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Safety assessment of dietary exposure to cooked taro leaves (*pinangat*) and smoked fish (*tinapa*) : Acute and 60-day toxicity studies in mice

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Abstract

The acute and sub-chronic toxicity of cooked taro leaves (*pinangat*) and smoked fish (*tinapa*) were assessed in mice**.** In the acute oral toxicity study, animals received a dose of *pinangat* and *tinapa* at 400, 800, 1600 and 3200 mg/kg bw. Corn oil served as the vehicle control. Results revealed no mortality in all the control and treated groups and the no observed adverse effect level (NOAEL) for *pinangat* is 400-1600 mg/kg whereas, NOAEL for *tinapa* is 400-800 mg/kg. Signs of toxicity as manifested in terms of sickness-like behaviour: lethargy and mild ataxia were observed in the group given with the highest dose of *pinangat* and medium dose of *tinapa*.

During the 60-d sub-chronic toxicity study, the animals were gavaged daily with graded doses of *pinangat* and *tinapa*. Biochemical analyses revealed significant increase in the total blood cholesterol, VLDL, and triglycerides of the *smoked fish* treated mice at 1500 mg/kg. Hepatorenal enzymes were also significantly elevated in all the treated groups compared to the control. Results of *pinangat* treatment revealed no significant change in the lipid profile and hepatorenal markers of all treated mice. However, creatinine (umol/L) was significantly elevated in the group given with 3500 mg/kg *pinangat*. The results of the study suggest food safety of the food products when taken at appropriate doses however, findings also confirm the adverse effects of excess dietary salt loading on kidney and liver.

Key words : Acute and sub-chronic toxicity, NOAEL, *pinangat, tinapa,* biochemical, hepatorenal

INTRODUCTION

Dietary safety assessment of common food items remains an interesting area for exploration since nutrition and nutritional factors are widely considered to be crucial for human health and well-being. The growing number of people adopting lifestyles and diets that are conducive to overweight and related non-communicable diseases (NCDs) is even correlated to economic growth in developing regions[1]. Epidemiological studies done on Asian population linked diet and nutrition uptake and its regulation to incidence of NCDs including cardiovascular diseases[2] diabetes[3], renal disease[4], and some types of cancers[5,6].

Pinangat, a vegetable dish made of taro (*Colocasia esculenta*) leaves cooked in coconut milk and *tinapa*, a fish usually made of blue mackerel scad (*Decapterus macarellus*) cured by smoking are considered as staple items served from meal to meal in the food tables of low to middle class household in the Bicol Region, Philippines. Since the raw materials for the food products are locally grown, harvested and processed in the region, *pinangat* and *tinapa* are popularly known as Bicol's *one town one product* (OTOP).

To date, there is a dearth of information regarding the adverse and non-adverse effects of regular and frequent consumption of these food products as well as, the highest exposure level that does not cause treatment related effects relevant to human health risk assessment. The present study examines how dietary intake of *pinangat* and *tinapa* relate to changes in the lipid profile and markers of hepatic oxidative metabolism in a mouse model.

MATERIALS AND METHODS

Test substance

Pinangat (Fig.1a) and *tinapa* (Fig.1b) were obtained from a local supplier in Albay and Camarines Sur (Philippines), respectively. In the laboratory, each of the food products was processed using Oster® blender with corn oil as vehicle to obtain a homogenous food paste having the different concentrations of the diet to be administered to the test animals. Each diet was prepared the day before study initiation and weekly thereafter. Prepared diets were stored protected from light in dark bottles at 4 °C.

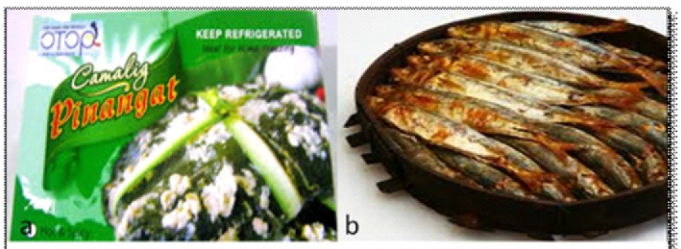

Fig.1. The OTOP food products used as test substance: a) *pinangat* and b) *tinapa*.

