# Effects of *Andrographis paniculata* leaf extract on gastrointestinal muscles

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**The aqueous extract of the leaves of *Andrographis paniculata* (Family: Acanthaceae) was prepared and tested on gastrointestinal smooth muscles of mice, rats and rabbits. The median lethal dose ($LD_{50}$) of the extract was determined. The effects of the extract on rat ileum, rabbit jejunum, rat stomach fundus strip and mice gastrointestinal motility were evaluated. The $LD_{50}$ value in mice was 950 mg/kg i.p. Low concentrations of the extract (1.0 – 4.0 mg/ml) relaxed the isolated rat ileum and rabbit jejunum but did not alter the intrinsic myogenic contraction of the isolated rat stomach fundus strip. Higher concentrations of the extract (8.0 – 32.0 mg/ml) produced a concentration – dependent contraction of the same tissues. Lower doses of the extract (16 – 32 mg/kg p.o.) reduced mice gastrointestinal motility while the higher doses (64 – 500 mg/kg p.o) increased the motility. The contractile effects of acetylcholine (5 ng/ml) and histamine (2.0 µg/ml; 0.04 mg/ml) were blocked by the lower concentrations of the extract (4.0 mg/ml; 2.0 mg/ml) on both rat ileum and rabbit jejunum. Atropine (0.25 – 1.0 µg/ml) reduced the contractile activity of the higher concentration of the extract (16.0 mg/ml) on rat stomach fundus strip while mepyramine (0.25 – 1.0 µg/ml) blocked the contractile activity of higher concentrations of the extract (16.0 mg/ml) on the same tissue. The studies suggest that the aqueous extract of *Andrographis paniculata* exhibited biphasic effects on the gastrointestinal smooth muscles which may be attributable to different mechanisms. These effects may influence the therapeutic dose(s) and application of *Andrographis paniculata* in the treatment of different ailments.**

**Key words:** *Andrographis paniculata*, rat ileum, rabbit jejunum, rat stomach fundus, gastrointestinal motility.

## INTRODUCTION

*Andrographis paniculata* (Burm.) Nees (Acanthaceae) is a herb widely used in ethno medicine (Chaturvedi, 1983; Chang, 1986; Medicinal Plants in Vietnam, 1990; Pharmacopoeia of the People's Republic of China, 1992; Standard of ASEAN herbal medicine, 1993; Singh, 1994). it's uses in the treatment of bacillary dysentery, colitis, diarrhoea of microbial origin, have also been reported (Thanagkul *et al.*, 1985; Chaichantipyuth *et al.*, 1986; Gupta, 1993). According to Gupta (1993), ethanol, chloroform and butanol extracts of *A. paniculata* (300 mg/ml) inhibited *Escherichia coli* enterotoxin-induced secretory response which caused a diarrhoeal syndrome in the rabbit and guinea pig ileal loop assay. Also, Thanagkul et al. (1985) and Chaichantipyuth (1986) reported the use of *A. paniculata* in the treatment of acute diarrhoea caused by microorganisms. The clinical trial of *A. paniculata* extracts for the treatment of HIV infection has also been reported (Calabrese *et al.*, 2000).

Reports have shown that diarrhoea is the cause of 4 – 5 million deaths throughout the world annually and ranges from a mild and socially inconvenient illness to a major cause of malnutrition among children in developing countries (Anonymous, 1979; Syder and Merson, 1982). It has also been reported that HIV/AIDS pandemic is among the most serious natural disasters in recent centuries (Adeyi *et al.*, 2006). According to Piot *et al* (1999), HIV has become endemic in parts of Africa and is a major public health problem on the same magnitude as malaria, diarrhoea and malnutrition. The report of Omalu *et al.* (2007) showed that most of the HIV/AIDS patients had chronic diarrhoea leading to severe weight loss.

The leading cause of morbidity and mortality by diarrhoea in developing countries prompted the World Health Organisation (WHO) to constitute diarrhoeal disease control programme (CDD) which includes studies of traditional medicinal practices, together with the evaluation of health education and prevention approaches to combat the problem of diarrhoea (Anonymous, 1979; Syder and Merson, 1982; Lutterodt, 1989).

Plants constitute one of the greatest resources of nature and have been playing a significant role in the healthcare of a large proportion of the population in the developing countries. In the developed countries also, the popularity of crude herbal products is on the increase. The consumers' preference is shifting from purely synthetic to natural based drugs and this is dictating the basis for the global resurgence in the utilization of such products (Eisenberg *et al.*, 1993, 1998; Goldbeck-wood *et al.*, 1996; Wambebe, 1998; Scimone and Scimone, 1998). This, therefore, justifies the need for scientific evaluation of plants and their products for efficacy and safety as recommended by WHO (1991/92). This study was designed to evaluate the pharmacological effects of the extract of *A. paniculata* on the gastrointestinal smooth muscles in order to account for its values in the treatment of ailments associated with diarrhoea such as HIV/AIDS and those associated with pains such as in colitis. Knowledge of the direct effect of the leaf extract of *A. paniculata* on gastrointestinal smooth muscles may also be used to guide future drug developmental effort from the plant.

