An analysis of the effects of Phoenix *dactilifera* crude extract on the GI Tract of Adult Albino Rats

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Abstract: *Phoenix dactilifera (P. dactilifera) has previously been used in the treatment of various ailments; therefore, a study aimed at histologically determining the anti-diarrheal effects of this plant on rats was necessitated. Thirty six (36) Wistar rats of both sexes (150-240g) were randomly assigned to six groups each of six animals and housed in separate cages. Group VI received only water, V received 10µg/g of Castor oil, while those in I-III were pre-treated with P. dactilifera (4µg/g, 8µg/g and 16µg/g) respectively orally before 10µg/g oil. The standard drug Loperamide was administered to group IV (5µg/g, orally). Following treatment with castor oil, the animals were placed in separate cages over clean white sheets and were inspected four hours for the presence of diarrheal droppings. The rats were thereafter sacrificed and the GIT were harvested and fixed. Standard histological techniques were employed and the resultant slides were analyzed with the aid of a light microscope. P. dactilifera significantly caused delay in passing stool, reduced mean stool weight, and decreased defecation frequency. Histologically, P. dactilifera comparably to Loperamide ameliorated oedema observed in the wall of the gut especially in the small intestine and also caused congestion of blood vessels with activation of the immune system through aggregations of lymphocytes. This agent returned the histoarchitecture of the GIT to normal and furthermore, induced variable levels of immune responses, through acute inflammation and activation of lymphoid aggregates. The protection was dose dependent. Therefore the result of this study revealed a histological basis for the anti-diarrheal curative effects for P. dactilfera.*
Key Words: *antidiarrheal, P. dactilifera, GIT, Castor Oil, Loperamide, Rats.*

I.   Introduction

Diarrhoea has been described as a medical condition involving passing at least three loose or liquid stools daily and had been the cause of about 40% of pediatric admission resulting from infections [1]. Although several factors have been attributed to the cause of this illness, the most important ones according to [2] are infectious agents, certain medications, plants and animal toxins hence making the disease one of the most deadly diseases and accounting for 4-5 million deaths annually [3]. Recently, WHO has included a programme which incorporated traditional herbal medicine in the treatment of some diseases thereby leading to some researches into herbal treatment of diarrheal diseases [4]. These programmes were acceptable because some studies were able to prove the safe, clinically effective, better patient tolerant, relatively less expensive and globally competitive nature of some of these herbs [1] [4] [5]. Therefore the role of traditional medicines in the solution of health problems is invaluable on a global level and cannot be over emphasized. Also, with the associated adverse effects of most modern drugs, herbal medications have become important and have been scientifically proven to be potent in the management of diseases. Date palm (Phoenix *dactilifera*) fruit is well consumed mostly by the Muslims during their fasting periods that usually lead to religious celebrations. This plant has been associated with the treatment of swollen limbs, cough, infections, ulcers, cancers and infertility problems [6] [7] [8] [9]. More recently, a study by [10] reported a statistically significant reduction in both castor oil intestinal transit time and frequency of diarrhoea in rats, although they failed to investigate the histological basis for its curative effects. In another study by [11], they observed a statistically significant reduction in frequency of defecation and gastrointestinal motility in rats' treated with high doses of the plant. Therefore this study was focused on the histological basis of these anti-diarrheal or curative effects of this agent in rats. The aim of the study was to histologically determine the effects of crude extracts of date palm fruit on the GIT of adult Wistar rats.

II.   Materials and Methods

Study Design
The study involved both the use of experimental and observational study design.

Experimental Animals

The study involved the use of thirty six (36) adult Wistar rats of both sexes (18 males and 18 females) that were obtained from the animal facility of the Department of Anatomy, University of Benin, Benin City. The animals were in good physical condition weighing 150-250g. The rats were kept in an iron cage with compartments and allowed to acclimatize for three weeks under favorable climatic conditions. They were fed with standard rat pellets and water Ad libitum.

Drugs Used for the Experiment

Castor oil and Loperamide were bought from a Pharmaceutical store in Benin and used for the experiment.

