

Advances in Natural and Applied Sciences

published by:

American-Eurasian Network for Scientific Information



ANAS

AN INTERNATIONAL OPEN FREE ACCESS PEER REVIEWED RESEARCH JOURNAL

ANNOUNCEMENTS & NEWS

12' Frequency



Our Editorial Board..

a detailed view.

PrevNext
1

Our Editorial Board..

a detailed view.

- Home (index.php)
- Editorial Board

Contact us for more info
(contact.html)



Regional Editors::

- Dr. Adya Singh Senior Scientist, Wood Processing,the joint forces of CSIRO & SCION (formerly Forest Research),Te Papa Tipu Innovation Park, 49 Sala Street,Private Bag 3020, ROTORUA,New Zealand.
- Dr. Attila Hegedus Corvinus University of Budapest, Faculty of Food Science, Department of Applied Chemistry,H-1118 Budapest, Hungary.Villányi út 29-31.
- Dr. Naveed Ahmed Khan School of Biological & Chemical Sciences,Birkbeck College, University of London,U.K.
- Dr. Amita Pal Plant Molecular & Cellular Genetics,Bose Institute, Kolkata - 700 054,India.
- Dr. Abdel-Sattar S. Hamad Elgazwy Department of Chemistry, Faculty of Science,Ain Shams University, Cairo,Egypt.
- Dr. Luisa Consiglieri Department of Mathematics/CMAF, FCUL (Univ. of Lisbon),1749-016 Lisboa,Portugal.
- Dr. Nicholas A. Nechval Department of Mathematics and Statistics,University of Latvia,Latvia.
- Dr. Manal Fawzy Department of Environmental Sciences, Faculty of Science, University of Alexandria,Moharram Bek 21511, Alexandria,Egypt.
- Dr. Surendra Prasad School of Chemical Sciences, Faculty of Science and Technology, The University of the South Pacific Suva, Fiji.
- Dr. Nidal Odat Department of Biology,Al-Hussein Bin Talal University,P.O.BOX 20, Ma'an,JORDAN.
- Dr. Mohamed Al-Harabsheh Vice Dean of Academic Research, Department of Mining Engineering, College of Mining and Environmental Engineering, Al-Hussein Bin Talal University, Ma'an, Jordan.
- DR. BENJARAM MAHIPAL REDDY Deputy Director, Inorganic and Physical Chemistry Division, Indian Institute of Chemical Technology (IICT), Uppal Road Hyderabad – 500 607, India.
- Prof. Dr. Hakan ARSLAN Department of Basic Pharmaceutical Sciences, Division of Chemistry, Faculty of Pharmacy, Mersin University, TR-33169 Mersin, Turkey.

Advisory Board::

- Prof. Myung-Hoon Kim Chemistry Professor, Science Department, Georgia Perimeter College Dunwoody, GA 30338, USA
- Dr. YASIR HASAN SIDDIQUE Human Genetics and Toxicology Lab, Section of Genetics, Department of Zoology, Faculty of Life Sciences, Aligarh Muslim University, Aligarh - 202002 (UP), INDIA.
- Prof. M. Husain Department of Physics, Jamia Millia Islamia (Central University), Jamia Nagar, New Delhi-110025, India.
- Dr. Tarik S.T.Ali Associate Professor, Department of Mathematical Sciences, College of Science, UAE Univ., Al-Ain, P.O. Box 17551, United Arab Emirates.
- Dr. Osaretin Albert T. Ebuehi Associate Professor of Biochemistry, Department of Biochemistry, College of Medicine, University of Lagos, PMB 12003 Lagos, Nigeria, West Africa.
- Dr. S. RAMACHANDRA SETTY Professor and Principal, S. C. S. COLLEGE OF PHARMACY, HARAPANAHALLI 583 131. INDIA.
- Prof. Ji-Huan He Modern Textile Institute, Donghua University, 1882 Yan'an Xilu Road, Shanghai 200051, China.
- Dr. Vikash Kumar Dubey Department of Biotechnology, Indian Institute of Technology Guwahati, Assam, India-781039.

