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Consumption of pomegranate juice combination with exercise effect on cholinesterase activity Irisin, Dopamine level and many biochemical parameters in stressed women in Kirkuk City-Iraq

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***ABSTRACT**--The present study is designed to evaluate the effects of consumption of pomegranate juice in combination with training exercise in activity of cholinesterase , concentration of Irisin , Dopamine and many biochemical parameters (Creatine , creatine kinase –CK- , Total protein , Glutathione –GSH- ,Ceruloplasmin , Malondialdehyde –MDA- and Catalase) Seventy-eight employed women age (25to 45) BMI (24±4.22 kg/m2) eighteen of these women as control while others about sixty whom women suffer from daily stress plus working in house for 12-15 h/day divided five groups: Group 1:(Control group) healthy employed working without kid for 5-7 h/day include 18 women. Group 2 –(Stressed group) employed women(have more than 2 kid) +working for 12-15 h/day include 20 women . Group 3- (S+CEG) Stressed employed women(have more than 2 kid)+Chronic Training Exercise Group (1 h/day/ 21 day#P$yChOsOcIaL@s) include 14 women. Group 4- (S+PJG) Stressed employed women (have more than 2 kid)+ consumption Pomegranate Juice PJ include 13women.Group 5-(S+CE+PJG) Stressed employed women (have more than 2 kid)+ Chronic Training Exercise (1 h/day/ 21 days) + PJ include 13 women.Samples were collected randomly from Kirkuk city from October 2018 to July 2019.The result of this study show positive effect for chronic exercise alone and in combination with pomegranate juice significantly increase Irisin ,Dopamine , GSH ,CP levels and CK ,CAT activities in stressed women compared with stressed women without treatment. While there was significant decrease in Ach E activity ,Creatine ,MDA in stressed women training exercise only or in combination with pomegranate juice compared with stressed group only .*

As in previous studies the protein level show no change in experimental groups and control group.

***Keywords** --Pomegranate juice , Training exercise , Cholinesterase , Irisin , Dopamine , antioxidant agents.*

I. INTRODUCTION

Physical activity and physical fitness are positively linked to the health status of the human being and on the opposed, staying out from exercise is a cause of increased probability of many chronic diseases such as cardiovascular disease and type 2 diabetes mellitus [1,2,3]

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The scientists have noticed that the muscles have a role as an exorcism as they secrete substances known as myokines, which are the substances that are excreted from the skeletal muscles in response to the activity of sports and the resulting positive health effects [4]. Irisin is one of the these mayokines free from skeletal muscles, which has effects in the metabolic processes of the body and this hormone irisin (12 kilodalton molecular weight) was discovered by Bostrom and his colleagues in 2012 , during its release from the skeletal muscles works to convert white fatty tissue to brown fatty tissue by stimulating unrelated proteins (UCP1) results through this conversion in tissues heat-generating , which enhances insulin resistance and increases the level of glucose in the blood [5].

This hormone irisin has the capability to make the brown type of fatty tissue through its effect of genetic encryption of white fatty tissue as it increases the rate of nutritional metabolism and reduces insulin resistance, irisin secretion is caused by the supporter agent to stimulate the reproduction of peroxisome peroxisome Peroxisome proliferator- 1 alpha (PGC-1 α).

This is the main regulatory factor for muscular metabolism in exercise as it was documented that increased genetic coding of this agent leads to an elevation in resistance to metabolic diseases by which the hormone irisin is made from the muscles as a protein analyser after fission the main source of it (Fibronectin type domain - containing protein 5) present in human and rat serum as now studies have found that the hormone irisin is secreted from adipocytes [6] ; in addition this hormone has a similar effect to certain hormones through its effect on metabolism and insulin resistance [7,8].

Several previous researches have been discovered the hormone irisin mechanism of action , and it has been noticed that the peroxisome proliferator-activated receptor (PPARφ) and PGC-1α have the capability to regulate multiple genes based on their response to the physiological and nutritional signals that are encoded in skeletal muscles during exercise are associated with reduced body weight and reduced levels of oxidative stress in the skeletal muscles and encryption improves insulin sensitivity by improving the efficiency of insulin pathways [9,10].