## MATERIALS AND METHODS

### Plant preparation and extraction

The plants were raised in the Medicinal Plant garden of National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria. They were harvested 6 – 7 months post-planting between November and December. They were authenticated at the Department of Medicinal Plant Research and Traditional Medicine, NIPRD, Abuja. The leaves were cleaned, air-dried and pulverized. 500 g of the pulverized leaves was extracted by adding boiling water in a 5 liter flask. The mixture was stirred and allowed to stand for 24 h at room temperature after which it was filtered. The filtrate was freeze-dried to give a grayish-brown solid (68.9 g). The percentage yield was 13.8% w/w. The freeze-dried extract was reconstituted with water to obtain desired concentrations for the studies.

### Experimental Animals

Swiss albino mice (20 – 26 g), adult Wistar rats (210 – 230 g) and male rabbits (2.3 -2.5 kg) of both sexes were used for the studies. They were obtained from the Animal Facility Centre (AFC), Department of Pharmacology and Toxicology, NIPRD, Abuja.The animals were maintained under normal environmental conditions with approximately normal 12 h day and night illumination cycle. They were fed *ad libitum* with NIPRD formulated feed and had free access to water except when starvation was needed in the course of the study.

### Chemicals

Atropine, acetylcholine, histamine (all from Sigma Chemical Company, USA), mepyramine (May and Baker) sodium chloride, potassium chloride, sodium dihydrogen orthophosphate, D-glucose monohydrate, sodium hydrogen bicarbonate, calcium chloride, magnesium chloride (All from BDH, Poole, England) and activated charcoal were used for the investigation.

### Phytochemical Screening

The aqueous extract of *A. paniculata* leaves was tested for the presence of phytochemical constituents such as alkaloids, flavonoids, glycosides, tannins and saponins using the standard procedures (Trease and Evans, 1983). The presence of steroids and terpenoids were also tested using Salkowskis test and Liebermann-Burchard test respectively.

### Acute Toxicity Study ($LD_{50}$)

The acute toxicity study was carried out according to the method of Lorkes (1983). The estimation was done in mice using intraperitoneal route. The method estimates the dose of the extract that will cause death of 50% of the animal population through the route of administration. In brief, the method involved the intraperitoneal (i.p). administration of graded doses (10 – 2000 mg/kg) of the extract to different groups of mice. The animals were then observed over 48 h period post treatment for toxicity signs such as nervousness, ataxia, excitement, writhing or death. The $LD_{50}$ value was then estimated using the geometric mean of the highest dose that did not cause death and the lowest dose that caused death.

### Studies on Isolated Rat Ileum

Adult Wistar rats used for the study were starved of feed for 18 h prior to the experiment but allowed access to water. They were sacrificed after a stun and abdomen of each cut open. About 2 cm piece of the rat ileum was removed from each rat, dissected free of adhering mesentery and mounted in a 20 ml organ bath containing aerated Tyrode physiological solution. The physiological solution was constituted in millimole (mM) as NaCl 136.8;

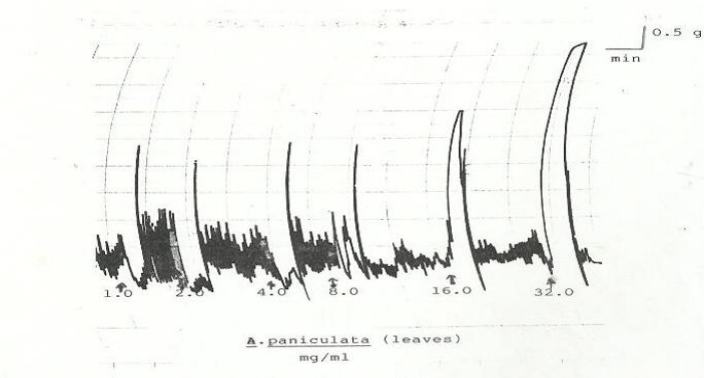


**Figure 1** The biphasic effects of aqueous extract of *Andrographis paniculata* leaves 1.0 - 32.0mg/ml) on rat ileum.

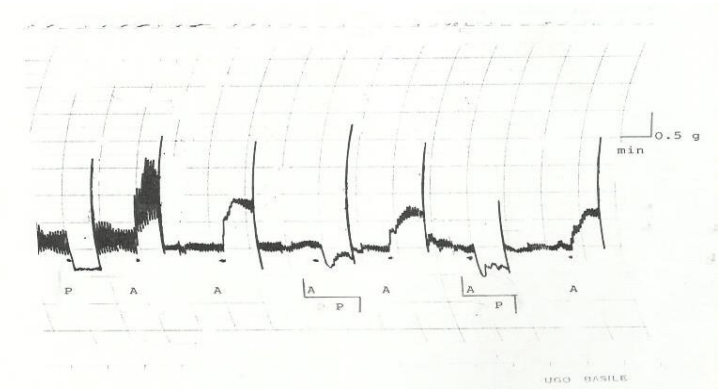


**Figure 2** Inhibitory effects of the aqueous extract of *Andrographis paniculata* leaves, **P** (4.0 mg/ml) on Acetylcholine, **A** (5.0 ng/ml)-induced contractions of the isolated rat ileum.

