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## Antidiarrheal and Antihepatic Effect of *Andrographis paniculata* Leaf Extract on Castor Oil Induced Diarrhea in Wistar Rats

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**Abstract** This study was carried out to investigate the effect of *Andrographis paniculata* leaf extract of ethanol, methanol and petroleum ether on serum electrolytes, urea, and serum enzymes as indices of toxicological importance in wistar rats. Eighty (80) female wistar rats weighing between 225-230 g were used for the study. Rats were divided into ten (10) study groups and designated as; non-diarrhea control (NDC), diarrhea control (DC), diarrhea orthodox control (DO) and diarrhea extract treated groups (DERE, DERM, DERP) receiving varying extract concentrations of 200mg/kg and 400mg/kg body weight respectively. Animals were sacrificed after seven (7) days of experimental period and blood was collected for biochemical analysis. The extracts were found to produce significant antidiarrheal activity in dose dependent manner. The ethanol and methanol extracts were found to be more effective against castor oil induced diarrhea on experiment rat at the dose of 400 mg/kg body weight. Body weight indices obtained from this study revealed a decrease ( $p < 0.05$ ) in body weight in the extract treated group when compared with non-diarrhea induced control with an attendant increase ( $P < 0.05$ ) when compared with diarrhea control. Phytochemical screening detected biological important metabolites including tannins and steroids while serum electrolyte level such as sodium and chlorine increase in the extract treated group when compared with the diarrhea control group (Group B) but significantly decrease ( $p < 0.05$ ) when compared to non-diarrhea control group (group A). Serum liver enzymes; ALT, AST, ALP, LDH, and GGT were increased in the extract treated group when compared to the non-diarrhea control group (Group A) but a statistically decrease ( $p < 0.05$ ) was observed in comparison with the diarrhea control group (Group B). In conclusion, data generated from this study show that *Andrographis paniculata* leaf extracts possesses anti-diarrhea anti-hepatic activities in castor oil induced diarrhea in rats.

**Keywords** *Phytochemical Analysis, Castor Oil, Serum Enzymes and Electrolyte Level*

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### Introduction

Medicinal plants have been identified and used throughout human history. In the last few decades there has been an exponential increase in the field of herbal medicine. It is getting popularized in developing and developed countries owing to its natural origin and lesser side effects. In developing countries like India, majority of the people who live in the rural areas exclusively rely on traditional medicines in treatment of some diseases, including diarrhea, malaria etc. It is necessary to establish the scientific basis for the therapeutic actions of traditional plants as these may serve as the source for the development of more effective medication. Plants have the ability to synthesize a wide variety of active ingredients that are used to perform important biological functions, and to defend against predators such as



insects, fungi, and herbivorous mammals. Chemical compounds in plants mediate their effect on humans in the same manner as conventional orthodox drug; thus herbal medicine do not differ greatly from conventional drugs in terms of their mechanism of action, but may have beneficial pharmacokinetics [1].

In ethnobotany, traditional medicine is recognized as an effective way to discover future medicines. In 2001, researchers identified 122 compounds used in modern medicine which were derived from “ethnomedical” plant sources; 80% of these had ethnomedical applications that are related to the current use of active elements of the plants (Fabricant and Farnsworth, 2001). Many of the pharmaceuticals available to physicians are long time derivatives of herbal remedies and aspirin, digoxin, quinine, digitalis, and opium are common examples [2].

The use of herbal medicine is almost universal among non-industrialized countries and the world in general because it is affordable than orthodox pharmaceuticals. The World Health Organization estimates that 80% of Asian and African countries use herbal medicine for primarily health care. Health records in the United States and Europe have shown that herbal medicine is less common in clinical settings, and more acceptable. The annual global report of pharmaceutical plants in 2011 accounted for over US\$2.2 billion [3]. More than two third of the world plant species have medicinal value are found in developing countries. At least 7,000 medical compounds in the modern pharmacopoeia are derived from plants. In many Medicinal and Aromatic Plants (MAPs), significant variations of plants characteristics have been ascertained with varying soil traits, and the selective recovery and subsequent release in food of certain elements have been demonstrated. Great attention must be paid to choose soil and cropping strategies, to obtain satisfactory yields of high quality and best-priced products in respect to their safety and nutritional value [4].

*Andrographis paniculata* is a herbaceous plant in the family *Acanthaceae*, native to India and Sri Lanka. It is widely cultivated in Southern and South eastern Asia, where it is used to treat infections and some diseases, often being used before antibiotic were discovered. Mostly the leaves and roots were used for medicinal purposes. *Andrographis paniculata* is an erect annual herb extremely bitter in taste in all parts of the plant body. The plant is known in north eastern India as *Maha-tita* literally “king of bitters”, and known by various vernacular names. As an Ayurveda herb it is known as *Kalmeghor Kalamegha*, meaning “dark cloud”. It is also known as *Bhuineem*, meaning “neem of the ground”, since the plant, being a small annual herb has a similar strong bitter taste as that of the large Neem tree (*Azadirachta indica*). In Malaysia, it is known as *Hempedubumi*, which literally means “bile of earth” since it is one of the bitterest plants that are used in traditional medicine. The genus *Andrographis* consist of 28 species of small annual shrubs essentially distributed in tropical Asia. Only a few species are medicinal, of which *Andrographis paniculata* is the most popular [5].



Figure 2: The *Andrographis paniculata* plant [5]

Castor oil has long been used commercially as a highly renewable resource for the chemical industry [6]. It is a vegetable oil obtained by pressing the seeds of the castor oil plant (*Ricinus communis* L.) that is mainly cultivated in Africa, South America, and India. Major castor oil-producing countries include Brazil, China, and India [7]. This oil is known to have been domesticated in Eastern Africa and was introduced to China from India approximately 1,400 years ago [7]. India is a net exporter of castor oil, accounting for over 90% of castor oil exports, while the United States, European Union, and China are the major importers, accounting for 84% of imported castor oil [8].



Approximately 86% of castor seed production in India is concentrated in Gujarat, followed by Andhra Pradesh and Rajasthan. Specifically, the regions of Mehsana, Banaskantha, and Saurashtra/Kutch in Gujarat and the districts of Nalgonda and Mahboobnagar of Andhra Pradesh are the major areas of castor oil production in India [9]. The economic success of castor crops in Gujarat in the 1980s and thereafter can be attributed to a combination of a good breeding program, good extension model, with access to well-developed national and international markets [10]. Castor is one of the oldest cultivated crops; however, it contributes to only 0.15% of the vegetable oil produced in the world. The oil produced from this crop is considered to be of industrial importance because it is the only commercial source of hydroxylated fatty acid. On average, worldwide consumption of castor oil increased at a rate of 7.32 thousand tons per year. In general, the current rate of castor oil production is not considered sufficient to meet the anticipated increase in demand [11].



Figure 2: Castor oil seed [12]

## Materials and Methods

### Chemicals

Methanol, Chloroform, Ethanol, Petroleum ether, and other chemicals of analytical grade were obtained from Famlab Nigeria limited and Lixok-k chemicals, Akure, Ondo state- Nigeria. Castor seed plant was obtained from Arewa market, Kebbi state, Nigeria and used for study.

