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A cross talk between adropin and possible metabolic syndrome disorders in experimental male albino rats

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***Abstract*---**This research is to study the relationship between adropin and possible metabolic syndrome disorders, also to assess the role of PPAR-α in these disorders in rats. 50 male rats were divided into 5 groups: I-Control group, II-Obese group, III-Obese diabetic group, IV-Adropin treated obese group, V-Adropin treated obese diabetic group. At the end, body weight, BMI, AC/TC ratio and lee index was measured, blood samples were collected for measuring glucose , insulin, HbA1C , HOMA-IR , HOMA-β , TC, TG, HDL-C , LDL-C, atherogenic index , ALT, AST & GGT. The liver was dissected for measuring PPARα expression, TNF-α, IL6 & iNOS. High fat diet produced a significant increase in all anthropometric measures in group (II) and (III) compared to control group. After adropin administration they were significantly decreased. The serum blood glucose, insulin, HbA1c and HOMA-IR were significantly increased, while B- cell function significantly decreased in group (II) and (III) compared to control, however after adropin administration they showed significant decrease while, HOMA-β was significantly decreased. Group (II) and (III) showed significant lipid profile deterioration compared to control group, after adropin treatment lipid profile was significantly improved. Group II and III showed significant increase in liver enzymes compared to control, while after administration of adropin they were significantly decreased. (PPAR-α) expression, TNF-α, IL-6 and iNOS showed significant increase in

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group (II) & (III) compared to control. However they were significantly decreased after adropin administration. We concluded that adropin might be a potential target for treatment of diabetes and hyperlipidemia

***Keywords*---**adropin, metabolic syndrome, streptozotocin, diabetes, PPARα.

Introduction

Obesity, elevated blood pressure, raised blood glucose levels, and hyperlipidemia are all symptoms of metabolic syndrome, which is connected to physical inactivity and a high-fat, high-calorie diet. Cardiovascular disease, type-2 diabetes (T2DM) and hypertension are all lifestyle-related disorders that may arise from metabolic syndrome. In addition, hyperlipidemia may cause damage to solid organs, such as liver and kidneys (Oruc et al., 2017). A high-fat diet promotes the formation of intra-abdominal fat deposits and hepatic steatosis, as well as insulin resistance and increased glucose levels, which leads to hyperinsulinemia. (Gao et al., 2015).

Adropin is a newly discovered peptide hormone that affects lipid metabolism. The Energy homeostasis associated (Enho) gene codes for adropin (Kocaoglu et al., 2015). Adropin is a highly conserved polypeptide that has been suggested to have a role in metabolic regulation, insulin sensitivity, and endothelial function as an endocrine factor. The specific physiological roles of this peptide, however, are unclear (Chang et al., 2018). Adropin is a well-known T2DM medicine however its effects and actions remain unclear. The literature on the relationship between adropin and T2DM is sparse (Sato et al., 2018).

The feeding of a high fat diet to wild-type mice causes increased hepatic expression of (peroxisome proliferator activated receptor-alpha) PPAR-α and PPAR target genes involved in fatty acid oxidation (Cave et al. 2016). PPAR-α is present all throughout the body, but it is particularly prevalent in the liver. This protein controls fatty acid absorption, beta oxidation, ketogenesis, bile acid synthesis, and triglyceride turnover (Pawlak et al., 2015). Adropin has been shown to control the expression of hepatic lipogenic genes as well as the PPAR receptor, which is a significant regulator of lipogenesis, in studies. However, our understanding of this intriguing protein, as well as its precise function and mode of action, is limited. (Ivanova et al., 2017).

Materials and Methods

The current study used 50 male Albino rats of a local breed. weighted (150-200gm) and the dead rats were replaced During the workday, the rats were kept in segregated animal cages (10 rats per cage) in the physiology department's research lab, where they were given unrestricted food and water and kept at room temperature (12-hour light-dark cycle) at 22-25°C. All procedures were accepted by ethical committee of faculty of medicine by code no (33214/7/19), Tanta University.

