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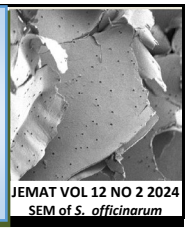

Evaluation of Phytochemical Components and Antidiarrheal Activity of Hydro-Methanolic Extracts of *Carica papaya* Seeds in Castor Oil-Induced Diarrhea in Wistar Rats

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ABSTRACT

Carica papaya, popularly known as pawpaw in English, is a plant that grows worldwide and bears leaves, fruits, and seeds with many medicinal properties, including anti-inflammatory, antidiarrheal, and antibiotic effects. The seeds of *Carica papaya* were collected at Dandagoro Garden and authenticated by a botanist at Ummaru Musa Yar'adua University, Katsina, with voucher number UMYUK/P/105.This study aimed to evaluate the antidiarrheal activity of the hydro-methanolic extract of *Carica papaya* seeds in Wistar rats using a negative and positive control. The seed extract was administered to Wistar rats (weighing 162 g) at different doses in each of the five groups, each containing five Wistar rats. Thirty minutes after the administration of the extracts, diarrhea was induced using castor oil. The quantitative determination of phytochemical constituents in *Carica papaya* seeds was performed using a UV spectrophotometric method. The results shows that alkaloids having the highest amount of (24.03±0.08 mg/100 g) whereas tannins having the lesser amount (0.02±0.01 mg/100 g), also the antioxidant activities *of Carica papaya* seed extract and its effects on some biomarkers where determined which includes the Intestinal Protein (IP), malondialdehyde (MDA), reduced glutathione(GSH), superoxide dismutase(SOD), alkaline phosphatase (AP), nitric oxide (NO) and Na^+-K^+ ATPase. The levels of IP, SOD and AP shows a significant decrease when compared to the negative control at ($p \leq 0.05$). The levels of NO, GSH, and Na+-K+ ATPase show a significant increase compared to the negative control ($p \leq 0.05$). The levels of MDA do not show any significant increase or decrease when compared to the negative control ($p \geq 0.05$). In conclusion, the findings of this study show that *Carica papaya* seeds have antidiarrheal properties, albeit in a lesser amount, which may be due to their lower flavonoid content. However, *Carica papaya* seeds can be used to control diarrhea before an absolute proper medication.

INTRODUCTION

Due to unsanitary living conditions, people in third-world countries are susceptible to a number of common diseases. According to the World Health Organization (WHO), diarrheal is the second leading cause of death in children under 5 years old [1]. During normal bowel movements, they are often altered, resulting in increased water content, volume, or stool frequency. The most common cause of diarrhea is an infection of the digestive tract with various bacteria, viruses, and parasites. This infection can be spread through food, water, and unsanitary environments.

Diarrhea is generally defined as the passage of abnormally liquid or unformed stools associated with increased frequency of defecation and abdominal pain [2]. In spite of improvements in morbidity and mortality worldwide, diarrhea still claims more than 2 million lives each year and is linked to delayed physical and intellectual growth in nations with poor resources [3].

This is especially true for infants and young children in underdeveloped nations, where diarrheal disease is thought to be the cause of 1.5 million deaths annually, or 17.5-21% of all pediatric fatalities. 78% of all infant diarrheal deaths occur in

Africa and Southeast Asia, placing a great financial burden on healthcare spending [4]. The United Nations Children's Fund and World Health Organization [5]. Defined diarrhea as having loose or watery stools at least three times per day or more frequently than normal for an individual [5]. According to [6], there are three main types of diarrhea episodes: acute watery diarrhea, which frequently causes varying degrees of dehydration. Persistent diarrhea is characterized by episodes that last for more than 14 days [7]. This type of diarrhea causes malabsorption, nutritional losses, and wasting in addition to bloody diarrhea [5].

According to [5] and [8], children who experience mild or acute diarrheal episodes lose a considerable amount of fluid and become severely dehydrated, which can be fatal. Bloody and mucous stools were classified as acute and dysentery by [9]. Dysentery is a kind of diarrhea that can occasionally be fatal. The organisms that cause diarrhea include bacteria, viruses, and protozoa, according to [5]. One of the main causes of diarrhea is the rotavirus, which accounts for 40% of hospital admissions among children under the age of five worldwide [10].

