

# Source details

## Pharmacognosy Journal

Scopus coverage years: from 2009 to Present

Publisher: Pharmacognosy Network Worldwide

ISSN: 0975-3575

Subject area: Pharmacology, Toxicology and Pharmaceutics: Pharmacology

Pharmacology, Toxicology and Pharmaceutics: Drug Discovery

Source type: Journal

CiteScore 2020

1.6



SJR 2020

0.268



SNIP 2020

0.775



[View all documents >](#)

[Set document alert](#)

[Save to source list](#)

[CiteScore](#) [CiteScore rank & trend](#) [Scopus content coverage](#)

### i Improved CiteScore methodology ×

CiteScore 2020 counts the citations received in 2017-2020 to articles, reviews, conference papers, book chapters and data papers published in 2017-2020, and divides this by the number of publications published in 2017-2020. [Learn more >](#)

CiteScore <sup>2020</sup> ▾

$$1.6 = \frac{1,368 \text{ Citations 2017 - 2020}}{852 \text{ Documents 2017 - 2020}}$$

Calculated on 05 May, 2021

CiteScoreTracker 2021 ⓘ

$$1.8 = \frac{1,747 \text{ Citations to date}}{946 \text{ Documents to date}}$$

Last updated on 06 April, 2022 • Updated monthly

### CiteScore rank 2020 ⓘ

Category	Rank	Percentile
Pharmacology, Toxicology and Pharmaceutics — Pharmacology	#214/297	28th
Pharmacology, Toxicology and Pharmaceutics — Drug Discovery	#112/145	23rd

[View CiteScore methodology >](#) [CiteScore FAQ >](#) [Add CiteScore to your site](#)



### Evidence Based Nursing Paper

Submit Your Nursing and Midwifery Research With Hinc

Hindawi

## Pharmacognosy Journal

**COUNTRY**

India

Universities and research institutions in India

**SUBJECT AREA AND CATEGORY**

Pharmacology, Toxicology and Pharmaceutics  
Drug Discovery  
Pharmacology

**PUBLISHER**

EManuscript Technologies

**H-INDEX**

21

**Telkomsel**

Koneksi internet cepat, pakai Orbit tetap hemat!

Beli paket sesuai kebutuhan, banyak tambahan paket dan potongan harga. Yuk, gabung bersama 250 ribu pengguna lainnya dan nikmati hematnya!

[Cek Promosi Hematnya](#)

**PUBLICATION TYPE**

Journals

**ISSN**

09753575

**COVERAGE**

2009-2020

**INFORMATION**

[Homepage](#)  
[How to publish in this journal](#)  
[editor@phcoj.com](mailto:editor@phcoj.com)

Koleksi

Ads by Google

[Stop seeing this ad](#) [Why this ad?](#)

Buka

SCOPE


Pharmacognosy Journal (Phcog J.) covers different topics in natural product drug discovery, and also publishes manuscripts that describe pharmacognostic investigations, evaluation reports, methods, techniques and applications of all forms of medicinal plant research

Join the conversation about this journal

Satu aplikasi untuk nikmati berjuta fungsi

Kontrol jumlah pembelian, pemakaian internet, transfer, hingga akses ke rekening, semua resmi!

[Cek Info Selengkapnya](#)



Quartiles

FIND SIMILAR JOURNALS

Unity Pro

E-BOOK

GET THE SCOOP ON TAKING YOUR GAMES TO MAC.

1 **Oriental Pharmacy and Experimental Medicine**  
USA

**82%**  
similarity

2 **Pharmacognosy Research**  
IND

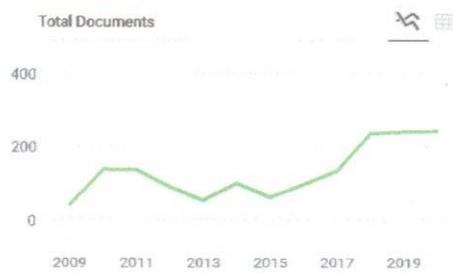
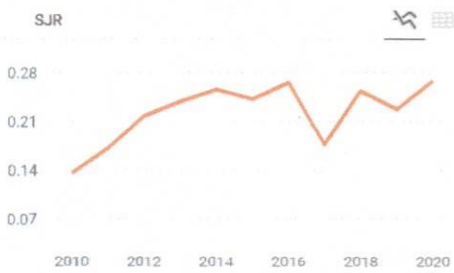
**81%**  
similarity

3 **Indian Journal of Natural Products and Resources**  
IND

**77%**  
similarity

4 **Journal of Herbs, Spices and Medicinal Plants**  
USA

**76%**  
similarity

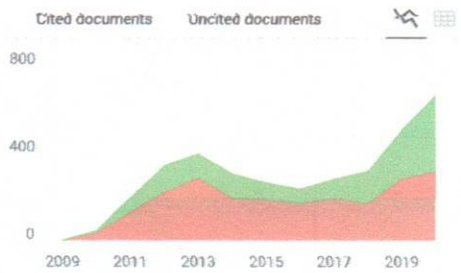
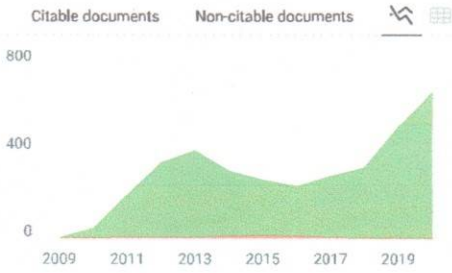
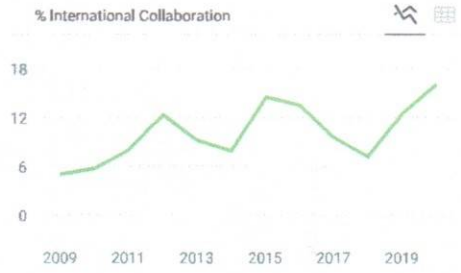


Total Cites    Self-Cites

Citations per document

Ads by Google

[Stop seeing this ad](#)    [Why this ad?](#)



**Pharmacognosy Journal** ← Show this widget in your own website

Q3 Drug Discovery  
best quartile

SJR 2020 0.27

powered by scimagojr.com

Just copy the code below and paste within your html code:

```
<a href="https://www.scimagojr.com" style="color: #e67e22; text-decoration: none;">

```

### SCImago Graphica

Explore, visually communicate and make sense of data with our new free tool.