KCl 2.7; $NaH_2$ $PO_4$ 0.14; D-Glucose 5.5; $NaHCO_3$ 12.0; $CaCl_2$ 1.3; $MgCl_2$ 0.5 dissolved in 1 litre of distilled water and was maintained at $37^0$C. A tension of 0.5 g was applied. The set up was connected to Ugo Basile micro dynamometer recorder 7050 set at sensitively of 3.0 mV and speed of 20 mm/min. At equilibration, the effects of acetylcholine, histamine and different concentrations of the extract were tested. The effects of acetylcholine, histamine, mepyramine and atropine on the tissue responses to the extract were also tested.

**Studies on isolated rabbit jejunum**

Each of the adult rabbits used for the study was starved of feed for about 18 h prior to the experiment. Each rabbit was then stunned by a blow on the head, sacrificed and the abdomen cut open. About 2 cm segment of the jejunum was cut and dissected free of adhering mesentery and was mounted in a 20 ml organ bath containing aerated Tyrode physiological solution (composition as above) maintained at $37^0$C. Tension of 0.5 g was applied. At equilibration, the effects of different concentrations of the extract were tested. The effects of acetylcholine and histamine on tissue responses to the extract were also tested. The responses were recorded on Ugo Basile Unirecorder 7050 set at sensitivity of 1.0 mV and speed of 20 mm/min.

**Studies on Isolated rat Stomach fundus Strip**

Wistar rats used for the study were starved of feed 18 h

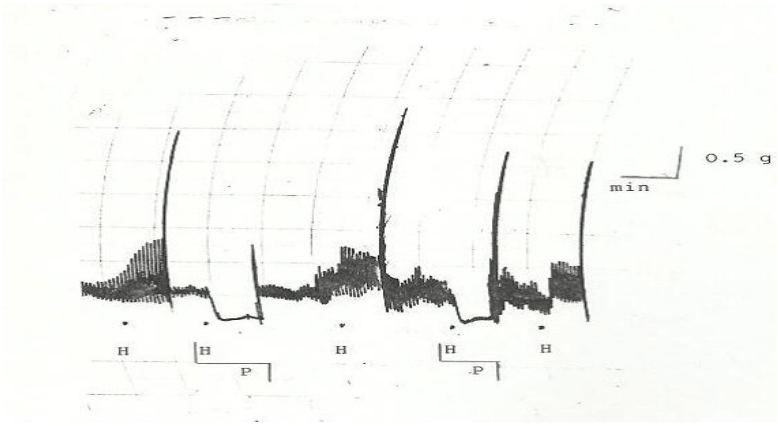


**Figure 3** Inhibitory effects of the aqueous extract of *Andrographis paniculata* leaves, P (4.0 mg/ml) on histamine, H (2.0 µg/ ml)-induced contractions of the isolated rat ileum.

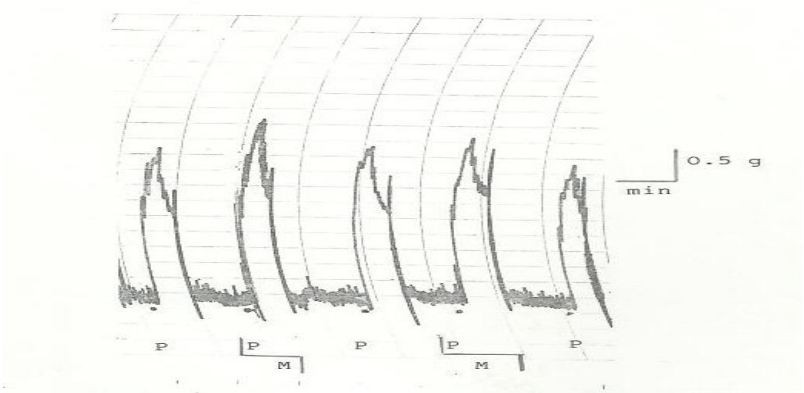


**Figure 4** Interaction of mepyramine, **M** (0.4 µg/ml) and aqueous extract of *Andrographis paniculata* leaves, **P** (16.0 mg/ml) on rat ileum**.**

