RESEARCH ARTICLE

STUDY THE EFFECT OF EXOGENOUS AND ENDOGENOUS IRISIN ON OBESITY AND TYPE II DIABETES MELLITUS IN MALE ALBINO RATS

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Manuscript Info

Abstract

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Irisin is a myokine secreted from muscle cells and other organs and it has multiple endocrinal effects on other body systems. The exact relationship of irisin in diabetes and obesity is still not completely understood.
Aim of the work: Is to study the effect of exogenous and endogenous irisin on obesity and type II diabetes mellitus in male albino rats.
Methods: 90 male albino rats divided into 3 groups. Control group;10 rats that fed with a regular diet. Obese group; 40 rats that fed with high fat diet for 8 weeks then subdivided into 4 equal groups 10 rats each. Control obese group; treated by intraperitoneal (ip) injection of 150 µl saline daily for 8 weeks. Irisin treated obese group;treated by ip injection of 150 µl of prepared irisin solution daily for 8 weeks. Exercise treated obese group;treated by moderate intensity swimming exercise for 30 min per day 5 days a week for 8 successive weeks. Irisin and exercise treated obese group;treated as before. Diabetic group; 40 rats that fed with high fat diet for 4 weeks then injected with a single ip streptozotocin (STZ) in a dose of 30 mg/kg body weight. After one week, diabetic group subdivided into 4 equal groups 10 rats each. Control diabetic group, irisin treated diabetic group, exercise treated diabetic group and irisin and exercise treated diabetic group;treated as before.
Results: Non treated obese and diabetic groups showed significant increase in body weight gain, body mass index, serum insulin level, fasting glucose level,(HOMA-IR), osteopontin level, triglycerides level and atherogenic index of plasma.While, showed significant decrease in serum nitrite/ nitrate level,HDL cholesterol level,irisin level and (HOMA-S) when compared to the control group.In contrast, irisin treated obese and diabetic groups showed significant decrease in body weight gain, body mass index, serum insulin level, fasting glucose level,(HOMA-IR), osteopontin level, triglycerides level and atherogenic index of plasma. Whileit showed significant increase in serum nitrite/ nitrate level, HDL cholesterol level, irisin level and (HOMA-S) when compared to the control obese and control diabetic groups respectively.Treated obese and diabetic groups by moderate intensity swimming exercise for 8 successive weeks showed; significant changes in all parameters as compared to control obese and control diabetic groups respectively and insignificant changes in all

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parameters as compared to irisin treated groups.Combination of irisin and exercise treated obese and diabetic groups showed insignificant change compared to control group and a significant change compared to either irisin treated groups or exercise treated groups. except in body weight gain and body mass index there is no significant difference as compared to control group. Our work also showed that; serum irisin level is negatively correlated with all studied parameters except; insulin sensitivity, serum HDL cholesterol level and serum nitrite/nitrate level is positively correlated.We can conclude that combination therapy of irisin and exercise produce better results as anti hyperglycemic, anti-hyperlipidemic and , antioxidant .

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Introduction:-

It has become increasingly recognized that skeletal muscle cells secrete signaling cytokines peptides referred as myokines which act in an autocrine, paracrine, and endocrine fashion in response to skeletal muscle contraction (e.g., exercise) and contribute to the immediate and chronic benefits of exercise (Mehrabian et al; 2016).

Irisin is a newly discovered hormonethat is related to energy homeostasis and obesity(Pedersen and Febbraio 2012). It has direct significant impactson fat tissues (Boström et al., 2012) and is apotential indicator of myocardial infarction (Kulogluet al., 2014). Irisin is secreted into the circulationby contracting skeletal muscle after cleavage fromthe membrane protein fibronectin type III domaincontaining protein 5 (FNDC-5) (Seo et al; 2014).This hormone increases energy consumption and eventually reduces weight by affecting whiteand brown fats (He et al ;2015).

Diabetes mellitus is the most common chronic disease characterized by hyperglycemia resulting from defects in insulin secretion and/or activity (Shaw et al; 2010).

Obesityis one of the most important factors in the developmentof diabetes through various mechanisms including increased circulating free fatty acids, secretion of cytokines by white adipose tissue which ultimately exacerbates insulin resistance and decreased adiponectin which is cytokine derived from white adipose tissue and it has been linked to insulin sensitizingactivity and cardiovascular protective properties (Choi et al;2013).

Regular physical activity is found to play a key role in reducing the risk of obesity by increasing energy expenditure (Yun et al.,2016).

Materials and Methods:-

Drugs and chemicals were obtained from (Sigma Aldrich co.) and all kits used for measurement of parameters were obtained from (Bio-diagnostic and fine test co.)

The rats were acclimatized for two weeks then categorized into:
Group I(Control group10 rats): Rats of this group were fed with a regular diet.
Group II (Obese group 40 rats):Rats of this group were fed with high fat. after 8 weeks this group was subdivided into 4 equal groups 10 rats each(da Rocha et al; 2016).
1. Control obese group: Rats were treated by intraperitoneal injection of 150 μl saline daily for 8 weeks.
2. Irisin treated obese group:Rats were treated by intraperitoneal injection of 150 μl of prepared irisin solution (100 ng/ml) daily for 8 weeks(Basil et al; 2014).
3. Exercise treated obese group:Rats were treated by moderate intensity swimming exercise. It was performed without a load in a barrel filled with water at 33–35 °C to a depth of 40–50 cm, which allowed free swimming (Li et al; 2012). The duration of the first swimming exercise was limited to 15 min then increased by 5 min daily up to 30 min. Rats in the exercise groups swam for 30 min a day, 5 days a week for 8 successive weeks) (Gobatto et al.,2001)
4. Irisin and exercise treated obese group:Rats were treated by irisin in addition to exercise treatment as before.

Group III (Type II diabetic group 40 rats): type II diabetes (T2DM) was induced by feeding rats with a high-fat diet (HFD) as obese group for 4weeks, then rats injected with a single intraperitoneal (i.p) administration of streptozotocin (STZ) in a dose of 30 mg/kg body weight(Sabitha et al; 2011).

One week after STZ injection, the rats with FBG levels above 200 mg/dL considered as diabetic. After that this group was subdivided into 4 equal groups 10 rats each:
1. Control diabetic group: Rats were treated by intraperitoneal injection of 150 µl saline daily for 8 weeks.
2. Irisin treated diabetic group:Rats were treated by irisin as before.
3. Exercise treated diabetic group:Rats were treated by moderate intensity swimming exercise as before.
4. Irisin and exercise treated diabetic group:Rats were treated by irisin in addition to exercise treatment as before.

