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# THE ANTIOXIDANT EFFECT OF COMPOUND E JIAO JIANG ON BALL GAME PLAYERS ENGAGING IN ENDURANCE TRAINING

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#### ABSTRACT

This study was designed to explore the antioxidant effect of compound E Jiao Jiang on ball game players engaging in endurance training, aiming to provide a theoretical basis for the effectiveness and security of compound E Jiao Jiang in the application of fatigue resistance of ball game players. Totally 50 ball game players, including 25 males and 25 females, were selected and divided into control group (placebo 3 times/day, one dose/time), experimental group (compound E Jiao Jiang 3 times/day, one dose/time) and high-dose group (compound E Jiao Jiang 3 times/day, two doses/time) using a double-blind design. Under the same training program, each group took compound E Jiao Jiang for 4 weeks according to prescription. Subjects' antioxidant indexes were measured before test, after 2 and 4 weeks oral administration respectively. Results revealed that, after 2 and 4 weeks of oral administration, methane dicarboxylic aldehyde (MDA) content decreased (P<0.01); superoxide dismutase (SOD) and catalase (CAT) content in experimental group and high-dose group increased as dosing time prolonged (P < 0.01, P < 0.05); MDA and carbonyl content in experimental group and high-dose group decreased more and more apparently after taking medication for 2 and 4 weeks (P < 0.01, P < 0.01). Besides, the antioxidant ability showed a time-response relation. After 2 weeks of administration, all subjects were found with reduced MDA content in experimental group and high-dose group (P < 0.01); SOD and haemoglobin (Hb) in male subjects in experimental group rose (P<0.05); MDA content in male subjects in high-dose group dropped (P<0.01); and CAT (P<0.05), Hb (P<0.01) and total antioxidant capacity (T-AOC) (P<0.05) in female subjects in experimental group increased. After 4 weeks of administration, the antioxidant ability was observed without dose-response relationship. Thus, it can be known that compound E Jiao Jiang is capable of enhancing Hb content and maximum oxygen uptake of athletes, improving athletes' oxygen carrying capacity as well as aerobic endurance and also resisting fatigue.

### 1. Introduction

An adequate and regular exercise, belonging to a special kind of stressor, is able to change shape and function of the heart positively and actively, increase the contractility of cardiac muscle and reduce the risks of the occurrence of cardiovascular disease. However, excessive exercise will damage morphological structure and function of the myocardial tissues (Veneroso et al., 2009; Berzosa et al., 2010; Comstock et al., 2013; Dolezal et al., 2000). Severe myocardial damage is likely to cause sudden death. Antioxidant substance existing at a low concentration can inhibit the oxidation reaction of free radicals effectively, which can directly act on free radicals, and also indirectly consume material easy to produce free radicals away, to prevent further reaction (Esmaeili and Sonboli, 2009). Human body produces free radicals, and meanwhile, generates antioxidants, so as to offset the oxidation attacks of free radicals to human cells. The stronger antioxidant ability of human body is likely to have better effect in eliminating free radicals. There are a variety of methods for enhancing antioxidant ability, improving the activity involving of antioxidant enzyme, increasing the amount, reducing the intake of materials easy to produce free radicals and increasing the intake of materials with antioxidant ability. A study (Hongzhong et al., 2007) reports that E Jiao Jiang supplements have significant effects in promoting red blood cells and haemoglobin (Hb) of ischemic animals and accelerating the differentiation proliferation and of hematopoietic stem cell (HSC). Compound E Jiao Jiang as a compound traditional Chinese medicine well promotes the hematopoietic function of marrow. Oxygen of each cell in the whole body increases and fatigue resistance strengthens with the improvement hemachrome, which of make athletes tolerance of high intensity exercise training (Maoxuan et al., 2014). Based on the current situation of traditional Chinese medicine tonic and sports fatigue, this study measures the antioxidant indexes of athletes after taking compound E Jiao Jiang, to confirm whether compound E Jiao Jiang can strengthen the antioxidant ability of body, thereby providing a theoretical foundation for the application of

oral administration of compound E Jiao Jiang in athlete's physical exercise.

## 2. Materials and methods

## 2.1. Research objects

Fifty ball game players were selected as research objects, including 25 males and 25 females, with the age ranging from 18 to 25 years. They were randomly divided into three groups applying double blind method, i.e., control group, experimental group and highdose group. All subjects ate and trained normally, athletes in control group were treated with oral administration of placebo that had the similar appearance and taste with compound E Jiao Jiang three times a day, one dose per time; experimental group took compound E Jiao Jiang three times a day, one dose per time; and athletes in high-dose group received the oral administration of compound E Jiao Jiang three times a day, two doses per time. All three groups took medication half an hour before meals. The qualified athletes were included as study subjects after they were screened based on grouping criteria, and research objects signed the informed consent.

