Effects of Polyherbal Formulation of *Allium sativum* and *Persea americana* Seeds’ Extracts on Postprandial Hyperglycemia and Sucrose Digestion in Acute Treatment of Normoglycemic Rats

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Received: September 4, 2017; Accepted: March 07, 2018; Published: March 14, 2018

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**Abstract**

This study was carried out on *Allium sativum* and *Persea americana* seeds’ extracts, to compare their alkaloid content and to evaluate the antihyperglycemic potentials solely and in combination, as well as the effect of the combination on sucrose digestion in normal Wistar rats. Aqueous extract (AE) and hydroethanolic extract (HEE) of both plants were prepared. Alkaloid content was determined. Antihyperglycemic activity was evaluated by oral glucose tolerance test and sucrose digestion for 120 min. Normoglycemic albino Wistar male rats of 150-220 g were used. Rats were distributed in five groups of six each: positive control group (water), four test groups (400 mg/kg b.w. HEE and AE of *A. s.* and *P. a.*), and reference group (4 mg/kg b.w., glibenclamide). All the groups received 2 g/kg b.w. of glucose solution. Similar design was followed to test formulation (1:1, AE of *A. s.* 200 and HEE of *P. a.* 200 extracts) and for sucrose (2 g/kg bw) digestion, using acarbose as reference drug. Glycemia was followed up for 30, 60, 90, and 120 min after administration of substrates. HEE Pa revealed eight folds of alkaloid content higher than *A. s.* extracts. Both the individual extracts (AE *A. s.*: 1563; HEE *P. a.*: 5270) and the formulation had lower area under the curve (AUC) values (combination: 2825 mg min/dL) compared to positive control (4015 mg min/dL; p > 0.05). However, AE As400 efficacy was comparable to glibenclamide (1163 mg min/dL; p > 0.05). The HEE Pa, AE As400, and the formulation (AEAs200 + HEEAs200) on sucrose digestion revealed higher AUC values compared to positive control. We concluded that AE As200 combined with HEE Pa200 ameliorates the glucose lowering potential of the HEE of *P. americana* but increases the sucrose digestion rate.

**Keywords:** Antihyperglycemia; *Allium sativum*; *Persea americana* seeds; Formulation; Potentiating effects

**Introduction**

Obesity and diabetes mellitus (DM) are among the most challenging metabolic disorders in the world. The clinical management of long-term complications associated to them is expensive for patients and governments [1]. Obesity and T2DM lead to reduction in both life expectancy and quality [2]. The global prevalence of DM in adults above 18 years was estimated at 422 million adults [3], Asia and Africa being the most at risk [4].

DM is a combination of heterogeneous disorders commonly presenting with glucose excursions and glucose intolerance, which arise because of derangements in the regulatory systems for storage and mobilization of metabolic fuels [2]. Hyperglycemia, one of the primary symptoms of T2D, gradually induces oxidative stress, insulin resistance, and impairment of insulin secretion [5]. Hyperglycemia, which can also arise from chronic overnutrition, is responsible for long-term complications such as macrovascular and microvascular damages [6,7]. Therefore, control of hyperglycemia, especially postprandial hyperglycemia, is critical in the treatment of diabetic patients and individuals with impaired glucose tolerance [8]. Unfortunately, conventional oral antihyperglycemic agents are not only expensive [6,9] but also have flatulence, diarrhea, abdominal bloating, nausea, headache, pancreatitis (rare), vomiting, sense of fullness, and hypoglycemia as side effects [10]. This justifies the growing interest toward combinations/ polyherbal therapies used as alternative treatment with improved efficacy [11,12]. These include *Andrographis paniculata* and *Gynura procumbens* ethnical extracts [13] and garlic and metformin [14].

Garlic is a member of the genus *Allium*. It is a spice belonging to the family of *Alliaceae* [15]. It possesses anticancer, antiviral, antioxidant, anti-inflammatory, and antiabetic properties [15]. *Avocado or Persea americana (Pa)*, another plant found in tropical and subtropical regions, belongs to the family Lauraceae [16]. *Avocado seeds* are used as natural antioxidants to treat gastrointestinal irregularities, anemia [17,18], arthritis, and hypertension [19,20].

Avocado leaf preparations have been used as long-term treatment of diabetes [21-23]. Moreover, in an acute treatment, they were shown to reduce blood glucose level after 5 h [24] to 6 h [25]. The timing could be improved with plant combinations, which offer multiple targets for treatment and may act via synergistic, antagonistic, or potentiating effects. This study was designed to study the effects of aqueous extract (AE) and hydroethanolic extract (HEE) of *Allium sativum* (*A.s.*) and *P. americana* seeds solely or in combination (formulation) on postprandial hyperglycemia, sucrose digestion, and to compare the alkaloid content of both plant extracts.

