Polyherbal extracts of *Vernonia amygdalina*, *Moringa oleifera*, *Gongronema latifolium* and *Ocimum gratissimum* may substitute insulin requirement in the long term management of type 1 diabetes


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**Abstract**
Diabetes mellitus is the most challenging metabolic disorder resulting in multiple complications. It cannot be cured but needs only to be managed.

**1. Introduction**
Diabetes mellitus (DM) is the most common endocrine disorder, which is characterized by a defective or deficient insulin secretory process, glucose underutilization and increased blood sugar (hyperglycemia). It is a congenital or acquired inability to transport sugar from the bloodstream into the cells (Prabhakar and Doble, 2008). DM is ranked seventh among the leading causes of death and is considered third when its fatal complications are taken into account Trivedi et al. (2004). The multifaceted etiologies are in recent times thought to be the reason why a range of conventional and chemotherapeutic agents, though in use for over several decades have failed to completely address the problem (Tiwari and Rao, 2002). As single agents, they act only in part of the pathogenic process and to a partial extent only (Luna and Feinglos, 2001).

Although some very potent drugs have been developed to manage this illness, research is still on, as to how this illness can be totally...
cured, and several plants have been identified as having anti-diabetic properties and available literature indicate that there are more than 800 plant species showing hypoglycaemic activity (Rajagopal et al., 2008). Some of these plants are considered in this research work. They include M. oleifera, V. amygdalina, G. latifolium and O. gratissimum. According to the traditional system of Indian medicine, a combination of substances is used to enhance the desired activity and eliminate unwanted side effects (Patel et al., 2009). Thus, the need to investigate the polyherbal outcome of the four plants extract.

Moringa oleifera is an edible plant. A wide variety of nutritional and medicinal virtues have been attributed to its roots, bark, leaves, flowers, fruits, and seeds (Anwar et al., 2007; Kumar et al., 2010). Phytochemical analyses have shown that its leaves are particularly rich in potassium, calcium, phosphorous, iron, vitamins A and D, essential amino acids, as well as such known antioxidants such as β-carotene, vitamin C, and flavonoids (Amaglo et al., 2010; Gowrishankar et al., 2010). In Indian ethnotherapeutic system of medicine M. oleifera is reported to possess hypoglycemic activity (Bhishagratna, 1991; Kar et al., 2003).

Vernonia amygdalina (Compositae) also called bitter leaf in Nigeria because of its bitter taste, is a shrub that grows predominantly in Tropical Africa. The leaves have found relevance in traditional folk medicine as an antihelminth, a laxative herb and an antimalarial as they are known as quinine substitute (Farombi, 2003; Ugwu et al., 2011). Vernonia amygdalina is used traditionally in the management of diabetes in the African sub–Region and Asia respectively. The antihyperglycemic action of the plant (Nimenibo-Uadia, 2003; Akah et al., 2004) and hypoglycemic effect (Gyang et al., 2004; Atangwho, et al., 2007) have been reported.

G. latifolium is a tropical rainforest plant which has been traditionally used in the South eastern part of Nigeria for the management of diseases such as diabetes, high blood pressure etc (Ugochukwu et al., 2003). Aqueous and ethanolic extracts of G. latifolium have shown hypoglycemic properties according to Ugochukwu and Babady (2003).

O. gratissimum, Africa basil/sweet basil, is a plant belonging to Lamiaceae. The Ocimum species are widely found in tropical and subtropical regions and commonly used as food spice and traditional herb (Lee, 2011). Recently, its hypoglycemic efficacy (Aguiyi et al., 2000; Mohammed et al., 2007) and safety (Egesie et al., 2006) have been reported. Preliminary studies on holy basil and hairy basil have shown that the leaf and seed may help people with type 2 diabetes control their blood sugar levels (Rai et al., 1997).

It was therefore considered worthwhile to investigate the combined ethanolic extracts of M. oleifera, V. amygdalina, G. latifolium and O. gratissimum leaves for their glycemetic potential by evaluating their effects on fasting blood glucose, serum glucose and hepatic glucose levels of normal and diabetic rats. Their effects on serum c-peptide and insulin were also examined in the diabetic models. Thus, this is the first reporting of hypoglycemic effects of ethanolic extracts of the four leaves.

2. Objective of Research

The objective of the study was to investigate the effects of the combined administration of V. amygdalina, G. latifolium, O. gratissimum and M. oleifera leaves extracts on serum glucose, hepatic glucose, serum insulin and c-peptide levels diabetic rat models. These parameters were used to access the glycaemic index of the experimental rats.

