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Evaluation of Anti-Diarrhoeal Properties of Methanol Extract of *Napoleonae Imperialis* Leaves

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Abstract:
The methanol extract of the leaves of Napoleonae imperialis was tested for its anti-diarrhoeal properties using the castor oil-induced antidiarrhoeal studies, intestinal fluid accumulation (enteropooling) and intestinal transit as the models. The rats were divided into five (5) groups. Groups 1, 2 and 3 served as the test groups and were given the methanol extract of the Napoleonae imperialis leaves at doses of 250, 450 and 650 mg/kg body weight respectively. Group 4 served as the positive control group and was given the standard antidiarrhoeal drug, loperamide at a dose of 3mg/kg body weight and group 5 served as the negative control untreated group and was given 1ml/kg of normal saline. The plant extract exhibited significant antidiarrhoeal effect by reducing the watery texture and the number of faecal droppings in the treated groups by 3.83±0.74, 2.33±0.45 and 1.66±0.42 when compared with the normal saline untreated group 6.33±0.49 over a period of 5 hours. The intestinal transit antidiarrhoeal experiment of activated charcoal meal produced percentage inhibition of 65.39%, 72.39%, and 83.20% in the treated groups when compared with the untreated groups which produced a percentage inhibition of 54.11%. Result of the intestinal fluid accumulation showed a significant($p<0.05$) reduction in the volume of the intestinal contents of the treated groups 1, 2 and 3 by 1.48±0.08, 1.11±0.13, 0.92±0.02 respectively when compared with the untreated group 3.25±0.98.The electrolytes that were determined from the serum are : Calcium(Ca^{2+}), Inorganic phosphate (PO_3^-), Bicarbonate (HCO_3^-), Iron (Fe^{2+}), Chloride(Cl^-), Potassium (K^+) and Zinc (Zn^{2+}) and results indicated reduced serum electrolyte concentration when compared with control (untreated) group. The liver function enzymes AST and ALT were also determined. The results of AST and ALT activities were significantly ($p<0.05$) lower in the test groups when compared with the control untreated groups which have the highest value. This study has shown that the methanol extract of the leaf of Napoleonae imperialis possesses antidiarrhoeal effect.

Keywords: *Napoleonae imperialis, Enteropooling, Intestinal transit, Volume of intestinal content, Serum electrolytes, AST, ALT.*

1. Introduction

Medicinal plants are of utmost importance to the health of individuals and the community. In developing countries, Nigeria inclusive, millions of people use herbal medicines because they are readily available and easily prescribed by traditional medicine practitioners. The World Health Organisation in 2007 reported that about 80% of the world population relies heavily on the use of traditional medicine which are mainly based on plant materials. Over 90% of Nigerians in the rural areas and 40% in the urban areas depend partly or wholly on traditional medicines for their health care (Alabi et al, 2005). This trend is due mainly to the cheapness, accessibility and availability of plant-derived medicines (Larrey, 1994). However, their use is limited because many of the claimed medicinal values have not been scientifically evaluated and their safety profiles are uncertain (Ernst 2005). One such plant, *Napoleonae imperialis,* which belong to the family *Lecythidaceae*,has been reported by traditional medicine practitioners to have such effects that range from anti-inflammatory to antidiarrhoeal activities. It is an evergreen non-timber plant that grows abundantly in bush fallows, secondary bushes and marginal lands in most of the tropical humid zones of West Africa (Koppel, 1990). The plant is variously called Utum in Ikwuano (Abia State) (Ukpabi et al, 2003), Ike Mkpudu in Mbaise (Imo State), and Odure by the Nsukka people of Enugu State (Aloh, 2015. Private communications).

Diarrhoea, an important health problem worldwide especially in developing countries accounts for more than 5-8 million deaths every year in infants below 5 years of age (Mujundar et al, 2005). World Health Organization in 2009 defined it as having three or more watery or loose bowel movements in a 24- hour period. In Nigeria, it is considered the number one

killer disease among children under 5 years (Magaji et al, 2010). Infectious agents, gastrointestinal disorders like efflux of electrolytes, plant and animal toxins, and substances that increase gastrointestinal tract secretions can cause it. The key remedies for diarrhoea are the use of antibiotics and Oral rehydration therapy and in spite of these measures; the incidence of the disease has not been on the decline, while ORT treatment often fails where there is high stool output (Brijesh et al, 2006). Hence, there is a dire need for researchers to delve into the study of plants with antidiarrhoeal potentials.

