# EFFECTS OF CARVEDILOL ON STREPTOZOTOCIN INDUCED DIABETIC CARDIAC AUTONOMIC NEUROPATHY IN WISTAR RATS


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**(14/64KJ004)**


**MAY, 2021**

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**By**

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**A THESIS WRITTEN IN THE DEPARTMENT OF PHYSIOLOGY, FACULTY OF BASIC MEDICAL SCIENCES, UNIVERSITY OF ILORIN, ILORIN, NIGERIA, IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF DOCTOR OF PHILOSOPHY (Ph.D.) IN PHYSIOLOGY**

**MAY, 2021**

## APPROVAL PAGE

This thesis has been read and approved as having met the partial fulfilment of the requirements of the Department of Physiology and postgraduate school, University of Ilorin, Ilorin, Nigeria, for the award of the degree of Philosophy in Physiology (Ph.D.).

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## DEDICATION

This research is dedicated to the Almighty God for the strength, inspiration and enablement to research into the mysteries of his creation thereby advancing science and medical care.

## DECLARATION

I AKINLADE, Olawale Mathias declare that this thesis titled "Effects of Carvedilol on Streptozotocin induced Diabetic Cardiac Autonomic Neuropathy in Wistar Rats" is a record of my research work. It has neither been presented nor accepted in any previous application for a higher degree. All sources of information have been specifically acknowledged.

This research has been approved by the University ethical review committee of the University of Ilorin with approval number: UERC/ASN/2019/1912.

…….. ……..

AKINLADE, Olawale Mathias Date

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AKINLADE, Olawale Mathias

2021

## ABSTRACT

The prevalence of diabetic cardiac autonomic neuropathy (DCAN) is very high among patients with diabetes mellitus (DM). Despite intensive research on mechanism and treatment of DCAN, it still remains a major cause of mortality. Although carvedilol has been recommended for the treatment of heart failure, its role in the management of DCAN is yet to be established. This study investigated the effects of carvedilol on streptozotocin-induced DCAN rats. The objectives of the study were to determine the: (i) outcome of DM and DCAN induction in rats; (ii) effects of carvedilol on biochemical markers of autonomic nervous system in streptozotocin-induced DCAN rats; (iii) effects of carvedilol on heart rate variability (HRV) indices in streptozotocin-induced DCAN rats; and (iv) effects of carvedilol on cardiac histology and nerve densities (ND) in streptozotocin-induced DCAN rats.

Eighty-four Wistar rats were assigned into two groups in the induction phase; type 1 DM model (single high dose Streptozotocin (STZ) at 50mg/kg body weight (bw)) and type 2 DM model (high fat diet feeding for 8 weeks, thereafter 25mg/kg bw of STZ daily for five days) respectively. In the interventional phase; each model was sub-divided into five sub-groups comprising; i) a sham control sub-group; ii) three DCAN sub-groups receiving carvedilol at 0.1, 1 and 10mg/kg bw respectively for 28 days; and iii) a DCAN control sub-group without carvedilol. Blood glucose, C-peptide, insulin, homeostatic model for assessment of insulin resistance and pancreas histology were adopted in assessing DM induction. Holter electrocardiogram was used to access HRV indices. Antioxidant markers, autonomic biomarkers, cardiac histology and ND were evaluated using standard methods. Data, expressed as means ± standard errors of mean were analysed using one way analysis of variance and Bonferonni's *Post-hoc* test at $p<0.05$.

The findings of the study were that:

(i) STZ-induced DM rats had significantly ($p<0.05$) higher blood glucose (22.50 ± 7.53 vs. 4.68 ± 0.59 mmol/L) and lowered insulin and C-peptide. Advanced glycated end products (AGEs) (63.54 ± 2.09 vs. 23.04 ± 5.17 ng/ml and 91.61 ± 9.09 vs. 32.04 ± 6.37 ng/ml) and noradrenaline (528.21 ± 279.48 vs. 119.06 ± 57.88 pg/ml and 889.76 ± 299.48 vs. 111.56 ± 57.65 pg/ml) were significantly higher in both DM models respectively;

(ii) choline acetyl tranferase (choactase) and nerve growth factor (NGF) were significantly reduced after STZ treatment;

