Antidiarrheal Potential of Methanol Extract of *Combretum dolichopetalum* and its Fractions in Wistar Albino Rats

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Aim: The study investigated the antidiarrheal potential of methanol extract of *Combretum dolichopetalum* and its solvent fractions. **Method:** Leaves of *Combretum dolichopetalum* were extracted using methanol as solvent. This was classified as the crude methanol extract (MECD). The crude extract was further fractionated using column chromatographic techniques to get various fractions. Castor oil was used to induce diarrhea in the animals, following atropine and the plant extracts to confirm any antidiarrheal activity. Fecal excretion and absorption/secretion ratio, enteropooling, small intestinal transit, glucose estimation, free fatty acid estimation and fecal osmolarity were employed to serve as antidiarrheal biomarker. **Result:** result showed improvements in all studied biomarkers compared to the negative control. The extract worked similarly like the standard drug used atropine. **Conclusion:** From the result of this study, it can be concluded that the methanol extract of *Combretum dolichopetalum* and its fractions contains compounds with antidiarrheal potentials, and this justifies its use in ethnomedicine.

Keywords: *Combretum dolichopetalum*; Diarrhea; Castor oil; Electrolyte

Diarrhoea is an abnormal faecal discharge characterized by frequent and fluid stool and/or the presence of blood and mucus with increased neutrophil polymorphs in the stool; usually due to disease of the small intestine and involving fluid and electrolyte loss. Epidemiologically, it is the passage of three or more loose or watery stool. Diarrhoea is found to best correlate with an increase in stool weights, stool weights above 10_{0z} (300g) *per day*. This increase is mainly due to excess water, which

makes up 60-85% of faecal matter. However, no universally accepted definition of clinical values as guidelines for the more commonly used determinants of clinically meaning diarrhoea elevated stool output (>200 – 250g/day); this measure varies greatly in dietary fibre intake; watery, difficult to control bowel movements (qualitative assessment); and bowel movement frequency exceeding three bowel movements *per day* ^[1]. Therefore, diarrhoea is a symptom of the secretory response of the gastrointestinal mucosa to a wide variety of stimuli. The increased secretion increases the watery content and fluidity of the stool. If the primary disease causes inflammation or ulceration of the intestinal mucosa, particularly of the large intestine and rectum, blood and mucus may be present in the stool. A wide variety of conditions including enteric infections, alteration in digestion and absorption of food, a variety of hormonal factors or even a response to a parenteral infection can result in the symptom of diarrhoea. Nevertheless, the mechanism leading to this increased fluid content of the stool may be acute or chronic and may represent a symptom of gastrointestinal infection that will respond differently to pharmacological therapy. Acute infective diarrhoea is the major clinical problem in tropical countries ^[2]. Diarrhoea is an important cause of under nutrition as patients eat less with reduced absorption of nutrients; nutrient requirements are increased as result of infection. It is often the underlying reason for malnutrition changing from marginal to severe in a child. It is also an important cause of growth retardation because children with diarrhoea suffer from loss of appetite, restriction and malabsorption ^[1].

Materials and Methods

Collection of Plant Material

The leaves of *Combretum dolichopetalum* were collected between the months of January and February from Orba, Udenu Local Government Area of Enugu State. The Botanical Identification was confirmed by Mr. A. Ozioko of Bioresources Development and Conservation Programme (BDCP). The voucher specimen was deposited in the BDCP herbarium (BDCP 0094).

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Extraction of the Plant Material

The leaves were air dried for 5-7 days. The leaves were ground to coarse powder which was extracted by macerating in methanol (1:10 w/v). The filtrate was dried *in vacuo* to obtain the Methanolic Extract of *Combretum dolichopetalum* (MECD). The MECD was partitioned using solvents of different polarities in column chromatography to obtain the fractions used in the study. The various yields of the extract and the fractions were calculated using the formular:

$$\frac{Ye \times 100}{We} = \text{Percentage Yield of the Extract (fractions)}$$

Where *Ye* is the weight (g) of Methanolic Extract and the fractions respectively; and
We is the weight (g) of the plant material or the methanolic extract.