Animals and treatment

Acute oral toxicity and repeated dose 60-day oral toxicity were conducted at Bicol University College of Science (Legazpi City, Philippines). The tests are in compliance to the testing procedures set by the Organisation for Economic Cooperation

and Development (OECD) guidelines. The animals (male ICR mice, 30 g) used were obtained from the Food and Drug Administration (FDA, Philippines) and were cared for in accordance with the Guide for the Care and Use of Laboratory Animals set by the Philippine Association for Laboratory Animal Science (PALAS) Code of Practice. Animals were allowed free access to standard feed and water. The animals were acclimated to facility conditions 7 days prior to use.

Oral acute toxicity

Mice were randomly divided into five groups of three animals per group. Graded doses of each diet (400, 800, 1600, and 3200 mg/kg) were administered to the animals orally by gavage. The control group was administered 0.5 ml corn oil orally. Mice were observed for 24-48 h post-treatment for mortality, behavioural changes (restlessness, dullness, agitation) and signs of toxicity.

Sub-chronic toxicity study

Animals were randomly distributed to four groups of five animals per group for each diet and orally administered by gavage of *pinangat* at doses of 775, 1550 and 3100 mg/kg daily and *tinapa* at doses of 375, 750 and 1500 mg/kg daily for 60 days. The control group was orally administered 0.5 ml of corn oil daily. The mice were weighed weekly throughout the course of the experiment. The animals were closely observed for behavioural such as restlessness, hyperactivity, dullness and general morphological changes.

Collection of blood sample

The animals were anaesthesized with Zoletil® (10 mg/kg, IM) on the 61st day of the experiment and blood samples were collected via cardiac puncture with the aid of a tuberculin syringe into EDTA tubes for blood chemistry analysis.

Biochemical parameters

The biochemical analysis was done at Central Link Laboratory (Albay, Philippines). Serum concentration of total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), very low density lipoprotein (VLDL), and triglyceride were measured by enzyme-assay using commercial kits. The serum activities of liver enzymes alanine aminotransferase (ALT), and aspartate aminotransferase (AST) creatinine and blood urea nitrogen (BUN) were estimated using automated analyser.

Statistical analysis

Results were expressed as mean± standard error of the means (SEM). The data were subjected to one-way analysis of variance (ANOVA) test and differences between samples were determined by Tukey's multiple comparison test, using the SPSS (statistical) software. Results were considered to be significant at $p \leq 0.05$.

RESULTS

Oral acute toxicity study

The diets did not produce any mortality when administered orally at various doses of 400 mg/kg to 3200 mg/kg, but reduced locomotion and dullness were noted in some animals treated with higher doses of 3200 mg/kg *pinangat* and 1600 mg/kg *tinapa*. Signs of toxicity as manifested in terms of sickness-like behaviour : lethargy and mild ataxia were observed. No other gross toxicities, adverse pharmacological effects or abnormal behaviors were seen in the rest of the animals during the 48-hour observation period. These results suggest that the no observed adverse effect level (NOAEL) for *pinangat* is 400-1600 mg/kg whereas, NOAEL for *tinapa* is 400-800 mg/kg.

Sub-chronic toxicity study

Throughout the 60-day treatment duration it was observed that all animals given with the varying doses of *pinangat and tinapa* were generally normal. Daily cage-side observations did not reveal any physical changes in the skin, fur, eyes, respiratory system and general behavioural patterns. The results of the blood chemistry analysis are summarized in Tables 1A-2B.

