ANTI - DIARRHOEAL EFFECTS OF NAPOLEONAEA IMPERALIS LEAF EXTRACTS

Aloh, G.S.1, *Obeagu, Emmanuel Ifeanyi2, Odo Christian Emeka1, Kanu, Stella Ngozika3, Okpara, Kingsley Ezechukwu4, Nnennam,M. Nwankwo5,
Obeagu Getrude Uzoma6

1Department of Biochemistry, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.
2Diagnostic Laboratory Unit,University Health Services, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.
3Abia State University Teaching Hospital,Aba, Abia State,Nigeria.
4Rivers State College of Health Technology, Port Harcourt.
5Department of Biochemistry, Ebonyi State University, Abakaliki, Nigeria.
6School of Nursing Science, ESUT Teaching Hospital, Parklane, Enugu, Nigeria.

ABSTRACT

Water, methanol and Chloroform extracts of Napoleonaea imperialis were tried for their effects in arresting ion losses due to diarrhoea induced with magnesium sulphate. The ions whose levels were monitored for two days. were Na+, K+, Cl− and HCO3−. After one hour of the induction of diarrhoea the Na+ levels in the three (H2O, Methanol and chloroform or B, C, D) groups dropped from 125.7-102.7mmol/L 12^102.7mmol/L and 126.3-103.3mmol/L respectively K+ levels dropped from 2.77-1.70mmol/L, 2.70-1.73mmol/L and 2.20-1.53mmol A. respectively. The levels of chloride reduced from 74.70mmol/L - 49.70mmol/L, 74.00 -53.3mmol/L and 71-5230mmol/L in each of the three groups, while the recorded HCO3− levels reduced from 24.30 -13.00mmol/L respectively. After one hour of the administration of the extracts, all the animals still recorded father Ton losses^ probably due to the time log needed for their uptake across gut Walls. The greatest drop of up to 41- 46% was recorded for K+ ion and may have accounted for the observed reduced activities /Vigours of the animals. Within two
days of continued administration of the extracts, the levels of all the ions had been restored to near normal values relative to their levels before the induction of diarrhoea. Each of the extracts was found to be efficacious in restoring ion losses due to diarrhea.

**KEYWORDS**: Water, methanol and Chloroform extracts of *Napoleonaea imperialis*.

**INTRODUCTION**

Electrolytes are minerals found naturally in the body and potassium, calcium sodium and magnesium among others. They are needed to keep the body’s balancer of fluid at the proper level and to maintain such normal functions as heart rhythm, muscle contraction and brain function (Christopher et al., 2002). If the body's electrolytes are not in proper balance, it may lead to seizures, regular heart, beat, muscle weakness, oedema etc. Electrolyte imbalances can be caused by a variety of health conditions, such as chronic heart or kidney disease, endocrine disease (such as problem with the adrenal pituitary, thyroid, or parathyroid glands) malnutrition, or bone disorders. Any condition that caused the body to lose too much water, such as diarrhoea, vomiting, fever, or taking medications (called diuretics) can also lead to an electrolyte imbalance. In this work therefore, the effects of various extracts of *Napoleonaea imperialis* in treating or arresting diarrhea rabbits will be investigated.

Picked up from food contaminated with these organisms is ingested along with the food and later causes inflammation spasm of the intestinal muscles and other symptoms. Other types of gastroenteritis are caused by intestinal parasites, such as Giardia Amoebae both of which are usually contracted by eating contaminated food or drinking unsterile water (Ramzi et al., 1999). Initial heating inadequate to destroy spores.

Spores contaminate raw or unprocessed food, especially meat Storage without rapid Toxin – mediated chiaHariaerobi Conditions, viable bacteria multiply+++ Rapid reheat, heat shock, Enteritis: 6 hours post-ingestion. le -diarrhoea proper. Bacteria speculate and release Enterotoxin.

