AVERRHOA CARAMBOLA (STAR FRUIT) INDUCES HEPATIC ALKALINE PHOSPHATASE ACTIVITY IN RATS

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Abstract

The objective of the study is to examine the *in vivo* effect of star fruit juice at different storage conditions on the activity of alkaline phosphates in female Sprague Dawley (SD) rats. A total of 20 healthy female rats weighing 180-200g were used for the experiment. All animals were divided into four groups with five animals per group (n=5). First control was served as control group. Second, third and forth groups were orally treated with a single dose daily of freshly prepared star fruit juice, juice stored for 1-hour and juice stored for 3-hour, respectively for 14 days. All animals were sacrificed at day-15 and various organs such as liver, kidney and heart were removed. All organs were homogenised and centrifuged to prepare cytosolic fraction as a source of alkaline phosphatase enzyme. Protein content of each organ was determined. *p*-nitrophenol phosphate was used as a substrate to determine the activity of alkaline phosphatase using spectrophotometer. All results were analysed using Dunnett's test. Based on the results obtained, a significant increase ($P<0.01$) of the activity of alkaline phosphatase in rat liver was observed in all star fruit juice treatment groups when compared to control rats. In conclusion, star fruit juice at different storage times was selectively induced the activity of alkaline phosphatase in rat liver but not in the heart and kidney.

Keywords: *p*-nitrophenol phosphate, liver cytoslic fraction, star fruit juice.

Introduction

The star fruit, *Averrhoa carambola*, is from the Oxalidaceae family.[1] Star fruit is also known as belimbing batu or belimbing besi (Malaysia), yang tao (China), and five fingers or five-corner fruit (Europe). The young fruit appears green and turns golden-yellow when riped. A transverse cut across the fruit shows a five pointed star shape, hence its common name. The *carambola* fruit is crunchy, and has a slightly tart, acidic, sweet taste, reminiscent of pears, apples and sometimes grapes. Star fruit has been reported to have high antioxidant that effectively scavenged free radicals.[2] When the star fruit is served as a fruit juice, the residue of the star fruit is normally discarded as waste or used as a low-value by-product. The residue of the star fruit was reported to contain higher antioxidant activity than the extracted juice.[3] Star fruit also acts as a

hypoglycemic and hypocholesteremia agent.[4,5] These effects of star fruit attracted many people to consume it as it can help to improve their health.

Recently there was a report in the local media of a patient with kidney problem who went into a coma after consuming the star fruit.[6] This attracts our interest to investigate the *in vivo* effect of star fruit since star fruit is considered as one of the most popular edible fruits in Malaysia. In the recent study, we had reported that star fruit juice could inhibit the activity of acetylcholinesterase in rat liver.[7] Other researchers reported the interaction of star fruit with medazolam by inhibiting the CYP 3A4 activity. Thus, the present study aims to evaluate the effect of 14 days oral administration of star fruit juice at different storage times on the activity of alkaline phosphatase (ALP) in various organs of female Sprague Dawley rats. ALP is the enzyme that catalyses the release of phosphate ions from organic phosphates.

Materials and Methods

Chemicals : *p*-nitrophenol phosphate, *p*-nitrophenol, were supplied by Sigma Aldrich.

Animal Selection: Female Sprague Dawley (SD) rats bred in the Animal Holding Room, UCSI University were used throughout the experiment. A total of 20 healthy female rats weighing between 180-220g were selected for the experiment. All rats were randomly assigned into four groups with five rats per group (n=5). All rats were kept *ad libitium*.

Star fruit juice preparation: Semi ripe star fruits weighing 120-150g were used. Star fruits were kept in the fridge to maintain its freshness. Approximately 100g of semi ripe star fruit was used to extract the juice before the experiment. Three preparations of star fruit juice were prepared, i.e. freshly prepared juice, juice stored at 1-hour and juice stored 3-hour.

