Aim of the Work

This work was a continuation of the work published by ***Abd EL-Wahab et al. (2014)*** who observed that disintegrin /like domain, isolated from ***Cerastes cerastes*** crude venom was able to enhance homing of labeled bone marrow mesenchymal stem cells into CCl_4 induced injured liver.

a) The current study aimed to replicate the previous work to proof its validity with extending the time of work from one week to three weeks. This could give better therapeutic effect for the injured liver.

b) In the same time, the study investigated the possible mechanism by which disintegrin /like domain could enhance the homing of labeled bone marrow mesenchymal stem cells.

Snake Venoms

Snake venoms are natural sources of many types of non-lethal and lethal biologically active compounds ***(Marcinkiewicz, 2013).***

The venomous snakes are classified depending on their morphological characteristics into five families ***(Tu, 1977*).**

- **Crotalidae**: comprises 6 genera; Crotalus, Sistrurrus, Agkistrodon, Bothrops, Lachesis and Trimersurus.
- **Colubridae**: It is the principle snake family which comprises an enormous group of about 1400 species including roughly 2/3 of world's snakes.
- **Elapidae**: comprises 50 genera, including the well-known Cobras, the African Mambas and the Kraits.
- **Hydrophidae:** represent sea snakes.
- **Viperidae**: this family comprises the genera Viper, Atractapis, Bitis, Causus, Cerastes, Echis, Adenorhinos, Atheris, Eristicophis, Pseudocerastes and Azempios.

The genus ***Cerastes*** includes both ***Cerastes cerastes*** and ***Cerastes vipera***; both comprise the most venomous snakes in Egypt ***(Marx, 1968)***.

Toxic effects of viper venoms on the tissues include severe local necrosis and hemorrhage. Integrins are the most important cellular receptors responsible for cell/ECM interaction. Viper venoms contain antagonists of integrins, which are classified according their structure as disintegrins and C-type lectin proteins (CLP) ***(Marcinkiewicz, 2013).***

The most common pathological effects in Viperidae venomation are edema, erythema, hemorrhage, blistering, and tissue necrosis ***(Fry, 2015).***

Hemorrhagic activity of viperid snake venoms induced by hemorrhagic metallo-proteinases (SVMPs) that act by initially hydrolyzing key substrates at the basement membrane (BM) of capillaries. This degradation results in the weakening of the mechanical stability of the capillary wall ***(Gutiérrez et al., 2016).***

Snake venoms components can be classified into two main components ***(Tu, 1977)****:*

Non protein components: contain both inorganic and organic constituents. Inorganic constituents include sodium, potassium, zinc, calcium and iron. These metals act as cofactors for many enzymes. Organic constituents include carbohydrates, lipids and nucleic acids ***(Torres et al., 2003)***.

Protein Components: Snake venoms are mix of enzymatic and non-enzymatic proteins ***(Kang et al., 2011).***

- **Non-enzymatic protein:** They include more than 1000 proteins which have been characterized including serine proteinase inhibitors, lectins, nerve growth factors, disintegrin, neurotoxins, cardiotoxins and myotoxins ***(Torres et al., 2003 and Gawade, 2004)***.

- **Enzymatic protein:** The most common snake venom enzymes involve phospholipases A_2, L-amino acid oxidases, acetylcholinesterases, serine proteinases and metalloproteinases ***(Kang et al., 2011).***

There are certain components of snake venoms which are platelet aggregation inducers and others are platelet aggregation inhibitors ***(El-Asmer et al., 1986, Farid et al., 1990, Nasser et al., 1991, Basheer et al., 1995, Salam et al., 1995 and Marrakchi et al., 1997).***

Cerastes cerastes is widely found in Arabian Peninsula and the Sahara. It is a small snake, about 30 cm in length, and is usually characterized by the presence of 2 small horns-like appendages above the eyes. Sometimes the horn may be short or absent so ***Cerastes cersates*** may be horned or not **(*Schneemann et al., 2004*)** as shown in figure (1).

