COMPARATIVE EFFECTS OF NEEM LEAVES (*Azadirachta indica*) SUSPENSION AND TOLTRAZURIL AGAINST COCCIDIOSIS IN SONALI CHICKEN

A THESIS
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MASTER OF SCIENCE (MS)
IN
PHARMACOLOGY

DEPARTMENT OF PHYSIOLOGY AND PHARMACOLOGY
HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR-5200

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Submitted to the
Department of Physiology and Pharmacology
Hajee Mohammad Danesh Science and Technology University,
Dinajpur-5200, Bangladesh
In Partial fulfillment of the requirements for the degree of

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DEDICATED

TO MY

BELOVED PARENTS

ACKNOWLEDGEMENT

At first, all praises are due to the Almighty God, the creator and supreme authority of universe, who empowers me to complete the research work successfully and to materialize the dream for the degree of Master of Science (MS) in Pharmacology.

I wish to express the deepest sense of gratitude, sincere appreciation, indebtedness and best regards to my respected teacher and research supervisor Dr. Md. Mahmudul Hasan, Assistant Professor, Department of Physiology and Pharmacology, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur for his scholastic and dynamic guidance, constant inspiration, cordial consistence, innovative suggestions, affectionate feeling, helpful comment, inspiration, sympathetic supervision and constructive criticism in all phases of this study and preparing the manuscript.

I express deep indebtedness to my Co-supervisor, Dr. Fahima Binthe Aziz, Associate Professor, Department of Physiology and Pharmacology, Hajee Mohammad Danesh Science and Technology University, Dinajpur for her scholastic guidance, untiring assistance and advice in preparing the thesis.

I express my cordial respect and profound gratitude to my teacher, Professor Dr. Rakibul Islam, Dr. Md. Bazlar Rashid, Associate Professor, Dr. Mst. Misrat Masuma Parvez, Assistant Professor, Dr. Sumon Sarkar Setu, Lecturer, Department of Physiology and Pharmacology, Hajee Mohammad Danesh Science and Technology University, Dinajpur for their helpful advice and cooperation in providing facilities to conduct the experiments.

I like to express profound gratitude and thanks to my all reverend teachers of the Faculty of Veterinary and Animal Science, Hajee Mohammad Danesh Science and Technology University, Dinajpur for their kind help, co-operation, encouragement and valuable suggestions.

With due pleasure I wish to acknowledge the healthy working relationship of the staff of the Department of Physiology and Pharmacology, Hajee Mohammad Danesh Science and Technology University, Dinajpur.

I like to express cordial thanks to the Ministry of National Science and Technology, for funding this experiment.

Finally, I am very much grateful to my beloved parents, brother and sister for their sacrifice, inspiration, encouragement and endless love and continuous blessing for educating myself up to the postgraduate level.

The Author *June 2019*

ABSTRACT

Coccidiosis is recognized as the parasitic disease with the greatest economic impact on poultry industries worldwide. Sonail chicken commonly affected by coccidiosis in Bangladesh. Recently, the concerns about possible drug resistance have aroused great caution in the usage of drug in the animal industry. As one of its alternative nowadays herbal extracts are used and Neem leaves (*Azadirachta indica*) have anti-coccidial property. This work was conducted to evaluate the comparative efficacy of Neem leaves suspension and toltrazuril on coccidial load, blood constituents, growth performance of sonali chicken infected with *Eimeria spp*. The location was in Laboratory and experimental shed under the department of physiology and pharmacology, HSTU, Dinajpur, 2nd January to 3rd February, 2019. Eighty (80) sonali chicken of seven days old were randomly divided into four groups named T_0, T_1 T_2 and T_3 are each group contained 20 birds. All groups were supplied *E. spp* orally except T_0 group and after 3 days T_2 group was treated with Neem Leaves Suspension (5%) for 15 days and T_3 groups was treated with toltrazuril (Coxitril ®) solution (1 ml/lit) for 2 day. Results showed that protozoal load significantly ($P<0.01$) decreased in T_2 & T_3 groups whereas significantly ($P<0.01$) increased in T_0 and T_1 group. In case of hematological parameters total erythrocyte count and hemoglobin in different treatment groups were almost similar and the differences were statistically non-significant ($P>0.05$) except total leukocyte count which was statistically significant ($P<0.01$). The body weight of T_0, T_1, T_2 and T_3 group at 0^{th} day were 332.65, 269.81, 269.81, 489.22 gm respectively which is statistically significant ($P<0.01$) and highest body weight gain was recorded from Neem Leaves Suspension treated T_3 group. In conclusion, neem leaves suspension have significant effects on protozoal load, body weight and no significant effect on heamatological parameters except total leukocyte count which is statistically significant.

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LIST OF ABBREVIATIONS AND SYMBOLS

Abbreviations	Full meanings
%	Percentage
° C	Degree celcius
/	Per
&	And
:	Ratio
@	At the rate of
<	Less than
>	Greater than
±	Plus minus
ANOVA	Analysis of Variance
Av.	Average
Contd.	Continued
e.g.	For example
et al.	And others
Fig.	Figure
FAO	Food and Agriculture Organization
DLS	Department of Livestock Service
gm	Gram
HSTU	Hajee Mohammad Danesh Science and Technology University
i.e.	That is
Kg	Kilogram
NLS	Neem leaves suspension
OPG	Oocyst per gram

LIST OF ABBREVIATIONS AND SYMBOLS (Contd.)

Mg	Milligram
Mm	Millimeter
Ml	Mililitre
Lit	Litre
No.	Number
NS	Non-significant
OPG	Oocyst per gram
Ppm	Parts per million
T	Treatment
R	Replication
Sq.	Square
SE	Standard Error
E. spp	*Eimeria species*
TEC	Total Erythrocyte Count
TLC	Total Leukocyte Count
Hb	Hemoglobin
mm^3	Cubic Millimeter
Ltd.	Limited
B. wt.	Body Weight
No.	Number
Contd.	Continued
Conc.	Concentration

CHAPTER-I
INTRODUCTION

Bangladesh is an agriculture based tropical country where more than 80% of the country's people (150 million) are living in rural areas and highly dependent on agriculture. Poultry farming is one of the major activities in rural areas as it provides immense employment opportunities to the local communities especially for youth and women that helps in poverty reduction, ensures food security and improves the nutritional status of the people. Poultry industry can produce very high quality proteins for human nutrition as well as a source of income for the community in many countries, therefore poultry production plays very important role in economic development of any country (Tarhyel *et al*., 2012). Poultry is a promising sector in Bangladesh which is increasing day by day. Poultry production is hindered by many problems among which various diseases namely bacterial, viral and parasitic infections are the most important (Ojok, 1993).

Sonali chickens are well adapted in the environmental conditions of Bangladesh, as it requires less care and attention as compared to other breeds. It is also easier for women to rear (Saleque and Saha, 2013). In spite of many positive reasons behind rearing of Sonali chickens, there are some causes that hamper in optimum production of Sonali chicken by smallholder households in Bangladesh (Biswas *et al*. 2006). Poultry diseases are one of the major constraints for developing poultry industry in Bangladesh (Islam and Samad, 2004). Prevalence of diseases in a particular area depends on various factors like geo-climatic condition, management practices, immunization status, and social awareness.