Control group

This group of rats was given a regular chow diet for four weeks. The typical chow diet consists of: (14 percent fat, 26 percent protein and 60 percent carbohydrates).Then they received distilled water for 10 days (I.P) *(Akrabawi and Salji, 1973).*

Obese group

The rats of this group were fed (HFD) for 4 weeks. Then they received distilled water for 10 days (I.P). HFD: Is composed of (10% protein, 20% carbohydrates and 70% fat). It was consisted of (green vegetables, bread, full cream milk and cooked cow fat), then they received distilled water for 10 days (I.P) *(Srinivasan et al., 2005).*

Obese Diabetic group

Obesity was produced in these rats for 4 weeks in the same way as it did in group II *(Srinivasan et al., 2005).* The mice were starved overnight and a single dose of streptozotocin (35 mg/kg body weight) was given intra-peritoneal (I. P), after 3 weeks. One week after the STZ injection, fasting blood glucose levels were assessed. in all creatures by utilizing a portable glucometer and diabetes was confirmed blood glucose level above 350 mg/dl and the rats having lower levels were excluded from the study. Then they received distilled water for 10 days (I.P) *(Srinivasan et al., 2005).*

Adropin treated obese group

Obesity was induced in these rats as in group II, then adropin was daily injected (I.P) in a dose of (2.1µg/kg/day) dissolved in distilled water for 10 days *(Lovren et al., 2010).*

Adropin treated Obese Diabetic group

Obese diabetic group was done as in group III then adropin was injected as in group IV (Loven et al., 2010). The livers of all rats were thoroughly dissected after they were anaesthetized with diethyl ether and murdered via cervical decapitation and the blood samples were collected. (Artwohl et al., 2006).

Anthropometric measures

- Body weight (Nascimento et al., 2008), rat length, abdominal circumference (AC), thoracic circumference (TC) and AC/TC ratio, body mass index (BMI) (Novelli et al., 2007) and Lee index (Angeloco et al., 2012).
- The following parameters were measured:
  blood glucose level (Tietz., 1986), serum insulin level (Chevenne et al., 1994), glycated hemoglobin (HbA1C) (Blanc et al., 1981), insulin resistance (HOMA-IR) (Matthews et al., 1985), β-cell function (HOMA-β) (Haffner et al., 1996), serum total cholesterol, triglycerides (TG) (Mc Gowan et al., 1983), High Density Lipoproteins Cholesterol (HDL-C) (Grove, 1979), Low Density

Lipoproteins Cholesterol (LDL-C) (Assmann et al., 1984), Atherogenic index (Dobiasova, 2004), Alanine Aminotransferase (ALT), Aspartate amino transferase (AST) (Huang et al., 2006) & Gamma Glutamyl Transferase (GGT) (Thapa and Anuj, 2007).

The liver of all animals were dissected and prepared for determination of

PPARα receptor (peroxisome proliferator-activated receptor alpha gene) (Berger et al., 1999), Tumor necrosis factor α (TNF-α) (Sawada et al., 1989), Interleukin-6 (IL6) (Sharif et al., 1993) & Inducible nitric oxide synthase (iNOS) (Dawson et al., 1991).

Statistical analysis

Results were expressed as Mean ± SD and all statistical rapprochements were made by one-way ANOVA test and p values less than 0.05 were considered statistically significant. The analysis was done by statistical package for the social science software (SPSS version 22.0). Pearson correlation coefficient (Pearson r test): was done to determine strength and relation among variables.

Results

The Fasting Blood Glucose, Insulin Level, glycated hemoglobin (HbA1c)%, insulin resistance (HOMA-IR), B-cell function (HOMA-β)(%) among studied groups as in table (1): When obese group II & obese diabetic group III were compared to the control group, serum levels of blood glucose, insulin, HbAc, and the HOMA-IR index all rised considerably, but B-cell activity declined dramatically. Intra-peritoneal injections of adropin resulted in substantial reductions in serum levels of blood glucose, insulin, glycated hemoglobin, and HOMA-IR index, as well as a significant increase in B-cell activity, as compared to groups II and III. Group (IV) & (V) had a substantial rise in serum blood glucose and HbA1c percent compared to the control group, but no change in serum insulin, HOMA-IR index, or pancreatic-cell function.

