Asian Journal of Poultry Science

ISSN 1819-3609

Academic Journals Inc.

www.academicjournals.com

OPEN ACCESS Asian Journal of Poultry Science

ISSN 1819-3609
DOI: 10.3923/ajpsaj.2018.1.6

Research Article
Effects of *Phyllanthus niruri* and *Curcuma longa* on Liver and Renal Function of *Escherichia coli* Infected Layers

[1]Sri Hartati, [2]Tri Untari, [3]Bambang Sutrisno and [4]Ida Fitriana

[1]Department of Veterinary Clinical Science, Faculty of Veterinary Medicine, Gadjah Mada University, Yogyakarta, Indonesia
[2]Departement of Microbiology, Faculty of Veterinary Medicine, Gadjah Mada University, Yogyakarta, Indonesia
[3]Departement of Pathology, Faculty of Veterinary Medicine, Gadjah Mada University, Yogyakarta, Indonesia
[4]Departement of Pharmacology, Faculty of Veterinary Medicine, Gadjah Mada University, Yogyakarta, Indonesia

Abstract

Background and Objective: Colibacillosis caused by *Escherichia coli* (*E. coli*) and can cause economic losses in the poultry industry. One of the failures to overcome colibacillosis is the occurrence of bacterial resistance to some antibiotics. This study aimed to determine the effect of *Phyllanthus niruri* and *Curcuma longa* on the function of liver and renal layer chicken infected *E. coli*. **Methodology:** Fifty layers (DOC) were used in this study. After having infected *E. coli*, the layers were grouped into 5 and labeled with groups A, B, C, D and E. Group A was those who were treated with *Curcuma longa* 300 mg kg^{-1}, group B was treated with *Phyllanthus niruri* 500 mg kg^{-1}, group C was treated with equal dosage (1:1) of *Curcuma longa* and *Phyllanthus niruri*, group D was treated with *Curcuma longa* and *Phyllanthus niruri* whose dosages were 2: 1 and group E was without herbal treatment. Blood and organ samples were collected at 3 weeks post-herbal-treatment. Blood sample were used for examination of Alanine Transaminase (ALT) and creatinine concentration. Liver and renal samples were for histological examination. The results were analyzed by one-way ANOVA test. **Results:** Concentration of group B ALT was significantly different with group A, D and E ($p<0.05$). Group A had the highest ALT concentration i.e., 23.77 ± 0.44 IU L^{-1} and Group E (without herbal treatment) had the highest creatinine concentration i.e., 0.38 ± 0.02 mg dL^{-1}. Creatinine concentration of group B was significantly different with all treatment groups ($p<0.05$). Histological observation of liver organs of group A showed infiltration of heterophile cells in the kiernan trigonum areas. Histological observation of renal organs showed that all groups were normal and there was no pathology. **Conclusion:** *Phyllanthus niruri* does not cause toxic effects for 21 days, so it can be alternative in the treatment of infectious disease of diarrhea caused by *E. coli*.

Key words: Alanine transaminase, colibacillosis, creatinine, *Curcuma longa*, *Phyllanthus niruri*

Citation: Sri Hartati, Tri Untari, Bambang Sutrisno and Ida Fitriana, 2018. Effects of *Phyllanthus niruri* and *Curcuma longa* on liver and renal function of *Escherichia coli* infected layers. Asian J. Poult. Sci., 12: 1-6.

Corresponding Author: Ida Fitriana, Department of Pharmacology, Faculty of Veterinary Medicine, Universitas Gadjah Mada, Jl. Fauna No. 2, Sleman, 55821 Yogyakarta, Indonesia Tel: +62 85728836789

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Poultry farms have excellent prospects on both large scale and small scales (community farms). This is supported by the easy access to input production and rapid capital turnover and increasing demand for chicken meat. Based on data of Association of Indonesian Breeding Poultry in 2012, it is predicted that carcass consumption per capita will increase from 8.6 kg/capita in 2013 and will reach 14.49 kg/capita in 2017. Similarly, it is predicted to increase over the next 5 years, for example, egg consumption was 74 kg/capita in 2012 and will increase to 162 kg/capita by 2017[1].

