Protective effect of papain from *Carica papaya* (papaya) extracts to caerulein-induced acute pancreatitis in Sprague Dawley rats

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Abstract: Acute pancreatitis is an inflammatory condition of the pancreas characterized by increased serum level of digestive enzymes, amylase and lipase. Caerulein, as frequently used in other studies, can induce acute pancreatitis. Studies have shown that *Carica papaya* contains papain, an enzyme effective in controlling both edema and inflammation associated with surgical operations. In this study, the effects of papain from unripe *C. papaya* fruit extract in caerulein-induced acute pancreatitis in Sprague Dawley rats were determined. Four groups of rat models (control group, caerulein group, and treatment groups administered with 100 mg/kg and 200 mg/kg) were subjected to biochemical and histopathologic examinations. The lethal dose of papain was 400 mg/kg. The obtained amylase levels were 1717.50 ± 31.12 mg/dL and 1842.50 ± 18.87 mg/dL for Groups III and IV, respectively. On the other hand, the lipase levels were 6.25 ± 0.48 mg/dL and 3.00 ± 0.82 mg/dL, respectively. Both results were lower than the caerulein group which proves the ability of papain to reduce the levels of the said enzymes. Using Tukey and Dunnett tests, the obtained p-values (P=0.000) for all the groups were highly significant; thus, supporting the biochemical assay results. Histopathological findings proved that only the 200 mg/kg papain has protective activity against caerulein-induced pancreatitis since degenerative changes and apoptotic effect were observed in the group treated with 100 mg/kg papain. Therefore, papain from unripe *C. papaya* extract is evident to produce a protective effect against acute pancreatitis at 200 mg/kg concentration. Thus, *C. papaya* can be an alternative agent to the expensive treatment for acute pancreatitis.

Keywords: *Carica papaya*, papain, acute pancreatitis, caerulein

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INTRODUCTION

Acute pancreatitis (AP) is the inflammation and perivascular infiltration in the pancreas which is usually considered as an autodigestive disease. It is an inflammatory condition that usually begins with gradual or sudden pain in the upper abdomen extending to the back. It is characterized by tissue edema, acinar necrosis, hemorrhage, fat necrosis and leukocyte infiltration. Causes of acute pancreatitis include high triglyceride levels in the blood, severe alcohol consumption and patients' drug intake. Drugs considered to be able to cause acute pancreatitis include thiazide diuretics, furosemides, estrogen, azathioprine, L-asparginase, methyldopa, sulfonamides, tetracycline and procainamide. Worldwide incidence of acute pancreatitis ranges from 4.9 and 73.4 cases per 100,000 populations. Its prevalence rate from the 1988 to 2003 National Hospital Discharge Survey showed that hospital admissions for acute pancreatitis increased from 40 per 100,000 in 1998 to 70 per 100,000 in 2002 (Tenner, Baillie, DeWitt & Vege, 2013). However, Kim, Cuthbertson and Christophi (2006) added that acute pancreatitis has a relative frequency ranging from 5 to 80 cases per 100, 000 populations in the Western world. Biliary tract disease and alcoholism account for 80 to 90% of patients given the diagnosis of acute pancreatitis; with alcoholism being the most common cause in inner city areas. The other 10 to 20% of the patients with acute pancreatitis had this disease in the absence of any other known processes (Steer, 1993). Other mechanisms such as oxidative stress have also been shown to be involved in the development of acute pancreatitis (Eşrefoğlu, Gül, Ateş, Batçıoğlu & Selimoğlu, 2006).To diagnose AP, blood tests, physical examination and abdominal imaging are necessary. Biochemical tests are the most used methods for the diagnosis of acute pancreatitis. Serum amylase is used as a very sensitive method of determining pancreatic inflammation but it was regarded to have insufficient specificity. Serum lipase is considered to be the primary biochemical diagnostic marker because of its high sensitivity and specificity for acute pancreatitis (Cappell, 2008). Despite dozens of randomized trials, no medication has been shown to be effective in treating AP. However, an effective intervention has been well described as aggressive intravenous hydration which requires side-by-side monitoring and guide with the use of hematocrit, BUN and creatinine as surrogate markers. Although some human trials have shown a clear benefit to aggressive hydration, other studies have suggested that aggressive hydration may be associated with increased morbidity and mortality. Thus, in spite of these several advances in care medicine for the disease, its diagnosis and treatment is still inadequate to decrease the worldwide incidence of acute pancreatitis.