At the end of the experimental period, the following parameters were measured from all animals:Body weight gain, Body mass index (BMI).The animals fasted overnight then, all rats were anesthetized by i.p injection of pentobarbital and blood samples were obtained and centrifuged at 3000 resolution per minute (rpm) for 15 minutes and the separated sera were then stored in aliquots at -30 ^{o}C till be used. Samples were thawed at room temperature at the time of assay measurement for estimation of the following parameters:
1. Serum insulin level; measured by radioimmunoassay (Kao et al., 1994).
2. Fasting blood glucose (FBG);measured according to method of (Tietz, 1986).
3. Homeostatic model assessment of insulin resistance (HOMA-IR); calculated using the equation described by(Matthews et al; 1985).
4. Homeostatic model assessment of insulin sensitivity (HOMA-S);calculated using the equation described by(Matthews et al., 1985).
5. Serum Irisin; measured by enzyme linked immunosorbent assay (ELISA) (Kohl and Ascoli 2017).
6. Serum Osteopontin;measured by ELISA (Kohl and Ascoli 2017).
7. Serum high density lipoprotein (HDL) cholesterol level; measuredaccording to method of (Grove, 1979).
8. Serum triglycerides (TG)level; measured by glycerol phosphate dehydrogenase (GPO) enzymatic. method(Mcgowan et al., 1983)
9. Atherogenic index; Atherogenic index of plasma (AIP); log (triglycerides TG / high density lipoprotein cholesterol HDL-C) (Dobiásová, 2004).
10. Nitrite/ Nitrate level: Was measured by colorimetric procedure according to the method described by (Montgomery and Dymock, 1961).

Statistical Analysis:
The data were analyzed using Statistical Program for Social Science (SPSS) version 22.0. Quantitative data were expressed as mean ± stander deviation (SD).The following tests were done; Independent- samples t-test of significance when comparing between two means. Correlation: to measure how strong a relationship is between two variables.Probability (P-value) less than or equal 0.05 was considered significant.

Results:-
Non treated obese and diabetic groups showed significant increase in body weight gain, body mass index,serum insulin level, fasting glucose level,(HOMA-IR), osteopontin level, triglycerides level and atherogenic index of plasma.While, showed significant decrease in serumnitrite/ nitrate level,HDL cholesterol level, irisin level and (HOMA-S) when compared to the control group.

In contrast, treated obese and diabetic groups by irisin showed significant decrease in body weight gain, body mass index, serum insulin level, fasting glucose level,(HOMA-IR), osteopontin level, triglycerides level and atherogenic index of plasma. While, showed significant increase in serum nitrite/ nitrate level, HDL cholesterol level, irisin level and (HOMA-S) when compared to the control obese and control diabetic groups respectively.

Treated obese and diabetic groups by moderate intensity swimming exercise for 8 successive weeks showed; significant changes in all parameters as compared to control obese and control diabetic groups respectively and insignificant changes in all parameters as compared to irisin treated groups.

Treated obese and diabetic groups by combination of both irisin and exercise showed insignificant change compared to control groupand a significant change compared to irisin treated groups and exercise treated groups. This effect may be due to the potentiation between the effect of irisin and the effect of exercise in all parameters except in body weight gain and body mass index.

Our work also showed that; serum irisin level is negatively correlated with all studied parameters except; insulin sensitivity, serum HDL cholesterol level and serum nitrite/nitrate level is positively correlated.

Table 1:- The mean value and standard deviation of measured parameters in obese groups.

Group \ Parameter	Control group	Control obese group	Irisin treated obese group	Exercise treated obese group	Irisin and exercise treated obese group
Body weight gain in (gm)	83.7 ±39.28	149.4[a] ±54.91	22.5[a,b] ±41.13	27.7[a,b] ±33.36	-18.1[a,b] ±53.17
Body mass index (BMI) (gm/cm^2)	0.596 ±0.106	0.950[a] ± 0.140	0.667[b] ± 0.141	0.681[b] ± 0.142	0.579[b] ± 0.141
Serum insulin level (μIU /ml)	12.2 ±1.7	18.6[a] ±2.0	13.9[a,b] ±1.2	14.0[a,b] ±1.3	12.4[b,c,d] ±1.9
Serum FBG (mg/dl)	88.4 ±8.1	148.7[a] ±10.1	106.4[a,b] ±14.5	110.3[a,b] ±12.3	83.9[b,c,d] ±8.4
HOMA-IR	2.65 ±0.28	6.84[a] ±0.86	3.77[a,b] ±0.38	3.79[a,b] ±0.30	2.55[b,c,d] ±0.36
HOMA-S	0.329 ±0.005	0.290[a] ± 0.004	0.315[a,b] ±0.007	0.313[a,b] ±0.003	0.331[b,c,d] ± 0.008
Serum irisin level (ng/ml)	37.9 ±3.0	28.8[a] ±3.4	69.7[a,b] ±4.4	72.9[a,b] ±4.6	84.6[a,b,c,d] ±4.6
Serum osteopontin level (ng/ml)	36 ±3.4	76.5[a] ±4.4	63.7[a,b] ±5.9	66.7[a,b] ±5.5	38.1[b,c,d] ±3.8
Serum HDL cholesterol level(mg/dl)	41.1 ±4.0	24.0[a] ±4.1	34.9[a,b] ±4.2	36.4[a,b] ±4.1	41.7[b,c,d] ±3.9
Serum triglycerides level(mg/dl)	137.5 ±7.5	176.7[a] ±9.8	147.3[a,b] ±9.8	145.3[a,b] ±7.3	134.3[b,c,d] ±7.7
Atherogenic index of plasma (AIP)	0.525 ±0.049	0.871[a] ±0.080	0.662[a,b] ±0.040	0.602[a,b] ±0.042	0.508[b,c,d] ±0.037
Nitrite/ Nitrate level (μmol/L)	15.9 ±1.921	9.3[a] ±1.23	11.5[a,b] ±1.56	11.1[a,b] ±1.32	15.4[b,c,d] ±1.77

a, b, cand dDenote statistical significance at p ≤ 0.05.
aVs control group.
bVs control obese group.
c Vs irisin treated obese group.
dVs exercise treated obese group.

Table 2:- The mean value and standard deviation of measured parameters in diabetic groups.