Get it



Metrics based on Scopus® data as of April 2021

N **Najib** 10 months ago

Is this journal still indexed by scopus or have been discontinued?

reply



**Melanie Ortiz** 10 months ago

Dear Najib,  
Thank you very much for your comment.

All the metadata have been provided by Scopus /Elsevier in their last update sent to

SCImago Team

Ads by Google

Stop seeing this ad Why this ad?



# Pharmacognosy Journal (/)

An Open Access, Peer Reviewed Journal in the field of Pharmacognosy

Enter terms then hit Search...



[Articles In Press \(/inpress\)](#)

[Current Issue \(/v14/i1\)](#)

[Archives \(/archives\)](#)

[RSS Feeds \(/rss.xml\)](#)

[Submit Article \(https://www.phcogj.info\)](https://www.phcogj.info)



[\(http://www.facebook.com/ehvito8.com/#!/\)](http://www.facebook.com/ehvito8.com/)

[HOME \(/\)](#) / [EDITORIAL BOARD \(2020-21\)](#)

[View \(/editorial-board-2020-21\)](#)

[What links here \(/node/25/backlinks\)](#)

Time to read  
1 minute

## Editorial Board (2020-21)

Share



**Dr. Djemli Samir**  
Department of Biology , Applied Neuroendocrinology Laboratory  
Bach Mokrani Annaba University  
<https://www.facebook.com/sharer/sharer.php?u=https%3A%2F%2Fphcogj.com%2Feditorial-board-2020-21&t=Editorial+Board+%282020-21%29>



**Dr. Raghava Naidu, Ph.D**  
Department of Human Oncology,  
University of Wisconsin,  
1111, Highland Ave, Madison,  
Wisconsin 53705, USA  
<https://plus.google.com/share?url=https%3A%2F%2Fphcogj.com%2Feditorial-board-2020-21>  
**Dr. Karim Raafat**  
Associate Professor of Pharmacognosy and Phytochemistry,  
Pharmaceutical Sciences Department,

2020- Faculty of Pharmacy,  
21) Beirut Arab University (BAU),  
Beirut 115020, Lebanon



(<http://twitter.com/EditorialBoard2020-21>)  
<https://phcogj.com/Editorial-board-2020-21>  
 text=Editorial+Board+%282020-21%29&url=https%3A%2F%2Fphcogj.com%2Feditorial-board-2020-21

board-

2020- **Janib Achmad**  
21) Lecturer of Faculty of Fisheries and Marine Science,  
University of Khairun Ternate  
Kampus 2 Jalan Pertamina, Kelurahan Gambesi,  
Ternate Selatan

Print

a- a+

**Muammar Fawwaz, Ph.D**  
Department of Pharmaceutical Chemistry  
Faculty of Pharmacy  
Universitas Muslim Indonesia  
Makassar 90231, South Sulawesi, Indonesia

**Hany Ezzat Khalil**  
Associate Professor,  
College of Clinical Pharmacy,  
King Faisal University,  
KSA

**Emad Yousif**  
Department of Chemistry  
College of Science  
Al-Nahrain University  
Baghdad, Iraq

**Sughosh Upasani**  
R.C Patel Institute of pharmacy,  
Shirpur, Dist-Dhule, Maharashtra,  
India.

**Gurusiddaiah suresh kumar**  
Scientist  
Dept of biochemistry  
CSIR-CFTRI  
Mysore, Karnataka, INDIA

**Arjun Patra**  
Assistant Professor  
School of Pharmaceutical Sciences

**Guru Ghasidas Central University**

Koni, Bilaspur - 495 009

Chattisgarh, India

**Francis O. Atanu, Ph.D**

Department of Biochemistry

Faculty of Natural Sciences

Kogi State University

Anyigba, Nigeria.

**Vijay Kumar Chattu**

Faculty of Medical Sciences

University of the West Indies

St. Augustine, Trinidad &amp; Tobago.

**Dr.Kunle Okaiyeto, PhD**

Applied and Environmental Microbiology Research Group (AEMREG)

Department of Biochemistry and Microbiology

University of Fort Hare

Alice campus

5700, Alice

South Africa.

**Dr. Srisailam Keshetti, Ph.D**

Principal, University College of Pharmaceutical Sciences, Satavahana University

Karimnagar 505001

Telangana

INDIA

**Dr. Gayathri M Rao**

Associate Professor

Department of Biochemistry

Kasturba Medical Collge, Mangaluru.

**Shuge Tian**

Experimental Teaching Demonstration Center of TCM in Xinjiang Medical University

Department of traditional medicine ,TCM

Xinjiang Medical University

Xinjiang CHINA 830054

**Dr. Ramachandra Setty Siddamsetty,**

Professor, Govt College of Pharmacy,

Mission Road, Bengaluru, INDIA







**Distinctions:** The most widely read, cited, and known Pharmacognosy journal and website is well browsed with all the articles published. More than 50,000 readers in nearly every country in the world each month

**ISSN :** 0975-3575 ; Frequency : Rapid at a time publication (6 issues/year)

**Indexed and Abstracted in :** SCOPUS, Scimago Journal Ranking, Chemical Abstracts, Excerpta Medica / EMBASE, Google Scholar, CABI Full Text, Index Copernicus, Ulrich's International Periodical Directory, ProQuest, Journalseek & Genamics, PhcogBase, EBSCOHost, Academic Search Complete, Open J-Gate, SciACCESS.

**Rapid publication:** Average time from submission to first decision is 30 days and from acceptance to In Press online publication is 45 days.

**Open Access Journal:** Pharmacognosy Journal is an open access journal, which allows authors to fund their article to be open access from publication.

## Submit your Next Article

- Online submission
- Highly indexed and abstracted
- 10 years of successful publishing
- Wider visibility though open access
- Higher impact with wider visibility
- Prompt review

## Submit your next article to Phcog J

and be a part of many successful authors.

Create free account (<http://phcogj.info>)

(<https://www.phcogj.com/submissions/index.php/phcogj/index>)

/ Login

---

Copyright © 2020 Pharmacogn J. All rights reserved.

