



---

**Effects of Red Dragon Fruits (*Hylocereus polyrhizus*)  
Powder and Swimming Exercise on Inflammation,  
Oxidative Stress Markers, and Physical Fitness in Male  
Obesity Rats (*Sprague dawley*)**

Tonny C. Maigoda<sup>a\*</sup>, Ahmad Sulaeman<sup>b</sup>, Budi Setiawan<sup>c</sup>, I Wayan T. Wibawan<sup>d</sup>

*<sup>a</sup>Bengkulu Health Polytechnic, Ministry of Health, Jl. Indra Giri No.3 Padang Harapan,  
Bengkulu 38225. INDONESIA*

*<sup>b,c</sup>Department of Community Nutrition, Faculty of Human Ecology, Bogor Agricultural University (IPB),*

*<sup>d</sup>Lecturer of Veterinary Faculty, Bogor Agricultural University (IPB), Bogor 16680, Indonesia*

*<sup>a</sup>E-mail: tonnymaigoda@yahoo.com*

**Abstract**

Studying childhood obesity is essential to identify early or initial triggers for cardiovascular disease. Inflammation and oxidative stress are known to be key factors in the pathogenesis of cardiovascular disease. Consuming fresh fruits, vegetables and regular physical activity may protect against some chronic diseases caused by oxidative stress and inflammation. Red dragon fruit contains vitamins, minerals and phytochemical properties that are needed for human health. This study was intended to evaluate effects of red dragon fruits (*Hylocereus polyrhizus*) powder and swimming exercise on inflammation, oxidative stress, and physical fitness in male obesity rats (*Sprague dawley*). There were 24 rats divided by two groups, 20 rats induced obesity by giving high fat diet, and 4 rats given standard diet for 19 weeks.

---

\* Corresponding author.

Another 4 weeks 20 rats were already obese divided into 5 groups intervention as follows : standard diet (SD), high fat diet (HFD), high fat diet plus swimming exercise (HFD+SE), red dragon fruit powder plus high fat diet (RDFP+HFD), and red dragon fruit powder plus high fat diet plus swimming exercise (RDFP+HFD+SE). Blood samples and physical fitness test were taken before and after intervention. Red dragon fruit powder contained  $171.79 \pm 2.01$  mg/100gr total of flavonoid,  $157.34 \pm 0.08$  mg/100gr total phenolic acid,  $88.17 \pm 1.98$  mg/100gr total vitamin C,  $47.76 \pm 0.55$  mg/100gr total anthocyanin,  $35.92 \pm 1.44$  mg/100gr total alkaloid,  $11.12 \pm 0.35$  mg/100gr total dietary fiber, and  $0.25 \pm 0.04$  mg/100gr carotenoid. TNF- $\alpha$  (tumor necrosis factor-alpha) and SOD (Superoxide dismutase) were significant among intervention group ( $p < 0.05$ ). Red dragon fruit powder and swimming exercise were able to inhibit MDA (malondialdehyde) and to enhance immune system by decreasing TNF- $\alpha$  concentration. Red dragon fruit powder contained rich of bioactive compounds (antioxidants) and nutritional substances that were good as a functional food ingredient.

**Keywords:** inflammation; oxidative stress; physical fitness; obesity; and red dragon fruit powder.

## **1. Introduction**

Rates of obesity are rising alarmingly in many parts of the world, and this trend is not restricted to adults only. Child and adolescent obesity is becoming a significant health problem. Obesity in children and adolescents is a major concern, not only because of health and social problems in the short-term, but also due to a high risk it may continue into adulthood and affect long-term health. The global epidemic of obesity is a major public health problem because of its comorbidities, such as : cardiovascular disease, type II diabetes, and cancer. Nevertheless, the prevalence of obesity has drastically escalated by nearly 57% over the previous two decades [1]. The National Health and Nutrition Examination survey reported that 36% of US adults are currently classified as obese, while 16% represent indices of severe cases [2,3].

In Indonesia, the prevalence of obesity or overweight with a body mass index (BMI) over 30 to the population aged > 15 years was 18.8% and in 2010 increased to 21.7%. Moreover, in 2013 visceral obesity in adolescent was 26,6 % and estimated will increase per year [4]. Individuals with central obesity is more at risk of metabolic syndrome compared to those who have peripheral body fat distribution [5]. Obesity is associated with increased cardiovascular mortality and morbidity. Because of the comorbidities commonly found in obese adults, studying childhood obesity is essential to identify early or initial triggers for cardiovascular disease [6].

Inflammation and oxidative stress are the main factors in the pathogenesis of cardiovascular disease, contributing both to the early stages and to the development of atherosclerosis. Many of the pro-inflammatory and proatherogenic disorders associated with vascular disease in adults have also been demonstrated in obese children, including elevated inflammatory markers such as of leptin, interleukin-6 (IL-6), C-reactive protein (CRP), tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ), fibrinogen, vascular adhesion molecules and decreased adiponectin level, increased oxidative stress markers such as malondialdehyde (MDA), F2-isoprostanes, and advanced oxidation protein products [7,8].

Handling of overweight and obesity can be done with a variety of approaches. An example is to combine

physical exercise interventions, nutritional counseling and provision of functional foods containing antioxidants. Consuming a healthy diet and regular physical activity is key in the prevention and treatment due to increased risk of obesity in children and adolescents [9,10].

Healthy diet should contain fruits and vegetables as source of vitamins, minerals and other nutritional substances including antioxidants. Consuming fresh fruits and vegetables may afford protection against some chronic diseases caused by oxidative stress such as cardiovascular disorders [11] and different types of cancer [12]. This is mainly contributed to their antioxidant properties, especially vitamin c, carotenoid, phenolic, flavonoids, tannins and anthocyanins which are potential to scavenge free radicals and inhibit peroxidation [13].

Fruits become the main subject for researchers to investigate for their bioactive compounds that are beneficial for human health and red dragon fruit is a new promising one. Dragon fruit contains a source of vitamins and minerals. The content of vitamin B1 reaches 0.3 mg per 100 g of flesh. It also contained phytochemicals that were good for the body, such as polyphenols, flavonoids. The total content of polyphenols in red dragon fruit flesh as much as  $86.129 \pm 17.016$  (mg / 0.5 g gallic acid), total flavonoid content of  $2.3 \pm 0.20$  (mg / g catechins) [14].

Many previous study have been conducted on dragon fruits much more focus on its' economics value, cultivation, nutrients content and antioxidant activity through in vitro. While the potential applications of dragon fruit and its effect on human health is still relatively rare.

In addition, in vivo studies that assess to what extent the potency of red dragon fruit powder in boosting the immune system, and inhibit oxidative stress are still remain unknown. The objectives of this study were to examine the effect of the red dragon fruit powder and aerobic exercise on inflammation, oxidative stress and physical fitness on rats with obesity.

