Cardioprotective Potential Of Vernonia Amygdalina And Ocimum Gratissimum Against Streptozotocin (Stz) – Induced Diabetes In Wistar Rats.

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Citation

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Abstract

Diabetes mellitus is characterized by disordered metabolism and abnormally high blood sugar resulting from insufficient levels of insulin. It is a disease of the developed world that is gradually creeping into developing countries, especially in Africa where Westernized diets are imbibed. Vernonia amygdalina and Ocimum gratissimum have been used singly for the management of diabetes in Nigeria, especially in the South Eastern part. The acceptance of the use of polyherbal therapy necessitated the research of the combination of these two herbs in the management of diabetes. Forty Wistar rats of an average weight of 140g were used for this study and were randomly divided into five (5) groups of eight (8) rats each. Groups A and B served as the normal and diabetic controls respectively and were given placebo treatment. Groups C, D and E were the experimental groups administered with 200mg/kg body weight of the extracts by gastric intubations for 28 days. Groups C and D received extracts of Vernonia amygdalina and Ocimum gratissimum respectively. Group E received a combination of both extracts. Fasting blood glucose (FBG) level was checked on a weekly basis. Results showed a significant lowering of FBG level of animals in the treated groups, while the diabetic control group recorded a significant increase in FBG. The normal control group had FBG level within the normal range. Histological examination revealed a normal cytoarchitecture in the group A animals. The diabetic control group B animals showed degeneration of myocytes, loss of nuclei, loss of cross striation of the cardiac muscles and an increase in myofibre diameter. These conditions were greatly reversed in the treated groups that received the extracts. It can therefore be concluded that Vernonia amygdalina and Ocimum gratissimum may be effective and safe in the proper management of cardiomyopathy, one of the numerous complications of diabetes mellitus.

INTRODUCTION

Diabetes is an age long, serious metabolic disorder with micro-vascular and macro- vascular complications that results in significant morbidity and mortality[1]. Chronic hyperglycemia during diabetes causes glycation of body protein which in turn leads to secondary complications that affect the eyes, kidneys, nerves and arteries[2]. These complications may be delayed, lessened or prevented by maintaining blood glucose levels close to normal [3]. Cardiovascular disease is the major cause of mortality and morbidity in modern societies, among the numerous complications of diabetes [4]. Long standing diabetes has been documented to cause structural and functional cardiac impairment, which was found to lead to ischemic heart disease, cardiomyopathy and congestive heart failure [5]. It has been postulated that endothelial dysfunction, endomyocardia fibrosis; direct toxic effect of hyperglycemia on cardiomyocytes and autonomic neuropathy play an important role in diabetic cardiomyopathy [6]. Diabetes has been identified by the World Health Organization (WHO) as an epidemic underway, since about 164 million people Worldwide were afflicted in 2000 and a possible increase to 366 million by 2030 has been projected [7]. In Nigeria, the prevalence of diabetes according to a National survey reveals that 2.2 per cent (%); that is, 2-3 in every 100 person are suffering from the disease [8]. The populations of people with this disease are found mostly in urban areas due to their sedentary life style [9]. Despite these alarming figures and statistical data, a final therapy for now does not exist. However, therapeutic drugs have been in use since the accidental discovery of the hypoglycemic action of sulphonamides[10]. Consequently, herbal medicine, a new promising approach has emerged. It has been established that 40-60% of diabetics use non-conventional treatments, and 23% of this number use botanicals alone[11]. WHO in its

general assembly authenticated this approach[12, 13]. Application of natural remedies for diabetes treatment has been further strengthened due to the belief that herbs can provide some benefits over allopathic medicine, which allow users to feel that they have control in their choice of medication[14, 15, 16] . The use of traditional medicine and medicinal plants in most developing countries as a normative basis for the maintenance of good health has been widely observed[17]. An increasing reliance on the use of medicinal plants in the industrialized societies has been traced to the extraction and development of several drugs and chemotherapeutics from these plants as well as from traditionally used rural remedies[18].Vernonia amygdalina and Ocimum gratissimum are among the traditionally used herbal plants to treat different ailments. Vernonia amygdalina has been found useful in the ethno therapy of asthma, schistosomiasis, malaria, measles, diarrhoea, tuberculosis, abdominal pain and fever[19, 20]. Ocimum gratissimum is commonly used in cooking due to its minty aromatic flavour [21]. Traditionally, Ocimum gratissimum has been used for the treatment of headache, diarrhoea, wart worms and kidney infections [22]. The leaves of the African varieties of Ocimum gratissimum are said to contain thymol oil which has been found to be highly antiseptic and also used to prevent mosquito bite [23]. Vernonia amygdalina and Ocimum gratissimum have been found to have antidiabetic properties [3, 24, 25]. This research was carried out to investigate the combination therapy of these plant extracts on the heart of streptozotocin (STZ)-induced diabetes in Wistar rats.