Despite the efforts of international organizations to control this disease, the incidence of diarrhea remains very high [11]. Some antibiotics are used as anti-infective agents, but these drugs sometimes have side effects, and microorganisms tend to develop resistance to them [12]. Therefore, the search for safer and more effective plant-derived agents continues to be an important area of active research. However, plants have long been an important source of new medicines.

Many plants have been studied for their medicinal properties. For the treatment of diarrheal, medicinal plants are potential sources of anti-diuretic drugs [13]. The World Health Organization (WHO) estimates that 80% of the population in certain Asian and African countries currently uses herbal medicines for some aspect of primary healthcare. Studies in the United States and Europe have shown that their use is less common in clinical settings, but has become increasingly important in recent years as scientific evidence of the effectiveness of herbal medicines mounts. Pharmaceuticals are increasingly popular [14].

BACKGROUND OF THE STUDY

Carica papaya, commonly known as pawpaw in English, Papitain in Hindi, and Gwandain in Hausa, belongs to the Caricaceae family, which consists of four genera. The tree originates from southern Mexico [15]. *Carica papaya* is a large, single-stem tree growing up to 5–10 m tall, with spirally arranged leaves limited to the top of the stem. The truck below has obvious scars where the leaves and fruit were. The leaves are large, 50 to 70 [20 to 28 inches] in diameter, deeply lobed and palm-shaped with seven lobes [10]. Every part of *Carica papaya* has economic value, and its uses range from nutrition to medicine [16]. The fruit is commonly used to make juice and wine, and it is also cooked as a vegetable [17].

The seeds are medically important in the treatment of sickle cell anemia and disorders related to poisoning. Tea or leaf extracts are said to have tumour-killing and anti-cancer effects. Fresh green tea has antiseptic properties, while dried brown papaya leaves are best used as a tonic and blood purifier [18]. Traditionally, it is used to treat yellow fever, diarrhea, eczema, skin rashes, and stomach pain [16]. In Nigeria, papaya is one of the most popular, inexpensive, and economically valuable fruit crops grown and consumed due to its nutritional content and medicinal properties [17]. *Carica papaya* contains the enzyme papain, which is found in the fruit, stem, and leaves [17]. It contains bioactive compounds such as chymopain and papain that aid digestion [16].

To date, many traditional medicinal plants claiming medicinal value are awaiting scientific verification of their effectiveness and safety. Accordingly, this study was conducted to evaluate the antidiarrheal activity of hydromethanolic extract of *Carica papaya* seeds in Castor oil-induced diarrheal. Diarrhea is commonly defined as the passage of abnormally loose or poorly formed stools, accompanied by increased frequency of defecation and abdominal pain [1]. Although morbidity and mortality have decreased worldwide, disease remains responsible for more than 2 million deaths each year and is associated with impaired physical and cognitive development.

Knowledge in countries with limited resources [2,3]. This is more important in the case of infants and young children. For example, in developing countries, diarrheal diseases account for about 17. 5-21% of all deaths in children under 5 years old, equivalent to 1.5 million deaths each year. Of all childhood deaths due to diarrheal diseases, 78% occur in the African and Southeast Asian regions, placing enormous economic pressure on health care costs [4].

MATERIAL AND METHODS

Collection and Identification of Plant Material

Fresh *Carica papaya* seeds were collected locally in Katsina State from a market by fruit sellers on October 25, 2023. The tree was identified and confirmed by a botanist from the Department of Biological Sciences at Al-Qalam University in Katsina.

Preparation of Plant Extract

After *Carica papaya* seeds were collected, they were washed and left to dry at room temperature for 3 days at the Biochemistry Research Laboratory of Al-Qalam University, Katsina. Dried *Carica papaya* seeds are then ground into coarse powder using a pestle and mortar.One hundred grams of crushed *Carica papaya* seeds were soaked in a 50% methanol and 50% distilled water solution for 48 hours, stirring occasionally. The soaked, crushed seeds were filtered using Whatman filter paper (type no:101).The residue is removed, and the filtrate is evaporated using a rotary evaporator. The extract was stored in the refrigerator for later analysis [19].

Preliminary phytochemical investigations

Preliminary phytochemical studies were conducted using the hydromethanol extract of C. papaya seeds to determine the presence of phytochemical constituents in the extract. All chemicals and reagents used were of analytical grade. [20].

