

INDUCIBLE NITRI COXIDE
SYNTHASE (INOS) EXPRESSION
IN THE BURSA OF ABRICIUS OF
BROILER INFECTED WITH
VIRULENT GUMBORO VIRUS
AND PROVIDES EXTRACTS
Annona squamosa (Sweetsop)

by Bimo Aksono

Submission date: 19-Dec-2022 10:44AM (UTC+0800)

Submission ID: 1984184564

File name: Inducible-Bimo.pdf (954.94K)

Word count: 505

Character count: 2722

INDUCIBLE NITRIC OXIDE SYNTHASE (iNOS) EXPRESSION IN THE BURSA OF FABRICIUS OF BROILERS INFECTED WITH VIRULENT GUMBORO VIRUS AND PROVIDES EXTRACTS *Annona squamosa* (Sweetsop)

E. Bimo Aksono, H. W.

*IbIKK - Institute of Tropical Disease, Airlangga University
Faculty of Veterinary Medicine, Airlangga University
baksono@yahoo.com

ABSTRACT

The bursa of fabricius serves as an important tissue in the process of gumboro virus (infectious bursal diseases/IBD) pathogenesis, since B cells of the bursa harbor the cytolitic phase of gumboro replication cycle. In the present study, inducible nitric oxide synthase (iNOS) associated with gumboro virus infection in the bursa of fabricius of broilers and provides extracts *Annona squamosa* (Sweetsop) as a leading immunostimulant were investigated.

This research using the 40 individuals one-day-old broiler chickens (DOC). Broiler chicken experiment was obtained from livestock breeding. The cage was used multiple systems Cages measuring 20 x 15 x 10 cm, each unit consisting of one head so that the number of cages all 40 units. Extraction of *Annona squamosa* (Sweetsop) is done at the laboratory of the Faculty of Pharmacy, Widya Mandala University. *Annona squamosa* (Sweetsop) used as test material was the result of reflux extraction methods using ethanol as solvent. The extract *Annona squamosa* (Sweetsop) with a dose of 10 mg/kg body weight, administered orally at the age of 7 days until the end of the experiment, namely the age of 35 days, given gumboro virus infection at the age of 20 days in a peroral dose of EID₅₀ 107. The design was as follows: Group A: given the extract of *Annona squamosa* (Sweetsop) without infected gumboro virus; Group B: without a given extract *Annona squamosa* (Sweetsop) and without infected gumboro virus; Group C: given the *Annona squamosa* (Sweetsop) and infected with gumboro virus; Group D: without any extract *Annona squamosa* (Sweetsop) and given infected with gumboro virus. iNOS expression examination by immunohistochemistry method performed at the Laboratory of Faculty of Medicine, Airlangga University. The study began in June 2010 to October 2010.

From these results prove that the expression of iNOS was higher in the bursa of gumboro virus infected broilers without any extract *Annona squamosa* (Sweetsop) when compared to uninfected contrpls and provides *Annona squamosa* (Sweetsop) (p<0.05).

Keyword : iNOS, IBD, *Annona squamosa*, Bursa fabricius

INTRODUCTION

Gumboro virus is a non-enveloped, double-stranded (ds) RNA virus consisting of two segments, segment A (3.2 kb) and B (2.9 kb), encoding five proteins and belongs to the Birnaviridae family (Dobos *et al*, 1979; Kibenge *et al*, 1988; Mundt *et al*, 1997). Gumboro virus mainly affects young chickens from 3-6 weeks of age (Hoffmann and Lade, 1972). Although viral antigen has been detected in other organs within the first few hours of infection, the most extensive virus replication takes place primarily in the bursa of fabricius (Dobos *et al*, 1979). Activated dividing B lymphocytes that secrete IgM+ Terve as target cells for the virus (Sharma *et al*, 2000; Hirai and Calnek, 1979). Viral infection results in lymphoid depletion of B cells and the destruction of bursal

tissues (Kaufer and Weiss, 1980), leading to an increased susceptibility to other infectious diseases and poor immune response to vaccines (Kibenge *et al.*, 1988).

