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Acetylcholinesterase inhibitory activity of star fruit and its effect on serum lipid profiles in rats

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Abstract: **:** The objective of this study was to examine the effects of different storage conditions of star fruit (*Averrhoa carambola)* juice on the activity of acetylcholinesterase in various organs of Sprague Dawley (SD) rats. The effect of oral administration of star fruit on serum lipid profiles was also examined in this study. A total of 15 female rats were assigned into three groups with five animals per group (n=5). The first group served as control group and given only distilled water (vehicle) while the other two groups were given different star fruit preparations, i.e. freshly prepared star fruit juice and after 3 hours storage, respectively. From the results obtained, a significant decrease in the hepatic acetylcholinesterase activity was observed in rats treated with star fruit juice. In conclusion, the star fruit juice at different storage conditions is selectively targeted on the acetylcholinesterase activity in rat liver but not in kidney and heart.

Keywords: acetylcholinesterase, *Averrhoa carambola*, lipid profile, liver, rats

Introduction

The star fruit, *Averrhoa carambola,* is from the Oxalidaceae family. Star fruit is also known as *belimbing batu* or *belimbing besi* (Malaysia), *yang tao* (China), and *five fingers* or *five-corner fruit* (Europe). There are 2 varieties of star fruit in Malaysia, one of which is acidic and the other is sweet (Carolino *et al*., 2005). The tart variety can be identified by the narrowly spaced ribs while the sweet variety usually has thick fleshy ribs. This plant probably originated from Sri Lanka and the Moluccas and has been introduced to many other tropical countries, including Malaysia, for over several hundred years. Star fruits are served as juice drinks or eaten directly and are a good source of vitamin C, which help to ward off winter colds and flus. They are also very good for health as they are rich in antioxidants and flavonoids. In addition to its low calories, it also contains sufficient amount of nutrients such as vitamin A, vitamin C, potassium, calcium, fibre, iron, thiamine, riboflavin, niacin and tryptophan (Keith, 2007). Star fruit has been used in traditional medicine to treat headache, vomiting, coughing and restlessness (Carolino *et al*., 2005).

Recently, it has been reported in the media that a patient with kidney problem went into a coma after consuming star fruits (*Palaniswamy et al*., 2004). This led to the researcher's suspicion that the star fruit may contain a neurotoxin which may affect patients with renal failure. The convulsant activity and neurochemical alterations induced by a fraction obtained from *Averrhoa carambola* fruit have been previously studied and the neurotoxic fraction (AcTx) had been identified and its effect on GABAergic and glutamatergic transmission systems studied (Carolino *et al*., 2005). Acetylcholinesterase (AChE; E.C 3.1.1.7) is chosen to investigate the effect of star fruit on nervous system. Acetylcholinesterase is always the target of many Alzheimer dementia drugs and insecticides. Ellman's reaction is commonly used to screen the activity of AChE. AChE is synthesised mainly in hepatocytes which will then released the enzyme into the blood which distributes it to its target sites. AChE terminates the action of acetylcholine post-synaptically (Garcia-Ayllon *et al*., 2006). The inhibition or induction of AChE activity will cause changes in acetylcholine concentration in the body which can lead to the adverse effect. Inhibition of the AChE will cause high concentration of acetylcholine to accumulate in the body whereas induction of the acetylcholine will cause more hydrolysis of the acetylcholine into acetate and choline which reduce the concentration of the acetylcholine in the body. The toxicity of acetylcholine are manifested by muscarinic and nicotinic signs and symptoms such as marked miosis, ocular pain, hypotension, tightness of chest, bradycardia, confusion, ataxia, slurred speech, loss of reflexes, generalized convulsions, coma and central respiratory paralysis (Lassiter *et al*.,

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2003). However, as the acetylcholine concentration decreased or the acetylcholine receptor blocked, it may cause prolonged apnea, cardiovascular collapse, presence of latent myasthenia gravis, malignant hyperthermia and respiratory paralysis (Gian *et al*., 1997).

