

Full Length Research Paper

Anti-motility and reductions in the concentrations of gut electrolytes: Mechanisms for the anti-spasmodic use of the seeds of avocado (*Persea americana* Mill) in folk medicine

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The seeds of avocado (*Persea americana* Mill) are used in traditional medicine to treat, allay or prevent some spasm-related disorders, for instance, diarrhoea. The chloroform and methanol fractions of the chloroform-methanol extract of the seeds 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 in Wistar rats. The qualitative and quantitative phytochemical studies of the chloroform and methanol fractions showed the presence and amounts of alkaloids (2.92 ± 0.14 g/100 g and 2.81 ± 0.08 g/100 g respectively), flavonoids (3.43 ± 0.19 g/100 g and 3.11 ± 0.16 g/100 g, respectively), tannins (2.64 ± 0.13 g/100 g and 2.85 ± 0.14 g/100 g, respectively), steroids (1.51 ± 0.07 g/100 g and 1.27 ± 0.04 g/100 g, respectively), saponins (2.35 ± 0.08 % and 2.47 ± 0.09%, 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). The results indicate that the chloroform-methanol extract of the seeds of *P. americana* contains compounds with anti-spasmodic effect.

Key words: *Persea americana*, spasm-related, castor oil, gastro-intestinal motility and electrolytes.

INTRODUCTION

Diarrhoea is characterised by increased frequency of bowel movement, wet stool and abdominal pain (Nitinkumar et al., 2010). 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, diarrhoeal infection remains the number one killer disease

among children under the age of five, while 7-12 month old babies remain the most susceptible (Sule et al., 2009). Nigeria, the fourth largest economy in Africa with an estimated per capita income of \$350 has over half of its population living in poverty (WHO, 2007). 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 (Nitinkumar et al., 2010). 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) is an evergreen tree belonging to the laurel family, Lauraceae. Various parts of the plant have been shown to possess medicinal properties. The edible fruit pulp contains up to 33% oil rich in monounsaturated fatty acids that are believed to modify the fatty acid contents in cardiac and renal membranes and enhance the absorption of alpha/beta-carotene and lutein (Salazar et al., 2005). The carotenoid content has been reported to play significant role in cancer risk reduction (Lu et al., 2005). Other properties of the oil include wound-healing and hepatoprotection (Nayak et al., 2008). The aqueous leaf extract has analgesic and anti-inflammatory activities (Adeyemi et al., 2002). The seeds of *P. americana* have diverse applications in ethno-medicine, ranging from treatment for diarrhoea, dysentery, toothache, intestinal parasites, skin treatment and beautification (Alhassan et al., 2012). In this study, we report the phytochemical and anti-spasmodic activity of the chloroform-methanol extract of the seeds of *P. americana* in normal and castor oil-induced diarrhoeal rats.

MATERIALS AND METHODS

Plant

Fresh fruit of *P. americana* were harvested from trees at various points in Iheapku-Awka, Igbo Eze South Local Government Area of Enugu State, Nigeria. The fruit seeds were identified by Prof. (Mrs.) May Nwosu of the Department of Botany, University of Nigeria, Nsukka.

Preparation of the extract

The fresh fruit were split open with a knife and the seeds removed. The seeds were washed with distilled water and sliced with knife. The sliced seeds were spread on a clean mat in a well-ventilated room with regular turning to enhance even drying and avoid decaying. The sliced seeds were shade-dried for 8 weeks. The shade-dried sliced seeds were milled with an electric blender and 1380 g of the milled seeds 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% its volume to obtain two 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.

Phytochemical analyses

Qualitative phytochemical analyses were carried out on both the methanol and the chloroform fractions according to the procedures outlined by Harborne (1998) and Trease and Evans (1989). Quantitative phytochemical analyses were carried out to determine the concentration of the following: alkaloids and flavonoids by the methods of Harborne (1998); tannins by the method of Swain (1979); steroids by the method of Okeke and Elekwa (2003) and saponins by the method of Obadoni and Ochuko (2001).

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 adhered to. The experimental protocol was approved by the University Animal Research Ethical Committee.

Chemicals and reagents

The chemicals used for this study were of analytical grade. They included the following: hyoscine butylbromide [standard anti-diarrhoeal drug (Sigma-Aldrich, Inc., St. Louis, USA)], methanol and chloroform, 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), 3% (v/v) tween 80, Dragendorff's reagent, Mayer's reagent, Wagner's reagent, Millon's reagent, Fehling's solution, 5% (w/v) ferric chloride solution, aluminium chloride solution, lead subacetate solution, ammonium solution, Molisch's reagent, filtrate reagent, acid reagent, sodium colour reagent, sodium standard, potassium reagent and potassium standard.