Infectious bursal disease have not found the right treatment, because the gumboro virus attacks the lymphoid organs (thymus and bursa fabricius). Gumboro disease is preventable by vaccine, but the prevention of gumboro with any vaccine has not been efficient. It can be concluded that the vaccine can only handle 85-90% of gumboro cases (Hair *et al.*, 2000). The damage caused is usually permanent, because this lymphoid organ, initially had hypertrophic later developed into atrophy. Where is the result of atrophy of lymphoid organs are experiencing this performance of the organ to be not optimal.

The herb is one of the alternative precaution against gumboro. The difference between the vaccine and prevention through the use of herbal medicines, which are both situated on the effects. Medicinal plants with their natural properties will improve patient endurance especially in the immune system. Triadisti (2005) proved that infusa *Annona squamosa* has the ability as an antiviral against New Castle Disease Virus. *Annona squamosa* is also known to contain polyphenolic compounds, flavonoids, tannins, alkaloids and saponins, and is reported to have antiviral activity against EBV Early Antigen. Some chemical components in plants can be extracted *Annona squamosa* with ethanol, so ethanol extract of antiviral research needs to be done.

Avian macrophages play an important role as part of the innate immune system by producing cytokines and exerting phagocytic functions (Qureshi *et al.*, 2000). In natural gumboro infection, infected macrophages associated with the respiratory system are suggested to carry gumboro from the site of initial infection to the bursa of fabricius (Barrow *et al.*, 2003). Thus, it is possible that macrophages were involved in inhibition of gumboro replication. It is also possible that macrophages may have played a role in clearing gumboro-infected cells by phagocytosis (Djeraba *et al.*, 2000). Since in the present study the expression of iNOS was significantly higher in the bursa of gumboro infected chickens and correlated significantly with macrophage counts in the bursa, macrophages may have curtailed gumboro replication through NO production as has been described previously (Xing and Schat, 2000).

Despite this research the influence of ethanol extract of *Annona squamosa* leaves against the body's defense system overview broilers infected with gumboro virus (Infectious like HIV) particularly associated with the expression of iNOS has not been widely reported.

MATERIALS AND METHODS

This research using the 40 individuals one-day-old broiler chickens (DOC). Broiler chicken experiment was obtained from livestock breeding. The cage was used 40 systems Cages. Ethical Clearance for the present study was obtained by The Ethics Committee of the FKH Unair. Extraction of *Annona squamosa* (Sweetsop) is done at the Laboratory of the Faculty of Pharmacy, Widya Mandala University. *Annona squamosa* (Sweetsop) used as test material was the result of reflux extraction methods using ethanol as solvent. The extract *Annona squamosa* (Sweetsop) with a dose of 10 mg/kg body weight, administered orally at the age of 7 days until the end of the experiment, namely the age of 35 days, given gumboro virus infection at the age of 20 days in a peroral dose of EID50 10⁷. The design was as follows: Group A: given the extract of *Annona squamosa* (Sweetsop) without infected gumboro virus; Group B: without a given extract *Annona squamosa* (Sweetsop) and without infected gumboro virus; Group C: given the *Annona squamosa* (Sweetsop) and infected with gumboro

virus; Group D: without any extract *Annona squamosa* (Sweetsop) and given infected with gumboro virus. iNOS expression examination by immunohistochemistry methods performed at the Laboratory of Faculty of Medicine, Airlangga University. The study began in June 2010 to October 2010.

RESULTS AND DISCUSSION

From table 1 shows that the average expression of iNOS as the influence of *A. squamosa* in broiler chickens infected with gumboro (like HIV) differ significantly ($p < 0.05$), respectively, the group D ($27,00^a \pm 0,73$), group C ($15,00^b \pm 0,87$), group A ($7,00^c \pm 0,67$), group B ($5,00^c \pm 0,83$).

