



## The effect of aqueous extract of *Morinda lucida* on haematological parameters and intestinal ATPases, lactase activity and histopathology studies in lactose induced diarrhea in Wistar rats

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### Abstract

Diarrhea remains a major health burden in developing society till date. Efforts aimed at ameliorating this condition is very imminent. In this study, the anti-diarrheal effect of aqueous extracts (dried and fresh forms) of *M. lucida* leaves was evaluated in Wistar rats. 25% lactose enriched diet was used to induce diarrhea. 25 Wistar rats of an average weight of 150g were divided equally into five groups labeled A-E. The diet was admonished for 72 h to rats in groups B-E. Fresh aqueous extract of *M. lucida*, dried aqueous extract of *M. lucida* and loperamide were then administered to groups C-E respectively for the next seven days. The animals were then sacrificed and blood collected for lactase, intestinal ATPases, and some haematological parameters were assayed using standard laboratory procedures. Histopathological examination of the small intestine was also examined. Our results showed a significant increase in the intestinal lactase activity of diarrheic rats. However, the dried extract was able to restore parity with the control. We observed a significant increase in the activities of Na<sup>+</sup>/K<sup>+</sup>-ATPase and Mg<sup>2+</sup>-ATPase while a decrease in the activity of Ca<sup>2+</sup>-ATPase in lactose induced diarrheic rats when compared with the control. The extracts of *M. lucida* further increased significantly the activity of Na<sup>+</sup>/K<sup>+</sup>-ATPase and Mg<sup>2+</sup>-ATPase when compared with the control and the untreated group. However, no significant effect of the extracts on Ca<sup>2+</sup>-ATPase was observed. On haematological parameters, we observed increase in hematocrit and hemoglobin. Treatment with fresh extract and loperamide was able to reverse this increase. Furthermore results of the differential white blood cell count revealed higher lymphocyte count in the group given dried extract of *M. lucida*. The extracts were able to ameliorate the aberrations observed in the architecture of the intestinal lumen.

**Keywords:** *Morinda lucida*, Aqueous extracts, Lactose, Wistar rats, Diarrhea

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### 1. Introduction

Evidence from research findings have confirmed that to truly deal with the modern-day plaques, disease and increasing toxins in our environment, we must return to nature and use the abundant plant resources around us. The earliest humans recognized their importance and have used them as medicine and by all cultures throughout history (Alabi *et al.*, 2005). Since orthodox medicine has been inadequate in the management of

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health problems, the modern man is going back to the age of green medicine (Williamson *et al.*, 2002). Diarrhea, a condition in which there is a frequent passage of abnormally loose and watery stool is the most common disease causing infant death. Poor hygiene, consumption of contaminated foods and close proximity to infected animals and humans all contribute to easy and frequent acquisition of pathogens that cause this condition. These pathogens could be bacteria such as *E. coli*, *Clostridium difficile*, *Shigella species*, *Salmonella species*, *Staphylococcus aureus* and *Campylobacter jejuni*, among many others (Adebolu *et al.*, 2007).

