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EFFECT OF CORN SILK, PUMPKIN SEEDS, AND CREATINE ON OXIDATIVE STRESS OF MALE EXERCISED RATS

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Key Words: Corn silk, Pumpkin seeds, creatine supplementation, creatine kinase, lactate dehydrogenase, treadmill, oxidative stress.

ABSTRACT

The present study was conducted to investigate the effect of corn silk, pumpkin seeds and creatine supplementation on oxidative stress biomarker as well as liver function and glycogen content of adult male exercised rats. Fifty male albino rats were fed on basal diet for one week for adaptation. During the adaptive week all rats (except negative control group) were exposed to day after day forced treadmill running exercise for 10 min. Rats were divided into two main groups, the first main group (n=10 rats) was fed on basal diet only and served as negative control group. The second main group (exercised rats) were divided into 4 subgroups (10 rats each) as followed:-**Subgroup 1**: were fed on basal diet and had run for 10, 15, 20 and 25minutes (15 days for each time period), respectively and served as positive control group. The **Subgroups (2-4)** as the same of positive control subgroup and were fed on basal diet supplemented with 4% corn silk powder, 4% pumpkin seeds powder and 4% creatine powder, respectively. At the end of the experimental period (60 day), 5 rats of each subgroup were exposed to the exhausting treadmill test and the run time was recorded, then all experimental rats were scarified. Findings of these study prove that corn silk and pumpkin seeds were a good source of flavonoid which help to scavenge the oxidative stress substances, and improve liver functions , while creatine recorded the largest glycogen quantity in gastrocnemius muscles among supplemented groups, but it causing an elevation ammonia, and AST levels on the group which exposure to exhaustive time test. It could be recommended that corn silk and pumpkin seeds have the ability to eliminate physical fatigue and prolong the training period without fatigue

INTRODUCTION

Biomarker discovery and validation is a critical aim of the medical and scientific community. Research into exercise and diet-related biomarkers aims to improve health, performance, and recovery in military personnel, athletes, and lay

Persons. Exercise physiology research has identified individual biomarkers for assessing health, performance, and recovery during

exercise training. However, there are few recommendations for biomarker panels for tracking changes in individuals participating in physical activity and exercise training programs **(lee *et al* .,2017).** The diversity of nutrients or compounds from food factors or medical herbs could be investigated for their possible effect on exercise physiology and for understanding the different bioactivities that could be used for health promotion **(Ching *et al*., 2015).** Physical activity modifies the balance between oxidative stress and antioxidant defense mechanisms. For both athletes and fitness enthusiasts, the combination of regular physical activity and antioxidant supplement may have important restorative effects on the body's oxidation reduction or redox balance (**Araújo *et al*., 2013)**.

Corn silk (*Maydis stigma*) is a waste material from corn cultivation, available in abundance, and possesses antioxidant, anti-fatigue, anti-tumor and anti-fungal activities (**Ebrahimzadeh *et al*., 2009 and Hu *et al*., 2010**).

The seeds of pumpkin are generally considered to be agro-industrial wastes and discarded. In some parts of the world, the seeds are consumed raw, roasted or cooked, but only at the domestic scale. With the discovery of their richness in protein, fibers, minerals, polyunsaturated fatty acids and phytosterols, they are being regarded valuable for the food industry. Also, their beneficial effects on blood glucose level, immunity, and cholesterol, liver, prostate gland, bladder, depression, learning disabilities and parasite inhibition are being validated **(Patel, 2013).** Dietary supplementation with creatine (CrS) is popular in the sports and fitness industry. While certain mechanisms of action involved in improved physical exercise performance with CrS have been established **(Gama, 2011).**

Therefore, the aim of the study was to investigate the oxidative stress effect of corn silk, pumpkin seeds and creatine supplementation on adult male trained rats and their exercise performance.

MATERIALS AND METHODS

Materials:

Corn silk and Pumpkin seeds were purchased from Field Crops Research Institute, Ministry of Agriculture Giza, Egypt. **Chemicals**: Creatine powder, casein, vitamin mixture, mineral mixture and cellulose were purchased from El-Gomhoria Pharmaceutical Company, Cairo, Egypt. **Rats**: fifty young adult male albino rats of Sprague- Dawely Strain, weighting (150 ±5 g) were obtained from Animal House Colony of Vacsera, Helwan, Egypt. **Kits** for blood analysis were purchased from Gama Trade Company for Chemicals, Cairo, Egypt. **The Treadmill** was purchased from Sports Equipment Store, Cairo, Egypt (YY-T900D Luxury electronic treadmill, Motor power 3,0HP Quality in Taiwan with

an adjustable speed (0, 8 -14.8 Km/h), Running surface 1230*430mm, and expand dimensions 1620* 700*1200 mm enabling forced exercise training and accurate testing of fatigue in rats.

Methods:

Preparation of Dried Plants:-Fresh corn silk and pumpkin seeds were washed by using tap water and were dried using solar energy at the National Research Centre, Giza, Egypt in the sun at 50°C for two days then grounded to get a fine powder .The mild temperature (45-55°C) enable the dried product to retain its nutrients as described by **(Andritsos *et al*., 2003).**

Active components (phenolic-flavonoids) and antioxidant activity of corn silk and pumpkin seeds were determined chemically according to the method described by **(Brand-Williams *et al*., 1995)**

Experimental Design:-

This study was carried out at the Animal House of Home Economic Faculty, Helwan University. Fifty male albino rats, were housed in well aerated cages under hygienic condition and were fed on basal diet for one week for adaptation. During the adaptive week all rats (except negative control group) were exposed to day after day forced treadmill running exercise for 10 min by using treadmill according to the method of (**Davis, 2009)**.

