UNIVERSITI SAINS MALAYSIA
GERAN PENYELIDIKAN UNIVERSITI PENYELIDIKAN
LAPORAN AKHIR

EXPRESSION OF BONE-SPECIFIC GENES AND PROTEINS IN HUMAN FETAL OSTEOBLAST CELL LINE (HFOB 1.19) TREATED WITH SEMI-PURIFIED FRACTION OF QUERCUS INFECTORIA

PENYELIDIK

DR. HERMIZI BT. HAPIDIN

2019

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FINAL REPORT OF FUNDAMENTAL RESEARCH GRANT SCHEME (FRGS)

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TITLE:
EXPRESSION OF BONE-SPECIFIC GENES AND PROTEINS IN HUMAN FETAL OSTEOBLAST CELL LINE (hFOB 1.19) TREATED WITH SEMI-PURIFIED FRACTION OF *Quercus infectoria*

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KEMENTERIAN PENDIDIKAN MALAYSIA

FINAL REPORT
FUNDAMENTAL RESEARCH GRANT SCHEME (FRGS)
Laporan Akhir Skim Geran Penyelidikan Fundamental (FRGS)
Pindaan 1/2014

A

RESEARCH TITLE:
EXPRESSION OF BONE-SPECIFIC GENES AND PROTEINS IN HUMAN FETAL OSTEOBLAST CELL LINE (hFOB 1.19) TREATED WITH SEMI-PURIFIED FRACTION OF *Quercus infectoria*

PHASE & YEAR:
2 & 2014

START DATE:
1 December 2014

END DATE:
30 November 2017

EXTENSION PERIOD 1 (DATE):
1 December 2017 to 31 May 2018

EXTENSION PERIOD 2 (DATE):
1 June 2018 to 31 August 2018

PROJECT LEADER:
Dr. Hermizi binti Hapidin

PROJECT MEMBERS:
(including GRA)
1. Assoc. Prof. Dr. Hasmah binti Abdullah
2. Prof. Dr. Ima Nirwana binti Soelaiman
3. Assoc. Prof. Dr. Rapeah binti Suppian
4. Amira Raudhah binti Abdullah (GRA)

B PROJECT ACHIEVEMENT (*Pencapaian Projek*)

ACHIEVEMENT PERCENTAGE			
Project progress according to milestones achieved up to this period	**0 - 50%**	**51 - 75%**	**76 - 100%**
Percentage (please state #%)			**100%**

RESEARCH OUTPUT		
Number of articles/ manuscripts/ books *(Please attach the First Page of Publication)*	**Indexed Journal**	***Non-Indexed Journal***
	2	1
Conference Proceeding *(Please attach the First Page of Publication)*	**International**	***National***
	1	1
Intellectual Property *(Please specify)*	-	

HUMAN CAPITAL DEVELOPMENT

Human Capital	**Number**				**Others** (please specify)
	On-going		**Graduated**		
Citizen	Malaysian	Non Malaysian	Malaysian	Non Malaysian	1) Amira Raudhah binti Abdullah (PhD – full research) 2) Nor Munira binti Hashim (Master – mixed mode) 3) Nur Afiqah Amalina binti Romli (undergraduate) 4) Fairuza Munira binti Mazlan (undergraduate)
PhD Student	1	-	-	-	
Master Student	1	-	-	-	
Undergraduate Student	-	-	2	-	
Total	2	-	2	-	

C EXPENDITURE (*Perbelanjaan*)

D ADDITIONAL RESEARCH ACTIVITIES THAT CONTRIBUTE TOWARDS DEVELOPING SOFT AND HARD SKILLS (*Aktiviti Penyelidikan tambahan yang menyumbang kepada pembangunan kemahiran insaniah*)

International		
Activity	Date (Month, Year)	Organizer
International Conference on Innovations in Research and Regenerative Medicine (CRRM) 2017	10th to 13th September 2017	BioMedPress, Science and Technics Publishing House.

4th International Conference on Postgraduated Research (ICPR) 2017	7th to 8th December 2017	International Islamic University Collage Selangor (KUIS).
6th International Conference on Biotechnology for the Wellness Industry (ICBWI) 2016	16th to 17th August 2016	Universiti Teknologi Malaysia (UTM).

