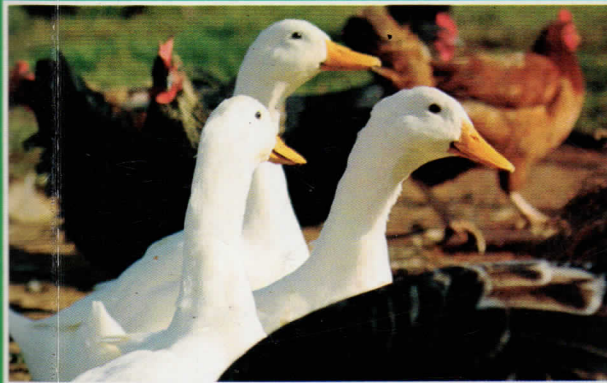


# PROCEEDING



*international seminar*

## STRATEGY TO MANAGE BIO-ECO-HEALTH SYSTEM FOR STABILIZING ANIMAL HEALTH & PRODUCTIVITY TO SUPPORT PUBLIC HEALTH



Surabaya-Indonesia, 19-20 June 2012  
JW Marriott Hotel Surabaya

**EDITORS:**

Michael P. Ward (Australia)

Faouzi Kechrid (Africa)

Montip Gettayacamin (Thailand)

Fedik Abdul Rantam (Indonesia)

Suzanita Utama (Indonesia)

FACULTY OF VETERINARY MEDICINE - UNIVERSITAS AIRLANGGA  
I-MHERE SUB-COMPONENT B.2.C PERFORMANCE BASED CONTRACT



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*This is to certify that*

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**International Seminar**

**STRATEGY TO MANAGE BIO-ECO-HEALTH SYSTEM  
FOR STABILIZING ANIMAL HEALTH AND PRODUCTIVITY  
TO SUPPORT PUBLIC HEALTH**

*19-20 June 2012*

*JW Marriott Hotel, Surabaya -Indonesia*

Dean of the Faculty of Veterinary Medicine  
Universitas Airlangga,

A handwritten signature in blue ink, appearing to be 'R. Sidik'.

Prof. Hj. Romziah Sidik, DVM., Ph.D.



INDONESIA-Managing Higher Education for Relevance and Efficiency  
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FACULTY OF VETERINARY MEDICINE - UNIVERSITAS AIRLANGGA  
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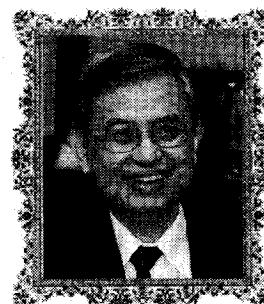
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## MESSAGES

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**PROF. DR. H. FASICH, APT.**  
Rector of Universitas Airlangga



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### **International Seminar STRATEGY TO MANAGE BIO-ECO-HEALTH SYSTEM FOR STABILIZING THE ANIMAL HEALTH AND PRODUCTIVITY TO SUPPORT PUBLIC HEALTH**

Assalamu'alaikum warahmatullahi wabarakatuh

Praised be to Allah SWT for His love and compassion that today we all gather for an important and interesting international seminar on healthy animal management system.

As we all aware, up to the present time, the world is still struggling to overcome various zoonotic diseases that have been threatening human being all over the world for centuries. Through the development of modern information and technology, we also know that those diseases weaken and decrease human quality of life sooner or later.

Therefore, concern over the problem is needed, especially in the region where people live below poverty line. As nothing can be expected from a nation with low quality of human life, it is about time for us to work hand-in hand to eradicate and prevent the outbreak of all kinds of diseases. Also, every human being is in need of good quality of domestic animals which provide plenty of protein for healthy people.

Time has come for all parties, including institutions of science to recognize the essential link between human, domestic animals and wildlife health, and the threat disease poses to people, their food supplies and economies, the biodiversity essential to maintaining the healthy environments and functioning the ecosystems.