Prevalence of infectious diseases is considered for establishing a commercial poultry farm. The northern part of Bangladesh is considered as a poultry hub especially of Sonali chicken, which is used as meat purpose. Biswas *et al*. (2006) reported that about 28% mortality of Sonali chickens in southeastern coast of Bangladesh due to outbreak of several infectious diseases. However, very few studies have been reported on prevalence of infectious diseases of chickens of northern part of Bangladesh. Thus, this study was aimed at investigate the prevalence of various infectious diseases of Sonali chickens in the northern part of Bangladesh.

Avian coccidiosis is a parasitic disease of intestinal tract caused by single cell protozoan parasite belonging to genus Eimeria. It causes massive destruction of the epithelial cells, which leads to bloody diarrhea, reduced weight gain and temporary reduction in egg production (Dalloul and Lillehoj, 2005). Coccidiosis has been considered as a very harmful disease affecting growth and performance of birds in the intense poultry (Lin *et al*., 2006). Thus coccidiosis is probably the most expensive and wide spread infectious disease in commercial poultry systems. Control of coccidiosis mostly depends upon the chemoprophylaxis by using anticoccidial drugs, however, managerial skills are also important to get maximum anticoccidial effect of these drugs (Tewari and Maharana, 2011). Chemical anticoccidial feed additives has played a vital role in the growth of the poultry industry and has also facilitated better availability of affordable and good quality poultry meat. Most knowledge on coccidiosis has been Obtained from poultry, in which the disease has been studied most intensively as in this species the parasite causes the most devastating losses due to the large number of birds per flock and high stock densities The economic importance of coccidiosis can be related to decreased profit caused by higher feed conversion, growth depression, increased mortality and the costs of prevention and treatment. Worldwide the annual costs inflicted by coccidiosis to commercial poultry have been estimated at € 2 billion, stressing the urgent need for more efficient strategies to control the parasite (Williams, 1999).

In Bangladesh, a number of drugs are available for the treatment and prevention of chicken coccidiosis. Among these sulphaclozine sodium are used extensively throughout the country. In addition, ionophorous antibiotic i.e., maduramycin and lasalocid and some other drugs like amprolium, toltrazuril are also used in the field. It has been found that the efficacy of various anti-coccdial drugs varies considerably against coccidisis (Lee, 1978).Therefore, the present study was undertaken with the objective for the evaluation of the sensitivity and effect of sulphaclozine sodium and toltrazuril on the body weight against experimentally produced coccidiosis in sonalis.

Toltrazuril is a symmetrical triazinetrione compound and 2.5% oral solution has been shown to be effective against all species of Eimeria infecting chickens (Mehlhorn *et al*., 1988). It is active against all intracellular developmental stages.

Including those of schizogony and gametogony. Toltrazuril has chemoprophylactic and therapeutic effects against coccidiosis and does not interfere with the development of immunity Chemoprophylaxis with Toltrazuril enhances immunity against poultry

coccidiosis. It has been proved that therapeutic medication with Toltrazuril protects the birds from clinical coccidiosis (Ramadan *et al*., 1997).

So, we need to quickly replace of antic0ccidial with other options. These new options should be inexpensive and available in everywhere as well as healthy for human society. It is thought that medicinal plants are a good alternative for this purpose. Since ancient times medicinal plants play important role in health management of traditional poultry production (Eevuri and Putturu, 2013). In recent years, the growing demand for herbal product has led to a quantum jump in volume of plant materials traded within and across the countries.

In recent years, Secondary plant metabolites (Phytochemicals), previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents (Krishnaraju *et al*., 2005). Thus it is anticipated that phytochemicals with adequate anticoccidial efficacy will be used for the treatment of the protozoal infections. Also, in contrast to anticoccidial, most active components of medicinal plants are readily absorbed in intestine and have short half life.

Neem elaborates a vast array of biologically active compounds that are chemically diverse and structurally complex. More than 140 compounds have been isolated from different parts of Neem. All parts of the Neem tree-leaves, flowers, seeds, fruits, roots and bark have been used traditionally for the treatment of inflammation, infections, fever, skin diseases and dental disorders. In general, Neem leaves extract may be used therapeutically to control respiratory problems, constipation and also as health promoter (Pandey *et al*. 2018). Aqueous extract of Neem leaves extract has a good therapeutic potential as anti-hyperglycemic agent, antibacterial agent and could be used for controlling airborne bacterial contamination in the residential premise (Mishra *et al*. 2013). The medicinal utilities and wide range of pharmacological activities have been described especially for Neem leaf.

Neem leaves and its constituents have been demonstrated to exhibit immunomodulatory, anti-inflammatory, antihyperglycaemic, antiulcer, antimalarial, antifungal, antibacterial, antiviral, antioxidant, antimutagenic and anticarcinogenic properties (Rahmani *et al*. 2018). The leaves extract containnimbin, nimbinene, 6-desacetylnimbiene, nimbandiol, nimbolide and quercetin (Shareef *et al*. 2018). Leaves are carminative and aid in digestion. Since Neem leaf increases immunity against some common diseases which assists to reduce mortality, it is likely that the growth pattern of sonali chicks will proceed uninterrupted.

So based on these medicinal properties of Neem a study was planned to provide Neem leaf extract (NLE) in drinking water to investigate the effect of Neem leaves suspension (NLS) on the performance of sonali chicks.

Considering the above facts the present study was undertaken with the following objectives:

1. To determine the therapeutic efficacy of Neem leaves suspension and toltrazuril by counting the coccidial Oocyst
2. To evaluate the Neem leaves suspension (NLS) & tolrazuril on live body weight and haematological parameters.

CHAPTER II
REVIEW OF LITERATURE

Karim *et al.*(2016) studied this study was undertaken to investigate the effect of sulphaclozine sodium and toltrazuril for the treatment of experimentally induced caecal coccidosis. Day-old chicks (n=96) reared in a coccidia free condition divided into six Groups (A, B, C, D, E and F) each having 4 subgroups of 4 chicks in each. At the age of 23 days the chicks were infected with 104 sporulated oocysts of *E. spp* except control Group F and treated for 4 consecutive days from 3 days post infection. Then treatment was given in Group A (2 gm/L), B (3 gm/L) with sulphaclozine sodium and in Group C (1 ml/L), D (1.5 ml/L) with toltrazuril. Group E was maintained as infected, but untreated and Group F as negative control. After treatment Group A gained almost similar weight with Group F. A significantly higher ($P<0.05$) weight gain was recorded in Group A and B compared with Group C and D.The infected untreated chicks passed a large number of oocysts from day 5 till the end of the experiment. Chicks receiving sulphaclozine sodium (Groups A and B) had large oocysts count compared with the Groups C and D treated with toltrazuril but lower than the untreated chicks. At the end of experiment the lowest oocysts count was found in Group D (31.32 x 106) and highest in Group E (53.59 x106). Hence this study recommends to use sulphaclozine sodium and toltrazuril for chicken coccidiosis.

