

Original Research Article

INFLUENCE OF METHANOLIC LEAF EXTRACTS OF *Vernonia amygdalina* ON CASTOR OIL INDUCED DIARRHEA IN RATS

ABSTRACT

Diarrhea is a common cause of death in developing countries and second most common cause of infant deaths worldwide, this is because the loss of fluids through diarrhea can cause dehydration and electrolyte imbalances. In this study, the effect of *Vernonia amygdalina* in treating diarrhea induced rats was investigated. Twenty-five male albino rats weighing between 120-227g grouped into A, B, C, D and E, with five rats in per group were induced with diarrhea. Group A served as the negative control group, Group B served as the positive control group administered with 2 mg/kg body weight (bwt) loperamide, Groups C, D and E induced with 1 ml castor oil and treated with 100mg/kg bwt, 200mg/kg bwt and 400mg/kg bwt of methanolic extract of *Vernonia amygdalina* respectively. The diarrheal episodes were observed for 1-7 hour and the cumulative frequency of wet and formed stools were noted on the 4th-7th hour. It was observed that 400mg/kg body weight of the extracts reduced the fecal spot compared to 200mg/kg and 100mg/kg body weight. *Vernonia amygdalina* extracts were found to be non-toxic. The phytochemical screening of the methanolic extract of *Vernonia amygdalina* revealed the presence of some bioactive components which may be responsible for the antidiarrhea properties observed in this study. The above findings suggest that the methanolic extracts of *Vernonia amygdalina* contain active constituents that have antidiarrhea activities that may lead to its use in treating diarrhea.

Keywords: *Vernonia amygdalina*, diarrhea, castor oil, loperamide

1.0 Introduction

Diarrhea disease is the cause of almost three million deaths mainly among children younger than five years of age [1]. Worldwide the annual global burden of diarrhea is enormous, involving 3 to 5 billion cases and nearly 2 million deaths, accounting for almost 20% deaths in children below five years [2]. In Africa, the diarrhea-specific mortality in children has been estimated at

about 106 per 1000 [3]. Available reports in Nigeria indicate that more 315,000 deaths of preschool age children are recorded annually as a result of diarrhea disease [4]. Diarrhea is related to a wide range of bacteria (such as *Escherichia coli*, *Salmonella spp.*, *Shigella spp.*, *Vibrio cholerae*), enteroparasites (such as *Giardia lamblia*, *Cryptosporidium spp.*, *Entamoeba histolytica*), and viruses (such as adenovirus and rotavirus) [5,6,] The contribution of the various pathogens to diarrhea may differ substantially between regions depending on local meteorological, geographic and socio-economic conditions [7]. Underlying reasons for the spread of diarrhea diseases are found in poor hygiene and sanitation, limited access to safe drinking water as well as in adequate education of health care providers and recipients [8] Diarrhea symptoms are characterized by increased number of loose or watery stools, fluid loss and dehydration [9,10] Diarrhea is treated mostly with antimicrobial drugs, but this treatment is generally ineffective [11,12,13]. In this study Loperamide is used as a positive control, it is a commonly use opioid antidiarrhea agent which acts by increasing colonic phasic segmenting activities through inhibition of presynaptic cholinergic nerves in the sub-mucosal and myenteric plexuses. These effects result in fecal water absorption thus reducing the frequency of defecation [14]. There are increase in antibiotic resistance among enteric pathogens which is a major concern now since the empirical treatment has lead to risk of persistent diarrhea [9]. Therefore, there is need for a quick development of antibacterial drugs that will be more effective than those currently in use. *Vernonia amygdalina* commonly known as bitter leaf is a shrub that peaks in height around 3 meters. It grows in several parts of Africa, including the tropics and particularly South Africa, Zimbabwe and Nigeria [15-17]. *Vernonia amygdalina* may be effective against amoebic dysentery [18]; gastrointestinal disorders [19-20]; microbial and parasitic activities [21-22]; hepatotoxicities [23]; and cancer [24-29]. This study was based to explore the possibility of using herbs such as *Vernonia amygdalina* for treatment of diarrhea. The aim of this study is to determine the effect of *Vernonia amygdalina* methanolic extracts as anti diarrhea agents and to determine if they posses any toxicological effects as a proof of ascertaining their safety.

2.0 Materials and Methods

2.1 Collection, Preparation and Extraction of *Vernonia amygdalina*

The *Vernonia amygdalina* was obtained from Masaka market a suburb of Abuja, Nigeria, it was identify and authenticated by Mrs Tarfa D. Florence, The H.O.D, Medicinal Chemistry and Quality Control, National Institute of Pharmaceutical Research and Development, Abuja, Nigeria. Voucher specimen number of the plant NIPRD/09/014. These leaves were dried for fifteen days at ambient laboratory temperature (20-28°C). It was milled to a fine powder using commercial blender (Blendtec), and stored in the dark at laboratory temperature in closed containers until required. It was then dried in room temperature (37%), for about a month. The powdered sample of *Vernonia amygdalina* leaves were weighed using electric balance. 22.8g *Vernonia amygdalina* were used for the methanolic extraction, using soxhlet apparatus. The Methanolic extract was dried invacuo using rotary evaporator.