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Caerulein, which is a cholecystokinin-pancreozymin analogue, has been effectively used to cause acute pancreatitis in several experimental models. Acute pancreatitis is induced by stimulating pancreatic amylase with caerulein which can cause an increase in serum amylase level and its pancreatic water content. Acute caerulein pancreatitis is proven to be efficient for the investigation of pancreatitis pathogenecity (Medeiros, Lemos, Fagundes, Montenegro & Camara, 2014). Caerulein's action leads to the activation of NADPH oxidase, a major source of reactive oxygen involved in nuclear factor-KB activation, cytokine expression, apoptosis in the rat pancreatic acinar cells and pathogenesis of pancreatitis (Kim, 2008).

In developing countries, where medications and medical supplies are often in short supply, they usually used a cheap alternative in treating certain disease. Moreover, there is no limitation due to seasonality as the papaya is available almost round the year. It is the most conventional fruit in the family of Caricacea. It is an erect, fast growing and usually unbranched tree or shrub. It originated from Central America and also distributed into many parts of the tropics. Many parts of the plant like leaves, roots and fruit have health benefits such as protection against heart disease, rheumatoid arthritis, macular degeneration in the digestive system, immune support and anti-inflammatory effect (Owoyele, Adebukola, Funmilayo & Soladoye, 2008). It was proven to reduce the amount of granuloma in rats with paw edema and arthritis. It also produces beneficial effects in patients with inflammatory disorders of certain body parts like intestine, liver and eye (Elgadir, Salama & Adam, 2013). *C. papaya* contains many enzymes. One of these is papain which is recognized as effective natural medicine in controlling both edema and inflammation associated with surgical operations.

Figure 1.*C. papaya* unripe fruit

According to Amri and Mamboya (2012), papain has significant analgesic and anti-inflammatory activity against symptoms of acute allergic sinusitis like headache and toothache pain without producing side effects. Papain also has been successfully used to overcome the allergies associated with leaky gut syndrome, hypochlorhydria (insufficient stomach acid)and intestinal symbiosis like gluten intolerance. It is used in combating dyspepsia and other digestive disorders and disturbances of the gastrointestinal tract (Huet et al., 2006). Papain belongs to cysteine protease family which can be

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seen in minor constituent of papaya fruit latex (Elgadir, et al., 2013). Former researchers have proven that papain enzyme has a long history of being used to treat sports injuries and control inflammation associated with surgical operations. Though it has been proven to have many significant uses in the field of medicine, its protective effect to acute pancreatitis was not yet studied. The aim of this study is to evaluate the protective effect of papain in vivo as an anti-inflammatory agent against induced AP in rat models. The researchers would like to: 1) determine the acute toxicity of doses of papain in the fruit extract, 2) determine the effect of *C. papaya* extract on amylase and lipase activity and compare it to the control group, and3) determine the protective effect of the extract in the histopathologic examination of pancreas. This study hypothesized that papain in the fruit extract would give a significant effect in decreasing the pancreatic toxicity level of caerulein-induced acute pancreatitis in rats. This study is done to prove that through the anti-inflammatory effect of papain from *C. papaya* extract, it can give protection to the researchers' target organ, pancreas, even after the induction of pancreatitis. This could be a cheap alternative and preventive measure to avoid the progression of the disease and decrease the cases of acute pancreatitis worldwide since diagnosis and treatment is still not very established.

MATERIALS AND METHODS

Plant

The fruits of *C. papaya* were used in this study. The plants were collected in San Pascual, Batangas City. Vouchers of the plant specimen were submitted to the Botanical Herbarium, Department of Biology in University of the Philippines - Los Baños, Laguna for authentication.

Extraction

Using 83 unripe papaya fruits, collection of latex was obtained through making 3-4 vertical incisions of about 2 mm on the papaya peel utilizing sharp knives. After the incision, the latex was collected with a crucible and immediately begun to coagulate. It was oven dried at 40°C for 14 hours. Using a laboratory mortar and pestle, it was ground to form a greenish or grey powder (Adu, Akinboye & Akinfem, 2009). The obtained dried powder is considered as papain. (Amri and Mamboya, 2012).