Animals

White albino rats (90- 240 g) and white albino mice (17 –26 g) of either sex bred in the animal house of Faculty of Biological Sciences and Veterinary Medicine were used for the study. They were acclimatized in the animal house for five days with free access to water and food before the start of the experiment. The rats and mice were fed with standard pellets (Guinea Feeds, Plc, Nigeria). The proximate compositions of the normal rat diet are shown in appendix 2.1. The animals were maintained under standard 12-hour light-dark cycle throughout the duration of the study. The rat and mice were randomly assigned to different control and treatment groups in the course of the experiments.

Column Chromatography

The initial material (MECD) used for the column chromatography weighed 551 g. This was mixed thoroughly with adequate quantity of silica gel (particle size 0.063-0.200 *i.e* 70 – 200 Mesh) to produce free flowing powder. The powder was placed on top of the dry packed column (4 x 80 cm diameter by length) and eluted first with hexane (HxE), until the eluents became clear. The hexane was allowed to drip-dry overnight. 10% methanol-methylene Chloride (MMCE) was further used to elute the constituents. This was followed by the elution with ethyl acetate (EToAcE), butanol (BuE) and water (AqE).

Evaluation of Effect on faecal excretion and Absorption/secretion Ratio

Faecal excretion was assessed according the method of Crocci *et al.* [3]. The rats (100- 160 g) used for the study were acclimatised individually in grid floor cages in 12 hrs cycle for 5 days and allowed free access to food and water. Prior to the studies, the food was withdrawn and 3 h later the rats were given castor oil (1 ml/100 g body weight). The individual groups of the animal (*n*=5) received various treatments; first, second and third received *intraperitonally* 2 ml/kg of 10% ethanol in normal saline (ET₁₀), nothing and 3 mg/kg of atropine respectively and were considered negative and positive controls. The remaining groups (4 -12) were given various doses (100, 200 and 400 mg/ml *p.o*) of the different extracts (BuE, MMCE, EToAcE, and HxE) respectively. However, the extracts were given 1 hour before castor oil was administered. Gentle thumb pressure was applied to the perianal to expel any faecal pellet from the rectum immediately after the castor oil was given. The pellets discharged during the next 210 minutes were collected and weighed immediately (wet weight) and after drying for 18 h at 50 °C to constant weight (dry weight). Any action of intestinal secretion and absorption of fluid was scored as the ratio of wet to dry faecal weights. The basal ratio was assessed in the 2 h before treatment. The incidence of diarrhoea was assessed as the number of animals presenting unformed stool.

Effect of the Extracts on Castor Oil-Induced Enteropooling

Intraluminal fluid accumulation was determined by the modified method of Robert *et al.*, [4]. Overnight fasted albino mice were grouped as above (section 2.13) in the faecal excretion and absorption/secretion ratio. The individual groups of the animal (*n*=5) received various treatments; first group received *intraperitonally* 3 mg/kg of atropine and 2 ml/kg of 10% ethanol in normal saline (ET₁₀), second and third groups. These were considered negative and positive controls respectively and the remaining groups (4 -12) were given various doses (100, 200 and 400 mg/ml *i.p*) of the different extracts (BuE, EToAcE, HxE and MMCE,) respectively 1h before the oral administration of castor oil. Two hours later the rats were sacrificed the small intestine was removed after tying the ends with thread and weighed. The intestinal content was collected by milking into a graduated tube. The intestine was reweighed and the difference between full and empty intestine was calculated and expressed as a percentage inhibition.