The use of medicinal plants for treatment of microbial diseases is well-known and has been documented since ancient times. Plants synthesize many components, which act as defensive agents, helping to protect them from microbial infection and other predators. Those compounds are bioactive and can be medicinal, intoxicating or toxic depending on circumstances. In different parts of Nigeria, different varieties of plants are used in the treatment of different types of diseases. Roots, barks or leaves of *Newbouldia laevis* are used in the treatment of scrotal elephantiasis, dysentery, ringworm, syphilis, sore eyes and ear ache (Azoro, 2002). Also, the stem bark and leaves of *Anthocliesta djalonensis* are used as antipyretic and in the treatment of stomach ache, gonorrhoea and fever (Azoro, 2002). Shoots of *Phyllanthus pentandrous* is used in treating boils and backache. Herbalists in Soha-Zaria use bark of the stem of *Jacaranda mimosoides* in the treatment of diarrhoea (Azoro, 2002). Different substances have been identified in medicinal plants which are believed to be the antimicrobial agents and these include; different forms of alkaloids, sesquiterpenes, diterpenes, triterpenes saponins, triterpenaglycos, flavonoids, sterols, coumarin, quinines, monoterpenes, different forms of other proteins as well as lipids and tannins (Sofowora, 1993). *Escherichia coli* normally colonize an infant's gastrointestinal tract within 40 h of birth, arriving with food or water or with the individuals handling the child. In the bowel, it adheres to the mucus of the large intestine. It is the primary facultative organism of the human gastrointestinal tract (Todar, 2007). Amongst the Igede People in Benue State of Nigeria, it has been reported that decoction of *Morinda lucida* leaf is used twice or thrice daily for treating diarrhea, and also employed in the treatment of infertility in women (Igoli *et al.*, 2005). Most medicinal plants are known to possess more than one therapeutic properties. *Morinda lucida* may likely be one of such plants. Moreover, its relative abundance and wide spread has drawn the interest of researchers towards investigating its anti-diarrheal properties as previously reported apart from the already known ones (Bernstein *et al.*, 2006).

## 2. Materials and Methods

### 2.1. Collection and Identification of Plants and Parts

Fresh leaves of the plant (*Morinda lucida*, Benth.) were collected at Ekpoma in Esan West local Government area of Edo State Nigeria. The plant was identified in the Botany Department, Ambrose Alli University, Ekpoma.

### 2.2. Experimental Animals

Male Wistar rats weighing between 120-150 g were obtained in the Animal House of the Department of Anatomy, University of Benin. The animals were housed in the Department of Biochemistry in a cage made up of 40 compartments (1 per each compartment). Healthy animals with an average body weight of 150 g were considered for the experimental purposes after acclimatization for a period of two weeks. Animals were provided with Pellet rodent diet and water *ad libitum*. To use Animals for this research, The National Council for Animal Experiments Control (CONCEA) was contacted. Handling of animals was carried out in accordance with the recommended international standard.

### 2.3. Lactose - Induced Diarrhea in Wistar Rats

Albino Wistar rats (25) with average weight (150 g  $\pm$  5) g were used for this purpose. They were divided into five groups of five animals each. Aluminum plates were placed under the cages for the collection of stool. Earlier, two weeks of adaptation period was allowed. Each rat in the groups apart from the normal control group were orally administered 25% lactose rich diet.

### 2.4. Preparation of Lactose-Base Diet

75 g of pellet grower's mash was weighed and added to 25 g of commercially made lactose. The mixture was moisture with water and pelleted using a 5 ml syringe. They diet was then air dried and stored in an air tight container until required for use.

## 2.5. Preparation of Extracts

The leaves were washed with clean water to remove debris and dried under shade for three weeks and pulverized into coarse powdered form in the Department of Pharmacognosy, University of Benin, Benin city. The powder plant material was stored in an air-tight container until used. 500 g of the pulverized plant material was extracted with three liters of distilled water for 48 h. The suspension was shaken occasionally and then filtered with muslin cloth, to yield the aqueous extract. The filtrate was concentrated in rotatory evaporator at 60 °C. The concentrate was then freeze-dried at the Energy Centre in the University of Benin, Benin City and kept in air-tight container until required. The fresh leaf of the plants, collected fresh were washed with clean water and its contents were squeezed out, freeze dried and stored in air-tight container.

## 2.6. Treatment of Lactose-Induced Diarrhea Rats

Group A: Normal control.

Group B: Lactose-induced rats + Normal saline

Group C: Lactose-induced rats + 500 mg/kg fresh leaf extract

Group D: Lactose-induced rats + 500 mg/kg dried leaf extract

Group E: Lactose-induced rats + 3 mg/kg Loperamide

The rats were treated for seven days. The animals were sacrificed on the eighth day and samples collected for analysis.

## 2.7. Sacrifice of Experimental Animals and Collection of Tissues and Organs

The animals were sacrificed by cervical dislocation and blood was collected through cardiac puncture using a 5ml syringe and placed into EDTA bottles for haematological and biochemical assays.