(iii) carvedilol significantly ($p<0.05$) increased gluthathione level, total anti-oxidant capacity, choactase activity (1.21 ± 0.04 vs. 0.71 ± 0.02 ng/ml and 1.14 ± 0.03 vs. 0.69 ± 0.02 ng/ml) and NGF whereas it reduced the AGEs and noradrenaline levels in both DM models;

(iv) carvedilol significantly ($p<0.05$) increased high frequency power spectral (14,096.7 ± 238.5 vs. 5,403.5 ± 125.7 $ms^2$ and 8,123.9 ± 167.3 vs. 3,057.8 ± 101.8 $ms^2$ respectively) and other autonomic heart rate variables but reduced the low frequency power spectral in both models of DM; and

(v) carvedilol had no significant ($p>0.05$) effect on cardiac axonal dystrophy and cardiac ND in both models.

The study concluded that carvedilol ameliorated cardiac autonomic neuropathy in both DM animal models. The study recommended multicenter randomized clinical trials to ascertain the role of carvedilol in DCAN patients.

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| AACE | American Association of Clinical Endocrinologists |
| ABPM | Ambulatory blood pressure measurement |
| ACE | American College of Endocrinology |
| ACCORD | Action to control cardiovascular risk in diabetes |
| ACE | Angiotensin-converting enzyme |
| ADA | American Diabetes Association |
| ADP | Adenosine diphosphate |
| AGE | Advanced glycated end product |
| AIDS | Acquired immunodeficiency syndrome |
| ALA | Alpha-lipoic acid |
| AMD | Italian Association of Clinical Diabetologists |
| AMP | Adenosine monophosphate |
| AMPK | AMP activated protein kinase |
| ANOVA | Analysis of variance |
| ANS | Autonomic nervous system |
| ATP | Adenosine triphosphate |
| AUC | Area under curve |
| AV | Atrioventricular |
| BP | Blood pressure |
| BSA | Body surface area |
| C | Carvedilol |
| CAN | Cardiovascular autonomic neuropathy |

| | |
|---|---|
| CART | Cardiovascular autonomic reflex test |
| CAT | Cathecholamines |
| CD | Cluster of differentiation |
| CHAT | Choline acetyltransferase |
| Choactase | Choline acetyltransferase |
| CGL | Congenital generalized lipodystrophy |
| CV | Cardiovascular |
| DAG | Diacylglycerol |
| DCAN | Diabetic cardiac autonomic neuropathy |
| DCCT | Diabetes control and complication trials |
| DEND | Developmental delay epilepsy and neonatal diabetes |
| DF | Dilution factor |
| DGLA | Dihomo-γ-linolenic acid |
| DHAP | Dihydroxyacetonephosphate |
| DIAD | Detection of ischaemia in asymptomatic diabetics |
| DKA | Diabetic ketoacidosis |
| DM | Diabetes Mellitus |
| DNA | Deoxyribonucleic Acid |
| DPP | Diabetes Prevention Program |
| DPP-4 | Dipeptidyl-peptidase 4 |
| ECG | Electrocardiogram |
| EDIC | Epidemiology of Diabetes Interventions and Complications |
| eGFR | Estimated glomerular filtration rate |

| | |
|---|---|
| ELISA | Enzyme-linked immunosorbent assay |
| ER | Endoplasmic reticulum |
| FFA | Free fatty acids |
| FBS | Fasting blood sugar |
| FPG | Fasting plasma glucose |
| FPLD | Familial partial lipodystrophy |
| G-6-P | Glucose-6-phosphate |
| GAD | Glutamic acid decarboxylase |
| GDM | Gestational diabetes Mellitus |
| GI | Gastrointestinal disorder |
| GIT | Gastrointestinal tract |
| GLP-1 | Glucagon like peptide 1 |
| GLUT | Glucose transporters |
| Gly3P | Glycerol-3-phosphate |
| GSH | Glutathione |
| GSK | Glaxosmithkline |
| HbA1C | Glycated haemoglobin |
| HCL | Hydrocloric acid |
| HDL | High density Lipoprotein |
| H & E | Haematoxylin and eosin |
| HED | Hydroxyephedrine |
| HF | High frequency |
| HFD | High fat diet |