MATERIALS AND METHODS

Forty (40) Wistar rats of an average weight of 140g were bred in the animal house, Department of Human Anatomy, University of Calabar. Approval was given by the ethical committee before commencement of the experiment. The animals were randomly divided into five groups (A-E), were housed in well ventilated cages under controlled conditions. The rats were fed with rat chow obtained from Agrofeeds (Nig) Limited. Water was given ad libitum.

EXTRACTION

Fresh matured leaves of Vernonia amygdalina (VA) and Ocimum gratissimum (OC) were harvested from the Endocrine research farm, University of Calabar. They were rinsed and dried under shade. The dried leaves were grounded with the aid of an electric blender into powder. 450g of each blended plant was suspended in 2500mls of ethanol and homogenated for about 10 minutes. The homogenates were left for about 12 hours in a refrigerator with a temperature of 4^{o}c. it was first filtered with a chess cloth and later with Whitman's No 1 filter paper. The filtrate was concentrated in vacuo using a rotary evaporator to 10% of the original volume at 40^{o}c. The concentrate was allowed to stand in a water bath at 37 ^{o}c for complete dryness, giving a yield of 157.5g of the crude extract. The extracts were re-constituted in distilled water before administration.

EXPERIMENTAL DESIGN

The animals were divided into five groups (A-E) of 8 rats each. Group A served as the non- diabetic positive control, group B served as diabetic negative control. These two groups were given distilled water. Groups B-E was induced with diabetes mellitus using STZ as vehicle. This was done by single intraperitoneal injection of 65mg/kg of STZ into 12 hours fasted rats. Group C rats were treated with 200mg/kg body weight of VA; group D received 200mg/kg OC, while group E received a combination of VA & OC at a dose of 100mg/kg body weight each to give a total dose of 200mg/kg. The extracts were administered orally using an orogastric tube for 28 days. The weights of the rats were taken every four days. The rats were allowed to fast overnight after the administration of extracts and were sacrificed on the 29th day. The blood from the aorta was collected for FBG test. The hearts were excised and fixed in 10% buffered formalin for tissue processing.

RESULTS

There was marked variation in the mean body weight, FBG and behavioral tendencies across the groups. Group A animals showed a rapid increase in body weight maintaining an agile and healthy state with their FBG within normal range. Group B showed a decline in body weight which lasted throughout the experiment. There was a high increase in their FBG. They showed greater food and water intake. They exhibited decreased locomotive activity and sensitivity to touch as well as increase in defecation and urination. In groups C-E, treated with extracts of VA & OC, rats maintained a steady increase in body weight but not as observed in group A rats. There was improvement in their FBG as values were directed towards normal. The mean body weights and FBG levels are represented in table1 below.

HISTOLOGICAL

The results of the groups varied considerably. Group A showed a normal cardiac architecture represented by the

presence of cardiac muscles cells; myocytes, having its own nucleus. The junction between adjoining cardiac myocytes is seen as darkly stained transverse line running across the muscle fibres, signifying the presence of intercalated discs. The myofibres formed cross striation with network of blood capillaries around the muscle fibres (fig i). The diabetic control group B rats showed a complete distortion of cells. There was cellular hypertrophy and mycytolytic necrosis. Microscopic fibrosis with distribution of interstitial connective tissue was observed throughout the myocardium. High level of myofibrillar disorganization with significant loss of cross striation and an increase in myofibre diameter were observed compared to group A (fig ii). Group C rats treated with extracts of VA showed cardiac muscle fibres having a granular slightly branded cytoplasm with less cross striations compared to normal control. Myocytes were seen with stained nuclei, few capillary networks were also seen closely located within the muscle fibres. The section shows heart under going repairs (fig iii). Group D rats treated with extract of OG showed cardiac muscle fibers exhibiting cross striations with centrally placed nuclei. Blood capillaries were seen lying around muscle fibers. The extensive cytoplasm was seen branching, to give an appearance of a continuous three dimensional network as seen in group A (fig iv). Group E rats given a combination of VA and OG exhibited cross striation with closely packed muscle fibres as well as an increased capillary network around muscle fibres. (fig v)

Figure 1

TABLE 1: Effect of extracts on mean body weight and fasting blood glucose (FBG) level.