Collection and Preparation of Plant Material

Date palm (Phoenix *dactilifera*) fruit was bought from Hausa merchandise at Hausa quarters, Ring- road Benin City, Nigeria. The fruit was authenticated at forest research institute of Nigeria (F.R.I.N) Ibadan.

Processing of the Plant Material

The fruits were spread on the laboratory table to dry for 3 days; thereafter they were further dried in the oven maintained at 50°C. After this treatment, the dried fruits were grounded to powdered-form using a laboratory electric milling machine (Chris Norris, England). The powder was kept in air tight container for subsequent use.

Preliminary Phytochemical Screening

Phytochemical screenings were done using standard procedures previously used by [12] and [13].

Extraction of the Plant Material

About 2.0kg of the powdered fruit of Phoenix *dactilifera* was macerated with 4.5L methanol for 72 hours at room temperature with occasional shaking and stirring. Each of the material was then filtered and filtrate concentrated to dryness using rotary evaporator maintained at 40°C.

Experimental Protocol

The method described by [14] was employed though modified to suit experimental needs. Wister albino rats (weighing 150-240g) were randomly placed into six groups each of six animals and housed in separate cages. Groups' I-III was treatment groups while IV- VI was control groups. Animals of group V and VI were pre-treated with 4ml/Kg water, those in groups I, II and III were pre-treated with Phoenix *dactilifera* crude extract ($4\mu g/g$, $8g/\mu g$, and $16\mu g/g$ respectively) while IV was pre-treated with the standard drug Loperamide ($5\mu g/g$, orally) orally using the orogastric cannula. After 30 minutes of these pre-treatments, groups I-V received castor oil ($10\mu/g$ orally) while VI received water only using the orogastric cannula. Following these treatments, each animal was then placed in separate compartment over clean white cardboard paper and were inspected up to six hours (by an observer unaware of the particular treatment) for the presence of the characteristic diarrheal droppings; their absence was recorded as a protection from diarrhea [15] and the percentage protection calculated [16].

Methods of Sample Collection

At the end of the experiment the rats were sacrificed by chloroform anaesthesia. The stomach, small intestine and large intestine were harvested for histological evaluation. The organs were preserved with 10% formol saline (8.5g NaCl, 900ml distilled water and 100ml formalin) for 48 hours. The tissues were processed using standard manual tissue processing techniques. Sections of about $3-5\mu m$ were cut by a rotary microtome, stained with haematoxylin and eosin by a standard staining method and examined under light microscope [17]. The slides were viewed and captured on a Brunel light microscope (Brunel SP35 Digital Trinocular).

Ethical clearance

Approval for this study was obtained from the Research Ethics Committee of the Faculty of Medicine, University of Benin and was carried out in strict accordance with the guidelines for the care and use of animals for research [18].

III. Results

Yields of the Fruit Materials

The 2kg of the powdered sample of Phoenix *dactilifera* was observed to yield 95.55g of the methanol extract corresponding to 4.78%. Partitioning of the methanol extract of the fruit gave 42.57g (2.13%) of the aqueous and 28.55g (1.43%) of the chloroform fraction respectively.

Table I: Result of Phytochemical Screening

Constituents	Results
Test for carbohydrate	+
Test for Morlish	+
Test Anthraquinone	+
Test for Flavonoid	+
Test for Tannins	+
Test for Saponins	-
Test for Alkaloid	+
Test for Steroid	+