Table (1):- The Fasting Blood Glucose, Insulin Level, glycated hemoglobin (HbA1c)%, insulin resistance (HOMA-IR), B-cell function (HOMA-β)(%) among studied groups (Mean value ±SD)

	Control group I	Obese group II	Obese diabetic group III	Adropin treated obese group IV	Adropin treated obese diabetic group V
The Fasting Blood Glucose (mg/dl):	90.8±8.36	211.8±10.27*	355.5±23.51*#	116.3±7.09*#&	124±6.99 *#&
Insulin Level (μIU/ml):	8.21±0.59	28.5±1.25 *	27.6±1.87*	6.54±0.55#&	6.21±0.72#&
Glycated hemoglobin (HbA1c) (%):	5.1±0.13	7.9±0.42*	8.88±0.46*#	6±0.28*#&	5.6±0.42 *#&
Insulin resistance	1.5±0.28	14.8±1.04*	14.01±0.89*	1.72±0.198#	1.67±0.21#&

(HOMA-IR):				&	
B-cell function (HOMA-β)(%)	71.8±4.9	31.5±5.26 *	20.6±1.9 *#	68.7±3.8 #&	68.1±5.1 #&

* Denotes statistical significance at P≤0.05 compared to group.
# Denotes statistical significance at P≤0.05compared to group II.
& Denotes statistical significance at P≤0.05 compared to group III.

Serum total cholesterol, Triglycerides (TG), High density lipoproteins cholesterol (HDL-C), Low density lipoproteins cholesterol (LDL-C) and Atherogenic index among studied groups, as in table (2) as: Obese group and obese diabetic group showed significant increase in serum levels of TC, LDL-C, TG, atherogenic index and significant decrease in serum HDL-C as compared to control group, In comparison to group II and III, adropin administration to obese rats in group (IV) and obese diabetic rats in group (V) resulted in large decreases in blood levels of TC, LDL-C, and TG, as well as a significant increase in serum HDL-C. However, group IV and V experienced substantial increases in serum TC, LDL-C, and significant reductions in serum HDL-C compared to the control group, but no significant changes in serum TG.

Table (2):- Serum total cholesterol, Triglycerides (TG), High density lipoproteins cholesterol (HDL-C), Low density lipoproteins cholesterol (LDL-C) and Atherogenic index among studied groups: Mean value ±SD)

	Control group I	Obese group II	Obese diabetic group III	Adropin treated obese group IV	Adropin treated obese diabetic group V
Total cholesterol (mg/dl):	71.6±13.20	232.5±9.78*	271±10.49*#	90.9±9.79*#&	100.5±11.02 *#&
Triglycerides (mg/dl):	70.1±6.79	165.2±11.71 *	193.3±18.59 *#	73.8±7.54 #&	75.9±6.15#&
High density lipoproteins cholesterol (HDL-C) (mg/dl):	51±6.52	28.9±4.73*	22.2±2.3*#	39.6±3.41*#&	37.3±3.53 *#&
Low density lipoproteins cholesterol LDL-C (mg/dl):	74.2±9.102	146.8±13.17*	173.3±12.84*#	89±6.18*#&	89.9±5.57*#&
Atherogenic index:	0.05±0.026	0.40±0.028 *	0.47±0.069 *#	0.10±0.015 *#&	0.11±0.037 *#&

* Denotes statistical significance at P≤0.05 compared to group.
# Denotes statistical significance at P≤0.05compared to group II.
& Denotes statistical significance at P≤0.05 compared to group III.

Liver peroxisome proliferator activated receptor- alpha (PPARα) Relative gene expression, Tumor necrosis factor (TNF-α), Interleukin 6 (IL6) and inducible nitric oxide synthase (iNOS) among studied groups, as in table (3): In this research, the relative gene expression of (PPAR-α) TNF-α, IL-6 and iNOS was shown to be significantly higher in the obese (II) and obese diabetic (III) groups as compared to the control group. Adropin administration resulted in a considerable reduction in

PPAR relative gene expression, TNF-α, IL-6 and iNOS in groups (IV) and (V) compared to groups (II) and (III), although it was still considerably higher than the control group.

