Effects of Capsules of *Vernonia amygdalina* Combined with Glibenclamide or Metformin on Blood Glucose Levels of Diabetic Rats.

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ABSTRACT

This study focused on the formulation of *Vernonia amygdalina* (VA) capsules combined with standard anti-diabetic drugs (glibenclamide (GLI) or Metformin (MET)). Capsules of semi-purified VA and their combinations were prepared using conventional capsule preparation techniques. The capsules were evaluated for various physico-chemical properties such as weight uniformity, disintegration time, dissolution test and stability. Investigation on anti-diabetic effect of VA capsules (individual and combined) on streptozotocin-induced diabetic rats was carried out using a glucometer. Weight uniformity tests of all the capsules showed percentage deviation of less than 10%. Disintegration of all the capsules was within 4 minutes. Drug release profile showed that peak drug release occurred at 15 minutes for all capsules. The maximum anti-diabetic dose for capsules of semi-purified VA was 100mg/kg. The antidiabetic activity of VA and its combinations (VA + GLI and VA + MET) capsules were 75.68±16.3, 69.58±12.08 and 65.05±15.03 respectively. Combined VA and standard drugs had no significant effect on anti-diabetic activity compared with VA alone (p = 0.813). Quantitative determination of C-peptide for individual extract capsules showed significantly increased effect (p<0.05) than the combinations with standard. This work has shown that capsule of VA combination with standards had no advantage in antidiabetic activity over individual VA. Capsules of individual and combinations VA pass compendia requirements.

Keywords: Capsules, *Vernonia amygdalina*, Diabetes, Combination with standards.

INTRODUCTION

Medicinal plants have provided very interesting areas of research in the history of man. The traditional use of medicinal plants in the treatment of many diseases is associated with cultures of many countries. There is a great variety of compounds that can be extracted and characterized from plants. One example is the harmaline, one of the indole alkaloids found in *Peganium harmalan* (Zygophyllaceae) used in the treatment of dermatosis1. Diabetes mellitus (DM) is a chronic disorder of carbohydrate, protein and lipid metabolism characterized by persistent elevations of fasting blood sugar over 126 mg/dl, due to insufficient or complete cessation of insulin synthesis or secretion and/or peripheral resistance to insulin action2. The non-pharmacological means (diet and exercise) and the pharmacological means (insulin and oral hypoglycemic) may be used in the management of diabetes mellitus. The use of pharmacological agents is restricted by their pharmacokinetic properties, secondary failure rates and accompanying side effects such as insulin resistance3 and this necessitated a search for alternatives. A number of medicinal plants have been studied for the management of diabetes mellitus. Ethnobotanical information indicates that more than 800 plants are used in traditional remedies for the treatment of diabetes due to their effectiveness, less side effects and relatively low costs4. *Vernonia amygdalina* extracts and isolated chemical constituents have been studied for their potential pharmacological effects, including: antioxidants, anthelmintic anti-parasitic properties5, anti diabetic6 and enhanced chemotherapy sensitivity7. Due to side effects of synthetic drugs namely gastro-intestinal disturbances, weight gain and cardiovascular and liver risks and proven advantages of natural products, this study aims to combine standard (glibenclamide and Metformin) and VA semi purified in capsule formulations.

MATERIALS AND METHODS

Plant collection

The leaves of the plant *Vernonia amygdalina* (VA) were collected from Enugu in Enugu North Local Government Area, Enugu State, Nigeria. The leaves were authenticated by Mr. A.O. Ozioko, a consultant taxonomist with the International Center for Ethnomedicine and Drug Development (Inter CEDD) Nsukka and the voucher specimen (no. PC98033) is preserved in the Pharmacognosy Herbarium, University of Nigeria, Nsukka. The fresh leaves were washed and air-dried in a room temperature. They were pulverized into a fine powder using a blender, sealed in polythene bags and stored at 4°C.

Materials

Methanol, chloroform, hexane, ethyl acetate (all from Sigma Aldrich), lactose, (Santa Cruz USA), TLC silica gel 60 F254 (Merck, Darmstadt Germany), Silica gel (QualiKems, China), potassium acetate, potassium iodide (all from...
Labto, China), ammonium nitrate (Avis Chemical, China). Metformin HCl (Sigma Aldrich, Germany), glibenclamide (Santa Cruz, USA), streptozotocin mixed anomers SO130 (Sigma Aldrich Co, St Liou, USA).

