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The Veterinary Medicine International Conference (VMIC)

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Published: 29 November 2017
ISSN: 2413-0877
# Table of Contents

**The Veterinary Medicine International Conference (VMIC)** | pages 1-9

**Toll-Like Receptors (TLRs) Play Role in Adaptive Immunity in Rabbits Immunized by Sarcopes scabiei Proteins**

Nunuk Dyah Retno Lastuti, Fedik Abdul Rantam, Poedji Hastutiek, Dony Chrismanto

*The Veterinary Medicine International Conference (VMIC)* | pages 10-20

**Preservation Effect of Grouper (Epinephelus sp) Fillet Against Survival of Anisakidae**

Hartanto M. Raharjo, Setiawan Koesdarto, A.T Soelih Estoepongestie, Kusuma Wardhani L.D

*The Veterinary Medicine International Conference (VMIC)* | pages 21-27

**Acanthocephalan in Xenochrophis piscator Snake in Sidoarjo Indonesia**

Inggarsetya Syah Audini, Lucia Tri Suwanti, Setiawan Koesdarto, Emmanuel Djoko Poetranto

*The Veterinary Medicine International Conference (VMIC)* | pages 28-33

**The Identification Blood Parasites On Pig (Sus domesticus) In Polewali Mandar District**

Silvana Arfin, Lucia Muslimin, Adryanis ris

*The Veterinary Medicine International Conference (VMIC)* | pages 34-40

**Spirometra in Ptyas mucosus Snake in Sidoarjo, Indonesia**

Garindra Tiara Pranashinta, Lucia Tri Suwanti, Setiawan Koesdarto, Emmanuel Djoko Poetranto

*The Veterinary Medicine International Conference (VMIC)* | pages 41-47

**Toxicity of Citrus mitis, Citrus aurantifolia, and Citrus maxima leaf extract toward mortality of Aedes aegypti larvae (Diptera: Culicidae)**

Hamidah Hamidah, Hebert Adrianto

*The Veterinary Medicine International Conference (VMIC)* | pages 48-61

**An In Vitro Antibacterial Activity Test of Meniran Herbs’ (Phyllanthus Niruri L.) Ethanol Extract Against Mycoplasma gallisepticum causes Chronic Respiratory Disease (CRD) in Broiler Chickens**

Emy Koesianti Sabdoningrum, Sri Hidana, Retno Sri Wahjuni, Sri Chusnati, Arimbi Arimbi

*The Veterinary Medicine International Conference (VMIC)* | pages 62-68

**Bioremediation of Mercury (II) Contaminated Seawater Using the Diatom Skeletonema costatum**

Thin Soedarti, Tini S., Sucipto H., Eko P. Kuncoro

*The Veterinary Medicine International Conference (VMIC)* | pages 69-76

**BMP-2 Expression of Post Tooth Extraction that Catfish Oil Application**

Theresia Indah B., Bambang Sumaryono, Ketut Suardita, Amelia Putri R.

*The Veterinary Medicine International Conference (VMIC)* | pages 77-83

**Inhibition of Apoptosis in Retinal of Newborn Mice Due to Congenital Toxoplasmosis**

Lucia Tri Suwanti, Mufasirin Mufasirin, Hani Plumeriastuti
Effect of Spirulina Platensis on The Number of Spermatogenic Cells in The Seminiferous Tubules of Rat (Rattus Norvegicus) with Excessive Physical Exercise

Rahmah Wahyu Rosidawati, Rimayanti Rimayanti, Koesnoto Supranianondo
The Veterinary Medicine International Conference (VMIC) | pages 93-104

Phytochemicals, Antioxidant and Antifungal Properties of Acorus calamus, Curcuma mangga, and Allium sativum

Bayyinatul Muchtaromah, Mujahidin Ahmad, Emy Koestanti S, Yuni Ma’rifatul A, Velayati Labone A
The Veterinary Medicine International Conference (VMIC) | pages 105-110

Shark Species on Export Products from East Java and Bali by Dna Barcoding Based on Internal Transcribed Spacer-2 (Its-2) Locus in Mitochondrial

Eduardus Bimo Aksono
The Veterinary Medicine International Conference (VMIC) | pages 111-124

Bioactivity of human Menopausal Gonadotrophin (hMG) and Deglycosylated hMG (hMGdG) from Urine of Post-Menopausal Women On invitro Bovine Embryonic cleavage

Herry Agoes Hermadiv
The Veterinary Medicine International Conference (VMIC) | pages 125-138

The Effect of Frequency Acoustic Stimulation Sound on Intrauterine Weakening of Pregnant Sheep

Djamir Suherman, Hermanto Tri Joewono, I Komang Wiarsa Sardjana
The Veterinary Medicine International Conference (VMIC) | pages 139-152

The Potency of AF 508-T Gen Mutant the Coding of Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) As Prototype at the Congenital Bilateral Absence of Vas Deferens (CBAVD) Disease in Indonesia