Confirmatory Test for the Presence of Papain in *C. papaya*

To ten ml of distilled water, 20 ml of powdered skim milk and a drop of diluted acetic acid were mixed. The solution was maintained to a pH of 5.5, to which 0.01g of the collected sample was added and

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was incubated to 37ºC until the liquid coagulated. The solution of papain exhibited maximum absorption at a wavelength of 270-280 nm with 3.79, 3.80 and 3.79 absorbance respectively (Seenivasan, Roopa & Geetha, 2010).

Lethal Dose of Papain (LD50)

The animals in their cages, six in each cage (A-E), received papain from *C. papaya* administered intraperitoneally with the range of 50, 100, 200, 400 and 800 mg/kg, respectively. The negative control group (Group I) received 0.25 ml/kg of normal saline IP. The toxicity symptoms such as twitching, dizziness and weakness, and, or death were observed within 24 hours and recorded. Any dead animal was being removed from the cage right away. The LD50 was calculated as the probity of the minimum dose of the extract that killed half the number of the animals in the study group or the mean of two doses where applicable (Amazu, Azikiwe, Njoku, Osuala, Nwosu, et al., 2010).

Laboratory Animals

Rat models including mice were obtained from the Department of Science and Technology, Taguig (DOST) as the subject of the study due to administration of drugs and extract in vivo. The chosen rat models were composed of 16 male Sprague Dawley rats and 36 male albino mice. The animals were housed in temperature-controlled (23 ± 2°C) room with a 12 hour light-dark cycle. The rats had free access to water and all rats were fed with standard laboratory diet until an overnight fasting before the experiment (Jong-Kil & Jeong-Sang, 2012). All protocols using laboratory animals were subjected to ethical review by the Institutional Animal Care and Use Committee of Lyceum of the Philippines University - Batangas.

Experimental Design, Administration of Fruit Extract and Induction of Pancreatitis

This study is designed to determine the appropriate dose of the *C. papaya* fruit extract for the protective effect in pancreas. The rats were randomly divided into four groups containing six animals each:

Group I: Control Group
Group II: 50 ug/kg caerulein
Group III: 100 mg/kg papain from *C. papaya* and 50 µg/kg caerulein
Group IV: 200 mg/kg papain from *C. papaya* and 50 µg/kg caerulein

Rats were given caerulein, which was obtained from Bellman Laboratories, Quezon City. Acute Pancreatitis was induced by 4

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subcutaneous injections of 50 μg/kg body weight of caerulein within 4 hours upon hourly intervals and rats were killed after 6 hours (Akyuz, Sehirli, Topaloglu, Ogunc, Cetinel, & Sener, 2000). Group I is composed of 4 Sprague Dawley rats, and is considered as the control group administered with saline only. Group II is administered with caerulein. Group III is administered with 100 mg dose of fruit extract before the start of caerulein administration. The last group is administered with 200 mg dose of fruit extract before the start of caerulein administration. Treatment of fruit extract was done orally to the Sprague Dawley rats. Through administering different doses, the protective effect of *C. papaya* in caerulein-induced acute pancreatitis was tested (Jong-Kil & Jeong-Sang, 2012).

Biochemical Assay

Blood samples were collected six hours after the last caerulein administration. Samples were submitted to Advanced Diagnostic Veterinary Laboratory located at Ayala Alabang, Muntinlupa City, Philippines for the determination of amylase and lipase levels. Serum was used to detect amylase and lipase levels. Kinetic method was used for the determination of amylase using Abcam's α-amylase assay kit while lipase was measured through Abcam's Lipase Assay Kit colorimetric method (Carvalho, Morais, Melo, Brito, Andrade, et al.,2010).

Histopathologic Studies

Portions of the pancreas were fixed overnight at room temperature in a neutral pH, phosphate-buffered, 10% formalin solution. It was sent to Advanced Diagnostic Veterinary Laboratory located at Ayala Alabang, Muntinlupa City, Philippines for pathologic diagnosis and microscopic examination (Jong-Kil & Jeong-Sang, 2012).