| | |
|---|---|
| HHS | Hyperosmolar hyperglycaemic state |
| HIV | Human immunodeficiency virus |
| HLA | Human luecocyte antigen |
| HOMA-IR | Homeostasis model assessment for insulin resistance |
| HR | Heart rate |
| HRP | Histidine rich protein |
| HRV | Heart rate variability |
| HT | Hypertension |
| IAA | Anti-insulin antibodies |
| IAPP | Islet amyloid polypeptide |
| IBD | Inflammatory bowel disorders |
| ICA | Islet cell autoantibodies |
| ICCA | Islet cell cytoplasmic antibodies |
| ICSA | Islet cell surface antibodies |
| IDDM | Insulin dependent diabetes Mellitus |
| IDF | International Diabetes Federation |
| IFG | Impaired fasting glucose |
| IGT | Impaired glucose tolerance |
| IGGTT | Intragastric glucose tolerance test |
| IL | Interleukins |
| IPGTT | Intra-peritoneal glucose tolerance test |
| IR | Insulin resistance |
| IVGTT | Intravenous glucose tolerance test |

| | |
|---|---|
| KG | Ketoglutarate |
| LA | Lipoic acid |
| LADA | Latent autoimmune diabetes of adulthood |
| LDL | Low density lipoprotein |
| LF | Low frequency |
| LF/HF | Low frequency to high frequency |
| MALA | Metformin associated lactic acidosis |
| MHC | Major histocompatibility complex |
| MIBG | Metaiodobenzylguanidine |
| MIDD | Maternally inherited diabetes and deafness |
| MODY | Maturity-onset diabetes of the young |
| MSNA | Muscle sympathetic nerve activity |
| NAD | Nicotinamide adenine dinucleotide |
| NADH | Nicotinamide adenine dinucleotide hydrogen |
| NAFLD | Non-alcoholic fatty liver disease |
| NC | Normoglycaemic control |
| NGF | Nerve growth factor |
| NGSP | National Glycohemoglobin Standardization Program |
| NIDDM | Non-insulin dependent diabetes Mellitus |
| NOD | Non-obese diabetes |
| NPDR | Non-proliferative diabetic retinopathy |
| OAA | Oxaloacetic acid |
| OADs | Oral anti-diabetic agents |

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|---|---|
| OD | Optical density |
| OH | Orthostatic Hypotension |
| OGGT | Oral glucose tolerance test |
| OS | Oxygen species |
| OSAS | Obstructive sleep apnoea syndrome |
| PBS | phosphate buffer solution |
| PCOS | Polycystic ovary syndrome |
| PDE | Phosphodiesterase |
| PDR | Proliferative diabetic retinopathy |
| PI3-K | Phosphatidylinositol-3 kinase |
| PKC | Protein kinase C |
| PNN50 | Percentage of successive normal sinus R-R intervals >50 ms |
| PNDM | Permanent neonatal diabetes |
| PS | Parasympathetic |
| PUFA | Polyunsaturated fatty acid |
| PVD | Peripheral vascular disease |
| RCAD | Renal cysts and diabetes |
| RMSSD | Root mean square of successive differences in normal-to-normal R-R intervals |
| RNS | Reactive nitrogen species |
| ROS | Reactive oxygen species |
| RPG | Random plasma glucose |
| S | Sympathetic |
| SA | Sinoatrial |

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|---|---|
| SDNN | Standard deviation of normal to normal R-R intervals |
| SD | Sorbitol Dehydrogenase |
| SDH | Sorbitol Dehydrogenase |
| SGLT2 | Sodium glucose transporter 2 |
| SID | Italian Society of Diabetology |
| SMI | Silent myocardial ischaemia |
| STZ | Streptozotocin |
| T1DM | Type 1 DM |
| T2DM | Type 2 DM |
| TAC | Total antioxidants capacity |
| TC | Total cholesterol |
| TCA | Tricarboxylic acid |
| TCR | Toll-like cell receptor |
| TG | Triglycerides |
| TGF | Transforming growth factor |
| TNDM | Transient neonatal diabetes |
| TNF | Tumor necrosis factor |
| TREG | Regulatory T cells |
| UERC | University of Ilorin ethical review committee |
| USA | United states of America |
| Vm | Voltage membrane |
| WHO | World Health Organisation |