- Dr. Alper BABA Canakkale Onsekiz Mart University, Engineering and Architecture Faculty, Geological Engineering, 17020- Canakkale/ Turkey.
- Prof. Khalil Ahmad Professor, Department of Mathematics, Jamia Millia Islamia, New Delhi - 25.
- Dr. i-Ping Liang Chair, Division of Systematic & Evolutionary Zoology, Institute of Zoology, Chinese Academy of Sciences, Datun Road, Chaoyang District, Beijing 100101, PR China.
- Assoc. Prof. Kemal Büyükgüzel Manager of University-Industrial Cooperation Development Center (UICDEC) and Director of School of Natural and Applied Science, Zonguldak Karaelmas University, Faculty of Science and Arts Faculty, Biology Department, 67100 Incivez- Zonguldak, Turkey.
- Dr. Ali Gazanchian Department of Genetic and Physiology, Agricultural and Natural Resources Research Center of Khorassan, Addresses: Mashhad, Razavi Khorassan Province, Iran. Box P.O.: 91735-1148, Mashhad, Iran.
- Prof. Anwar Ali Department of Chemistry, Jamia Millia Islamia (Central University), New Delhi - 110 025 (India).
- Professor Siamak E. Khadem Tarbiat Modares University faculty member and Managing Director of Khadem Sound & Vibration Technology Ltd.#205, No 140 Mehr Trade Bldg., Moghaddas Ardebili Ave, Tehran, Iran.
- Dr. Akuro ADOKI EPG-VHSI, Health, Safety & Environment, Shell Petroleum Development Company Ltd, I.A. Prodeco Block F, Room 8, Old Aba Road, Port Harcourt.
- Dr. Mohammad Kamil Associate Professor and Head- Deptt. of Pharmacognostic Sciences and Ethno botany, Research Coordinator, Zayed Complex for Herbal Research & Traditional Medicine, P.O. Box 29300, Abu Dhabi, UAE.
- Dr. Rachid ROUABHI Cellular toxicology, AJBR Editorial Board Member, Biology Department, Tebessa University, Algeria.
- Dr. Wellington Riach Liamba Masamba Senior Research Fellow, Harry Oppenheimer Okavango Research Centre, University of Botswana, P/Bag 285 Botswana.
- Dr. Ekwenye Uchechi Nnembiue (Oji) Michael Okpara University of Agriculture, P.M.B. 7267, Umuahia.
- Dr. BOVERA FULVIA Dip. Scienze Zootechniche e Ispezione degli alimenti, Via F. Delpino, 1 – 80137 Napoli.
- Prof. DUDUKU KRISHNAIAH Chemical Engineering Programme, School of Engineering & Information Technology, Universiti Malaysia Sabah, 88999 Kota Kinabalu, Sabah. MALAYSIA.
- Assoc. Prof. Dr. Nazan DARCAN Dept. of Animal Sci., Faculty of Agriculture, Cukurova Univ. 01330 Adana-Turkey.
- Asst. Prof. Dr. Zafer OLMEZ Artvin Coruh University, Faculty of Forestry, 08000-Artvin/TURKEY.
- Dr. Fahrettin Tilki Artvin Coruh University, Faculty of Forestry, 08000-Artvin, Turkey.
- Dr. SANJAY MISHRA Professor, Department of Biotechnology, College of Engineering and Technology, IFTM Campus, Moradabad, U.P., India.
- Prof. Ibijola Emmanuel Adeolu Department of Mathematical Sciences, University of Ado-Ekiti, Ado-Ekiti, Ekiti State, Nigeria.
- Dr. Hatil Hashim EL-Kamali Associate Professor (by Research), Head Department of Botany, Faculty of Science and Technology, Omdurman Islamic University, P.O. Box # 382, Omdurman, Sudan.
- Dr. Ideisan I. Abu-Abdoun Chemistry Department, University of Sharjah, Sharjah - P.O.Box 27272, United Arab Emirates.
- Dr. Akuro Adoki Incident Management & Regional LFI Coordinator, Shell Petroleum Development Company of Nigeria Ltd, P.O. Box 263, Old Aba Road, Port Harcourt, Nigeria.
- Dr. Abdellatif KHAMLICHI Résidence Al Ismailia, Appt. B 15,33, Avenue des FAR, Tetouan, Morocco.
- Dr. Dmytro Malyskyy Carpathian Branch of Subbotin Institute of Geophysics, Department of Seismotectonic Researches, 3-b, Naukova st., 79060 Lviv, Ukraine.
- Prof. Hoda Salama Ibrahim Professor of Nutrition, Nutrition and Food Sciences. Dept. Helwan University, Cairo, Egypt.
- Dr. Sevil Toroğlu Kahramanmaraş Sütçü İmam Üniv., Science and Arts Fac., Department of Biology, 46045, Aşar Campus, K. Maraş.
- Dr. Zeinab Ahmed Elsady Professor of Mathematics, Al-Azhar University, Faculty of science, Department of Mathematics, Nasr City, Cairo Egypt.
- Dr. Amani Mohamed Dory EL-Ahwany 681 El Horria St. Gianaklis, Alexandria, Egypt.
- Dr. Nasser S. Awwad Prof. Assis. of Radiochemistry, Hot Labs center, Atomic Energy Authority.
- Dr. Narinder Kumar Reader, Department of Statistics, Panjab University, Chandigarh-160014 (INDIA).
- Dr. Lissinda du Plessis Unit for Drug Research and Development, Northwest-University - Potchefstroom campus, Private bag X6100, Internal box 36, Potchefstroom, South Africa. 2520
- Dr. Ganeshchandra Narharao Shinde Principal, Reader & PG Teacher, Research Guide In Physics, Electronics & Computer Sc., Indira Gandhi (Sr.) college, CIDCO, Nanded-431 603 Maharashtra, INDIA.
- Dr. Ayse KAPLAN Zonguldak Karaelmas University, Faculty of Arts-Sciences, Department of Botany, 67100, Incivez, Zonguldak, TURKIYE.
- Dr. Khalid Mahmood Khawar Associate Professor, Agricultural Biotechnology Section, Department of Field Crops, Faculty of Agriculture, University of Ankara, 06110 Diskapi-Altindag, Ankara, Turkey.
- Nabila BENHAMMOU Laboratory of the Natural products, Department of molecular and cellular Biology, Faculty of Science, University Abou Bekr Belkaid, Tlemcen, Algeria.
- Dr. Federica Migliardo Dipartimento di Fisica, Università di Messina, C.da Papardo, S.ta Sperone 31, 98166 Messina,
- Prof. Geronikaki Aristotelian University of Thessaloniki, School of Pharmacy, Department Pharm. Chemistry, Thessaloniki, 54006, Greece.
- Dr. Naveed Ahmed Khan Senior Lecturer in Microbiology, School of Biological & Chemical Sciences, Birkbeck College, University of London, Malet Street, London WC1E 7HX, England, U.K.
- Dr. Alaa Salem Professor of Analytical Chemistry, Department of Chemistry, Faculty of Science, UAE University, Al-Ain P.O.Box 17551, U.A.E.
- Prof. Dr. Hoda Hanem Mohammed Fadel Head of Chemistry of Flavour and Aroma Department, National Research Centre, AL-Behos Street, Dokki, Cairo, Egypt.
- Dr. Sausan Al Kawas Head of Oral & Craniofacial, Health Sciences Department, College of Dentistry, University of Sharjah, P.O.Box 27272 Sharjah, U.A.E.