### Experimental Animals

Adult eighty (80) female albino rats weighing between 225-230g were purchased from a disease free stock of Ladoko- Akintola University, Osogbo, Nigeria and used for the study. The rats were randomly assigned into ten (10) study groups based on their body weight. Normal feeds and tap water were given to the rats ad-libitum while feed and water intake were noted. They were kept in wooden cages of eight (8) rats per cage, placed in a well-ventilated animal house of Joseph Ayo Babalola University (JABU) at normal temperature of 30-35°C. The cages were cleaned daily and rats were treated according to the international guidelines for the care and use of laboratory animals [13]. The animals were allowed for a week of acclimatization and their weights were measured before treatment commenced.

### Source of standard drug

Loperamide Hydrochloride (2mg) marketed by Trumax pharmaceutical, was obtained from a registered pharmacist in Skymax pharmacy, Ilesa, Osun State, Nigeria and used for the study.

### Plant materials and preparation

Fresh and healthy plants of *Andrographis paniculata* (king's of bitter) were obtained from premises of Federal College of Agriculture Ijabo Akure, Ondo State, Nigeria. The plant sample was taken to the department of Botany, Obafemi Awolowo University (O.A.U) for identification and authentication.

### Preparation of plant extracts

*Andrographis paniculata* leaves were sorted out, washed under running tap water to remove adhering dirt's and dust particles and air-dried under shade for three weeks, during January period. The dried materials were homogenized



using mechanical grinder and weighed on an analytical weighing balance. The powder form was kept in an airtight container and stored at 4°C until when needed for further analysis.

### Extraction procedures

Twenty seven grams (27g) of the powdered extract was soaked in different solvents, 250ml of 98% Ethanol, 98% Methanol and 98% Petroleum ether at room temperature for 72 hours after which the mixture was filtered. The filtrate was air-dried at room temperature into flat aluminum plates for each and then the filtrates were scrapped out and kept in an air tight container. The residue obtained also, was weighed and kept in a clean black nylon bag, at normal room temperature for further use.

### Enzyme Assay

#### Determination of alanine aminotransferase activity

Activity of the enzyme was Colorimetric method. Alanine aminotransferase was measured by monitoring the concentration of pyruvate hydrazine formed with 2, dinitrophenylhydrazine.

#### Principle



#### Determination of aspartate aminotransferase activity

The AST activity was measured by colorimetric method. Aspartate aminotransferase activity was measured by monitoring the concentration of oxaloacetate hydrazine formed with 2,4-dinitrophenylhydrazine.

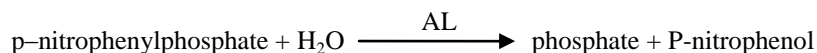
#### Principle:



#### Determination of alkaline phosphatase activity:

**Method:** colorimetric method.

#### Principle:



#### Determination of Gamma Glutamyl Transferase ( $\gamma$ -GT) Activity:

Activity of the enzymes was measured by Colorimetric method.

**Principle:** At pH 7.8  $\gamma$ -GT in sample was made to catalyze the transfer of glutamyl terminal residue from  $\gamma$ -glutamyl-p-nitroanalide to the acceptor, glycylglycine gave yellow a coloured  $\gamma$ -glutamyl-glycylglycine and the rate of formation of product monitored at 405nm was proportional to the enzyme activity.

**Urea determination:** Urease Berthelot method was used.

**Determination of electrolytes level:** Ion Selective Electrodes (ISE) was used to determined the ion level.

### Statistical analysis

The data are represented as mean value  $\pm$  SEM (Standard error of mean). All results will be mean of 10 data samples and the statistical analysis will be carried out using students' t-test. The values will be considered at probability level of  $p < 0.05$ ; all the values of  $p < 0.05$  will be considered statistically significant.

### Results and discussion

Table 1: Phytochemical screening for the different solvent extracts of *Andrographis paniculata* leaves

Phytochemicals	Extracts		
	Ethanol	Methanol	Petroleum ether
Alkaloids	++	+	--
Flavanoids	+++	++	+
Steroids	+	+	--
Glycosides	++	++	+
Carbohydrates	--	--	--
Tannins	+++	++	+
Saponins	+++	++	-
Terpenoids	+	+	--

+++ , strong; ++, moderately; +, poor presence; --, absence



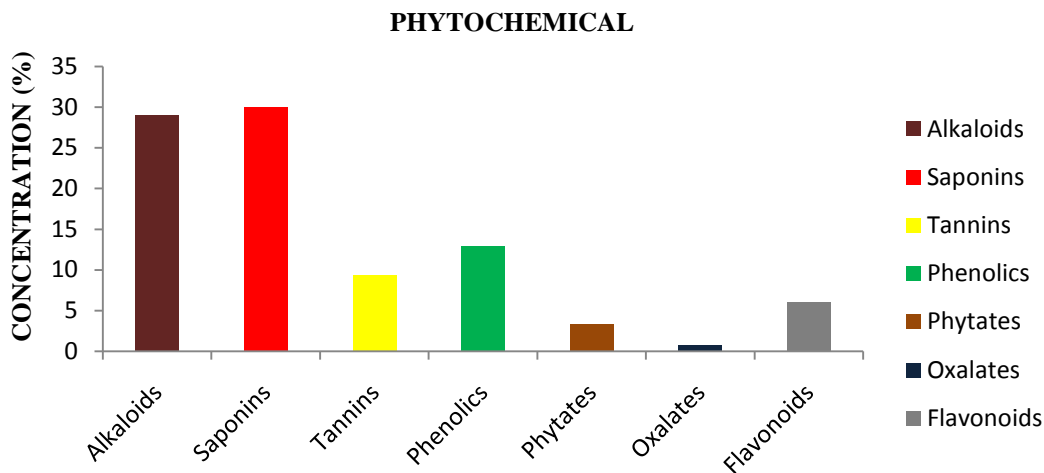


Figure 3: The result of quantitative phytochemical analysis of *A. paniculata* leaf