Masliahah Mfuchati, Mas’ud Harijadi, Widjiati Widjiati, Boerhan Hidayat
The Veterinary Medicine International Conference (VMIC) | pages 153-165

A Retrospective Study of Canine Pyometra in Segar Veterinary Hospital, Kuala Lumpur, Malaysia Year 2012-2016

Ng Xin Hui, Mas’ud Harijadi, Hardany Primarizky
The Veterinary Medicine International Conference (VMIC) | pages 166-174

Oocyte Quality and Subsequent In Vitro Maturation of Sheep Oocyte-Cumulus Complex from Ovary with Presence and Absence of Corpus Luteum

Rini Widyastuti, Mas Rizky A.A. Syamsunarno, Takdir Sali, Arief Boediono
The Veterinary Medicine International Conference (VMIC) | pages 175-188

The Effect of Insulin-Like Growth Factor-I of Pregnant Crossbreed Mare Serum and Insulin-Like Growth Factor-I Recombinant Mouse on Estrous Cycles and Litter Sizes of Mice (Mus musculus)

Tjuk Imam Restiadi, Imam Mustofa, Suzanita Utama, Sri Mulyati
The Veterinary Medicine International Conference (VMIC) | pages 189-196
Adding of L-Arginin Amino Acid in Skim Milk Diluent to Maintain Quality of Buck Sperm in Cold Temperature

Tri Wahyu Suprayogy, Suberni Susilowati, Tatik Hernawati
The Veterinary Medicine International Conference (VMIC) | pages 197-204

Profile of Crude Protein Tyrosine Kinase on Plasma Membrane of Merino Sheep Spermatozoa Using the Method of SDS-Page (Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis)

Vilda Carlenia Wardani, Sri Pantja Madyawati, Poedji Hastutiek
The Veterinary Medicine International Conference (VMIC) | pages 205-211

Comparison of Morula and Blastula Embryo Vitrification by Using Cryoprotectant Ethylene Glycol, Propanediol, DMSO and Insulin Transferrin Selenium

Widjiati Widjiati, Epy Muhammad Luqman, Portia Sumarsono
The Veterinary Medicine International Conference (VMIC) | pages 212-223

Effect of L-Arginine on the Thickness Iliac Arteries Wall Post Fogarty Balloon Embolectomy Catheter in Rabbit (Oryctolagus cuniculus)

Gavrila Amadeu Pusupitarani, Ngakan Made Rai Widjaja, Hardany Primarizky
The Veterinary Medicine International Conference (VMIC) | pages 224-233

Ellagic Acid Activity in Healing Process of Incision Wound on Male Albino Rats (Rattus norvegicus)

Hardany Primarizky, Wiwik Misaco Yunarti, Bambang Sektiai Lukiswanto
The Veterinary Medicine International Conference (VMIC) | pages 234-240

Control and Preventive Study of Brucellosis by Using

Rahmahani J, Handijatno D, Tyaningsih W, Suwarno Suwarno
The Veterinary Medicine International Conference (VMIC) | pages 241-251

Histopathology of Coronary artery of male rat (Ratus Norvegicus) with high fat diet after being given ethanol extract of Indian acalypha (Acalipha indica, L)

The Veterinary Medicine International Conference (VMIC) | pages 252-265

Immunohistochemical Detection of Porcine Reproductive and Respiratory Syndrome Virus Antigen in Formalin-Fixed, Paraffin-Embedded Tissues with Correlation to Clinicopathologic Data

Lavina Gracia G. Manzano
The Veterinary Medicine International Conference (VMIC) | pages 266-277

Anthelmintic Activity of Ocimum sanctum Linn. Leaves Ethanol Extract Against Fasciola gigantica in vitro

Mesia Margi Mahardika, Sri Agus Sudjarwo, Setiawan Koestarto
The Veterinary Medicine International Conference (VMIC) | pages 278-285

Scabiosis in Rabbit

Miyayu Soneta Sofyan, Doni Chrismanto
The Veterinary Medicine International Conference (VMIC) | pages 286-295
Detection of Newcastle Disease Virus by Immunohistochemistry on the Brains of Laying Birds with Clinical Signs Torticollis and Curled Toe Paralysis
Ocie Harum Wulan, Niken Yunita, Hastari Wuryastuty, Raden Wasito
The Veterinary Medicine International Conference (VMIC) | pages 296-307

Implementation of Meniran Extract (Phyllanthus Niruri Linn) on the Performance of Broiler Chickens Infected by Mycoplasma gallisepticum Caused Chronic Respiratory Disease
Sri Hidanah, Emy Koestanti Sabdiningrum, Retno Sri Wahjuni, Arimbi Arimbi
The Veterinary Medicine International Conference (VMIC) | pages 308-315

Blood Parasite Infection Prevalence in Kampong Chicken Breeder’s Group in Garut
Djoko Legowo, Syifa Husnul Khotimah, Lucia Tri Suwanti
The Veterinary Medicine International Conference (VMIC) | pages 316-326

