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GARCINIA MANGOSTANA L. RIND EXTRACT AND PHYSICAL TRAINING REDUCE OXIDATIVE STRESS IN WISTAR RATS DURING MAXIMAL PHYSICAL ACTIVITY

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ABSTRACTS

Oxidative stress is a condition of imbalance between the production of free radicals or reactive oxygen species (ROS) and antioxidants, in which the levels of free radicals higher than antioxidants. One causes of oxidative stress is the maximum physical activity. Oxidative stress can be reduced by antioxidants. One source of antioxidants is mangosteen rind (*Garcinia mangostana* L). This study aimed to investigate the role of mangosteen rind extract and physical training in reducing Malondialdehyde (MDA, increasing Superoxide dismutase (SOD) and Glutathione Peroxidase (GPx).

This study used a randomized block design with factorial pattern of 6 x 2 with four repetitions. The first treatments are mangosteen rind extract with doses; 0, 50, 100; 200; 300, and 400 mg/kg bodyweight/day for four weeks. The second treatments are the physical training consists of; without and with physical training. MDA, SOD and GPx were assessed in the end of treatment. Data were analyzed with GLZ.

The results showed that average of MDA, SOD and GPx different significantly ($p<0.05$) after administration of extracts as well as physical training. Extracts and physical training concurrently also showed effect significant ($p<0.05$). However, at doses 0 until 300 mg/kg bodyweight, MDA levels more higher while SOD and GPx lower significantly ($p<0.05$) at training group compared with without training. Whereas at dose 400 mg/kg bw, MDA lower at training ($p>0.05$), while SOD and GPx were recorded significantly ($p<0.05$) higher than the group without training.

Overall, it could be concluded that physical training with administration of mangosteen rind extract reduce oxidative stress through reduction of MDA, as well as increased both SOD and GPx.

Keywords: *Garcinia mangostana* L, Physical Training, oxidative stress, MDA, SOD and GPx

INTRODUCTION

Oxidative stress is a condition of imbalance between the production of free radicals or reactive oxygen species (ROS) and antioxidants, in which the levels of free radicals higher than antioxidants.[1] Free radicals can be derived from outside the body, can also be formed in the body as an integral part of physiologic processes such as during the formation of energy in the mitochondria through oxidative phosphorylation.[2]

In the body, the major source of ROS is oxidation phosphorylation during maximum physical activity. During physical activity, ROS are formed as byproducts of oxidation phosphorylation reaction to form energy (ATP) in the electron transport chain in mitochondria. The process requires O_2, but not all O_2 binds with hydrogen to form water, approximately 4% to 5% of the oxygen that were consumed is transformed into ROS.[3-5]

Reactive oxygen species can be reduced by administration of antioxidants, but the use of antioxidants in the exercise was still considered unable to improve athlete performance.[6] Studies on the use of antioxidants showed different results. For example, the use of vitamin E 450 mg per day for eight weeks did not decrease MDA, carbonyl protein and creatine kinase significantly after physical activity.[7] Meanwhile, Traber showed that vitamin E and C can prevent an increase in F2-isoprostane of blood, but cannot prevent inflammation, DNA damage and muscle damage after following a marathon race.[8] Research conducted in mice

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by administering of antioxidants alfa-lipoic acid 100 mg / kg during the training period (five days per week for six weeks) can lower MDA of blood and liver of Sprague-Dawley rats were significantly.[9]

Synthetic antioxidants also have been believed have side effects, so that exploration of natural ingredient that has antioxidant capability has been done intensively. One source of natural antioxidants is mangosteen rind (Garcinia mangostana L.). Mangosteen rind has known have antioxidant capacity in-vitro.[10-18] However, *in-vivo* studies in particular the use of mangosteen rind extracts in exercise is still rarely done. Mangosteen rind has not been used optimally and discarded as agricultural waste. Therefore, the use of mangosteen rind extracts as a source of antioxidants in exercise still needs to be further investigated. This research aimed to investigate the role of mangosteen rind extract and physical training in reducing the levels of MDA, increase SOD and GPx.