Chromatographic separation of methanolic extract of VA leaf
A 200 g of VA powder was immersed in aqueous methanol (80 %) for 48 h and shaken every 2 h. The solvent was distilled off in the rotary evaporator to obtain a solid residue. The methanol extracts was fractionated by chromatographic methods using gradient solvent system (n-hexane, chloroform, ethyl acetate and methanol) to obtain fractions F1 – F 9. These fractions were subjected to anti-diabetic activity.

Determination of some physical properties of powder blend of semi purified and excipients
Some physical properties of powder blends of semi-purified VA and excipients were determined by calculating the bulk density, tapped density, Hausner ratio, Carr’s index and angle of repose using standard methods.

Formulation of capsule dosage form
The formula for the semi purified capsules and combination with standard are shown in Table 1. The powder composed of excipients namely lactose and talc were used as diluents and lubricant respectively. The powder composed of drugs and excipients were mixed in a glass mortar for 20 min. The powder blend was filled into No 2 hard gelatin capsules (260 mg) using a semiautomatic capsule-filling machine (Capsul CN, China) as reported in USP8.

Table 1: Formula for Vernonia amygdalina semi-purified individual and combination capsules

<table>
<thead>
<tr>
<th>Materials</th>
<th>Qty/1 cap (mg)</th>
<th>Qty/12 cap (g)</th>
<th>Qty/120 cap (g) Combination (GLI)</th>
<th>Qty/120 cap (g) Combination (MET)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VA (100 mg/kg) Rat wt 130 g</td>
<td>13</td>
<td>1.560</td>
<td>1.560</td>
<td>1.560</td>
</tr>
<tr>
<td>Lactose</td>
<td>144.6</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>D.F</td>
<td>116</td>
<td>13.920</td>
<td>13.889</td>
<td>8.95</td>
</tr>
<tr>
<td>Talc</td>
<td>72</td>
<td>8.640</td>
<td>8.640</td>
<td>4.47</td>
</tr>
<tr>
<td>Glibenclamide GLI (2mg/kg)</td>
<td>0.26</td>
<td>-</td>
<td>0.031</td>
<td>-</td>
</tr>
<tr>
<td>Metformin Met (500 mg/kg)</td>
<td>65</td>
<td>-</td>
<td>-</td>
<td>7.8</td>
</tr>
</tbody>
</table>

Key
VA = Vernonia amygdalina semi-purified
D.F = Displacement factor

Evaluation of VA capsule dosage forms

Physical evaluation
Physical examination of all the capsules was carried out visually.

Weight uniformity test
Weight uniformity test was performed for 20 capsules. The percent weight deviation was calculated as described in literature9.

In-vitro disintegration time
In vitro disintegration time was determined for various batches of capsules using the disintegration test apparatus (Logan Instrument, Germany). A capsule was placed in each of six tubes of the apparatus and one disc was added to each tube. The distilled water was maintained at a temperature of 37 ± 0.5°C and the time taken for complete disintegration of the capsules with no palpable mass remaining in the apparatus was noted.

In-vitro release studies for the capsules
The capsules were subjected to in-vitro dissolution studies in 900 ml of distilled water, pH 7 for 2 h using a USP XXIII dissolution apparatus (Bender and Hoban perLaborfachach, Germany) at 50 rpm maintained at 37± 0.5°C. A 5 ml quantity of the dissolution medium was withdrawn every 10 min and filtered through Whatmann filter paper 2, diluted ten folds and analyzed using UV-visible double beam spectrophotometer at 277 nm. Equal amounts of fresh dissolution medium were replaced immediately after withdrawing 5 ml.

Stability studies
The drugs were subjected to stability studies so as to evaluate the chemical stability and physical characteristics. The capsules contained in amber-coloured containers were stored in various saturated salt solutions. The saturated solutions of potassium iodide and ammonium nitrate gave varying relative humidity and temperature viz 30°± 2°C / 75% ± 5% RH and 25°± 2°C / 60% ± 5% RH respectively10. The stability was assessed by evaluating the percentage of the initial concentration remaining after specific periods of time (0, 3, 6 months). A difference in concentration of ± 10% was a notable change.