Amino Acid Analysis of Fusion (F) Gene and Prediction of Epitope B-Cell Newcastle Disease Surabaya Isolate As Vaccine Candidate
Indah Laili Rahmawati, Fedik Abdul Rantam, Wiwik Tyasningsih
The Veterinary Medicine International Conference (VMIC) | pages 327-337

Mini Review: Liver Fibrosis Mechanism
Wiwik Misaco Yuniarti, Hardany Primarizky
The Veterinary Medicine International Conference (VMIC) | pages 338-343

The Nutrients Contents, Dry Matter Digestibility, Organic Matter Digestibility, Total Digestible Nutrient, and NH3 Rumen Production of Three Kinds of Cattle Feeding Models
M. Anam Al-Arif, Lucia Tri Suwanti, AT Soelih Estoeangepang, Mirni Lamid
The Veterinary Medicine International Conference (VMIC) | pages 344-355

Immunogenicity of Bone Graft Using Xenograft Freeze-Dried Cortical Bovine, Allograft Freeze-Dried Cortical New Zealand White Rabbit, Xenograft Hydroxapatite Bovine, And Xenograft Demineralized Bone Matrix Bovine In Bone Defect Of Femoral Diaphysis White Rabbit Experimental Study In Vivo
Ferdiansyah Ferdiansyah, Dwikora Novembri Utomo, Heri Suroto
The Veterinary Medicine International Conference (VMIC) | pages 356-368

Genotyping Analysis of Mycobacterium leprae isolated in Water Environment of Leprosy Endemic Places in Lamongan, East Java
Cita Rosita Sigit Prakoeswa, Nanny Herwanto, Ratna Wahyuni, Iswahyudi Iswahyudi, Dinar Adriaty, Indropo Agusni, Shinzo Izumi
The Veterinary Medicine International Conference (VMIC) | pages 369-377

Iron Overload Reduces Cholesterol and Triglyceride Serum of Mice
Devi Agustin Setiawati, Mas Rizky A.A. Syamsunarno, Pandji Irani Fianza, Nur Atik, Neni Anggreaeni, Mohammad Ghozali, Ratu Safitri, Ramdang Panigoro
The Veterinary Medicine International Conference (VMIC) | pages 378-385
Measurement of Alkaloids Achyranthes Aspera Linn Level Using Thin Layer Chromatography Method and High-Performance Liquid Chromatography

Dewa Ketut Meles, Wurlina Wurlina, Dewa Putu Anom Adnyana
The Veterinary Medicine International Conference (VMIC) | pages 386-394

Propolis Potential Toward the Amount of Limphoblast and Spleen Diameter of Male Mice (Mus musculus)

Werstant Adhityananda Rinaldi, Eka Pramythra Hestianah, Sri Mumpuni Sosiawati, Lita Rakhma Yustinasari
The Veterinary Medicine International Conference (VMIC) | pages 395-403

Identification the Gene Nucleotide Sequence of Outer Membrane Protein Aeromonas Hydrophila Bacteria from East Java Local Isolates Using Polimerase Chain Reaction

M. Gandul Atik Yuliani, Didik Handijatno, Sri Pantja Madyawati
The Veterinary Medicine International Conference (VMIC) | pages 404-412

Postmortem Interval Estimation Time from Algormortis Temperature of Rats Expressed by MARS Model Approach

Dwi M. Syabani, Hana Eliyani, Suharsono Suharsono, Fedik A. Rantam, Anwar Ma’ruf
The Veterinary Medicine International Conference (VMIC) | pages 413-421

Aqueous Extract of Neem Leaves (Azadirachta Indica) Decrease Expression of Immunoglobulin E (IgE) and Interleukin 4 (IL-4) in Gingiva Tissue of BALB/c Mice Injected by Ovalbumine

I Dewa Ayu Ratna Dewanti, I Dewa Ayu Susilawati, Pujiana Endah Lestari, Roedy Budirahardjo, Erawati Wulandari, Risty Widya, Sunlip Wibisono
The Veterinary Medicine International Conference (VMIC) | pages 422-435

Experimental Models Point Mutations In Plasmodium falciparum ptpase6 Gene Exposed to Recuring Artemisinin In Vitro

Lilik Maslachah, Yoes Prijatna Dachlan, Chairul A. Nidom, Loeki Enggar Fitr
The Veterinary Medicine International Conference (VMIC) | pages 436-442

Sinesetin-Rich Fraction Solid Dispersion Inhibits Cancer Cell Cycle

Lusiana Arifianti, Sukardiman Sukardiman, Mulia Hadi Santos
The Veterinary Medicine International Conference (VMIC) | pages 443-449

Blunted Expression of PPARa in Mice with FABP-4 and -5 Deficiency under Acute Cold Exposure

Mas Rizky A. A Syamsunarno, Mirasari Putri, Tatsuya Iso, Rini Widyaastuti, Ramdan Panigoro, Masahiko Kurabayashi
The Veterinary Medicine International Conference (VMIC) | pages 450-459

Increased Iron in Pediatric β-Thalassaemia Major Associates with CD3+, Not 76 Lymphocytes

