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Subacute Effects of Edible Film from Modified Sago Starch in Rats

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ABSTRACT

The effects of edible film from modified sago starch were determined on female Sprague-Dawley rats where the aim was to develop a basic guideline for safe use of edible films as food packaging. Rats were fed with 2 g kg^{-1} (low dose) and 5 g kg^{-1} (high dose) of body weight edible film for 28 days and the control rats only received normal rat pellet. The study showed that repeated administration of high dose edible film to rats did produce significant change in the liver function (total bilirubin) and renal function parameters (creatinine). Other metabolic parameters tested were: aspartate transaminase, alanine transaminase and alkaline phosphatase, blood lipid profile; cholesterol, high-density lipoprotein, low-density lipoprotein and triglyceride, total protein and glucose concentrations, however, they were not significantly changed with respect to control animals. In conclusion, edible film from modified sago starch at 2 g kg^{-1} is less likely to develop toxicity as observed in the sub-acute toxicity study.

Key words: Sago, starch, sub-acute, toxicity, edible film

INTRODUCTION

Over the past 30 years, edible films and coatings have received increasing attention in the research field and in food industry as a potential alternative for food packaging. Several works on the potential of so-called green packaging have been described recently (Sobral *et al*., 2001; Donatella *et al*., 2003; Parra *et al*., 2004; Phan *et al*., 2009).

Edible films use renewable sources which is biodegradable and convenient due to lower environmental consequences compared to synthetic filmswhich are mostly petrochemical-based, therefore, non-biodegradable and persist in nature for a long time (Tharanathan, 2003; Ramesh *et al*., 2004; Bourtoom, 2008; Chillo *et al*., 2008). It is reported that over 25 million tons of non-biodegradable plastics are being accumulated in the environment yearly (Ramesh *et al*., 2004). Additionally, food additives such as antimicrobials, antioxidants, nutrients and colorants are easily incorporated with edible but not to synthetic films to improve structural integrity and handling of foods (Sothornvit and Krochta, 2001; Chillo *et al*., 2008; Ozdemir and Floros, 2008).

Edible films can be prepared from protein, polysaccharide and/or lipid material. Though lipid and protein-based films are available, they show poor flexibility and exhibit allergic responses to

some individuals. Polysaccharides such as starch, cellulose and pectin are available in abundance and non-toxic (Ramesh *et al.*, 2004). High amylose starch and hydroxypropylated high amylose starch have been used to form self-supporting films by casting from aqueous solution (Pagella *et al.*, 2002).

Sago is a starch extracted form sago palm stems *Metroxylon* sp. which is known as 'rumbia' in Malay. The palm grows well in humid tropical lowland forest and freshwater swamps across South East Asia and Oceania. The world producers of sago starch and/ or conversion to animal food or to ethanol are Malaysia, Indonesia and Papua New Guinea. Sago is the main carbohydrate source in East Malaysia with a high yield and low production cost as compared to other starches such as cassava and maize (Singhal *et al.*, 2008).

The usage of sago starch is not limited in food industry; employed together with other starches, it was used in production of noodles, monosodium glutamate and soft drinks (Suraini, 2002). But a new use of sago starch in edible film production is a key material input for producing biodegradable plastics and edible coatings. Although sago has been consumed for decades, the safety assessment of this modified sago starch for edible film has yet been studied extensively (Sorrentino *et al.*, 2007). Subacute toxicity study has been used previously to evaluate the safety aspects of any food or edible materials (Thanabhorn *et al.*, 2006). Liver function tests such as total bilirubin, aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) and renal function tests (creatinine and blood urea nitrogen) are commonly accessed in toxicity assessment. With an extensive safety assessment done, consumer has to be assured about the safety of key material used as ingredient in the edible film. Hence, the objective of the present study is to evaluate the toxicity level of modified sago starch and determine the safe dose for oral use to be recommended.

MATERIALS AND METHODS

The sub-acute study for 28 days was performed from October 5th to November 2nd, 2009 at Malaysia Agriculture Research and Development Institute, Serdang, Selangor, Malaysia.

Preparation of edible film: Edible films were prepared through casting process with some modifications. Modified sago starch is isolated from sago palm (*metroxylon* sp.) which is better known as "rumbia" and it has been distributed throughout South East Asia (Ahmad *et al.*, 1999). It was mixed suspended in 1000 mL sodium acetate buffer, pH 5.0 (20% w/v, starch slurry). Pullulanase enzyme (20% v/w of starch) was added and the starch suspensions were incubated at and homogenized until starch gelatinized at 95°C. The gelatinized starch was then cast on Thin Layer Chromatography (TLC) plate prior to dry and peeled-off (Tharanathan, 2003; Ozdemir and Floros, 2008; Vascones *et al.*, 2009).