Escherichia coli is considered as a member of the normal micro-flora of the poultry intestine, except certain strains, such as those designated as APEC, spread into various internal organs and cause colibacillosis characterized by systemic fatal disease[2,3]. Colibacillosis is an infectious disease, which is considered as one of the principal causes of morbidity and mortality, associated with heavy economic losses to the poultry industry by its association with various disease conditions[4].

Antibacterials therapy is an important tool in reducing both the incidence and mortality associated with colibacillosis. This has increased resistance to commonly used antibacterials both in the public health and veterinary sectors. Antibacterials-resistant *E. coli* and others pathogenic bacteria can be transferred from animals to humans through consumption of contaminated food and food products so that such a thing presents a public health risk[5,6]. Improper use of antibacterials also lead to pathogenic bacteria resistant to one antibacterial (antibacterial-resistance) or some type of antibiotic (multiple drug resistance)[7]. It causes the difficulty of treating infection by bacteria.

Phyllanthus niruri have a wide range of pharmacological activities like antimicrobial, antiviral, hepato-protective, antioxidant, anticancer, anti-inflammatory, anti-plasmodial and diuretic[8]. The study of Ajibade and Egbebi[9] explained that the alkaloid extract of *Phyllanthus niruri* can be used as a therapy in colibacillosis. This thing was proved with the result showing that concentration of white blood cells (WBC) and neutrophils increased, hemoglobin decreased and lymphocytes, more over there are no changes in enzyme concentration[9]. Meanwhile, *Curcuma longa* has an indication to have a great variety of pharmacological activities, which exhibit anti-inflammatory, anti-human immunodeficiency virus, anti-bacteria, antioxidant effects and nematocidal activities[10,11]. According to previous studies, *Curcuma longa* has an antibacterial effect against *Staphylococcus aureus* and *E. coli*[11-14]. Also, based on another/other references, *Phyllanthus niruri* and *Curcuma longa* can be used as an alternative therapy for colibacillosis. Therefore, this study aimed to know activity of *Phyllanthus niruri* and *Curcuma longa* on liver and renal function of layer chicken infected by *E. coli*.

MATERIALS AND METHODS

Materials: This study used powder of simplicial *Phyllanthus niruri* and *Curcuma longa*, Newcastle disease (ND) vaccine, avian influenza (AI) vaccine and cells of *Escherichia coli*.

Ethical approval: This experiment has received approval from the Ethical Clearance Commission of Faculty of Veterinary Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia, with number: 0006/EC-FKH/Int./2017.

Animal and experimental design: Fifty layers of day-old-chicken (DOC) were used in this study. One week after adaptation, all chickens were given ND vaccine and 1 week later were vaccinated against AI. One week after vaccination, all treatment groups were treated orally with *E. coli* at a dose of 10^8 CFU mL^{-1}. Having infected *E. coli*, each layer was orally given herbal treatment. The layers were randomly divided into 5 treatment groups: Colibacillosis group treated with *Curcuma longa* 300 mg kg^{-1} (group A), colibacillosis group treated with *Phyllanthus niruri* 500 mg kg^{-1} (group B), colibacillosis group treated with equal dose (1:1) of *Curcuma longa* and *Phyllanthus niruri* (group C), colibacillosis group treated with *Curcuma longa* and *Phyllanthus niruri* whose doses were 2:1 (group D) and colibacillosis group which was without herbal treatment (group E).

Sample collection: Blood and organ samples were collected at 3 weeks after herbal treatment. Blood sample were used for examination of Alanine Transaminase (ALT) and creatinine concentration using Semi-Auto Chemistry Analyzer tool. The layers were euthanized by intra-cardiac administration of saturated $MgSO_4$ solution[2]. A thorough post-mortem-examination of all dead layers was carried out. Liver and renal samples were collected and fixed with formalin. Organ samples were processed with paraffin-embedded method and visualized by using hematoxylin-eosin stain.

Statistical analysis: Alanine Transaminase and creatinine concentration were analyzed quantitatively by one-way ANOVA test. Statistical calculations used SPSS v.16 (SPSS, Chicago, IL). Histology of liver and renal samples were analyzed qualitatively.