Fig 2.1 -The description of food contaminated with the causative organisms of diarrhoea, that finally lead to diarrhoea (Christopher et al 2002). Other types of gastroenteritis are caused by intestinal parasite, such as Giardia and Amoebae both of which are usually contracted by eating contaminated food or drinking unsterile water (Farthing, 2001).
CLINICAL PRESENTATIONS OF DIARRHOEA

The main symptoms of gastro-enteritis are diarrhoea and or jumbig (Farthing, 2001). The severity of diarrhoea or -omffing suffered varies among individual sufferers, and depends on tier ages and the types of causative organism (Ramzi et al., 1999). In addition top diarrhoea and vomiting, softie .infections can cause other symptoms such as stomach cramps, fever, blood in the motions and general debility (Farthing, 1994). As a general rule simple mat gastro - enteritis tends to cause diarrhoea and vomiting without the other symptoms (Farthing, 2001). Infections due to food poisoning by organisms other than viruses, example bacteria and other intestinal parasites, present with more severe conditions, such as stomach cramps and rectal haemorrhage.

Prolonged diarrhoea and vomiting leads to dehydration which in adults presents as a dramatic reduction in the volume of urine produced and associated with assignificant feeling of thirst, general. Lethargy, a dryness of the tongue and loss of elastcity of the skin.

Babies and children in addition to these symptom present with a sunken fontanelle (soft spot on the top of the head) and sometimes becomes sunken -eyed when significantly dehydrate , a general rule the younger the child the more quickly they are likely to become dehydrated because their fluid reserves are less than those of adults and this obviates the need for early medical attention . Which short Id incorporate a fluid replacement regime to arrest or forestall dehydration (Ramzi et al., 1999).

The different types of infection are caught in different ways. These infections are contracted spread by different modes. A major route is the faeco - oral route through which diarrhoea a causing organisms are contracted, especially by bottle -fed babies who get them from poorly sterilized bottles, and by older children who contract air-born organisms such as viruses as they come into contact with infected persons (Farthing, 2001).

Depending on the part of the world visited, foreign travels constitute another mode of spread of diarrhoea and vomiting . As many as 7.02million (39%) of the estimated 18 million Nigerians that travel to foreign lands contract "travellers diarrhea even through the risk of gastro - enteritis varies from one country to another, with European countries constituting the lowest risk of contracting it from such organisms as campylobacterial and food Poisoning, while African countries constitute more than forty five percent of such infected travelers.
BIOCHEMISTRY OF DIARRHOEA

In diarrhea patient, the contents of the lower digestive tract is evaporated the fluidity of the contents of the small and large intestines in increase (Farthing, 2001). Active transport of Na⁺ back into the gut initiates a reverse sodium transport. This causes both Cl⁻ and HCO₃⁻ to follow passively, as well as water. Now in the intestines, The water dilutes toxins as well as triggers contractions of the intestine due to increase in intestinal distension (Farthing, 2001). contractions push the contents of the lower gastro intestine tract towards and out of the and canal. Medications such as loperamide are designed to prevent such contraction in response to the distension, and should not be used to diarrhoea. Such inhibition actually prolongs the infection or irritation, and can cause a Worsening over time because the evacuation of the bowel contents has been delayed (Farthing, 2001). In diarrhoea mechanism infectious diarrhoea occurs as a result of two major disturbances in normal intestinal physiology; Increased intestinal secretion of fluid and electrolytes, predominantly in the small intestine as; and 2 decreased absorption of fluid, electrolytes and sometimes nutrient that can involve the small and large intestine (Farthing, 2001).

INCREASED INTESTINAL SECRETION

Intestinal secretary process can be activated by infection with bacteria and viruses. Secretary enterotoxins are the major cause of increased intestinal secretion in infective diarrhoea. Cholera toxin (CT) is the “prototype” enterotoxin and its mechanism of action has been extensively researched.

