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Effect of Tomato and Guava juices on Oxidative Stress in Rats after Strenuous Exercise

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Abstract

Oxidative stress is thought to play an important role in the pathogenesis of numerous degenerative and chronic diseases. Some antioxidants in tomato and guava juices were found to have a powerful antioxidant effect. Sixty adult male albino rats were used to compare the effect of tomato and guava juices supplementation on oxidative stress. Xanthine oxidase (XO), Glutathion reductase (GR), Vitamin C, Malondialdehyde (MDA), Myeloperoxidase (MPO) and some parameter of liver and kidney functions were measured in groups of rats subjected to strenuous exercises fed on basal diet supplemented with different doses of tomato or guava juices compared with non supplemented rats as a control group. This study found that guava juice is more effective than tomato juice in impairment of oxidative damage caused by strenuous exercise leading to significant decrease in plasma and muscle XO, MDA as well as muscle MPO after exercise comparing with tomato juice. The difference in plasma vitamin C level between the group that given guava juice was significantly higher compared with group that given tomato juice (P<0.05).

Key Words : Tomato juice, guava juice, oxidative stress, antioxidant**.**

1. Introduction

Oxygen is an element obligatory for life, living systems have evolved to survive in the presence of molecular oxygen and for most biological systems. Oxygen has double-edged properties, being essential for life; it can also aggravate the damage within the cell by oxidative events (Shinde *et al.,* 2006). Physical exercise is characterized by an increase in oxygen consumption particularly by muscles. The increase in oxygen uptake is associated with a rise in the production of reactive oxygen species which lead to an increase in lipid peroxidation and impairment of antioxidant defense systems of target tissues and blood (Clarkson and Thompson, 2000). Oxidative stress is thought to play an important role in the pathogenesis of numerous degenerative and chronic diseases. Oxidative stress is characterized by an imbalance between antioxidant capacity and reactive oxygen species (ROS) generation. Over- production of (ROS) increased during aging and contributed to many pathological events such as cancer and cardiovascular disease (Moroni *et al.*, 2004).

Xanthine oxidase is an important source of oxygen free radicals and can catalyses the reduction of oxygen, leading to formation of superoxide (O_2) and H_2O_2 which is proposed as a central mechanism of oxidative injury in some tissues (Mc Cord, 1985). Myeloperoxidase is a marker for neutrophil infiltration which is associated with strenuous exercise-induced tissue damage. A part from its host defense, involvement of (MPO) has been described in numerous non infectious diseases such as atherosclerosis, lung cancer, Alzheimer and multiple sclerosis (Tidball, 2005). The reaction of polyunsaturated fatty acids with activated oxygen species result in primary lipid peroxidation production (lipid hydroperoxide). Lipid peroxidation production degraded to secondary lipid peroxidation product like malondialdehyde (MPO) which is used as marker of lipid peroxidation. (MPO) can react with DNA leading to DNA aberration with altered gene product and peptide bonds are broken through the impact of (MPO) in proteins (Sarkar *et al*., 1997).

Lycopene, a carotenoid found in tomato juice which is a powerful antioxidant with a single oxygen quenching capacity 100, times greater than vitamin E and has been hypothesized to be responsible for health benefits of tomatoes (Dimasicio *et al.,* 1989).

Recent epidemiologic studies focusing on tomato and tomato products associated their intake with a reduced risk of degenerative diseases (Giovannacci, 2002). Guava (*Psidium guajava*) is an important tropical fruit and widely

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cultivated in Egypt, mostly consumed fresh. The fruit contains saponin combined with oleanolic acid and flavonoids, guaijavarin (Arima and Danno, 2002). Guava fruit is considered a highly nutritious fruit because it contains a high level of ascorbic acid (50–300 mg/100 g fresh weight), which is three to six times higher than oranges. Guava contain both carotenoids and polyphenols the major classes of antioxidant and pigments giving them relatively high potential antioxidant value among plant foods (Jordan *et al*., 2003). Phenolic compounds found in guava juice were gallic acid, catechin, vanillic acid, trans-cinnamic acid and ferulic acid (Zabidah *et al.*, 2011). Guava contains antioxidant (quercetin) which block enzymes that are responsible for building of sorbitol and quercetin also combats free radical produced during metabolism and aids in preventing age related chronic diseases such as al-zheimers, cataract, heart disease and rheumatoid arthritis (Thaipong *et al.*, 2006).