Group	Control group	Control diabetic	Irisin treated diabetic	Exercise treated	Irisin and exercise

Parameter		group	group	diabetic group	treated diabetic group
Body weight gain in (gm)	83.7 ±39.28	154[a] ±80.61	28.8[a,b] ±33.43	33.3[a,b] ±35.21	-13.5[a,b] ±30.55
Body mass index (BMI) (gm/cm^2)	0.596 ±0.106	0.856[a] ±0.181	0.646[b] ±0.082	0.644[b] ±0.101	0.601[b] ±0.185
Serum insulin level (μIU /ml)	12.2 ±1.7	19.3[a] ±1.8	14.7[a,b] ±1.2	14.7[a,b] ±1.4	11.9[b,c,d] ±1.9
Serum FBG (mg/dl)	88.4 ±8.1	276.1[a] ± 17.1	180.5[a,b] ±18.5	185.0[a,b] ±12.7	83.9[b,c,d] ±8.4
HOMA-IR	2.65 ±0.28	13.1[a] ±1.45	6.55[a,b] ±0.49	6.74[a,b] ±0.96	2.49[b,c,d] ±0.53
HOMA-S	0.329 ±0.005	0.268[a] ±0.003	0.291[a,b] ±0.002	0.291[a,b] ±0.005	0.333[b,c,d] ±0.010
Serum irisin level (ng/ml)	37.9 ±3.0	30.3[a] ±2.4	68.7[a,b] ±3.9	70.8[a,b] ±4.2	92.6[a,b,c,d] ±3.3
Serum osteopontin level (ng/ml)	36 ±3.4	79.2[a] ±4.7	65.5[a,b] ±4.8	65.5[a,b] ±4.2	37.2[b,c,d] ±3.3
Serum HDL cholesterol level(mg/dl)	41.1 ±4.0	25.7[a] ±3.8	35.7[a,b] ±4.5	36.2[a,b] ±5.5	40.2[b,c,d] ±4.7
Serum triglycerides level(mg/dl)	137.5 ±7.5	170.9[a] ±9.4	145.7[a,b] ±9.4	145.7[a,b] ±7.3	133.4[b,c,d] ±6.8
Atherogenic index of plasma (AIP)	0.525 ±0.049	0.825[a] ±0.074	0.612[a,b] ±0.052	0.607[a,b] ±0.063	0.522[b,c,d] ±0.061
Nitrite/ Nitrate level (μmol/L)	15.9 ±1.921	7.6[a] ±0.99	9.8[a,b] ±1.04	9.6[a,b] ±1.22	15.5[b,c,d] ±2.23

a, b, cand dDenote statistical significance at p ≤ 0.05

aVs control group.

bVs control diabetic group

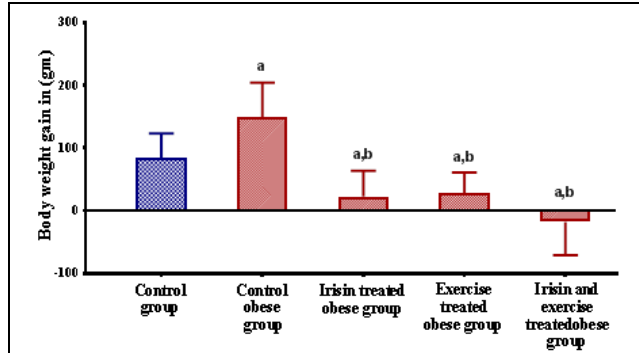cVs irisin treated diabetic group.

dVs exercise treated diabetic group

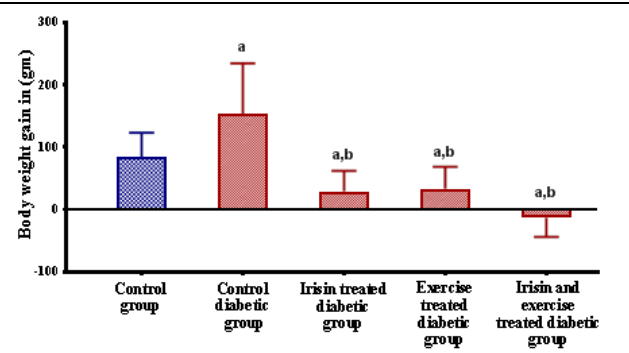**Figure 1a:** Body weight gain in (gm) of obese groups

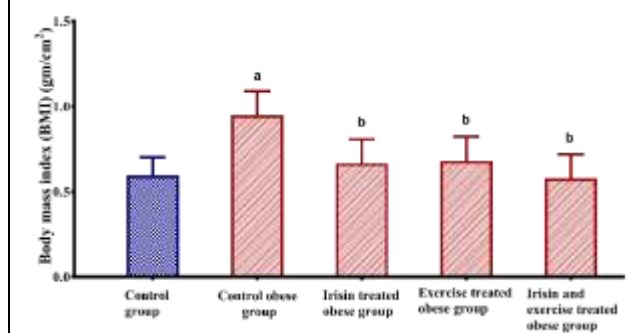**Figure 1b:** Body weight gain in (gm) of diabetic groups

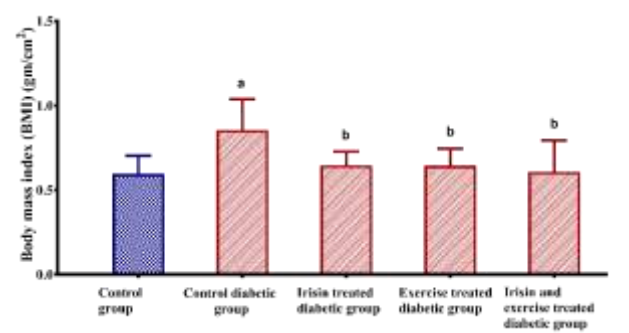**Figure 2a:** Body mass index (BMI) (gm/cm^2) in obese groups

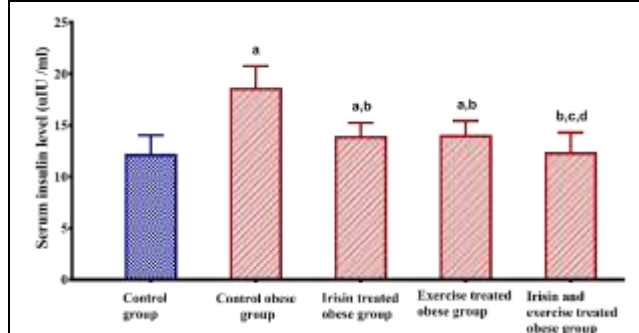**Figure 2b:** Body mass index (BMI) (gm/cm^2) in diabetic groups

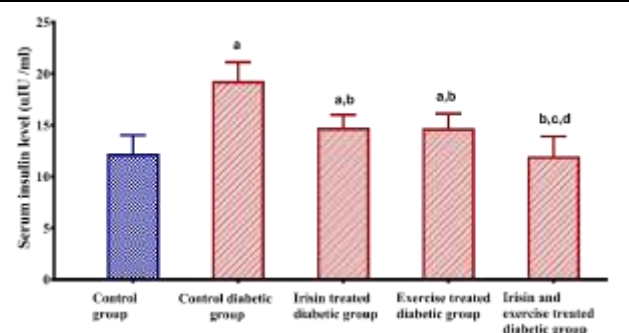**Figure 3a:** Serum insulin level (µIU /ml) in obese groups