GROUP(n=8)	TREATMENT	WEIGHT(g)	FBG(mg/dl)
A	Non-diabetic control	198.88 ± 1.70	86.4 ± 3.27
B	Diabetic control	116.12 ± 4.65	285.2 ± 14.86
C	200mg/kg VA	173.3 ± 1.89	108.6 ± 4.54
D	200mg/kg OG	174.9 ± 3.15	106.4 ± 3.93
E	200mg/kg VA+ OG	179.06 ± 2.12	98.2 ± 2.08

Figure 2

Fig i: Micrograph of the heart of a control rat showing normal cardiac features. N-Nucleus, ID- Intercalated disc, C-Capillaries. H&E X 100.

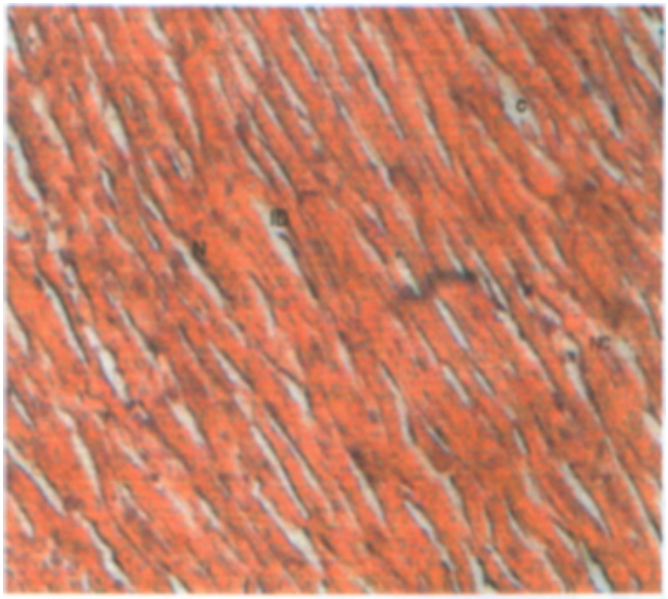


Figure 3

Fig ii: Micrograph of heart of diabetic control rat showing distortion in cytoarchitecture. F- Fibrous tissue, Ni- Loss of nuclei. H & E X 100.

Figure 4

Fig iii: Micrograph of diabetic rat treated with showing presence of Cardiac muscles. H & E X 100.

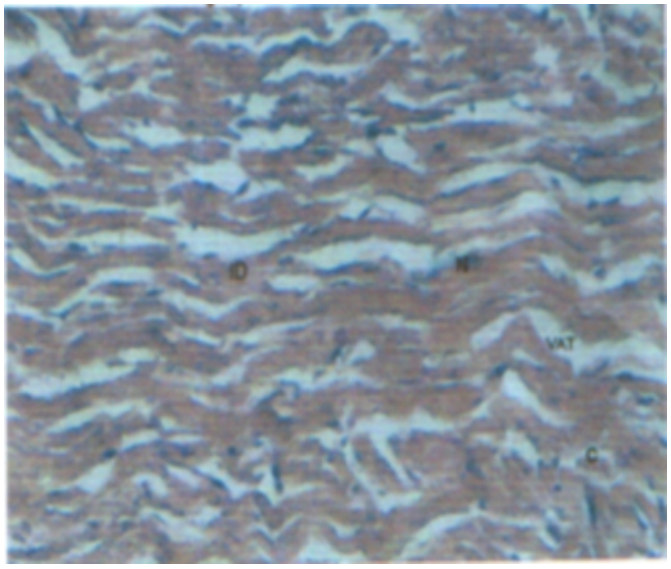


Figure 5

Fig iv: Micrograph of heart of diabetic rat treated with showing Restoration of striated cardiac muscles. H & E X 100.

