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# Evaluation of the Acute Toxicity and Antidiarrhoeal Effect of the Aqueous Extract of *Acacia nilotica* Pods in Wistar Rats

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***Abstract***: *Plant parts are utilized in conventional herbal therapy practice for illness management and cure. Many of these plants are extremely helpful to the pharmaceutical industry in the synthesis of modern drugs. Acacia nilotica is a member of the Fabaceae family. It's a medium-sized, prickly tree with compressed, long, grey pods between seeds and yellow flowers.This study investigated the phytochemical constituents, acute toxicity as well as antidiarrhoeal activity of the aqueous extract of Acacia nilotica pods in Wistar strain albino rats. The phytochemical components of the extract were evaluated by qualitative and quantitative test. The study revealed that saponins was found to be the most abundant phytochemical (133.92 ± 1.80 µg/ml) followed by tannins (102.45 ± 0.40 µg/ml), flavonoids (93.44 ± 2.12µg/ml), phenols (81.24 ± 0.93 µg/ml) and terpenoids (46.31 ± 0.58 µg/ml). Alkaloid content of the sample had the least quantity (18.91 ± 0.22 µg/ml). The $LD_{50}$ of the extract was found to be ≥ 5000 mg/kg body weight of the rats as no mortality was observed. The results showed that the extract produced a statistically significant ($p<0.05$) reduction in the frequency of diarrhoea produced by castor oil, castor oil-induced intestinal fluid accumulation and transit of charcoal meal at all doses tested. The presence of the bioactive compounds may be responsible for some of the pharmacological activities including antidiarrhoeal activity of the pods of Acacia nilotica and thus could be of considerable interest to the development of new drugs.*

***Keywords:*** *Phytochemicals, Acute Toxicity, Antidiarrhoeal Activity, Wistar Rats.*

## INTRODUCTION

Potential pharmaceuticals have been hidden away in the plant kingdom since ancient time. The significance of medicinal plants have come to light more and more in recent years. Plant based pharmaceuticals are broadly accessible, less costly, safe, effective, and hardly have negative side effects. According to Pritish *et al*. (2015), chemical entities for synthetic drugs, food supplements, folk remedies, nutraceuticals, and traditional and modern medical systems' medica tions. The totality of knowledge, skills, and practices based on ideas, opinions, and personal experiences indigenous to various traditions, whether they are explicable or not , used in the maintenance of health as well as in the prevention, diagnosis, improvement, or cures for physical and mental conditions" is therefore referred to as traditional medicine (WHO, 2000).

Diseases and life are correlated: in environments with life, diseases also exist. Plants provide food, fiber, and shelter for both humans and animals. Moreover, plants have long been utilized as medicines, a safe and effective way to treat and manage illness. Native Americans have discovered a variety of herbs that are used to treat illnesses on occasion. For medical care, more than 25% of the world's population primarily uses plants and plant extracts (Verma, 2016). Approximately thirty percent of all plant species were utilized medicinally at some point (Verma, 2016).

Increased frequency of bowel movements and abdominal pains are the hallmarks of diarrohea. It's among the primary causes of malnutrition and deaths for children of the world's underdeveloped countries (Nitai *et al.,* 2011).

Herbal medicines have garnered significant attention in recent times as a means of treating various disorders. Traditional healers use a variety of medicinal plants that have antidiarrhoeal qualities extensively. Nevertheless, many of these traditional remedies for diarrhoea have not had their efficacy objectively assessed (Chaulya *et al.,* 2010).