Dash *et al.* (2017) found that Plants have the facility to endanger diverse variety of phytochemical and biochemical compounds which can be acclimated to perform different biological functions. Many of these phytochemicals have salutary effects on long-term health when consumed by the human and can be efficaciously used to treat human diseases. The current research paper deals with the various phytochemical and biochemical analysis of *Azadirachta indica.* The analysis was carried out utilizing standard methods and protocols.

Phytochemical analysis of methanolic leaf extracts of *Azadirachta indica* has shown the presence of biological compounds like, Alkaloids, Flavonoids, Saponins, etc which are then compared to aqueous leaf extracts of the plant. Biochemical analysis includes the estimation of chlorophyll content, carbohydrate content and proline content. The result suggests that the *Azadirachta indica* extracts contain plenty of phytochemicals with antimicrobial, anti-inflammatory and antioxidant properties.

Vongpakorn *et al.* (2013) tested that Hematological, biochemical and histopathological alterations caused by coccidiosis in broiler chickens from an outbreak of bloody coccidiosis in a flock on a small-scale broiler farm, Debre Zeit, Ethiopia, were evaluated. *Eimeria spp* and *E. brunetti* were identified. Anemia caused by these species was characterized by a decreased number of red blood cells (RBC) and decreased packed cell volume (PCV). Differential leukocyte counts revealed monocytosis, lymphocytosis, heterophilia and eosinophilia. Serum biochemical analysis showed decreases in alanine aminotransferase/ glutamic pyruvic transaminase (ALT/GPT) and aspartate aminotransferase/glutamic oxalacetic transaminases (AST/GOT), and a marked increase in alkaline phosphatase (ALP) activities. Histopathological examinations of the affected caeca also demonstrated excessive tissue damage, hemorrhage, the presence of clusters of large schizonts and merozoites in the tissue, and coccidian oocysts in the lumen. The study demonstrated changes in the hematology, histopathology and blood chemistry of broilers caused by *E. spp* and *E. brunetti*.

Khan M. N (2012) conducted the objective of the present study was to evaluate the anticoccidial effect of different concentrations of the herbal complex of 4 plants (leaves of *Azadirachta indica* and Nicotiana tabacum, flowers of Calotropis procera and seeds of Trachyspermum ammi) in broiler chickens in comparison with amprolium anticoccidial. Three concentrations (2 g, 4 g and 6 g) of herbal complex were given to the experimental groups once a day and amprolium (at the dose rate of 125 ppm) was given orally in drinking water from the 14th to the 21st days of age. One group was kept as infected, non-medicated control and one as non-infected, non-medicated control. All groups were inoculated orally with 75,000 sporulated oocysts on the 14th day of age except the non-infected, non-medicated control. Among herbal complex medicated groups, the maximum anticoccidial effect was seen in the group medicated with 6 g herbal complex followed by 4 g and 2 g herbal complex medicated groups. Treatment with 6 g of the herbal complex significantly reduced the negative performance and pathogenic effects associated with *Eimeria spp* challenge at a level that was comparable with amprolium when using a largely susceptible recent field isolate. In summary, concentration-dependent anticoccidial activity of the studied herbal complex suggests its use as an alternative anticoccidial agent to chemotherapeutic drugs for *Eimeria spp* control.

Raja Ratna Reddy *et al.* (2013) found that Screening of medicinal plants for bioactive compounds leads to development of less expensive new antimicrobial agents with improved

safety and efficacy. *Azadirachta indica* (Neem) is a multipurpose tree with multiple health benefits. Different parts of the plant are shown to exhibit antimicrobial effects against a wide variety of microorganisms. In the present study we compared the antimicrobial efficacy of aqueous extracts of leaf, bark and seeds of A. Indica against human pathogenic bacteria (Staphylococcus aureus, Enterococcus feacalis, Proteus mirabilis and Pseudomonas aeuroginosa) and fungi (Aspergillus fumigatus and Candida albicans). Agar well diffusion method and micro-broth dilution methods were used to determine the minimum inhibitory concentration (MIC). Results showed that leaf extract exhibited strong antimicrobial activity against bacteria and fungi at all the concentrations tested (500, 1000 and 2000µg/ml).

Antimicrobial activity of bark extract was found to be moderate on bacteria and fungi (effective at 1000 and 2000µg/ml), whereas seed extract exhibited least antimicrobial activity. Minimum inhibitory concentration (MIC) of leaf and bark extract was found to be in the range of 500 to 2000µg/ml for all the tested microorganisms, where as the seed extract did not inhibit the microorganisms at all the concentrations tested except Candida albicans (1000µg /ml). Our results suggest that aqueous extracts of *Azadirachta indica* leaf and bark exhibit high antimicrobial activity.

Abdullahi *et al*. (2017) recorded that the phytochemical properties and the antibacterial activity of aqueous extract of Neem twigs ( ) on the bacterial species isolated from human mouth was investigated. The Neem tree was observed to be devoid of any infection prior to cutting the twigs for laboratory analysis. The mouths of fifteen (15) students were swabbed using a sterile swab stick in the morning before each of them washes their mouth at Federal University Wukari. Bacterial isolation was done using nutrient agar. Identification of the bacteria isolates were done using standard techniques. Agar-well diffusion method was used to determine the antibacterial effect of the extract on a prepared nutrient agar. The twigs extracts showed antibacterial activity against all the test isolates at different concentrations. The zone of inhibition increased with increased concentrations of the extracts. Phytochemical extraction was done using GC-MS QP2010 PLUS machine. Polyphenols were extracted at a very large amount; Saponnins and Flavonoides were found to be in a large quantity while Glycosides and Alkaloids were found to be low.

However, Phylobatanins, Anthraguinones, Hydroxymethyl Anthraguinones and Tannins were found to be absent. Most of these compounds extracted from Neem twigs have antibacterial activity. Therefore, these results, further confirm the belief and other scientific

studies on the importance of the traditional use of Neem twigs in maintaining oral hygiene. Again, some of the isolates from the human mouth are potential pathogens and may pose a public health hazard to the community. Therefore, routine oral hygiene are strongly advocated to prevent oral diseases.

Mathis *et al.* (2004) conducted a 56-day floor pen study to determine the appropriate time to administer toltrazuril (BaycoxReg.) for control of coccidiosis in broiler chickens. They reported that the final performance for the salinomycin (SAL/SAL) was significantly less compared to all toltrazuril and nicarb / salinomycin (NIC/SAL) birds. All toltrazuril treatments at days 2-3 provided good coccidiosis control with accompanying performance. The absence of clinical coccidiosis relapse during the last third of the growout along with moderate oocyst counts and low lesions was indicative of unimpaired coccidiosis immunity.