Statistical Analysis

To gather accurate information for the correct interpretation of results, observational collection of data and results was employed. Results collected were analyzed using one-way analysis of variance (ANOVA) with Tukey's test and Dunnett test to compare the level of significance between control and experimental groups. The values of $p<0.05$ were considered significant for each group determining if results were different from other groups. All computations are done using SPSS.

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RESULTS AND DISCUSSION

I. Papain from C. papaya latex

A greenish gray powder was obtained after 14 hours of oven-drying. The papain obtained is similar in appearance with the papain extracted by Adu, Akingboye and Akinfemi (2009). Total amount yield was 3.4 g.

II. Confirmatory Test for the presence of Papain in *C. papaya*

The protein content measured from 0.01g of extract was 3.79 mg/dL, which is nearly similar with the yield (4 mg)) obtained by Seenivasan, Roopa & Geetha (2010).

Figure 2. Papain from C. papaya latex

III. Lethal Dose Toxicity

Table 1 shows the lethal dose of papain in albino mice. After 24 hours of observation, mice administered with 50 mg/kg and 100 mg/kg of the papain from *C. papaya* exhibited signs and symptoms like twitching, ataxia and dizziness. Deaths of the mice were observed at 400 mg/kg and 800 mg/kg. Upon analyzing the results, the intraperitoneal LD50 of the papain extract was estimated to be 400 mg/kg. It also shows that upon administration of 50 mg/kg, 100 mg/kg and 200 mg/kg of papain from *C. papaya* fruit extract to the mice, none died. The signs of toxicity like weakness, twitching and dizziness were observed in all test animals that received treatment. However, death occurred at 400 mg/kg and 800 mg/kg of papain.

Table 1
Lethal Dose of Papain from *Carica papaya* extract treated in Albino Mice

Dosage	Signs of toxicity	Number of Deaths
50 mg	Weakness	0
100 mg	Twitching and weakness	0
200 mg	Twitching, dizziness and weakness	0
400 mg	Twitching, dizziness, weakness and death	4
800 mg	Twitching, dizziness, weakness and death	6

This is comparable to the toxicity study of Amazu, et al. (2010) where the highest dose that caused the death of less than half of the mice in a group was 400 mg/kg and the highest dose that killed more than half of the mice in a group was 800 mg/kg. This implies that the concentrations of 50 mg/kg, 100 mg/kg and 200 mg/kg of the extract papain being administered can cause signs of toxicity but were

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non-lethal. In addition, dosages of 400 mg/kg and 800 mg/kg extract could produce toxic effects that lead to the death of the mice. The highest non-lethal dose extract was exhibited by 200 mg/kg dose and the least dosage that gave lethal toxicity was 400 mg/kg dose. Thus, of the doses that the researchers tested for toxicity, the highest dose that can be both safe and protective is 200 mg/kg.

III. Biochemical Analysis

Serum amylase and lipase are two commonly used serum parameters in detecting disorders of pancreas. In caerulein-induced acute pancreatitis, marked elevations in the value of the serum amylase and lipase were considered significant indications of renal impairment with serum amylase being more potent indicator than serum lipase (Jong-Kil & Jeong-Sang, 2012).

Table 2
The Mean Serum Amylase and Lipase Levels

	Amylase (mg/dl)	**Lipase (mg/dl)**
Group I Control Group	1397.50 ± 26.15	7.50 ± 0.65
Group II Caerulein Group	2669.50 ± 43.03	364.00 ± 16.67
Group III 100 mg of papain from *C. papaya* + Caerulein	1717.50 ± 31.12	6.25 ± 0.48
Group IV 200 mg of *papain from C. papaya* + Caerulein	1842.50 ± 18.87	3.00 ± 0.82

**Values are mean ± SEM. P<0.05 was considered to be statistically significant*.

Table 2 shows the mean serum amylase and lipase levels. Group I resulted to serum amylase and lipase levels of 1397.50±26.15 mg/dL and 7.50±0.65 mg/dL, respectively. As the control group, administered with saline only, Group I served as the basis of comparison with the other groups. On the other hand, the levels of amylase and lipase for Group II resulted to 2669.50±43.03 mg/dL and 364.00±16.67 mg/dL which showed elevated levels of the enzymes. The results prove that the pancreas of the involved mice in the group was damaged by caerulein. This implies that caerulein had caused acute pancreatitis in mice. These results were interrelated to the study conducted by Jong-Kil in 2012 where in single injection of 50 ug/kg of caerulein induced 4 times in hourly interval gave pancreatitis with the increase of parameters like amylase, lipase, TNF-α and IL-6 cytokine.