Table I showed a summary of the result from phytochemical screening of the plant. Out of all the active ingredients tested for in the plant, only saponins were absent from the plant.
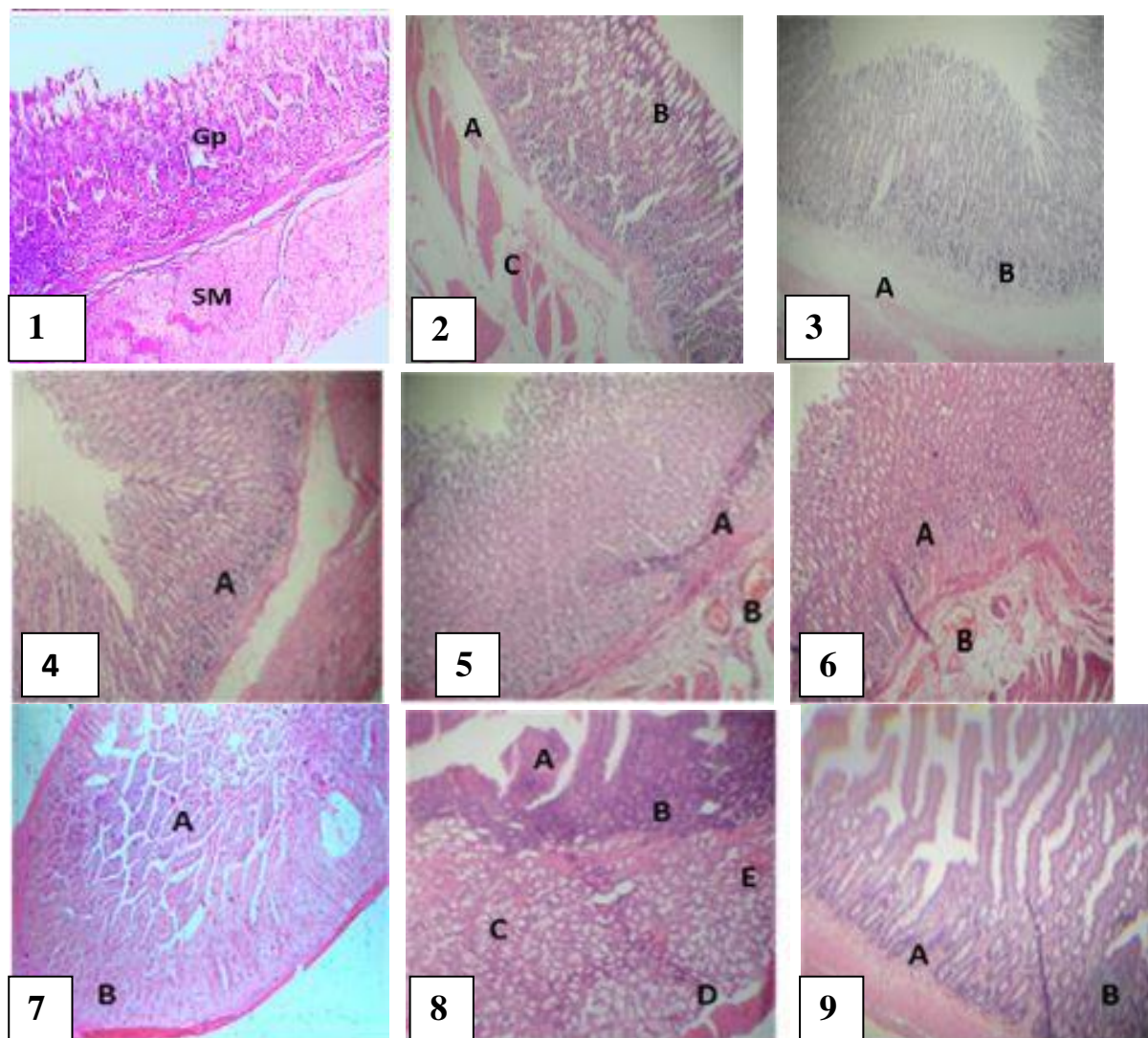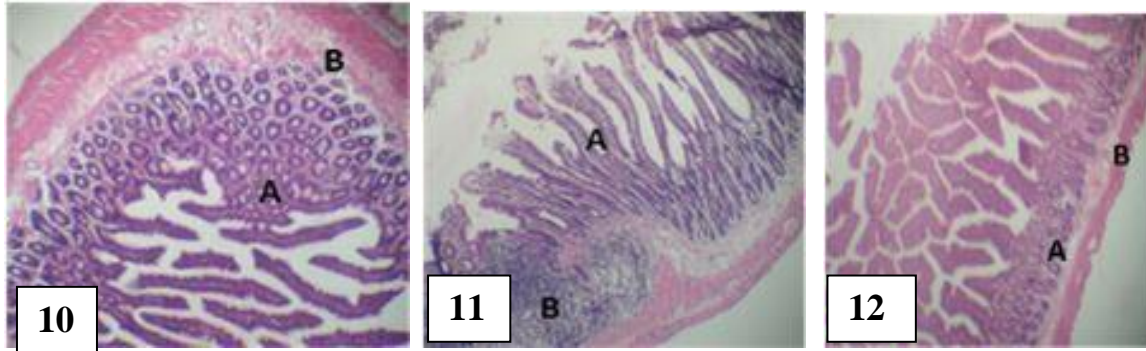
Fig 1 normal rat stomach (H&E x 10); Gp= gastric pit, Sm= submucosa. **Fig 2** Rat Stomach treated with Castor oil for 8 hrs (H&E, x5); A= oedema, B= separated glands. **Fig 3** Rat Stomach treated with Castor oil and Loperamide for 8 hrs (H&E, x5); A= oedema, B= increased basophilia of gland. **Fig 4** Rat Stomach treated with 4mg P. *dactilifera* and Castor oil for 8 hrs (H&E x5); A= increased basophila of gland. **Fig 5** Rat Stomach treated with 8mg P.*dactilifera* and Castor oil for 8 hrs (H&E x5); A= increased basophila of glands, B= vascular congestion. **Fig 6** Rat Stomach treated with 16mg P.*dactilifera* and Castor oil for 8 hrs (H&E, x5); A= increased basophila of glands, B= vascular congestion. **Fig 7** normal rat small intestine (jejunum); B = submucosa, A = villi (H&E x100). **Fig 8** Rat Small Intestine treated Castor oil for 8 hrs (H&E, x5); A= mucosal fold, B= serous glands, C= submucosal mucous glands, D= muscle layer. **Fig 9** Rat Small Intestine treated with Castor oil and Loperamide for 8 hrs (H&E x5); A= increased basophilia of mucosal gland, B= lymphoid aggregate. **Fig 10** Rat Small Intestine treated with 4mg P. *dactilifera* and Castor oil for 8 hrs (H&E, x5); A= increased basophilia of epithelia, B= vascular congestion. **Fig 11** Rat Small Intestine treated with 8mg P. *dactilifera* and Castor oil for 8 hrs(H&E x 5); A= attenuated mucosa, B= activated lymphoid aggregate. **Fig 12** Rat Small Intestine treated with 16mg P. *dactilifera* and Castor oil for 8 hrs(H&E, x5); A= increased basophilia, B= vascular congestion.