The focus of this study was on the evaluation of antidiarrhoeal properties of *Napoleonae imperialis* leave to ascertain its scientific efficacy. This was achieved through the following objectives: methanol extract of *Napoleonae imperialis* leaves; intestinal transit time; enteropooling; serum electrolyte concentrations and liver function enzymes AST and ALT.

2. Experimental Methods

2.1. Collection and Identification of Napoleonae Imperialis Leaves

Fresh leaves of *Napoleonae imperialis* were collected at Lude in Ahiazu Mbaise and later identified and authenticated by a taxonomist Dr Garuba Omosun of Plant Science and Biotechnology Department, Michael Okpara University of Agriculture, Umudike. The leaves were deposited in the herbarium for reference purposes.

2.2. Preparation of the Extracts

This was carried out using the method of (Al-Qarawi *et al*, 2004). Fresh leaves of *Napoleonae imperialis* were collected and washed with distilled water, blended and soaked in methanol and allowed to stand overnight with constant shaking. It is filtered, concentrated at a mild temperature $\leq 30^{\circ}C$ and stored in a cool dry condition until used. The mixture was thereafter filtered, concentrated in a rotary evaporator, dried in a boiling water bath and weighed.

2.3. Acute Toxicity Test

Acute toxicity study was carried out using the method of (Lorke, 1983). Nine (9) rats were randomly divided into three groups of three rats each. Each group was given 200, 500 and 1000mg/kg doses body weight of extract by oral gavages respectively. The rats were observed for signs of adverse effects and death for 24 hours

2.4. Phytochemical Screening of Napoleonae Imperialis Leaves

The phytochemical screening was carried out by (Onyegbule et al, 2011) using the method of (Trease and Evans, 1983). The test was carried out on various phytochemical constituents.

2.5. Antidiarrhoeal Studies

The antidiarrhoeal study was carried out according to the method described by (Sunil et al., 2001). Thirty rats were starved for a period of 18hrs prior to the commencement of the study and were divided into five groups of six animals each. Diarrhoea was induced by administering 1ml of castor oil orally to rats. Groups 1, 2 and 3 served as the test groups and were given the methanol extract of *Napoleonae imperialis* at doses of 250, 450 and 650mg/kg, respectively. Group 4 served as the positive control and was given loperamide at a dose of 3mg/kg while group 5 served as negative control and was given 1ml/kg, of normal saline. All administrations were given by gavage. 1ml of Castor oil was given orally to all the rats an hour before the treatment. The rats were housed in a cage over a clean white paper and numbers of both wet and dry diarrhoeal droppings were counted every hour for a period of 5 hours. Total weight of the faeces and percentage inhibition was recorded.

2.6. Small Intestinal Transit

Small intestinal transit time was done according to the method of (Qnais et al., 2005) and (Meite et al., 2009). The rats were fasted for 18 hrs and divided into five groups of six animals each. The extract of *Napoleonae imperialis* was given at doses of250, 450, and 650 mg/kg body weight of the rats respectively. Loperamide, the antidiarrhoeal drug that had served as positive control was given to group 4 at a dose 3mg/kg. Group 5 served as a negative control and received 1ml of normal saline per kg body weight. All administrations were made orally by gavage. The rats were given 1m of marker (10% charcoal suspension in 5% gum acacia) orally 1 hour after castor oil treatment. The rats were sacrificed after 1h and the distance travelled by charcoal meal from the pylorus was measured and expressed as percentage of the total length of the intestine from the pylorus to caecum.

2.7. Castor Oil-Induced Intestinal Fluid Accumulation

Intraluminal fluid accumulation was determined by the method of (Robert et al., 1976). Overnight fasted rats were divided into five groups of six animals each. Groups 1, 2 and 3 received the *N. imperialis* extracts at a dose of 250, 450 and 650 mg/kg intraperitoneally respectively. Group 4 received loperamide as the standard drug at a dose of 3mg/kg body weight while group 5 received normal saline at a dose of 1mg/kg. Castor oil (1ml) was given orally to the rats after an hour. Two hours later the rats were sacrificed, the small intestine was removed after tying the ends with thread and weighed. The intestinal contents were collected by milking into a graduated tube and their volumes measured. The intestine was reweighed and the difference between full and empty intestines was calculated.