Accordingly, the purpose of the present study was to compare the effect of tomato and guava juices supplementation on oxidative stress, lipid peroxidation and anti –oxidant substance in plasma and tissues of rats after strenuous exercises.

The present work is the first study aimed to compare the effect of tomato and guava juices on oxidative stress by strenuous exercise, as protective for oxidative damage.

2. Subject and Methods

2.1. Preparation of Tomato and Guava

Tomato (*Lycopersicon esculentum)* and Guava (*Psidium guajava*) were Egyptian species and collected from local market. After sorting and grading operation only red tomato and yellow guava were chosen. They were then sliced with a slicer. The seeded portion of tomato and guava was removed and the fresh flesh was collected. The flesh was cut into small pieces and blended in electric blender to get fine juice. Then the juices were diluted by 25% of distilled water.

2.2. Animals

Sixty adult male albino rats of local species weighing 230-250 grams were used in the present study. All rats were given normal diet and water *ad libitum* and housed in room maintained at 25±5 ^{o}C and a 12 h light-dark cycle. Tomato (*Lycopersicon esculentum)* and Guava (*Psidium guajava*) added to rat's basal diet with different doses were obtained from Egyptian species.

Rats were divided into four equal groups:

Group I: (10 rats) sedentary control group (control negative).

Group II: (10 rats) subjected to strenuous exercises (control strenuous exercises).

Group III: (20 rats) subjected to strenuous exercises plus low doses (25 g/day) of tomato juice (10 rats) or guava juice (10 rats) for 30 days.

Group IV: (20 rats) subjected to strenuous exercises plus high doses (75 g/day) of tomato juice (10 rats) or guava juice (10 rats) for 30 days.

2.3. Exercises Protocol

On the morning day of samples collection, the exercise groups were introduced to motorized treadmill running device .According to Brook and White 1978, rats warmed up for 15 minute running at speed of 15 m/min, then rats progressed to running for 15 minute at 20 m/Min., and 30 minute at 25 m/min., finally, rats ran to exhaustion at a final speed 30m/Min. The point of exhaustion was determined as when the rats were unable to right it when placed on its back.

2.4. Samples Preparation and Measurement

The rats in the sedentary group were anaesthetized with fluothane and sacrificed after 12 h fasting. Then, the rats in the exercise groups were sacrificed immediately after strenuous exercise. Blood samples were collected from abdominal aorta, then heparinized and centrifuged at 3000 rpm for 15 minute. Plasma was used for determination of plasma parameters but for erythrocyte content (GR), erythrocyte washed four times with ice-cold saline for lysis of RBCs, centrifuged at 10000 xg for 10 minutes. Then, supernatant was collected (erythrocyte lysate) and stored with plasma at – 70^{o}C till the assay time. For vitamin C, Blood samples were drawn into vacutainers without anticoagulants, stored for 30 min before centrifugation and acidification, then centrifuged at 2000 *xg* for 10 min for generation of serum ,all samples were treated immediately with an equal volume of 10% metaphosphoric acid (MPA), centrifuged at 3000 x*g* for 15 min and the supernatant frozen at – 70^{o}C till analysis of vitamin C.

*Skeletal muscle tissues***:** The muscle tissues were perfused prior to dissection with a PBs (phosphate buffer saline) solution, pH 7.4 to remove any blood cells and clots, 200mg of different muscle tissues were homogenized in 5-10 ml of cold buffer (i.e 50 mM potassium phosphates, pH 7.5, 1 mM EDTA). Then the mixture was centrifuged at 10000 xg for 10 minutes, the supernatant fluid was removed and stored at – 70 ^{o}C till the assay time.

Plasma alanine aminotransferase (ALT), aspartate aminotransferase (AST), Lactate dehydrogenase (LDH), creatin kinase (CK), and uric acid (UA) were measured using a kit from BioMerieux (France). The concentrations were determined spectrophotometrically (Hitachi, Japan).

Xanthine oxidase (XO): Skeletal muscle and plasma (XO) were determined by ELISA kit (Cayman's xanthine oxidase, USA), The assay is multi step enzymatic reaction in which xanthine first produce H_2O_2 during oxidation of hypoxanthine, in the presence of horseradish peroxidase , H_2O_2 react with ADHP (10-acetyl-3-7-Dihydroxyphenoxazine) to produce highly fluorescent compound which can be measured at wave length from 520-550 nm.