Sherein *et al.* (2010) reported that Oocysts of Eimeria species were collected from 8 Native breed chicken flocks aged 7-8 weeks. These chickens were suffering from bloody dropping, loss of weight, low conversion rate and variable mortalities 3-12% in 6-10 days. Eimeria species' oocysts were sporulated and tested for their infectivity and pathogenicity in male commercial chicks aged 14 days old. The infected chicks showed general signs of ruffled feathers, off food, huddling together with loose dropping and/or bloody dropping with total mortality reached to 90%. The post mortem examination showed hemorrhagic foci in the duodenum, hemorrhagic mucosa in mid intestine and bloody caecal core in two caeci. Eimeria species developmental stages in duodenum, intestine and caecum were histopathologically detected at the 6th day post infection.

The obtained sporulated oocysts were identified according to morphological features, and the calculated shape index were 1.14, 1.19, 1.25 and 1.23 suggestive to be *E. spp*, *E. necatrix*, *E. acervulina* and *E. praecox*; respectively. Chicks kept individually in a wire cage were inoculated with one sporulated oocyst for obtaining pure isolate from morphologically identified 10 isolates and for detection the site of infection and histopathological features. Egyptian four local isolates in a pure form were obtained.

These isolate, including *E. spp*, E. acervulina, *E. necatrix*, and *E. praecox*. This isolates were passed in the chicks 14th day old from increasing their number. [M.M. Amer, M.H.H. Awaad, Rabab M. El-Khateeb, Nadia M.T.N. Abu-Elezz, A. Sherein-Said, M.M. Ghetas and M.A. Kutkat. Isolation and Identification of Eimeria from Field Coccidiosis in Chickens.

Bhowmik *et al*. (2010) reported thatThe Neem tree (Azadirachta indica) has been known as the wonder tree for centuries in the Indian subcontinent. It has become important in the global context today because it offers answers to the major concerns facing mankind.Neem (*Azadirachta indica*) is considered harmless to humans, animals, birds, beneficial insects and earthworms, and has been approved by the US Environmental Protection Agency for use on food crops. Azadirachtin and other active ingredients in the Neem seed have insecticidal properties that are effective against a broad spectrum of insects, many mites and nematodes, and even snails and fungi, and do not seem to generate resistance in the pests they affect.

Nowadays, Neem and its extracts are used in numerous herbal and allopathic medicines. What's more, even Neem contraceptives are available in the market these days.Neem extract which have Nimbinin, nimbandiol as active constituents, alcoholic extract of the leaves was found to possess a significant blood sugar lowering effect, which are very useful against diabetes. Neem is used in Dermatitis Eczema, Acne, Bacterial, Fungal infections and other skin disorders. It has demonstrated its effectiveness as a powerful antibiotic. Neem also has shown antiviral, anti-fungal and anti-bacterial properties. It helps support a strong immune system and is used in cases of inflammatory skin conditions. Traditionally Neem has been used for skin and blood purifying conditions. Neem not only helps in curing diseases, but it also provides us with the strength of fighting diseases by enhancing our immunity.

Asomaning (2011) studied that the seeds of *Azadirachta indica* A. Juss. popularly known as Neem collected from five cities of Accra metropolis was studied. Trees with wide girth and different seed weight were observed. Maximum residual oil content was noticed in trees from Haatso. Weight of the seeds had no effect on the oil yield. Seed oil content in most of the cities was not significantly correlated with morphological parameters of seeds. A.indica seed oil extracted was analyzed for their physicochemical properties such as viscosity at 28 oC (0.07 kg/ms), pH (5.7), acid value (1.102 ml/g), iodine value (71.0 gI2/100g) and free fatty acid value (48.35 ml/g).

The maximum mean percentage oil obtained (52.5 %) makes the commercialization of the seeds of *Azadirachta indica* in Ghana a possible and profitable venture. The result also confirms the oil to be good quality and can find application in industrial purposes.

Hadas *et al*. (2014) stated that a cross sectional study was conducted from October 2013 up to April 2014 in Gondar town to determine the prevalence of poultry coccidiosis and to identify the associated risk factors. Fecal sample from a total of 384 chickens were taken from three selected areas and flotation method was employed to harvest coccidial oocysts. The result revealed that out of the 384 chickens, 165 (43%) of the chickens were found positive. the prevalence was higher in Private Farms (43.6%) followed by Markets (43%) and University of Gondar Veterinary Clinic (41%). The prevalence was found highly statistically significant ($P<0.05$) across age groups, in which 68.1% <3 months old chickens and 37.5% in chickens of greater or equals to three months old. A statistically significant difference ($P<0.05$) in prevalence of coccidiosis was also noted across breeds of chickens, with the prevalence of 48.7%, 44.2% and 26.8% in local, white leghorn and brown cucar respectively. There was a significant difference ($P<0.05$) among the different housing types with the prevalence of 49.1% in Floor, 45.7% in Backyard and 25.6% in Cages. There was no statistically significant difference ($P<0.05$) on the prevalence between male (44.3%) and female (42.4%) chickens.

The effect of body condition on the disease prevalence was assessed and relatively high prevalence was recorded in those chickens which have poor body condition (43.8%) than those chicken which have good body condition (42.7%) but not significant ($P>0.05$). This study showed that coccidiosis was prevalent in the study area and this signifie the need for intervention through awareness creation among farmers and veterinarians.

Dholi *et al*. (2011) found that *Azadirachta indica* has been used medicinally throughout history by many different cultures. Many compounds have been found in the exudates of the, *Azadirachta indica* plant that have been used medically by humans. We have examined the pharmacological hypoglycemic action of *Azadirachta indica* in diabetic rats. After treatment for 24 hrs, *Azadirachta indica* 250mg/kg (single dose study) reduced glucose (18%), cholesterol (15%), triglycerides (32%), urea (13%), creatinine (23%), and lipids (15%). Multiple dose study for 15days also reduced creatinine, urea, lipids, triglycerides and glucose. In a glucose tolerance test in diabetic rats with Neem extract 250 mg/kg demonstrated glucose levels were significantly less compared to the control group. *Azadirachta indica* significantly reduce glucose levels at 15th day in diabetic rats. *Azadirachta indica* serves as an important alternative source in the management of diabetes mellitus involved in reducing

increased blood glucose during diabetes which should be examined further by oral hypoglycemic therapy.

Kotsch *et al.* (2013) reported thatin the present study, efficacy of the toltrazuril treatment for prevention of coccidiosis and necrotic enteritis was tested. Ninety-six 14-day-old commercial broiler chickens were caged and divided into eight groups (n12), designated groups 1 to 8. Chickens of groups 1 to 6 were inoculated orally at 18 days of age with 25,000 oocysts of *Eimeria spp* and 75,000 oocysts of *Eimeria brunetti*. At 22 days of age, chickens of groups 1 to 6 were infected with 109 colony-forming unit Clostridium perfringens. Chickens of group 1 were treated with 75 parts/106 toltrazuril in drinking water for 8 h on two consecutive days up to 12 h before Eimeria infection, while chickens of groups 2 to 5 were treated with the same dose of toltrazuril at 12 h, 36 h, 60 h and 84 h after Eimeria infection, respectively. The non-treated group 6 served as a positive control. Chickens in group 7 were treated with toltrazuril at 17 and 18 days of age, and those of group 8 remained uninfected and non-treated as a negative control. The feed conversion ratio was higher in the positive control compared with other groups. The mortality rates were 16.8% and 41.7% in the late toltrazuril-treated (at 84 h) and infected non-treated chickens, respectively. Lesions scores of necrotic enteritis or coccidiosis in infected, non-treated chickens were significantly more severe compared with negative controls (PB0.01) and late toltrazuril-treated (at 84 h) chickens (PB0.05). In conclusion, application of toltrazuril before Eimeria challenge protected chickens from coccidiosis and indirectly from successive necrotic enteritis caused by *C. perfringens* infection.