Small Intestinal Transit

Mice were fasted for 18 h were divided into fourteen groups ($n=5$). Groups one (1) through twelve (12) were treated with various extracts (BuE, MMCE, EToAcE, and HxE) orally in a dose dependent (100, 200 and 400 mg/kg) manner respectively while groups thirteen (13) and fourteen (14) were given 2 ml/kg 10% ethanol in normal saline (ET₁₀) and 3 mg/kg (*i.p*) body weight respectively 1 h before oral administration of castor oil. One ml of a marker (10% charcoal suspension in 5% gum acacia) was administered orally 1 h after castor oil treatment. The mice were sacrificed after 1 h and the distance travelled by charcoal from the pylorus was measured and expressed as a percentage of the total length of the intestine from the pylorus to caecum [5].

Effect on Castor oil-induced Diarrhoea

The castor oil test was performed as described by Offiah and Chikwendu [6]. Fifty-five albino rats of both sexes were divided into eleven groups of five animals per group. The animals were housed singly in a metabolic cage and starved 18 hours prior to the commencement of the experiment, but had free access to water. Those in groups three (3) through eleven (11) were each pre-treated orally with 100, 200 and 400 mg/kg of the different extracts (HxE, EToAcE, and MMCE), respectively, while the rats in groups 1 and 2 received (*i.p*) 2 ml/kg 10% ethanol in normal saline (ET₁₀) and 3 mg/kg of atropine respectively. One (1) hour later, all the rats were subsequently treated with 1 ml of castor oil orally. The animals were then kept and observed over a period of 4 h for the consistency of faecal frequency of passing watery (wet) and unformed stool. The numbers of both dry and wet diarrhoeal droppings were counted every hour for the 4 h period. Mean number of the stools passed by the treated was compared with that of control (2 ml/kg ET₁₀) [7]. The animals were sacrificed thereafter and the plasma assayed for lipase activity and free fatty acid levels. The blood glucose was taken before and after the four-hour period. The faecal and blood samples were analysed for the Na⁺ and K⁺ levels.

Glucose Estimation

One touch ultra-glucometer was used in determining the glucose level in the blood.

Lipase Activity

The spectrophotometric determination of liberated free fatty acid in an olive oil suspension was used to determine the lipase activity in the plasma. One ml of the incubation mixture (5 ml of 0.05 M sodium deoxycholate and 45 ml of triethanolamine buffer) and 0.1 ml of plasma placed in a test-tube was incubated at 30 °C in a water bath for 10 minutes and transferred to another water bath at 70 °C for one minute to stop the reaction. The stoppered tubes were shaken for 20 minutes and centrifuged at 1000 x g for 5 minutes. The chloroform phase was carefully transferred into a clean test-tube to avoid contamination. A 2.5 ml of diethyldithiocarbamate was added and the absorbance read at 436 nm. Inactivated plasma at 70 °C for 2 minutes served as a sample blank and 5 ml of chloroform as the reagent blank [8].

The volume of the activity is calculated as follows:

$$\text{Volume activity} = \frac{E_{\text{lipase}}}{E_{\text{standard}}} \times \frac{250}{10 \times v}$$

Where $v = 0.1$ ml (sample volume)

Free fatty acid Estimation

One-half (0.5) ml of the test solution thus obtained was added to glass stoppered pyrex test tube (16 x 150 mm) containing 4 ml of chloroform and 0.5 ml of phosphate buffer (0.5M, pH 7). The tube was then shaken for 80 seconds. After 30 minutes, the upper layer was carefully taken off by suction with a fine-tipped pipette, and to the residual chloroform layer was added 2 ml of a Cu-triethanolamine (Cu-TEA). This solution consisted of 1M triethanolamine-1N acetic acid-6.45% Cu (NO₃)₂·3H₂O in 9:1:10 (v/v/v). The tube was shaken 20 times, and after 15 minutes, the upper layer was filtered into another test tube as the colour was developed by addition of 1.5 ml of 0.5% solution of diethyldithiocarbamate in butanol and absorbance was read at 436 nm in a spectrophotometer [8].

Effect of extracts on Faecal and Blood Na⁺ and K⁺ Level

The quantity of Na⁺ and K⁺ on the faecal and blood samples was assayed using the atomic absorption spectrophotometer (INSRUCHEM TAS-986 w/ PC ISO9001 & 14001). The Na⁺ and K⁺ were released from the blood and faecal samples by wet and dry digestion methods respectively.