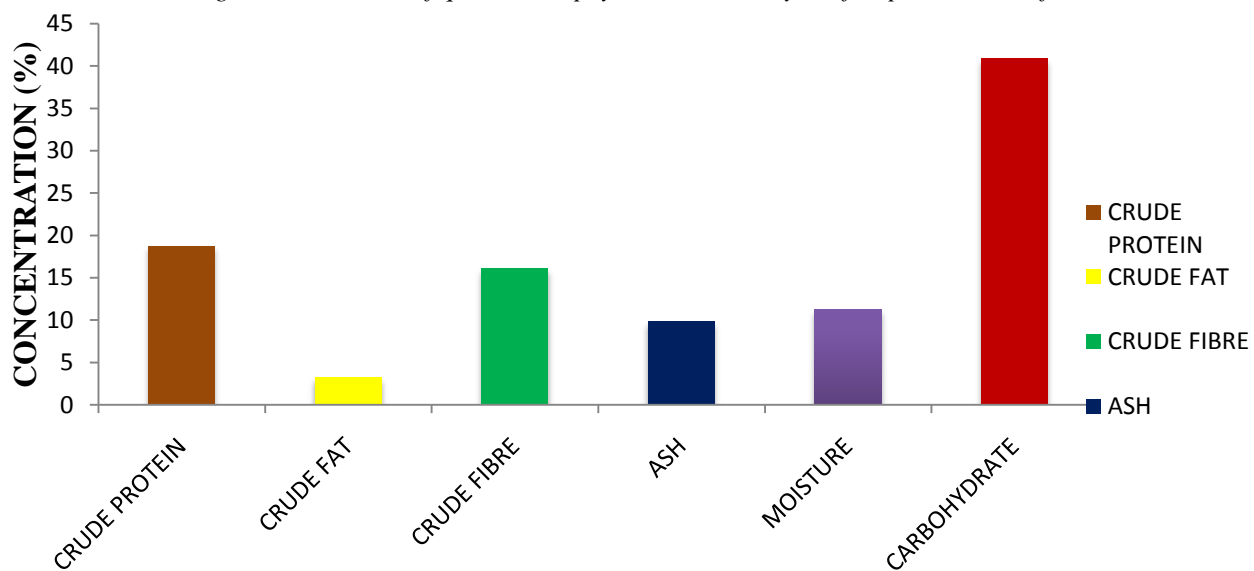


Figure 4: The result of proximate analysis of *A. paniculata* leaf

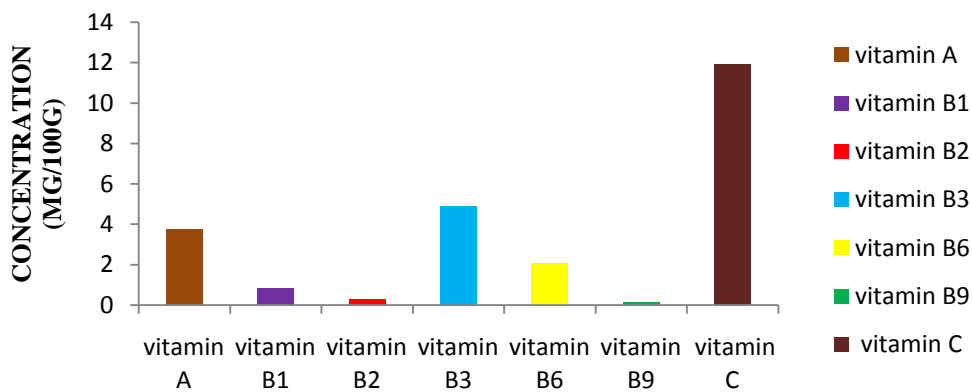


Figure 5: The result of vitamin analysis of *A. paniculata* leaf.

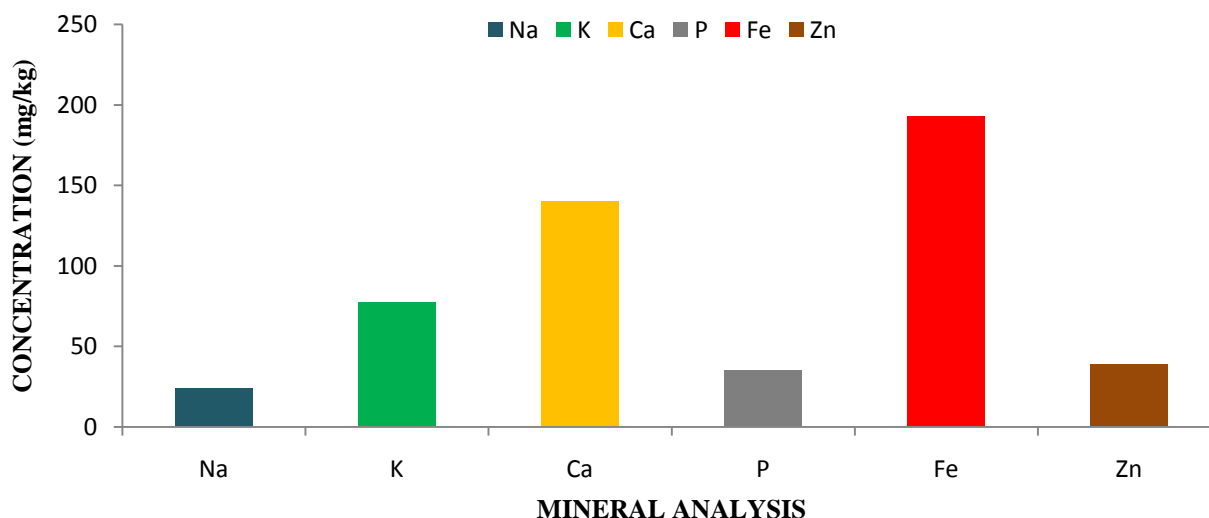
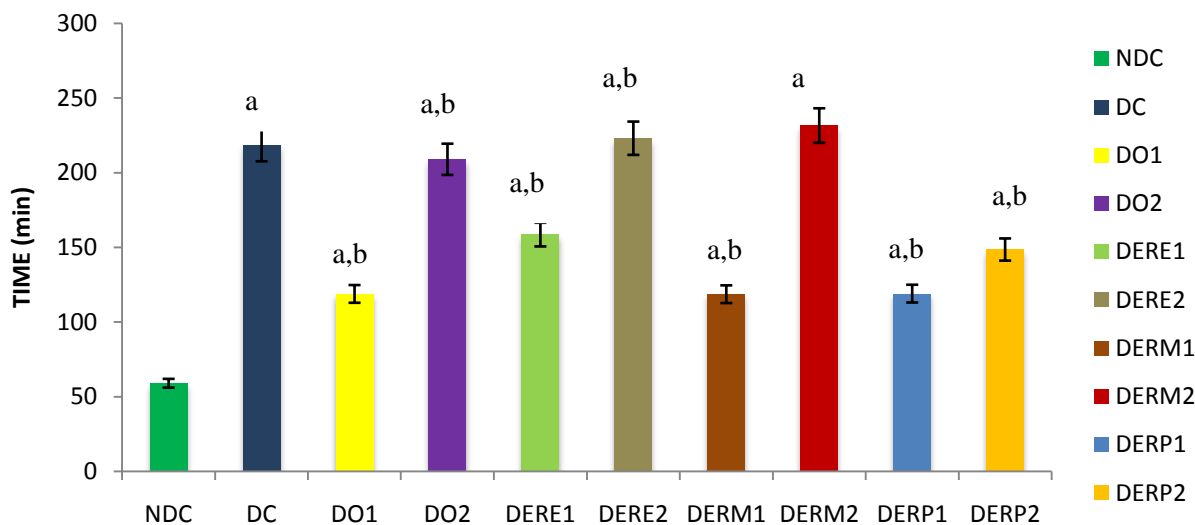


Figure 6: The result of mineral analysis of *A. paniculata* leaf



Values are expressed in means  $\pm$  S.E.M of 8 determinations  
 Figure 7: The result of castor oil induced diarrhea in wistar rats

**KEY:**

NDC = Non diarrhea control group

DC = Diarrhea control group

DO 1 = Diarrhea orthodox group treated with loperamide (200mg/kg body weight)

DO 2 = Diarrhea orthodox group treated with loperamide (400mg/kg body weight)

DERE1 = Treated Diarrhea with Methanol (200mg/kg body weight)