I belief and hope that today's seminar will be able to strengthen our collaborations and networking, as an important step in reaching a safe and healthy life.

To all participants, I would like to thank you for making this seminar a success and the organizing committee for a wonderful work.

Thank you very much.

Wassalamu'alaikum warahmatullahi wabarakatuh.

## MESSAGES

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**PROF. HJ. ROMZIAH SIDIK, PH.D., DVM.**

Dean of the Faculty of Veterinary Medicine  
Universitas Airlangga, Surabaya - Indonesia



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Assalamu'alaikum warahmatullahi wabarakatuh

Dear Sir/Madam

On behalf of Organizing Committee the International Seminar entitled “STRATEGY TO MANAGE BIO-ECO-HEALTH SYSTEM FOR STABILIZING ANIMAL HEALTH AND PRODUCTIVITY TO SUPPORT PUBLIC HEALTH”, I would like to say thank you to the honorable: Rector Airlangga University, The Chairman of Academic Senate-Airlangga University, East Java Governor, Director General Animal Health and Husbandry-Indonesia, Chairman Bank Rakyat Malaysia, President of World Veterinary Association, President of Indonesian Veterinary School Association, President of Indonesian Veterinary Medicine School Association, Chairman of Animal Husbandry- East Java Province and Surabaya City. And also to all our special guest: The Dean VetAgro Sup Nationale Veterinary School of Lyon from France, The Chairman of Veterinary Public Health and Food Safety The University of Sydney Australia, Former Dean School of Animal and Veterinary Sciences – Faculty of Sciences The University of Adelaide Australia, Representatives Envoy School of Veterinary and Biomedical Sciences Murdoch University Australia, Former Dean Institute of Veterinary – Animal and Biomedical Science Colleges of Sciences Massey University, Regional Director for Southeast Asia, AAALAC International, Chairman of Animal BSL – 3 Airlangga University, and all best colleague the Deans of Veterinary Schools in Indonesia (Institute Pertanian Bogor, Gajah Mada University, Universitas Airlangga, Udayana University, Syahkuala University, Brawijaya University, HasanudinUniversity. University Nusa Cendana, University Wijayakusuma and University of Nusa Tenggara Barat), also the Deans comes from the other Faculty of Universitas Airlangga. I also would like to say thank you to the partnership Institutions and Industries, and I proudly to all the academic staffs and students and guest participants.

Welcome to the event in Surabaya, the city which located in East Java province. As one of Indonesia's leading prefectural capital, Surabaya is keenly aware of the need to promote into a cosmopolitan city. East Java is settled between two world class tourist destinations, Jogjakarta and Bali. East Java, a province rich of tropical sights and cultural heritage is easily fitted to your journey from Jogjakarta to Bali. As natural lovers would expect an agritourism, the smoking volcano “Mt. Bromo” and the “G-Land” as a surfer paradise are the East Java's breathtaking tourist attractions that should not be missed. East Java also have several National Park that protected bulls (*Bos javanicus*) and turtles (Chelonians), and the specific centre of commodities livestock, as a plan in the future will promote Timorensis deer as a potential meat animal product. For art lovers special dance, you can see the magical dance

of Reog Ponorogo. We are pleased to introduce you these tourism objects of splendorous, scenic and cultural. The best to know this spectacular East Java is to come and see yourself.

I am very great pleasure for your attended to the International Seminar that hosted by Faculty of Veterinary Medicine, Airlangga University. It because of the God bless and love to us, therefore we could arranged the peach among Veterinary School in Asia region and in the World for Strengthen and Establishment, Benchmarking and branding our Veterinary School by global link.

Alhamdulillah robba a'lammin, thank you so much for The God.