Fig 2.2 - Mechanism of action of cholera toxin. It is the paradigm for enterotoxin mediated diarrhoea CT Switches on secretion without any macro or microscopic damage to the enterocyte. Other secretary enterotoxins have also been well characterized and include the closely related E. coli heat labile toxin (LT) and the structurally distinct E. coli heat stable toxin (ST). Since the discovery of these toxins, other prosecretory enterotoxins have been more recently discovered enterotoxing have been less well characterized. Accessory cholera enterotoxin increase short circuit in using chambers, although its precise mode of action has not been defined (Farthing, 2002). Zonular occludens toxin, which is produced by V. cholerae 01, increase the permeability of the small intestine by intereacting with the cytoskedeton and altering the structure of intercellular tight junctions (Farthing, 2002). It is now evident that secretary diarrhoea may be mediated by other mechanisms of secreton, as well as the classical enterocyte interaction. Multiple extracellular factors
regulate epithelial iron transport; paracrine, immunologic, neural, and endocrine factors. There is extensive overlap and interplay between these systems that a single superregulatory system has been termed PINES (paracrine-immuno-neuro-endocrine system) (Frathing, 2002). Secretory diarrhoea may be mediated by a variety of secretagogues, including prostaglandins, 5-hydroxytryptamine (5-HT), Substance P, and vasoactive intestinal peptide (VIP).

Neuronal pathways are involved in the amplification of the effects of enterotoxins (Sears & Kaper, 1996). Cholerastoxin (CT) has been shown to release 5-HT from enterochromaffin cells, which through to then activate the afferent limb of a neuronal reflex. The effector limb of the neuronal pathway by releasing the neurotransmitter VIP (Smears and Kaper, 1996). This binds to specific receptors on the basolateral membrane and activates adenylate cyclase -cAMP intracellular secretory pathways. Interneurons propagate the secretory effects of CT distally in the small intestine. The importance of S-HT in mediating CT induced secretory diarrhoea has been confirmed by the use of 5-HT2 and 5-HT3 receptor antagonists, which decrease secretion in the rat and human intestine (Smears and Kaper, 1996). Substance P antagonists also reduce CT induced fluid secretion in mammalian small intestine, suggesting that it may be a key neurotransmitter in the sensory afferent limb or interneuron of the neuronal reflex (Sears and Kaper, 1996). Hence CT affects the epithelium directly but also recruits other components in PINES, including enteric neurons, enterochromaffin cells, and multiple mediators to produce a complex secretory response. There may also be distant effects in the small intestine and a reflex secretory response in the colon (Farthing, 2001). LT and ST also activate neural secretory reflexes but 5-HT does not appear to be involved in the secretory pathways of these toxins.

Rotavirus has been assumed to elicit diarrhoea by damaging absorptive cells but evidence is emerging that rotavirus intestinal infection can evoke fluid and electrolyte secretion by activation of the enteric nervous system (Farthing, 2001).

**DECREASED INTESTINAL ABSORPTION**

The other major mechanism by which enteric pathogens cause Diarrhea is impaired intestinal absorption. This is usually accompanied by macroscopic and microscopic injury to the intestine (Sears and Kaper, 1996). Diarrhea due to impaired intestinal absorption can be due to:

- impaired epithelial transport processes - that is, impaired fluid, electrolyte, and nutrient
absorption in the small intestine;
i. Osmotic diarrhoea due to the appearance of incompletely absorbed mitnents in the colon.
i. impaired water and sodium reabsorption by the colon due to direct involvement of the
colic absorptive process (Farthing, 2001). Intestinal absorption is also dependent on the
duration of time allow for digestion and contact with the epithduim, and therefore any
alteration in small intestinal and whole gut transit times may RESULT in impaired absorption
(Farthing, 2001).