*Acacia nilotica* is a member of the *Fabaceae* family, commonly called: Bagaruwa (Hausa), Kangar (Kanuri) and Gawari (Fulani). It's a medium-sized, prickly tree with compressed, long, grey pods between seeds and yellow flowers that resemble mimosas. There are black fractures in the bark and branches. The branches have two-centimeter-long spikes. According to Desphande and Kadam (2013), the leaves are thin and heavily hairy, with three to six pairs of pinnate leaflets that are small, have parallel margins, and are rounded at the tip with a central midrib that is densely packed. The plant's powdered bark, which contains very little salt, is used to cure acute diarrhoea. Furthermore it is broadly utilized to cure leucoderma, colds, cough, diarrhoea, and bleeding piles (Del, 2009). Teeth brushes are made from the delicate twigs (Meena *et al.,* 2006).

## EXPERIMENTAL SECTION

### Sample Collection and Identification

The pods of *Acacia nilotica* was collected from the natural environment growing around the Federal Polytechnic Damaturu, Yobe State, Nigeria. It was identified and authenticated by a Botanist from the Science Laboratory Technology Department of the Polytechnic; Dr. Ibrahim Babale Gashua. It was washed to remove unwanted adhering material and shade-dried at room temperature. It was pulverized using pestle and mortar to fine powder. The fine powder was then sieved with a 0.01 mm sieve size and kept in a dried grease free polyethylene bag for further analysis.

### Extract Preparation

Crude powder (500 g) was subjected to a cold extraction process with distilled water at room temperature for 7 days. The extraction was done in aliquot. The solution was filtered with Whatman No.1 filter paper and the filtrate was evaporated to dryness using a rotary evaporator at a temp. range of 40-50 $^{o}$C. The concentrate (extract) was weighed, percentage yield calculated, labelled, and stored at 4$^{0}$C in a refrigerator until required (Azwaninda, 2015).

**Phytochemical Studies**

**Qualitative Phytochemical Analysis**

**Qualitative phytochemical analyses of the extract were carried out according to the methods described by** (Sofowara 1984), (Harbone, 1998), (Awe and Sodipo, 2001), (Trease and Evans, 2002) and (Edoga, 2005).

**Quantitative Phytochemical Analysis**

**Quantitative phytochemical analyses of the extract were carried out according to the methods described by** (Krishnaiah *et al*., 2009), (Rasool, *et al*., (2010), (Matthias *et al*., 2015) and (Velavan, 2015).

**Experimental Animals**

**White Wistar strain albino rats of both sexes weighing between 150–200 g were acquired from the animal house of the Department of Biochemistry,** Yobe State University, Damaturu**, Nigeria. All animals were used after 1 week of acclimatization, they had free access to water and food.** The guidelines and protocol review of the Canadian Council on Animal Care contain the well recognized standards for the use and care of laboratory animals (1993) was duly observed.

**Acute Oral Toxicity ($LD_{50}$)**

The extract's median lethal dose ($LD_{50}$), which serves as a safety indicator, was ascertained using the two-phase method outlined by Lorke (1983). In phase one, nine (9) male Wistar albino rats were randomly split into three groups of three rats each and denied access to food but not water. The extract was given orally to the animals in groups 1, 2, and 3 at doses of 10 mg/kg, 100 mg/kg, and 1000 mg/kg, respectively. The animals were observed for behavioral changes, toxicity symptoms, and mortality for two hours, four hours on occasion, and for the next twenty-four hours following treatment. Three male rats were split into three groups of one rat each at random for the second phase of the experiment, which was inferred from the first. The extract was given to the rats in groups 1, 2, and 3 at a dose of 1600 mg/kg, 2900 mg/kg and 5000 mg/kg respectively. Every treatment was administered orally, and the results were observed as previously mentioned. The rats were further observed for up to 14 days following treatment for any sign of toxicity and mortality.