Fig 1 showed a section of the rat stomach treated with just water as placebo composed of gastric glands which were thrown into folds of rugae in some foci. The underlining lamina propria, submucosa and muscle wall were intact (H&E, x10).

Fig 2 showed a section of the rat stomach treated with Castor oil for 8 hrs. There was observed oedema fluid cross the wall (A) of the stomach, with separation of the glands (B) and muscle layer (C). There was also a mild increased basophilia of mostly the lower mucous glands. These features were marked in this section suggestive of a diarrheal gut (H&E x **5**).

Fig 3 showed a section of the rat stomach treated with Castor oil and Loperamide (5mg/kg) for 8 hrs showing mild oedema across the wall (A) and marked increased basophilia of the lower mucosal glands (B) as well as mild acute inflammation in the Sub-mucosa. The muscular layer was compacted and intact just as observed in **Fig 1** above (H&E x **5**).

Fig 4 showed a section of the rat stomach treated with 4mg P. *dactilifera* and Castor oil for 8 hrs. There was an observed mild compacting of the architecture of the tunics compared to **Fig 2** with increased basophilia of the lower mucosal glands (A) (H&E x **5**).

Fig 5 showed a section of the rat stomach treated with 8mg P. *dactilifera* and Castor oil for 8 hrs. There was mild increase in the number of mucosal glands, thickening of the muscularis mucosa and submucosal congestion with increased basophilia of the lower glands (A) and moderate vascular congestion (B) (H&E x **5**).

Fig 6 showed a section of the rat stomach treated with 16mg P. *dactilifera* and Castor oil for 8 hrs. The features noticed were moderate increase in the number of gastric glands, increased basophilia of the lower mucosal glands (A) and mild acute inflammation, and moderate vascular congestion (B) (H&E x **5**).

Fig 7 showed a section of the rat small intestine composed of both long and short villi (V) that were lined by simple columnar epithelium with brush border. The underlining lamina propria (Lp) was intact. The submucosa (SM) was composed of two layers of smooth muscles arranged perpendicular to each other and showed normal architecture.

Fig 8 showed a section of the rat small intestine treated with Castor oil for 8 hrs. Features observed in this section include mucosal folds (A), serous glands (B), submucosal mucous glands (C) and muscle layer (D) separated by oedema fluid with mild congestion of surrounding blood vessels (E) (H&E x **5**).

Fig 9 showed a section of the rat small intestine treated with Castor oil and Loperamide (5mg/kg) for 8 hrs. The small intestine showed a compact architecture and focal aggregation of lymphocytes (B) in the Mucosa.

Fig 10 showed a section of the rat small intestine treated with 4mg P. *dactilifera* and Castor oil for 8 hrs. Compact gastric walls with increased basophilia of the lower mucosal glands (A) were observed and mild vascular congestion (B).

Fig 11 showed a section of the rat small intestine treated with 8mg P. *dactilifera* and Castor oil for 8 hrs. There was compact architecture of the tunics with moderate activation of lymphoid aggregate (B) and increased basophilia of the lower mucosal glands. The section also showed mildly attenuated Mucosa folds (A).

Fig 12 showed a section of the rat small intestine treated with 16mg P. *dactilifera* and Castor oil for 8 hrs. The small intestine showed a compact architecture of its wall with increased basophilia of the lower mucosal glands (A) and mild vascular congestion (B), as well as mild acute inflammation.

Table 1: descriptive statistics for duration of protection against diarrhea (Minutes)

Groups	Treatment	Number of Animals	Minimum	Maximum	Mean ± SE	% Inhibition (100%)
1	4µg/g of extracts + 10µg/g castor oil	6	29.00	70.00	51.00±5.37*	20
2	8µg/g of extracts + 10µg/g castor oil	6	43.00	116.00	63.17±10.77*	26.32
3	16µg/g of extracts + 10µg/g castor oil	6	20.00	240.00	83.33±34.21*	34.72
4	5µg/g of Loperamide + 10µg/g castor oil	6	240.00	240.00	240.00±.00**	100
5	Water + castor oil	6	10.00	69.00	38.83±9.36	16
6	Placebo (Water only)	6	15.00	100.00	50.00±13.52*	20.83

n= 6; *= $p < 0.05$; ***= $p < 0.001$
Significant difference when compared with control (group 5) using a multiple comparison analysis.

Table 2: descriptive statistics for frequency of stool (count)

Groups	Treatment	Number of Animals	Minimum	Maximum	Mean ± SE
1	4µg/g of extracts + 10µg/g castor oil	6	1	5	3.33±.61*
2	8µg/g of extracts + 10µg/g castor oil	6	1	3	2.17±.31**
3	16µg/g of extracts + 10µg/g castor oil	6	0	4	2.17±.60**
4	5µg/µg of Loperamide + 10µg/g castor oil	6	0	0	0.00±.00**
5	Water + 10µg/g castor oil	6	3	6	4.67±.42
6	Placebo (water)	6	1	4	2.50±.50*

n= 6; *= $p < 0.05$; ***= $p < 0.001$
Significant difference when compared with control (group 5) using a multiple comparison analysis.