Figure 3b: Serum insulin level (µIU /ml) in diabetic groups
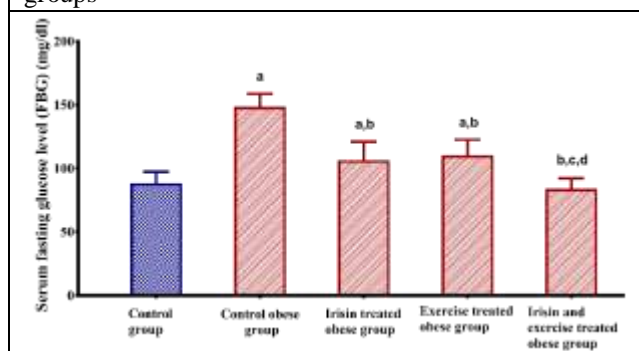
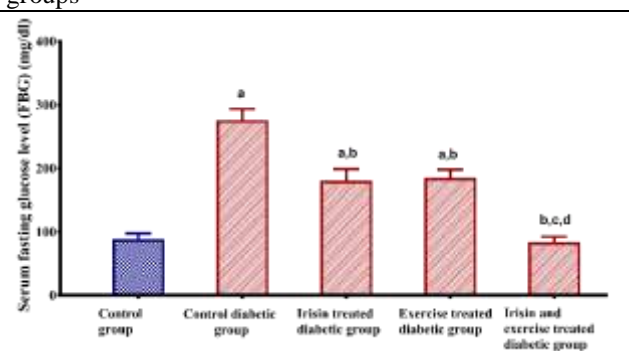**Figure 4a:** Serum fasting glucose level(FBG) (mg/dl) in obese groups

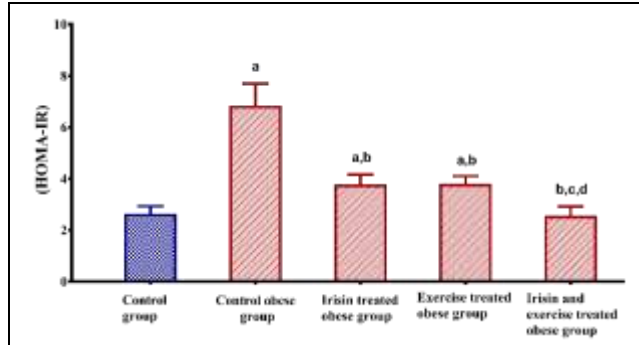**Figure 4b:** Serum fasting glucose level(FBG) (mg/dl) in diabetic groups

Figure 5a: Homeostatic model assessment of insulin resistance (HOMA-IR) in obese groups
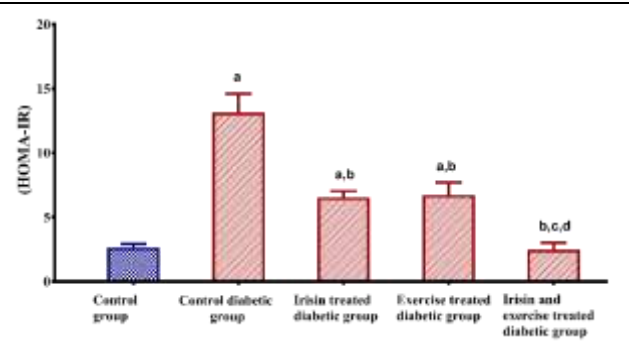
Figure 5b: Homeostatic model assessment of insulin resistance (HOMA-IR) in diabetic groups
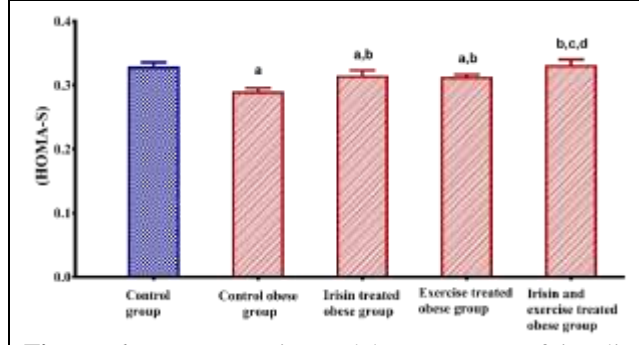
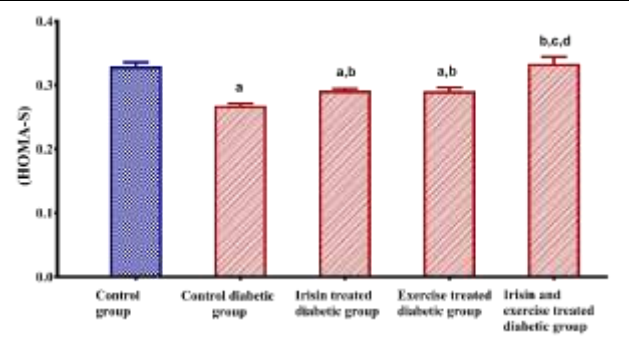**Figure 6a:** Homeostatic model assessment of insulin sensitivity (HOMA-S) in obese groups

Figure 6b: Homeostatic model assessment of insulin sensitivity (HOMA-S) in diabetic groups

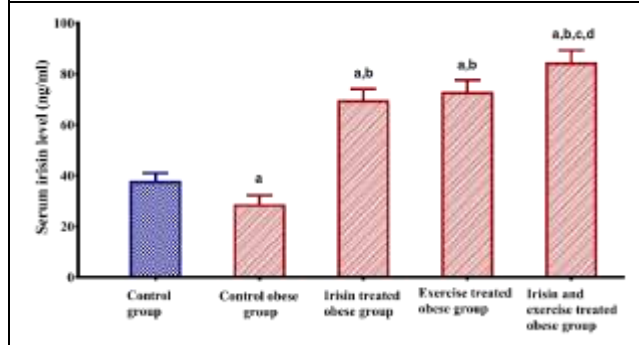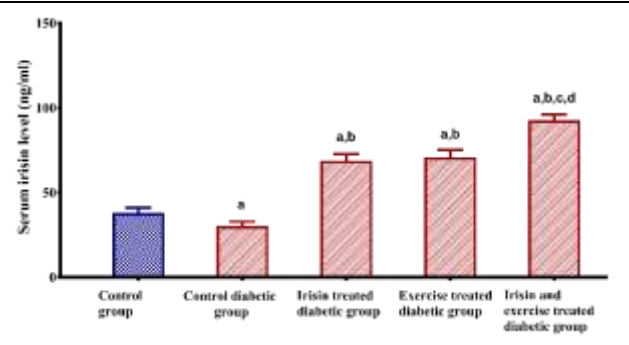**Figure 7a:** Serum irisin level (ng/ml) in obese groups