MATERIALS AND METHODS

Research Design

This research was use a randomized block design with factorial pattern of 6 x 2 with four repetitions. The first factors are mangosteen rind extract with doses; 0, 50, 100; 200; 300, and 400 mg/kg bodyweight/day for four weeks. The second factors are the physical training consist of without and swimming (30 min, 5 days a week, four weeks period). MDA, SOD and GPx were assessed from blood plasma in the end of treatment. Data were analyzed with GLZ.

Animals

Forty-eight of male Wistar rats (*Rattus norvegicus*) age of 12 weeks, weight 216 g until 258 g used in this research. Rats were housed four per cage, water and fed ad libitum. The animals and study protocol was approved by the local ethics committee.

Rats were divided into 12 treatments. Five treatments were given mangosteen rind extract by gastrogavage at dose of 50, 100; 200; 300, and 400 mg/kg bodyweight /day for four weeks. Five treatments were given mangosteen rind extract at dose of 50, 100; 200; 300, and 400 mg/kg bodyweight /day for four weeks, as well as the swimming (30 min, 5 days a week, four weeks period). One treatment was swum only (30 min, 5 days a week, four weeks period). One treatment again was given mangosteen rind extract at dose of 0 mg/kg bodyweight /day for four weeks. Twenty four hours after last treatment, all rats were made to swim until exhaustion, then blood were taken immediately from medial cantus sinus orbital for analysis of MDA, SOD and GPx.

Extraction

Mangosteen rind extract were obtained by maceration using 96% ethanol for 48 hours, and remaceration twice. Macerate were filtered with Whatman filter paper No. 40. The filtrate was evaporated in a rotary vacuum evaporator at 45^{o}C and then dried using a freeze dry.

Assessment of MDA, SOD, and GPx

MDA were measured according to the method of Wuryastuti.[19] Briefly, 0.75 ml phosphoric acid pipetted into polypropylene 13 ml, and then 0.05 ml samples of blood plasma were added. The mixture was then added 0.25 ml of thiobarbituric acid (TBA) 40 mM, followed by 0.45 ml of water and then mixed well and covered tightly. After heated in a water bath for 60 minutes to 100°C, the mixture cooled to 30^{o}C, and then inserted into the column Sep-Park C_{18}. Before use, the column was washed with 5 ml of methanol and water and then discarded. The next, sample inserted into the column and discarded too. TBA were eluted from the column with 4 ml of methanol and collected in cuvet tube. Absorbency of sample was measured spectrophotometrically at 532 nm wavelength. As standard were used 1.1.3.3 tetraetoksipropana (TEP).

SOD analysis, 0.06 ml of plasma was reacted with a mixture consisting of 2.70 ml of sodium-carbonate buffer containing 0.1 mM EDTA (pH 10), 60 µl xanthine 10 mM, 0.5% bovine serum albumin (BSA) 30 µl, and 30 µl NBT 2.5 mM. Then, xanthine oxidase (0.04 units) was added. After 30 min, absorbency was measured at 560 nm wavelength. SOD was calculated using equation: (B-A/B) x100%, where A was absorbency of sample solution, B was absorbency of the control solution.[20-22]

GPx analysis, 0.2 mL of plasma was added 0.2 mL phosphate buffer 0.1 M (pH 7.0) which contain 0.1 mM EDTA, 0.2 mL reduced glutathione (GSH) 10 mM and 0.2 mL of glutathione reductase enzyme. Then incubated at 37°C for 10 min, 0.2 mL NADPH 1.5 mM was added and incubated again for 3 minutes at the same temperature, 0.2 mL hydrogen peroxide 1.5 mM was added. Absorbency was measured between one to two minutes with a spectrophotometer at of 340 nm wavelength.[21-22]

RESULTS

Levels of MDA, SOD and GPx after treatment mangosteen rind extract

The results showed that average levels of MDA, SOD and GPx differ significantly (p<0.05) between treatments as presented in Table 1.