Epithelial injury in the small intestine and colon occurs in association with many
enteropathogen; bacteria, parasites, and viruses. The nature of the injury can occur at many
levels; from discrete damage to the microvillus membrane during the attachment of E. coli
and cryptosporidium parvum, to the mucosal inflammatory response to invasive pathogens
for example, shigella spp, salmonella spp, and Entamoeba histolytica, usually involving the
lesease of cytolethal cytotoxins reslting in epithelial.cells loss and ulceration (Christopher ef
a/ 2002). Rotavirus, another invasive enteropathogen, directly invades the epithelial cells In
middle and H>per portion of the viilus, with rapid epithelial cell death and acute «*xjs
trophy, invasive enteropathogens also produce an acute inflammatory response within the
mucosa.recruiting proinflammatory mediators such as prostaglandins and leukotrienes,
resulting in both impaired intestinal absorption and the initiation of a prosecretary state in the
intestine (Smears and kaper, 1996).

Invasive enteropathogens also promote the synthesis and release chemokunes such as
interleukin (IL) -8, by intestinal epithelial cells. IL-8 is a known potent chemoattractant for
polyprohenuclear leucocytes that enhance the inflammatory cascade and produce further
mucosal and epithelial damage by release of reactive oxygen species (Ramzi et al.,1999).
Neutrophils also release 5¹- AMP, which Is a potent secretagogue acting though the
adenosine A2 receptor on

MATERIALS AND METHODS

MATERIAL

BIOLOGICAL MATERIALS

Fifteen rabbits.

Fresh leave of Napoleonaea imperialis super starter feed (growers mesh) and Elephant
grasses.
METHODS

COLLECTION OF BIOLOGICAL SAMPLES

PLANT USED
The fresh leaves of Napoleonaea imperialis were collected from Nuskka town.

ANIMAL USED
Fifteen rabbits were used. The rabbits which contained both male and female (none pregnant) rabbits were obtained from the Animal House, Ebonyi State University, AbakaUki, Ebonyi State. The rabbits were divided into five different groups, labeled A, B, C, D, and E (control).

PREPARATION OF EXTRACT
Eighty grammes (80g) of fresh leaves of Napoleonaea imperialis were ground with 200ml of each extraction solvents methanol, water and chloroform. After grinding, the mixture was filtered. The filtrate was administered to the animals after the induction of diarrhoea.

PREPARATION OF WORKING REAGENT 3.2.3.1 PREPARATION OF MAGNESIUM SULPHATE SOLUTION
Five grammes (5g) of magnesium sulphate (Mgso₄) were dissolved in 25ml of water.

DETERMINATION OF WEIGHT OF ANIMALS
The rabbits were weighed using weighing balance. Their different weight were recorded and used to determine the amount of extract.

ADMINISTRATION OF EXTRACT SULPHATE.
The Fifteen rabbits were divided into groups; A, B, C, D and E Which was the Control. In group A, B, C, and D, diarrhoea was induced by administering orally 5ml of magnesium sulphate. Group A was used as positive control by not administering the extract after the induction of diarrhoea with MgSO₄ while Groups B, C and D received appropriate quantities of the extract. The animal in Group B were given water extract of the Napoleonaea imperialis, while Group C. and D received chloroform and methanol extract after evaporation and dissolution in water. The plasma electrolyte levels of the animals were measured before and four hours after the administration of MgSO₄, and for once daily for the next three day 5ml of each extract was administered three times daily to the appropriate group of animals.
except those in group E in which diarrhoea was not induced.

**COLLECTION OF BLOOD SAMPLE**

The blood sample was collected by venopuncture, Centrifuged at 3000xg for 10 minutes and the plasma used for the assays.

**3 MEASUREMENT OF PLASMA SODIUM CONCENTRATION PRINCIPLE**

This was done by and modifications of the methods of Rnaruna (1958) and Trinder (1951) sodium is precipitated as the triple salt, sodium magnesium uranyl acetate, With the excess uranium then being reacted with ferrocyanide, producing a chromophore whose absorbance varies inversely with the concentration of sodium in the test specimen.