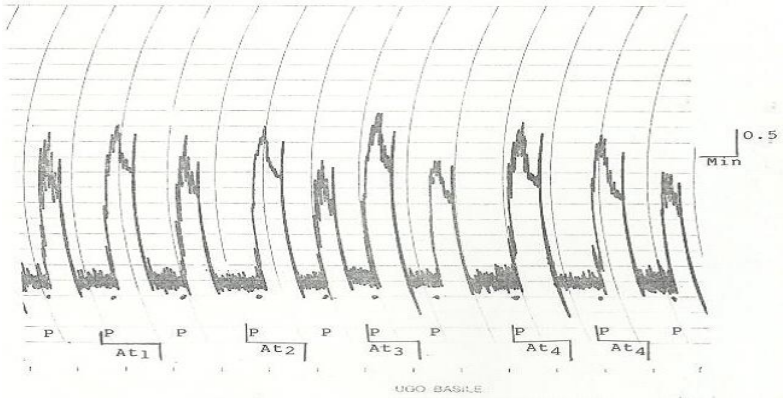


**Figure 5** Interaction of atropine, **At$_1$** (0.1 µg/ml), **At$_2$** (0.2 µg/ml), **At$_3$** (0.4 µg/ml) **At$_4$** (0.8 µg/ml) and aqueous extract of *Andrographis paniculata* leaves**, P** (16.0 mg/ml) on rat ileum.

prior to the experiment. They were stunned by a blow on the head, sacrificed and abdomen of each cut open. The stomach was excised, freed of feed content and a strip of the stomach fundus cut and mounted in a 20 ml organ

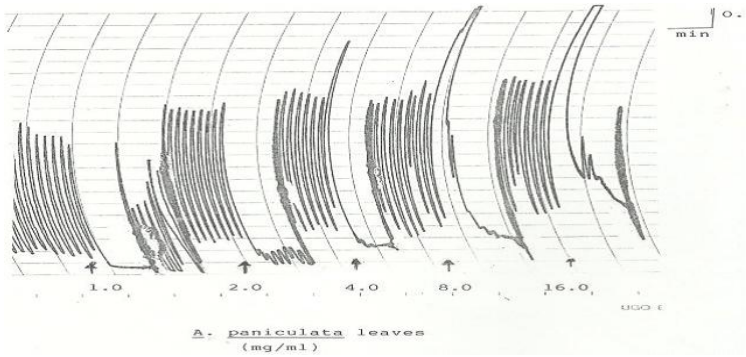


**Figure 6** The biphasic effects of aqueous extract of *Andrographis paniculata* leaves (1.0 – 16.0 mg/ml) on rabbit jejunum.

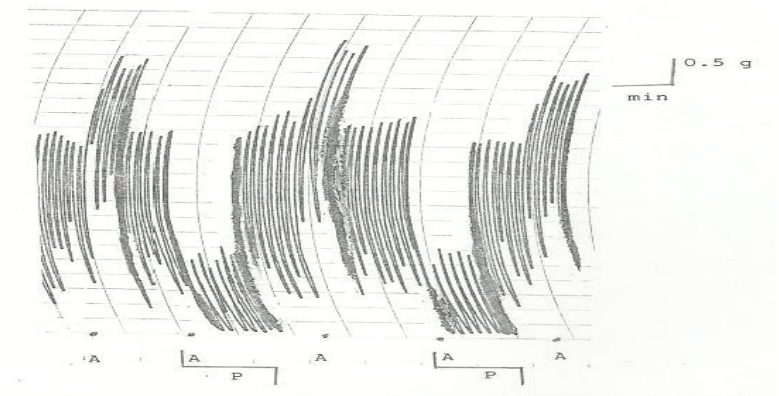


**Figure 7** Inhibitory effects of the aqueous extract of *Andrographis paniculata* leaves, **P** (2.0 mg/ml) on acetylcholine, **A** (5.0 ng/ml)-induced contractions of the isolated rabbit jejunum

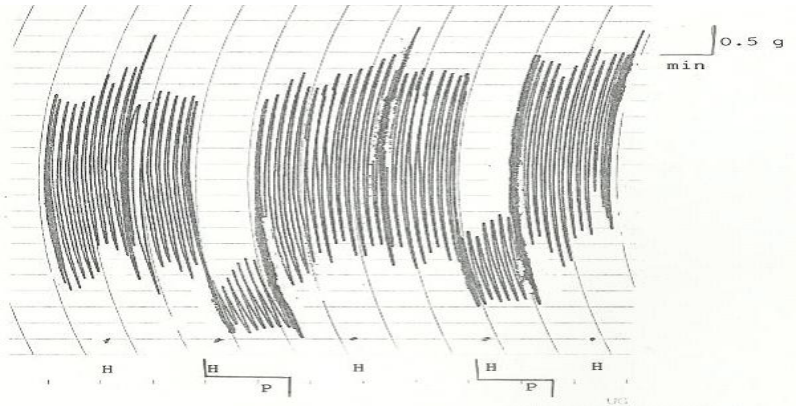


**Figure 8** Inhibitory effects of the aqueous extract of *Andrographis paniculata* leaves, **P** (2.0 mg/ml) on histamine, **H** (0.04 mg/ml)-induced contractions of the isolated rabbit jejunum.

bath containing aerated Tyrode solution maintained at $37^0$C under a tension of 0.5 g.

The effects of graded concentrations of the extract were tested at equilibration. The effects of mepyramine and atropine on the tissue response to the extract were also tested. The responses were recorded on Ugo Basile unirecorder 7050 set at sensitivity of 3mV and speed of 20 mm/min.