Levels of MDA, SOD and GPx after Physical Training

The results showed that average of MDA, SOD and GPx differ significantly (p <0.05) between treatments. MDA levels were higher in the treatment with physical

training compared to no training, while SOD and GPx vice versa, as shown in Table 2.

Table 1
Average Levels of MDA, SOD and GPx after Treatment of Mangosteen Rind Extracts

DOSE (mg/kg bw)	MDA (nmol/ml)	SOD (%)	GPx (U/ml)
0	8.50±0.30^{a}	52.40±0.39^{a}	14.62±0.11^{a}
50	7.50±0.26^{b}	57.62±0.42^{b}	16.41±0.12^{b}
100	4.77±0.17^{c}	63.90±0.47^{c}	25.81±0.19^{c}
200	3.91±0.14^{d}	71.98±0.53^{d}	29.10±0.21^{d}
300	3.49±0.12^{e}	75.90±0.56^{e}	31.04±0.23^{e}
400	2.78±0.10^{f}	81.35±0.60^{f}	34.97±0.25^{f}

Value within the same columns with dissimilar superscript, differ significantly at level of 0.05

Levels of MDA, SOD and GPx after Treatment of Mangosteen Rind Extracts and Physical Training

This research was found that administration of mangosteen rind extract and physical training influenced to MDA, SOD, and GPx were significantly ($p < 0.05$). Average of MDA decreased, while SOD and GPx increased with increasing doses of the extract which were given concurrently with physical training. However, at dose of 0 until 300 mg/kg bw, MDA was higher, whereas SOD and GPx lower significantly at physical training groups were compared to without training. Meanwhile, at 400 mg/kg bw MDA lower at training group, but not significantly, while SOD and GPx recorded higher significantly when compared with without training (Table 3).

Table 2
Average Levels of MDA, SOD, and GPx after Physical Training

Treatment	MDA (nmol/ml)	SOD (%)	GPx (U/ml)
Without	3.85±0.08^{a}	72.09±0.31^{a}	29.87±0.13^{a}
Training	5.88±0.12^{b}	61.17±0.26^{b}	19.43±0.08^{b}

Value within the same columns with dissimilar superscript, differ significantly at level of 0.05

DISCUSSION

MDA is a dialdehydes compound with the molecular formula of $C_3H_4O_2$, which can be generated from the oxidation of unsaturated fatty acids by free radicals. Changes in MDA levels can be used as a biomarker of damage of cell membranes. Results of this research showed that mangosteen rind extract can reduce MDA levels significantly ($p < 0.05$) (Table 1). This is probably caused by the compounds contained in mangosteen rind extract worked as an antioxidant by donating electrons to the free radicals. The results of this study are supported by some of the results of previous in vitro studies that say, mangosteen rind extract (Garcinia mangostana L.) has the ability as an antioxidant [10-18]. Thus, the result of this study confirms that mangosteen rind extract can reduce oxidative stress formed during maximum physical activity.

Table 3.
Average Levels of MDA, SOD and GPx after Treatment of Mangosteen Rind Extracts and Physical Training

Variable	Dose mg/kg bw)	Physical Training	
		Without	Training
MDA	0	6.,42±0.32^{aA}	11.25±0.55^{aB}
	50	5.86±0.29^{aA}	9.60±0.47^{bB}
	100	3.39±0.17^{bA}	6.73±0.33^{cB}
	200	2.89±0.14^{cA}	5.30±0.26^{dB}
	300	3.04±0.15^{bcA}	4.02±0.20^{eB}
	400	2.89±0.14^{cA}	2.68±0.13^{fA}
SOD	0	62.11±0.65^{aA}	44.20±0.46^{aB}
	50	64.70±0.67^{bA}	51.31±0.53^{bB}
	100	71.62±0.75^{cA}	57.00±0.59^{cB}
	200	77.59±0.81^{dA}	66.78±0.70^{dB}
	300	78.45±0.82^{deA}	73.44±0.76^{eB}
	400	80.10±0.83^{eA}	82.61±0.86^{fB}
GPx	0	24.79±0.25^{aA}	8.62±0.09^{aB}
	50	26.64±0.27^{bA}	10.11±0.10^{bB}
	100	30.75±0.32^{cA}	21.67±0.22^{cB}
	200	31.92±0.33^{dA}	26.53±0.27^{dB}
	300	32.70±0.34^{deA}	29.46±0.30^{eB}
	400	33.51±0.34^{eA}	36.50±0.37^{fB}