**Pharmacognosy Journal** and its contents are licensed under a Creative Commons Attribution-Non Commercial-No Derivs 4.0 License. Permissions beyond the scope of this license may be available with [editor@phcogj.com](mailto:editor@phcogj.com) (<mailto:editor@phcogj.com>)

[Home \(/\)](#)

[Advertise with us \(/\)](#)

[Home \(/\)](#)[About Journal](#) ▾[Editorial Board \(/editorial-board-2020-21\)](#)[For Authors](#) ▾[Contact Us \(/contact-us\)](#)

# Pharmacognosy Journal (/)

An Open Access, Peer Reviewed Journal in the field of  
Pharmacognosy

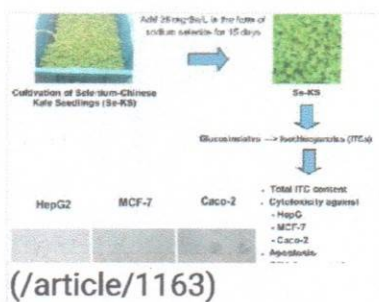
Enter terms then hit Search...

[Articles In Press \(/inpress\)](#)[Current Issue \(/v14/i1\)](#)[Archives \(/archives\)](#)[RSS Feeds \(/rss.xml\)](#)[Submit Article \(https://www.phcogj.info\)](https://www.phcogj.info)

[HOME \(/\)](#) / PHARMACOGNOSY JOURNAL,  
VOL 12, ISSUE 4, JUL-AUG 2020

## Pharmacognosy Journal, Vol 12, Issue 4, Jul-Aug 2020

### RECENT ARTICLES



Original Article

### Cytotoxicity of Selenium-Enriched Chinese Kale (*Brassica oleracea* var. *alboglabra* L.) Seedlings Against Caco-2, MCF-7 and HepG2 Cancer Cells (/article/1163)

Vijitra Luang-In, Worachot Saengha, Benjaporn Buranrat, Anut Chantiratikul, Nyuk Ling Ma

Pharmacognosy Journal, 12(4):674-681

DOI: 10.5530/pj.2020.12.99

Published: Mon, 15-Jun-2020

[Read More \(/article/1163\)](#)

Original Article



## The Extract of Kincung Flower (*Etilingera elatior* (Jack) R.M.Sm.) Activity to Decrease IL-4 and IgE Levels in Type I Hypersensitivity White Male Mice (/article/1164)

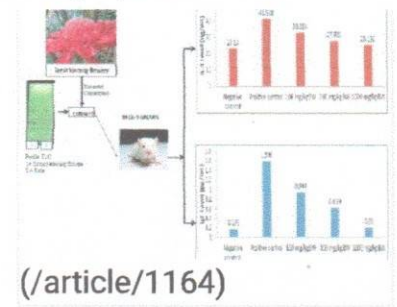
Elidahanum Husni, Relin Yesika, Yufri Aldi

Pharmacognosy Journal, 12(4):682-686

DOI: 10.5530/pj.2020.12.100

Published: Mon, 15-Jun-2020

[Read More \(/article/1164\)](#)

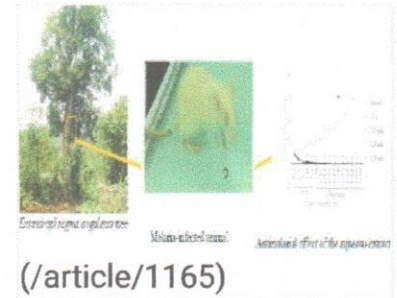


[\(/article/1164\)](#)

Original Article

## Antimalarial Effects of the Aqueous Extract of *Entandrophragma angolense* Bark on *Plasmodium berghei* Infection in Mice (/article/1165)

Raceline Gounou Kamkumo, Abel Narcisse M. Betene, Patrick Valere Tso Fokou, Jean Hubert Donfack, Marius Jaurès Ts Nangap, Albertine Ngako, Roberto Fokou, Mariscal Brice Tcha Tali, Florence Ngueguim Tsofack, Théophile Dimo, Fabrice Fekam Boyom



[\(/article/1165\)](#)

Pharmacognosy Journal, 12(4):687-698

DOI: 10.5530/pj.2020.12.101

Published: Mon, 15-Jun-2020

[Read More \(/article/1165\)](#)

Original Article

## Assessment of the Impact of Wild Stinkhorn Mushroom Extracts on Different Cancer Cell Proliferation and Study of Primary Metabolites (/article/1166)

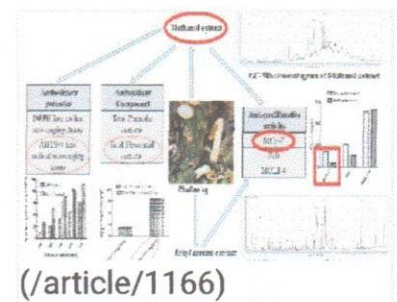
Ribhu Ray, Amrita Pal, Santanu Paul

Pharmacognosy Journal, 12(4):699-708

DOI: 10.5530/pj.2020.12.102

Published: Mon, 15-Jun-2020

[Read More \(/article/1166\)](#)



[\(/article/1166\)](#)

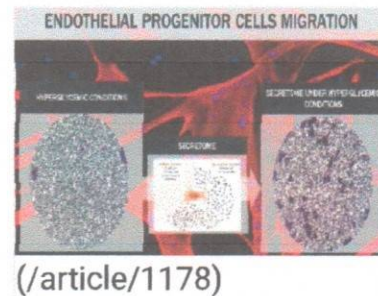
Original Article

**DOI:** 10.5530/pj.2020.12.112**Published:** Mon, 15-Jun-2020[Read More \(/article/1176\)](#)

Original Article

**Human Umbilical Cord Blood-derived Secretome Enhance Endothelial Progenitor Cells Migration on Hyperglycemic Conditions (/article/1178)**

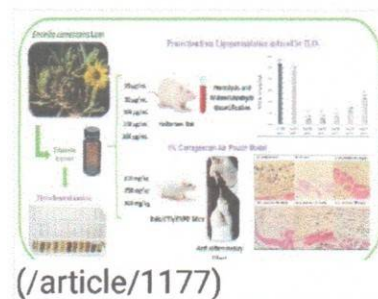
Yudi Her Oktaviono, Melly Susanti, Achmad Lefi, Ferry Sandra

[\(/article/1178\)](#)**Pharmacognosy Journal**,12(4):793-797**DOI:** 10.5530/pj.2020.12.113**Published:** Mon, 15-Jun-2020[Read More \(/article/1178\)](#)

Original Article

**Protection of Erythrocytes against Lipoperoxidation and Antiinflammatory Effects of Ethanolic Extract of Encelia canescens Lam Leaves in Mice (/article/1177)**