Wet Digestion

The samples (1.2 g) were weighed into 100 ml digestion flask and 10 mls of the *aqua regia* was added. The mixture was heated in a fume chamber for 30 minutes. The solutions were made up to 100 mls with deionised water. The signal of filtrate was read using the atomic absorption spectrophotometer (INSRUCHEM TAS-986 w/ PC ISO9001 & 14001).

Dry Digestion

The samples (1.2 g) were weighed into a platinum crucible on an analytical balance. The crucibles were placed in a muffle furnace (Model JMF-1500) at 650 °C for two and half (2½) hrs for proper ashing. The ashed samples were cooled in a dessicator for 30 minutes. 2 mls of *aqua regia* was added to each of the ashed samples to dissolve it completely. The solution was transferred differently into 100 ml plastic container and made up to 50 ml with deionised water. The ash was dissolved in deionised water and filtered. The filtrate signal was read using the AAS.

The sample signals were converted into concentration using the formula:

$$\frac{\text{Conc. Of the Standard}}{\text{Standard Signal}} \times \text{sample signal} = R$$

$$\frac{R \times \text{Volume of the sample}}{\text{Weight of the sample}} = \text{concentration of the Element (ppm)}$$

R= elemental signal

To convert ppm to mg/100 g divide by 10.

Faecal Osmolarity

The faecal osmolarity was calculated as

$$\text{Osmolarity (mOsmol/kg)} = 290 - 2 \times (\text{Sodium Concentration [Na}^+ \text{]} + \text{Potassium Concentration [K}^+ \text{]})$$

Statistical Analysis

Results were expressed as mean $\pm$ Standard Error of Mean (SEM) using SPSS version 12.00. Data obtained were analysed using one way analyse of variance (ANOVA) and LSD post Hoc test. Difference between mean values of the treated groups were compared to the controls regarded as significant at $p < 0.05$.

RESULTS

Normal Fecal Excretion and Absorption/Secretion Ratio

The effects of the cholinergic receptor antagonists, atropine, on fecal excretion and diarrhea induced by castor oil were compared with those of the extracts. The figure 1 and 2 shows the basal and diarrheal

(wet/dry) incidence ratios in the various treatments. The result indicates a difference between the activities of BuE, EToAcE and HxE in basal and diarrheal dry weight. Also, the BuE indicated a difference in basal fecal ratio. The BuE partially antagonized the effect of castor oil in reducing diarrhea without affecting the increase in faecal water content. The MMCE, but at substantially higher doses are as effective as atropine in preventing the actions of castor oil. HxE although potent, only partially reduced diarrhea and fecal mass.

Figure 1: Effect of varying concentrations of the extracts on the fecal excretion and diarrhea induced by castor oil.

Figure 2: Effect of varying concentrations of the extracts on the dry fecal weight and diarrhea induced by castor oil.

Effect of extracts on Castor oil – induced Enteropooling

Castor oil caused accumulation of water and electrolytes in intestinal loop. Figure 3 shows effect of the various extracts in reducing water and electrolyte accumulation in the intestinal loop due to castor oil induction of diarrhoea. Each dose of the extracts produced significant ($p < 0.05$) variable reduction in the intestinal content weight.

Figure 3: BuE, HxE, EToAcE and MMCE on reducing water and electrolyte accumulation in the intestinal loop due to castor oil induction of diarrhea.

Effect on the Small Intestinal Transit

Figure 4 indicates the inhibition of gastrointestinal motility by the various extracts in movement of charcoal meal as percentage of intestinal length. The percentage inhibition of small intestinal transit was increased with castor oil but was subsequently reduced significantly ($p < 0.05$) with various concentrations of the extracts except for 100 and 400 mg/kg of BuE and EToAcE respectively. The various concentrations of MMCE, HxE and some of BuE and EToAcE produced a much-marked reduction more than atropine, a standard antispasmodic agent. There was no significant difference when inhibitory effect of EToAcE, HxE and MMCE were compared to atropine, indicating comparable efficacy. However, there was significant difference in efficacy when the effect of BuE was compared with atropine, a sign of their relative lack of inhibitory potency.

