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Effect of *Butea monosperma* on egg per gram of faeces of artificially induced *Ascaridia galli* infection in indigenous chicken

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Abstract
The experiment was carried out to assess the efficacy of ethanolic, hydroethanolic and aqueous extracts of *Butea monosperma* leaves on *Ascaridia galli* infection in indigenous birds. A total of sixty chicks were randomly selected that were divided into three groups twelve groups with 6 birds in each group. The birds were then artificially infected with *Ascaridia galli* @ 1000 eggs/bird. Ethanolic extract of *Butea monosperma* was orally fed to Group I, II, and III at 100, 500 and 1000 mg/kg body weight, hydroethanolic extract of *Butea monosperma* was orally fed to Group IV, V, and VI at 100, 500 and 1000 mg/kg body weight and aqueous extract of *Butea monosperma* was orally fed to Group VII, VIII, and IX at 100, 500 and 1000 mg/kg body weight respectively while Positive control group was left untreated that served as control. A standard Piperazine group was also maintained. The faecal egg count (FEC) was conducted on a weekly basis. The pre-treatment values of FEC in all the groups were found negative from day 0 to 44 after artificially inducing infection. The FEC post-treatment values of all the groups were significantly (p<0.05) decreased as compared to the control. It was concluded that *Butea monosperma* was effective in controlling the *Ascaridia galli* infection in chickens.

Keywords: Anthelmintics, *Ascaridia galli*, *Butea monosperma*, indigenous chicken

Introduction
The intestinal parasitic nematodes cause severe diseases in poultry birds in developing countries. *Ascaridia galli* is the most common and important nematode that causes sustains economic losses to poultry birds. Birds infected with *A. galli* usually suffer from severe diarrhoea, and anaemia leading to the loss of body weight resulting in heavy economic losses in poultry farming (Permin & Raving, 2001) [17] therefore their timely control may help bear better economic benefits. Mostly synthetic compounds are used for controlling the internal parasites. The use of these compounds has a negative impact on the health of the bird as well as public health issues with residues of drugs in poultry meat leading to carcinogenesis (Butaye *et al*., 2003) [6]. These compounds also develop resistance against the causative agents. However, the high cost of anthelmintics diverted attention towards alternative control practices, including the usage of medicinal plants in traditional remedies. The World Health Organization reported that eighty percent of the rural population confides on herbal remedies for their basic health care. From the days of human civilization, numerous plants are being used in conventional medicine. The data on the usefulness of the majority of plants are unavailable. However, researchers are going on to confirm the efficacy of various plants (Nayak *et al.*, 2011; Sofowora *et al.*, 2013) [16, 20]. The herbal remedies are cost effective, have minimum toxicity with reduced health hazards and are easily available in the market as compared to synthetic medicines (Khandaker *et al.*, 2016) [12]. In the current study, the efficacy of ethanolic, hydroethanolic and aqueous extract of *Butea monosperma* leaves on *Ascaridia galli* infection in indigenous birds was evaluated.

Materials and Methods
The study was carried out at Krishi Vigyan Kendra, Golaghat and the Department of Veterinary Pharmacology & Toxicology, Assam Agriculture University, Khanapara, Guwahati.

Sample size and Experimental birds

Ten days old, thirty chicks were brought from M/S B. S. Poultry market and placed into 12 groups comprising 6 birds. The birds were reared in low cost experimental sheds with uniform managemental conditions. The birds were vaccinated against Gumboro and New Castle Disease.

Collection of the *A. galli* eggs

Mature and live *A. galli* worms were collected from freshly slaughtered birds from Golaghat town. The lumen of the intestine was opened longitudinally and adult *A. galli* were collected with a needle. Female parasites were identified under the microscope by size, shape and colour of the shell using the key provided by Soulsby (1983) [21] and the gravid female worms were selected, washed in tap water several times and rinsed in normal saline (Riedel, 1951) [19]. Uteri of these long and gravid female worms were taken out in a Petri dish and the eggs were teased out of each uterus with a soft brush so that the eggs so obtained were not damaged. Only the eggs in the portion of the uterus proximal to the vagina were used because Ackert (1931) [4] has shown this part of the uterus contains a high percentage of fertile eggs. This thin suspension of *Ascaridia* ova was then transferred into a clean and sterile Petri dish and some more tap water was added into the Petri dish so that the layer of this suspension was only 0.5 mm in height. The suspension was then incubated at 22-26 °C until the development of second stage larvae (L2). About half of the Petri dishes were kept open to allow aeration for the development of eggs and the upper meniscus of fluid was maintained regularly to prevent desiccation. After the development of L2 stage, the culture media was washed several times in tap water through centrifugation at 2000 rpm for seven minutes and finally counted for *in vivo* assay (Choudhury, 2008) [7].