Determination of the percentage yield of the crude extract

$$\text{Percentage yield (\%)} = \frac{\text{Weight of the plant extract}}{\text{Weight of the dried plant sample used}} \times 100$$

2.2 Phytochemical screening of *Vernonia amygdalina*

The methanolic extracts of *Vernonia amygdalina* leaves were screened in the presence of phytochemical compounds as described by Evans [30]

Test for alkaloids

1.0ml of the methanolic extracts, few drops of dragendorff reagent were added. An orange colour developed, indicating the presence of alkaloid.

Test for Flavonoids

1.0ml of the methanolic extract was heated with 10ml of ethyl acetate over a steam bath for 3 minutes. The mixture was filtered and 4ml of the filtrate was shaken with 1ml of dilute ammonia solution. A yellow colouration was observed indicating a positive test for flavonoids

Test for Tannins

82 Ferric chloride test:-1.0ml of the methanolic extract was diluted with 4.0ml of distilled water (in
83 ratio 1:4) and few drops of 10% ferric chloride solution were added. Blue or green precipitate or
84 colouration formed, indicating the presence of tannins.

85 **Test for saponin**

86 To 0.5ml of the methanolic extract, 5.0ml distilled water was added vigorously for 2 minutes.
87 Then few drops of olive oil were added. Formation of emulsion showed the presence of saponin.

88 **Test for steroids**

89 Two ml of acetic anhydride was added to 0.5ml of the methanolic extracts with 2 ml H_2SO_4 . The
90 colour changed from violet to blue indicating the presence of steroids.

91 **Test for terpenoids (Salkowski test)**

92 0.5ml of the methanolic extracts was mixed in 2 ml of chloroform and concentrated H_2SO_4 (3 ml)
93 was carefully added to form a layer. A reddish brown colouration of the inner face was formed
94 indicating the presence of terpenoids.

95 **Test for Anthraquinones:**

96 0.5ml of the methanolic extract was boiled with 10ml H_2SO_4 and filtered while hot. The filtrate
97 was shaken with 5ml of chloroform. The chloroform layer was pipette into another test tube and
98 1ml of dilute ammonia was added. The resulting solution was observed for a greenish-yellow for
99 *Vernonia amygdalina* was extracts.

100 **Test for Cardiac Glycolyses (Keller-Killani Test):**

101 1ml of the methanolic extracts was treated with 2ml of glacial acetic acid containing one drop of
102 ferric chloride solution. This was underlayered with 1ml of concentrated sulphuric acid. A brown
103 ring at the interface indicated deoxysugar characteristics of cardenolides.

104 **2.3 Animals**

105 Experimental rats were purchased from the animal house of the Bingham University. The
106 animals were housed in steel cages and kept at room temperature; the animals were allowed to

107 stabilize for 21 days prior to the commencement to the experiment. All the rats were given water
108 and pelleted standard laboratory feed, *ad libitum*. Food was withheld for 24 hours prior to each
109 experiment.

110 2.3.1 Experimental Design

111 Twenty five (25) white male albino rats of body weight ranging between 120-227g were induced
112 with castor oil for development of diarrhea. The animals were divided groups; A, B, C, D and E,
113 containing five rats in each group. The rats of group sub group A served as the negative control
114 group (no treatment, castor oil administered only), group B served as the positive control
115 group(administered with a standard drug for diarrhea, loperamide), groups C, D and E were all
116 induced with the diarrheal dose of castor oil. The rats in each group were placed singly in cages.
117 The diarrheal episodes were observed for 1-7 hour and the cumulative frequency of wet and
118 formed stools were noted on the 4th-7th hour. Treatments with plant samples (100mg/kg body
119 weight, 200mg/kg body weight and 400mg/kg body weight) then followed.

120 **Table 1: Experimental design**

GROUP	DESCRIPTION	TREATMENT
A	Control	Normal saline
B	Standard control	Administered with 2 mg/kg body weight of loperamide
C	Treatment group	Administrated with 1 ml castor oil induced Diarrhea and treated with 100mg/kg body weight extract 6 hours
D	Treatment group	Administrated with 1 ml castor oil induced Diarrhea and treated with 200mg/kg body weight extract for 6 hours
E	Treatment group	Administrated with 1 ml castor oil induced Diarrhea and treated with 400mg/kg body weight extract for 6 hours

121

122 2.4 Induction of Diarrhea

Diarrhea was induced by single dose (1ml) oral administration using the castor oil induced model in all the five groups of the white male albino rats. Anti diarrhea activity of the extract was evaluated using the castor oil induced diarrhea model in rats

2.5 Toxicity test

To evaluate the level of toxicity in both extracts, animals were randomly divided into three groups of three rats with different sizes after being weighed and then extracts were given orally ranging from 600mg/kg and 1000mg/kg body weight. Acute toxicity study was carried out according to the modified method described by Lorke