Phytochemical analyses

In order to determine the presence of specific phytochemicals in *Carica papaya* seed extracts, a qualitative phytochemical analysis was conducted using standard methods (unpublished results). Once the presence of the phytochemical had been determined, the quantification of its quantity was then conducted.

(i) Test for flavonoids

About 1.0 g of each of the five extracts of *Carica papaya* seed was diluted with 200 µL of distilled water separately, followed by the addition of 150µL of sodium nitrate (5%) solution. The mixture was then incubated for 5 minutes and 150 µL of ammonium chloride (10%) solution was added and made up to 5ml with distilled water. The mixture was shaken well and left for 15 minutes at room temperature. The absorbance was measured at 510 nm. The total flavonoids were expressed as rutin

equivalent (mg RE)/g extract on a dry weight basis using the standard curve [20].

(ii) Test for alkaloids
A total of 100 mL of 20% acetic acid was added to 2.5 g of each *Carica papaya* seed extract in a 250 mL beaker and covered to stand for 4 hours. The mixture was then filtered, and the volume was reduced to one-quarter using a water bath. A concentrated solution of ammonium hydroxide was then added dropwise to the sample until the precipitate was complete. The whole solution was then allowed to settle, and the precipitate was collected by filtration and weighed [24]. The percentage of total alkaloid content was calculated as: Percentage of total alkaloids (%) = (weight of residue (g) x 100) / weight of sample taken.

(iii) Test for saponins
This was estimated based on vanillin-sulphuric acid colorimetric reaction with some modifications. 50 µg of each extract was added with 250 µL of distilled water. To this, 250 µL of vanillin (800 mg of vanillin in 10 mL of 99.5% ethanol) was added. Then, 2.5 mL of 72% sulfuric acid was added, mixed thoroughly, and the solution was kept in a water bath at 60 °C for 10 minutes. Afterward, it was cooled in ice-cold water, and the absorbance was read at 544 nm. The values were expressed as diosgenin equivalents (mg DE/g extract derived from the standard curve [25].

Test for Total Phenolic
The total phenol content of *Carica papaya* was estimated using the Folin-Ciocalteau reagent by the method described in [21]. About 20 µg of *Carica papaya* seed extracts was taken separately and made up to 1 mL with distilled water. Then 500µL of distilled Folin-phenol reagent (1:1ratio with water) and 2.5 mL of sodium carbonate NaCO (20%) was added. The mixture was shaken well and incubated in the dark for 40 minutes to develop color. After incubation, the absorbance was measured at 725nm. A calibration curve for gallic acid was constructed, and linearity was observed in the range of 10-50 µg/mL. The total phenolic content in the *ficussycomorous* extracts was expressed as mg of gallic acid equivalent (mg GAE/g extract) by using the standard curve.

Test for Tannins
Tannins content of *Carica papaya* extract was estimated by the method of [21]. A total of 500 µL of the extract was taken in a test tube separately and treated with 100 mg of polyvinyl polypyrrolidone and 500 µL of distilled water. This solution was incubated at 4°C for 4 h. Then, the sample was centrifuged at 5000 rpm for 5 min, and 20 µL of the supernatant was taken. This supernatant has only simple phenolic-free tannins (the tannins would have been precipitated along with the polyvinyl polypyrrolidone). The phenolic content of the supernatant was measured at 725 nm and expressed as the content of free phenolic on a dry matter basis from the above result, the tannin content of the extract was calculated as follows:
Tannins (mg GAE/g extract) =Total phenolic (mg GAE/g extract)-Free phenolic (mg GAE/gextract).

Experimental Animals
Sixty (60) healthy male and female Wistar rats (Rattus norvegicus) weighing (120+2.33) g were obtained from the Nigerian Trypanosomosis Research Institute, Kaduna, Kaduna State, in Nigeria. Animals are kept in clean plastic cages, placed in well-ventilated barn conditions (temperature: 25–27 °C; photoperiod with a light-dark cycle of approximately 12 hours; relative humidity: 45%–50%). Animals were acclimatized for two weeks and allowed unrestricted access to clean mouse pellets and tap water. The cages are cleaned daily. This study involving animals was conducted in accordance with internationally accepted ethical standards for the use of laboratory animals, including the guidelines and principles outlined by the National Committee for Research Ethics in Science and Technology. The institution where the research was conducted does not have a formal animal ethics committee. However, all experimental procedures were carried out by qualified personnel with prior training in animal handling and welfare.