The objectives of this study were to examine the effect of oral administration of star fruit at different storage conditions on the acetylcholinesterase activity and serum lipid profiles in rats. The activity of acetylcholinesterase in liver, heart and kidney was examined using acetylcholine iodide as probe substrate. Serum lipid profiles i.e. total cholesterol, high density lipoproteins and triacylglycerol were also determined.

Materials and Methods

Chemical

Disodium hydrogen phosphate (Na_2HPO_4), sodium dihydrogen phosphate (NaH_2PO_4), Potassium hydroxide (KOH), Sodium carbonate (Na_2CO_3), Copper sulphate ($CuSO_4$), Sodium potassium tartrate, Folin reagent, Acetylcholine iodide, 5,5-dithio-bis-2-nitrobenzoic acid (DTNB) were supplied by Merck, Darmstadt, Germany. All chemicals used in the present study were at analytical grade.

Preparation of star fruit juice

Semi ripe star fruit purchased from a local hypermarket were used in the present experiment. The star fruits were kept in the refrigerator at 5^{o}C to retain its freshness. Approximately 100g of the star fruit was weighted every morning and then cut into smaller pieces to enhance the efficiency of extracting the juice. The star fruit juice was extracted by using the fruit blender and filtered to obtain the clear star fruit juice. About 40-50ml of the star fruit juice was extracted from100g of the star fruit. After extraction, the star fruit juice was kept in the beaker and covered with parafilm under room temperature (25^{o}C) to prevent it from oxidising. The star fruit juice was used to feed the animals as freshly prepared juice or after storage for 3 hours stored respectively, to the different groups of treated animals.

Animal grouping

A total of 15 healthy young Sprague Dawley (SD) rats (7±1 weeks old and 180±20g body weight) have been obtained from the Animal House Unit of UCSI University. All rats were randomly assigned into three groups with five animals per group (n=5). First group was served as control group, received only distilled water. Second and third groups were orally received freshly prepared fruit juice and juice after 3 hours storage respectively. The animals were force-fed with the different juice types, according to their body weights, for 14 days. After every treatment, the cage-side observation was conducted for the first 4 hours to detect any toxic signs or abnormalities. The animals were handled and observed based on the approved design of the experiments. Food consumption, water intake and animal body weight was recorded weekly

Blood sampling

After overnight fasting, all animals were subjected to cardiac puncture to collect blood for serum lipid profiles examination. All blood serum samples were sent for analysis within the day of blood sampling. Three lipid parameters, i.e. total cholesterol, high density lipoprotein and triacylglycerol were determined.

Preparation of liver cytosolic fraction

The rat was anaesthetised with diethyl ether for about 5 minutes after which it was dissected and several organs such as the liver, kidney, spleen and heart from each experimental group were removed. Each organ was washed with distilled water and weighed. Organ homogenate (1 g organ: 4 ml of 0.25 M phosphate buffer) was prepared by using a homogeniser. Differential centrifugation technique was used to prepare the subcellular organ cytosolic fractions. Protein concentration of all organ cytosolic fractions was determined by the method of Lowry (1951). The standard curve was constructed by plotting the value of absorbance versus concentration of BSA, µg/ml.

Determination of Acetylcholinesterase activity

The determination of acetylcholinesterase activity was conducted according to the method described by Ellman *et al* (1961). 100 µg/ml of organ sample was used as AChE enzyme source in the presence of 0.5 M Acetylcholine iodide as substrate. Absorbance was recorded at 412 nm using spectrophotometer.

Data analysis

All data obtained were expressed as mean ± standard deviation and analysed using Dunnett test. $P<0.05$ and $P<0.01$ were considered significant or very significant, respectively, as compared to the control group.

Results

The 14 days cage-side observations showed that oral administration of star fruit juice did not

produce significant changes in behaviour, breathing or nervous responses of the animals. The different storage time of the star fruit juice did not show any toxic signs to the animals for the 14 days treatment. No significant change was detected in the mean body weight, food consumption and water intake as well as compared to the control group (Table 1). Fourteen days administration of freshly prepared star fruit juice or after 3 hours storage to SD rats had no significant effects on the organ weights such as heart, liver, kidney, lung and spleen (Table 2).