Figure 7b: Serum irisin level (ng/ml) in diabetic groups
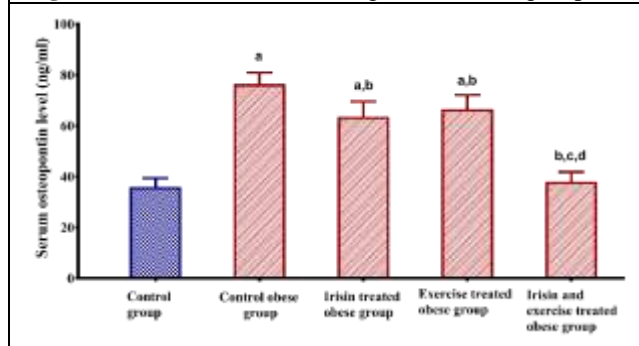
Figure 8a: Serum osteopontin level (ng/ml) in obese groups
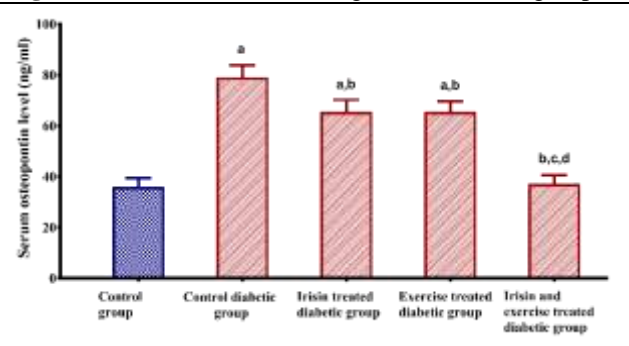
Figure 8b: Serum osteopontin level (ng/ml) in diabetic groups
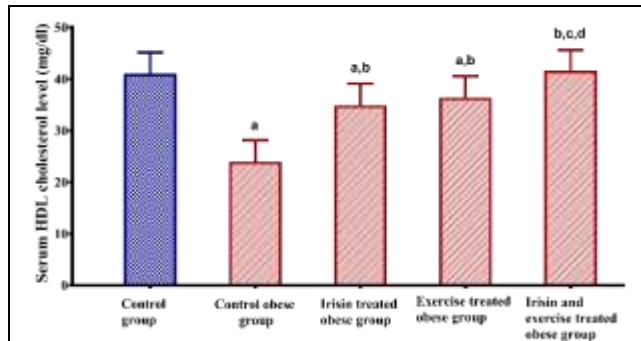
Figure 9a: Serum HDL cholesterol level (mg/dl) in obese groups
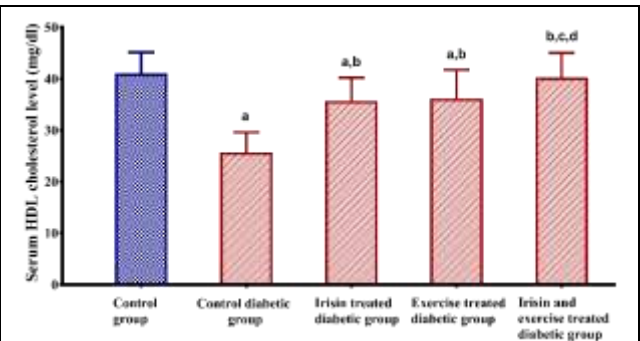
Figure 9b: Serum HDL cholesterol level (mg/dl) in diabetic groups
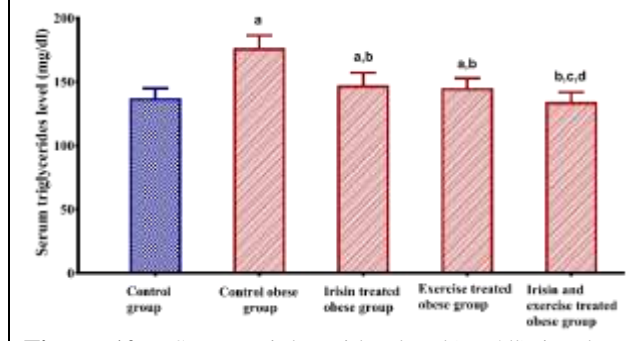
Figure 10a: Serum triglycerides level(mg/dl) in obese groups
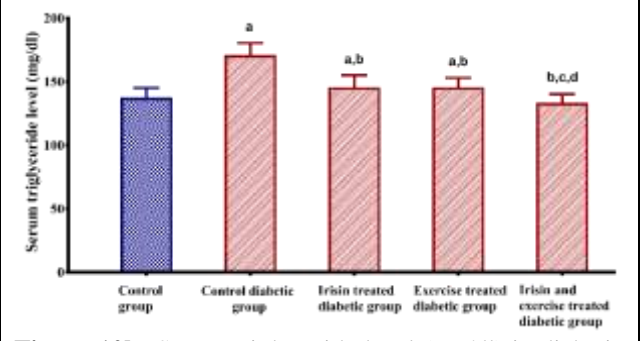
Figure 10b: Serum triglyceride level (mg/dl) in diabetic groups
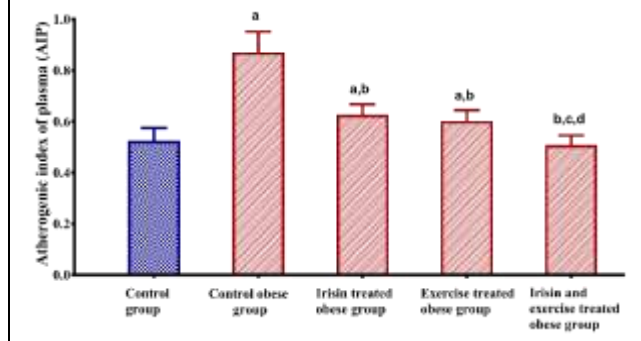
Figure 11a: Atherogenic index of plasma (AIP) in obese groups
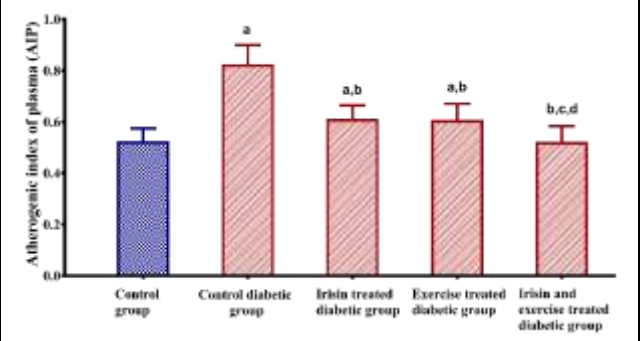
Figure 11b: Atherogenic indexof plasma (AIP) in diabetic groups
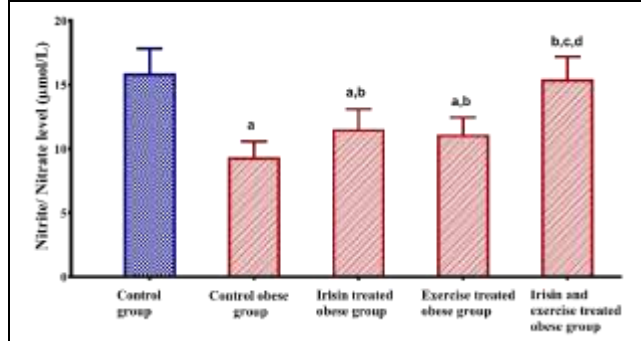
Figure 12a: Nitrite/ Nitrate level (µmol/L) in obese groups
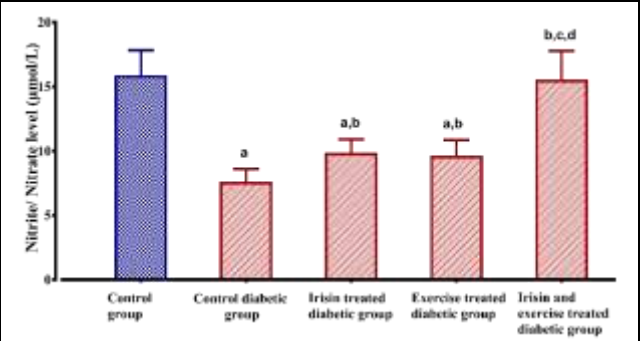
Figure 12b: Nitrite/ Nitrate level (µmol/L) in diabetic groups

