Gross and Histopathological Evaluation of the hepatoprotective and Nephroprotective Properties of Native Guava (*Psidium guajava* Linn. var. native guava) Leaf Extract on CCL4-Fed White Mice (*Mus musculus*)"

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ABSTRACT

The Native Guava (*Psidium guajava* Linn. var. native guava) Leaf Extract was evaluated for its hepatoprotective and nephroprotective properties after induction of acute liver and kidney damage in laboratory mice using carbon tetrachloride (CCl4). The mice were orally administered with 1ml/200g body weight 3% CCl4mixed in corn oil and after 24 hours, the mice were sacrificed and the liver and kidney were harvested for gross and histopathologic evaluation.

It was found that oral supplementation of native guava leaf extract (NGLE) at a concentration of 200mg/kg body weight prevented enlargement of both liver and kidney. Evaluation of macroscopic or gross lesions in both organs showed marginal or minimal presence of lesions. Histopathological evaluation of liver and kidney showed fewer numbers of observed cellular lesions with lesser degree and extent of tissue damage when compared with animals that did not receive any NGLE supplementation. This study was able to show that NGLE has hepatoprotective and nephroprotective properties and can be used to prevent kidney and liver damage.

INTRODUCTION

Herbal medicine is still the mainstay of about 75-80% of the world population, mainly in the developing countries for primary health care (Akande *et al.*, 2010). It is generally known that the consumption of a variety of local herbs and vegetables by man contribute significantly to the improvement of human health, in terms of prevention, and or cure of diseases because plants have long served as a useful and natural source of the therapeutic agents (Uboh *et al.*, 2010).

Psidium guajava contains a number of chemical constituents, which are reported to possess antibacterial, antidiarrhoeal, antimycobacterial, antihyperglycemic, antimalarial, cytotoxic and antioxidant activities. The major constituents associated with the antioxidant activity of *Psidium guajava* are caryophyllene oxide, caryophyllene, and tannins (Chanchal *et al.*, 2006).

Changes in lifestyle and diet have increased our risk of developing chronic degenerative diseases such as liver and kidney diseases since these organs have critical roles in metabolism. Different products were developed and are marketed as hepatoprotective and nephroprotective dietary supplements. However, their efficacy is yet to be proven by

further clinical tests.

This study aimed to determine the efficacy of native guava leaf extract as hepatoprotective and nephroprotective agent. Specifically, it: 1) determined the effect of administration of NGLE at a concentration of 200 mg/kg BW on liver and kidney organ weights; 2) assessed the number and extent of gross/macroscopic kidney and liver lesions; and 3) evaluated the severity and extent of histopathologic/ microscopic kidney and liver lesions of mice after induction of acute kidney and liver damage using carbon tetrachloride (CCl_4).

MATERIALS AND METHODS

Eighteen 8-week old ICR mice (9 male and 9 female) with weights ranging from 15-40 g were randomly distributed to 3 treatments replicated three times. One male and female animal was assigned per replicate. The experiment was laid out in a completely randomized design with the following treatments:

Treatment 1 - Native Guava Leaf Extract (200 mg/kg BW for 7 days)
Treatment 2 - Native Guava Leaf Extract (200 mg/kg BW for 7days) plus 3.3% CCl_4 (7th day)
Treatment 3 - 3.3% CCL4 (7th day)

The dose of NGLE extract used was 200mg/kg as this level was found to be effective in similar study conducted by Uboh *et al.*, (2010).

The experimental animals were individually housed in glass cages and were provided with feeder and waterer. Commercially available rabbit pellet feeds and were given. Clean fresh water was given and replaced everyday.

Preparation of Native Guava Leaf Extract

Two hundred fifty (250) grams ofmature native guava leaves were oven-dried to contain approximately 5% moisture content. The dried guava leaves were ground to obtain a fine powder. The powdered leaves were placed in a beaker and soaked in a solution containing distilled water (450 ml) and chloroform (50 ml) for seven days, then filtered using a sterilefilter. The solution was then evaporated using hot water bath for 4 hours then stored in an airtight container in the refrigerator until used.

