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Effect of Temulawak (*Curcumin xanthorrhiza Roxb*) Extract on Reduction Of MDA (*Malondialdehyde*) Levels of Football Athletes

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Abstract: Temulawak has a role as antioxidant to prevent oxidative stress. This study aimed to assess the effect of temulawak extract on the MDA level (*Malondialdehyde*) of athletes. The double blind control trials were used, conducted on thirty five football athletes aged 14-18 years of Student Education and Exercise Center. The students were grouped and each received one of five different treatments for 21 days as follows: capsules of temulawak extract which contain of (1) curcumin of 250 mg (2) curcumin of 500 mg (3) curcumin of 750 mg, (4) capsule of multivitamin and mineral (which contain 5000 IU of beta carotene, 200 IU of vitamin E, 500 mg of vitamin C, Zn of 15 mg, selenium of 50 mcg) and (5) placebo, which received a capsule contain of cellulose (avizel). The characteristics of samples of five treatments were not significantly different ($p>0.05$). Before intervention were no significantly different on mean of MDA and SOD in five treatment groups ($p>0.05$). After intervention, the MDA level in all groups were decreased, except in placebo group. ANOVA test showed that there were mean differences of MDA level on five groups of interventions ($p<0.05$). ANCOVA test showed that mean reduction of MDA level on placebo group were significantly different with group received capsules which contain curcumin of 250, 500 and 750 mg/day and MVM ($p<0.05$). Temulawak extract had capability to reduce MDA level, significantly. The highest reduction of MDA level was done by administration of temulawak extract which contain curcumin 750 mg.

Key words: Temulawak, MDA (*Malondialdehyde*), SOD (superoxide dismutase), athletes

INTRODUCTION

Negative impact of strenuous physical activities is the development of free radical. This escalation was the consequences of oxygen consumption that increase, mobilization and activation of leukocyte, cytosol calcium proliferation, the increase of body temperature, hypohydration, exposure to air pollutant, inflammation, and the escalation of adrenaline secreation (Halliwell and Gutteridge, 1999; Pedersen and Hoffman, 2000; Nethery *et al*., 1999; Oh-Ishi *et al*., 1997; Suryohudoyo, 1999). The imbalance of free radical amount which was made inside the body with the natural antioxidant capacity to overcome them can trigger oxidative stress. The oxidative stress that caused by those free-radicals can be determined by measuring one of the parameters which is *malondialdehyde* (MDA). High MDA level in plasma shows cell has suffered for oxidative stress (Valko *et al*., 2006). Previous studies showed that MDA level on blood, liver, heart, and colon were significantly increase after physical activities administration were on maximal level to mice (Jawi *et al*., 2006) and human (Kiyatno, 2008). Oxidative stress on strenuous physical activities also been reported can affect athlete's physical fitness because it may cause an increase in muscle damage and soreness (Astuti, 2011; White *et al*., 2008; Cooke *et al*., 2010; Clarkson and Thompson, 2000; Udani and Singh, 2009) after intense exercise or competition, erythrocyte damage (Senturk *et al*., 2001) and hemoglobin level reduction (Senturk *et al*., 2005).

In normal condition, free radical occurrence will be balanced by endogen antioxidant. Body produces endogen antioxidant as SOD (*superoxide dismutase*), GPx (glutation peroxidase), and catalase. SOD is natural antioxidant in the shape of enzyme, very strong effect, and it is first body defense against free radical invasion. The more high SOD level the more optimal body defense against free radicals on cells and body organs. SOD also has a role to activate and drives the power of entire antioxidant defense system, including secondary antioxidant. To reduce and prevent the chain reaction of free radicals, body needs exogenous antioxidant from outside the body. Exogenous antioxidant such as vitamin E, vitamin A, vitamin C, Cu, Zn, Mn (Gordon, 1994; Simanjuntak, 2007; Winarsi, 2011).

Temulawak (*Curcumin xanthorrhiza Roxb*) already known as herb of traditional foods and medicines

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(Herman, 1985). Evidently, temulawak extract has antioxidant effect. Result of some studies showed that bioactive substance in temulawak rhizome were curcumin and demethoxycurcumin (Kunchandy and Rao, 1990; Tonnesen and Greenhill, 1992). Active ingredient curcuminoid inside temulawak has higher antioxidant effectiveness compared to active ingredient in each curcumin and demethoxycurcumin (Sutrisno *et al*., 2008). Another study showed that curcumin was more active than vitamin E, beta carotene and lipoic acid proven was by the phenolic, the methoxyl, the 1,3 diketone system and the enolisable styryl keton make significant contributions to the antioxidant properties of curcumin (Rao, 1985). This study aimed to assess the effect of temulawak extract administration to MDA level reduction in football athletes after physical exercise of running 5000 meters.