Preparation of Ethanolic, Hydroethanolic and Aqueous Extracts

Powdered plant materials were extracted with ethanol, hydroethanol (1:1) and distilled water respectively as per the procedure of Prasad (1965). Finely powdered plant powders were soaked individually for 72 hours, three times, with intermittent agitation. The extracts were then double filtered using a muslin cloth and Whatman No. 1 filter paper. The filtrate obtained was concentrated in a Soxhlet apparatus under reduced pressure and completely dried over a regulated water bath maintained at 50 °C. The percentage yield of each of the fifteen extracts was calculated. The extracts were refrigerated at 4 °C until the experiments for screening were done. Standard procedures (Lateef *et al.* (2003) [15]; Sujon *et al.*, 2008; Krimpen *et al.*, 2008) [22, 13] were used with a few modifications.

Inoculation of experimental birds

All the experimental birds were artificially infected orally with 1000 *A. galli* eggs. The fecal samples of all birds were collected and checked on a regular basis for confirmation of *A. galli* eggs. On day 44th (after confirmation of infection) the groups were treated with ethanolic, hydroethanolic and aqueous extracts of *Butea monosperma* @ 100,500 and 1000 mg/kg b.w. respectively. Positive control group received no treatment and served as control. The fecal examination was continued up to 28 days and the Fecal Eggs Counts - FEC (quantitative examination) was carried out by McMaster Technique according to the method given by Urquhart *et al.*, (1996) [24].

Statistical analyses

Results were expressed as Mean ± SEM. Statistical analysis was performed by MS-excel to calculate the mean, standard error of Mean (SEM), analysis of variance (ANOVA), and coefficient of correlation (r) values. All statements of significance were based at least on 95% confidence limits (*i.e.* $p<0.05$).

Results

Table 1 represents the data of all the experimental birds shedding *A. galli* eggs from day 0 to 28 recorded on a weekly basis post treatment with *B. monosperma*. All the groups showed no egg shedding from day 0 to 44 days post inoculation. However, the egg shedding started from day 44th and the pretreatment fecal egg count (FEC) of all the groups recorded are shown in Table 1. It was further revealed that the egg shedding was significantly ($p>0.05$) reduced after 14 days post-treatment. The ethanolic, hydroethanolic and aqueous extracts of *Butea monosperma* brought down the average EPG from 7th day onwards but the change was not significant ($p<0.05$). Some minor fluctuations in EPG values were seen with hydroethanolic extract. Hydroethanolic extracts of *B. monosperma* showed a good reduction of EPG at 100 mg/kg than 500 and 1000 mg/kg, which means the therapeutic effect may not be dose-dependent. A higher egg count may not necessarily indicate the level of worm infection (Tarpoff, 2010). Weekly EPG did not show any significant difference ($p>0.05$). Piperazine hydrate brought down the mean EPG from 733.33±268.22 at pre-treatment to 106.67±23.33 on 28th day post-treatment.