DERE2 = Treated Diarrhea with Methanol (400mg/kg body weight)

DERM 1 = Treated Diarrhea with Ethanol (200mg/kg body weight)

DERM 2 = Treated Diarrhea with Ethanol (400mg/kg body weight)

DERP 1 = Treated Diarrhea with Petroleum ether (200mg/kg body weight)

DERP 2 = Treated Diarrhea with Petroleum ether (400mg/kg body weight)

a= shows significant difference compared with positive control group (Group A) ( $p < 0.05$ )

b= shows significant difference compared with negative control group (Group B) ( $p < 0.05$ )





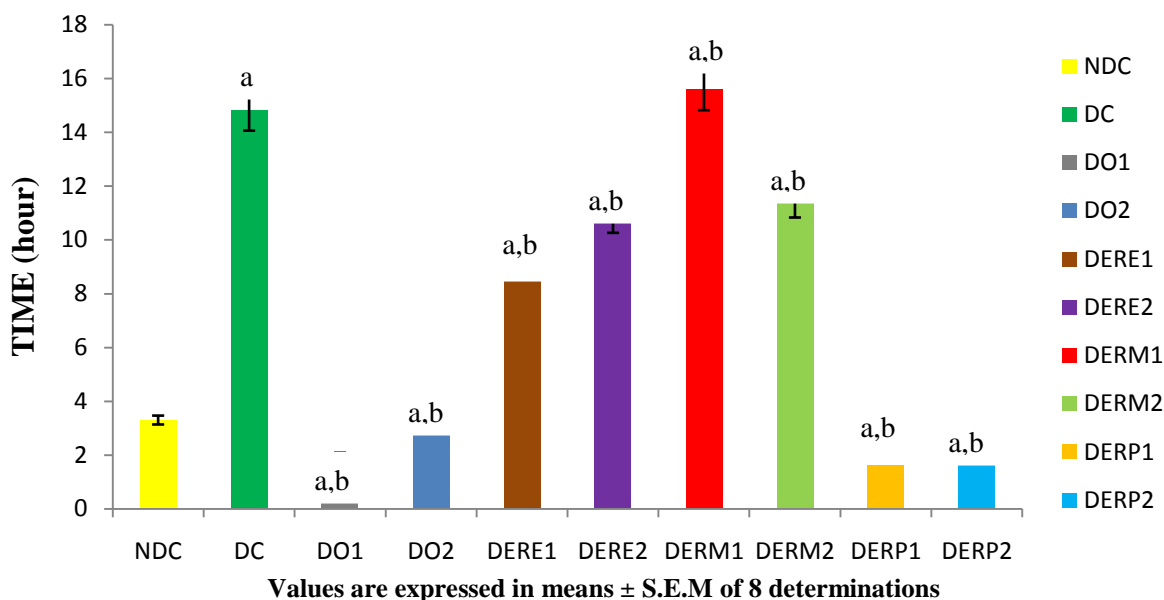


Figure 8: The result showing the frequency of defecation in 4 hours on castor oil induced diarrhea in wistar rats

KEY:

NDC = Non diarrhea control group; DC = Diarrhea control group

DO 1 = Diarrhea orthodox group treated with loperamide (200mg/kg body weight)

DO 2 = Diarrhea orthodox group treated with loperamide (400mg/kg body weight)

DERE1 = Treated Diarrhea with Methanol (200mg/kg body weight)

DERE2 = Treated Diarrhea with Methanol (400mg/kg body weight)

DERM 1 = Treated Diarrhea with Ethanol (200mg/kg body weight)

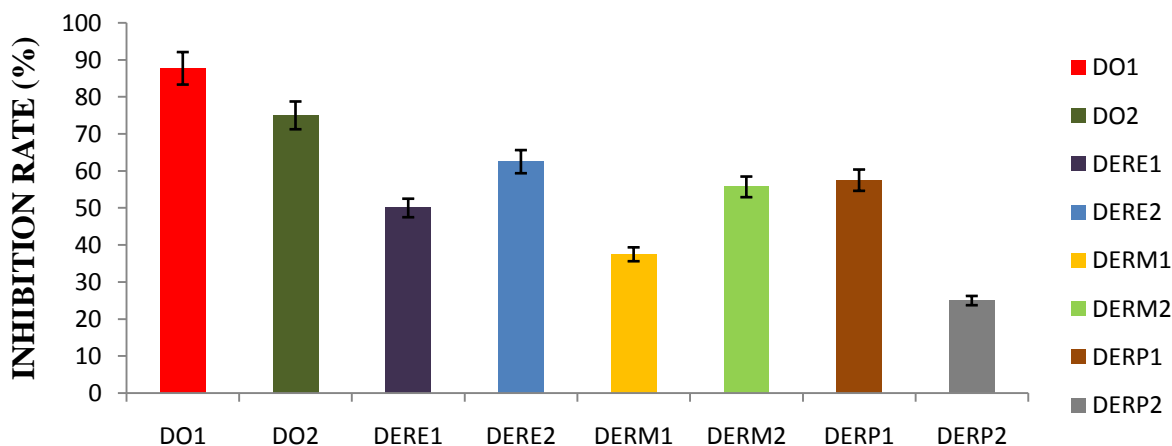
DERM 2 = Treated Diarrhea with Ethanol (400mg/kg body weight)

DERP 1 = Treated Diarrhea with Petroleum ether (200mg/kg body weight)

DERP 2 = Treated Diarrhea with Petroleum ether (400mg/kg body weight)

a= shows significant difference compared with positive control group (Group A) ( $p < 0.05$ )

b= shows significant difference compared with negative control group (Group B) ( $p < 0.05$ )



Values are expressed in means ± S.E.M of 8 determinations.

Figure 9: The result showing the percentage inhibition of defecation of castor oil induced diarrhea in wistar rats.

## KEY:

DO 1 = Diarrhea orthodox group treated with loperamide (200mg/kg body weight)

DO 2 = Diarrhea orthodox group treated with loperamide (400mg/kg body weight)

DERE1 = Treated Diarrhea with Methanol (200mg/kg body weight)

