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RAMMOHAN COLLEGE

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E-mail : rmc.tic85b@yahoo.in, rmc.principal@gmail.com

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Date.....20

Collaboration with Sports Authority of India,
Kolkata
and
Sports and Exercise Physiology Laboratory,
Department of Physiology, University of Calcutta



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Deputy Registrar (Acting)
University of Calcutta



University of Calcutta

Senate House
87/1, College Street
Kolkata - 700 073
Phone : +91-33-2241-0071/4984
Fax : +91-33-2241-3222/88
eMail : phdcaluni@yahoo.co.in

Letter: 10232/Ph.D.(Sc.)

Dated: 30.Dec.2022

TO WHOM IT MAY CONCERN



This is to certify that **Sri Surojit Sarkar** of 57/1, Shri Dhar Roy Road; **Kolkata-700039** bearing Aadhaar card no. **2856 1791 1882** has submitted a thesis entitled

“Effect Of Antioxidant Vitamin Supplementation On High-Intensity Training Induced Alteration In Muscle Damage, Oxidative Stress And Fitness Profile Parameters In Post-Adolescent Male Endurance Athletes.”

on **28th December 2022** under **Ph.D. Regulations 2016** of University of Calcutta for consideration of the University for award of the Ph.D. degree in **Physiology**

Name of Supervisor: **Dr. Gouriprosad Datta, Associate Professor, Rammohan College, Kolkata.**
Name of Joint Supervisor: **Dr. Swapan Kumar Dey, Consultant Scientist, Sports Authority Of India, Kolkata.**
Name of Associate Supervisor: **Dr. Amit Bandyopadhyay, Assistant Professor, C.U.**

S. Das
30.12.22
Deputy Registrar (Acting)
②



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Collaboration with Sports and Excercise Physiology Laboratory, Department of Physiology, University of Calcutta



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Kolkata - 700 073
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eMail : phdcaluni@yahoo.co.in

Dated: 21st April 2022

Letter: 02712/Ph.D.(Sc.)Pro

To
Dr. Gouriprosad Datta
Dept. of Physiology,
Rammohan College,
102/1, Raja Rammohan Sarani, Kolkata-700009.
eMail: dattagp@yahoo.co.in

Subject : Inclusion of Joint Supervisor

Dear Sir / Madam,
This is in reference to your letter dated 2nd February 2022, regarding Inclusion of Joint Supervisor for the Ph.D. programme in "Physiology", being carried out by Smt Pritha Roy.

In this connection, I am desired to inform you that Dr. Amit Bandyopadhyay has been appointed Joint Supervisor for the said Ph.D. programme under your supervisorship.

Yours faithfully,

Sd/-

Deputy Registrar (Acting)

Letter No: 02713/Ph.D.(Sc.)Pro dated 21st April 2022

Copy forwarded to: Dr. Amit Bandyopadhyay
Dept. of Physiology, C.U; 92, A.P.C.Road, Kolkata-700009; eMail:
bamit74@gmail.com; bamit74@yahoo.co.in.

Sd/-

Deputy Registrar (Acting)

Letter No: 02714/Ph.D.(Sc.)Pro dated 21st April 2022

Copy forwarded to: **Smt Pritha Roy**
Krishna Apt., Block-B, Flat No.-203; 17/5, Ramcharan Sett Road, Ramrajatala;
Howrah-11104.

Deputy Registrar (Acting)



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Date.....20



University of Calcutta
Senate House, Kolkata - 700073

Date of Enrollment : 13th August 2019

Registration Number : 07435/Ph.D.(Sc.)Proceed/2021

Date of Registration : 24th November 2021

Date of Letter : 8th December 2021

(Please quote the above Number and Date in all future Correspondence)

From:

Deputy Registrar (Acting)
University of Calcutta

To:

Smt Pritha Roy
Krishna Apt., Block-B, Flat No.-203,
17/5, Ramcharan Sett Road, Ramrajatala,
Howrah-11104.



Madam,

I am desired to inform you that you have been granted registration for the Ph.D. programme under this University in **Physiology** in terms of **6.6** of the Regulations for the Degree of Doctor of Philosophy (Ph.D.), C.U., framed under UGC Guidelines, **2016**.

This registration shall remain valid for next six years with effect from the date of enrolment as indicated above.

You are to comply with the usual rules of migration in case you have passed the qualifying examinations for the Ph.D. programme from a University/Institute other than the University of Calcutta.

Title of Thesis

"Comparative Study Of Orphan And Non-Orphan Children: A Socio-Physiological And Nutritional Approach."

Name of the Supervisor : Dr. Gouriprosad Datta

Name of the Joint Supervisor : X

Name of the Associate Supervisor : X

Yours faithfully,

Deputy Registrar (Acting)
Deputy Registrar (Acting)
University of Calcutta

N.B. Please see the instructions overleaf.



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Date.....20

Collaboration with Department of Physiology,
Raja Narendra Lal Khan Women's College,
Medinipur, West Bengal
and
Sports and Excercise Physiology Laboratory,
Department of Physiology, University of Calcutta



S Sanyal
Principal
Rammohan College
Kolkata-9



श्रद्धावान् लभते ज्ञानम्

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Date.....20


University of Calcutta
Senate House, Kolkata - 700073

Date of Enrollment : **25th August 2017**
Registration Number : **01880/Ph.D.(Sc.)Proceed/2019**
Date of Registration : **12th March 2019** Date of Letter : **20th March 2019**
(Please quote the above Number and Date in all future Correspondence)

From:
Dy. The Registrar (Actg.),
University of Calcutta

To:
Smt Mousumi Mitra
A/2, Safal Bhaban,
Mithumasjid Road, Habibpur,
Paschim Medinipur, Pin-721101.

Madam,

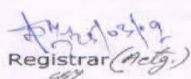
I am desired to inform you that you have been granted registration for the Ph.D. programme under this University in **Physiology** in terms of **6.6** of the Regulations for the Degree of Doctor of Philosophy (Ph.D.), C.U., framed under UGC Guidelines, **2016**.

This registration shall remain valid for next six years with effect from the date of enrolment as indicated above.

You are to comply with the usual rules of migration in case you have passed the qualifying examinations for the Ph.D. programme from a University/Institute other than the University of Calcutta.

Title of Thesis
Green Synthesis Of Gold Nanoparticles Using Bark Extract Of *Terminalia arjuna* And Its Protection Against Hepato-Renal Dysfunctions On Experimentally Induced Rats.

Name of the Supervisor : **Dr. Gouriprosad Datta**
Name of the Joint Supervisor : **Dr. Dilip Kumar Nandi**
Name of the Associate Supervisor : **Dr. Amit Bandyopadhyay**

Yours faithfully,

Dy. Registrar (Actg.)

N.B. Please see the instructions overleaf.



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बसु विज्ञान मंदिर
BOSE INSTITUTE

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(An Autonomous Institute of Department of Science & Technology, Govt. of India)

मुख्य कैंपस / Main Campus :

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93/1, Acharya Prafulla Chandra Road, Kolkata-700 009
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फैक्स / Fax : 91-33-2350-6790

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P-1/12, C.I.T. Scheme VII-M, Kolkata-700054
फोन / Phone : 2355-7434 (निदेशक / Director), 2355-0595 (रजिस्ट्रार / Registrar)
इपीएबीएक्स / EPABX : 2355-9416/9219/9544, 2569-3271, फैक्स / Fax : 91-33-2355-3886

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फोन / Phone : 2355-7434 (निदेशक / Director)
इपीएबीएक्स / EPABX : 2569-3123/28, फैक्स / Fax : 91-33-2569-3127

संदर्भ सं. / Ref. No. _____

दिनांक / Date : 30.10.2023

To whom it may concern

This is to declare that I, Dr. Kuladip Jana, Principal Scientist, Division of Molecular Medicine, Bose Institute, has acted as Joint Supervisor in research collaboration with Dr. Gouriprosad Datta, Associate Professor, Dept. of Physiology, Rammohan College for the Ph.D. program of Ms. Moumita Das (Reg No. 8646/Ph.D.(Sc.)Proceed/2014) on the thesis entitled "Nutritional Profiling and Pharmacognostic Evaluation of Green Capsicum (Capsicum annum L.) against Alcohol Induced Oxidative Stress".

Kuladip Jana
30.10.2023

डॉ. कुलादीप जना, एम एस पीएच डी / Dr. Kuladip Jana, M.S., Ph.D.
प्रधानाचार्य वैश्वविज्ञानिक / Principal Scientist
आणविक औषधि, बसु विज्ञान मंदिर / Div. of Molecular Medicine
बसु विज्ञान मंदिर / Bose Institute
कोलकाता / Kolkata
भारत सरकार विज्ञान एवं प्रौद्योगिकी विभाग
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Date.....20

Collaboration with Department of Physiology, University of Calcutta, Kolkata

UNIVERSITY OF CALCUTTA
Department of Physiology

Dr. Amit Bandyopadhyay M.Sc., Ph.D., FICN, FPSI
Assistant Professor

University Colleges of Science and Technology
92 A.P.C. Road, Kolkata: 700009, West Bengal, India.



Phone : +91 33 23508386/6396 (Extn. 317)
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bamit74@gmail.com

Residence:
Flat No. 3, First Floor, Urmila Apartment
C-51/2, Brahmapur More, Kolkata: 700096, India.

Date: 20th October 2023.

To whom it may concern

This is to declare that I, Dr. Amit Bandyopadhyay, Assistant Professor, Sports and Exercise Physiology Laboratory, Department of Physiology, University of Calcutta, have acted as an Associate Supervisor in research collaboration with Dr. Gouriprosad Datta, Associate Professor, Dept. of Physiology, Rammohan College for the Ph.D. programme of Mr. Surojit Sarkar, Ms. Monalisa Debnath, and Ms. Mousumi Dutta .

Also, I am presently associated as a Joint Supervisor in research collaboration with Dr. Gouriprosad Datta, Associate Professor, Dept. of Physiology, Rammohan College for the Ph.D. programme of Ms. Pritha Roy (Reg No. 07435/Ph.D.(Sc.)Proceed/2021), on the thesis entitled "Comparative Study of Orphan and Non-Orphan Children: A Socio-Physiological and Nutritional Approach"

(Amit Bandyopadhyay)



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Gope Palace, Midnapore, Dist. - Paschim Medinipur, PIN - 721102 (W.B.)

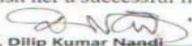
Ref. 1421/Fv.D...MM/2021 Date 11/01/2021


TO WHOM IT MAY CONCERN

It is my delight pleasure to give my utmost possible recommendation for **Mousumi Mitra**, D/O Shri Subrata Mitra and Smt. Monika Mitra, will submit her final copy of thesis in next few days under our Guidance. She had successfully completed her pre-submission seminar of Ph.D work in September 2020. The title of her Thesis is "**Green synthesis of gold nanoparticles using bark extract of *Terminalia arjuna* and its protection against hepato-renal dysfunctions on experimentally induced rats**". I have gone through her whole thesis which is hereby being approved for submission.

She had also achieved as JRF a major research project under Department of Science and technology, Government of west Bengal, under my supervision. She had finished her work under scheduled time by fulfilling ethical guidelines. She possesses potential ability to read the literature by herself and produce appealing hypothesis with problem solving capabilities. She had published several research articles in different international and national journals. She has brilliant communication skills with clear and concise view. Moreover, she is a disciplined, studious, hard working, reliable, well mannered, person with pleasant personality with good team work skill.

I wish her a successful life ahead.


Dr. Dilip Kumar Nandi
Associate Professor & Head,
Dept. of Human Physiology,
Raja N. L. Khan Women's College (Autonomous), Midnapore, West Bengal,
Residential Address: Barmanikpur, Midnapore, Paschim Medinipur, 721101.
Email: dilipnandi2004@yahoo.co.in Mobile: 9434229882



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Collaboration with Sports Authority of India, Kolkata and Sports and Exercise Physiology Laboratory, Department of Physiology, University of Calcutta



Vitamin C and E supplementation and high intensity interval training induced changes in lipid profile and haematological variables of young males

Surojit Sarkar^a, Swapan Kr Dey^b, Gouriprosad Datta^a, Amit Bandyopadhyay^{c,*}

^a Department of Physiology, Rammoohan College, 102/1, Raja Ram Mohan Sarani, Batihakkhana, Kolkata, 700 009, West Bengal, India

^b Department of Sports Science, University of Calcutta, University Colleges of Science and Technology, 92, A.P.C. Road, Kolkata, 700009, West Bengal, India

^c Sports and Exercise Physiology Laboratory, Department of Physiology, University of Calcutta, University Colleges of Science and Technology, 92, A.P.C. Road, Kolkata, 700009, West Bengal, India

ARTICLE INFO

Keywords:

Sprint interval training
Lipid profile
Haematological indices
Haemolysis
Athletes

ABSTRACT

High intensity interval training (HIIT) causes oxidative stress and haematological alteration. Present study was aimed to evaluate the effect of 8 weeks' supplementation of vitamin C and E on HIIT induced changes in lipid profile parameters and haematological variables. Hundred six male adolescent players were randomly assigned into five age-matched groups, i.e., Control (no exercise + placebo), HIIT (placebo), HIIT + vitamin-C (1 000 mg/day), HIIT + vitamin-E 400 IU/day) and combined HIIT + vitamin C and E. Morning and evening sessions (90 min) of HIIT included 4 phases (15 min each) with 3 sets (4 min each). Each 4 min HIIT set consisted of 2 min intense sprint workout (90%–95% of heart rate maximum [HR_{max}]) followed by 1 min active recovery (60%–70% HR_{max}) followed by 1 min of complete rest (1:1 work-rest ratio). Lipid profile parameters, haematological variables, endurance capacity and vertical jump were evaluated by standard protocols. Significant decrease in body weight, fat%, total cholesterol, triglyceride, Total Cholesterol/High Density Lipoprotein-Cholesterol and significant increase in High Density Lipoprotein-Cholesterol, maximal oxygen consumption, vertical jump were observed for all four intervention groups. White blood cell count, red blood cell count, haemoglobin percentage and haematocrit values were significantly decreased while platelet count and platelet-to-leukocyte ratio (PLR) ratio were increased significantly only for HIIT group. Blood level of tocopherol and ascorbic acid was significantly increased (values were within the normal range) in all the respective vitamin supplemented groups. Supplementation of vitamin C and E secures health protection with suppressed haemolysis and improved inflammatory blood variables with enhanced explosive leg strength and lipid profile parameters without any concomitant change in endurance capacity.