Gastro-intestinal motility test

Gastro-intestinal motility was evaluated using the method of Mascolo et al. (1994) with little modification.

Determination of the concentrations of sodium and potassium ions

The concentrations of sodium and potassium ions were determined by the method of Tietz (1994).

Statistical analysis

The data obtained from the laboratory 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). Analysis was performed using Statistical Package for Social Sciences (SPSS), version 16.

Table 1. Qualitative phytochemical constituents of the chloroform and the methanol fractions.

Phytochemical constituent	Chloroform fraction	Methanol fraction
Alkaloids	++	+
Flavonoids	+++	++
Glycosides	ND	+
Proteins	+	+
Carbohydrates	+	+
Reducing sugars	ND	ND
Saponins	+++	+++
Tannins	+++	++
Fats and oil	+	ND
Resins	ND	ND
Terpenoids	++	++
Steroids	++	++
Acidic compounds	ND	ND

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

Table 2. Amounts of alkaloids, flavonoids, tannins, steroids and saponins in the chloroform and the methanol fractions of *P.americana* seeds.

Phytochemical constituent	Chloroform fraction	Methanol fraction
Alkaloids (g/100 g)	2.92 ± 0.14	2.81 ± 0.08
Flavonoids (g/100 g)	3.43 ± 0.19	3.11 ± 0.16
Tannins (g/100 g)	2.85 ± 0.14	2.64 ± 0.13
Steroids (g/100 g)	1.51 ± 0.07	1.27 ± 0.04
Saponins (%)	2.35 ± 0.08	2.47 ± 0.09

Values are expressed as means of five determinations ± SD.

RESULTS

The qualitative and quantitative phytochemical composition of the chloroform and the methanol fractions

The chloroform fraction of the extract contained higher amounts of alkaloids (2.92 ± 0.14 g/100 g), flavonoids (3.43 ± 0.19 g/100 g), tannins (2.85 ± 0.14 g/100 g) and steroids (1.51 ± 0.07 g/100 g) than the methanol fraction while the methanol fraction contained higher amount of saponins (2.47 ± 0.09 %) than the chloroform fraction (Tables 1 and 2).

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 Figure 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 60.25 ± 1.37, 55.25 ± 1.50, 56.25 ± 1.61 and 33.25 ± 1.75 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 (72.75 ± 1.98) 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 Figure 1.

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

Result of the intestinal fluid sodium ion concentration test as shown in Figure 2, shows that the rats of the castor

