EVALUATION OF CARDIOVASCULAR DISEASE RISK FACTORS IN DIABETIC RATS ADMINISTERED ETHANOL EXTRACT OF *Cucumis sativus* FRUIT.

*1Abu O.D., 2Awhin E.P. & 1Ozedu M.E.*

1Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin City, Nigeria.  
2Department of Medical Biochemistry, Faculty of Basic Medical Sciences, Delta State University, Abraka, Delta State, Nigeria.  
*Correspondent author’s email: osahon.abu@uniben.edu; Tel: +2347086427636*

**ABSTRACT**

It is estimated that by the year 2030, the number of persons with diabetes mellitus (DM) would increase to 366 million. Diabetes mellitus is primarily defined by the level of hyperglycemia which causes micro- and macro-vascular damage. This study evaluated cardiovascular disease risk factors in diabetic rats administered ethanol extract of *Cucumis sativus* fruit. Male Wistar rats (n = 25, mean weight = 215 ± 15 g) were used. The rats were randomly assigned to five groups (5 rats per group): normal control, diabetic control, metformin, 200 mg/kg body weight (BWT) extract and 300 mg/kg BWT extract groups. Diabetes mellitus was induced via intraperitoneal injection of streptozotocin (STZ, 50 mg/kg BWT). The diabetic rats were then treated for 21 days with metformin (50 mg/kg BWT) or the extract at doses of 200 and 300 mg/kg BWT, respectively. The results showed that STZ-induced DM significantly increased plasma concentrations of total cholesterol (TC), triacylglycerol (TG), very low-density lipoprotein cholesterol (VLDL-C), low-density lipoprotein cholesterol (LDL-C), atherogenic index of plasma (AIP), atherogenic coefficient (AC) and cardiac risk ratio (CRR), but it significantly reduced high-density lipoprotein cholesterol (HDL-C) (p < 0.05). However, treatment of diabetic rats with ethanol extract of *C. sativus* fruit led to significant reductions in circulating levels of lipid profile (except HDL-C, which increased) as well as AIP, AC and CRR (p < 0.05). These results suggest that the medicinal plant extract has the capacity to protect against cardiovascular events in diabetic rats.

**Keywords:** Atherogenic coefficient, Cholesterol, Diabetes mellitus, Lipid profile, Streptozotocin.

**Abbreviations:** DM (diabetes mellitus); BWT (body weight); STZ (streptozotocin); AIP (atherogenic index of plasma); AC (atherogenic coefficient); CRR (cardiac risk ratio).
INTRODUCTION
The heart is a muscular organ which pumps blood through the blood vessels of the circulatory system (Benson et al., 2001). Blood provides the animal’s body with oxygen and nutrients as well as aid the removal of metabolic wastes. In humans, it is located in the middle compartment of the chest between the lungs (Guyton and Hall, 2006; Moore et al., 2009; Taber and Venes, 2009). The heart is effectively a syncytium, a meshwork of cardiac muscle cells interconnected by contiguous cytoplasmic bridges (Maton et al., 1993; DuBose, 1996; MacDonald, 2009). Cardiac injury induced by drugs and other chemical substances is becoming an increasingly important concern, since it constitutes serious risk to human health (Dessalvi et al., 2018). Cardiac dysfunction caused by cardiotoxic agents such as STZ may lead to heart failure, myocardial ischemia, arrhythmias, hypertension, myocarditis, pericarditis, and thromboembolism (Anabel et al., 2010; Colombo et al., 2013).

Diabetes mellitus (DM) is a major cause of heart disease and stroke. Death rates for heart disease and the risk of stroke are about 2 – 4 times higher among adults with diabetes than among those without the disease (CDC, 2011). In DM, macrovascular complications result from damage to large blood vessels and are mainly cardiovascular diseases such as premature atherosclerosis, ischemic heart disease (myocardial infarction) and stroke (WHO, 2009).