Introduction

High-intensity interval training (HIIT) is a time-efficient strategy and an efficient alternative to traditional endurance training among athletes to develop both the aerobic and anaerobic systems within a short period.¹ But strenuous exercises like eccentric intervals/high-intensity training inflict metabolic and mechanical stress due to the need for excessive energy in a very short time. This higher need for energy increase oxygen consumption leading to the generation of mitochondrial reactive oxygen species (ROS).^{2,3} Studies depict that high-intensity exercises elicit detrimental effects on skeletal muscle^{2,4} and increase circulatory

proinflammatory cytokines (interleukin-6 [IL-6] and tumour necrosis factor-alpha [TNF-α]) in proportion to ROS generation.^{5,6}

High-intensity/eccentric exhaustive training induces oxidative stress and alters the haematological profile by facilitating haemolysis along with a decrease in ferritin, haemoglobin (Hb) content, and haematocrit value (HCT). However, the erythrocyte-related changes occur simultaneously with decreased leukocyte count, increased platelet count, and platelet-to-leukocyte ratio (PLR) due to the effect of HIIT.⁷ Examination of the literature revealed that antioxidant vitamins (e.g., vitamin A, vitamin C and vitamin E) are effective in preventing exercise-induced inflammation-like responses and adverse haemorrhagic changes.^{8,9}

Vitamin C and vitamin E are the most prevalent vitamin supplements

* Corresponding author. Sports and Exercise Physiology Laboratory, Department of Physiology, University of Calcutta, University Colleges of Science and Technology, 92, A.P.C. Road, Kolkata, 700009, India.
E-mail address: abphys@caluniv.ac.in (A. Bandyopadhyay).

<https://doi.org/10.1016/j.smhs.2023.03.006>

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Please cite this article as: Sarkar S et al., Vitamin C and E supplementation and high intensity interval training induced changes in lipid profile and haematological variables of young males, Sports Medicine and Health Science, <https://doi.org/10.1016/j.smhs.2023.03.006>



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ORIGINAL ARTICLE

TRENDS in
Sport Sciences
2023; 30(1): 21-28
ISSN 2299-9590
DOI: 10.23829/TSS.2023.30.1-3

Reference interval for oxidative stress markers in young football and hockey players

SUROJIT SARKAR¹, SWAPAN KUMAR DEY², GOURIPROSAD DATTA¹, AMIT BANDYOPADHYAY³

Abstract

Introduction. Malondialdehyde (MDA), superoxide dismutase (SOD), glutathione (GSH), and glutathione peroxidase (GPx) are widely accepted as biological markers for checking the redox balance and antioxidant status. **Aim of Study.** The purpose of the study was to frame the reference interval for antioxidant variables (MDA, SOD, GSH and GPx) in the young athletic population of various sports discipline. **Material and Methods.** 190 young male players [i.e., football (n = 89), and hockey (n = 101)] were recruited for the study (mean age = 18.3 ± 2.01 yrs). Assay of MDA, SOD, GSH and GPx was done by using the standard enzymatic protocol. Reference interval was calculated by following the Clinical and Laboratory Standard Institute (CLSI) C28-A3 guideline and MedCalc software (version 19) with a 90% confidence interval. **Results.** Serum MDA range was from 23.75-36.19 µmoles/100ml serum with mean of 30.29 ± 3.24 µmoles/100 ml serum and median around 30.43. Serum SOD ranged from 0.05-0.14 U/min/mg protein with mean of 0.08 ± 0.01 U/min/mg protein and median around 0.08. The GSH was ranging from 43.21-55.55 mg/100 ml serum with mean of 46.43 ± 2.11 mg/100 ml serum and median around 46.10. The GPx was ranging from 9.04-14.33 µmol/min/mg protein with mean of 11.35 ± 1.38 µmol/min/mg protein and median around 11.05. **Conclusions.** Present study confers 24.55-35.58 µmoles/100 ml serum, 0.06-0.13 U/min/mg protein, 43.27-51.86 mg/100 ml serum, and 9.07-14.12 µmol/min/mg protein as the reference interval values for MDA, SOD, GSH, and GPx respectively. The present finding will guide the researchers to avoid misinterpretation of antioxidant biomarker values during any phase of competitive training of sports person.

KEYWORDS: lipid peroxidation, glutathione, reference interval, antioxidant biomarkers, endurance team-game.

Received: 30 August 2022
Accepted: 13 March 2023

Corresponding author: bamit74@yahoo.co.in

¹ Rammohan College, Department of Physiology, Kolkata, India
² University of Calcutta, Department of Sports Science, Kolkata, India
³ University of Calcutta, University Colleges of Science and Technology, Department of Physiology, Sports and Exercise Physiology Laboratory, Kolkata, India

Introduction

An exercise-induced oxidative stress condition following a high-intensity training session was (i.e., eccentric or reaped works) hypothesized to be metabolic, mechanical or both in nature during the temporary hypoxic condition that leads to excess reactive oxygen species (ROS) generation [17, 23]. The exercise-induced overproduction of ROS creates oxidative stress and challenge redox equilibrium, which further disrupts cellular homeostasis and leads to a rise in lipid peroxidation [13, 23]. The presently studied summary data of MDA, SOD, GSH, and GPx in reference to endurance team-games such as football and hockey have no game specific references in terms of antioxidant variables, which might due to the nature of energy requirements for the game and the high demand of recovery with a higher level of endurance capacity with a high burst of intense energy for short running sprints [1, 17]. However, a single high-intensity exercise and/or even a long duration moderate-high intensity training of endurance team-game such as football and hockey were observed to induce oxidative stress via



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RESEARCH ARTICLE



DOI: 10.34256/ijpefs2225

Reference Interval of Muscle Damage Indices and Cortisol in Young Athletes of Various Sports Discipline

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Abstract: Creatine kinase (CK), lactate dehydrogenase (LDH) and cortisol are widely accepted as biological markers. The purpose of the study was to frame the reference interval for muscle damage indices (CK, LDH) and cortisol in the young athletic population of various sports disciplines. 260 young male players [i.e., football (n=62), hockey (n=60), gymnastics (n=36), swimming (n=28), table tennis (n=25), sprint-jump-throw (n=36) and middle-long distance running (n=13)] were recruited for the study (mean age = 15.6±1.59 yrs). Assay of LDH, CK and cortisol was done using the standard enzymatic protocol. The reference interval was calculated by following the Clinical and Laboratory Standard Institute (CLSI) C28-A3 guideline and "MedCalc" software (version 19) with a 90% confidence interval. Serum LDH range was from 148.00-324.00 IU/L with a mean of 233.2±34.74 and a median around 236.25. Serum CK ranged from 17.00-43.50 IU/L with a mean of 28.93±5.23 IU/L and a median around 28.00. Cortisol ranged from 4.99-15.78 µg/dl with a mean of 9.31±2.09 µg/dl and a median around 8.90. The present study confers 165.63 - 303.43 IU/L, 19.00 - 40.09 IU/L and 6.07-14.15 µg/dl as the reference interval values for LDH, CK and cortisol, respectively. The present finding will guide the researchers to avoid misinterpretation of muscle damage indices values during any phase of competitive training of sports person.

Keywords: Reference Interval, Creatine Kinase, Lactate Dehydrogenase, Cortisol, Sports Discipline.

About the Authors



Mr. Surojit Sarkar has pursued both B.Sc (Physiology) in 2013 and M.Sc (Physiology) in 2015 from the University of Calcutta, India, and now he is pursuing a Ph.D. at the same university. Mr. Sarkar has also completed various courses, i.e., Workshop course on Statistics (from ISI, Kolkata) and Advance Proteomics course (from IIT, Kharagpur). Mr. Sarkar has experience working with many sophisticated high-end sports science techniques and molecular biology techniques. He is currently working as Physiologist Gd-III (Lead) at Sports Authority of India. He was awarded 'National Fellowship in Sports' in 2016 under the Ministry of Youth Affairs and Sports (MYAS), Govt of India and conducted the Fellowship under the Sports Authority of India.



Dr. Swapan Kumar Dey was the senior scientist of the Sports Authority of India (SAI). Presently, he is a visiting professor in the Department of Sports Science, University of Calcutta. He has done his master's and Ph.D. from Calcutta University in 1979 and 1988, respectively, in Sports, Exercise and Cardio-respiratory Physiology. Dr. Dey has more than 35 years of research and 30 years of teaching experience in the field of Sports and Exercise Physiology at graduate and post graduate levels. He teaches Sports Anthropometry and Sports Nutrition and Physiology to the students of various courses undertaken by SAI and post graduate physiology and sports science students. He is an active member of the Indian Science Congress Association, the Physiological Society of India and the Indian Association of Sports Medicine. He was attached as a Physiologist with the All India Football Federation (AIFF) of AFC's development program in India and a member of the



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Collaboration with Department of Sports Science, Sports and Exercise Physiology Laboratory, Department of Physiology, University of Calcutta and Sister Nivedita University

Apunts Sports Medicine 56 (2021) 100352



ORIGINAL ARTICLE

Effect of high intensity interval training on antioxidant status, inflammatory response and muscle damage indices in endurance team male players



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Received 10 November 2020; accepted 4 February 2021

KEYWORDS

Athletes;
Muscular damage;
Oxidative stress;
Sprint interval training

Abstract

Introduction: High-intensity interval training (HIIT) has previously been reported having the effect of training period on altering oxidant status, muscle damage and performance. The present study was aimed to understand and evaluate the adaptive response of 8 weeks HIIT on muscle damage indices, inflammatory markers, oxidative stress variables and physical fitness parameters.

Methods: Forty young endurance male players [i.e., football ($n=20$) and field hockey ($n=20$)] were recruited under two groups i.e., control and HIIT. 8 weeks long 3h/day of sprint-HIIT was intervened for thrice/week. HIIT workouts includes total 4 sets/session (divided into 2 phase \times 2 sets \times 2 min) of all-out sprint workout (at 90–95% of HR_{max} with work: rest = 1:1). Muscle damage indices (CK and LDH), inflammatory markers (IL-6 and TNF- α), oxidative stress variables (MDA, SOD, GSH and GPx) and physical fitness variables (VO_{2max}, W_{peak} and VJ) were assessed via following standard protocols.

Result: The HIIT resulted to significantly ($p < 0.001$) increase BMI (1.1%), LDH (15.0%), CK (14.4%), cortisol (9.4%), IL-6 (15.7%), TNF- α (18.2%), MDA (29.5%), VO_{2max} (13.6%), W_{peak} (11.6%), VJ (11.2%) and GPx (0.4%) along with significant ($p < 0.001$) reduction in BF% (7.6%), SOD (11.1%), GSH (10.8%) content of athletes.

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Effect of high intensity interval training on antioxidant status, inflammatory response and muscle damage indices in endurance team male players



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SPECIAL ISSUE ORIGINAL ARTICLE

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Renoprotective effect of *Capsicum annuum* against ethanol-induced oxidative stress and renal apoptosis

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Abstract

The present study explored the ameliorative potency of aqueous extract of *Capsicum annuum* (AqCA), against oxidative imbalance and renal toxicity induced by ethanol. Randomly grouped male Wistar rats ($n = 6$), were marked as ethanol-treated (2 g/kg bw, i.p.), CA₁₂₅ (125 mg/kg bw, i.p.), CA₂₅₀ (250 mg/kg bw, i.p.), ethanol pre-treated with CA (similar doses), and control (0.5 ml normal saline, i.p.), and treated for 30 consecutive days. Biochemical analysis of tissue and serum parameters was performed, along with histopathological and histochemical studies. Also, we performed TUNEL assay and western blotting for our experimental groups. Statistical analysis revealed significant ($p \leq .001$) alteration in the levels of antioxidant enzymes, serum urea, creatinine, pro-inflammatory cytokines, and cleaved caspases, along with histopathological alterations in the ethanol-treated group. Prior treatment with AqCA prevented ethanol-induced alterations in tissue and serum parameters. These findings indicate that the extract of CA can protect renal cells from ethanol-induced damage by inhibiting oxidative stress, inflammatory response, and apoptosis.

Practical applications

Chronic alcohol consumption is a major public health concern that leads to various diseases and social problems as well. It affects both the affluent and non-affluent society equally. Alcohol (ethanol) is a renowned hepato-toxicant and a well-documented risk factor for oxidative stress, with less known effect on the kidney. Thus, it is essential to investigate the effect of alcohol metabolism on the kidney to find a remedy to prevent it. The present investigation depicts the anti-oxidative and anti-inflammatory role of *Capsicum annuum* against ethanol-induced renal damage. The outcome of this study can be utilized in the future for phytotherapeutic herbal drug formulation. Besides, the bioactive components identified in the study can be further explored by researchers or pharmaceutical corporates for potential therapeutic purpose against renal impairment.

Abbreviations: AI, apoptotic index; BUN, blood urea nitrogen; CA, *Capsicum annuum* L.; Caspase, cysteine aspartic acid-specific protease; Cont, control; Cu-Zn SOD, copper zinc superoxide dismutase; DAPI, 4, 6-diamidino-2-phenylindole; EtOH, ethanol; G6PD, glucose 6-phosphate dehydrogenase; GPs, glutathione peroxidase; GR, glutathione reductase; GSH, reduced glutathione; GS-SG, oxidized glutathione; GST, glutathione-S-transferase; H-E, hematoxylin-eosin; IL-6, interleukin-6; MDA, malondialdehyde; Mn-SOD, manganese-superoxide dismutase; PBS, phosphate buffer saline; ROS, reactive oxygen species; SODs, superoxide dismutases; TBARS, thiobarbituric acid reactive substance; TNF- α , tumor necrosis factor- α ; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling.