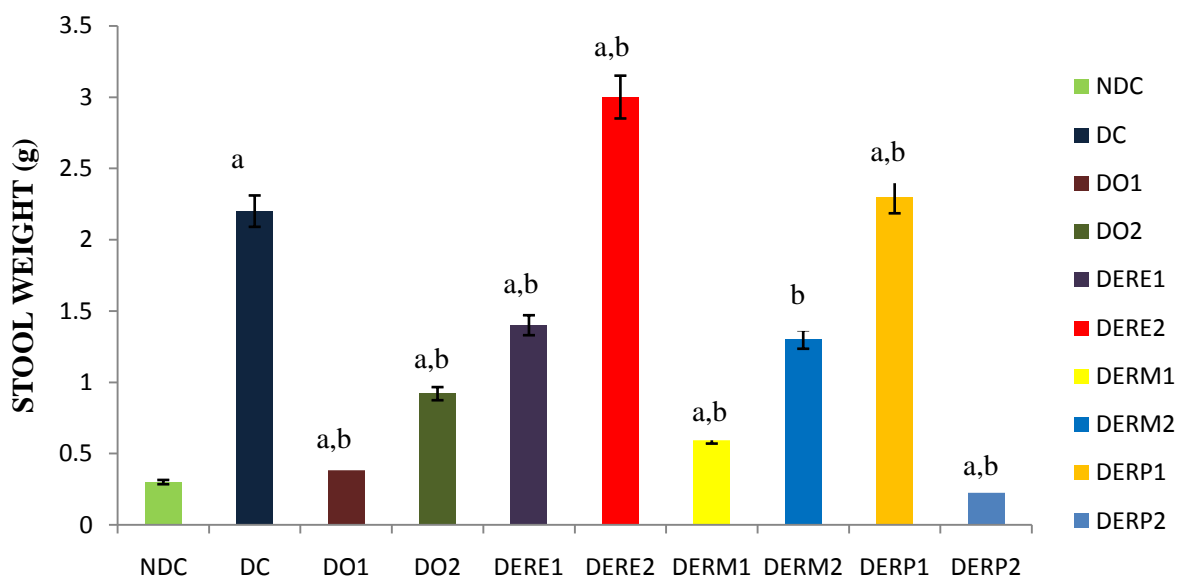
DERE2 = Treated Diarrhea with Methanol (400mg/kg body weight)

DERM1 = Treated Diarrhea with Ethanol (200mg/kg body weight)

DERM2 = Treated Diarrhea with Ethanol (400mg/kg body weight)

DERP 1 = Treated Diarrhea with Petroleum ether (200mg/kg body weight)

DERP 2 = Treated Diarrhea with Petroleum ether (400mg/kg body weight)



Values are expressed in means  $\pm$  S.E.M of 8 determinations.

Figure 10: The result showing the weight of stool of castor oil induced diarrhea in wistar rats

## KEY:

NDC = Non diarrhea control group

DC = Diarrhea control group

DO 1 = Diarrhea orthodox group treated with loperamide (200mg/kg body weight)

DO 2 = Diarrhea orthodox group treated with loperamide (400mg/kg body weight)

DERE1 = Treated Diarrhea with Methanol (200mg/kg body weight)

DERE2 = Treated Diarrhea with Methanol (400mg/kg body weight)

DERM1 = Treated Diarrhea with Ethanol (200mg/kg body weight)

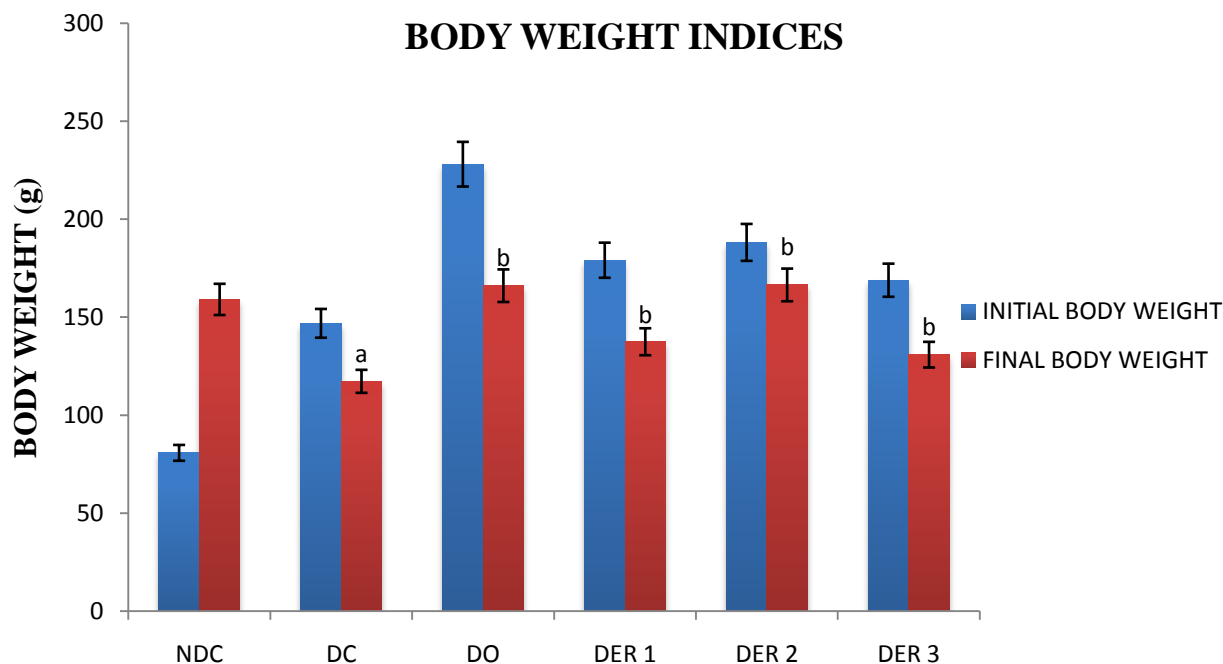
DERM2 = Treated Diarrhea with Ethanol (400mg/kg body weight)

DERP 1 = Treated Diarrhea with Petroleum ether (200mg/kg body weight)

DERP 2 = Treated Diarrhea with Petroleum ether (400mg/kg body weight)

a= shows significant difference compared with positive control group (Group A) ( $p < 0.05$ )b= shows significant difference compared with negative control group (Group B) ( $p < 0.05$ ).





Values are expressed in means ± S.E.M of 8 determinations

Figure 11: The result of body weight indices of experimental rats before and after the experiment