Pana *et al.* (2010) showed that Coccidiosis is an old parasitic disease, prevalent all over the country and has a significant impact on poultry production. In this paper, economic loss to poultry industry has been estimated considering the major economic parameters. The estimation has revealed that commercial broiler industry is a major sufferer due to coccidiosis wherein 95.61 per cent of the total economic loss occurs due to the disease.

The commercial layer industry shares 3.53 per cent economic loss, mainly due to cost of chemoprophylaxis and reduced egg production. A comparison across economic traits has revealed that loss is maximum due to reduced body weight gain, followed by increased FCR (23.74%) and chemoprophylaxis (2.83%) in the total loss due to coccidiosis in broiler industry of India. The overall comparison of economic traits for all the types of poultry sector

it has shown that reduced body wt gain and increased FCR are the major parameters from which 68.08 per cent and 22.70 per cent annual loss has occurred in the total loss from coccidiosis in India during the year 2003-04. The total loss due to coccidiosis has been found to be of Rs 1.14 billion (approx) for the year 2003-04. The study has observed that generation of this data across different geographical regions will be helpful to conclude about the global economic loss due to coccidiosis in the poultry industry.

Gesek *et al.* (2014) found that Coccidiosis is the most predominant parasitic disease affecting Japanese quails (Cotumix cotumix japonica) in commercial farms. Coccidiosis as a subclinical infection is difficult to diagnose without parasitological examinations. Oocysts of two Eimeria species, E. bateri and E. tsunodai, were determined in the analysed quail flock. Infected birds were administered Baycox2.5% at the dose of: group 1-7 mg toltrazuril/kg BW per day provided in drinking water (1.5 ml/D.5 I H20) that was available 24 h for 2 days, group II - 14 mg/k:gBW (3 ml/D.5 I H20), and group III - 24.5 mg/k:gBW (5 m1/D.5 IH20); in groups II and III, the solutions were available 8 h/24 h for 2 days. After the first day of the treatment, the number of excreted oocysts (OPG - oocysts per gram) increased, a steady decrease in oocyst counts began on the second day of Baycox administration and lasted until a three-day period when no oocyst were determined in faecal sampies. .Regardless of the dose applied, toltrazuril (Baycox) completely eliminated *E. bateri coccidia* and led to a highly significant reduction in the number of *E. tsunodai oocysts*. The results suggest that the effectiveness of toltrazuril varies depending on coccidia species and developmental stages of the parasite. From the clinical point of view, the treatment applied significant1yreduces the number of coccidia oocysts in commercial flocks of Japanese quails.

CHAPTER III
MATERIALS AND METHODS

This study was conducted in collaboration with the department of physiology and pharmacology, department of Pathology and parasitology and department of dairy and poultry science, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur to evaluate the anti-coccidial effect of Neem leaves suspension and toltrazurilin *Eimeria spp* infection, heamatological parameters and growth performance in Sonali. The following procedures were followed for conducting this study.

3.1 Research site

The experiment was conducted at the experimental shed under the department of physiology and pharmacology, body weight determination was performed at the dairy and poultry science laboratory of the department of dairy and poultry science and microbial test was performed at microbiology laboratory of the department of microbiology, HSTU. The experiment was performed for a period of 80 days from 2ndjanuary to 3rdFebruary, 2019 to investigate the effects of Neem leaves and Toltrazuril liquid suspension on *E. spp* infected sonali.

3.2 Preparation of house

First the room as well as the wire cages were washed by sweeping with tap water using hose pipe connected with the tap. The room was disinfected with a phenolic disinfectant and allowed to dry leaving the room unused with the electric fan and the bulb switched on overnight. The room was properly ventilated. All the utensils required for the experiment such as feeder, water pot, beakers, pestle and mortar, syringe, needle etc. were collected and the experimental shed was properly designed.

3.3 Collection of feed

Polli mash commercial feed were collected from Griholokkhi Poultry Feed, Kalitola, Dinajpur from a reputed Sonali feed exporter. Mash and water were provided *ad-libitum* during the whole experimental period.

Fig 3.1. Sonali feed

3.4 Experimental sonali chicken

Eighty (80) chicks at the age of 10 days were obtained from Bahadur bazar, Dinajpur. All the birds were kept in the wire cages of the experimental shed. Proper ventilation and lighting was maintained inside the shed.

Fig. 3.2:Experimental Birds

3.5 Acclimatization of sonali

Immediately after reaching the destination the sonali were shifted to wire cages. They were fed with Griholokkhi Poultry Feed, Kalitola, Dinajpur and drinking water *ad-libitum*. Glucose and vitamin C were supplied with drinking water for first three days to overcome the transportation stress. sonalis were allowed to acclimatize in their new environment for 13 days before the commencement of the experiment.

3.6Lighting

During the whole experimental period, all Sonalis were exposed to a 16 hours continuous photoperiod (natural light plus artificial light) in an open sided house. Electrical bulbs were used for additional light at night.

3.7Routine management

Sonalis were provided to similar care and management in all groups throughout the experimental period. Adequate hygiene and sanitation were maintained properly.

3.8 Experimental birds grouping

Eighty (80) Sonalis were used to evaluate the dietary effect of Neem leavessuspension and toltrazuril on *Eimeria spp* infection, heamatological parameters and growth performance. The Sonalis were assigned into four groupscontaining 20 Sonalis in each groups.20Sonalis kept in each group were considered as an experimental unit (group). Sonalis were randomly distributed in every groups. The groups were designated and maintained as follows:

Group T_0: The Sonalis were fed normal diet and given water *ad-libitum* and their body weight was recorded at every 15 days interval. Body weights, blood parametesr and *Eimeria spp* protozoal load were measured at the times when that of other groups were measured. This group was served as "**Negative control**" group.

Group T_1: The Sonalis were supplied with protozoa after acclimatization to induce *Eimeria spp* infection in this group. No antiprotozoal treatment was done against *Eimeria spp* in T_1 group. Adequate feed and drinking water was given. This group served as "**Positive control"** group.

Group T_2: Sonalis were supplied with *Eimeria spp* protozoa after acclimatization to induce *Eimeria spp* infection as like as T_1 group. This group left as about 24 hours for establishment of *Eimeria spp* infection. After 24 hours, this group was treated with Neem leaves suspension as herbal antiprotozoal agent at a dose rate of 0.25 ml per 100 ml of drinking water. This group served as "**Neem leaves suspension**" group to find the effect of formulation as antiprotozoal drug, growth promoter, blood parameters.