Table 3: descriptive statistics for weight of wet stool

Group	Treatment	Number of Animals	Minimum	Maximum	Mean ± SE
1	4µg/kg of extracts+ 10µg/g castor oil	6	280	1690	733.33±215.71*
2	8µg/g of extracts + 10µg/g castor oil	6	50	750	373.33±96.87**
3	16µg/g of extracts + 10µg/g castor oil	6	0	1000	300.00±149.31**
4	5µg/g of Loperamide + 10µg/gcastor oil	6	0	0	0.00±.00**
5	Water + castor 10µ/g oil	6	50	1810	798.33±310.12
6	Placebo (water)	6	90	1020	500.00±171.33*

n= 6; *= $p < 0.05$; ***= $p < 0.001$
Significant difference when compared with control (group 5) using a multiple comparison analysis.

IV.   Discussion

Diarrhoea from noninfectious causes such as induced in this study can lead to inflammatory bowel diseases and irritable bowel syndrome [19] with specific features as observed in the present study. Diarrhoea induced by castor oil, occurs when there is hydrolysis of the oil by intestinal lipases resulting in the release of ricinoleic acid which in turn caused an irritating reaction on the wall of the intestine thereby enhancing the peristaltic activity of the small intestine and also reducing the net absorption of water and electrolytes [20]. Histological analysis of the GIT of the rats treated with castor oil revealed separation of gastric water (oedema) as well as mild congestion in the stomach. These features have been understood to accompany secretory and

inflammatory diarrhoea often caused by substances that usually increase gastrointestinal tract secretions [2]. In most cases, much of the pathology of secretory and inflammatory diarrhea was reported to be due to inflammatory responses of infected intestinal epithelia through secretion of pro-inflammatory cytokines [19] which could have resulted to the congestion observed in the GIT.

Loperamide, a drug widely used in the management of diarrheal disorders has been reported to be effective in the prevention of diarrhoea induced by castor oil, prostaglandins, and cholera toxin [21]. The pharmacological effects of loperamide were observed to be due to its anti-motility and anti-secretory properties [22]. From our investigation, it is likely that the plant extract mediated its effects through similar mechanisms. Prostaglandins have earlier been implicated in the patho-physiology of diarrhoea [23] due to its inflammatory responses mostly on blood vessels. According to [24], flavonoids were known to modify the production of cyclo-oxygenase 1 and 2 (COX-1, COX-2) and lipo-oxygenase (LOX) there by inhibiting prostaglandin production. Also, they observed that the activation of LOX was induced by fatty meals while COX1and COX-2 was by diarrhea-genic agents. Phytochemical analysis carried out on P. *dactilifera* revealed it contained flavonoids. Although several other ingredients were present in the extract, it was most likely flavonoids, singly or in combination with tannins, that was responsible for the observed anti-diarrheal effects. Most of the ameliorating changes observed were dose-dependent and were comparable with those noticed in loperamide treated rats.

Other finding from this study was the inhibition of diarrhea that was also dose-dependent. The mean inhibition time of the extract was very significantly (p< .001) lower than that of the drug, although the extract was observed to have caused significant delay (p< .05) of stooling in the rats. Frequency of stooling was significantly lower (p< .05) in rats treated with the extract compared to the placebo group but was not dose-dependent. Also, observed in our study was a dose-dependent significant (p< .05) decrease in weight of wet stool of rats treated with P. *dactilifera* when compared with the diarrheal control (group 5) suggesting an increased reabsorption of fluid within the GI lumen. Our result conforms to that of [12] which reported reduced frequency of defecation as well as decreased gastrointestinal motility in rats treated very large doses of P. *dactilifera* although their findings did not suggest any dose-dependency. Also, [11] had earlier reported that spathe aqueous extract of this plant produced a statistically significant reduction in both intestinal transit time and frequency of diarrhea in rats. However, the physiological features observed in the index study showed that loperamide was a far better antidiarrheal drug than this agent.

V.   Conclusion

This study revealed that the aqueous extract of Phoenix *dactylifera* contained pharmacologically active substance(s) with antidiarrheal properties thereby making it an effective drug for management of diarrhea mostly in cases where orthodox drugs are contraindicated or not available.

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