Glutathion reductase (GR): Erythrocyte and skeletal muscle tissues glutathione reductase were measured by ELISA kit (Cayman's Glutathion reductase assay kits, USA), which measure the rate of NADPH oxidation.

$$GSSG + NADPH + H^{+} \rightarrow 2\ GSH + NADP^{+}$$

The oxidation of NADPH to $NADP^{+}$ is accompanied by a decrease in the absorbance at 340 nm and is directly proportional to (GR) activity in erythrocytes and tissues.

*Vitamin C***:** Plasma vitamin C concentrations estimated with a fluorometric assay by Bio-Assay system, USA kit.

*Malondialdehyde (MDA)***:** MDA in plasma and tissue was measured by HPLC with fluorescence detection using commercial kit (Immunodiagnostic kit, Germany).

Myeloperoxidase (MPO): Myeloperoxidase in muscle tissue was measured by ELISA using commercial kit (Immunodiagnostic kit, Germany). The assay utilizes the two-site sandwich technique with two selected polyclonal antibodies that bind to MPO.

2.5. Statistical Analysis

The SPSS (10.00) soft ware was used to data management and analysis, while Microsoft Excel was used for charts .The results were expressed as mean ± standard deviation of the mean. Statistical analysis was carried out using student *t*- test. $P < 0.05$ was accepted as significant.

3. Results

As shown in (Table 1 and 2), plasma levels of AST, ALT, LDH, CK and UA were significantly increased ($P<0.05$) in strenuous exercise group (control exercise (group II)) as compared with sedentary control group (group I). However, there were no significant difference for these parameters in group III and group IV that subjected to strenuous exercises and fed on low and high doses of tomato and guava juices, as compared with group II that subjected to strenuous exercises (control exercise).

From data present in (Table 1 and 2), it was observed that, the liver functions, LDH, CK and uric acid were significantly increased after the exercise. Moreover, both concentrations of tomato or guava juices had no significant effect on decreasing their concentrations near to control negative level for rats subjected to strenuous exercises.

As shown in (Table 3), XO and MDA of plasma and muscle in group II (control strenuous exercise) were significantly increased ($P<0.05$) as compared with group I (sedentary control). However, the muscular XO, MDA and plasma MDA were significantly lower with strenuous exercise fed on low dose (group III) and strenuous exercise fed on high dose (group IV) of tomato juice, as compared with group II.

Also, MPO activity in muscle showed significant increase ($P<0.05$) in control strenuous exercise group (group II) as compared to sedentary control group (group I). However, muscular MPO activity was significantly decrease ($P<0.05$) with strenuous exercise that fed on low dose (group III) and strenuous exercise that fed on high dose (group IV) of tomato juice, as compared with group II (control strenuous exercise).

There was no significant difference in the GR concentration in strenuous exercise group (group II), as compared to sedentary control group (group I). Also, no significant changes in GR concentration in group III and group IV as compared to group II (control strenuous exercise).

There was no significant difference in plasma and muscle parameters in strenuous exercise group with high dose of tomato juice (group IV) as compared to strenuous exercise group with low dose (group III).

The same finding for tomato juice was reported in guava juice (Table 4), except the significant effect of low and high doses of guava juice (group III and IV) in decreasing plasma xathine oxidase, as compared to group II (control strenuous exercise) ($P<0.05$).

As shown in (Table 3 and 4) , the decrease in plasma level of vitamin C in group (II) (control strenuous exercise) was not significant as compared with group (I) (control sedentary group). However, plasma level of vitamin C was significantly increased with strenuous exercise that fed on low dose of tomato or guava juices(group III) and strenuous exercise that fed on high dose of tomato or guava juices (group IV), as compared with group II (control strenuous exercise) ($P<0.05$).