2.8. Determination of Serum Potassium Ion Concentration

The concentration of serum potassium ion (K^+) was determined using the turbidometric method as described by (Henry *et al*., 1974).

Na-Tetraphenylborate + $k^+ \Longrightarrow$ k-Tetraphenylborate + Na^+

2.9. Determination of Serum Calcium Ion Concentration

Serum calcium concentration was determined using the colorimetric method as described by (Faulker and Meites, 1982).

Calcuim + O-Cresolphthalein Complexone $\Longrightarrow$ Calcium-Cresolphthalein Complexone Complex (purple color)

2.10. Determination of Serum Bicarbonate Ion Concentration

Serum bicarbonate ion was determined using enzyme spectrophotometric procedures as described by (Forrester *et al*., 1976).

Phosphoenol pyruvate + HCO_3^- $\Longrightarrow$ PEPC oxaloacetate + H_2PO_4

Oxalate + NADH MDH Malate + NAD $\Longrightarrow$

2.11. Determination of Serum Inorganic Phosphate Concentration

Serum inorganic phosphate concentration was determined using the method as described by (Ochei and Kolhatkar, 2008).

2.12. Determination of Serum Zinc Concentration

Estimation of serum zinc level was done by the method of (Johnsen and Eliasson, 1987).

2.13. Determination of Serum Iron Concentration

Determination of serum iron concentration was done according to the method of (Henry, 1984)

$4\ Fe_3+ + 2\ NH_2OH{\cdot}HCl^- \longrightarrow 4\ Fe_2^+ + N_2O + 4\ H^+ + H_2O$

2.14. Determination of Serum Chloride Concentration

Serum Chloride was determined using the method of Tietz, N.W (1976)

2.15. Determination of Aspartate Aminotransferase (AST) Activity

The determination of aspartate aminotransferase in whole blood according to the method of (Reitman and Frankel, 1957) was done using Randox limited commercial kits.

L− aspartate +α−ketoglutarate $\longrightarrow$ AST oxaloacetate + L−glutamate

Oxaloacetate + NADH+H^+ MDH Malate +NAD^+ $\longrightarrow$

2.16. Determination of Serum Alanine Aminotransferase (ALT) Activity

The determination of alanine aminotransferase in whole blood according to the method of (Reitman and Frankel, 1957) was done using Randox limited commercial kits.

3. Statistical Analysis

All the data were expressed in appropriate data presentation methods mean standard error of mean (S.E.M.). The significant of differences among the groups were assessed using one way and multiple way of analysis of valance (ANOVA). SPSS statistical software version 2.0 was used for the processing and analysis of the data.

4. Results

4.1. Antidiarrhoeal Studies

As depicted in figure 6 below, *N. imperialis* extract at the doses of 250mg/kg, 450mg/kg and 650mg/kg body weights significantly ($p<0.05$), reduced the mean stool scores and the weights of wet faeces, when compared with the negative control. The standard drug, loperamide, significantly reduced the mean stool score and the weight of wet faeces when also compared to the negative control. There was a significant reduction in the number of defaecations over the five-hour period starting usually from the 2nd hour with percent inhibitions of 39.49%, 63.19% and, 73.77% for 250, 450, & 650mg/kg doses respectively. The standard drug also showed a marked reduction in the frequency of defecation by 78.98% which is a little bit higher when compared with other groups 39.49%, 63.19%, 73.77%.