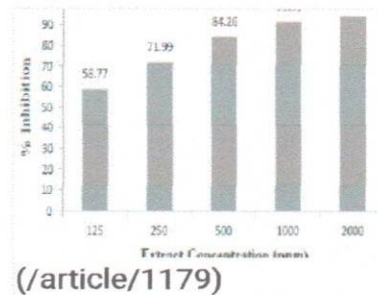
Fernández-Flores N, Rojas-Cardenas NF, Vásquez-Quispe AD, Chávez-Flores Juana E, Justil-Guerrero Hugo J, Parreño-Tipian JM, Silva-Correa Carmen R, Villarreal-La Torre Víc E

[\(/article/1177\)](#)**Pharmacognosy Journal**,12(4):798-804**DOI:** 10.5530/pj.2020.12.114**Published:** Mon, 15-Jun-2020[Read More \(/article/1177\)](#)

Original Article

**Antidiabetic Activity with N-Hexane, Ethyl-Acetate and Ethanol Extract of Halodule uninervis Seagrass (/article/1179)**

Ace Baehaki, Susi Lestari, Muhammad Hendri, Febri Ariska

[\(/article/1179\)](#)**Pharmacognosy Journal**,12(4):805-808**DOI:** 10.5530/pj.2020.12.115**Published:** Mon, 15-Jun-2020[Read More \(/article/1179\)](#)



- Higher impact with wider visibility
- Prompt review

**Submit** your next article to Phcog J

and be a part of many successful authors.

**Create free account** (<http://phcogj.info>)

(<https://www.phcogj.com/submissions/index.php/phcogj/index>)

/ Login

---

Copyright © 2020 Pharmacogn J. All rights reserved.

**Pharmacognosy Journal** and its contents are licensed under a Creative Commons Attribution-Non Commercial-No Derivs 4.0 License. Permissions beyond the scope of this license may be available with [editor@phcogj.com](mailto:editor@phcogj.com) (<mailto:editor@phcogj.com>)

[Home \(/\)](#)

[Advertise with us \(/\)](#)

[Privacy Statement \(/\)](#)



# Human Umbilical Cord Blood-derived Secretome Enhance Endothelial Progenitor Cells Migration on Hyperglycemic Conditions

Yudi Her Oktaviono<sup>1,\*</sup>, Melly Susanti<sup>1,\*</sup>, Achmad Lefi<sup>1</sup>, Ferry Sandra<sup>2,3</sup>

Yudi Her Oktaviono<sup>1,\*</sup>, Melly Susanti<sup>1,\*</sup>, Achmad Lefi<sup>1</sup>, Ferry Sandra<sup>2,3</sup>

<sup>1</sup>Department of Cardiology and Vascular Medicine, Faculty of Medicine, Universitas Airlangga, Prof Moestopo Street 6-8, Surabaya, INDONESIA.

<sup>2</sup>Prodia Stem Cell Laboratory, Kramat 7 No. 11 Street, Jakarta, INDONESIA.

<sup>3</sup>Department of Biochemistry and Molecular Biology, Faculty of Dentistry, Universitas Trisakti, Kyai Tapa Street No.260, Jakarta, INDONESIA.

## Correspondence

Yudi Her Oktaviono

Department of Cardiology and Vascular Medicine, Faculty of Medicine, Universitas Airlangga, Prof Moestopo Street 6-8, Surabaya, INDONESIA.

E-mail: yoktaviono@gmail.com

## History

- Submission Date: 02-04-2020;
- Review completed: 30-04-2020;
- Accepted Date: 19-05-2020.

DOI : 10.5530/pj.2020.12.113

## Article Available online

<http://www.phcogj.com/v12/i4>

## Copyright

© 2020 Phcogj.Com. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International license.

## ABSTRACT

Hyperglycemia state is harmful to body's homeostasis. Uncontrolled hyperglycemic patients, especially patients with diabetes mellitus have a higher mortality risk of heart disease 2 to 4 times compared to non-hyperglycemic patients. Vascular endothelial impairment always been observed and found as a key feature of hyperglycemia state, which is correlated with reduced numbers and dysfunction of endothelial progenitor cells (EPCs). **Objective:** This paper aims to investigate the effect of hUCB-MSCs derived secretome treatment on the EPCs migration under hyperglycemia state. **Material and Method:** EPCs were isolated and cultured from peripheral blood samples and cultured for three days. Cultured EPCs were cultivated in 6-well plates until confluence and incubated with high glucose for 5 days, then placed in the modified Boyden chamber at the upper chamber with basal media. The lower chamber was supplemented with basal media and secretome at 2%, 10%, and 20% concentration and VEGF treated group as a control. EPCS migration was evaluated using a Boyden chamber assay. Statistical analysis was performed using SPS 25.0. **Results:** EPCs migration were significantly higher when hUCB-MSCs-derived secretome was given in high glucose concentrations compared to the and control group ( $79.80 \pm 5.07$  vs  $51.00 \pm 5.15$ ,  $p < 0.000$ ). This study also showed that hUCB-MSCs-derived secretome increase EPCs migration under high glucose concentrations in a dose-dependent manner ( $p < 0.05$ ). **Conclusion:** hUCB-MSCs-derived secretome enhances EPCs migration under hyperglycemic state. This result may be of relevance for cell-free and regenerative therapeutic modality for a diabetic patient with coronary artery disease (CAD).

**Key words:** Mesenchymal stem cells, Endothelial progenitor cells, Secretome, Migration, Hyperglycemia state.

## INTRODUCTION

Cardiovascular disease, especially coronary heart disease has been always correlated with the long-term prognosis of patients with uncontrolled hyperglycemia, especially diabetes mellitus patients. Uncontrolled hyperglycemic patients have a higher mortality risk of heart disease ranging from 2 to 4 times higher compared to non-hyperglycemic patients.<sup>1</sup> Vascular endothelial impairment has been well observed and found as the main culprit of coronary heart disease in uncontrolled hyperglycemic patients.<sup>2-6</sup> In uncontrolled hyperglycemic patients, endothelial dysfunction is caused by low levels and dysfunction of circulating endothelial progenitor cells (EPCs). Disruption of EPC function was found and observed in both of DM types, DM type 1<sup>3</sup> and 2. This finding proved that there is a correlation between uncontrolled hyperglycemia conditions and EPC dysfunction.<sup>4</sup>

After being recruited from the bone marrow, the circulating EPCs have the capability to differentiate into mature endothelial cells and also promote endothelial repair. It has been documented that EPCs plays major roles in both the stimulation of angiogenesis and vasculogenesis in human body.<sup>5</sup> Hence, improvement in EPCs function may be very

beneficial in reducing cardiovascular complications, especially coronary heart disease in uncontrolled hyperglycemic patients.<sup>6</sup>

Human mesenchymal stem cells (hMSCs) are multipotent stem cells that can be isolated from various tissue, such as bone marrow, adipose tissue, and postnatal tissue<sup>7</sup> as used on this study is the human umbilical cord blood (hUCB-MSCs). The therapeutic effect of hMSCs is believed to originate from the soluble factors or cytokines known as secretome, which shown to increase neovascularization and angiogenesis.<sup>8-11</sup> But its capability under hyperglycemia conditions has not yet been illustrated.