MATERIALS AND METHODS

This study conducted in Student Education and Exercise Center, Salatiga, Central Java, using double blind randomized controlled trial, which the inclusion criteria were male athlete, aged 14-18 years, healthy on physical examination and laboratory and did not suffer from either acute or chronic illness, non-anemic (≥13g/L), had normal nutritional status (>18,5-25,0), no consume of coffee, cigarette, alcohol (drink) and drugs, also antioxidant vitamin or other supplement at least two months before study, no exercise and games activity in addition to the program from training manager during research and maintain the recommended exercises to maintain fitness levels (conditions) the same during the study and agreed to become subject of the study by signed the informed consent. This study got the approval from Health Ethical Committee of Medical Faculty of Semarang Diponegoro University, no. 214/EC/FK/RSDK/2012.

Subject of the study was 35 athletes which grouped into 5 group interventions, as follows: group which received capsules of temulawak extract with curcumin content (ETKK) of (1) 250 mg/day, (2) 500 mg/day, (3) 750 mg/day, (4) group which received capsule of Multi Vitamin Mineral (MVM), consist of beta carotene 5000 IU, vitamin E 200 IU, vitamin C 500 mg, Zn 15 mg, Se 50 mcg (commercial product) and 5) placebo group which received capsules of cellulose (*avizel*). All groups receive each treatment for 17 days. A study from Kiyatno (2008) mentioned that the exact time of antioxidant administration was done after physical activity. After physical exercise (running 5000 m), they still received temulawak extract capsules and multivitamin mineral for four days.

Subject screening was conducted two months before intervention. Blood MDA measurement was conducted 48 h after physical activity of running 5000 m (Leeuwenburgh and Heinecke, 2001). *Superoxide dismutase* (SOD) measurement was done as soon as the intense physical activity test. On day sixth, temulawak extract capsule and multi vitamin mineral treatment was started to be given throughout 17 days. Dosage and duration of temulawak extract capsule administration was based on Davis *et al*. (2007), Soni and Kuttan (1992) along conversion factor from mice to human. Food intake data collection was conducted two days before treatment and two days during treatment. On day 23rd, it was conducted another physical activity of running 5000 m. Likewise, MDA examination was conducted 48 h after physical activity test and SOD measurement after physical activity test.

Characteristic of subjects including name, place and date of birth, hometown, parents' name and history of sport field occupied parents. Athletes' characteristics data were collected by interview and questionnaires. Medical checkup were conducted by sport doctor. Those data including physical and laboratory assessment along hospital sheet anamnesis. Nutritional status data was conducted by anthropometry assessments which were body weight and body height. Body weight was measured by using ACIS digital body weight with capacity of 150 kgs and accuracy of 0.1 kg. Body height was measured by using *somatometre microtoise* with accuracy of 0.1 cm. Food intake assessment was collected by using food record method and weighed food record. The food record and weighed food record were conducted two days before treatment and two days during treatment. Physical activity data were based on diary of daily activities that was stated in PAL (Physical Activity Level). All foods ingredients were known the raw weight and nutrients content were translated using Indonesian Food Composition Table. The conversion to nutrients was using NUTRSOFT software. Physical activity data was based on diary of daily activities. SOD measurement was conducted by using activity assay method using commercial kit of BioVision *Superoxide Dismutase* (SOD) Activity Assay Kit (Catalogue #K 335-100). MDA assessment was assessed by using ELISA method using commercial kit of Cusabio Human *Malondialdehyde* (MDA) ELISA Kit (Catalog No. CSB-E08557h). Hemoglobin blood level was measured using *cyanmethemoglobin* method.

Data which were collected was analyzed by univariate, bivariate and multivariate. Univariat test were presented in mean value, standard deviation, highest and lowest value. ANOVA statistical test was used to analyze effect of supplementation based on variables; MDA, SOD, vitamin A, C, E, Zn, Cu, Mn, Fe: before, during and after treatment to all five groups. Before the analysis, normality test of biomarker data was conducted using *Kolmogorov-Smirnov* test. Criteria of the analysis were $p>0.05$ accepting null hypothesis (Ho) that data had Mean of all samples' hemoglobin level before treatmentnormal distribution. Paired t-test was used to

analyze the difference between before and after treatment. ANCOVA test was used to adjustment confounding variable influence with MDA. Significant level that be used was 5% (Munro, 1997; Santoso, 2002).

