EFFECT OF NUTMEG (*Myristica fragrans*) ON OXIDATIVE STRESS IN ALLOXAN-INDUCED DIABETIC IN WISTAR ALBINO RATS

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ABSTRACTS

Plants contain many important and potential therapeutic agents for treating several diseases of man. In this study, we investigate and compare the hypoglycemic with lipid profile evaluations of nutmeg (*Myristica fragrans*) seed on Alloxan-induced diabetic albino rats with the aim of alleviating oxidative stress. Twenty matured male wistar albino rats were acclimatized for one week. The animals were grouped into 5 groups (1, 2, 3, 4 and 4) containing four rats each. Group 1(normal) was fed with rat pellets, Group 2 (negative control) was fed with normal rat feed, Group 3 animals was treated with 0.5ml of 30% aqueous extract of nutmeg (*Myristica fragrans*), while group 4 and 5 animals were treated with 5% and 10% formulated feed respectively. Blood samples were collected for glucose, total cholesterol, low density lipoprotein (LDL), high density lipoprotein (HDL) and triglyceride determination. The result of this work revealed that 30% aqueous extract, 5% and 10% supplemented nutmeg (*Myristical fragrance*) caused increased weight and high density lipoprotein (HDL) concentration and decrease in blood glucose, low density lipoprotein (LDL), triglyceride and total cholesterol. Thus, from this study we conclude that nutmeg (*Myristical fragrance*) diet exhibited significant anti-hyperglycemic in alloxan-induced diabetic rats.

KEYWORDS: Therapeutic agent, Anti-diabetic activity, Myristical fragrans, Intra-peritoneally.
INTRODUCTION

Nutmeg has been used across the world as a ground spice in cooking for centuries. In the tribal areas of India, where there is a lack of access to conventional medicine, nutmeg is also used for treating diabetes, as well as other ailments such as diarrhea, mouth sores, and insomnia.\(^1\) Nutmeg has been one of the major sources of drug for the treatment of diabetes mellitus in Indian system of medicine and other ancient systems in the world.\(^2\) The importance of antidiabetic plants in the development of economic and effective treatment for diabetes, currently estimated to affect over 30 million people worldwide, has been recognized by the World Health Organization.\(^3\)

Diabetes mellitus (DM), commonly referred to as diabetes, is a group of metabolic diseases in which there are high blood sugar levels over a prolonged period.\(^4\) Symptoms of high blood sugar include frequent urination, increased thirst and increased hunger. If left untreated, diabetes can cause many complications.\(^5\) Acute complications include diabetic ketoacidosis and non-ketotic hyperosmolar coma. Serious long-term complications include cardiovascular disease, stroke, chronic kidney failure, foot ulcers, and damage to the eyes.\(^4\)

Prevention and treatment involve a healthy diet, physical exercise, not using tobacco and being a normal body weight. Blood pressure control and proper foot care are also important for people with the disease. Type 1 diabetes must be managed with insulin injections. Type 2 diabetes may be treated with medications with or without insulin. Insulin and some oral medications can cause low blood sugar.\(^6\) Weight loss surgery in those with obesity is an effective measure in those with type 2 DM.\(^7\) Gestational diabetes usually resolves after the birth of the baby.

As of 2014, an estimated 387 million people have diabetes worldwide, with type 2 diabetes making up about 90% of the cases. This represents 8.3% of the adult population, with equal rates in both women and men. From 2012 to 2014, diabetes is estimated to have resulted in 1.5 to 4.9 million deaths each year. Diabetes at least doubles a person's risk of death. The number of people with diabetes is expected to rise to 592 million by 2035. The global economic cost of diabetes in 2014 was estimated to be $612 billion USD.\(^4\)

In view of the wide spread biological uses of nutmeg and little information about the hypoglycemic with lipid profile and marker enzymes evaluations, hence, this study aimed at
determining and comparing the hypoglycemic effects with lipid profile evaluations of nutmeg on alloxan-induced wistar albino rats.

MATERIALS AND METHODS

Sample collection and preparation

Fresh samples of the *Nutmeg* were purchased from Oja-Bisi market in Ado-Ekiti, Ekiti State, Nigeria. The sample was air dried and powdered for aqueous extract used in this work.

Animal treatment

Twenty matured male wistar albino rats with weights ranging from 180 to 200g were used for this experiment. The animals were acclimatized for a period of one week to the laboratory conditions prior to the experiment at the Animal House of College of Medicine, Ekiti-State University. Rats were housed in cages at room temperature with 12hours light and dark cycle with free access to drinking water and rat feeds.