Table 1A: A. Lipid profile of mice administered with *tinapa* for 60d

Smoked fish (mg/kg)	Total Cholesterol (mmol/L)	HDL (mmol/L)	LDL (mmol/L)	VLDL (mmol/L)	Triglycerides (mmol/L)
0	4.77 ± .150	0.556 ± .170	3.41 ± .118	31.30 ± 3.66	1.80 ± .2078
375	4.38 ± .000	0.640 ± 0.00	2.96 ± 0.00	30.24 ± 0.00	1.74 ± 0.00
750	3.99 ± .000	0.540 ± 0.00	2.81 ± 0.00	24.82 ± 0.00*	1.43 ± 0.00*
1500	5.83 ± .476*	0.970 ± .017*	3.87 ± .456	38.07 ± .1789*	2.19 ± .0115*

Values are expressed as mean ± standard error of means, N=5
*significantly different compared to control (p=0.05)

Table 1B: A. Lipid profile of mice administered with *pinangat* for 60d[ns]

Pinangat (mg/kg)	Total Cholesterol (mmol/L)	HDL (mmol/L)	LDL (mmol/L)	VLDL (mmol/L)	Triglycerides (mmol/L)
0	4.30 ±.150	0.733 ±.098	2.80 ± .243	29.95 ± .667	1.72 ± .037
775	4.90 ± 436	0.746 ± .192	3.31± .253	32.63 ± 4.28	1.87 ± .245
1550	4.31 ± .305	0.813 ± .132	2.75 ± .105	28.93 ± 3.79	1.66 ± .218
3100	4.67 ± .646	0.740 ±.211	3.08 ± .338	32.98 ±5.09	1.90 ± .293

Values are expressed as mean ± standard error of means, N=5
ns = not significantly different compared to untreated group

Table 2A: Hepatorenal markers in mice administered with *tinapa* for 60d

Smoked fish (mg/kg)	AST (U/L)	ALT (U/L)	BUN (mmol/L)	Creatinine* (umol/L)
0	14.60 ± 2.598	14.25 ± 2.626	3.10 ± .115	52.15± 1.934
375	22.80 ± 0.00	22.10± .00	4.10 ± .00	66.90 ± .00*
750	28.80 ± 0.00*	28.50 ± .00*	4.00 ± .00	59.90 ± .00*
1500	34.30 ± 2.021*	32.10 ± 2.829*	4.65 ± .14723	66.65 ± .83716*

Values are expressed as mean ± standard error of means, N=5
*significantly different compared to control (*p*=0.05)
Abbreviations: AST = Aspartate Aminotransferase; ALT = Alanine Aminotransferase; BUN =Blood Urea Nitrogen

Table 2B: A. Hepatorenal markers in mice administered with *pinangat* for 60d

Pinangat (mg/kg)	AST (U/L)	ALT (U/L)	BUN (mmol/L)	Creatinine (umol/L)
0	14.07 ± 3.53	15.60 ± 2.97	3.63 ± .379	54.47 ± 3.98
775	25.56 ± 6.79	25.93 ± 5.25	4.47 ± .202	53.37 ± 3.36
1550	26.33 ± 3.14	24.90 ± 2.15	4.73 ± .408	62.30 ± 5.97
3100	24.83 ± 8.79	25.93 ± 9.27	4.34 ± .517	69.30 ± .929*

Values are expressed as mean ± standard error of means, N=5
*significantly different compared to control (p=0.05)
Abbreviations: AST = Aspartate Aminotransferase; ALT = Alanine Aminotransferase; BUN = Blood Urea Nitrogen

Tables 1A-1B present the change in total cholesterol, HDL, LDL, VLDL and triglycerides in mice given with *tinapa and pinangat* for 60 days. Results revealed that long-term consumption of *tinapa at* 1500 mg/kg dose significantly ($p<0.05$) increase the total blood cholesterol, VLDL, and triglycerides of the treated mice compared to control. No significant change was observed in the lipid profile of mice given with the graded doses of *pinangat*.