**Antidiarrhoeal Activity Studies**

**Effect of Aqueous Extract of *Acacia nilotica* Pods on Castor Oil induced Diarrhoea in Wistar Rats**

*In-vivo* antidiarrhoeal activity was studied using castor oil induced model in albino Wistar rats. The animals were grouped into five groups after random selection, each group contained five albino rats of Wistar strain (6-8 weeks) of age of either sex in separate cages having paper placed below for collection of faecal matter. One hour before oral administration of castor oil (2 ml/rat), Group I received normal saline orally (5 ml/kg body weight) and served as the control. Group II animals received loperamide (5mg/kg, oral) while groups III, IV and V received the extract at doses of 200, 400 and 800 mg/kg body weight via oral intubation respectively. The number of both dry and wet faecal droppings were counted each hour for 6 hours and the papers were changed every hour and then antidiarrhoeal activity was determined in terms of percentage inhibition using the formula below;

$$\text{Percentage Inhibition} = \frac{\text{mean number of (faeces of control group – faeces of test group)}}{\text{mean number of faeces of control group}} \times 100$$

**Effect of Aqueous Extract of *Acacia nilotica* Pods on Intestinal Propulsion (transit time) of Charcoal Meal (CM) in Wistar Rats**

The rats were divided into five groups of five animals each and fasted for 18 h but water was freely provided. The first group (control group) received normal saline (5 ml/kg body weight) orally, while the second, third and fourth groups were given the plant extract (orally) in doses of 200, 400, and 800 mg/kg body weight, respectively. The fifth group received the standard drug, loperamide (5 mg/kg body weight) orally. Thirty (30) min later, each animal was given 1 ml/rat of charcoal meal (10% activated charcoal in 5% gum acacia) via the oral route. All animals were sacrificed 30 min thereafter and the distance covered by the charcoal meal in the intestine, from the pylorus to the caecum was measured and expressed as percentage of distance moved (Shettima *et al.,* 2012).

**Effect of Aqueous Extract of *Acacia nilotica* Pods on Castor Oil Induced Fluid Accumulation (Enteropooling) in Wistar Rats**

Intraluminal fluid accumulation was determined. The rats were divided into five groups of five animals each, one hour before oral administration of castor oil (2 ml/rat). Group 1 received normal saline orally (5 ml/kg body weight) and served as the control. Group 2 animals received loperamide (5mg/kg, oral) while groups 3, 4 and 5 received the extract at doses of 200, 400 and 800 mg/kg body weight via oral intubation respectively. Two hours later, the rats were sacrificed and the small intestine from the pylorus to the caecum was isolated. The intestinal contents were collected by milking into a graduated tube and their volume measured (Shettima *et al.,* 2012).

**Statistical Analysis**

Data generated was analysed using Completely Randomized Design (CRD). All values were expressed as Mean ± S.D. Duncan's multiple range test was employed to test for the difference between the means utilising computer aided software called Statistical Package for Social Sciences (SPSS) version 20.0. Values of $p \leq 0.05$ was considered statistically significant.

**Results**

**Phytochemical Analysis**

**Qualitative Phytochemical Constituents of Aqueous Extract of *Acacia nilotica* Pods**

The phytochemical constituents of the *Acacia nilotica* aqueous pods extract (table 1) revealed the presence of alkaloids, cardiac glycosides, flavonoids, phenols, saponins, tannins and terpenoids. However, steroids were not detected in the extract.

**Table 1: Qualitative Phytochemical Constituents of Aqueous Extract of *Acacia nilotica* Pods**

| S/No | PhytochemicalConstituents | Test | Inference |
|---|---|---|---|
| 1. | Alkaloids | Mayer's Test | + |
| 2. | Cardiac glycosides | Liebermann Buchard's Test | + |
| 3. | Flavonoids | Shinoda's Test | + |
| 4. | Phenols | Ferric Chloride Test | + |
| 5. | Saponins | Frothing Test | + |
| 6. | Steroids | Salkowski's Test | _ |
| 7. | Tannins | Ferric Chloride Test | + |
| **8.** | Terpenoids | Salkowski's Test | + |

**Keys: +** = Present - **=** Absent

**Quantitative Phytochemical Determination of Aqueous Extract of *Acacia nilotica* Pods**

The results of the quantitative phytochemical determination of *Acacia nilotica* aqueous pods extract revealed that saponins was found to be the most abundant phytochemical (133.92 ± 1.80 µg/ml) followed by tannins (102.45 ± 0.40 µg/ml), flavonoids (93.44 ± 2.12µg/ml), phenols (81.24 ± 0.93 µg/ml) and terpenoids (46.31 ± 0.58 µg/ml). Alkaloid content of the sample had the least quantity (18.91 ± 0.22 µg/ml).