No significant change was detected in the the activity of AChE in the heart and kidney of the treatment groups when compared with the control group. However, the activity of AChE in liver was found to be significantly lower from the control group (Table 3). No significant difference in the serum lipid profiles was observed between treatment groups and control group (Table 4).

Discussion

The use of serum cholinesterase activity as a specific indicator of liver dysfunction have been suggested rather than the conventional liver function parameters such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Any liver dysfunction or damage will decrease the activity of serum cholinesterase due to reduced synthesis (Orgunkeye and Roluga, 2006). In addition, liver damage often leads to fat accumulation in hepatocytes (Lombardi, 1966). Changes in plasma lipids only become more obvious when a dysfunction is developed to a serious extent. The level of hepatic enzymes such as triacylglycerol lipase will be reduced in acute hepatic injury and thus will elevate plasma triacylglycerol with a concomitant decrease in cholesterol. Serum lipid measurements may provide information on the potential development of fatty liver (Alpers *et al*., 1993). Serum lipid profiles are not affected by star fruit ingestion. Insignificant change in the relative organ weights and no lethality was observed during treatment duration. There was no general toxic sign shown by rats treated with star fruit juice during the experiment. However, further examination on the liver or kidney functions using serum biochemical analysis and histopathology needs to be carried out to confirm the safe use of star fruit juice in rats.

Different storage times of star fruit juice were chosen to examine their effect on the activity of acetylcholinesterase in various organs of rats. Based on one research reported by Taiwanese researchers, the methanol content in star fruit juice was significantly increased as storage time increases (Shui and Leong, 2005). The methanol content in the star fruit juice after 3 hours storage at 30^{o}C was increased to approximately 164.17% (14.2 mg/100ml) when compared to the freshly prepared juice. In general, excessive ingestion of methanol could produce toxic effect to human by hepatic metabolism into formaldehydes, formic acid (toxic), and carbon dioxide through sequential oxidative steps (*Palaniswamy et al*., 2004). Accumulation of formic acid and formaldehyde in liver causes intoxication. The oxalate in star fruit has also been reported to cause renal failure in experimental animals. The details of the oral toxicity and its action on nervous system either in humans or animals are still limited and remain to be elucidated.

Based on the results obtained, oral administration of star fruit juice was found to affect the AChE activity in the rat liver only and the inhibition was greater in star fruit after storage for 3 hours than the freshly prepared fruit juice. Liver is always the main organ involved in the metabolism of exogenous compounds ingested through oral. Star fruit has been previously reported to inhibit the hepatic CYP3A activity in rats as well as in humans (Hidaka *et al*., 2004). The concentrations of the star fruit juice that enter into heart and kidney to give their action could be lower when compared to livers. The dose taken by the animals in this experiment is 10ml/kg based on the OECD guideline (OECD, 2001). In the present study, 100g of the star fruit could produce approximately 40ml of the star fruit juice. The average dose of star fruit juice for the rats is 2ml which is equivalent to 5 g of star fruit per day. Previously, cholinesterase inhibitors are the only approved drugs used to treat Alzheimer Disease (AD) patients. The report has shown a decline in ACh with age, which is reflected by the ACh level in the brain (Kaur *et al*., 2008). The study indicated that the body and brain need an optimal balance between cholinergic neurotransmission and cognitive performance (Papandreou *et al*., 2009). The AChE inhibitors (AChEI) have been used to inhibit the AChE activity thus increasing the acetylcholine level in the AD-affected brain and thereby enhancing cholinergic function (Lopez *et al*., 2002). A variety of synthetic medicines such as tacrine and donepezil used for treatment of cognitive dysfunction and memory loss associated with AD have been reported to have side effects including gastrointestinal disturbances (Oh *et al*., 2004; Schulz, 2003). Many natural resources such as *Bacopa monniera* and *Gingko biloba* have been proven to have significant AChE inhibitory activity (Das *et al*., 2002). The major classes of compound reported to AChE inhibitory activity are