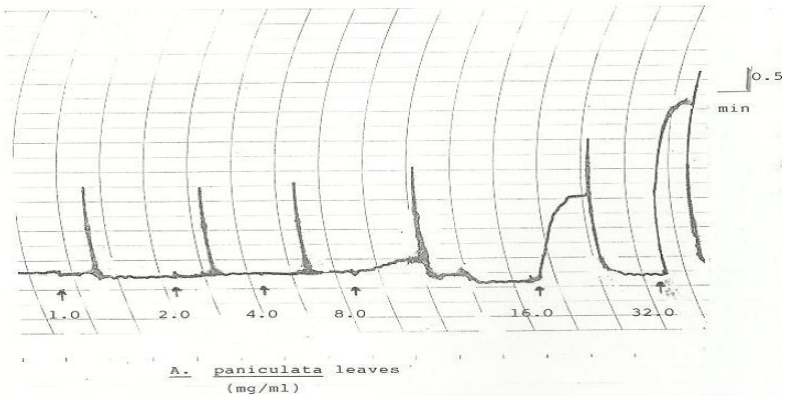


**Figure 9** Dose-dependent contraction of isolated rat stomach fundus strip by higher concentrations (8.0 - 32.0 mg/ml) of aqueous extract of *Andrographis paniculata* leaves.

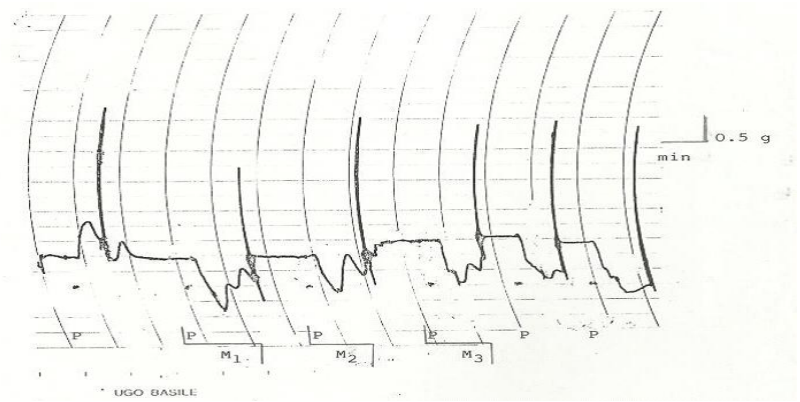


**Figure 10** Inhibitory effects of mepyramine, $\mathbf{M_1}$ (0.25 µg/ml), $\mathbf{M_2}$ (0.5 µg/ml) and $\mathbf{M_3}$ (1.0 µg/ml) on contraction caused by the aqueous extract of *Andrographis paniculata* leaves, **P** (16.0 mg/ml) on rat stomach fundus strip.

### Studies on Gastrointestinal Motility in Mice

This *in vivo* study was done according to the method of Akah *et al.* (1998). Adult Swiss albino mice used for the study were starved of feed 24 h prior to the study but were allowed free access to water. The mice were divided into seven groups (n = 4). The first group received normal saline (10 ml/kg p.o.) and served as the control. Groups two, three, four, five, six and seven were pre-treated orally with 16.0, 32.0, 64.0, 125.0, 250.0 and 500.0 mg/kg of the extract respectively. 5 min post treatment, 0.5 ml of a 5% charcoal in a 10% suspension of tragacanth powder was administered orally to each mouse. 30 min later, all the mice were sacrificed and their abdomen opened. The entire length of the small intestines were dissected out, straightened on a flat surface and measured.

The distance traveled by the charcoal meal from the Pylorus was also measured. The distance traveled by the charcoal plug in the small intestine (from the pylorus) was expressed as a percentage of the distance from the pylorus to the ileocaecal junction (Akah, 1989).

### Statistical Analysis

All values were expressed as mean ± SEM. One-way analysis of variance (ANOVA) was used for the statistical analysis of data. Dunnet test was then used for post hoc analysis. P-values < 0.05 were taken to be statistically significant.

### Compliance with Good Laboratory Practice (GLP)

The studies were carried out in accordance with Good

**Table 1** Effect of aqueous extract of *Andrographis paniculata* leaves on mice intestinal motility.

| Treatment Group | Mean Intestinal length (cm) | Mean distance travelled by charcoal (cm) | Movement of charcoal as percentage of intestinal length |
|---|---|---|---|
| **Normal saline 10 ml/kg p.o** | 32.5 ± 1.0 | 25.4 ± 1.5 | 78.2 |
| ***A. paniculata* (mg/kg p.o)** | | | |
| 16.0 | 36.7 ± 1.1 | *26.9* ± 2.0 | 73.3 |
| 32.0 | 37.0 ± 0.4 | *26.2* ± 2.3 | 70.8 |
| 64.0 | 34.2 ± 1.7 | *27.5* ± 2.4 | 80.4 |
| 125.0 | 32.6 ± 1.6 | *28.5* ±2.4 | 87.4 |
| 250.0 | 30.2 ± 1.2 | *28.6* ± 2.0 | 94.7* |
| 500.0 | 32.0 ± 2.0 | *31.0* ± 1.2 | 97.0* |

*Significant (P<0.05) increase in motility, one-way ANOVA.