Fig. (1): Cerastes cerastes snake ***(Schneemann et al., 2004).***

Cerastes cerastes venom has many components with different activities as inhibition of angiogenesis both in vivo and in vitro, induction of platelet aggregation and activation of prothrombin and factor X in coagulation cascade ***(Kessentini-Zouari et al., 2010)***. A coagulant component has been purified from Cerastes cerastes venom. This compound is believed to be primarily a factor X activator ***(El-Asmar et al., 1986).*** Purification and characterization of a platelet aggregation inhibitory compound from crude ***Cerastes cerastes*** venom was investigated by ***(Bassyouni, 2000).*** Metalloproteinase/ disintegrin- like component is purified from crude venom of ***Cerastes cerastes*** with platelets aggregation inhibiting activity by ***(Louka, 2001).***

Snake Venom Metalloproteinases

Snake venom metallo-proteinases (SVMPs) are abundant in the venoms of vipers and rattle snakes, playing important roles for the snake adaptation to different environments, and are related to most of the pathological effects of these venoms in human victims. The most evident effect of SVMPs is hemorrhage due to their functional diversity, targeting important physiological proteins or receptors in different tissues and in the coagulation system ***(Moura-da-Silva et al., 2016).*** They are monozinc endo-peptidases varying in size from 20 to 100 kDa ***(Sarray et al., 2013).***

SVMPs are grouped into several subclasses according to their domain organization:

1) **P-I SVMPs** are the simplest class of enzymes that contain only a metalloproteinase (M) domain.

2) **P-II SVMPs** contain a metalloproteinase (M) domain followed by a disintegrin (D) domain.

3) **P-III SVMPs** contain metalloproteinase (M), disintegrin-like (D) and cysteine-rich (C) domains. Based on their distinct post-translational modifications **P-III SVMPs** are further divided into subclasses, such

as **(P-IIIc)** obtained from dimerization or **(P-IIIb)** obtained from proteolytic processing. A new **(P-IIId)** subclass is a hetero-trimeric SVMPs with an additional snake C-type lectin-like domain ***(Sarray et al., 2013).***

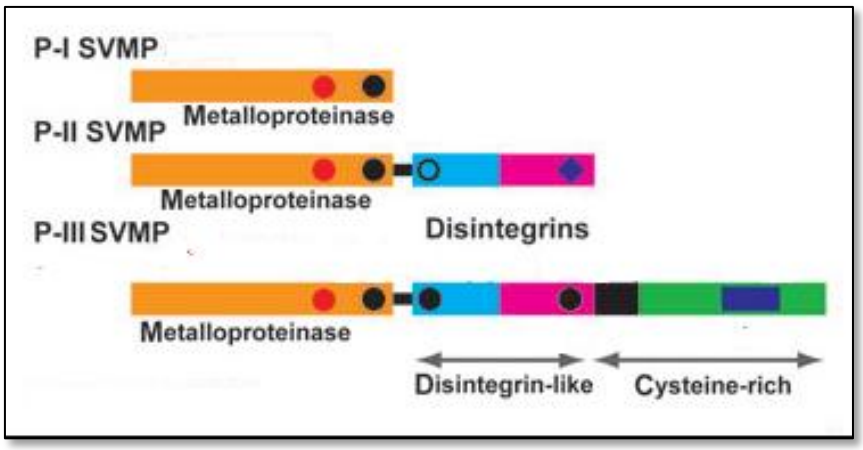

Fig. (2): the classification and structure of SVMPs ***(Kang et al., 2011).***

Snake venom metallo-proteinases (SVMPs) cause hemorrhage by disrupting the interactions between endothelial cells and the basement membrane through the degradation of endothelial cell membrane proteins (e.g., integrin, cadherin) and basement membrane components (e.g. fibronectin, laminin, type IV collagen) ***(Takeda et al., 2012)***. Large number of the P-III SVMPs can inhibit platelet aggregation, thus enhancing the hemorrhagic state ***(Sarray et al., 2013)***. Their disintegrin-like domain is devoid of the RGD motif ***(Moura-da-Silva et. al., 2007).***