RESULTS

Figure 1 explains the concentration of ALT in the blood layers in each treatment group. Statistical analysis with one-way ANOVA test showed that ALT concentration of group B was significantly different with groups A, D and E ($p<0.05$). The results showed that the colibacillosis group treated with *Curcuma longa* (group A) had the highest ALT concentration (i.e., 23.77±0.44 IU L^{-1}) and significantly different compared with the colibacillosis group treated with *Phyllanthus niruri* 500 mg kg^{-1} (group B) and colibacillosis group treated with 1:1 combination of *Curcuma longa* and *Phyllanthus niruri* (group C) ($p<0.05$).

Blood creatinine concentration determines the effect of combination of herbal medicine on renal function. A statistical test with one-way ANOVA showed that creatinine concentration of group B was significantly different with all treatment groups ($p<0.05$). Group E (without herbal treatment) had the highest creatinine concentration i.e., 0.38±0.02 mg dL^{-1} (Fig. 2).

The results of histological examination visualized by using hematoxylin-eosin stain of liver showed that group B in the kiernan trigonum areas was clean (Fig. 3a). Group A showed infiltration of heterophile cells in the kiernan trigonum areas (Fig. 3b). Groups C, D and E showed the thickening of the Glisson's capsule with heterophilic inflammatory cells, macrophages and fibrin deposits. The results of histological examination of renal organs are known that all groups appear normal (Fig. 3c).

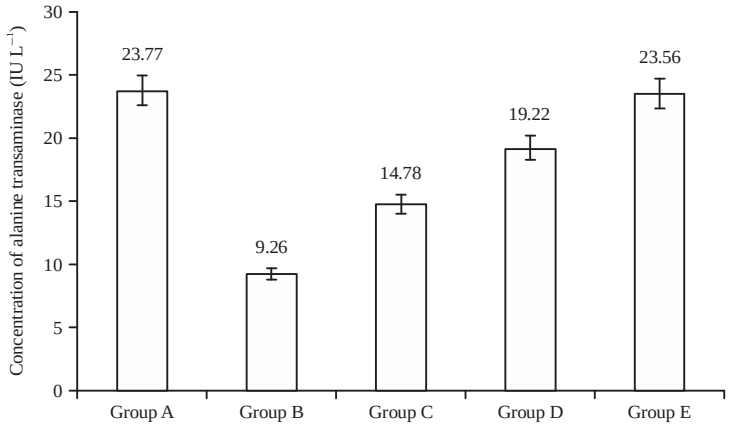

Fig. 1: Concentration of alanine transaminase (IU L^{-1})
Values are represented in Mean±SD

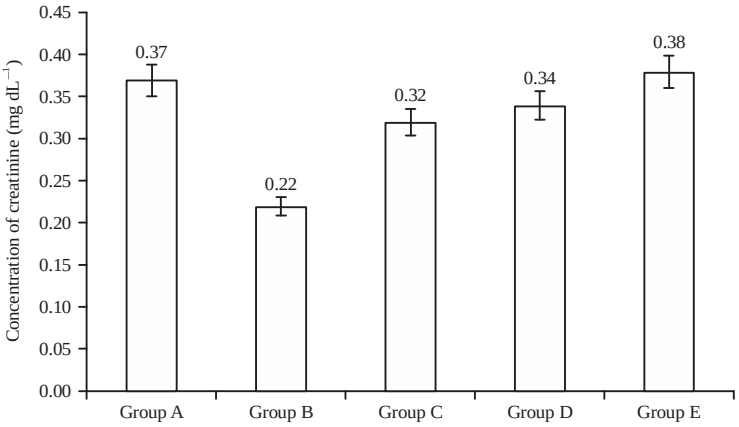

Fig. 2: Concentration of creatinine (mg dL^{-1})
Values are represented in Mean±SD

