Prolonged Nutmeg Extract Usage As Potent As Glibenclamide In Diabetes Treatment

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ABSTRACT

Comparative antidiabetic potentials study of the effect of ethanolic and aqueous extract of readymade nutmeg with glibenclamide was carried out on thirty (30) male and female albino rats and thirty (30) male and female albino mice for the period of 7, 14, 21 and 35 days. The animals were given low, medium and high doses of extract and 5mg/kg per body weight of glibenclamide for the period. The group administered with high dose of extract of nutmeg had significant reduction in blood glucose level than (p<0.05), control. The aqueous extract of nutmeg showed significant reduction in blood glucose level on the days with low dose (p<0.05) compared to control. A combination of nutmeg extract and glibenclamide showed a drastic significant (p<0.05) reduction in blood glucose level. The LD50 of the extract was 2738.1 mg/kg. The phytochemical analysis of the extract showed the presence of alkaloids, flavonoid, tannins, cardiac glycosides and saponins. But flavonoid concentration was very significant. It is showed in the study that prolong usage of aqueous extract of readymade nutmeg is very potent as glibenclamide in the treatment of diabetes. However, ethanolic extract of nutmeg showed higher potency than aqueous extract in the study.

Key words: Ethanolic, aqueous, extract, nutmeg, glibenclamide, diabetes.
INTRODUCTION
Medicinal plants are gradually being accepted as alternative therapy in the treatment of diseases. Such plants are also available in the treatment of diabetes,\(^1\). The search for the alternative is that the current orthodox drugs for diabetic treatment are full of shortcomings particularly inadequate potency, side effects, and other limitations findings\(^2\). Glibenclamide is often prescribed as oral hypoglycemic drug\(^3\).

It is called glyburide with sulfonylurea compound of 5-chloro-N-(2) – cyclohexyl-carbon n-sulfonyl-phenyl) ethyl (7-2 – methoxy benzamide\(^4\). It increases insulin release from beta cells by it action on pancreas,\(^5\). It acts on the beta cells by binding on the ATP-sensitive potassium channels (KATP) inhibitory sub unit and uninhibitory subunits of sulfonyl urea receptor 1 (SURI),\(^6\). This will cause cell membrane depolarization and opening of voltage dependent channels leading to an increase intracellular calcium in the beta cells and hence the release of insulin\(^7\). Comparative studies of the potency of this drug has been compared with herbal drug with antidiabetic properties\(^8\). Myristica fragrans also known as nutmeg as powder form is of the family myristicaceae. It is a plant with seeds which when ground into powder form is called nutmeg. The plant family is placed taxonomically between Annonaceae and Lauraceae\(^9\). The dried nutmeg is grayish brown, oval and with furrowed surface. It has the height of 10-20m and indigenous to India and Indonesia and Srlanka\(^10,11\). It is a tropical plant found in tropical countries including Nigeria. Nutmeg is used as flavouring agent, and as spice in preparing confectionaries, puddings, seasonings of meat and vegetables, sauces, milk flavouring, dishes baked foods. It is used as stimulant medically\(^12\) and in pregnancy and lactation with side effects of an intoxication \(^13\). The active ingredient in nutmeg is myrostone with cytotoxic and apoptotic effects\(^14\). It also consists of oils, cellulose called nutmeg butter which is reddish in colour and the butter by weight) is about 75% called trimyristin\(^15\). Other health benefits of nutmeg include relieving of pains, improving recognition function, detoxification of the body, boosting of skin quality, strengthening of immune system, improve blood circulation, prevents leukemia.\(^6\). It is used traditionally in the treatment of paralysis, respiratory problems, libido, stomach ache\(^11\). Anti-thrombotic, antifungal, antidysentery anti-inflammatory properties and sexual functional properties of nutmeg have been reported . But importantly, the strong side effects of hallucination have been reported which may be risky for children consumption as cooking additive\(^17\). However, the n-hexane extract of nutmeg have been found to have memory enhancing effect in mice \(^18\). Diabetes is still a debilitating disease and as at 2015 an estimated 415 million people had diabetes globally\(^19\) with type 2 diabetes being about 90% and represents 8.3% of the adult population sharing equally in male and female in rates \(^20\).
This paper is aimed at finding radical cure for diabetes and hence the comparative study of the nutmeg extract with glibenclamide. Glibenclamide have been compared with Yoyo bitters in previous study.

MATERIALS AND METHOD

Ethical committee Approval: This study was approved by the ethical committee of the Faculty.