Preparation of rat-feed edible film powder: The edible film from modified sago starch was ground to powder and incorporated with standard commercial rat feed (powder form) as 2 g kg^{-1} body weight (low dose) and 5 g kg^{-1} body weight (high dose) of edible film.

Experimental animals: Twenty four Sprague Dawley female rats (24-28 weeks old) each weighing between 220 and 240 g, were held for about one week to acclimatize to the animal room conditions (temperature 21±2°C and light 12/12 light/dark cycle). They were maintained on a standard pellet diet with water offered *ad libitum*. The total number of animals were 24

where, 8 rats/group was randomly assigned for two treatment (low dose and high dose) and one control groups. The animals were housed in stainless steel wire-bottomed cages containing wood shavings for bedding, feed and water were supplied according to the treatment protocol and *ad libitum*. The research was conducted in accordance with the internationally accepted principles for the use and care of laboratory animals as found with the WHO guidelines (WHO, 1993).

Sub-acute toxicity: The study was conducted in accordance with the guidelines published by the Organization for Economic Cooperation and Development (OECD) No. 407, Issue Date 7/27/95 (Michael and Hollinger, 2003). Control rats were fed with commercial standard rat pellet and the two treatment groups of edible film in their feed for 28 days. Rats were then observed daily for mortality, signs of gross toxicity and behavioral changes.

Blood chemistry: After 4 weeks of the treatment, the rats were either fasted overnight or at least 15-16 h prior to blood collection. Then, they were anesthetized with ethyl ether and for each rat approximately 2 mL of blood were collected from the posterior vena cava. The blood samples were centrifuged at 4°C (3000 rpm, 5 min) to obtain the serum fraction. The liver function tests were determined by the analysis of serum AST, ALT and ALP levels using standard methods of the manufacturer using a blood chemistry analyzer (Selectra E, Vitalab-Sweden) for the clinical blood chemistry analyze and urea, uric acid and creatinine were for assayed for kidney function.

Statistical analysis: Significant differences between the control and treatment groups were determined using statistics software (SAS version 9.1,Cary, NC, USA) for Least Significant Difference (LSD) test. The data was considered significant if the probability level of $p<0.05$. All significant treatment means were then subjected to Duncan New Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Sub-acute toxicity: During the study, no fatal outcomes were observed for animals from both treated and untreated rat groups. Repeated oral administration of edible film from modified sago starch for 28 days did not induce any mortality up to 5 g kg^{-1} body weight. The modified film from sago starch did not affect rat behavior as there were no signs of behavioral changes observed. An acute toxicology study by Ramesh *et al.* (2004) on carboxymethylated starch in rat observed no ill effects and all rats survived throughout the period. This study also revealed that sago is safe with minimal reactions. Besides, incorporated chitosan with starch as food additive has also been found to be safe in mice and rats. Minami *et al.* (2004) reported that oral LD_{50} of chitosan in rats is over 1.5 g kg^{-1} body weight.

Effects of edible film on kidney function: Kidney functions are evaluated by measuring plasma creatinine, urea and uric acid concentrations. Increments in these parameters in the blood indicate impaired excretion by the kidney. The results of the present study indicate that there were no significant differences in urea and uric acid concentrations for all the groups of rats (Table 1). However, the creatinine concentrations were significant lower ($p<0.05$) in high dose group compared to low dose and control groups (Fig. 1). This may suggest good effect of sago starch in increasing excretion of creatinine in urine.

Effects of edible film on lipid profile: The concentrations of plasma cholesterol, LDL, HDL and triglycerides were not affected by the repeated administration of edible film in all rats (Table 2).

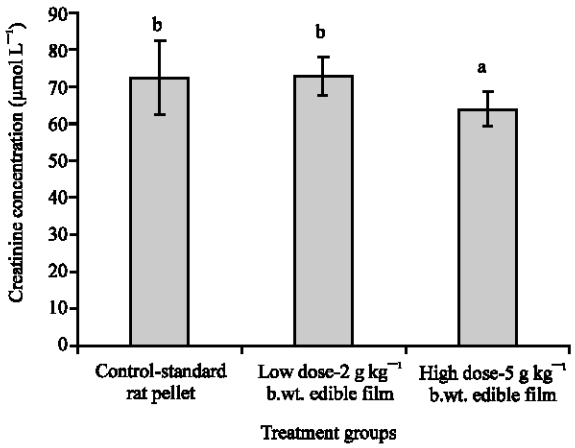

Fig. 1: Effect of edible film from modified sago starch on creatinine. Each point represents means of creatinine concentration. Bar with different letters differ significantly at $p<0.05$ according to Duncan Multiple New Range test(DMRT)