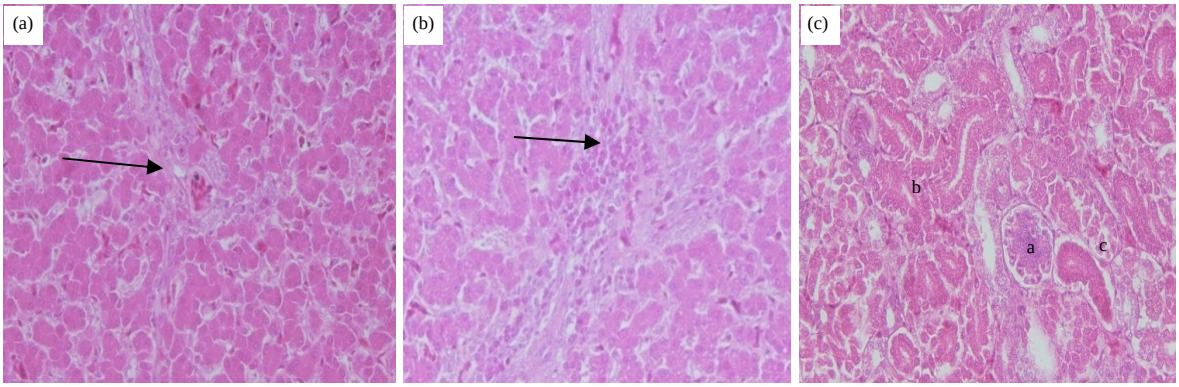

Fig. 3(a-c): Histological observation of liver and renal organs. Histopathologic liver of group colibacillosis treated with (a) *Phylanthus niruri*, trigonum area kiernan liver looks clean (arrow), (b) Histopathologic liver of colibacillosis group treated with *Curcuma longa*, trigonum area kiernan liver organ infiltration of heterophile cells (arrow) and (c) Histopathologic kidney organ of all groups showed normal, glomerulus showed clean and no cell reaction
a: Bowman space still looks empty, b: Epithelial and lumen convulatus tubules are still visible and c: In interstitial tissue, there is no inflammatory cell reaction

DISCUSSION

Effects of drug therapy, especially natural medicines, on liver function can be examined based on alanine-transaminase (ALT) levels in blood, whereas, renal function can be observed with creatinine levels. High creatinine levels in animals indicate a decrease in the excretion of a possible impaired renal function. Liver is a vital organ that plays a role in the detoxification and metabolism of proteins, materials that will be toxic, bio-transformation, conjugation and destruction. Liver parenchymal cells damaged both necrosis and increased membrane permeability leading into an increase of liver parenchymal cells in the enzymes produced by the liver including ALT[15].

Alanine transaminase is a transaminase enzyme that catalyzes the transfer of amino groups from alpha amino acids to alpha keto acids. Alanine transaminase is a cytoplasmic enzyme so that it can cause cytoplasmic components get into the blood circulation and serum enzyme concentration increased if there is interference permeabilities membrane of liver cells. The absolute amount of the ALT enzyme is lower than that of Aspartate Transaminase (AST). This enzyme is widely found in liver cells and is effective for diagnosing hepatocellular destruction. This enzyme in small amounts is found in the heart muscle, renal and skeletal muscle. In general, the ALT test score is higher than AST in acute liver parenchymal damage, whereas in chronic process, it is found lower. An elevated ALT concentration more than 20 times of concentration normal may occur in cases on acute viral hepatitis, hepatic necrosis (drug or chemical toxicity)[16]. Such elevated ALT levels indicate liver damage.

The normal concentration of ALT of chicken blood were 5.0-13.0 IU L^{-1} [15]. Based on Fig. 1, it was known that only colibacillosis group treated with *Phyllanthus niruri* has normal ALT concentration (i.e., 9.26±0.24 UI L^{-1}). It is in accordance with the results of histological examination of liver organs of colibacillosis group treated with *Phyllanthus niruri*. The results showed that trigonum area of kiernan liver organ was clean (Fig. 3a), they indicate cure of infection *E. coli*. Colibacillosis therapy with *Curcuma longa* showed that it can increase ALT levels in chicken blood infected with *E. coli*. It is in accordance with the results of histological observation of liver treatment group colibacillosis with *Curcuma longa* that showed trigonum kiernan liver organ happened heterophile cells infiltration (Fig. 3b), they indicated not yet recovered from *E. coli* infection.

