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Phospholipid Profile of the Stomach and Duodenum of Normal Rabbits Fed with Supplements of Unripe Pawpaw (*Carica papaya*) and Unripe Plantain (*Musa sapientum*) Extract

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Abstract: This study reports the effect of unripe pawpaw and/or unripe plantain extracts on the phospholipid profile of the stomach and duodenum of normal rabbits. Supplementation of chow with unripe pawpaw or plantain extract significantly reduced ($p<0.05$) the weight gained by rabbits compared with the control. The total phospholipids content in the stomach and duodenum were significantly increased ($p<0.05$) in the test groups compared to the control. In the stomach and duodenum, phosphatidylcholine (PC), phosphatidylethanolamine (PE) and sphingomyelin (SGM) were significantly increased ($p<0.05$) in the test groups compared to the control. Pawpaw occasioned a decrease in the PE/PC ratio in both tissues as against an increase observed in the SGM/PC ratio. The results presented suggests that unripe pawpaw meal and unripe plantain extract alter the phospholipid profile of the stomach and duodenum in ways which may affect membrane fluidity of these tissues and would have profound effect on the gastro-duodenal mucosa and thus have implication(s) for gastric and duodenal ulcers in rabbits.

Key words: Ulcer, phospholipid, plantain and pawpaw extracts

INTRODUCTION

Several plants and plant products have been used in the treatment/management of ulcers. Raw cabbage juice that is high in glutamic acid is very effective in the healing of ulcer (Fullick, 1994). Aloe vera has been shown to ameliorate irritable bowel symptoms and constipation, enhance digestion and absorption of food and also reduce bacterial putrefaction in the gut (Miller, 1997). Extracts of unripe plantain (*Musa sapientum*) and unripe pawpaw (*Carica papaya*) can alleviate or completely heal peptic ulcers and other gastrointestinal tract disorders (Best *et al*., 1984; Lewis *et al*., 1999). Several other studies have tried to elucidate the active ingredients and the mechanism of action of unripe plantain. Recently, it has been established that the active ingredient for the anti-ulcerogenic properties of plantain banana has been identified as the flavonoid leucocyanidin (Lewis and Shaw, 2001; Lewis *et al*., 1999). *Carica papaya* has been shown to have antimicrobial and antifungal effects (Ghosh *et al*., 1998; Osato *et al*., 1993).

The gastro-duodenal mucosa constantly secretes mucin which acts as a barrier that prevents self-digestion by enzymes and secretions such as acids (Konturek, 1985; Turnberg, 1985) and in ulcer, this protective cover is compromised. A change in the phospholipid profile of membranes has long been implicated in the development of diseases (Barenholz and Thompson, 1980). It is becoming accepted that reducing the hydrophobicity of the mucous gel layer by changing the action of surface-active phospholipids is a cause of ulcers and the ability of membrane phospholipids to prevent the back diffusion of hydrogen ions protects the mucilage cover (Akaydin *et al*., 1991; Turnberg, 1985). Report of Litchenberger (1983) demonstrated the protective effect of phospholipid addition into gastric lumen of rats subjected to ulcerogenic dose of acid. Lysophosphatidylcholine and bile salts have been shown to have a weakening effect on the mucosa barrier by disturbing the membrane lipids (Konturek, 1985; Duane *et al*., 1986). It has been shown that in patients with Helicobacter pylori infection in peptic ulcer, phosphatidylcholine and phosphatidylethanolamine concentration in the gastric mucosa is decreased and that the eradication of the bacteria normalized the levels of these phospholipids (Ishibashi *et al*., 2002).

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A change in the composition of the lipid component of membrane would thus play a significant role in breaking down the barrier, based on the pathophysiology of ulcers. There is a dearth of information on changes in other members of the membrane phospholipids profile of the mucosa membrane in ulcer and the effect of medicinal plants that have been shown to have anti-ulcerogenic effect. The use of unripe pawpaw and unripe plantain in the management of ulcers is common here in Nigeria, however the practice is to administer extracts of boiled whole unripe plantain (with its peels) and unripe pawpaw. This present work is thus aimed at evaluating the effect of extracts of boiled unripe plantain and unripe pawpaw meal on stomach and duodenal membrane phospholipids profile of rabbits, with a view to determining the possible protective effect of these supplementations in ulcers.