Supplementation of Native Guava Leaf Extract

Native Guava Leaf Extract (NGLE) was used as a protective agent on the liver and kidney of mice. NGLE was prepared with a final concentration of 50mg/ml and orally administered once a day for 7 days at a dose of 200mg/kg body weight.

Induction of Liver and Kidney Damage

Carbon tetrachloride (CCl_4) was used to induce acute liver and kidney damage in the experimental animals. Carbon tetrachloride (3.3%) was mixed in corn oil and was given orally on the 7th day, after one week of treatment with NGLE. A single dose of 1ml/200grams body weight was used to induce acute liver and kidney damage.

Necropsy and Histopathological Evaluation

Necropsy was conducted 24 hours after induction of liver and kidney damage. The experimental animals were euthanized via cervical dislocation. External examination was performed before evisceration. After physical examination, vertical ventral midline incision was done using scissors to remove the skin, from the neck topubis then organs were eviscerated. The kidneys and liver were weighed, evaluated, and graded based on the severity of lesions.

The organs were placed in individual containers filled with 10% buffered formalin solution then forwarded after 1 week tothe Philippine Kidney Dialysis Foundation (PKDF) for histopathologic slide preparation, and to

Table 1. Severity of the macroscopic/gross lesion*

Severity	Macroscopic proportion of liver/kidney affected	Grade	Quantifiable finding
Marginal or minimal	Very small amount	1	1-2 foci
Slight or few	Small amount	2	3-6 foci
Moderate or several	Medium amount	3	7-12 foci
Marked or many	Large amount	4	>12 foci
Severe	Very large amount	5	Diffuse

* (Modified from Hardisty and Eustis 1990; WorldHealth Organization 1978; Derelanko 2000)

the University of the Philippines Los Banos-College of Veterinary Medicine- Department of Paraclinical Sciencesfor the reading and interpretation of histopathologic slides.

The severity of the macroscopic/gross lesions was graded from 1-5 (Table 1).

Statistical Analysis

Data collected was statistically analysed using the Analysis of Variance (ANOVA) in a Completely Randomized Design. Differences among treatment groups were determined using the Least Significant Difference (LSD).

RESULTS AND DISCUSSION

Organ Weights

Comparison of the liver and kidney weights in table 2 showed that NGLE has significant hepatoprotective and nephroprotective properties by preventing organ enlargement in animals supplemented with the extract before induction of acute liver and kidney damage. The mean organ weights of the different groups were measured and compared.

There was no significant difference between the mean liver weights of the treatments 1 and 2. However, the increase in the mean liver weight of treatment 3 was significantly different from the treatments 1 and 2.

There is no significant difference between the mean kidney weights of the treatments 1 and 2. However, the increase in the mean kidney weight of treatment 3 was significantly different from the treatments 1 and 2.

This means that supplementation of NGLE prevented the enlargement of the liver and kidney, showing its hepatoprotective and nephroprotective effects.

This result is similar to the toxicity study conducted by Chanchal *et al.,* (2006), where rats supplemented with different concentrations of aqueous guava leaf extract before induction of acute liver damage showed lesser liver enlargement with minimal elevation in liver function enzyme indicator.

Macroscopic/Gross Lesions

Macroscopic evaluation was done by counting the number of visible lesions on the surface of the liver and kidney. The severity of the lesions was graded using the grading system shown in table 1.

Grading of the liver lesions showed that the treatments 1 and 2 and 3 are significantly different from each other. Treatment 1 showed the lowest grade with a mean grade of 1.000 or very minimal presence of lesions. Treatment 2 had a mean grade of 1.667. Treatment 3 showed the highest grade with a mean grade of 5.00.