Table 1. Effect of different doses of tomato juice supplementation on plasma ALT, AST, LDH, CK and UA levels after strenuous exercise

Parameters / Groups (N=10)	ALT U/L	AST U/L	LDH U/L	CK U/L	UA mg/dL
Group I	20±2.1	40±3.3	108±4.5	52±2.9	4.5 ±2.9
Group II	33±8.2 *P<0.05*	70±4.3 *P<0.05*	200±6.3 *P<0.05*	240±6.3 *P<0.05*	12.4±2.6 *P<0.05*
Group III	32±2.8 *P>0.05*	67±4.1 *P>0.05*	191±4.6 *P>0.05*	231±10.1 *P>0.05*	11.5±2.1 *P>0.05*
Group IV	31±2.9 *P>0.05*	64±4.7 *P>0.05*	185±6.2 *P>0.05*	225±9.3 *P>0.05*	10.8±5.1 *P>0.05*

N = number of animals in each group values given are mean ± standards deviation Group II compared to group I (control group) group III and IV are compared to group II P<0.05 is significant

Table 2. Effect of different doses of guava juice supplementation on plasma ALT, AST, LDH, CK, and UA levels after strenuous exercise

Parameters / Groups (N=10)	**ALT** U/L	**AST** U/L	**LDH** U/L	**CK** U/L	**UA** mg/dL
Group I	20±2.1	40±3.3	108±4.5	52±2.9	4.5 ±2.9
Group II	33±8.2 *P<0.05*	70±4.3 *P<0.05*	200±6.3 *P<0.05*	240±6.3 *P<0.05*	12.4±2.6 *P<0.05*
Group III	31±3.2 *P>0.05*	65±5.2 *P>0.05*	187±6.7 *P>0.05*	229±8.4 *P>0.05*	11.0±2.1 *P>0.05*
Group IV	30±3.9 *P>0.05*	63±3.9 *P>0.05*	185±7.9 *P>0.05*	226±7.5 *P>0.05*	9.9±2.1 *P>0.05*

N = number of animals in each group values given are mean ± standards deviation Group II compared to group I (control group) group III and IV are compared to group II P<0.05 is significant.

Table 3. Effect of tomato juice on lipid peroxidation product and antioxidant

Parameters / Group N=10	Plasma				Muscle			
	XO uU/ml	**MDA** Umol/l	**GR (Erythrocyte)** Umol/g	**Vitamin C** Umol/l	**XO** uU/g	**MPO** ng/ml	**MDA** Umol/g	**GR** Umol/g
Group I	5.2±0.9	9.3±1.8	26.1 ±3.7	35.3 ±8.4	0.5±0.3	1.6±0.1	21.4 ±2.9	58.7 ±4.6
Group II	8.3±1.2 *P<0.05*	18.4±3.4 *P<0.05*	25.3±4.5 *P>0.05*	33.4±5.3 *P>0.05*	1.4±0.1 *P<0.05*	2.8±0.3 *P<0.05*	40.3±4.7 *P<0.05*	55.5±6.4 *P>0.05*
Group III	8.0±1.4 *P>0.05*	12.1±1.7 *P<0.05*	23.7±2.9 *P>0.05*	42.3±6.2 *P<0.05*	0.9±0.2 *P<0.05*	2.1±0.4 *P<0.05*	30.6±3.5 *P<0.05*	53.6±4.3 *P>0.05*
Group IV	7.9±1.6 *P>0.05*	10.4±2.3 *P<0.05*	24.2±2.3 *P>0.05*	45.4±5.3 *P< 0.05*	0.87±0.7 *P<0.05*	1.9±0.2 *P<0.05*	28.6±2.8 *P<0.05*	55.9±3.9 *P>0.05*

Table 4. Effect of guava juice on lipid peroxidation product and antioxidant

Parameters / Group N=10	Plasma				Muscle			
	XO uU/ml	MDA umol/l	GR (Erythrocyte) Umol/g	Vitamin C Umol/l	XO uU/g	MPO ng/ml	MDA umol/g	GR Umol/g
Group I	5.2±0.9	9.3±1.8	26.1 ±3.7	35.3 ±8.4	0.5±0.3	1.6±0.1	21.4 ±2.9	58.7 ±4.6
Group II	8.3±1.2 *P<0.05*	18.4±3.4 *P<0.05*	25.3±4.5 *P>0.05*	33.4±5.3 *P>0.05*	1.4±0.1 *P<0.05*	2.8±0.3 *P<0.05*	40.3±4.7 *P<0.05*	55.5±6.4 *P>0.05*
Group III	5.9±0.7 *P<0.05*	9.8±2.2 *P<0.05*	26.4±3.7 *P>0.05*	50.8±5.6 *P<0.05*	0.62±0.4 *P<0.05*	1.7±0.6 *P<0.05*	23.8±2.5 *P<0.05*	56.2±4.6 *P>0.05*
Group IV	5.5±2.3 *P<0.05*	9.5±1.3 *P<0.05*	27.5±2.4 *P>0.05*	52.4±3.9 *P<0.05*	0.58±0.3 *P<0.05*	1.5±0.4 *P<0.05*	22.7±3.6 *P<0.05*	56.5±3.7 *P>0.05*