RESULTS

Samples Characteristic: Age of the samples were in the range between 14-18 years with a mean of 16.65±0.84 years. All five treatment group had relatively similar age range. Anova statistical test showed that the age of five treatment group were not significantly different (p>0.05). Mean of body weight from all samples before treatment was 62.09±5.17 kg with range between 52-76.9 kg. Among five treatment group, athletes' body weight were relatively similar. ANOVA statistical test showed that there were no difference among five treatment group (p>0.05). Mean of body height from five treatment group were 171.90±5.47 cm with range between 161-181 cm. Those five groups had relatively similar body height (p>0.05). Mean of samples' BMI was 21.01±1.43 kg/m^2 with range between 18.98 - 24.41 kg/m^2. Those five groups had relatively similar BMI. Anova statistical test showed that there were no significantly difference among five group interview (p>0.05).

Overall mean PAL (Physical Activity Level) of samples of the study in one day had was 1.93±0.02 (medium activity) with range between 1.90-1.98. Based on treatment groups, it was found exercise activity, other activity, and total activities per day relatively similar. Anova statistical test between other activity and total activities per day among treatment groups were not show significant difference (p>0.05).

Result of the study showed thet all samples in Student Education and Exercise Center, Salatiga, Central Java had heredity factor of athletes either from father, mother, or both. Most of them had from father side (62.9%).

Coffee consumption habit was found in 13 samples (37.1%). In a week, mean of coffee consumption was 1.38±0.77 times. Antioxidant supplementation consumption habit was found in 20 athletes (57.1%). All athletes in MVM group of treatment consumed antioxidant supplement. From 20 athletes who consumed antioxidant supplement, 15 of them (75%) consumed multivitamin and mineral. On placebo group, all athletes were found consumed antioxidant such as multivitamin and mineral. This research did not find cigarette habit, alcohol drink habit, drugs, and anti inflammation on athletes. Medical checkup also did not find any athletes who had illness.

Capsules consumption compliance: Capsules were given 6 morsels every day, each was consumed two morsels in the morning, afternoon, and night time. The color of capsules was identical which bright red. Capsules were packed in transparent plastic contain of two capsules. Those packages were labeled in accordance with the athletes' name, medication time and date. Capsules were given to all athletes for 21 days. During intervention, athletes consumed 126 morsels. To monitor the compliance, each athlete was given compliance form, were filled each time capsule was consumed and signed it. After intervention, it was found that athletes' compliance of capsules consumption was 100%.

Antioxidant source food consumption: The highest consumption of vitamin A was 531.13±183.89 RE on treatment of temulawak extract with curcumin content of 250 mg/day and the lowest was 386.61±104.25 RE on treatment of temulawak extract with curcumin content of 750 mg/day. Anova test showed mean of vitamin A consumption before treatment on five treatment groups were not significantly different (p>0.05). Vitamin A consumption during intervention was increased as compared to before intervention (10.35%). If it was specified based on treatment group, then all treatment groups increased consumption of vitamin A. Nevertheless, it was still found decreased vitamin A consumption on athletes who were given temulawak extract with curcumin content of 250 mg of 37.35 RE (6.22%). After it was tested with anova statistical test, it was found that mean of vitamin A consumption during treatment on five treatment groups were not significantly different (p>0.05).

Vitamin C consumption during intervention was decreased 4.49%. The highest mean consumption of vitamin C was 81.71±10.01 mg on treatment of MVM and the lowest was 74.01±7.79 mg on treatment of temulawak extract with curcumin content of 500 mg/day. If it was specified based on treatment group, only group of MVM treatment was increased consumption of vitamin C of 7.71%. After it was tested with anova statistical test, it was found that mean of vitamin C consumption during treatment on five treatment groups were not significantly different (p>0.05).