### 2.7.1. Preparation of homogenates (post mitochondria fraction)

1 g of a section of the small intestines was homogenized at 4 °C in 10 ml of phosphate buffer. The homogenate was centrifuged with 80-2 electric centrifuge made in Germany at 8000 g for 10 min. The supernatant was carefully withdrawn and stored in refrigerator until the assays for total, Ca<sup>2+</sup> and Mg<sup>2+</sup> ATPases were done.

## 2.8. Biochemical Assays

### 2.8.1. Assay of ATPase (Mg<sup>2+</sup>, Ca<sup>2+</sup>, and Na<sup>+</sup>/K<sup>+</sup>-ATPase) activities

The assay for total ATPases activity was based on the procedures reported by [Adam-Vizi and Seregi \(1982\)](#) with slight modifications.

### 2.8.2. Assay of lactase activity

The lactase activity assay was carried out as reported by [Craven et al. \(1965\)](#) with slight modifications.

### 2.8.3. Evaluation of haematological parameters

The haematological test was done using an ERMA PCE-210 autohematological analyzer made in Japan.

## 2.9. Statistical Analysis

To test the level of significance, data was expressed as mean ± standard error of the mean and was subjected to analysis of variance (ANOVA). Significant differences between treatment means were determined at 5% level using the Duncan Multiple Range test.

## 3. Results

The effect of *M. lucida* on intestinal lactase activity in lactose induced diarrhea in Wistar rats is shown in Table 1. The intestinal lactase activity of lactose induced diarrhea rats was significantly increased relative to control during induction. A similar trend was observed in induced rats treated with fresh *M. lucida* extract, while no significant change was observed in induced rats treated with the other treatment groups relative to control.

Each value represented the Mean ± SEM of *n* = 5 readings. The *p* < 0.05 value was taken as statistically significant.

Group	Lactase Activity
A	7.06 ± 0.54 <sup>a</sup>
B	12.34 ± 0.67 <sup>a</sup>
C	13.26 ± 0.96 <sup>b</sup>
D	8.20 ± 0.64 <sup>c</sup>
F	9.88 ± 0.36 <sup>d</sup>

**Note:** Each value represented the Mean ± SEM of n=5 readings. The  $p < 0.05$  value was taken as statistically significant; a, b, c, d shows the order of significance of the  $p < 0.05$  values.