Mohammad Ghozali, Ulrike Panjaitan, Adi Imam Cahyadi, Reni Ghrahani, Lelani Reniarti, MRAA. Syamsunarno, Ramdan Panigoro
The Veterinary Medicine International Conference (VMIC) | pages 460-467

Low Serum Cholesterol in Mice Pre-treated with Imperata cylindrica L. after Acute Olive Oil Gavage
Neni Anggraeni, Mas Rizky A.A Syamsunarno, Ghina Rahmadiani Mukarromah, Almira Zada, Rima Destya Triatin, Yunisa Pamela, Diah Dhanawaty
The Veterinary Medicine International Conference (VMIC) | pages 468-475

The Role Of IL-6 In TMPD-Treated Lupus Arthritis Mice
Niken Indriyanti
The Veterinary Medicine International Conference (VMIC) | pages 476-485

Immunopathological Approach for Avian Influenza Virus Detection in Brain of Laying Bird with Clinical Signs of Torticollis and Curled Toe Paralysis
Niken Yunita, Ocic Harum Wulan, Hastari Wuryastuty, Raden Wasito
The Veterinary Medicine International Conference (VMIC) | pages 486-496

The Efficacy of Permot (Passiflora foetida Linn.) Leaves Crude Extract Ointment on the Healing of Skin of Rabbit with Scabies
Poedji Hastutiek, Hana Eliyani
The Veterinary Medicine International Conference (VMIC) | pages 497-512

The Effect of Sappan Wood Extract (Caesalpinia sappan), Wheat grass and Vitamin E Treatment on the Liver Structure of Iron overload of Rat (Rattus norvegicus)
Ratu Safitri, Lelani Reniarti, Maditah Maditah, Lila Delia, Mas Rizky A.A Syamsunarno, Ramdan Panigoro
The Veterinary Medicine International Conference (VMIC) | pages 513-519

The Effectiveness of Antibiotics and Hematopoietic Stem Cell Treatment in Periodontitis Rat Model Toward TNF α Expression
Retno Indrawati R, Indeswati DIYatri, Dwi Rahmawati
The Veterinary Medicine International Conference (VMIC) | pages 520-527

Construction Hybrid immunoglobin All Four Dengue serotype Using Mesenchymal Stem
Rofiqul A’la, Rahaju Ernawati, Nunuk Dyah Retno L, Muhasirin Muhasirin, Anwar Ma’ruf, Fedik A. Rantam
The Veterinary Medicine International Conference (VMIC) | pages 528-535

The Potential of Black Gluten and Red Rice in Rations on the Biological Values and Ideally Body Score of Healthy “Mini Rex Rabbit”
Romziah Sidik
The Veterinary Medicine International Conference (VMIC) | pages 536-548

Antiviral Activity Effect of Silver Nanoparticles (AgNO3) Solution Against the Growth of Infectious Bursal Disease Virus on Embryonated Chicken Eggs with Elisa Test
Rosa Pangestika, Rahaju Ernawati
The Veterinary Medicine International Conference (VMIC) | pages 549-556

Identification and Characterization Indigenous of Lactobacillus sp from Bovine Rumen Fluid of Slaughterhouse
Tri Nurhajati, Koesnomo Soepranianondo, Widya Paramita Lokapirnasari, Adriana Monica Sahidu
The Veterinary Medicine International Conference (VMIC) | pages 557-565
Protective Effect of Propolis Extract Against Lead Acetate Toxicity in Mice (Mus Musculus) Testes

Tuti Widawati, Sri Agus Sudjarwo, Harry Agoes Hermadi
The Veterinary Medicine International Conference (VMIC) | pages 566-578

Detection of Antibiotic Residues and Concentration in Raw Milk from Lembang Small Holder Dairy Farm

Virgianty Vivi
The Veterinary Medicine International Conference (VMIC) | pages 579-587

Potency of Bacillus cereus WPL 415 to Increase Crude Protein and Decrease Crude Fiber of Animal Feed Stuff

Widya Paramita Lokapirnasari, Adriana Monica Sahidu, Tri Nurhajati, Koesnito Soepranianondo, Andreas Berny Yulianto
The Veterinary Medicine International Conference (VMIC) | pages 588-695

Acute Toxicity Tests of Alkaloid Pare (Momordica Charantia) Fruit on The Histopathology of Liver

Wurlina Wurlina, Dewa Ketut Meles, Sunarni Zakaria, Imam Mustofa, Suheri Susilowati, I Dewa Putu Anom Adnyana
The Veterinary Medicine International Conference (VMIC) | pages 696-602

Teratogenic Effect of Congenital Toxoplasmosis in Chicken Embryo

Lucia Tri Suwanti, Mufasirin Mufasirin, Hani Plumeriastuti, Erma Safitri
The Veterinary Medicine International Conference (VMIC) | pages 603-608

Case Study: Dystocia on Beef Cattle in Kunir Regency of Lumajang District, East Java, Indonesia in 2015 and 2016