Table (3): Liver peroxisome proliferator activated receptor- alpha (PPARα) Relative gene expression, Tumor necrosis factor (TNF-α), Interleukin 6 (IL6) and inducible nitric oxide synthase (iNOS) among studied groups (Mean value ±SD)

	Control group I	Obese group II	Obese diabetic group III	Adropin treated obese group IV	Adropin treated obese diabetic group V
PPARα Relative gene expression (%):	1±0	1.30±0.016*	1.34±0.013*#	1.11±0.015*#&	1.12±0.020*#&
Tumor necrosis factor (TNF-α) (ng/l):	13±1.37	77.3±6.96*	89.6±6.33*#	16.3±2.3#&	16.8±2.7#&
Interleukin-6 (IL6) (pg/ml):	12.7±0.79	66.6±3.73*	85.8±6.16*#	16.8±1.77#&	17.1±1.89#&
inducible nitric oxide synthase (iNOS) (ng/ml):	18.9±0.79	73.2±5.26*	88.9±7.42*#	19.02±1.31#&	18.9±2.6#&

* Denotes statistical significance at P≤0.05 compared to group.
# Denotes statistical significance at P≤0.05compared to group II.
& Denotes statistical significance at P≤0.05 compared to group III.

Anthropometric measures (Body weight gain, Body mass index BMI, Abdominal circumference /thoracic circumference AC/TC ratio, final lee index) among studied groups as in figure (1): The result of this work revealed that rats in groups II and III acquired a large amount of weight over time, as well as a significant increase in BMI, AC/TC ratio, and Lee index, indicating obesity, as compared to the control group. Surprisingly, adropin therapy lowered body weight, BMI, AC/TC ratio, and lee index in groups IV and V, compared to groups II and III. They did, however, exhibit a considerable increase when compared to the control group,

