Effect of aqueous extract of *Anacardium occidentale* (L) stem bark on sodium and chloride transport in the rabbit colon

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Excessive secretion of fluid and electrolytes elicited by enterotoxins is a major cause of mortality in Africa. This study investigated the traditional use of the stem bark of *Anacardium occidentale* (cashew) in the treatment of diarrhea by studying its effect on sodium and chloride transport in the rabbit colon. A segment of the rabbit colon was cannulated and perfused *in situ* with iso-osmotic medium at 0.3 ml/min. The plant extract was administered intraperitoneally at a dose of 20, 40 and 80 mg/kg respectively. Perfusates were collected at 10 min interval for 1 h. The amount of sodium and chloride ions in the perfusates was analyzed with a flame photometer. The results indicated that the plant extract dose dependently stimulate sodium absorption (P < 0.05) and has no effect on chloride transport in the rabbit colon. In conclusion, this study indicates that *A. occidentale* stimulate sodium and water absorption and therefore may be effective in the treatment of diarrhea in developing countries.

Key words: *Anacardium occidentale*, chloride ion, colon, diarrhea, rabbit, sodium ion.

INTRODUCTION

Absorption of sodium (Na⁺) and chloride (Cl⁻) in the colon is necessary for water conservation by the body in health and become critical in disease condition characterized by excessive fluid loss from the body. Enterotoxins produced by invading pathogenic microorganisms can alter ion transport across the intestinal epithelial cells. Alteration of ion transport can cause secretory and inflammatory diarrhea which may result in life-threatening dehydration by excessive salt and water loss (Field et al., 1989a; Field et al., 1989b; Field and Semrad, 1993; Hyams, 2000; Paton et al., 2000).

Secretory diarrhea is the leading cause of infant mortality and has devastating effects on adults in Nigeria and most of the developing countries. Effective treatment of diarrhea involves rapid replacement of lost ions and fluid either intravenously or orally with a solution (Oral Rehydration Solution or ORS), the recipe for which is approved by World Health Organization (WHO, 2000). ORS is only effective in preventing dehydration and not the purging rate or duration of the diarrhea (Goodman and Percy, 2005). Therefore, new drug approaches are continually being pursued.

The use of herbal preparations in the treatment of diarrhea is a common practice in many African countries including Nigeria because Western pharmaceuticals are beyond the financial reach of the people. *Anacardium occidentale* (Linn) commonly called Cashew in English, Kasu in Hausa, Okpokpo in Ibo and Kaju in Yoruba, is a multipurpose tree whose leaves, stem and bark extracts are used extensively for the treatment of diarrhea, dysentery and colonic pain. It has also been reported to possess anti-diabetic, anti-bacterial, anti-inflammatory and anti-ulcerogenic (Laurens, 1999; Akinpelu, 2001). The mechanisms of these beneficial effects remain elusive. The bark of *A. occidentale* is a rich source of tannins, a group of plant chemicals with documented biological activity (Laurens, 1987) and other unique chemicals among which are; lauric acid, leucine, linoleic acid, methylglucronic acid, oleic acid and salicylic acid (Kubo et al., 1994).

Since the use of herbal preparations is growing with...
many as 4 in 10 Nigerians using various botanical remedies in a given year, the present study was undertaken to investigate the effects of aqueous stem bark extract of *A. occidentale* on \( \text{Na}^+ \) and \( \text{Cl}^- \) transport in the rabbit colon *in vivo* in order to explore the mechanism underlying its use as an ethnomedicine.

**MATERIALS AND METHODS**

**Chemicals**

All drugs and salts used in the preparation of Tyrode's solution were of analytical grade and were purchased from Sigma Chemical Co (St Louise MO).

**Animals**

Male, white New Zealand rabbits weighing 0.8 - 1.2 kg were obtained from the Animal House of the Department of Physiology, Ahmadu Bello University, Zaria, Nigeria. The animals were housed within the animal care facility at the Department of Physiology, Ahmadu Bello University and fed *ad libitum* with standard pellet chow and tap water. The animals were randomly distributed into different experimental groups with 5 rabbits per group. They were deprived of food but not water 18 h before surgery. All experiments were approved by the Institution Animal Care Committee in accordance with National guidelines on use of animals for studies before commencement of the study.