During these two days, the event programs include four main programs, such as plenary lectures, the scientific paper session, International Standardization Veterinary Scholl Curriculum base on OIE recommendation with possibly to arrange Twinning and Double Degree Study Program with France, New Zealand and Australia Veterinary School, and to construct Indonesian Veterinary Medicine Council supported by Indonesian Veterinary School Association and Stake Holders. We hope all of you could follow the programs by pleased, savor and it will be beneficial for us.

In this moment, I would like to say thank you to:

Chairman of Indonesia-Managing Higher Education Relevance and Efficiency (IM-HERE) Project-Sub Component B.2.c. Performance Based Contract for supporting and funding the International Seminar.

Rector of Universitas Airlangga for supporting and funding the International Seminar based on Annual Budgeting Plan 2012 of Airlangga University.

The sponsorships that supporting to the event.

All Keynote speakers and invited speakers.

All participants.

Finally, thank you very much for all the distinguish guest for your kindly and closely to all the participants, please have a nice time to enroll the event.

Thank you very much.

Wassalamu'alaikum Warahmatullahi Wabarakatuh.





## MESSAGES

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**DR. ANWAR MA'RUF, DVM., M.KES.**

Chairman



---

Assalamu'alaikum warahmatullahi wabarakatuh

Ladies and Gentleman

I have the honour of welcoming, delegates and speakers to Surabaya and the 2012 International Seminar.

Organised under the theme "Strategy to Manage Bio-Eco-Health System for Stabilizing the Animal Health and Productivity to Support Public Health" this program was aimed to provide a forum for all those interested in sharing and discussing common concerns and up to date research in the physical, biological, social and economical changes that it happen in the environment which generates for human health.

It is only through exchange of information that we can carefully develop the strategic and medical intervention in managing bio eco health system to increase health and reproduction animal for supporting public health. So I hope you will take advantage of the many opportunities this program provides to network with colleagues from around Indonesia and overseas.

The successful organization of this program has required the dedication and time of all committee members. Much work went into preparing the program. I am very grateful for the financial support we have received from our sponsors which are recognized in this book. It would not be possible to hold this program without their support.

I do hope that the seminar will be fruitful for all of us and please enjoy your stay in Surabaya.

Thank you very much.

Wassalamu'alaikum warahmatullahi wabarakatuh.

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# ANTI NECRO-INFLAMMATORY EFFECT OF STANDARDIZED PUNICA GRANATUM EXTRACT (40% ELLAGIC ACID) ON LIVER FIBROSIS INDUCED BY BILE DUCT LIGATION IN RATS

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## ABSTRACT

The aim of this study was to evaluate the anti necro-inflammatory effect on liver fibrosis treated by standardized punica granatum extract. A total of thirty two albino rats (*Rattus Norwegicus*), male, Wistar strain were divided into 4 groups namely sham operated group (group I) and three main bile duct ligation groups (BDL) (group II = BDL, non-treated; group III = BDL, treated by 60 mg/kg body weight/day ellagic acid and group IV = BDL, treated by 150 mg/kg body weight/day standardized punica granatum extract respectively). The results showed a significative decrease of the necro-inflammatory score and fibrosis score for group III and group IV compared to group II but higher than group I ( $p < 0.05$ ). The necroinflammatory value were higher in the group III compared to others group ( $p < 0.05$ ). It can be concluded that the administration of 150 mg/kg body weight/day standardized punica granatum extract possessed hepatoprotective activity and avoided progression of fibrosis by its anti necro-inflammatory activity.

**Keywords:** anti necroinflammatory, liver fibrosis, punica granatum extract, bile duct ligation (BDL), hepatoprotective

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## INTRODUCTION

Liver fibrosis is the excessive accumulation of extracellular matrix proteins (ECM) in the liver (Bataller and Brenner, 2005). Patients with liver fibrosis can be asymptomatic for 15-20 years with morbidity and mortality only occurring after progression to cirrhosis (Friedman, 2003; Bataller and Brenner, 2005). Liver fibrosis was historically thought to be a passive and irreversible process due to the collapse of the hepatic parenchyma and its substitution with a collagen-rich tissue (Albanis and Friedman, 2001).

