UNIVERSITI TEKNOLOGI MARA

AMELIORATIVE EFFECT OF GLUTATHIONE ADMINISTRATION ON TESTICULAR AND SPERM PARAMETERS IN STZ-INDUCED DIABETIC MICE

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ABSTRACT

Diabetes mellitus (DM) is known to cause reproductive impairment. In men, it has been linked to altered sperm quality and testicular damage. Oxidative stress (OS) plays a pivotal role in the development of DM complications. Glutathione (GSH) is a part of a non-enzymatic antioxidant defence system that protects lipid, protein and nucleic acid from oxidative damage. However, the protective effects of exogenous GSH on the male reproductive system have not been comprehensively examined. This study determined the impact of GSH administration in ameliorating the adverse effect of DM on testicular and sperm parameters in C57BL/6NTac STZ-induced diabetic mice. The mice were divided into four groups; non-diabetic control group (Group C), diabetic control group (Group D), GSH-treated non-diabetic group (Group GSH) and GSH-treated diabetic group (Group DGSH15). Administration of GSH at a dose of 15 mg/kg body weight was given for 6 consecutive weeks to Groups GSH and DGSH15 mice. Diabetic mice showed significant impairment in testicular and sperm parameters compared to non-diabetic mice. The administration of GSH at 15 mg/kg body weight improved testicular and sperm parameters in diabetic mice. Histological examination of testes also showed a significant difference in the diameter of seminiferous tubules, epithelial height, and diameter of the lumen in GSH-treated group, compared to diabetic control. The apoptotic index calculated using the fluorescent TUNEL assay was lower in GSH-treated compared to diabetic control group, showing the ability of GSH to improve diabetes-related reproductive impairment. Malondialdehyde (MDA) and 8-hydroxy-2'-deoxyguanosine (8-OHdG) were significantly lower in the GSH-treated group than in the diabetic control group. The expression of 17 different genes were examined using RT-qPCR. The antioxidative actions of GSH were seen in GSH-treated mice, supported by the upregulations of *Bcl-2*, *Sod1*, *Gpx1*, *Mfn1*, *Ndufs1*, *Ndufs2* and *Uqcrfs1*. Five Caspase pathway genes were further examined using ELISA for protein expression. The Bax/Bcl-2 ratio was found to be lower in the GSH-treated group, indicating reduced OS with GSH intervention. In conclusion, GSH at 15 mg/kg body weight was adequate to reverse the effect of DM on sperm quality and testicular damage in C57BL/6NTac mice. The determination of the effective dose of GSH in this study is expected to provide a baseline for further exploration into the mechanisms underlying the possible protective effects of GSH and its development for clinical application.

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CHAPTER ONE
INTRODUCTION

1.1 Background of Study

Diabetes mellitus (DM) is a metabolic disorder characterized by hyperglycaemia. It is caused by defects in insulin secretion, insulin action or both. Failure to metabolize glucose leads to its accumulation in blood, resulting in various systemic complications including male infertility. Hyperglycaemia may affect spermatogenesis, sperm maturation and the reproductive function of sperm. Excessive free radical production induced by hyperglycaemia in the testis and epididymis has been recognized as an important factor contributing to sperm damage in most diabetic models. Over the years, as a result of social and economic transformations that include increasing food availability, a westernized diet, and decreased physical activity the younger generation has become more susceptible to DM.

In the context of this study, the increasing prevalence of DM in young males is concerning as it increases subfertility. The rising occurrence of diabetes at the reproductive age significantly alters fertility rates. Oxidative stress (OS) plays a pivotal role in the development of diabetes complications. In diabetic males, high amounts of OS may result in decreased fertility due to impaired spermatogenesis. A significant decrease in total antioxidant levels among DM patients was reported by various studies. Intracellular glutathione (GSH) concentrations have been found to decrease in poorly managed DM patients compared to nondiabetic patients. Glutathione deficiency may lead to instability of the midpiece, resulting in defective motility and morphology of the sperm.

Glutathione is an antioxidant that is naturally found in humans, animals, fungi, and bacteria. It protects lipids, proteins, and nucleic acids against oxidative damage. It is a tripeptide containing a gamma peptide bond between the glutamate carboxyl group and the cysteine amine group. It is a major antioxidant defence mechanism against reactive oxygen species (ROS) and electrophiles. It is generated *de novo* in two successive enzyme processes that require ATP. The first step involves the reaction of cysteine with glutamate to create γ -glutamylcysteine. This initial reaction is the rate-limiting step in the synthesis of GSH and is controlled by the availability of