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Research Article

Open Access

Protective Role of Green Synthesized Gold Nanoparticles Using *Terminalia arjuna* against Acetaminophen Induced Hematological Alterations in Male Wistar Rats

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Abstract

Background: The present study aim to investigate on the characterization of green synthesized gold nanoparticles (AuNPs) and to evaluate whether this herbal nanoparticle can increase the efficiency of herb for alteration of hematological indices against acetaminophen induced toxicity in male Wistar rats.

Methods: Bark extract of *Terminalia arjuna* was used for the green synthesis of AuNPs and then characterization of the nanoparticles were done. Then experiment was conducted on 24 healthy male Wistar rats. The animals were divided into four groups, each group having six rats. Group-1: Control; Group-2: acetaminophen treated (500 mg / kg) for 14 days; Group-3: Co-administration of acetaminophen (500 mg/kg/day) along with *Terminalia arjuna* bark extract (175 µg/kg/day) for 14 days; Group-4: Co-administration acetaminophen (500 mg/kg/day) along with green synthesized AuNPs (175 µg/kg/day) for 14 days. Hematological indices were measured using standard hematological techniques.

Results: The green synthesized AuNPs were characterized by UV-visible spectroscopy, FESEM, HRTEM, EDX, FTIR, XRD, DLS analysis. UV-visible spectroscopy showed SPR band at 524 nm. FESEM, HRTEM and XRD analyses revealed that green synthesized AuNPs were spherical shaped, crystalline in nature with size ranging between 20 and 40 nm. Hematological analysis revealed that there was significant decrease in Red Blood Cells (RBCs), Hemoglobin (HB), Hematocrit (HCT)%, Lymphocyte percentage and Platelet Distribution Width (PDW)% with acetaminophen treatment but White Blood Cells(WBCs), Red blood cell Distribution Width (RDW)% and Platelets (PLTs) significantly increases with acetaminophen administration. Then after co-administration with green synthesized AuNPs along with acetaminophen showed effective significant recovery in the hematological alterations.

Conclusions: Overall the results highlighted the promising effect of green synthesized AuNPs against acetaminophen induced hematological alterations in male Wistar rats.

Keywords: *Terminalia arjuna*; Gold nanoparticles; FESEM; HRTEM; Hematological indices

Introduction

Development in the field of nanotechnology has embossed the necessity of utilizing therapeutic nanoparticles for the detection and treatment of diseases. Among the metallic nanoparticles gold nanoparticles (AuNPs) has great importance because of its wider applications in drug delivery [1], biomedical [2], biosensor [3], anticancer [4], antioxidant [5] due to its biocompatibility well defined size, shape, stability and can be easily synthesized [6]. Chemical synthesis method of AuNPs is hazardous to the environment toxic to the biological system. Green synthesis of nanoparticles by using plants and its extract have received much interest due to its eco-friendliness [7,8], less biohazardous, non-toxicity, cost effectiveness and easily scalable [9]. From different studies it has been reported that flavones, polyols, terpenoids, polysaccharides and proteins are involved in the bioreduction and stabilization of the metal ions during nanoparticles synthesis using plant [10]. In last few years, for the development of nanotechnology based drugs many pharmaceutical companies have got approval from the US Food and Drug Administration (FDA) as there is a great urge for large investment in developing new nanotechnology based medical tools for therapeutics [11].

Investigations in the area of green synthesis of gold nanoparticles using living plants [12] were first reported by Gardea-Torresdey and his co-workers. Scientific research reports demonstrated that several

plants were used for biosynthesis of nanoparticles, which includes *Sida acuta* leaf extract [13], *Beta vulgaris* [14], crude extract of *Syzygium aromaticum* [15], *Piper nigrum* [16]. Synthesis of AuNPs using several plants have been reported which includes *Terminalia arjuna* [17], *Morinda citrifolia* L. [18], *Murraya koenigii* [19], *Terminalia chebula* [20], *R. tuberosa* & *P. acidus* [21], and *Gnidia glauca* [22]. From environmental issues it is clear that the green synthesis meets the significant potential in using of safe, harmless, renewable materials for nanoparticle synthesis. In this current study bark extract of *Terminalia arjuna* is used for the green synthesis of gold nanoparticles. Different bioactive constituents such as triterpenoid, saponin, tannin, ellagic acid, gallic acid and proanthocyanidines are present in *Terminalia arjuna* bark extract had been reported [23]. In ayurveda *Terminalia arjuna* is considered as miracle herb used for the treatment of cardiovascular and

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ORIGINAL SCIENTIFIC PAPER

Prediction of Athletic Performance through Nutrition Knowledge and Practice: A Cross-Sectional Study among Young Team Athletes

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Abstract

The present study was conducted to assess the nutrition knowledge, practice, and status and to identify the nutritional and body composition factors predicting athletes' performance. Young team athletes including 40 footballers and 50 hockey players were recruited in this study (age 16.48±1.5) to assess the nutrition knowledge (NK), nutrition practice (NP), and 24-hour dietary recall using a semi-structured questionnaire. Physical characteristics, including height, weight and body mass index (BMI), along with static strength—handgrip and relative back strength, were recorded. Fat mass (FM), fat-free mass (FFM), muscle mass (MM), basal metabolic rate (BMR) and glycogen store was determined using a bioelectrical impedance analyser. Aerobic capacity (VO₂max) was measured with a beep test. The majority of the athletes with good NK scores were found to have good NP scores as well and vice versa ($\chi^2=23.861$, $p=0.000$). Their mean recorded scores for NK and NP were found to be 11.13±3.6 and 7.30±2.0 out of a total of 20 and 12, respectively. Daily consumption of protein ($\beta=0.336$; p value=0.004), sodium ($\beta=0.273$; p value=0.006) and dietary fibre ($\beta=0.220$; p value=0.002) were found to be the best predictors for nutritional practice. Nutrition knowledge and practice had significant positive correlation with BMR (0.314***; 0.419***), calcium intake (0.248*; 0.482***), iron intake (0.303***; 0.221*) and VO₂max (0.331***; 0.428***), respectively. Daily calorie consumption ($\beta=0.144$, $p=0.029$), BMR ($\beta=0.304$, $p<0.001$ ***), MM ($\beta=0.213$, $p=0.020$), calcium ($\beta=0.275$, $p=0.001$) and iron intake ($\beta=0.240$, $p=0.001$) were the significant predictors of athletic performance. Therefore, good nutrition knowledge may improve the nutritional habits and dietary pattern of athletes. Body composition and nutrient intake can predict athletic performance. Intervention studies should emphasize nutrition education aiming for improved athletic performance.

Key words: basal metabolic rate, bioelectrical impedance analysis, body composition, dietary pattern, aerobic capacity

Introduction

Optimal fuelling is an essential requisite for athletes to excel to their best ability (Maughan & Burke, 2011; Kerksick et al., 2008). Apart from nutrition playing an influential role in enhancing on-field performance; it also promotes muscle growth, prevents injury, accelerates recovery, and supports re-

habilitation (Mahan & Stump, 1998). Undoubtedly, athletes' daily diet and fluid intake affect their health, body composition, and substrate availability during exercise as well as recovery time (American Dietetic Association, 2009). Adequate nutrition, which can be reached through sufficient nutrition knowledge (NK), is an integral part of a training programme



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Hepatoprotective effects of green *Capsicum annuum* against ethanol induced oxidative stress, inflammation and apoptosis in rats

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ABSTRACT

Ethnopharmacological relevance: *Capsicum annuum L.* (CA) is used extensively as a spice and is a rich source of antioxidant vitamins. It has long been used in Indian, Native American, and Chinese traditional medicine as a carminative and an appetizer that normalizes liver function. However, its hepato-protective activity has so far not been studied.

Aim of the study: The present study was undertaken to evaluate the efficacy of aqueous extract of CA at two different doses (125 mg/kg body weight and 250 mg/kg body weight), against ethanol induced oxidative stress and apoptosis in liver tissue.

Materials and methods: Adult male Wistar rats, weighing 150–200 g, were randomly grouped (n = 6) and treated with ethanol (2 g/kg bw, i.p.), CA₁₂₅ (125 mg/kg bw, i.p.), CA₂₅₀ (250 mg/kg bw, i.p.), ethanol with CA (similar doses), and control (0.5 ml normal saline, i.p.) for 30 days. Lipid peroxidation (LPO) and reduced glutathione content (GSH) in tissue homogenate, along with catalase (CAT), superoxide dismutase (Cu-Zn-SOD & Mn-SOD), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione-S-transferase (GST) and glucose-6-phosphate dehydrogenase (G-6-P-D) activity were evaluated. Serum levels of alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphate (ALP), triglyceride (TG), total cholesterol (CHLS), high density lipoprotein (HDL), low density lipoprotein (LDL) very low density lipoprotein (VLDL), tumour necrotic factor alpha (TNF- α) and interleukin 6 (IL-6) were also measured using ELISA kits. Histopathological evaluation of the hepatic tissue was performed by hematoxylin and eosin (H&E) and periodic acid-schiff (PAS) staining. TUNEL assay was performed for apoptosis detection.

Results: Ethanol significantly (p < 0.001) increased ALT, AST, ALP, TNF- α , IL-6, LPO, Cu-Zn-SOD, GST, GPx, TG, CHLS, LDL, VLDL levels, along with significant (p < 0.001) decrease in HDL, Mn-SOD, CAT, GSH, GR and GPx activity. Co-administration of CA along with ethanol alleviated changes in the above parameters (p < 0.001) in a dose-dependent manner and also reduced the number of apoptotic death cells. Histopathological and histo-chemical studies of liver sections also ascertained the outcomes of this study.

Conclusion: Thus, it can be concluded that the aqueous extract of green CA can exert a protective effect against ethanol induced hepato-toxicity. The possible mechanism may be by acting as an antioxidant, preventing ethanol induced apoptosis and reducing pro-inflammatory cytokine levels.

1. Introduction

Hepatotoxicity is one of the common complaints leading to several metabolic disorders (Patel et al., 2008) and at times can even be fatal. Ethanol being a xenobiotic is metabolized primarily in the liver and excess consumption of ethanol results in acute hepatic toxicity.

Ethanol has long been consumed by most people of all socio-economic strata in the form of alcohol. It is a commonly consumed recreational beverage of modern society and when in excess, is responsible for causing Alcoholic Liver Disease (ALD). Study of literature suggests that the underlying mechanism of ethanol induced hepatotoxicity is oxidative stress and endotoxin mediated activation of Kupffer

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SPECIAL ISSUE ORIGINAL ARTICLE

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Renoprotective effect of *Capsicum annum* against ethanol-induced oxidative stress and renal apoptosis

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Abstract

The present study explored the ameliorative potency of aqueous extract of *Capsicum annum* (AqCA), against oxidative imbalance and renal toxicity induced by ethanol. Randomly grouped male Wistar rats ($n = 6$), were marked as ethanol-treated (2 g/kg bw, i.p.), CA₁₂₅ (125 mg/kg bw, i.p.), CA₂₅₀ (250 mg/kg bw, i.p.), ethanol pre-treated with CA (similar doses), and control (0.5 ml normal saline, i.p.), and treated for 30 consecutive days. Biochemical analysis of tissue and serum parameters was performed, along with histopathological and histochemical studies. Also, we performed TUNEL assay and western blotting for our experimental groups. Statistical analysis revealed significant ($p \leq .001$) alteration in the levels of antioxidant enzymes, serum urea, creatinine, pro-inflammatory cytokines, and cleaved caspases, along with histopathological alterations in the ethanol-treated group. Prior treatment with AqCA prevented ethanol-induced alterations in tissue and serum parameters. These findings indicate that the extract of CA can protect renal cells from ethanol-induced damage by inhibiting oxidative stress, inflammatory response, and apoptosis.

Practical applications

Chronic alcohol consumption is a major public health concern that leads to various diseases and social problems as well. It affects both the affluent and non-affluent society equally. Alcohol (ethanol) is a renowned hepato-toxicant and a well-documented risk factor for oxidative stress, with less known effect on the kidney. Thus, it is essential to investigate the effect of alcohol metabolism on the kidney to find a remedy to prevent it. The present investigation depicts the anti-oxidative and anti-inflammatory role of *Capsicum annum* against ethanol-induced renal damage. The outcome of this study can be utilized in the future for phytotherapeutic herbal drug formulation. Besides, the bioactive components identified in the study can be further explored by researchers or pharmaceutical corporates for potential therapeutic purpose against renal impairment.

Abbreviations: AI, apoptotic index; BUN, blood urea nitrogen; CA, *Capsicum annum* L.; Caspase, cysteine aspartic acid-specific protease; Cont, control; Cu-Zn SOD, copper zinc superoxide dismutase; DAPI, 4', 6-diamidino-2-phenylindole; EtOH, ethanol; G6PD, glucose 6-phosphate dehydrogenase; GPx, glutathione peroxidase; GR, glutathione reductase; GSH, reduced glutathione; GSSG, oxidized glutathione; GST, glutathione-S-transferase; H-E, hematoxylin-eosin; IL-6, interleukin-6; MDA, malondialdehyde; Mn-SOD, manganese-superoxide dismutase; PBS, phosphate buffer saline; ROS, reactive oxygen species; SODs, superoxide dismutases; TBARS, thiobarbituric acid reactive substance; TNF- α , tumor necrosis factor- α ; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling.