Figure 4: Effect of HxE, EToAcE and MMCE on the average inhibition of gastrointestinal motility by the various extracts in movement of charcoal meal as percentage of intestinal length.

Effect of the Castor Oil induced Diarrhoea

The various extracts HxE, MMCE and EToAcE showed varying level of inhibition compared with the control. However, the effects of EToAcE and MMCE indicated a dose – dependent inhibition of post absorptive fecal weight against castor oil induced diarrhea. The HxE was effect was a little more significant ($p < 0.05$) compare to the control at 400 mg/kg.

Figure 5: Effect of HxE, EToAcE and MMCE on the inhibition of four (4) hours post-absorptive fecal weights against castor oil induced diarrhea.

Effect on Extracts on Plasma Glucose Concentration

The result indicates the mean plasma glucose level of the pre- and post castor oil-induced diarrhoea in the blood of the animals are as shown in the figure 6. The EToAcE showed a significant post castor oil induced diarrhea glucose level. The HxE and MMCE showed insignificant dose dependent decrease of the post absorptive glucose level. The insignificant decreases compared to atropine indicate comparable efficacy. However, the post absorptive four (4) hour castor oil induced glucose level was maintained above the blood threshold in all treated animals.

Figure 6: Effect of the HxE, EToAcE and MMCE on the blood glucose concentration.

Effect of extract on Plasma lipase activity and free fatty acid concentration

The result indicates significant ($p < 0.05$) increase in the plasma lipase activity of the EToAcE and MMCE treated animals. Both figures 7a and b showed a dose-dependent increase in plasma lipase activity. These independent and collective increases of both extracts were better compared to that of atropine. Also, the plasma free fatty acid concentration showed a dose dependent increase in HxE and significant ($p < 0.05$) dose dependent decreases in the EToAcE. The MMCE significantly ($p < 0.05$) depressed the plasma free fatty acid concentration compared with the control. There is an observed inverse relationship in plasma activity and free fatty acid concentration in EToAcE.

Figure 7a: Effects of HxE, EToAcE and MMCE on the plasma lipase activity

Figure 7b: Effects of HxE, EToAcE and MMCE on the free fatty acid concentration

Effect of extract on fecal and blood $[Na^+]$ and $[K^+]$

The figure 8a and b indicate the effects of various extracts on the fecal and blood $[Na^+]$ and $[K^+]$ in all treated animals. All extracts indicated a significant ($p < 0.05$) reduction in fecal $[Na^+]$ and $[K^+]$ in the treated animals compared to the normal (negative) control while the blood $[Na^+]$ and $[K^+]$ showed an insignificant ($p > 0.05$) varied response in the tested doses of the extracts. There is an overall increase of

blood and faecal $[Na^+]$ compared to the $[K^+]$ in all the treated animals.

Figure 8a: Effects of HxE, EToAcE and MMCE on the faecal $[Na^+]$ and $[K^+]$

Figure 8b: Effects of HxE, EToAcE and MMCE on the blood $[Na^+]$ and $[K^+]$

Effect of the extracts on the fecal osmolarity

The effects of the various doses of the extracts on the faecal osmolarity of the animals induced with diarrhoea is shown in figure 8c. The faecal osmolarity was found to be within the normal range (>250 mMol) but for the insignificant reduction observed in the HxE. The faecal osmolarity effect of HxE showed a

dose-dependent increase slightly below the normal range.