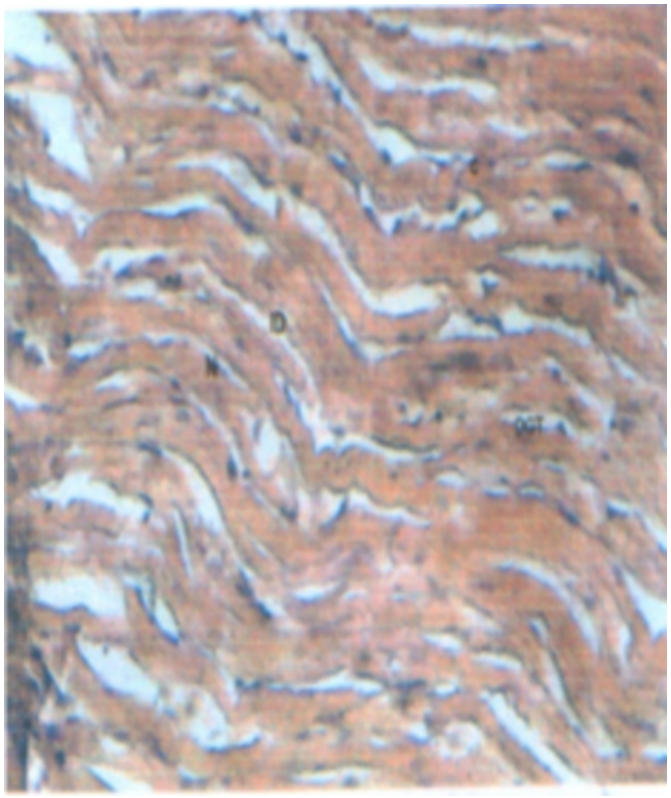
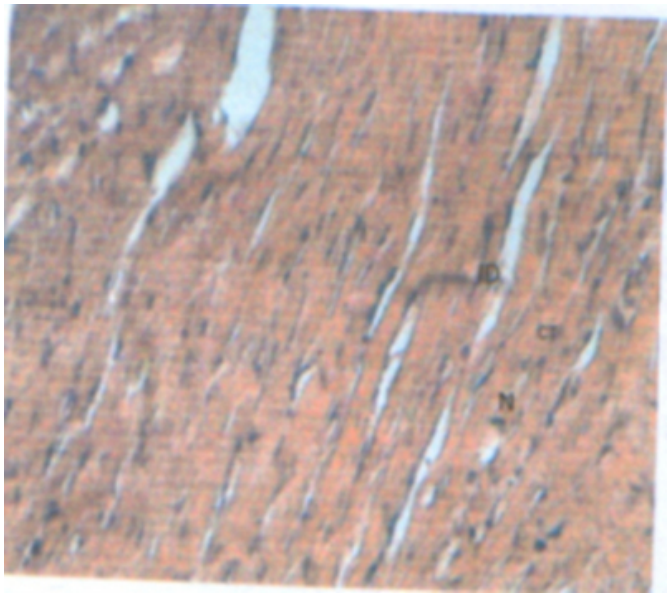


Figure 6

Fig v: Micrograph of heart of diabetic rat treated with combination of And Ocimum showing almost near to normal cardiac features. H & E X 100



DISCUSSION

Diabetes cardiomyopathy is well known to develop in human in the absence of coronary or hypertensive diseases. Endothelial dysfunction, endomyocardial fibrosis- a direct toxic effect of hyperglycemia on cardiomyocytes and autonomic neuropathy, play an important role in the mechanism of diabetic cardiomyopathy development[6] . These cardiovascular complications compromise cardiac performance ultimately resulting in cardiac failure, seen in cardiovascular complication individuals with diabetic cardiomyopathy[26] . The STZ diabetic induced rats are reminiscent of a model of uncontrolled hyperglycemia due to the direct pancreatic beta cell destruction and resulting insulin deficiency. The death of some rats in the course of the experiment may be as a result of acidosis. Earlier studies have shown that STZ and product of its reduction, establishes a redox cycle with formation of superoxides radicals resulting in the formation of highly reactive hydroxyl radical within the pancreas, bringing about acidosis and subsequent death[27] . Lowering of the FBG level in the experimental groups is consistent with earlier reports on the hypoglycemic and antihyperglycemic actions of the extracts[24, 28, 29] . The chemical base, tannin, present in both plants might have played a vital role in the action of the extracts, since it has been reported to suppress oxidation [30] . The myocardial salvaging effect of OC was more potent than those treated with VA. This is in accordance with other

results obtained using herbs[31-38]. How OG carries out this action is not known, but it might be due to the volatile oils it contains. The polyherbal therapy however produced a more impressive result. The mechanism through which this is done is not well known, it may be likely due to the presence of phytochemicals in the plants that led to synergism of actions rather than been antagonistic to each other. It may also be as a result of augmentation of endogenous antioxidants and suppression of oxidative stress by the various constituents of the plants acting together to give a better effect. The study of cardiomyopathies in small animals will contribute to the understanding of cardiac pathophysiology and an evaluation of experimental treatment strategy. We therefore conclude that extracts of Vernonia amygdalina and Ocimum gratissimum apart from their hypoglycemic actions could protect the heart against impairment and complete destruction due to diabetes.

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