Original Article

Anti-motility and reductions in the concentrations of gut electrolytes: Bases for the anti-spastic use of the leaves of Persea americana in folk medicine

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Abstract
Background and Aim: The leaves of Persea americana are used in traditional medicine to treat, allay or prevent some spasm-related disorders, for instance, diarrhoea. The study was aimed at providing the basis for the anti-spastic use of the leaves of P. americana in folk medicine.

Methods: The chloroform and methanol fractions of the chloroform—methanol extract of the leaves of P. americana were investigated for their qualitative and quantitative phytochemical constituents as well as effects on gastro-intestinal motility (transit) and castor oil-induced intestinal fluid sodium ion (Na⁺) and potassium ion (K⁺) concentrations.

Results: The qualitative and quantitative phytochemical studies of the chloroform and methanol fractions showed the presence and amounts of alkaloids (2.67 ± 0.13% and 2.57 ± 0.06% respectively), flavonoids (3.20 ± 0.17% and 2.95 ± 0.14% respectively), saponins (2.15 ± 0.08% and 2.23 ± 0.09% respectively), tannins (2.48 ± 0.11% and 2.73 ± 0.13% respectively), steroids (1.36 ± 0.04% and 1.10 ± 0.03% respectively), terpenoids, proteins and carbohydrates in both fractions. Fats and oil were present only in the chloroform fraction. At the two doses (100 and 200 mg/kg body weight), the chloroform and methanol fractions produced significant (p < 0.05) and dose-related decreases in the gastro-intestinal motility and concentration of the intestinal fluid potassium ions but only the chloroform fraction at the dose of 200 mg/kg body weight significantly (p < 0.05) decreased the concentration of the intestinal fluid sodium ions. Results of the fractions were comparable with those of the standard anti-diarrhoeal drug, hyoscine butylbromide (3 mg/kg body weight).

Conclusion: The results indicate that the chloroform—methanol extract of the leaves of P. americana contains compounds with anti-spastic effect.

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1. Introduction

Diarrhoea is characterised by increased frequency of bowel movement, wet stool and abdominal pain.1 Diarrhoea remains one of the commonest illnesses of children and one of the major causes of infant and childhood mortality in developing countries. It is estimated that 3.3 million deaths occur each year among children under five-year-old. In Nigeria, diarrhoea infection remains the number one killer disease among children under the age of five, while 7–12 month old babies remain the most susceptible.2 Nigeria, the fourth largest economy in Africa with an estimated per capita income of $350 has over half of its population living in poverty. This implies that not very many persons can afford orthodox medicine in curing diseases. In addition, many synthetic chemicals like diphenoxylate, loperamide and antibiotics are available for the treatment of diarrhoea but they have some side effects. Also, the natural drugs are used as anti-diarrhoeal drugs which are not always free from adverse effects. Thus, the search for safe and more effective agents has continued to be a vital area of active research. Since ancient times, diarrhoea has been treated orally with several medicinal plants or their extracts based on folklore medicine.

**Persea americana** (avocado or alligator pear) is an almost evergreen tree belonging to the laurel family Lauraceae. It is indigenous to Central and South America but is now cultivated in the United States, Asia, parts of Europe and tropical Africa. The plant is a tall evergreen tree that can grow up to 65 feet in height. The leaves are alternate, dark green and glossy on the upper surface, whitish on the underside; variable in shape (lanceolate, elliptic, oval, ovate or obovate) and 7.5─40 cm long. The fruit of **P. americana** Mill is eaten in many parts of the world. In recent years, researches have focused on various parts of the plants.3 It is alleged to stimulate and regulate menstruation. The leaf decoction is taken as a remedy for diarrhoea, sore throat and haemorrhage.4 In this study, I report the phytochemical and anti-spastic activity of the chloroform—methanol extract of the leaves of **P. americana** in normal and castor oil-induced diarrhoeal rats.

2. Materials and methods

2.1 Plant

Fresh leaves of **P. americana** were got from their trees at various points in Iheapku-Awka, Igbo Eze South Local Government Area of Enugu State, Nigeria. The leaves were identified by Mr. A. Ozioko of Bioresource Development and Conservation Programme (BDCP) Research Centre, Nsukka.