# 2.2. Experimental methods

This experiment collected and detected samples three times, i.e., before oral administration, after 2 and 4 weeks of oral administration respectively, lasting 4 weeks.

# (1)Collection of blood samples

Blood samples were collected from fingertip and vein of subjects with an empty belly in the early morning, finger-tip blood was used for Hb measurement, and venous blood was preserved in the refrigerator at - 80 °C for future application after serum was separated through stewing and centrifuging and serum samples were marked.

(2)Measurement of maximum oxygen uptake and blood antioxidant

Maximum oxygen uptake was measured through Astrand-Ryhnuiy method to predict the maximum oxygen uptake of subjects, and the data was recorded. Blood antioxidant indexes were detected using relevant antioxidant index kit as per the instructions. performed Biochemical test was on corresponding indexes, and data was measured and recorded.

### 2.3. Documentation methods

To acquire the current situation of the field and the latest studies, relevant literatures on colla corii asini, ginseng, radix rehmanniae praeparata, compound E Jiao Jiang, antioxidant, fatigue resistance, index of athletes' body function were collected, sorted and analyzed by searching PUBMED, China National Knowledge Infrastructure (CNKI) and other professional websites, so as to provide a theoretical foundation for writing this thesis.

### 2.4. Statistics

Data was statistically processed using SPSS 17.0 software and expressed as mean  $\pm$ 

standard deviation (SD). Test indexes measured before oral administration, after 2 and 4 weeks of oral administration received normal distribution test, and all of them were observed to be normally distributed (P>0.05). Bonferroni multiple comparison method from analysis of variance methods was considered as the statistical method for measuring the design repeatedly. The difference was considered to be statistically significant if P<0.05 and extremely significant in statistics if P<0.01.

### **3. Results and discussions**

### 3.1. Analysis of general materials

Stature, weight, systolic pressure, diastolic pressure and average training year of athletes are displayed in Table 1. Before the test, natural conditions of subjects were collected to make the following comparison between some variables more comparable.

	Gender	Stature	Weight	Systolic	Diastolic	Heart rate	Average
		(cm)	( <b>kg</b> )	pressure	pressure	(times/min)	training
				(mmHg)	(mmHg)		year
Control	Male	168.35±5.53	76.78±14.4	118.24±7.54	74.76±5.76	60.53±7.71	6.3
group			6				
	Female	164.63±2.43	62.23±11.6	107.18±3.35	71.50±2.68	66.42±11.20	6.5
			6				
Experim	Male	169.53±5.96	71.92±12.0	117.23±10.8	74.75±5.76	60.64±7.72	6.7
ental			1	3			
group	Female	163.42±2.39	58.73±6.75	112.01±8.05	72.64±6.95	72.49±10.62	6.6
High-	Male	169.43±5.85	67.34±12.0	117.28±11.3	71.26±4.96	63.85±11.73	6.7
dose			3	4			
group	Female	162.65±2.53	59.46±9.86	107.50±6.82	70.53±2.54	78.01±6.72	6.9

Table 1. Basic physical information of subjects

# **3.2. Test results of antioxidant indexes in serum**

Before the experiment, antioxidant indexes measured in this experiment, such as catalase (CAT), superoxide dismutase (SOD), methane dicarboxylic aldehyde (MDA), carbonyl and Total antioxidant capacity (T-AOC) received mean T test. Results showed that CAT, SOD, MDA and T-AOC had no difference in gender, while carbonyl had, and the content of carbonyl in male athletes was significantly higher than in female athletes (P<0.05) (Table 2). Only when plasma concentration achieved an effective level as drug entered into blood,

could drug take effect by acting on the target point. Test results of male and female players

were set as follows because plasma concentration was affected by gender.

	Females	Males
SOD (U/ml)	52.815±6.382	52.934±4.418
CAT (U/ml)	2.621±0.265	2.564±0.217
MDA (nmol/ml)	4.985±0.62	4.971±0.812
T-AOC (U/ml)	16.654±1.852	16.925±1.031
Carbonyl (mg/ml)	0.349±0.067*	0.428±0.104

**Table 2.** Difference of antioxidant indexes in gender before test (mean  $\pm$  SD)

Note: \*: P<0.05 while comparing female athletes with male athletes, suggesting a statistical significance.