Rats were divided into two main groups, the first main group (n=10 rats) were fed on basal diet only and served as negative control group. The second main group (exercised rats) were divided into 4 subgroups (10 rats each) as followed:- **Subgroup 1**: were fed on basal diet and will be run for 10, 15, 20 and 25minutes (15 days for each time period), respectively and served as positive control group. The **Subgroups (2-4)** as the same of positive control subgroup and were fed on basal diet supplemented with 4% corn silk powder, 4% pumpkin seeds powder and 4% creatine powder, respectively.

The exhausting time protocol:

At the end of the experimental period (60 day), 5 rats of each subgroup were exposed to the exhausting treadmill test and the run time was recorded, then all experimental rats were scarified. **Blood samples** were collected and centrifuged to obtained serum which was used for the biochemical analysis. Liver and gastrocnemius muscle were collected from each rat to determine glycogen content (**Banchroft *et al.*, 1996)**

Biochemical analysis of serum:

Serum samples were used for the determination of glucose described by **(Trinder, 1969** and **Weissman and Klein, 1958),** creatine kinase **(Tietz, 1976),** lactate dehydrogenase (**Vassault *et al.*, 1986),** Aspartate aminotransferase (AST) **(Schumann *et al.*, 2002),** Alanine aminotransferase (ALT) was determined in serum according to

(Sherwin, 1984)., Serum malondialdehyde (MDA) was determined by the method of **(Ohkawa *et al*., 1979),** Serum Glutathion-s-trasferase was determined by the method of **(Habig et al., 1974)**.

Statistical analysis:-

The results was evaluated by statistically analyzed using computerized program SPSS .results were expressed as mean ± SD .differences among groups were analyzed of analysis of variance (ANOVA) using Duncan's test .A $p<0.05$ was considered statistically according to **(Sendecor and Cochran, 1986)**

RESULTS

The total phenolic content of corn silk and pumpkin seeds are presented in Table (1). The results indicate that corn silk contained mild amount of myricetin acid ,benzoic acid , Salicylic acid , Neringein , Kampherol and mean value was 16.79, 23.84, 34.75, 61.87, 75.57 mg / kg respectively. The Ellagic recorded the largest amount of phenolic content in corn silk with mean value 417.10 mg / kg. On the other hand, pumpkin seed contained mild amount of Chlorogenic, Vanillin, Caffeine, benzoic acid, and Neringein with mean values 7.34, 8.12, 10.167, 13.47, and 22.88 mg / kg respectively . The largest amount of phenolic contents in pumpkin seeds was Ellagic and Catechol which recorded 71.91 and 76.84 mg/kg respectively.

Table (1): Corn silk and pumpkin seeds total phenolic content

Phenolic Compounds	Corn Silk(mg / kg)	Pumpkin seed(mg / kg)
Syringic acid	1.49	1.04
Vanillic acid	2.06	8.12
p- Coumaric acid	2.25	1.52
Quercetin	2.74	--
Cinnamic acid	3.63	--
Rutin	4.13	--
Chlorogenic	4.30	7.34
Ferulic acid	4.82	--
Caffeic acid	5.02	2.29
Caffeine	5.15	10.16
Myricetin acid	16.80	--
Benzoic acid	23.85	13.47
Salicylic acid	34.75	5.19
Neringein	61.88	22.88
Kampherol	75.58	1.93
Ellagic	417.10	71.91
Quinol	--	1.12
Gallic acid	--	1.72
Pyrogallol	--	1.78
Catechol	--	76.84

The data in table (2) shown the result of radical scavenging activity for corn silk and pumpkin seeds which recorded high antioxidant activity 99.68% at 5% concentration compared to corn silk which recorded 84.07 at 5%concentration.

Table (2):The Antioxidant activity of corn silk and pumpkin seed

SN	% DPPH Radical-Scavenging Activity			
	0.5%	1.0%	2.5%	5%
Corn Silk	20.20	41.02	60.33	84.07
Pumpkin Seed	31.79	46.53	88.50	99.68

Table (3) recorded the effect of corn silk, pumpkin seeds, and creatine on FI, BWG, and FER in male rats which exposure to exhaustive test according to the experimental training treadmill protocol. Results indicate that positive control group which fed on basal diet throughout the experiment and was trained day after day for (60) days non significantly increased on FI level compared to (-ve) control group. Corn silk and creatine groups significantly increased FI compared to +ve group, otherwise pumpkin seeds group non significantly changed FI compared to (+ve) group. The highest feed intake was recorded at the creatine group. Rats in +ve control group had significant decrease ($P<0.05$) in BWG compared to –ve control group. Corn silk group and pumpkin group significantly decreased BWG compared to (+ve) control, on the other side, creatine group significantly increased BWG compared to (+ve) group and recorded the best group findings. Regarding to FER of rats in +ve control group was significantly decreased compared to –ve control group. Corn silk and pumpkin seeds significantly decreased FER compared to +ve control group, inversely creatine group was significantly increased FER compared to +ve control group. The best result in FER of all tested group was creatine group.