## **2. Materials and Method**

This study was approved by the Research Ethics committee of the Faculty of Veterinary Medicine at SKEH Animal Ethics Approval Certificate Number: 020 / KEH / SKE / II / 2015, Bogor Agricultural University

### **2.1 Materials**

#### **2.1.1 Raw Material**

Local red dragon fruits used to make powder obtained from "Sabisa Farm" , located in Sindang Barang, Bogor. Red dragon fruit was grown using natural fertilizer and without pesticide.

#### **2.1.2 Diet**

There were three formulation experimental diet for rats used in this study such as : standard diet, high fat diet, and red dragon fruit powder plus high fat diet. Composition of standard diet and high fat diet (HFD) modified

from the formula of [15].

**Table 1:** Composition of experimental diet

Composition	Standard Diet per 100 gr	High Fat Diet per 100 gr
Corn	55	15
Pollard	17	19
Corn Gluten Meal	19	25
Crude Palm Oil	1	0
Tapioka powder	5	3
CaCO <sub>3</sub>	2	2
DCP	1	1
Salt	0.2	0,2
Premix	0.3	0.3
Tallow (Fat from Cow)	0	35
Amount	100	100

The third formula was red dragon fruit powder plus high fat diet. It was prepared based on flavonoid daily requirement of human. It was estimated 240 mg [16] and converted to rats become 45 mg flavonoid / 200gr weight of rats. Total Flavonoid content of red dragon fruit powder was 171.79 mg  $\pm$  2,01 /100 gr. The average estimation consumption of rats was 30 grams per day. Therefore, the rats fed approximately 25 gr of red dragon fruit powder and rest of 5 gr came from high fat diet. So, the formula red dragon fruit powder plus high fat diet contained 25 gr red dragon fruit and 5 gr high fat diet (5 :1).

### 2.1.3 Experimental Animals

Twenty four white male rats strain *Sprague Dawley* aged 2 weeks and weighted 80-120 gr were used and obtained from National Agency of Drug and Food Control (NADFC) Jakarta, Indonesia. 20 rats were used for induction of obesity by fed high fat diet, and 4 rats fed standard diet as a control group. All animals were housed individually in plastic cage size 36 x 28 x 12 cm<sup>3</sup>, made of plastic material with a covered rectangular wire netting at the top that can be closed and opened, and given a bottle for each cage, bottle filled with water and drink ad libitum. The rats were kept individually and each rats had a tag number. They were kept under standard environmentally controlled, clean-air room with temperature 24 $\pm$ 5<sup>0</sup> C, illumination 12 hours light/12 hours dark cycle, a relative humidity of 60  $\pm$  4%.

Body weight, body length, abdomen circumference were measured once a week during induction of obesity. In the intervention period, beside weight, body length and abdominal circumference were also calculated food consumption. Food consumption was calculated daily at the same time by subtracting the amount of food left over in each cage with the amount food provided at the previous day (gm/day/rat).

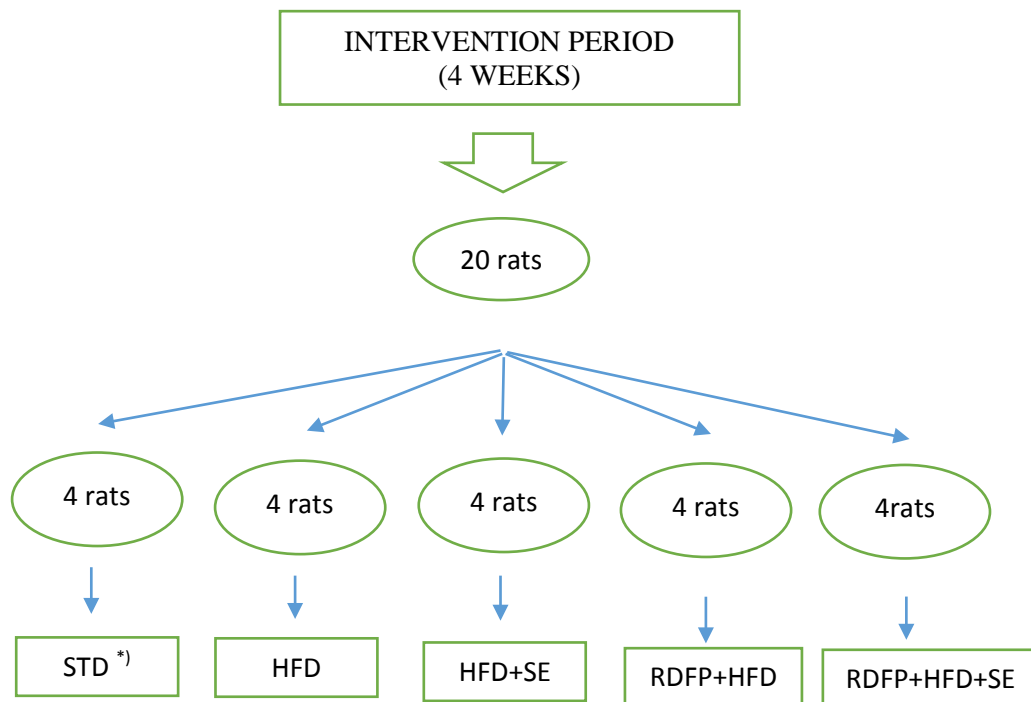
### 2.1.4 Physical Fitness and Swimming Exercise equipments

Physical fitness was measured by Rotarod Instruments for rats, followed the protocol of ARR (*accelerating rota rod*) [17]. Swimming exercise equipment was made by using Acrylic material box sized 37 x 30 x 50 in water depth 50 cm, equipped by water flow machine, and divided by two side. This equipment was designed and modified from the design of animal exercise by [18].

## 2.2 Methods

### 2.2.1 Experimental design and animal grouping

This study was divided by two periods : (1) rats induced obesity and (2) intervention. In rats induced obesity period, 24 rats were randomly assigned into two groups : 4 rats fed standard diet and 20 rats fed high fat diet. In the intervention period for 4 weeks, 20 rats have been achieved obesity were randomly assigned into 5 groups based on their weight (Figure 1).



**Figure 1:** Intervention grouping STD : Standard Diet, HFD : High Fat Diet, HFD+SE : High Fat Diet + Swimming Exercise, RDFP+HFD: Red Dragon Fruit Powder + High Fat Diet, RDFP+HFD+SE: Red Dragon Fruit Powder + High Fat Diet + Swimming Exercise

### 2.2.2 Steps of Processing Red Dragon Fruit Powder

Red dragon fruit powder was prepared using fresh red dragon fruit through the following steps: sorting, bark peeled and removed, cutting, pouring into slurry, adding 30% maltodextrin stirring thoroughly, then dried with a vacuum evaporator for two hours, and milling with a size of 40 mesh.