Editors::

- Dr. Ismail Sahid School of Environmental and Natural Resource Sciences, Faculty of Science & Technology, Universiti Kebangsaan Malaysia, 43600 UKM, Bangi, Selangor, MALAYSIA.
- Dr. Ali Mahmud ATEIWI Dean Faculty of Science, Department of Mathematics and Statistics, Al- Hussein Bin Talal University, Ma'an, Jordan.

Associate Editors::

- Dr. Eduardo A. Castro INIFTA, Theoretical Chemistry Division, Diag. 113 y 64, Suc.4, C.C. 16, La Plata 1900, Buenos Aires, Argentina.
- Dr. Qutaibeh Katatbeh Department of Mathematics and Statistics, Jordan University of Science and Technology, Irbid, Jordan.
- Dr. Khalid Majid Fazili Department of Biotechnology, Science Block Bldg., University of Kashmir, Srinagar - Hazratbal campus 190 006, Jammu and Kashmir, India.

- Dr. Mahmut DOGAN Erciyes University College of Engineering, Food Engineering Department 38039-Kayseri, Turkey.
- Dr. Ahmed Hassan El-Ghorab Flavors and Aroma Dept. National Research Center, Tahrir St. Dokki, Cairo, Egypt.
- Dr. Madhuban Gopal National Fellow, Division of Agricultural Chemicals, IARI, New Delhi-110012, India, Tel: 91-11-25848706
- Dr. Shaban Sharaf El-Deen Head of Microbial Genetics Dept. National Research Centre, Cairo, Egypt.
- Dr. Fernando Castaño Faculty of Agronomy-UNMdP, UIB - Unidad Integrada Balcarce, PO Box 276, B 7620 BKL Balcarce, Argentina.
- Dr. Athina Geronikaki Athina Geronikaki Aristotelian University of Thessaloniki, Greece.
- Dr. N.K. Gupta Department of Plant Physiology, Rajasthan Agricultural University, SKN College of Agriculture, Jobner 303 329, India.
- Dr. Anil Vyas Department Of Botany, J.N.V. University, Jodhpur-342005, Rajasthan, India.
- Dr. Gwendolyne Peraza Mercado Camino a Escudero 2251-1012, Cd. Juárez, Chihuahua, 32545, 11041 Sunshine Ct., El Paso, TX, 79936, PO Box 26574, El Paso, TX, 79926
- Dr. Hassan Salehi Department of Horticulture Science, College of Agriculture, Shiraz University, Shiraz, Iran.
- Dr. Siham Abdul Muhsen Al-Kadeeb Girls College of Education in Riyadh, Scientific Department, P.O. Box 100683, Riyadh, 11645, Saudi Arabia.
- Dr. Fahrettin Tilki Artvin Coruh University, Faculty of Forestry, 08000-Artvin, Turkey.
- Dr. Mohammed Taleb. Akidat Department of Statistics, Yarmouk University, Irbid, Jordan.
- Dr. Hoda HM Fadel Chemistry of Flavour and Aroma Dept., National Research Center, Dokki, Cairo, Egypt.
- Dr. Ahmed Musa Chair, Department of Computer Engineering, Al-Hussein Bin Talal University, P.O. Box 20 Maan, Jordan.
- Dr. Ahmed Fawaz Al-Jamel Chair, Department of Physics, Al-Hussein Bin Talal University P.O. Box 20 Maan, Jordan.
- Dr. Faisal Ababneh Dept. of Maths & Stats, Al-Hussein Bin Talal University, Jordan.
- Dr. Mohsen Jahanshahi Head of Nanobiotech. Research Lab., Faculty of Chemical Engineering, Babol University of Technology, P.O. Box: 484, Babol, Iran.
- Dr. Senthilkumar Sivaprakasam Research Associate, Chemical Engineering Department, Central Leather Research Institute, Adyar, Chennai -20, India,
- Dr. Kamal Ghasemi Bezd Biotechnology Res. Fellow, Cotton Res. Inst., Iran, Beheshti St, 49175-483, Gorgan, Iran.
- Dr. Han Dai Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, 250 Longwood Ave., SGM. 123 / Boston, MA 02115
- Dr. Savita Chaurasia Department of Biotechnology, IILM Academy of Higher Learning, 18, Knowledge Park II, Greater Noida 201306.
- Dr. Abdelouaheb DJILANI City Rym, 350 logts, appt. N°35, G.S. 4; Annaba.
- Dr. Mostofi Sarkari Agricultural Engineering Research Institute, Kara Ilran.
- Dr. Selim Zedan Heneidy Professor of Applied Ecology & Flora, Department of Botany and Microbiology, Faculty of Science, Moharam Bey 21511, Alexandria University, Alexandria, Egypt.
- Dr. Mehlika Benli Ankara University, Faculty of Science, Department of Biology, 06100, Ankara, Turkey.
- Dr. Ing Agr Miriam E. ARENA Laboratorio de Recursos Agronómicos, Centro Austral de Investigaciones Científicas (CADIC-CONICET) B. Houssay 200 (9410) Ushuaia, Tierra del Fuego.
- Dr. Nisha Mary Joseph P.O. BOX - 3142 School of pharmacy College of health sciences Mekelle University, Mekelle, Ethiopia.
- Dr. Shafiqur Rahman Scientific Officer, Bangladesh Fisheries Research Institute, Establishment of Shrimp Research Center in Bagerhat District Project Bagerhat- 9300, Bangladesh.
- Dr. ABDEL-AZIZ ALI FAYED Botany Department, Faculty of Science, Assiut University, Assiut, Egypt.
- Dr. MOHAMMAD MAHMOUD EFFAT ABDEL Veterinary division building, Microbiology and Immunology dept., National Research Center, Ministry of higher education and scientific research, EL-Tahrir st., Dokki, Cairo, Egypt.
- Professor Viroj Wiwanitkit, M.D. Wiwanitkit House, Bangkok, Bangkok Thailand/ Professor of Tropical Medicine, Visiting Professor, Hainan Medical College, Hainan China.
- Dr. Monir M. EL-Husseini Professor of Biological Control, Faculty of Agriculture, Cairo University, Egypt.
- Dr. (Mrs.) USHA DIXIT SCIENTIST 'C' SEED DIVISION DEPARTMENT OF SCIENCE & TECHNOLOGY TECHNOLOGY BHAWAN NEW MEHRAULI ROAD NEW DELHI-110016, India.
- Dr. Sunita Dashrath Bansod Rajeshree Shahu College, Dept. Biotechnology, Latur, Maharashtra, India
- Dr. Pronobesh Chattopadhyay College of Pharmacy, IFTM Lodhipur Rajput Moradabad- 244001, U.P, India.

