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Research Article

MOMORDICA CHARANTIA PROTECTS AGAINST CARDIAC DAMAGE IN STREPTOZOTOCIN-INDUCED DIABETIC WISTAR RATS

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ABSTRACT
Diabetes mellitus is one of the most important world health problems, especially in developing countries where prevalence and incidence rates are highest. Diabetic patients are particularly prone to cardiovascular diseases including hypertension, atherosclerosis, diabetic cardiomyopathy, congestive heart failure and cardiac autonomic neuropathy. The present study investigated the effects of *Momordica charantia (M. charantia)* on histological changes of the left ventricle of the heart in streptozotocin-induced diabetic Wistar rats. Forty healthy adult Wistar rats of both sexes were randomly assigned into five groups A, B, C, D and E of eight rats each. Group A were the control (normal rats); B were the experimentally-induced diabetic rats; C were diabetic rats treated with methanolic extracts of *M. charantia* for two weeks (withdrawal group); D were diabetic rats treated with methanolic extracts of *M. charantia* for four weeks. E was diabetic rats treated with glimepiride for four weeks. Tissues were harvested, processed routinely in paraffin wax and stained with routine and special stains. Histological studies revealed disorganization of myofibril in the left ventricle of diabetic rats. Histochemical analysis also revealed abnormal deposition of glycogen in left ventricle of diabetic rats. *M. charantia* and glimperide attenuated the morphological alterations and reduced the glycogen deposits.
Keywords: Diabetes, *Momordica charantia*, Histology, Heart, Left ventricle

INTRODUCTION

Diabetes mellitus is a serious metabolic disorder with micro and macro-vascular complications that result in a significant morbidity and mortality. Increasing proportion of the aging population, consumption of calorie rich diet, obesity and sedentary lifestyle have led to a tremendous increase in the number of diabetics worldwide[1]. Diabetes mellitus is one of the most important world health problems, especially in developing countries where prevalence and incidence rates are highest. Diabetic patients are particularly prone to cardiovascular diseases including hypertension, atherosclerosis, diabetic cardiomyopathy, congestive heart failure and cardiac autonomic neuropathy[2]. Coronary atherosclerosis and cardiomyopathy occur as a result of the metabolic abnormalities associated with diabetes[3]. These physical changes require years to develop in human following the onset of chronic hyperglycemia[4]. Bradycardia has also been noted to be a consistent feature when evaluating diabetic rat models[5,6,7]. Heart rate variability in diabetes is commonly attributed to associated neuropathy[8]. Carnitine deficiency is often encountered in Diabetes mellitus[9,10]. Structural changes in myocardial mitochondria have been associated with diabetes and concomitant carnitine deficiency[5].

Momordica charantia (Linn Family: Cucurbaceae) is one of the popular herbs that grow in different regions of Nigeria. It is commonly called Bitter melon, Bittergoud, Balsam pear. Bittergourd is known in some tribes of Nigeria as Ejirin wewe (Yoruba) Okban, Ndeme (Igbo) and Garafun (Hausa). It's a slender, climbing annual vine with long-stalked leaves and yellow, solitary male and female flowers borne in the leaf axils. The fruit looks like a warty gourd, usually oblong and resembling a small cucumber. The young fruit is emerald green, turning to orange-yellow when ripe. At maturity, the fruit splits into three irregular valves that curl backwards and release numerous reddish-brown or white seeds encased in scarlet arils. The Latin name *Momordica* means "to bite," referring to the jagged edges of the leaves, which appear as if they have been bitten. All parts of the plant, including the fruit, taste very bitter[11].

