



EFFECT OF METHANOL, N-HEXANE AND AQUEOUS EXTRACT OF CARICA PAPAYA ROOT ON CASTOR OIL-INDUCED DIARRHOEA IN ALBINO RATS

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ABSTRACT

Paw paw root (*Carica papaya*) is commonly used locally to treat diarrhoea. The present study evaluated the anti-diarrhoea effect of this plant extract on albino rats induced with castor oil. Fresh tender root of this plant was collected, air-dried, powdered and percolated in n-hexane, methanol and aqueous solvents. Diarrhoea was induced with castor oil on albino rats using standard procedures. The extract (100, 250 and 500 mg/kg) in a dose dependent manner, significantly protected the rat against diarrhoea induced experimentally with castor oil in terms of nature and number of stool droppings within 3hrs of administration of extract, there was no significant difference when compared with the control drug (Loperamide) ($P > 0.05$). The study justifies the ethno pharmacological uses of this medicinal plant for treatment of diarrhoeal disease.

KEYWORDS: Diarrhoea, Castor oil, *Carica papaya*.

INTRODUCTION

Diarrhoea is a condition characterized by an abnormal increase in liquidity, frequency of defecation and stool weight exceeding 200 g daily and containing 60-95% water. It is a common disease in tropics and in some parts of the world (Weber, 1976). It is a prime cause of illness and mortality especially in infant and children (Weber, 1976). Diarrhoea occurs at all ages of human, but its incidence is higher in children between 6 months and 3 years (WHO, 1993). On a worldwide basis, 750 million cases are reported in children below 5 years in Asia, Latin America and Africa resulting in 4-5 million deaths (Abdullahi et al., 2001).

However, the incidence of diarrhoeal diseases varies greatly with the seasons and a child's age. The youngest children are most vulnerable. Incidence is highest in the first two years of life and declines as a child grows older. Mortality from diarrhoea has declined over the past two decades from an estimated 5 million deaths among children under 5 years to 1.5 million deaths in 2004 (WHO, 2004). In an effort to tackle the problems of diarrhoea, the World Health Organization (WHO) has established diarrhoea disease control programme (DDC)

which includes studies of traditional medicinal practices together with the evaluation of health education and prevention approaches (WHO, 2014). In most parts of the developing countries, particularly Africa, the use of herbal remedies in management of diarrhoea is a common practice. Papaya (*Carica papaya* L.) belongs to the family Caricaceae grown in Australia, Hawaii, Philippines, Sri Lanka, South Africa, India, Bangladesh, Malaysia and other countries in tropical America (Anuara, 2008). Papaya is a polygamous species and it is difficult to identify a plant whether it is male, female or hermaphrodite. It is a small unbranched tree, single stem growing up to 5-10 m tall. The leaves are large, 50-70 cm in diameter, deeply palmate lobed with 7 lobes. The fruits are rich in vitamin A and vitamin C. The fruits, leaf, latex of *Carica papaya* are used for the treatment of *Carica papaya* are used for the treatment of typhoid fever, wound infection, asthma, fever, diarrhea, boils, hypertension, and so on (Kurian J.C 2001, Zakaria Z.A, 2006). Recently, antifertility (Lohiya et al., 1999) antihelminthic, (Satrija et al., 1994) and anti-inflammatory activity (Oladunmoye M.K and Osho I.B 2007) have been reported. *Carica papaya* is identified by various names such as Pawpaw in English, Okwuru ezi

in Igbo, Gwanda in Hausa, and Ibepe or Gbebere in Yoruba parts of Nigeria.

The variability of the phytochemical compounds of each plant may explain why traditional medicine uses the combination of both plants to increase the antioxidant activities. (Koevi et al., 2015).

Okorundu et al. (2013) observed that the inhibitory action of the plant extracts could be attributed to the presence of the phytochemical constituents in the plant extracts such as alkaloid, flavonoid and saponin. Castor oil is a ricinoleate (McKeon et al., 1999) and its diarrhoea inducing property is known to be due to its active metabolite ricinoleic acid which increases peristaltic activity and alters permeability of the intestinal mucosa membrane to electrolytes.

The objective of the study was to determine the therapeutic effect of the plant extract in rats induced with castor oil.

MATERIALS AND METHODS

Plant Collection: Carica papaya root was collected from Lilo town in Ihiala L.G.A of Anambra State, Nigeria. The plant was identified and authenticated in the Department of botany, Nnamdi Azikiwe University, Awka Nigeria where the sample was deposited.

The roots spread out and dried on a clean surface under a shade at room temperature to exclude direct Sunlight in order to prevent the active constituents of the roots from being degraded due to photochemical reactions.

It was air dried for some days after which, it was observed to be dried. The dried roots were gathered, and crushed with grinder.