Acetylcholine is one of the neurotransmitters that helps transmit nerve impulses through neurons and neural synapses – high levels of choline estres improve cognitive function and memory [11]. Cholinesterase (AchE) and Butyrylcholine (BuchE) are composed of large and complex proteins of four peptide chains and are present in most organisms, in mammals in the liver and plasma in high-density lipoprotein (HLD), and nerve tissue in choline clamp sites where acetylcholine is analyzed at the site of neuromuscular association, neuromuscular connections, body of neurons, nerve axis, central nervous system (spinal cord and brain), muscles, serum, red blood cells, platelets, T lymphocytes, pancreas, motor end plate and nerve fibres , acetylcholine is released from the neuromuscular link as well as from the pre-node nerve fibres of the autonomic nervous system, and therefore it is involved in physical movement, when you start exercise, levels of acetylcholine in the brain increase, especially in these hippocampus and cortex regions. The elevation in acetylcholine enhances and passes the neural signal during the neural synapses and the formation of memory [12,13].

While only one study in rodents referred to changes after exercise in acetylcholine levels , it was most likely due to the minimum exercise protocol used (walking at a rate of 2.3 meters per minute for 5 minutes). In humans, it was found that choline in plasma, an introduction to acetylcholine, decreased by 40% after marathon running, and besides serotonin and dopamine, although acetylcholine is involved in cognitive and cognitive processes.

There is also interest in supplements that can improve athletic performance, and athletes often use supplements to increase their metabolic ability, delay signs of fatigue, improve muscle swell and shorten the recovery period. They take dietary supplements to handle with the decline in immune system functions due to intense and frequent exercises, which become more susceptible to upper respiratory infections.

In addition , exercise shows physiological pressure on the body, requiring a coordinated response by the cardiovascular, pulmonary, and nervous systems to raise blood flow and oxygen supply for skeletal muscle work. At rest, muscles receive about 20% of total blood flow, but, during exercise, this elevate to more than 80% [14,15].

Punica granatum L. (Punicaceae) is generally consumed as pomegranate juice (PJ). Polyphenols, present in PJ, have powerful antioxidants, which participate in its anti-inflammatory and anti-atherosclerotic properties.

Dietary supplementation of polyphenols potentially play a therapeutic role in protecting against oxidative stress by which polyphenols act as scavengers of oxygen radical species and raise the concentrations of the antioxidant glutathione by the induction of glutamate cysteine ligase.

The combination polyphenols with phytochemicals such as ellagic acid synergistically enhances the larger antioxidant properties of PJ.

Furthermore, one of important anti-inflammatory properties of polyphenols are the inhibition of nuclear factor kappa B (NF-κB) expression/activation, interleukin (IL)-8 release, cyclooxygenase-2, and heme oxygenase [16,17,18].

The current study aims to clarify the effect of Pomegranate juice administration during chronic training exercise in compare with the regular walking in employed stressed women working daily addition to raise their children and take care from and its main role in increase the production and release of the hormone irisin, which is secreted from the skeletal muscles ,The study also aims to elucidate the role of the hormone in improving mental and cognitive abilities through its effect as a natural temporary inhibitor of cholinesterase so it give the time to accumulation of acetylcholine in the neural synapses to transport the signals ,addition to increasing the concentration of acetylcholine and dispensing as much as possible with medication or excessive treatment without resorting to consulting a doctor This is because these supplements, specifically are an important natural requirement for the human body in general and particular for stressed employed women in Kirkuk city .

II. MATERIAL AND METHODS

2.1Patients and control

Seventy -eight stressed employed women age (25to 45) BMI (24±4.22 kg/m2) these women suffer from daily stress addition to her responsibility from their kids and finished working house for 12-15 h/day divided five groups:

Group 1: (Control group) healthy employed working without kid for 7-9 h/day include 18women.

Group 2 : (Stressed group) employed women(have more than 2 kid) +working for 12-15 h/day include 20 women .

Group 3: (S+CEG) Stressed employed women (have more than 2 kid)+Chronic Training Exercise Group (1 h/day/ 21 days) include 14women.

Group 4: (S+PJG) Stressed employed women (have more than 2 kid)+ consumption Pomegranate Juice PJ include 13 women.

Group 5: (S+CE+PJG) Stressed employed women (have more than 2 kid)+ Chronic Training Exercise (1 h/day/ 21 days) + PJ include 13 women.

Samples were collected randomly from Kirkuk city from October 2018 to June 2019.

2.2. Sample collection

5 ml of blood were collected from the vein from healthy employed working without kid ,and from other experimental groups and kept in plain tube ,after allowing the blood to clot at room temperature (25 c)for 15 min.blood sample were centrifuged at 2500rpm for 15 minute , serum were separated divided into aliquots and frozen at -20 c for estimation of the activity of cholinesterase concentration of markers of Hormonal ,biochemical parameters.

2.3. Plant materials

Fruits were washed and hand-peeled. Arils were squeezed using a commercial blender (moulinex, France). The extract juice was centrifuged at 15000 rpm for 15 min. Then the supernatant was recovered and lyophilized.