The test tubes were labeled, thus; blank standard, control and patient 1. 0ml of the filtrate reagent were pipetted to all the tubes, also 50ML of sample was added to all the tubes and to the blank, distilled water to make the volume of 0.05ml All the tubes were shaken vigorously, centrifuged at high speed (1,500G) for 10 minutes; The supernatant fluids as described below, were taken not to disturb the protein precipitate. Also test tubes were labeled corresponding to the above filtrate tubes. Also added to all the tubes were reagent 50pl of supernatant are mixed thoroughly and 50µ of rolour reagent (Chromophore) and also mixed thoroughly. The absorbance of each reaction mixture was read at 550nm against a water black.

**MEASUREMENT OF PLASMA POTASSIUM CONCENTRATION PRINCIPLE**

If a serum sample is treated with trichloracetic acid (TCA) in an alkaline medium, potassium ions precipitate upon treatment with sodium tetraphenylboron (Na -TPB) giving rise to a turbid and stable potassium tetraphenylborate suspension. The turbidity produced is proportional to the potassium concentration of the sample.

Aqueous solution of potassium equivalent to 5mmOl/L Equal parts of 0.3m Na - TPB and 0.15m NaoH were mixed and let stand for 10 minutes. Mixed well and centrifuged at 2000xg for 5 minutes. The supernatant was stored for use. The working reagent was constituted by mixing 2.0mi each of the sample and standard reagent, centrifuging.
Obeagu et al.  

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<table>
<thead>
<tr>
<th>Blank</th>
<th>Sample</th>
<th>Standard</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>ml</td>
<td>ml</td>
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<tr>
<td></td>
<td>0.1</td>
<td>-</td>
</tr>
<tr>
<td>Supernatant</td>
<td>-</td>
<td>0.2</td>
</tr>
<tr>
<td>Standard</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Mixed thoroughly to obtain a homogenous turbidity. Read after 5-10 minutes. Also mixed once again prior to reading. The reading were taken under the wavelength of Hg 578nm, 580nm. SAO. D. \( X \ 5 = \text{mmol potassium/L STD} \).

**MEASUREMENT OF PLASMA CHLORIDE CONCENTRATION. PRINCIPLE.**

\[
2\text{CL} + \text{Hg (SON)}_2 \rightarrow \text{Hg Cl}_2 + 2\text{SCN} \rightarrow \text{Fe}^{3+} \rightarrow \text{Fe (SCN)}_3
\]

**METHOD**

The reagents contains

A - 2x 100ml of thiocyanate reagent.
B - 1x 5ml of Blank reagent
C - 1x5ml of standard.

Aqueous solution of chloride equivalent to 100mEq/L (100mmol/L).

The reagent compositions is as follows Reagent A.

Mercuric thiocyanate-0.4mM
Iron (iii) nitrate-21.8mM
Stabilizers
Reagent B
Nacl-30.7mM

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<thead>
<tr>
<th>BLANK</th>
<th>SAMPLE</th>
<th>STANDARD</th>
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<tr>
<td>ml</td>
<td>ml</td>
<td>ml</td>
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<tr>
<td>Standard</td>
<td>-</td>
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</tr>
<tr>
<td>Sample</td>
<td>-</td>
<td>0.01</td>
</tr>
<tr>
<td>Blank reagent</td>
<td>0.01</td>
<td>-</td>
</tr>
<tr>
<td>Reagent A</td>
<td>1.00</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Mixed and let to stand for S minutes at room temperature (15 - 25°C).

The reading were taken under the wavelength of 450nm. Thiocyanate was used as the blank reagent /
MEASUREMENT OF PLASMA BICARBONATE CONCENTRATION PRINCIPLE

Phosphoenolpyruvate + HCO₃⁻ PEPC^⁰xalate + H⁺ po₄

Oxalate + NADH MD^⁰ Malate + NAD⁺ Phosphenol pyruvate carboxylase (PEPC) Catalyse the reaction between phosphoenolpyruvate and carbon dioxide (bicarbonate) to form oxaloacetate and phosphate ion. Oxaloacetate is reduced to malate with simultaneous oxidation of an equimolar amount of reduced nicotinamide adenine dinucleotide (NADH); to oxidized nicotinamide adenine dinucleotide (NAD⁺) the reaction is catalysed by malate dehydrogenase (MDH). This result in a decrease in absorbance at 340nm, which is directly proportional to CO₂ concentration in the sample.