DISINTEGRINS

The term disintigrin was first used in 1990 ***(McLane et al., 1998).*** Disintegrins were discovered and isolated from the venom of snakes and were given their name because of their biological function of binding to integrins ***(Amiryan, 2011)***. Disintegrins are small cysteine-rich non-enzymatic proteins (40-90 amino acids) that are created by the proteolytic processing of larger precursor of snake venom metallo-proteinases (**SVMP**s) ***(Takeda et. al., 2012).*** Most disintegrins represent the C-terminal domain of SVMPs PII by proteolytic cleavage and a minority of these proteins from the class of SVMPs PIII ***(Sarray et al., 2013).*** They are produced by every snake studied from four families: Atractaspididae, Elapidae, Viperidae and Colubridae **(*McLane and Paquette-Straub, 2008).***

The first disintegrin **(Trigramin)** was discovered and purified from the venom of ***Trimeresurus gramineus*** by ***Huang et al.*** in ***1987*** as a strong platelet aggregation inhibitor. Hundreds of new disintegrin molecules have been identified belonging to different structural and functional families ***(Walsh and Marcinkiewicz, 2011).***

Disintegrins contain a characteristic tripeptide motif, e.g. Arg-Gly-Asp (RGD), which is vital for binding integrins. Disintegrins typically possess RGD sequence which can inhibit integrin mediated platelet aggregation and cell–matrix interactions ***(Takeda et. al., 2012).***

Disintegrins were first known as inhibitors of platelet aggregation. Many studies on disintegrins have revealed new uses in the diagnosis of cardiovascular diseases and the design of therapeutic agents in arterial thrombosis, osteoporosis, and angiogenesis related tumor growth and metastasis ***(Sarray et al., 2013).***

Functional classification depends on presence of particular tripeptide integrin-binding motifs. Three functional classes of disintegrins have been identified containing **RGD**, **MLD** or **KTS** motifs.

- **The RGD family** of disintegrins is considered as the most investigated and largest family. They are present in some homo-dimeric disintegrins, such as **contortrostatin**.

Two relatively new families of snake venom disintegrins were identified:

- **The MLD family**, present only in heterodimeric disintegrins, mediates binding of these disintegrins to α4β1, α4β7 and α9β1 integrins. It was first characterized with the heterodimeric disintegrin, **EC3** which is isolated from **Echis (carinatus) sochureki** venom.

- **The KTS family** is monomeric molecules with 41 amino acids in its polypeptide chain. **Obtustatin** was the first reported member of the KTS disintegrins which is obtained from **Vipera lebetina obtuse** venom ***(Walsh and Marcinkiewicz, 2011)***.

EL-Asmar et al., (2007) cloned and sequenced disintegrin/like domain from ***Cerastes cerastes*** venom gland. The sequenced domain has 70 percent identification prediction with many disintegrins domains from different snakes like ***Trimeresurus flavoviridis, Glodius halys***, ***Agkistrodon halys*** and ***Trimeresurus macrosquamatus.*** It contains RGE domain rather than RGD domain.

Zaki et al. (2011) studied the value of the purified metalloproteinase / disintegrin/like domain from crude venom of ***Cerastes cerastes*** as a hepato-protective agent in white albino mice model treated with CCl_4. This hepato-protection role could be through up-regulation of TNF-α and HO-1 genes expression in the liver.

Abd EL-Wahab et al. (2014) explored that purified disintegrin/ like domain obtained from ***Cerastes cerastes*** crude venom has potential stimulatory effect on homing of mesenchymal stem cells to the liver tissue of CCl_4 treated white albino mice.

Arruda-Macêdo et al. (2015) discussed that Integrins regulate diverse functions in cancer pathology and in tumor cell development and contribute to important processes such as cell shape, survival, proliferation, transcription, angiogenesis, migration, and invasion. A number of snake venom proteins have the ability to interact with integrins. Among these are the disintegrins. Disintegrin can target specific integrins and as such it is conceivable that they could interfere in important processes involved in carcinogenesis, tumor growth, invasion and migration.