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Research Article

PROTECTIVE ROLE OF CRUDE EXTRACT OF *AMORPHOPHALLUS CAMPANULATUS* AGAINST ETHANOL-INDUCED OXIDATIVE RENAL DAMAGE

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ABSTRACT

Objective: The current study investigates the nephroprotective effect of *Amorphophallus campanulatus* against chronic alcohol-induced oxidative stress and tissue damage.

Methods: The rats were simultaneously supplemented with ethanolic extract of *A. campanulatus* along with ethanol (40% w/v) 2 g/kg body weight/day for 30 days to evaluate the nephroprotective effect against alcohol toxicity. Renal antioxidant enzymes, serum urea, creatinine, and pro-inflammatory cytokines were assayed biochemically. Histomorphological and histochemical alterations were detected by Hematoxylin and Eosin, periodic acid Schiff, and Feulgen and Picrosirius stain, respectively. The degree of apoptotic cell death was examined by terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay technique.

Results: Serum urea, creatinine, pro-inflammatory cytokines, tissue TBARS, and activity of glutathione metabolizing enzymes were significantly ($p < 0.01$) elevated, whereas cytosolic and mitochondrial superoxide dismutase, catalase, and levels of reduced glutathione were significantly ($p < 0.001$) decreased in the EtOH group compared to control. However, ethanolic extract of *A. campanulatus* (ACE) supplementation to the EtOH rats reversed these effects to normal levels. Furthermore, degenerative changes in renal cells with alcohol treatment were minimized to nearness in architecture by ACE supplementation. Glycogen and deoxyribonucleic acid depletion, excess fibrosis due to collagen deposition, and increased apoptotic cell number were also restricted by ACE supplementation, with the higher dose being more promising.

Conclusion: Thus ethanol-induced nephrotoxicity was attenuated by ACE treatment by the antioxidative and antiapoptotic property of the extract. Such effects of the extract may be due to the probable presence of different bioactive components in the tuber. Hence, it can be used as a regular nutrient or therapeutic agent to protect the renal cells.

Keywords: Apoptosis, Fibrosis, Nephrotoxicity, Oxidative stress, Pro-inflammatory cytokines, TUNEL.

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INTRODUCTION

Consumption of alcoholic beverages is considered as a usual habit in most societies around the world. Alcoholism is a serious human health ailment that can disturb the important defense systems in the body, including kidney tissue. The liver is the primary organ responsible for the oxidation of ingested alcohol, but other tissues, including the kidney, may contribute to alcohol metabolism as well [1]. Regular alcohol consumption raises the blood pressure, which *per se* is a risk factor for renal damage [2]. Besides, excess alcohol intake increases free radical or reactive oxygen species (ROS) production and causes oxidative stress by compromising the antioxidant defense system, production of the reactive product acetaldehyde, damage to mitochondria, and altered cytokine production [3-5]. ROS-induced altered antioxidant system causes continued damage to the vital biomolecules, and this condition ultimately gives way to impaired kidney function [6].

In the recent time, many natural products are being used to protect the tissues from various drugs or chemical-induced toxicities. The use of plants as food and medicinal remedies since ancient times is partially attributed to the biological efficacy of secondary metabolites that possess antioxidant activities such as phenolic compounds, Vitamins C and E, and carotenoids [7].

Currently, research interest has been focused on the role of antioxidants as well as antioxidant enzymes, in the treatment and prevention of the diseases mentioned above. The most commonly used antioxidants at present are vitamins, butylated hydroxyanisole, butylated

hydroxytoluene, propyl gallate, and tert-butylhydroquinone. However, they are suspected of being responsible for liver damage and acting as carcinogens in laboratory animals. Therefore, the development and utilization of more effective antioxidants of natural origin are desirable [8].

In Southeast Asian countries, besides vegetables, tuber crops also contribute to a major part of the staple diet. They are of immense importance because of their high calorific value. One such popular tuberous crop in India, especially the south and eastern region, is *Amorphophallus campanulatus* commonly known as "suran" in Sanskrit and elephant yam in English. *A. campanulatus* has its mention in Ayurveda.

Recently, from our laboratory, we reported the *in vitro* antioxidant potential of a hydroethanolic extract of *A. campanulatus* against DPPH, hydroxyl, and superoxide radical [9]. Besides, we have also studied the various bioactive components in the extract by GC-MS analysis and found that the extract had several bioactive components with antioxidant potency along with good source of components such as hexadecanoic acid and its methyl and ethyl esters, heptadecanoic acid, linoleic acid and its ester, oleic acid, stigmasterol, 1, 3, 5, benzenetriol, 4H-pyran-4-one, 2, 3-dihydro-3, 5-dihydroxy-6-methyl-, squalene, and Vitamin E [10]. None the less, hepatoprotective activity of the hydroethanolic extract against ethanol-induced oxidative stress in albino rats has also revealed an upregulation of *in vivo* antioxidant defense system and simultaneous attenuation in the level of tissue lipid



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To Whom It May Concern

This is to declare that Dr. Kaustav Dutta Chowdhury, Assistant Professor (Stage-II), Dept. of Zoology, Rammohan College, Kolkata, West Bengal, India, is doing collaborative research with my research group since 2018 on alteration in pulmonary tissues and its protection by Hexadecanoic acid, ethyl ester. The facilities of both institutions are utilized for this purpose.

The collaboration helps us to exchange our scientific idea/s. Till date, the association has produced 4 international paper/s in reputed journal/s.


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Biotechnological Communication

Effect of Calcium Carbide Exposure Through Inhalation in Lungs of *Mus musculus*

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ABSTRACT

Study on occupational injuries indicates the industrial exposure to air-pollutants, asthmagens, carcinogens, and noise for extended hours as leading risk factors directing to death. This exposure generally occurs by inhalation, ingestion, or via dermal contact. Out of which inhalation is the most rapid route of uptake through breathing in the air that is contaminated with particulate matter/dust, vapours of volatile or semi-volatile contaminants and aerosols due to outdoor and indoor industrial activities. Irritational lung injury, asphyxia, respiratory depression, tachycardia, pulmonary edema may develop as long-lasting systemic effects even after completion of the working life of a worker. Most occupational lung diseases are caused by repeated, long-term exposure. Therefore, our study was conducted to analyze the effect of 40 days of chronic calcium carbide exposure in a close chamber through inhalation in lung of Swiss-albino mice. ALT, AST, SOD and catalase activities were estimated spectrophotometrically. Spectrofluorimetric estimation was performed for reactive oxygen species determination. Flow cytometric analysis was performed to examine cell death and cell cycle. Pro-apoptotic and anti-apoptotic protein levels were estimated by immunoblot. Data demonstrated altered body homeostasis as marked by AST/ALT assay. 3gm CaC₂ exposure indicated activation of antioxidant enzymes, increased cell death causing sustained animal survivability. 5gm and significantly 7gm CaC₂ exposure displayed antioxidant enzymatic activities along-with decreased cell death and animal survivability. While in 9gm CaC₂ exposure total antioxidant enzymes were collapsed with increased cell death leading to probably maintenance of animal survivability to some-extent in the said group.

KEY WORDS: CaC₂, CELL DEATH, LUNGS, MICE, ROS.

INTRODUCTION

Recent time witnessed an increase in respiratory distress due to environmental pollution, lifestyle as well as occupational exposures. In this context, the lung is the most affected organ due to its delicate endothelial network being constantly involved in gaseous exchange with the environment. Report suggests that 1 in 20 people suffers from chronic respiratory diseases (CRDs) globally, attributing CRDs as the third leading cause of death in the world (Momtazmanesh et al., 2019).

Amongst all other causes of CRDs, professional hazard (i.e., breathing in chemicals, dust or noxious gases in

industrial zones), is the most overlooked and neglected one. Occupational lung diseases may take a long time to develop and may have lasting effects on lungs even after the worker stops working. According to the World Health Organization (WHO), 125 million people worldwide are exposed to asbestos at work. According to global estimates, at least 90,000 people die each year from asbestosis, asbestos-related lung cancer and mesothelioma (Chen et al., 2022). Despite all efforts to prevent silicosis, it still afflicts tens of millions of workers and kills thousands of people every year, all over the world (Hoy et al., 2022).

Calcium carbide (CaC₂) also known as calcium acetylide being a source of acetylene and other noxious gases is considered as hazardous by the OSHA Hazard Communication Standard (29 CFR 1910.1200). It is mainly used to manufacture acetylene and other industrial compounds. Pure CaC₂

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Protective role of Decylubiquinone against secondary melanoma at lung in B16F10 induced mice by reducing E-cadherin expression and ameliorating ROCKII-Limk1/2-Cofilin mediated metastasis

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Pulmonary metastatic melanoma

ABSTRACT

Melanoma is one of the most consequential skin cancer with a rising death incidences. Silent but belligerent nature of metastatic sprouting is the leading cause of melanoma related mortality. Invasion of metastatic cells and re-expression of E-Cadherin play the crucial role in the establishment of secondary tumor at distal sites. Thus, manipulation of tumor cell invasion in parallel to regulation of E-Cadherin expression can be considered as potential anti-metastatic strategy. Evidences suggested key role of reactive oxygen species associated ROCK activities in the modulation of metastatic invasion via F-actin stabilization. Here, we first-time report Decylubiquinone, a dietary Coenzyme Q₁₀ analog, as an effective attenuator of pulmonary metastatic melanoma in C57BL/6 mice. Current study depicted detailed molecular interplay associated with Decylubiquinone mediated phosphorylation of ROCKII at Tyr722 along with reduced phosphorylation of ROCKII Ser1366 leading to suppression of Limk1/2-Cofilin-F-actin stabilization axis that finally restricted B16F10 melanoma cell invasion at metastatic site. Analysis further deciphered the role of HNF4 α as its nuclear translocation modulated E-Cadherin expression, the effect of reactive oxygen species dependent ROCKII activity in secondarily colonized B16F10 melanoma cells at lungs. Thus unobscuring of related signal orchestra represented Decylubiquinone as a potential remedial agent against secondary lung melanoma.

1. Introduction

Melanoma is reported as one of the virulent dermatological cancer [1]. According to GLOBCAN 2020, this fatal disease was responsible for >57,043 deaths and in most cases metastatic spreading is responsible for the same [2]. Now a days, only 15% patients with distant metastasis were survived after five years of diagnosis [1]. Thus, inhibition of metastasis is the main key for improving melanoma related

survivability.

Metastasis is a multi-step process involving epithelial to mesenchymal transition (EMT), loss of cell adhesion and dissolving ECM via metalloprotease activity leading to extravasation [3]. Following extravasation from a primary tumor, migrating cancer cells invade into local as well as distant organ, carry out mesenchymal-epithelial transition (MET) and finally proliferate to generate new metastatic tumors [4]. Studies on breast cancer pulmonary metastasis also suggested

Abbreviations: Dub, Decylubiquinone; DMEM, Dulbecco's Modified Eagle's Media; DMSO, Dimethyl sulphoxide; HNF, Hepatocyte nuclear factor; EDTA, Ethylenediaminetetraacetic acid; ID-1, Inhibitor of DNA binding 1; FBS, Foetal bovine serum; IL, Interleukin; ROCK, Rho-associated coiled-coil containing protein kinase; MMP, Matrix metalloproteinase; PBS, Phosphate buffered saline; TGF β , Tumor growth factor β ; VEGF, Vascular endothelial growth factor; Smad, Sma-and Mad-related protein; LIMK, LIMK domain kinase 1; ECM, Extracellular matrix; MT, Metastatic tumor bearing mice; BAL, Bronchoalveolar lavage; NAC, N-Acetyl Cysteine.

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Activity of ROCKII not ROCKI promotes pulmonary metastasis of melanoma cells via modulating Smad2/3-MMP9 and FAK-Src-VEGF signalling

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VEGF regulatory axis
KD-025

ABSTRACT

Rho-associated coiled-coil kinase (ROCK) inhibition decreases tumorigenic growth, proliferation and angiogenesis. Multifaceted evidences are there about the role of ROCK in cancer progression, but isoform specific analysis in secondary pulmonary melanoma is still unaddressed. This study explored the operating function of ROCK in the metastasis of B16F10 mice melanoma cell line. Inhibition by KD-025 indicated dual wielding role of ROCKII as it is associated with the regulation of MMP9 activity responsible for extra-cellular matrix (ECM) degradation as well as angiogenic invasion as an effect of Src-FAK-STAT3 interaction dependent VEGF switching. We found the assisting role of ROCKII, not ROCKI in nuclear localization of Smads that effectively increased MMP9 expression and activity ($p < 0.01$). This cleaved the protein components of ECM thereby played a crucial role in tissue remodeling at secondary site during establishment of metastatic tumour. ROCKII phosphorylation at Ser¹³⁶⁶ as an activation of the same was imprinted essential for oncogenic molecular bagatelle leading to histo-architectural change of pulmonary tissue with extracellular matrix degradation as a consequence of invasion. Direct correlation of pROCKII^{ser1366} with MMP9 as well as VEGF expression in vivo studies cue to demonstrate the importance of pROCKII^{ser1366} inhibition in the context of angiogenesis, and metastasis suggesting ROCKII signaling as a possible target for the treatment of secondary lung cancer specially in metastatic melanoma.

1. Introduction

Melanoma is a type of cutaneous neoplasia which is originated from the pigment-producing cells known as melanocytes [1]. Disease primarily develops in the skin but may rarely occur in the nose, eyes and sometimes inside the body such as in the mouth, throat even in the intestine [2]. It is known for its aggressive nature with a least chance of prognosis until tumours become mature and metastasize at variety of atypical locations [3]. Median overall survival of malignant melanoma

(MM) is only 5.3 months and the mean survival is 9.2 months [3]. Clinical studies identify lung as the most common metastatic site (18–36%) for melanoma [4] and only 5–19% of patients are generally survived after five years of diagnosis [3].