Rosiana Febrianila, Widya P Lokapirnasari, Tjuk I Restiadi, Imam Mustofa, Harry A Hermadi, Erma Safitri
The Veterinary Medicine International Conference (VMIC) | pages 609-618

The Effectiveness of Honey in Physiological NaCl to Maintain of Viability and Motility of Spermatozoa

Elsa Agustinia, Harry Agoes Hermadi, Hario Puntodewo S, Tatik Hernawati, Indah Norma Triana, Erma Safitri
The Veterinary Medicine International Conference (VMIC) | pages 619-626

Utilization of Sumbawa Tropical Forest Honey Apis Dorsata to Improve Fertility of Indonesia Oriental Magpie Robin (Copsychus saularis) as Effort Animal Population Increasement

Abdullah Hasib, Risaldi Muhamad, Talita Yuanda Reksa, Alvina Ulimaz Artha, Erma Safitri
The Veterinary Medicine International Conference (VMIC) | pages 627-632

Utilization of Honey Apis dorsata as Antiosteoporosis on Requirements of Bone Calcium Ash Density on Ovariolysectomized White Rat (Ratus norvegicus)

Muhammad Huda Ramadhan Ibrahim, Abdullah Hasib Hasib, Siti Nur Rohmah, Salsabilla Abani, Samsi Yordan, Ira Sari Yudanriyanti
The Veterinary Medicine International Conference (VMIC) | pages 633-641

Increased Integrity of Plasma Membrane and Acrosome Cap Spermatozoa Limousin Cattle at Post Thawing in Frozen Media by adding Seawater Extract

Nur Faidah, Tatik Hernawati, Mirni Lamid, Ishmudiono Ishmudiono, Tri Wahyu Suprayogi, Sri Mulyati
The Veterinary Medicine International Conference (VMIC) | pages 642-649

The Relation of Body Temperature and Vaginal Cytology Examination in Time Artificial Insemination Rate Fat-tailed Sheep (Ovis Aries) in The District Sidoarjo East Java

Rhendyka Prasetya Anggriawan, Suzanita Utama, Hana Eliyani
The Veterinary Medicine International Conference (VMIC) | pages 650-657

Effect of Laser Acupuncture Shoot on Ova Point of Male Mojosari Duck (Anas plathyrynchos) on The Number of Sertoli and Leydig Cells

Yuanara AR Adikara, Abdul Samik, Ira S Yudaniayanti, Tatang S Adikara, Eka P Hestianah, Suzanita Utama
The Veterinary Medicine International Conference (VMIC) | pages 658-667

Insulin-Like Growth Factor-I (IGF-I) from Crossbred Pregnant Mare Serum to Increase Follicle Number of Mice (Mus musculus)

Abdullah Abdullah, Tjuk IRestiadi, Nunuk DR Lastuti, Tita Damayanti, Wurlina Wurlina, Erma Safitri
The Veterinary Medicine International Conference (VMIC) | pages 668-676

Morphological Identification Nematodes Tanqua tiara Found on Gastric Varanus salvator at East Java

Alfiana Laili Dwi Agustin, Setiawan Koesdarto, Bambang Sakti Lukiswanto, Lucia Tri Suwanti, Zainal Arifin, Emmanuel Djoko Putranto
The Veterinary Medicine International Conference (VMIC) | pages 677-683

Effect of Propolis on Spermatogenic Cells Number and Diameter of Seminiferous Tubules in Male Mice (Mus musculus)

Dona Astari Nurkarimah, Eka Pramythta Hestianah, Retno Sri Wahjuni, Mas'ud Hariadi, Suryo Kuncorojakti, Harry Agoes Hermadi
The Veterinary Medicine International Conference (VMIC) | pages 684-693

The Effect of Blue Green Algae (Spirulina platensis) Extract in White Rat (RattusNorvegicus) Treated with Excessive Physical Exercise on Leydig Cell Number and Seminiferous Tubules Diameter

Dimas Yuzrifar Rhavindra Lazuardi, Rimayanti Rimayanti, Hardany Primarizky, Sri Agus Sudjarwo, Suzanita Utama, Kadek Rachmawati
The Veterinary Medicine International Conference (VMIC) | pages 694-701

The Effect of Mahkota Dewa (Phaleria macrocarpa) Pulp Extract by Peroral Administration Toward The Percentage of Capacitation and Acrosome Reaction in Rat (Rattus norvegicus)

Dhanang Estu Bagyo, Budi Utomo, Rudy Sukamto Setiabudi
The Veterinary Medicine International Conference (VMIC) | pages 702-711

Protection of Dayak Onion Tuber Extract (Eleutherine Palmifolia) Against Kidney Histopathological Appearance of Albino Male Rat Strain Wistar which was Induced by Alloxan

Dwi Gayatri Nurcahyawati, Hani Plumeriastuti, Lilik Maslachah
The Veterinary Medicine International Conference (VMIC) | pages 712-717

Test Various Estrus Detection Device Against Pregnancy Rates on Dairy Cows in Cooperative Tunas Setia Baru Kabupaten Pasuruan