Table 1: Effect of ethanolic, hydroethanolic and aqueous extract of *Butea monosperma* on egg per gram faeces after *A. galli* infection in *Gallus domesticus*

Group	Post Treatment Days				
	0	7	14	21	28
Negative Control (NC)	$0.00\pm0.00^{a}{}_{A}$	$0\pm0.00^{a}{}_{A}$	$15.67\pm10.27^{a}{}_{A}$	$13.33\pm13.33^{a}{}_{A}$	$23.33\pm23.33^{a}{}_{A}$
Positive Control (PC)	$966.67\pm101.38^{b}{}_{A}$	$983.33\pm202.76^{b}{}_{A}$	$1206.67\pm166.97^{b}{}_{AB}$	$1390.00\pm221.89^{b}{}_{BC}$	$1563.33\pm370.24^{b}{}_{C}$
Group I (BME-100)	$706.67\pm179.47^{bc}{}_{A}$	$550.00\pm155.24^{c}{}_{AB}$	$463.33\pm167.07^{cd}{}_{AB}$	$256.67\pm41.77^{a}{}_{AB}$	$240.00\pm75.72^{a}{}_{B}$
Group II (BME-500)	$490.00\pm133.17^{c}{}_{A}$	$403.33\pm127.06^{c}{}_{A}$	$320.00\pm47.26^{acd}{}_{A}$	$213.33\pm26.67^{a}{}_{A}$	$200.00\pm25.17^{a}{}_{A}$
Group III (BME-1000)	$616.67\pm189.77^{bc}{}_{A}$	$540.00\pm65.06^{c}{}_{A}$	$540.00\pm17.32^{c}{}_{A}$	$280.00\pm50.00^{a}{}_{A}$	$246.67\pm34.80^{a}{}_{A}$
Group IV (BMH-100)	$690.00\pm78.10^{bc}{}_{A}$	$380.00\pm162.89^{c}{}_{AB}$	$110.00\pm11.55^{a}{}_{B}$	$130.00\pm55.08^{a}{}_{B}$	$103.33\pm23.33^{a}{}_{B}$
Group V (BMH-500)	$630.00\pm164.62^{bc}{}_{A}$	$416.67\pm87.62^{c}{}_{AB}$	$320.00\pm72.11^{ac}{}_{AC}$	$233.33\pm37.12^{a}{}_{BC}$	$296.67\pm46.31^{a}{}_{AC}$
Group VI (BMH-1000)	$590.00\pm204.04^{c}{}_{A}$	$510.00\pm125.03^{c}{}_{A}$	$546.67\pm72.19^{ce}{}_{A}$	$280.00\pm32.15^{a}{}_{A}$	$253.33\pm21.86^{a}{}_{A}$
GroupVII (BMA-100)	$673.33\pm212.63^{bc}{}_{A}$	$500.00\pm175.59^{c}{}_{AB}$	$176.67\pm63.33^{ade}{}_{BC}$	$136.67\pm42.56^{a}{}_{C}$	$140.00\pm80.83^{a}{}_{C}$
Group VIII (BMA-500)	$530.00\pm205.99^{c}{}_{A}$	$456.67\pm84.13^{c}{}_{A}$	$326.67\pm35.28^{ac}{}_{A}$	$230.00\pm23.09^{a}{}_{A}$	$260.00\pm81.45^{a}{}_{A}$
Group IX (BMA-1000)	$673.33\pm199.36^{bc}{}_{A}$	$576.67\pm182.70^{c}{}_{AB}$	$530.00\pm145.72^{c}{}_{AB}$	$273.33\pm96.15^{a}{}_{B}$	$226.67\pm44.10^{a}{}_{B}$
Standard (Piperazine)	$733.33\pm268.22^{bc}{}_{A}$	$536.67\pm193.25^{c}{}_{AB}$	$256.67\pm80.90^{ac}{}_{BC}$	$153.33\pm49.10^{a}{}_{C}$	$106.67\pm23.33^{a}{}_{C}$

* All values are Mean ± S.E.M (n=6)

**Means with different superscripts within a column (small letters) and within arow (capital letters) differ significantly ($P<0.05$)