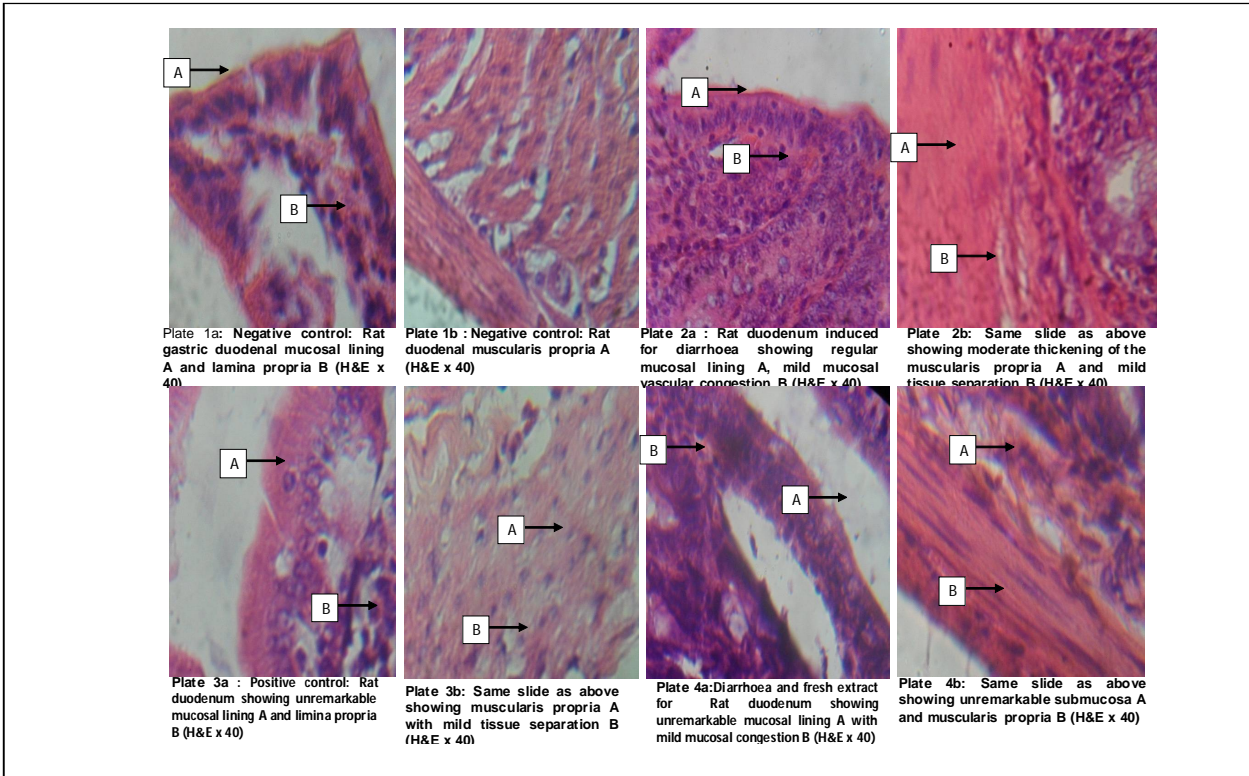
Group	Parameter			
	Na <sup>+</sup> /K <sup>+</sup> ATPase	Ca <sup>2+</sup> -ATPase	Mg <sup>2+</sup> -ATPase	Total-ATPase
A	3.16 ± 0.16 <sup>a</sup>	5.60 ± 0.20 <sup>e</sup>	3.24 ± 0.21	12.00 ± 0.21
B	4.80 ± 0.25	3.46 ± 0.41 <sup>d</sup>	4.52 ± 0.12	12.78 ± 0.12
C	5.44 ± 0.50	2.48 ± 0.39 <sup>d</sup>	4.02 ± 0.45	11.94 ± 0.45
D	5.24 ± 0.58 <sup>b</sup>	2.85 ± 0.90 <sup>d</sup>	3.74 ± 0.43	11.83 ± 0.43
E	4.85 ± 0.43	2.73 ± 0.41 <sup>d</sup>	4.36 ± 0.37	11.94 ± 0.37

**Note:** a, b, c, d shows the order of significance of the  $p < 0.05$  values.

Results from the present study in Table 2 showed the effect of *M. lucida* on intestinal ATPases activity in lactose induced diarrhea in Wistar rat. Our findings show a significant increase in the activities of Na<sup>+</sup>/K<sup>+</sup>-ATPase and Mg<sup>2+</sup>-ATPase while a decrease in the activity of Ca<sup>2+</sup>-ATPase in lactose induced rats when compared with the control. The extracts of *M. lucida* leaves further increased significantly activity of Na<sup>+</sup>/K<sup>+</sup>-ATPase and Mg<sup>2+</sup>-ATPase. When compared with the control and the induced group without treatment. However, no significant effect of the plant leaf extract on Ca<sup>2+</sup>-ATPase was observed.