Dose-related anti-diabetic response of semi-purified VA capsules
Thirty-five rats of male sex weighing between 120 – 150 g were randomly divided into 7 groups (1 –7) of five rats per group and fasted for 14 h. Each rat in each group was intraperitoneally injected with accurately determined volume of streptozotocin (STZ) (65 mg /kg) in distilled water. The animals were fed with standard pellet diet (growers feed, Vital and Nigeria) and water ad libitum for 48 h. Diabetes was confirmed after 48 h in rats that showed fasting blood glucose (FBG) levels of > 240mg/dl. The purified VA capsule was orally administered at doses of 50, 75 and 100 mg/kg to groups 2 - 4 using an improvised method. Then standard glibenclamide (2 mg/kg) and Metformin (500 mg/kg) were administered to groups 5 and 6. The control group (1) and diabetic group (7) received 2 ml/kg of normal saline. Blood glucose...
of the treated rats was measured at 0, 1, 2, 4 and 8 h using Accu-check glucometer. The percentage glucose reduction was calculated.

**Delivery of the capsules**

A 1 ml syringe was used as improvised device (Figure 1) for delivering the capsules into the stomach of the rats. The tip where the needle is normally inserted was cut off. The capsule was placed at the cut end of the syringe. The rat was restrained by holding the neck muscle. The device with the inserted capsule was placed inside the mouth at the dorsal part of the tongue (roof of the tongue), pushing the plunger and delivered the capsule inside the stomach through the oesophagus. Water was then given to the animal.

**Effect of combination of semi-purified VA with standard capsules on streptozotocin (STZ) induced diabetes**

Forty-eight healthy male albino rats (100-150 g) were randomly selected and housed in eight groups (1-8). The animals were fed with standard pellet diet and water ad libitum. The animals were fasted 14 h before induction of diabetes (2 - 8 groups) by intraperitoneal (ip) injection of a single dose of streptozotocin (STZ) (65 mg/kg body weight). The weighed STZ was dissolved in distilled water and injected immediately within few minutes to avoid degradation. Diabetes was confirmed after 48 h in rats that showed fasting blood glucose (FBG) levels of > 240 mg/dl. The animals in groups 1 and 8 were designated normal and diabetic controls and received 2 ml/kg of normal saline. Test groups 2 - 5 received VA (100 mg/kg), VA and GLI (100:2 mg/kg), VA and MET (100:500 mg/kg) VA and GL (100:400 mg/kg) respectively. In groups 6 and 7, standard drug; glibenclamide (2 mg/kg) and Metformin (500 mg/kg) were given respectively. The blood glucose of all the rats was measured at predetermined times of 0, 1, 2, 4 and 8 h. The percentage glucose reduction was calculated.

**Quantitative Determination of Serum C-peptides of STZ induced rats treated with semi purified VA (Individual and Combination) capsules**

Quantitative determination of C-peptide was carried out using commercial C-Peptide rat Elisa kits obtained from ALPCO USA. All the standards, samples and controls were run concurrently in duplicate.

**RESULTS AND DISCUSSION**

**Antidiabetic activity of fractions of VA methanol leaf extract**

VA methanol leaf extract was separated into nine fractions (F1 - F9) by column and thin layer chromatographic methods. Antihyperglycemic screenings of various fractions of VA methanol leaf extract are presented in Figure 2. Out of the nine fractions, fraction seven (F7) has the highest percentage blood glucose reduction (78%) as shown in Figure 2. The lowest activity was found to be F1 and F8. F7 has no significant difference with the standard drugs metformin and glibenclamide (P>0.05). Fraction 7 was extracted with polar solvents, ethyl acetate and methanol. This is in accordance with the previous work. In their study, F6 polar fraction exhibited potent anti-hyperglycemic activity.

**Pre formulation studies**

**Physical properties of powder blend of extract and excipients**

Some physical properties of the powder blend of extracts and excipients are presented in Table 2. Blends had Hausner ratio < 1.25 and Carr’s index of < 20 %. Hausner ratio is related to interparticulate friction. Powders with low interparticulate friction have ratios of < 1.2, which indicate good flow; whereas more cohesive, less free-flowing powders have Hausner ratios greater than 1.5.