National

Activity	Date (Month, Year)	Organizer
Health Sciences Symposium (HSS) 2018	19th May 2018	Universiti Sains Malaysia (USM).
31st Scientific Meeting of Malaysian Society of Pharmacology & Physiology (MSPP) 2017	18th to 19th August 2017	Health Campus, Universiti Sains Malaysia and Malaysian Society of Pharmacology & Physiology.
7th Malaysian Symposium of Biomedical Science 2017	14th to 15th May 2016	Universiti Putra Malaysia (UPM).

PROBLEMS / CONSTRAINTS IF ANY *(Masalah/ Kekangan sekiranya ada)*

E

1) Difficult to obtain Quercus infectoria (QI) from overseas (India and China) due to customs issue. Thus, we used QI obtained from the local market to optimise the purification method.
2) Difficult to establish compound separation.
3) *In vitro* study (cultures) tend to get contaminated easily.
4) Optimisation problem when performing RNA extraction and analysis.

RECOMMENDATION *(Cadangan Penambahbaikan)*

Setting up a systematic clean room and proper ventilation for cell culture laboratory to avoid persistent cell contamination.

RESEARCH ABSTRACT – Not More Than 200 Words *(Abstrak Penyelidikan – Tidak Melebihi 200 patah perkataan)*

The study aim to investigate the effect of established *Quercus infectoria* (QI) semi-purified fractions (QIsm-F) and combination of established QIsm-F fraction with readily available osteoporotic drug (pamidronate) on proliferation, differentiation and mineralisation of osteoblast as well as to delineate the molecular mechanism of QIsm-F that enhanced bone formation by employing human foetal osteoblast (hFOB 1.19) cell model. A total of 13 QIsm-F were isolated by chromatographic technique and tested in series of bio-guided assay by MTT assay to determine the most potent fraction. Three most potent fractions; Fraction A (FA), Fraction B (FB) and Fraction C (FC) were used throughout this study. Polyphenolic content of each QIsm-F were identified by Liquid-Chromatography-Mass-Spectrometry (LCMS). The cells were treated on day 1, day 3 and day 7 for assessments of mineralisation by Alizarin Red S staining for calcium (Ca) depositions and von Kossa staining for phosphate (P) depositions as well as evaluation of cellular morphology by using inverted microscope. Detailed assessment of bone specific markers were conducted by RT-PCR and western blot along with immunofluorescence staining. The LCMS analysis of each FA, FB and FC reveals that each fraction consists of mainly polyphenolic compounds (gallic acid, digallate, ellagic acid, syringic acid and theogallin). The mineral deposition per viable cell increases with treatment of FA, FB and FC as well as combined treatment of FA, FB and FC with pamidronate on hFOB1.19 cells in a time-dependent manner. The Ca depositions appeared as red spot meanwhile P depositions appeared as black spot. Interestingly, from day 3 until day 7; quantification of investigated bone specific genes (Runx2, *Osx*, BMP-2, BSPII, BGLAP, and TGF-β1) and proteins (Runx2, *Osx* and BSPII) on hFOB1.19 cells were highest in cells treated with combined treatment of FA, FB and FC with pamidronate and at peak in cells treated with combined treatment of FC with pamidronate compared to hFOB 1.19 cells treated with single individual treatment. Protein analysis results were consistent with investigation of Runx2 and *Osx* marker in immunofluorescence staining. Therefore, these finding demonstrated that polyphenols presence in each FA, FB and FC enhanced mineral deposition along with expression of investigated bone marker as well as acknowledge the potential therapeutic effects when combining each QIsm-F with pamidronate through increasing efficiency of pamidronate acting on osteoblast cells by stimulating osteoblast proliferation, differentiation and mineralisation.

Date : 17/7/2018
Tarikh

Project Leader's Signature:
Tandatangan Ketua Projek

Senior Lecturer, Biomedicine Programme
School of Health Sciences
Universiti Sains Malaysia
16150 Kubang Kerian, Kelantan.

COMMENTS, IF ANY / ENDORSEMENT BY RESEARCH MANAGEMENT CENTER (RMC)
(Komen, sekiranya ada/ Pengesahan oleh Pusat Pengurusan Penyelidikan)

H .....

.....