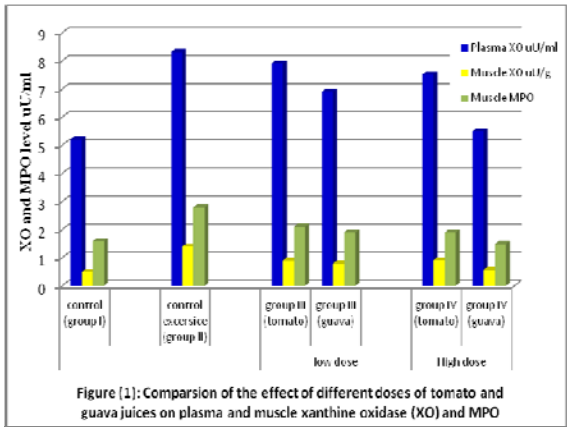

Figure (1): Comparsion of the effect of different doses of tomato and guava juices on plasma and muscle xanthine oxidase (XO) and MPO

As shown in figure 1 ,the plasma levels of XO, muscle XO and MPO were significantly decreased in rats with strenuous exercise and fed on high dose of guava juice (group IV), as compared with rats that strenuous exercised and fed on high dose of tomato juice (group IV) (P<0.05).

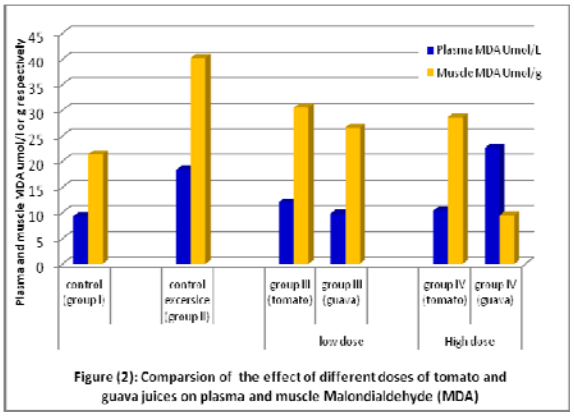

Figure (2): Comparsion of the effect of different doses of tomato and guava juices on plasma and muscle Malondialdehyde (MDA)

As shown in figure 2 ,the MDA for plasma and muscle was significantly decreased in rats with strenuous exercise and fed on high dose of guava juice (group IV), as compared with rat that strenuous exercised and fed on high dose of tomato juice (group IV) (P<0.05).

As shown in figure 3, the plasma levels of vitamin C in group III and IV of rats fed on guava juice were

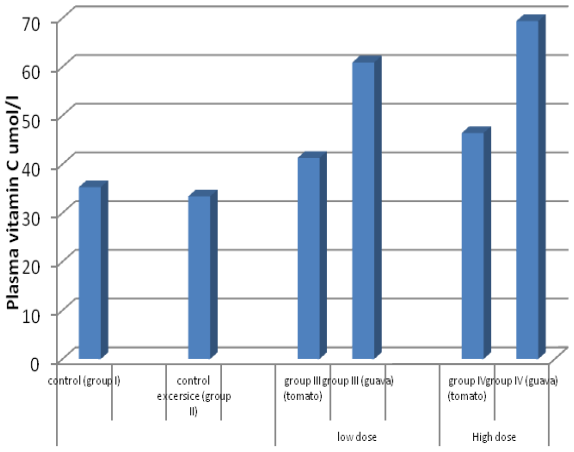

Figure (3): Comparsion of the effect of different doses of tomato and guava juices on plasma vitamin C

significantly increased when compared with the same groups of rats fed on tomato juice (P<0.05).

No significant difference was detected for GR between rats fed on tomato juice group III and IV, as compared with the same groups of rats fed on guava juice (P >0.05).

4. Discussion

A single bout of physical exercise has been shown to induce formation of reactive oxygen species, nitrogen species and the related oxidative damage. On the other hand, regular training is known to increase the resistance against reactive oxygen species induced lipid peroxidation, and to decrease oxidative protein and DNA damage (Quindry *et al*., 2003).

