Int.J.Curr.Microbiol.App.Sci (2020) *9*(10): 4035-4041

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 9 Number 10 (2020)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2020.910.463

Evaluation of Anticoccidial Effect of *Piper betel* Leaves Extract against *Eimeria tenella* in Broiler Chicken

K. Saranya[1], R. Velusamy[1]*, N. Rani[1] and A. Arulmozhi[2]

[1]Department of Veterinary Parasitology, [2]Department of Veterinary Pathology
Veterinary College and Research Institute- Namakkal,
Tamil Nadu Veterinary and Animal Sciences University, Tamil Nadu, India

**Corresponding author*

Keywords

Anticoccidial effect, Piper betel, Broiler

Article Info

Accepted:
04 September 2020
Available Online:
10 October 2020

A B S T R A C T

To evaluate the anti-coccidial effect of *Piper betel* leaves aqueous extract (PBE) on *Eimeria tenella* infection in broilers, 96 day old Ven Cobb-400 broiler chicks were divided into six groups, each with two replicate (n=16). The six groups were blank control group (BC), negative control group (NC), positive control group (PC) and three PBE treated groups. The birds in the three PBE treated groups were supplemented with PBE at 5 (T1), 10 (T2) and 15 (T3) percent in drinking water while the birds in the control groups (BC, NC) were not supplemented with PBE and PC group birds were supplemented with Salinomycin in the feed. At 21 days of age, the birds in the NC, PC and the three PBE groups were inoculated with sporulated oocysts (20,000 oocysts / bird) of *Eimeria tenella.* The results showed that PBE significantly ($P<0.05$) lower the oocysts per gram (OPG) and the higher (73%) oocysts reduction rate in T1 group and values were comparable with PC group. Similarly, the Intensity of caecal lesions due to *E.tenella* was mild in T1 group. The current results show that aqueous leaves extract of *Piper betel* having anticoccidial activity and the effect was not increased with level of supplementation. Hence it can be safely used as prophylactic against *E. tenella* infection in broiler chicken.

Introduction

In broiler industry, the growth rate is considered as the most important economic trait. But it is often affected by many bacterial, viral and protozoan diseases. Of which coccidiosis is considered as one of the most economically important protozoan diseases and caused by different species of *Eimeria*. Among the *Eimeria spp.* affecting chicken, *Eimeria tenella* is the most common and highly pathogenic in broilers (Alzahrani *et al.,* 2016). It causes great economic losses to the poultry farmers and remains a big concern for the commercial broiler production, because of the high cost involved in the control of this deadly disease (Abbas *et al.,* 2012). Currently, the poultry industry is largely depends on chemotherapy and live vaccines for control of coccidiosis (Allen and

Fetterer, 2002). The routine use of synthetic drugs in the feed or water has led to the development of resistant strains (Abbas, 2011) and on the other hand, consumer health issues due to drug residues (metabolites) in poultry products (Shivaramaiah *et al.*, 2014).

Therefore, more effective, environment friendly, residues free and safe anti-coccidial drugs are necessary to control this insidious coccidiosis under field condition (Giannenas *et al.*, 2012). One of the potential alternative to synthetic drugs is the use of botanical supplements as they are not only the natural products but also have novel therapeutic molecules to which resistance is not yet developed (Masssoud *et al.*, 2010 and Almeida *et al.*, 2012). Several herbal and medicinal plants have been used as coccid-iostat in traditional medicine in different parts of the world (Nwosu *et al.*, 2011; El-Khtam *et al.*, 2014 and Nghonjuyi *et al.*, 2015).

The betel (*Piper betel*) leaves are commonly used as masticatory in Asian countries. Leaves of the betel possess various types of therapeutic properties like anti-inflammatory, immunomodulator, anthelmintic and anti-coccidial effect. The betel leaves possess antioxidant properties (Dasgupta and De, 2004), which potentially decrease the oocysts production in the intestinal mucosa and sporulation of oocysts in the faeces (Naidoo *et al.*, 2008). Hence, the present study was undertaken to evaluate the effect of aqueous extract of betel leaves on oocysts production and caecal lesion development in broiler chicks experimentally infected with *Eimeria tenella* .