KEY: NDC = Positive control; DC = Negative control; DO = Diarrhea orthodox group

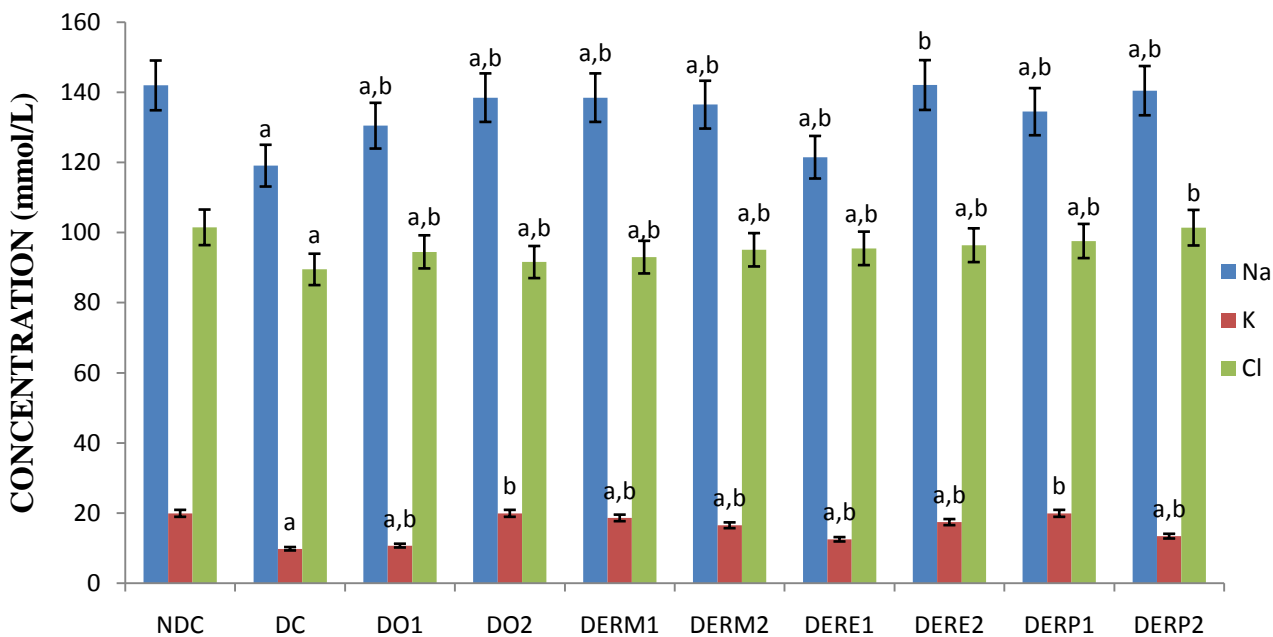
DER 1 = Treated Diarrhea group 1

DER 2 = Treated Diarrhea group 2

DER 3 = Treated Diarrhea group 3

a= shows significant difference compared with positive control group (Group A) ( $p < 0.05$ )

b= shows significant difference compared with negative control group (Group B) ( $p < 0.05$ ).



Values are expressed in means ± S.E.M of 8 determinations

Figure 12: The result showing the electrolyte levels after experimental period in rats

KEY: NDC = Non diarrhea control group

DC= Diarrhea control group

DO 1 = Diarrhea orthodox group treated with loperamide (200mg/kg body weight)

DO 2 = Diarrhea orthodox group treated with loperamide (400mg/kg body weight)

DERM 1 =Treated Diarrhea with Methanol (200mg/kg body weight)

DERM 2 =Treated Diarrhea with Methanol (400mg/kg body weight)

DERE 1 = Treated Diarrhea with Ethanol (200mg/kg body weight)

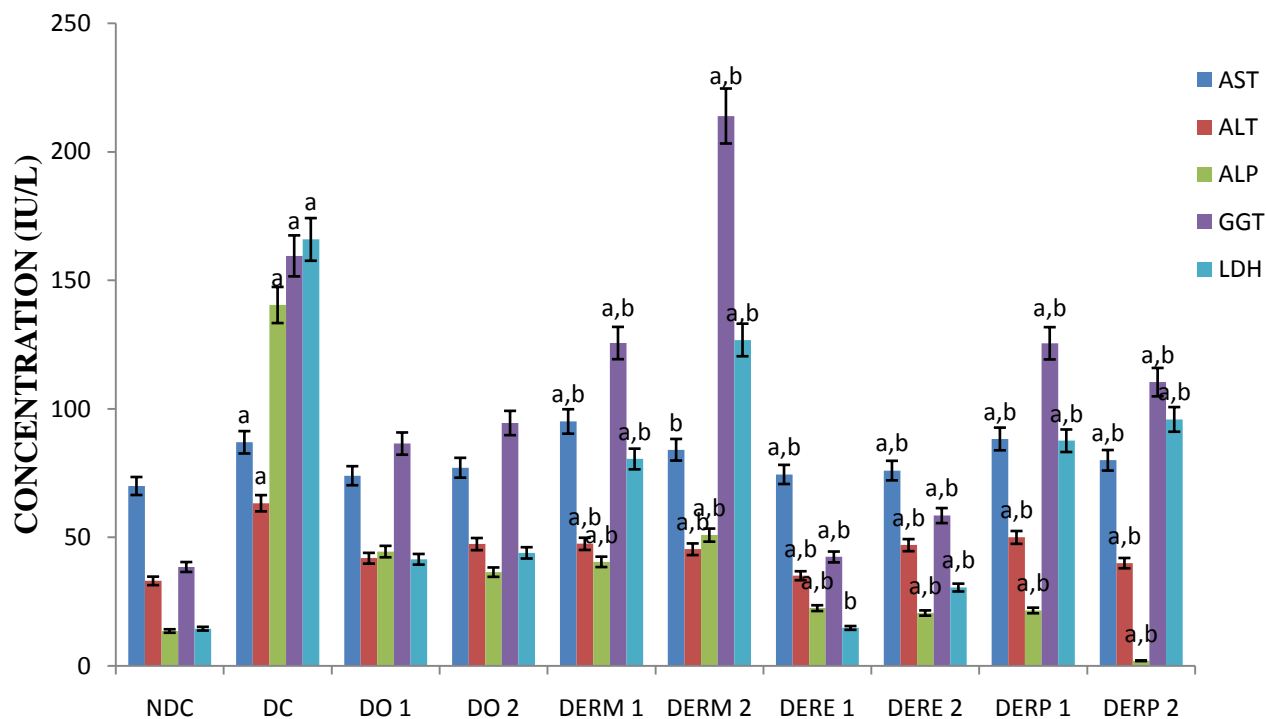
DERE 2 = Treated Diarrhea with Ethanol (400mg/kg body weight)

DERP 1 = Treated Diarrhea with Petroleum ether (200mg/kg body weight)

DERP 2 = Treated Diarrhea with Petroleum ether (400mg/kg body weight)

a= shows significant difference compared with positive control group (Group A) ( $p < 0.05$ )

b= shows significant difference compared with negative control group (Group B) ( $p < 0.05$ ).



Values are expressed in means  $\pm$  S.E.M of 8 determinations

Figure 13: The result showing serum enzyme levels after experimental period in rats

KEY:

NDC = Non diarrhea control group

DC = Diarrhea control group

DO 1 = Diarrhea orthodox group treated with loperamide (200mg/kg body weight)

DO 2 = Diarrhea orthodox group treated with loperamide (400mg/kg body weight)

DERM 1 = Treated Diarrhea with Methanol (200mg/kg body weight)

DERM 2 = Treated Diarrhea with Methanol (400mg/kg body weight)

DERE 1 = Treated Diarrhea with Ethanol (200mg/kg body weight)

DERE 2 = Treated Diarrhea with Ethanol (400mg/kg body weight)

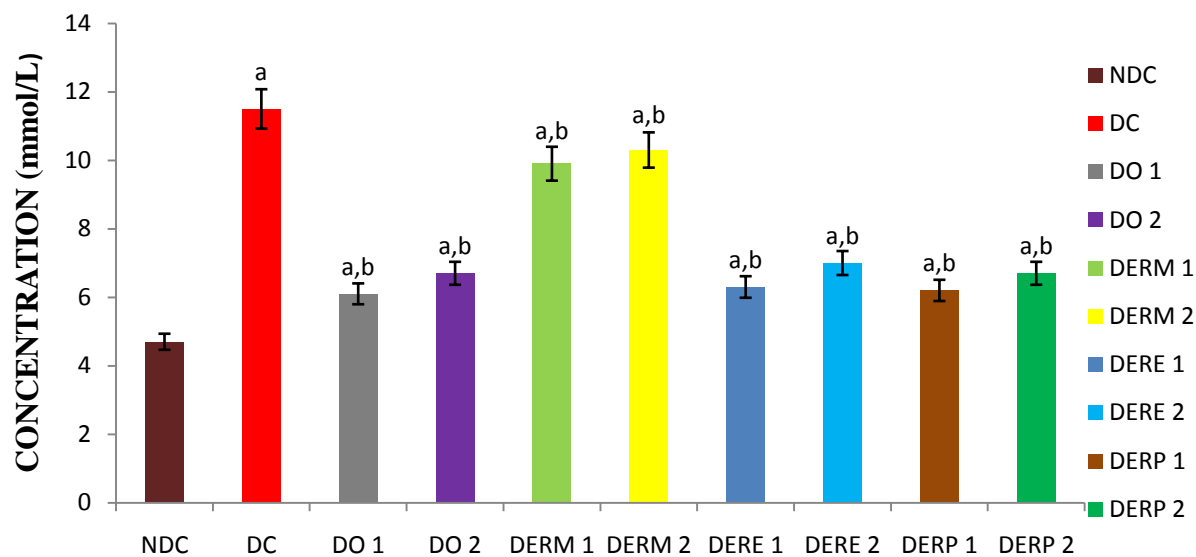
DERP 1 = Treated Diarrhea with Petroleum ether (200mg/kg body weight)