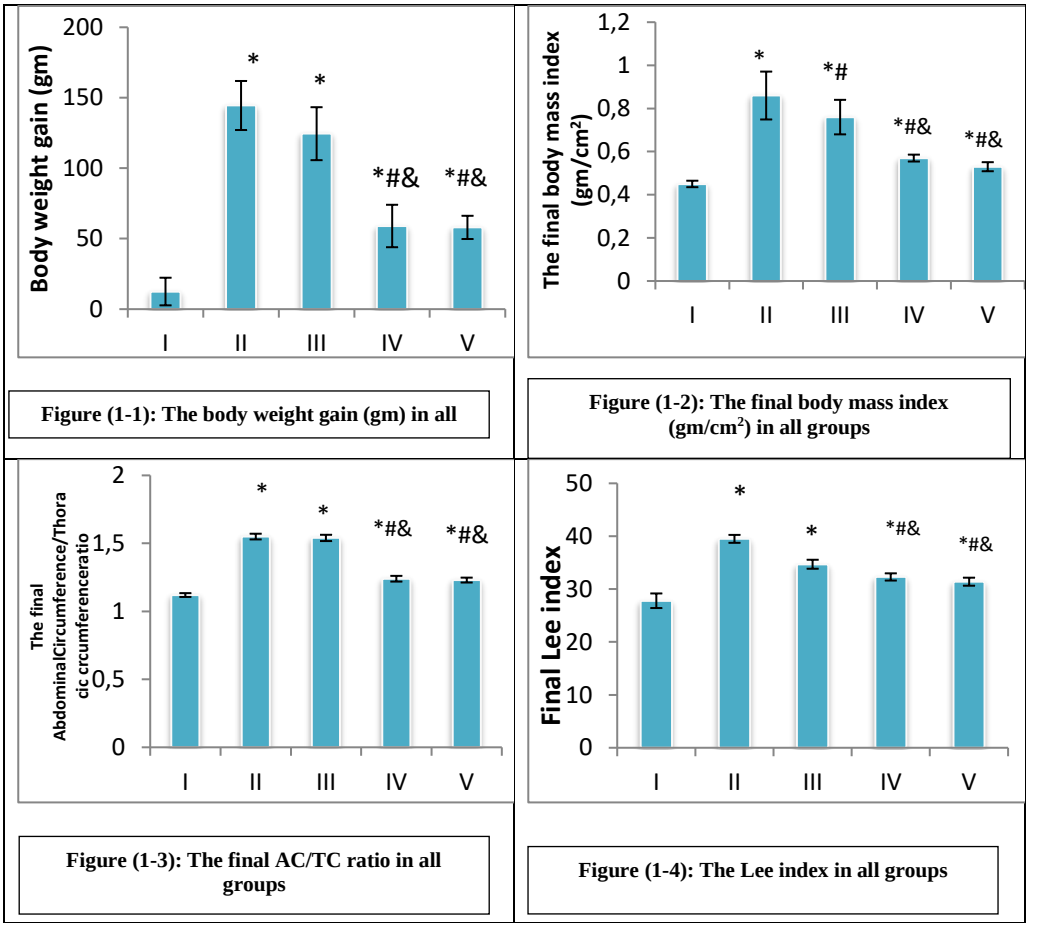

Figure (1-1): The body weight gain (gm) in all

Figure (1-2): The final body mass index (gm/cm^2) in all groups

Figure (1-3): The final AC/TC ratio in all groups

Figure (1-4): The Lee index in all groups

Fig (1):- Anthropometric measures (Body weight gain, Body mass index BMI, Abdominal circumference /thoracic circumference AC/TC ratio, final lee index) among studied groups

* Denotes statistical significance at P≤0.05 compared to group.

# Denotes statistical significance at P≤0.05compared to group II.

& Denotes statistical significance at P≤0.05 compared to group III .

Serum Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Gamma glutamyl transferase (GGT) among studied groups, as in figure (2): Group II and III showed significant increase in serum levels of ALT, AST, and GGT in comparison to control group. After administration of adropin, group (IV) and (V) showed significant decrease in serum levels of ALT, AST, and GGT in as compared to group (II) and (III) respectively. Group (IV) and (V) showed also significant increase in GGT level, while they showed insignificant change in serum levels of ALT, AST when compared to control group.