Contact Us

-  Editor in Chief - Dr. Abdel Rahman Tawaha
-  Address: Amman-Jordan
-  +962-795016606
-  Aensieditor@Gmail.Com (mailto:Aensieditor@Gmail.Com)
-  Jasaeditor@Gmail.Com (mailto:Jasaeditor@Gmail.Com)
-  abdeltawaha74@gmail.com (mailto:abdeltawaha74@gmail.com)



[| Home](#) | [Journals](#) | [Special Issues](#) | [Conferences](#) | [Contact us](#) |

ANAS Volume 8, Number 8: July, 2014

Analysis of International Marketing Strategy among Malaysian Exporters

Saad Dubayyan Alshammari and Rabiul Islam

1-13

Investigating Task-Technology Fit and Peer Acceptance on Usage and Performance of Collaborative Systems for Research

Halimi Zakaria and Associate Professor Dr Norzaidi Mohd Daud

14-24

The Relationship between HbA1c, Insulin Resistance and Changes of Insulin Secretion in Indonesian Type 2 Diabetic Subjects

Srihardyastutie, A, D.W.Soeatmadji, Fatchiyah and Aulanni'am

25-30

Diagnostic Analysis of National Parks in Georgia in context of Ecotourism

Tamari Poladashvili, Dr., António Cardoso, Ph.D., Álvaro Cairrão, Ph.D.

31-41

Hypoxic Preconditioning For Viable and Self Renewing Mesenchymal Stem Cells (Mscs) As the Regeneration of Spermatogenesis Process

Erma Safitri, Suzanita Utama, Candra Bumi, Sri Wigati Mardi Mulyani, Endang Retnowati, Purwati, R. Heru Prasetyo, Mas'ud Hariadi, Aulani'am, Ferdiansyah Mahyudin, Fedik Abdul Rantam

42-46

The Relationships among Service Quality, Customer Satisfaction and Brand Advocates in the Context of Fine Dining Restaurant Services

Mee, L.Y., Ariffin, A.A.M., Rahman, M.R.Z.A.

47-52

Effective Battery Management Controller with FIC Based Charging Controller for Solar-Diesel Hybrid Power System

P. Raju and Dr.S. Vijayan,

53-61

Methanolic Extract Of Luffa Cylindrica Fruits Show Antihyperglycemic Potential In Swiss Albino Mice

Fahima Akther, Ashif Rahman, Jajiratul Jannat Proma, Md. Zahirul Kabir, Prashanta Kumer Paul, Mohammed Rahmatullah

62-65

Effect of Allium Sativum Leaf Extracts on Glucose Tolerance in Glucose-Induced Hyperglycemic Mice

Diponcor Ghosh, Indrani Mandal, Jannatul Ferdous Rumi, Ummay Kawchur Trisha, Humayra Jannat, Mousumi Ahmed, Mohammed Rahmatullah

66-69

Antihyperglycemic and Antinociceptive Activity Evaluation of Methanolic Extract of *Trichosanthes Anguina* Fruits in Swiss Albino Mice

Mahfuza Akter, Israt Zebin Mitu, Jajiratul Jannat Proma, Sk. Mizanur Rahman, Md. Rashedul Islam, Shahnaz Rahman, Mohammed Rahmatullah

70-74

The Causality between Energy Consumption, Co2 Emissions and Economic Growth in Nigeria: An Application of Toda and Yamamoto Procedure

Chindo Sulaiman

75-81

The Characteristics of Shiraz School of Architecture; A Study on the Architecture of Fars in 13th to 15th Centuries