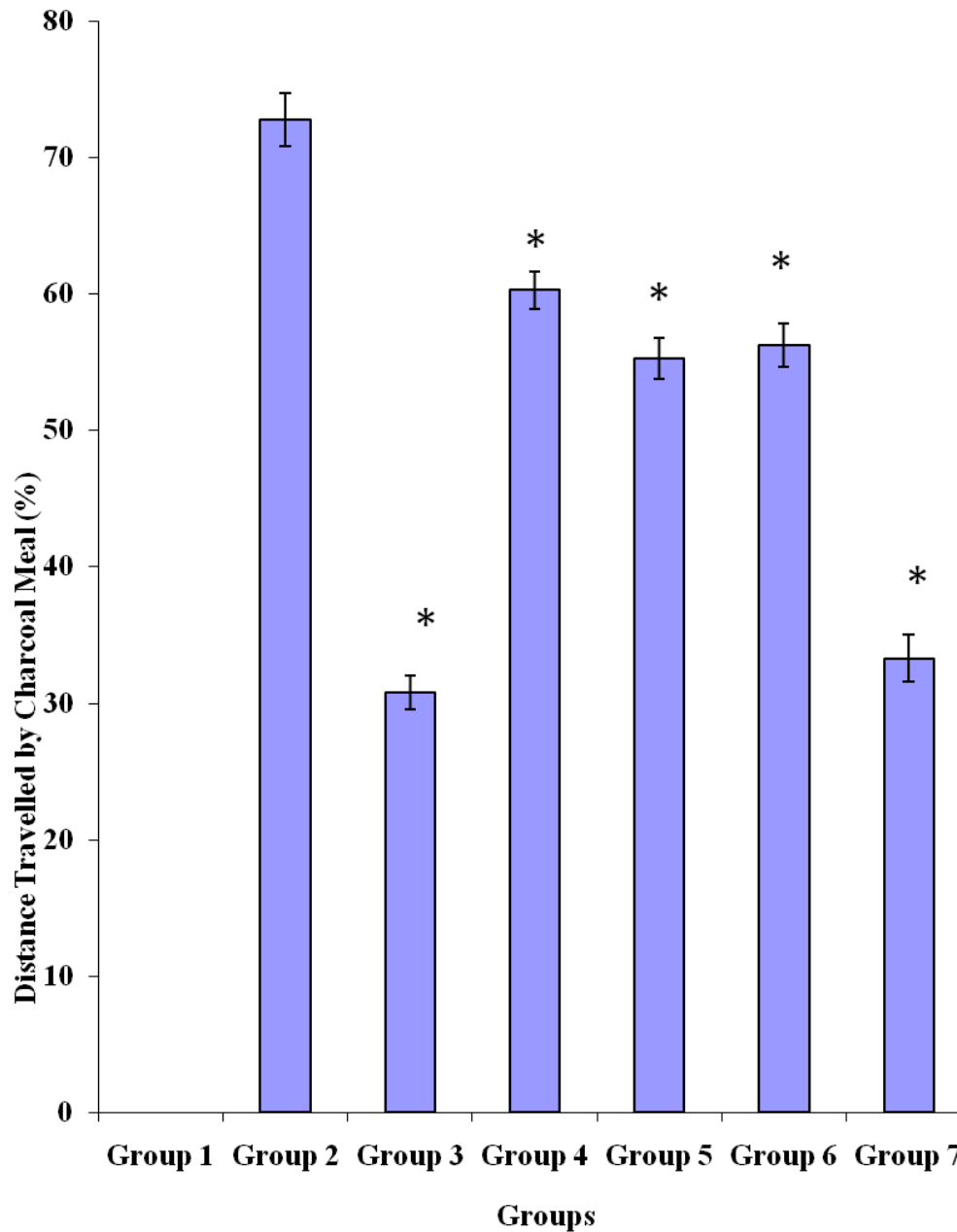


Figure 1. Effects of the methanol and the chloroform fractions of the chloroform-methanol seed extract of *Persea americana* on the gastro-intestinal motility. Data represented as mean \pm SD; *, significantly ($p < 0.05$) lower compared to that of group 2). Group 1, 5 ml/kg b.w of 3% v/v tween 80 (vehicle); group 2, vehicle + 1 ml of castor oil (CO); group 3, 3 mg/kg b.w of hyoscine + 1ml of CO; group 4=100mg/kg b.w of methanol fraction of *P. americana* +1ml 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 chloroform fraction of *P. americana* +1 ml of CO.

oil-treated control group (group 2) had significantly ($p < 0.05$) increased intestinal fluid sodium ion concentration (236.00 ± 5.79) when compared to the value (192.75 ± 4.82) 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 (201.00 ± 4.90) when compared to the value (236.00 ± 5.79) obtained for rats in the castor oil-treated control group.