This study aims to investigate the migration capability of endothelial progenitor cells (EPCs) under hyperglycemia conditions, which is expected to mimic the metabolic disturbance that occurs in patients with type 2 diabetes mellitus.

## MATERIALS AND METHODS

### Sample criteria

This is an in-vitro study, with true experimental post-test only control group design. The blood sample was obtained from CAD patients in Dr. Soetomo General

**Cite this article:** Oktaviono YH, Susanti M, Lefi A, Sandra F. Human Umbilical Cord Blood-derived Secretome Enhance Endothelial Progenitor Cells Migration on Hyperglycemic Conditions. *Pharmacogn J.* 2020;12(4):793-7.

Hospital, Surabaya, Indonesia. The inclusion criteria were male, aged 40–59 years old, history of chronic ischemic heart disease as proven by coronary angiography results that showed >50% stenosis of left main coronary artery or >70% of other coronary arteries. The exclusion criteria were a history of the percutaneous coronary intervention procedure, coronary artery bypass grafting surgery, acute coronary syndromes, diabetes mellitus and anaemia. This study protocol has an ethical clearance from the Health Research Ethics Committee of Dr. Soetomo General Hospital, Surabaya (No.1567/KEPK/X/2019, approved on October 8<sup>th</sup>, 2019). The included subject has signed informed consent before subject recruitment.

### The hUCB-MSCs-derived secretome preparation

Preparation of hUCB-MSCs-derived secretome was conducted as described in a previous study.<sup>12</sup> hUCB-MSCs (3H Biomedical AB, Uppsala, Sweden) was cultured in Mesencult media (StemCell Technologies Inc., Vancouver, Canada) containing penicillin and streptomycin. Upon reaching 80% confluency, the media was replaced with supplement-free media and incubated for 24 hours. The media was collected and centrifuged. Supernatant was used as a conditioned medium contained with hUCB-MSCs-derived secretome.

### EPCs treatment

Cultured EPCs were treated with high glucose and various concentrations of hUCB-MSCs-derived secretome (2%, 10% and 20%). The control group was given VEGF 100ng/mL.

### EPCs isolation and culture

EPCs were isolated from mononuclear cells (MNCs) of the peripheral blood of CAD patient. 40 ml of blood was withdrawn and put into conical tubes and diluted with Phosphate buffer saline with 2% fetal bovine serum. Afterwards, Ficoll histopaque was added to the mixtures. And then centrifugation was done until peripheral blood MNCs layer was formed. And then  $5 \times 10^5$  cells/mL PBMCs were cultured with basal Stemline II hematopoietic stem cell expansion medium (Sigma-Aldrich, St. Louis, MO, USA) supplemented with 15% fetal bovine serum and several growth factors in the fibronectin-coated 6-well plates. The culture was maintained at 37 C with 5% CO<sub>2</sub> in a humidified atmosphere. Non-adherent cells were discarded, and fresh medium was added, after two days of culture. After three days of EPCs isolation and culture, EPCs were confirmed using FITC-labeled anti-human CD34 antibody (Biolegend, USA) staining and examined with immunofluorescence microscopy.

### Migration assay

This experiment was performed using Costar® Transwell® Permeable Support (Corning, USA) with a 3.0 µm pore size membrane. EPCs migration was calculated using a Boyden chamber assay method. Isolated EPCs were detached using Trypsin EDTA solution (Sigma-Aldrich, USA). A total of  $5 \times 10^5$  EPCs were incubated with high glucose (25 mmol/L D-Glucose, Sigma-Aldrich, USA). After 5 days, cells were placed in the modified Boyden chamber at the upper chamber with basal media. The lower chamber was supplemented with basal media and secretome at 2%, 10%, and 20% concentration. The culture was incubated at 37° C for 24 hours. The non-migratory cells were removed manually. Meanwhile, the migratory EPCs below the upper chamber were fixed with 3.7% paraformaldehyde and permeabilized with methanol. Migrated EPCs were stained with Crystal Violet stain and calculated.

### Statistical analysis

Statistical analyses were conducted using SPSS Statistics 24.0 from IBM to detect significance level at  $p < 0.05$ . Data were evaluated for normal

distribution using the Kolmogorov Smirnov test and compared between groups using a one-way ANOVA test for migration and proliferation. Correlation between variables was obtained using Spearman correlation followed by a linear regression test.

## RESULTS

### EPCs characteristics

EPCs were successfully confirmed from peripheral blood MNCs, marked by positive CD34 on immunofluorescence. Light microscope view showed spindle-shaped cells which also characterize EPCs (Figure 1).

### Effect of hUCB-MSCs-derived Secretome on EPC migration

hUCB-MSCs-derived secretome under non-glucose conditions induced a higher migration rate of EPC in a dose-dependent manner, but when compared to VEGF treated group, the migration was not significant.

### The hUCB-MSCs-derived Secretome improved EPCs migration under high glucose conditions

As shown in Figure 2, EPCs migration was significantly higher treated with secretome of 20% concentrations under high glucose concentrations compared to VEGF treated EPCs ( $79.80 \pm 5.07$  vs  $51.00 \pm 4.85$ ,  $p < 0.000$ ). EPCs migration was improved with a dose-dependent manner on the treatment with secretome alone and high glucose conditions. EPCs migration was significantly higher in secretome treated under high glucose concentrations groups compared to the secretome alone at 2% ( $40.40 \pm 4.72$  vs  $17.20 \pm 1.92$ ,  $p < 0.000$ ), 10% ( $56.00 \pm 5.34$  vs  $27.00 \pm 4.00$ ,  $p < 0.000$ ), and 20% concentrations ( $79.80 \pm 5.07$  vs  $51.00 \pm 5.15$ ,  $p < 0.000$ ).

Treatment with secretome under high glucose concentrations group had a moderate correlation with EPCs migration ( $r = 0.401$ ;  $p < 0.000$ ). The linear regression test showed an r-square of 0.638. This suggested that the treatment with secretome under high glucose concentrations is responsible for 63.8% of EPCs migration.