**Materials and Methods**

All the chemicals used were of analytical grade and included ethanol, FeCl₃, HCl, and phenanthroline, purchased from Sigma Co., Louis, MO, USA.
Plant materials and preparation

*Allium sativum* and *Pa* fruits were bought from a local market in Buea (Cameroon) in February 2016. They were identified at the national herbarium as belonging to the families Alliaceae and Lauraceae, respectively. The peels of the *A.s* bulb were removed, and the bulb was shade dried in open air for one month. In addition, the *Pa* fruits were allowed to get ripe; the seeds were removed and were cut into small pieces. Then, they were shade dried in open air for one month. The dried materials were ground to obtain powder, from which the different extracts were prepared as previously described [26].

Preparation of aqueous extract

One hundred grams (100 g) of either *A.s* or *Pa* seeds were placed in 800 mL of water for maceration for 48 h. After filtration, the filtrate was evaporated at 50°C using an oven to obtain the AEs.

Preparation of hydroethanolic extract

One hundred grams (100 g) of either *A.s* or *Pa* seeds were placed in 800 mL of water/ethanol (95%) in a ratio of 1:1 (v/v) for maceration for 48 h. After filtration, the filtrate was evaporated at 50°C to obtain the HEEs.

Determination of alkaloid content

Alkaloid content was evaluated following the method reported by Singh et al. [27]. One hundred milligrams of the powder were mixed in 10 mL of ethanol (80%). The supernatant was used for the estimation of the total alkaloid content. The reaction mixture contained 1 mL of extract, 1 mL of 0.025 M FeCl$_3$ in 0.5 M HCl, and 1 mL of 0.05 M of 1,10-phenanthroline in ethanol. The mixture was incubated for 30 min using a water bath at the temperature of 70°C. Absorbance of red colored complex developed was read at 510 nm against reagent blank. Alkaloid content was expressed as microgram (µg) equivalence of quinine/milliliter (mL) of extract.

Animals and treatments

Thirty-five adult male albino *Wistar* rats weighing between 150 and 220 g were obtained from the Laboratory of Biochemistry, University of Yaoundé I. The study protocol was approved by the institutional review board of the University of Yaoundé. Animals were housed in clean and dry cages, ad libitum. They were fed with a standard diet and normal tap water under standard laboratory conditions (room temperature with dark/light cycle 12/12 h). They were fed with a standard diet and normal tap water ad libitum.

Study of antihyperglycemic activity: Oral glucose tolerance test

Oral glucose tolerance test (OGTT), an *in vivo* acute test was conducted as described by Al-Malki [28], to compare the effect of combination (200 mg/kg b.w HEE *Pa* + 200 mg/kg b.w. AE *Pa*), to individual constituent HEE *Pa* and AE *A.s*, in preventing the absorption and the rise of glycemia. After 12 h overnight fasting, blood glucose at baseline (T0) was measured from the tails of all the rats using a glucometer and test strips (One touch Gluco-plus). The rats were randomly distributed into the following groups:

- **Group 1**: Positive control (PC) group was given water orally.
- **Group 2**: Aqueous extract of *Allium sativum* (AEAs) (400 mg/kg body weight).
- **Group 3**: Hydroethanolic extract of *Allium sativum* (HEEAs) (400 mg/kg b.w.).
- **Group 4**: Aqueous extract of *Persea americana* seeds (AEPs) (400 mg/kg b.w.).
- **Group 5**: Hydroethanolic extract of *Persea americana* seeds (HEEPA) (400 mg/kg b.w.).
- **Group 6**: Glibenclamide (GB, 4 mg/kg b.w).
- **Group 7**: Formulation 400 mg/kg b. w. (combination 200 mg AE of *A. sativum* and 200 mg of HEE of *P. americana* seeds). The combination included the poorer (HEE of *P. americana*) extract and the most efficacious (AE of *A. sativum*) extract. Thirty (30) minutes after administration of extracts, animals in all the groups were given an overload glucose solution (2 g/kg body weight). Blood glucose levels were determined with a glucometer by tail pricking at 30, 60, 90, and 120 min after glucose loading. Administration of extracts and glucose was done orally in water 5 mL/Kg body weight.

Results

Determination of alkaloids content of different plant extracts

It was observed that the extract obtained from a mixture of water/ethanol (1:1) contains higher amount of alkaloids than water alone. In addition, HEE of *Pa* seeds contains eight folds of alkaloids than HEE of *A.s* (Table 1).