During treatment, vitamin E consumption were increased, however there was one treatment group which also decreased the consumption of vitamin E. The treatment group which decreased was temulawak extract with curcumin content of 250 mg of 0.53 mg. The highest increase of vitamin E consumption during treatment was on group of temulawak extract with curcumin content of 500 mg of 1.84 mg. The highest mean of vitamin E consumption during treatment was on group of temulawak extract with curcumin content of 500 mg by 6.37±0.68 mg and the lowest was on group of temulawak extract with curcumin content of 750 mg by 4.89±1.98 mg. Anova test found that there were no differences of mean of vitamin E consumption during treatment among five treatment groups (p>0.05). Inadequacy of vitamin E was also found during treatment on either group of temulawak extract with curcumin content of 250 mg, 500 mg, 750 mg, or MVM and

placebo. The increase of vitamin E adequacy were occured on all treatment groups, except for the group that received temulawak extract with curcumin content of 250 mg were decreased by 3.56% of RDA. The highest escalation of vitamin E adequacy was on group of temulawak extract with curcumin content of 500 mg by 12.26% of RDA, while the lowest was on MVM treatment group by 2.36% of RDA. Based on Anova statistical test, there were no differences of vitamin E consumption among treatment groups during treatment (p>0.05).

During intervention, zinc adequacy level was found increasing on all treatment groups. If it was specified based on treatment group, it was found that only one group included in inadequate category, which was treatment of temulawak extract with curcumin content of 750 mg by 69.53±19.55% of RDA. The highest escalation of Zinc adequacy was on group of placebo by 18.37% of RDA, while the lowest was on group of temulawak extract with curcumin content of 250 mg by by 6.58% of RDA. Anova statistical test showed that there were no differences of mean of Zinc adequacy during treatment among five groups (p>0.05).

During treatment, mean of cuprum consumption in all treatment groups were increasing. The highest escalation was on treatment of MVM by 35.21%, while the lowest was on temulawak extract with curcumin content of 750 mg by 7.23%. The highest mean consumption of Cu during treatment on treatment was on MVM group by 1.92±0.24 mg and the lowest was on group of temulawak extract with curcumin content of 500 mg by1.74±0.18 mg. Anova statistical test found that there were no difference of mean of cuprum consumption among five treatment groups (p>0.05).

Mean consumption of mangan during intervention were decrease almost in all groups, except for MVM group. Consumption of mangan was increased by 9.37%. The highest reduction was on treatment of temulawak extract with curcumin content of 750 mg by 12.99%, while the lowest escalation was on group of temulawak extract with curcumin content of 500 mg by 6.09%. After they were tested using anova statistical test, it was found that there were no difference of mean of mangan consumption during treatment of five groups (p>0.05).

Mean consumption of iron during treatment were increase in all five groups. The highest increase was on group of temulawak extract with curcumin content of 250 mg by 3.29 mg (24.64%), while the lowest was on group of placebo treatment by 1.45% (10.48%). Anova statistical test showed that there were no difference of mean iron consumption among five group (p>0.05).

Superoxide dismutase (SOD) level : Before intervention, it was found that mean of SOD level on all treatment group was 74.51±11.67 U/mL range between 49.42-98.45 U/mL. The highest SOD level was on group of temulawak extract with curcumin content of 750 mg by 79.31±12.62 U/mL and the lowest SOD level was on group of temulawak extract with curcumin content of 250 mg by 70.37±12.84 U/mL. Based on anova statistical test, there were no difference of mean of SOD level among five groups before treatment (p>0.05).

SOD level after intervention was decrease on group of temulawak extract with curcumin content of 250 mg and MVM by -0.27±25.33 U/mL and -12.51±33.86 U/mL, respectively. The highest increase of SOD level (△SOD) was found in group of temulawak extract with curcumin content of 750 mg by 13,07±16,74 U/mL, while the lowest was on group of temulawak extract with curcumin content of 750 mg by 3.21±11.04 U/mL. After it was tested with anova statistical test, it was found that mean of SOD level on five treatment groups were not significantly different (p>0.05).

***Malondialdehyde* Level (MDA) :** Mean of MDA level before intervention on all treatment groups was 953.65±355.76 ppm ranged between 388.76-2100 ppm. The highest mean of MDA level was on group of temulawak extract with curcumin content of 750 mg by 1160.95±516.58 ppm and the lowest was on group of temulawak extract with curcumin content of 250 mg by 821.02±236.43 ppm. Based on anova statistical test, there were no differences of mean of MDA level among five groups before intervention (p>0.05).