Kazem Me'mar Zia, and Hossein Abhar

82-100

Immunogenic Protein from Salivary Gland of *Aedes aegypti* Against to Human Sera

Rike Oktarianti, Kartika Senjarini, Fatchiyah Fatchiyah, Aulanni'am

101-107

Moderated Mediation Using Covariance-Based Structural Equation Modeling with Amos Graphic: Volunteerism Program

Asyraf Afthanorhan, Sabri Ahmad, Shuhaili Safee

108-115

Vehicular Traffic Re-Routing For Avoiding Traffic Congestion in VANET

N. Kirthiga, M. Dhivyashree, Dr. S. Karthik, Dr. K. Srihari

116-120

Antecedents of Service Quality in the Insurance Industry

Alawni Mohammed Saad, Rushami Zien Yusoff and Rabiul Islam

121-130

Phytochemical study of *Anacyclus pyrethrum* (L.) of Middle Atlas (Morocco), and *in vitro* study of antibacterial activity of pyrethrum

Hanane Elazzouzi, Aminata Soro, Fatima Elhilali, Amar Bentayeb, Mohamed Alaoui El Belghiti, Touriya Zair

131-140

Enzymatic and Functional Properties of Lactic Acid Bacteria Isolated From Algerian Fermented Milk products

Mechai Abdelbasset, Debabza Manel, Menasria Taha and Kirane Djamilia

141-150

Effect of the Antecedent Factors on the Degree of Adaptation the Marketing Mix in the Malaysian Export Companies

Saad Dubayyan Alshammari and Rabiul Islam

151-162

Oral Glucose Tolerance test (OGTT) with three Common Spices of Bangladesh: Onion, Garlic and Ginger

Erina Islam Erin, Helal Uddin Sumon, Jahan Islam, Md. Azizur Rahman, Wasifa Ferdous Disharee, Mohammed Rahmatullah

163-168

Antinociceptive activity Studies with Methanol Extract of Onion, Garlic and Ginger in Mice

Farah Farjana, Nazmus Sakib, Md. Minto Hasan, Poulomi Das, Ahamed Ismail Hossain, Mohammed Rahmatullah

169-174

The Use of Biochar Fortified Compost on Calcareous Soil of East Nusa Tenggara, Indonesia: 1. Evolution of organic matter and nitrogen on composting of farm yard manure (FYM) and Siam weed (*Chromolaena odorata* L.) biomass added with BIOCHAR as a bulking agent.

M.S.M. Nur, W.H. Utomo, E. Handayanto, W.H. Nugroho and T. Islami

175-182

Predicting Financial Stress and Earning Management Using Ratio Analysis

Normah Omar, Zuraidah Mohd Sanusi, Zulaikha, Amirah Johari, Intan Salwani Mohamed

183-189

Auxiliary Stabilizer of STATCOM based on the State Feedback Theory

Reza Saki, Somayeh Yarahmadi, Faranak Nikabadi, Vahid Chegeni

190-195

Power System Stabilizer Design based on the Full State Feedback Method

Reza Saki, Somayeh Yarahmadi, Faranak Nikabadi, Vahid Chegeni

196-200



AENSI Journals

Advances in Natural and Applied Sciences

ISSN:1995-0772 EISSN: 1998-1090

Journal home page: www.aensiweb.com/ANAS



Hypoxic Preconditioning For Viable and Self Renewing Mesenchymal Stem Cells (Mscs) As the Regeneration of Spermatogenesis Process

^{1,2}Erma Safitri, ¹Suzanita Utama, ^{2,3}Candra Bumi, ^{2,4}Sri Wigati Mardi Mulyani, ⁵Endang Retnowati, ^{2,6}Purwati, ⁷R. Heru Prasetyo, ¹Mas'ud Hariadi, ⁸Aulani'am, ^{2,9}Ferdiansyah Mahyudin, ^{2,10}Fedik Abdul Rantam

¹ Department of Veterinary Reproduction Faculty of Veterinary Medicine Airlangga University

² Stem Cells Research Division of Institute Tropical Disease (ITD), Airlangga University

³ Department of Epidemiology and Biostatistics Public Health, Jember University

⁴ Department of DentoMaxillo Facial Radiology, Faculty of Dentistry, Airlangga University

⁵ Department of Clinical Pathology, Faculty of Medicine, Airlangga University

⁶ Department of Internal Medicine, RSUD Dr. Soetomo Surabaya

⁷ Departement of Parasitology, Faculty of Medicine, Airlangga University

⁸ Department of Biochemistry Faculty of Life Sciences, Brawijaya University

⁹ Departemen of Orthopedic & Traumatology RSUD Dr. Soetomo Surabaya

¹⁰ Department of Vet. Microbiology and Virology Faculty of Vet. Med, Airlangga University

ARTICLE INFO

Article history:

Received 2 April 2014

Received in revised form

13 May 2014

Accepted 28 May 2014

Available online 27 June 2014

Keywords:

mesenchymal stem cells, hypoxic preconditioning, viable, self renewal

ABSTRACT

The aim of this research was to obtain MSCs that were viable and self renewing for spermatogenesis process by a treatment of hypoxic precondition in vitro culture. In this research, hypoxic precondition was the use of 1% O₂ concentration which was compared to those of culture under normoxic (21% O₂) condition. Flowcytometric analysis showed that in MSCs culture under 1% O₂ concentration, the level of CD90⁺ and CD34⁻ were not altered (remained undifferentiated), meanwhile under 21% O₂ concentration, cells have experienced alteration (became differentiated), that was indicated by the down regulation of CD90⁺ and up regulation of CD34⁻. Immunocytochemical and immunofluorescence analysis showed that under 1% O₂ concentration, MSCs culture expressed transcription factors, such as OCT4 and SOX2, meanwhile under 21% O₂ concentration, the transcription factors OCT4 and SOX2 (self renewal function), were not expressed. In conclusion, this research showed that hypoxic preconditioning with 1% O₂ concentration very supported MSCs to remain viable before transplantation for spermatogenesis disorder, because the cells still undifferentiated and self renewal capacity was maintained.