3. Experimental

Diabetes mellitus is serious metabolic disorder with micro and macrovascular complications that results in significant morbidity and mortality (Efiong et al., 2013). It cannot be cured but needs only to be managed. The management of diabetes mellitus is considered a global problem and successful treatment is yet to be discovered. The modern drugs, including insulin and oral hypoglycemic agents, control the blood sugar level as long as they are regularly administered and they also produce a number of undesirable effects (Kumar, 2010). The adverse effects include hypoglycemia at higher doses, liver problems, lactic acidosis and diarrhoea (Suba et al., 2004). Even when effective glycaemic control is achieved, the use of these drugs is restricted by their pharmacokinetic properties, secondary failure rates and accompanied undesirable effects (El-Nagar et al., 2005; Egwim, 2005). Since the therapy is life long, therapeutic agents devoid of side effects would be appreciated and one of such approaches is the use of alternative system of medicine comprising herbal products (Pari and Saravanan, 2004).
Thus, the need to investigate scientifically the effects of the four plants *Vernonia amygdalina* (VA), *Moringa oleifera* (MO), *Gongronema latifolium* (GL) and *Ocimum gratissimum* and of even more importance is to investigate the polyherbal effects of the four herbal extracts when compared with the orthodox antidiabetic drug insulin.

The work plan included the following stages: animal grouping, induction of diabetes using 40 mg/kg bw of streptozotocin, testing for diabetes, administration of extracts, sacrifice of experimental animal, collection of serum for laboratory analysis and analysis of sera and interpretation of results.

2.1 Collection of plant materials
The fresh leaves (800g) of the shrubs were collected from the Endocrine Research farm, University of Calabar, Calabar.

2.2 Chemicals used
All chemicals and drugs used were obtained commercially and of analytical grade.

2.3 Preparation of extract
Fresh leaves of *M. oleifera*, *V. amygdalina*, *G. latifolium* and *O. gratissimum* were collected, macerated and allowed to stand in 80% alcohol at room temperature for 48 hours. The filtrate was evaporated in a rotary evaporator and allowed to concentrate in a water bath at 36° C. A greenish paste was obtained. The extraction of *V. amygdalina*, *O. gratissimum*, *G. latifolium* and *O. gratissimum* leaves was done in the Department of Biochemistry, University of Calabar. The obtained leaves extract were stored at 4° C.

2.4 Experimental animals
Forty eight rats weighing between 120-180 g, were obtained from the department of biochemistry animal house, University of Calabar divided into eight groups of 6 rats each. Before the experiment, the rats were allowed to acclimatize to the animal house for 7 days. Standard environmental conditions such as temperature (26-2° C), relative humidity (45-55%) and 12 hrs dark/light cycle were maintained. All the animals were fed with standard rat chow and water was allowed *ad libitum* under strict hygienic conditions.

2.5 Acute toxicity test (LD50)
The oral acute toxicity of the ethanol extract (EE) was determined in mice as described by Lorke (1983).

2.6 Induction of diabetes
STZ was prepared in citrate buffer (0.1 M, pH 4.5). STZ solution was injected intraperitoneally at a concentration of 40 mg/kg of body weight in a volume of 0.5 ml/rat. Diabetic condition (type I) was confirmed in fasting rats from blood glucose level more than 150 mg/100 ml determined after 72 hours after day of injection.

2.7 Experimental design
48 adult male and female wistar albino rats weighing 120-180 g were grouped into eight (8) as follow:

<table>
<thead>
<tr>
<th>Table 2.1: Animal Grouping</th>
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<tbody>
<tr>
<td><strong>Groups</strong></td>
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<tr>
<td>1</td>
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<td>7</td>
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<td>8</td>
</tr>
</tbody>
</table>

Treatment was administered twice daily (12 hourly) for 28 days.

2.8 Statistical analysis
Blood glucose levels were expressed in mg/dL as Mean±SEM. The data were statistically analyzed using ANOVA with multiple comparisons versus control group by Dunnett’s method. The values of p<0.05 were taken as significant.

2.9 Justification of research
It was important to carry out this research in order to ascertain the antidiabetic impact of polyherbal therapy of the four plants when compared with insulin in a bit to providing a safer treatment option for the management of type 1 diabetes. It was significant to carry out the study in order to validate or invalidate the use of herbs in our traditional society in the management of diabetes. Therefore, the results from this work will be a milestone in this scientific domain as it will provide information for further research into the
utilization of the polyherbal therapy in diabetes management when compared with insulin.

3. Results and Discussion

3.1 Effects of treatment on Fasting Blood Sugar

Figure 3.1: Comparison of fasting blood glucose concentration of the different experimental groups. Values are mean + SEM

From fig. 3.1, Fasting Blood Glucose remained raised in the diabetic control group when compared with the normal control group, while the reverse was observed for the individual plant extracts with Fasting Blood Glucose levels comparable with the normal control at the end of the experimental period of 28 days thus, showing antidiabetic activity. The FBG for the individual plants were lower than for the standard drug INS. This result is in conformity with results by Mohammed et al., 2007; Edet et al., 2009; Mbikay, 2012 and Ebong et al., 2006 for Ocimum gratissimum, Gongronema latifolium, Moringa oleifera and Vernonia amygdalina respectively. The possible mechanism of hypoglycemic action observed in these herbal extracts may be by increasing either the pancreatic secretion of insulin from β-cell of islet of langerhans or its release from bound form, inhibition in the intestinal absorption of glucose and increase in glucose metabolism. Also, from the preliminary phytochemical screening, it is confirmed that the herbal extracts are rich sources of flavonoids and tritepenoids (Igile et al., 2002). These compounds are known to possess free radical scavenging effect and rejuvenating potential (Ugochukwu and Babady, 2002). Therefore, the hypoglycemic activity of the extracts may be due to presence of these active constituents (Atangwo et al., 2009).