### 3.2.1. Test results of CAT content

(1) Dose-response relationship.

The content of CAT in all athletes after taking compound E Jiao Jiang increased to some extent in the same test cycle, but had no statistical significant difference. Details are shown in Figure 1.



Figure 1. CAT content in all athletes

After male athletes took compound E Jiao Jiang, the pairwise comparison between control group, experimental group and high-

dose group in the same test cycle was not statistically significant Figure 2).



Figure 2. CAT content in male athletes

Comparing female subjects in experimental group with control group after 2 weeks of oral administration, CAT content



increased, and the difference had a statistical significance (P < 0.05), as shown in Figure 3.

Figure 3. CAT content in female athletes

(2)Time-response relationship

After all athlete subjects took compound E Jiao Jiang, the content of CAT in experimental group after 2 and 4 weeks of oral administration increased significantly as time went on in comparison with before experiment (P<0.01). However, the content in high-dose group was in an increasing trend, without statistical significance. CAT content of male subjects in high-dose group rose gradually with time, and increased after 4 weeks of oral administration, the difference was statistically significant (P<0.05); while the content in control group and experimental group had a tendency to increase slightly, but without statistical significance. Compared with before experiment, CAT content of female subjects in experimental group increased notably after 2 weeks of administration with time, and there was a statistical significance in the differences (P<0.05), and it increased more markedly after 4 weeks of administration (P<0.05). However, as time went on, the content only showed a certain rising trend in high-dose group, and without statistical significance.

(3)Influence of gender on CAT content

Data indicated that the content of CAT in male and female players taking the same dosage in the same time had no difference (P>0.05) (Table 3).

	Before experiment		After 2 weeks of oral		After 4 weeks of oral	
	_		administration		administration	
	Male	Female	Male	Female	Male	Female
Control group	2.557±0.125	2.625±0.244	$2.674 \pm 0.605$	2.451±0.301	2.958±0.513	2.497±0.365
Experimental	2.553±0.215	2.670±0.185	2.756±0.419	3.272±0.574	3.065±0.474	3.274±0.585
group						
High-dose group	2.584±0.302	2.546±0.360	2.678±0.365	2.933±0.664	2.945±0.505	3.012±0.831

**Table 3.** Comparison of CAT content in male and female (mean  $\pm$  SD)

Compared with subjects sharing the same gender and taking compound E Jiao Jiang at the same time in control group, all subjects in

3.2.2. Test results of SOD content

experimental group and high-dose group were observed with very significantly increased SOD content after 4 weeks of oral administration, and there was a verv significant difference (P < 0.01). Comparing male in experimental group with control group after 2 weeks of oral administration, SOD content increased obviously, with a significant difference (P < 0.05); and the content in male in experimental group and high-dose group after 4 weeks of oral administration increased very notably, with a statistical significance (P < 0.01). In comparison with subjects of the same sex taking compound E Jiao Jiang at the same dose before experiment, SOD content in all subjects in high-dose group increased very markedly after 4 weeks of oral administration (P < 0.01), the content in male subjects in experimental group increased significantly after 4 weeks of oral administration (P < 0.01), and the content in female subjects in experimental increased group verv significantly after 2 and 4 weeks of oral administration (*P*<0.01). After female subjects in high-dose group took compound E Jiao Jiang for 4 weeks, SOD content increased obviously (P < 0.05). Details are displayed in Table 4.

		Before experiment	After 2 weeks of oral administration	After 4 weeks of oral administration
Control group	Male (n=8)	50.225±3.554	45.812±5.714	44.664±4.253
	Female (n=8)	53.688±4.611	56.295±7.564	54.172±5.418
	All of them (n=16)	51.954±4.362	51.042±8.447	49.415±6.801
Experimental	Male (n=8)	54.514±2.625	62.131±9.248*	63.994±5.427** ##
group	Female (n=8)	53.861±4.902	56.815±5.818##	56.331±6.048##
	All of them (n=16)	54.184±3.828	59.454±7.979	60.162±6.826**
High-dose group	Male (n=8)	53.835±5.826	54.445±6.852	57.904±7.265**
	Female (n=8)	50.775±6.612	54.002±3.486	57.813±4.898#
	All of them (n=16)	52.301±6.225	54.204±5.245	57.854±5.987** ## 00

Table 4	Test results	of SOD content	(mean + SD)
1 aut 4.	1 CSt ICSUILS	OI SOD COMEN	$(\Pi \cup a \Pi \perp s D)$

Note: compared with subjects sharing the same gender and taking compound E Jiao Jiang at the same time in control group, \*: P<0.05, suggesting a significant difference; \*\*: P<0.01, suggesting a very significant difference; compared with subjects of the same sex taking compound E Jiao Jiang at the same dose before experiment, #: P<0.05, suggesting a significant difference; ##: P<0.01, suggesting a very significant difference; compared with subjects sharing the same gender and taking compound E Jiao Jiang at the same dose for 2 weeks,  $\circ: P<0.05$ , suggesting a significant difference;  $\circ: P<0.01$ , suggesting a very significant difference.