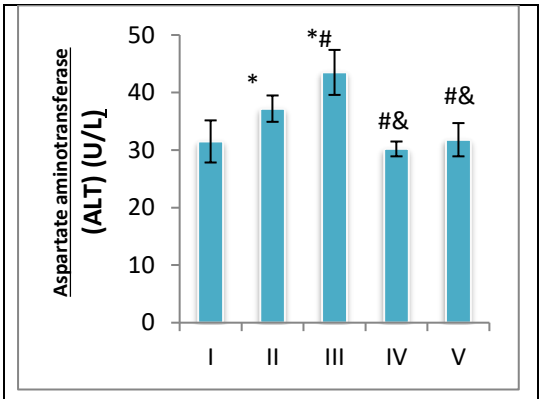

Figure (2-1): Serum Alanine Aminotransferase (U/L) in all groups

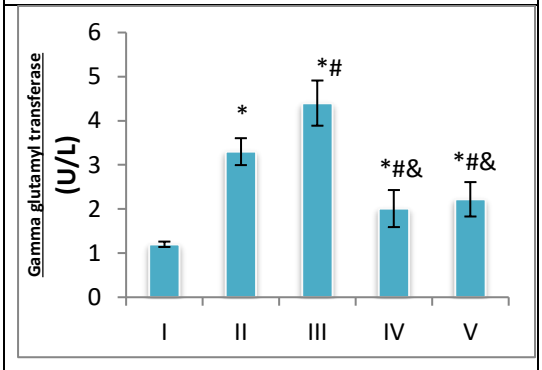

Figure (2-2): Serum Gamma Glutamyl Transferase (U/L) in all groups

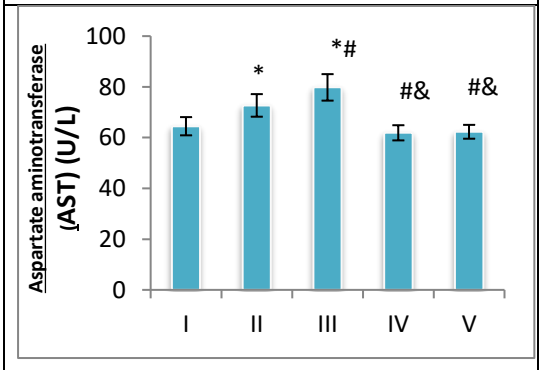

Figure (2-3): Serum Aspartate amino transferase (U/L) in all groups

Fig-(2):- Serum Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Gamma glutamyl transferase (GGT) among studied groups.
* Denotes statistical significance at P≤0.05 compared to group.
# Denotes statistical significance at P≤0.05compared to group II.

& Denotes statistical significance at P≤0.05 compared to group III.

Correlation between PPAR-α &all parameter (the body weight gain, final rat length, BMI, AC/TC, lee index, fasting blood glucose, serum insulin, HbA1c, HOMA-IR, total cholesterol, LDL-C, serum TG, atherogenic index, ALT, AST, TNF-α, IL6, iNOSB- Cell function and HDL-C in all groups, as in figures (3): There were significant positive correlations between PPAR-α relative gene expression and the body weight gain, final rat length, BMI, AC/TC, lee index, fasting blood glucose, serum insulin, HbA1c, HOMA-IR, total cholesterol, LDL-C, serum TG, atherogenic index, ALT, AST, TNF-α, IL6 and iNOS in all groups. While there were negative correlations between PPAR-α relative gene expression and B- Cell function and HDL-C in all groups.