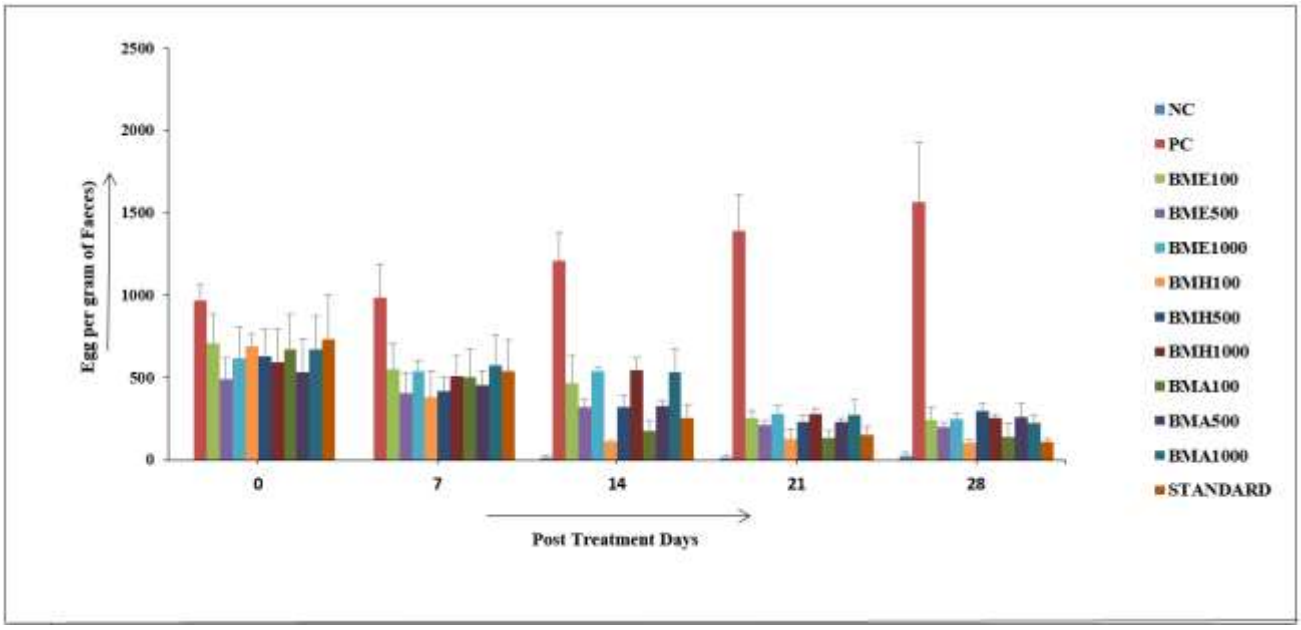

Fig 1: Effect of ethanolic, hydroethanolic and aqueous extract of *Butea monosperma* on egg per gram faeces after *A. galli* infection in *Gallus domesticus*

Discussion

The use of medicinal plants against various diseases of humans and animals is widely documented. Various parts of medicinal plants show anti-parasitic characteristics and are usually applied as a remedy against internal and external parasites (Bauri *et al*., 2015) [5]. Many workers demonstrated the medicinal effect of numerous plants, including papaya and neem, against A. *galli* infection in poultry and some other animals (Akhter & Riffat, 1984; Hammond *et al*., 1997; Lateef, 2002; Hordegen *et al*., 2003; Hordegen *et al*., 2006; Ali, 2006; Das *et al*., 2006; Ayub *et al*., 2011) [1, 9, 14, 10, 11, 2, 8, 3]. During the present study, the anthelmintic activity of *Butea monosperma* was evaluated *in-vivo* against *A. galli* by giving oral treatment of ethanolic, hydroethanolic and aqueous extracts of these plants in infected birds at 100, 500 and 1000 mg/kg and was compared with untreated control and Piperazine standard. The successive control of *A. galli* by all the extracts of the plant was observed. Results revealed that on day 44th of the artificial inoculation of *A. galli*, the infection was established and EPG was determined. On the same day, the treatment with the extracts was given to all the groups respectively and a significant (p<0.05) decrease in parasitic burden was recorded in the following days while in group PC (control without treatment), a significant increase in infection was observed. The EPG in the Standard group was found to decrease significantly by the 14th day. It was shown that the ethanolic, hydroethanolic and aqueous extracts of *Butea monosperma* were effective against *A. galli* in chicken.

Conclusion

It is concluded that ethanolic, hydroethanolic and aqueous extracts of *Butea monosperma* were effective against the *A. galli* infection in chicken. More research towards particular active compounds against the control of gastrointestinal parasites in poultry is suggested here.

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Conflict of Interest

The author declared no conflict of interest.

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Author's Contribution

All the authors read and approved the final manuscript.

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