Various parts of *M. charantia* such as the seed, fruit and even the whole plant has been reported to have beneficial effects in prevention and treatment of many diseases in folkloric medicine, especially in the treatment of DM in individuals with non-insulin dependent diabetes[12,13]. It has hypoglycaemic properties as it significantly suppressed the rise in blood glucose concentrations in albino rats[12,14]. The first clinical study into the influence of the fresh juice of bittergourd on the management of DM was by Akhtar[15]. These findings suggested the intervention would effectively treat all symptoms of diabetes including polyuria, polydipsia, and polyphagia. Sarkar *et al.,*[16] and Miura *et al.,*[17] indicated that the fresh bitter-gourd juice caused a significant reduction in plasma glucose concentration, and an improvement in the response to an oral glucose load. Bitter melon contains an array of biologically active plant chemicals including triterpenes, proteins and steroids. In addition, a protein found in bitter melon, momordin, has clinically demonstrated anticancerous activity against Hodgkin's lymphoma in animals. Other proteins in the plant, alpha- and beta-momorcharin and cucurbitacinB, have been tested for possible anticancerous effects[18]. In numerous studies, at least three different groups of constituents found in all parts of bitter melon have clinically demonstrated hypoglycemic (blood sugar lowering) properties or other actions of potential benefit against diabetes mellitus[19]. These chemicals that lower blood sugar include a mixture of steroidal saponins known as charantins, insulin-like peptides, and alkaloids. The hypoglycemic effect is more pronounced in the fruit of bitter melon where these chemicals are found in greater abundance.

The present study examined the effects of *M. charantia* on histological changes of the left ventricle of the heart in streptozotocin-induced diabetic Wistar rats and compared the

effects with those of glimepiride, an oral blood-glucose-lowering drug of the sulfonylurea class.

MATERIALS AND METHODS

Animal care

Forty healthy adult Wistar rats of both sexes, with average weight of 134.4g were used for the experiment. The rats were bred in the animal holding of College of Health Sciences, Obafemi Awolowo University, Ile-Ife. They were maintained on standard rat pellet (Capsfeed**,** Ibadan, Nigeria) and water was provided *ad libitum*.

The animals were randomly assigned into five groups A, B, C, D and E of eight rats each. Group A were the control normal rats administered intraperitoneally with 0.1M sodium citrate buffer, group B were the rats induced with single dose of 65mg/kg streptozotocin and administered with 10% tween 80 for four weeks after four weeks of diabetic stabilization, group C and D were the experimentally-induced diabetic rats treated with methanolic extracts of *Momordica charantia* (100 mg/kg/day) dissolved in 10% tween 80 for two weeks (withdrawal group) and four weeks respectively after four weeks of diabetic stabilization, group E were the diabetic rats treated with a standard diabetic drug (2 mg/kg/day of glimepiride) dissolved in 10% tween 80 for four weeks after four weeks of diabetic stabilization.

The animals received humane treatment as outlined in the "Care and Management of Laboratory Animals" published by the National Institute of Health[20] with ethical clearance number (IRB/IEC 00005422)

Plant material:

Matured leaves of *Momordica charantia* (Cucurbitaceae) were collected during the raining season from suburban villages of Ile-Ife metropolis in Osun State of Nigeria. The leaves were taken to the Herbarium in the Department of Botany, Obafemi Awolowo University, Ile -Ife to confirm identification and a voucher specimen number (UHI 16510) was placed in the Herbarium.

Preparation of methanolic extract of *M. charantia*:

Leaves of *Momordica charantia* were air dried and powdered in a warring blender. A 765 g of the powdered leaves were extracted in 1.95 L of absolute methanol for 72 hours with intermittent shaking and filtered. The filtrate were concentrated *in vacuo* at 35^0C using a vacuum rotary evaporator (Bűchi Rotavapor R110, Schweiz). The extract were partitioned between water and dichloromethane, the dichloromethane fraction (5.94%) was oven-dried at 37^oC and stored until it is ready to be used. Aliquot portions of the extract were weighed and dissolved in 10% tween 80 for use on each day of the experiment.