Materials and Methods

This study was conducted after necessary approval from the Institutional Animal Ethics Committee (IAEC) of Veterinary College and Research Institute, Namakkal.

Chicks

In this study, 96 day old VenCobb-400 broiler chicks were purchased from commercial hatchery.

The birds were randomly divided into six groups, each with two replicate (n=16). The birds were reared under standard conditions in a cage system and allowed free access to water and feed. The duration of the experiment lasted for 32 days.

Preparation of PBE

The betel leaves were collected and washed thoroughly in tap water to remove the debris. Aqueous extract was prepared by mixing finely cut fresh leaves of papaya (100 g) or betel (100 g) with water (100 ml) (1:1, W/V) and considered as a concentration of 100 per cent.

Various concentrations *viz*., 5, 10 and 15 per cent of extract were prepared freshly by mixing 50, 100 and 150 ml of leaves extract with 950, 900 and 850 ml of water respectively.

Experimental design and diet

The six groups were blank control group (BC), negative control group (NC), positive control group (PC) and three PBE treated groups.

The birds in the three PBE treated groups were supplemented with freshly prepared PBE at 5 (T1), 10 (T2) and 15 (T3) percent in drinking water while the birds in the control groups (BC, NC) were not supplemented with PBE and PC group birds were supplemented with Salinomycin (0.01%) in the feed.

The diet was prepared as per the standard of BIS (2007). Broiler pre starter ration was fed

to broiler chicks for first two weeks of age, starter feed for 3 to 4 weeks and finisher ration was used after that.

Experimental infection

All the chicks were reared in cage system up to 32 days of age under standard condition. At 21 days of age, the birds in the NC, PC and the three PBE groups were inoculated with sporulated oocysts (20,000 oocysts / bird) of *Eimeria tenella* which was propagated in the Department of Veterinary Parasitology, VC & RI, Namakkal during the initial phase of the experimental trial.

Oocysts per gram (OPG)

To determine the anticoccidial effect the OPG in all the groups were determined. OPG was examined in samples of faeces collected daily from each replicate, daily from 5 to 10 days post infection based on methods described by Holdsworth *et al.*, (2004).

The faecal oocysts concentration reduction rate was determined by using the formula as per Arlette *et al.*, (2019).

$$\text{Faecal oocysts concentration reduction rate (\%)} = \frac{\text{Initial mean OPG - Final mean OPG}}{\text{Initial mean OPG}} \times 100$$

Cecal lesion score

To assess the severity of caecal lesions, the birds were sacrificed on the 7th of post infection six birds from each group (totally 12 birds per treatment) were randomly selected and sacrificed.

The caeca were collected and gross lesions score (0 to 4) for each group was determined as per the method suggested by Johnson and Reid (1970).

Statistical analysis

The data were subjected to Analysis of Variance (ANOVA) and the differences between means were tested by Duncan's multiple range tests (Duncan, 1955).

Results and Discussion

Oocysts per gram

As shown in Table 1, the OPG number shedded was statistically ($P>0.05$) similar in PBE supplemented groups at all the three concentrations (5, 10 & 15%) relative to NC group. No oocysts were observed in the BC group. The highest OPG was observed on 7 days post infection (DPI) and then gradually decreased on 8 to 10 DPI. The percentage of oocysts reduction rate was significantly ($P<0.05$) higher in PBE (5%) group than in the other groups.

The decreased OPG in PBE groups suggests that betel leaves extract may be suitable for prevention of avian coccidiosis in chicken farms. Among the plant extract supplemented groups, less number of oocysts in the droppings was observed in birds supplemented with betel (5%). The anti-coccidial effect of this plant could be attributed to the effect of antioxidant present in the leaves. This finding is akin to the observation of Allen and Danforth (1998) and Khaliq *et al.*, (2015).