## **3.3. Free radical injury markers**

### 3.3.1. MDA test results

Compared with subjects sharing the same gender and taking compound E Jiao Jiang at the same time in control group, all subjects in experimental group and high-dose group were observed with very significantly decreased MDA content after 2 and 4 weeks of oral administration (P<0.01). Comparing male in high-dose group with control group after 2 weeks of oral administration, we found MDA content was reduced obviously (P<0.05); and the content after 4 weeks of oral

administration dropped notably very (P < 0.01). Female subjects in high-dose group after 4 weeks of oral administration were found with obviously decreased MDA content (P < 0.05). In comparison with subjects of the same sex taking compound E Jiao Jiang at the same dose before experiment, MDA content in all subjects declined after 2 and 4 weeks of oral administration, and the difference was very statistically significant (P < 0.01), the content in male subjects in experimental group decreased very significantly after 4 weeks of oral administration (P < 0.01); the content

decreased significantly after 2 and 4 weeks of oral administration at high dose, and the difference had a very statistically significance (P < 0.01); female subjects in experimental group and high-dose group were observed with a very notably declined MDA content after 2 and 4 weeks of oral administration (P < 0.01). The MDA content in high-dose group decreased very significantly after subjects took compound E Jiao Jiang for 4 weeks compared with subjects sharing the same gender and taking compound E Jiao Jiang at the same dose for 2 weeks (P < 0.01), and female subjects in high-dose group had a markedly dropped MDA content after 4 weeks of oral administration (P < 0.05).

### 3.3.2. Carbonyl test results

Compared with subjects sharing the same gender and taking compound E Jiao Jiang at the same time in control group, all subjects in experimental group and high-dose group were observed with decreased carbonyl content after 4 weeks of oral administration, and the difference was very statistically significant (P<0.01). Female subjects in experimental group had a markedly dropped carbonyl content after 2 weeks of oral administration, and the difference was significant (P<0.05). In comparison with subjects of the same sex taking compound E Jiao Jiang at the same dose before experiment, carbonyl content in

experimental group and high-dose group declined significantly after 4 weeks of oral administration (P<0.01); female subjects in high-dose group were observed with a notably dropped carbonyl content after 4 weeks of oral administration, with a statistical significance (P<0.05). The carbonyl content in female subjects in experimental group decreased markedly after 4 weeks of oral administration compared with subjects sharing the same gender who took compound E Jiao Jiang at the same dose for 2 weeks (P<0.05). No statistical significance was found in the remaining pairwise comparison.

### **3.4. T-AOC**

Table 5 shows the T-AOC results detected three times from subjects sharing different genders and taking compound E Jiao Jiang at various doses. Compared with subjects sharing the same gender and taking compound E Jiao Jiang at the same time in control group, all athletes' T-AOC in experimental group were observed to be significantly increased after taking compound E Jiao Jiang for 4 weeks, and the difference was statistically significant (P < 0.05). After taking compound E Jiao Jiang for 2 weeks, females subjects in experimental group had notably increased T-AOC in comparison with control group (P < 0.05). The rest of pairwise comparison had no statistical significance.

		Before experiment	After 2 weeks of	After 4 weeks of
			oral administration	oral administration
Control group	Male (n=8)	17.221±0.574	17.905±0.784	17.277±1.612
	Female (n=8)	15.795±1.818	14.752±2.785	14.032±1.922
	All of them (n=16)	16.504±1.522	16.324±2.566	$15.644 \pm 2.402$
Experimental	Male (n=8)	16.685±1.552	17.424±1.435	18.085±1.398
group	Female (n=8)	17.671±1.533	16.722±1.948*	17.642±1.616
	All of them (n=16)	17.174±1.569	17.063±1.695	17.861±1.485*
High-dose	Male (n=8)	16.867±0.663	17.127±1.042	17.172±1.185
	Female (n=8)	16.392±1.983	15.681±2.093	16.345±1.872
Broup	All of them (n=16)	16.631±1.428	16.415±1.762	16.761±1.574

**Table 5.** Test results of T-AOC content (mean  $\pm$  SD)

Note: compared with subjects sharing the same gender and taking compound E Jiao Jiang at the same time in control group, \*: P < 0.05 suggesting a significant difference; \*\*: P < 0.01, suggesting a very significant difference.