It has been asserted that a large population of people in developing countries use herbal medicine for some aspects of their basic health care (Luper, 1998; Thyagarajan et al., 2002). Medicinal plants have proven health benefits (Abu et al., 2020, 2021a, 2021b, 2022a and 2022b). Cucumis sativus is a medicinal plant used ethno-botanically to treat various ailments (Heidari et al., 2012; Abu et al., 2023a, 2023b, 2023c, 2023d and 2023e). This study evaluated cardiovascular disease risk factors in diabetic rats administered ethanol extract of C. sativus fruit.

MATERIALS AND METHODS
Chemicals
All chemicals and reagents used in this study were of analytical grade and they were products of Sigma-Aldrich Ltd. (USA).

Plant Extraction
Freshly harvested Cucumis sativus fruits were purchased from a major fruit/vegetable market in Benin City, Nigeria and identified in the Department of Plant Biology and Biotechnology, University of Benin. They were thereafter washed, and air-dried for about 4 weeks at the Department of Biochemistry, University of Benin. The dry plant was ground with a mechanical blender. The pulverized sample was cold macerated in absolute ethanol for three days (72 h) in a bell jar and filtered using Whatmann filter paper No. 42 (125 mm). The ethanol extract was thereafter concentrated using rotary evaporator and freeze-dried using a lyophilizer (Abu et al., 2015, 2017 and 2019).

Experimental Animals
Mature male Wistar albino rats (n = 25, mean weight = 215 ± 15 g) were bought from the Department of Anatomy, University of Benin, Nigeria and housed in wooden cages. They were acclimatized for two weeks before commencement of the study, and had free access to feed and water.
Experimental Design
The rats were randomly assigned to five groups (5 rats/group): normal control, diabetic control, metformin, extract (200 mg/kg bwt) and extract (300 mg/kg bwt) groups. Diabetes mellitus was induced in the rats via intraperitoneal injection of 50 mg/kg bwt STZ. The diabetic rats were then treated with either the standard antidiabetic drug metformin (50 mg/kg bwt) or the extract at doses of 200 and 300 mg/kg bwt, respectively, for 21 days.

Blood Sample Collection and Preparation
At the end of day 21 of treatment, the rats were euthanized under mild chloroform anaesthesia after an overnight fast. Blood was drawn via cardiac puncture into heparinized sample bottles and centrifuged at 2000 rpm for 10 min to obtain plasma which was used for biochemical analyses.

Biochemical Analyses
Lipid profile and cardiovascular disease risk parameters were determined in plasma (Friedewald et al., 1972; Lopes-Virella et al., 1977; Reiser et al., 1985; Tietz et al., 1990; Frohlich and Dobiášová, 2003; Abu et al., 2022c).

Data Analysis
Data are expressed as mean ± SEM (n = 5). One-Way Analysis of Variance (ANOVA) was performed using SPSS (version 20). Groups were compared using Duncan multiple range test. Statistical significance was assumed at p < 0.05.

RESULTS AND DISCUSSION
Effect of Ethanol Extract of C. sativus on Cardiovascular Disease Risk Factors
Streptozotocin (STZ)-induced DM significantly increased plasma concentrations of TC, TG, VLDL-C, LDL-C, AIP, AC and CRR, but it significantly reduced HDL-C (p < 0.05). However, treatment of diabetic rats with ethanol extract of C. sativus fruit led to significant reductions in circulating levels of lipid profile (except HDL-C, which increased) as well as AIP, AC and CRR (p < 0.05; Figures 1 to 4).

People with Type-2 diabetes mellitus are at risk for both micro- and macrovascular complications (CDC, 2011). The toxicity produced by STZ impart negatively on organs such as liver, kidneys, heart and lung. The exact molecular mechanism underlying the cytotoxic effect of STZ is not well-understood, however studies suggest that the cytotoxicity could be via production of reactive oxygen species (ROS) thus inducing oxidative stress, causing DNA damage with resultant necrosis due to the DNA methylating activity of the methyl nitroso urea moiety of the drug, release of nitric oxide (NO) which inhibits aconitase activity resulting in mitochondrial dysfunction, or via inhibition of O-linked β-N-acetylglucosaminase (O-GlcNAcase) (Anabel et al., 2010).

