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The therapeutic effect of camel mesenchymal stem cells on experimentally induced diabetes in rats.

Thesis
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Abstract

This study was carried out to investigate the possible therapeutic effect of camel Wharton jelly mesenchymal stem cells (WJ-MSCs) on induced diabetes in rats and compare it with the therapeutic effect of rat bone marrow mesenchymal stem cells (BM-MSCs) on diabetic rats. The MSCs were successfully isolated from camel Wharton's jelly and rat bone marrow. Induction of diabetes in rats was initiated by streptozotocin (STZ 60 mg/kg). Labeled MSCs with pkh26 stain were injected intravenously by dose of (3×10^6 cells) in the diabetic rats. Blood glucose and insulin levels were measured every two weeks as indicator for diabetes. Tissue samples from pancreas, liver and kidneys were collected for histopathological studies. Detection of insulin1, Smad-2 and PDX-1 genes in pancreatic tissues was done by quantitative RT-PCR. Serum was collected for liver and kidney function tests. The insulin level showed significant increase in

diabetic group treated with camel WJ-MSCs at 4 weeks post treatment (P.T) and reach near normal values at the end of experiment. But insulin level in diabetic group treated with rat BM-MSCs began to increase at 6 weeks (P.T) and still exhibit less values than control and camel WJ-MSCs groups at the end of experiment .The results of quantitative RT-PCR revealed significant increased in the three genes in Camel WJ-MSCs group but the Smad-2 gene in rat BM-MSCs group showed decreased values than in control and camel WJ-MSCs group. The histopathological results denote that the diabetic group treated with camel WJMSCs retains the cellular integrity of islets of Langerhans and pancreatic acini. Improvement of STZ side effects in the liver and kidneys was recorded supported by decrease of ALT, AST and Urea levels than diabetic group. In conclusion, camel WJ-MSCs possessed a good therapeutic effect against induced diabetes in rats than rat BM-MSCs. They reduced the side effects of STZ on the liver and kidneys. Their effects were more rapid than rat BM-MSCs.

Key words: Camel WJ-MSCs – rat BM-MSCs – STZ - diabetes.

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

أَفَلَا يَنظُرُونَ إِلَى الْإِبِلِ كَيْفَ خُلِقَتْ (17)

صدق الله العظيم
سورة الغاشية

Dedication

To my dear parents for their great help, guidance, encouragement and continuous support.

Thanks a lot...

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CONTENTS

List of abbreviations

ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BM-MNCs	Bone marrow mononuclear cell fraction
BM-MSCs	Bone marrow Mesenchymal stem cells
CD	Cluster differentiation
D	Diabetic
D+B	Diabetic+ Bone marrow MSCs
D+W	Diabetic+ Wharton's Jelly MSCs
DM	Diabetes mellitus
DMEM	Dulbecco's modified Eagle's medium
ESCs	Embryonic stem cells
FBS	Fetal bovine serum
IDDM	Insulin dependent diabetes mellitus
IDF	International Diabetes Federation's
IPCs	Insulin-producing cells
IPSCs	Induced pluripotent stem cells
MHC	Major histocompatibility complex
MSCs	Mesenchymal stem cells
NIDDM	Non-insulin dependent diabetes mellitus
NO	Nitric oxide
OH	Hydrogen peroxide
P.I	Post induction
P.T	Post treatment
qRT-PCR	quantitative Real-time polymerase chain reaction
STZ	Streptozotocin
T h	T helper cells
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus
TGF	Transforming growth factor
UC-PSCs	Umbilical cord primitive stromal cells
WJ-MSCs	Wharton's Jelly Mesenchymal stem cells
β-cells	Beta cells