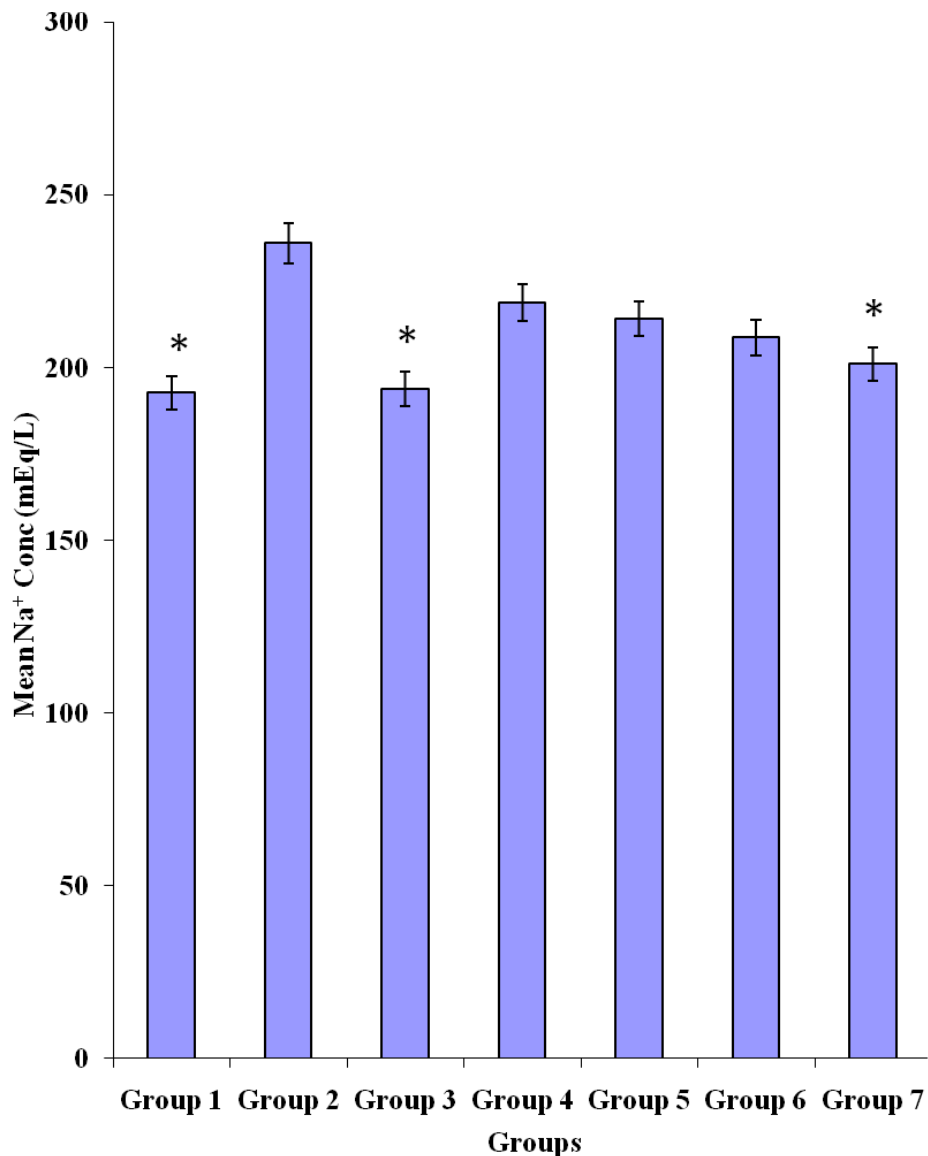


Figure 2. Effects of the methanol and chloroform fractions of the chloroform-methanol seed extract of *Persea americana* on intestinal sodium ion (Na⁺) concentration. Data represented as mean \pm SD; *, significantly ($p < 0.05$) lower compared to that of group 2. Group 1, 5 ml/kg b.w of 3% v/v tween 80 (vehicle); group 2, vehicle + 1 ml of castor oil (CO); group 3, 3 mg/kg b.w of hyoscine + 1 ml of CO; group 4=100mg/kg b.w of methanol fraction of *P. americana* +1ml 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 chloroform fraction of *P. americana* +1 ml of CO.

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

As shown in Figure 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 5.95 ± 0.56 , 6.00 ± 0.55 , 6.00 ± 0.42 and 5.45 ± 0.32 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 (11.90 ± 0.78) obtained for rats in the castor oil-treated control group (group 2). The effects of the methanol and the chloroform