Recently, there is growing interest in understanding the role and mechanism of the phytochemicals: polyphenolics, flavonoids and phenyl propanoids as inhibitors of oxidative stress. Among all phytochemicals, ellagic acid (EA) has been receiving the most attention because of its wide array of biological properties, such as radical scavenging, chemopreventive, antiviral and antibacterial properties. It is mostly abundant in berries, walnuts, pecans, pomegranate, cranberries and other plant foods in the forms of hydrolysable tannins called ellagitannins (Devipriya *et al.*, 2007). The aim of this study was to evaluate the degree of necroinflammatory activity on liver fibrosis treated by standardized punica granatum extract in order to find new potential sources of hepatoprotective and antifibrotic agent.

## MATERIALS AND METHODS

### Chemicals used

Standardized punica granatum extracts (40% ellagic acid) and ellagic acid 90% were obtained from Xi'an Biof Bio-Technology Co., Ltd. (Room 1-1111, High-tech Venture Park, No. 69 Jinye Road, Gaoxin Distric of Xi'an, People Republic of China). Standardized punica granatum extract was administered at a dose level of 150 mg/kg body weight/day and ellagic acid 60 mg/kg body weight/day.

## Experimental design

Male Wistar albino rats (200–250 g) were housed in a room at a mean constant temperature of 37° C with a 12 hour light-dark cycle, and free access to standard pellet chow and water. Biliary cirrhosis was induced surgically through double ligation and division of the common bile duct under combination of ketamine H Cl and diazepam (100 mg/ml: 5 mg/ml) at dose of 1 ml/kg body weight.

After 2 days from surgery 32 animals were divided into 4 groups (each group contain of 8 rats); sham operated group (group I) and three main bile duct ligated groups (BDL) (group II = BDL, non-treated; group III = BDL, treated by 60 mg/kg body weight/day ellagic acid and group IV = BDL, treated by 150 mg/kg body weight/day standardized punica granatum extract respectively). All these treated groups were treated for 3 weeks.

### Histological studies

Small liver slices were rapidly removed, fixed in buffer formalin 10% fluid and stained with hematoxylin and eosin for histological examination under light microscopy. For analysis, ten power fields per each liver section were done at X 10 and 40 (10 and 40 objective X 10 ocular). Necro-inflammatory scoring was taken by algorithm for necro-inflammatory activity evaluation in Metavir System (Brunt, 2000). The degree of necro-inflammatory and fibrosis staging has been analysed by using the Kruskal–Wallis one-way analysis of variance.

## RESULTS AND DISCUSSION

As shown in Table 1 necro-inflammation score was significantly higher in BDL rat treated with EA compared to the other groups ( $p < 0.050$ ). While punica granatum extract administration in BDL (group IV) reduced this values significantly near of group I ( $p < 0.05$ ). In BDL rat, score of fibrosis significantly increased compared to group I, III and IV ( $p < 0.05$ ), while there was no significant difference between group III and IV (Table 1).