Induction of diabetes:

Diabetes mellitus was experimentally-induced in groups B, C, D and E by a single intraperitoneal injection of 65 mg/kg body weight of streptozotocin (Tocris Bioscience, UK) dissolved in 0.1M sodium citrate buffer (pH 6.3). Diabetes was confirmed in animals 48 hours after induction, by determining fasting blood glucose level using a digital glucometer (Accu-chek® Advantage, Roche Diagnostic, Germany) consisting of a digital meter and the test strips using blood samples obtained from the tail vein of the rats. Animals in group A were given equal volume of citrate buffer used in dissolving streptozotocin intraperitoneally.

Administration of extract and anti-diabetic drug:

Methanolic extracts of the leaves of *M. charantia* (100 mg/kg) was dissolved in 10% tween 80 and administered daily (orally) by gastric intubation to the rats in groups C and D for 2 and 4 weeks respectively. The standard antidiabetic drug (glimepiride 2 mg/kg) was administered daily to group E rats for four weeks [21], while the rats in group B were administered with the vehicle used in dissolving the extract and glimperide (10% tween 80).

Sacrifice of the animals:

At the end of the experimental period, all the animals were physically observed and anesthesized by chloroform inhalation. A midline incision was performed at the thoracic region. The heart was harvested and the left ventricle was excised, fixed in 10 % Formol saline and 10% neutral buffered formalin for 72 hours.

Histology, Histochemistry and Photomicrography:

Tissues were processed routinely in paraffin wax embedding. Sections of 6µm thick were cut and stained using heamatoxylin and eosin (H&E) procedure, Verhoeff - Van Gieson (VVG) staining procedure for elastic fibre and Periodic Acid Schiff (PAS) staining procedure for demonstration of glycogen. The sections were examined under a LEICA research microscope (LEICA Dm750, Switzerland) with a digital camera attached (LEICA ICC50). Digital photomicrographs of stained sections were taken.

RESULTS

The control section showed a normal histological outline of the left ventricle characterized by well organized myofibrils as against disorganized myofibrils in the diabetic group (Fig. 1). These alterations were greatly attenuated with administration of *M. charantia* and glimepiride for four weeks. This effect was better improved with *M. charantia* than glimepiride. Withdrawal of extract only showed mild improvement in the histological outline. Evidences from Verhoeff-vanGieson stain revealed that the elastic fibers were greatly reduced in the myocardium of the left ventricle in the diabetic group. With the commencement of extract administration and glimepiride for four weeks, there were significant improvements in the restoration of elastic fibers as evident in the staining intensity. Withdrawal of extract treatment slightly improves the deposition of elastic fibers (Fig. 2). Histochemical findings showed accumulation of glycogen deposit in the left ventricle of diabetic rats (Fig. 3). Treatment of diabetic rats with *M. charantia* and glimepiride for four weeks reduced the PAS positive staining intensity. The group treated with *M. charantia* for two weeks, revealed a mild reduction in the PAS positive staining intensity.