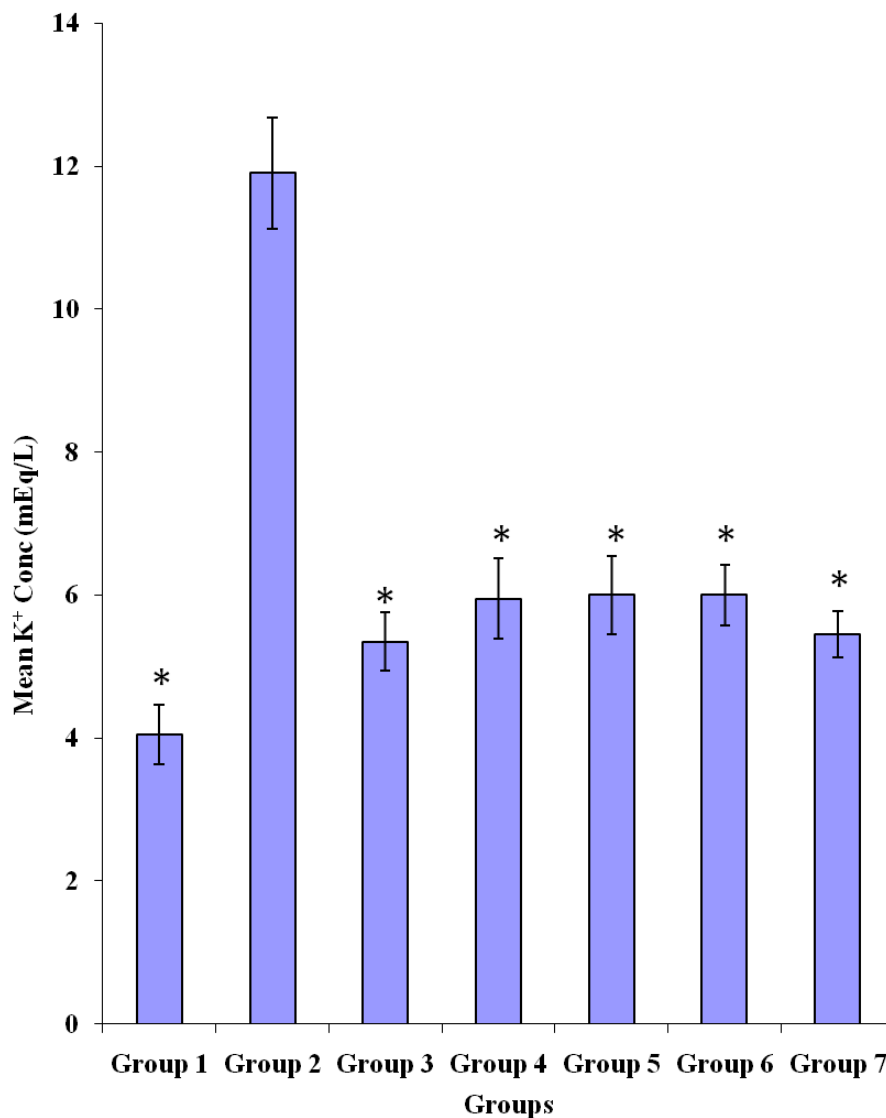


Figure 3. Effects of the methanol and chloroform fractions of the chloroform-methanol seed extract of *Persea americana* on intestinal potassium ion (K⁺) concentration. Data represented as mean \pm SD; *, significantly ($p < 0.05$) lower compared to that of group 2. Group 1, 5 ml/kg b.w of 3% v/v tween 80 (vehicle); group 2, vehicle + 1 ml of castor oil (CO); group 3, 3 mg/kg b.w of hyoscine + 1 ml of CO; group 4=100mg/kg b.w of methanol fraction of *P. americana* +1ml 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 chloroform fraction of *P. americana* + 1 ml of CO.

fractions of the extract at the tested doses were comparable to that of the standard anti-diarrhoeal agent (hyoscine butylbromide) as shown in Figure 3.

DISCUSSION

The results of the qualitative and quantitative phytochemical analyses of the chloroform and the methanol fractions of the chloroform-methanol extract of

the seeds of *P. americana* showed, in both fractions of the extract, the presence and amounts of alkaloids (2.92 ± 0.14 and 2.81 ± 0.08 g/100 g in the chloroform and the methanol fractions, respectively), flavonoids (3.43 ± 0.19 and 3.11 ± 0.16 g/100 g in the chloroform and the methanol fractions, respectively), tannins (2.85 ± 0.14 and 2.64 ± 0.13 g/100 g in the chloroform and the methanol fractions, respectively), steroids (1.51 ± 0.07 and 1.27 ± 0.04 g/100 g in the chloroform and the methanol fractions, respectively) and saponins ($2.35 \pm$

0.08 and $2.47 \pm 0.09\%$ in the chloroform and the methanol fractions, respectively). This indicates that the bioactive constituents present in the chloroform-methanol extract of the seeds 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 shown in the present study could be, in part, due to the presence of tannins, alkaloids, saponins, flavonoids and steroids. In other words, it is possible that flavonoids and steroids, acting dually or in combination with other phytochemicals, produced the observed anti-diarrhoeal effect of both fractions of the chloroform-methanol extract of the seeds of *P. americana*. Previous studies showed that anti-dysenteric and anti-diarrhoeal properties of medicinal plants were mostly due to tannins, alkaloids, saponins, flavonoids, sterol and triterpenes (Longanga et al., 2000). While 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 (Havagiray et al., 2004). Also, extracts of plants that contain flavonoids are known to modify the production of cyclo-oxygenase 1 and 2 (COX-1 and COX-2) and lipoxygenase (LOX) thereby inhibiting the production of prostaglandins (Sule et al., 2009). Steroids are also useful for the treatment of diarrhoea and may also enhance intestinal absorption of sodium ion (Na^+) and water (Anup et al., 2007).

Anti-motility along the GIT was demonstrated by both fractions of the chloroform-methanol extract of the seeds 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 seeds of *P. americana*. This might be due to the ability of both fractions 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 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 agreement with the finding of Sule et al. (2009) 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 seeds 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 seeds 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 (Gerald et al., 2007). This is achieved through its dual effects on gastro-intestinal motility as well as transport of water and electrolytes (decreasing Na^+ and K^+ absorption) across the intestinal mucosa (Rouf et al., 2003).

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

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