Antibacterial of *Curcuma longa* is caused by the main chemical content curcuminoid and essential oils. *Curcumin* is a phenolic compound that can inhibit bacterial growth by altering the permeability of cytoplasmic membranes so that cell membranes are damaged and cell metabolism is disrupted[10-14]. The natural product curcumin has gained considerable attention for its multiple pharmacological activities but more efforts are needed to understand how curcumin can get these pharmacological effects because of considering its low bioavailability[17]. Exactly, this is due to water

insoluble curcumin, the poor solubility and wettability of curcumin leads to poor dissolution[18]. According to the results of this study, the treatment of colibacillosis with curcumin peroral did not provide a good therapeutic effect.

Creatinine is a product of muscle mass that is produced from the breakdown of creatine phosphate. It is generally produced in the body in a fixed amount and released into the blood. It is filtered by the glomerulus in the renal and if there is a disturbance in renal filtration function, then the creatinine level in the blood will increase and this increase can be used as an indicator of impaired renal function. An elevated creatinine concentration always indicates a decrease in excretion caused by impaired renal function. Because blood creatinine levels can be used to diagnose kidney failure by measuring glomerular filtration rate. Creatinine is filtered by the glomerulus without reabsorption. Because creatinine is not reabsorbed by the renal tubules, the creatinine value in the blood may be a feature of the renal glomerular capability in the glomerular filtration process.

The data of creatinine concentration presented in Fig. 2 can be seen that all treatment groups are within the normal range of creatinine concentration of chickens. The normal concentration of creatinine is 0.20-0.80 mg dL^{-1} [15]. It is in accordance with the results of histological examination of renal organs of all treatment groups. Histological observation of renal organs of all groups is normal and there is no pathology (Fig. 3C). The colibacillosis group treated with *Phyllanthus niruri* had the lowest creatinine concentration, whereas, the group of colibacillosis which was without herbal treatment had the highest creatinine concentration.

The phytochemical studies of *Phyllanthus niruri* were characterized and the presence of various compounds such as lignans, phyllanthin, hypophyllanthin, flavonoids, glycosides and tannins were mentioned. The extracts of *Phyllanthus niruri* have a wide range of pharmacological activities like antimicrobial, antiviral, hepato-protective, antioxidant, anticancer, anti-inflammatory, antiplasmodial and diuretic[8]. The previous studies explain that the alkaloid extract of *Phyllanthus niruri* has a therapeutic effect on *E. coli*[9].

CONCLUSION

Based on the results of the study, it can be concluded that the provision of *Phyllanthus niruri* does not cause damage to liver's and kidney's function of chicken layers infected with *E. coli*. This is proved with the concentrations of ALT and creatinine which are still within normal ranges. Therapy with *Phyllanthus niruri* does not cause toxic effects and has shown an opportunity in the treatment of intractable infectious disease of diarrhea caused by *E coli*.

SIGNIFICANCE STATEMENT

This study discovers the basic principle of natural materials such as *Phyllanthus niruri* and *Curcuma longa* can be used as an alternative therapy for colibacillosis. Herbal medicine is very beneficial because the raw material is very cheap, easy to obtain and can be applied simply. This study will help researchers to uncover the critical area that herbal medicine as well as a breakthrough in overcoming the existence of multiple-drug resistant to *E. coli*.

ACKNOWLEDGMENTS

This study was fully supported by the Grant for Scientific Research (PUPT UGM 2017) from the Directorate General of Higher Education (DIKTI), Ministry of Research, Technology and Higher Education of Indonesia, with grant number 001/SP2H/LT/DRPM/IV/2017.