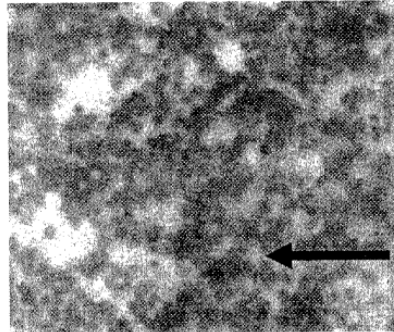


Figure 1. Immunohistochemistry analysis of the expression of iNOS of bursa fabricius of chickens infected gumboro virus (pembesaran 400X)



Figure 2. Immunohistochemistry analysis of the expression of iNOS of bursa fabricius of chickens without infected gumboro virus (pembesaran 400X)

Table 1. The average expression of iNOS as the influence of *A. squamosa* in broiler chickens infected with gumboro (like HIV)

Treatments	Average expression of iNOS
A : given the extract of <i>Annona squamosa</i> (Sweetsop) without infected gumboro virus	7,00 ^c ± 0,67
B : without a given extract <i>Annona squamosa</i> (Sweetsop) and without infected gumboro virus	5,00 ^c ± 0,83
C : given the <i>Annona squamosa</i> (Sweetsop) and infected with gumboro virus	15,00 ^b ± 0,87
D : without any extract <i>Annona squamosa</i> (Sweetsop) and given infected with gumboro virus	27,00 ^a ± 0,73

Different letters on the same column indicate significant differences (p <0.05)

Utilization of natural materials is one alternative to seeking new antivirals. It has been reported that the extract of *Annona squamosa* L. contains RIP (ribosome-Inactivating Protein) (Sulistiyani *et al.*, 2009) because it can breakdown the DNA is supercoiled (Sismindari *et al.*, 1998). RIP shown to have antiviral effects as, in both plant and animal viruses (Barbieri *et al.*, 1993; Sulistiyani *et al.*, 2009). Some chemical components in plants can be extracted *Annona squamosa* L. with ethanol, so ethanol extract of antiviral research needs to be done (Sulistiyani *et al.*, 2009). RIP on some plants have been known to have antiviral activity with several possible mechanisms, including changing the permeability and facilitate entry of RIP into the infected cells, inactivation of ribosomes of infected cells to block protein synthesis and reduces viral replication (Barbieri *et al.*, 1993). Tannins in some plants also can inhibit the interaction of the host cell surface proteins and viral proteins, thus inhibiting viral attachment and penetration of virus into the plasma membrane (Moreira *et al.*, 2005 cited by Sulistiyani *et al.*, 2009) or the tannins will bind well with viral proteins and host cell proteins to form complexes, thus preventing the virus adsorption process (Jasim and Naji, 2003 cited by Sulistiyani *et al.*, 2009).

CONCLUSION

From this study proves that by giving *A. squamosa* will iNOS production decrease is due to the amount of virus replication has been declining in number due to the RIP (ribosome-Inactivating Protein) as an antiviral mechanism

REFERENCES

- Barbieri, L., Battelli, M.G., and Stirpe, F., 1993, Ribosome-Inactivating Protein from Plants, *Biochem et Biophys Acta*, 1154, 237-282.
- Barrow, A.D., Burgess, S.C., Baigent, S.J., Howes, K., Nair, V.K., 2003. Infection of macrophages by a lymphotropic herpesvirus: a new tropism for Marek's disease virus. *J. Gen. Virol.* 84, 2635-2645