Experimental Design: All animal works were conducted according to the animal ethic codes. First group was served as control rats, received distilled water only. 2nd, 3rd and 4th groups were orally treated up to 14 days with (i) freshly prepared star fruit juice, (ii) juice after 1-hour storage at room temperature and (iii) juice after3-hour storage at room temperature, respectively. 10ml/kg of the respective star fruit juice was fed to all treatment animals based on the body weight. The organs such as liver, kidney and heart were removed at day-15. All organs were homogenised (in 1 g tissue to 4ml of phosphate buffer solution) and the cytosolic fraction of each organ was prepared by differential centrifugation at 10,000 X rpm for 22 minutes at 4^{o}C. The protein content of each organ was determined according to Lowry method, with serum bovine albumin as standard protein.[8]

Alkaline Phosphatase (ALP) Assay:

The activity of ALP was determined using substrate known as *p*-nitrophenol phosphate (*p*-NPP). The formation f yellowish product, *p*-nitrophenol was determined based on the absorbance at 415nm using spectrophotometer with slight modification from the method described by Martins and co-workers (2001).[9] The amount of product was calculated based on the standard calibration curve of *p*-nitrophenol.

Statistic Analysis: All data were reported as mean ± standard deviation (SD). All results were analysed using Dunnett's test. $P<0.01$ and $P<0.05$ was considered as

significantly difference as compared to the control group.

Results

No any toxic sign and abnormal behavioral was observed in all treatment rats during the experimental duration. Repeated oral administration of star fruit juice at different storage times was significantly induced ($P<0.01$) the activity of ALP in the female rat liver (Figure 1). However, no significant change of ALP activity was observed in the kidney and heart when compared to the control group. The activity of ALP in liver was found to be the highest, followed by heart and kidney.

Discussion

Alkaline phosphatase (ALP, E.C. 3.1.3.1) is the enzyme that catalyses the hydrolysis of phosphate monoesters to produce an inorganic phosphate and alcohol.[10] ALP plays an important role in mineralization. Human ALP isoforms have been categorised electrophoretically into four groups, i.e. hepatic, intestinal, bone and placenta.[11] Studies on the mechanism of ALP revealed that ALP was responsible for bone growth and homeostasis in rats and humans.[12] The level of serum ALP could be used as a clinical parameter for diagnosing the liver functions. The elevation of serum ALP is generally related to the various bone disorders and obstructive liver diseases. As reported by Koyama *et al* (1987), the elevated activity of liver isoform in blood serum was observed during cholestasis in rats.[13] The blockage of bile ducts will block the release of ALP into hepatic system which later will be released into circulation system.

From the data obtained in the present study, star fruit juice was selectively targeted on the hepatic ALP but not from heart and kidney. Only the activity of ALP in rat livers was induced when compared to other organs. However, the data obtained from the figure 1 indicated that the storage duration was not the main factor affecting the activity of ALP in the rat liver as no significant dose-dependent differences were observed between star fruit juice at different storage conditions. Liver is the main organ exposed to the dietary foods and exogenous compounds especially ingested through oral route. However, compounds that responsible for the *in vivo* induction of ALP activity in rat livers remain unknown and need to be further investigated. One of the compounds in star fruit which is known as oxalate has been previously reported to cause toxicity to the renal epithelium cells. As reported by Taiwanese researchers, the concentration of methanol contained in the star fruit juice was significantly increased after three hours of storage.[14] High level of methanol is able to cause liver injury due to the release of free radical from formaldehyde. Previous study demonstrated that star fruit juice was able to modulate the rat liver functions. A significant of 18 % elevation of serum alanine aminotransferase (ALT) was observed in those animals treated with star fruit juice stored three hours after preparation. However, the serum ALP remains in the normal range.[15]