Group III, treated with 100 mg/kg extract of *C. papaya*, produced results of 1717.50±31.12 mg/dL for amylase and 6.25±0.48 mg/dL for lipase. The results were lower than that of Group II. This can be attributed to the effect of papain. Group IV treated with 200 mg extract, produced results of 1842.50±18.87 mg/dL for amylase and

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3.00±0.82 mg/dL for lipase which when compared to Group II, have shown to be effective in giving lower enzyme levels, comparable to Group I (Control Group). Comparing the amylase and lipase levels of these two groups to Group I and Group II, the amylase levels were lower than the level for AP but is still higher than the normal pancreatic condition meaning that the papain had lessened the amylase levels but was not able to be at par normal pancreatic conditions. The lipase levels of both groups were greatly reduced when compared to Group II and also, to Group I, which implies that the extract managed to lower the lipase levels even up to match a normal pancreas' lipase level. This infers that papain from *C. papaya* administered to Group III and Group IV really contributed to yield lower amylase levels when compared to Group II and lower lipase levels when compared to Group I and Group II.

Table 3 shows the effect of amylase to Groups II, III and IV as compared to Group I. It is noticeable that the obtained p-values for Groups II, III and IV were the same (p=0.000) which are highly significant.

Table 3
Effect of Amylase and Lipase Levels as Compared to Control Group

Dependent Variable	Group	p-value	Interpretation
Amylase	Group II (Caerulein Group)	0.000	Highly Significant
	Group III (100 mg + Caerulein)	0.000	Highly Significant
	Group IV (200 mg + Caerulein)	0.000	Highly Significant
Lipase	Group II (Caerulein Group)	0.000	Highly Significant
	Group III (100 mg + Caerulein)	0.999	Not Significant
	Group IV (200 mg + Caerulein)	0.963	Not Significant

**Significant p-value = <0.05.*

This reveals that there is a highly significant differences in the values obtained for amylase from the three groups when compared with Group I. As their amylase activity was significantly increased in the caerulein-treated group, both treated with pomegranate freeze-dried powder (PFDP) and pomegranate seeds extract (PSE), which is caused significant reduction in amylase activity. It can be implied that the difference is due to the effect of papain on the caerulin-induced mice. These findings are related to the study of Minaiyan et al. (2014) which shows the significant reduction of lipase levels on Sprague Dawley rats,

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it is also stated that the concentration of the dose does not meaningfully exert a decline in the serum levels of amylase and lipase. This implies that there was a highly significant difference between Groups I and II lipase levels since they serve as controls for acute pancreatitis and normal pancreas, respectively. This also suggests that there was no significant difference between Groups I, IV and III lipase levels since they were of the same range of values meaning that the treatment of papain before caerulein induction greatly helped in stabilizing the lipase levels of Groups III and IV as compared to Group I. This was in accordance with the study of Bulut, Ozkan, Ekinci, Dulundu, Topaloglu et al. (2011), in which the increase in lipase was markedly reduced in caerulein-induced Sprague Dawley rats after pretreatment with alpha lipoic acid.

The study provides the first evidence that papain in the fruit extract has the ability to reduce the levels of amylase and lipase. These findings support the view that papain contained in the extract could provide a protective effect on the pancreas and can reduce the degree of acute pancreatitis by the amylase and lipase activity to near normal pancreatic conditions when compared to the control group.