### 2.2.3 Analysis nutritional substances and bioactive compounds of red dragon fruit powder

- Proximate analysis of basic nutritional substances

Proximate analysis was conducted for red dragon fruit powder in order to know the basic nutritional substances. Proximate analysis consists of water content (gravimetric method), ash content (dry ash method), fat content (soxhlet method) that was done according to the national standards of SNI 01-2891-1992 [19], protein content (Microjeldahl) according to the standard of [20], and carbohydrate content used different method.

- Bioactive Compounds Analysis

Bioactive compounds were analyzed in this research including: anthocyanin, phenolic acid, caroten, vitamin C, alkaloid and dietary fiber. Total of flavonoid content was measured by colorimetric assay developed by [21], total amount of phenolic acid was measured by spectrophotometer the method performed by [22], with some modifications. The anthocyanins were quantified using spectrophotometric method. The total amount of carotenoids was determined using a spectrophotometer (Specord 210, model Analytikjena), at 450 nm. Total dietary fiber is calculated using enzymatic methods. Total amount of Vitamin C was determined using titration methods. Alkaloid total was measured by Gravimetric.

## 3. Blood Sampling

Before and after intervention venous blood sampling was collected from *venous retro orbital* using *micro hematokrit capillary tubes*, and put in EDTA containing tubes. Centrifuge for 10 minutes at 1,000 x g within 30 minutes of collection. Assay immediately or store plasma samples at  $< -70^{\circ}\text{C}$ . The amount of blood plasma taken by rats was 1 ml, each 1 ml of blood contains 480 microliters of plasma. Each elisa assay takes 100 microliters of blood plasma, so the blood plasma needed for 4 elisa assay was 400 microliters.

### 3.1 Analysis of Inflammation

#### 3.1.1 TNF- $\alpha$ (Tumor Necrosis Alpha- $\alpha$ ) marker

TNF- $\alpha$  marker was measured referring to the user manual of Legend Max<sup>TM</sup> ELISA Kit with Pre-coated Plates manufactured by BioLegend Inc. San Diego, CA 92121

#### 3.1.2 CRP (C Reactive Protein) marker

CRP marker was measured referring to the user manual of ELISA KIT Elabscience manufactured by Elabscience Biotechnology Co.,Ltd (Guandong Science Science and Technology Industry Park, Wu Han,P.R.C.)

### 3.2 Analysis of Oxidative Stress

#### 3.2.1 Superoxide Dismutase (SOD) activity

SOD activity was measured referring to the user manual of ELISA KIT Elabscience manufactured by Elabscience Biotechnology Co.,Ltd (Guandong Science Science and Technology Industry Park, Wu Han,P.R.C.) and [23].

### **3.2.2 Malondialdehyde (MDA) marker**

Malondialdehyde (MDA) is a compound with a molecular formula  $C_3H_4O_2$  dialdehydes produced from the oxidation of unsaturated fatty acids by free radicals in the body. Measuring blood plasma of MDA referred to [24].

## **4. Training and physical fitness protocol**

The HFD+SE and RDFP+HFD+SE group were submitted to swimming exercise in double side tanks containing water at  $33 \pm 1^{\circ}C$  [18] for five minutes per day, three days per week, for four weeks during intervention period.

Physical fitness test was measured by counting the length of time rats survived on the rotarod and how many times rats falling from rotarod in five minutes. Fitness test with Rota Rod conducted before and after intervention.

## **5. Statistical Analysis**

Data processing was conducted using Microsoft Excel 2010 and SPSS 17.0 for windows. Analysis was carried out by using Two Way ANOVA. If there was any significant difference, duncan test was conducted for further analysis. Values of  $p < 0,05$  were regarded as significant. Data was presented in tables and figure as mean  $\pm$  standard deviation.

## **6. Result and Discussion**

### **6.1 Nutritional substances and bioactive compounds of Red Dragon Fruit Powder**

Proximate composition of three kinds diet formula that were contained nutritional substances used in this study (Table 2).

High fat diet contained 501.70 kkal/100 gr was the highest energy that came from fat, carbohydrate, and protein. In order to induce rats obesity, the diet should contain 30-50 % fat from total kalori. According to [25] standard rat diets contain less than 10% of kcal fat, whereas high-fat diets and very high-fat diets contained 30%-50% and more than 50% of kcal fat, respectively.

Bioactive compounds were measured in this study such as : total content of flavonoid, anthocyanin, phenolic acid, alkaloids, vitamin C, dietary fiber, and caratenoid in red dragon fruit powder. Total content of flavonoid was  $171,79 \pm 2.01$  gr higher than phenolic acid, vitamin C, anthocyanin, alkaloid, dietary fiber and carotenoid. Based on this result, red dragon fruit powder had many biactive compounds such as phenolic acid, vitamin C, anthocyanin, and alkaloid.

High flavonoids content contributed towards its high antioxidative activity against the free radical [26]. Flavonoids, also known as nature's tender drugs, possess various biological/pharmacological activities including anticancer, antimicrobial, antiviral, antiinflammatory, immunomodulatory, and antithrombotic activities [27].

**Table 2:** Proximate Composition of Three Feeding Formula For Rats

Parameters	Standard Diet		High Fat Diet	RDFP+ HFD
	Unit	Amount	Amount	Amount
Energy total	kkal/100g	365.24	501.70	399,65
Energy from Fat	kkal/100g	68.04	286.02	69,21
Water Content	%	12.26	8.16	7,78
Fat	%	7.56	31.78	7.69
Protein	%	20.97	21.68	5.73
Carbohydrate Total	%	53.33	32.24	76.88

