppers, Chutra leaves and patent drug on PCV% in chicks

Group	PCV level on different days (% In 30 min.) of treatment and post treatment period						
	Treatment period					Post treatment period	
	Day 0	Day3	Day 6	Day 9	Day 12	Day 3	Day6
A	28.53 ± 0.13	28.23 ± 0.12	27.56b	27.50c			
B	29.56 ± 0.32	29.15 ± 0.15	38.50 ± 0.62	30.58b*	31.16b	31.56b	31.82b*
C	28.36 ± 0.36	28.78 ± 0.16	29.26a ± 0.23a	29.67a ± 0.21a	29.83a ± 0.09a	30.26a ± 0.36a	30.79a ± 0.26a
D	27.56 ± 0.25	28.12 ± 0.16	28.56 ± 0.23	29.23 ± 0.56	29.56 ± 0.21	30.83 ± 0.56	30.03 ± 0.73

The values above are mean ± SE of 4 chicks unless otherwise stated. a= values are mean ± SE of 3 chicks, b= values are mean of 2 chicks, c= value of 1 chick, \* significant decrease ( $p < 0.01$ ), Death of all chicks

Coccidiosis is a ubiquitous intestinal protozoan infection of poultry seriously impairing the growth and feed utilization of infected bird. Conventional disease control strategies are now experiencing resistance resulting searching for alternative strategy to control the disease. There is a huge demand to investigate natural products to compete coccidiosis in poultry. The alcoholic extracts of whole of Black Peppers and leaves of Chutra at the dose rate of 9 ml/kg body wt. were given orally to group B and group C chicks respectively. Two chicks of group B died within 4-7 days of medication. Rest two chicks became apparently normal within 7-10 days of treatment. Oocyst disappeared completely 6 day onward of medication. Administration of alcoholic extracts of leaves of Chutra at the dose rate of 9 ml/kg body wt. in group C chicks gave 75% protection. In this group one chick died within 6 days of medication and rest three chicks survived. Oocyst disappeared completely from the faecal sample from 3<sup>rd</sup> day onward of medication. On the other hand, administration of Esb3 at the dose rate of 1 gm / litre of water afforded 100% protection in group D chicks. The oocyst disappeared completely from 3<sup>rd</sup> day onward of medication.

A number of medicinal plants and herbal preparation have been successfully used to control coccidiosis (Du and Hu, 2004; Dkhil et al., 2011; Almeida et al., 2014). Black pepper have been successfully used to reduced calcium level (Yoon et al., 2015), antimicrobials (Nassan & Mohamed, 2014), antioxidant (Obou et al., 2013), anti-obesity (Neyrinck et al., 2013) and anti-inflammatory (Ying et al., 2013). However, no data of Black Pepper is yet available used it in coccidiosis. Therefore, the data could not be compared. But, it is observed that anti-inflammatory, anti-oxidant and antimicrobial properties could play a vital role as anti-coccidiosis. Chutra leaves have been shown effective as antidiabetic, hypolipidemic, and liver and renal damage recovering effects (Abedi Gaballu et al., 2015). Our data with Chutra leaves could not be compared as any data with coccidiosis treatment is available in published literature. However, this leaves are used as antidepressant and memory boosting (Patel & Udayabanu, 2013), hypoglycaemia (Patel & Udayabanu, 2013), anti-inflammatory (Hajhashemi & Klooshani, 2013) and antibacterial study (Körpe et al., 2013).

Therefore, it can be concluded that alcoholic extracts of Chutra leaves at the dose rate of 9 ml/kg was found to be most effective (75%) nearer to patent drug Esb3 in treating the chicks experimentally infected with coccidiosis. Alcoholic extracts of whole of Black Peppers proved less effective (50%) than those of alcoholic extracts of chutra leaves and patent drug Esb3. Further study should commence to investigate the mechanism of action of Black Peppers and Chutra leaves as anticoccidial in poultry.

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