MATERIALS AND METHODS

Twenty rabbits of the New Zealand breed were purchased from the University of Benin Animal Farm and used for this study. The animals were acclimatized to our Laboratory conditions for a period of 1 week and thereafter sorted into four different groups of five rabbits each such that the weight difference between the groups was about 5.0 g. One group of animals were fed the chow only and acted as the control. Another group was fed with the chow and given unripe plantain (*Musa sapientum*) extract orally by the gavage method. The third group was fed with the chow supplemented with unripe pawpaw (*Carica papaya*) and the last group was fed with the chow with unripe pawpaw and also given the unripe plantain extract. The unripe plantain extract was prepared freshly daily by boiling the unripe plantain with the bark (100 g) in 100 mL of water for 25 min and the extract was collected by filtering with a clean white cloth and allowed to cool before administering (1 mL kg^{-1} body weight). Unripe pawpaw (4-5 weeks old) was peeled, washed with distilled water and chopped into small bits/cubes and administered (2 g kg^{-1} body weight). The animals given the unripe plantain extract and the unripe pawpaw were treated like the ones given the extract or the pawpaw alone. The rabbits were allowed the chow and water freely after been fed with their respective regime. The animals were again acclimatized with their respective treatment for 1 week and then continued on this treatment regime in the experimental period which lasted for 4 weeks. Prior to the commencement of the study period the weights were taken and weight changes were recorded weekly. The animals were handled in accordance with the principles of laboratory animal care as contained in NIH guide for laboratory animal welfare. At the end of the study period, the animals were sacrificed after an overnight fast and the stomach and duodenum excised. The tissues were then washed free of debris using normal saline and the biochemical assays were immediately carried out. Where the tissues could not be immediately assayed; they were stored frozen at -20°C until required for biochemical assays, which was within a few hours. The proximal stomach, distal stomach and duodenum were excised for phospholipids analysis.

Extraction of lipids and assays: The total lipids of a known weight of the proximal stomach, distal stomach and duodenum (1 g) were extracted by the modified method of Bligh and Dyer (1959) and the amount of the total phospholipid content and the fractions of the phospholipids were separated using thin layer chromatography as described by Cuzner and Davidson (1967). Spots corresponding to specific phospholipids were recovered by scrapping and quantified. For this quantification the method of Fiske and Subarrow (1925) was employed. Recovered phospholipid fractions and reference standards (containing between 1 to 5 μg P $tube^{-1}$) were digested with perchloric acid. The digests were then incubated with molybdate and ascorbic acid solutions and the absorbance was read at 800 nm.

Statistical analysis: The results are expressed as means±SEM. Analysis of variance was used to test for differences in the groups. Duncan's multiple range test was employed to test for significant differences between the means (Sokal and Rohlf, 1969).

RESULTS

The data on the food intake and weight changes in the experimental groups are presented shown in Table 1. Inclusion of pawpaw to the rabbit chow significantly ($p<0.05$) reduced the weight of the rabbits. Plantain extract also had a similar effect but the reduction failed to reach a statistically significant ($p>0.05$) level. In the rabbits given the combined pawpaw and the plantain extract, there was also a significant ($p<0.05$) decrease in weight gain compared with the control though the observed weight gain in these animals was significantly higher than that observed in the rabbits given pawpaw alone. The study shows that these plant supplementations affect weight of rabbits.

Table 2 shows the total phospholipid content in sections of the stomach and the duodenum of rabbits fed pawpaw and/or plantain extract for 4 weeks. Compared with the control, statistical analysis of the data revealed that feeding rabbits with either unripe plantain extracts or

Table 1: Weight changes in rabbit given supplementation of unripe pawpaw, unripe plantain extract or pawpaw and plantain extract for 4 weeks

	Groups			
Parameters	Control	Extract	Pawpaw	Pawpaw/extract
Initial weight	909.0±4.3	911.6±3.5	906.3±3.6	907.3±3.6
Final weight	1185.0±31.6	1054.5±25.6	950.7±31.2	1129.8±32.7
Wt. gain (g day^{-1})	7.9±1.3^{a}	6.4±1.2^{a}	1.3±0.8^{b}	4.1±1.3^{c}