Moreover, it can also be emphasized that plant extracts inhibited the development of *Eimeria* life cycle in the host cell before oocysts are released into host droppings, thus ultimately decreased *Eimeria* oocysts excretion and severity of infection (Dkhil *et al.*, 2011).

Table.1 Mean (± SE) Oocysts per gram (OPG No. × 10^5) of faeces and oocysts reduction rate in broilers from different experimental groups

Groups	Days after infection					Total	Oocyst Reduction rate (%)
	6	7	8	9	10		
***Piper betel* leaves extract (%)**							
5	0	3.88^{cd} ± 0.06	2.92^{cde} ± 0.08	1.61^{c} ± 0.04	1.04^{bc} ± 0.04	9.45^{c} ± 0.01	73.20^{def} ± 0.62
10	0	3.32^{c} ± 0.02	2.70^{cde} ± 0.19	2.76^{e} ± 0.19	1.26^{bc} ± 0.08	10.04^{cde} ± 0.06	62.03^{c} ± 2.64
15	0	3.98^{cd} ± 0.18	2.42^{cd} ± 0.01	2.72^{e} ± 0.11	1.48^{bcd} ± 0.04	10.59^{cde} ± 0.27	62.80^{cd} ± 2.3
***Positive control- PC**	0	2.31^{b} ± 0.24	1.14^{b} ± 0.14	0.79^{b} ± 0.21	0.40^{a} ± 0.11	4.64^{b} ± 0.47	82.00^{f} ± 6.64
****Negative control - NC**	0	7.16^{e} ± 0.25	6.09^{f} ±0.76	4.39^{f} ± 0.12	3.72^{e} ± 0.14	21.35^{f} ± 1.26	48.08^{b} ± 0.11
*****Blank control – BC**	0	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
SEM	-	0.038	0.029	0.031	0.020	1.103	3.134
P value	-	0.001	0.001	0.001	0.001	0.001	0.001

^{abcdef} Means with different superscripts in column differ significantly (P<0.05)
* Positive control - Infected and supplemented with salinomycin
** Negative control – Infected and without supplementation of salinomycin
*** Blank control – Uninfected and without supplementation of salinomycin

Table.2 Caecal gross lesion score of broilers in different experimental groups infected with *E. tenella* (7 DPI)

Treatment	Mean lesion score (± SE)	Lesion score distribution (%)				
		0	+1	+2	+3	+4
***Piper betel* leaves extract (%)**						
5	1.80 ± 0.13^{cd}	0	20	80	0	0
10	2.40 ± 0.22^{e}	0	10	40	50	0
15	2.20 ± 0.20^{de}	0	10	60	30	0
***Positive control- PC**	1.00 ± 0.00^{b}	0	100	0	0	0
****Negative control - NC**	3.17 ± 0.17^{f}	0	0	25	75	0
*****Blank control – BC**	0.00 ± 0.00^{a}	100	0	0	0	0
SEM	0.022	-	-	-	-	-
P value	0.001	-	-	-	-	-

^{abcdef} Means with different superscripts in column differ significantly (P<0.05)
* Positive control - Infected and supplemented with salinomycin
** Negative control - Infected without supplementation of salinomycin
*** Blank control - Uninfected without supplementation of salinomycin

The highest oocysts reduction rate of 73.20 ± 0.62 per cent in betel (5%) group was observed and was comparable with PC group (82.00 ± 6.64 %) in our study indicates that the efficacy of betel not increased with dose of extract increases.

This is in agreement with the work of El-Khtam *et al.,* (2014) and Biu *et al.,* (2006).

Cecal lesion score and mortality

Caecal gross lesion score in experimentally infected broilers with *E. tenella* (7 DPI) are presented in Table 2.