.....

Name:
Nama:

PROF. DR LEE KEAT TEONG
Director
Research Creativity & Management Office
Universiti Sains Malaysia

Signature:
Tandatangan:

Date:
Tarikh:

FORMAT PROFIL PENYELIDIKAN

Format Profil Penyelidikan adalah seperti berikut:-

i) Dalam versi Bahasa Inggeris.

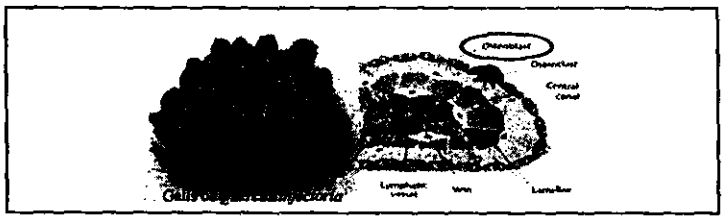
ii) Laporan tidak melebihi lima (5) muka surat bagi setiap satu projek penyelidikan dan perlu disertakan gambar berkaitan projek penyelidikan.

iii) Jenis dan saiz tulisan: Tajuk – Arial, 13
Laporan – Arial, 11

iv) Antara kriteria yang perlu ada:-

- Maklumat projek penyelidikan;
- Abstract - tidak melebihi 120 patah perkataan;
- Introduction;
- Research Methodology;
- Literature Review;
- Findings;
- Conclusion;
- Achievement;
- References;
- Appendixes

PROFIL PENYELIDIKAN



TITLE OF RESEARCH: EXPRESSION OF BONE-SPECIFIC GENES AND PROTEINS IN HUMAN FETAL OSTEOBLAST CELL LINE (hFOB 1.19) TREATED WITH SEMI-PURIFIED FRACTION OF *Quercus infectoria*

Name of Project Leader: Dr. Hermizi binti Hapidin

Name of co-researchers: **Assoc. Prof. Dr. Hasmah binti Abdullah, Prof. Dr. Ima Nirwana binti Soelaiman, Assoc. Prof. Dr. Rapeah binti Suppian & Amira Raudhah binti Abdullah (GRA)**

IPTA/ Faculty / School/ Centre/Unit: Universiti Sains Malaysia, School of Health Sciences, Biomedicine Programme

Field: Anatomy, Bone Metabolism, Natural Product

ABSTRACT (120 words)

The study aim to investigate the effect of established *Quercus infectoria* (QI) semi-purified fractions (QIsm-F) and combination of established QIsm-F fraction with readily available osteoporotic drug (pamidronate) on proliferation, differentiation and mineralisation of osteoblast as well as to delineate the molecular mechanism of QIsm-F that enhanced bone formation by employing hFOB 1.19 cell model. A total of 13 QIsm-F were isolated by chromatographic technique and tested in series of bio-guided assay by MTT assay to determine the most potent fraction. Three most potent fractions; Fraction A (FA), Fraction B (FB) and Fraction C (FC) were used throughout this study. Polyphenolic content of each QIsm-F were identified by LCMS. The cells were treated on day 1, day 3 and day 7 for assessments of mineralisation by Alizarin Red S staining [calcium (Ca) depositions] and von Kossa staining [phosphate (P) depositions] and also evaluation of cellular morphology by using microscope. Detailed assessment of bone specific markers were conducted by RT-PCR, western blot and immunofluorescence staining. The LCMS analysis of each FA, FB and FC reveals that each fraction consists of mainly polyphenolic compounds (gallic acid, digallate, ellagic acid, syringic acid and theogallin). The mineral deposition per viable cell increases with treatment of FA, FB and FC as well as combined treatment of FA, FB and FC with pamidronate on hFOB1.19 cells in a time-dependent manner. Interestingly, from day 3 until day 7; quantification of investigated bone specific genes (Runx2, *Osx*, BMP-2, BSPII, BGLAP, and TGF-β1) and proteins (Runx2, *Osx* and BSPII) on

hFOB1.19 cells were highest in cells treated with combined treatment of FA, FB and FC with pamidronate and at peak in cells treated with combined treatment of FC with pamidronate compared to hFOB 1.19 cells treated with single individual treatment. Protein analysis results were consistent with investigation of immunofluorescence staining. Therefore, these finding demonstrated that polyphenols presence in each FA, FB and FC enhanced mineral deposition along with expression of investigated bone marker as well as acknowledge the potential therapeutic effects when combining each QIsm-F with pamidronate through increasing efficiency of pamidronate acting on osteoblast cells.