**Table 3:** Total Content Bioactive Compounds of Red Dragon Fruit Powder

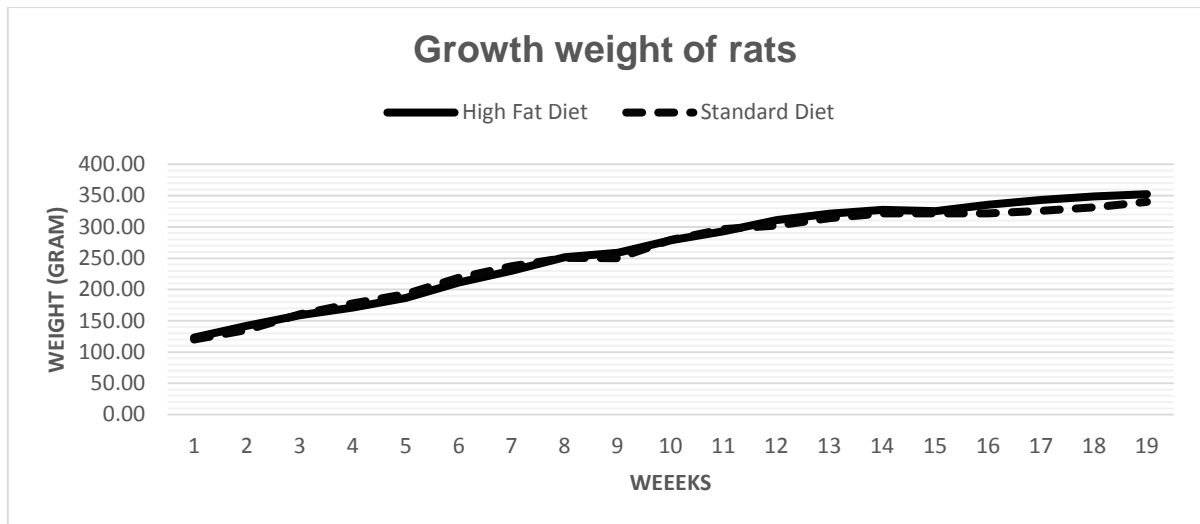
Bioactive Compounds	Amount	Unit
Total Flavonoid	171,79± 2,01	mg/100gr
Total Anthosianin	47,76±0,55	
Total Karoten	0,25±0,04	
Total Fenolic Acid	157,34±0,08	
Total Alkaloid	35,92±1,44	
Vitamin C	88,17±1,98	
Dietary Fiber	11,12±0,35	

In addition, high phenolic content correlated with high radical scavenging activity [28]. Moreover, red dragon fruit also contained high amount of vitamin C was 88,17±1,98 mg/100gr that was also useful for boosting immune system in the body.

### 6.2 Rats Induced Obesity

Rats obesity have been achieved in 19 weeks. Based on the final weight average at week 14<sup>th</sup> was 359,63 gr and body mass index average was 0,69 gr/cm<sup>2</sup>, which has already met the criteria of [29] BMI > 0.068 gr/cm<sup>2</sup>. The growht weight for 19 months presented in figure 2.





**Figure2:** Weight growth of rats during induction period

There was no significant difference weight gain between rats fed by high fat diet and standard diet. The same result also found by [30], there was no difference in final bodyweight of *Sprague Dawley* rats fed high fat diet for 3 weeks on NASH (Non Alcoholic Steatohue Hepatitis) model in rats. However, previous study pointed out that there was any difference of growth weight between rats fed by high fat diet and standard diet started 7 weeks until 28 weeks [31]. This growth of weight was lead to purified diet. In this research diets contain plant-derived ingredients which were subject to changes in the growing season and will vary in composition at the time of harvest. Thus, diet formulas may change based on the nutritional composition. Purified ingredients, on the other hand, are highly refined and contain just a single nutrient (ie. sucrose = carbohydrate). These ingredients have little variability and therefore provide consistency between batches [31].

### 6.3 Inflammation, Oxidative Stress and Physical Fitness

#### 6.3.1 Inflammation

- TNF- $\alpha$  (pg/ml) :

TNF- $\alpha$  (Tumor Necrosis Alpha) is a major cytokine with a polypeptide structure, produced by endothelial cells, tumor cells, vascular smooth muscle fibers, mast cells, NK cells, T and B cells and, predominantly by activated macrophages. It has multiple roles: proinflammatory, angiogenetic, proapoptotic, prooxidant, proatherogenic [32].

This reasearch showed that, the treatment group was significantly influence the inflammatory marker of TNF- $\alpha$  (pg / ml) at the level of  $p < 5\%$ . Inflammation marker TNF- $\alpha$  after the intervention did not differ significantly between treatment groups, only when compared with conditions before the intervention seen clearly that there was a decrease in TNF- $\alpha$  (pg / ml) in rats after being given the intervention of five treatment groups. Rats given a high-fat diet plus swimming exercise decreased the average of TNF- $\alpha$  by 7.82 (pg / ml) to 3.45 (pg / ml), as well as rats given red dragon fruit powder plus high fat diet and swimming exercise decreased level of TNF- $\alpha$  from the average of 8.21 (pg / ml) to 5.14 (pg / ml).

Inflammation is the most common aspect of tissue pathology and is implicated in the pathogenesis of many diseases, including cancer, diabetes, cardiovascular, neurodegenerative and other life-threatening and debilitating diseases [33]. Many studies demonstrated that effects of flavonoids on a variety of inflammatory processes and immune functions have been extensively reviewed, and it has been proved that they may inhibit several enzymes that are activated in certain inflammatory condition [34,35,36]. In this study, the group was given red dragon fruit powder plus high fat diet had potential anti-inflammatory compared with the group given high fat diet by inhibiting proinflammatory cytokine of TNF- $\alpha$ .

Another study by [37] conducted their study by exploring mechanism at the molecular level of flavonoid related to their anti-inflammatory properties in rats concluded that the mechanism of flavonoids ameliorate inflammation by upregulation of HO-1 mRNA expression with simultaneous reduction in the cytokine, TNF- $\alpha$  release.

Heme oxygenase-1 (HO-1) is an inducible enzyme that catalyzes the first and rate-limiting step in the oxidative degradation of free heme into ferrous iron, carbon monoxide (CO) and biliverdin, the latter being subsequently converted into bilirubin. HO-1 plays important role in host defense against oxidative injury. Therefore, HO-1 is an important therapeutic target in various disease models, and compounds that induce HO-1 possess great therapeutic potential against diseases associated with oxidative stress and inflammation [38].

Previous study concluded that in fructose fed rats, administration of flavonoid-containing red wine reduced adipose tissue weight and favored antiinflammation pathways by reducing resistin expression (proobesity) and increasing adiponectin expression as an antiobesity [39]. Another study also proved that in obese Zucker rats administered a 10-week of quercetin (10 mg/kg of body weight/day) increased plasma concentration of adiponectin, reduced TNF- $\alpha$  secretion and the expression of the proinflammatory iNOS (inducible nitric oxide synthase) in visceral adipose tissue [40].