Introduction

Diabetes mellitus (DM) is a chronic endocrine metabolic disorder characterized by inadequate production and use of insulin , resulting in abnormally high blood glucose levels which are responsible for complications such as blindness, kidney failure, cardiovascular disease, stroke, neuropathy and vascular dysfunction **(Georg and Ludvik, 2000) (Nyholm *et al*., 2000)**. Diabetes mellitus is classified as either type 1or type 2. Type 1 diabetes mellitus (insulin-dependent diabetes mellitus) results from the autoimmune destruction of the pancreatic beta cells, whereas type 2 diabetes mellitus (noninsulin-dependent diabetes mellitus) results from insulin resistance and impaired glucose tolerance **(Kaku, 2010 and Stanekzai *et al*., 2012).**

Diabetes is recognized as the world's fastest growing chronic disease, the number of people with diabetes is growing in each country. In 2013, the International Diabetes Federation's (IDF) estimates that, diabetes caused 5.1 million deaths globally. In 2015, (IDF) estimates that: One in 11 adults has diabetes (415 million) around world and (46.5%) of adults with diabetes is undiagnosed. In the Middle East and North Africa, four in ten adults with diabetes are undiagnosed. 542,000 children have type 1 diabetes, Europe has the highest prevalence of children living with type 1 diabetes .In 2040, IDF estimates that: One adult in ten will have diabetes (642 million) **(International Diabetes Federation's (IDF), 2015).**

Insulin injection and hypoglycemic drugs are currently used for Diabetes mellitus treatment. They can achieve adequate glycemic control but they don't prevent the development of diabetic complications **(Hussain and Theise, 2004).**

Islets transplantation is one of the promising therapies that may effectively prevent diabetic complications but there are several limitations as shortage of human donors and immune rejection **(Noguchi *et al*., 2010)** .

Stem cells having the ability to differentiate into functional insulin-producing cells, they become a promising source for insulin-producing cells **(Wu *et al*., 2010)**. Successful stem cell therapy can eliminate the cause of the disease and enhance the regeneration of pancreas **(Gholamrezanezhad, 2011)**.

MSCs isolated from Wharton's Jelly (WJ-MSCs) represent a potential cell source to treat diabetes. Systemic administration of WJ-MSCs results in recovery of pancreatic islet from insulitis, increased blood insulin secretion and correction of hyperglycemia **(Tsai *et al*., 2012 and Hu *et al*., 2013)**.

There were previous successful trails for isolation and differentiation of camel stem cells as Endothelial progenitor stem cells. They were successfully isolated from camel peripheral blood and differentiated in vitro to chondrocyte, osteoblast and neural cells**(El Miniawy *et al*., 2012)** . Also mesenchymal stem cells were successfully isolated and expanded from adipose tissues of camel and differentiated in vitro to chondrocyte and osteoblast **(Mohammadi-Sangcheshmeh *et al*., 2013)**.

Aim of the study is to:

1-Detect the ability of camel mesenchymal stem cells in treatment of diabetes mellitus induced experimentally in rats.

2-Compair between camel MSCs and rat bone marrow MSCs treatment effects on diabetes mellitus induced experimentally in rats.

Review of literature

(2.1.) Diabetes mellitus:

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by hyperglycemia due to disturbance in metabolism of carbohydrates, fat and protein. It results from defects in insulin secretion, action or both **(Nyholm *et al*., 2000)** . Insulin is the major regulatory hormone for intermediary metabolism, produced and secreted by β-cells of islet of Langerhans of the pancreas **(Partanen *et al*., 1995 and Butler *et al*., 1998)** . Pancreatic β-cells synthesize and secrete insulin in response to an increase of the blood glucose level in order to maintain it within the normal range, as a result of hyperglycemia glucose combines with longer-lasting proteins in blood vessel walls and interstitial tissue and turned into irreversible products **(Masaki *et al*., 2010)**. Accumulation of these products in blood vessel walls and interstitial tissue lead to serious complications like microangiopathy, nephropathy, neuropathy and retinopathy **(Crawford *et al*., 2009).**

Diabetes mellitus characterized by fasting hyperglycemia, glycosuria, osmotic polyuria, polydipsia, dehydration, increased appetite and often ketoacidosis, also the affected animals become either obese or wasted with increase susceptibility to infections **(Georg and Ludvik, 2000 and Ozougwu *et al*., 2013)**.

