

# **Role of some natural products in mobilization of stem cells in rats with acute myocardial infarction**

Thesis

Submitted for partial fulfillment of the master degree in Pharmaceutical Sciences (Biochemistry)

By

**Pharmacist: Maha Abdelmonem Mohammed Ahmed**

B.Sc. 2008 (Cairo University)

Demonstrator of Biochemistry

Faculty of Pharmacy

Cairo University

Supervised by

**Prof. Dr.**

**Amira AbdElmonem Shaheen**

Professor of Biochemistry

Faculty of Pharmacy

Cairo University

**Prof. Dr. Hala Gabr Metwally**

Professor of Hematology and

Immunology

Faculty of Medicine

Cairo University

**Dr. Samar Hassab Allah Kassem**

Lecturer of Biochemistry

Faculty of Physical Therapy

6- October University

**Faculty of Pharmacy**

**Cairo University**

**2016**

## **Introduction**

Myocardial infarction (MI) is one of the major causes of cardiovascular morbidity and mortality. MI results in loss of cardiomyocytes, scar formation, ventricular remodeling and eventually heart failure. Although current pharmacological and surgical interventions have led to improved survival of patients, they failed to regenerate dead myocardium and/or prevent deterioration of cardiac function. In last decade, stem cell (SC) therapy has emerged as a potential new strategy for incurable and life threatening MI. The ultimate goals of stem cell therapy are myocardial regeneration and neovascularization leading to clinical improvement without severe adverse effects. Mechanisms involved in the endogenous SC-associated myocardial regeneration include the mobilization of SCs from the bone marrow and other putative "niches" (such as skeletal and cardiac muscles), cytokine-guided homing with subsequent engraftment into the ischemic area, and finally the transdifferentiation into functional cardiomyocytes. These tissue-committed SCs circulate in peripheral blood at low number and can be mobilized by ischemia-related inflammatory and hematopoietic cytokines, such as granulocyte colony-stimulating factor (G-CSF), granulocyte macrophage colony-stimulating factor (GM-CSF), interleukin-8 (IL-8), vascular endothelial growth factor (VEGF), and stromal cell-derived factor-1 (SDF-1). The levels of these cytokines were found to be significantly higher in patients with acute myocardial infarction (AMI) and were correlated positively with the number of circulating CD34<sup>+</sup> SCs. However, such endogenous responses unfortunately do not

offer a sufficient regenerative solution of damaged myocardium. Therefore, the need for SC therapy is a must. Basically, the efficacy of SC therapy in regenerative medicine depends on sufficient recruitment of available cells (either exogenously administered populations or endogenously mobilized residents) to the target tissue.

Although SC transplantation is the most common means to replenish impoverished SC pools, their applications are restricted by the limited availability of SC sources, the excessive cost and the anticipated difficulties of clinical translation and regulatory approval. Thus, regenerative therapy should not be limited to this approach but should instead seek for a strategy that retrieves the initial healing capacity of a tissue. In this regard, pharmacological activation of endogenous SCs already present in a patient's body from either the blood or a tissue-specific niche and their homing into the injury sites, is a promising approach for therapeutic success. This technique has the potential to provide new therapeutic options for in situ tissue regeneration. Such options would be less costly and complex than approaches requiring ex vivo cell manipulation. In this context, using medicinal plant products for activation of endogenous SCs represents an emerging field of regenerative medicine in health and disease.

In the current study, two natural products namely *Avemar* and *Echinacea* were selected to investigate their possible role in supporting SC biology. *Avemar* is a product of industrial fermentation of wheat germ with a standardized content of benzoquinone and plant flavonoids that has been reported as safe and effective anticancer and

immunomodulatory adjuvant therapy for human cancer. Avemar also has a potential role to attenuate the cardiovascular symptoms induced by hypertension or diabetes. The proposed mechanisms were attributed to its actions in inhibition of cyclooxygenase isoforms and upregulation of endogenous antioxidants. On the other side, *Echinacea purpurea* is one of the *Echinacea* species which has been widely used for its anti-inflammatory and antioxidant activity in addition to a profound immunostimulatory action on a number of human immune cells, such as macrophages and peripheral blood mononuclear cells. Clinical trials confirmed the efficiency of *Echinacea purpurea* extracts in inflammatory diseases, including those of infectious nature. *Echinacea* proved to improve the regeneration process of damaged tissues where it can intensify the immunological angiogenesis which could be of high benefit in wound healing and in future cardiology as a preventive for myocardial infarction. Thus, we hypothesized that treatment with Avemar and *Echinacea* may help in regeneration of damaged myocardium via stimulating SCs. In this regard, the effect of these natural products on activation of endogenous SCs cannot be separated from their already known antioxidant, anti-inflammatory and immunomodulatory activities, as all of these activities are speculated to synergistically drive tissue repair and regeneration.