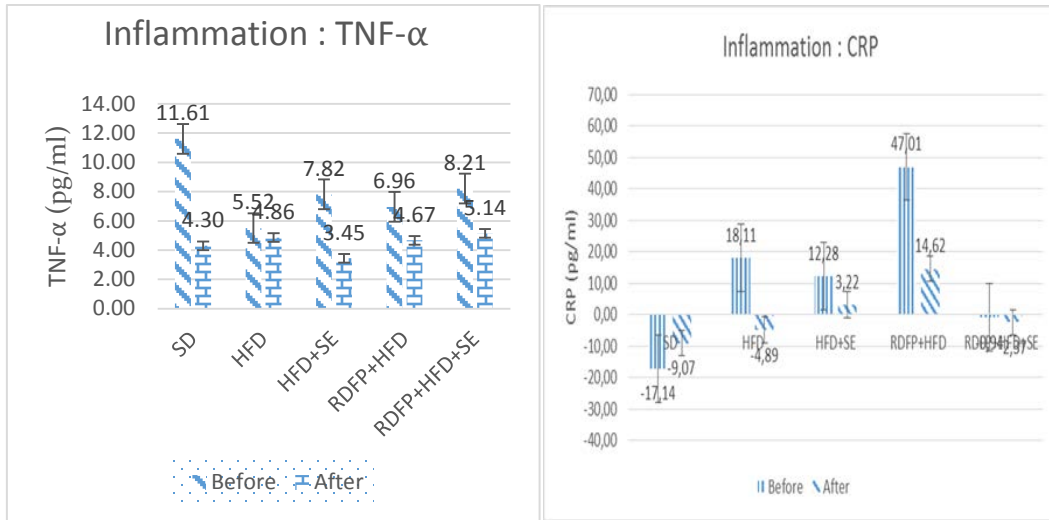
- C-Reactive Protein (CRP)

CRP is produced by hepatocytes as part of the acute-phase response and represents a sensitive, non-specific marker of inflammation [41]. Elevated serum C-reactive protein (CRP) and plasma homocysteine (Hcy) concentrations have been identified as contributing risk factors to CVD [42, 43, 44]. In the present study a marker of inflammatory CRP did not differ significantly before and after intervention.

Another study by [45] also found there was an inverse association of vitamins C and E and carotene intakes with the chance of elevated serum on CRP. Inflammation may depress antioxidant concentrations in blood and thereby mask the potential beneficial effect of antioxidants on CRP concentrations.

In addition, in this study has shown there was no relationship between exercise and concentrations of CRP. Numerous studies have been conducted on the relationship between exercise and biomarker of CRP [46,47,48]. These studies all demonstrate an inverse relationship between CRP concentration and physical activity. According to [49], one possible explanation for these conflicting results is that intervention duration of 4 weeks was too short change CRP levels. Ultimately, it seems that long-term exercise interventions (greater than 8

weeks) are effective to reduce the inflammation response and improve physical fitness.



**Figure 3:** Effects of red dragon fruit powder and swimming exercise on inflammation markers

### 6.3.2 Oxidative Stress

- SOD (ng/ml)

The treatment group were an inversely significant correlation with the antioxidant enzyme level of SOD (pg / ml) at the level of a gap of 5%. An inverse significant correlation occurred between the group treated High Fat Diet plus Swimming Exercise with SOD level from 0.44 ng / ml to 0.13 ng/ and another group of Red Dragon Fruit Powder plus High Fat Diet with SOD level from 0.55 ng / to 0.12 ng /ml before and after intervention.

Superoxide dismutase is the most important antioxidant enzyme, which neutralizes the harmful superoxide anion radical by converting it to hydrogen peroxide. In this research there was an inverse relationship between treatments group and SOD level. The early stage of obesity the SOD level for all treatments were high in order to neutralize the accumulation of stress oxidative from free radical in adipocyte cells. At the end of intervention, endogen enzyme antioxidant of SOD activity especially in group RDFP+HFD+SE remain constant at nearly the same level due to antioxidant exogen intake from red dragon fruit powder.

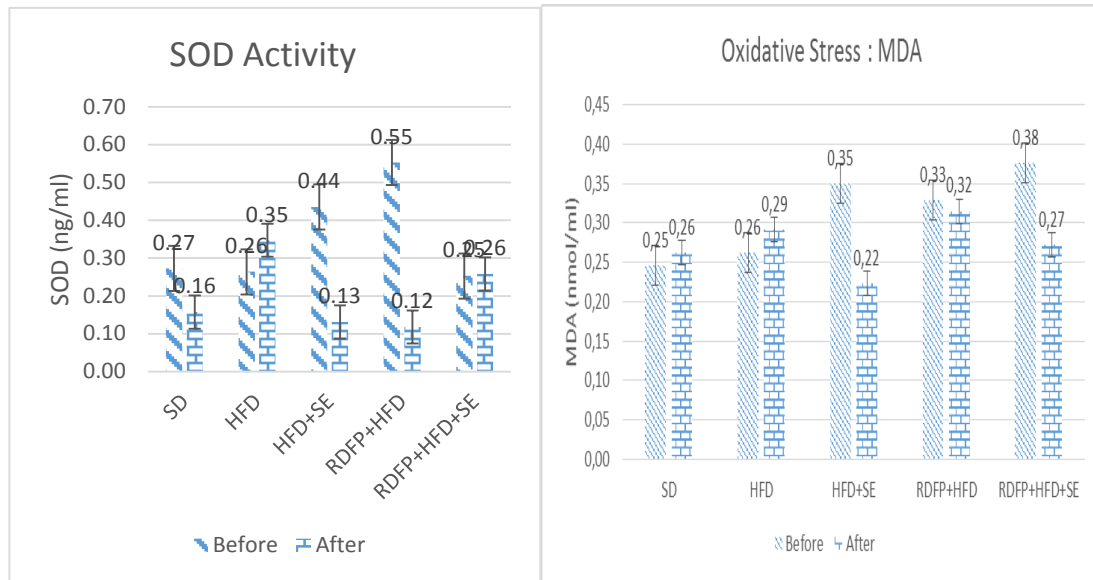
We also found that superoxide dismutase levels were inconsistently elevated after exposure to the red dragon fruit powder and swimming exercise. Previous study by [50] found the same result that was a polyherbal formulation influenced some biochemical parameters in wistar rats and they concluded SOD was inducible enzyme, and elevated SOD may indicate to the presence of oxidative stress.

- MDA (nmol/ml)

Malondialdehyde (MDA) is a compound with a molecular formula C<sub>3</sub>H<sub>4</sub>O<sub>2</sub> dialdehydes produced from the oxidation of unsaturated fatty acids by free radicals. MDA can be used as a biomarker of cell membrane

damage. The cell membrane is mainly composed of polyunsaturated fatty acids. Polyunsaturated fatty acids are more vulnerable to free radicals as compared to saturated fatty acids. Oxidation of polyunsaturated fatty acids will generate about 82% of MDA so that MDA is widely used as a biomarker of cell membrane damage [51].

This research showed that there was no relationship significant between five treatments and MDA marker level, however there was a trend of decreasing marker level of MDA.



**Figure 4:** Effects of red dragon fruit powder and swimming exercise on oxidative stress markers.

The current results showed that the potential of exogenous antioxidant from red dragon fruit powder such as flavonoid, anthocyanin, phenolic acid, carotenoid were approved to be able to inhibit MDA level in blood plasma of rats especially for groups that are given treatments of high fat diet plus swimming exercise, red dragon fruit powder plus high fat diet and red dragon fruit powder plus swimming exercise.