Group	Parameters								
	WBC (10 <sup>3</sup> /μ)	GR%	LY%	MO%	RBC (10 <sup>3</sup> /μ)	Hgb (g/dl)	HCT%	MCH%	MCHC (g/dl)
A	6.08 ± 1.19 <sup>c</sup>	9.49 ± 3.42 <sup>c</sup>	81.30 ± 8.24 <sup>b</sup>	9.16 ± 4.13 <sup>b</sup>	5.93 ± 0.40 <sup>b</sup>	10.70 ± 0.47 <sup>c</sup>	33.34 ± 1.38 <sup>b</sup>	80.37 ± 18.36 <sup>b</sup>	31.69 ± 0.28 <sup>a</sup>
B	5.23 ± 0.54 <sup>b</sup>	24.43 ± 1.11 <sup>b</sup>	66.88 ± 1.49 <sup>b</sup>	8.75 ± 0.77 <sup>b</sup>	7.98 ± 0.08 <sup>a</sup>	15.06 ± 0.06 <sup>a</sup>	45.04 ± 0.36 <sup>a</sup>	18.84 ± 0.13 <sup>a</sup>	33.40 ± 0.41 <sup>a</sup>
C	4.12 ± 0.33 <sup>b</sup>	17.96 ± 2.79 <sup>a</sup>	66.24 ± 4.26 <sup>b</sup>	15.78 ± 1.88 <sup>c</sup>	6.14 ± 0.46 <sup>b</sup>	10.74 ± 6.45 <sup>c</sup>	35.04 ± 1.36 <sup>b</sup>	62.48 ± 1.70 <sup>b</sup>	30.61 ± 0.31 <sup>a</sup>
D	11.72 ± 3.7 <sup>d</sup>	7.32 ± 1.04 <sup>a</sup>	84.38 ± 2.46 <sup>b</sup>	8.30 ± 1.58 <sup>b</sup>	7.60 ± 1.59 <sup>a</sup>	11.40 ± 2.36 <sup>a</sup>	72.70 ± 3.76 <sup>c</sup>	16.88 ± 2.21 <sup>a</sup>	30.63 ± 0.70 <sup>a</sup>
E	5.10 ± 3.26 <sup>b</sup>	12.38 ± 3.32	71.00 ± 6.92 <sup>b</sup>	16.60 ± 3.59 <sup>c</sup>	7.21 ± 0.81 <sup>a</sup>	12.78 ± 0.28 <sup>a</sup>	41.00 ± 0.63 <sup>a</sup>	56.88 ± 1.57 <sup>c</sup>	31.15 ± 0.21 <sup>a</sup>

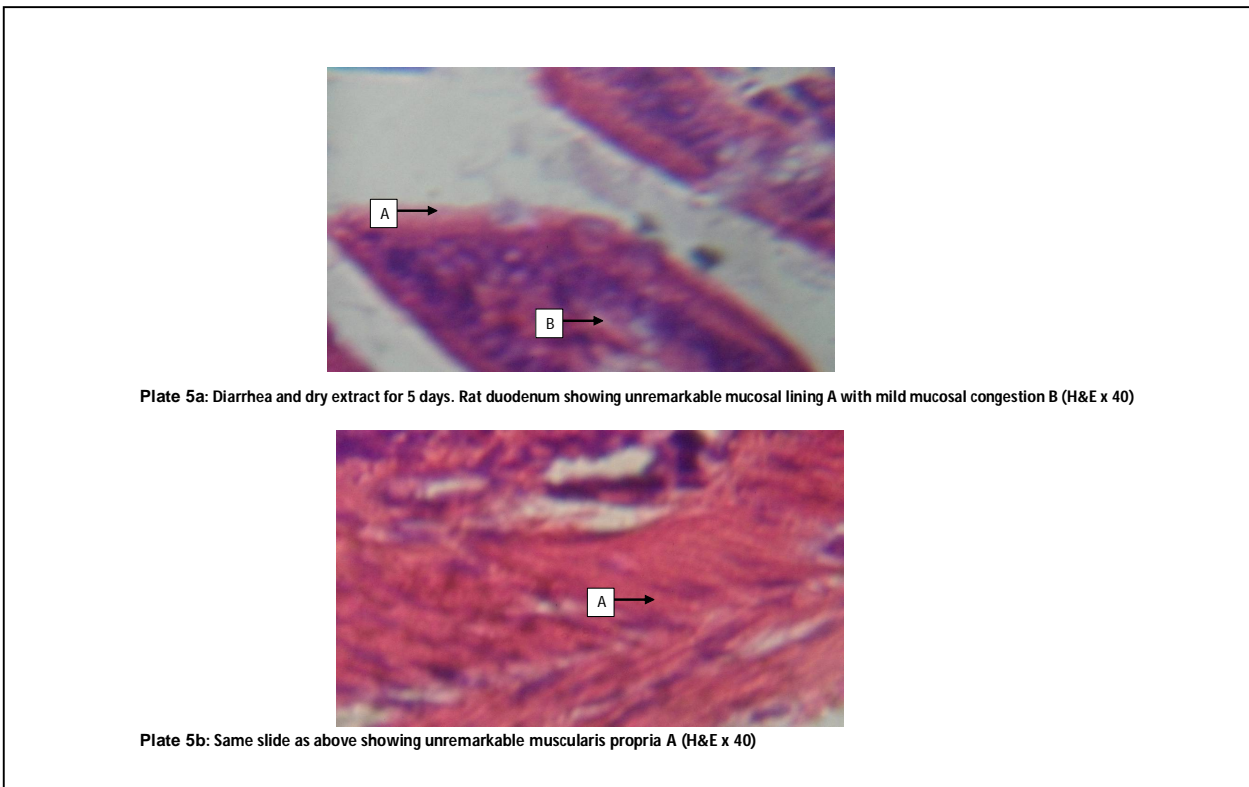
**Note:** a, b, c shows the order of significance of the  $p < 0.05$  values.