Value with dissimilar superscript, differ significantly at level of 0.05. Capital letters indicate significantly towards same line, whereas small letters toward same columns.

Several studies using extracts from plants also showed similar results. For example, the use of extracts or syrups purple cassava lower MDA levels of blood and liver of mice after administration of the maximal physical activity.[23] Supplementation of grape seed extract also decrease MDA levels in mice.[24] Mansouri et al.[25] which also use grape seed extract shown decrease of MDA levels in male Sprague-Dawley rats with diabetes mellitus. Chattopadhyay et al.[26] using Moringa oleifera seed extract to reduce levels of MDA of Wistar rat which were induced by Arsenic metal.

Antioxidant properties of the mangosteen fruit were associated with the presence of active ingredients mainly from the fruit peel. The active ingredients that have been identified from the mangosteen rind are a large number of xanthones compounds α-mangostin and γ-mangostin especially.[10] Presence of hydroxyl group (OH) on phenolic compounds such as xanthones allows these compounds work as antioxidants by donating electrons to the free radicals to form a stable

end product so there is no further initiation or propagation reaction.[13,27]

The results also showed that Mangosteen rind extract increases levels of SOD and GPx were significantly (Table 1). It is occur because the compounds in the mangosteen rind extract, not only working as an antioxidant by donating electrons to free radicals, but also as an inducer that triggers the expression of genes encoding antioxidants through activation of *Nuclear factor-erythroid 2-related factor* 2 (Nrf2).[28] Several similar studies have shown that polyphenols such as epicatechin compounds have been known capable to trigger the expression of genes encoding antioxidants through activation of Nrf2,[29,30] also Curcumin can reduce liver damage through the activation of Nrf2[31], broccoli seeds which containing glucosinolate can induce the formation of antioxidants and protein detoxication through activation of Nrf2 in mice[32]. These compounds activate Nrf2 directly or through a series of pathways that were mediated by interaction with specific proteins.[33] *Nuclear factor-erythroid 2-related factor* 2 is a *basic region-leucine zipper (bZIP) transcription factor* and member of *Cap 'n' Collar* (CNC) *family*, which mediate cellular response due to exposure to a variety of inducer such as oxidants or xenobiotic by binding to the elements promoter of cytoprotective genes.[34]

MDA levels were higher in treatment with physical training than without training. This is likely due to dose of training improperly, where the training intensity excessive while duration less so that more resembles the acute exercise. Acute exercise is an exercise that is only done incidentally, in other words not performed regularly or periodically. According to Bompa[35], the intensity of 70% of maximum capacity categorized as intermediate to medium intensity, and in accordance to concept of hormesis that low doses would have a stimulating effect while excessive doses would be toxic.[36] However, the intensity of 70% of maximum capacity appears to be too high resulting in the production of more free radicals. It is because free radicals can be formed as an integral part of the process of oxidation phosphorylation in mitochondria[4]. In addition, SOD levels and GPx lower in treatment with physical training than without training (Table 2). There are indications that dose of training improperly, where the training intensity excessive while duration less so that more resembles the acute exercise. These conditions resulted in the training cannot be used as an adaptation mechanism to trigger the expression of genes encoding antioxidants through activation of Nrf2,[33] so that antioxidant enzymes are not sufficient to reduce free radicals by the way catalyze into a more stable product. In other words, the effects of training cannot protect when given the maximum physical activity.