1. INTRODUCTION

Osteoporosis is a major health threat that occur as a results of imbalance production of osteoblast and osteoclast. Osteoblasts are specialised mesenchymal-derived cells that give rise to progenitor cells of restricted osteoblast lineage and may undergo a series of proliferation stages before expressing recognisable specific osteoblastic markers. The expression of bone-specific genes and proteins are valuable markers for demonstrating osteoblast phenotype *in-vitro*. Understanding the transcriptional regulation of the genes is a fundamental need in the regulation of osteoblast differentiation and function. Hence, elucidating the mechanism of the expression of osteoblast markers is crucial in exploring a future pharmacotherapeutic agent for osteoporosis. Presently, numerous classification of drug such as bisphosphonate, selective estrogen receptor modulator (SERM) and hormone replacement therapy (HRT) are readily available for osteoporosis treatment. Despite all the advancement, these drug perceived drawback and presented with many side effects. Hence, the motivation in developing alternative therapy aside for these readily available drug are as important. Meanwhile, research has pointed that the galls of *Quercus infectoria* (QI) contains various active phytochemical compounds that have the potential to stimulate bone formation due to its abundance polyphenolic content. However, the precise mechanism of the stimulation effect of QI on osteoblast has not been elucidated. Thus, this current study has been conducted to investigate the effects of herbal drug combination between established QI semi-purified fractions (QIsm-F) and readily available osteoporotic drug on hFOB 1.19 cells; towards development of new perspective in prevention and treatment of osteoporosis and at the same time enrich modern phytochemical knowledge.

2. RESEARCH METHODOLOGY

In this current study, the QI gall extract was subjected to vacuum liquid chromatography to obtain multiple semi-purified fractions based on chromatographic profile. The fractions was screened for proliferative activity towards hFOB 1.19 cell lines using MTT assay. Then, half maximal effective concentration (EC_{50}) value was calculated from the assay. The most active fraction (indicated by the lowest EC_{50} value) was chosen to be separated using column chromatography to produce sub-fractions. Thin layer chromatography (TLC) was used to determine the chemical finger printing in the sub-fraction. The sub-fractions with equal or almost similar finger printing patent were pooled and evaluated for proliferative activity. Those with potent proliferative activity was subjected to preparative thin layer chromatography in order to produce semi-purified fraction (QIsm-F). The QIsm-F with most potent proliferative activity was chosen for bone specific genes and protein expression studies. Concurrently the active QIsm-F was subjected to liquid chromatography–mass spectrometry (LC-MS) method to determine its chemicals profile in general. The expression of the bone-specific genes and proteins was

determined by reverse transcriptase polymerase chain reaction (RT-PCR), enzyme-linked immunosorbent assay (ELISA), immunocytochemistry (ICC) and western blot.

3. LITERATURE REVIEW

National Institutes of Health (NIH) defined osteoporosis as a systemic skeletal disorder characterised by reduced bone strength, resulting in an increased risk of fracture primarily as a results of depleted bone density and quality [1]. Typically, the events of bone loss transpire as results of imbalance production between osteoblast and osteoclast that is associated with increase in substantial morbidity, motility along with social cost [2,3]. The preponderance of osteoporosis in Asian country, mainly in multiracial country like Malaysia are affected by the bias of race whereby recent reported findings documented 44.8% incidence of osteoporosis in Chinese women in contrast to Indians and Malays population [4] with significant escalation in the older age group [5]. Meanwhile in China, preponderance of osteoporosis reported 25.41% higher in females than males [6]; in India from 8% to 62% Indian women suffer from osteoporosis cross countries [7]; and in Japan, documented reaching 12 million citizen agonise from osteoporosis [8]. The general preponderance of osteoporosis in Asian countries is greater than the western countries largely due to the physical character of Asian population with shorter height and lower body mass index [9].