The study was designed to minimize animal suffering, reduce the number of animals used, and ensure humane treatment throughout. The authors affirm that they accepted full responsibility for the ethical conduct of the study and confirm that no unnecessary harm or distress was caused to the animals. All applicable national regulations regarding animal experimentation were strictly adhered to. We acknowledge the absence of a formal ethics approval number due to the institutional context and are prepared to provide further clarification or documentation upon request [26].

Experimental Design

Effect of hydro-methanol seed extract of *Carica papaya* on castor oil-induced diarrhea in Wistar rats
A total of 25 Wistar rats, weighing between 120 ± 2.33 grams, were used. The rats were fasted for 6 hours prior to the experiment, but were allowed free access to water. The experimental rats were completely randomized into five groups of five animals each. The procedure described by [24].was adopted with slight modification. Animal in group A was treated with (1ml/kg) body weight of distilled water orally (negative control), while those in group B were treated with Ref. Drug Loperamide (positive control) 1 mL/kg body weight. Animals in groups C, D, and E were treated orally with 50 mg/kg, 100 mg/kg, and 200 mg/kg body weight of the hydro-methanol seed extract of *Carica papaya*, corresponding to 2.5 mg/kg body weight of loperamide. After 30 minutes, the rats were placed singly in a cage lined with a pre-weighed plain white sheet of paper, and each rat was treated orally with 1 mL of castor oil.

During the 6-hours observation period, the time of onset of diarrhea, the faecal parameters (total number of faeces, diarrheal faeces, fresh weight of faeces, and fresh water content of faeces), and percentage inhibition of diarrheal defecation in each group were taken. The weight of the faeces was obtained by subtracting the weight of the pre-weighed transparent paper from the fresh weight of the stool. The dry weight of the faeces was determined by drying the fresh faeces in a laboratory oven at 100 ∘C until a constant weight was achieved.

The difference between the fresh weight and dry weight of the faeces indicates the water content of the faeces. At the end of the 6-hour exposure period, the animals were sacrificed as described by (The small intestine supernatants prepared and assayed for the activities of Na^+/K^+-ATPase, superoxide dismutase (SOD), intestinal Protein (IP), alkaline phosphatase, malondialdehyde (MDA), nitric oxide (N.O), and reduced glutathione (GSH).

Preparation of Small Intestine Supernatants
The procedure described by [25] was adopted for the preparation of small intestine supernatants. Briefly, under anesthesia, the animals were dissected, and the small intestine was removed. Thereafter, the contents of the small intestines were squeezed out, blotted on blotting paper, and homogenized in a 0.25 M sucrose solution (1:4 w/v) using a Teflon homogenizer. The homogenate

was centrifuged at 894 × *g* for 15 minutes to obtain the supernatant. Afterward, the supernatant was aspirated and used within 12 hours of preparation for biochemical analysis.

Determination of Protein Content of the Supernatant
The protein concentration in the small intestine supernatant of the animals was determined using the Biuret reagent, as described by [26]. About 4.0 mL of Biuret reagent was added to 1.0 mL of the sample (appropriately diluted). This was mixed thoroughly by shaking and left undisturbed for 30 minutes at room temperature for colour development. The blank was constituted by replacing the sample with 1.0 mL of distilled water. The absorbance was read against a blank at 540 nm. The concentration of protein in the supernatants was extrapolated from the calibration curve for bovine serum albumin using the expression: Protein concentration (mg/mL)= Cs× F Where Cs=corresponding protein concentration from the calibration F=dilution factor

Determination of Na^+-K^+ ATPase Activity
The procedure described by [27] was adopted for the determination of the activity of Na^+-K^+ ATPase in the small intestine supernatant. 400 mL of 200 mM NaCl, 40 mM KCl, and 60 mM Tris (pH 7.4) were pipetted into a test tube. Thereafter, 20 mL of $MgCl_2$ $6H_2O$ (80 mM), 20 mL of 20 mM MEGTA, 240 mL of distilled water, and 20 mL of appropriately diluted supernatant of the small intestine were added. The mixture was incubated at 37 ºC for 5 minutes. A known volume (100 mL) of 8 mM ATP was added, mixed thoroughly, and incubated at 37 °C for 30 minutes.

