

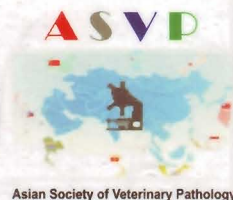
CONFERENCE MANUAL & PROCEEDINGS



The Joint Meeting of the 5th Conference and Congress of Asian Society of Veterinary Pathology (ASVP) 2011 & The 10th Scientific Symposium of Indonesia Society of Veterinary Pathology (ISVP) 2011

“The role of veterinary pathology in animal health for improving eco-health”

Organized by:





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Proceedings

**The Joint Meeting of Conference and Congress
of Asian Society of Veterinary Pathology
(ASVP) 2011
&
The 10th Scientific Symposium of Indonesian
Society of Veterinary Pathology (ISVP) 2011**

The Role of Veterinary Pathology Animal Health for
Improving Eco-Health

IPB International Convention Centre
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Bogor Agricultural University 2011

Faculty of Veterinary Medicine
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Contact Address
ASVP 2011 Organizing Committee
Division of Veterinary Pathology
Department of Veterinary Clinic, Reproduction & Pathology
Faculty of Veterinary Medicine
Bogor Agricultural University
Wing 6th & 7th , 1st Fl. Jl. Agatis, Campus IPB of Darmaga
Bogor 16680, INDONESIA
Phone/Fax. +62-251-8421807
E-mail : asvpbogor2011@gmail.com
Website : <http://asvpbogor2011.event.ipb.ac.id>

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PANCREATIC BETA CELLS EVALUATION AFTER TREATED BY *Phaleria macrocarpa* (Mahkota Dewa) FRUIT EXTRACT IN DIABETIC MONKEY

E. Sulistiawati¹, I. H. Suparto^{1,2}, M. Bintang³, I. Indraswari³, S.A. Prabandari¹

¹Primate Research Center, Bogor Agricultural University

²Department of Chemistry and ³Department of Biochemistry Faculty of Mathematics and Natural Science, Bogor Agricultural University

Keyword : *Phaleria macrocarpa*, Diabetes Mellitus, *Macaca fascicularis*

Introduction

Phaleria macrocarpa, is a medicinal plants originated from Papua. Empirically, it is capable to control various health problems including diabetes mellitus. There is growing evidence that excess generation of highly reactive free radicals largely due to oxidative stress (hyperglycemia) causing increase blood level. This further exacerbates the development and progression of diabetes and its complications. Based on previous studies, *Phaleria macrocarpa* contained antioxidant of phenolic glycoside (Oshimi, *et al.*, 2008) and lignans pinoresinol, lariciresinol, and matairesinol (Saufi *et al.*, 2008). The aim of this study was to evaluate the number of beta cell pancreas and to detect antigen-antibody reaction of β cells after treated *Phaleria macrocarpa* fruit extract in diabetic monkey (DM) induced with streptozotocin (STZ).

Materials and Methods

Pancreatic tissues from fifteen diabetic adults male *Macaca fascicularis* were collected at necropsy and preserved in 4% paraformaldehyde as fixative solution. Each pancreatic tissues were trimmed at three different areas; caput, corpus and cauda, processed for immunohistochemical staining, then evaluated and calculated under microscope. Monkeys were induced by single intravenous injection of STZ (55 mg/kg BW) to be DM. All DM were divided randomly into three groups (n = 5 animals). First group, DM treated only with distilled water as control, second and third groups were treated with *Phaleria macrocarpa* fruit extract of 1000 and 500 mg/kg BW, respectively. All experimental procedure on these animals were conducted in compliance with the guideline established by the Institutional Animal Care and Use Committee.

Results

Pancreatic β cells of the Langerhans Islets distributed randomly in three different areas, caput, corpus, and cauda pancreas. The mean of number β cells on the caput pancreas of the control DM was higher than both of treated DM group. While the mean of the number β cells on the corpus and cauda

pancreas of DM which had received extract of 500 mg/kgBW was higher than the control and animals with 1000 mg/kgBW (Figure. 1).

Immunohistochemical staining method showed various color intensity which depends on the concentration of antigen-antibody binding, tissue preparation and other factors. In this study, the brown color intensity indicated the amount of insulin that secreted by pancreatic β cells. Factors affecting the color intensity of the antibody-antigen binding specificity closely related to the concentration of primary antibody used in the process. Optimization of anti-insulin concentration was performed and revealed that the concentration of 1:1500 was the optimal concentration with unstained background, supported by Gobel (2011).