Although physical training increases levels of MDA, but physical training and administration of mangosteen rind extract was able to reduce levels of MDA were significantly, even at dose of 400 mg/kg bw MDA lower at treatment with physical training than without training, although not significant (Table 3). This means that mangosteen rind extract during physical training has a major role in reducing free radicals which were formed during training. This happens because the mangosteen rind extract contain compounds which works as an antioxidant by donating electrons to the free radicals so there is no initiation reaction or further propagation and formed a stable end product.[13,27]

The results also showed that administration of mangosteen rind extract which were given concurrently with the physical training up to a dose of 300 mg/kg bodyweight, treatment with physical training still had levels of SOD and GPx lower compared to without training, but it is opposite at doses of 400 mg/kg bodyweight the treatment with physical training was noted have levels of SOD and GPx were significantly higher compared with without training (Table 3). This means that the physical training with mangosteen rind extract will show good results at a dose of 400 mg/kb bodyweight because at this dose there is interaction effect between the training with extract for lowering the MDA and increasing SOD and GPx. Thus it can be said that although the use of antioxidants in the exercise is still unable to improve athlete performance[6], but this research suggest that physical training with administration of mangosteen rind extract concurrently, have a beneficial effect because it can reduce free radicals by donating electrons for free radicals and increasing the activity of antioxidant enzymes through Nrf2 activation mechanism.

CONCLUSION

Mangosteen rind extract decreases MDA, increases SOD and GPx. Physical training increases levels of MDA, decrease level of SOD and GPx. Physical training with mangosteen rind extract dose of 400 mg/kg bw reduced oxidative stress through reduction of MDA, as well as increased both SOD and GPx.