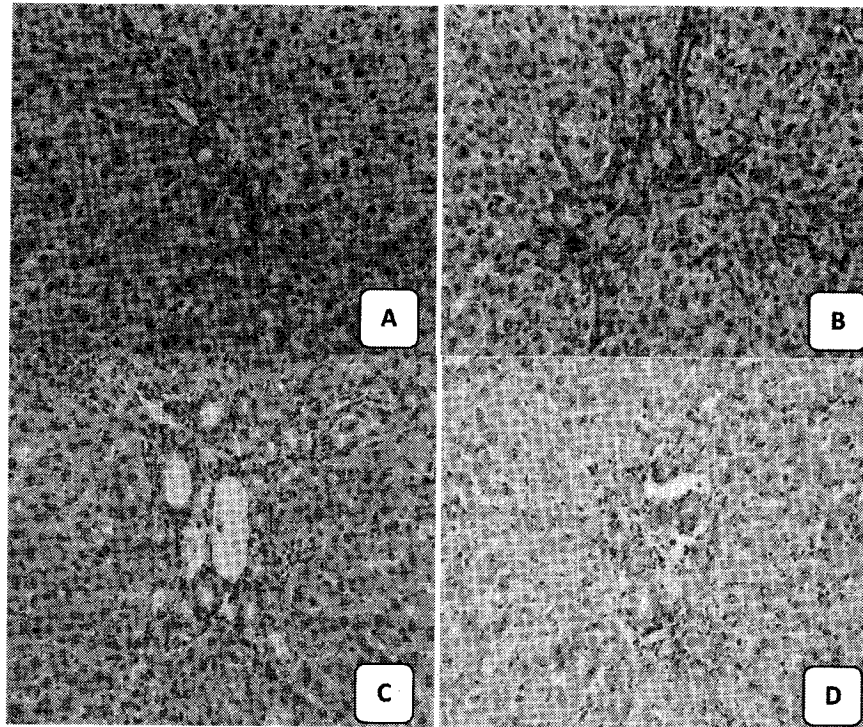
**Table 1.** The Average of Necro-inflammatory and Fibrosis Scores

Group	Necro-Inflammatory Score (Mean $\pm$ SD)	Fibrosis Score (Mean $\pm$ SD)
I (Sham Operation)	0.375 <sup>a</sup> $\pm$ 0.510	1.000 <sup>a</sup> $\pm$ 0.000
II. (BDL)	0.875 <sup>b</sup> $\pm$ 0.350	2.625 <sup>c</sup> $\pm$ 0.518
III (BDL + EA)	1.250 <sup>c</sup> $\pm$ 0.460	2.250 <sup>b</sup> $\pm$ 0.463
IV (BDL + PG)	0,500 <sup>a</sup> $\pm$ 0,530	1.875 <sup>b</sup> $\pm$ 0.667

Note: \*) BDL= Bile Duct Ligation; EA =Ellagic Acid; PG= Promegranate Extract  
The different superscript in the same column is significantly different ( $p < 0.05$ )

In sham operated groups, no histological alterations were detected (Fig.1A). On the other hand, all examined liver sections from BDL group show progression of fibrosis after 3 weeks. The hepatic architecture was completely distorted, liver lobules nearly disappeared and were replaced with wide fibrous tissue area admixed with highly proliferated and dilated bile ductules. Hepatic cells appeared with variable changes in their nuclei including hypertrophy, marginal chromatin, pyknosis, clumped, crescent shape, and irregular nuclear envelope. Mild to moderate inflammation and prominent enlarged Kupffer cells were seen in the fibrous tissue stroma (Fig 1B).





**Figure 1.** Liver section of: A: sham operated rat; B: Liver section of common bile duct ligated rat for 3 weeks; C: Section of liver of BDL rat treated with 60 mg ellagic acid/kg for 3 weeks and D: Section of liver of BDL rat treated with 150 mg standardized punica granatum extract/kg for 3 weeks (HE, X 100).

In BDL groups treated daily with 60 mg ellagic acid/kg for 3 weeks, the histological of liver sections still showing evidence of marked loss of hepatic lobular architecture. However, lobular architecture started to be distinguished, still show well organized hepatic lobular architecture with appearance of inflammatory cells and marked reduction in number of the proliferated bile ductules (Fig 1C).

Our study has demonstrated that oral administration of EA (60 mg/kg body weight) effectively decreased the degree of fibrosis but the necroinflammation degree of the liver still higher, so EA inhibit the progression of liver fibrosis by prolong the inflammatory process. This can be attributed to the antioxidant and the antiinflammation effect of EA.