Experimental procedure

Hyperglycemia was induced in rats using alloxan (40mg/kg body weight) intra-peritoneally (ip). Twenty wistar albino rats were fed with water and rat pellets for one week. Rats were fasted over night to determine their blood glucose level. Four of the animals were sacrificed to serve as the positive control (stage 1). The remaining animals were injected intra-peritoneally with 0.5ml/40mg/kg body weight alloxan which was observed for another week with normal feeding. These animals were confirmed diabetic after their fasting blood sugar were determined after 48hours. Four of these animals were sacrificed to serve as negative control (stage 2). The remaining animals were grouped into 3 groups (A, B and C) containing four rats each. Group A animals were treated with 0.5ml of 30% aqueous extract of nutmeg (*myristical fragrans*), while group B and C animals were treated with 5% and 10% formulated feed respectively. These animals (Group A, B and C) serve as stage 3. Blood samples were collected for glucose, total cholesterol, low density lipoprotein (LDL), high density lipoprotein (HDL) and tryglyceride determination.

Determination of Whole blood Glucose

Glucose concentration in mg/dL was measured in the animal with the aid of ON-CALL PLUS Glucometer using compatible glucose test strips according to prescribed instructions. Standard Randox kits were used to determine Triglycerides, Cholesterol and HDL-Cholesterol, LDL-Cholesterol.
Determination of Biochemical parameters

Serum Triacylglycerides was determined by Glycerol Phosphate Oxidase-Peroxidase (GPO-PAP) method of Randox.\textsuperscript{[8]}

Total cholesterol in all the samples collected from various subjects was measured by cholesterol CHOD-PAP method which is an enzymatic end point method.\textsuperscript{[9]}

High Density Lipoprotein Cholesterol (HDLC) in the sample was separated by precipitation through the procedure adopted by Lopes-Virella in 1977.\textsuperscript{[10]}

LDLC concentration in the sample was determined using the relationship described by Frederickson-Friedwald et. al., (1972).\textsuperscript{[11]} LDL Cholesterol (mg/dL) = Total Cholesterol – TG / 5 – HDLC.

RESULTS AND DISSCUSSION

Table I showed the effects of nutmeg (\textit{Myristica fragrans}) seed (aqueous extracts with 5\% and 10\% nutmeg-modified fed) treatments on the weights and blood glucose of alloxan-induced diabetic rats. It was observed in this study that alloxan induced diabetic rats had a marked loss in body weight. This would be expected as one of the effects of diabetes is body weight loss, an observation which was also made by Eleazu \textit{et al.}, (2010)\textsuperscript{[12]} that reported weight loss in diabetic rat before the administration of unripe plantain. Alloxan is known to destroy the cells of the pancreas that function in the regulation of insulin secretion and thus leads to an increase in the concentration of blood glucose. With the destruction of the pancreatic cells by alloxan, there is deficiency of insulin leading to increased synthesis of ketone bodies which are excreted in urine.

Table I: Effects of nutmeg (\textit{Myristica fragrans}) seed treatments on the weights and blood glucose level of alloxan-induced diabetic rats.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Weight(g)</th>
<th>Glucose(mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group(Group 1)</td>
<td>184.30 ±1.620</td>
<td>85 ± 0.032</td>
</tr>
<tr>
<td>Diabetic animals(Group2)</td>
<td>175.30 ±1.310</td>
<td>120 ± 0.351</td>
</tr>
<tr>
<td>Diabetic animals treated with nutmeg extract,(Group3)</td>
<td>180.50 ±1.422**</td>
<td>98 ± 0.036**</td>
</tr>
<tr>
<td>Diabetic animals treated with meal compounded with 5% nutmeg,(Group4)</td>
<td>179.30 ±1.361*</td>
<td>87 ± 0.023**</td>
</tr>
<tr>
<td>Diabetic animals treated with meal compounded with 10% nutmeg,(Group5)</td>
<td>182.20 ±1.483**</td>
<td>80 ± 0.021**</td>
</tr>
</tbody>
</table>