**Plant material**

Fresh Cashew stem bark was harvested from the tree in Botanical Gardens of Ahmadu Bello University, Zaria. Identification of the plant was done by the Herbarium Keeper (Mr. Musa Mohammed) of the Department of Biological Sciences, Ahmadu Bello University, Zaria -Nigeria. Voucher number 1117 is available in the same Herbarium. The fresh stem bark (300 g) was air dried for six days. The air dried bark was ground to a coarse powder which was macerated in distilled water. The solution was agitated over a mechanical shaker (SFI, Great Britain) for 24 h. The resultant mixture was purified by filtration and then the filtrate was concentrated into a residue over water bath (Brian and Turner, 1975). The yield was 9.8% (w/w). The extract was dissolved in saline on the morning of the experiment and used in the study.

**Intestinal perfusion technique**

A modification of the isolated perfused intestine method of Edgecombe et al. (2000) from the model described by Blanchard et al. (1990) and Windnmueller and Speth (1981) was adopted in this study. Briefly, the animals were anesthetized by a single intraperitoneal injection of urethane (0.2 ml/100g body weight of a 50% solution). The abdomen was opened through a 10 cm midline incision just below the xiphisternum. A segment (12 cm) of the ascending colon was isolated and cannulated with a polyethylene tube at the upper and lower ends. The inflow cannula was connected to a syringe pump. The perfusing fluid was a medium called Tyrode solution. Total concentration of \( \text{Na}^+ \) and \( \text{Cl}^- \) in the solution was calculated to be 267 mM and 355 mM respectively. The medium was continuously gassed with 95% O\(_2\) and 5% CO\(_2\) and maintained at 37°C and a pH of 7.4.

The outflow cannula was connected to a flow inducer (MIRE200, Waston-Marlow ltd, Cornwall, England). The rate of flow was maintained at 0.3 ml/min. Following placement of the cannula, the laparotomy was sutured closed to prevent desiccation and heat loss. After 1 h equilibration period post surgery, perfusate was collected from the distal cannula at 10 min interval for a total period of 60 min. unlike Blanchard et al. (1990) who re-circulated the perfusate, flow of perfusate in this experiment was a single pass and the out flow perfusate was collected.

**Study design**

The study evaluated the effect of 20, 40 and 80 mg/kg aqueous extract of *A. occidentale* stem bark administered intraperitoneally after two 10 min basal collection. The control animals received normal saline in place of *A. occidentale*. In another series of experiments, animals received 20 mg/kg Frusemide (a loop diuretic that selectively blocks the Na\(^+\) - K pump) alone or Frusemide 30 min before administration of the extract. Again perfusate was collected and treated as described for the other groups. Drugs were administered in 0.2 ml/kg of body weight i.p. The concentration of \( \text{Na}^+ \) and \( \text{Cl}^- \) ions was estimated for each animal and expressed as mM/10 min.

**Electrolyte analysis**

\( \text{Na}^+ \) and \( \text{Cl}^- \) concentration in the perfusate was determined by means of a Flame Photometer (model 400, Patterson Scientific, Luton, England) and Buchler-Cotlove Chloridometer (Buchler Instruments, Inc. Fort lee, N.J) respectively. The \( \text{Na}^+ \) or \( \text{Cl}^- \) absorbed was calculated by subtracting the concentration of \( \text{Na}^+ \) or \( \text{Cl}^- \) in the perfusate from the perfusing medium.

**Statistical analysis**

Data are expressed as mean ± SEM. The statistical significance of data was evaluated by student’s Student t-test and \( p < 0.05 \) was regarded as significant.

**RESULTS**

The net \( \text{Na}^+ \) and \( \text{Cl}^- \) absorbed during 1h perfusion period were 112.0 ± 1.16 and 199.0 ± 1.15 mM respectively. There was no significance difference (\( p < 0.05 \)) in absorption between the time points (Figures 1 and 2) indicating that our perfusion model was stable and suitable for the study.

**Effect of *A. occidentale***

Administration of extract of *A. occidentale* significantly increased \( \text{Na}^+ \) absorption from the lumen of the colon 20 min after administration (Figure 1; \( p < 0.05 \)). This response was more pronounced at 40 mg/kg. The volume of the outflow was also reduced significantly. The net \( \text{Na}^+ \) absorption in response to *A. occidentale* administration was significantly different when compared to control animals (\( p < 0.05 \)). There was no significant different in \( \text{Cl}^- \) absorption (Figure 2; \( p > 0.05 \)).