Thus, this study was directed for the first time to investigate the possible effect of Avemar or *Echinacea* on enhancing CD34<sup>+</sup>SC mobilization, homing in relation to

inflammatory and hematopoietic cytokines such as VEGF, IL-8 and GM-CSF in rat model of AMI.

## Abstract

Activation of endogenous stem cells mobilization can contribute to myocardial regeneration after ischemic injury. This study aimed to evaluate the possible role of Avemar or Echinacea extracts in inducing mobilization and homing of CD34<sup>+</sup> stem cells in relation to the inflammatory and hematopoietic cytokines in rats suffering from acute myocardial infarction (AMI). AMI was developed by two consecutive subcutaneous injections of isoprenaline (85 mg/kg). AMI rats were either post-treated or pre- and post-treated daily with oral doses of Avemar (121 mg/kg) or Echinacea (130 mg/kg). In whole blood, the number of CD34<sup>+</sup> cells was measured by flow cytometry and their homing to the myocardium was immunohistochemically assessed. Serum creatine kinase (CK), vascular endothelial growth factor (VEGF), interleukin-8 (IL-8) and granulocyte macrophage colony stimulating factor (GM-CSF) were determined on day 1, 7 and 14 after AMI. Sections of the myocardium were histopathologically assessed. Rats pre- and post-treated with Avemar or Echinacea exhibited substantial increases in the number of circulating CD34<sup>+</sup> cells peaking on the first day after AMI to ~ 13 and 15 fold respectively with a decline in their level on day 7 followed by significant increase on day 14 compared to their corresponding AMI levels. Only post-treatment with Echinacea caused time dependent increase in circulating CD34<sup>+</sup> cells on 7 and 14 days. Such increases in circulating CD34<sup>+</sup> cells were accompanied by increased homing to myocardial tissue 14 days after AMI. Interestingly, pre- and post-treatment with Avemar or Echinacea substantially increased serum CK on day 1, normalized its activity on day 7 and on continued treatment only Echinacea increased markedly its activity on day 14 compared to their corresponding AMI values. Moreover, both treatments modified differently the elevated serum VEGF and the lowered GM-CSF levels of AMI group but did

not affect IL-8 level. These results were supported histopathologically by reduced inflammatory reactions and enhanced neovascularization. In conclusion, Avemar and Echinacea extracts can effectively induce mobilization and homing of CD34<sup>+</sup> stem cells to the myocardial tissue and thus may help in stem cell-based regeneration of the infarcted myocardium.

**Key words:** CD34<sup>+</sup> Stem Cells - Myocardial Infarction - Avemar - Echinacea

## **Aim of the work**

In recent years, stem cell therapy has emerged as a potential new strategy for patients with myocardial infarction. Stem cell mediated cardiac repair involves stem cell differentiation into myocardial cells and release of soluble autocrine/paracrine factors which are involved in stem cell renewal and myocardial protection/neovascularization respectively. Likewise, in acute myocardial infarction patients, reactivation of endogenous stem cells may help the rejuvenation of damaged myocardium. Therefore searching for new therapeutic agents, especially natural products, that assist in the maintenance of stem cell niches and guide the mobilization of stem cells from these niches and their subsequent recruitment to the ischemic myocardium will represent a potential target for stem cell-mediated cardiac repair.

Therefore, the objective of the present study was directed to investigate the possible role of two safely and widely used natural products Avemar and Echinacea extracts, in inducing mobilization and homing of CD34<sup>+</sup> stem cells in relation to inflammatory and hematopoietic cytokines and thereby regeneration of damaged myocardium in rats with acute myocardial infarction. To fulfill this purpose, the frequencies of CD34<sup>+</sup> stem cells in blood as well as their homing to the myocardial tissue were measured. Serum creatine kinase activity and the level of certain cytokines (vascular endothelial growth factor (VEGF), interleukin-8 (IL-8) and granulocyte macrophage colony stimulating factor (GM-CSF)) were determined on day 1, 7 and 14 after AMI development in addition to histopathological investigation of the myocardial tissue.