Table 1. The effect of the star fruit juice on the body weight gained, food consumption and water intake in healthy female SD rats

	0 day	3rd day	7th day	14th day
Group		**Body weight (g)**		
Control	188.0±13.36	194.0±13.88	200.9±16.10	211.6±17.32
SFE 1	185.7±13.86	198.1±18.24	201.4±20.12	210.1±18.41
SFE 2	183.0±20.11	191.4±19.61	198.1±19.03	210.2±16.46
		Food consumption (g/rat/day)		
Control	12.4±2.83	12.9±2.14	13.6±1.18	13.1±3.10
SFE 1	13.2±2.16	13.2±0.47	13.8±0.08	12.4±0.70
SFE 2	14.2±0.52	14.7±1.88	14.0±1.82	14.6±0.04
		Water intake (ml/rat/day)		
Control	19.60±4.12	28.8±5.30	32.5±3.54	30.0±7.07
SFE 1	18.3±4.72	29.2±5.89	30.8±1.18	27.1±6.48
SFE 2	22.5±3.54	32.1±5.07	28.5±2.12	31.7±9.43

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

Table 2. The effect of star fruit juice on the relative organ weight in healthy female SD rats

	Relative organ weight (g/100g body weight)				
Group	**Heart**	**Liver**	**Kidney**	**Lung**	**Spleen**
Control	0.376±0.0243	2.690±0.255	0.640±0.0828	0.671±0.148	0.186±0.0233
SFE 1	0.378±0.0357	2.614±0.259	0.642±0.0354	0.763±0.165	0.206±0.0434
SFE 2	0.354±0.0400	2.761±0.262	0.626±0.0629	0.731±0.138	0.216±0.0242

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

Table 3. The Effect of Star Fruit on acetylcholinesterase (AChE) activity in rats

	AChE Activity (µmol/mg/min)		
Group	**Heart**	**Liver**	**Kidney**
Control	9.5±1.92	13.1±0.58	8.1±1.53
SFE 1	8.9±1.08	9.2±0.75*	7.5±0.54
SFE 2	6.7±1.28	6.1±1.39**	7.9±1.83

n=5; Results are expressed as mean ± S.D.
Analysed using Dunnett's test; *P<0.05 and ** P<0.01 significant 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 3 hours storage.

Table 4. The Effect of Star Fruit on serum lipid profiles in rats

	Serum Lipids (mg/dL)		
Group	**Total Cholesterol**	**High density lipoprotein**	**Triacylglycerol**
Control	55.7±6.8	29.3±3.5	80.6±13.2
SFE 1	40.0±6.9	21.7±0.6	68.0±2.6
SFE 2	53.5±5.0	32.0±8.9	88.0±15.6

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

the alkaloids, terpenoids, glycosides and coumarins (Mukherjee *et al*., 2007). Therefore, the identification and isolation of active ingredients in star fruit juice that responsible for the inhibition of AChE is worthy to be carried out to find better AChE inhibitors from natural resources such as fruits.

Conclusion

For conclusion, oral administration of freshly prepared star fruit juice and juice stored after 3 hours selectively inhibited the AChE activity in the rat liver but not from kidney and heart. Star fruit juice had no significant effect on the serum in rats. Further study needs to be conducted to elucidate the mechanism of action of star fruit on the hepatic AchE activity in rats.

References

Alpers, D.H., Sabesin, S.M. and White, H.M. 1993. Fatty acid: biochemical and clinical aspects. In: Schiff, E.R. (Ed). Diseases of the Liver, p. 825-871. Philadelphia: JB Lippincott Company.

Carolino, R.O.G., Beleboni, R.O., Pizzo, A.B., Vecchio, F.D., Garcia-Cairasco, N. and Moyses-Neto, M. 2005. Convulsant activity and neurochemical alterations induced by a fraction obtained from fruit Averrhoa carambola (Oxalidaceae: Geraniales). Neurochemistry International 46: 523-531.