Malignant melanoma at lung creates further complications since the prophecy of lung cancer is poor due to its asymptomatic nature at the initial phase [5]. In fact, the symptoms are often mistaken with infection or effect of smoking, which further delays diagnosis. Therefore, majority of metastatic lung melanoma cases are diagnosed at either stage III or IV,

Abbreviations: CDK, cyclin dependent kinase; DMEM, Dulbecco's Modified Eagle's Media; DMSO, dimethyl sulphoxide; ECM, extracellular matrix; EDTA, ethylenediaminetetraacetic acid; FAK, focal adhesion kinase; FBS, foetal bovine serum; IL, interleukin; JNK, Janus kinase; MMP, matrix metalloproteinase; PBS, phosphate buffered saline; ROCK, rho associated protein kinase or rho-associated coiled-coil kinase; STAT, signal transducer and activator of transcription; TGPF, tumour growth factor; VEGF, vascular endothelial growth factor; Smad, Sma-and Mad-related protein; CBP, CREB-binding protein; HMGB1, high mobility group box protein.

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Combinational Impact of Chelerythrine and S-Allyl Cystine on Metastasis melanoma of liver : An *In vivo* Analysis

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ABSTRACT

Metastatic melanoma, the highly fatal and aggressive disease, has yet to any effectual remedies. Several evidences suggested delicate responsibility of oxidative/cytotoxic stress in the modulation of tumor microenvironment leading to metastasis. Therefore, conditioning of reactive oxygen species in tumour and its adjacent arena may play a guardian role for restricting metastatic melanoma. Well-known active biocomponents like S-allyl Cysteine and Chelerythrine as nontoxic dietary phytochemicals are recently documented as potential anti-tumorigenic and anti-inflammatory therapeutics but their role in metastatic melanoma still remains elusive. Therefore, present study was carried out to investigate the efficacy of S-allyl Cysteine and Chelerythrine against metastatic melanoma to the hepatic tissue. Status of liver function was estimated by performing ALT, AST, GGT and ALKP assay. ROS accumulation was determined by estimating the altered DCF fluorescence in hepatic tissue lysates. GSH and TBARS content were measured as a marker of anti-oxidant and cytotoxicity level after the treatment. Analysis on the marker proteins like Caspases, CytochromeC, BCL₂, Bax, VEGF, MMP9 and NF- κ B depicted the triggering of p-p53 nuclear translocation and significant increase in Bax expression that in-turn induced CytochromeC-Caspase9-Caspase3 apoptotic axis after drug administration. Data also illustrated notable reduction in tumor nodules at liver along-with normalization of liver function as demarcated by the level of biomarkers in the treated groups. Restoration of enzymatic and non-enzymatic anti-oxidants as well as suppression of VEGF and MMP9 expression as an effect of attenuated NF κ B nuclear localization by S-allyl Cysteine and Chelerythrine effectively delimited extracellular matrix remodeling as well as angiogenesis, two major prerequisites for metastasis. Combinatorial administration of S-allyl Cysteine and Chelerythrine further portrayed better efficacy in metastatic tumor regression and tissue restoration by sustaining ROS/antioxidant balance and stabilization of p53 through its phosphorylation, that can be considered as future directives for the development of novel remedial strategy against metastatic melanoma in liver.

KEY WORDS: METASTATIC MELANOMA, ROS, ANTIOXIDANT, S-ALLYL CYSTEINE, CHELERYTHRINE.

ARTICLE INFORMATION

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INTRODUCTION

Melanoma, a predominant skin cancer, originates from melanocyte. Surgical removal followed by popular therapies with chemo/radiation-based drugs can cure primary melanomas. Due to its high aggressive nature and lack of complete effective therapeutic strategy, it can able to metastasize into local as well as distant organ following invasion and this in turn reduces the chances

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Cathepsin B mediated scramblase activation triggers cytotoxicity and cell cycle arrest by andrographolide to overcome cellular resistance in cisplatin resistant human hepatocellular carcinoma HepG2 cells

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HepG2
Cisplatin
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Scramblase
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ABSTRACT

Andrographolide regimen in single or in combination with anticancer drugs is a promising new strategy to reverse chemoresistance in hepatocellular carcinoma. Apoptosis inducing factor (AIF) may regulate a complementary, cooperative or redundant pathway, along with caspase cascades. Despite these findings, mechanisms underlying caspase-dependent and-independent signaling pathways in andrographolide-induced apoptosis in cisplatin-resistant human hepatocellular carcinoma cell line (HepG2CR) remain unclear. Andrographolide treatment effectively reduced NF- κ B nuclear localization by modulating protein kinase A- protein phosphatase 2 A- I κ B kinase (PKA/PP2A/IKK) axis that in turn maintains initiator caspase8 activity. Lysosomal distribution of tBid stimulates cytosolic cathepsin B resulting accumulation of truncated-AIF with induction in scramblase mediated phosphatidylserine exposure in HepG2CR cells. Andrographolide treatment thereby switch on subG1 phase arrest by modulating cellular check points (cyclin A, B, cyclin dependent kinase-1) cueing to the apoptosis event. Collectively, this study suggested antineoplastic potential of andrographolide through PKA/PP2A/IKK pathway in HepG2CR cells.

1. Introduction

Resistance is an evolutionary attributable cellular self-defense to protect cells from environmental stress and toxic effects (Pfeffer and Singh, 2015). Hepatocellular carcinoma (HCC) with its diversity in origin in biological and clinical characteristics thwarted the efficacy of chemotherapy (Samonakis and Kouroumalis, 2017) in part caused by multidrug resistance (MDR). Several mechanisms including vital roles of drug efflux pump, epithelial mesenchymal transition (EMT), hypoxia-inducible factor-1 α (HIF1- α) signaling and DNA damage repair govern MDR induction, in chemo-resistance of HCC (Wen et al., 2016). Combined chemotherapy based on cisplatin, recommended by international cancer organizations has become a potential line of

chemotherapy against liver cancer in recent times (Buendia and Neuvut, 2015) and continued to be a mainstay to treat HCC (Kim et al., 2017). Widespread use of platinum drugs led to a gradual design of escape route for tumor cell to build up resistance that reduces the effect of chemotherapy to a significant extent developing intense modifications at both molecular and cellular levels about cell survival/death, endocytosis, gene activation/silencing by regulating methylation and acetylation as well as mutations mediated by transcription factors/miRNAs (Shindo et al., 2018). Hence, the concept of using phyto-medicines warrants immediate attention to overcome drug resistance.

Protein phosphatase 2 A (PP2A) play dual role in keeping both pro-survival as well as pro-apoptotic signaling networks in check, maintaining a crosstalk with protein kinase A (via mitogen activated protein

Abbreviations: Andro, andrographolide; Cisp, cisplatin; cAMP, cyclic adenosine monophosphate; PKA, protein kinase A; PP2A, protein phosphatase2A; IKK, I κ B Kinase; cFLIP, cellular FLICE inhibitory protein; HCC, hepatocellular carcinoma; HepG2CR, cisplatin resistant HepG2 cell; FBS, fetal bovine serum; PEN-STREP, penicillin-streptomycin; AIF, apoptosis inducing factor; I κ B, inhibitory κ B

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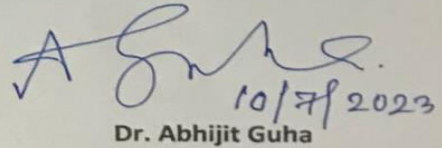
Date: 10/7/23

To Whom It May Concern

This is to certify that Dr. Madhab Ghosh, Assistant Professor, Department of Education, Rammohan College, Kolkata-700009, West Bengal is doing collaborative research work with my research activities since August, 2017 under the broad area of 'Education'. The facilities of both Institutions are utilized for this purpose.

The collaboration helps us to exchange our educational ideas. Till date, the collaboration has produced one M.Phil. Dissertation (jointly supervised) under Ramakrishna Mission Sikshanamandira, Belur Math, Howrah-711202 and two research articles published in UGC listed journal and edited book.

The collaboration has yielded satisfactory results and in the near future we look forward to have positive outcomes using the institutional facilities available.


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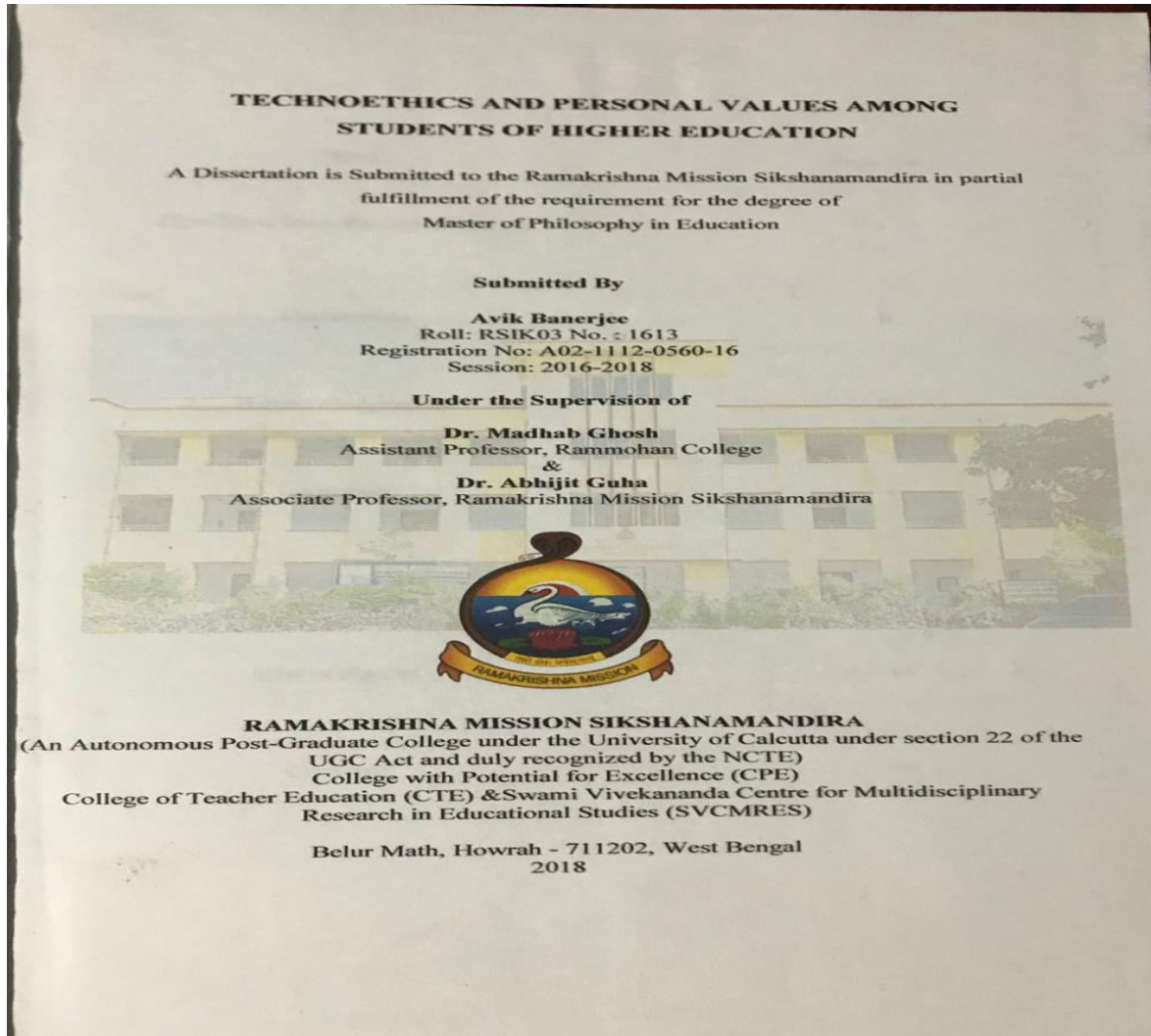
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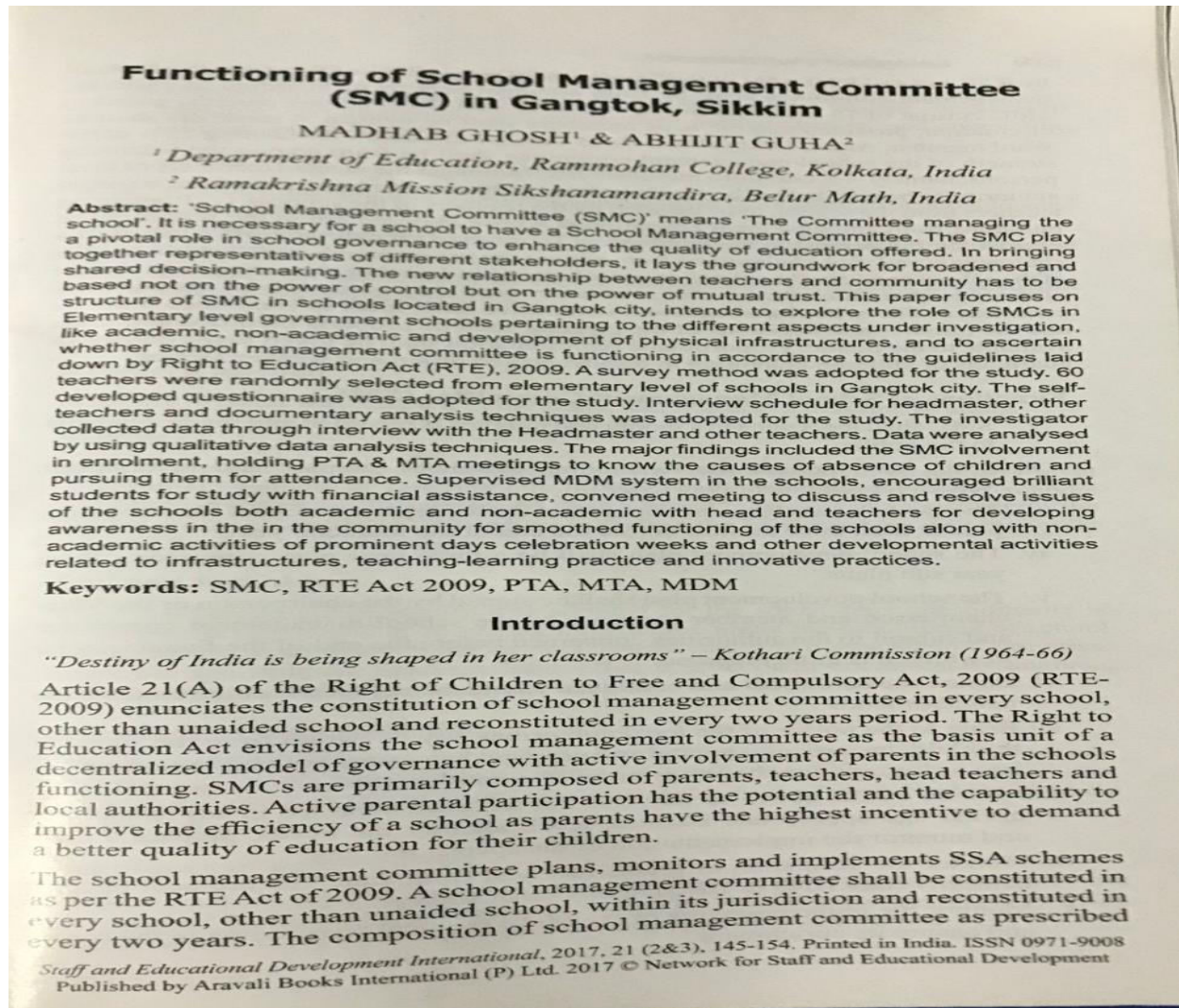
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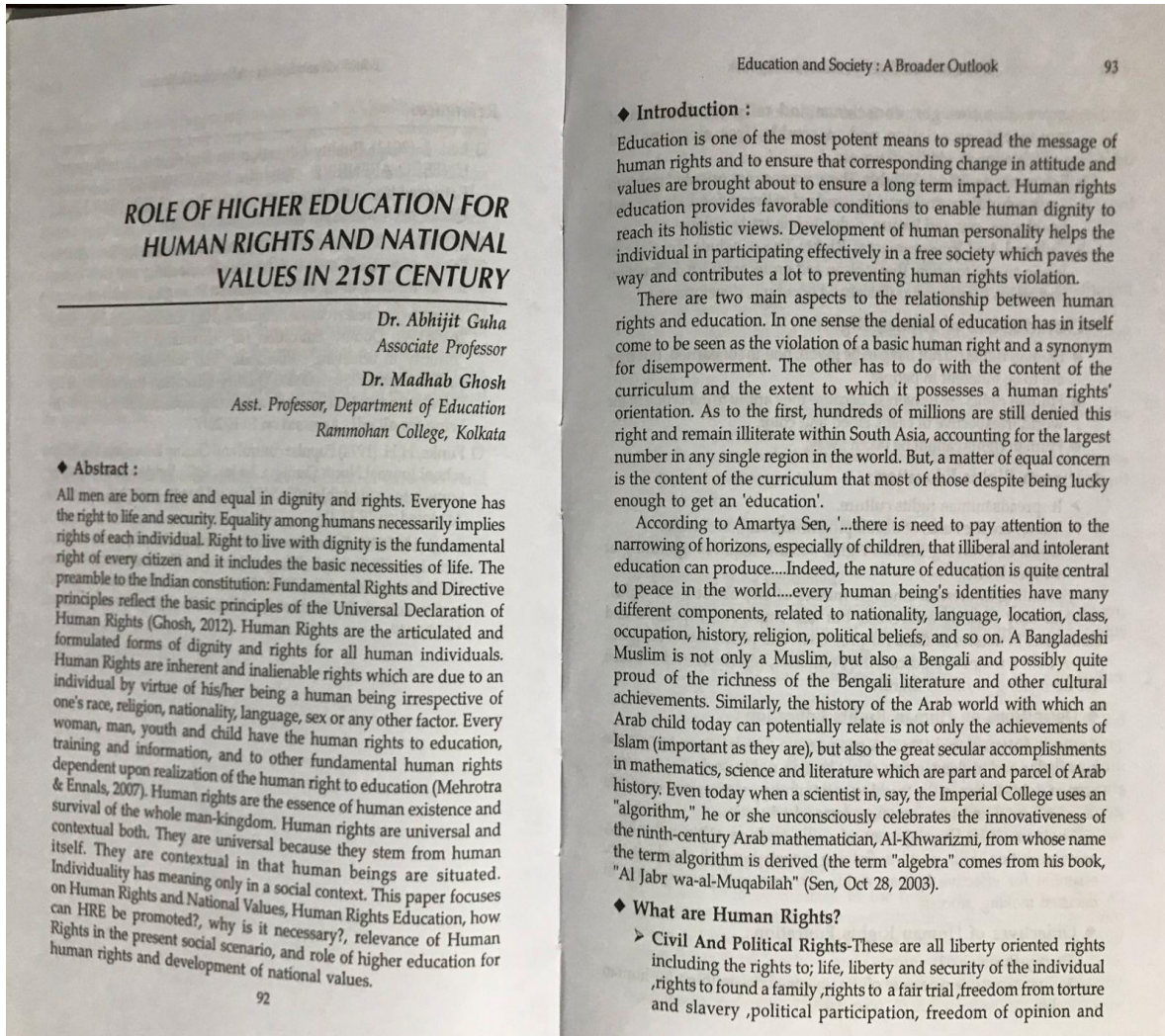
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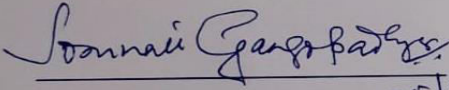
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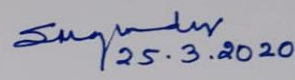
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To whom it may concern