Diabetes Mellitus presents in two forms either Type 1 (T1DM) or Type2 (T2DM) (Zhang ***et al*., 2001)**. Type 1 diabetes (T1DM) is an insulin-dependent diabetes affecting genetically predisposed individuals. In such type, insulin-secreting β -cells within pancreatic islets of Langerhans are selectively and irreversibly destroyed by autoimmune assault **(Stanekzai *et al*., 2012)**.T1DM is an organ-specific autoimmune disease characterized by inhibited insulin production as a result of T cell mediated destruction of the pancreatic β cells in the islets of Langerhans **(Suarez-Pinzon and Rabinovitch, 2001 and Danke *et al*., 2004).**

Type 2 diabetes (T2DM) is characterized by a gradual decrease in insulin sensitivity and insulin secretion **(Stanekzai *et al*., 2012)**. T2DM is a heterogonous disorder caused by combination of genetic factors related to impaired insulin secretion, insulin resistance and environmental factors such as obesity, over eating, lack of exercise, and stress as well as aging **(Kaku, 2010)**. T2DM is not an autoimmune disorder and the susceptible genes that predispose to non-insulin dependent diabetes mellitus (NIDDM) have not been identified **(Ozougwu *et al*., 2013)** .

(2.2) Diabetes mellitus in animals.

Horses develop all forms of diabetes as insulin dependent diabetes mellitus (IDDM) and non-insulin dependent diabetes mellitus . IDDM was reported mainly in young horses, but elderly horses are also affected (the age ranges between 5 and 18 years) **(Kronfeld, 2003)**. Affected individuals with IDDM develop rapid weight loss despite polyphagia, hyperglycemia, glucosuria, low insulin levels, polydipsia compensatory polyuria and hepatomegaly may occur due to hepatic lipidosis **(Wreiole, 2011)**.The pancreas exhibits segmental shrinkage, lobule demarcation by lymphocytic infiltrates and inflammatory population into the pancreatic islets which causes severe decrease of β-cells **(Giri *et al*., 2011).** Sometimes inflammation is not observed especially in old individuals but the number of β-cells being reduced and confined to the periphery of the islets **(Jonhson *et al*., 2005)**.

Non-insulin dependent diabetes mellitus is rarely diagnosed in horses as hyperinsulinemia due to insulin resistance may be maintained for years, without exhaustion of β-cell **(Jonhson *et al*., 2004)**.

Juvenile or IDDM has been recorded in dogs with less than 12 months of age and spontaneous cases of insulin-dependent diabetes mellitus also recorded in older dogs and females **(Davison *et al*., 2003)** . Several studies concluded that females are prone to diabetes (more than 70% of diagnosed cases **(Fall *et al*., 2007)**. Insulin deficiency diabetes in dogs is due to progressive destruction of β-cells which occur either idiopathically or due to immune mediated β-cell **(Fall, 2009).**The pancreas of these cases showed β-cell loss associated with exocrine pancreatic lesions as pancreatic necrosis and pancreatitis **(Kang, 2008)**. Insulin resistance diabetes (relative insulin deficiency) also recorded in dogs. It

produced by insulin antagonists or concurrent disorder like diestrus gestation or secondary to other endocrinopathies (acromegaly, hyperadrenocorticism) **(Davison *et al*., 2005)**. Obesity was found to play an important role in the incidence of Insulin resistance diabetes in dogs **(Catchpole, 2008)**.