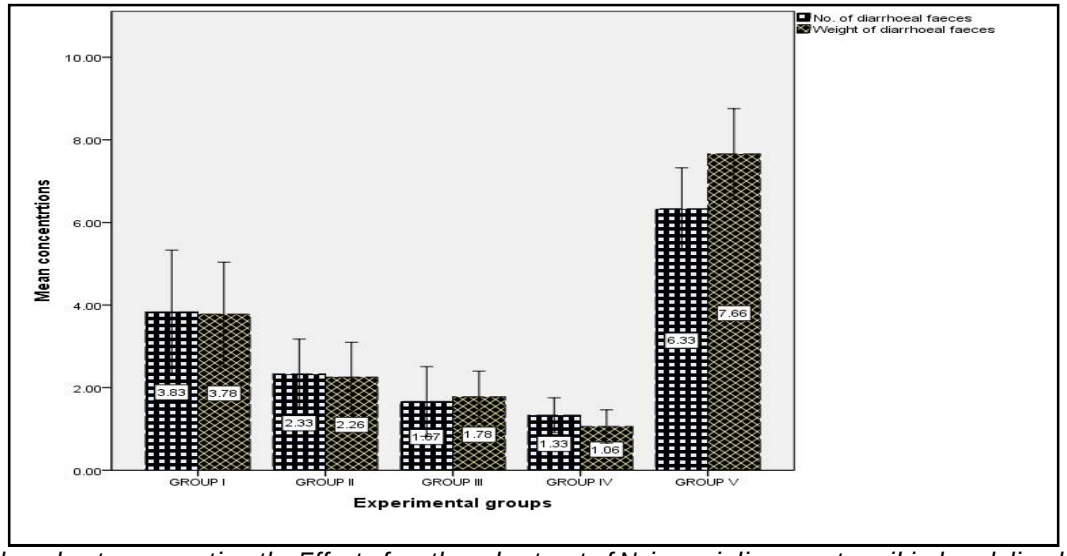

Figure 6: A bar chart representing the Effect of methanol extract of N. imperialis on castor oil induced diarrhoea in Rats

4.2. Castor Oil Induced Fluid Accumulation (Enteropooling)

As presented in figure 7, the doses of the extract significantly reduced the intestinal weight and volume in dose dependent manner. The 250mg/kg body weight dose of the extract significantly (P<0.05) produced, relative to the untreated control rats, 5.41% inhibition of intestinal weight content with significance (P<0.05). The 450mg/kg body dose weight of the extract was more as it produced19.58% inhibition of intestinal weight, while the extract. The 650mg/kg dose produced 22.16% inhibition of intestinal weight with significance (P<0.05)

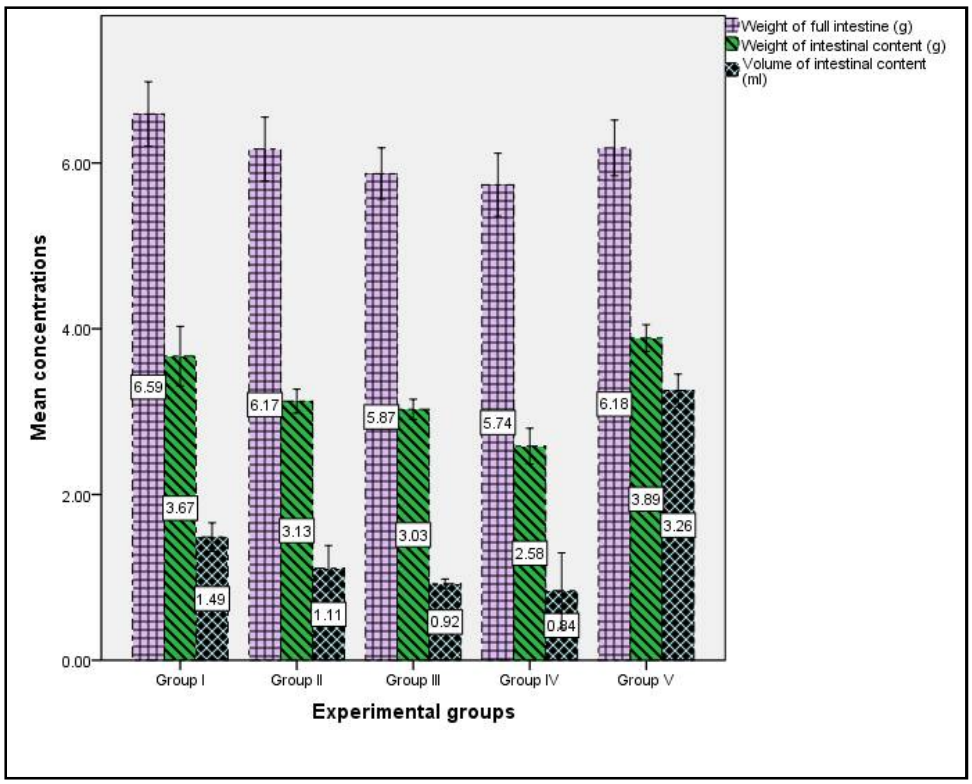

Figure 7: A bar chart representing the Effect of methanol extract of N. imperialis on castor oil induced enteropooling in Rats