© 2014 AENSI Publisher All rights reserved.

To Cite This Article: Erma Safitri, Suzanita Utama, Candra Bumi, Sri Wigati Mardi Mulyani, Endang Retnowati, Purwati, R. Heru Prasetyo, Mas'ud Hariadi, Aulani'am, Ferdiansyah Mahyudin, Fedik Abdul Rantam., Hypoxic Preconditioning For Viable and Self Renewing Mesenchymal Stem Cells (Mscs) As the Regeneration of Spermatogenesis Process. *Adv. in Nat. Appl. Sci.*, 8(8): 42-46, 2014

INTRODUCTION

Cell transplantation therapy of mesenchymal stem cells (MSCs) from bone marrow provides a very promising solution for the regeneration of spermatogenesis process in oligospermic patient (Kilani, 2009). However, the low viability of the transplanted MSCs for the regeneration of normal testis function to produce spermatozoa caused the limitation of efficacy of this therapy (Tang *et al.*, 2005; Kenichiro *et al.*, 2005). Studies on stem cell by Suzuki (2004), Geng (2003) and their co-workers revealed that 93-99 % of the stem cells injected died three to four days after injection which indicated that microenvironment in the degenerative tissue or body organs of patients were not conducive for the viability of the stem cells. The estimated mechanism accounted for the decreasing survivability of stem cells was the high amount of the stem cells underwent differentiation and senescence (not self renewing) prior to transplantation to patients. Therefore the retainment of undifferentiated state and self renewal capacity of MSCs before implantation were very important for stem cells' viability and subsequently the efficacy of stem cell therapy.

Therefore, preconditioning stem cells with hypoxia (1% O₂) during in vitro culture, which is an adjustment to the in vivo niche of the stem cells, need to be conducted in an attempt to increase viability after transplantation to oligospermic patient.

Corresponding Author: Erma Safitri, Department of Veterinary Reproduction Faculty of Veterinary Medicine Airlangga University
E-mail: rma_fispro@yahoo.com

Research Methods:

Procedure of rabbit MSCs isolation and culture:

Rabbit was premedicated and general anesthetized. MSCs from bone marrow was harvested by an aspiration at the middle of femur bone below the condylus. Aspirate contained MSCs from bone marrow was placed in heparinized tubes. Sample in tube was placed in thermos maintained at 4°C during transportation to be processed in stem cell laboratory.

Sample was transferred into 15 ml sterile blue capped tubes and then the tube was rinsed twice with 5 ml sterile Phosphate Buffered Saline (PBS). PBS was added up to a total volume of 10 ml. The diluted sample was loaded over a same volume of Ficoll in a separate 15 ml tube. Centrifugation at 1600 rpm was performed for 15 minutes at room temperature. After centrifugation, the cells were collected from Ficoll-PBS interface using sterile pasteur pipette and transferred into a 15 ml tube. The cells were then resuspended in PBS up to a total volume of 15 ml. The tube was inverted gently 5 times to homogenize the suspension. The suspension was then centrifuged again at 1600 rpm for 10 minutes. Supernatant and floating cells were discarded and cell pellet was resuspended in 6 ml of α MEM media. Mononucleated cells were plated in 10 cm² plates at 2.10^7 and incubated at 37°C in a humidified atmosphere containing 5% CO₂ for 24 hours to let the cells adhere. After 24 hours, media and non-adherent cells were discarded. Adherent cells were rinsed twice using 5 ml of PBS. Ten ml of fresh α MEM media was then added into dish and the dish was returned into the incubator. Culture was observed daily under an inverted microscope. Every 4 days medium was changed, preceded by a rinse using 10 ml PBS then 10 ml of fresh α MEM media were replaced. Culture was continued until approximately 75-80% confluence was attained. After confluence, cells were passaged into several dishes for subculture (Rantam *et al.*, 2009). Passage was conducted 3 times, then cells were divided into two hypoxic precondition treatments of 1% in hypoxic chamber inside a 5% CO₂ incubator while another treatment was the use of 21% oxygen (normoxia).

At the fourth day after hypoxic precondition treatments cells were analysed for the expression of surface marker CD90 and CD34 by flowcytometry, and for transcription factors OCT4 and SOX2 by immunocytochemistry and immunofluorescence microscopy. Flow cytometric analysis was performed using a flowcytometer (FACSCalibur) and CellQuest software. Antibodies used were monoclonal FITC-conjugated anti-rabbit CD90 (Biossusa) and PE-conjugated anti-rabbit CD34 (BD). Antibodies used for immunocytochemical and immunofluorescence analysis were polyclonal FITC-conjugated anti-rabbit OCT4/POU5F1 (BioLegend) and polyclonal FITC-conjugated anti-rabbit SOX2 (BioLegend).

Research Results and Discussion:

Flowcytometric analysis showed that under 1% O₂ concentration, the level of CD90⁺ and CD34⁻ cells in MSCs culture were not altered (still undifferentiated). Meanwhile under 21% O₂ concentration, cells have experienced alteration (became differentiated), that was indicated by the decrease of CD90⁺ and the increase of CD34⁻ cells (Figure 1 - 3).