## Summary

With the changes in lifestyle of population, the morbidity of hypertension, coronary heart disease, and other common cardiovascular diseases has shown continuous rising tendency. Current drug treatment can only improve symptoms without preventing the ventricular remodeling and the deterioration of the progressive heart function (**Mirotsoou et al., 2011**). Stem cell therapy represents a promising approach for patients with myocardial infarction (MI). The aim of stem cell-mediated cardiac repair embodies restoration of cardiac function by regeneration of healthy myocardial tissue, which is accomplished by neo-angiogenesis and cardiogenesis (**Vandervelde et al., 2005**). An alternative to stem cell transplantation is mobilization of cell populations already present in a patient's body, including stem/progenitor cells, which can be actively attracted to sites of injury. This technique has the potential to provide new therapeutic options for in situ tissue regeneration (**Chen et al., 2011**). In this context, dietary strategies for supporting stem cell biology represent an emerging field of nutritional medicine.

Avemar, a product of industrial fermentation of wheat germ with a standardized content of benzoquinone has been used as an anti-cancer and immunomodulatory dietary supplement. Avemar also has a potential role to attenuate the cardiovascular symptoms induced by hypertension, diabetes or the metabolic syndrome. Proposed mechanisms include antioxidant and anti-inflammatory actions (**Iyer & Brown, 2011**). *Echinacea purpurea* is one of the *Echinacea* species widely used for nutraceutical or pharmaceutical purposes. Various tissue extracts of this traditional medicinal plant have been reported to have immunostimulatory activity on a number of human immune cells, such as macrophages and peripheral blood mononuclear cells (**Wang et al., 2006**). Clinical trials confirmed the efficiency of *Echinacea purpurea* preparations in inflammatory diseases, including those of infectious nature (**Kapai et al., 2011**).

This study was performed to determine the possible role of Avemar or Echinacea in enhancing stem cell mobilization, recruitment and homing in an experimental AMI.

112 Rats were randomly divided into 6 groups as follows:

**Group 1: (Normal control group)** 8 rats were used to evaluate baseline values of various parameters in this study where rats received a subcutaneous dose of 0.1 ml saline for two consecutive days at 24 h. time interval.

**Group 2: (AMI group)** 24 rats received subcutaneous dose of ISP (85 mg/kg) dissolved in saline for two consecutive days at 24 hour time interval (for induction of myocardial infarction)

**Group 3: (AMI post-treated with Avemar)** 16 rats received daily an oral dose of Avemar 121 mg/kg in distilled water for 14 days after development of myocardial infarction as group 2.

**Group 4: (AMI pre- and post-treated with Avemar)** 24 rats received daily an oral dose of Avemar 121 mg/kg 14 days before induction of AMI and treatment continued for another 14 days after the development of AMI.

**Group 5: (AMI post-treated with Echinacea)** 16 rats received daily an oral dose of Echinacea extracts (130 mg/kg) in distilled water for 14 days after development of myocardial infarction as group 2.

**Group 6: (AMI pre- and post-treated with Echinacea)** 24 rats received daily an oral dose of Echinacea extracts (130 mg/kg) in distilled water 14 days before induction of AMI and treatment continued for another 14 days after the development of AMI.

Blood was collected as follows:

In group 1: one day after last injection.

In groups 2, 4 and 6: on day 1, 7 and 14 after induction of myocardial infarction (8 animals per time)

In groups 3 and 5: on day 7 and 14 after induction of myocardial infarction (8 animals per time)

The collected blood was divided into two halves. One half was used for separation of serum for determination of CK, IL-8, GM-CSF and VEGF. The other half (EDTA-blood) was used for determination of CD34<sup>+</sup> Cells frequency by flow cytometry.

Animal's hearts were isolated, washed with cold saline and kept in formalin for immunohistochemical and histopathological investigations.

The results of the present study revealed that induction of AMI using isoprenaline resulted in increased circulating CD34<sup>+</sup> cells. Interestingly, post-treatment with Echinacea and Pre- and post-treatment with Avemar or Echinacea resulted in more pronounced increase in circulating CD34<sup>+</sup> cells frequency. The underlying mechanism of this increase could be through the disruption of the interaction between the BM and stem cells or through increasing serum levels of chemotactic agents, thus providing gradient guiding stem cells out into the blood.