Over the recent years, various therapies are available for management of osteoporosis commonly are bisphosphonate, calcitonin and hormone replacement therapy (HRT) that work as an anti-resorptive agents [10]. A part from that, alternative endorsement to these therapies includes adequate calcium intake, vitamin D supplementation and weight bearing exercise to strengthen the bone [11]. Bisphosphonates are widely used drugs as standard treatment for prevention of fragility fractures primarily osteoporosis [12]. Whilst it is validate that bisphosphonate are effective by limiting bone loss, however there is growing concern over long term use of bisphosphonate which are linked to severe suppression of bone turnover as well as pose side effects which includes gastroesophageal irritation and osteonecrosis of the jaw (ONJ) [13]. Pamidronate, was the first of the second-generation bisphosphonates shown to prevent bone loss in patients with postmenopausal, idiopathic and glucocorticoid-induced osteoporosis [14,15]. Meanwhile, selective estrogen modulators (SERM) for example raloxifene possess the risk of deep vein thrombosis and might as well as cause leg cramps [16,17]. Thus, nevertheless the presence of variety osteoporosis therapies, there is a continuing need and a continuing search in this field as an alternative to helps convey this problem.

Development of osteoporotic therapy predominantly utilise the backbone of bone remodelling model procuring successful osteoporotic agent. Bone is an active tissue that undergoes constant remodelling in which old bone is degraded by osteoclasts and subsequently replaced by new bone formed by osteoblasts through bone remodelling process [18]. Osteoblasts pose a vital role in bone remodelling for the maintenance of the structural and metabolic capacity of the skeleton [19]. The metabolism of bone specific cell includes the steps of differentiation, proliferation and mineralisation that are regulated by several hormones, growth factors and mechanical pathway that act via connected signalling networks which resulting in the activation of specific transcription factors and, in turn, their target genes [20].

Important bone marker known as bone morphogenic protein-2 (BMP-2) is the key marker in controlling bone remodelling pathway whereby it promotes differentiation of mesenchymal cells into osteoblasts by controlling the expression and functions of Runt-related transcription factor 2 (Runx2), osterix (Osx) and bone sialoprotein (BSP) via the Smad signalling pathway [21]. Moreover, osteopontin (OPN) on the other hand is a type of marker expressed during the early stage of active proliferation and expressed maximally during mineralisation phase together with other several proteins which includes osteocalcin (OCN),

alkaline phosphatase (ALP), transforming growth factor β-1 (TGFβ-1) and many more [22]. Thus, quantitative or qualitative measurements of these important bone specific markers are the key into developing biomechanical strategies for the treatment of bone metabolic diseases [23].

Herbal medicine is the use of medicinal plants for prevention and treatment of diseases which ranges from traditional and popular medicines of every country to the use of standardised herbal extracts [24]. Traditional herbal medicines are getting significant attention in global health debates. Such as in China, traditional herbal medicine played a prominent role in the strategy to contain and treat severe acute respiratory syndrome (SARS) [25]. World Health Organisation (WHO) have made substantial research investments in traditional herbal medicines looking for promising medicinal herbs along with novel chemical compound in herbal plants. The extensive and successful research in herbal medicine urges the need to make use of these findings in development of new generation of medicine with minimal adverse effects to human. Furthermore, development of new generation of a combination therapies between natural herbal with synthetic drugs (phytopharmaceuticals) can helps to improve therapeutic efficacy through modern molecular biological methods subsequently minimise the adverse effect that pose by synthetic drug.

Polyphenols has been reported in several researches to promote bone formation. Yamaguchi *et al.,* 2001 [26], reported in his research that polyphenols in food had a potent anabolic effect on bone calcification in a study conducted on femoral-diaphyseal and metaphyseal tissues of rats *in vitro*. A study by Shen *et al.*, 2008 [27] proves that green tea polyphenols are promising agents for preventing bone loss in women. Moreover, polyphenols derived from dried plum also has been reported to enhance osteoblast activity and function by up-regulating Runx2 and Osx expression [28]. Our preliminary study [29] established the potential role of *Quercus infectoria* (QI) on the viability of human osteoblast cell; whereby the human osteoblast cell model increase in a concentration dependent manner after treatment with QI gall extract.