Ellagic acid, a as phenolic compound can act as scavenging free radicals (Gil *et al.*, 2000), an antioxidant and inhibits cell injury (Priyadarsini *et al.*, 2002; Jung *et al.*, 2010). Furthermore, it has been reported that EA decreases the liver marker enzymes during CCl<sub>4</sub>-induced toxicity (Thresiamma *et al.*, 1996). Hence, it could be suggested that hepatocellular injury is decreased by the antioxidant action of EA. Since ROS have been implicated in the development of various pathological conditions, EA has the ability to control these diseases through its potential antioxidant activity.

The antiinflammatory effects of ellagic acid have been demonstrated in various animal model studies. In addition, the anti-inflammatory activity of EA was also investigated by using a standardized pomegranate rind extract containing 13% EA and finally proved to possess anti-inflammatory activity (Panichayupakaranant *et al.*, 2010).

Examination of BDL rats treated with 150 mg standardized punica granatum extract/kg/day for 3 weeks revealed moderate improvement in the hepatic lobular architecture. Well formed hepatocellular lobulation with slightly dilated sinusoids was seen in all examined slide. The hepatic lobules were separated with only thin bands of fibrous tissue, infiltrated with moderately dense inflammatory

cells. Few bile ductules were occasionally seen in portal areas and most of the hepatocytes appeared with normal nuclei (Fig 1D).

Inflammation, the first physiological defense system in the human body, can protect against injuries caused by physical wounds, poisons, and so on. However, long-term over-inflammation might cause such dysfunctions of the regular physiology as asthma, liver cirrhosis and rheumatic arthritis (Lee *et al.*, 2010). Many studies have pointed to the antiinflammatory properties of pomegranate fruit (Lansky and Newman 2007; Shukla *et al.*, 2008; Larrosa *et al.*, 2010; Lee *et al.*, 2010).

The administration of 150 mg/kg body weight/day standardized punica granatum extract possessed hepatoprotective activity and inhibit progression of fibrosis by decreasing the necro-inflammatory activity. It is suggest that antioxidant and antiinflammatory activity of the punica granatum extract are better than EA. The components of pomegranate extract that contain of EA and another compound might appear to synergistically suppress inflammatory cytokine expression. More recently, a whole pomegranate methanol extract was also shown to inhibit, in a dose-dependent manner, the production and expression of TNF $\alpha$  in microglial cells (Jung *et al.*, 2006), IL-1-induced expression of matrix metalloproteinases in human chondrocytes *in vitro* (Ahmed *et al.*, 2005), the expression of vascular inflammation markers, thrombospondin (TSP), transforming growth factor- $\beta$ 1 (DeNigris *et al.*, 2007) and inhibited cytokine IL-8, prostaglandin PGE $_2$ , and nitric oxide secretion, due to the action of the EA that also present in pomegranate (Romier-Crouzet *et al.*, 2009).

The antioxidant activity of pomegranate components due to the diverse phenolic compounds present in pomegranate. These compounds are known for their properties to scavenge free radicals and to inhibit lipid oxidation *in vitro* (Gil *et al.*, 2000). However, Tzulker *et al.*, (2007) suggested that punicalagin originating from the peels is one of the major phytochemicals contributing to the total antioxidant capacity of pomegranate juice. Madrigal-Carballo *et al.*, (2009) suggested that phenolic hydroxyl groups in punica granatum extract donate hydrogen to reduce free radicals. However, Amarowicz *et al.*, (2004) suggested that the antioxidant activity of phenolic compounds is due to their ability to scavenge free radicals or chelate metal cations. Tzulker *et al.* (2007) reported that the homogenates preparation from the whole fruit exhibited an approximately 20-fold higher antioxidant activity than the level found in the aril juice.

## CONCLUSION

It can be conclude that the increase of the necro-inflammatory grade could accelerate fibrosis progression and the administration of 150 mg/kg body weight/day standardized punica granatum extract possessed hepatoprotective activity and inhibit fibrosis progression by decreasing the necro-inflammatory activity.

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