Silvia Rani Andriyanti, Mas'ud Hariadi, Roesno Darsono, Pudji Srianto
The Veterinary Medicine International Conference (VMIC) | pages 718-726

Effect of Laserpuncture Shoot on Reproduction Point of Male Mojosari Duck (Anas Platyrhynchos) on The Numbers of Spermatogonium Cells and Seminiferous Tubules Diameter
Melyandari Ayu Qomar, Rimayanti Rimayanti, Tri Nurhajati
The Veterinary Medicine International Conference (VMIC) | pages 727-733

Cases of Reproduction Disorder in Beef Cattle of Modo District, Lamongan in 2015
Azharuddin Anshoria, Tri Nurhajati, Budi Utomo
The Veterinary Medicine International Conference (VMIC) | pages 734-741

The Application of Equine Chorionic Gonadotropin (Ecg) and Prostaglandin F2α to Increase the Rate of Pregnancy in Bali Cattle at Buleleng, Bali
Yugenthri A/P Chandran, Herry Agoes Hermadi, Eka Pramyrtha Hestianah
The Veterinary Medicine International Conference (VMIC) | pages 742-752

Morphometry Study of Hemipenis Biawak Air Varanus Salvador on Length Measurement of Snout Vent Length (Svl) Andbody Weight
Ilham Adi Kusuma, Dicky Beo Alfiyanto, Pudji Sianto, Nusdianto Triakoso, Djoko Legowo
The Veterinary Medicine International Conference (VMIC) | pages 753-762

Effect of Polygonum Minus (Knotweed) Leaves Extract on the Histopathological Changes of Kidney in Mice (Mus Musculus) Induced by Mercuric Chloride
Winni Aprianti, Thomas Valentinus Widiyatno, Sri Agus Sudjarwo
The Veterinary Medicine International Conference (VMIC) | pages 763-775

Skin Stem Cell Resource Potential for Peripheral Nerve Repair Due to trauma of post regional anesthesia
Sumartono Christrijogo, Fedik A Rantam, Eddy Rahardjo, Martia R Tucharina
The Veterinary Medicine International Conference (VMIC) | pages 776-781

Public Awareness in ensuring Animal Originated Food Safety : A Review on “One Health” Approach in Veterinary Medicine
A. T. Soelih Estoe Pangestie
Conference Paper

Ellagic Acid Activity in Healing Process of Incision Wound on Male Albino Rats (Rattus norvegicus)

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Abstract

This study was conducted to prove whether ellagic acid can be used as a regulator of incision wound healing process in male albino rats as experimental animals. A total of 24 male healthy rats based on physical examination, aged at 3 months old and weight at 150-180 grams used in this study. Rats were divided into three groups and each group has 8 replications, group of control (P0) has no incision wound and no medication, treatment group (P1) has incision wound and treated with Betadine® and (P2) has incision wound and treated with 2.5 % ellagic acid. The variable that observed are histopathology changes (collagen, PMN, angiogenesis and fibrosis) from the male albino rats. The samples collection was taken at day 15 and data were collected and analyzed by Analysis of Variance (ANOVA) test. The result of collagen and PMN cells score in the control group (P0) were 1.80 ± 0.16 and 1.6 ± 0.08 with mild angiogenesis and no fibrosis. The results in the treatment (P2) group, where the collagen and PMN scores were 1.60 ± 0.08 and 1.5 ± 0.8, accompanied by mild angiogenesis and fibrosis. Meanwhile, in the treatment (P1) group, the results of scoring of collagen and PMN were lower than the other groups, 0.80 ± 0.08 and 0.6 ± 0.16 accompanied by moderate angiogenesis and fibrosis. There was a significant difference of collagen scoring, PMN cells number, fibrosis and angiogenesis among the treatment groups (p<0.05). P1 treatment group has significant different with treatment groups Po and P2 (p<0.05), but there is no significant difference between treatment groups Po and P2 (p>0.05). From the results, it can be concluded that the topical application of ellagic acid ointment was proved to provide healing process on incision wound. Topical administration of ellagic acid ointment for 14 days was able to improve the skin condition and promoted the wound healing.

Keywords: Ellagic acid; wound healing; collagen; angiogenesis; fibrosis; anti-inflammatory; antioxidant; antibacterial.
1. Introduction

Wound is a condition of tissue damaged continuity caused by trauma from sharp or dull objects, temperature changes, chemical materials, electricity, radiation or animal bites. An incision wound is a wound on the skin surface that frequently caused by contact with a sharp object [1-4].

Wound healing is a process of the body to repair the tissue damaged to be functioned again. The body tried to regulate the abnormal conditions as a result of healing process. The body responded if the skin integrity was damaged and presented an overlapped phase but biologically distinguishable. After wound occurred, the healing phase that involved are inflammation, proliferation and remodeling [18].