DERP 2 = Treated Diarrhea with Petroleum ether (400mg/kg body weight)

a= shows significant difference compared with positive control group (Group A) ( $p < 0.05$ )

b= shows significant difference compared with negative control group (Group B) ( $p < 0.05$ ).





Values are expressed in means  $\pm$  S.E.M of 8 determinations

Figure 14: The result showing urea levels of experimental rats after the experiment

#### KEY:

NDC = Non diarrhea control group

DC= Diarrhea control group

DO 1 = Diarrhea orthodox group treated with loperamide (200mg/kg body weight)

DO 2 = Diarrhea orthodox group treated with loperamide (400mg/kg body weight)

DERM 1 = Treated Diarrhea with Methanol (200mg/kg body weight)

DERM 2 = Treated Diarrhea with Methanol (400mg/kg body weight)

DERE 1 = Treated Diarrhea with Ethanol (200mg/kg body weight)

DERE 2 = Treated Diarrhea with Ethanol (400mg/kg body weight)

DERP 1 = Treated Diarrhea with Petroleum ether (200mg/kg body weight)

DERP 2 = Treated Diarrhea with Petroleum ether (400mg/kg body weight)

a= shows significant difference compared with positive control group (Group A) ( $p < 0.05$ )

b= shows significant difference compared with negative control group (Group B) ( $p < 0.05$ ).

#### Discussion

This study which is aimed at evaluating the antidiarrheal effect of *Andrographis paniculata* leaf extract in castor oil induced diarrhea rats. Much attention has been drawn by herbal medicine as an alternative medicine useful for treatment and prevention of lifestyle related disorders but there is inadequate knowledge on the pharmacokinetics of medicinal plants. There is a growing interest in the analysis of plant products which has stimulated intense research on their potential health benefits [14]. There are about 200 pure compounds from plants sources reported to show antidiarrheal effect. The compounds include; Saponins, Alkaloids, Carbohydrates, Amino acids, Flavonoids, Steroids, Phenols, Glycosides, Terpenoids, is as well as many other phytochemical constituents. People have customarily used the plant(s) or plant(s) derived preparations to combat diarrhoeal disorders. However, only few of them have been controlled clinically, or studied chemically and biologically to identify their active constituents [15], as supposed the compounds present in *Andrographis paniculata* which includes; Saponins, Flavonoids, Alkaloids, Tannins, Steroids, Cardiac glycosides, and Terpenoids.

The preliminary phytochemical screening showed that the different solvent extract of *Andrographis paniculata* contains important phytoconstituents such as; Saponins, Flavonoids, Alkaloids, Tannins, Steroids, Cardiac



glycosides, and Terpenoids in all the solvent extracts and carbohydrates absent in all the extracts. The ethanol extract yielded strongly, all the phytochemicals followed by methanol and petroleum ether. The flavonoid and tannin tend to appear poorly compared to others which were shown in (Figure 3). Moreover, tannins have been shown to be present in leaves and pods of *A. sieberiana* tree [16]. Tannins, flavonoids, saponins and steroids have been shown to possess antidiarrheal properties [17]. In present study, terpenoids are found to be the most abundant compounds in *A. paniculata* leaves. It has been found in chloroform, ethyl acetate and methanol extracts. Terpenoids compounds have been reported that it had antibacterial, antiviral, antidiarrhoeal. The most common terpenoid compounds that isolated from *A. paniculata* are diterpenoids lactones. One of the example of diterpenoids lactone is the andrographolide which has bitter taste, colourless and in crystal form [18].

The quantitative estimation of the proximate compositions of *A. paniculata* is shown in Figure 4. It was revealed that the *A. paniculata* contained moisture (11.25%), crude fat (3.19%), crude protein (18.74%), crude fibre (16.10%), ash (9.82%) and carbohydrate (40.91%) as shown in Figure 4, compared to *A. paniculata* containing moisture (73.02%), fat (0.98%), protein (2.85%) and carbohydrate (12.16%), [19] described the high-quality distribution of nutrients in the vegetables.

In this study, *A. paniculata* was found to contain 11.91mg/100mg vitamin C as illustrated in Figure 5 compared to *A. paniculata* found to contain 45.01mg/100mg vitamin C [20]. The deficiency of vitamin C causes scurvy in humans. Vitamin C facilitates wound healing, production of collagen, formation of red blood cells and boosts immune system. The recommended daily allowance of vitamin C is 75mg/day for women and 90mg/day for men. The results of the present study showed that the *A. paniculata* contain sufficient quantity of vitamin C which may help to control the physiological oxidative stress compared to other vitamins.

Some of the mineral constituents of the defatted leaves are given in figure 6. The sodium, calcium, potassium, phosphorous, iron, and zinc contents of *A. paniculata* were as follows 23.7mg/kg, 140.15mg/kg, 77.2mg/kg, 35.1mg/kg, 192.7mg/kg, and 38.9mg/kg respectively, dry weight. The observed data of mineral content of the plant species were found to be significant different compared to the mineral content of *A. paniculata* observed by [20]. The *A. paniculata* have fairly adequate concentrations of Potassium Zinc, Phosphorous, and Sodium, equivalent concentrations of Calcium and Iron.

In this study, body weight indices of the experimental groups, at the commencement of the experiment compared to its weight at the end of the experiment demonstrated a significant difference. This can be attributed to the administration of *Andrographis paniculata* extracts (200 and 400mg/kg) showed proportional decreased in body weight but at high concentration of the extracts (400mg/kg), the animals showed a significant decrease in body weight. This decrease was however more significant ( $P < 0.05$ ) in the *Andrographis paniculata* extracts treated groups as compared with other antidiarrheal plants which contains similar phytoconstituents as found in *Bixa orellana* leave extracts [21].

Castor oil induce diarrhea on experimental rats at the dose of 400 mg/kg body weight, the extract produced a significant decrease in the severity of diarrhea in terms of reduction in the rate of defecation and consistency of feces in Wister rats. At the same dose, the extract showed significant antidiarrheal activity showing 62.5% and 55.7% (ethanol and methanol) extracts reduction in diarrhea comparable to that of loperamide that showed 75% reduction in diarrhea (Figure 9). This experiment is in agreement with the antidiarrheal effect of *Bixaorellana* leaf extract on castor oil induced diarrhea in rat [22].