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REFERENCES

1. Kürkçü, R., Tekin, A., Özda, S., and Akçakoyun, F., 2010. The Effects of Regular Exercıse on Oxıdatıve and Antıoxıdatıve Parameters ın Young Wrestlers. *African Journal of Pharmacy and Pharmacology.* 4(5): 244-51.
2. Waris, G. and Ahsan, H. 2006. Reactive Oxygen Species: Role in The Development of Cancer and Various Chronic Condition. *Journal of Carcinogenesis.* 5 (14): 1-8.
3. Ngurah, I. B. 2007. Peranan Antioksidan pada olah raga. *Medicina.* 38 (1): 3-6.
4. Figueiredo, P. A., Mota, M. P., Appell, H. J., and Duarte, J. A. 2008. The Role of Mitochondria in Aging of Skeletal Muscle. *Biogerontology.* 9: 67-84
5. Marciniak, A., Brzeszczyńska, J., Gwoździński, K., and Jegier, A. 2009. Antioxidant Capacity and Physical Exercise. *Biology of Sport.* 26 (3):197-213.
6. Harjanto. 2006. Antioksidan dan Latihan Olahraga. *Jurnal Kedokteran Yarsi.* 14 (1): 070-7.
7. Gaeini, A. A., Rahnama, N., and Hamedinia, M. R. 2006. Effect of Vitamin E Supplementation on Oxidative Stress at Rest and After Exercise to Exhaustion in Athletic Students. *J. Sports Med. Phys. Fitness.* 46: 458-61.
8. Traber, M. G. 2006. Relationship of Vitamin E Metabolism and Oxidation in Exercising Human Subjects. *British Journal of Nutrition.* 96 (*Suppl.* 1): 34-7.
9. Kim, H. T. and. Chae, C. H. 2006. Effect of Exercise and α-Lipoic Acid Supplementation on Oxidative Stress in Rats. *Biology of Sport.* 23(2):114-53.
10. Jung, H. A., Su, B. N., Keller, W. J., Metha, R. G., and Kinghorn, A. D. 2006. Antioxidant Xanthones from The Pericarp of *Garcinia mangostana* (Mangosteen). *J. Agric. Food Chem.* 54: 2077-82.
11. Weecharangsan, W., Opanasopit, P., Sukma, M., Ngawhirunpat, T., Sotanaphun, U., and Siripong, P. 2006. Antioxidative and Neuroprotective Activities of Extracts from the Fruit Hull of Mangosteen (Garcinia *mangostana* L.). *Med.Princ. Pract*.15: 281-7.
12. Kosem, N., Han, Y. H., and Moongkarndi, P. 200. Antioxidant and Cytoprotective Activities of Methanolic Extract from *Garcinia mangostana* Hulls. *Science Asia.* 33: 283-92.
13. Zarena, A. S., and Sankar, K. U. 2009. Study of Antioxidant Properties from *Garcinia mangostana* L. Pericarp Extract. *Acta Sci. Pol. Technol. Aliment*. 8(1): 23-34.
14. Ngawhirunpat, T., Opanasopi, P., Sukma, M., Sittisombut, C., AtsushiKat, and Adachi, I. 2010. Antioxidant, Free Radical-scavenging Activity and Cytotoxicity of Different Solvent Extracts and Their Phenolic Constituents from The Fruit Hull of Mangosteen (*Garcinia mangostana*). *Pharmaceutical Biology.* 48 (1): 55-62.
15. Palakawong, C., Sophanodora, P., Pisuchpen, S. and Phongpaichit. 2010. Antioxidant and Antimicrobial Activities of Crude Extracts from Mangosteen (*Garcinia mangostana* L.) Parts and Some Essential Oils. *International Food Research Journal.*17:583-9.
16. Chomnawang, M. T., Surassmo, S., Nukoolkarn, V. S., and Gritsanapan, W., 2007. Effect of *Garcinia mangostana* on Inflammation Caused by *Propionibacterium acnes*. *Fitoterapia.* 78: 401–8.
17. Haruenkit, R., Poovarodom, S., Leontowicz, H., Leontowicz, M., Sajewcz, M., Kowalska, T., Delgado-Licon, E., Rocha-Guzmaan, N. E., Gallegos-Infante, J. A., Trakhtenberg, S., and Gorinstein, S. 2007. Comparative Study of Health Properties and Nutritional Value of Durian, Mangosteen, and Snake Fruit: Experiments In vitro and In vivo. *Journal of Agricultural and Food Chemistry. 55: 5842-9.*
18. Pothitirat, W., Chomnawang, M. T., and Grtsanapan, W. 2010. Free Radical and Anti-Acne Activities of Mangosteen Fruit Rind Extracts Prepared by Different Extraction Methods. *Pharmaceutical Biology.* 48 (2): 182- 6.
19. Wuryastuti, H. 2000. The Influence of Dietary Protein and Fats on Plasma Lipids in Sprague-Dawley Rats. *Indonesian Food and Nutrition Progress* 7 (2): 37- 41.
20. Sun, Y., Oberley, L., W. and Li, Y. 1988. A Simple Method for Clinical Assay of Superoxide Dismutase. *Clin.Chem* 34 (3): 497-500.
21. Kotan, E., Alpsoy, L., Anar, M., Aslan, A. and Agar, G. 2011. Protective Role of Methanol Extract of Cetraria islandica (L.) Against Oxidative Stress and Genotoxic Effects of AFB in Human Lymphocytes Invitro. *Toxicology and Industrial Health* 27(7): 599-5.
22. Wrasiati, L. P. 2011. *Karakteristik dan Toksisitas Ekstrak Bubuk Simplisia Bunga kamboja Cendana (Plumeria alba) dan Peranannya dalam Meningkatkan Aktivitas Antioksidan Enzimatis pada Tikus Sparague Dawley*. Disertasi. Program Pascasarjana. Universitas Udayana. Denpasar.
23. Jawi, I. M., Suprapta, D. N. and Subawa. A. A. N. 2008. Ubi Jalar Ungu Menurunkan Kadar MDA dalam Darah dan Hati Mencit Setelah Aktivitas Fisik Maksimal. *Jurnal Veteriner.* 9 (2): 65-72.
24. Shan, Y., Ye, X. H. and Xin, H. 2010. Effect of Grape Seed Proanthocyanidin Extract on The Free Radical and Energy Metabolism Indicators During The Movement. *Scientific Research and Essay.* 5 (2): 148-53.