QI gall is a small oak tree widely distributed in Greece, Asia Minor, Syria and Iran [30]. The galls has been reported of great medicinal value and widely been used as traditional medicine mainly as astringent and as anti-inflammation [31]. To date, pharmacological evaluation of galls has deciphered them to be astringent, anti-diabetic, anti-bacterial [32], anti-inflammatory and wound healing properties [33]. Earlier research has reported that the galls of QI contain mainly a mixture polyphenols; 50% to 70% of gallotannin, gallic acid, ellagic acid, starch, and glucose as principal constituents [34].

Generally in the current study, QI semi-purified fraction (QIsm-F) were acquire from a series of purification and isolation through chromatographic technique to secure the majority polyphenols content in QI gall and investigated for its effect on osteoblast proliferation, differentiation and mineralisation through several experimental procedures *in vitro* by using human fetal osteoblast (hFOB 1.19) cell line model and comparing it to different control groups in order to elucidate the potential role of QI on osteoporotic therapy. In addition, the potential role of combining established QIsm-F with readily available osteoporotic drug (pamidronate) was also investigated in contemplation to determine its effect on the osteoblast model used, as well as correlate its efficiency with individual QIsm-F.

4. FINDINGS

The current study reported the potential role of QIsm-FA, FB and FC alone as well as combination of QIsm-FA, FB and FC with pamidronate on promoting osteoblast cell proliferation and increasing the number of hFOB 1.19 cells particularly in cells supplied with QIsm-FC and combination of QIsm-FC with pamidronate treatment in comparison to other control groups. Moreover, cells enrich with QIsm-F treatment evidently indicate increasing levels of calcium and

phosphate deposition based on histochemical staining which demonstrated enhanced mineralisation mechanism.

This study also unfold the potential effects of QIsm-FA, FB and FC along with combined treatment of QIsm-FA, FB and FC with pamidronate through stimulation of extracellular protein runt related transcription factor 2 (Runx2), bone morphogenic protein-2 (BMP-2) and osteopontin (OPN) that plays a vital role in each osteoblast differentiation, proliferation and mineralisation providing initial leads on the potential role of these agents towards promotion of bone metabolism. In order to further support our finding, real-time PCR quantification on selected important osteoblast specific progenitor cell; Runx2, osterix (Osx), BMP-2, bone sialoprotein II (BSPII), osteoprotegerin (OPG) and transforming growth factor β-1 (TGF-β1) revealed up-regulation of researched genes prior to incubation on day 1, 3 and 7 which evidently indicate that QIsm-FA, FB and FC plays a role in bone metabolism pathway whereas interaction between QIsm-FA, FB and FC with pamidronate helps to promotes the efficacy of pamidronate acting on hFOB 1.19 cell model.

Evaluation of gene expression was supported by acquisition of protein expression. By selecting QIsm-FC and combined treatment of QIsm-FC with pamidronate as the choice of treatment that yields out the highest efficacy effects, expression of Runx2, Osx and BSPII were observed to be heightened represented by intense blot in western blotting analysis compared. Immunofluorescence staining then followed to confirm presence of Runx2 and Osx marker on the cell culture. Indubitably, heightened fluorescence intensity and higher cell number observed by culture treated with combination treatment of QIsm-FC with pamidronate followed with QIsm-C in comparison with pamidronate and tamoxifen treated groups via immunofluorescence staining against Runx2 and Osx which marked the role of QIsm-F and interaction of QIsm-F with pamidronate in metabolism of bone.

5. CONCLUSION

In conclusion, the polyphenols presence in each FA, FB and FC enhanced mineral deposition along with expression of investigated bone marker, as well as acknowledge the potential therapeutic effects when combining each QIsm-F with pamidronate through increasing efficiency of pamidronate acting on osteoblast cells by stimulating osteoblast proliferation, differentiation and mineralisation.