Based on the data of the color intensity of pancreatic β cell evaluation, the caput pancreas of the control DM had the lowest of color intensity compared to both treated groups. Corpus and cauda areas of animals treated with 500 mg/kg BW showed higher intensity compared to the other groups (Figure 2).

Discussion

Detection of pancreatic β cells in Langerhans Islet by immunohistochemistry indicate the presence of β cell damage in diabetes mellitus. Pancreatic tissue of untreated animals had lowest pancreatic β cell compared to treated animals. This may be caused by advanced β cell damaged due to cytotoxic effect of STZ. Glucose uptake through glucose transporters (GLUT-2) caused the STZ into β cells, resulting in DNA alkylation. DNA was fragmented so that activating poly (ADP-ribose) synthetase, the enzyme that polymerizes to form the ADP-ribose poly (ADP-ribose), and activation of the ATP and NAD⁺ reduction. Decreased production of ATP and NAD⁺ led to the opening of K⁺ channels and the plasma membrane hyperpolarization. Furthermore, closure of the gate voltage reduced Ca²⁺ concentration and insulin secretion resulting in β cell death (Elsner *et al.* 2000).

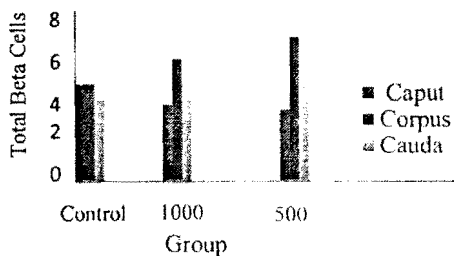


Figure 1 Mean of the number of β cells on three different areas of pancreas by immuno histochemical staining evaluation

Fruit extract improved total number of β cell, modestly higher in the lowest dosage (500 mg/kg BW). This was supported by Suparto *et al.* (2010), that *Phaleria macrocarpa* fruit extract increased insulin secretion on diabetic cynomolgus monkeys. This fruit extract was already available in the market with dosage of 500 mg/capsule. It revealed that dose of 500 mg/kgBW was more appropriate to improve hyperglycemic condition or to reduce blood glucose level.

Pancreatic β cells distribution in three different areas of untreated animals showed that the caput and the corpus pancreas had higher β cells than the cauda pancreas. In the highest dosage, the corpus area showed higher numbers of β cells than in the caput and caudal pancreas. Total number of β cells for the lowest dosage showed higher number in the corpus and the cauda compared to caput pancreas. This result was supported by Dahmiarti (2000), that the distribution of diabetic pancreas β cells located at the periphery of the islets of Langerhans. However, according to Sundler and Harkanson (1988), β cell distribution differed from each species and the composition of the islets of Langerhans differed from each area of the pancreas. The Langerhans islets located in the cauda pancreas seemed to have tendency to be more numerous.

The color intensity of pancreatic β cells were highest in treated animal with 500 mg/kg BW. This result was also indicated by increasing in the number of pancreatic β cells. A dark brown color on the Langerhans Islets was resulted from the affinity of anti-insulin with high insulin and vice versa. Affinity of antibody was the strength of the bond of a side of antibody binding (paratop) with antigenic determinants (epitopes) (Vara 2005). So, it can be stated that the number of pancreatic β cells was directly proportional to the color intensity obtained from the reaction of antigen and antibody binding by immunohistochemical staining methods.

Conclusion

Phaleria macrocarpa fruit extract with dosage of 500 mg/kgBW orally was the best dose to improve pancreatic beta cells damage in DM.

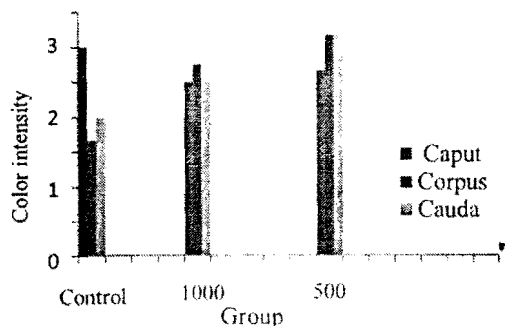


Figure 2 Mean of the color intensity of β cells on three different area of pancreas by immuno histochemical staining evaluation

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