Cecal lesion score was significantly higher in the NC group ($P<0.05$) than in the other groups. Salinomycin (PC) significantly reduced cecal lesion score ($P<0.05$) on 7 DPI. Birds supplemented with PBE reduced lesion scores compared to NC group ($P<0.05$). *Eimeria tenella* infections mainly damage the caecal mucosa, causing mucosal haemorrhage, inflammation and a thickened tunica muscularis, thus altering the integrity of the caecal mucosa. The lower lesion score in plant extracts supplemented groups in our study may be due to the decreased mucosal damage, suggesting that developmental stages of *E. tenella* might have been suppressed or delayed by the ingredients present in the plant extracts and exerts some palliative effect against coccidiosis (Blake and Tomley, 2014).

The lower lesion score observed in these group can be correlated with lower OPG and higher oocysts reduction rate recorded in our study.

No death occurred in all the groups. The absence of mortality in any of the groups of chicken in our study supports the findings of previous workers (Anosa and Okoro, 2011). The present study concluded that aqueous leaves extract of *Piper betel* having anticoccidial activity. Hence it can be safely used as prophylactic against *E. tenella* infection in broiler chicken. The effect was not increased with level of supplementation.

References

Abbas, R. Z., Z. Iqbal, D. Blake, M. N. Khan and M. K. Saleemi. 2011. Anticoccidial drug resistance in fowl coccidia: the state of the play revisited. *World Poult. Sci. J*., 67: 337 -350.

Abbas, R., Z. Iqbal, A. Khan, Z. Sindhu, J. Khan, M. Khan and A. Raza. 2012. Options for integrated strategies for the control of avian coccidiosis. *Int. J. Agric. Biol*., 14: 1014 -1020.

Allen, P. C and R. H. Fetterer. 2002. Recent advances in biology and immunobiology of *Eimeria* species and in diagnosis and control of infection with these coccidian parasites of poultry. *Clin. Microbiol. Rev*., 15: 58 - 65.

Allen, P. C., H. D. Danforth and P. C. Augustine. 1998. Dietary modulation of avian coccidiosis. *Int. J. Parasitol*., 28(7): 1131-1140.

Almeida, G. F., K. Horsted, S. M. Thamsborg, N. C. Kyvsgaard, J. F. S. Ferreira and J. E. John Hermansen. 2012. Use of *Artemisia annua* as a natural coccidiostat in free range broilers and its effects on infection dynamics and performance. *Vet. Parasitol*., 186(3-4): 178-187.

Alzahrani, F., E. M. Al-Shaebi, M. A. Dkhil and S. Al-Quraishy. 2016. *In vivo* anti-*Eimeria* and *in vitro* anthelmintic activity of *Ziziphus spinachristi* leaf extracts. *Pakistan J. Zoo*., 48: 409 - 413.

Anosa, G. M. and O. J. Okoro. 2011. Anticoccidial Activity of the methanolic Extract of *Musa*

paradisiaca root in chickens. *J. Trop. Anim. Health Prod.*, 43: 245- 248.

Arlette, N. T., N. A. C. Nadia, Y. Jeanette, M. T. Gertrude, W. P. Josue and M. Mbida. 2019. Anticoccidial effects of *Ageratum conyzoides* (Asteraceae) and *Vernonia amygdalina* (Asteraceae) leaves extracts on broiler chickens. *South Asian J.Parasitol*., 2(1): 1-10.

BIS, Bureau of Indian Standard. 2007. Poultry feeds specification. Bureau Manak of Bihavan, India.

Biu, A. A., S. D. Yusuf and J. S. Rabo. 2006. Use of neem (*Azadirachta indica*) aqueous extract as a treatment for poultry coccidiosis in Borno State, Nigeria. *African Scientist*., 7: 147-153.

Blake, D. P and F. M. Tomley. 2014. Securing poultry production from the ever-present *Eimeria* challenge. *Trends. Parasitol*., 30: 12-19.

Dasgupta, N and B. De. 2004. Antioxidant activity of *Piper betle* L. leaf extracts *in vitro*. *Food Chem*., 88(2): 219.