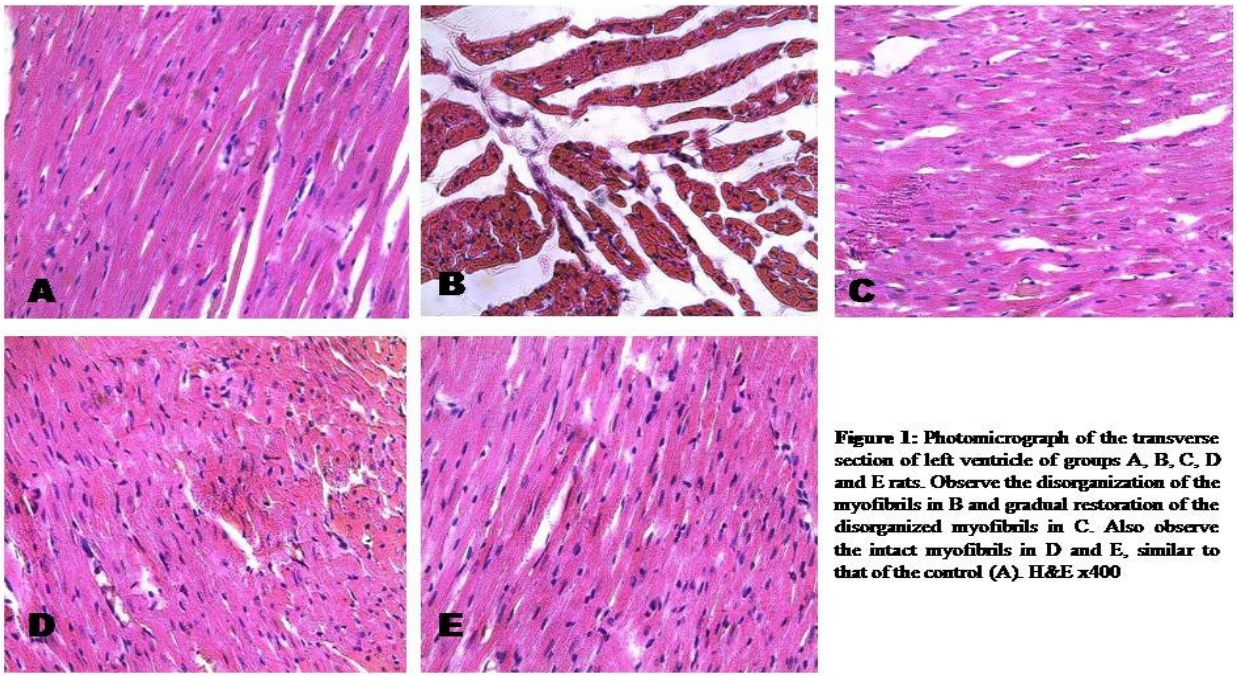

Figure 1: Photomicrograph of the transverse section of left ventricle of groups A, B, C, D and E rats. Observe the disorganization of the myofibrils in B and gradual restoration of the disorganized myofibrils in C. Also observe the intact myofibrils in D and E, similar to that of the control (A). H&E x400