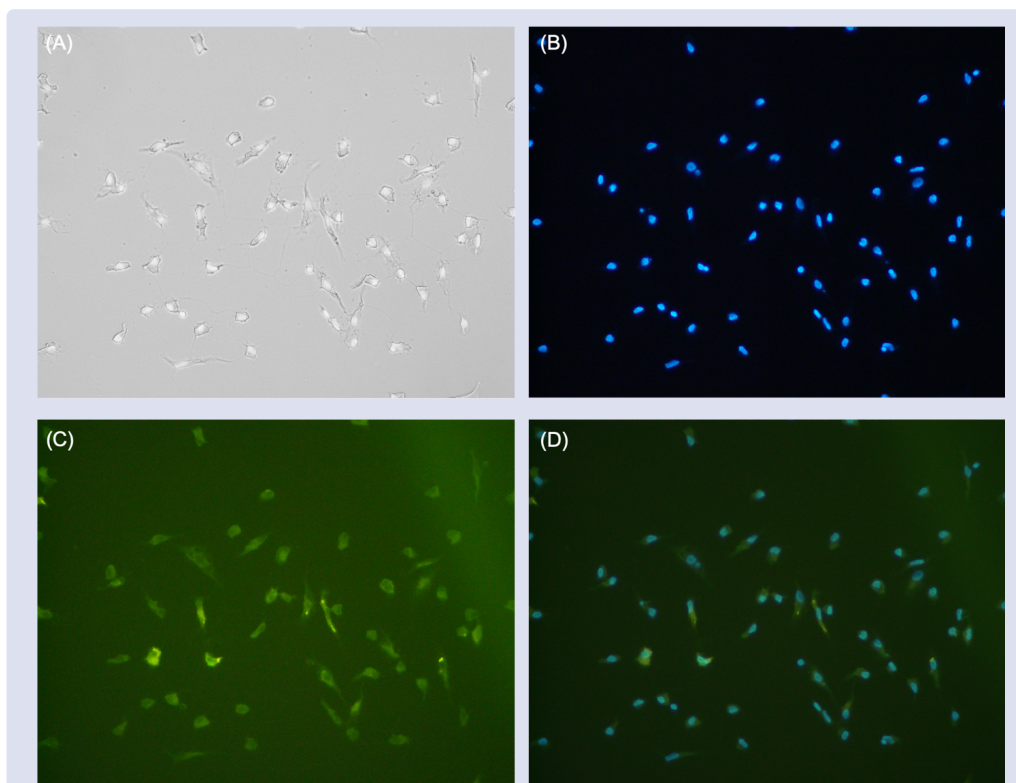
## DISCUSSION

In this study, we show that hUCB-MSCs-derived secretome has the capability of inducing the migration of EPC in a dose dependent manner under none and high glucose concentrations. Surprisingly, this effect statistically significant only with 20% concentrations of secretome and under high glucose concentrations, compared to VEGF treated group as a control.

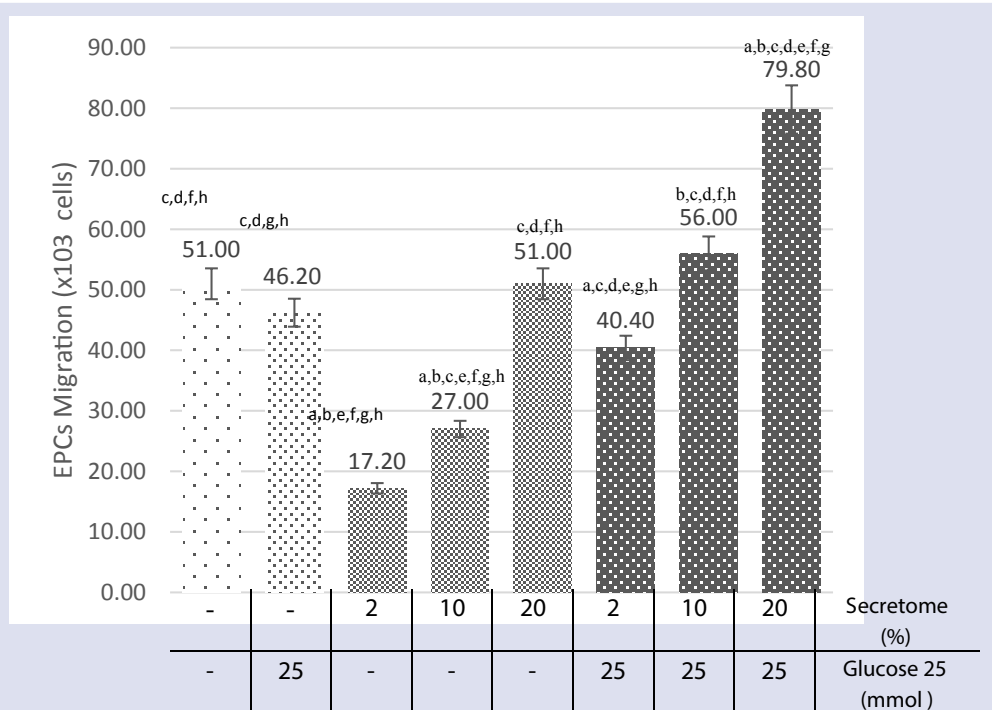
The impaired migratory capacity of EPC in diabetic patients compared to healthy subjects has been well demonstrated in the previous study<sup>3,13</sup>, it was also demonstrated the upregulated VEGF expression as a response of the low level of circulating EPCs. Unfortunately, the increased expression of VEGF was not followed by the improvement of EPCs number, thus therapeutic angiogenesis strategies by intensifying plasma VEGF may not be effective.<sup>13</sup>

The survival, migration, and angiogenesis capability of EPCs are known to be related by the expression and phosphorylation of endothelial nitric oxide synthase (eNOS).<sup>14-17</sup> Nitric oxide derived from eNOS plays a major role in vascular integrity by upregulating VEGF expression.<sup>15</sup> Hyperglycemia state was associated with inadequate production of eNOS activation, thus increasing the production of reactive oxygen species (ROS), which further impaired EPCs function.<sup>14-16</sup> However, the pathophysiology behind the impairment of EPC in diabetic patients, have been described by Chen *et al.*, which showed the reversal of EPC function after NO donor, not by antioxidants.<sup>14</sup>

Cytokine gradient has also been proposed as an essential mechanism behind the impairment of EPCs mobilization.<sup>8</sup> The stromal cell-derived



**Figure 1:** Microscopic finding of EPCs after three days incubation and culture (A) Light microscope view showing spindle shape of EPC (B) Blue fluorescent showing nuclear image of EPCs with diamidino-2-phenylindole (DAPI) staining (C) Immunofluorescence view of EPCs stained with FITC-labelled anti-CD34 antibody (D) Merged view of B and C.