Table 4
Multiple Comparisons on the Serum Amylase when controlled by Control Group After Treatment

	Other Groups	p-value	Interpretation
Group I	Group II	0.000	Highly Significant
	Group III	0.000	Highly Significant
	Group IV	0.000	Highly Significant
Group II	Group I	0.000	Highly Significant
	Group III	0.000	Highly Significant
	Group IV	0.000	Highly Significant
Group III	Group I	0.000	Highly Significant
	Group II	0.000	Highly Significant
	Group IV	0.000	Highly Significant
Group IV	Group I	0.000	Highly Significant
	Group II	0.000	Highly Significant
	Group III	0.000	Highly Significant

**Significant p-value = <0.05.*

Table 4 presents the multiple comparisons on the effect of papain in the fruit extract on the amylase levels of the four groups. This was carried out using Tukey test followed by Dunnett's Test to correlate the effect of each treatment when compared to control group. It shows that amylase levels gave statistically significant result as Group I (normal control) was compared with Group II (AP control) with p-value less than 0.05 (0.000). This result can be correlated with

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the pathological mechanisms involved in acute pancreatitis induced by caerulein in the study of Minaiyan et al. (2013). This also shows that there was a significant difference between Group I and Group II since they serve as basis for normal pancreatic condition and acute pancreatitis. Treatment with papain at 100 mg/kg in Group III and 200 mg/kg in Group IV showed significant difference compared to Group I as it was still elevated but much lower than Group II justifying that the amylase levels for these groups were reduced due to the activity of papain. The table presents the comparison of the amylase levels of all the groups to Group II. There were all highly significant results for the amylase levels, since they had lower levels of amylase compared to the amylase levels of Group II. This means that the levels of amylase for these Groups I, III and IV were significantly different and lower than the amylase level of Group II.

In the comparison of amylase levels of Groups III and IV to the other groups, they have also shown significant results when compared to the control groups. Groups III and IV, when compared to Group II, have much lower levels of amylase but when compared to Group I, have slightly higher amylase levels; thus, the significant differences between the groups. This was in accordance with the study of Bulut, Ozkan, Ekinci, Dulundu, Topaloglu, et al. (2011) It can be implied that amylase levels in the Groups III and IV were maintained at near normal values due to the administration of papain in the *C. papaya* fruit extract.

Table 5
Multiple Comparisons on the Serum Lipase when controlled by Control Group after Treatment

	Other Groups	p-value	Interpretation
Group I	Group II	0.000	Highly Significant
	Group III	1.000	Not Significant
	Group IV	0.980	Not Significant
Group II	Group I	0.000	Highly significant
	Group III	0.000	Highly Significant
	Group IV	0.000	Highly Significant
Group III	Group I	1.000	Not Significant
	Group II	0.000	Highly Significant
	Group IV	0.980	Not Significant
Group IV	Group I	1.000	Not Significant
	Group II	0.000	Highly Significant
	Group III	1.000	Not Significant

**Significant p-value = <0.05.*

Table 5 presents the multiple comparisons on the effect of papain enzyme. Lipase levels gave statistically significant result as Group I (normal control) was compared with Group II (AP control)

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with p-value less than 0.05 (0.000). This result can be correlated with the pathological mechanisms involved in acute pancreatitis induced by caerulein in the study of Minaiyan et al. (2013). The comparison shows that there is a highly significant difference between Group I and Group II lipase levels. Treatment with papain in the fruit extract at 100 mg/kg in Group III and at 200 mg/kg in Group IV showed no significant difference compared to Group I as their levels were almost the same when being compared to each other showing that there was a lowering effect of the extract on the lipase levels of the rats' pancreas even after the induction of caerulein. The table also presents the comparison of the lipase levels of all the groups to Group II. All results were found to be highly significant results for the lipase levels, since they had lower lipase levels than that of Group II. This means that the levels of lipase for Groups I, III and IV were significantly different and lesser than that from Group II. This is more likely due to the effect of papain on the caerulein-induced test animals.

In the comparison of lipase levels of Groups III and IV to other groups, they have shown highly significant difference when compared to Group II. However, both groups showed no significant difference when compared with each other and with Group I, which implies that Groups I, III and IV have comparatively near lipase levels.

Furthermore, oral administration of papain in the fruit extract at 100 mg/kg and 200 mg/kg provided no significant effect on the serum lipase levels when compared to Group I. This result implies that papain enzyme at a dosage of 100 mg/kg and 200 mg/kg administered simultaneously with caerulein (50 ug/kg) is effective in reducing the high lipase level in the test animals with acute pancreatitis.