**Plate A: Histology of the small intestine of lactose induced diarrheic rats treated with *M. lucida***

Each value represented the Mean ± SEM of n = 5 readings. The  $p < 0.05$  value was taken as statistically significant. The activities of the enzymes were expressed as ( $\mu\text{mole Pi released /min/mg protein}$ )  $10^{-3}$ .

The effect of aqueous extract of *M. lucida* on the haematological parameters in lactose induced diarrhea in



**Plates B: Histology of the small intestine of lactose induced diarrheic rats treated with *M. lucida*.**

Wistar rat is reported in Table 3. The results showed an increase in haematocrit and hemoglobin. Treatment with fresh extract and loperamide was able to reverse this; the group administered dried extract showed an extreme increase in haematocrit. Furthermore the values of the WBCs were reduced as shown below. Results of the differential white blood cell count revealed higher lymphocyte count in the group given dried extract of *M. lucida*. The concentrations of MCH and MCHC were not altered significantly. The effect of aqueous extract of *M. lucida* on the haematological parameters in lactose induced diarrhea in Wistar rat.

### 3.1. Results of Histological Analysis

Lactose induced diarrheic rats (Plates 1 and 2) produced thickening of muscularispropria as well as acute mucosa inflammation. The standard drug used (Plate 3) showed restoration of the tissue architecture of the gastrointestinal tract which was equally matched by the dried extract (Plates 5a and 5b). However, the fresh extract (4a and 4b) appeared slightly less potent.

## 4. Discussion

The effect of *M. lucida* on intestinal lactase activity in lactose induced diarrhea in Wistar rats is shown in Table 1. The intestinal lactase activity of lactose induced diarrhea rats was significantly increased relative to control during induction. A similar trend was observed in induced rats treated with fresh *M. lucida* extract, while no significant change was observed in induced rats treated with the other treatment groups relative to control. The induction of intestinal lactase may be responsible for the increase in lactase activity observed. This indicates that the rats adapted to the use of this carbohydrates. [Bolin et al. \(1971\)](#) reported an increase in intestinal lactase of rats as a result of chronic lactose feeding. In humans, [Jiang and Saviano \(1997\)](#) linked this adaptation with a progressive modification of the intestinal microflora. As a result, an increase in microbial glucosidase activity and a better capacity of fermentation of malabsorbed lactose was reported in their investigation. The withdrawal of lactose as showed in Table 1 led to a decrease in lactase activity. However, the groups administered the dried extract proved to be most effective. A plausible explanation to this may not be unconnected with the anti-diarrheal potential of the extract.