Clinical chemistry parameters in terms of alanine aminotransferase (ALT); aspartate aminotransferase (AST); blood urea nitrogen (BUN) and creatinine are shown in Tables 2A-2B. Statistically significant ($p<0.05$) increase in the liver enzymes ALT and AST were observed in all the animals given with *tinapa* at 775 mg/kg and 1500 mg/kg compared to the control group. No significant increase in liver enzymes was recorded in all *pinangat*-treated mice compared to control. Renal function test reported a non-significant change in the BUN level for all the treated groups given with both test diets compared to the control. There was a significant ($p<0.05$) increase in the creatinine level in all the animals fed with the increasing doses of *smoked fish* compared to the control group. Creatinine level was also elevated significantly ($p<0.05$) in the animals given with the highest dose (3500 mg/kg) of *pinangat*.

DISCUSSION

Food safety and security is one of the research priorities in the Philippine Higher Education Institutions. Considering the country's vulnerability to natural hazards and disasters and with its booming population, it is important for the people to be assured of affordable and safe food on their table[7].The present study aimed to carry out toxicity evaluation of two popular food products in the Bicol Region, Philippines. Acute and sub-chronic oral toxicity studies did not cause any mortality in all animal models suggesting food safety for repeated consumption.

Based on the results of acute oral toxicity study the toxic dose as manifested in terms of mild ataxia and lethargy was observed at ≥1600 mg/kg and ≥3200 mg/kg for *tinapa* and *pinangat*, respectively. Nutritional analysis revealed a much higher Na content in *tinapa* (209 mg/oz) compared to *pinangat* (52.38 mg/oz). Excess dietary sodium intake from *tinapa* and *pinangat* may have alleviated the impact of psychological stress in the laboratory mice. Studies have reported that high sodium levels in mice often manifests as weakness, as well as lethargy and agitation[8] and acute salt loading is anxiolytic and dampens stress responsiveness in laboratory rats [9].

The results from the 60-day sub-chronic oral toxicity study did not show any dose or time dependent adverse effects on behaviour and gross morphology of test animals. Blood chemistry analysis revealed a significant increase in the total blood cholesterol, HDL, and triglycerides of the *smoked fish* treated mice at 1500 mg/kg dose but a significant decrease in VLDL and triglycerides was noted in treated group given only with 750 mg/kg of the same diet. Results herein suggest a correlation between salt loading and amount of serum lipids. Previous work presented evidences that high and low sodium intake may increase or decrease cholesterol and triglycerides in human subjects[10]. Surprisingly, the graded doses (775, 1500 and 3100 mg/kg) of *pinangat* did not alter the lipid profile of the treated mice. The findings of the study support the health benefits of coconut milk which is the major ingredient of *pinangat.* A recent study showed that dietary supplementation of coconut milk to

human subjects did not cause a detrimental effect on the lipid profile and in fact was beneficial due to the decrease in LDL and rise in HDL of the healthy volunteers [11].

Alterations in blood biomarkers including ALT, AST and creatinine were observed in all the animals given with *tinapa* at 775 mg/kg and 1500 mg/kg. ALT and AST are good indices of liver and kidney damage. The present study showed a significant increase in the levels of ALT and AST, conditions which can be associated with liver cell inflammation or necrosis as well as significant rise in creatinine level, state due to impaired renal function or in acute renal failure. The relation of salt intake to chronic kidney disease is well documented in several studies: a study proved that salt restriction reduced renal hypertrophy and injury in *uninephrectomized* spontaneously hypertensive rats (*SHR*)[12] and similar works using *pregnancy induced nephrotoxicity* established the role of maternal salt intake in altering the kidney development of offsprings[13,14]. Further, kidney and digestive disorders involving the liver are included as major adverse effects of excessive salt (sodium) intake in addition to hypertension and cardiovascular diseases[15].