3.0 Results

Table 2: Number of fecal spot in experimental rats

GROUP	DESCRIPTION	NUMBER OF FETAL SPOT
A	Control	5.1±1.2 ^{a*}
B	Treatment with 2mg/kg body weight Loperamide	1.0±0.5 ^b
C	Treatment with 100mg/kg body weight of lemon grass	2.8±0.8 ^{d,b}
D	Treatment with 200mg/kg body weight of lemon grass	1.5±0.9 ^{e,b}
E	Treatment with 400mg/kg body weight of lemon grass	0.5±0.3 ^{f,b}

Values are expressed as mean ± standard deviation, *Superscripts that are different indicate significant differences

Table 3: Phytochemical screening of methanolic extracts *Vernonia amygdalina*

Phytochemical constituents	Inference
Tannins	+
Saponins	+
Flavanoids	+
Alkaloid	+
Glycosides	+
Cardiac glycosides	+
Phlbatannins	+
Steroids	-

Anthraquinones	ND
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137 **Acute toxicity study:** The animals were observed for forty-eight (48) hours for signs of toxicity,
 138 but no mortality was recorded in any of the three (3) groups thus indicating that the plant was not
 139 acutely toxic.

140 **4.0 Discussion**

141 Diarrhea was induced in the experimental rats by the administration of single dose of 1 ml castor
 142 oil. The castor oil model was chosen for these pharmacological studies, because of its chemical
 143 properties. The chemistry of castor oil is centered on its high content of ricinoleic acid, a product
 144 of ricinoleate which is reported to be responsible for the diarrhea inducing property of castor oil
 145 [31] Ricinoleic acid stimulates peristaltic activity in the small intestine, leading to change in the
 146 electrolyte permeability of the intestinal mucosa. Ricinoleic extracts contain flavonoids which
 147 modify the production of cyclo-oxygenase (COX-1 and 2) and lipoxygenase (LOX) [32] which
 148 are essential in prostaglandin biosynthesis. Ricinoleic acid action also stimulates the release of
 149 endogenous prostaglandin [33] which contributes to the pathophysiological functions in the
 150 gastrointestinal tract [34]. This release of prostaglandins is a major cause of rachidonic-induced
 151 diarrhea [35] which is characterized by an increase in intestinal transit time and an increase in
 152 wet faeces. In this study Loperamide is used as a positive control, it is a commonly use opioid
 153 antidiarrhea agent which acts by increasing colonic phasic segmenting activities through
 154 inhibition of presynaptic cholinergic nerves in the sub-mucosal and myenteric plexuses. These
 155 effects result in fecal water absorption thus reducing the frequency of defecation [36]. The
 156 biologically-active compounds of *Vernonia amygdalina* are saponins and alkaloids [37];
 157 terpenes, steroids, coumarines, flavonoids, phenolic acids, lignans, xanthonenes and
 158 anthraquinones [38-41]; edotides [42]; tannis and [43]; sesquiterpene lactone [44-46]. These
 159 compounds isolated from *Vernonia amygdalina* extracts using various solvents of different
 160 polarity indexes, have been attributed to specific biological activities such as antiplasmodial
 161 activity [40] antitumor activities in humans [43]. In this study, qualitative phytochemical analysis
 162 was carried out on methanolic extracts of *Vernonia amygdalina* (Tables 3) revealed the presence
 163 of some important bio-active components such as tannins, alkaloids, carbohydrates, flavonoids,
 164 glycosides, cardiac glycosides. Some of these components may be responsible for the

antidiarrhea properties of the methanolic extracts of *Vernonia amygdalina*. Flavonoids also possess anti-oxidant activity which is presumed to be responsible for inhibitory effects on several enzymes including those involved in arachidonic acid metabolism. Early studies have also reported that anti-diarrheal activity of medicinal plants may be due to alkaloids, saponins, tannins, sterols and reducing sugar [36]. Therefore, it is most likely that these constituents either single or in combination, contribute to the anti-diarrhea activities of the extracts. In the present study, on the 4th -7th hours after the castor oil challenge, the cages were inspected for the presence of characteristic diarrhea droppings. Methanol extracts of *Vernonia amygdalina* leaves at 100 mg/kg, 200 mg/kg and 400 mg/kg body weight was administered orally to the animals which reduced the fecal spots (Table 2.). It was observed that at 400mg/kg body weight of methanolic leaf extracts of *Vernonia amygdalina* there was drastic reduction of fecal spots compared to the 200mg/kg and 100mg/kg body weight respectively. The significance of this result indicates that more of the extracts can be taken for effective treatment of diarrhea since methanolic leaf extracts of *Vernonia amygdalina* were confirmed not to be toxic.

5.0 CONCLUSION

The anti diarrhea levels were analyzed in loperamide-treated control and extract-treated albino rats. The methanol leaf extract of *Vernonia amygdalina* neutralized the ricinoleic acid responsible diarrhea inducing property of castor oil under the normal treatment period of diarrhea, the treatment of diarrhea using the extract have been successful without any degree of toxicity to the experimental subject. Therefore, it can be concluded that *Vernonia amygdalina* may possess anti-diarrhea properties for effective treatment of diarrhea.

7.0 REFERENCES

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