25. Mansouri, E., Panahi, M., Ghaffari, M. A., and Ghorbani, A. 2011. Effects of Grape Seed Proanthocyanidin Extract on Oxidative Stress Induced by Diabetes in Rat Kidney. *Iranian Biomedical Journal* 15 (3): 100-6.
26. Chattopadhyay, S., Maiti, S., Maji, G., Deb, B., Pan, B., and Ghosh, D. 2011. Protective Role of *Moringa Oleifera* (Sajina) Seed on Arsenic-Induced Hepatocellular Degeneration in Female Albino Rats. *Biol. Trace. Elem Res*. 142: 200–12.
27. Middleton Jr, E., Kandaswami, C., and Theoharides, T. C. 2000. The Effects of Plant Flavonoids on Mammalian Cells: Implications for Inflammation, Heart Disease, and Cancer. *Pharmacological Review*. 52: 673–751.
28. Son, T. G., Camandola, S. dan Mattson, M. P. 2008. Hormetic Dietary Phytochemicals. *Neuromol Med*. 10: 236-46.
29. Granado-Serrano, A. B., Martı´n, M. A., Haegeman, G., Goya, L., Bravo, L., and Ramos, S. 2010. Epicatechin Induces NF-kB, Activator Protein-1 (AP-1) and Nuclear Transcription Factor Erythroid 2p45-related factor-2 (Nrf2) via Phosphatidylinositol-3-Kinase/Protein Kinase B (PI3K/AKT) and Extracellular Regulated Kinase (ERK) Signalling in HepG2 cells. *British Journal of Nutrition.* 103: 168-79.
30. Shah, Z. A., Li, R. C., Ahmad, A. S., Kensler, T. W., Yamamoto, M., Biswal, S., and Dore. S., 2010. The Flavanol (-)-Epicatechin Prevents Stroke Damage Through The Nrf2/HO1 Pathway. *Journal of Cerebral Blood Flow & Metabolism.* 30: 1951-61.
31. Farombi, E. O., Shrotriya, S., Na, H. K., Kim, S. H., and Surh, Y. J. 2008. Curcumin Attenuates Dimethylnitrosamine-Induced Liver Injury in Rats Through Nrf2-Mediated Induction of Heme Oxygenase-1. *Food and Chemical Toxicology*. 46: 1279–87.
32. McWalter, G. K., Higgins, L. G., McLellan, L. I., Henderson, C. J., Song, L., Thornalley, P. J., Itoh, K., Yamamoto, M., and Hayes, J. D. 2004. Transcription Factor Nrf2 is Essential for Induction of NAD(P)H:Quinone Oxidoreductase1, Glutathione S-Transferase, and Glutamate Cysteine Ligase by Broccoli Seed and Isothiocyanates. *The Journal of Nutrition.*134*(Supl.)*: 3499-506.
33. Mann, G. E., Niehueser-Saran, J., Watson, A., Gao, L., Ishii, T., Winter, P. de, and Siow, R. C. M. 2007. Nrf2/ARE Regulated Antioxidant Gene Expression in Endothelial and Smooth Muscle Cells in Oxidative Stress: Implications for Atherosclerosis and Preeclampsia. *Acta Physiologica Sinica.* 59 (2):117-27.
34. Baird, L., Albena, T., and Dinkova-Kostova. 2011. The Cytoprotective Role of the Keap1–Nrf2 Pathway. *Arch Toxicol*. 85:241–72.
35. Bompa, T. O. 1994. *Theory and Methodology of Training:The Key to Athletic Performance*. 3rd. ed. Iowa: Kendall/Hunt Publishing Company. p: 57-70.
36. Son, T. G., Camandola, S. and Mattson, M. P. 2008. Hormetic Dietary Phytochemicals. *Neuromol Med*. 10: 236-46