Professor Somnath Gangopadhyay, Professor and In-charge, Occupational Ergonomics Laboratory Department of Physiology ,University of Calcutta and Dr Sahana Mazumder Sen Associate Professor, Department of Physiology ,Rammohan College agree to work at all communication levels of research activities, including laboratory, study material, and intellectual exchange programme. during the period 2020 to 2025.


Prof. Somnath Gangopadhyay 25/3/2020


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Int. J. Nano Dimens., 14 (2): 178-190, Spring 2023

ORIGINAL ARTICLE

Efficacy of green synthesis of Silver nanoparticles from Tulsi (*Ocimum sanctum*) leaf aqueous extract and its antibacterial effect on clinical multidrug-resistant *Staphylococcus aureus* in West Bengal

Kartik Shaw^{1*}, Payel Das¹, Tamal Ghorai¹, Tapomoy Chatterjee¹, Somnath Gangopadhyay², Sahana Mazumder³

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Received 22 December 2022,

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Abstract

Rapid augmentation in the prevalence of multidrug-resistant (MDR) *Staphylococcus aureus* is a worldwide threat. Advising newer antibiotics may fail to reduce the chances of the emergence of newer drug-resistant *Staphylococcus aureus*. Very little shreds of evidence can be found to treat clinical MDR *Staphylococcus aureus* with biogenic silver nanoparticles (AgNPs) in West Bengal. To prepare AgNPs biogenically using aqueous tulsi leaf extract (TLE) and also to assess its antibacterial effect upon clinical MDR *Staphylococcus aureus*, biogenic synthesis of the AgNPs using aqueous TLE was done, characterized those with UV-Vis Spectrophotometer, dynamic light scattering, field emission scanning electron microscopy, Fourier transform infrared spectroscopy, and evaluated the antibacterial activity against the clinical MDR *Staphylococcus aureus*. ANOVA followed by LSD post hoc test was used to test the differences between the OD (optical density) of different experimental sets. The biosynthesized AgNPs were spherical, monodispersed, and of smaller size (9-23 nm) with the involvement of eugenol, quercetin, and oleanolic acid present in the tulsi leaf. A significant change in OD was observed in AgNPs (prepared using TLE) treated broth compared to only tulsi leaf extract treated culture. There was a significant similarity between the efficacies of AgNPs and clindamycin ($P < 0.05$). Our findings propose that AgNPs synthesized using TLE were fast and efficient to ameliorate the bacterial growth, which may be used as a potent antibacterial agent for the treatment of clinical MDR *Staphylococcus aureus* infection in near future.

Keywords: Ag Nanoparticles; Biogenic; Clindamycin; MDR; MRSA; *Staphylococcus aureus*; Tulsi.

How to cite this article

Shaw K., Das P., Ghorai T., Chatterjee T., Gangopadhyay S., Mazumder S. Efficacy of green synthesis of Silver nanoparticles from Tulsi (*Ocimum sanctum*) leaf aqueous extract and its antibacterial effect on clinical multidrug-resistant *Staphylococcus aureus* in West Bengal. *Int. J. Nano Dimens.*, 2023; 14(2): 178-190.

INTRODUCTION

From the origin of the concept of nanoparticles in 1954 by eminent scientist Paul Ehrlich [1] to the 21st century, there is an immense change in the craze of using nanoparticles in research work has been observed. Biologically prepared nanoparticles have the potential to lead us to find solutions to a wide range of issues that are being

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encountered nowadays. Chemically reduced silver nanoparticles have an adverse effect on human health as well as it gives low yield and requires high energy [2]. As an alternative, biogenic silver nanoparticles emerged as a good antibacterial, as well as an antifungal, and anticancer agent. Apart from this, the literature suggests the use of AgNPs (Silver nanoparticles) in different sectors like clothing [3], water treatment/purification

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Study of Haemoglobin Level and Tumour Growth on Mouse Ascites Tumour in Response to Combination Effect of 2-Methoxyestradiol and Cyclophosphamide

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Original Research Article

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Abstract: S-180 tumour bearing mice were subjected to 2-Methoxyestradiol (2ME) and Cyclophosphamide (CP) monotherapy and 2ME and CP combination therapy on 7th day of ascitic tumour cell transplantation when the tumour growth was at log phase. Then, the effect has been studied on host's system in respect to dead cell – living cell frequency, tumour volume, haemoglobin percentage, and differential count of WBC. In 2ME and CP combination therapy, a steady increase in the dead cell or non-living cell population was noted with the steady decrease in tumour volume. Haematological studies from peripheral blood revealed a drastic depletion in neutrophil count and elevation of lymphocyte population on the 12th day and 16th day of tumour transplantation in combination therapy series. Moreover, the haemoglobin concentration is more or less stable in combination therapy treatment series. So, the 2ME and CP combination therapy provides some protective compensatory mechanisms in the body of the host.
Keywords: Combination therapy, Differential count, Viable cell, Haemoglobin Percentage, 2-Methoxyestradiol, Cyclophosphamide.

INTRODUCTION

Cancer is a complex multistage genetic disease in which a group of normal cells transforms into metastatic malignant cells. At present, surgery, radiation therapy and chemotherapy are common methods of cancer treatment. Among these, chemotherapy has become much popular due to some reasons. Firstly, it prevents cell proliferation by interfering with their ability to replicate DNA and secondly, it can induce apoptosis in cancerous cells [1-4].

MATERIALS AND METHODS

Experimental animal

Swiss Albino adult mice (*Mus musculus*) with an average body weight of 20g were grouped and housed in normal laboratory condition for acclimatization at 24° - 25°C temperatures. Mice were provided standard mice food and water ad libitum.

Selection of animal tumour model

Sarcoma 180 (S-180), a well-known transplantable tumour, was maintained intraperitoneally in Swiss albino mice (1 × 10⁶ cells/ animal). All experiments were done in accordance to the guideline of Institutional Animal Ethics Committee (IAEC).

S-180 tumour transplantation

Freshly aspirated S-180 tumour cells were diluted with 0.9% normal saline under sterile condition and were injected intraperitoneally to normal mice for induction of ascitic tumour [14-15] for pursuing our experiments.

But this type of treatment has some toxic side effects on normal cells. Many chemotherapeutic agents may induce cytological abnormalities (i.e. chromosomal aberrations) as well as haematological abnormalities. Use of combination treatment is a novel idea to treat cancer as combination therapies may induce less toxic side-effects at cytological and haematological level. Moreover, good combination may protect the host from some undesirable effects. In the present study, 2-Methoxyestradiol (2ME)—an anti-angiogenic, anti-neoplastic [5-10] agent has been used in combination with an alkylating anti-tumour drug cyclophosphamide (CP). CP has been used in different cancer patient as monotherapy and combination therapy [11-13]. Different types of cytological effects of 2ME and CP have been reported in different animal tumour model systems [9,10] but its effect on host's hemopoietic system during the period of treatment has not been studied yet. So, the present study has been oriented to find out the effect of monotherapy of 2ME, CP and combination therapy of 2ME and CP at haematological level during the course of treatment using Sarcoma 180 tumour bearing mouse.

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Provisional Ph.D. Certificate

No. Ph.D./Physio./SM/012(30)/2018

December 19, 2018

This is to certify that on the recommendation of the Board of Examiners, the thesis submitted by *Smt. Srabantika Mallick*, for the award of *Ph.D. Degree* of this University and on the performance of his/her Ph.D. Open Viva vide Reg.13 (D.D.) & Reg.14 (D.D.) (as per Regulation 2016, K.U.), he/she has been admitted to the aforesaid degree on **13.12.2018** in *Physiology* under the faculty of *Science*.

The Ph.D. Degree has been awarded in accordance with the provisions of UGC (Minimum Standards and Procedure for Awards of M.Phil./Ph.D. Degree) Regulation, 2009.

Title of the Thesis:

"ANTIANGIOGENIC THERAPY FOR TREATMENT OF CANCER: EFFECT OF 2 METHOXYESTRADIOL IN COMBINATION WITH CYCLOPHOSPHAMIDE ON EXPERIMENTAL TUMOUR GROWTH IN MOUSE"

His/Her Degree will be conferred by the Hon'ble Chancellor at the next Convocation of this University.