Table (3):Effect of corn Silk, pumpkin seed and creatine on BWG, FI and FER of rats exposure to exhaustive test

Parameters / Groups		FI g/day	BWG g	FER
1	(-ve)	19.68 ± 1.16^{c}	62.25 ± 2.99^{b}	0.05 ± 0.001^{a}
2	(+ve)	23.59 ± 0.54^{c}	33.00 ± 2.88^{c}	0.02 ± 0.002^{b}
3	Corn Silk	26.99 ± 1.17^{b}	-56.00 ± 2.54^{e}	-0.03 ± 0.001^{d}
4	pumpkin	20.00 ± 0.88^{c}	-14.50 ± 1.58^{d}	-0.01 ± 0.001^{c}
5	Creatine	28.03 ± 0.47^{a}	72.75 ± 2.21^{a}	0.04 ± 0.001^{a}

All value represented as mean ± SD.

Means with different superscript are significantly different ($p<0.05$)

Table (4) illustrated the effect of corn silk, pumpkin seeds, and creatine on, FI, BWG, and FER in male rats which non exposure to exhaustive test (the end of experiment) in the experimental training

treadmill protocol. Positive control group which fed on basel diet while not exposure at the end of experiment to exhaustive treadmill test had significant increase in FI amount compared to (-ve) group. All tested groups had significant decrease in FI compared to +ve control group. Rats in + ve control group had significant decrease in BWG compared to –ve control group. Pumpkin seeds and corn silk group significantly decreased ($P<0.05$) BWG compared to +ve control group, however creatine group significantly increased compared to +ve control group. The best findings in all test groups was creatine group. Positive control group showed significant decreased in FER compared to –ve control group. Pumpkin seeds and corn silk significant decreased FER compared to +ve control group on the opposite creatine group significantly increased compared to +ve control group and the best result from all tested group was creatine.

Table (4): Effect of corn Silk, pumpkin seed and creatine on BWG, FI and FER of rats non exposure to exhaustive male rats

Parameters / Groups		FI g/day	BWG g	FER
1	(-ve)	19.68 ± 1.16^{b}	62.25±2.99^{b}	0.05 ± 0.01^{b}
2	(+ve)	22.25 ± 1.26^{a}	24.75±2.50^{c}	0.02 ± 0.003^{c}
3	corn Silk	20.50 ± 2.69^{b}	-11.20±0.69^{d}	-0.01 ± 0.03^{d}
4	Pumpkin	20.63 ± 2.18^{b}	-08.00±0.06^{a}	-0.007 ± 0.004^{a}
5	Creatine	20.83 ± 2.34^{b}	59.00±4.81^{b}	0.05 ± 0.001^{b}

All value represented as mean ± SD.

Means with different superscript are significantly different ($p<0.05$).

As shown in Table (5), the effect of corn silk, pumpkin seed and creatine on AST, which illustrated significant increase in (+ve) control group compared to (-ve) control group. Rats fed on corn silk non significantly decrease AST compared to (+ve) control group. Whereas, rats fed on pumpkin seeds and creatine recorded significant increase in AST level compared to (+ve) control group and. Corn silk group marked as the best group among all tested group. The concentration of ALT for (+ve) control group had significant decrease compared to (-ve) control group. Rats fed on corn silk had non-significant decrease compared to (+ve) control group. While rats which fed on pumpkin seeds and creatine noticed significant increase compared to (+ve) control group. Corn silk group recorded best findings among all tested group. Results indicate that positive control group had significant increase in ammonia level compared with (-ve) control group. While rats which fed on corn silk and pumpkin seeds were significantly decreased compared to (+ve) control group. Creatine group recorded significant increase among all tested group.

Table (5): Effect of Corn Silk, Pumpkin Seed and Creatine on liver functions and ammonia Level on rats exposure to exhaustive time test

Groups / Parameters		AST u/l	ALT u/l	Ammonia µg /dl
1	(-ve)	122.00 ± 2.83^{d}	41.50 ± 3.00^{b}	73.00 ± 10.61^{c}
2	(+ve)	128.20 ± 13.57^{c}	34.20 ± 3.19^{c}	104.20 ± 5.15^{b}
3	Corn Silk	127.25 ± 9.22^{c}	32.50 ± 7.33^{c}	70.25 ± 4.72^{c}
4	Pumpkin seed	148.50 ± 6.35^{b}	64.50 ± 3.32^{a}	51.00 ± 4.62^{d}
5	Creatine	177.00 ± 9.90^{a}	42.00 ± 0.81^{b}	133.65 ± 1.30^{a}

All value represented as mean ± SD.
Means with different superscript are significantly different (p<0.05)

Table (6) showed that rats in (+ve) control group had significant decrease in AST level compared to (-ve) control group. Rats fed on pumpkin seeds and creatine had significant increase in AST compared to (+ve) group. Corn silk group recorded significant decrease compared to (+ve) control group, and it was the best result among all tested group. Serum ALT of (+ve) control group was significantly decreased compared to (-ve) control group .Whereas rats fed on corn silk, pumpkin seed and creatine significantly increased compared to (+ve) control group. The best result for ALT at pumpkin seeds group. Results of Ammonia level of (+ve) control group was shown significant increase compared to (-ve) control group. Whereas corn silk group recorded nonsignificant decrease compared to (+ve) control group**.** But pumpkin seeds and creatine groups showed significant increase in ammonia level compared to (+ve) control group.