The current study demonstrated an increase in serum CK activity on day 1 after infarction development in AMI group. The explanation of this increase is based on the oxidative stress induced by isoprenaline injection which leads to loss of membrane integrity with subsequent leakage of cellular enzymes including CK. Administration of Avemar or Echinacea two weeks before induction of AMI caused further increase in serum CK activity on day 1 after MI development. This could be interpreted on the basis of stem cell based cardiogenesis which is associated with enhancement of CK circuits to meet increased demands for energetic communication and processing of cellular information. This interpretation is supported by the presence of significant positive correlation between the frequency of circulating CD34<sup>+</sup> cell and serum CK activity.

In AMI group, there was an increase in serum VEGF level which may be triggered through a mechanism involving hypoxia-induciblefactor-1 activation. VEGF level showed higher increases in groups pre- and post-treated with Avemar or Echinacea. These increases may be responsible for the elevated frequency of circulating CD34<sup>+</sup> cells. However, the increase in VEGF level was followed by significant decreases after two

weeks in these groups which may represent an additional benefit of Avemar or Echinacea therapy through improving the ventricular function and reducing the adverse myocardial remodeling. Moreover, we found a positive correlation between circulating CD34<sup>+</sup> cells and serum VEGF level in Avemar treated groups and between VEGF level and CK activity in both Avemar and Echinacea treated groups.

Regarding IL-8, our results showed moderate increase of serum IL-8 in AMI group which is supposed to be partially responsible for the previously described increase in circulating CD34<sup>+</sup> cells after AMI. Neither Avemar nor Echinacea treated groups showed significant differences from untreated group in IL-8 level.

The results of the current study revealed also significant decreases in serum GM-CSF in AMI group. Groups pre- and post-treated with Avemar or post-treated with Echinacea showed more pronounced decrease in GM-CSF level. This reduction in GM-CSF level might be attributed to its uptake by polymorph nuclear cells (PMNs). Reduction of GM-CSF level by Avemar or Echinacea is assumed to be beneficial in preventing left ventricular remodeling as GM-CSF may inhibit leukocyte apoptosis and enhance monocyte infiltration into the ischemic myocardial tissue. Moreover, low GM-CSF level is assumed to give a chance for more CD34<sup>+</sup> cells to differentiate into cardiomyocytes or endothelial cells rather than granulocytes or macrophages. On the other side, in group pre- and post-treated with Echinacea there was increase in GM-CSF level which might be attributed to the immune enhancing properties of Echinacea.

Interestingly, post- treatment with Echinacea and pre- and post- treatment with Avemar or Echinacea caused substantial increase in CD-34<sup>+</sup> cells number in myocardial tissue which indicates successful recruitment and homing of the mobilized stem cells to the diseased myocardium. The mechanisms of participation of these recruited CD34<sup>+</sup> cells in myocardial regeneration after AMI may involve direct incorporation of

the cells into the newly developing vasculature, production and secretion of angiogenic cytokines or transdifferentiation of CD34<sup>+</sup> cells to cardiomyocytes.

Histopathological sections obtained from the hearts of animals that received isoprenaline alone showed very severe inflammatory reaction which may be attributed to ROS formation. Avemar and Echinacea administration could reduce this inflammatory reaction efficiently. These results may be explained in case of Avemar by its anti-inflammatory effect through the inhibition of the cyclooxygenases 1 and 2 activity. Regarding Echinacea, this effect could be attributed to its ability to inhibit free radical production and lipid peroxidation.

## Conclusion

In the light of the previous results, it can be concluded that:

- AMI increases serum CK activity and VEGF level, decreases GM-CSF level and induces CD34<sup>+</sup> cells mobilization moderately.
- Avemar and Echinacea treatments substantially enhance mobilization, recruitment and homing CD34<sup>+</sup> stem cells to the ischemic myocardium.
- The mechanism of Avemar- and Echinacea-induced mobilization, recruitment and homing may be based on:

a) Increasing CK activity to meet the increased demands for energetic communication and processing of cellular information during stem cell based cardiogenesis.

b) Elevating serum VEGF level which may enhance CD34<sup>+</sup> stem cells homing capacity within the myocardium and their potential regenerative ability and promote endothelial cell proliferation and migration.

c) Reducing GM-CSF level which is assumed to give the chance for more CD34<sup>+</sup> cells to differentiate into cardiomyocytes or endothelial cells.

- Echinacea was superior to Avemar where treatment can be started after the development of myocardial infarction to enhance substantial mobilization and homing of CD34<sup>+</sup> stem cells.