Values are given as mean±SEM, n = 5. Means of the same row followed by different letter(s) differ significantly ($p<0.05$)

Table 2: Total phospholipid in sections of the stomach and the duodenum of rabbit given supplementation of unripe pawpaw, unripe plantain extract or pawpaw and plantain extract for 4 weeks

	Groups			
Tissues	Control	Extract	Pawpaw	Pawpaw/extract
Proximal stomach	13.2±1.8^{a}	21.1±2.9^{b}	20.1±2.3^{b}	22.0±3.8^{b}
Distal stomach	9.9±0.2^{a}	14.7±2.4^{b}	7.5±2.8^{a}	22.4±3.6^{c}
Duodenum	14.1±3.5^{a}	21.7±2.4^{b}	22.3±2.2^{b}	18.8±4.5^{b}

Values are given as mean±SEM, n = 5. Values are multiplied by 10, Means of the same row followed by different letter(s) differ significantly ($p<0.05$)

Table 3: Individual phospholipids content in the proximal stomach of rabbits given supplementation of unripe pawpaw, unripe plantain extract or pawpaw and plantain extract for 4 weeks

	Groups			
Parameters	Control	Extract	Pawpaw	Pawpaw/extract
PI+PS	2.40±0.2^{a}	5.70±0.6^{b}	5.80±0.9^{b}	2.60±0.2^{a}
SGM	4.10±0.3^{a}	6.00±0.6^{b}	4.90±0.8^{bc}	4.10±0.4^{c}
PC	7.30±0.7^{a}	8.80±0.8^{a}	14.80±1.0^{b}	8.40±0.7^{a}
PE	7.50±0.6^{a}	10.70±0.8^{b}	12.60±0.9^{c}	17.10±1.1^{d}
SGM:PC	0.60	0.70	0.30	0.50
PE:PC	1.02	1.22	0.85	2.04

Values are given as mean±SEM, n = 5. Values are multiplied by 10. Means of the same row followed by different letter(s) differ significantly ($p<0.05$), PI = Phosphatidylinositol, PS = Phosphatidylserine, SGM = Sphingomyelin, PC = Phosphatidylcholine, PE = Phosphatidylethanolamine

pawpaw or both significantly ($p<0.05$) increased total phospholipids in gastric and duodenal tissues except in the distal stomach of the animals fed with pawpaw alone where a significant reduction ($p<0.05$) in the total phospholipid was observed compared with the values obtained in the other groups. The observed increases in the phospholipids in the test animals were as high as 127.4% in the distal stomach of the rabbits fed with both supplementations and were only 49.2% in the distal stomach of the animals fed with plantain only. The decrease in the total phospholipid in the distal stomach of the animals fed with the unripe pawpaw only was about 24.2%. This study reveals that total phospholipid levels of rabbits are affected by unripe plantain extract and pawpaw.

Pawpaw caused a significant increase ($p<0.05$) in phosphatidylinositol (PI) and Phosphatidylserine (PS) compared with the other groups where a statistically similar ($p>0.05$) level of these phospholipids were observed. Sphingomyelin (SGM) and phosphatidylcholine (PC) were significantly raised by pawpaw, unripe plantain extract or both compared with the control. While pawpaw reduced SGM of the proximal stomach of rabbits, it increased PC compared with the plantain extract. Also in comparison, pawpaw reduced SGM/PC and PE/PC ratios in this tissue. As the SGM/PC and the PE/PC ratios can be used as index of disease development, aging, malignancy or membrane fluidity this study shows that these plant supplementation may affect these states (Table 3).

In the distal part of the stomach the different supplementations significantly ($p<0.05$) reduced PI and PS compared with the control. However, SGM, PC and PE were increased compared with the proximal part, hence there was a relatively higher SGM/PC and PE/PC ratios in the distal stomach compared with the proximal one (Table 4). Comparing the test supplementations, unripe plantain extract resulted in a higher SGM level and SGM/PC ratio in the distal stomach than pawpaw. The study reveals that the effect of pawpaw and unripe plantain extract on the phospholipid profile in the distal stomach differ from its effect in the proximal stomach.