Table (6): Effect of Corn Silk, Pumpkin Seed and Creatine on liver functions and ammonia level on rats non exposure to exhaustive time

Groups / Parameters		AST u/l	ALT u/l	Ammonia µg /dl
1	(-ve)	122.00 ± 2.83^{c}	41.50 ± 3.00^{b}	73.00 ± 10.61^{d}
2	(+ve)	110.75 ± 25.64^{d}	33.00 ± 3.16^{d}	93.50 ± 2.38^{c}
3	corn Silk	99.82 ± 4.01^{e}	42.40 ± 5.3^{b}	91.60 ± 2.07^{c}
4	pumpkin	137.75 ± 3.86^{b}	36.50 ± 1.73^{c}	125.05 ± 3.59^{a}
5	Creatine	147.00 ± 7.81^{a}	45.20 ± 3.96^{a}	109.84 ± 3.40^{b}

All value represented as mean ± SD..
Means with different superscript are significantly different (p<0.05)

Table (7) showed the effect of corn silk, pumpkin seeds, and creatine on, glucose, CK, and LDH level in male rats which exposure to exhaustive test. The result indicated that glucose level at (+ve) control

group was significantly increased compared to (-ve) control group. Corn silk group and pumpkin group recorded significant decreased in glucose level compared to (+ve) group, however creatine group recorded significant increase in glucose compared to (+ve) control group. The best result was recorded at corn silk group. Data of creatine kinase revealed that (+ve) control group non-significantly decreased compared with (-ve) control group. The finding indicated that corn silk group, pumpkin seed, and creatine supplementation caused significant increase CK compared with (+ve) control group, the best result among all tested groups was creatine group. LDH level in (+ve) control group shown significant decrease compared to (-ve) control group, but corn silk group, pumpkin seed, and creatine recorded significant decreased compared to (-ve) control group. The best result was corn silk group.

Table (7): Effect of Corn Silk, Pumpkin Seed and Creatine on Glucose, CK and LDH level on rats exposure to exhaustive time

Groups	Parameters	Glucose mg / dl	CK u/l	LDH u/l
1	(-ve)	106.01 ± 5.42^{c}	24.50 ± 5.74^{d}	2207.75 ± 84.16^{a}
3	(+ve)	115.02 ± 11.14^{b}	18.20 ± 3.96^{d}	1416.40 ± 91.50^{b}
5	Corn Silk	96.50 ± 01.91^{c}	676.25 ± 18.89^{b}	787.25 ± 3.77^{e}
6	Pumpkin seed	97.00 ± 03.83^{c}	1185.00 ± 84.67^{a}	965.00 ± 19.51^{d}
9	Creatine	165.05 ± 14.14^{a}	45.65 ± 2.36^{c}	1301.50 ± 52.80^{c}

All value represented as mean ± SD.
Means with different superscript are significantly different ($p<0.05$).

Table (8) showed the effect of corn silk, pumpkin seeds, and creatine on, glucose, CK, and LDH level in male rats which non exposure to exhaustive test according to the experimental training treadmill protocol. The results indicate that (+ve) control group which fed on basal diet and non-exposure to exhaustive time had significant increase in glucose level compared to (-ve) control group. Corn silk and creatine group recorded non-significant increase in glucose level compared with (+ve) control group. However pumpkin seed group shown significant increase compared with (+ve) control group, and also pumpkin seed group considered as the best group among all tested group. Concerning CK concentration in serum, the data indicated that rats in (+ve) control group had non-significant decrease compared with (-ve) control group, furthermore corn silk and pumpkin seed groups shown significant increase in CK level. Creatine group was the best group among all tested group. Results indicate that LDH level was significantly decreased in (+ve) control group when compared with (-ve) control group but in corn silk group, LDH was significantly decreased when compared with (+ve)

control group. The result pumpkin seeds and creatine groups indicated significant increased values when compared with (+ve) control group.

Table (8): Effect of Corn Silk, Pumpkin Seed and Creatine on Glucose, CK and LDH level of rats non-exposure to exhaustive time

Groups / Parameters		Glucose mg / dl	CK u/l	LDH u/l
1	(-ve)	106.01 ± 5.42^{c}	24.50 ± 5.74^{c}	2207.75 ± 84.16^{b}
2	(+ve)	110.06 ± 11.17^{b}	17.25 ± 0.96^{c}	1465.50 ± 74.93^{d}
3	corn Silk	117.60 ± 01.82^{b}	621.60 ±106.78^{a}	922.40 ± 31.16^{e}
4	Pumpkin seed	158.01 ± 05.77^{a}	50.09 ± 5.83^{b}	2380.50 ± 91.22^{a}
5	Creatine	124.20 ± 04.82^{b}	20.60 ± 2.30^{c}	2142.20 ± 168.50^{c}

All value represented as mean ± SD.
Means with different superscript are significantly different (p<0.05).