Furthermore, 200 mL of sodium dodecylsulphate (5%) and 200 mL of reagent C (mixture of ammonium molybdate/sulphuric acid solution [reagent A] and 9% ascorbic acid [reagent B] in ratio 4:1 v/v) were added. The mixture was left undisturbed for 30 minutes at room temperature for colour development. The blank was constituted in the same manner, except that the small intestine supernatant was replaced with 20 mL of distilled water.

The absorbance of the test solution was read against that of the blank at 820 nm, and then the concentration of inorganic phosphate was determined by extrapolating the absorbance obtained from the calibration curve for phosphate. Thereafter, the specific activity of Na^+-K+ ATPase was computed using the following expression: Where [P] = concentration of inorganic phosphate in moles (obtained from the calibration curve) 2 = factor introduced to obtain the amount of P released per hour 1000 = factor introduced to convert the P released to moles.

Determination of Nitric Oxide concentration
The procedure described by [28] was used to determine the concentration of nitric oxide in the supernatants of the animals' small intestines. A known volume (0.5 mL) of the supernatant was added to 2 mL of a 75 mmol/L ZnSO4 solution and 2.5 mL of a 55 mmol/L NaOH solution. The solution was mixed thoroughly, adjusted to a pH of 7.3, incubated for 10 minutes, and then centrifuged at 504 × g for 10 minutes. The blank was constituted in a similar manner to the test, except that 0.5 mL of supernatant was replaced by 0.5 mL of distilled water. Furthermore, 1 mL of glycine-NaOH buffer was added to the test sample and blank.

A known volume (2 mL) of deproteinized solution was added to the test and blank, and the volume was adjusted to 4.0 mL with deionized distilled water. The reaction was initiated by the addition of freshly activated cadmium granules and, after 60 minutes, 2.0 mL each of test and blank was added to tubes containing 2.5 mL of ethylenediaminetetraacetic acid solution, 3.0 mL of 1.0 mol/L HCl, and 0.3 mL of 1.0 g/L fuchsin acid solution, mixed thoroughly and incubated for 2 minutes. Next, 0.2 mL of 0.05 mol/L resorcinol and 3.0 mL of 1.0 mol/L NH_4OH were added. The absorbance of the test solution was read against the blank at 436 nm. The concentration of serum nitrite was extrapolated from the calibration curve of nitrite.

Determination of Superoxide Dismutase
The method described by [29] was used to assay the activity of superoxide dismutase in the supernatant of the animals' small intestine. Tissue homogenate of 0.5 mL was diluted in 4.5 mL of distilled water (1:10) dilution factor. An aliquot of 0.2 mL of diluted supernatant was added to 2.5 mL of 0.05 M carbonate buffer (pH 10.2) in a spectrophotometric cuvette, and the reaction was initiated by adding 0.3 mL of substrate (0.3 mM Epinephrine) and 0.2 mL of distilled water. The increase in absorbance at 480 nm was monitored every 30 seconds for 150 seconds. Increase in absorbance per minute =A1–A0 where A0 = absorbance after 30 seconds. A1= absorbance after 150 seconds. df= dilution factor
0.1=volume in mL of tissue homogenate 50% = inhibition of the rate of cytochrome C reduction as per unit definition 1000 = the factor introduced to enable enzyme activity to be expressed in mmol/min/mL. The specific enzyme activity was calculated using the formula.

Determination Of Reduced Glutathione (GSH)
THE level of GSH in the liver homogenate was determined using the procedure described by [30]. Briefly, 1.0 mL of small intestine homogenate was added to 0.1 mL of 25% trichloroacetic acid (TCA), and the precipitate was removed by centrifugation at 5,000 g for 10 min. Supernatant (0.1 mL) was added to 2 mL of 0.6 mM DTNB prepared in 0.2 M sodium phosphate buffer (pH 8.0). The absorbance was read at 412nm.

Determination of malondialdehyde
The concentration of MDA was quantified according to the method of [31], as outlined below: A portion of TBA reagent (2 mL of 0.7% and 1 mL of TCA) was added to 2 mL of the sample. The mixture was thoroughly heated in a water bath at 100 ºC for 20 minutes. It was then cooled and centrifuged at 78 g (4000 rpm) for 10 minutes. The absorbance of the supernatant was read at a wavelength of 540 nm against a reference blank of distilled water after an additional 10 minutes of centrifugation. Extinction Coefficient of MDA = 1.56 x 10^5 $nm^{-1}cm^{-1}$.TBA:0.7% 1.e0.7 g in 100 mL. TCA:20% i.e. 20 g in 100 mL.