METHOD

CO₂ reagent was prepared according to the makers instruction. The test tubes were labeled: Blank, standard, control and patient. 1.0 ml of carbon dioxide reagent place in each tubes and incubated for 3 minutes at 37°C. The spectrophotometer wavelength was set at 340nm, temperature to 37°C and the absorbance reading to zero with water as blank. 0.05ml of water, standard, and sample were pipetted into the cuvette labeled blank, standard and patient respectively mixed gently by inversion and incubated for 5 minutes. The readings were taken at 340nm and recorded the CO₂ (HCO₃⁻) Concentration was obtained from the relation:

$$\text{CO}_2 \text{ content} = \frac{\text{Abs. blank} - \text{Abs. Sample}}{\text{Tvbs T Standard}}$$

RESULTS

The mean plasma concentration of electrolytes before induction of diarrhea (Mmol/L)

<table>
<thead>
<tr>
<th></th>
<th>Na⁺</th>
<th>K⁺</th>
<th>cr</th>
<th>HC03⁻</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>125.3±1.53</td>
<td>2.7±0.20</td>
<td>73.7±2.52</td>
<td>24.7±1.53</td>
</tr>
<tr>
<td>Group A</td>
<td>125.3±2.52</td>
<td>2.7±0.21</td>
<td>72±2.65</td>
<td>24.3±152</td>
</tr>
<tr>
<td>Group B</td>
<td>125.7±0.12</td>
<td>2.77±0.12</td>
<td>74.7±2.52</td>
<td>24.3±2.Q8</td>
</tr>
<tr>
<td>Group C</td>
<td>126±3.Q</td>
<td>2.70±0.1Q</td>
<td>74±1.73</td>
<td>24.7±2.Q8</td>
</tr>
<tr>
<td>Group D</td>
<td>126.3±2.08</td>
<td>2.70±0.17</td>
<td>71 ±3.0</td>
<td>25±1.0</td>
</tr>
</tbody>
</table>

The level of electrolytes after an hour of induction of the diarrhoea (the levels reduced drastically about 20 - 50% lower than the normal values).
The level of electrolytes after one hour of extract administration.

<table>
<thead>
<tr>
<th></th>
<th>Na⁺</th>
<th>K⁺</th>
<th>Cr</th>
<th>HCO₃⁻</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>102. ±1.53</td>
<td>1.77*0.15</td>
<td>49.7*152</td>
<td>13*2.0</td>
</tr>
<tr>
<td>Group B</td>
<td>102.7±2.08</td>
<td>1.70*0.10</td>
<td>53.3±3.06</td>
<td>.12.7*152</td>
</tr>
<tr>
<td>Group C</td>
<td>102.7±3.06</td>
<td>1.73*0.12</td>
<td>53±2.0</td>
<td>1-17*153</td>
</tr>
<tr>
<td>Group D</td>
<td>103.3±2.08</td>
<td>1.53*0.06</td>
<td>52.3±3.06</td>
<td>11*10</td>
</tr>
</tbody>
</table>

The level of electrolytes after Two hours of the extract administration was the same with the level after one hour, which shows that the diarrhoea has slopped.

<table>
<thead>
<tr>
<th></th>
<th>Na⁺</th>
<th>K⁺</th>
<th>Cr</th>
<th>HCO₃⁻</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>8131153</td>
<td>1.4±0.10</td>
<td>4131153</td>
<td>9.311.53</td>
</tr>
<tr>
<td>Group B</td>
<td>100,712.52</td>
<td>1.57*0.06</td>
<td>50.3±2.08</td>
<td>10.311.53</td>
</tr>
<tr>
<td>Group C</td>
<td>100.713.06</td>
<td>1610.10</td>
<td>50.3±0.58</td>
<td>1011.0</td>
</tr>
<tr>
<td>Group D</td>
<td>99.3±2.08</td>
<td>1.43±0.06</td>
<td>4912.65</td>
<td>9.2410.65</td>
</tr>
</tbody>
</table>

The level of electrolytes after 2days of extract administration.