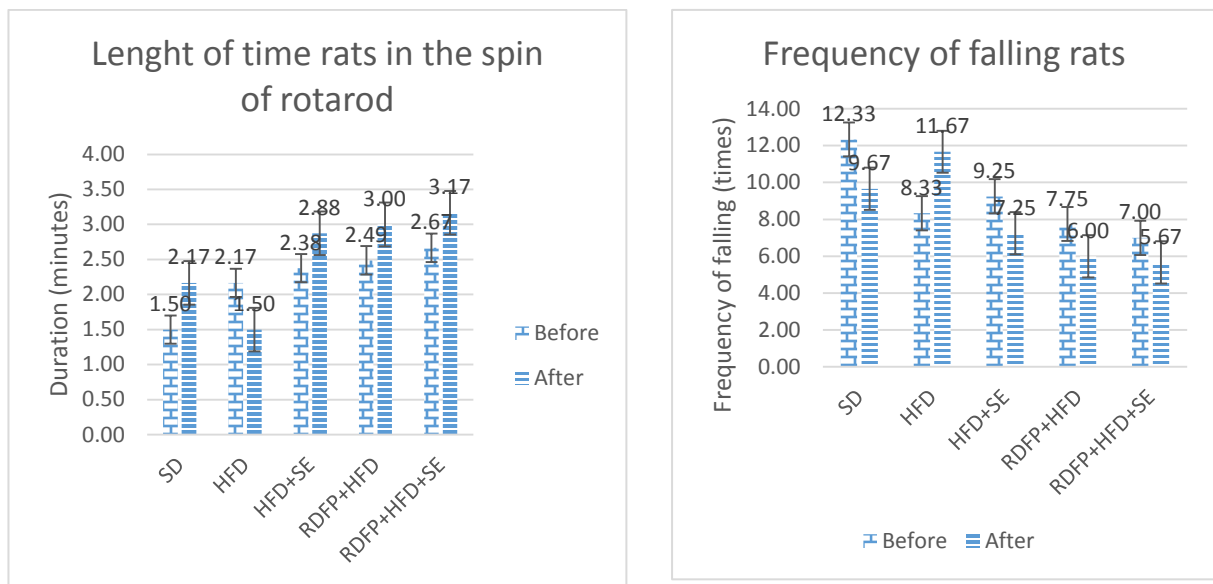
Consumption of flavonoids found in red dragon fruit flour and swimming exercise routinely were able to suppress oxidative stress. Mechanism of action of flavonoids as antioxidants may directly or indirectly. Direct mechanism is to donate hydrogen ions to neutralize the toxic effects of free radicals. Meanwhile, as an antioxidant indirectly by increasing endogenous antioxidant gene expression. This mechanism via activation of nuclear factor erythroid 2-related factor 2 (Nrf2) resulting in increased expression of genes involved in the synthesis of endogenous antioxidant enzymes [52].

### 6.3.3 Physical Fitness

The ARP protocol (accelerating rota rod) or setting the pace on the rota rod, the rats were placed on a rotating spin that can be accelerated between 4-40 rpm for 5 minutes, three times a week. Measurements carried out by measuring the length of time each rat can survive on a rotating drum, and the number of times the rats fell from rotarod during exercise for 5 minutes [53]. The limitation of this study does not use VO<sub>2</sub> max as a gold standard to evaluate aerobic physical fitness, and may have influenced in our result.

The physical fitness test was not significant among all group. However, the length of time of rats survived in a rotarod spin gradually increased especially in group HFD+SE, RDFP+SE and RDFP+SE before and after intervention. In addition, the frequency of falling rats in five minutes during test were gradually decrease especially in group SD, HFD+SE, RDFP+HFD and RDFP+HFD+SE. This data indicated that the effect of swimming exercise and red dragon fruit powder treatments were able to increase aerobic capacity of rats.

In the current study, regular exercise was able to prevent plasma oxidative stress by the reduction of lipid peroxidation, evaluated by malondialdehyde (MDA) levels, and by the increment of plasma of total SOD activity, thus reinforcing the antioxidant action of training.



**Figure 5:** The result of physical fitness test

Previous study by [54] concluded that antioxidants supplementation and moderate intensity physical exercise cause a significant increase in aerobic exercise capacity compared to physical exercise of the same intensity without supplementation.

Physical activity has long been associated with improvements in aerobic capacity [55], strength, muscle growth, and body composition. It is now widely accepted that chronic physical activity enhance immune function and attenuates the likelihood of chronic disease, such as CVD, diabetes, and obesity [56]. Moreover, these benefits to immune function in relation to regular exercise include decreased levels of proinflammatory cytokines TNF- $\alpha$  [57], IL-6 [58], and CRP [46].

In this study, we can suggest that swimming exercise at moderate intensity, could be an important intervention strategy for both the prevention and treatment of the inflammatory state of obesity and associated chronic diseases.

Therefore, regular physical activity appears to act as a natural antioxidant and anti-inflammatory strategy for preventing obesity-associated complications: it improves glucose-insulin homeostatis, endothelial function and

antioxidant defenses, while lowering circulating triglycerides [59].

## **7. Conclusion**

The result of this study suggest that consuming red dragon fruit powder and swimming exercise for obese rats were able to inhibit oxidative stress mainly MDA, to enhance immune system by decreasing TNF- $\alpha$  concentration. Physical fitness was increased by consuming red dragon fruit powder and conducted regular exercise. This study also suggest that the combination treatment between red dragon fruit powder and swimming exercise were able to decrease the accumulation of fat in the liver organ of obese rats. Although change in oxidative stress was slight, however this study can be clinically important.

Red dragon fruit flour containing rich of bioactive compounds (antioxidants) and nutritional substances that is good as a functional food ingredient.

It is recommended that important to continue this research by exploring flavonoid identification and other kind of bioactive compounds which is mostly powerful to inhibit inflammation and oxidative stress. This study can be used as a basic research and to be continued using humans as a clinical trial.

## **Acknowledgement**

This study was financially supported by Ministry Of National Education and Culture Secretariate General Seameo Seamolec, Southeast Asian Regional Centre for Tropical Biology (SEAMEO BIOTROP) by the year 2015.