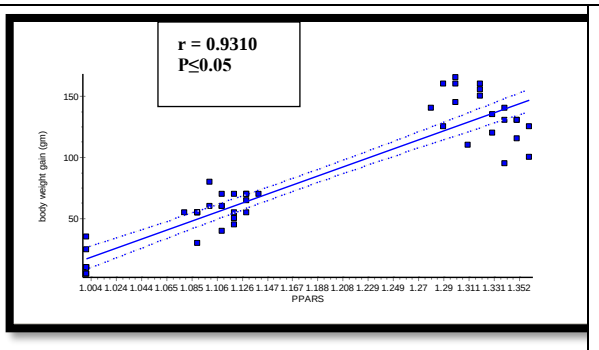

Figure (3-1): Correlation between PPAR-α & body weight gain

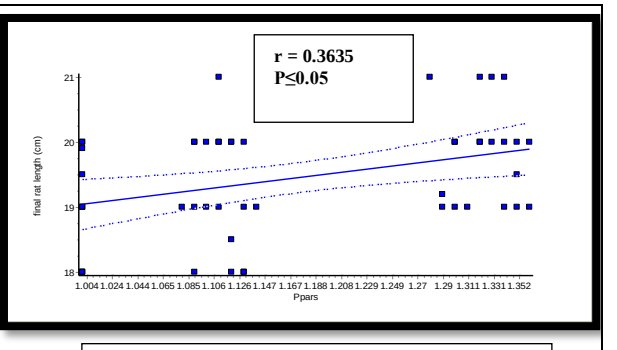

Figure (3-2): Correlation between PPAR-α &

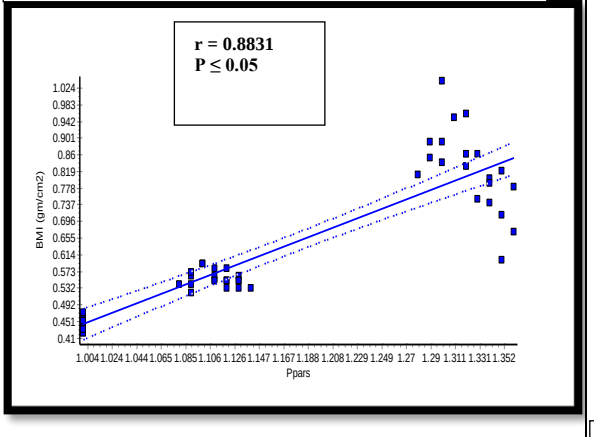

Figure (3-3): Correlation between PPAR-α & BMI

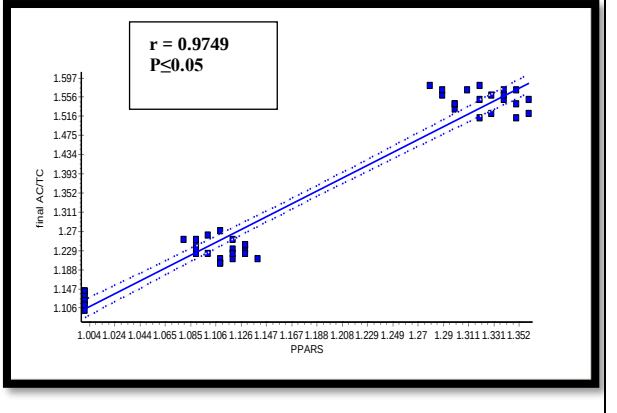

Figure (3-4): Correlation between PPAR-α & final AC/TC

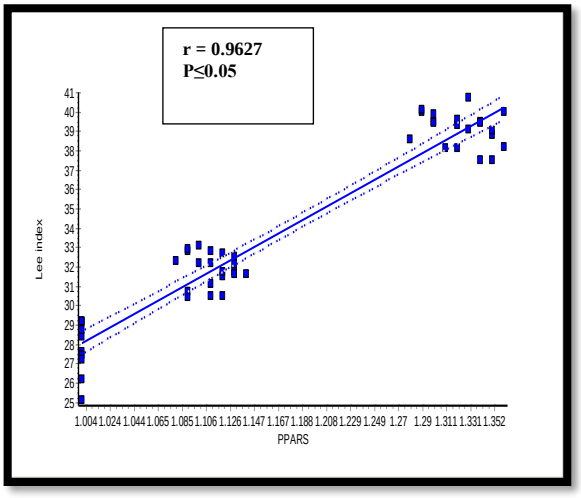

Figure (3-5): Correlation between PPAR-α & lee index

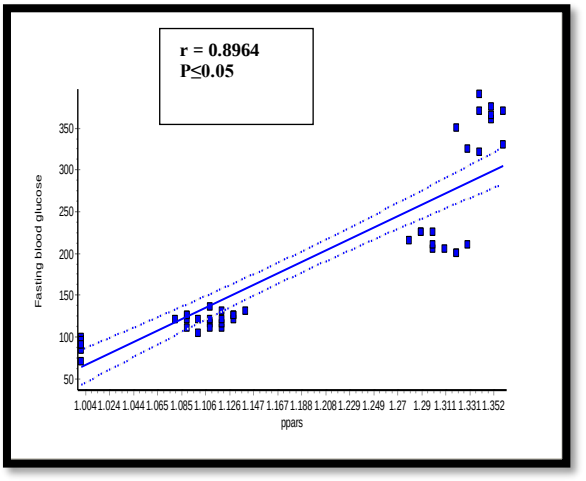

Figure (3-6): Correlation between PPAR-α & fasting blood glucose