Feeding of unripe plantain extracts and pawpaw either alone or combined significantly ($p<0.05$) increased individual phospholipids in duodenal tissues. However, the increase did not manifest in the level of phosphatidylinositol and phosphatidylserine in the rabbits fed with the unripe plantain extract alone. The results also showed that there was no significant ($p>0.05$) difference in Sphingomyelin level observed in the pawpaw fed group and the pawpaw and plantain extract fed group.

Table 4: The individual phospholipids content in the distal stomach of rabbits given supplementation of unripe pawpaw, unripe plantain extract or pawpaw and plantain extract for 4 weeks

	Groups			
Parameters	Control	Extract	Pawpaw	Pawpaw/extract
PI+PS	2.70±0.3^{a}	4.40±0.6^{b}	5.90±0.6^{c}	5.20±0.7^{c}
SGM	4.20±0.4^{a}	6.50±0.4^{b}	5.40±0.5^{c}	5.90±0.6^{bc}
PC	8.80±0.7^{a}	11.10±0.9^{b}	11.90±0.9^{b}	11.50±0.9^{b}
PE	8.40±0.8^{a}	12.30±0.9^{b}	15.50±0.7^{c}	13.50±0.8^{b}
SGM:PC	0.48	0.59	0.45	0.51
PE:PC	0.95	1.11	1.41	1.17

Values are given as mean±SEM, n = 5. Values are multiplied by 10. Means of the same row followed by different letter(s) differ significantly ($p<0.05$), PI = Phosphatidylinositol, PS = Phosphatidylserine, SGM = Sphingomyelin, PC = Phosphatidylcholine, PE = Phosphatidylethanolamine

Table 5: The individual phospholipids content in the duodenum of rabbits given supplementation of unripe pawpaw, unripe plantain extract or pawpaw and plantain extract for 4 weeks

	Groups			
Parameters	Control	Extract	Pawpaw	Pawpaw/extract
PI+PS	5.10±0.6^{a}	5.10±0.5^{a}	6.70±0.8^{b}	5.80±0.9^{ab}
SGM	5.40±0.8^{a}	7.90±0.6^{b}	8.90±0.8^{b}	9.60±1.1^{b}
PC	9.80±0.7^{a}	16.20±1.1^{b}	13.90±1.4^{c}	16.50±1.3^{b}
PE	10.50±0.7^{a}	15.90±0.9^{b}	12.20±1.3^{c}	16.60±1.5^{b}
SGM:PC	0.55	0.49	0.64	0.58
PE:PC	1.07	0.98	0.87	1.01

Values are given as mean±SEM, n = 5. Values are multiplied by 10. Means of the same row followed by different letter(s) differ significantly ($p<0.05$), PI = Phosphatidylinositol, PS = Phosphatidylserine, SGM = Sphingomyelin, PC = Phosphatidylcholine, PE = Phosphatidylethanolamine

In the pawpaw combined with plantain extract fed rabbits, there was a significantly ($p<0.05$) increased the PE and PC content compared with those given the extract alone. A high SGM/PC ratio was observed in the group given the extract compared with the control group. The PE/PC ratio was quite the opposite to the SGM/PC ratio where the PE/PC ratio had a higher value in the plantain extract fed rabbits and a lower value was recorded in the pawpaw fed ones (Table 5).

DISCUSSION

In some Nigerian traditional medical practice, extracts from boiled unripe plantain and unripe pawpaw are administered to sufferers of ulcers to alleviate the problem. The use of unripe plantain is common in other areas of the world and the active ingredient for the anti-ulcerogenic properties of plantain banana has even been identified as the flavonoid leucocyanidin (Lewis and Shaw, 2001; Lewis *et al*., 1999). Whether this flavonoid is stable in extract of boiled plantain is not certain, however, changes in phospholipids have been reported in ulcers. This study attempts to provide data on the changes in the phospholipid profile associated with the feeding of boiled plantain extract and unripe pawpaw in normal rabbits. Though in this study, animals in which ulcers have been induced were not used, but the changes reported would have a relationship with ulcer. The study relies on cell membrane phospholipid composition for any of such relationship since membrane phospholipid had been shown to be altered in ulcers disease (Konturek, 1985; Duane *et al*., 1986; Ishibashi *et al*., 2002), though a larger spectrum of phospholipids have not been investigated with these extracts.