Data Analysis
The results were expressed as mean ± standard deviation (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for all pairwise comparisons or Dunnett's test for comparison with the control group. A p-value < 0.05 was considered statistically significant. All analyses were conducted using Minitab software, version 18.

RESULTS

The quantitative analysis of the hydro-methanol extract of *Carica papaya* seeds revealed the quantity and presence of classes of compounds with potential antidiarrheal activities (**Table 1**). Alkaloids, flavonoids, tannins, saponins and Total phenolic were detected and quantified.

Table 1. The phytochemical content of hydro-methanolic extract of *Carica papaya* seed (Pawpaw Seed).

Parameters	Concentration (mg/100 g
Alkaloid	24.0333±0.0800
Flavonoid	0.1000±0.0500
T. Phenolic	0.1000±0.0500
Saponins	0.71533±0.0094
Tannins	0.000200±0.0001

KEY: values are means of triplicate ± Stdev,

The hydro-methanol extract of *Carica papaya* seed significantly prolonged the time for diarrhea induction when compared to the standard drug (Loperamide) in control rats. The latent periods were observed to increase with an increase in drug dose. At 200 mg/kg, the purging indices and the inhibition percentages of defecation and wet stool for the hydro-methanol extract of *Carica papaya* seed were comparable to those of the reference drugs loperamide at 2.5 mg/kg. The hydro-methanolic crude extract of *Carica papaya* seed at 50, 100 and 200 mg/kg doses showed statistically significant ($p \leq 0.05$) defecation and weight difference of the fluid content of the faces compared to the positive controls (**Table 2**).

Ricinoleic acid causes irritation and inflammation of the intestinal mucosa, leading to the release of prostaglandins, which in turn increase the net secretion of water and electrolytes into the small intestine. Inhibitors of prostaglandin biosynthesis delay castor oil-induced diarrhea. Compared to the negative control, the three serial doses (50, 100, and 200 mg/kg) of the extract significantly reduced the total number of fecal outputs within 6 hours of observation ($P < 0.05$). The mean number of defecations for the negative control group was 1.180±0.305, while in the extract-treated group, this value was found to be 1.039±0.208, 0.346±0.085, 0.486±0.544, and 5.17±0.54 at the doses of 50, 100, and 200 mg/kg body weight, respectively, and 1.273±0.500 for the positive control group. Loperamide showed a higher degree of reduction than all three serial doses of the extracts ($P < 0.05$) (**Table 2**).

Table 2. Effect of hydro-methanolic seed extract of *Carica papaya* on Castor oil-induced diarrhea in Wistar Rats.

Treatment doses (mg/kg)	Onset of diarrhea (minutes)	Weight of fresh fecal drop (g)	Weight of dried fecal drop (g)
Distilled water (control)1ml/kg	166	1.180±0.305^{a}	0.330±0.240^{a}
Control drug (loperaminde) 1ml/kg	42.5	1.273±0.500^{a}	0.960±0.520^{b}
HMECPS 50 mL/kg	328	1.039±2.208^{b}	0.176±0.037^{c}
HMECPS 100 mL/kg	170.5	0.346±0.085^{c}	0.250±0.167^{ab}
HMECPS 200 mL/kg	130.5	0.486±0.544^{c}	0.143±0.145^{c}

KEY: values are means of triplicate ± Stdev, n=5, *p≤0.05 when compared with control using ANOVA. Figures with different superscripts along the horizontal array are significant. HMCPS = hydro-methanolic extract of *Carica papaya* seed.

Table 3 demonstrates a single oral administration of hydro-methanolic *Carica papaya* seed extract (CPSE) on intestinal biochemical parameters in Wistar rats. The results show a significant differences ($p \leq 0.05$) were observed in the levels of intestinal protein (IP), superoxide dismutase (SOD), nitric oxide (NO), reduced glutathione (GSH), and alkaline phosphatase (ALP) across treatment groups receiving 50 mg/kg, 100 mg/kg, and 200 mg/kg of CPSE, as well as the reference drug loperamide, compared to the control group. More specifically, intestinal protein levels were markedly raised in all treated groups, with the highest increase at 100 mg/kg. SOD levels varied significantly, with a notable decrease at 100 mg/kg and a substantial increase at 200 mg/kg.