2.2 Preparation of the extract

Fresh leaves of **P. americana** were plucked and washed with distilled water. The leaves were spread on a clean mat in a well-ventilated room with regular turning to enhance even drying and avoid decaying. The leaves were shade-dried for 3 weeks. The shade-dried leaves were pulverised with an electric blender and a known weight (1380 g) of the pulverised **P. americana** leaves was macerated in 5 volumes (w/v) of chloroform—methanol (2:1) for 24 h. The mixture was separated with Whatman No 1 filter paper. The filtrate of the macerate was shaken with distilled water that measured 20 percent its volume to obtain two (2) fractions. The upper fraction (methanol fraction) was separated from the lower fraction (chloroform fraction). The methanol and the chloroform fractions were concentrated in a rotary evaporator, dried in a boiling water bath and weighed.

2.3 Phytochemical analyses

Qualitative phytochemical analyses were carried out on both the methanol and the chloroform fractions according to the procedures outlined by.5,6 Quantitative phytochemical analyses were carried out to determine the concentration of the following: alkaloids and flavonoids5; saponins7; tannins8 and steroids.9

2.4 Animals

Adult male Wistar rats of between 8 and 12 weeks old with average weight of 125 ± 25 g were obtained from the Animal house of the Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka. The rats were acclimatised for one week under a standard environmental condition with a 12 h light and dark cycle and maintained on a regular feed and water ad libitum. The Principles of Laboratory Animal Care were followed. The University Animal Research Ethical Committee approved the experimental protocol used.

2.5 Chemicals and reagents

The chemicals used for this study were of analytical grade and procured from reputable scientific shops at Nsukka. They included the following: hyoscine butylbromide [standard anti-diarrhoeal drug (Sigma—Aldrich, Inc., St. Louis, USA)], methanol and chloroform (both supplied by BDH Chemicals Ltd., Poole, England), 45% (v/v) ethanol (BDH Chemicals Ltd., Poole, England), dilute tetraoxosulphate(vi) acid, 2% (v/v) hydrochloric acid, 1% (w/v) picric acid, methyl orange, activated charcoal, gum acacia, castor oil (laxative) and 3% (v/v) Tween 80 (vehicle for dissolving the extract), Dragendoff’s reagent, Wagner’s reagent, Fehling’s solution, 5% (w/v) ferric chloride solution, aluminium chloride solution, lead sub acetate solution, ammonium solution, Molisch’s reagent, filtrate reagent, acid reagent, sodium colour reagent, sodium standard, potassium reagent and potassium standard.

2.6 Gastro-intestinal motility test

Gastro-intestinal motility was evaluated using the method of10 with slight modification.

2.7 Determination of the concentrations of sodium and potassium ions

The concentrations of sodium and potassium ions were determined by the method of11.
2.8. Statistical analysis

The data obtained from the laboratory results of the tests were subjected to One Way Analysis of Variance (ANOVA). Significant differences were observed at \( p \leq 0.05 \). The results were expressed as means of five replicates \( \pm \) standard deviations (SD). This analysis was done using the computer software known as Statistical Package for Social Sciences (SPSS), version 16.

3. Results

3.1. Qualitative phytochemical composition of the chloroform and the methanol fractions

The qualitative phytochemical analyses showed, the presence of saponins in very high concentration in both fractions of the chloroform–methanol extract of the leaves of \( P. \) americana (Table 1). Flavonoids were found to be present in very high concentration and moderately high concentration in the chloroform and the methanol fractions respectively. Tannins, terpenoids and steroids were found to be present in moderately high concentrations in both fractions as shown in Table 1.

3.2. Quantitative phytochemical composition of the chloroform and the methanol fractions

The phytochemical constituents of the chloroform and the methanol fractions of the chloroform–methanol extract of the leaves of \( P. \) americana are summarised in Table 2. The chloroform fraction of the extract contained higher percentages of alkaloids (2.67 \( \pm \) 0.13\%), flavonoids (3.20 \( \pm \) 0.17\%) and steroids (1.36 \( \pm \) 0.04\%) than the methanol fraction while the methanol fraction contained higher percentages of saponins (2.15 \( \pm \) 0.08\%) and tannins (2.48 \( \pm \) 0.11\%) than the chloroform fraction (Table 2).

<table>
<thead>
<tr>
<th>Phytochemical constituents</th>
<th>Chloroform fraction</th>
<th>Methanol fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>Glycosides</td>
<td>ND</td>
<td>+</td>
</tr>
<tr>
<td>Proteins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Reducing sugars</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Saponins</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Fats and oil</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td>Resins</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Acidic compounds</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND = not detected; + = present in low concentration; ++ = present in moderately high concentration; +++ = present in very high concentration.

Table 2 – Percentages of alkaloids, flavonoids, saponins, tannins and steroids in the chloroform and the methanol fractions.