(1) Collection and Identification of Myristica Fragrance (Nutmeg):
The plant was bought from a popular market in Uyo Metropolis, Akwa Ibom State, Nigeria. It was identified by a Professor of Taxonomy in the Department of Botany and Ecological Studies, University of Uyo, Akwa Ibom State, Nigeria.

(2) Preparation of Ethanolic Extract of Myristica Fragrance
Dried Myristica fragrance weighing 1kg was made to powder using pulverizer and macerated and soaked in 60% ethanol for 72 hours. It was then filtered and the filtrate obtained was concentrated to dryness using rotary evaporator. The extract was put in a bottle and stored in a refrigerator for use in the studies.

(3) Preparation of Aqueous Extract of Readymade Myristica Fragrance (Nutmeg):
Five sachets of powdered readymade nutmeg weighing 30.99g each were purchased from the some popular market in Uyo Metropolis, Akwa Ibom State, Nigeria. The five sachets were macerated in 100ml of distilled water and kept for 72 hours. It was stirred at regular interval and then filtered and the filtrate was concentrated at 45⁰C in the water bath and the concentrate weighed 22.33g. It was stored in the refrigerator at 4⁰C till used for the studies.

(4) Phytochemical Screening: Methods of Sofowora 1993 was used.
Alkaloid: 0.5g of the extract was added to 5ml of 5% hydrochloric acid on steam bath and filtered. 1ml of filtrate was heated with a few drops of Meyer’s reagent. Another 1ml was treated similarly with Dragendorff reagent. Turbidly was indicated another 1ml of the extract was treated with a few drops of picric acid, yellow colour indicated presence of alkaloids;
Saponins: (Frothing test) 0.5g of extract was added to 10ml of distilled water, shaken and boiled frothing occurred. Indicating presence of saponins.
Tannins: 0.5g of extract was added to 10ml of distilled water stirred, filtered and the filtrate added to Fehlings solution and a blue black precipitate formed showed presence of tannins.
**Flavonoids:** 0.5g of the extract was added to few pieces of magnesium. Formation of orange colour showed the presence of flavonoids.

**Cardiac Glycosides:**
0.5g of the extract was dissolved in 2ml of chloroform and concentrated sulphuric acid added by running it down the side of the tube. A reddish brown colour at the interphase indicated presence of glycosides.

(5) **Animal Care and Use:**
Thirty (30) albino rats male and female of weight between 80g – 130g and thirty (30) male and female albino mice weighing between 17g-30g were used for the study. The animals were kept in a ventilated animal house of the Faculty of Pharmacy, University of Uyo, Akwa Ibom State, Nigeria and fed with clean water and pellets. The animals were maintained according to the regulation of institute for animal ethical committee (IAEC) of Helsinki, 1964.

**Acute Toxicity LD50 Test:**
This was done according to methods of Lorke, 1983\(^2\)\(^1\). Thirty albino mice weighing between 17g-30g were used. The animals were divided into two phases for the test. In phase 1, 3 mice were allotted in each group of 6, total 18 and intraperitoneal doses of 5000mg/kg, 3000mg/kg and 1000mg/kg were administered. In this group 100% mortality was recorded. In phase 2, 3 mice were allotted into 4 groups, total 12, 1500mg/kg, 2000mg/kg and 2500mg/kg were administered to the mice and there was no mortality recorded (0% mortality).

Therefore \(LD_{50} = \sqrt{A \times B}\)

Where

\(A= Maximum \ dose \ that \ produces \ 0\% \ mortality \ (2500mg/kg)\)

\(B = Minimum \ dose \ that \ produces \ 100\% \ Mortality \ (3000mg/kg)\)

\(LD_{50} = \sqrt{2500 \times 3000}\)

\(= 2738.61mg/kg.\)

\(= 2738.61mg/kg\)

This was further taken to percentage dosages;

10% of \(LD_{50} = 273.86mg/kg\) (low dose)

20% of \(LD_{50} = 547.72mg/kg\) (medium)

30% of \(LD_{50} = 821.58mg/kg\) (high dose)

**Induction of Diabetes:**
A solution of alloxan monohydrate 150mg/kg was administered intravenously on rats weighing 80g to 130g. The rats were allowed 72 hours before the blood glucose levels were determined, methods of Jaraid 2013 was used.