After intervention, MDA level was reduce on all of treatment groups, except on placebo group. The increase of MDA level on placebo group was 104.36±207.07 ppm. The highest reduction of MDA level was on group of temulawak extract with curcumin content of 750 mg by 243.49±170.18 ppm, while the lowest on group of temulawak extract with curcumin content of 250 mg by 77.08±104.77 ppm. Anova statistical test showed that there were differences of mean of MDA level among five groups (p<0.05). MDA level on placebo group was significantly difference with temulawak extract with curcumin content of 500 mg, 750 mg, and MVM (p<0.05), while placebo with temulawak extract with curcumin content of 250 mg; temulawak extract with curcumin content of 500 mg with temulawak extract with curcumin content of 250 mg, 750 mg, MVM, each; temulawak extract with curcumin content of 750 mg with temulawak extract with curcumin content of 250 mg, 500 mg, each; and MVM with temulawak extract with curcumin content of 750 mg, were not significantly different (p>0.05).

All covariate variables which had possibilities effecting MDA level were included into analysis. Those variables were MDA level (before intervention), adequacy of vitamin A, vitamin C, vitamin E, Zn, Cu, Fe, Mn (during intervention), and SOD (after intervention). Ancova statistical test showed that Δ MDA level after intervention was influenced by MDA level before intervention and treatment variation (p<0.05), however other variables were not significantly effected (p>0.05). Post-hoc test, LSD, showed that mean reduction of MDA level

Table 1: Characteristic of samples before treatment

Characteristic of samples	Placebo (n = 7)	ETKK* 250 mg (n = 7)	ETKK* 500 mg (n = 7)	ETKK* 750 mg (n = 7)	MVM (n = 7)	p[1]
Age (year)	16.23±1.16[a]	16.75±0.54[a]	16.41±0.69[a]	17.25±0.91[a]	16.60±0.63[a]	0.196
Body weight (kg)	59.14±4.14[a]	64.29±1.60[a]	60.47±14.54[a]	64.56±8.50[a]	63.43±3.31[a]	0.163
Body height (cm)	169.74±4.89[a]	174.86±4.39[a]	172.43±6.78[a]	168.99±5.92[a]	173.57±3.95[a]	0.205
BMI (kg m^{-2})	20.54±1.03[a]	21.05±0.92[a]	20.40±1.85[a]	22.40±1.83[a]	21.07±1.19[a]	0.096
Hemoglobin (g dL^{-1})	13.92±0.83[a]	14.74±0.48[a]	14.17±0.88[a]	14.11±1.08[a]	13.89±0.63[a]	0.302
Total activities per day	1.94±0.02[a]	1.93±0.01[a]	1.93±0.01[a]	1.93±0.02[a]	1.92±0.02[a]	0.530

*ETKK: temulawak extract with curcumin content (Ekstrak Temulawak dengan Kandungan Kurkumin)

[1]p-value of ANOVA result on the s[a]me row

[a,b,c]same alphabet in one row showed that there were no significantly difference among treatments (anova test, p>0.05)

Table 2: Samples distribution based on ancestry, habit, antioxidant supplement consumption habit, coffee drinking before treatment

Characteristic of samples	Placebo (n = 7)	ETKK* 250 mg (n = 7)	ETKK* 500 mg (n = 7)	ETKK* 750 mg (n = 7)	MVM (n = 7)	p[1]
Ancestry						
Father	3 (42.9%)	6 (85.7%)	5 (71.4%)	3 (50%)	5 (71.4%)	0.875
Mother	2 (28.6%)	0 (0%)	1 (14.4%)	1 (16.7%)	1 (14.4%)	
Both parents	2 (28.6%)	1 (14.3%)	1 (14.3%)	2 (33.3%)	1 (14.3%)	
Antioxidant supplement consumption habit					0.028	
Yes	2 (28.6%)	5 (71.4%)	4 (57.1%)	2 (28.6%)	7 (100%)	
No	5 (71.4%)	2 (28.6%)	3 (42.9%)	5 (71.4%)	0 (0%)	
Type of antioxidant supplement						0.319
Vitamin	0 (0%)	0 (0%)	2 (50%)	1 (50%)	2 (28.6%)	
Multivitamin Mineral	2 (100%)	5 (100%)	2 (50%)	1 (50%)	5 (71.4%)	
Coffee drinking habit						0.017
Yes	1 (14.3%)	5 (71.4%)	1 (14.3%)	5 (71.4%)	1 (14.3%)	
No	6 (85.7%)	2 (28.6%)	6 (85.7%)	2 (28.6%)	6 (85.7%)	

*ETKK: Temulawak extract with curcuma content (Ekstrak Temulawak dengan Kandungan Kurkumin)