The powder was weighed using an electric weighing balance by Kern ALS 220 – 4. The powder was then stored in an air tight bag at room temperature and used for further extraction.

Preparation of plant extract

The ground roots was prepared in three ways to get the extracts.

Aqueous Extract (Maceration Method)

Maceration method was used for aqueous extraction powdered root of Carica papaya was used. 150 g of the plant was weighed and put in 375 ml of distilled water and allowed to stand for 48 hrs, agitate or shake for 45 mins. The extract was filtered using british standard mesh filter and first muslin cloth and Concentrated by using air drying under constant air current and water bath at 50 °C. The extract was then transferred into a clean container and stored in the refrigerator until required for use.

Organic solvent extraction by maceration

This was carried out at Pharmacognosis department, faculty of Pharmaceutical Sciences, Agulu. 150 g of the plant sample was transferred into 1000 ml volumetric flask, then 375 mls of solvent (methanol and n-hexane) were added. This was covered and allowed for 48 hrs with continuous shaking, filtered and transferred to rotary evaporator for concentration. The extract was then transferred into a clean container and stored in the refrigerator until required for use.

Extraction by Soxhlet Method

This method was carried out by continuously extracting a sample with a non-polar organic solvent for about 4-6 hrs.

Experimental Animal and Diet

In the study, thirty-three (33) of about 8 weeks old healthy rats (*Rattus norvegicus*) weighing 92 – 138 g were used. The rats were kept in standard metal cages obtained from the faculty. Controlled conditions of temperature (25 ± 20 °C), relative humidity ($50 \pm 15\%$) and normal photoperiod (12 – 12 hrs light – dark cycle) were maintained. Free access to standard pellet and water was ensured. Acclimatization of the rats to the metal cages (4 – 6 rats/cage) was done for seven days and maintained according to the NIH guideline for care and use of laboratory Animals (Saha et al., 2001).

Drug: Loperamide (2mg) was obtain from pharmacy department Nnamdi Azikiwe University Hospital, Nnewi, Anambra State Nigeria.

Anti-Diarrhoea Assay

Test Protocol (Castor oil induced diarrhoea)

The plant extract with three different solvents (n-hexane, aqueous and methanol) were used for the study. Eleven^[11] cages allotted three albino rats each, out of the eleven cages, two were used as negative control and positive control, nine cages were used as test groups.

The animals were fasted for 12 hrs and weighed. 2 mls of castor oil were given intragastrically to each rat in each group to induced diarrhea, (Yegnanarayan and Shostri 1982). While papers were dropped underneath of the cages. Faecal materials were checked for the nature of stool passage.

30 mins after administration of castor oil, diarrhoea was established, 2 mls of water was given to negative control and 2 ml of anti-diarrhoea (Loperamide) was given to positive control and volume equivalent of plant extract (100, 250 and 500 mg/kg) were given to test groups. Faecal materials were checked for consistency/frequency every 30 min for the characteristic droppings. The presence/absence of fluid material in the stool that stained the absorbent paper placed beneath the cages and nature of stool passed during 3 hrs period was recorded to determine the effect of plant extract on castor oil induced diarrhoea. Their absence was recorded as a good

protection from diarrhoea, also reduction in diarrhoea (watery Stool) is a protection.

Statistical Analysis

The Statistical method used was 2- way ANOVA. This was done at $P < 0.05$ level of significance.

RESULTS

Table 1 – 3, show the results of effect of methanol, n-hexane and aqueous extracts of *Carica papaya* roots on castor oil induced diarrhoea. After administration of castor oil, the rats were defecating watery stools (ws), then after administration of extracts, the watery stooling stopped. The methanol and aqueous extracts of *Carica papaya* show that in high doses (500 mg/kg) the number of stool droppings within 3 hrs of administration of extract was one (1) while the number of no stool dropping (No defecation) within 3 hrs of administration of extract was five (5), when compared with the anti-diarrhoea drug (loperamide) ($P < 0.05$) that had the same result.

Then, the n-hexane extract of *Carica papaya* shows that in all doses (100, 250 and 500mg/kg) the number of stool droppings within 3 hrs of administration of extracts were two (2), while the number of no stool dropping (no defecation) within 3 hrs of administration of extracts were four (4). In other ways, the methanol and aqueous extracts of *Carica papaya* were more effective than the n-hexane extract of *Carica papaya* at high doses (500 mg/kg). Moreover, the methanol, aqueous and n-hexane extracts had the same number of stool dropping (2) and the number of no stool dropping (4) within 3hrs at low (100 mg/kg) and middle (250 mg/kg) doses. Table 1 - 3.