Laboratory Practice (GLP)Regulations of Organization for Economic Cooperation and Development –OECD (UNDP/World Bank/WHO, 2001).

## RESULTS

### Phytochemical Screening

The study revealed that aqueous extract of *A. paniculata* contains saponin and terpenes. Glycosides, anthracene, tannins, alkaloids, flavonoids and volatile oil were not detected.

### Acute Toxicity Study ($LD_{50}$)

The experimental mice given doses of 1000 – 2000 mg/kg i.p. manifested so much distress few minutes post drug administration. There was shivering, ataxia, writhing reflexes, panting and death within 24 h of drug administration. Those administered doses below 1000 mg/kg i.p. survived. The $LD_{50}$ was calculated to be 950 mg/kg i.p in mice.

### Studies on Isolated rat Ileum

Aqueous extract of *A. paniculata* leaves showed biphasic effects on rat ileum. The lower concentrations (1.0 – 4.0 mg/ml) relaxed the tissue while the higher concentrations (8.0 – 32.0 mg/ml) caused a concentration – dependent contraction of the same tissue (Figure 1).

The contractile effects of acetylcholine (5 ng/ml) and histamine (2.0 µg/ml) on the tissue were reversibly blocked by the extract (4.0 mg/ml; Figure 2 and Figure 3 respectively). Mepyramine (0.4 µg/ml) and atropine (0.1 – 0.8 µg/ml) did not block the contractile effect of the extract (16.0 mg/ml) on the tissue. (figure 4 and figure 5).

### Studies on Isolated rabbit Jejunum

The aqueous leaf extract of *A. paniculata* exhibited biphasic effects on isolated rabbit jejunum. The first phase comprised of the lower concentrations of the extract (1.0 – 2.0 mg/ml) which caused relaxation of the tissue while the second phase involved the higher concentrations (4.0 – 16.0 mg/ml) which caused a transient and concentration – dependent contraction of the tissue (Figure 6). The contraction caused by acetylcholine (5.0.ng/ml) and histamine (0.04 mg/ml) on the tissue were reversibly blocked by the extract (2.0 mg/ml: Figure 7 and Figure 8 respectively).

### Studies on Isolated rat stomach fundus strip

The lower concentrations of the aqueous extract of *a Paniculata* (1.0 – 4.0 mg/ml) produced no overt effect on rat fundus strip. However, the higher concentrations (8.0 – 32.0 mg/ml) caused a concentration – dependent

contraction of the tissue (Figure 9). Mepyramine (0.25 – 1.0 µg/ml) blocked the contractile effect of the extract (16.0 mg/ml) on the tissue (Fig 10) while atropine (0.25 – 1.0 µg/ml) reduced the contractile amplitude of the extract (16.0 mg/ml; tracing not shown).

**Studies on Gastrointestinal Motility in Mice**

The lower doses of the aqueous leaf extract of *A. paniculata* (16.0 – 32.0 mg/kg p.o) dose – dependently reduced the mice gastrointestinal motility while the higher doses of the extract (64.0 – 500.0 mg/kg p.o) significantly and dose-dependently increased the mice gastrointestinal motility (Table 1)

**DISCUSSION**

The relaxation and contraction of the isolated tissues caused by the lower and higher concentrations of *A. paniculata* leaf extract respectively indicate that the extract has biphasic effects on the gastrointestinal smooth muscles. This may suggest that the extract of *A. Paniculata* possesses both spasmolytic/antimotility and spasmogenic/motility-enhancing activities. These *in vitro* effects observed on the isolated gastrointestinal smooth muscles support the decreased and increased intestinal motility observed *in vivo* in charcoal meal fed mice following administration of the lower and higher doses of the extract respectively. This also manifested in the acute toxicity study ($LD_{50}$) where mice treated with high doses ($\geq$ 1000 mg/kg i.p) showed writhing reflexes and other distress signs, the effects / signs that were not observed in mice treated with lower doses (< 1000 mg/kg i.p) of the extract.

The introductory report showed that *A. paniculata* is used ethno medicinally in the treatment of colitis. Reports also showed that the extract inhibited diarrhea of microbial origin (Thanagkul *et al*, 1985; Chaichantipyuth, 1986; Gupta, 1993). The present study, however, showed that direct effect of the lower doses of the extract on the gastrointestinal muscles has the potential of inhibiting diarrhea and possibly, ameliorating colitis. According to Dinesh *et al.,* (1999), advantage can be taken of agents that reduce intestinal motility, gastric secretary effect and are antispasmodic as adjunctive treatment in non-ulcer dyspepsia, irritable bowel syndrome and diverticular disease. Antispasmodics are of value for treating abdominal cramps associated with diarrhea while anti-motility drugs relieve diarrhea. This may therefore suggest that *A. paniculata* extract has the potential of being developed into antispasmodic and/or anti-motility agent only at lower doses.