Results from the present study in Table 2 showed that *M. lucida* leaf extract significantly increased the activity of  $\text{Na}^+/\text{K}^+$ -ATPase and  $\text{Mg}^{2+}$ -ATPase. However, no significant effect of the plant leaf extract on  $\text{Ca}^{2+}$ -ATPase was observed. This observation further substantiated the importance of the afore-mentioned parameters in diarrhea and that increasing the activity of  $\text{Na}^+/\text{K}^+$ -ATPase and  $\text{Mg}^{2+}$ -ATPase can serve as one of the treatment routes for diarrhea. [Ewe \(1988\)](#) found that Sodium absorption is abolished and  $\text{Cl}^-$  secretion reduced when  $\text{Na}^+/\text{K}^+$ -ATPase is inhibited as Both  $\text{Cl}^-$  secretion and  $\text{Na}^+$  absorption are dependent on the function of  $\text{Na}^+/\text{K}^+$ -ATPase in the basolateral membrane ([Mark et al., 2002](#)). Not surprisingly, multiple groups have detected decreased activity of epithelial  $\text{Na}^+/\text{K}^+$ -ATPase in inflamed tissue in inflammatory bowel disease (IBD) and infectious enteritis ([Ejderham et al., 1989](#)) these findings suggest that down regulation of  $\text{Na}^+/\text{K}^+$ -ATPase is an important factor in the diarrhea associated with intestinal inflammation.

Although the actual mechanism of action of *Morinda lucida* leaf extract and its specific anti-diarrheal agent (s) is presently poorly known, the results of this study showed that the extract exhibited anti-diarrheal effect, as well as prompt reduction of the watery texture of diarrheal stools and increase in the activity of key enzymes (like  $\text{Na}^+/\text{K}^+$ -ATPase) necessary for proper intestinal absorption of water, electrolytes and other intestinal materials.

Table 3 shows the effect of lactose induced diarrhea on haematological parameters. Haematocrit changes in fluid balance cause increase or decrease in the concentration of red cells. Plasma loss may be monitored by frequent haematocrit measurements. Loss of ECF due to gastroenteritis or other causes similarly increases haematocrit. Conversely fluid overload causes a fall in haematocrit due to dilution. ([Dileep et al., 2013](#)). Our result showed an increase in haematocrit and hemoglobin. This is attributed to dehydration as a result of fluid loss. Treatment with fresh extract and loperamide was able to reverse this; the group administered dried extract showed an extreme increase in haematocrit. This possibly could be the result of its ability to enhance erythropoiesis. This in agreement with the report of [Asuzu and Chineme \(1990\)](#) that *M. lucida* leaf extracts increases packed cell volume and haemoglobin concentration. The reduced WBC and lymphocytes observed in Table 3 may be due to malnutrition since there is deprivation of some essential nutrients due to maladsorption. Episodes of diarrhea has been reported to impair cellular immunity ([Sazawal et al., 1995](#)). The rats treated with dried extract of *M. lucida* significantly ( $p > 0.05$ ) increase the concentration of WBC. This is corroborated in the work done by [Nworu et al. \(2012\)](#); who reported the immunostimulating properties of *M. lucida*. Granulocytes and monocytes did not show any gross deficiencies. Lymphocytes are the main effectors cells of the immune system ([Alli et al., 2011](#)). Results of the differential white blood cell count revealed higher lymphocyte count in the group given dried extract of *M. lucida*. The concentrations of MCV and MCHC were not altered significantly.

The efficacy of the extracts were further probed with histopathological investigations and result from the study indicates a positive outcome of the effect of *M. lucida* in repair of intestinal tissue injury caused by lactose.

## 5. Conclusion

The results of this study have provided very compelling evidence to infer that aqueous extracts of *Morinda lucida* has antidiarrhea effect. This study therefore has further set the stage for characterization of the plant and the determination of the active components of the aqueous extract.

## Ethical Approval for Animal Use for Experiments

To use Animals for this research, The National Council for Animal Experiments Control (CONCEA) was contacted for approval. Handling of animals was carried out in accordance with the recommended International Standard.

## Conflicts of Interest

There is no conflict of interest as regard to this work and publishing the article.

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