The extracts significantly protected rats against castor oil- induced diarrhoea when compared with the control as evident in the nature and prolongation of onset. Loperamide (2 mg/kg) also significantly protected rats against castor oil-induced diarrhoea. The extracts effect was dose dependent.

Table 1: Effect of methanol extract of *Carica papaya* on castor oil induced diarrhea.

Nature of stool, 30 min after administration of castor oil.	Extract doses (mg/kg)	Weight (g)	Volume Equivalent to dose administered (ml).	Nature of stool at time interval (30 minutes).						Number of stool dropping within 3 hrs of adm. Of extract.	Number of no stool dropping within 3 hrs.
				0 - 30	31- 61	62- 92	93-123	124-154	155-185		
Watery stool	100	135	0.10	SWS	ND	ND	ND	ND	NS	2	4
Watery stool	250	119	0.29	ND	ND	ND	ND	NS	NS	2	4
Watery stool	500	129	0.64	ND	ND	ND	ND	ND	HS	1	5
Watery stool	Loperamide	121	0.41	ND	ND	ND	ND	ND	HS	1	5

Key: WS = watery stool
 SWS = semi – watery stool
 HS = Hard stool
 NS = Normal stool
 ND = No defecation.

Table 2: Effect of n-hexane extract of *Carica papaya* on castor oil induced diarrhea.

Nature of stool, 30 min after administration of castor oil.	Extract doses (mg/kg)	Weight (g)	Volume Equivalent to dose administered (ml).	Nature of stool at time interval (30 minutes).						Number of stool dropping within 3 hrs of adm. Of extract.	Number of no stool dropping within 3 hrs.
				0 - 30	31- 61	62- 92	93-123	124-154	155-185		
Watery stool	100	100	0.11	ND	NS	ND	NS	ND	ND	2	4
Watery stool	250	114	0.28	NS	ND	ND	ND	ND	HS	2	4
Watery stool	500	112	0.50	NS	ND	ND	ND	ND	NS	2	4
Watery stool	Loperamide	121	0.41	ND	ND	ND	ND	ND	HS	1	5

Key: WS = watery stool
 SWS = semi – watery stool
 HS = Hard stool
 NS = Normal stool
 ND = No defecation.

Table 3: Effect of aqueous extract of *Carica papaya* on castor oil induced diarrhea.

Nature of stool, 30 min after administration of castor oil.	Extract doses (mg/kg)	Weight (g)	Volume Equivalent to dose administered (ml).	Nature of stool at time interval (30 minutes).						Number of stool dropping within 3 hrs of adm. of extract.	Number of no stool dropping within 3 hrs.
				0 - 30	31- 61	62- 92	93-123	124-154	155-185		
Watery stool	100	91	0.09	ND	NS	ND	NS	ND	ND	2	4
Watery stool	250	117	0.29	NS	ND	ND	ND	ND	HS	2	4
Watery stool	500	114	0.57	ND	ND	ND	ND	ND	HS	1	5
Watery stool	Loperamide	121	0.41	ND	ND	ND	ND	ND	HS	1	5

Key: WS = watery stool
 SWS = semi – watery stool
 HS = Hard stool
 NS = Normal stool
 ND = No defecation.

DISCUSSION

The results of the present study suggested that the methanol, n-hexane and aqueous extracts of *Carica papaya* had significant anti-diarrhoea effects. Castor oil is ricinoleate (Mckeen et al., 1999) and its diarrhoea inducing property is known to be due to its active metabolic ricinoleic acid which increases peristaltic activity and alters permeability of the intestinal mucosa membrane to electrolyte, particularly Na^+ , Cl^- (Adzu et al., 2003) and water. (Capasso et al., 1994).

Castor oil is also reported to induce diarrhoea by increasing the volume of intestinal content by prevention of the reabsorption of water.

The secretory diarrhoea is associated with an activation of Cl^- channels, causing Cl^- efflux from the cell, the efflux of Cl^- results in massive secretion of water into the intestinal lumen and profuse watery diarrhoea (Longanga et al., 2000).

Diarrhoea was established 30 mins after administration of castor oil to the rats at volume equivalent to their weights. The results revealed that the extract was able to stop the diarrhoea in the rats within 3 hrs of therapy, showing that they exhibited anti-diarrhoeal effects. Effect of the extracts on castor oil induced diarrhoea in rat also showed protection ability of the extract was high. In 2004 Abdulrahman et al reported the same. The above findings are therefore, suggestive of non-specific mechanism of action of anti-diarrhoeal activity of the extract. Moreso, Unaeye, et al., 2017 also suggested that the plant had anti diarrhoea effect.

CONCLUSION

In conclusion, the results of this study indicated that the plant extracts had an anti-diarrhoeal effects.

Declaration of Interest

The authors declare that there are no conflicts of interest, including any financial or personal interest.

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