The wound healing process can be disrupted by the causes from the body itself (endogenous) and from external causes (exogenous). The most important endogenous causes are impaired coagulation and immune system disorder. Both of these conditions will inhibit wound healing with changes of body reactions to injury, necrosis and contamination. Exogenous causes that can affected wound healing process include radiance ionization, cytostatic agents’ administration and immune suppressant drug like corticosteroid [6].

Nowadays the patent medicines are served to optimized wound healing process like burn wounds, incision wounds or any other wounds. The available drugs has many disadvantages such as expensive price and not affordable for everyone. Furthermore, the consideration of synthetic chemical substances addition have some side-effects that influenced other vital organs. That consideration made some researcher did studies about natural herbs as an alternative for medical treatments [7].

One of the most widely studied medicinal plants for health problems is pomegranate or Punica granatum Linn (PGL). The antioxidant and anti-inflammatory activity from Pomegranate are due to the very high content of polyphenols. Polyphenols that contained in pomegranates are ellagic acid (EA), gallotannins, antocyanins and others [8].

Ellagic acid is the active ingredient that widely studied and it’s a polyphenol that has much activities as an antioxidant, anti-inflammatory and as a regulator on fibrosis process with decreasing the excess collagen level on the chronic lesion tissues [9, 10].

This study was conducted to prove whether ellagic acid can be used as a regulator of incision wound healing process in male albino rats as experimental animals.

2. Materials and Methods
2.1. Materials

The materials that used in this study were 90% ellagic acid produced by Xi’an Biof. Bio-Technology Co., Ltd., Ketamine® and Diazepam for anesthesia, sterile aquadest, vaselin flavum, Betadine®, 70% ethanol, pellet feed, 10% buffered formalin for organ fixation to avoid damage, Entellan® for mounting cover glass, absolute alcohol solution for dehydration stage (96%, 80%, 70%), xylol for clearing phase, and HE staining for the histopathological staining.

The tools for this study were ACIS® digital scales for weighing albino rats, surgical equipment for making an incision wound are gloves, scissors, sterile cotton, plastic spoon, cotton bud, tissue forceps, artery forceps, sterile gauze, scalpel no.3, blade no.10 and tissue container. The tools for making ointment such as stamper and mortar also needed, then the tools for histopathological slide such as microtome, embedding set, slides, cover slides, staining set and microscope for observation and camera.

2.2. Methods

This study used male albino rat (Rattus norvegicus) as an experimental animal. A total of 24 male with rats were healthy based on physical examination, aged at 3 months old and weight at 150-180 grams used in this study. 24 male albino rats (Rattus norvegicus) as an experimental animal with one week adaptation for preparing and checking the health status. After a week of adaptation, the rats divided into three groups and each group has 8 replications. The variable that observed are histopathology changes (collagen, PMN, angiogenesis and fibrosis) from the male albino rats. Control group animals (P0) has no incision wound and no medication. Treatment groups (P1) has incision wound and treated with Betadine® and (P2) has incision wound and treated with 2,5% ellagic acid. Animals then were euthanized and skin samples on day 15 were harvested.

After 1 week of adaptation, the albino rats were shaved on their dorsal body of 3 cm x 2.5 cm to facilitate the incision process. For that procedure, anaesthetic agents were administered with combination of Ketamin® and Diazepam (100mg/ml : 5 mg/ml) at dose level of 1 ml/kg body weight [11]. The incision wound made along 3 cm, 0.25 cm depth and 0.01 cm width in shaved skin region. The skin fixation was made and scalpel blade placed on skin surface with 30-40 angle then incision done with moved the scalpel blade caudally [12]. Treatment was done by applying Betadine® and Ellagic acid ointment with cotton bud, three times daily with 8 hours interval for 14 days [13]. Skin tissue sampling was done at day 15 with euthanasia procedure using Ketamin®.
Table 1: The comparison of healing process in each treatment groups based on collagen and PMN scores.

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>Collagen</th>
<th>PMN Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>P0</td>
<td>1.80 ± 0.16a</td>
<td>1.6 ± 0.08a</td>
</tr>
<tr>
<td>P1</td>
<td>0.80 ± 0.08a</td>
<td>0.6 ± 0.16a</td>
</tr>
<tr>
<td>P2</td>
<td>1.60 ± 0.08a</td>
<td>1.5 ± 0.8a</td>
</tr>
</tbody>
</table>

Note: different superscript (*a, b*) indicate significant difference (p < 0.05). P0: control group; P1 and P2: treatment group.

Skin tissue samples was taken at 0.5 cm depth and 1 cm extra on four sides for better sample quality [6].

The observation of histopathological slides was done with scoring technique that are defined as: Collagen: normal bundle: 2, unorganized / oedema: 1, amorphous: 0; PMN: 0-10: 2, 11-40: 1, > 40: 0; angiogenesis in 3 degrees ie. mild, moderate and severe; presence of fibrosis by measuring the collagen bundles thickness in 3 degrees ie. mild, moderate and severe [14].

All of data results from histopathology changes of collagen and PMN cells are taken at day 15 and analyzed with Kruskal-Wallis test continued with Mann-Whitney U test. The result of collagen and PMN cells numbers in the slides were calculated for individual groups also with the presence of angiogenesis and fibrosis.