Non-insulin dependent diabetes mellitus (NIDDM) is one of the most frequently encountered endocrinopathy in cats, many research studies concluded that diabetic cat mimic some of the clinical and pathological features of human diabetes type 2 , its occurrence is in obese, indoor confined, middle-aged and old individuals (highest incidence in cats with 8 years of age) **(Scott-Moncrieff, 2010)**. In such cases, the pancreas showed deposition of amyloid in islets of Langerhans associated with loss of β-cells and onset of complications such as peripheral neuropathy and retinopathy **(Hoenig, 2002 and Reusch, 2010)**.The risk of disease results from obesity which induced low sensitivity of tissues to insulin**(Laflamme, 2011).**Juvenile diabetes is rarely described in cats **(Zini, 2009).**

(2.3.) Induction of diabetes mellitus.

Experimental diabetes mellitus has been induced in laboratory animals by chemical induction, surgical (pancreatectomy) and genetic manipulations in several animal species. Chemical induction of diabetes mellitus was the most widely used procedure, genetic and surgical procedures were rarely used because they required highly technical skills and the percentage of animals lost during the procedures were higher **(Etuk and Muhammed, 2010).**

(2.3.1.) Chemical induction of diabetes mellitus:

The diabetogenic drugs used for induction of diabetes mellitus are alloxan monohydrate, streptozotocin (STZ) with or without nicotinamide, ferric nitrilotriacetate, ditizona and anti-insulin serum **(Attia, 2009)**. STZ is preferred than alloxan to induce diabetic rat for its higher inductive rate, lower toxicity and mortality rate of animals is less than alloxan. It also preferred as its higher specificity as STZ with lower dose gives the same diabetic effect than alloxan with higher doses **(Szkudelski, 2001)**. In addition to that STZ is a simple, unexpensive and available method **(Holemans *et al*., 1997 and Thulesen *et al*.,**

1997). On the other hand induction of diabetes mellitus with ferric nitrilotriacetate is rarely used procedure, and there are no reports for using ditizona or anti-insulin serum for induction of diabetes mellitus **(Etuk and Muhammed, 2010)**. So researchers around the world have used streptozotocin to create experimental diabetes.

STZ (2-deoxy-2-(3-methyl-3-nitrosourea)-1-D-glucopyranose) is a broad-spectrum antibiotic produced by Streptomyces achromogenes. The diabetogenic effect of STZ was detected after injection of a single intravenous dose in rats and dogs **(McNeill, 1999).** Streptozotocin (STZ) is well known for its selective pancreatic islet cell toxicity, so it extensively used for induction of diabetes mellitus in animals **(Akhtar and Ali, 1984)**. STZ prevents cellular reproduction with a much smaller dose than the dose needed for inhibition enzymes involved in DNA synthesis **(Holemans and Vanassche, 2003)**, so it induced rapid and irreversible necrosis of β-cells **(Rerup *et al*., 1970)**. In addition to that, it causes alterations in blood insulin and glucose concentrations, two hours after injection hyperglycemia is observed with a temporary drop in blood insulin about six hours later hypoglycemia occurs with high levels of blood insulin, finally hyperglycemia develops and blood insulin levels decrease **(West *et al*., 1996)**. These changes in blood glucose and insulin concentrations reflect abnormalities in β cell function as STZ impairs glucose oxidation **(Bedoya *et al*., 1996)** and decreases insulin biosynthesis and secretion **(Bolaffi *et al*.,1987 and Nukatsuka *et al*., 1990b)** .

Some studies reported that STZ enter cells via a glucose transporter (GLUT 2) **(Schnedl *et al*., 1994 and Thulesen *et al*., 1997)**. The Intracellular action of STZ results in changes of DNA in pancreatic β cells comprising its fragmentation **(Yamamoto et *al*., 1981 and Morgan *et al*., 1994)**. Recent experiments have proved that the main reason for the STZ-induced β cell death is the alkylation of DNA **(Delaney *et al*., 1995 and Elsner *et al*., 2007)**. Moreover, STZ is a nitric oxide (NO) donor as it liberated when STZ metabolized inside cells **(Kröncke *et al*., 1995)**. It contributes in DNA damage **(Morgan *et al*., 1994 and Kröncke *et al*., 1995)**. NO is not the only molecule responsible for the