a, b, c and d Denote statistical significance at $p \leq 0.05$. a Vs control G., b Vs control obese G., c Vs irisin treated obese G. and d Vs exercise treated obese G.	a, b, c and d Denote statistical significance at $p \leq 0.05$. a Vs control G., b Vs control diabetic G., c Vs irisin treated diabetic G. and d Vs exercise treated diabetic G.

Correlation between serum irisin level (ng/ml) and other parameters in obese and diabetic groups:
Figures (**13 and 14**) showed that; serum irisin level is negatively correlated with body weight, body mass index, serum insulin, serum fasting blood sugar, insulin resistance, serum osteopontin, serum triglycerides and atherogenic index. While it is positively correlated with insulin sensitivity, serum HDL cholesterol level and serum nitrite/nitrate level in both obese and diabetic groups.
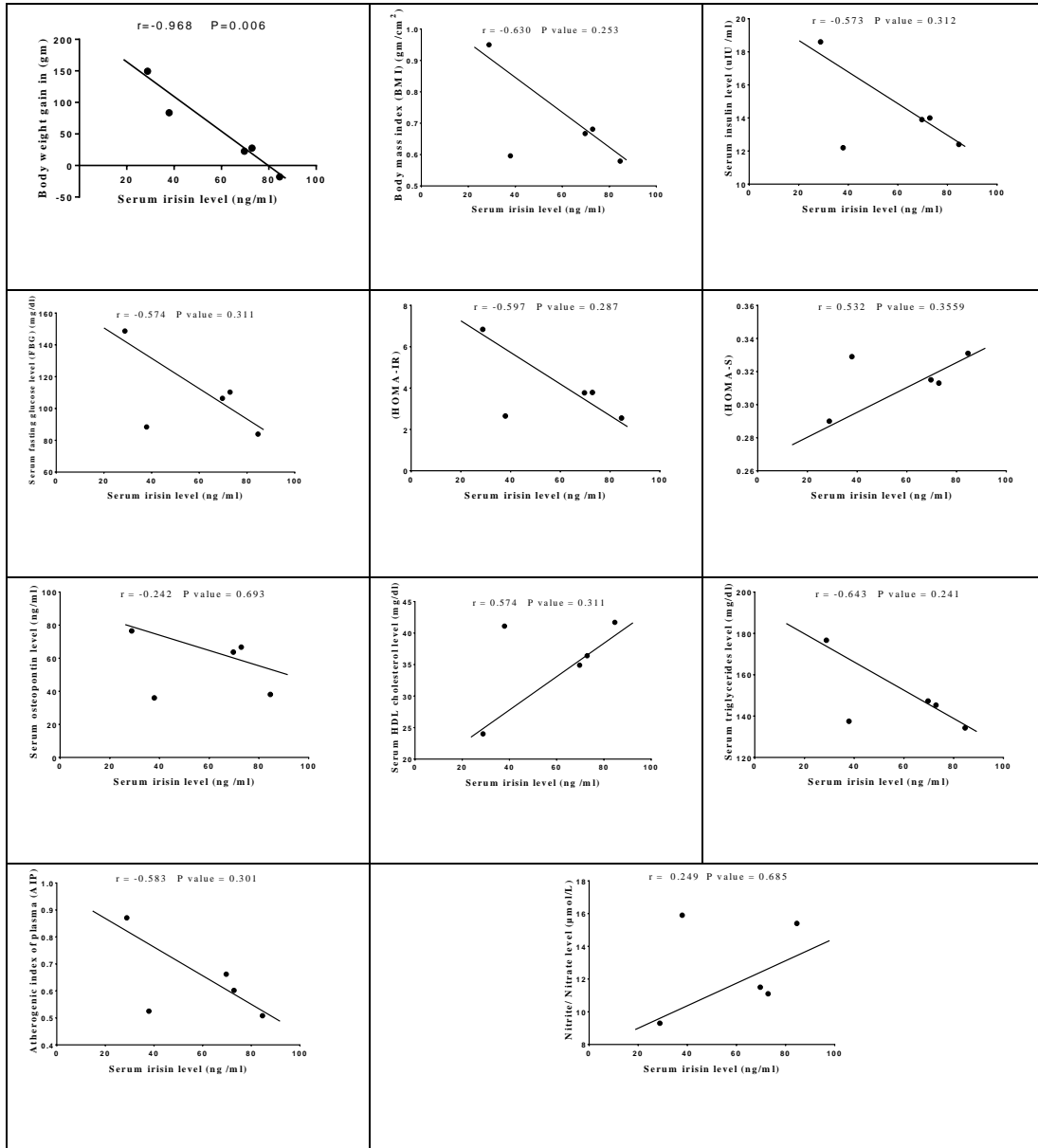
Figure (13):- Correlation between serum irisin level (ng/ml) and other parameters in obese groups.
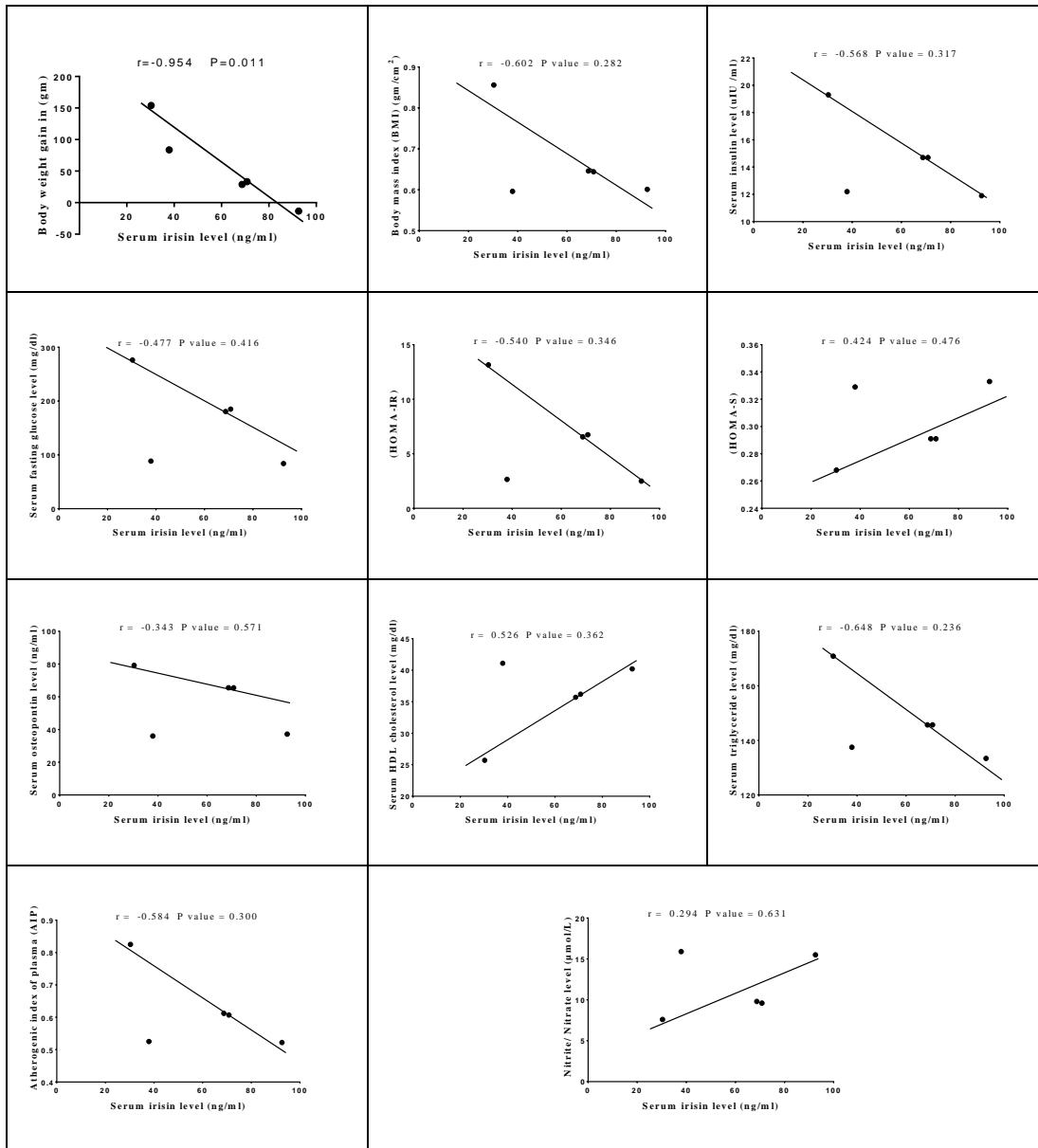
Figure14:- Correlation between serum irisin level (ng/ml) and other parameters in diabetic groups.