<table>
<thead>
<tr>
<th>Phytochemical constituents</th>
<th>Chloroform fraction</th>
<th>Methanol fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>2.67 ( \pm ) 0.13</td>
<td>2.57 ( \pm ) 0.06</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>3.20 ( \pm ) 0.17</td>
<td>2.95 ( \pm ) 0.14</td>
</tr>
<tr>
<td>Saponins</td>
<td>2.15 ( \pm ) 0.08</td>
<td>2.23 ( \pm ) 0.09</td>
</tr>
<tr>
<td>Tannins</td>
<td>2.48 ( \pm ) 0.11</td>
<td>2.73 ( \pm ) 0.13</td>
</tr>
<tr>
<td>Steroids</td>
<td>1.36 ( \pm ) 0.04</td>
<td>1.10 ( \pm ) 0.03</td>
</tr>
</tbody>
</table>

Values are expressed as means of five determinations \( \pm \) SD.

3.3. Effects of the methanol and the chloroform fractions on gastro-intestinal motility

The charcoal meal (gastro-intestinal motility) test was used to determine the propulsive movement along the gastro-intestinal tract (GIT) of rats. As shown in Fig. 1, the methanol and the chloroform fractions of the extract at the tested doses (100 and 200 mg/kg body weight of each) significantly \( (p < 0.05) \) decreased the percentage distance travelled by the charcoal meal along the gastro-intestinal tract of rats in groups 4, 5, 6 and 7 when compared to the value obtained for rats in the charcoal meal-treated control group (group 2). The observed effects were dose-dependent with percentage distance travelled by charcoal meal as 62.25 \( \pm \) 4.57, 57.25 \( \pm \) 1.50, 58.25 \( \pm \) 2.22 and 35.25 \( \pm \) 2.36 for rats in the 100 and 200 mg/kg body weight of the methanol fraction-treated groups (groups 4 and 5), 100 and 200 mg/kg body weight of the chloroform fraction-treated groups (groups 6 and 7) respectively when compared to the value \( (70.25 \pm 3.30) \) obtained for rats in the charcoal meal-treated control group (group 2). The effects of the methanol and the chloroform fractions of the extract at the tested doses were comparable to that of the standard anti-diarrhoeal agent (hyoscine butylbromide) as shown in Fig. 1.

3.4. Effects of the methanol and the chloroform fractions on the intestinal fluid sodium ion \( (Na^+) \) concentration

Result of the intestinal fluid sodium ion concentration test as shown in Fig. 2, shows that the rats of the castor oil-treated control group (group 2) had significantly \( (p < 0.05) \) increased intestinal fluid sodium ion concentration \( (227.00 \pm 3.46) \) when compared to the value \( (192.75 \pm 11.62) \) obtained for rats in the group that received only the vehicle (group 1). The chloroform fraction of the extract at the dose of 200 mg/kg body weight, like the standard anti-diarrhoeal agent (hyoscine butylbromide), caused a significant \( (p < 0.05) \) reduction in the intestinal fluid sodium ion concentration of rats in group 7 \( (209.00 \pm 11.40) \) when compared to the value \( (227.00 \pm 3.46) \) obtained for rats in the castor oil-treated control group.

3.5. Effects of the methanol and the chloroform fractions on the intestinal fluid potassium ion \( (K^+) \) concentration

As shown in Fig. 3, the methanol and the chloroform fractions of the extract at the tested doses (100 and 200 mg/kg body weight of each) significantly \( (p < 0.05) \) reduced the intestinal fluid...
potassium ion concentration of rats in groups 4, 5, 6 and 7 when compared to that of the rats in the castor oil-treated control group (group 2). The effects observed were dose-related with the intestinal fluid potassium ion concentration as $6.15/C6^{1.75}$, $6.20/C6^{1.70}$, $6.20/C6^{1.23}$ and $5.65/C6^{1.05}$ for rats in the 100 and 200 mg/kg body weight of the methanol fraction-treated groups (groups 4 and 5), 100 and 200 mg/kg body weight of the chloroform fraction-treated groups (groups 6 and 7) respectively when compared to the value ($11.40/C6^{2.98}$) obtained for rats in the castor oil-treated control group. The effects of the methanol and the chloroform fractions of the extract at the tested doses were comparable to that of the standard anti-diarrhoeal agent (hyoscine butylbromide) as shown in Fig. 3.