## **References**

- [1] Flegal K.M, Carroll .D, Kit B.K, and Ogden C.I. 2012. Prevalence of obesity and trends in the distribution of body mass index among US adults, 1999-2010, *Journal of the American Medical Association*, vol 307, no.5, pp. 491-497, 2012
- [2] Wang Y, Beydoun M.A, Liang L, Caballero and Kumanyika S.K. 2008, "will all americans become overweight or obese? Estimating the progression and cost of the US obesity epidemic".*Obesity*, vol.16, no. 10, pp.2323-2330,2008
- [3] Finkelstein E.A, Khavjou O.A, Thompson H. 2012. Obesity and severe obesity forecasts through 2030" *American Journal of Preventive Medicine*,vol.42, no.6, pp.563-570
- [4] Health Research Report. 2007, 2010, 2013 Ministry of Health Department Republic of IndonesiaDepartment Of Health Republic of Indonesia. 2013
- [5] Steinberger J, Daniels S.R. 2003. Obesity, Insulin resistance, diabetes, and cardiovascular risk in children. *American Heart Association,Inc. Circulation*. 2003;107:1448-1453

- [6] Montero D, Walther G, Perez-Martin A, Roche E, and A Vinet (2012). Endothelial dysfunction, inflammation, and oxidative stress in obese children: markers and effect of lifestyle intervention. *Obesity review* (2012) 13.441-455eth.
- [7] Pena As, Wiltshire E, MacKenzie K et al.2006. Vascular endothelial and smooth muscle function relates to body mass index and glucose in obese and nonobese children. *J Clin Endocrinol Metab* 2006; 91:4467-4471
- [8] Giordano P, Del Vecchio GC, Cecinati V et al. 2011. Metabolic, Inflammatory, endothelial and haemostatic markers in a group of Italian obese children and adolescents. *Eur J Pediatr* 2011; 170:845-850
- [9] Elmaghoub BC, Lambers S, Stegen S, Laethem CV, Cambier D, Calders P. , 2009. The Influence of combined exercise training on indices of obesity, physical fitness and lipid profile in overweight and obese adolescents with retardation. *Eur J Pediatr* (2009) 168:1327-1333 DOI 10.1007/s00431-009-0930-3
- [10] Ribeirio MM, Silva AG, Santos NS et al 2005. Diet and exercise training restore blood pressure and vasodilatory responses during physiological maneuvers in obese children. *Circulation* 2005; 111:1915-1923
- [11] Srinath R.K, and Katan, M.B. 2004. Diet, Nutrition and the prevention of hypertension and cardiovascular diseases. *Public Health Nutrition*, 7, 167-186
- [12] Frydoonfar, H.R, McGrath, D.R, & Spiegelman, A.D. 2003. The variable effect on proliferation of a colon cancer cell line by the citrus fruit flavonoid Naringenin. *Colorectal Disease*, 5, 149-153
- [13] Simirgiostis, M.J., & Schmeda-Hirschmann, G. 2010. Determination of phenolic composition and antioxidant activity in fruits, rhizomes and leaves of the white strawberry (*fragaria chiloensis* spp. *Chiloensis* form *chiloensis*) using HPLC-DAD-ESI-MS and free radical quenching techniques. *Journal of Food Composition and Analysis*, 23, 545-553
- [14] Rebecca OPS, Zuliana R, Boyce AN, Chandran S (2008). Determining pigment extraction efficiency and pigment stability of dragon fruit (*Hylocereus polyrhizus*). *J. Biol. Sci.* 8(7): 1174-1180.
- [15] Ulman E.A, 2006. Open Formula Purified Diets Lab Animal. Research Diets, Inc.,New Brunswick,USA
- [16] Knab A.M, Nieman D.C, Gillitt N.D, Shanely A, Ciaidella-Kam L, Hanson D.A, and Sha W. 2013. Effects of a Flavonoid-Rich Juice on Inflammation, Oxidative Stress, and Immunity in Elite Swimmers: A Metabolic-Based Approach. *International Journal of Sport Nutrition and Exercise Metabolism*,2013, 23, 150-160
- [17] Shiotsuki H., Yoshimi K., Shimo Y., Funayama M., Takamatsu Y., Ikeda K., Takahashi R., Kitazawa S., Hattori N. 2010. A rotarod test for evaluation of motor skill learning. *Journal of neuroscience methods* 189 (2010) 180-185 doi:10.1016/j.jneumeth.2010.03.026
- [18] Kregel K.C., Allen D.L., Booth F.W., Fleshner M.R., Henriksen E.J., Musch T.I., O'Leary D.S., Parks C.M., Poole D.C., Ra'anana A.W., et al. 2006. Resource Book for the Design of Animal Exercise Protocols.

American Physiology Society.2006

- [19] Badan Standarisasi Nasional. "Cara Uji Makanan dan Minuman SNI 01-2891-1992".Indonesia, 1992
- [20] AOAC (1984). Official Methods of Analysis, 14th ed., (Williams, S., ed.). Washington, DC: Association of Official Analytical Chemists
- [21] Zhishen, J., Mengcheng, T., & Jianming, W. 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food chemistry*, 64, 555-559
- [22] Singleton, V.L., & Rossi, J.A. Jr. 1965. Colorimetry of total phenolic with phosphomolibdic-phosphotungstic acid reagents. *American journal of enology and viticulture*, 16,144-158
- [23] Wood et al. 2003. Improved antioxidant and fatty acid status of patients with cystic fibrosis after antioxidant supplementation is linked to improve lung function. *Am J.Clin Nutr.*77:150-159
- [24] Capeyron et al.2002. A diet cholesterol and deficient in vitamin E induced lipid peroxidation but doesnot enhance antioxidant enzyme expression in rats. *J. Nutr Biochem.* 13: 296-301
- [25] Fellmann L, Nascimento AR, Tibirica E, Bousquet P. 2013. Murine models for pharmacological studies of the metabolic syndrome. *Pharmacol Ther* 2013; 137:331-340 [PMID: 231785510 DOI: 10.1016/j.pharmthera.2012.11.004]
- [26] Koba, K.; Matsuoka, A.; Osada, K.; Huang, Y.-S. 2007. Effect of loquat (*Eriobotrya japonica*) extracts on LDL oxidation. *Food Chemistry*: 2007, 104, 308–316.
- [27] Havsteen B. 1983. Flavonoid, a class of natural products of high pharmacological potency. *Biochem Pharmacol.* 1983; 32:1141-1148
- [28] Wu, L.-C.; Hsu, H.-W.; Chen, Y.-C.; Chiu, C.-C.; Lin, Y.-I.; Ho, J.-A. 2006. Antioxidant andantiproliferative activities of red pitaya. *Food Chemistry* 2006, 95, 319–327
- [29] Novelli, E.L.B., Diniz, Y.S., Galhardi, C.M., Ebaid, G.M.X., Rodrigues, H.G., Mani, F., Fernandes, A.A.H., Cicogna, A.C., Filho, J.L.V.B.N. 2007. Anthropometrical parameters and markers of obesity in rats. *Laboratory Animals*; 41: 111-119.
- [30] Kucera O, Cervinko Z. 2014. Experimental models of non-alcoholic fatty liver disease in rats. *World J Gastroenterol* 2014 July 14; 20(26): 8364-8376
- [31] Dieman V.V, Trindale E.N, Trindale M.R.M. 2006. Experimental model to induce obesity in rats. *Acta Cir'urgica Brasileira* Vol. 21(6) 2006
- [32] Sprague AH and Khalil RA., 2009. Inflammatory cytokines in vascular dysfunction and vascular disease.