<table>
<thead>
<tr>
<th>Materials</th>
<th>Aqueous extract</th>
<th>Hydroethanolic extract</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Allium sativum</em></td>
<td>1.10 ± 0.04$^a$</td>
<td>1.92 ± 0.13$^b$</td>
</tr>
<tr>
<td><em>Persea americana</em> seeds</td>
<td>9.77 ± 0.25$^c$</td>
<td>15.36 ± 0.17$^d$</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SD. Alkaloids are expressed in equivalence of quinine. For each plant (lines) and each solvent (column), variables with different letters a, b, c, and d associated are significantly different at $p < 0.05$.

Table 1: Alkaloid content of extracts
Glucose lowering potential of *Allium sativum* extracts on normoglycemic rats

It was observed that there was an increase of blood glucose ($p < 0.05$) in all the groups in the first 30 min of the experiment, followed by a decrease in all the groups (Figure 1).

Following adjustment from baseline (Figure 2), it was observed that, during the first 30 min, the PC group receiving (water + glucose) only had a similar profile with HEE of *A.s*: 70% increase from the baseline. Thereafter, blood glucose dropped for 40% in control (PC), while in HEE As400 group, the BG level remains higher than in all including the group receiving glibenclamide, a reference drug. Glibenclamide group (GB 3 mg/kg) showed the lowest increase all through the experiment with a regulation to baseline after 82 min. The efficacy of AE As400 in lowering BG was closely similar to glibenclamide ($p > 0.05$), making it the more efficacious extract to be used in the combination.

Glucose lowering potential of *Persea americana* seed extract on normoglycemic rats

It clearly appears that glibenclamide can prevent the increase in glucose absorption 30 min after administration, better than AE and HEE of *P. americana*. After 60 min, it was observed that all the tested groups exhibit blood glucose higher than reference and control groups. Only glibenclamide could regulate glucose increase after 80 min. HEE Pa400 was poorer in reducing blood glucose, making it part of the combination to be tested in Group 7 (Figure 3).

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Figure 1: Effect of *Allium sativum* extracts on glucose absorption

Figure 2: Effect of *Allium sativum* extracts on postprandial glucose adjusted from baseline
Effect of formulation (AE As200 + HEE Pa200 combination) of extracts on OGTT

Given that Pa and As extracts have been traditionally used to treat diabetes and other metabolic disorders, we then studied the effect of the most hyperglycemic extract of HEEPa and the most efficient or lowering extract of Allium sativum (AEAs).

Results of Figure 4 revealed that there is a glycemic peak in all the groups after 30 min with the highest peak associated with the positive control group (71 mg/dL, \( p > 0.05 \)), the lowest peak associated with the reference group (21 mg/dL), and the group receiving the AE of A. sativum having the lowest peak (27 mg/dL) among the test groups. At time 60 min, the combination is observed to have the lowest peak (16 mg/dL), thus challenging the reference group. From time 60 min till the end of the experiment, both the reference group and the group receiving AE of A. sativum have peaks lower than the positive control; meanwhile, the HEE of P. americana has a peak higher than that of the positive control. In addition, it is only after 90 min that the combination...
Effect of combination of extracts on sucrose digestion

Thirty minutes after oral overload of sucrose solution, the blood glucose level in all the groups attained a peak. Then, the level progressively slows down over time (Figure 6). However, the highest peak was observed with the group receiving the combination.

Effect of extracts on glucose absorption

It clearly appears that AE As200 potentiates the efficacy of HEE Pa200.

Efficacy of extracts on postprandial glycemia

From the above graphs, AUC, which provides a better expression of the efficacy of the extracts, was computed. Moreover, the result reveals that the combination has a lower AUC value compared to the positive control and the group receiving the HEE of *P. americana*. Thus, it is confirmed that, in the formulation, AE As potentiates or improves the effect of HEEPa by lowering the AUC value of the HEE of *P. americana*, although not comparable to the reference drug, glibenclamide (Figure 3).

### Figure 5: Efficacy of extracts on glucose absorption

Bar graphs with different letters a, b, c, and d associated are significantly different at \( p < 0.05 \).

### Figure 6: Glycemic increment after administration of sucrose adjusted from baseline

PC: positive control, acarbose (reference drug), A.s: *Allium sativum*, P.a: *Persea americana*, combination (AE of *A. sativum* and HEE of *P. americana* seed). Variables with different letters a, b, and c associated at each time are significantly different at \( p < 0.05 \).