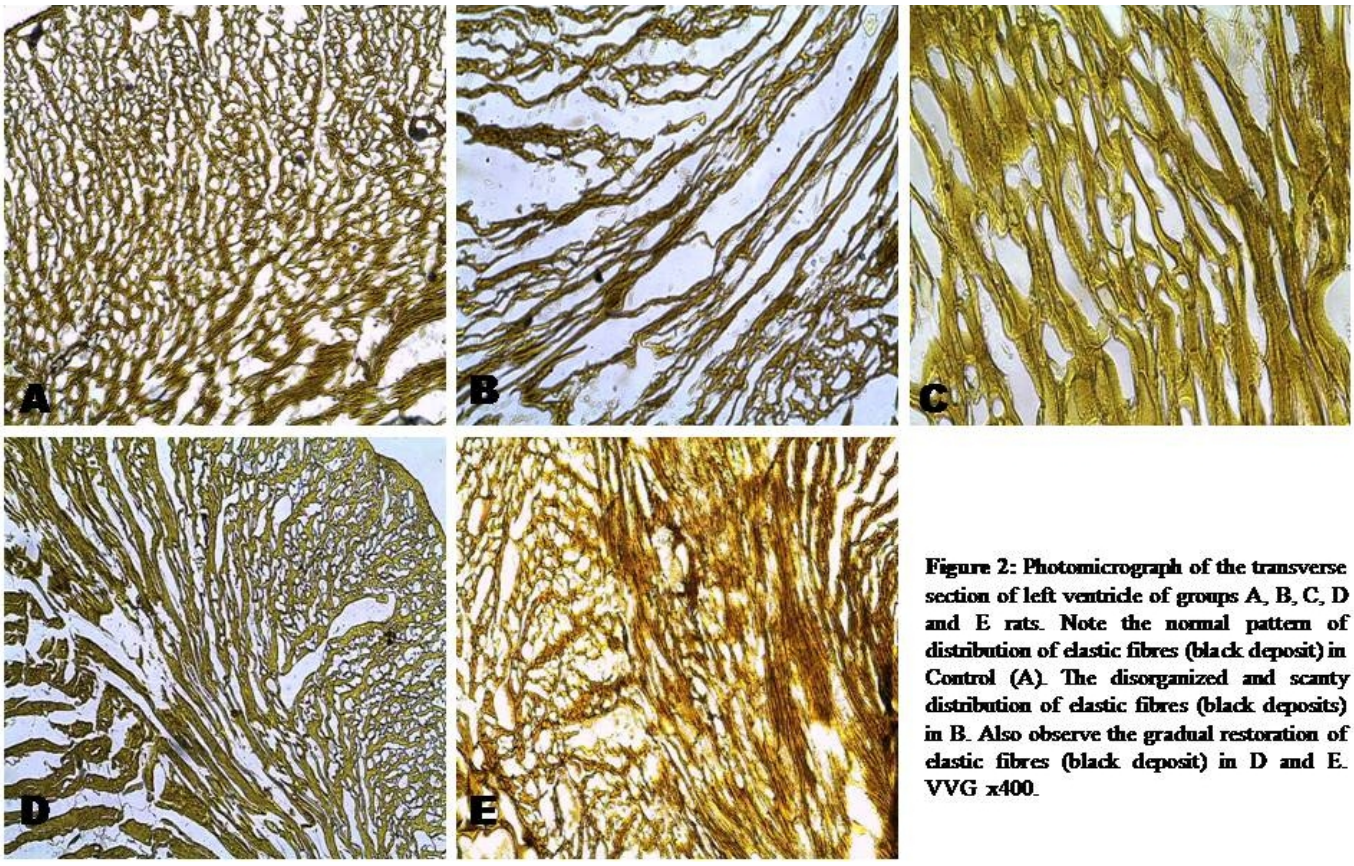

Figure 2: Photomicrograph of the transverse section of left ventricle of groups A, B, C, D and E rats. Note the normal pattern of distribution of elastic fibres (black deposit) in Control (A). The disorganized and scanty distribution of elastic fibres (black deposits) in B. Also observe the gradual restoration of elastic fibres (black deposit) in D and E. VVG x400.