The data presented in Table 1 show significant reduction in the weight of test rabbits fed supplementations of pawpaw and/or plantain extract. This observation agrees with those of an earlier study which also reported weight loss with the feeding of plantain (Goel *et al*., 2001). This implies that unripe pawpaw and/or plantain extract may be beneficial in clinical conditions where weight gain poses a problem.

Increase in total phospholipids content of the gastro-duodenal mucosa (Table 2), might be of clinical significance and may possibly explain the alleviative effects of unripe pawpaw and/or plantain extracts on ulcers of gastric or duodenal origin. Studies have shown that an increase in phospholipids protects the mucosa from damage (Litchenberger, 1983). In that study, addition of phospholipids to the lumen of rats protect the animals from ulcer. A similar report has been made by Oner *et al*. (1989). So as the unripe plantain extract and the unripe pawpaw increase total phospholipid content of both the stomach and the duodenum, it would be beneficial in ulcers.

The present study observed significant increases in phosphatidylcholine, phosphatidylethanolamine and sphingomyelin of the gastric and duodenal tissues of rabbits given unripe plantain extract and unripe pawpaw (Table 5). Studies show that there is normalization of phosphatidylcholine and phosphatidylethanolamine concentration in the gastric mucosa of patients with

Helicobacter pylori infection when the bacteria are eradicated in peptic ulcer in which the phospholipids are decreased (Ishibashi *et al*., 2002). In view of the study of Ishibashi *et al*. (2002), the plantain extract and unripe pawpaw may protect rabbits from gastric and duodenal ulcers since they increased the individual phospholipids except the phosphatidylinositol and phosphatidylserine content, which was reduced (Table 5). This effect of the treatments on gastric and duodenal mucosa phosphatidylinositol and phosphatidylserine may be connected to the conversion of phosphatidylinositol to prostaglandins. Phosphatidylinositol is the primary source of arachidonic acid required for the biosynthesis of eicosanoids including prostaglandins via the action of phospholipase A_2 (Vance and Vance, 2002). Also animal phosphatidylethanolamine tend to contain higher proportions of arachidonic acids (Bogdanov and Dowhan, 1999). Prostaglandins have long been known to protect the gastro-duodenal mucosa. If the observed reductions in phosphatidylinositol indicate increased mobilization of this phospholipid for the synthesis of prostaglandins, then the unripe plantain extract and the unripe pawpaw may be able to protect the rabbit gastro-duodenal mucosa.

The reduction in the sphingomyelin/ phosphatidylcholine ratio in the stomach and duodenum tissues of rabbits fed pawpaw only (Table 5) may suggest a significant fluidizing effect on these membranes. Phosphatidylcholine, particularly phosphatidylcholine rich in polyunsaturated fatty acids, has a marked fluidizing effect on cellular membranes. Decreased cell membrane fluidization and breakdown of cell-membrane integrity, as well as impairment of cell-membrane repair mechanisms are associated with disease (Barenholz and Thompson, 1980; Vance and Vance, 2002). Thus this data may also suggest that the beneficial effect of unripe plantain extract and pawpaw may not only be mediated by their effect on the levels of some phospholipids but also through their effect of membrane fluidity.

The report of present study on the effect of unripe plantain extract which show elevation in sphingomyelin level in stomach and duodenum tissues of rabbits (Table 5) do not agree with those of Mukhopadhyaya *et al*. (1987) which reported reduction in sphingomyelin with the feeding of rabbits with unripe plantain. It is pertinent though to stress that 0.2 mL kg^{-1} body weight of plantain extract was used in this study and was administered orally once daily for 4 weeks, while Mukhopadhyaya *et al*. (1987) used 0.5 g kg^{-1}, twice daily for 3 days. Also in their study they fed whole unripe plantain while in present study, we administered the water extract. These differences in approach may account the differences.

In conclusion, unripe plantain extract and unripe pawpaw alter the phospholipid profile of the stomach and duodenum in ways which may affect membrane fluidity of these tissues. This would have profound effect on the gastro-duodenal mucosa and thus have implication(s) for gastric and duodenal ulcers in rabbits.

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