**Figure 2:** Comparison of EPCs migration effects among all treatment groups (see text). a: Significant difference compared to the control group ( $p < 0.000$ ), b: Significant difference compared to the 25 mmol glucose group, ( $p < 0.002$ ), c: Significant difference compared to the 2% secretome group, ( $p < 0.002$ ), d: Significant difference compared to the 10% secretome group, ( $p < 0.002$ ), e: Significant difference compared to the 20% secretome group ( $p < 0.001$ ), f: Significant difference compared to 2% secretome under 25 mmol glucose group, ( $p < 0.001$ ), g: Significant difference compared to 10% secretome under 25 mmol glucose group, ( $p < 0.002$ ) and h: Significant difference compared to 20% secretome under 25 mmol glucose group, ( $p < 0.000$ ).



factor 1 (SDF-1 $\alpha$ ), through stimulation of its receptor (CXCR4) on the EPCs membrane, is a noteworthy chemokine that responsible for the mobilization of EPC from bone marrow to ischemic tissues.<sup>8,15</sup> Diabetes has been associated with the impaired production of SDF-1 $\alpha$  in the ischemic tissue. In contrast, production increased in bone marrow, which leads to a reduced chemoattractant gradient to mobilized EPCs from the bone marrow.<sup>15</sup>

Incorporating findings from previous studies, we hypothesize that improving the production of NO and increasing the concentration of chemoattractants, especially SDF-1 $\alpha$ , may improve the EPCs migration capability.

The hUCB-MSCs exceptionally secrete higher amounts of important cytokines and growth factors, *via* either direct secretion or exosomes and microvesicles, compared to bone marrow derived MSCs.<sup>18</sup> In terms of improving EPCs migration, secreted factors like G-CSF, GM-CSF, SDF1, insulin-like growth factor 1 (IGF1), placental growth factor (PIGF), PDGF- $\beta$ ,<sup>8,18,19</sup> and enhancing expression of iNOS through the secretion of extracellular vesicles,<sup>20</sup> may play a major role in the mechanism of the hUCB-MSCs secretome capability.

## CONCLUSION

In this study, we report for the first time, the paracrine effect associated with secreted factors from hUCB-MSCs, collectively known as secretome, have the capability of inducing EPCs migration under high glucose concentrations, thus may be of relevance for cell-free and regenerative therapeutic modality for a diabetic patient with CAD.

## ACKNOWLEDGEMENTS

The authors are thankful to Christian Jonatan, MD., Suryo Ardi Hutomo, MD., Christian Pramudita, MD., Ilma Alfia Isaridha, MD., and Dwi Fachrul MD., for invaluable support in this project.

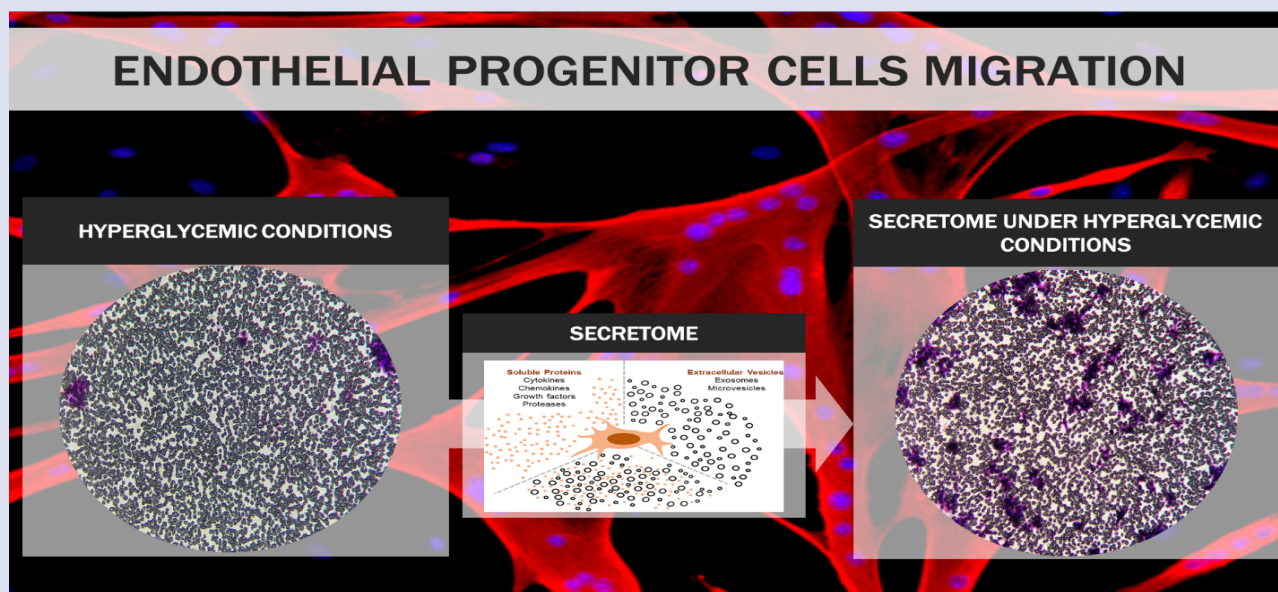
## FUNDING

This research was funded by Indonesian Ministry of Research and Technology – National Research and Innovation Foundation (Kementerian Riset dan Teknologi-Badan Riset dan Inovasi Nasional).