3. Result

The statistical analysis result showed that ellagic acid gave an effect on healing process of an incision wound on albino rats as an experimental animals. This study also showed that there is a significant difference of collagen scoring, PMN cells number, fibrosis and angiogenesis among the treatment groups (p < 0.05). P1 treatment group has significant different with treatment groups P0 and P2 (p < 0.05), but there is no significant difference between treatment groups P0 and P2 (p > 0.05).

In the P0 group, where the skin of mice was in good health, the collagen and PMN cells scores were 1.80 ± 0.16 and 1.6 ± 0.08 with mild angiogenesis and no fibrosis. The results were observed in the P2 group, where the collagen and PMN scores were 1.60 ± 0.08 and 1.5 ± 0.8, accompanied by mild angiogenesis and fibrosis. Meanwhile, in the P1 group, the results of scoring of collagen and PMN were lower than the other groups, 0.80 ± 0.08 and 0.6 ± 0.16 accompanied by moderate angiogenesis and fibrosis. The comparison collagen and PMN scores on Table 1.

Based on data observed, the presence of angiogenesis and fibrosis are also obtained histopathological slide, as shown in Table 2.
Table 2: The presence of angiogenesis and fibrosis in each groups.

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>Angiogenesis</th>
<th>Fibrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>P0</td>
<td>Mild</td>
<td>None</td>
</tr>
<tr>
<td>P1</td>
<td>Moderate</td>
<td>Moderate</td>
</tr>
<tr>
<td>P2</td>
<td>Mild</td>
<td>Mild</td>
</tr>
</tbody>
</table>

From the results, it seem that the topical application of ellagic acid ointment was proved to provide healing process on incision wound. Topical administration of ellagic acid ointment for 14 days was able to heal the skin consistently healthy.

4. Discussion

The process of wound healing is a series of characterized condition that performed by combinations of biological processes that occurred in certain conditions and time. This process is marked by the restructuring of the damaged tissues to original condition. The natural response that occurred in healing process was repaired quite fast, and ensured the damaged tissues has completely returned to normal conditions [15].
Figure 2: Histopathological cross section (shown by black arrow) of a skin with incision wound and used Betadine® treatment (P1 group). (A) Collagen score, (B) PMN score and (C) presence of moderate angiogenesis and (D) presence of moderate fibrosis.

Figure 3: Histopathological cross section of a skin (shown by black arrow) of 15 days treatment with incision wound and used 2.5% of ellagid acid cream treatment (P2 group). (A) Collagen score, (B) PMN score and (C) presence of mild angiogenesis and (D) presence of mild fibrosis.
In this study, the group treated with topical ellagic acid ointment (P2), showed a better wound healing process when compared to the P1 group. There are several reasons that may cause the condition.

Ellagic acid, is a part of flavonoids which usually produced by various plants in tannin formation that known as ellagitannin. Ellagic acid two groups of lactons and 4 hydroxyl groups, where the hydroxyl groups have antioxidant activities so it prevent the lipid peroxidase and protect the tissue damages from oxidative stress. At this time, ellagic acid gets more attention because it has been shown to have antioxidant activity, chemo preventive and antiapoptosis, antimutagenetic, anti-inflammatory, anti-atherosclerosis, antifibrosis, antibacterial and anti-HIV replication [16].

Oxidative stress is one of the factors that inhibits wound healing process. Research on topical administration of natural ingredients that have antioxidant activity proved to improve the process of wound healing and prevent tissue damage due to oxidative stress [17, 18].

Inflammmation is a protective process that happened to eliminated foreign materials then initiated the healing process. However, the excessive or imbalanced inflammatory process can delay the wound healing. Therefore, intervention was necessary to prevent the delayed of wound healing with administered of anti-inflammatory agents [19].

Infection can delay the process of wound healing by various mechanisms, such as lowering the blood supply, causing dysfunction of the leucocyte function, prolonging inflammatory and debridement processes and producing proteolytic enzymes. Therefore, infection is a complication of various types of wounds and antibiotics have an important role in wound healing processes [20–22].

Herbs contain tannins or ellagic acid in the form of ellagitannin has a therapeutic effect if used on wounds, either burns or other types of wounds. This is because of antioxidant activities, anti-bacterial and anti-inflammatory activities [23]. Ellagitannin or ellagic acid can help the precipitation of proteins in damaged tissues, so it can provide the scabs formation rapidly. This activity will also reduce the edema and exudative fluids on wound surface through decreasing of capillaries permeability [20, 24]. Beside that activity, other activities are thought to play a role in wound healing process from its ability as an angiogenic agent [25].

Based on the results of the research, to get the results of a good wound healing process, 2.5% of ellagic acid ointment should be given at the beginning of the initial phase of injury.
5. Conclusion

This study concluded that the application of 2.5% ellagic acid has an activity to promote healing process on incision wound. Therefore, to show the best result it suggested to use it frequently for 14 days.

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