Discussion:-

The adverse effects of obesity and diabetes have been extensively studied in experimental animals.Low dose streptozotocin (STZ) and a high fat diet (HFD) are an ideal animal model for type 2 diabetes. This model simulates the natural disease progression and metabolic characteristics typical of type 2 diabetes. Treatment with the β- cell toxin STZ results in a severe reduction in functional β- cell mass.Thismodelmimics the pathology of type 2 diabetes on a shorter time scale than found in the human condition (Skovsø 2014).

It is evident from the results of our work that non treated obese and diabetic groups showed significant increase in body weight and body mass index comparing to control group.This rise in the mentioned parameters may be attributed to the high caloric diet (contain 60% fat) andpositive energy balance leading to augmented mass of body fat and fat accumulation in adipose tissue (Hashem et al. 2018).

In contrast, treated obese and diabetic groups by irisin showed significant decrease in body weight and body mass index comparing to control obese group and control diabetic group respectively, which is consistent with previous

studies (Boström et al., 2012).It has been established that irisin plays a significant role in energy metabolism and glucose tolerance. After beingreleased from muscle following exercise or externally injected, it circulates and exerts its function as a hormone that stimulates the browningof white adipose tissue which translates into burning more calories and therefore increasing oxygen consumption and thermogenesis in adipocytes (Pardo et al., 2014).Also, irisin increase uncoupling protein 1 (UCP1) which is a key protein to the thermogenic capacity of adipose tissues that enables the separation of lipid oxidation from ATP production, allowing a higher metabolic rate and the conversion of nutritional energy to heat (Xiong et al. 2015).

Treated obese and diabetic groups by moderate intensity swimming exercise showed significant decrease in body weight and body mass index comparing to control obese group and control diabetic group respectively, which is consistent with previous studies (Seo et al.,2014). The reduction of theanthropometric parameters in the exercise treated groups is mainly attributable to exercise-induced energy consumption (Lu et al.,2016).

The exogenous administration of irisin has the same effect of exercise. Hence, obese individuals who cannot lose weight with or without exercise due to various conditions may potentially benefit from this exogenous administration of irisin(Ercan et al.,2018).

Eight weeks consumption of HFD inducehyperinsulinemia and altered glucose homeostasis due to insufficient compensation by the beta cells of the pancreatic islets(Czech 2017). Non treated obese and diabetic groups showed that; serum insulin level, serum fasting glucose level, insulin resistance (HOMA-IR) significantly increased and homeostatic model assessment of insulin sensitivity (HOMA-S) significantly decreasedcompared to control group. Obesity and diabetes are associated with a chronic low-grade pro-inflammatory metabolic state that stimulate signals from specialized cells ;(adipocytes, macrophages, and T-cells) and initiate the inflammatory responses that induce alterations in metabolic homeostasis through the secretion of numerous cytokines and adipokines (Guilherme et al. 2012).

On the other hand treated obese and diabetic groups by irisin showed that; serum insulin level, serum fasting glucose level, insulin resistance (HOMA-IR) significantly decreased and homeostatic model assessment of insulin sensitivity (HOMA-S) significantly increasedcomparing to control obese group and control diabetic group respectively. These results are consistent with previous studies (Moreno-Navarrete et al. 2013) that prove the effect of irisin therapy in preventing the occurrence of DM by reducing the risk of insulin resistance, lowering serum glucose and reducing the weight in rats which are under HFD.Irisin administration result in improvement in glucose homeostasis which was achieved by increased glucose uptake combined with reduced gluconeogenesis in the liver (Pósa et al., 2015).

The results of exercise treated groups are nearly similar to irisin treated groups and this proves that irisin mimics the exercise effect in theimprovement of obesity related disorders, which is consistent with(Basil et al. ,2014).Exercise prevent chronic lowgrade inflammatory state and decrease secretion of TNF-alpha and increase level of IL-10 thus preventing the deleterious effect on glucose homeostasis (Guilherme et al., 2012). The effect of exercise also, may be attributed to an increased expressionand/or activity of certain proteins involved inglucose metabolism and insulin signaling in skeletalmusclessuch as glycogen synthase and glucosetransporter 4 (GLUT4) (Moustafa and Marwa2017) (Ercan et al., 2018).