Grading of the kidney lesions showed that there was no significant difference between treatments 1 and 2 but they were significantly different from treatment 3. Treatment 1 and 2

Table 2. Comparison of Liver and kidney weights among the different treatment groups expressed in grams

Treatment	Mean (grams)	
	Liver	Kidney
1 Native Guava Leaf Extract	1.133[a]	0.3167[a]
2 Native Guava Leaf Extract + Carbon Tetrachloride	1.200[a]	0.4000[a]
3 Carbon Tetrachloride	1.650[b]	0.6000[b]
Normal Range(in grams)	1.273-1.407	0.284-0.472
F-computed	11.68	20.82
Significance	**	**
cv (%)	15.16	17.81

Means with the same letter are not significantly different.
** highly significant

Table 3. Grades based on number of lesions of liver and kidney of the different treatments after gross/macroscopic evaluation

Treatment	Mean (grams)	
	Liver	Kidney
1 Native Guava Leaf Extract	1.000[a]	1.000[a]
2 Native Guava Leaf Extract + Carbon Tetrachloride	1.667[b]	1.000[a]
3 Carbon Tetrachloride	5.000[c]	5.000[b]
Normal Range(in grams)	1.273-1.407	0.284-0.472
F-computed	248	∞
Significance	**	**
cv (%)	13.04	0

Means with the same letter are not significantly different.
** highly significant

both had a mean grade of 1.000 while treatment 3 has a mean grade of 5.000.

Results of the gross examination based on liver and kidney mean grades (Table 3) show the hepatoprotective and nephroprotective properties of the native guava leaf extract.

Destruction or necrosis of the liver and kidney tissue will manifest as points of pale foci on the surface of the organ. The severity of the insult can be assessed based on the number of the lesions, and its distribution on the surface of the organ. The appearance of fewer lesions on both liver and kidney can be due to the presence of high levels of antioxidants in the native guava leaf extract. Antioxidants prevented cellular damage by stabilizing lysozomal membranes of the cells of the liver and kidney.

Histopathological Evaluation

Tables 4 and 5 show the summary of the microscopic lesions found during the histopathologic evaluation of liver and kidney in mice respectively. It shows that mice administered with CCl_4 (Treatment 3) had the greatest damage based on the degree, extent,