**Fasting blood glucose level:**

The blood glucose levels were determined using glucometer. It was switched on and the test strip inserted. Blood was collected from the rats by cutting the tail and a drop of it was allowed to drip into the glucose sensor of the test strip, and the blood glucose level measured in mg/dl for 7, 14, 21, 28 and 35 days. The blood glucose above 120mg/dl was considered to be diabetic, (Jaraid, 2013).

**Administration of Extract Nutmeg and Glibenclamide**

**Preparation of Stock Solution:** 1g of extract was dissolved in 10ml of distilled water

\[
\begin{align*}
1g/10ml & = 1000mg/10ml & = 100mg/ml = stock
\end{align*}
\]

\[\text{Dosage} = \frac{\text{Weight of animal} \times LD_{50}}{\text{Stock solution (100mg/kg)}}\]

**Grouping of Animals and Administration of Extract and Glibenclamide**

Thirty (30) albino rats weighing between 120-150g were randomly assigned into six (6) groups with five (5) rats in each group as follows: Group 1 was control without diabetes induction.

**Group 2:** Diabetes induced treated with low dose of nutmeg extract (273.86mg/kg)

**Group 3:** Diabetes induced treated with medium dose of nutmeg extract (547.72mg/kg)

**Group 4:** Diabetes induced treated with high dose nutmeg extract (821.58mg/kg).

**Group 5:** Diabetes induced treated with 5mg/kg of gibenclamide.

**Group 6:** Diabetes induced treated with 5mg/kg gibenclamide and low medium dose of nutmeg extract.

The drug and extract were given orally using canula by-passing the esophagus to the stomach, (Robert, 1979) for the period of 7, 14, 21, 28 and 35 days.

**Statistical Analysis:**

Data were expressed as mean plus standard error of mean (M+SEM) and analysed using analysis of variance (ANOVA). Significant difference between mean was assessed by student – Newman-Keuls post-hoc test. Ninety five (95%) level of significance. (P<0.05) was used for statistical significant. Microsoft Excel 2010 package was used for graphs and bars.
RESULTS

The study has shown differences in the effects of ethanolic extract of nutmeg, aqueous and the drug glibenclamide on blood glucose levels in the treatment of induced diabetes, Table 1, figure 1, 2, 3, 4, 5, 6, 7. On day 1 the initial fasting mean blood glucose level (MBGL) was obtained and ranged from (88.20 ±5.06 to 92.00 ± 5.09mg/dl across the six groups, which was within the normal range (80-120mg/dl). After induction of diabetes by alloxan the blood glucose levels ranged from 181.20± To 353.40±45.88mg/dl confirming diabetes and was significantly (p<0.05) different from that of day 1. On day seven (7) the mean blood glucose level in groups 2, 3, 4 & 5 were as follows, Table 1, figure 1-7

<table>
<thead>
<tr>
<th>Groups</th>
<th>Initial Glucose before induction (mg/dl)</th>
<th>Blood glucose level after induction (mg/dl)</th>
<th>Blood glucose on day 7 (mg/dl)</th>
<th>Blood glucose on day 14 (mg/dl)</th>
<th>Blood glucose on day 21 (mg/dl)</th>
<th>Blood glucose on day 28 (mg/dl)</th>
<th>Blood glucose on day 35 (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>89.00±4.35</td>
<td>90.40±3.84</td>
<td>94.40±2.65</td>
<td>97.80±3.34</td>
<td>92.20±3.42</td>
<td>99.60±1.42</td>
<td>97.60±1.88</td>
</tr>
<tr>
<td>Low dose Nutmeg Extract</td>
<td>88.20±5.06</td>
<td>182.00±16.06</td>
<td>169.20±33.99</td>
<td>129.20±3.70</td>
<td>121.60±54.70</td>
<td>115.80±51.46</td>
<td>110.00±3.74</td>
</tr>
<tr>
<td>Middle dose Nutmeg Extract</td>
<td>91.60±4.56</td>
<td>353.40±02</td>
<td>204.80±98.73</td>
<td>153.80±19.19</td>
<td>127.20±57.82</td>
<td>96.60±4.04</td>
<td>109.00±2.06</td>
</tr>
<tr>
<td>High dose Nutmeg Extract</td>
<td>91.60±4.56</td>
<td>181.20±94.88</td>
<td>126.20±3.02</td>
<td>109.20±49.03</td>
<td>109.60±2.37</td>
<td>96.60±4.04</td>
<td>93.00±1.41</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>92.00±5.09</td>
<td>220.80±11.00</td>
<td>118.40±3.85</td>
<td>85.20±26.90</td>
<td>81.20±9.15</td>
<td>64.60±4.01</td>
<td>62.80±4.04</td>
</tr>
<tr>
<td>Glibenclamide+Nutmeg</td>
<td>91.20±4.08</td>
<td>222.80±70.29</td>
<td>118.00±9.24</td>
<td>99.40±12.87</td>
<td>92.60±6.80</td>
<td>82.20±7.99</td>
<td>77.60±8.19</td>
</tr>
</tbody>
</table>