Table 3: Mean of antioxidant source vitamin consumption based on treatment group before and during treatment

Antioxidant vitamin	Placebo (n = 7)	ETKK* 250 mg (n = 7)	ETKK* 500 mg (n = 7)	ETKK* 750 mg (n = 7)	MVM (n = 7)	P[1]
Vitamin A consumption						
Before						
Intake (mg)	446.27±103.81[a]	531.13±183.89[a]	407.70±87.72[a]	386.61±104.25[a]	444.34±101.79[a]	0.241
RDA (%)	74.38±17.30[a]	88.52±30.65[a]	67.95±14.62[a]	64.44±17.37[a]	74.06±16.97[a]	0.410
During						
Intake (mg)	510.07±148.67[a]	493.78±207.51[a]	585.91±62.76[a]	450.14±182.52[a]	486.68±108.97[a]	0.554
RDA (%)	85.01±24.78[a]	82.30±34.58[a]	97.65±10.46[a]	75.02±30.42[a]	81.11±18.16[a]	0.554
Vitamin C consumption						
Before						
Intake (mg)	85.80±7.08[a]	82.56±5.39[abc]	78.77±7.01[abc]	87.05±11.34[b]	74.70±6.03[c]	0.030
RDA (%)	103.14±9.16[a]	91.73±5.99[b]	92.14±7.13[bc]	96.72±12.60[abcd]	85.17±6.58[bcd]	0.008
During						
Intake (mg)	80.05±8.69[a]	77.65±8.74[a]	74.01±7.79[a]	75.67±9.83[a]	81.71±10.01[a]	0.511
RDA (%)	96.77±15.33[a]	86.27±9.71[a]	86.47±7.08[a]	84.07±10.92[a]	92.88±8.28[a]	0.166
Vitamin E consumption						
Before						
Intake (mg)	4.96±1.15[a]	5.90±2.04[a]	4.53±.97[a]	4.30±1.16[a]	4.94±1.13[a]	0.241
RDA (%)	33.06±7.69[a]	39.34±13.62[a]	30.20±6.50[a]	28.64±7.72[a]	32.91±7.54[a]	0.241
During						
Intake (mg)	5.54±1.62[a]	5.37±2.26[a]	6.37±.68[a]	4.89±1.98[a]	5.29±1.184[a]	0.554
RDA (%)	36.96±10.77[a]	35.78±15.04[a]	42.46±4.55[a]	32.62±13.23[a]	35.27±7.90[a]	0.554

*ETKK: Temulawak extract with curcuma content (Ekstrak Temulawak dengan Kandungan Kurkumin)

[1]p-value of anova test in the same row

[a,b,c]Same alphabet in one row showed that there were no significant differences among treatment (anova test, p>0.05)

(adjusted) on placebo group was significantly different with temulawak extract with curcumin content of 250 mg, 500 mg, 750 mg, and MVM (p<0.05), while within other treatment groups were not significantly different (p>0.05).

DISCUSSION

Oxidative stress condition usually occurred when number of free radical inside the body is higher than number of antioxidant system. Oxidative stress which caused by those free radicals can be determined by

Table 4: Mean of antioxidant source mineral consumption based on treatment group before and during treatment