Accordingly, Avemar and Echinacea consumption could be used as a complementary therapy in cardiovascular and endothelial dysfunction. Their use effectively triggers mobilization and recruitment and assist homing of CD34<sup>+</sup> stem cells to the infarcted myocardium thus they can help in stem cell-based regeneration of the myocardial tissue.

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استمارة معلومات الرسائل التي تمت مناقشتها

القسم: الكيمياء الحيوية

الكلية/المعهد: الصيدلة

1- الدرجة العلمية : ماجستير  نعم  لا  دكتوراه

2- بيانات الرسالة:

عنوان الرسالة باللغة العربية : دور بعض المنتجات الطبيعية في تحريك الخلايا الجذعية في الجرذان المصابة باحتشاء عضلة القلب الحاد

عنوان الرسالة باللغة الأجنبية:

**Role of some natural products in mobilization of stem cells in rats with acute myocardial infarction**

التخصص الدقيق: كيمياء حيوية

تاريخ المناقشة: 26/1/2016

3- بيانات الطالب:

الاسم: مها عبد المنعم محمد احمد

الجنسية: مصرية

النوع: أنثى

العنوان: 15 ش نصر الاسلام - امبابه - الجيزة

رقم التليفون: 01226981937

جهة العمل: كلية الصيدلة - جامعة القاهرة

رقم الفاكس:

البريد الإلكتروني: moha\_abdelmonem@yahoo.com

4- المشرفون على الرسالة:

الاسم	القسم	الكلية	الجامعة
1- أ.د/ أميرة عبد المنعم شاهين	الكيمياء الحيوية	الصيدلة	القاهرة
2- أ.د/ هالة جبر متولي	أمراض الدم والمناعة	الطب	القاهرة
3- د/ سمر حسب الله قاسم	الكيمياء الحيوية	العلاج الطبيعي	6 أكتوبر
4-			

## 5- مستخلص الرسالة (Abstract) 1-5 باللغة العربية .:

يعد مرض احتشاء القلب الحاد من أهم الأسباب المؤدية إلى الوفاة في جميع انحاء العالم. وقد اتجهت الأنظار في الأونة الأخيرة إلى الدور الذي يمكن ان تلعبه الخلايا الجذعية في تجديد خلايا القلب بعد حدوث احتشاء القلب الحاد. تهدف هذه الدراسة إلى بحث امكانية استخدام خلاصة نبات الاكنيشيا و خلاصة جنين القمح لزيادة عدد الخلايا الجذعية بالدم و تحسين وظائف القلب في الجرذان المستحدث بها احتشاء عضلة القلب الحاد. تم استحداث مرض احتشاء القلب الحاد عن طريق حقن الجرذان بمادة أيزوبروترينول ( 85 مجم/كجم ) تحت الجلد ليومين متتاليين . وقد عولجت الجرذان بخلاصة جنين القمح أو خلاصة نبات الاكنيشيا بعد حدوث الاحتشاء أو قبل وبعد حدوث احتشاء القلب الحاد. تم بعد ذلك قياس عدد خلايا CD34<sup>+</sup> بالدم وكذلك تم التحليل المناعي لخلايا CD34<sup>+</sup> بأنسجة القلب ، بالإضافة للفحص المجهرى لأنسجة القلب. كما تم قياس نشاط إنزيم الكرياتين كيناز ومستوي VEGF و انترليوكين 8 و GM-CSF بمصل الدم وذلك في اليوم الاول والسابع والرابع عشر بعد حدوث احتشاء القلب الحاد. وقد اثبتت النتائج قدرة خلاصة جنين القمح و خلاصة نبات الاكنيشيا على تحريك الخلايا الجذعية CD34<sup>+</sup> من مكانها و زيادة عددها في الدم وكذلك في أنسجة القلب. كما تمكنت هاتين الخلاصتين من زيادة نشاط انزيم الكرياتين كيناز والتأثير على مستوى VEGF و تقليل مستوى GM-CSF في مصل الدم، وكذلك تقليل الالتهابات المصاحبة لمرض احتشاء القلب الحاد ; مما قد يكون له دور في تعويض أنسجة القلب التالفة وتكوين أوعية دموية جديدة و منع حدوث الاختلال الوظيفي لعضلة القلب والذي يعقب الاحتشاء الحاد.