Das, A., Shanker, G., Nath, C., Pal, R., Singh, S. and Singh, H.K. 2002. A comparative study in rodents of standardized extract of *Bacopa monniera* and *Gingko biloba* anticholinesterase and cognitive enhancing activities. Pharmacology Biochemical Behavioral 73: 893-900.

Ellman, G.L., Gourtney, D., Andres, V. and Featherstone, R.M 1961. A new rapid colorimetric determination of acetylcholinesterase activity.
Biochemical Pharmacology 7:88-95.

Garcia-Ayllon, M.S., Silveyra, M.X., Candela, A., Compan, A., Claria, J. and Jover, R. 2006. Changes in Liver and Plasma Acetylcholinesterase in Rats With Cirrhosis Induced by Bile Duct Ligation. Hepatology 43(3): 444-453.

Gian, S., Javier, S., Konrad, B., Colin, L.M. and David, H.S. 1997. The Amyloid [beta]-Protein of Alzheimer's Disease Increases Acetylcholinesterase Expression by Increasing Intracellular Calcium in Embryonal Carcinoma P19 Cells. Journal of Neurochemistry 69(3): 1177-1184.

Guidance document on acute oral toxicity testing. Paris: Organisation for Economic Co-operation and Development (OECD); 2001 June. p.12.

Hidaka, M., Fujita, K., Ogikubo, T., Yamasaki, K., Iwakiri, T. and Okumura, M. 2004. Potent inhibition by star fruit of human cytochrome P450 3A (CYP3A) activity. Drug Metabolism Disposition 32:581-583.

Kaur, T., Pathak, C.M., Pandhi, P. and Khanduja, K.L. 2008. Effects of green tea extract on learning, memory,behavior and acetylcholinesterase activity in young and old male rats. Brain Cognition 67: 25-30.

Keith, M.A. Hillsborough County: Star Fruit Season Starts Now 2007.

Lassiter, T.L., Marshall, R.S., Jackson, L.C., Hunter, D.L., Vu, J.T. and Padilla, S. 2003. Automated measurement of acetylcholinesterase activity in rat peripheral tissues. Toxicology 186: 241-253.

Lombardi, B. 1966. Considerations on the pathogenesis of fatty liver. Laboratory Investigation, 15: 1-20.

Lopez, S., Bastida, J., Viladomat, F. and Codina, C. 2002. Acetylcholinesterase inhibitory activity of some Amaryllidaceae alkaloids and *Narcissus* extracts. Life Science 71: 2521-2529.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. 1951. Protein measurement with the folin phenol reagent. Biology Chemistry 193: 265-275.

Mukherjee, P.K., Kumar, V., Mal, M. and Houghton, P.J. 2007. Acetylcholinesterase inhibitions from Plants. Phytomedicine 14: 289-300.

Orgunkeye, O.O. and Roluga, A.I. 2006. Serum cholinesterase activity helps to distinguish between liver disease and non-liver disease aberration in liver functions tests. Pathophysiology 13: 91-93.

Oh, M.H., Houghton, P.J., Whang, W.K. and Cho, J.H. 2004. Screening of Korean herbal medicines used to improve cognitive function for anti-cholinesterase activity. Phytomedicine 11:544-548.

Palaniswamy, U.R., Bible, B.B. and McAvoy, R.J. 2004. Oxalic acid concentrations in Purslane (Portulaca oleraceae L.) is altered by the stage of harvest and the nitrate to ammonium ratios in hydroponics. Science Horticulture 102 (2): 267-275.

Shui, G. and Leong, L.P. 2005. Residue from star fruit as valuable source for functional food ingredients and antioxidant nutraceuticals. Food Chemistry 23: 277-284.

Papandreou, M.A., Dimakopoulou, A., Linardaki, I., Cordopatis, P., Klimis-Zacas, D. and Margarity, M. 2009. Effects of a polyphenol-rich wild blueberry extract on cognitive performance of mice, brain oxidant markers and acetylcholinesterase activity. Behavioral Brain Research 198: 352-358.

Schulz, V. 2003. Gingko extract or cholinesterase inhibitors in patients with dementia: what clinical trial and guidelines fail to consider. Phytomedicine 10:74-79.