Table 1: Renal function parameters at termination of the treatments

Dose (g mL^{-1})	Creatinine (mmol L^{-1})	Urea (mmol L^{-1})	Uric aid (mmol L^{-1})
Control (Standard rat pellet)	72.33[a]	6.66[a]	0.19[a]
Low dose (2 g kg^{-1} b.wt.)	72.56[a]	5.59[a]	0.26[a]
High dose (5 g kg^{-1} b.wt.)	63.52[b]	5.41[a]	0.26[a]

Mean values with the same letter within each column are not significantly different ($p\leq0.5$) according to Duncan Multiple New Range test (DMRT). The relative standard deviation was less than 6% for all samples

Table 2: Blood lipid profiles at termination of the treatments

Dose (g mL^{-1})	HDL (mmol L^{-1})	Triglycerides (mmol L^{-1})	Cholesterol (mmol L^{-1})	LDL (mmol L^{-1})
Control (Standard rat pellet)	0.64[a]	0.80[a]	2.14[a]	1.14[a]
Low dose (2 g kg^{-1} b.wt.)	0.63[a]	0.66[a]	2.05[a]	1.21[a]
High dose (5 g kg^{-1} b.wt.)	0.58[a]	0.65[a]	1.83[a]	0.95[a]

Mean values with the same letter within each column are not significantly different ($p\leq0.05$) according to Duncan Multiple New Range test (DMRT). The relative standard deviation was less than 5% for all samples

There were no statistically significant changes in all groups. Some polysaccharides have been explored as possible ingredients in the development of "functional foods" because of their ability to reduce plasma cholesterol and consequently contribute to the reduction of the risk of cardiovascular disease (Castro *et al*., 2003).

Effects of edible film on liver function: Serum enzymes, including AST, ALT and ALP are mainly used in the evaluation of hepatic damage. The results of the present study indicate that there were no significant differences in all serum enzymes of the treated rats (Table 3). Interestingly, total protein was reduced by approximately 33% with edible film ingestion. Lower total protein may indicate liver or kidney failure but the serum enzymes of liver and kidney functions were within normal range and statistically similar to the control. However, a significantly higher ($p<0.05$) concentration of total bilirubin was found with high dose treatment compared to control group (Fig. 2). A small elevation in plasma bilirubin is an important indicator of cholestatic liver damage in laboratory animals or could be a sign of billiary duct obstruction (Woodman, 1996).

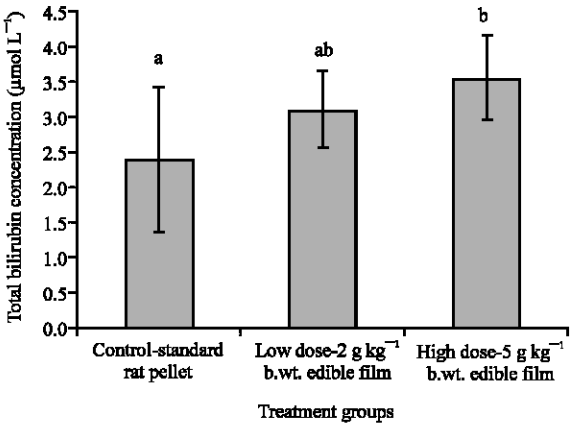

Fig. 2: Effect of edible film from modified sago starch on total bilirubin Each point represents means of total bilirubin concentration Bar with different letters differ significantly at $p<0.05$ according to Duncan Multiple New Range test (DMRT)

Table 3: Liver function parameters at termination of treatment

	Dose (g mL^{-1})		
Parameters	Control (Standard rat pellet)	Low dose (2 g kg^{-1} b.wt.)	High dose (5 g kg^{-1} b.wt.)
Total protein (g L^{-1})	80.70^{a}	53.87^{b}	59.74^{a}
Albumin (g L^{-1})	43.90^{a}	42.00^{a}	42.50^{a}
Total bilirubin (µmol L^{-1})	2.38^{a}	3.09^{ab}	3.53^{b}
Globulin (g L^{-1})	37.00^{a}	36.00^{a}	33.00^{a}
AST (U/L)	144.00^{a}	120.00^{a}	113.00^{a}
ALT (U/L)	68.00^{a}	55.00^{a}	62.00^{a}
ALP (U/L)	62.00^{a}	81.00^{a}	82.00^{a}

Mean values with the same letter within each row are not significantly different ($p \leq 0.05$) according to Duncan Multiple New Range test (DMRT). The relative standard deviation was less than 7% for all samples

CONCLUSION

In conclusion, edible film from modified sago starch consumed at the rate of 2 g kg^{-1} body weight is a less likely chance of developing toxicity as observed throughout the period of sub-acute study.

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