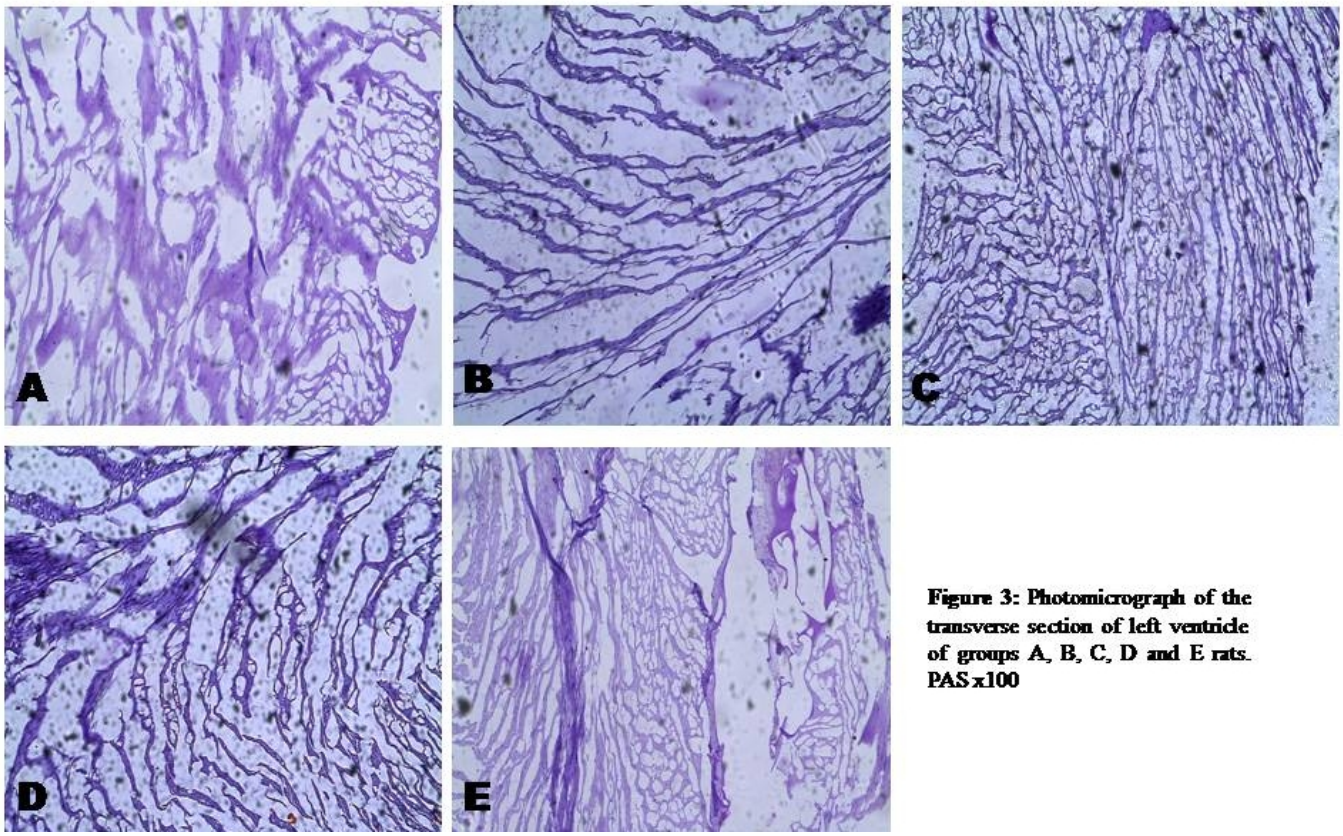

Figure 3: Photomicrograph of the transverse section of left ventricle of groups A, B, C, D and E rats. PAS x100

DISCUSSION

Diabetes has been reported to be associated with profound alterations in biochemical and normal histology leading to an increased risk of coronary heart disease[22,23,24,25]. Diabetic cardiomyopathy characterized by diastolic dysfunction and left ventricular hypertrophy is usually the terminal condition of heart in diabetes[26].

Histologically, there was evidence of architectural alteration in the myocardium of diabetic animals. This observation is consistent with previous study[27]. These effects were abrogated with the administration of *M. charantia* extract and glimepiride for four weeks. This observation may have been possible due to a reduction in blood glucose level leading to enhanced peripheral glucose utilization. Tripathi and Chandra *et al* [28] also reported that *M. charantia* extracts potentiate the insulin effect by rejuvenation of damaged pancreatic β cell.

Distributions of elastic fibres were observed to be sparsely distributed as evident by the staining intensity in the left ventricle of diabetic rats when compared with the control group. This observation suggests a reduction in the tensile strength and elasticity of the heart. Administration of *M. charantia* and glimperide gradually restored the integrity of these fibres there by reducing the susceptibility to cardiovascular complications such as stroke syndrome, myocardial infarction and hypertension[26]. Histochemical findings suggest accumulation of glycogen deposit in diabetic rats. In general, glycogen accumulation is an anatomic indicator of a deranged carbohydrate metabolism in alloxan-diabetes which probably reflects the hyperglycaemia[29]. Glycogen is synthesized in the body in a reaction beginning with glucose-1- phosphate which is converted to uridine diphosphate glucose (UDPG) and then to glycogen in reactions catalysed by the enzyme UDPG glycogen-transferase, and the branching enzyme (amylo-l, 4 >1, 6 transglucosidase). The reduction in the PAS staining intensity is reflective of reduced glycogen content in the group treated with *M. charantia* and glimepiride for four weeks[29]. All these evidences suggest cardio-protective effects of *M. charantia* against anatomical derangements observed in the diabetic group. Thus the present results showed that the methanolic extract of *M. charantia* has a promising ameliorative effects on the associated cardiac complications implicated in STZ induced diabetes in rats.

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