Group T_3: After acclimatization to induce *Eimeria spp* infection, Sonalis of this group were supplied with *Eimeria spp* protozoa as like as T_1& T_2 group. This group left as about 24 hours for establishment of *Eimeria spp* infection. After 24 hours, this group was treated with antiprotozoal drug (toltrazuril liquid @ 1 ml per 2lit water.This group served as **"Toltrazuril"** group to find the effect of formulation as antiprotozoal drug, growth promoter, blood parameters.

3.9 Experimental design

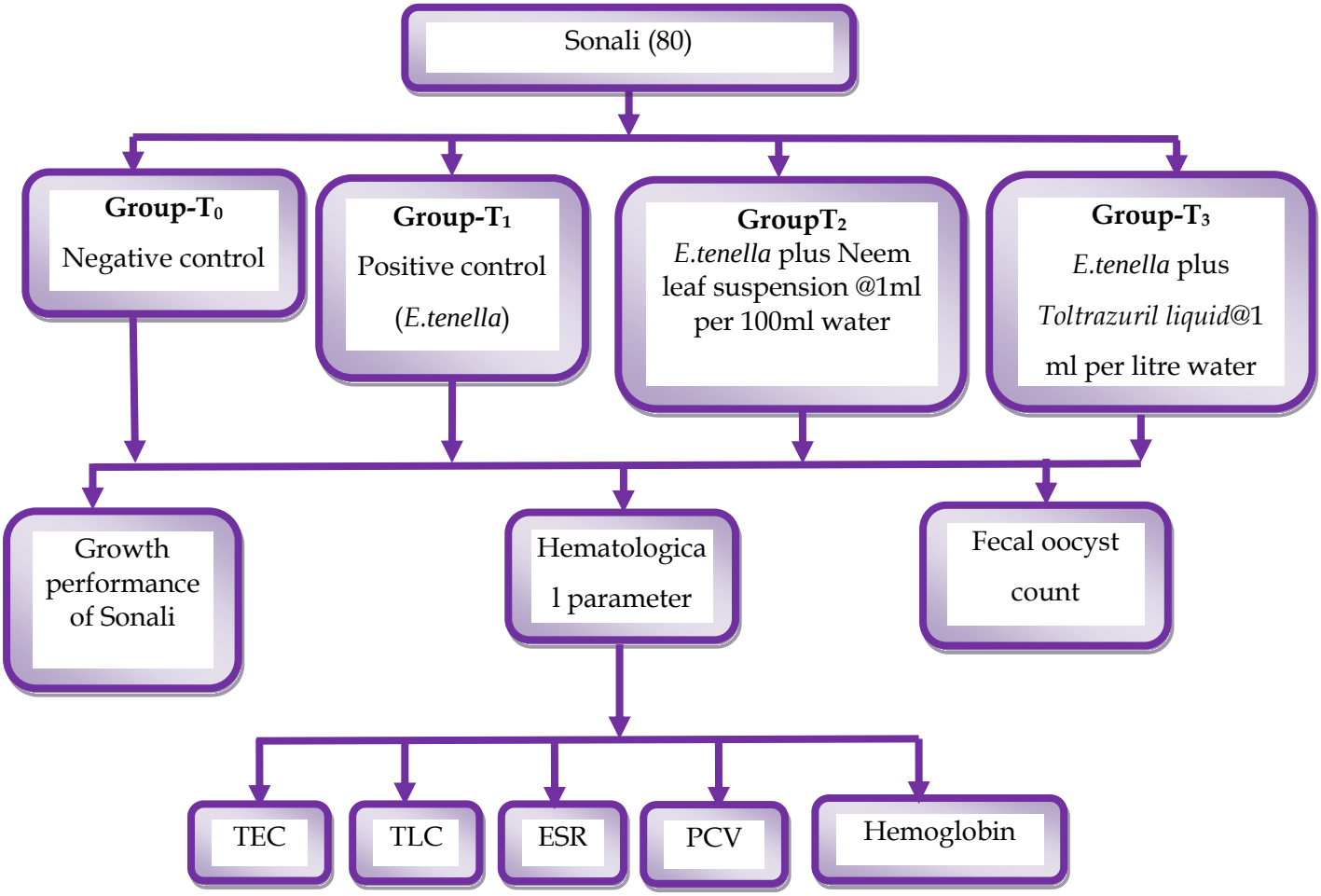

Fig 3.3: Layout of the Experiment

3.10 Collection and processing of neem leaves

Neem leaves were collected from the HSTU, Dinajpur. Young Neem leaves were collected and washed with fresh water. Before chopping it into small pieces, it was soaked with cotton for removing the adhesive water. Then the leaf was chopped into small pieces and was mashed with the help of pestle and mortar. Leaf juice was collected by squeezing mashed leaf. Then it had been produced 0.25% of suspension of grinded Neem leaf with distilled water.

Fig 3.3: Processing of Neem leaf

3.11Collection, preservation and administration of toltrazuril

Coxitril liquid was collected from Square pharmaceuticals Ltd. It was preserved in a dry place at room temperature and was administered at a dose rate of 1ml per 2 litre of drinking water for 3-7 days.

Fig. 3.4 Coxitril (toltrazuril 2.5%)

Composition: Each 100 ml solution contains toltrazuril BP 2.5g

Indications: Coxitril is an anticoccidial with activity against Eimeria species in poultry: Chicken: *Eimeria acervulina*, *E. brunetti*, *E. mitis*, *E. necatrix*, *E. praecox*, *E. spp* & *E. maxima*. Turkey: Eimeria adenoides, *E. meleagrimitis*.

Dosage & Administration: 1 ml of Coxitril per liter of the drinking water for 2 consecutive days, which can be repeated after 5 days in case of severe infections. Or as directed by the Veterinarian.

Contraindication: Don not use in laying bird

Side Effects: At the recommended dose, side effects are not found.

Withdrawal Period: The drug should be withdrawal 14 days prior to slaughter.

Precautions: Toltrazuril is an alkaline solution, wash off contaminated skin or eyes immediately.

Presentation: 100 ml, 500 ml, 1 Liter Bottle

3.12 Recording of body weight

The Sonalis were weighed at start (initial body weight) and then at the 7 days interval. Last body weight was recorded at 30th day of the experimental period with the help of digital balance.

Fig 3.5: Recording of body weigh

3.13 Collection and preparation of test organism

The test organism (*Eimeria spp*) was collected from the Gopalgonj local farm area, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur.

3.14 Collection and examination of feces for parasitic egg investigation

Feces were examined by two different types of qualitative tests; namely direct smear and flotation techniques were used to examine the fecal samples to identify the morphological features of eggs, cyst and oocysts (Hendrin and Robinson, 2006; Soulsby, 1982). The direct smear technique was conducted by mixing a drop of water with little bit of feces using an applicator stick on glass slide, covered with cover slip and examined under microscope.