CONCLUSION

The data presented in the study demonstrate the effect of two locally processed food products on lipid profile and biochemical parameters in mice. The results suggest safety of *pinangat* and *tinapa* for repeated but moderate consumption. However, more toxicity tests should be carried out in order to be in a position to suggest new recommendations on the permissible daily intake for these food products. To date, there is no official record for the recommended daily intake or allowable daily intake for the salted and processed foods in the Philippines.

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REFERENCES

1. FAO. Economic growth is necessary but not sufficient to accelerate reduction of hunger and malnutrition. The State of Food Insecurity in the World 2012: Rome, FAO.

2. Lipoeto NI, Agus Z, Oenzil F, Wahlqvist ML and Wattanapenpaiboon N. Dietary intake and the risk of coronary heart disease among the coconut-consuming Minangkabau in West Sumatra, Indonesia. *Asia Pac J Clin Nutr. 2004:13 (4):377-384.*

3. Agrawal S, Millett CJ, Dhillon PK, Subramanian SV, and Ebrahim S. Type of vegetarian diet, obesity and diabetes in adult Indian population. Nutrition Journ. 2014: 13:89.

4. Van den Berg E, Hospers FA, Navis G, Engberink MF, Brink EJ, Geleijnse JM, van Baak MA, Gans RO and Bakker SJ. 2011. Dietary acid load and rapid progression to end-stage renal disease of diabetic nephropathy in Westernized South Asian people. J Nephrol.2011:24(1):11-7.

5. Sinha R, Anderson DE, Mc Donald SS and Greenwald P. Cancer Risk and Diet in India. J of Postgrad Med. 2003:49 : 222-228.

6. Yee YK, Tan VPY, Chan P, Hung IFN, Pang R and Wong BCY. 2009. Epidemiology of colorectal cancer in Asia. Journal of Gastroenterology and Hepatology, 2009: 24: 18101816.

7. CHED. Commission on Higher Education : Priority area food safety and security. National Higher Education Research Agenda 2, 2009: CMO 42 s.2010. NCR Manila Philippines.

8. Morris MJ, and Na ES. Johnson, A.K. Salt craving: The psychobiology of pathogenic sodium intake. *Physiol Behav*.2008: August 6; 94(5): 709721.

9. Frazier CJ, Pati D, Hiller H, Nguyen D, Wang L, Smith JA. Acute hypernatremia exerts an inhibitory oxytocinergic tone that is associated with anxiolytic mood in male rats. Endocrinology, 2013: 154:245767.

10. Graudal NA, Hubeck-Graudal T, and Jurgens G. Effects of Low-Sodium Diet vs. High-Sodium Diet on Blood Pressure, Renin, Aldosterone, Catecholamines, Cholesterol, and Triglyceride (Cochrane Review). Special Review. American Journal of Hypertension, 2011:1-15.

11. Ekanayaka RAI, Ekanayaka NK, Perera B, and De Silva PGMSM. Impact of a traditional dietary supplement with coconut milk and soya milk on the lipid profile in normal free living subjects. Hindawi Publishing Corporation Journal of Nutrition and Metabolism, 2013: 1-11. http://dx.doi.org/10.1155/2013/481068.

12. Benstein JA, Feiner HD, Parker M. Superiority of salt restriction over diuretics in reducing renal hypertrophy and injury in uninephrectomized SHR. Am J Physiol.1990: 258, F1675F1681.

13. Koleganova N, Piecha G, Ritz E. Both high and low maternal salt intake in pregnancy alter kidney development in the offspring. Am J Physiol Renal Physiol. 2012: 301: F344F354.

14. Reynolds CM, Vickers MH, Harrison CJ, Segovia SA, and Gray C. High fat and/or high salt intake during pregnancy alters maternal metainflammation and offspring growth and metabolic profiles. Physiological Reports, 2014: 2 DOI: 10.14814/phy2.12110.

15. Kenney JJ. Diet, Hypertension and Salt Toxicity. Food & Health Communications, Inc. 1998, 67 pp.