4.3. Small Intestinal Percentage Transit Time

As shown in figure 8, the percent intestinal transit time was elevated with castor oil to (40.61± 1.45cm) but it was reduced by each of the different doses of the extract and much more markedly by loperamide (8.04 ± 0.31cm). 250mg/kg of the extract produced 30.8 ± 0.60 and intestinal transit induced by castor oil. 450mg/kg of the extract produced 24.83 ± 0.5 intestinal transit induced by castor oil. 650mg/kg of the extract produced 15.00 ± 0.73 & intestinal transit induced by castor oil.

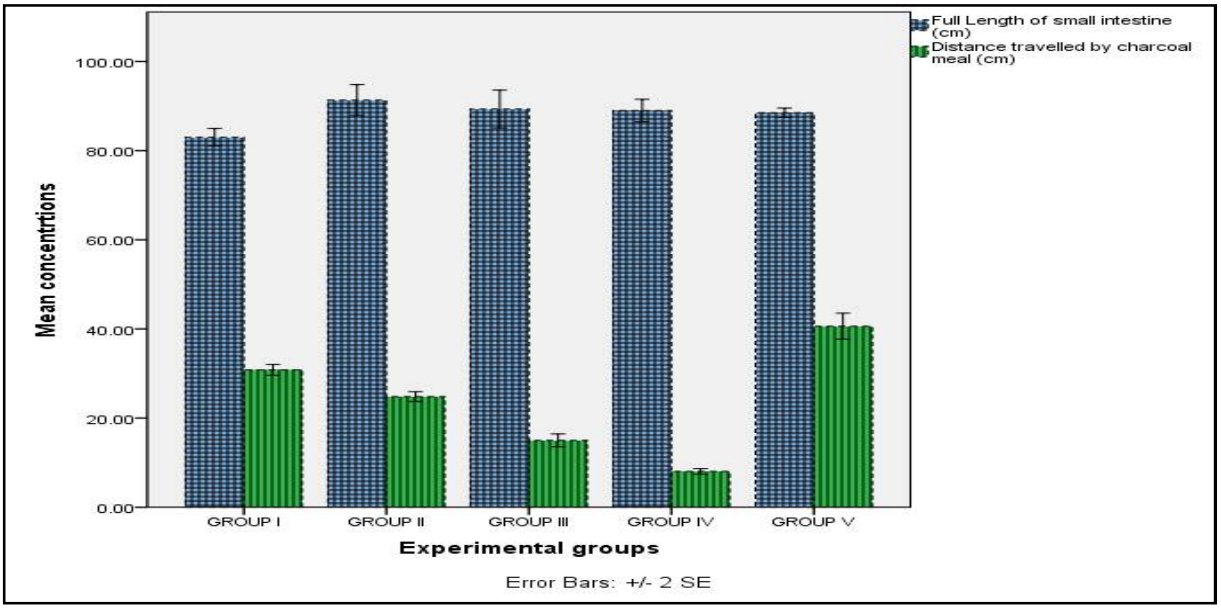

Figure 8: A bar chart representing the Effect of methanol extract of N. imperialis on castor oil induced intestinal transit time in Rats

4.4. Results for the Concentration of Serum Electrolytes and Liver Enzymes (Ast & Alt) on Rats Treated with N. Imperialis on Castor Oil-Induced

The rats administered with the *Napoleonae imperialis* extract showed a significant (P<0.05) decrease in the serum electrolytes when compared with the values gotten from untreated groups. The results obtained from the rats administered with the antidiarrhoeal drug (loperamide) showed the greatest significant (P<0.05) decrease when compared with the values obtained from the rats in the untreated group. However, the castor oil induced rats showed a significant increase (p<0.05) in serum ALT and AST activities as compared to the normal control. The treatment of castor oil induced rats with the methanol leaf extract of *N. imperialis* leaves significantly decreased the elevated serum ALT and AST levels. The results of AST and ALT activities being significantly lower in the test groups 1,2 and 3 when compared with the control group 5 which has the highest value. Figure 9 below shows the results as explained.