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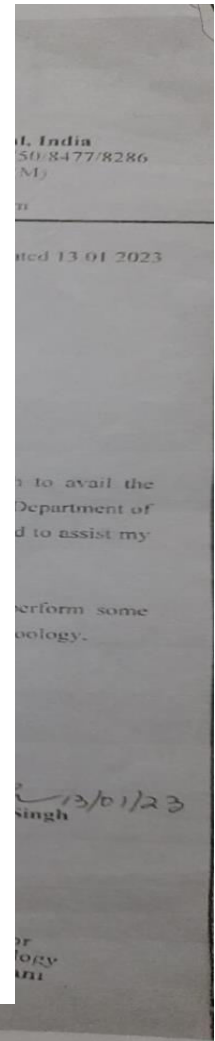
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To Whom It May Concern

This is to declare that Dr. Kaustav Dutta Chowdhury, Assistant Professor (Stage-II), Dept. of Zoology, Rammohan College, Kolkata, West Bengal, India, is doing collaborative research with my research group since 2018 on alteration in pulmonary tissues and its protection by Hexadecanoic acid, ethyl ester. The facilities of both institutions are utilized for this purpose.

The collaboration helps us to exchange our scientific idea/s. Till date, the association has produced 4 international paper/s in reputed journal/s.


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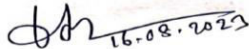
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This is to declare that Dr. Samik Acharjee, Assistant Professor, Department of Zoology, Rammohan College, Kolkata, West Bengal, India is doing collaborative research with my research group since 2022 on Biodiversity conservation and Proteomics studies in different freshwater fishes. The facilities of both institutions are utilized for this purpose.


The collaboration helps us to exchange our scientific idea/s. Till date, the association has produced 1 research paper in reputed journal.


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Research Article

Ethnomedicinal Knowledge of Bishnupriya Manipuri Community of Unakoti District of Tripura, North East India



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ABSTRACT

The present study deals with indigenous ethno-medicinal knowledge of Bishnupriya Manipuri community of Unakoti district of Tripura, Northeast India. The ethno-medicinal exploration reveals the usage of different plant and herb species in a particular concoction that has not been documented till date. The study comprises of 15 plants and herb species mixed in a preparation locally known as 'Khulleigulli' that is used as an excellent primary treatment for sore throat, cough, cold, fever and also has been claimed to miraculously reduce the severity of upper respiratory symptoms of COVID 19. The concoction could possibly a better alternative with no known side effects as modern day allopathic medicines. There is a need of further critical phytochemical analysis of the formulation.

1. Introduction

Northeast India comes under the lower Himalayan range and is known for its extraordinary biodiversity. The region including Tripura is home to huge number of bioresources and is ranked 8th out of the 234 Bio-diversity hotspots in the world. [1, 2] Out of 450 tribes found in the country, about 225 of them hail from the region of Northeast India. [3] This magnificent region has the richest reservoir of plant diversity and it supports around 50% of India's biodiversity. [4] Tripura is a small state located at the Indo-Bangladesh border and it also shares the boundary with Mizoram and Assam. Tripura is home to many communities such as Bengalis, Reang, Chakma, Tripuri, Bishnupriya Manipuri and many others. Bishnupriya Manipuri, an original community of Manipur had to relocate themselves after they lost control over Manipur to the rival clan of Meiteis. [5] In Tripura, the community resides in parts of Unakoti district, North Tripura and parts of West Tripura. Unakoti is a beautiful district in the northern part of Tripura and the district is named after a magnificent archeological site called Unakoti nestled in the hills of Tripura with coordinates as 24.1781° N, 92.0273° E. It is believed to have one less than a crore marvellous rock carvings of Lord Shiva, his followers, Lord Ganesha, MaaDurga and many other Gods and Goddesses. The site has rich plant diversity.

Over the time, the community has gathered knowledge of utilizing the vast flora diversity found in the region and uses different ethno-botanical plants as medicines based on their belief and practices in curing diseases and ailments. The community still prefers traditional medicines before reaching out to the modern pharmaceutical ones. The people of the community prefer the herbal concoction as they are non-toxic and works miraculously in relieving common cold-like symptoms including fever, sore throat etc. The objective of the study is to survey and understand the use of herbal concoction called as 'Khulleigulli' by the Bishnupriya community since no record is available with regard to it so far. The use of this concoction is however declining because of the modernization and due to the decease of the knowledgeable person. This miraculous concoction is alien to the outside world and has not been under study. Also, the plants used in this concoction have not been studied empirically in detail for the active chemical compounds in it. A detail study on the 'khulleigulli' would be helpful as an alternative to several allopathic medicines. Immediate documentation of such valuable knowledge is important as we are gradually missing precious ethno-medicinal knowledge with increasing impact of modern western pharmaceutical medicines.

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To Whom It May Concern

This is to declare that Dr. Samiran Mondal, Assistant Professor (Stage-II), Dept. of Chemistry, Rammohan College, Kolkata, West Bengal, India, and I, Dr. Naznin Ara Begum, Associate Professor, Dept. of Chemistry, Siksha-Bhavana, Visva-Bharati (A central University) are doing collaborative research on small-molecule-based drug development. The facilities of both institutions are utilized for this purpose. This collaboration helps us to exchange our scientific knowledge and expertise. This is highly important as it helps us understand small molecules' molecular functioning as drugs for cancer and other fatal diseases. Thus our collaborative endeavour will be noteworthy in the future in the field of Cancer Biology/Chemical Biology.

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RESEARCH ARTICLE

Curry Leaf and its Antioxidant Potential: A Systematic Study to Enhance its Activity in Aqueous Medium

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Abstract: Background: We have done a systematic study on the antioxidant activity of the methanol and petroleum ether (60-80°C) extracts (MEC and PEC respectively) of Curry leaves (*Murraya koenigii* Spreng. Family: Rutaceae) using various in-vitro chemical methods.

Method: Both of these two extracts were found to be highly efficient in the formation of Ag and Au nanoparticles. So we have explored their ability to form the nanoparticles to study their antioxidant activity. In all the assay systems, MEC showed higher activity over PEC in aqueous medium. This may be due to the higher solubility of MEC and its active components, like polyphenols and flavonoids in the aqueous medium. PEC contains lesser amount of these water soluble active components but PEC was rich in carbazole types of alkaloids which are hydrophobic in nature. So, to enhance the antioxidant activity of PEC and its carbazole constituent, like 2-hydroxy carbazole and mahanimbine, we have encapsulated these in the biopolymeric matrix of the mucilage isolated from an edible vegetable, *Abelmoschus esculentus* L. (commonly known as Lady's finger, family: Malvaceae).

Result: It was interesting to note that, PEC and its carbazole compounds showed better antioxidant activity (ferrous ion chelation and ferric reducing antioxidant activity) in aqueous medium after this encapsulation process.

Conclusion: The protocols used in the present study were very simple and can be implemented in any lab set-up. In future, this work can be extended to evaluate antioxidant potentials of other plant based materials.

Keywords: *Abelmoschus esculentus* L., antioxidant activity, curry leaves, encapsulation, lady's finger, *Murraya koenigii* Spreng.

1. INTRODUCTION

Edible leaves of various medicinal plants have a long history of use in the traditional medicine of various countries, including India [1]. Locally available and edible plant-based sources, like fruits and leafy vegetables are thus note-worthy as these are low-cost, effective and have minimal side effects [2-4].

Curry leaves are widely used in Indian cuisine as spice and condiment. Moreover, there is a long history of the use of these leaves in the Indian traditional medicine [5]. These leaves show various pharmacological activities, such as,

anti-tumours, anti-viral, anti-inflammatory, anti-convulsant, diuretic and antioxidant activities [5]. Curry leaves are collected from the Indian medicinal plant and Indian curry leaf plant (scientific name: *Murraya koenigii* Spreng., family: Rutaceae). It is a small tropical tree, widely cultivated in India and is famous for its aromatic leaves (commonly known as Curry leaves).

Curry leaves have been identified as rich sources of polyphenolics (e.g. myricetin-3-galactoside, quercetin-3-rutinoside, quercetin-3-glucoside, kaempferol-3-O-caffeoylate, 5-caffeoyl-quinic acid, tannic acid, gallic acid, caffeic acid, cinnamic acid, chlorogenic acid, ferulic acid and vanilic acid etc.), free amino acids, carbazole alkaloids, flavonoids and terpenoids [5]. Leaves of these plants are the richest sources of carbazole alkaloids (e.g. mahanimbine, koenigine etc.) [5].

Aqueous extract of Curry leaves and the carbazole alkaloids isolated from these leaves show hepatoprotective activity, hypoglycemic activity along with antioxidant activity

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Article

Unfolding the Role of a Flavone-Based Fluorescent Antioxidant towards the Misfolding of Amyloid Proteins: An Endeavour to Probe Amyloid Aggregation

Abhijit Karmakar, Tamanna Mallick, Chandrani Fouzder, Alpana Mukhuty, Samiran Mondal, Anup Pramanik, Rakesh Kundu, Debabrata Mandal, and Naznin Ara Begum*

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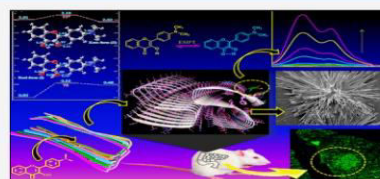
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ABSTRACT: 4'-N,N-Dimethylamino-3-hydroxyflavone (DMAHF), a synthetic fluorescent flavone analogue with potent antioxidant activity, was explored as a molecular rotor-like fluorophore for amyloid aggregations, a causative factor in Alzheimer's disease, Parkinson's disease, type-2 diabetes, etc. During its interactions with (human) insulin amyloid aggregation (IAA), its microenvironment was changed. This instigated a drastic change in its excited-state intramolecular proton transfer-based dual emission behavior, which was tracked to monitor its amyloid probing activity. Thus, the amyloid probing potential of DMAHF was originated from its interactions with IAA, which were studied by various spectroscopic techniques and molecular docking and quantum-mechanical calculations. Morphological changes of the IAA in the presence of DMAHF were studied by scanning electron microscopy. DMAHF also probed efficiently the islet amyloid polypeptide deposition in the pancreatic β -cells of diabetic mice. DMAHF showed significant sensitivity and specificity towards amyloid aggregation without having any complexity in its photophysical behavior. This indicates its potential as an ideal bio-friendly and cost-effective fluorophore for amyloid proteins.



INTRODUCTION

Amyloid aggregation has long been suspected as a major key factor in various incurable neuro-degenerative and metabolic diseases, for example, Alzheimer's disease, Parkinson's disease, Type-2 diabetes, etc. Amyloids represent a broad class of proteins having minimal primary sequence similarity that can self-assemble into β -sheet-rich un-branched fibrillar structures, which are termed as amyloid plaques/fibrils.^{1,2} Such misfolded amyloid proteins are the pathological traits for these fatal diseases, like amyloid- β ($A\beta$) peptide and tau protein are related to Alzheimer's disease, whereas islet amyloid polypeptide (IAPP or amylin) and α -synuclein (α -s) are associated with type-2 diabetes and Parkinson's disease, respectively.¹⁻⁶

Nowadays, researchers are struggling to shed light on the etiology of the amyloid aggregation related diseases. However, until now, we do not have drugs or therapeutic agents that can delay and/or prevent the progression of Alzheimer's or other amyloidosis-induced diseases.¹⁻⁸ The reasons behind this lacuna may be the complexity in the amyloid structure and difficulty in understanding its mechanism of formation. Increased knowledge in this direction can immensely help to us develop the diagnostic and therapeutic tools for combating these incurable diseases. Scientists have taken various strategies to achieve such knowledge. One such strategy is based on the

inhibition or reversal of the amyloid aggregation. But to achieve this goal, early detection/diagnosis of amyloid aggregation is necessary. It is noteworthy that the studies on the interactions of various small molecules with amyloid fibrils are extremely relevant and necessary in developing the efficient amyloid diagnostic probes as well therapeutic agents. In this context, several small molecules having characteristic chromophoric/fluorophoric behavior, for example, dyes based on azobenzene, benzothiazole, and benzimidazole moieties, are put into trial for detecting amyloid oligomers, for example, $A\beta$ and corresponding aggregates.^{5,6,9-14} Over half a century, the most widely used amyloid fluorescence probe or fluorophore is Thioflavin-T (ThT), which is a small-molecule-based fluorescent molecular rotor having a benzothiazole framework (Figure 1).

The fluorescence responses of ThT in the presence of amyloid aggregations are monitored to probe the amyloid, and

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Exploring the Propensities of Fluorescent Carbazole Analogs toward the Inhibition of Amyloid Aggregation in Type 2 Diabetes: An Experimental and Theoretical Endeavor

Tamanna Mallick, Abhijit Karmakar, Alpana Mukhuty, Chandrani Fouzder, Jishu Mandal, Samiran Mondal, Anup Pramanik, Rakesh Kundu, and Naznin Ara Begum*

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ABSTRACT: Amyloid aggregation is a pathological trait observed in many incurable and fatal neurodegenerative and metabolic diseases associated with misfolding and self-assembly of various proteins. Noncovalent interactions between these structural motifs and small molecules can, however, prevent this aggregation. Herein, five structurally different synthetic (Cz1–Cz4) and naturally occurring (Cz5, mahanimbine) fluorescent carbazole analogs are explored for their comparative amyloid aggregation inhibitory activities. Cz3 inhibited the amyloid deposition on the pancreatic β -cells of diabetic mice. Moreover, Cz3 and Cz5 also showed efficacy as the fluorescent cell (MIN6) imaging agents. Further structural modifications of these carbazoles may lead to development of low-cost and nontoxic therapeutic agents for Type 2 diabetes and other amyloidosis-related diseases.