FBG for the polyherbal group remained raised at the end of the experiment. The raised FBG for the polyherbal group (4EXT) may have been as a result of an antagonistic action of the plant extracts secondary metabolites or the reduction of antioxidant activity. According to Muligo et al., 2013, co-occurrence of alkaloids and saponins significantly reduced antioxidant activity and the antagonistic relationship among phytochemicals would affect the efficacy of crude extracts as used in traditional medicine. However, it would be observed that there was a sustained fall in FBG for the 4EXT group. This will suggest that normoglycaemic condition may be reached with prolonged exposure to the polyherbal therapy. Hence, the polyherbal combination of Gongronema latifolium, Moringa oleifera, Ocimum gratissimum and Vernonia amygdalina may be the preferred chronic treatment over INS in the management of diabetes mellitus.

Hepatic glucose levels for the individual as well as the polyherbal group recorded a significant decrease when compared with insulin (fig. 3.2). From table 3.1, it is observed that the polyherbal therapy showed a marked reduction in serum glucose which showed better activity than individual plants and insulin and compared well with the normal control. This result is in tandem with previous findings from our laboratory (Ebong et al., 2009). Results of this research shows an increase in serum C-peptide and insulin levels for the single herbal treated groups when compared with DC and insulin and corroborates with a similar finding by Malini et al., 2011; Mandade and Sreenivas, 2011. The increase in both parameters for the polyherbal treated groups was better than that obtained for the standard drug insulin and was comparable with NC. On the basis of these results it can be said that the single and polyherbal extracts exerts antidiabetic effects with a more significant effect observed for the polyherbal therapy when compared with insulin. This could have
Table 3.1: Effects of treatment on serum glucose, insulin and c-peptide

<table>
<thead>
<tr>
<th>Groups</th>
<th>Glucose(mg/dl)</th>
<th>Insulin(U/ml)</th>
<th>C-Peptide(ng/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>28.57±10.36</td>
<td>3.67±0.81</td>
<td>0.04±0.01</td>
</tr>
<tr>
<td>DC</td>
<td>153.59±12.22*</td>
<td>1.50±0.32</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>INS</td>
<td>58.27±28.04</td>
<td>2.00±0.00</td>
<td>0.03±0.01</td>
</tr>
<tr>
<td>VA</td>
<td>84.43±10.56</td>
<td>8.50±2.58</td>
<td>0.14±0.03</td>
</tr>
<tr>
<td>MO</td>
<td>44.36±27.78</td>
<td>2.50±0.50</td>
<td>0.07±0.00</td>
</tr>
<tr>
<td>GL</td>
<td>91.94±31.10*</td>
<td>2.60±1.13</td>
<td>0.06±0.01</td>
</tr>
<tr>
<td>OG</td>
<td>59.21±21.07, a</td>
<td>11.30±4.74, b</td>
<td>0.28±0.16, a,b</td>
</tr>
<tr>
<td>4EXT</td>
<td>29.20±6.95, a</td>
<td>4.35±0.04</td>
<td>0.05±0.00</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM.
* significantly different from NC at p<0.05
a = significantly different from DC at p<0.05
b = significantly different from INS at p<0.05
4EXT = M.O+V.A+O.G+G.L

been as a result of different secondary metabolites in the different plant extracts possessing different mechanisms of action. Sequel to this, combination of plants extract should be encouraged. However, it cannot be concluded that the polyherbal therapy may have synergistic effects only as observed in the FBG results. Nevertheless, the polyherbal therapy may be a better and long term supplementary treatment option in the safe management of diabetes mellitus because a safe treatment for diabetes mellitus should be aimed at ameliorating hyperglycaemia.

Research Highlights

• Single extracts administered to the diabetic rats reversed hyperglycaemia and was comparable with the normal control group.
• Fasting Blood Glucose for the polyherbal therapy showed gradual reduction while its serum glucose was reduced to normoglycaemic level which was lower than insulin.
• Serum insulin and c-peptide levels were increased for the treated groups with polyherbal levels higher than that of insulin and compared well with the normal control group, thus, signifying a regeneration of the beta cells of the pancreas.

Limitations

The limitation to this research was the number of days the extracts were administered to the rats. This did not give the true picture of the rats’ response to the polyherbal therapy on a long term.

Recommendations

To address this limitation, it is recommended that the number of days for the experimental work be increased in order to ascertain the long term impact of the polyherbal therapy.

Justification of Research

From the assessment of the hypoglycaemic parameters from this work, it has been possible to ascertain the rats’ glycaemic response to the treatments after induction of diabetes with streptozotocin.

Conclusion

The polyherbal formulation showed significant improvement in the glycaemic alteration produced in Type 1 diabetes mellitus when compared with the orthodox antihyperglycaemic agent insulin and it could be effectively used as a safer long term nutraceutical substitute over insulin in the management of diabetic patients.

Conflict of Interests

The authors declared no conflicts of interest.

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