# **3.5. Indexes of oxygen carrying capacity and aerobic endurance**

Physical signs measured in this study included Hb and maximum oxygen uptake, both of which were influenced by gender differences. Compared with subjects sharing the same gender and taking compound E Jiao Jiang at the same time in control group, male athletes in experimental group had obviously increased Hb content after taking compound E Jiao Jiang for 2 weeks (P<0.05), and female athletes in experimental group had very obviously increased Hb content after taking compound E Jiao Jiang for 4 weeks (P<0.01). In comparison with subjects sharing the same gender and taking compound E Jiao Jiang at the same dose before experiment, female athletes in high-dose group were observed with significantly increased Hb content after 2 weeks of oral administration (P<0.05) and very markedly increased Hb content after 4 weeks of oral administration (P<0.01). Comparing female athletes in high-dose group taking compound E Jiao Jiang for 4 weeks with 2 weeks, Hb content increased notably (P<0.05). No statistical significance was found in the rest of pairwise comparison.

# 4. Conclusions

# **4.1. Influence of compound E Jiao Jiang on antioxidant enzyme**

CAT, a marker enzyme of peroxysome (Seyhan et al., 2013), existing in the peroxide body of red blood cell and other tissues, mainly helps H2O2 decomposed into oxygen and water, eliminates H2O2 inside the body, avoids the damage of H2O2 to cells and restricts the generation of hydroxyl radicals resulted from oxygen and hydrogen peroxide under the action of iron chelates. Hydroxyl radical is a kind of lively active oxygen, which has strong destructive effect as it is capable of reacting with most

of organic materials in the cell rapidly (Liang and Ramesh, 2010; László et al., 2014). It could be seen by analyzing results that the CAT content in athletes of different genders increased to some extent after taking compound E Jiao Jiang, and the increasing degree showed no gender differences. A study (Yee et al., 2014) that rehmannia glutinosa proves polysaccharide is able to increase the content of CAT and compound E Jiao Jiang can enhance the quantity of red blood cell as well as hematokrit, which directly increase the carrier of CAT. This is in accordance with the test results acquired in this study. SOD as an important antioxidant enzyme inside the biological body plays a crucial role in balancing oxidation and antioxidant of the body. It is effective in eliminating superoxide anion free radicals and protecting cells from damage (Alcely et al., 2003; Daizoh et al., 2001). By observing dose-response relationship of compound E Jiao Jiang, results indicated that SOD content in experimental group was significantly higher than in high-dose group after taking compound E Jiao Jiang. Thus, it could be seen that compound E Jiao Jiang had no dose-response relationship with SOD content. Through observing time-response relationship of compound E Jiao Jiang, SOD content was always in a rising trend as time went on from an overall perspective. Therefore, there was a time-response relationship between compound E Jiao Jiang and SOD content.

# **4.2. Influence of compound E Jiao Jiang on maximum oxygen uptake**

Maximum oxygen uptake refers to body's oxygen intake per unit time when cardiopulmonary function and muscle using oxygen reach a limitation after human body does long-time strenuous exercise involving a lot of muscles. It reflecting the ability of body to inhale, transport and use oxygen is one of the important indexes evaluating the aerobic working capacity of human body (Shephard, 2008). A variety of factors affect maximum oxygen uptake, including Hb content in the red blood cells, the ability to carry and transport oxygen; heart pump function, that is to say, the influence of cardiac output; the influence of pulmonary ventilation and gas exchange function, as well as the ability of muscle to use oxygen; genetic factors; age and gender; training level, etc (Shephard, 2008). Study results obtained by observing the time-response relationship between compound E Jiao Jiang and maximum oxygen uptake demonstrated that compound E Jiao Jiang could enhance maximum oxygen uptake. Besides, female athletes had obviously higher maximum oxygen uptake than male athletes as maximum oxygen uptake was affected by gender which was the key factor impacting drug sensitivity. However, no dose-response relationship was found between compound E Jiao Jiang and maximum oxygen uptake.

All in all, the study results indicate that compound E Jiao Jiang is able to improve the antioxidant ability of athletes, inhibit the production of free radicals and enhance the ability of body carrying and transporting oxygen, thereby improving the aerobic working ability as well as aerobic endurance. Eventually, it takes effect in resisting fatigue.

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