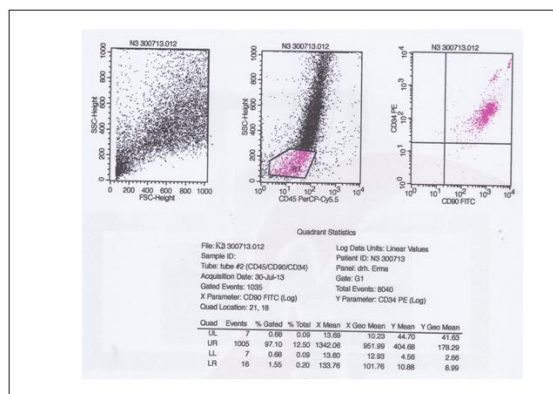


Fig. 1: Flowcytometric analysis of MSCs culture before hypoxic preconditioning (Control) showing positive expression of CD90 (98.66%), and negative expression of CD34 and CD45 (0.68% and 0.68% respectively).

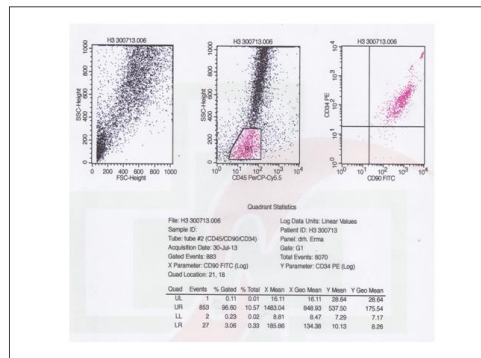


Fig. 2: Flowcytometric analysis of MSCs culture under hypoxic precondition (1% O₂ concentration) showing positive expression of CD90 (99.66%) and negative expression of CD34 and CD45 (0.11% and 0.23%).

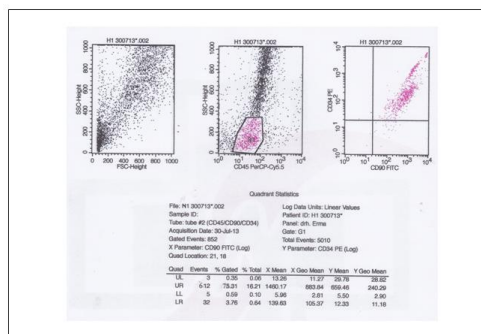


Fig. 3: Flowcytometric analysis of MSCs culture under 21% O₂ concentration (normoxia) showing positive expression of CD90 (79.07%) and negative expression of CD34 and CD45 (0.35% and 0.59%).

Immunocytochemical staining showed the expression of transcription factor (self renewal function) such as OCT4 and SOX2 in the MSCs culture. Meanwhile under 21% O₂ concentration, transcription factor OCT4 and SOX2 were not expressed (undetected by immunocytochemistry and immunofluorescence staining) (Figure 4 - 7).

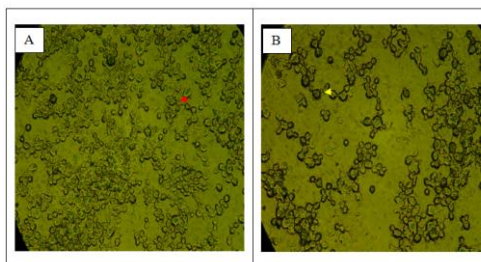


Fig. 4: Immunocytochemical analysis of MSCs culture under hypoxic precondition (1% O₂ concentration). **A.** Positive expression of OCT4 (red arrow head). **B.** Positive expression of SOX2 (yellow arrow head).

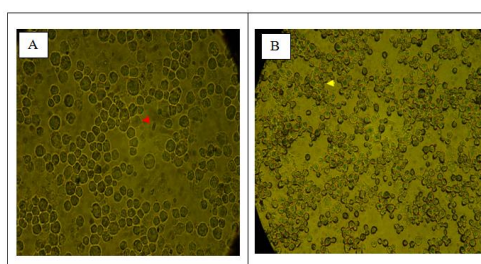


Fig. 5: Immunocytochemical analysis of MSCs culture under 21% O₂ concentration (normoxia). **A.** Negative expression of OCT4 (red arrow head). **B.** Negative expression of SOX2 (yellow arrow head).

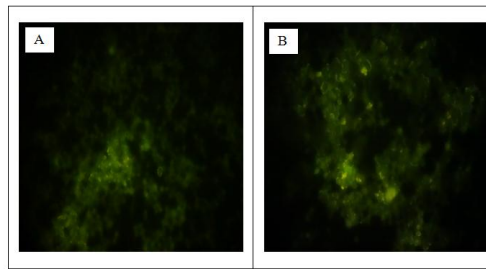


Fig. 6: Immunofluorescence analysis of MSCs culture under hypoxic precondition (1% O₂ concentration). **A.** Positive expression of OCT4 showing green fluorescence. **B.** Positive expression of OCT4 showing green fluorescence.

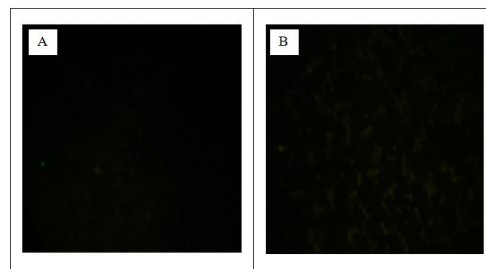


Fig. 7: Immunofluorescence analysis of MSCs culture under normoxic (21% O₂) condition. **A.** Negative expression of OCT4. **B.** Negative expression of SOX2.