Plants are at the center of Traditional Medicine. Their use in disease management is as old as man. Medicinal plants serve as cheap alternative to orthodox medicine since they are readily available (Abu et al., 2022d, 2022e and 2023c). A variety of indigenous medicinal plants can effectively manage diabetes mellitus. One of the most significant benefits of medicinal plants is their ready availability and minimal side effects (Abu et al., 2022f, 2022g and 2022h).
Cucurbits are vegetable crops, belonging to the family Cucurbitaceae, which primarily comprised species consumed as food worldwide. Cucurbits are an excellent fruit in nature having composition of all the essential constituents required for good health (Yawalkar, 1985). They are consumed as vegetables and salads because of availability at low cost. Cucumbers are botanically categorized as berries, which are available in different sizes, shapes and colours. They range from thick, stubby little fruits (10 - 12 cm long) to Dutch greenhouse varieties (of up to 50 cm long) (Yawalkar, 1985). The most popular variety is the long smooth salad cucumber which has a smooth, dark-green skin. Its little brother, the "gherkin" is actually a cucumber that has been harvested when little and pickled in brine. The parts of this medicinal plant which are traditionally used are leaves, flowers, seeds, fruits, and bark. These parts contain bioactive compounds responsible for particular pharmacological activity (Patil et al., 2012). This study evaluated cardiovascular disease risk factors in diabetic Wistar albino rats administered ethanol extract of C. sativus fruit. The results showed that STZ-induced DM significantly increased plasma concentrations of TC, TG, VLDL-C, LDL-C, AIP, AC and CRR, but it significantly reduced HDL-C. However, treatment of diabetic rats with ethanol extract of C. sativus fruit led to significant reductions in circulating levels of lipid profile (except HDL-C, which increased) as well as AIP, AC and CRR. These results have further strengthened the observation that medicinal plants are effective in ameliorating complications of some diseases (Ebhohon et al., 2019a and 2019b; Okpiabhele et al., 2018; Omoregie et al., 2020; Abu et al., 2022i, 2022j, 2022k and 2022l).

Table 1: Comparison of Organ and Relative Organ Weights

<table>
<thead>
<tr>
<th>Group</th>
<th>Weight of Heart (g)</th>
<th>Organ/Body Weight Ratio x 10^3</th>
</tr>
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<tbody>
<tr>
<td>Normal Control</td>
<td>0.56 ± 0.04</td>
<td>3.03 ± 0.26</td>
</tr>
<tr>
<td>Diabetic Control</td>
<td>0.47 ± 0.06a</td>
<td>2.89 ± 0.15a</td>
</tr>
<tr>
<td>Metformin</td>
<td>0.52 ± 0.04b</td>
<td>3.54 ± 0.09b</td>
</tr>
<tr>
<td>Extract (200 mg/kg bwt)</td>
<td>0.54 ± 0.04b</td>
<td>3.21 ± 0.24b</td>
</tr>
<tr>
<td>Extract (300 mg/kg bwt)</td>
<td>0.64 ± 0.05b</td>
<td>4.51 ± 0.52b</td>
</tr>
</tbody>
</table>

Data are weights of rat organs and are expressed as mean ± SEM (n = 5).

Values with superscript “a” are significantly different from the normal control group.

Values with superscript “b” are significantly different from the diabetic control group.

Figure 1: Concentrations of TC and TG in the Rats
Figure 2: Concentrations of LDL-C and HDL-C in the Rats

Figure 3: Concentrations of VLDL-C and AIP in the Rats

Figure 4: CRR and AC of Rats
CONCLUSION

The results of this study suggest that the medicinal plant extract has the capacity to protect against cardiovascular events in STZ-induced diabetic rats.

REFERENCES