2.4.Mesurments

2.4.1. Estimation of Irisin level in serum of human by using Irisin assay kit supplied from My BioSource (USA) company, it was measured by commercial enzyme-linked immune absorbent assay (ELISA)(ELISA) kits. (Young-Pearse *et al.*,2007) (19)

2.4.2. Estemation of Total Protein level in human serum by using ready TP assay kit supplied from BIOLABO ,SA:France (20)

2.4.3. Estimation of creatine kinase activity in serum by using CK assay kit supplied from Roche Company,Germany (21)

2.4.4. Estimation of creatinin in serum by using CK assay kit supplied from Roche Company,Germany (22)

2.4.5. Estimation of Glutathione Serum using the modified method used by the researchers (Sedlak and Lindsay,1968)(23)

2.4.6. Measurement of plasma peroxidation levels Malondialdehyde(MDA) asecondary product of lipid peroxidation was based on the colorimetric reaction eith thiobarbituric acid TBA (Guidet&Shah,1989)(24)

2.4.7. . Estimation of Ceruloplasmin of serum ceruloplasmin concentration using the modified method of the researchers. In pH: 5.45, ceruloplasmin oxidizes the colourless paraffanilin di-amine reagent to produce a violet coloured product,The rate of formation of the coloured oxidation product depends on the serum plasma concentration of human serum

2.4.8. Determination of Catalase activity assay kit (Beers & Sizer 1952)(25)

2.4.9. Determination of Dopamine: it was measured by commercial enzyme-linked immune absorbent assay (ELISA) dopamine (DA) were purchased from Sigma-Aldrich- USA)

2.4.10 . Determination of Acetylcholine esterase activity in serum by Ellman method This method can be accomplished via using spectrophotometer,

III. RESULTS AND DISCUSSION

Table 1: Estimation of acetylcholine esterase Activity , Irisin and Dopamine level in blood serum in Control and Experimental groups.

Parameters Groups	Ach E µmol/min/ml	Irisin ng/ml	Dopamine pg/ml
Control Group	0.053±0.06	49.75±3.25	25.17±1.20
Stressed Group	0.280±0.02	27.07±1.10	11.55±5.07
S+CEG	0.041±0.28	50.11±4.21	27.51±1.23
S+PJG	0.070±0.10	45.06±3.01	21.03±1.15
S+CE+PJG	0.092±0.041	52.22±3.21	28.10 ±1.00

Acetylcholine esterase activity shows significant increased ($p<0.05$) in stressed women (0.280±0.02) compared with control group (0.053±0.06) , while other experimental groups show significantly decrease ($p<0.05$) in AchE activity compared with stressed group. These results are in agreement with the study of Szwajgier and Borowiec (2012) [26] who noticed such anti-acetylcholine esterase activity , the mechanism of anticholinesterase activity of pomegranate is due to antioxidant power of its bioactive compounds such as flavonoids by three powerful mechanisms of protection of neural cell from oxidative stress including preventing the influx of Ca2+ what ever high levels of ROS,increasing intracellular GSH and directly lowering levels of ROS [27].

Irisin levels indicate significant decrease ($p<0.05$) in stressed women (27.07±1.10) in contrast with control group(49.75±3.25) while the experimental group show significant increase in irisin level compared with stressed group.

Irisin decrease in stressed women group occurred due to oxidative stress resulting from stress while Irisin increase in the experimental group and this take place because of antioxidative effects of both exercises and pomegranate juice which contains highly effective antioxidant components like Ellagitannins , ellagic acid, punicalagin and polyphenols [28] ; furthermore raised irisin itself in experimental group show powerful antioxidant properties by which it remove harmful hydroxyl groups and play a role in the increased expression of key enzymes involved with antioxidative stress pathways including SOD, GSH-Px and Catalase [29].

As well as our study find significant decrease in Dopamine level ($p<0.05$) in stressed women (11.55±5.07) compared with control group(25.17±1.20) while experimental groups show increase in dopamine level compared with stressed group. Stress lead to oxidative stress which have important roles in the dopaminergic neurodegeneration leading to dopamine decreasing , polyphenols component in Pomegranate juice reduce neuronal death due to its antioxidant and anti-inflammatory actions [30].