**Effect of Frusemide**

Frusemide, a diuretic that selectively inhibits NaCl absorption by inhibiting Na\(^+\)/K\(^+\)/2Cl\(^-\) transport significantly reduced the amount of \( \text{Na}^+ \) and \( \text{Cl}^- \) absorbed as expected.
Figure 1. Effect of intraperitoneal injection of *A. occidentale* (20, 40 and 80 mg/kg) on Na\(^+\) absorption from the rabbit colon. The colon was perfused with an iso-osmotic solution (Tyrode solution) at 37°C. *A. occidentale* or Frusemide (20 mg/kg) was injected after two 10 min basal collection (arrow). Data are presented as mean ± SEM of values from 5 rabbits per group. * P < 0.05 Vs Control.

**DISCUSSION**

The epithelial layer covering the mammalian colon absorbs Na\(^+\) and Cl\(^-\) ions and secretes K\(^+\) and HCO\(_3\)\(^-\). Many investigators of colonic function have assumed that electrolyte transport processes are uniform throughout the colon (Dawson, 1999; Heintze et al., 1983). Thus in vivo luminal perfusion experiments, in human and experimental animals have frequently been performed without regards to the possibility that electrolyte movement may differ in one or more segments (Foster et al., 1986). Enterotoxins produced by infectious organisms such as *vibrio cholerae* can disturb ion transport resulting to secretory diarrhea which can cause life threatening dehydration and excess loss of salts and water (Field et al., 1989a; Field et al., 1989b). Thus, treatment of secretory or inflammatory diarrhea has involved the development of drugs that blocks ion transport proteins to inhibit intestinal fluid secretion.

*A. occidentale* is a tree that grows up to 15 m high with thick tortuous trunk and woody branches (Paramashivappa et al., 2001). The plant produces many resources and products of importance, among which the bark, leaves and stem are used medicinally to treat diarrhea, dysentery and colonic pain (Bilcalho, 2001). This study reports the verification of the traditional claims of the use of the plant in treatment of diarrhea by investigating the effects of the stem/bark extract of *A. occidentale* on Na\(^+\) and Cl\(^-\) transport across the epithelial layer of the rabbit colon in vivo.

The results obtained from the study indicated that the extract significantly increased Na\(^+\) and fluid absorption which was not inhibited by frusemide, a Na\(^+\)/K\(^+\)/2Cl\(^-\) blocker. The plant did not significantly affect Cl\(^-\) transport. Bulk transport of Na\(^+\) and Cl\(^-\) is known to enter epithelial cells from the lumen via electrogenic Na\(^+\) channels (ENaC), sodium-dependent substrate co-transporter, electroneutral sodium-chloride co-transport, coupled sodium/proton exchangers (Na\(^+\)/H\(^+\)) and Cl/HCO\(_3\)\(^-\) exchangers (Goodman and Percy, 2005). The contribution of transcellular/paracellular absorption of Cl\(^-\) is also important but it is limited by para-cellular shunt resistance.
Figure 2. Effect of intraperitoneal injection of *A. occidentale* (20, 40 and 80 mg/kg) on Cl\(^-\) absorption from the rabbit colon. The colon was perfused with an iso-osmotic solution (Tyrode solution) at 37°C. *A. occidentale* or Frusemide (20 mg/kg) was injected after two 10 min basal collection (arrow). Data are presented as mean ± SEM of values from 5 rabbits per group. * P < 0.05 Vs Control.

(Gitter et al., 2000). Since the action of the extract was not influenced by frusemide, it suggests that the extract affected Na\(^+\) absorption by activating the electro-neutral (NHE) Na\(^+\)/H\(^+\) or sodium substrate co-transporters. This may explain the observed decrease in Na\(^+\) and water content of the outflow when compared to the inflow. Na\(^+\) must have been absorbed out of the intestinal from the perfusing fluid and may have led to the absorption of water molecule osmotically. These results suggest that *A. occidentale* may promote re-hydration of an individual suffering from secretory diarrhea through a mechanism associated with activation of ENaC or NHE transporters. Thus, it appears that the ethno-medicinal reports of *A. Occidentale* in the treatment of diarrhea may involve selective activation of Na\(^+\) channel and can be viable as an anti-diarrhea agent. The efficacy of the extract is perhaps better explained by the array of chemicals acting in concert rather than a single chemical found in the plant.

**Conclusion**

*A. occidentale* may be effective in the treatment of diarrhea by reducing intestinal fluid secretion and by selective activation of ENaC or NHE co-transporters. With further studies, the potential of this plant in managing diarrhea and other related gastrointestinal disease will be placed in proper perspective.

**REFERENCES**