Table (9), showed the effect of corn silk, pumpkin seeds, and creatine on MDA and GST level in male rats which exposure to exhaustive test according to the experimental training treadmill protocol. Rats in (+ve) control group which fed on basal diet, recorded significant increase in MDA concentration compared to (-ve) control group . While rats in corn silk group, pumpkin seed group, and creatine group had significant decrease when compared to (+ve) control group. Creatine group consider as the best result among all tested group. The results revealed that GST was significantly decreased in (+ve) control group when compared to (-ve) control group, however rats fed on corn silk, pumpkin seed and creatine shown significant increase in GST level when compared with (+ve) control group. The best result recorded in creatine group among all tested group.

Table (9): Effect of Corn Silk, Pumpkin Seed and Creatine on MDA and GST level on rats exposure to exhaustive time

Groups / Parameters		MDA nmol/ml	GST u/l
1	(-ve)	14.40 ± 1.09^{b}	122.58 ± 20.08^{b}
2	(+ve)	16.13 ± 0.98^{a}	95.60 ± 35.33^{d}
3	Corn Silk	13.83 ± 1.00^{b}	167.00 ± 7.86^{a}
4	Pumpkin	13.48 ± 0.74^{b}	111.03 ± 17.57^{c}
5	Creatine	11.28 ± 0.84^{c}	114.30 ± 6.65^{c}

All value represented as mean ± SD.
Means with different superscript are significantly different (p<0.05)

Table (10) showed the effect of corn silk, pumpkin seeds, and creatine on MAD and GST level in male rats which non-exposure to exhaustive test. Results indicated that MDA level was decreased

significantly in (+ve) control group when compared with (-ve) control group, while rats fed on corn silk or creatine had non-significant decrease in MDA compared to (+ve) control group, while rats which fed on pumpkin seed recorded a significant reduction compared to (+ve) control group. The result revealed that GST was significantly decreased in (+ve) control group compared to (-ve) control group. Although corn silk group, pumpkin seed group, and creatine group caused a significant increase compared to (+ve) control group. Creatine group was the best result among all tested group.

Table (10): Effect of Corn Silk, Pumpkin Seed and Creatine on MDA and GST level on rats non exposure to exhaustive time

Groups \ Parameters		MDA nmol/ml	GST u/l
1	(-ve)	14.40 ± 1.09[a]	122.58 ± 10.08[c]
2	(+ve)	13.83 ± 1.14[b]	100.00 ± 14.49[d]
3	corn Silk	12.16 ± 1.21[b]	166.80 ± 13.01[b]
4	Pumpkin	10.18 ± 0.97[c]	123.56 ± 13.37[c]
5	Creatine	12.24 ± 0.13[b]	189.60 ± 13.34[a]

All value represented as mean ± SD.
Means with different superscript are significantly different ($p<0.05$).

Table (11) recorded influences of tested groups on exhaustive treadmill time test of exercised male rats. The result indicated that rats in corn silk group and pumpkin seed recorded 35, 45 min respectively, whereas (+ve) control group which fed on basal diet recorded 20 minute, these results illustrated an increase in exhaustive time test when compared to +ve group.

Table (11): Influence of corn silk, pumpkin seeds, and creatine on exhausting test (min) of male rats

Groups	(-ve)	(+Ve)	Corn Silk	Pumpkin seeds	Creatine
Time (min)	—	20	35	45	25

The result in table (12) indicated that rats in (-ve) control group had sever glycogen content in liver and was recorded the highest level compared to all groups. The rats treated with either corn silk, pumpkin or creatine and exposed to exhaustive treadmill test had nil glycogen content as compared to control groups. While rats had nil, mild, sever glycogen content at non exposure protocol and treated with creatine, pumpkin , corn silk respectively. On the other hand, the glycogen content in gastrocnemius muscle for the rats that exposed to an exhaustive treadmill test was nil, mild, sever for the rats that treated with corn silk, pumpkin, creatine respectively. While non exposure protocol

caused moderate, sever, moderate increase in glycogen content at the groups fed on corn silk, pumpkin, creatine respectively.

Table (12) Effect of corn silk, pumpkin seeds, and creatine on glycogen content in liver and gastrocnemius muscles exposure and non-exposure to exhaustive treadmill test

organs	groups	(-ve)	(+Ve) EX	Corn Silk EX	Pumpkin EX	Creatine EX
Liver	exhaustive	+++	+	—	—	—
	Non exhaustive	+++	++	+++	+	—
Gastrocnemius muscle	exhaustive	+	++	—	+	+++
	Non exhaustive	+	+	++	+++	++

+++ Sever, ++ Moderate, + Mild, - Nil

DISCUSSION

Mammalian skeletal muscle is capable of producing a large number of free radicals and it is well recognized that a major source of this free radical production happened during oxygen flux through the mitochondria. Which can increase 100-fold, potentially resulting in an increased risk of 'oxidative stress', muscle injury and fatigue. However, skeletal muscle is well equipped to deal with this oxidative stress by possessing a number of antioxidant species including both endogenous and exogenous antioxidants. It is only when there are an increased number of free radicals, or a depletion of antioxidant levels that 'redox balance' is disturbed and the cell becomes vulnerable to free radical attack (**Spurway and Maclaren, 2007)**

In this experimental study, albino male rats were used to examine the oxidative stress effect of corn silk ,pumpkin seed ,and creatine after exercised by using treadmill reached the exhaustive time test as well as , to determine the changes in body weight , liver enzymes and oxidative stress biomarker.

The palatability of corn silk, pumpkin seed, and creatine powder affected the consumption and partly accounts for the trend in the final body weights of the animals.