Table 2: Estimation of Creatine, Creatine kinase activity and total protein level in control group and experimental groups

Parameters Group	Creatine mg/dl	CK IU/L	Total protein g/ml
Control group	0.52±0.01	74.22±12.07	7.01±1.21
Stressed Group	1.77±0.25 a	38.20±5.01 a	7.18±1.33
S+CEG	0.62±0.06	74.11±12.03	7.03±1.05
S+PJG	0.74±0.12	66.42±17.22	7.00±1.43
S+CE+PJG	0.66±0.04	71.04±9.11	7.12±1.02

The result show significantly increase in creatine level in stressed group (1.77±0.25) compare with control group (0.52±0.01), whereas creatine level decrease in S+CEG, S+PJG and S+CE+PJG compare with stressed group ,also stressed women observed high decrease in CK activity(38.20±5.01) as compare with control group(74.22±12.07) ; while Ck activity increase in S+CEG (74.11±12.03) ,S+CE+PJG (71.04±9.11) and S+PJG (66.42±17.22) compare with stressed group. These results in agreement with the study of Achraf et al. (2018) [31] who noticed during the exercise duration increase in muscle enzyme releasing including CK . Supplementation with pomegranate juice combined with exercises improve muscle recovery, prevent oxidative stress because pomegranate polyphenols are able to upregulate endogenous antioxidant capacity via the Nrf2/antioxidant response element (ARE) pathway. Nrf2 is a master regulator of the antioxidant response through the regulation of a wide range of antioxidant and phase II detoxification genes , polyphenols also seem to possess anti-inflammatory properties by which inhibit cyclo-oxygenase enzymes, COX1 and COX2 , reduce NF-ƘB expression signalling and inhibit nuclear translocation of NF-ƘB , further more polyphenols improves vascular function, specifically endothelium-dependent vasodilation reduced superoxide production and thus decreased conversion of NO to peroxynitrite contributes to the increased vasodilation response by improving NO bioavailability [32].

As according to previous study total protein level showed no change in studied groups in compare with healthy group.

Table 3: Concentration of GSH, CP , MDA level and Catalase activity in control group and experimental groups

Parameters Groups	Glutathione µmol/L	Ceruloplasmin µmol/L	Malondialdehyde µmol/L	Catalase IU/ml
Control G	12.43±0.25	249.1±6.02	4.06±1.22	46.1±0.83
Stressed Group	3.07±1.72	179.12±2.1	12.08±7.73	23.02±0.45
S+CEG	11.55±0.54	247.03±6.0	3.18±1.05	40.90±1.11

S+PJG	8.23±1.19	240±4.27	3.77±2.32	28.5±0.82
S+CE+PJG	11.02±0.33	244±4.05	6.04±4.05	41.11±0.66

Our study find Glutathione had been noticed significant decrease in stressed group (3.07±1.72) compare with control group (12.43±0.25) , while there was increase in GSH in S+CEG and S+CE+PJG compare with stressed group (11.55±0.54) , (11.02±0.33) ,(3.07±1.72) respectively.

These results are in agreement with the study of Naghizadeh-Baghi *et al.* (2015) [33] who demonstrated that after sever physical activity because pomegranate juice induce endogenous antioxidant defence mechanisms by which pomegranate act as a potent source of flavonoids which provide an additional support to the elevation of GSH levels [34].

Same results showed in Ceruloplasmin level in stressed group (179.12±2.1) compare with control group (249.1±6.02) , but experimental group showed significantly increase compare with stressed group.

These results coincide with the study of Mazani *et al.* (2014) [35] who studied that following exhaustive exercise in young healthy males and this occur due to the positive effects of pomegranate juice in increasing the levels of antioxidant enzymes including Ceruloplasmin [36].

MDA level show significant increase in stressed Group compare with control group (12.08±7.73), (4.06±1.22) respectively , whereas S+CEG and S+PJG witnessed significantly decrease in MDA level in compare with stressed group only .

These results supported by the study of Fuster-Muñoz , *et al.* (2016) [37] who showed that in endurance-based athletes , this can be explained because of antioxidant actions of pomegranate juice components like poly-phenols due to their affinity to plasma lipid molecules protecting them from lipid peroxidation occur as result of oxidative stress during exercise so this lead to significant lowering in MDA levels [38].

In our study find decrease in catalase activity in stressed group in compare with control group ,whereas S+CEG and S+CE+PJG has high increase in catalase compare with stressed group . in other hand S+PJG has no different in compare with stressed group.

These results are in agreement with the study of Ammar *et al.* (2017) [39] who noticed such effect following weightlifting exercise , this occur because pomegranate juice prevented the reduction in the antioxidant enzymes due to positive effects of different classes of pomegranate juice polyphenols and flavonoids on antioxidant enzyme activities causing a significant increase in CAT for example some polyphenols are able to modulate the transcrip-tion and expression of proteins involved in endogenous antioxidant defence, by interacting with antioxidant re-sponse elements in the promoter regions of protein-coding genes [40].

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