This agrees with the confirmed pancreatitis by highly significant values of pretreatment group of S-Propargyl-Cysteine to the caerulein-induced group which gave the same p-value (<0.05) of amylase levels from the study of Sidhapuriwala, Siaw, & Bhatia (2009). According to Owoyele, et a.l (2008), it was suggested that papain in the *C. papaya* fruit extract gives an anti-inflammatory mechanism; thus, papain was used in the reduction of serum parameters for pancreatitis and attenuating the inflammation of acinar cells of the pancreas.

IV. Histopathologic Examination

One sample per group was subjected for histopathologic examination. Findings revealed that there is no indication of pancreatitis to Group I. The microscopic examination mentioned that the section consists of serous acini of the exocrine pancreas and Islet of Langerhans that are within normal limits proving that Group I (Control group) is free of AP throughout the study. In Group II, the sample gave a final pathological diagnosis of pancreatitis ranging from mild to

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moderate. Majority of the serous acini have mild degenerative changes in the cells lining the ducts and there are few lymphoid aggregates around blood vessels, visually showing caerulein's apoptotic effect on the rats' pancreatic acinar cells.

The treatment Groups III and IV showed pathological diagnosis of mild to moderate pancreatitis and moderate pancreatitis respectively. Group III showed the same microscopic exam with the caerulein group; however, Group II showed the presence of increased numbers of vacuolations scattered within the exocrine pancreas. This could be due to the absence of treatment with the extract in Group II; thus, more vacuolations presented than Group III. This must be due to the low dosage of extract used, or there was not enough time for evidences of protection in the pancreas to show and that it was not only papain that acted upon the target organ. Hence, the protective effect of papain in the fruit extract was not manifested immediately histopathologically.

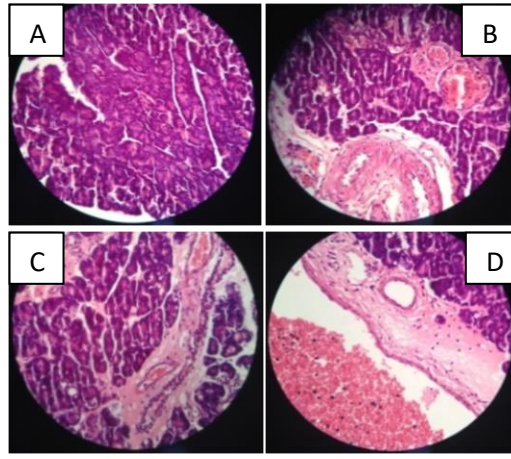

Figure 3. Histopathologic assessment.(A) Group I control group; (B) Group IIcerulein induced group; (C) Group III 100 mg dose treatment group; (D) Group IV 200 mg dose treatment group.

Those findings were in agreement with the results of Minaiyan, et al. (2013) as their saline group did not manifest any tissue injury and the caerulein-induced group showed edematous with severe leukocytic infiltration in pancreas. However, their pretreatment group of *C. intybus* showed that the severity of the edema and leukocytic infiltration was significantly reduced in compared to this study as the

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histopathologic result still gives a positive of occurrence of pancreatic edema that ranges from mild to moderate.

CONCLUSION

Biochemical test results revealed that the effect of papain of the *C. papaya* fruit extract was protective of the rats' pancreas. The researchers conclude that the effect of the papain from *C. papaya* extract gave safe and protective results at 100 mg/kg on lipase levels but not as much on amylase levels. It is proven that by increasing the dose by 200 mg/kg papain from *C. papaya* will give much protective effect on both lipase and amylase levels when measured in the experiment using control groups as basis. However, in histopathologic examination, the protective effect of the enzyme was not manifested.

RECOMMENDATION

It is recommended that further study must be conducted using higher dosage below the minimum toxicity of the enzyme for the positivity of its effect in the tissue and the pancreas. Also, isolation of the papain is suggested to view its distinct effect to the pancreas in acute pancreatitis. The researchers also recommend prolonging the duration time for the study to allow manifestation of the fruit extracts to the pancreatic tissue in histopathologic examination. Baseline data for amylase and lipase levels must be obtained.

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