NO levels showed a dose-dependent increase and peaked at 200 mg/kg. GSH concentrations significantly increased at 100 mg/kg and 200 mg/kg compared to the control. ALP activity showed a significant decrease in the 50 mg/kg group and varied across higher doses. However, the level of malondialdehyde (MDA), a marker of lipid peroxidation, did not show any statistically significant difference across treatment groups ($p \geq 0.05$).

DISCUSSION

Traditionally, people use plant(s) or plant-derived preparations, considering them to be efficacious against diarrheal disorders without any scientific basis [32]. The present study was undertaken to substantiate the scientific rationale behind the local use of *Carica papaya* in the treatment of diarrhea. Diarrhea can be described as the abnormally frequent defecation of feces of low consistency, which may be due to a disturbance in the transport of water and electrolytes in the intestines. Instead of the multiplicity of etiologies, (i) increased electrolyte secretion (secretory diarrhea), (ii) increased luminal osmolarity (osmotic diarrhea), (iii) deranged intestinal motility causing a decreased transit time, and (iv) decreased electrolyte absorption may be responsible for pathophysiology [33,34].

Some studies claim that nitric oxide in castor oil is responsible for the diarrheal effect, although it is evident that ricinoleic acid, the most active component of castor oil, produces diarrhea through a hypersecretory response [35,36]. Several mechanisms have been proposed to explain the diarrheal effect of castor oil, including the inhibition of intestinal Na+-K+-ATPase activity, which consequently reduces normal fluid absorption [37, 38], the activation of adenylate cyclase or mucosal cAMP-mediated active secretion [39], and the stimulation of prostaglandin formation and platelet-activating factor [40]. Usually, castor oil is metabolized into ricinoleic acid in the gut, which causes irritation and inflammation in the intestinal mucosa, resulting in the release of inflammatory mediators (e.g., prostaglandins and histamine).

The released prostaglandins initiate vasodilatation, smooth muscle contraction, and mucus secretion in the small intestines. In experimental animals as well as in humans, prostaglandins of the E series are considered to be potent diarrheagenic agents. The inhibitors of prostaglandin biosynthesis are therefore considered to delay the Castor oil-induced. The result of the present study showed that the hydromethanolic extract of Carica payaya at 50, 100 and 200 mg/kg body weight produced a statistically reduction in the severity and frequency of diarrhea produced by castor oil when compared with negative control and the antidiarrheal effect of the plant extract was comparable to the standard drug loperamide which at present is one of the most efficacious and widely employed antidiarrheal drug. Loperamide effectively antagonizes diarrheal activity induced by castor oil.

Table 3. The mean value of the antidiarrheal effect of single oral administration of hydro-methanolic extract of *Carica papaya* seed of Wistar rats.

	Groups (Dosage)				
Parameters	A (Ref. Drug)	B (50 mg/kg)	C(100 mg/kg)	D (200 mg/kg)	E (Water)
Intestinal protein	8.740±11.390^{a}	28.747±1.059^{b}	77.40±30.600^{c}	31.190±0.424^{b}	47.023±0.843^{d}
Superoxide dismutase	22.570±6.760^{a}	26.780±4.540^{a}	6.293±0.3930^{b}	39.390±9.400^{c}	20.090±9.830^{a}
Nitric oxide	191.97±13.72^{a}	244.40±64.60^{b}	330.50±24.10^{c}	382.800±18.10^{d}	250.00±1.800^{ba}
Na-K ATPase	0.2667±0.075^{a}	0.353±0.0057^{a}	0.1900±0.072^{b}	0.3166±0.0057^{a}	0.223±0.0057^{a}
MDA	4.173±0.393^{a}	3.243±1.193^{a}	7.880±4.9200^{b}	4.4230±0.3180^{a}	3.166±0.0057^{a}
GSH	70.95±3.340^{a}	73.83±2.330^{a}	99.210±7.210^{b}	88.903±0.855^{c}	86.870±3.920^{c}
Alkaline phosphatase	93.90±33.30^{a}	21.05±4.140^{b}	71.00±24.10^{c}	51.700±2.320^{d}	59.10±20.600^{d}

KEY: values are means of triplicate + Stdev, n=5, *p<0.05 when compared with control using ANOVA.
Figures with different superscripts along the horizontal array are significant different at (p<0.05).
CPSE= *Carica papaya* extract. MDA = Malondialdehyde GSH = Reduced Glutathione.