At  $P < 0.05$ , there was a statistically significant increase in the ALP activity in the serum of the rats in the treated groups as compared to the non-diarrhea control group (Group A) but a decrease was observed compared to the diarrhea control group (Group B). An increase in ALP levels in the serum indicates large bile duct obstruction, intra-hepatic cholestasis or other diseases of the liver [23]. However, in group F (400mg/kg) (Diarrhea effected rats treated with petroleum ether at 400mg/kg), there was a decrease in ALP levels as compared to diarrhea control group, hence, providing optimum efficiency to eradicate other complementary diseases associated with diarrhea in relation to ALP level. An ALP level in the diarrhea control group (Group B) is high compared to all the experimental groups because of the diarrhea condition of the rats in this group and hence hepatocellular damage results causing an inevitable increase in serum enzyme, ALP. This decrease was more noteworthy, at  $P < 0.05$ , in the



groups treated with the *Andrographis paniculata* extracts (200 and 400mg/kg) (Group D, E and F) when compared to the negative control group (Group B) than in the orthodox drug- treated group (Group C).

Hepatic serum enzymes AST and ALT are commonly used as markers of hepatocellular damage [24]. The extent of aminotransferase elevations in the serum depends on the severity of the damage, which will invariably cause an increase in cell permeability. Comparing the ALT and AST levels of the non- diarrheal control group (Group A) with the treated groups (Group D, E, and F) in different concentrations 200 and 400mg/kg, a sharp increase was observed statistically insignificant at  $P < 0.05$ . In the negative control, there was an increase in the levels of ALT and AST when compared to positive control. This is due to the diarrhea condition of the rats in this group resulting in hepatocellular damage and hence, amount to increase in serum enzymes (ALT and AST) levels. The treated groups when compared to the negative control group showed a significant decrease at  $P < 0.05$ . This can be attributed to the ability of *Andrographis paniculata* extracts (200 and 400mg/kg) (Group D, E and F) to ameliorate the diarrhea condition in this rats and hence, restoring the body system of the rats back to normal as compared to the positive control group. A more significant decrease was observed in Group E (200mg/kg) when compared to the negative control group (Group B).

At  $p < 0.05$ , there was a statistically significant increase in the LDH activity in the serum of the rats in the treated groups as compared to the non-diarrheal control group (Group A) but a decrease was observed compared to the diarrhea control group (Group B). An increase in LDH levels in the serum indicates hemolytic anemia, renal diseases, obstructive jaundice [25]. However, in group D (400mg/kg), there was an increase in LDH level as compared to all other experimental groups because of the diarrhea condition of the rats in this group and hence hepatocellular damage results, which tend to inevitably increase the level of LDH in serum enzyme in the groups treated with *Andrographis paniculata* leave extract (Groups D, E, and F) when compared to the negative control group (Group B) than in the orthodox drug-treated group (Group C), however, in diarrhea treated group (Group E)(200mg/kg), there was a decrease in the LDH level as compared to diarrhea control groups; Hence, providing optimum efficiency to eradicate other complementary diseases associated with diarrhea in relation to LDH level.

Serum GGT are commonly used to imply that there is an elevated level of ALP [25]. The extent of GGT elevations in the serum depends on the severity of the damage comparing the GGT levels of the non-diarrhea control group (Group A) and diarrhea control group (Group B) with the treated group (Group D)(400mg/kg), as shown in figure13, a sharp increase was observed statistically insignificantly at  $p < 0.05$ . This is due to the diarrhea condition of the rats in this group resulting in hepatocellular damage and hence, amount to increase in serum enzyme (GGT) level. The treated groups, when compared to the negative control group showed a significant decrease at  $p < 0.05$ . This can be attributed to the ability of ethanolic extract of *A. paniculata* to ameliorate the diarrhea condition in these rats and hence, restoring the body system of the rats back to normal as compared to the positive control group. A more considerable decrease was observed in Group E (200mg/kg) when compared to the negative control group (Group B) as shown in figure 13. However, there was also a decrease observed in GGT levels of the rats in the orthodox drug-treated group (Group C) when compared to the negative control group, this decrease was relatively less evident when compared to the groups treated with ethanolic leaf extract of *A. paniculata*.

In this study, there was a statistically significant decrease in  $\text{Na}^+$  ion levels in the serum of rats in the treated groups as compared to the non-diarrhea group (Group A), but an increase was observed compared to the diarrhea control group (Group B). A decrease in  $\text{Na}^+$  ion level indicate hyponatraemia which may lead to specific conditions like; gastrointestinal loss, kidney disease [26]. However, in groups E and F (400mg/kg), there was an increase in  $\text{Na}^+$  ion level as compared to non-diarrhea control group (Group A), this increase is suspected to be due to the inability of the kidney to excrete adequate  $\text{Na}^+$  from the tubular fluid or the *A. paniculata* leaf extract may contain some  $\text{Na}^+$  based compounds as seen in figure 6. These may have led to the excess  $\text{Na}^+$  ion levels in the tested rats. This observation is in agreement with [27] concluded that hypernatremia almost always indicates water depletion.

The results in figure 12 shows that at  $p < 0.05$  there was a statistical significant difference in the *A. paniculata* leaf extracts treated groups (Groups D, E, and F) as compared to the diarrhea control group (Group B); Potassium ion levels were however non-significant ( $p < 0.05$ ) compared to non-diarrhea control group (Group A) as shown in group F (200mg/kg) and orthodox drug-treated group (Group C)(400mg/kg).



Chloride levels also showed a statistically significant different ( $p < 0.05$ ) lower than the diarrhea control groups values (Groups B, C, D, E, and F). It was observed that there was a statistically significant difference of the diarrhea control group (Group F) (400mg/kg) compared to the non-diarrhea control group (Group A). This indicates the effectiveness of the *A. paniculata* leaf extracts in group F.

Urea clearance is used to determine the glomerular filtration rate of the kidneys. Although not commonly done anymore, they remain useful tests for renal function. Thus, plasma concentrations of urea could be used as indicators of nephrotoxicity. Urea indicates a diminished impaired ability of the kidneys to filter these waste products from the blood and excrete them in urine. As their clearance values decrease, their blood levels increase causing uremia, an excess of nitrogenous wastes in the blood which causes diarrhea [28]. In this study, there was a significant increase in the urea level ( $p < 0.05$ ) in the extract treated groups when compared to the non-diarrhea control group (Group A) but with attendant decrease when compared with diarrhea control group ( $p < 0.05$ ).

### Conclusion

In this study, we discovered that *Andrographis paniculata* leaf extracts showed significant anti-diarrheal activities in dose-dependent ratio on animal models as depicted in the positive activity of *Andrographis paniculata* leaf in castor oil diarrhea induced rats. So, the anti-diarrhea activity of *Andrographis paniculata* leaf extract has been confirmed and validated. The preliminary phytochemical screening of *Andrographis paniculata* leaf extracts showed the presence of alkaloids, tannins, flavonoids, terpenoids, steroids and saponins. These constituents may be responsible for the *in vivo* anti-diarrhea and antihepatic activities.

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