## REFERENCES

1. Aronson, D. & Edelman, E. R.: Coronary artery disease and diabetes mellitus. *Cardiology Clinics* 32, 439–55 (2014).
2. Saboor, M., Moinuddin, Ajmal, M. & Ilyas, S.: Functional status of vascular endothelium in diabetes mellitus. *J Ayub Med Coll Abbottabad* 26, 239-43 (2014).
3. Tepper OM, Galiano RD, Capla JM, *et al.*: Human endothelial progenitor cells from type II diabetics exhibit impaired proliferation, adhesion, and incorporation into vascular structures. *Circulation* 106, 2781–6 (2002).
4. Brunner S, Scherthner GH, Satler M, Elhenicky M, Hoellerl F, Schmid-Kubista KE, Zeiler F, Binder S, Scherthner G.: Correlation of different circulating endothelial progenitor cells to stages of diabetic retinopathy: first in vivo data. *Invest Ophthalmol Vis Sci* 50, 392–8 (2009).
5. J. Wils, J. Favre, and J. Bellien.: Modulating putative endothelial progenitor cells for the treatment of endothelial dysfunction and cardiovascular complications in diabetes. *Pharmacol Ther* 170, 98-115 (2017).
6. Fadini GP, Sartore S, Albiero M *et al.*: Number and function of endothelial progenitor cells as a marker of severity for diabetic vasculopathy. *Arterioscler Thromb Vasc Biol*, 26: 2140–46 (2006).
7. Jin HJ, Bae YK, Kim M, Kwon SJ, Jeon HB, Choi SJ, *et al.*: Comparative analysis of human mesenchymal stem cells from bone marrow, adipose tissue, and umbilical cord blood as sources of cell therapy. *Int J Mol Sci* 14 (9), 17986–18001 (2013).
8. Gallina C, Turinetti V, Giachino C.: A New Paradigm in Cardiac Regeneration: The Mesenchymal Stem Cell Secretome. *Stem Cells Int*. 2015.
9. Konala VBR, Mamidi MK, Bhonde R, Das AK, Pochampally R, Pal R.: The current landscape of the mesenchymal stromal cell secretome: A new paradigm for cell-free regeneration. *Cytotherapy* 18(1), 13–24 (2016).
10. Vizoso F, Eiro N, Cid S, Schneider J, Perez-Fernandez R.: Mesenchymal Stem Cell Secretome: Toward Cell-Free Therapeutic Strategies in Regenerative Medicine. *Int J Mol Sci* 18(9), 1852 (2017).
11. Phelps J, Sanati-Nezhad A, Ungrin M, Duncan NA, Sen A.: Bioprocessing of Mesenchymal Stem Cells and Their Derivatives: Toward Cell-Free Therapeutics. *Stem Cells Int* iii, 1–23 (2018).
12. Sandra F, Sudiono J, Sidharta EA, Sunata EP, Sungkono DJ, Dirgantara Y, *et al.*: Conditioned Media of Human Umbilical Cord Blood Mesenchymal Stem Cell-derived Secretome Induced Apoptosis and Inhibited Growth of HeLa Cells. *Indonesia Biomed. J* 6 (1), 57 (2014).
13. Chen, M. C., Sheu, J. J., Wang, P. W., Chen, C. Y., Kuo, M. C., Hsieh, C. J., Chen, J. F. & Chang, H. W.: Complications impaired endothelial progenitor cell function in Type 2 diabetic patients with or without critical leg ischaemia: implication for impaired neovascularization in diabetes. *Diabet Med* 26, 134-41 (2009).
14. Chen YH, Lin SJ, Lin FY, Wu TC, Tsao CR, Huang PH *et al.*: High glucose impairs early and late endothelial progenitor cells by modifying nitric oxide-related but not oxidative stress-mediated mechanisms. *Diabetes* 56, 1559–68 (2007).
15. Yiu KH, Tse HF.: Specific role of impaired glucose metabolism and diabetes mellitus in endothelial progenitor cell characteristics and function. *Arterioscler Thromb Vasc Biol* 34(6), 1136–1143 (2014).
16. Fadini GP, Albiero M, Vigili de Kreutzenberg S, *et al.*: Diabetes impairs stem cell and proangiogenic cell mobilization in humans. *Diabetes Care* 36(4), 943–9 (2013).
17. Bornique S, Garcia-Quintans N, Valle I, Olmos Y, Wild B, Martínez-Granero F, Soria E, Lamas S, Monsalve M.: Inactivation of Foxo3a and subsequent downregulation of PGC-1 alpha mediate nitric oxide-induced endothelial cell migration. *Mol Cell Biol* 30, 4035–44 (2010).
18. Arutyunyan I, Elchaninov A, Makarov A and Fatkhudinov T. Umbilical Cord Blood Prospective Source for Mesenchymal Stem Cell-Based Therapy. *Stem Cells Int*. (2016)
19. Kamprom W, Kheolamai P, U-Pratya Y, Supokawej A, Wattananapitch M, Laowtam-mathron C and Issaragrisil S. Effects of Mesenchymal Stem Cell-Derived Cytokines on the Functional Eur. J. Cell Biol. (2016)
20. Tan CY, Lai RC, Wong W, Dan YY, Lim SK, Ho HK.: Mesenchymal stem cell-derived exosomes promote hepatic regeneration in drug-induced liver injury models. *Stem Cell Res Ther* 5(3), 76 (2014).

## GRAPHICAL ABSTRACT



## ABOUT AUTHORS



**Yudi Her Oktaviono** work as an interventional cardiologist in the Department of Cardiology and Vascular Medicine Soetomo General Hospital and Faculty of Medicine, University of Airlangga. Previously, He led the Indonesian Heart Association chapter Surabaya and currently he is the vice president of the Indonesian Heart Association. His research interests include complex interventional cardiology, endothelial progenitor stem cells, usage of natural compound for cardiac disease and detection of early cardiac markers.



**Melly Susanti** is a cardiology resident in the Department of Cardiology and Vascular Medicine Soetomo General Hospital and Faculty of Medicine, University of Airlangga. Her research interests are stem cells, genetic polymorphism of cardiovascular risk and clinical cardiology.



**Achmad Lefi** work as senior cardiologist and echocardiography consultant in the Department of Cardiology and Vascular Medicine Soetomo General Hospital and Faculty of Medicine, University of Airlangga. He is the president of the Indonesian Heart Association chapter Surabaya and Chief of Medical Committee in Dr. Soetomo General Hospital Surabaya. His research interests include endothelial progenitor stem cells, cardiovascular molecular and cellular biology and noninvasive cardiac imaging.



**Ferry Sandra** is a professor in the Department of Biochemistry and Molecular Biology Dental Faculty, Trisakti University Jakarta. He is also responsible to led the the BioCORE Laboratory. Previously, he was the director of Stem Cells and Cancer Institute (SCI). His research interests includes stem cells, cancer, inflammation and dentistry.

**Cite this article:** Oktaviono YH, Susanti M, Lefi A, Sandra F. Human Umbilical Cord Blood-derived Secretome Enhance Endothelial Progenitor Cells Migration on Hyperglycemic Conditions. *Pharmacogn J.* 2020;12(4):793-7.