INTRODUCTION

Amyloid aggregation is a pathological trait observed in more than 30 serious neurodegenerative and metabolic diseases in human beings, e.g., Alzheimer's disease (AD), Parkinson's disease, Type 2 diabetes, etc.^{1–4} Misfolding and self-assembly of a wide range of proteins with little structural similarity in their primary sequence give rise to highly ordered (β -sheet rich) toxic fibrillar assemblies, known as amyloid aggregation.^{3,4} There is a quest for the novel therapeutic approaches, which can specifically target amyloid aggregation and delay or prevent its propagation.

Over the years, large numbers of research are being carried out to shed light on the etiology of Type 2 diabetes. Islet amyloid polypeptide (IAPP) or amylin is co-secreted with insulin from the pancreatic β -cells, and along with the insulin, it plays an important role in controlling blood glucose levels.^{5–9} However, apart from the body's insulin resistance, the misfolding of IAPP (triggered by factors like cellular oxidative stress, mitochondrial dysfunction, chromatin condensation, etc.) is considered as one of the key factors of Type 2 diabetes. The extracellular deposition of amyloid fibrils of IAPP on pancreatic β -cells causes their dysfunction.^{8,6,10–12} On the other hand, hyperinsulinemia is associated with Type 2 diabetes and other than IAPP, amyloids in the islet cells can also be formed by the excess secretion of insulin, which is amyloidogenic in nature.^{8,13–16} Thus, the identification of the external agents that can delay and prohibit the islet amyloid

aggregation can be a potential therapeutic strategy for Type 2 diabetes.^{7,8}

Nowadays, small molecules of natural product origin (secondary metabolites) with remarkable structural diversity, intense biological activities, and reduced toxicity are showing efficacy in preventing the aggregation of various amyloidogenic proteins, viz., A β , IAPP, TTR, etc.^{5,10,17–20} In this connection, it is noteworthy that carbazoles have attracted great attention as A β amyloid aggregation inhibitors.^{21–25} However, extensive studies on their activity toward the inhibition/prevention of islet amyloid aggregation are still rare;²⁶ despite this, several carbazole analogs, especially the carbazole alkaloids like mahanine, koenidine, and mahanimbine, isolated from the leaves of the plant *Murraya koenigii* Spreng. (commonly known as Indian Curry Leaf plant, Fam. Rutaceae), showed efficacy as antidiabetic agents in *in vitro* and in mice model.^{27,28} These naturally occurring carbazoles also showed efficiency toward the improvement of insulin resistance, i.e., activation of the insulin-stimulated glucose uptake pathway to control glucose homeostasis.^{27–29}

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Exploring the efficacy of naturally occurring biflavone based antioxidants towards the inhibition of the SARS-CoV-2 spike glycoprotein mediated membrane fusion

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ABSTRACT

Molecular docking studies were done to show the inhibitory effect of two naturally occurring biflavone based anti-HIV agents, hinokiflavone and robusatflavone against the SARS-CoV-2 spike (S) protein mediated attack on the human ACE2 receptors via membrane fusion mechanism. Nefamostat, a FDA approved drug, well-known as a serine protease inhibitor for MERS-CoV infection, was used as the reference compound. Both the biflavones, showed potential as inhibitors for SARS-CoV-2 S protein-mediated viral entry. The binding affinities of these naturally occurring biflavones for RBD-S2 subunit protein of SARS-CoV-2 were explored for the first time. Such binding affinities play a critical role in the virus-cell membrane fusion process. These biflavones are able to interact more strongly with the residues of heptad repeat 1 and 2 (HR1 and HR2) regions of S2 protein of SARS-CoV-2 compared to nefamostat, and thus, these biflavones can effectively block the formation of six-helix bundle core fusion structure (6-HB) leading to the inhibition of virus-target cell-membrane fusion.

1. Introduction

By the end of 2019, scientists came to know about a novel Corona virus, SARS-CoV-2 [Severe Acute Respiratory Syndrome-Corona virus-2] causing COVID-19 (Corona Virus Disease-19). This initially affected people of Wuhan city of China. Later, this virus became the root cause of deaths and untold sufferings of millions of people around the globe due to the unavailability of specific medicine/vaccine or therapeutic strategies.

Corona viruses (CoVs) are a family of RNA viruses, responsible for mild as well as a range of severe respiratory disease outbreaks and epidemics in human in last two decades e.g. Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS) (World Health Organization, 2019; Masters, 2006; Corman et al., 2019; Lu et al., 2015; WHO, 2004; WHO, 2016). Like, SARS-CoV and MERS-CoV, the very deadly SARS-CoV-2 belongs to β genus of CoVs containing positive-strand RNA (Wu et al., 2020). The size of the genome of SARS-CoV-2 falls in the range of ~30 kb involving 6 to 11 open ring frames (ORFs) (Song et al., 2019). Approximately, 67% of the entire genome is mainly located in the first ORF (ORF1a/ORF1b) which processes two polyproteins, pp1a and pp1ab and also encodes 16–17

non-structural proteins (NSPs) e.g. 3-chymotrypsin-like protease (3CL^{pro}), papain-like protease (PL^{pro}), helicase and RNA-dependent RNA polymerase (RdRp) (Dömling and Gao, 2020). The remaining ORFs encode accessory and structural proteins (Cui et al., 2019). Though SARS-CoV-2 genome has large size (characteristic of RNA virus), it genome encodes for fewer structural proteins; among which four major structural proteins are worth of mentioning: the structural spike (S) glycoprotein, small envelop (E) protein, nucleocapsid (N) protein and membrane (M) protein. These are essential for reproduction of a structurally complete virus particle (Dömling and Gao, 2020).

The spike (S) glycoprotein of CoVs, is responsible for the crown-like shape of the virus (Scheme 1 (a)) and belong to class-I viral fusion proteins, which facilitates the viral entry process into host cells through the binding with the receptors of the host cells, host tropism and pathogenesis (Lu et al., 2015; Millet and Whittaker, 2014). The binding of viral S protein through its receptor-binding domain (RBD) to the host cells instigates various vital steps necessary for viral infections e.g. fusion of viral and host membranes (Li, 2016; Zhu et al., 2018). The S proteins attacks the angiotensin-converting enzyme2 (ACE2) receptors of the host via its RBD and triggers a cascade of inflammation in the lower respiratory tract (Ksiazek et al., 2003; Kuba et al., 2005). Trimeric

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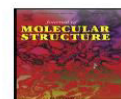
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Understanding the Role of Flavonoid Based Small Molecules in Modulating the Oncogenic Protein-Protein Interactions: A Quest for Therapeutic Arsenal

Abhijit Karmakar^a, Tamanna Mallick^a, Chandrani Fouzder^b, Alpana Mukhuty^b, Samiran Mondal^c, Rakesh Kundu^b, Naznin Ara Begum^{a,*}

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ABSTRACT

We explored the anticancer activity of two synthetic flavonoid-based small molecules, HMDC and HMDF, with bioactive methylenedioxy functionality. HMDF inhibited the proliferation of the p53 wild-type (NCIH460 and A549), and p53 null (NCIH299) non-small cell lung cancer and breast cancer (MCF-7) cells more potently than HMDC without significant cytotoxic effects on the normal lung-epithelial (L132) and macrophage (Raw 264.7) cells. HMDF mediated reduction of the cell proliferation occurred due to its attachment at the p53-binding domain of MDM2 (also evident from molecular docking analysis), which induced the disruption of the p53-MDM2 interactions. Ultimately, a higher expression of p53 in the NCIH460 cells was observed. The up-regulated p53 level instigated apoptosis of cancer cells. However, MDM2 expression level remained unaltered. The docking studies further indicate that HMDF can suppress the anti-apoptotic activity of Bcl-2 protein by blocking its BH3 domain.

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1. Introduction

The diverse range of protein-protein interactions (PPIs) greatly influences a broad spectrum of vital biological processes indispensable for the survival of living organisms [1–5]. However, the disruption of the PPI network is the root cause of many human diseases, most commonly, multiple forms of cancer [6]. Therefore, the identification and modulation, i.e., either inhibition or stabilization of the aberrant PPIs and associated transcription factors that regulate the signaling cascades [3–7], are essential for developing efficient anti-cancer therapeutic agents with lesser side effects.

The murine double minute 2 (MDM2) gene encodes a negative regulator of the tumor suppressor protein 53 (p53) that plays a fundamental role in regulating the cell cycle, apoptotic cell death, DNA repair mechanism, and innate immunity [8]. p53 is the master regulator of several cellular signaling pathways, and it also encodes a redox-sensitive transcription factor which generates a beneficial anti-cancer effect towards the genotoxic DNA damage [9]. Tumor suppressor p53 turns out to be inactive in almost 50% of human cancers, including non-small-cell lung cancer (NSCLC), due to its

mutation or deletion [9–11]. Here, it is noteworthy that lung cancer is the most fatal and critical factor of cancer-related deaths worldwide [12]. Therefore, PPIs involving MDM2 and p53 are among the most widely studied areas of cancer research.

MDM2 effectively suppresses the p53 activity through three mechanisms. Firstly, MDM2 binding to p53 at its trans-activation domain blocks the p53 transcription activity. Secondly, MDM2 can promote the nuclear export of p53, and lastly, MDM2 acts as an E3 ubiquitin ligase triggering the proteasome-mediated degradation of p53 [13–15]. Therefore, the maintenance and revival of the function of p53 with simultaneous inhibition of the MDM2 activities are emerging as promising therapeutic strategies for developing effective anti-cancer drugs [16].

Nowadays, many pieces of research are carried out to shed light on the therapeutic potentials of small molecules towards the modulation of intracellular PPIs. Small molecules are being extensively explored as PPI modulators due to their (i) ability to bind to a specific bio-target, e.g., protein or nucleic acid, and altering its function; (ii) access to a wide range of organs with high cell-penetrating effects and active site-specificity; (iii) ability to modulate multiple targets simultaneously as well as reversibly and (iv) high metabolic stability.

Plant-derived secondary metabolites, e.g., flavonoids, are well-known examples of naturally occurring small molecules with po-

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TO WHOM IT MAY CONCERN

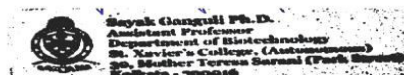
This is to state that my lab is in active collaboration with **Dr. Santi Ranjan Dey** and his group at Rammohan College, Kolkata and we are currently working on the following aspects:

1. Digital Key and database development for *Neuroptera*.
2. Mango Germplasm conservation using metagenomics approaches.
3. Computational Analysis of viral genomes having direct impact on crop yield.

Till date, the collaboration has yielded satisfactory results and in the near future we look forward to more fruitful outcomes using the institutional facilities available.

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IN SEARCH OF CONSERVED RNA MOTIFS OF DENGUE GENOME OF ALL SEROTYPE: A BIOINFORMATIC APPROACH

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Abstract: RNA viruses use small genomes that contain information in both their core sequences and higher-order structures to hijack cellular metabolism and encourage their own replication. By identifying particular sequences that are conserved throughout a collection of related viruses, the majority of functional structures that have been discovered to date. We effectively find numerous hitherto unannotated motifs crucial for viral fitness by flipping the traditional technique, which defines RNA structures first before checking for conservation of these motifs. In addition to identifying possible motifs helpful in the development of antiviral medicines and vaccines, this work demonstrates the ability of RNA structure as a tool for discovering functional elements in viruses. It also paves the way for additional functional element identification in big viral messenger as well as non-coding RNAs. A virus known as dengue virus 1 (DEN-1) was isolated by Walter Schlesinger and Albert B. Sabin. The four closely related viruses that cause dengue diseases are DEN-1, DEN-2, DEN-3, and DEN-4. They are known as serotypes because the antibodies in human blood serum react differently with each of these four viruses. The four dengue viruses are related and share roughly 65% of their genomes despite the fact that there is a great deal of genetic heterogeneity within a single serotype. Despite these variations, all dengue serotype infections result in the same illness and a set of same clinical symptoms. In this research, we looked for a specific or conservative RNA pattern that could be used to neutralize the DENGUE virus, by targeting RNA in future.

Key Words: Dengue, RNA, Serotype, RNA-motif, Dengue-protein

I. INTRODUCTION

A wide range of living things, including bacteria, plants, and animals, can become infected by viruses, which are little agents. The dengue virus is an ultra microscopic entity that can only replicate inside a host organism, like other viruses. The family Flaviviridae's genus Flavivirus contains the dengue viruses. This genus contains a variety of additional viruses that cause human infections and are spread by ticks and mosquitoes in addition to the dengue virus. Yellow fever, West Nile, Japanese encephalitis, and tick-borne encephalitis viruses are all classified as flaviviruses. REN KIMURA and SUSUMU HOTTA discovered the dengue virus in 1943.

These two researchers were looking at blood samples taken from patients in Nagasaki, Japan, during the 1943 dengue epidemic. A year later, the dengue virus was separately isolated by Albert B. Sabin and Walter Schlesinger. The virus that is now known as dengue virus 1 (DEN-1) had been isolated by both teams of researchers. The DEN-1, DEN-2, DEN-3, and DEN-4 viruses are four closely related viruses that cause dengue illnesses. Because each of these four viruses interacts differently with the antibodies in human blood serum, they are referred to as serotypes. Even while there is considerable genetic variation within a single serotype, the four dengue viruses are similar and share about 65% of their genomes. All dengue serotype infections cause the same sickness and similar set of clinical signs, despite these differences.

All four serotypes were discovered in Southeast Asia in the 1970s and both DEN-1 and DEN-2 were discovered in Central America and Africa. The four serotypes were, however, widely dispersed geographically by 2004. Currently, all four dengue serotypes coexist in tropical and subtropical areas of the world (Fig. 1). The four dengue serotypes have similar geographic and ecological niche. Scientists hypothesize that the dengue viruses evolved in nonhuman primates and jumped from these primates to humans in Africa or Southeast Asia between 500 and 1,000 years ago.

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