الكلمات الدلالية: الخلايا الجذعية CD34<sup>+</sup>، احتشاء القلب الحاد، خلاصة جنين القمح ، الاكنيشيا

## 2-5 باللغة الأجنبية:

Activation of endogenous stem cells mobilization can contribute to myocardial regeneration after ischemic injury. This study aimed to evaluate the possible role of Avemar or Echinacea extracts in inducing mobilization and homing of CD34<sup>+</sup> stem cells in relation to the inflammatory and hematopoietic cytokines in rats suffering from acute myocardial infarction (AMI). AMI was developed by two consecutive subcutaneous injections of isoprenaline (85 mg/kg). AMI rats were either post-treated or pre- and post-treated daily with oral doses of Avemar (121 mg/kg) or Echinacea (130 mg/kg). In whole blood, the number of CD34<sup>+</sup> cells was measured by flow cytometry and their homing to the myocardium was immunohistochemically assessed. Serum creatine kinase (CK), vascular endothelial growth factor (VEGF), interleukin-8 (IL-8) and granulocyte macrophage colony stimulating factor (GM-CSF) were determined on day 1, 7 and 14 after AMI. Sections of the myocardium were histopathologically assessed. Rats pre- and



post-treated with Avemar or Echinacea exhibited substantial increases in the number of circulating CD34<sup>+</sup> cells peaking on the first day after AMI to ~ 13 and 15 fold respectively with a decline in their level on day 7 followed by significant increase on day 14 compared to their corresponding AMI levels. Only post-treatment with Echinacea caused time dependent increase in circulating CD34<sup>+</sup> cells on 7 and 14 days. Such increases in circulating CD34<sup>+</sup> cells were accompanied by increased homing to myocardial tissue 14 days after AMI. Interestingly, pre- and post-treatment with Avemar or Echinacea substantially increased serum CK on day 1, normalized its activity on day 7 and on continued treatment only Echinacea increased markedly its activity on day 14 compared to their corresponding AMI values. Moreover, both treatments modified differently the elevated serum VEGF and the lowered GM-CSF levels of AMI group but did not affect IL-8 level. These results were supported histopathologically by reduced inflammatory reactions and enhanced neovascularization. In conclusion, Avemar and Echinacea extracts can effectively induce mobilization and homing of CD34<sup>+</sup> stem cells to the myocardial tissue and thus may help in stem cell-based regeneration of the infarcted myocardium.

**Key words:** CD34<sup>+</sup> Stem Cells - Myocardial Infarction - Avemar - Echinacea

#### **6- أهم النتائج التطبيقية التي تم التوصل إليها:**

1-6 أن لخلصة جنين القمح وخلصات نبات الاكنيشيا القدرة على تحريك الخلايا الجذعية CD34<sup>+</sup> من مكانها و زيادة عددها في الدم وكذلك في انسجة القلب  
2-6 تمكنت هاتين الخلاصتين من زيادة نشاط انزيم الكرياتين كيناز والتأثير على مستوى VEGF و تقليل مستوى GM-CSF في مصل الدم  
3-6 أدى تناول خلاصة جنين القمح وخلصات نبات الاكنيشيا إلى تقليل الالتهابات المصاحبة لمرض احتشاء القلب الحاد

7- ما هي الجهات التي يمكن أن تستفيد من هذا البحث:

1-7

2-7

3-7

4-7

8- هل توجد علاقة قائمة بإحدى هذه الجهات :  
في حالة نعم اذكر هذه الجهات :

لا

نعم

1-8

2-8

3-8

ما هي طبيعة العلاقة :

مشروع بحثي

تعاون أكاديمي

(

مشروع ممول من جهة ثالثة ( اذكر ما هي :

( تذكر )

أخري

9- هل توافق على التعاون مع جهات مستفيدة من خلال الجامعة :

لا  (لماذا)

نعم  نعم

(أ) لتطبيق البحث :  نعم

(ب) لاستكمال البحث:  نعم

(ج) أخرى :  (تذكر )

10- هل تم نشر بحوث مستخرجة من الرسالة في مجلات أو مؤتمرات علمية

Stem cell research and therapy 14 September 2015 1-10

11<sup>th</sup> National Conference of Biochemistry and Molecular Biology 2-10

From 30/3/2014 to 1/4/2014

11- هل سبق التقدم لتسجيل براءات اختراع

لا

12- هل توافق على إعطاء البيانات المذكورة في هذه الاستمارة لجهات أخرى

لا

نعم

توقيع المشرفين:

- أ.د/ أميرة عبد المنعم شاهين

- أ.د/ هالة جبر متولي

- د/ سمر حسب الله قاسم

توقيع الطالب:

مها عبد المنعم محمد

التاريخ :

وكيل الكلية( المعهد) للدراسات العليا و البحوث :