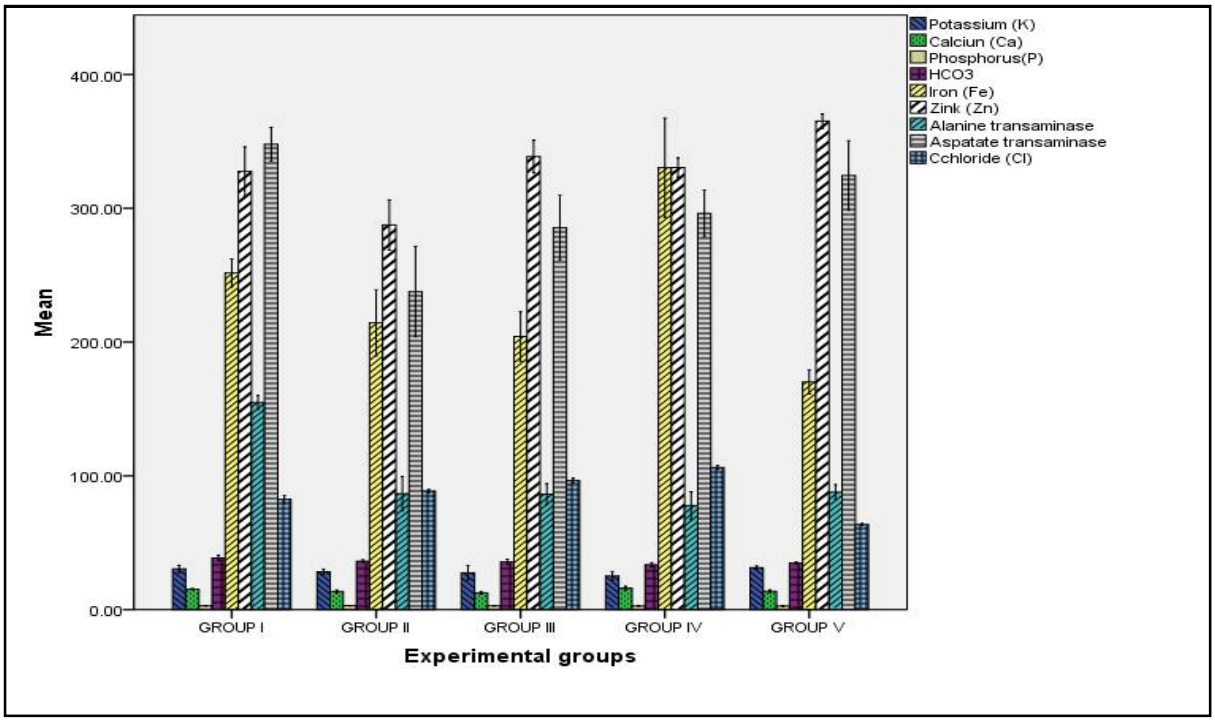

Figure 8: A bar chart showing results of serum electrolyte & liver enzymes (AST & ALT) concentration

4.5. Discussion

This study investigated the antidiarrhoeal properties of the methanol extract of *Napoleonae imperialis* leaf extract. In the antidiarrhoeal studies, treatment with loperamide and the three doses of methanol extract significantly ($p<0.05$) reduced the mean weight of diarrhoeal faeces when compared with group 5 (the negative control group) in which diarrhoea was induced but not treated. The reduction in the stool volume was marked in the loperamide-treated group while 250mg/kg, 450mg/kg and 650mg/kg body weight of the extract produced dose-dependent effect. The percentage inhibition values in the mean weight of faeces was highest in the loperamide-treated group (78.98%) followed by the 650mg/kg (73.77%) and 450mg/kg (63.19%) extracts and least in the 250mg/kg - (39.49%) treated groups. The high percentages of inhibition of transit the different doses of *N. imperialis* which compare favourably with those of loperamide justifies it to be ascribed with anti-diarrhoea property as recorded in this study.