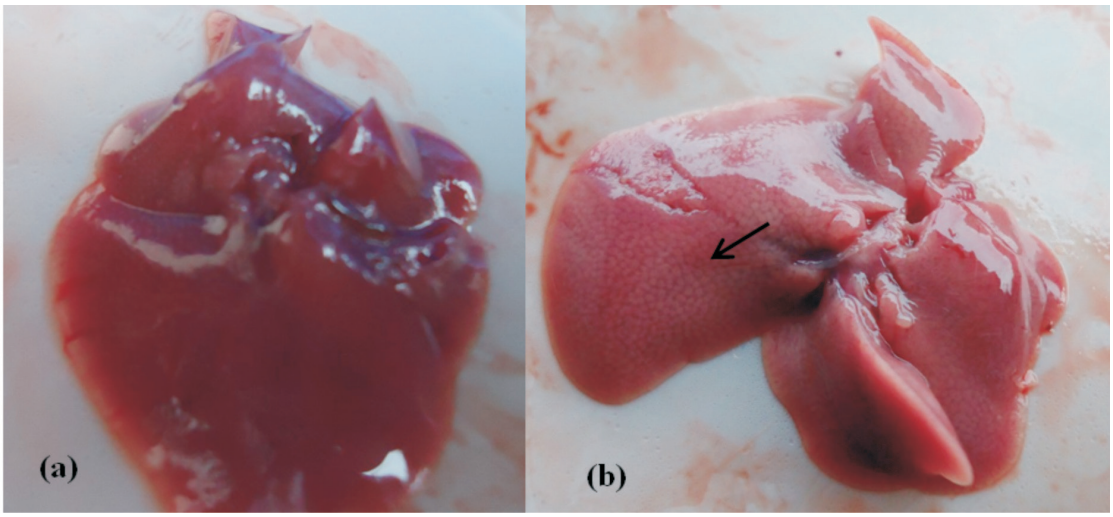

Figure 1. (a) Liver sample from the treatment group which received NGLE for one week before induction of acute liver damage. Mild congestion and some pale foci on the surface of the lobewas observed. (b) Liver sample from the mice group which received CCl_4 only. Note the numerous pale foci (arrow) found all over the organ. The liver is also greatly enlarged and friable after induction of damage using CCl_4

Table 4.Summary of observed microscopic hepatic lesions in treatment groups

Microscopic Lesion	Treatments		
	T_1 Native Guava Leaf Extract	T_2 Native Guava Leaf Extract + Carbon Tetrachloride	T_3 Carbon Tetrachloride
Congestion	+	+	+
Centrilobular degeneration	+	-	-
Cytoplasic disintegration	+	+	+
Centrilobular necrosis	-	+	+
Slight necrosis of hepatocyte	+	+	+
Inflammatory infiltrates	-	+	+
Focal hepatocellular necrosis	-	+	-
Bridging Necrosis	-	+	+
Mild acute coagulation necrosis	-	+	-
Moderate acute coagulation necrosis	-	+	-
Severe acute coagulation necrosis	-	-	+
Massive hepatocellular necrosis	-	-	+

Legend: (+) Positive lesion (-) Negative lesion

Table 5. Summary of observed microscopic kidney lesions in treatment groups

Microscopic Lesion	Treatments		
	T_1 Native Guava Leaf Extract	T_2 Native Guava Leaf Extract + Carbon Tetrachloride	T_3 Carbon Tetrachloride
Cortical congestion	+	+	+
Cortical and Medullary congestion	+	+	+
Non-well defined Bowman's space	-	+	+
Mild tubular coagulation necrosis	-	-	+
Non-uniform glomerular size	-	+	+
Moderate to severe multifocal glomerular and tubular necrosis of cells	-	+	+
Proliferative glomerulonephritis	-	+	-
Slight proteinaceous leakage in between renal tubules	-	+	+

Legend: (+) Positive lesion (-) Negative lesion

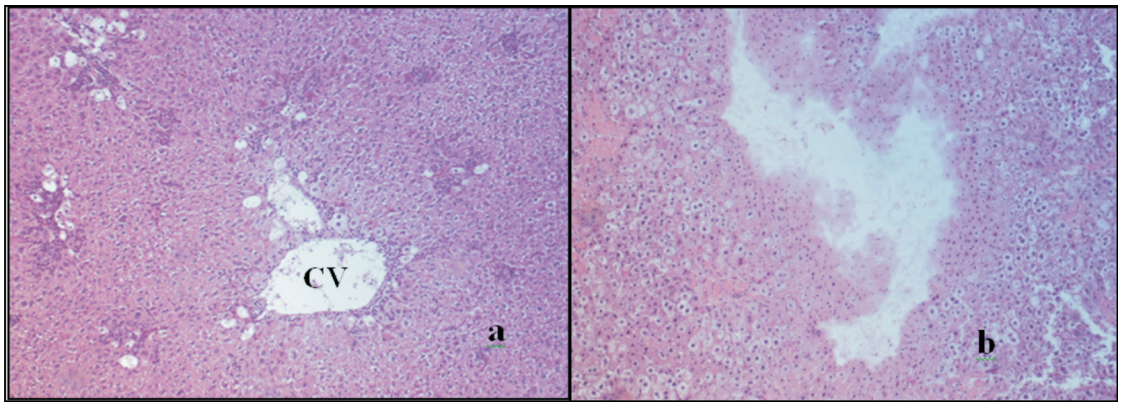

Figure 2. (a)Liver sample from treatment 2 (NGLE + CCl_4).Mild to moderate acute coagulation necrosis of hepatocytes especially around the central vein (CV) characterized by degradation of cytoplasmic proteins which gives the necrotic cell a pale appearance with few pyknotic cells.Majority of the hepatocytes are normal and arranged radially. (Magnification 100X). (b)Liver sample from treatment 3 (CCl_4). Massive hepatocellular degeneration was observed or the entire population of hepatocytes has undergone necrosis. (Magnification 100X)