Based on the results of this research, it could be explained that hypoxic precondition of 1% O₂ concentration caused the inhibition of Prolyl hydroxylases (PHDs) enzyme expression which caused the formation of Hypoxia Inducible Factor-1 (HIF-1) complex. This HIF-1 complex caused cell cycle arrest gene expression. Furthermore, 48 hours after cell cycle arrest genes expression, caused the expression of transcription factor (pluripotency genes) like OCT4, SOX2, NANOG and REX-1. Transcription factor is a component that is capable to stimulate stem cells to proliferate to be themselves (self renewal), therefore these cells are always young and do not undergo senescence (aging).

Transcription factor which is the pluripotency genes (capable to differentiate into whatever cells needed by the damaged body) was estimated to be activated by Hypoxia Inducible Factor 2 α (HIF 2 α) after the initiation by Hypoxia Inducible Factor 1 α (HIF 1 α). The pluripotency genes which were observable through the expression of transcription factors such as OCT4 dan SOX2 in this research could influence MSCs to be always self renewing themselves.

Therefore hypoxic preconditioning using 1% O₂ concentration for MSCs culture can support to maintain viability and self renewal potency until transplantation to oligospermic patient with spermatogenesis disorder. This is caused by the remain undifferentiated and self renewing stem cells.

Hypoxic preconditioning using 1% O₂ concentration caused HIF-1 α release from van Houpel Lindau (vPL) which was then accumulated in nucleus. The high level of HIF-1 α would inhibit Reactive Oxygen Species (ROS) that acted as free radical. The inhibition of ROS would inhibit the expression of protein genes P53 and P21. Therefore, cell cycle arrest genes were sensitized, which ended up with the slow proliferation and maintenance of stem cells. This maintenance was also supported by the reduced ROS by the role of HIF-1 α therefore p53 gene expression was inhibited. The inhibition of p53 gene expression caused an inhibition of the opening of mitochondrial membrane pt pore. Therefore, cytochrome C that acted as apoptotic protease activating factor-1 (APAF-1) caused inhibition of the release of various caspases (Caspase 9 and Caspase 3) as apoptotic cascade. The inhibition of P53, cytochrome C and caspases would cause the inhibition of cell death of the cultured stem cells. Meanwhile, the decreased P21 caused an inhibition of the active cycling cell which prevented cell senescence process from happening.

Conclusion:

From the research results, it could be concluded that hypoxic preconditioning with 1% O₂ concentration was very supportive to MSCs in maintaining cells viability before transplantation for spermatogenesis disorder therapy, because cells remained undifferentiated and had the potency for self renewal.

REFERENCES

- Cavallini, G., 2006. Male idiopathic oligoasthenoteratozoospermia. *Asian J Androl*, 8: 143-157.
- Geng, Y.J., 2003. Molecular Mechanisms for Cardiovascular Stem Cell Apoptosis and Growth in the Hearts with Atherosclerotic Coronary Disease and Ischemic Heart Failure. *Science. Ann NY Acad*, 1010: 687-697.
- Gadomska, I.S., V. Zayat, L. Buzanska, 2011. Influence of Low Oxygen Tension on Expression of Pluripotency Genes in Stem Cells. *Acta Neurobiol Exp*, 71: 86-93.
- Hamanaka, R.B. and N.S. Chandel, 2010. Review. Mitochondrial Reactive Oxygen Species Regulate Cellular Signaling and Dictate Biological Outcomes. *Trends in Biochemical Sciences*, 35: 505-513.
- He, S., D. Nakada and S.J. Morrison, 2009^a. Mechanisms of Stem Cell Self-Renewal. *Annu Rev Cell Dev Biol*, 25: 377-406.
- Kolf, C.M., E. Cho, R.S. Tuan, 2007. Review Mesenchymal Stromal. Biology of Adult Mesenchymal Stem Cells : Regulation of Niche, Self-Renewal and Differentiation. *Arthritis Research & Therapy*. 9:204 (doi:10.1186/ar2116). Available online <http://arthritis-research.com/content/9/1/204>.
- Rantam, F.A., Ferdiansyah, Nasronudin and Purwati, 2009. Stem Cell Exploration. Methods of Isolation and Culture. First Ed. Airlangga University Press. Surabaya.
- Suda, T., K. Takubo and G.L. Semenza, 2011. Cell Stem Cell Review. Metabolic Regulation of Hematopoietic Stem Cells in Hypoxic Niche. *Cell Stem Cell*, 9: 296-310.
- Suzuki, K., B. Murtuza and J.R. Beauchamp, 2004. Dynamics and Mediators of Acute Graft Attrition After Myoblast Transplantation to the Heart. *FASEB J.*, 18:1153-1155.
- Takubo, K., N. Goda, W. Yamada, H. Iriuchishima, E. Ikeda, Y. Kubota, S. Haruko, R.S. Johnson, A. Hirao, M. Suematsu and T. Suda, 2010. Regulation of the HIF-1 Level is Essential for Hematopoietic Stem Cells. *Cell Stem Cell*, 7: 391-402.
- Yamanaka, S., 2007. Strategies and New Developments in the Generation of Patient-Specific Pluripotent Stem Cells. *Cell Stem Cell Review*. 1 July 2007 @2007 Elsevier Inc. DOI 10.1016/j.stem.2007.05.012.