In the enteropooling studies, the result revealed that the mean volumes of the intestinal contents were significantly ($p<0.05$) decreased in the animals treated with loperamide (0.84±0.22ml); 650mg/kg (0.92±0.02ml) and 450mg/kg (1.11±0.13ml). Although, the mean volume obtained with loperamide was higher than the ones obtained with *N. imperialis* extract, the latter should be considered efficacious when compared with the negative control (3.25±0.98ml).

However, the mean weights of intestinal contents were markedly elevated in the *Napoleonae imperialis* extract groups 250mg/kg (3.67±0.17g); 450mg/kg (3.12±0.72g); 650mg/kg (3.02±0.6g); followed by the loperamide treated group (2.58±0.10g) when compared with the induced-untreated control group. The significant ($p<0.05$) increase in the mean weights of intestinal contents of rats treated with the extracts suggests the presence of compounds that could inhibit the action of ricinoleic acid thereby promoting the inhibition of the release of prostaglandins.

A comparison of the result revealed that the administrations of loperamide (8.04±0.31cm), and *Napoleonae imperialis* extract 650mg/kg (15±0.73cm), 450mg/kg (24.83±0.54cm), and 250mg/kg (30.8±0.60cm) body weight significantly reduced the mean distance travelled by the charcoal meal when compared with the induced-untreated control group (40.61±1.45cm). The decrease in the mean distance travelled by charcoal meal was dose-dependent in the groups treated with the extract at the indicated doses. The 650mg/kg body weight dose of the extract had a profound anti-motility effect but the standard drug (loperamide) had the greatest anti-motility effect. The percentage inhibition was highest in the animals treated, with loperamide followed by the highest dose of the extract (650mg/kg body weight) in a dose dependent fashion. The pattern of inhibition in distance travelled by charcoal meal is as follows: loperamide (90.96%)>650mg/kg extract (83.20%); > 450mg/kg (72.81%) extract > 250mg/kg extract (65.39%).

The result on the intestinal fluid accumulation showed that the extract significantly ($P<0.05$) reduced both the weight and volume of the intestinal contents. These effects are the direct consequences of reduced electrolyte and water absorption into the small intestine and this indicates that *N. imperialis* extract enhances electrolyte absorption into the small intestine. Electrolyte absorption, according to (Duggan et al, 2002), determines the efficiency of nutrients. The extract caused absorptive efflux of the electrolytes and antagonized the ion transport alteration effects of castor oil on electrolyte fluxes. As reported by (Malomo, 2000), assay of enzyme activities in tissues and body fluids is an important aid in disease investigations. This study revealed that the induction of diarrhoea with castor oil caused hepatoxicity manifested biochemically by a significant increase of serum AST and ALT levels. The significance ($P<0.05$) decrease in AST/ALT ratio at the administration of the *N. imperialis* extract showed that the extract did not cause hepatocellular damage.

5. Conclusion

Results from this research work showed the indications of antidiarrhoeal effects of the methanol leaf extracts of *N. imperialis* with the 650mg/kg body weight being the most effective. The antidiarrhoeal effect of the castor oil induced diarrhea, castor oil induced enteropooling, castor oil induced intestinal transit and castor oil induced increases in the serum concentrations of potassium, sodium, calcium, bicarbonate, zinc, chloride ions. The antidiarrhoel effect was also accompanied by reduction of the castor oil induced elevations of AST and ALT, markers of hepatocellular damage. The results this work agrees with, provide biochemical bases for the use of the leaves of *N. imperialis* in folk medicine in the treatment for diarrhoea.

5.1. Suggestions for Further Research

Further studies are required to fully investigate the mechanisms responsible for the observed antidiarrhoeal activity of the methanol extract of *Napoleonae imperialis leaves,* and to determine the exact active ingredient responsible for its antidiarrhoeal activity. It is necessary also to investigate the possible antidiarrhoeal activity of other solvent extract of *Napoleonae imperialis leaves* and other parts of the plant. Further studies are required to harmonize the results obtained from the comparison of the various treatments' antidiarrhoeal activity for the formulation of an efficacious therapeutic and/ or prophylactic dose.

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