**Table 2: Quantitative Phytochemical Determination of Aqueous Extract of *Acacia nilotica* Pods**

| S/N | Phytochemicals | Concentration (µg/ml) |
|---|---|---|
| 1. | Alkaloids | 18.91 ± 0.22 |
| 2. | Flavonoids | 93.44 ± 2.12 |
| 3. | Phenols | 81.24 ± 0.93 |
| 4. | Saponins | 133.92 ± 1.80 |
| 5. | Tannins | 102.45 ± 0.40 |
| 6. | Terpenoids | 46.31 ± 0.38 |

Data are expressed as mean ± S.D of three replicates

**Acute Toxicity Test**

The median lethal dose of the aqueous extract of *Acacia nilotica* pods was determined to be greater than 5000 mg/kg as the rats did not exhibit any indicators of weakening or mortality during the period of the test.

**Table 3: Acute Lethal Effect of Aqueous Extract of *Acacia nilotica* Pods**

**In Wistar Albino Rats**

| Experiment (mg/kg bw) | Dose after 24hrs | Number of Dead Rats |
|---|---|---|
| Phase-1 | 10 | 0/3 |
| | 100 | 0/3 |
| | 1000 | 0/3 |
| Phase 2 | 1,600 | 0/1 |
| | 2,900 | 0/1 |
| | 5,000 | 0/1 |

**Effect of Aqueous Extract of *Acacia nilotica* Pods on Castor oil Induced Diarrhoea in Wistar Albino Rats**

Table 4 shows the effect of aqueous extract of *Acacia nilotica* pods on castor oil induced diarrhoea in Wistar albino rats. The extract exhibited a considerable level of activity against castor oil-induced diarrhoea in experimental rats at various doses of 200, 400 and 800 mg/kg body weight. All the doses significantly decreased ($p < 0.05$) the total number of faeces produced by administration of castor oil (5.50 ± 0.74 at the dose of 200 mg/kg, 4.60 ± 1.05 at the dose of 400 mg/kg and 2.40 ± 0.85 at the dose of 800 mg/kg) as compared to the castor oil-treated control group (13.0 ± 1.35). The percentage inhibition of castor oil-induced diarrhoea in the extract treated rats was 57.69, 64.62 and 81.54 % respectively at 200, 400 and 800 mg/kg doses of extract.

**Table 4: Effect of Aqueous Extract of *Acacia nilotica* Pods on Castor oil Induced Diarrhoea in Wistar Albino Rats**

| Treatment | Dose (mg/kg) | Total number of Faeces | Percentage Inhibition (%) |
|---|---|---|---|
| Gp I (Control) Normal saline | 10ml | 13.0 ± 1.35 | 0 |
| Gp II (Standard) Loperamide | 5 | 0.6 ± 0.13 | 95.40 |
| Gp III (Extract) | 200 | 5.50 ± 0.74[a] | 57.69 |
| Gp IV (Extract) | 400 | 4.60 ± 1.05[ab] | 64.62 |
| Gp V (Extract) | 800 | 2.40 ± 0.85[b] | 81.54 |

Data are expressed as means ± SD of five replicates. Values with different superscript (alphabets) along each row are statistically significantly different ($p < 0.05$) relative to normal control.