<table>
<thead>
<tr>
<th></th>
<th>Na⁺</th>
<th>K⁺</th>
<th>Cr</th>
<th>HCO₃⁻</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>51.7±1.53</td>
<td>0.83±0.06</td>
<td>29i1.0</td>
<td>4.3±0.58</td>
</tr>
<tr>
<td>Group B</td>
<td>118.7*1.53</td>
<td>2.67±0.06</td>
<td>70.3i1.53</td>
<td>21i1.0</td>
</tr>
<tr>
<td>Group C</td>
<td>119.7i1.53</td>
<td>2.57*0.06</td>
<td>71.3i1.53</td>
<td>22±2.65</td>
</tr>
<tr>
<td>Group D</td>
<td>118.7±2.08</td>
<td>2.57±0.12</td>
<td>69. ±2.65</td>
<td>22i1.0</td>
</tr>
</tbody>
</table>

The level of electrolyte of those in group A that was not treated with the extract after two four hours of diarrhoea induction.
After the 4th hours the rabbit died because of the severe diarrhoea that lead to dehydration and excess lost of ions (that is due to salt and water depletion).

CHAPTER FOUR B RESULTS

The mean plasma levels of electrolytes after one hour of induction.

<table>
<thead>
<tr>
<th></th>
<th>Na⁺</th>
<th>K⁺</th>
<th>cr</th>
<th>HCO₃⁻</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>18.04%</td>
<td>34.44%</td>
<td>30.97%</td>
<td>46.50%</td>
</tr>
<tr>
<td>Water</td>
<td>18.30%</td>
<td>38.63%</td>
<td>28.65%</td>
<td>47.74%</td>
</tr>
<tr>
<td>Methanol</td>
<td>18.49%</td>
<td>35.93%</td>
<td>28.38%</td>
<td>52.63%</td>
</tr>
<tr>
<td>Chloroform</td>
<td>18.21%</td>
<td>43.33%</td>
<td>26.33%</td>
<td>56%</td>
</tr>
</tbody>
</table>

The mean plasma levels of electrolytes after one hour of extract administration.

<table>
<thead>
<tr>
<th></th>
<th>Na⁺</th>
<th>Na⁺</th>
<th>cr</th>
<th>HCO₃⁻</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>35.12%</td>
<td>48.15%</td>
<td>42.64%</td>
<td>46.50%</td>
</tr>
<tr>
<td>Group B</td>
<td>13.20%</td>
<td>46.56%</td>
<td>10.74%</td>
<td>31.50%</td>
</tr>
<tr>
<td>Group C</td>
<td>14.20%</td>
<td>46.63%</td>
<td>9.34%</td>
<td>42.47%</td>
</tr>
<tr>
<td>Group D</td>
<td>11.78%</td>
<td>41.96%</td>
<td>10.82%</td>
<td>39.09%</td>
</tr>
</tbody>
</table>

The mean plasma levels after a day of extract administration.

<table>
<thead>
<tr>
<th></th>
<th>Na⁺</th>
<th>K⁺</th>
<th>cr</th>
<th>HCO₃⁻</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>25.12%</td>
<td>33.13%</td>
<td>40.50%</td>
<td>41.63%</td>
</tr>
<tr>
<td>Group B</td>
<td>3.21%</td>
<td>16.03%</td>
<td>26.21%</td>
<td>21.39%</td>
</tr>
<tr>
<td>Group C</td>
<td>4.09%</td>
<td>10.30%</td>
<td>29.64%</td>
<td>31.74%</td>
</tr>
<tr>
<td>Group D</td>
<td>6.94%</td>
<td>26.60%</td>
<td>27.07%</td>
<td>14.48%</td>
</tr>
</tbody>
</table>