Biochem Pharmacol 2009;78(6):539-52

[33] Lawrence T, Willoughby DA and Gilroy DW. 2002. Anti-inflammatory lipid mediators and insights into the resolution of inflammation. *Nature Reviews Immunology* 2:787-795

[34] Gonzales R, Ballester I, Lopez-Posadas R, Suarez MD, Zarzuelo A, Martinez O, et al. 2011. Effects of flavonoids and other polyphenols on inflammation. *Critical Reviews in Food Science and Nutrition* 51(4): 331-362

[35] Katsori AM, Chatzopoulou M, Dimas K, Kontogiorgis C, Patsilnakos A, Trangas T, et al. 2011. Curcumin analogues as possible anti-proliferative & anti-inflammatory agents. *European Journal of Medicinal Chemistry* 46:2722-2735

[36] Nair MP, Mahajan S, Reynolds JL, Aalinkeel R, Nair H, Schwartz SA, et al. 2006. The flavonoid quercetin inhibits proinflammatory cytokine (Tumor necrosis alpha) gene expression in normal peripheral mononuclear cells via modulation of the NF-KB system. *Clinical and vaccine immunology* 13:319-328

[37] Heeba G.H, Mahmoed M.E, and Hanafy A.A.E. 2014. Anti-inflammatory potential of curcumin and quercetin in rats: Role of oxidative stress, heme oxygenase-I and TNF- $\alpha$ . *Toxicology and Industrial Health* 2014. Vol. 30(6) 551-560 DOI: 10.1177/0748233712462444

[38] Abraham NG, Kappas A. 2008. Pharmacological and clinical aspects of heme oxygenase. *Pharmacological Reviews* 60: 179-127

[39] Vasquez-Prieto, M.A. et al. 2011. Effect of red wine on adipocytokine expression and vascular alterations in fructose-fed rats. *Am J. Hypertens.* 24:234-240

[40] Rivera L. et al. 2008. Quercetin ameliorates metabolic syndrome and improves the inflammatory status in obese Zucker rats. *Obesity (Silver Spring)* 16:2081-2087

[41] Pepys MB & Hirschfield GM (2003) C-reactive protein: a critical update. *J Clin Invest* 111, 1805–1812.

[42] Mora S, Musunuru K & Blumenthal RS (2009) The clinical utility of high-sensitivity C-reactive protein in cardiovascular disease and the potential implication of JUPITER on current practice guidelines. *Clin Chem* 55,219–228.

[43] Stanger O, Herrmann W, Pietrzik K et al. (2003) DACHLIGA homocystein (German, Austrian and Swiss Homocysteine Society): consensus paper on the rational clinical use of homocysteine, folic acid and B-vitamins in cardiovascular and thrombotic diseases: guidelines and recommendations. *Clin Chem Lab Med* 41, 1392–1403.

[44] Buckley DI, Fu R, Freeman M et al. (2009) C-reactive protein as a risk factor for coronary heart disease: a systematic review and meta-analyses for the US Preventive Services Task Force. *Ann Intern Med* 151, 483–495.

- [45] Floegel A, Chung S-J, Ruesten A.V, Yang M, Chung S.E, Song Wo, Koo SI, Pischon T and Chun O.K. 2011. Antioxidant intake from diet and supplements and elevated serum C-reactive protein and plasma homocysteine concentrations in US adults: a cross-sectional study. *Public Health Nutrition*: 14(11), 2055-2064 doi:10.1017/51368980011000395
- [46] Kasapis C and Thompson P.D., 2005. The effects of physical activity on serum C-reactive protein and inflammatory markers: a systematic review”, *journal of the American College of cardiology*, vol.45, no. 10,pp.153-1569,2005
- [47] Mattusch F, Dufaux B, Heine O, Mertens I, and Rost. 2000. Reduction of the plasma concentration of C-reactive protein following nine months of endurance training, *International Journal of Sport Medicine*, vol. 21, no. I, pp. 21-24, 2000
- [48] Woods J.A, Viera V.J, and Keylock K.T. 2009. Exercise, Inflammation, and Innate Immunity. *Immunology and allergy clinics of North America*, vol. 29, no.2 ,pp.21-24,2000
- [49] Huang C.J, Zourdos M.C, Jo E, and Ormsbee M.J. 2013. Influence of physical activity and nutrition on obesity-related immune function. *The scientific world journal* Vol. 2013, Article ID 752071, 12 pages
- [50] Adeyemi OS, Fambegbe M, Daniyan OR, Nwajei I. 2012. Yoyo Bitters, a polyherbal formulation influenced some biochemical parameters in Wistar rats. *J Basic Clin Physiol Pharmacol* 2012;23:135-138
- [51] Marciniak, A., Brzeszczyńska, J., Gwoździński, K., dan Jegier, A., 2009. Antioxidant Capacity and Physical Exercise. *Biology of Sport*. 26 (3):197213.
- [52] 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: Implication for Atherosclerosis and Preeclampsia. *Acta Physiologica Sinica*. 59 (2):117-27
- [53] Monville C, Torres E.M, Dunnet S.B. 2006. Comparison of Incremental and accelerating protocols of the rotarod test for the assessment of motor deficit in the 6-OHDA model. *OI:10.1016/J.jneumeth.2006.01.001*
- [54] Lucia vandan. , A. Moldovan R. Tache S (2012) Antioxidant complex supplementation and aerobic exercise capacity in rats. *Palestrica of the third millennium – Civilization and Sport* Vol. 13, no. 3 no. 3, July-September 2012,182187
- [55] Warburton, D.F.R., Nicol, C.W., Bredin, S.S.D (2006) Health Benefit of physical activity: the evidence, *Canadian Medical Association Journal*, vol.174, no.6, pp. 801-809, 2006
- [56] Colbert L.H., Visser, Simonsick E.M. et al., 2004. Physical activity, exercise, and inflammatory markers in older adults: findings from the health, aging and body composition study,”*Journal of the American Geriatrics Society*, vol. 52, no. 7, pp.1098-1104,2004

[57] Starkie R., Ostrowski S.R., Jauffred S., Febbraio M., and Pederson B.K., 2003. Exercise and IL-6 infusion inhibit endotoxin-induced TNF- $\alpha$  production in humans, *The FASEB Journal*, vol.17, no.8, pp. 884-886,2003

[58] Jankord R and Jemiolo B., 2004. Influence of physical activity on serum IL-6 and IL-10 levels in healthy older men, *Medicine and science in sports and exercise*, vol. 36, no. 6, pp.960-964,2004.

[59] Savini I, Catani M.V., Evangelista D, Gasperi V and Avigliano L. 2013. Obesity-Associated Oxidative: Strategies Finalized to Improve Redox State. *Int. J. Mol. Sci.* 2013, 14, 10497-10538; doi:10.3390/ijms140510497