**Effect of Aqueous Extract of *Acacia nilotica* Pods on Intestinal Propulsion (transit time) of Charcoal Meal (CM) in Wistar Rats**

Table 5 shows the effect of the aqueous extract of *A. nilotica* pods on intestinal propulsion (transit time) of charcoal meal in Wistar rats. The extacts (200-800 mg/kg) given orally significantly ($P < 0.05$) reduced the gastrointestinal distance travelled by the charcoal meal in rats compared with control group. The extract produced a dose dependent decrease of gastrointestinal transit in rats. The gastrointestinal transit of charcoal meal produced by loperamide (5 mg/kg) was similar to that of 800 mg/kg of *A. nilotica* pods extract.

**Table 5: Effect of Aqueous Extract of *Acacia nilotica* Pods on Intestinal Propulsion (transit time) of Charcoal Meal (CM) in Wistar Rats**

| Treatment | Dose (mg/kg) | Mean Intestinal Length | Mean Distance travelled by CM | Percentage Distance travelled by CM (%) |
|---|---|---|---|---|
| Gp I (Control) Normal saline | 10ml | 82.31 ± 5.6 | 58.32 ± 2.61 | 70.85 |
| Gp II (Standard) Loperamide | 5 | 97.51 ± 4.8 | 41.32 ± 5.20 | 42.38 |
| Gp III (Extract) | 200 | 98.81 ± 8.3 | 51.21 ± 4.21[a] | 51.82 |
| Gp IV (Extract) | 400 | 89.38 ± 3.8 | 48.58 ± 2.20[b] | 54.35 |
| Gp V (Extract) | 800 | 80.85 ± 6.5 | 38.50 ± 3.21[c] | 47.62 |

Data are expressed as means ± SD of five replicates. Values with different superscript (alphabets) along each row are statistically significantly different ($p < 0.05$) relative to normal control.

**Effect of Aqueous Extract of *Acacia nilotica* Pods on Castor Oil Induced Fluid Accumulation (Enteropooling) in Wistar Rats**

Table 6 shows the findings on the effect of aqueous extract of *A. nilotica* pods on castor oil-induced enteropooling. When compared to the negative control (Gp I), studies on this model revealed that the test extract (Gp III, IV and V) significantly ($p < 0.05$) reduced the intraluminal volume of fluid accumulation at 200, 400 and 800 mg/kg respectively. The decrease in the volume of fluid accumulation was dose dependent. The percentage inhibition of the intestinal fluid accumulation from the extracts (200, 400 and 800 mg/kg dose) was 65.62 %, 78.48 % and 86.15 % respectively. The percentage inhibition also showed a dose dependent activity. Loperamide produced 100 % inhibition of the intestinal fluid with 0.00 mean volume of intestinal fluid. The inhibition produced by the extract treated rats are significantly different ($P<0.05$) relative to the control rats and the loperamide-treated rats.

**Table 6: Effect of Aqueous Extract of *Acacia nilotica* Pods on Castor Oil Induced Fluid Accumulation (Enteropooling) in Wistar Rats**

| Treatment | Dose (mg/kg) | Mean Volume of Intestinal Content (ml) | Percentage Inhibition of Intestinal Fluid (%) |
|---|---|---|---|
| Gp I (Control) Normal saline | 10ml | 3.81 ± 0.25 | 0.00 |
| Gp II (Standard) Loperamide | 5 | 0.00 ± 0.0 | 100 |
| Gp III (Extract) | 200 | 1.31 ± 0.42[a] | 65.62 |
| Gp IV (Extract) | 400 | 0.82 ± 0.21[b] | 78.48 |
| Gp V (Extract) | 800 | 0.68 ± 0.18[bc] | 86.15 |

Data are expressed as means ± SD of five replicates. Values with different superscript (alphabets) along each row are statistically significantly different ($P < 0.05$) relative to normal control.