In the present study, the strenuous exercise showed significant increase in liver ALT and AST produced by liver and other tissues as compared with sedentary control group; this result is in agreement with finding by (Bowers *et al*., 1978) who showed that strenuous exercises increase the severity of liver damage. In additional, human study showed that, the liver function parameters, AST and ALT, were significantly increased for at least 7 days after the exercise (Pettersson *et al.,* 2007). Also, in the present study, the plasma LDH activity of strenuous exercise group was significantly increased as compared with sedentary control group. This result is similar to the one found by (Chieh-chung *et a.l*, 2005) which showed that the plasma level of LDH increases three fold with strenuous exercise. Researchers speculated that free radicals resulting from strenuous exercise cause cardiac injury which lead to the release of LDH from cardiocytes into the blood (Vina *et al.*, 2000). However, tomato juices supplementation did not display any significant changes in plasma level of LDH as compared with strenuous exercise group.

In the present study, the plasma CK of strenuous exercise group compared with sedentary control group, was significantly increased. This result corresponds with many researchers (Marquez *et al.*, 2001 and Zajac *et al.*, 2001) who reported that strenuous exercise elevates CK activity in skeletal muscle. Also, the plasma level of uric acid was significantly increased with strenuous exercise group compared with sedentary control group. Goto *et al.*, (1989) reported that strenuous exercise affects the renal function resulting in reduction of glomerular filtration rate and uric acid excretion.

In the present study, the MDA concentration in plasma and muscle was significantly increased in strenuous exercise group as compared with sedentary control group. This result agrees with (Chieh-chung *et al.*, 2005) who reported that MDA in plasma and muscle significantly increased by 0.8 and l.l fold respectively with strenuous exercises caused oxidation damage, increasing lipid peroxidation and decreasing antioxidant. Huang *et al.*, (2008) reported that strenuous exercise elevate MDA, XO and MPO levels of myocardial, muscular, hepatic, pulmonary and renal tissues. In the current research, tomato and guava juices supplementation significantly decreases MDA level in plasma and muscular tissues. In addition, guava juice has more effect for reduction of MDA than tomato juice. The current results were consistent with another study which confirmed that, guava juice had the highest inhibitory effect on MDA formation among the almost fruit juices (Zabidah *et al*., 2011). However, the lycopene supplement through guava or tomato juices did not affect GR levels, both in the erythrocytes and muscle. Moreover, in another study, guava juice caused significant increase for both glutathione peroxidase and glutathione reductase and may reduce the risk of disease caused by free radical activities (Asmah *et al*., 2006).

Also, this study found that, the muscular MPO activity was significantly increased in strenuous exercise group comparing with sedentary control group. Morozov *et al.*, 2003 found that the strenuous exercise lead to a raise of MPO from neutrophils and then induced severe oxidative damage. Wan-teng *et al.* (2006) showed that strenuous exercise increase XO, MPO and lipid peroxidation end product (MDA) levels in myocardial tissues compared with control group. Sean and Kelvin (2007) reported that strenuous exercise generated free radical from mitochondria, neutrophils and XO.

This study demonstrated that tomato and guava juices supplementation with low and high doses significantly decreased muscular MPO activity as compared with strenuous exercise group. This result is similar to (Reifen *et al*., 2004) who reported that MPO activity in muscle was decreased by lycopene administration provided by tomato juice.

In other studies study, Guava juice showed anti-hypertensive (Ayub *et al*., 2010) and lipid-lowering properties (Norazmir and Ayub, 2010).

The recent study demonstrated that the level of plasma vitamin C is significantly increased in guava juice fed rats in low and high doses compared with rats fed on the same doses of tomato juice. The results obtained in this study clarified that, guava juice is more effective than tomato juice in impairment of oxidative damage caused by strenuous exercise by lowering plasma and muscle XO, MDA as well as muscle MPO after exercise more than tomato juice. It may be due to higher content of antioxidant in guava juice more than tomato juice which concede with the previous study published by Thaipong *et al*. (2006) which reported that guava juice is a good source of phenolic compounds much better than other fruits juice .

5. Conclusion

This study indicated that guava juice may have a potential to be introduced as functional food product more than tomato juice because of its highly antioxidant properties. No effect of supplementation of guava or tomato in lowering plasma levels of ALT, AST, CK, LDH and UA but still needs further investigations. Further studies are recommended to demonstrate the role of guava as a good source of vitamin C and anti oxidants in prevention or treatment of chronic degenerative diseases in humans.

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