The study, on the other hand, showed that higher concentrations and doses of *A. paniculata* leaf extract increased gastrointestinal smooth muscle contractility and motility respectively. According to Brunton (1990), some standard purgatives bring about their effects by increasing gastrointestinal motility, causing decreased absorption of salt and water secondary to decreased transit time. Purgative effects will lead to enhanced gastric and intestinal emptying. This may suggest that *A. Paniculata* has the potential of being developed into a purgative at higher doses.

These observations indeed showed the biphasic effects of the leaf extract of *A. paniculata* on the gastrointestinal tract. This information is very vital considering that Calabrese *et al* (2000) reported that *Andrographis paniculata* extract was already undergoing clinical trial for the treatment of HIV infection and also knowing that most of the HIV/AIDS patients have chronic diarrhea which lead to severe weight loss (Omalu *et al*, 2007). This, therefore, calls for the need to strike a balance between the dose to be used for inhibiting diarrhea and the dose for HIV treatment, and indeed other ailments for which *A. paniculata* may be indicated.

Although the blockade of the contractile effects of acetylcholine (5.0 ng/ml) by the lower concentrations of the leaf extract (2.0 – 4.0 mg/ml) on both rat ileum and rabbit jejunum may be viewed as physiological antagonism, the reduction of the contractile amplitude of the higher concentration of the extract (16.0 mg/ml) by atropine (0.25 – 1.0 µg/ml) on rat stomach fundus strip may suggest that the extract probably contains substances that act through muscarinic cholinergic mechanism. Cholinominetics such as acetylcholine are known to stimulate the smooth muscles of the gut through the muscarinic receptors and this stimulatory effect is blocked by atropine.

Similarly, the blockade of the contractile effects of histamine (0.04 mg/ml – 2.0 µg/ml) by the lower concentrations of the leaf extract (2.0 – 4.0 mg/ml) on both rat ileum and rabbit jejunum may suggest physiological antagonism. However, the total blockade of the contractile effect of the higher concentration of *A. paniculata* extract (16.0 mg/ml) by mepyramine (0.25 – 1.0 µg/ml) on the isolated rat stomach fundus strip probably indicates that the extract also has components that act through the histaminergic mechanism. This is premised on the knowledge that histamine acts on $H_1$ receptors to stimulate the gut (Ash *et al*, 1966) and this effect is inhibited by $H_1$ blockers such as mepyramine. However, the use of more specific antagonists and agonists in subsequent studies will further elucidate the possible mechanism(s) of action.

Also revealed in this study were the toxicity signs associated with the higher doses of the extract ($\geq$ 1000 mg/kg i.p). These manifested signs in the acute toxicity study ($LD_{50}$) included overt distress, shivering, ataxia, writhing reflex, panting and even death. According to Lorkes (1983), $LD_{50}$ value > 1g are generally considered safe for all practical purposes in the laboratory. Therefore, the toxicity signs exhibited by the mice treated

with doses ≥ 1000 mg/kg i.p probably indicate that the extract may not be relatively safe at those doses. This also means that this should be considered during drug development and also suggests the need to carry out repeated low dose toxicity studies in order to determine the cumulative effects of the extract on the body systems.

According to the World Health Organization, WHO (1991/92), the most critical assessment of herbal medicine is the safety evaluation. The WHO, in recognition of the immense value of herbal medicine to Primary Health-Care, advocated for the proper identification, sensible exploitation, scientific development and appropriate utilization of herbal medicines which provide safe and effective remedies.

In conclusion, the aqueous extract of *A. paniculata* possibly has spasmolytic/antimotility and spasmogenic/motility-enhancing effects on gastrointestinal muscles as exhibited at different concentrations and doses of the extract. Great caution should therefore be exercised in choosing therapeutic doses for different ailments for which they are used.

## ACKNOWLEDGEMENT

The authors are grateful to C.U. Menegbe, G. Gamaniel, A. Watsun, S. Dzarma, D.D. Akumka, M.J. Sini, U.O. Udoh, O. Iyonobhe, G.J. Audam for their technical assistance. They are also grateful to J.I. Atogwe of NIPRD Medicinal Plant Garden and A.O. Ohaeri for authentication of the plant of study.

### REFERENCES

Adeyi O, Kanki P, Odutolu O, Idoko JA (2006): AIDS in Niger., a nation on the threshold. Cambridge: Havard Center for Population and Development Studies. Asia-Pacific Conference on Library Information, Education and Practice.

Akah PA (1989): Purgative Potentials of *Euphorbia heterophylla*. *Fitoterapia* 60: 45 – 48.

Akah PA, Orisakwe OE, Gamaniel KS, Shittu A (1998): Evaluation of Niger.Traditional Med.; II Effects of some Niger. folk remedies on peptic ulcer. J . Ethnopharmacol. 62: 123 – 127.

Anonymous (1979): Diarrhoeal Disease Control Programme, Weekly Epidemic Record, 16: 121.

Ash AFS, Schild HO (1966): Receptors mediating some actions of histamine. British J. Pharmacol. 27: 427 – 439.