Figure 8c: Effects of HxE, EToAcE and MMCE on the fecal osmolarity

Discussion

The enhancement of colonic motility by the gastro-coli reflex, activated by gastric distention, and contraction of the rectum with relaxation of the internal anal sphincter by rectal reflex, caused by the ingression of faeces into the rectum are thought to be involved in the normal defaecation. These reflexes are controlled by parasympathetic nerves [9]. However the intestinal effects of castor oil have been associated with mucosa injury and release of several endogenous mediators, such as prostaglandins [10], platelet activating factor [11] and nitric oxide [12], which produces changes in transport of water and electrolytes resulting in hypersecretory responses. Results of normal faecal excretion and absorption ratio indicates incidence of diarrhoea (colonic motility) which MMCE, EToAcE, HxE and BuE are consistent with their ability to prevent castor oil diarrhoea. In animals not given castor oil (ET₁₀ *i.p.*), did not affect colonic contraction and any resultant defaecation. Under these conditions, drug-free control animals defaecate only occasionally and giant migrating contraction seldom occur [13]. However, the frequency of these propulsive contraction significantly increased after castor oil treatment; this effect was almost prevented by EToAcE, MMCE, the cholinergic receptors antagonist, atropine, and partially by HxE and BUE. To a lesser extent EToAcE and HxE, potently prevented diarrhoea and significantly ($p < 0.05$) reduced faecal mass output and

water content after the administration of the castor oil. These effects expressed as decrease for diarrhoeal faecal ratio and diarrhoeal dry faecal weight indicates partial intestinal absorption as well as metabolic activity as regulating the bioavailability. This was further substantiated by effects of these extracts on local electrical and mechanical activities of ileal circular muscles or castor oil induced intraluminal fluid accumulation of fluid by indirect enteropooling assay.

Diarrhoea results from an imbalance between the absorptive and secretory mechanisms in the intestinal tract, resulting in an excess loss of fluid in the faeces [14]. In some diarrhoea the secretory component predominates while others are characterised by hypermotility. The significant effect of the various extracts in reducing fluid accumulation and weight of intestinal content (water and electrolyte accumulation) in the intestinal loop due to castor oil induction of diarrhoea in the enteropooling assay indicates a possible reabsorption of the NaCl and water from stimulation of intestinal secretion by the cathartic agent.

The result of the small intestinal transit indicates that various concentrations of MMCE, HxE and some concentrations of BuE and EToAcE produced significant inhibition of gastrointestinal movement in mice more than atropine, a standard antispasmodic agent. Previous study indicates that activated charcoal avidly absorbs drugs and chemical on the surface of the charcoal particle thereby preventing absorption [15]. Thus gastrointestinal motility test with activated charcoal was carried out to evaluate effect of the extracts on peristaltic movement. The results also show that the extracts suppressed the propulsion of charcoal meal thereby increased the absorption of water and electrolyte. Drugs affecting motility, frequency and consistency of diarrhoea also affect secretion. These extracts probably act through inhibition of prostaglandins and propulsive movement of small intestinal tract. The extracts appear to act on all parts of the intestine, thus it reduced the intestinal propulsive movement in the charcoal meal treated model. The inhibitory effect of these extracts justifies its use in folklore medicine and as an antidiarrhoeal agents. The underlying mechanism for treatment of diarrhoea appear to be spasmolytic that may be attributed to some phytochemical constituents. Flavonoids, tannins, alkaloids, saponins, reducing sugars and sterols and/or terpenes has long been used

in the management of diarrhoea [16]. Since the giant migrating contraction in the distal colon are the one that best reflect faecal excretion in rats [3]; the scant ability of BuE to prevent their increased incidence of diarrhoea and relatively decreased intestinal propulsive movement in the charcoal meal by castor oil might explain why the extract had less antidiarrhoeal action than atropine. BuE presented an effective *in vitro* agent (histamine evoked response) but the inhibitory effect of BuE on peristaltic activity was significant ($p < 0.05$) as evident from distance travelled by charcoal meal. However, this significant difference in efficacy when the effect of BUE was compared with atropine is a sign of their relative lack of inhibitory potency. The MMCE and HxE reduced the intestinal propulsive movement in the charcoal meal treated model more than atropine. Probable extract increased the reabsorption of NaCl and water by increased intestinal transit time possibly due to its cholinergic effect. The antidiarrhoeal activity of the extracts may also be due to the presence of denatured protein forming protein tannates, which makes the intestinal mucosa more resistant and reduce secretion [17].