Dkhil, M. A., A. S. Abdel-Baki, F. Wunderlich, H. Sies and S. Al-Quraishy. 2011. Anticoccidial and anti-inflammatory activity of garlic in murine *Eimeria papillata* infections. *Vet. Parasitol*., 175: 66-72.

Duncan, D. B. 1955. Multiple range and multiple F-tests. *Biometrics*., 4: 11-42.

El-Khtam, A. O., A. A. El Latif, M. H. El-Hewaity. 2014. Efficacy of turmeric (*Curcuma longa*) and garlic (*Allium sativum*) on *Eimeria* species in broilers. *Int. J. Basic and Appl. Sci*., 3(3): 349- 356.

Giannenas, I., E. Papadopoulos, E. Tsalie, E. Triantafillou, S. Henikl, K. Teichmann and D. Tontis. 2012. Assessment of dietary supplementation with probiotics on performance, intestinal morphology and microflora of chickens infected with *Eimeria tenella. Vet. Parasitol*., 188(1-2): 31-40.

Holdsworth, P. A., Conway, D. P., McKenzie, M. E., Dayton, A. D., Chapman, H. D., Mathis, G. F., Skinner, J. T., Mundt, H. C., Wil-liams, R. B. 2004. World Association for the Advancement ofVeterinary Parasitology (WAAVP) guidelines for evaluating the efficacy of anticoccidial drugs in chickens and turkeys. Vet. Parasitol. 121: 189–212.

Johnson, J and W. M. Reid. 1970. Anticoccidial drugs: lesion scoring techniques in battery and floor pen experiments with chickens. *Exp. Parasitol*., 28: 30-36.

Khaliq, T., Mumtaz, F., Rahman, Z. U., Javed, I. and Iftikhar, A. (2015) Nephroprotective potential of *Rosa damascene* mill flowers, *Cichorium intybus* linn roots and their mixtures on gentamicin 283 induced toxicity in albino rabbits. *Pakistan Vet. J*., 35:43-47.

Massoud, A., R. M. El Khateeb and M. A. Kutkat. 2010. Efficacy of myrrh in controlling coccidiosis in chickens. *J. Egypt Soc. Parasitol*., 40(3): 751-758.

Naidoo,V., L. J. McGaw, S. P. R. Bisschop, N. Dunca and J. N. Eloff. 2008. The value of plant extracts with antioxidant activity in attenuating coccidiosis in broiler chickens. *Vet. Parasitol*., 152: 214-219.

Nghonjuyi, N. W., C. K. Tiambo, H. K. Kimbi, C. N. Manka, R. S. Juliano and F. Lisita. 2015. Efficacy of ethanolic extract of *Carica papaya* leaves as a substitute of Sulphanomide for the control of coccidiosis in Kabir chicken in Cameroon. *J. Ani. Health. Prod*., 3 (1): 21-27.

Nwosu, C. O., K. Yahayah and O. I. Igbokwe. 2011. Toxicity and anticoccidial efficacy of some plants used in the traditional treatment of avian

coccidiosis in semi-arid North Eastern Nigeria. *Res. J. Parasitol*., 6(1): 18-30.

Shivaramaiah, C., J. R. Barta, X. Hernandez-Velasco, G. Tellez and B. Hargis. 2014. Coccidiosis: recent advancements in the immunobiology of *Eimeria species*, preventive measures, and the importance of vaccination as a control tool against these apicomplexan parasites. *Vet. Med. Res. Repo*, 5: 23–34.

How to cite this article:

Saranya, K., R. Velusamy, N. Rani and Arulmozhi, A. 2020. Evaluation of Anticoccidial Effect of *Piper betel* Leaves Extract against *Eimeria tenella* in Broiler Chicken. *Int.J.Curr.Microbiol.App.Sci.* 9(10): 4035-4041. doi: https://doi.org/10.20546/ijcmas.2020.910.463