Brunton LL (1990): Agents affecting gastro-intestinal water flux and motility. Digestants and Bile acids. In: Gilman GA, Rall TW, Nies AS Taylor, P (ed), The Pharmacol. Basis of Therapeutics, Permamon Press, pp. 914 – 932.

Calabrese C, Berman SH, Babish JG, Ma X, Shinto L, Dorr M, Wells K, Wenner CA, Standish LJ (2000): A phase 1 trial of andrographolide in HIV positive patients and normal Volunteers. Phytotherapie Res. 14 (5): 333 – 8.

Chaichantipyuth C, Thanagkul B (1986): *Andrographis paniculata* Nees as antidiarrhoeal and antidysentery drug in Thailand. Asian J . Pharmacy (Suppl) 6: 59 – 60.

Chang HM, Butt PH (1986): Pharmacol. and Applications of Chinese Materia Med., vol 1,Singapore, World Scientific. pp 918 – 928.

Chaturvedi GN (1983): Clin. Studies on Kalmegh (*Andrographis paniculata*) in infectious Hepatitis. J. the Int. Institute of Ayurveda. 2: 208 – 211.

Dinesh KM, Martin J, Furniss L, Hargreaves AM, Jordan B, Patterson CA, Ryan SM, Shah SA, Whitley LJ(1999): *British National Formulary (BNF)*, 37: William Clowes, Britain. Pp 31 – 53.

Eisenberg DM, Kessler RC, Foster C, Norlock FE, Calkins DR, Delbanco TL (1993): Unconventional med. in the United States: prevalence, costs and patterns of use. New England J. Med, 328: 246 – 252.

Eisenberg DM, Davis RB, Ettner SL, Appel S, Wilkey S, Van Rompay M, Kessler RC (1998): Trends in alternative med. use in the United States, 1990 – 1997: results of a follow-up na.l survey. J. the Ame.Med. Association, 280: 1569 –1575.

Goldbeck-wood, S., Dorozynski, A., Lie, I.G., Yamauchi, M., Zinn, C., Josefson, D. and Ingram, M. (1996): Complementary med. is booming worldwide. British Med. J., 313: 131 – 133.

Gupta S (1993): Antisecretory (antidiarrhoeal) activity of Indian Med. Plants against *Escherichia coli* enterotoxin-induced secretion in rabbit and guinea pig ileal loop models, International J. Pharmacognosy, 31: 198 – 204.

Lorke D(1983): A new approach to acute toxicity testing. Archives of Toxicol., 54: 275 – 287.

Lutterodt GD (1989): inhibition of gastrointestinal release of acetylcholine by quercetin as a possible mode of action of *Psidium guajava* leaf extracts in the treatment of acute diarrhea disease. J. of Ethnopharmacol. 25, 235 – 247.

Med. Plants in Vietnam (1990): Manila, Philippines, World Health Organization Regional Office for the Western Pacific (WHO Regional Publications Western Pacific Series No. 2).

Omalu I, Duhlinska D, Anyanwu G, Pam V, Inyama P (2007): Seroprevalence of Microsporidiosis in immunocompromised patients in Kano, Niger. The Internet J. of Parasitic Diseases 1: 2.

Pharmacopoeia of the People's Republic of China, English Edition (1992). Guangdong Scien. And Technol. Press, Guangzhou, China. The Pharmacopoeia Committee of PRC.

Piot P Kapita B, Nguigi E(1999): AIDS in Africa, a manual for Physicians. World Health Organisation,150.

Scimone provide initial , Scimone A (1998): US sees the green in herbal supplements. *Chem. Market Reporter*, 254, FR3 – FR4.

Singh VK, Ali ZA (1994): Folk Med. in Primary Health Care: Common plants used for the treatment of fevers in India. Fitoterapia, 65: 68 – 74.

Standard of ASEAN herbal med. (1993): vol 1., ASEAN Countries, Jakarta, Indonesia.

Syder JD, Merson MH (1982). The magnitude of the global problem of acute diarrhoeal disease: a review of active surveillance data. Bulletin of the World Health Organisation 60: 605 – 613.

Thanagkul B, Chaichantipayut C (1985) Double – blind study of Andrographis paniculata Nees and tetracycline in acute diarrhoea and bacillary dysentery. Ramathibodi Med. J. 8: 57 – 61.

Trease GE, Evans MC (1983): Textbook of Pharmacognosy, 12th ed. Bailliere, Tindal, London. Pp 343 383.

UNDP/World Bank/WHO (2001): Introduction of the OECD principles of GLP. Special Programme for Res. and Training in Tropical Diseases (TDR) – Good Laboratory Practice Training Manual for the Traiee. Pp 3 – 19.

Wambebe C (1998): Development of standardized Phytomedicines in Africa (Review). J of Pharmaceutical Res. and Development, 3(1): 1 – 11.

WHO (1991): Guidelines for the assessment of Herbal Med. WHO/TRM/9104.

WHO (1992): Guidelines for the assessment of Herbal Med. WHO/TRM/91.4.