The mean plasma levels of electrolyte after two days of extract administration.
Table:<br><br| Group | Na⁺ | K⁺ | Cl⁻ | HCO₃⁻ |
<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>35, 12%</td>
<td>48.15%</td>
<td>42.64%</td>
<td>61.73%</td>
</tr>
<tr>
<td>Group B</td>
<td>5.57%</td>
<td>3.61%</td>
<td>5.89%</td>
<td>13.58%</td>
</tr>
<tr>
<td>Group C</td>
<td>5%</td>
<td>7.04%</td>
<td>3.65%</td>
<td>10.91%</td>
</tr>
<tr>
<td>Group D</td>
<td>6.02%</td>
<td>7.04%</td>
<td>2.82%</td>
<td>12%</td>
</tr>
</tbody>
</table>

**DISCUSSION/CONCLUSION**

The anti-diarrhoeal effects of water, methanol and chloroform extracts of Napoieonaea imperialis were evaluated in rabbits in which diarrhoea was induced with excess dosage of magnesium sulphate (MgSO₄). The levels or concentrations of four electrolytes Na⁺, K⁺, Cl⁻ and HCO₃⁻ before, during administration of each extract to the groups of rabbits were used as indices of alleviation or deterioration of diarrhoea in those animals following the administration of each extract.

Within one hour of the inducing of diarrhoea, drastic drops in the levels of both intracellular (K⁺) and extracellular (Na⁺, Cl⁻ and HCO₃⁻) electrolytes were observed after measurement. Within this short length of time, sodium level dropped by about 18% in all the groups of animals prior to the administration of the extracts. Within the same period too, K⁺ loss was of the order of 34 - 43%, Cl⁻ loss was 26 - 30% while the highest loss of 46 -56% was recorded for bicarbonate ions up to one hour of administration of water extract of *N. imperialis* the levels of both the intra (IC) and extracellular (Na⁺, Cl⁻ and HCO₃⁻) ions continued to drop. The level of potassium ions dropped by 46.50% from 1.77 mmol/L to 1.57 mmol/L. The same order loss was also recorded for other extracellular ions namely, Na⁺ which dropped by 13.20%, Cl⁻ by 10.74% and HCO₃⁻ by 33.50%.

This further ion loss also occurred on other groups of animals that received methanol and chloroform extracts. However, the greatest loss of 41 - 46% was noted in K⁺ and this could explain the reduced activities or vigours of the animals since this ion (K⁺) is needed for nervous transmission. Furthermore, the recorded further ion losses even after one hour of the administration of the extracts could be attributed to the delay in the uptake/absorption of the drugs (extracts) from the gut walls since they were administered orally.

After one day of the administration of the extracts, slight rises in the levels of all the ions relative to their levels after one hour of drug administration and pre-drug administration were recorded within this 6.94 in all the groups by 3.2-694% relative to their levels one hour after the induction of diarrhoea. Also within one day, the levels of chloride rose by 14-315 in all the
animals. Strikingly, those of HCO₃⁻ recorded the highest increase of 26-29% while a moderate rise of ID- 26% was recorded for plasma K⁺. By the second day of the administration of the extracts the levels of all the ions had virtually been restored to "near - normal" values.

In absolute terms, those treated with water extract had their Na⁺ levels elevated to mean values of 118.7mmol/W  11.7mmol/L and 118. 7mmol/L when compared with their levels of 125.7mmol/L,126mmol/L and 126.3mmol/L respectively before the induction of diarrhea.

In the same vein, K⁺ levels had rise to 2.67mmol/L 2.57mmol/L (in both methanol and chloroform extracts groups) which were very close to their levels of 2.77mmol/L and 2.7mmol/L before the induction of diarrhoea. The levels of Cl⁻ and HCO₃⁻ were equally elevated to near - normal levels. Although the leaf extracts were found to be effective in arresting ion losses, the length of time required for the active ingredients to exert their effects is a major setback. Perphaps this problem will be resolved if a different route of administration such as intravenous infusion is adopted.

REFERENCES