**Discussion**

Medicinal plants are possible sources of antidiarrhoeal medications. As a result, Global organizations especially the World Health Organization (WHO), have encouraged research using traditional medical methods to treat and prevent diarrhoeal illnesses. Diarrhoeal infections can be a significant cause of various diseases and deaths in underdeveloped nations, especially in small children and young animals (Attia and Mouneir, 2004). Seeds from the Ricinus communis (Euphorbiaceae) tropical shrub are used for the production of castor oil. It is well known that this oil increases the amount of aqueous luminal matter that passes easily via the large and small intestines through altering the permeability of water and electrolytes in intestinal mucosal membranes. Ricinoleic acid causes the epithelial cells in the small intestine to contract, stretching the tight junctions. By letting the intestinal lumen to absorb electrolytes and allowing the food's contents to become more hydrated, this mechanism induces diarrhoea (Mohamed *et al*., 2023). Phytochemical analysis of *Acacia nilotica* aqueous pods extract revealed the presence of alkaloids, flavonoids, phenols, saponins, tannins and terpenoids, their presence were also reported by Umaru *et al.,* (2011). Antidiarrhoeal properties of medicinal plants can be attributed to their phytochemical constituents; studies have related antidiarrhoeal properties to the presence of tannins, alkaloids, saponins, flavonoids, sterols and/or triterpenes (Longanga-Otshudi, 2000; Muazu and Usman, 2020). However, a number of investigations have shown that tannins, terpenoids, flavonoids, and saponins have antidiarrhoeal qualities in a range of experimental animal models (Abubakar *et al.*, 2015). According to recent studies, plant extracts containing alkaloids and flavonoids change the synthesis of cyclo-oxygenase 1 and 2 (COX-1, COX-2) and lipo-oxygenase (LOX), which inhibits the synthesis of prostaglandins and autacoids (Abubakar *et al.*, 2015). Flavonoids are a large class of compounds composed of polyphenols

having a vast biological actions, including anti-inflammatory, antispasmodic, and antioxidant qualities. They can also demonstrate antidiarrhoeal activity through lessening the amount of hydro-electrolytic secretions and intestinal motility (Alam *et al.*, 2021).

The median lethal dose ($LD_{50}$) determination of the *Acacia nilotica* pods extract showed no animal deaths within 24 hours of the treatment. From the lowest dose (10 milligram per kilogram body weight) to the maximum dose (5000 milligram per kilogram body weight ), no considerable toxicity symptoms such as breathing difficulties, appetite loss, or general weakness, were reported. As recommended by Lorke (1983), the $LD_{50}$ is regarded as safe if it is greater than 5000 mg/kg b.w. The plant extract was therefore considered to be safe at doses ≤ 5000mg/kg. This is in relation to the study conducted by Umaru *et al*., (2015).
The present study investigated the anti-diarrhoeal effects of the aqueous extract of *Acacia nilotica* pods using various methods, which included castor oil-induced diarrhoea, intestinal transit time and intestinal fluid accumulation. The results showed that the extract produced a statistically significant ($p < 0.05$) reduction in the frequency of diarrhoea produced by castor oil. It was also noted that the extract significantly inhibited ($p < 0.05$) castor oil-induced intestinal fluid accumulation and transit of charcoal meal. The result obtained is similar to the study conducted by Umaru *et al*., (2011). The activity of the extract to inhibit fluid accumulation and gastrointestinal transit in animal models suggests that the part of this plant (pod) may possess antidiarrhoeal agents.

**Conclusion**

In conclusion, experimental evidence obtained in the study indicated that *Acacia nilotica* pods extract may possess constituents that have antidiarrhoeal activity, which could be due to its inhibitory effect on castor oil-induced diarrhoea, gastrointestinal transit and fluid accumulation. The secondary metabolites detected could be responsible for the plant's anti- diarrheal activity through a variety of mechanisms of action. The results provided have justified the claim by locals of the use of this plant as natural antidiarrhoeal agent. However, more research is required to determine the extract's specific mechanism of action and to isolate and identify the active ingredient responsible for that activity.

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