- Djeraba, A., Bernardet, N., Dambrine, G., Quere, P., 2000. Nitric oxide inhibits Marek's disease virus replication but is not the single decisive factor in interferon-gamma mediated viral inhibition. *Virology* 277, 58-65.
- Dobos P, Hill BJ, Hallett R, Kells DT, Becht H, Teninges D. 1979. Biophysical and biochemical characterization of five animal viruses with bisegmented double-stranded RNA genomes. *J Virol*, 32:593-605
- Hair-Bejo, M., S. Salina, H. Hafiza and S. Julaida, 2000. In ovo vaccination against infectious bursal disease in broiler chickens. *J. Vet. Malaysia*, 12: 63-69.
- Hoffmann FG and Lade R. 1972. [Post-hatching development and involution of the Bursa Fabricii in the chicken (*Gallus domesticus*)]. *Z Zellforsch Mikrosk Anat* 1972, 124:406-418.
- Hirai K and Calnek BW. 1979 In vitro replication of infectious bursal disease virus in established lymphoid cell lines and chicken B lymphocytes. *Infect. Immun*, 25:964-970.
- Kaufer I and Weiss E. 1980 Significance of bursa of fabricius as target organ in infectious bursal disease of chickens. *Infect Immun*, 27:364-367.
- Kibenge FS, Dhillon AS, Russell RG. 1988. Biochemistry and immunology of infectious bursal disease virus. *J Gen Virol*, 69 (Pt 8):1757-1775.
- Mundt E, Kollner B, Kretzschmar D. 1997. VP5 of infectious bursal disease virus is not essential for viral replication in cell culture. *J Virol*, 71:5647-5651.
- Qureshi, M.A., Heggen, C.L., Hussain, I., 2000. Avian macrophage: effector functions in health and disease. *Dev. Comp. Immunol.* 24, 103-119.
- Sharma JM, Kim IJ, Rautenschlein S, Yeh HY. 2000. Infectious bursal disease virus of chickens: pathogenesis and immunosuppression. *Dev Comp Immunol*, 24:223-235.
- Sismindari, Hussana, A., dan Mubarika, S., 1998, Pematangan DNA Superkoil Untai Ganda Secara In Vitro oleh Ekstrak Gubal *Annona squamosa* L., *Majalah Farmasi Indonesia*, Vol. 9 No.4.
- Sulistiyani, N; I. Azizah; M. Kuswandi. 2009. Aktivitas antiviral ekstrak etanolik biji srikaya (*Annona squamosa* L.) terhadap virus *newcastle disease* pada telur ayam berembrio. *Majalah Farmasi Indonesia*. 20 (2). 62-67
- Triadisti, N., 2005, Uji Daya Antiviral Infus Biji Srikaya (*Annona squamosa* L.), Pada Embrio Telur Ayam Dengan Virus Newcastle Disease, *Skripsi*, F.Farmasi, Universitas Muhammadiyah Surakarta, Surakarta.
- Xing, Z., Schat, K.A., 2000. Inhibitory effects of nitric oxide and gamma interferon on in vitro and in vivo replication of Marek's disease virus. *J. Virol.* 74, 3605-3612.

INDUCIBLE NITRI COXIDE SYNTHASE (INOS) EXPRESSION IN THE BURSA OFF ABRICIUS OF BROILER SINFECTED WITH VIRULENT GUMBORO VIRUS AND PROVIDES EXTRACTS *Annona squamosa* (Sweetsop)

ORIGINALITY REPORT

8%

SIMILARITY INDEX

2%

INTERNET SOURCES

8%

PUBLICATIONS

0%

STUDENT PAPERS

PRIMARY SOURCES

- 1** V. Balamurugan, J. M. Kataria. "Economically Important Non-oncogenic Immunosuppressive Viral Diseases of Chicken —Current Status", *Veterinary Research Communications*, 2006
Publication 3%
 - 2** Preeti Jain, Rani Singh, V.K. Saxena, K.B. Singh, K.A. Ahmed, A.K. Tiwari, M. Saxena, N.R. Sundaresan. "In vitro rapid clearance of infectious bursal disease virus in peripheral blood mononuclear cells of chicken lines divergent for antibody response might be related to the enhanced expression of proinflammatory cytokines", *Research in Veterinary Science*, 2013
Publication 3%
 - 3** www.biosky.org
Internet Source 2%
-

Exclude quotes Off

Exclude matches Off

Exclude bibliography On

INDUCIBLE NITRI COXIDE SYNTHASE (INOS) EXPRESSION IN THE BURSA OF ABRAHAM OF BROILER INFECTED WITH VIRULENT GUMBORO VIRUS AND PROVIDES EXTRACTS Annona squamosa (Sweetsop)

GRADEMARK REPORT

FINAL GRADE

GENERAL COMMENTS

/0

Instructor

PAGE 1

PAGE 2

PAGE 3

PAGE 4

PAGE 5