The flotation technique was done by exploiting the density of the parasites, particularly eggs; it allows the parasites to float to the top of a dense solution (specific gravity about 1.20) and can then be skimmed from the top of the tube. For this purpose, 5g of feces were mixed with 5ml of saline. Then the mixture was filtered through sieve and the suspension was centrifuged at 1500 rpm for five minutes. The sediment mixed thoroughly with 5ml of saturated sodium chloride solution and centrifuged for one minute at 2500 rpm. Then the tube placed in a rack in a vertical position and slowly added enough saturated sodium chloride solution with a dropper to fill the tube so that an inverted meniscus forms. Without shaking the tube, a cover slip placed on top of the tube so that its underside rests on the meniscus and allowed to suspend on top for ten minutes. Then the cover slip was removed, applied to a glass slide and examined under microscope using a 10X objective.

3.15 Hematological parameters

Blood samples were collected from the wing vein of chicken of both control and treated groups at pre-feeding and during feeding (28 days) period at 7 days interval to study the effect of the Neem leaves extract and toltrazuril. The following parameters were observed:

- Total Erythrocyte Count (TEC)
- Hemoglobin Estimation (Hb)
- Total Leukocyte Count (TLC)

Total Erythrocytes Count (TEC), Erythroctes Sedimentation Rrate (ESR), Packed Cell Volume (PCV) and Total Leukocyte Count (TLC) were performed as per methods described by Schalm (1967). Hemoglobin estimation was perfomed as per method described by Coffin (1955).

3.15.1 Total Erythrocyte Count (TEC)

The tip of the dry and clean red pipette was placed on the blood sample.

- The blood was sucked up until it reached the exactly 0.5 mark and carefully wiped the tip of the pipette with a piece of absorbent cotton.
- Then the tip of the pipette placed immediately in the diluting fluid and filled the pipette exactly upto 101 mark.
- The number tube around the tip of the pipette was stretched and held with thumb and finger at each end.
- The contents of the pipette was shaked thoroughly with 8 knot or twisting motion for 1-2 minutes.
- Then the counting chamber with cover glass was placed under the microscope and made visible the finely rolled area with low power objective.
- After discarding 2 or 3 drops, a small drop from the pipette was placed to the end of the polished surface of the counting chamber containing the ruling and allowed the space to fill the area under the cover glass.
- The chamber was allowed to stand for 2 minutes to settle the erythrocytes and counted the cells on the four corner squares and one center square with high power objective(100x).
- The number of RBC was calculated as follows:
- Number of RBC = No. of cell counted x 10000
- The result was expressed in million/ cu.mm.

3.15.2 Determination of Hemoglobin

Following procedures were taken for determination of Hemoglobin.

- N/10 HCl was placed into the perfectly clean and dry diluting tube upto 2 mark.
- Then the blood sample was drawn into the sahli pipette exactly upto 20 mark and the side of pipette wiped to remove sticking blood to its outside with absorbent cotton.
- The blood of the pipette was transferred immediately into the diluting tube containing N/10 HCl acid and rinsed the pipette 2 or 3 times by sucking water into the pipette and added these washing to the solution in the tube.
- Using the dropper, water was added drop by drop, each time mixing the solution with a stirrer until the color of the solution matched the standard.
- After 5 minutes of first noting time, the result was read in day light from the scale of the measuring tube by observing the graduation mark at the lower edge of the meniscus at the top of the liquid column.
- The result was expressed in gm%.

3.15.3 Total Leukocyte Count (TLC)

The principles involved in enumeration of Total Leukocyte Count were almost same to those of erythrocytes. Here the leukocyte diluting fluid was N/10 HCl solution. Well mixed blood was drawn upto the 0.5 mark of white blood cell pipette. The diluting fluid was filled up to the 11 mark of the pipette and the contents were thoroughly mixed for 2 minutes. 2-3 drops of content were discarded and counting chamber was then filled in the same way as in the red blood cell count.

The counting chamber was placed under the microscope and examined under low power objective (10x). The leukocytes in the 4 large squares (each 1 square mm.) of the counting chamber were counted x 50 and expressed the result in thousand per cu.mm.

3.16 Adverse Effects

After Treatment there were no adverse effects on treated chickens.

3.17 Statistical analysis

The data were analyzed statistically between control and treated groups of chicken by the well-known statistical-‘t’ test.

CHAPTER IV
RESULTS AND DISCUSSION

To perform the experiment eighty sonalis randomly divided into four groups with three replications each containing ten birds. They were fed with *E. spp* organism for induction of infection. Group T_0 birds were kept as control (non infected) without giving *E. spp* and any other treatment whereas T_1 treated with *E. spp* and next two groups (T_2 &T_3) were treated with 0.25% Neem leaves suspension and Coxitril liquid suspension a dose of 1 gm/2lit water respectively. In this experiment, the efficacy of % Neem leaves suspension on protozoal load, blood constituents and growth performanc of sonali infected with *E. spp* were investigated. The results of this study are discussed under following headings.

4.1 Anticoccidial effect of Neem leaves suspension and toltrazuril liquid

Coccidial load in feces of *E.spp* infected sonali was presented in table 4.1. This study showed that, coccidial load in feces was significantly differed among the treated groups. Coccidial load significantly ($P<0.01$) increased in T_0 (negative control) and T_1 (positive control) group. Coccidial load significantly ($P<0.01$) decreased in T_2 group supplied with 1% Neem leaves suspension and Toltrazuril liquid suspension. Karim *et al.* (2016) stated that significantly higher ($P<0.05$) weight gain was recorded in Group A and B compared with Group C and D.The infected untreated chicks passed a large number of oocysts from day 5 till the end of the experiment. Chicks receiving sulphaclozine sodium (Groups A and B) had large oocysts count compared with the Groups C and D treated with toltrazuril but lower than the untreated chicks. At the end of experiment the lowest oocysts count was found in Group D (31.32 x 106) and highest in Group E (53.59 x106). Hence this study recommends to use sulphaclozine sodium and toltrazuril for chicken coccidiosis.

Table 4.1. Estimation of Oocyst per gram (OPG) during experimental period

Days	T_0	T_1	T_2	T_3	Significant level
1 day	0.00 ± 0.00^{a}	319.31 ± 1.67^{b}	412.48 ± 0.92^{c}	431.48 ± 1.25^{d}	*
15 day	0.00 ± 0.00^{a}	390.89 ± 1.81^{c}	190.36 ± 1.56^{b}	0.00 ± 0.00^{a}	*
30 day	0.00 ± 0.00^{a}	387.28 ± 1.62^{c}	55.68 ± 1.09^{b}	0.00 ± 0.00^{a}	*

Values are expressed as mean ± standard error of means.

a, b, c Means in each row with different superscript are significantly different at (0.05).

* = Significant at 1% level of significance.

*** T_0 =** Control (Basal diet)

* **T_1 =** (Basal diet plus *E. spp.*)

* **T_2 =** (Basal diet plus *E. spp.* plus0.5% neem leaves suspension*)*

* **T_3 =** (Basal diet plus *E. spp.* plus toltrazuril @ 1 ml per 1 litre drinking water)

4.2 Hematological parameter

Effect of Neem leaves suspension and toltrazuril liquid suspension on blood profile of sonali infected with *E.spp* is given below:

Total Erythrocyte Count:

Total Erythrocyte Count (TEC) is presented in table 4.1. The values of TEC in all treated groups and control group were more or less similar and within the normal range at 0th, 15th and 30th day of experimental period. These values showed a little fluctuation were not statistically significant (P>0.05) Meskerem *et al.*(2013). Hematological, Biochemical and Histopathological Changes Caused by Coccidiosis in Chickens.