Exercise of sufficient intensity and duration can lead to the formation of reactive oxygen and nitrogen species (**Fisher-Wellman and Bloomer, 2009)**, which when produced in amounts that overwhelm the antioxidant defense system may lead to a condition of "oxidative stress". Corn silk and pumpkin total phenolic contents which were seen in Tables

(1 and 2) may be exhibited antioxidant activity by several mechanisms including a reduction in ischemia-reperfusion-induced oxidative stress (**Loffredo *et al.*, 2007and Volek *et al.*, 2002),** a reduction in xanthine oxidase activity **Spierin *et al.*, 2007**)), and a free radical-scavenging activity as shown in Table (2) (**Vanella *et al.*, 2000).**

Fiber in pumpkin seeds content makes these seeds ideal for weight loss. This dense and heavy snack can keep the body satiated for quite a longer period of time. The fiber takes longer to digest, leaving you full for longer and further keeps you away from bingeing on fattening foods. Other than this, pumpkin seeds is quite a good source of zinc, which can further help in boosting the body's metabolism. A sluggish metabolism can hinder body's weight loss efforts to a great extent. Hence, it is important to keep body's metabolism up and running (**Sarine, 2018).** These results were consistent with the results of this current research at tables (3 and 4)**.**

In the line with the research results **Lee *et al.*, (2016)** reported that experimental animals that received high-fat diet along with 100 mg/kg of high maysin corn silk extract for 8 weeks showed significant reduction of body weight compared to the high-fat diet only group. Additionally, kidney fat and epididymal fat pad weights significant decreased, demonstrating high maysin corn silk extract had a weight-reducing effect by decreasing fat accumulation in the body. **Min et al., (2011)** reported that administration of 100 mg/kg BW and 400 mg/kg BW of corn silk extract significantly lowered body weight after 2 weeks of intake in mice, which is consistent with the weight-reducing effect.

Results of the present study were in a line with **Kreider *et al.*, (1998)** which indicate that 28 d of creatine supplementation ($15.75 g \cdot d^{-1}$) during resistance/agility training promoted significantly greater gains in fat/bone-free mass. These findings support previous reports that creatine supplementation may increase total body weight and/or lean body mass, as seen at tables (3 and 4).

The most sensitive and profitable diagnostic enzymes of the liver are aminotransferases including AST and ALT. Generally, certain amounts of these enzymes are produced by liver cells, yet after liver damage, the liver cells increase the secretion of the enzymes and their plasma levels, whose increased level is a sign of liver damage **(Terohid *et al.*, 2015).** The incidence of oxidative stress in the body has other negative effects, such as muscle fatigue, early restlessness impaired, decreased immune function, and muscle oxidation **(Bloomer and**

Goldfarb, 2004). The results showed that 60 days of training protocol had slightly alteration effect on serum levels of ALT and AST on +ve and corn silk feeding groups exposed to exhaustive test. The lack of alteration is inconsistent with the findings of **(Kim *et al.*, 2007 and Ghasemnian *et al.*, 2020).** Acute strenuous exercise induces oxidative stress and/or tissue damage in several tissues including skeletal muscle, liver, and kidney (**Suzuki *et al* 2003; Aoi *et al*., 2004 and Suzuki, 2018**). It is well known that antioxidant capacity is increased by acute or chronic exercise **(Yada *et al.*, 2020).** This research results were in a line with above findings at tables (5 and 6) especially corn silk group showed great improvement compared with other treated groups.

Ammonia can cross the blood–brain barrier and has the potential to affect central neurotransmitter levels and central neural fatigue **(Hargreaves and Lawrence, 2020)**. An increase in ammonia concentration occurs predominantly after high-intensity exercise, where ammonia aids in stimulating glycolysis. With increasing fatigue, the level of ammonia biomarker increase, reflecting the physiological status of the athlete (**Kantanista *et al.*, 2016).**These results agree with the present study findings. As shown in the research results, corn silk play a whiteness role in the ammonia values impairment higher than pumpkin and creatine. The action of corn silk extract on mice was by increasing insulin level and recovering injured beta cells, and not via increasing glycogen or inhibiting gluconeogenesis. The results suggest that in modern pharmacological study, corn silk extract may be used as a hypoglycemic food or medicine for hyperglycemic people **(Guo *et al.*, 2009).** Pancreatic enzyme synthesis and secretion may change with physical exercise **(Minato, 2000).** In rats, endurance running training increases pancreatic protein content, pancreatic enzyme activity, and basal amylase secretion (**Minato, 1997),** this finding was in the same line with the present study of glucose level and agreement with **(praphatsorn *et al.*, 2010)** which found that high-intensity exercise at 75% and 90% VO2max caused an increase of biochemical parameters in liver and pancreas. The levels of exercise also caused histopathology changes in the liver and pancreas.