\( n = 5 \) in each group, values are mean±SEM
The increased synthesis of ketone bodies coupled with increased lipolysis leads to a severe body weight loss. However, the diabetic rats treated differently with *Myristica fragrans* aqueous extracts had a remarkable gain in body weight. The concentration of fasting blood glucose was increase in the alloxan induced diabetic rats in this study, which was in correlation with Edoga et al., (2013)*"[^13] who also reported increased glucose concentration in diabetics. However, the glucose concentration significantly reduced in the diabetic rats placed on *Myristica fragrans* seed aqueous extracts. This is in agreement with earlier works done by Edoga et al., (2013)*"[^13]; Dolly et al., (2009)*"[^14] and Mohammed et al.,(2009)*"[^15] who earlier reported a hypoglycemic actions in their various works when diabetic animals were treated with some plants extracts like watermelon and moringa oleivera. Somani and Singhai, (2008)*"[^16] had also reported hypoglycemic activity of *Myristica fragrans* extract in alloxan induced diabetic rats and this is due to the bioactive constituents of nutmeg seeds such as flavonoids, tannins and alkaloids including some mineral and other antioxidant components.

**Table II: Effects of nutmeg (*Myristica fragrans*) seed treatments on the plasma lipid profile concentration in plasma of alloxan-induced diabetic rats.**

<table>
<thead>
<tr>
<th></th>
<th>TC (mmol/l)</th>
<th>TG (mmol/l)</th>
<th>HDL (mmol/l)</th>
<th>LDL (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control group (Group 1)</strong></td>
<td>0.91±0.025</td>
<td>0.55±0.006</td>
<td>1.95±0.161</td>
<td>2.97±0.188</td>
</tr>
<tr>
<td><strong>Diabetic animals (Group 2)</strong></td>
<td>5.37±0.035</td>
<td>2.78±0.025</td>
<td>0.86±0.170</td>
<td>5.07±0.060</td>
</tr>
<tr>
<td><strong>Diabetic animals treated with nutmeg extract (Group 3)</strong></td>
<td>3.45±0.046*</td>
<td>1.88±0.025*</td>
<td>0.41±0.281*</td>
<td>3.70±0.058*</td>
</tr>
<tr>
<td><strong>Diabetic animals treated with meal compounded with 5% nutmeg (Group 4)</strong></td>
<td>2.11±0.004*</td>
<td>0.98±0.139*</td>
<td>0.29±0.103*</td>
<td>2.53±0.027*</td>
</tr>
<tr>
<td><strong>Diabetic animals treated with meal compounded with 10% nutmeg (Group 5)</strong></td>
<td>1.68±0.189**</td>
<td>0.42±0.006**</td>
<td>0.37±0.180**</td>
<td>2.06±0.002**</td>
</tr>
</tbody>
</table>

*n= 5 in each group, values are mean±SEM
*p>0.5 compared to diabetic group and **p>0.1 compared to diabetic group (ANOVA).*

It is known that the factors influencing the glucose metabolism under various physiological conditions do influence lipid metabolism as well.*[^17] It has also been revealed that triglyceride accumulation increase considerably in diabetes mellitus.*[^18] Hypercholesterolemia and...
hypertriglyceridemia have been reported to occur in diabetic\textsuperscript{[19][20]} and a significant increase in cholesterol and triglyceride was observed in this experiment in accordance to these studies.

A significantly higher ($P<0.05$) serum levels of total cholesterol, triacylglycerides, LDLC, TC and lower level of HDLC were observed in diabetic rats when compared with nutmeg treated subjects (Table II). This study is consistent with previous cross sectional study conducted by Maha et al., (2012)\textsuperscript{[21]}, Sasmita et al., (2012)\textsuperscript{[22]}, Samatha et al., (2012)\textsuperscript{[23]} and Hasan et al., (2014)\textsuperscript{[24]} where increase prevalence of dyslipidemia was found to be the major contributor of cardiovascular diseases. In diabetes, many factors may affect blood lipid levels, this is because carbohydrates and lipid metabolism are interrelated to each other, if there is any disorder in carbohydrate metabolism, it also leads to disorder of lipid metabolism. Life style, environment, occupation and level of education may account for these differences in serum lipid fractions in different individuals.\textsuperscript{[25]} The most important of these bioactive constituents of plants are flavonoids, tannins, alkaloids including other minerals component which prevent metabolism disorder in body system.\textsuperscript{[26]}

**CONCLUSION**

This study has been able to demonstrate the alloxan and hypoglycemic potentials of nutmeg seeds on alloxan-induced diabetic rats. All the biochemical analysis showed nutmeg effectively protected pancreatic cells death especially, the diabetic rats fed with 10% formulated feeds compare to 5% compounded meal.

**ACKNOWLEDGEMENTS**

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**REFERENCES**