Table: 4.2 TEC during experimental period

Days	T_0	T_1	T_2	T_3	Significant level
15 day	2.97±.18	3.19±.21	3.14±.16	3.28±.06	NS
30 day	3.15±.22	3.47±.09	3.35±.07	3.34±.06	NS

NS= Non significant

Note: Values are expressed as mean ± standard error of means. Means within column and between column are not statistically significant (P>0.05). NS=Not significant.

Total Leukocyte count (TLC):

The values of TLC are presented in table 4.2. At 15 days interval the values of TLC of T_0 (Basal diet) and T_3 (*E.spp* plus Coxitril liquid) group are more or less similar followed by same superscripts in the same column. But in case of T_1 (Basal diet plus *E.spp*) and T_2 (*E. spp* plus Neemleaves suspension) group the values are differed followed by different superscripts in the same column. The values are also differed between columns among the treated groups.

Table: 4.3 TLC during experimental period

Days	T_0	T_1	T_2	T_3	Significant level
15 day	$6.39±.05^{b}$	$6.27±.05^{d}$	$6.83±.14^{c}$	$6.58±.21^{a}$	*
30 day	$6.35±.07^{d}$	$6.09±.28^{b}$	$6.75±.12^{a}$	$6.66±.07^{c}$	*

Values are expressed as mean ± standard error of means.

a, b, c Means in each row with different superscript are significantly different at (0.05).

* = Significant at 1% level of significance.

*** T_0 =** Control (Basal diet)

* **T_1 =** (Basal diet plus *E. spp.*)

* **T_2 =** (Basal diet plus *E. spp.* plus0.5% neem leaves suspension*)*

* **T_3 =** (Basal diet plus *E. spp.* plus toltrazuril @ 1 ml per 1 litre drinking water)

Estimation of Hemoglobin:

Hemoglobin (Hb) is presented in table 4.4. The values in all treated groups and control group were more or less similar and the values were within normal range. They showed a little fluctuation but were not statistically significant (P>0.05). This indicates that there was no dietary effect of Neem leaves suspension and Coxitril liquid suspension on Hb.

Table: 4.4Estimation of Hemoglobin during experimental period

Days	T_0	T_1	T_2	T_3	Significant level
15 day	7.67±.44	7.54±.16	7.90±.02	8.04±.06	NS
30 day	8.05±.05	7.53±.25	8.25±.07	8.41±.03	NS

NS= Non significant

Note: Values are expressed as mean ± standard error of means. Means within column and between column are not statistically significant (P>0.05). NS=Not significant.

4.3 Body Weight

Data of Table 4.3 indicated that there were a significant (P<0.01) increase in body weight in T_2 (Neem leaf suspension) and T_3 (Coxitril liquid) group than the T_0 (Basal diet) and T_1 (*E.spp*). Birds treated with Neem leaves suspension (T_2 group) had the highest body weight gain compared to other groups. Lakkundi *et al.* (2002) studied that effect of toltrazuril and amprolium on body weight and feed efficiency of broiler chicken experimentally infected with *Eimeria spp.*

Table: 4.5 Estimation of Body Weight during experimental period

Days	T_0	T_1	T_2	T_3	Significant level
1 day	180.84±.51	182.01±0.93	152.43±0.94	182.41±0.87	NS
7 day	$223.47±.05^{d}$	$210.54±1.43^{a}$	$250.31±0.71^{b}$	$261.92±1.42^{c}$	*
14 day	$253.50±1.18^{b}$	$264.23±0.84^{c}$	$343.72±1.55^{d}$	314.11±0.43a	*
21 day	$375.70±0.4^{d}$	$351.33±1.49^{c}$	$497.37±1.89^{b}$	$362.42±1.64^{a}$	*
30 day	$432.65±0.71^{b}$	$469.81±1.23^{c}$	$548.86±1.29^{d}$	$489.22±1.33^{a}$	*

Values are expressed as mean ± standard error of means.

a, b, c Means in each row with different superscript are significantly different at (0.05).

* = Significant at 1% level of significance.

*** T_0 =** Control (Basal diet)

* **T_1 =** (Basal diet plus *E. spp.*)

* **T_2 =** (Basal diet plus *E. spp.* plus0.5% neem leaves suspension*)*

* **T_3 =** (Basal diet plus *E. spp.* plus toltrazuril @ 1 ml per 1 litre drinking water)

*. Significant at the 0.01 level,

NS= Non significant

CHAPTER V
SUMMARY AND CONCLUSION

The experiment was conducted at the experimental shed under the department of physiology and pharmacology to evaluate the comparative effect of *neem leaves suspension and toltrazuril* on protozoal load, blood constituents and growth performance of sonali chicken infected with *E. spp.* This experiment was performed in collaboration with the department of physiology and pharmacology, department of pathology and parasitology, HSTU, Dinajpur. Eighty (80) sonali chicken of 7 days old were divided into 4 groups with 20 birds in each groups. Birds were randomly allowed to 4 dietary treatments: T_0 (control), T_1 (*E. spp*), T_2 (0.5% *neem leaves suspension*) and T_3 (toltrazuril). In the present study, supplementation of *neem leaves suspension* to birds significantly ($P < 0.01$) decreased the population of *E. spp* compared to T_0 and T_1 group. In case of hematological examination, TEC and Hb in different treatment groups were almost similar and the differences were statistically non-significant except TLC. TLC values were statistically significant ($P<0.01$). Body weights were increased significantly ($P<0.01$) in T_2 (*neem leaves suspension*) and T_3 (toltrazuril) *group* than the T_0 (Basal diet) and T_1 *(E. spp).* Highest body weight gain was recorded in T_2 group treated with *neem leaves suspension*. In this experiment the fecal oocyst count also performed to investigate the protozoal infestation and number of oocyst were statistically significant ($P<0.01$).

From the current experimental data, it may concluded that neem leaves (*Azadirachta indica*) suspension and toltrazural have anticoccidial effect and effects on growth performance of sonali chicken with *E. spp.* As toltrazuril shows drug resistance and other side effects, so neem leaves suspension is more preferable than toltrazuril but toltrazuril is more effective than *neem leaves suspension* against coccidiosis. There is no adverse effect found in herbal use of this neem leaves (*Azadirachta indica*) suspension at the dose rate of 0.5%during this experimental period but toltrazuril has some adverse effects. In conclusion, this experiment supports the traditional usage of neem leaf (*Azadirachta indica*) for the control of coccidiosis. It can also be considered as a good growth promoter, leukocyte enhancer in sonali chicken. It may be stated that the neem leaf (*Azadirachta indica*) may provide a new therapeutic avenue against *E. spp* because of its availability in our country and effectiveness. To draw a definite conclusion in this regard it demands elaborate study of this herbal medicine.

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