There is significant decrease in serum irisin level in nontreated obese and diabetic groups in comparison with control group. Our results are in consistent with (Yang et al.,2015) they showed significant negative correlation between obesity and serum irisin level. Lower levels of circulating irisin in obese and T2DM could be explained by impaired (PGC- 1a) expression and functions in the muscle and adipose tissue(Huh et al., 2012).

The groups received exogenous irisin(obese and diabetic) or exercised showed increased levels of serum irisin. Serum levels were higher in the exercise groups compared with the exogenous irisin injected groups, but the difference did not reach statistical significance. These results are consistent with some authors, who found that chronic exerciseinduce a significant increase inserum irisin level in the exercised rats when compared withsedentary ones(Ercan et al. 2017).

Osteopontin (OPN) plays an important role in infiltration and accumulation of macrophages in adipose tissue in the early stages of obesity and increased macrophage-related inflammatory activity could be a link between inflammation and insulin resistance (Kamei et al. 2006).

The results of our work showed that, the level of OPN is significantly increased in obese and type II diabetic rats as compared to control group. Our results are consistent with previous work of (Ahmad et al., 2013).

In contrast, the treated obese and diabetic groups by irisin showed significant reduction in serum OPN level compared to control obese and diabetic groups. Also treated obese and diabetic groups by moderate intensity swimming exercise showed the same results as treated irisin groups.

Blood lipids disorders are critical risk factors for cardiovascular diseases. Lipid abnormalities, often termed "dyslipidemia". Lipid abnormalities are common in obese and diabetic (Bhowmik et al. ,2018).

In our study we found that serum triglycerides leveland atherogenic index of plasma in obese and diabetic groups was significantly higher than control group. In contrast serum HDL cholesterol level in obese and diabetic groups was significantly lower than control group. Our results are similar to previous studies reporting that HFD and induced type 2 diabetes cause dyslipidemia in rodents (Gao et al. ,2018). The hepatocyte expression of transcription factors sterol regulatory element binding protein-1c (SREBP-1c) and nuclear factor-$\kappa\beta$ (NF$\kappa\beta$), and their target genes found to be upregulated in HFD fed obese and diabetic rats compared to healthy animals. Upregulation of these factors regulate fatty acid and cholesterol metabolism genes (Khadke et al. ,2019).

Treated obese and diabetic groups by irisin showed that; serum triglycerides leveland atherogenic index of plasma significantly reduced comparing to control obese group and control diabetic group respectively. In contrast serum HDL cholesterol levelin irisin treated group significantly increased comparing to control obese group and control diabetic group.In adipocytes, irisin enhanced basal lipolysis which prevented by inhibition of adenylate cyclase or protein kinase A (PKA); Irisin increased hormone-sensitive lipase (HSL) expression and phosphorylation; it increased PKA activity, and cAMP and HSL mRNA levels, but reduced perilipin expression. These results indicate thatirisin ameliorates lipid metabolic derangements. So, irisin enhance lipolysis via cAMP–PKA–HSL/perilipin pathway. Perilipin is a protein localized on the surface of lipid droplets that serves as a gatekeeper and inhibits lipolysis. The phosphorylation of HSL causes the activation of HSL, and the phosphorylation of perilipin induces the translocation of HSL from the cytosol to the surface of lipid droplets for converting stored triglycerides to glycerol and free fatty acids (FFAs). Irisin-induced down-regulation of perilipin and up-regulation of HSL promote the lipolysis (Xiong et al. ,2015).

Also treated obese and diabetic groups by moderate intensity swimming exercise showed that; serum triglycerides leveland atherogenic index of plasma significantly reduced comparing to control obese group and control diabetic group respectively. In contrast serum HDL cholesterol levelin exercise treated group significantly increased comparing to control obese group and control diabetic group. These finding is consistent with previous studies (liu et al., 2016) they confirm the effectiveness of swimming exercise in improving the lipid profile. Also, study done byMoustafa and Marwa 2017 reported that aerobic exerciselead to a significant improvement in lipid profile.Exercise increase blood lipid consumption and hence decrease lipids levels. Mechanisms may involve the increased activity of lipoprotein lipase (LPL) that responsible of hydrolysis of circulating triacylglycerol (TAG) moiety of TAG-rich lipoproteins; the chylomicrons and very-low-density lipoprotein (VLDL) generating free fatty acids (FFA) that are either oxidized in the muscles or re-esterified in the adipose tissues forming glycerol that is returned to the liver (Wang and Xu 2017).

In present study nitrite/ nitrate are significantly reduced in obese and diabetic rats compared to control group. Decreased nitric oxide (NO) signaling due to reduced endogenous formation or increased metabolism is a risk factor for development of obesity and diabetes and associated comorbidities. The mechanisms that explain this pathological alteration are reduced nitric oxide (NO) production and low NO bioavailability. Diminished levels of NO in obese and diabetic states may be due to decreased expression of nitric oxide synthase (NOS), impairments in NOS activity, or by the reaction of NO with reactive species. Decreased NOS expression commonly occurs in obese and diabetic states (Sansburyand Hill 2014).

Treated obese and diabetic groups by irisin shows significant increase in serum nitrate level in comparison with control obese and diabetic groups respectively. These results are consistent with previous study (Fang et al., 2015).

The mechanism for this increase in serum nitrate level seemed to be related to AMP-activated protein kinaseendothelial NO synthase (AMPK-eNOS) signaling pathway activation. High FFA levels released from excess white adipose tissue could impair eNOS phosphorylation through inhibiting this signaling pathway in obesity. Irisin turn white adipocytes into brown adipocytes and reduce circulating FFA levels from adipose tissue and prevent the effect of FFA (Wang et al. ,2012). Thus, irisin plays an important role in increasing NO production and activating the AMPK- Akt- eNOS pathway (Fu et al. ,2016).

Also, treated obese and diabetic groups by moderate intensity swimming exercise have the same results of significant increase in serum nitrate level compared to control obese and diabetic groups as irisin treated. Nitric oxide production increased in skeletal muscle in response to physical activity. Moderate exercise increasing activity of the entire NOS pool (the cumulative activity of endothelial NOS, neuronal NOS, and inducible NOS). Enhanced NO release in physical exercise is mediated through eNOS gene transcription (Dyakova et al., 2015

In our worktreated obese and diabetic groups by combination of both irisin and exercise showed a significant change compared to irisin treated groups and exercise treated groups. This was noticed in all parameters except in body weight gain and body mass index.There is no significant difference between the results when using this regimen of combination and control group. This effect may be due to the potentiation between the effect of irisin and the effect of exercise.

Conclusion:-
Irisin and exercise showed anti hyperglycemic, anti-hyperlipidemia, antioxidant effect with potentiation effect when used together.This study recommended that the use of irisin is promising and deserve further investigation and evaluation in clinical practice in treating and prevention of metabolic syndrome

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