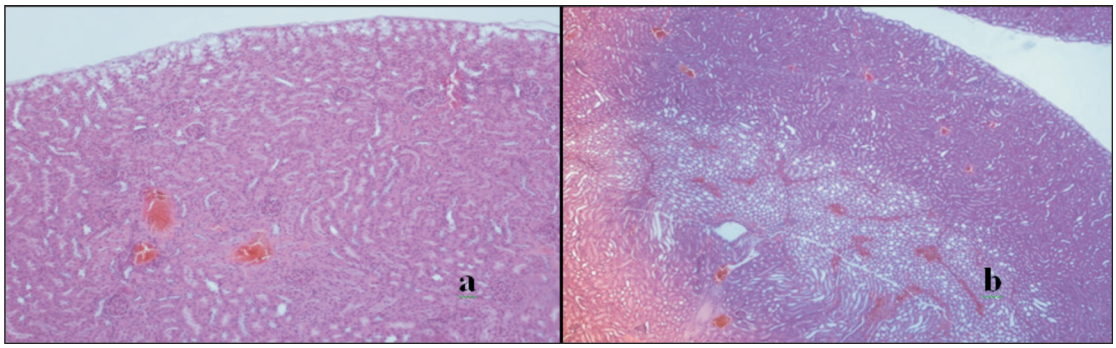

Figure 3. (a)Kidney sample from treatment 2 (NGLE + CCl_4).Both cortical and medullary congestion are observed. In some glomerulus, the space or the Bowman's space is non-well defined.(b)Kidney sample from treatment 3 (CCl_4). Moderate to severe glomerular and tubular necrosis of cells found in the cortex, characterized by pyknoric cells and pale cytoplasm. Size of the glomerulus are not uniform due to necrosis of some glomerulus (small). (Magnification 100X)

and type of observed lesions in both the kidney and liver. The kidney and liver of mice that were supplemented with NGLE prior to CCl_4 (Treatment 2) administration showed damage but had a milder degree, lesser extent, and fewer types of observed lesions. Mice that were given NGLE only (Treatment 1) have very few microscopic lesions which might be caused by other factors. Similar results were obtained by Chanchal *et al.*, (2006) was Uboh *et al.*, (2010) which shows that there is a correlation of the gross lesions obtained and the severity of microscopic lesions observed in mice that were subjected to liver damage with and without supplementation of guava leaf extracts.

The result of the histopathologic evaluation concurred with the previous observations that Native Guava Leaf Extract (NGLE) has significant hepatoprotective and nephroprotective properties which are attributed to the presence of high levels of antioxidants. Antioxidants prevented cellular damage by stabilizing lysozomal membranes of the hepatocytes.This study shows that guava can be a source of locally available, and effective health supplement to prevent liver and kidney damage.

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

Native guava leaf extract (NGLE) administration successfully prevented organ enlargement and produced minimal presence of gross lesions which are typical signs of organ damage proving that it is a hepatoprotective and nephroprotective agent. The gross lesions and microscopic lesions were also less severe in animals that received the supplementation. Results of the different parameters showed that native guava leaf extract can be used as alimentotherapy to prevent liver and kidney damage.

Recommendations

Oral NGLE supplementation at a dose of 200mg/kg body weight is found to be effective in preventing liver and kidney damage. However, further studies are recommended to gain more information. Areas of focus for further research are: (1) establishment of the minimum and maximum dose of administration;(2)effects of long term administration; and (3) its clinical application in domestic animals.

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