ACHIEVEMENT

i) Name of journal articles published:

a) Abdullah Amira Raudhah, **Hapidin Hermizi** & Abdullah Hasmah. (2018). Combination treatment of bisphosphonate (pamidronate) and *Quercus infectoria* semi-purified fraction promote proliferation and differentiation of osteoblast cell via expression of Osterix and Runx2 marker. *Asian Pac. J. Trop. Biomed.* **8(5)**: 261-267. doi:10.41032221-1691.233007.

b) Amira Raudhah Abdullah, **Hermizi Hapidin** & Hasmah Abdullah. (2018). The Role of Semipurified Fractions Isolated from Quercus infectoria on Bone Metabolism by Using hFOB 1.19 Human Osteoblast Cell Model. *Evid.-Based Complementary Altern. Med.* **Volume 2018**, Article ID 5319528, 13 pages, doi:10.1155/2018/5319528.

c) Amira Raudhah Abdullah, **Hermizi Hapidin** & Hasmah Abdullah. (2017). Phytochemical analysis of *Quercus infectoria* galls extract using FTIR, LC-MS and MS/MS analysis. *Res. J. Biotech.* **12(12)**: 55-61.

ii) Title of Paper presentations (international/ local):

a) Amira Raudhah Abdullah & **Hermizi Hapidin**. (2018). A potential herbal-drug interaction between *Quercus infectoria* semi-purified fraction and pamidronate for management of osteoporosis. Health Sciences Symposium (HSS) 2018.

b) Amira Raudhah Abdullah, **Hermizi Hapidin** & Hasmah Abdullah. (2017). The effect of polyphenolic compound isolated from *Quercus infectoria* on mineralisation and proliferation of human foetal osteoblast (hFOB1.19) cell line. *4th International Conference on Postgraduate Research (ICPR)* 2017, p.54.

c) Amira Raudhah Abdullah, **Hermizi Hapidin** & Hasmah Abdullah. (2017). *Quercus infectoria* semi-purified fractions promoted BMP-2, Runx2 and osteopontin expression in human fetal osteoblastic cells line. *International Conference on Innovations in Cancer Research and Regenerative Medicine, Biomed Res Ther.* 4(6):S85.

d) **Hermizi Hapidin**, Fairuza Munirah Mazlan, Wan Nurhidayah Wan Hanaffi, Wan Amira Raudhah Abdullah & Hasmah Abdullah. (2017). Effect of semipurified fractions from the galls of *Quercus infectoria* on mineralization of human fetal osteoblast (hFOB 1.19) cells. *31st Scientific Meeting of MSPP (Malaysian Society of Pharmacology & Physiology) 2017.* P22, p.76.

e) Amira Raudhah Abdullah, **Hermizi Hapidin** & Hasmah Abdullah. FTIR, LC-MS and MS/MS Analysis of *Quercus infectoria* Galls Extract. *6th International Conference on Biotechnology for the Wellness Industry (ICBWI) 2016.* SP5.

f) Fairuza Munirah Mazlan, **Hermizi Hapidin**, Wan Nurhidayah Wan Hanaffi, Amira Raudhah Abdullah & Hasmah Abdullah. (2016). Effects of *Quercus infectoria* semi-purified fractions on ALP activity and Morphological Changes of Human Fetal Osteoblast cell Line (hFOB 1.19). *7th Malaysian Symposium of Biomedical Science 2016.* p. 207.

iii) Human Capital Development:

a) **Amira Raudhah binti Abdullah** (PhD - research mode). Investigating the proliferation and differentiation mechanism of human osteoblast cell line hFOB 1.19 upon treatment with *Quercus infectoria* semi-purified fraction. Main-Supervisor. Status: waiting for viva voce.

b) **Nor Munira binti Mashim** (Master - mixed mode). 2018. Differentiation and Proliferation Activities of Human Foetal Osteoblast (hFOB 1.19) Cells Treated with a Polyphenol, Tannic Acid alone or in Combination with Pamidronate. Main Supervisor. Status: thesis writing.

c) **Nur Afiqah Amalina binti Romli** (Undergraduate - FYP). Effects of Tannic Acid on Human Fetal Osteoblast Cell (hFOB 1.19) Viability and It's Morphological Evaluation by Scanning Electron microscope (SEM). Main Supervisor. Status: graduated 2017.

d) **Fairuza Munira binti Mazlan** (Undergraduate - FYP). Effects of *Quercus infectoria* Semi-purified Fractions on Morphological Changes, Mineralisation and Alkaline Phosphatase (ALP) Activity of Human Fetal Osteoblast Cell Line (hFOB1.19). Main Supervisor. Status: graduated 2016.

iv) Awards/ Others -

v) Others -