Avocado seeds have been considered for long time as waste products because of its high content of antinutrient constituents such as tannins, phytic acid, and alkaloids [36]. This study demonstrated that P. americana is very rich in alkaloids compared to A. sativum (Table 1). Alkaloids are known to enhance glucose-induced insulin [4] to induce relatively high glucose uptake and to possess good antioxidant potential at low dosages [37]. They could also activate PI3K/Akt and suppress PTP1B, protein tyrosine phosphatase, thereby promoting the synthesis of glycogen from glucose [38]. This can justify why aqueous extract AEPa400 was more efficient in reducing sugar than HEE Pa400 compared to positive control after 60 min (Figure 4). Previous studies on acute administration of pear leaves’ extracts showed that effective time for glucose level regulation is only at 6 h after a single dose of the extract was administered, producing 60.02 ± 6.83% reduction in blood glucose level [25]. This could explain why in this study after 2 h the capacity of P. americana extracts to reduce hyper-postprandial glycaemia (HPPG) remains relatively low. The moderate activity of pear seeds can be attributed to bioactive molecules belonging to triterpenoids, tannins, flavonoids, saponins, and polyphenols [32,39,40]. Polyphenols like smeathxanthone A from Garcinia smeathmanii [41] and bioactive molecules’ extracts from Nypa fruticans extracts [42] have been used to justify glucose lowering potentials.

Plant formulation involved combinations of extracts preparation for polyherbal therapy. It offers the advantage to have diverse pharmacological principles, capable to produce maximum therapeutic efficacy with minimum side effects [11,12].

In this study, formulation based on HEE Pa400 with the poorer efficacy (Figure 3) and AE As400 As with best efficacy showed that AEs were capable to improve HEEPα efficacy after 2 h (Figure 4) instead of 6 h as reported with leaves. Thus, the combination of extracts ameliorates the activity of the extract of P. americana seeds, which alone showed only dampening effect on blood glucose level after 2 h. AEs200 potentiates the efficacy of HEE Pa200 (Figure 5). Such improvement in efficacy function of time can prevent crisis often observed with patients like comas [43]. A combination A.s + Pa can better regulate high blood sugar than HEE Pa and reduce complications mediated by free radicals such as cardiovascular disease, retinopathy, nephropathy, neuropathy,
leg ulcers, and gangrene [7,44,45]. A. sativum may improve the efficacy of Pa through complexity of bioactive molecules in the mixture on reducing free radicals in the pancreas, activation of β-cells in the pancreas for insulin secretion [3], and increase the rate of glucose clearance from the blood stream, thereby reducing the rise in postprandial glycemia [4,46]. Many formulations of herbal extracts with improved efficacy on hyperglycemia have been previously reported with synergistic effects [13,47]. Other formulations between pure or herbal/pure molecules have been reported. These include metformin and rosiglitazone [47,48] and garlic and metformin [14].

Given the formulation based on (AEAs200 + HEE Pa200) potentiates the effects of HEEPa on postprandial glucose (Figure 5), there should be awareness when such formulation interacts with sucrose, a disaccharide found in diverse meals. Neither P. americana nor A. sativum extract could inhibit sucrose with efficacy comparable to acarbose (Figures 6 and 7). Instead, combination (1:1) of both showed activation of sucrose. Sucre binds to the active site on sucrase, and this puts stress on the bond between the two sugars that make up sucrose. The bond breaks, thereby releasing glucose and fructose. The activation of enzyme by the combination contradicts the study by Malunga et al. [1] in which inhibition of digestive enzyme constitutes a therapeutic approach. Increased activity of sucrose triggered by combination can be due to the change brought in pH environment of Gastro-intestinal tract (GIT), promoting ion protonation or stimulating alkaline cations [49]. Such sucrose activation can be very dangerous for diabetic in critical situation such as comas. The use of formulations for diabetes treatment is on high demand [14,50]. However, care on good posology should then be taken when using polyherbal formulations.

Conclusion

Postprandial hyperglycemia remains a therapeutic target of interest in the management of DM and its associated complications. A. sativum at the dose 400 mg/kg b.w. lowered postprandial glucose better than HEE of Pa at 400 mg/kg b.w. In a formulation from the two plants (AEAs200 + HEE Pa400), A. sativum improves glucose lowering capacity of Pa seeds. However, the formulation instead activates sucrose digestion.

Authors’ Declaration

There is no conflict of interest in this work.

Authors’ Contributions

BGKA conceived, designed, and supervised the study and drafted the manuscript; WTK, GNT, EAT, and VMT prepared extracts and analyzed the data; JLN and JO supervised the work and corrected the manuscript.

References