Antioxidant mineral	Placebo (n = 7)	ETKK* 250 mg (n = 7)	ETKK* 500 mg (n = 7)	ETKK* 750 mg (n = 7)	MVM (n = 7)	P[1]
Zinc consumption						
Before						
Intake (mg)	11.50±3.00^{abc}	13.43±4.10^{ac}	10.57±1.14^{abc}	8.72±2.18^{b}	11.81±2.18^{c}	0.040
RDA (%)	66.84±17.00^{abc}	79.03±24.12^{ac}	61.83±7.08^{abc}	51.28±12.82^{b}	69.01±12.82^{c}	0.039
During						
Intake (mg)	14.61±3.44^{a}	14.55±3.83^{a}	12.27±4.41^{a}	11.82±3.32^{a}	12.97±3.46^{a}	0.508
RDA (%)	85.21±20.43^{a}	85.61±22.50^{a}	71.69±25.74^{a}	69.53±19.55^{a}	75.73±20.03^{a}	0.515
Cuprum consumption						
Before						
Intake (mg)	1.63±0.13^{a}	1.57±0.10^{abc}	1.50±0.13^{abc}	1.66±0.22^{ab}	1.42±0.11^{c}	0.030
RDA (%)	108.96±8.99^{a}	104.84±6.85^{abc}	100.03±8.91^{abc}	110.54±14.40^{ab}	94.86±7.66^{c}	0.030
During						
Intake (mg)	1.88±0.20^{a}	1.82±0.21^{a}	1.74±0.18^{a}	1.78±0.23^{a}	1.92±0.24^{a}	0.511
RDA (%)	125.27±13.61^{a}	121.51±13.67^{a}	115.82±12.18^{a}	118.41±15.38^{a}	127.87±15.67^{a}	0.511
Mangaan consumption						
Before						
Intake (mg)	8.58±0.71^{a}	8.26±0.54^{abc}	7.88±0.70 abc	8.70±1.13^{ab}	7.47±0.60^{c}	0.030
RDA (%)	379.99±27.72^{a}	358.95±23.45^{abc}	344.46±27.74^{abc}	378.48±49.29^{ab}	328.76±23.82^{c}	0.022
During						
Intake (mg)	8.00±0.87^{a}	7.76±0.87^{a}	7.40±0.78^{a}	7.57±0.98^{a}	8.17±1.00^{a}	0.511
RDA (%)	354.99±40.81^{a}	337.59±37.98^{a}	323.54±31.03^{a}	328.97±42.72^{a}	359.35±39.88^{a}	0.347
Iron consumption						
Before						
Intake (mg)	13.83±1.18^{a}	13.35±0.77^{a}	13.03±1.21^{a}	12.91±0.83^{a}	13.95±0.63^{a}	0.181
RDA (%)	83.53±9.82^{a}	89.02±5.10^{a}	84.53±12.43^{a}	86.08±5.51^{a}	87.40±10.14^{a}	0.799
During						
Intake (mg)	15.28±1.66^{a}	16.54±2.66^{a}	14.94±2.36a	14.17±0.77a	16.27±2.64a	0.243
RDA (%)	91.70±4.58^{a}	110.26±17.72^{a}	96.95±19.24a	94.47±5.11a	101.61±18.65a	0.173

*ETKK: Temulawak extract with curcuma content (Ekstrak Temulawak dengan Kandungan Kurkumin)

[1]p-value of anova test in the same row

[a,b,c]Same alphabet in one row showed that there were no significant differences among treatment (anova test, p>0.05)

measuring one of parameters of malondialdehid (MDA). High MDA level inside plasma shows cell was having oxidative stress (Volko *et al*., 2006). MDA level before intervention compared with MDA level after intervention in all four treatments (temulawak extract with curcumin content of 250 mg, 500 mg, 750 mg, and MVM) were decreased compared to control. It showed that temulawak extract as source of natural antioxidant. In temulawak which contain curcuminoid consist of two compounds, which are curcumin and bisdemethoxycurcumin. Curcumin has the highest antioxidant activity compared to demethoxycurcumin (Jayaprakasha *et al*., 2005).

The highest reduction on mean of MDA level of five treatments was on administration of temulawak extract with curcumin content of 750 mg. This finding was in line with a study from Rao (1985) that curcumin was more active compared to vitamin E and beta carotene. Curcumin's role as antioxidant to counteract free radicals can not be separated from the structure of curcumin's compound. Curcumin has important role in the proses of antioxidant. Curcumin's structure consists of fenolic-hidroxy and ß diketon fraction. Fenolic-hidroxy fraction was function as free radical snare on first phase of antioxidative mechanism. In structure of curcumin's compound, there were 2 fenolic fractions; hence one curcumin molecule can avert two free radicals. Franction of ß diketon was function as free radical snare on next phase. Those structures of curcumin have significant contribution to the nature of curcumin's antioxidant (Tonnesen and Greenhill, 1992; Majeed *et al*., 1995). The tendency of MDA reduction in this study was in accordance with study from Hussein and Zinadah (2010) on wistar mice which had diabetes with curcumin administration during seven weeks. As well as study from Kalpravidh *et al*. (2010) on patients of thalacaemia, there was tendency of MDA plasma reduction after being given 500 mg curcuminoid during 12 months. Thus, curcuminoid can be used to repair oxidative damage on patient of thalacaemia.