4. Discussion

The results of the qualitative and quantitative phytochemical analyses of the chloroform and the methanol fractions of the chloroform–methanol extract of the leaves of P. americana showed, in both fractions of the extract, the presence and percentages of such bioactive constituents as: alkaloids ($2.67/C6^{0.13}$% and $2.57/C6^{0.06}$% in the chloroform and the methanol fractions respectively), flavonoids ($3.20/C6^{0.17}$% and $2.95/C6^{0.14}$% in the chloroform and the methanol fractions respectively), saponins ($2.15/C6^{0.08}$% and $2.23/C6^{0.09}$% in the chloroform and the methanol fractions respectively), tannins ($2.48/C6^{0.11}$% and $2.73/C6^{0.13}$% in the chloroform and the methanol fractions respectively) and steroids ($1.37/C6^{0.04}$% and $1.10/C6^{0.03}$% in the chloroform and the methanol fractions respectively). This indicates that the bioactive constituents present in the chloroform–methanol extract of the leaves of P. americana resided more in the chloroform fraction than in the methanol fraction. Reducing sugars, resins and acidic compounds were found to be absent in both fractions of the extract. The anti-diarrhoeal effect of both fractions of the extract
diarrhoea and may also enhance intestinal absorption of sodium ion (Na⁺) and water.14

Anti-motility along the gastro-intestinal tract (GIT) was demonstrated by both fractions of the chloroform–methanol extract of the leaves of *P. americana* as there was dose-dependent reduction in the percentage distance travelled by the charcoal meal along the GIT in the charcoal meal-treated rats. Pre-treatment with both fractions of the extract suppressed the propulsive movement of the charcoal meal as observed by the decrease in the motility of charcoal meal along the GIT. Suppression of the propulsive movement of the charcoal meal along the GIT by both fractions of the extract at least, in part, indicates an anti-diarrhoeal effect of the leaves of *P. americana*. This might be indicative of the likely ability of both fractions of the extract to reduce peristaltic activity and ultimately bring about a reduction in the gastro-intestinal motility. Decrease in intestinal motility might have led to increased re-absorption of water and electrolytes from faeces and additionally, might have contributed to the reduction in the watery texture of the faeces. It is also possible that both fractions of the extract suppressed the propulsive movement of the charcoal meal along the GIT by anti-cholinergic mechanism in a manner similar to the action of the standard anti-diarrhoeal drug, hyoscine butylbromide. This is in consonance with the finding of2 who reported that anti-diarrhoeal agents increase intestinal transit time by anti-cholinergic effect.

Study of the effects of both fractions of the chloroform–methanol extract of the leaves of *P. americana* on intestinal fluid sodium ion (Na⁺) and potassium ion (K⁺) concentrations showed that both fractions of the extract markedly and dose-dependently caused reductions in the concentrations of these electrolytes. These observed effects in part, imply that the leaves of *P. americana* possess anti-diarrhoeal effect. The anti-diarrhoeal effect evidenced here, might be due to the fact that both fractions of the extract probably enhanced the absorption of the electrolytes from the intestinal lumen, while suppressing the rate of their secretion into the small intestine. It has been shown that castor oil causes motility and secretory diarrhoea. This is achieved through its (castor oil) dual effects on gastro-intestinal motility and water.14 While the flavonoids are known to inhibit intestinal hyper-motility and hydroelectrolytic secretion, tannins denature proteins in the intestinal mucosa by forming protein tannates which make intestinal mucosa more resistant to chemical alteration and reduce secretion. Also, extracts of plants that contain flavonoids2 are known to modify the production of cyclo-oxygenase 1 and 2 (COX-1 and COX-2) and lipo-oxygenase (LOX) thereby inhibiting the production of prostaglandins.13 Steroids are also useful for the treatment of diarrhoea and may also enhance intestinal absorption of sodium ion (Na⁺) and water.14

Fig. 3 — Effects of the methanol and chloroform fractions of the chloroform–methanol leaf extract of *Persea americana* on intestinal potassium ion (K⁺) concentration (Data represented as means ± SD; * = significantly (p < 0.05) lower compared to that of group 2). Group 1: 5 ml/kg body weight (b.w) of 3% v/v tween 80 (vehicle). Group 2: Vehicle + 1 ml of castor oil (CO). Group 3: 3 mg/kg b.w of hyoscine butylbromide + 1 ml of CO. Group 4: 100 mg/kg b.w of methanol fraction of *P. americana* + 1 ml of CO. Group 5: 200 mg/kg b.w of methanol fraction of *P. americana* + 1 ml of CO. Group 6: 100 mg/kg b.w of chloroform fraction of *P. americana* + 1 ml of CO. Group 7: 200 mg/kg b.w of chloroform fraction of *P. americana* + 1 ml of CO.

In conclusion, the present experimental findings thus, justify the use of the leaves of *P. americana* as an anti-spastic agent by the traditional medicine practitioners.

**Conflicts of interest**

The author has none to declare.

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