Recombinant disintegrin, vicrostatin (VCN); structure based on the contortrostatin (CN) sequence is considered as potent anti-tumor and anti-angiogenic agent by blocking the function of integrins on the surface of cancer cells and endothelial cells ***(Markland et al., 2010).*** Recombinant disintegrin, mojastin 1 (r-mojastin 1) from Mohave rattle snake could be a new anti-tumor agent through its ability to inhibit tumor migration, cell adhesion, and invasion ***(Lucena et al., 2011).***

Different disintegrins have been shown to possess antiproliferative activity for a variety of tumor cells. Lebein, a RGD heterodimeric disintegrin isolated from ***Macrovipera lebetina*** snake venom, inhibits colon tumor growth in vivo ***(Zakraoui et al., 2017)*** and was able to affect melanoma cell viability ***(Hammouda et al., 2016).***

Contortrostatin (CN), a disintegrin containing Arg-Gly-Asp, isolated from ***Agkistrodon contortrix contortrix*** venom, interacts with different epithelial carcinoma and endothelial cell surface receptors. The anticancer potential of CN was demonstrated because CN recognizes integrins αIIbβ3, α5β1, α5β3and α5β5 ***(Calderon et al., 2014).***

Stem Cells

Stem cells are a population of precursor cells that are capable of developing into many different cell types in the body. When a stem cell divides, each new cell has the potential either to remain a stem cell or differentiate into another type of cell with a more specialized function.

Stem cells are distinguished from other cell types in the body by capability of self- renewal (the ability to go through numerous cycles of cell division while maintaining the undifferentiated state) and under certain conditions induced to differentiate into specific cells ***(Dubie et al., 2014).***

Stem cells can be divided based on potency ***(Zhang and Cheng, 2013).*** Potency is the capacity to differentiate into specialized cell types.

Based on the potency, stem cells can be divided into five groups.

- **The first type** is the totipotent stem cells. These cells can differentiate into embryonic and extra-embryonic cell types. These cells are produced by fusion of an egg and sperm cell.

- **The second type** is pluripotent stem cells. These cells can differentiate into almost all cells except extra-embryonic cell types.

- **The third type** is the multi-potent stem cells which can differentiate into a number of cells, but only those of a closely related family of cells.

- **The fourth type** is the oligo-potent stem cells. These cells can differentiate into only a few cells, such as lymphoid or myeloid stem cells.

- **The fifth group** is the uni-potent cells.

Stem cells can also be classified based on their source, as embryonic, fetal, adult, amniotic cord blood and Induced pluripotent,

- **Mesenchymal stem cells (MSCs):**

Mesenchymal stem cells **(MSCs)** are spindle-shaped fibroblast-like cells with ability of self-renewal. These cells can be obtained from bone marrow, adipose tissue and umbilical cord blood ***(Zhao et al., 2012).*** First, MSCs must be plastic-adherent when maintained under standard culture conditions. Second, ≥ 95% of the MSC population must express CD105, CD73 and CD90, and lack the expression (≤ 2% positive) of CD45, CD34, CD14 or CD11b, CD79α or CD19 and HLA class II surface molecules. Third, MSCs

must differentiate into osteoblasts, adipocytes and chondroblasts under standerd in vitro differentiating conditions ***(Berardis et al., 2015).***

Mesenchymal stem cells **(MSCs)** can be differentiated into osteogenic, chondrogenic, adipogenic, myogenic, cardiomyogenic, and hematopoietic supporting tissue ***(Zhao et al., 2012).***MSCs secrete factors, including IL-6, M-CSF, IL-10, HGF, and PGE2, that promote tissue repair, stimulate proliferation and differentiation of endogenous tissue progenitors, and minimize inflammatory and immune reactions ***(Li et al., 2013).***

Bone marrow derived mesenchymal stem cells **(BM-MSCs)** can markedly decrease the induced liver injury by CCl_4 in albino rats, offering a hope to patient waiting for liver transplantation ***(Ahmed et al., 2014).***

Mechanism of action of mesenchymal stem cells (MSCs) in improving liver fibrosis include the following:

1) Trans-differentiation into hepatocyte-like cells.
2) Suppression of immune reactions.
3) Release of trophic factors which suppress the activated hepatic stellate cells (HSCs) and immune cells resulting in decrease fibrosis as well as increase the proliferation