All segment of the intestine from duodenum to distal colon have mechanisms for both absorbing and secreting water and electrolytes. The gastrointestinal tract propels ingested materials and waste products along its length for nutrient absorption and waste elimination. This vital role is accomplished through regulated and coordinated contractions but deregulation of these process leads to alterations in the speed of material transit through the gut. These extracts, EToAcE and MMCE, showed dose-dependent percentage inhibition of four (4) hours post-absorptive faecal weights against castor oil induced diarrhoea while HxE was significant ($p < 0.05$) at the 100 and 200 mg/kg respectively compared with atropine. The dose-dependent reduction of MMCE and EToAcE are similar to the preliminary antidiarrhoeal assay result with MECD indicating significant interplay of phytochemicals in a possible dose-limiting presence of flavonoids. Also a comparable efficacy of the insignificant effect of the extract on blood glucose concentration compared with atropine indicates increased post-absorptive material transit through the gut for nutrient absorption and waste elimination. However the insignificant pre- and post-blood glucose level and regulation of glucose concentration to normal (4 mM,) indicates nutrient

absorption and comparable effect in the intestinal active transport for glucose. On the other hand, food intake and glucose metabolism is regulated by many factors, at least in part, by glucosensors in the hypothalamus and neuroendocrine cells [18].

The effect of extract on plasma lipase activity and free fatty acid concentration shows EToAcE and MMCE as having significant ($p < 0.05$) increase in the plasma lipase activity that is somewhat reversed in plasma free fatty acid concentration. This inverse relationship established in fatty acid concentration and lipase activity with a fairly insignificant relationship in blood glucose level suggest the extracts exaggerated mobilisation of the fatty acids for maintaining vital organs such as the brain (100 g/day) through gluconeogenesis when compared with the control (ET₁₀). Nonetheless stimulating hydrolysis of triacylglycerol by inducing plasma lipase with the resultant fatty acid and monoacylglycerol being eventually depleted to sustain vital organs. Studies have highlighted the role of short chain fatty acids (SCFA) coupled Na⁺ in the colonic salvage of carbohydrate, Na⁺ and water [19].

Many diarrheal deaths are caused by dehydration. The use of low osmolarity ORS solution which, is absorbed in the small intestine even during copious diarrhea replaces the water and electrolytes (Na⁺, Cl⁻, K⁺ & HCO₃⁻) lost in faeces [20]. Clinically the use of blood or serum electrolytes in diarrhoea disorders is usually not helpful or often misinterpreted; leading to inappropriate treatment of diarrhoea [20]. The proximate composition of the normal rat diet shows a 0.15 % relative quantity of sodium in diet. Since the animals used in this study were starved 18 hrs prior to the diarrhoeal induction, the low [Na⁺] and high [K⁺] in the blood and the significantly reduced effect of the extracts on faecal [Na⁺] and [K⁺] indicates a possible neural control of the intestinal ion transport and paracellular permeability that is altered by nutritional status. Earlier studies suggested roles of neural influences in enhanced secretory responses to *Escherichia coli* heat-stable enterotoxin after fasting or malnutrition [21]. Nonetheless, neural regulation of active transport and paracellular permeability in piglet small intestine indicates that tonic neural activity modulates paracellular permeability in the fed state and the putative neurotransmitter; acetylcholine contributes to this process [22]. The effect of MMCE on the faecal [Na⁺] and [K⁺] is constant and significant within the tested doses. The EtoAcE

showed greatly reduced effect on both electrolytes' concentrations. These faecal concentrations, insignificant faecal osmolarity and the blood [Na⁺] and [K⁺] suggests a functional involvement of Na-K pump in process maintenance of an osmotic balance between fluid in the bowel and extracellular fluid in the intestinal tissue.

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