Some studies have demonstrated the stimulation of ALP in rat liver was mediated by cyclic AMP acts as inducer. The increment of hepatic ALP in rats was reported to be mediated by the induction of adenyl cyclase cyclic AMP with the rise of cyclic AMP as evident in the rats after injecting with cholera enterotoxin.[16] Another study demonstrated that the

induction of phosphotidylcholine excretion in bile was associated with the elevation of hepatic ALP due to the functions of ALP to hydrolyse phosphitidylcholine to choline to pass across the bile canalicular membrane.[17] Some drugs including simvastatin, propanolol and atenolol tended to activity of heart ALP in rats. On the other hand, aspirin, theophylline and several polyphenol enriched beverages such as red wine, green and black teas have shown significant *in vitro* inhibition on the activity of ALP in rat heart homogenate.[18]

Conclusion

Star fruit juice was selectively induced the activity of ALP in rat liver but not in the kidney and heart. The mechanism of action of star fruit juice and the active ingredient that responsible for the induction of hepatic ALP enzyme need to the elucidated.

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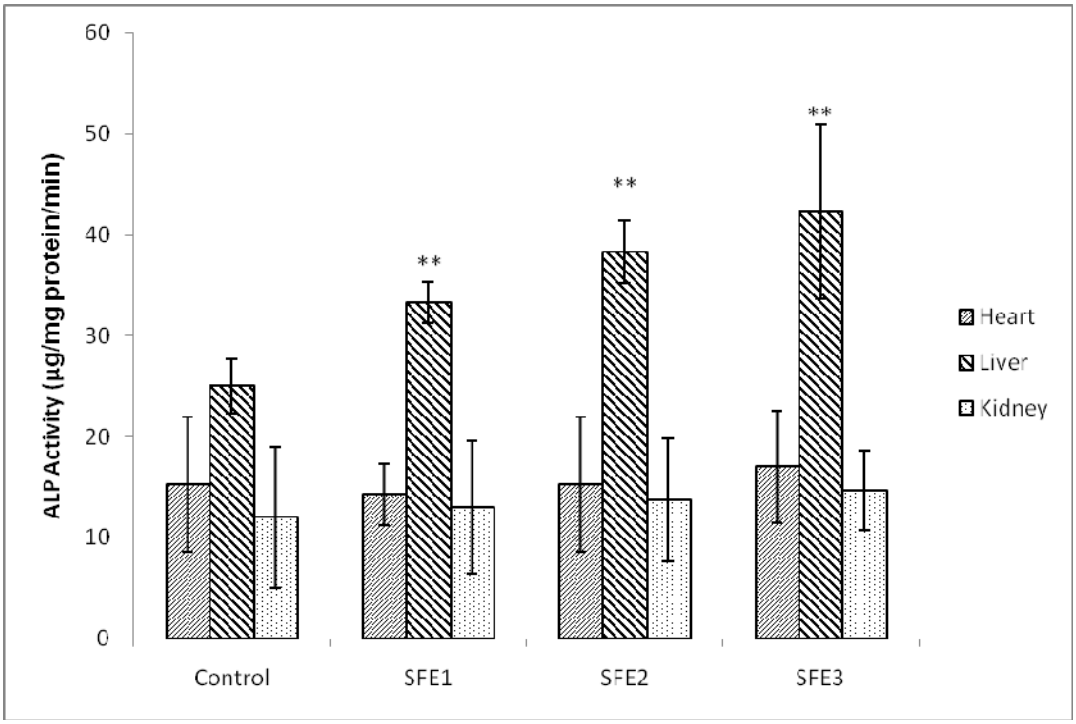
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Figure 1: Effect of *Averrhoa carambola* (star fruit) on the activity of alkaline phosphatase (ALP) in female rats.



n=5; Results are expressed as mean ± S.D; Analysed using Dunnett's test; **P<0.01 as compared to control.
Control = treated with distilled water.
SFE 1 = treated with freshly prepared star fruit juice.
SFE 2 = treated with star fruit juice after 1 hour storage.
SFE 3 = treated with star fruit juice after 3 hours storage.