It was also observed from the results of the study that the hydromethanolic extract of *Carica papaya* seed could in a dose – dependent manner, controls castor oil-induced diarrhea as well as the number of diarrheal feces and total weight of feces, which could be taken as antidiarrheal activities before an absolute proper medication is done, but higher dosages might result to laxative effects. According to some studies, the antidiarrheal properties of medicinal plants were found to be due to tannins, flavonoids, alkaloids, saponins, and phenols. *Carica papaya* leaves and fruits have been reported to exhibit antidiarrheal properties.

A similar study [41] from the Department of Biochemistry at the University of Maiduguri demonstrated that the leaf extract significantly reduced diarrheal symptoms in rats and was considered safe at a dose of 200 mg/kg. However, these findings are slightly inconsistent with the outcomes of this current research. Tannins, alkaloids, phenols, flavonoids, and saponins may be responsible for the mechanism of antidiarrheal activity of the hydromethanolic extract of *Carica papaya* seed [29,41]. These provide a scientific basis for the potential use of *Carica papaya* seed in gastrointestinal disorders, such as diarrhea [17, 41]. Biomarkers and biological antioxidants were also assayed, which is between the negative control group and the groups treated with various doses of the *Carica papaya* seeds extract (50 mg, 100 mg, 200 mg), as well as loperamide; there were statistically significant increase/decrease in the concentrations of intestinal protein (IP), superoxide dismutase (SD), nitric oxide (NO), reduced glutathione (GSH), and alkaline phosphate (AP) [41].

The variations indicate that, compared to the negative control group, the *Carica papaya* seeds extract and the reference drug loperamide significantly affected these indicators [2,41]. There was no discernible increase/decrease in the levels of malondialdehyde (MDA) among any of the groups. This means that, compared to the negative control group, neither the *Carica papaya* seed extract at various doses nor the reference drug, loperamide, had a statistically significant impact on MDA levels [41]. To put it another way, MDA levels remained largely constant in all groups. Intestinal Protein (IP): According to the findings, the levels of intestinal protein significantly changed in response to the various doses of the extract and the reference drug loperamide as compared to the standard control [41]. This implies that the concentration of intestinal proteins during diarrhea was significantly affected by the extract and the reference drug loperamide. \

The particular effects would depend on how these proteins function in the digestive tract. Superoxide Dismutase (SOD) levels were significantly altered, indicating that the extract and the reference drug, loperamide, had an impact on the body's antioxidant defenses [41]. This may be crucial for controlling the oxidative damage that can result from diarrhea [35]. According to the findings, nitric oxide levels have significantly changed. Given that nitric oxide is involved in both the immunological and inflammatory responses of the body, this suggests that both the extract and the reference drug, loperamide, may interfere with these processes [35,37].

Reduced Glutathione (GSH), changes in reduced glutathione levels can also have implications for the antioxidant and detoxification systems in the body during diarrhea [41]. Na^{+}/K^{+} ATPase: Significant differences in Na^{+}/K^{+} ATPase levels. This enzyme is linked to fluid balance, intestinal transit, and maintaining ion balance in cells [2,41]. Alterations in alkaline phosphatase levels suggest that the extract and reference drug loperamide had an impact on this marker, which may be related to gastrointestinal health and nutrient absorption [41]. Malondialdehyde (MDA) levels were constant across all groups, indicating that neither the extract nor the reference drug, loperamide, appreciably affected lipid peroxidation or oxidative damage that occurs during diarrhea [36,41]. Ultimately, the results suggest that both the reference drug loperamide and the extract have significant effects on several key biochemical indicators related to the digestive and antioxidant systems during diarrhea. These effects may help people with diarrhea manage their symptoms and improve their overall health [2,35,41].

CONCLUSION

In conclusion, the findings of this study provide convincing evidence that the hydromethanolic extract of Cacrica papaya seed (CPS) possesses appreciable antidiarrheal activity; however, a high dosage of it becomes laxative. The antidiarrheal effect is rapid, long-lasting, and statistically significant at both 100 and 200 mg/kg doses in controlling diarrhea. Determining the antidiarrheal effect in other models, as well as its impact on gut motility, may provide a clearer understanding of the mechanism(s) underlying antidiarrheal activity. However, further pharmacological studies are required to determine the laxative and abortive effects of this plant.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

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