Seeds from contain bioactive compounds which have antifatigue activity and can elevate exercise performance. The seeds were said to have bioactivities such as hepatoprotection, anti-diabetes, anticancer, and anti-obesity properties (**Wang, 2012).** According to **Bharti *et al.*, (2013),** tocopherol isomers (α, β, γ, and δ) from raw pumpkin seeds has been

reported to be effective in the alleviation of diabetes through its antioxidant activities. In another studies also on alloxan-induced diabetic rats demonstrate that polysaccharide from pumpkins has hypoglycemic effect (**Xiong and Cao, 2001)**.This polysaccharide (50 mg/kg) administered orally in alloxan-induced diabetic increase the body weight, reduce water intake, and blood glucose levels of diabetic mice group compared with diabetic control group (**Perez Gutierrez**, **2016).** Furthermore, the administration of fruit powder for a month in diabetic rats significantly reduced C-reactive protein (CRP), cholesterol , glucose, Triglycerides while insulin was increased in diabetic rats **(Sedigheh *et al* ., 2011)**. **Gualano *et al*., (2010)** showed that creatine supplementation combined with exercise alleviated glycemic conditions in T2DM.

Release of CK from muscle cells during exercise corresponds to the degree of permeability of cell membranes and their damage resulting, among other things, from an increase in the amount of free radicals formed during physical activity, leading to the peroxidation of cell membrane lipids (**Banfi et al., 2012).** After muscle-damaging exercise, the enzyme CK leaks from the muscle into the circulation. It is typical for athletes to have elevated CK during training, with reference ranges of 82–1,083 u/l in male and 47–513 u/l in female athletes suggested as athletic norms (**Mougios, 2007).**

In the present study table (7) showed increasing in creatine kinase in pumpkin seed group among others groups ,those results in the similar line with **(AL-Zuhair *et al* ., 1997)** which suggested that pumpkin seeds oil was recorded ameliorated in creatine kinase levels ,while creatine group recoded lowest creatine kinase level , this finding in the same line with (**Bassit *et al*., 2010)** observed a decrease in several markers of muscle damage (creatine kinase, lactate dehydrogenase, aldolase, glutamic oxaloacetic acid transaminase and glutamic pyruvic acid transaminase) in 4 athletes after an iron man competition who supplemented with 20 g/d plus 50 g maltodextrin during a 5 d period prior to the competition.

During exercise, when muscles exhaust the oxygen, pyruvate gets catalyzed into lactic acid by the lactate dehydrogenase enzyme. In erythrocytes also pyruvate is not further metabolized due to the absence of mitochondria but remains within the cytoplasm, finally converting to lactate. In this reaction, NADH oxidizes to NAD+. The availability of high intracellular concentrations of NAD is necessary to carry out the preparatory phase of glycolysis. The net ATP production

of anaerobic glycolysis is only 2 ATP per glucose molecule as compared to oxidative phosphorylation, which produces 36 ATP per glucose molecule. LDH can also catalyze the dehydrogenation of 2-hydroxybutyrate, but it is the less preferred substrate for **LDH** than lactate **(Adeva-Andany *et al.*, 2014),** our result in table (8) showing increasing in LDH in creatine group which prove the previous hypothesis.

Table (9) showing decreasing in MDA level with corn silk, pumpkin seed, and creatine groups compare with positive control group which reflect the great amount of antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases (**Hu and Deng, 2011**),this met agreement with **(Bouzid *et al*., 2015)** who found that MDA level was higher during the recovery period in the high fitness level as compared with the others groups,which concluded that both low and high physical fitness levels help maintain better antioxidant defenses in older adults. However, a higher physical fitness level, rather than a lower physical fitness level, could increase lipid peroxidation. MDA is one the most popular oxidative stress markers, and due to its toxicity.

Biomarkers to evaluate the antioxidant capacity include glutathione, glutathione peroxidase, catalase, and the total antioxidant capacity **(Finsterer, 2012)** GSTs are postulated as important detoxifying enzymes that catalyse reduced glutathione-dependent reactions involved in cellular protection against OS and toxic chemicals (**Liavanera *et al.*, 2020)**, the present study GST results agree with the previous study and (**Ghahremanloo *et al* ., 2017)** which demonstrate that Pumpkin ameliorated oxidative stress and dyslipidemia in obese rats, leading to decrease cardiovascular disease risk in obesity. However, some studies showed different flavonoid synergists as most effective in hepatoprotection. Moreover, it has been reported that corn silk effectively increases antioxidant enzyme levels such as sodium dismutase and glutathione peroxidase (**Hu and Deng, 2011**).

The data in table (11) explained the major findings of the present study were that rats fed on pumpkin seeds and exposure to exhaustive test recorded (45 min) the longest period among all tested groups, this reflect Pumpkin seed and seed oil are a rich natural source of phytosterols, proteins, polyunsaturated fatty acids, antioxidant vitamins, carotenoids and tocopherols, and various elements, due to these

components are attributed providing many health benefits, **(Perez Gutierrez, 2016).** High energy consumption during intense exercise may cause an imbalance between the oxidation and anti-oxidation systems, resulting in an increase in ROS and a reduction in antioxidant activities. These behaviors lead to enhanced ROS production. Oxidative stress is involved in both chronic fatigue and other fatigue related disorders (**Barclay and Hansel, 1991)** Extreme physical stress could lead to excessive generation of ROS in the skeletal muscle which, in turn, results in peripheral fatigue (**Allen *et al*., 2008).**

Glycogen is an important energy material that enables movement and provides adequate energy for muscle contraction. Energy use reduces glycogen; meanwhile, an increase in hepatic glycogen can improve exercise endurance (**Anand, 2012)**