REFERENCES

1. Suwarti, S.A., 2015. Pengaruh faktor sosial ekonomi rumah tangga terhadap konsumsi produk peternakan di kecamatan kasihan kabupaten bantul. M.Sc. Thesis, Universitas Gadjah Mada, Yogyakarta, Indonesia.
2. Matin, M.A., M.A. Islam and M.M. Khatun, 2017. Prevalence of colibacillosis in chickens in greater Mymensingh district of Bangladesh. Vet. World, 10: 29-33.
3. De Carli, S., N. Ikuta, F.K.M. Lehmann, V.P. da Silveira, G. de Melo Predebon, A.S.K. Fonseca and V.R. Lunge, 2015. Virulence gene content in *Escherichia coli* isolates from poultry flocks with clinical signs of colibacillosis in Brazil. Poult. Sci., 94: 2635-2640.
4. Kabir, S.M.L., 2010. Avian colibacillosis and salmonellosis: A closer look at epidemiology, pathogenesis, diagnosis, control and public health concerns. Int. J. Environ. Res. Public Health, 7: 89-114.
5. Aggad, H., Y.A. Ammar, A. Hammoudi and M. Kihal, 2010. Antimicrobial resistance of *Escherichia coli* isolated from chickens with colibacillosis. Global Vet., 4: 303-306.
6. Hasan, B., R. Faruque, M. Drobni, J. Waldenstrom and A. Sadique *et al*., 2011. High prevalence of antibiotic resistance in pathogenic *Escherichia coli* from large-and small-scale poultry farms in Bangladesh. Avian Dis., 55: 689-692.
7. Sola-Gines, M., K. Cameron-Veas, I. Badiola, R. Dolz and N. Majo *et al*., 2015. Diversity of multi-drug resistant Avian Pathogenic *Escherichia coli* (APEC) causing outbreaks of colibacillosis in broilers during 2012 in Spain. PloS One, Vol. 10, No. 11. 10.1371/journal.pone.0143191.

8. Narendra, K., J. Swathi, K.M. Sowjanya and A.K. Satya, 2012. *Phyllanthus niruri*: A review on its ethno botanical, phytochemical and pharmacological profile. J. Pharm. Res., 5: 4681-4691.
9. Ajibade, V.A. and A.O. Egbebi, 2011. Effect of alkaloid extract of *Phyllanthus niruri* on rabbits infected with enteropathogenic *Escherichia coli*. Int. J. Trop. Med. Public Health, 1: 33-39.
10. Araujo, C.A.C. and L.L. Leon, 2001. Biological activities of *Curcuma longa* L. Memorias Instituto Oswaldo Cruz, 96: 723-728.
11. Cikrikci, S., E. Mozioglu and H. Yilmaz, 2008. Biological activity of curcuminoids isolated from *Curcuma longa*. Records Natl. Prod., 2: 19-24.
12. Voravuthikunchai, S., W. Lortheeranuwat, W. Jeeju, T. Sririrak, S. Phongpaichit and T. Supawita, 2004. Effective medicinal plants against enterohaemorrhagic *Escherichia coli* O157: H7. J. Ethnopharmacol., 94: 49-54.
13. Lourenco, T.C., E.P. Mendonca, P.C. Nalevaiko, R.T. Melo, P.L. Silva and D.A. Rossi, 2013. Antimicrobial effect of turmeric (*Curcuma longa*) on chicken breast meat contamination. Rev. Bras. Cienc. Avic., 15: 79-82.
14. Afrose, R., S.K. Saha, L.A. Banu, A.U. Ahmed and A.S. Shahidullah, 2015. Antibacterial effect of *Curcuma longa* (Turmeric) against *Staphylococcus aureus* and *Escherichia coli*. Mymensingh Med. J., 24: 506-515.
15. Dawson, W.R. and G.C. Whittow, 2000. Regulation of Body Temperature. In: Sturkie's Avian Physiology, Whittow, G.C. (Ed.). Academic Press, New York, USA., ISBN: 978-0-12-747605-6, pp: 343-390.
16. Kee, J.L., 2007. Pedoman Pemeriksaan Laboratorium and Diagnostik. EGC., Jakarta.
17. Shen, L. and H.F. Ji, 2012. The pharmacology of curcumin: Is it the degradation products? Trend Mol. Med., 18: 138-144.
18. Modasiya, M.K. and V.M. Patel, 2012. Studies on solubility of curcumin. Int. J. Pharm. Life Sci., 3: 1490-1497.