This shows that all the powders exhibited good flow properties.
Table 2: Some physical properties of powder blend of extracts and excipients

<table>
<thead>
<tr>
<th>Powder Blend</th>
<th>Bulk Density (g/cm³)</th>
<th>Tapped Density (g/cm³)</th>
<th>Hausner ratio</th>
<th>Carr’s Index</th>
<th>Angle of Repose (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VAPE</td>
<td>0.465</td>
<td>0.565</td>
<td>1.215</td>
<td>17.7</td>
<td>18.41</td>
</tr>
<tr>
<td>VAPE+GLI</td>
<td>0.544</td>
<td>0.620</td>
<td>1.140</td>
<td>12.25</td>
<td>24.44</td>
</tr>
<tr>
<td>VAPE+MET</td>
<td>0.530</td>
<td>0.637</td>
<td>1.202</td>
<td>16.80</td>
<td>24.67</td>
</tr>
</tbody>
</table>

Evaluation of Capsules

Weight uniformity of capsules

The weight uniformity of capsules prepared with individual and combined semi-purified extract capsules was found to be within the pharmacopoeia limits. None had percentage deviation of 10%. The weight uniformity depicts that capsules have content uniformity of active ingredient.

Disintegration time of capsules

The disintegration time of the capsules was 3 min. This is within the BP specification of 30 min. To determine the ability of the formulated capsules to release their drug content in the gastrointestinal tract, disintegration time of the capsules were investigated. Capsules that failed to break up into smaller aggregates and primary particles may not release the active ingredient for absorption.

Drug release profile of semi-purified *Vernonia amygdalina* (VAPE) and in combination with metformin and glibenclamide

Data recorded showed sharp peaks of drug release profiles of VAPE combination with metformin or glibenclamide after 10 min (Figure 3). However, the combination of VA and standard negatively affected the rate of release of VA.

Dose related anti-diabetic response of semi-purified VA capsules

The mean blood glucose time graph for VA capsules is presented in Figure 4. A 100 mg/kg dose of VA capsules had the most lowering effect on blood glucose in STZ induced diabetic rats.

**Figure 3:** Drug release of *V. amygdalina* (VAPE) and its combination with standard

**Figure 4:** Mean blood glucose time graph for VA capsules

**Figure 5:** Combination effect of VA with standards on STZ-induced diabetic rat
C-Peptide content of individual and combined semi-purified capsules treated-diabetic animals

Data obtained from quantitative determination of C-peptides level in the VA semi-purified individual and combined capsules showed combination of semi-purified and standards was comparatively lower than individual semi-purified capsules (Table 3).

C-peptide is an important part of the insulin assembly process of beta cells in the pancreas. When insulin is made in the body, it is first formed as a long chain of 110 amino acids. The chain is folded and relocated within the beta cell. The chain proteolytically cleaved into three parts- the insulin A chain, the insulin B chain, and C-peptide in the middle. The two short insulin A and B chains bind to form a single insulin protein and then both entities (the insulin protein and the short C-peptides) are released equimolar by beta cells into circulation. Recently, the role of C-peptide as a measure of natural insulin production had made measure of insulin easier. The measurement of C-peptide has been reported to be a valuable index of insulin secretion rather than insulin alone16. The increase in C-peptide levels was mainly an indication of continuing beta cell function and its relation to long-term control for diabetics. These had effects on proliferation of the islet cells and other cells of the pancreas.

Stability study

The physical examination of the capsules stored at varying temperature and humidity showed that all the capsules had no colour and odour change. The powder blend flow well and capsules were not brittle. All the drug content of the various capsules in varying storage conditions were ≥ 95 % after 3 months.

**Table 3:** The C-peptide content of individual and combined VAPE semi-purified capsules on STZ induced diabetic rats

<table>
<thead>
<tr>
<th>Drugs</th>
<th>C-peptide (pM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VAPE</td>
<td>312.21±0.58</td>
</tr>
<tr>
<td>VAPE + MET</td>
<td>169.9 ± 1.20</td>
</tr>
<tr>
<td>VAPE+ GLI</td>
<td>23.52±1.66</td>
</tr>
<tr>
<td>GLI</td>
<td>32.58 ± 1.54</td>
</tr>
<tr>
<td>MET</td>
<td>33.98 ± 1.54</td>
</tr>
<tr>
<td>Normal</td>
<td>2111.72±1.45</td>
</tr>
<tr>
<td>Diabetic</td>
<td>0</td>
</tr>
</tbody>
</table>

**CONCLUSION**

This work focused on formulation and evaluation of semi purified VA individual and combination with standards capsules. All capsules passed pharmacopeia requirements. The maximum anti-diabetic dose of semi-purified VA capsules was 100 mg/kg. Combination of semi-purified VA with standard capsules had no better activity against individual drugs. The stability studies of VA and VA combination with glibenclamide capsules were up to 95% content in varying storage conditions after three months. Further investigation on alternative formulation technique should be designed to improve anti-diabetic activity of VA semi-purified combinations with standard drugs.

**REFERENCES**


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