In treatment group of MVM there was reduction of MDA level before compared to after intervention. Thus antioxidant defence system of vitamin mineral is capable to against oxidative stress that occurred. It also indicates that administration of MVM can provide protection against exercise-induced oxidative stress. This result was in line with study from Kiyatno (2009), that there was effect of multi vitamin mineral administration to MDA level reduction on student of FKIP-JPOK UNS, Surakarta. Vitamin antioxidant administration after physical activities leads to smaller plasma MDA level or smaller muscle damage. High MDA level indicate muscle

Table 5: Mean of SOD level based on treatment group before and after intervention

SOD Level (U mL^{-1})	Placebo (n = 7)	ETKK* 250 mg (n = 7)	ETKK* 500 mg (n = 7)	ETKK* 750 mg (n = 7)	MVM (n = 7)	P[1]
Before	72.29±13.73[a]	70.37±12.84[a]	79.18±5.45[a]	79.31±12.62[a]	75.42±13.29[a]	0.699
After	76.97±18.66[a]	70.10±19.06[a]	82.39±12.59[a]	88.37±6.20[a]	62.90±27.75[a]	0.107
Deviation	4.68±13.57[a]	-0.27±25.33[a]	3.21±11.04[a]	13.07±16.74[a]	-12.51±33.86[a]	0.301

*ETKK: Temulawak extract with curcuma content (Ekstrak Temulawak dengan Kandungan Kurkumin)

[1]p-value of anova test in the same row

[a]Same alphabet in one row showed that there were no significant differences among treatment (anova test, p>0.05)

Table 6: Mean of MDA Level Based on Treatment Group Before and After Intervention

MDA Level (ppm)	Placebo (n = 7)	ETKK* 250 mg (n = 7)	ETKK* 500 mg (n = 7)	ETKK* 750 mg (n = 7)	MVM (n = 7)	P[1]
Before	991.32±318.90[a]	821.02±236.43[a]	897.15±222.93[a]	1160.95±516.58[a]	897.82±404.20[a]	0.456
After	1095.68±265. 77[a]	743.94±189.67[a]	757.52±69.13[a]	917.46±560.06[a,2]	690.70±181.37[a]	0.106
Deviation	104.36±207.07[a]	-77.08±104.77[ab]	-139.63±221.01[b]	-243.49±170.18[b]	-207.12±270.6[b]	0.026

*ETKK: Temulawak extract with curcuma content (Ekstrak Temulawak dengan Kandungan Kurkumin)

[1]p-value of ANOVA test in the same row

[a,b]Same alphabet in one row showed that there were no significant differences among treatment (ANOVA test, p>0.05)

[2]significantly different before intervention (t-test, p<0.05)

damage is also large. This damage is due to the increase of free radical improvement or unbalance antioxidant formation with free radical development (Kim *et al*., 1996).

In the control group without addition of either temulawak extract or multi vitamin mineral MDA level were increased. According to Suroyo (2001), there was no protection mechanism for tissue against free radical attack that was formed, therefore the more lipids were attacked by free radicals to produce MDA.

Mean of MDA level reduction after being corrected (adjusted) on group of placebo treatment was significantly different with temulawak extract with curcumin content of 250 mg, 500 mg, 750 mg, and MVM ($p<0.05$), whereas among other treatment groups were not significantly different ($p>0.05$). Result's study of Setiawan and Ernawati (2007), showed that administration of curcumin 200 mg/kg BW and 400 mg/kg BW was able to reduce plasma MDA level on rats under stress (they were given swim activity). Study of Shen *et al*. (2012) showed the similar result, curcumin decrease MDA accumulation and lipid peroxide on drosophila. Study from Hosseinzadeh *et al*. (2013) on rats, showed that curcumin supplement was able to decrease plasma MDA level significantly after they were given treadmill treatment five times a week throughout eight weeks (15-22 m/minute, 25-64 minute).

In this study, the existence of SOD was not able to give significant contribution to MDA reduction. Similarly, exogenic sources of antioxidant from daily foods also needed to minimize oxidative stress such as vitamin C, vitamin E, vitamin A, Zn, Cu, Mn, and Fe. Therefore, the existence of vitamin C, vitamin E, vitamin A, Zinc, Cuprum, Mangan, and Fe, through foods was not able to give significant contribution to MDA reduction. It was due to food and nutrition consumption during intervention and among five treatment groups relatively equal either group of temulawak extract with curcumin content, MVM, or control. The increase of food and nutrition consumption not only occurred on treatment groups but also on control group. Temulawak extract was able to reduce atheletes' MDA level significantly. The highest decrease of MDA was on administration of temulawak extract with curcumin content of 750 mg.

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