* The mean difference is significant at the 0.05 level.
Figure 1: Initial glucose level before induction of alloxan (mg/dl)

<table>
<thead>
<tr>
<th>Condition</th>
<th>Glucose Level (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>59</td>
</tr>
<tr>
<td>Low dose Nutmeg</td>
<td>88.2</td>
</tr>
<tr>
<td>Middle dose Nutmeg</td>
<td>91.6</td>
</tr>
<tr>
<td>High dose Nutmeg</td>
<td>91.6</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>92</td>
</tr>
<tr>
<td>Glibenclamide + Nutmeg</td>
<td>91.2</td>
</tr>
</tbody>
</table>

Figure 2: Glucose level after induction of alloxan (mg/dl)

<table>
<thead>
<tr>
<th>Condition</th>
<th>Glucose Level (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>94.4</td>
</tr>
<tr>
<td>Low dose Nutmeg</td>
<td>182.16</td>
</tr>
<tr>
<td>Middle dose Nutmeg</td>
<td>353.4</td>
</tr>
<tr>
<td>High dose Nutmeg</td>
<td>181.2</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>220.8</td>
</tr>
<tr>
<td>Glibenclamide + Nutmeg</td>
<td>222</td>
</tr>
</tbody>
</table>
Figure 3: Glucose level on day 7 (mg/dl).

Figure 4: Glucose level on day 14 (mg/dl).
Figure 5: Glucose level on day 21 (mg/dl).

Figure 6: Glucose level on day 28 (mg/dl).
The result of photochemical screening of the spice (Nutmeg) revealed the presence of alkaloids, flavonoids, tannins, saponins and cardiac glycoside.

DISCUSSION

The results have shown that the ethanolic and aqueous extract of nutmeg reduced the blood glucose level in rats induced with diabetes. But the ethanolic extract showed a more potent reduction in the blood glucose level than the aqueous extract. The high hypoglycemic properties of the ethanolic extract is in consonant with the inherent properties of ethanol for the inductions of active ingredients in extract, 22. However, the high potency of the prolong usage of extract of nutmeg is of benefit to the consumers. Nutmeg is sold commercially in sachets and it dissolvable characteristics in water and in cooked meals as spice enhance it potentials. However, ethanolic alcohol has detrimental effect on the body cells though by it evaporation technique in concentrating extract, it is hoped that the ethanolic content is totally removed by freeze drying23, and methanol extraction is said to remove the poisonous substance in the plant extract which may cause harm in consumption. The weight of effects between removal of poisons with alcoholic solvent extract and the effects of such in the body system is one critical assessment for the application of the methods and that of water with likely intact poison. But the potency of the aqueous extract in the study is in line with previous studies24, 25. The high dose of nutmeg actually
evoked a near high hypoglycemic, activities on day 35. The potential for the drastic reduction in blood glucose level compares this extract with glibenclamide, the orthodox drug in the treatment of diabetes. It is seen in the combination therapy of this extract with glibenclamide that the blood glucose level reduction was close to that of glibenclamide effect only. It is very certain that if the study would have increased to days 42 and above, the hypoglycemic activities and the antidiabetic potential of the extract of nutmeg would have been even greater than that of glibenclamide. However, glibenclamide exhibited high hypoglycemic activity than the extract of nutmeg. Such potential is stem from its sulfonyl compound content which stimulates the pancreatic beta cells to produce more insulin and increasing glycogen depositions in the liver, (Seena, 2017)\textsuperscript{26}. The presence of saponins, flavonoids, tannins in the extract enhanced the reduction of blood glucose level in the study\textsuperscript{27,28,30}. Also flavonoids have been shown to exert potent antioxidant activity against superoxide radicals and inhibition of low density lipoprotein (LDL) oxidation has been attributed to the dietary supplement intake of flavonoids.

CONCLUSION

Aqueous extract of nutmeg is therefore recommended for consumption by diabetic patients as it reduces blood glucose level. But caution need be taken not to indulge in over consumption due to the risk of hypoglycemia.

REFERENCES