The our histopathological examination to determine the glycogen contents in liver and muscles variation between all rat groups which exposure to exhaustive treadmill test indicated that rats in creatine group were depleted glycogen content from liver but muscle stored it in sever amount, unlike the rats in corn silk group were depleted glycogen content from liver and muscle our result have supported from (**Ivy *et al*., 2002)** who tested the hypothesis that a carbohydrate-protein supplement would be more effective in the replenishment of muscle glycogen after exercise compared with a carbohydrate supplement of equal carbohydrate content or caloric equivalency when supplementing immediately and 2- hours post exercise. The results indicate that the co-ingestion of protein with carbohydrate will increase the efficiency of muscle glycogen storage when supplementing at intervals greater than 1-hour apart, or when the amount of carbohydrate ingested is below the threshold for maximal glycogen synthesis. These results have important implications for athletes who wish to limit their carbohydrate intake in an effort to control body weight and for those athletes who participate in sports that have very short recovery periods during competition such as basketball, ice hockey and soccer.

Steensberg *et al*., (2002) suggest that pre-exercise glycogen content may Influence glucose uptake during subsequent exercise. However, this is only the case when delivery of substrates and hormones remains constant. When delivery of substrates and hormones is altered, the potential effect of glycogen on glucose uptake is negated. **(Evans *et al*., 2019)** suggest that the type, duration, and intensity of the resistance

training program are important factors in determining the effects of resistance training on skeletal muscle glycogen content.

On the other hand at the present study, the supplemented groups which not exposure to exhaustive treadmill test preserves the glycogen content either in liver or muscle, and that met agreement with hypothesis that Decreased use of muscle causes it to become much less metabolically efficient; unfortunately, this de adaptation becomes apparent within a few days after cessation of exercise. Other factors induced by endurance training include changes in cardiac output, increases in capillary density, and increases in glycogen stores (**Baynes and Dominiczak ,2019).**

Findings of these study prove that corn silk and pumpkin seeds were a good source of flavonoid which help to scavenge the oxidative stress substances, creatine recorded the largest glycogen quantity in gastrocnemius muscles among supplemented groups, but it is causing an elevation ammonia, and AST levels on the groups which exposure to exhaustive time test, so more studies for creatine efficacy on skeletal muscle is recommended. Moreover, it could be recommended that corn silk and pumpkin seeds have the ability to eliminate physical fatigue and prolong the training period without fatigue.

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تاثير حرير الذرة ،بذور اليقطين و الكرياتين على الاجهاد التاكسدى لفئران الذكور المدربة

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اجريت الدراسة الحالية للتحقق من مدى تاثير التدعيم بحرير الذرة ،بذور اليقطين والكرياتين على الاجهاد التاكسدى كمؤشر حيوى مثل وظائف الكبد ومحتوى الجليكوجين على ذكور الفئران المدربة البالغة .خمسون فاراً من ذكور الالبينو تم تغذيتهم على الغذاء الاساسى وتهيئتهم لمدة اسبوع .خلال اسبوع التهيئة جميع الفئران (ماعدا المجموعة الضابطة السالبة)تم تعريضهم الى تدريب الجرى الاجبارى بالمشاية الكهربائية يوم بعد يوم لمدة 10 دقائق . تم تقسيم الفئران الى مجموعتين رئيسيتين ،المجموعة الرئيسية الاولى (عدد 10 فئران) تم تغذيتهم على الغذاء الاساسى فقط وصنفت كمجموعة ضابطة سالبة .المجموعة الرئيسية الثانية (الفئران المدربة)حيث قسمت الى 4 مجموعات فرعية (10 فئران فى كل مجموعة) كالتاالى:- **المجموعة الفرعية 1**:-تم تغذيتهم على الغذاء الاساسى وتم الجرى لفترة 25,20,15,10 دقيقة على التوالى (15 يوم لكل فترة زمنية) وصنفت كمجموعة ضابطة موجبة. المجموعة الفرعية (2-4):-مثل المجموعة الفرعية الموجبة وتغذت على الغذاء الاساسى مدعم بـ4%مسحوق حرير الذرة ،4%مسحوق بذور اليقطين ،4%مسحوق الكرياتين على التوالى .فى نهاية فترة التجربة (60 يوم)5 فئران من كل مجموعة فرعية تم تعريضهم الى اختبار الاجهاد المستنفذ بالمشاية الكهربائية وتم تسجيل وقت الجرى ،بعد ذلك تم ذبح كل فئران التجربة .برهنت نتائج هذه الدراسة على ان حرير الذرة وبذور اليقطين مصدر جيد للفلافونات والذى يساعد فى ازلة نواتج الاجهاد التاكسدى وتحسين وظائف الكبد، على الرغم ان سجل الكرياتين الكمية الاكبر من محتوى جليكوجين عضلة الساق بين كل المجموعات المدعمة ولكنه سبب ارتفاع فى مستويات الامونيا و AST فى المجموعة التى تعرضت الى اختبار وقت الاجهاد المستنفذ . يمكن التوصية بحرير الذرة وبذور اليقطين لقدرته على تقليل الارهاق البدنى وإطالة فترة التتمرين دون حدوث ارهاق.

الكلمات المفتاحية:- حرير الذرة ، بذور اليقطين، مكملات الكرياتين ،كرياتين كاينيز ، لاكتات دى هيدروجينيز ،مشاية كهربائية ،الاجهاد التاكسدى .