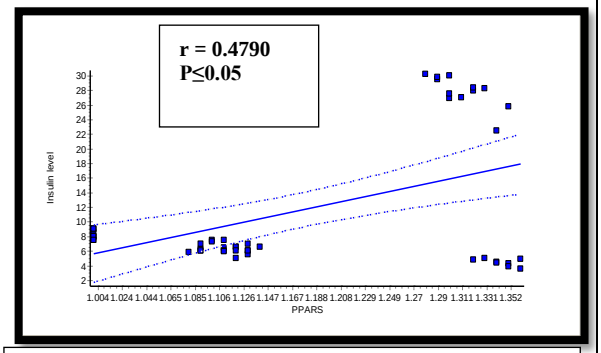

Figure (3-7): Correlation between PPAR-α & serum insulin

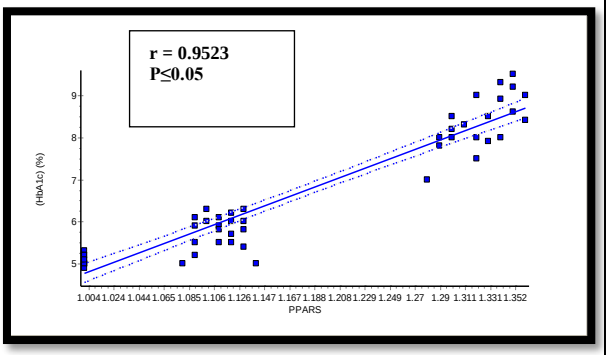

Figure (3-8): Correlation between PPAR-α & HbA1c %

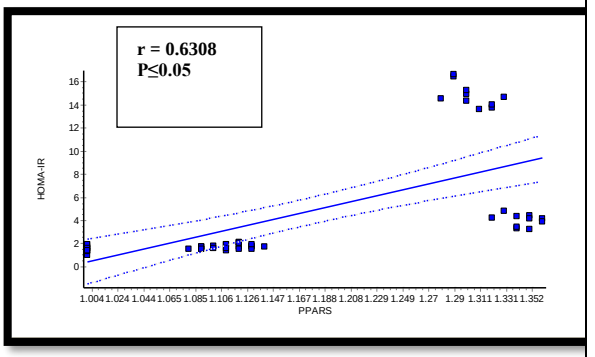

Figure (3-9): Correlation between PPAR-α & HOMA-IR

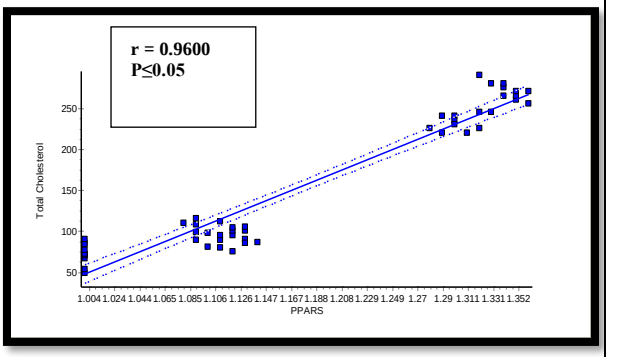

Figure (3-10): Correlation between PPAR-α & total cholesterol

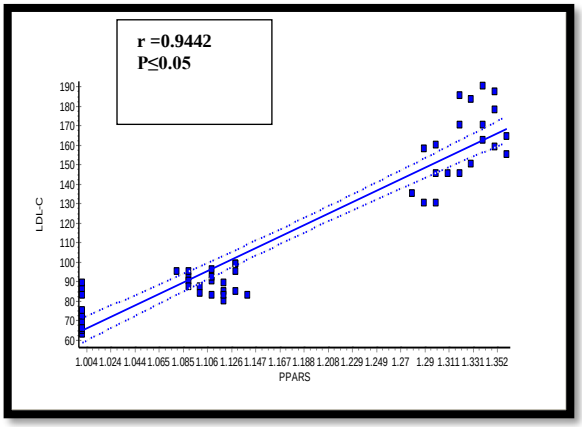

Figure (3-11): Correlation between PPAR-α & LDL-C

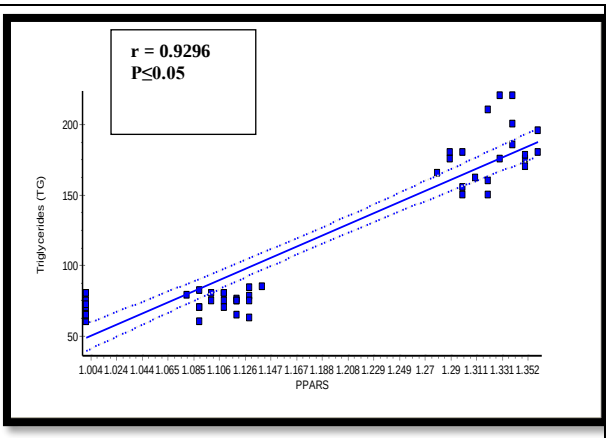

Figure (3-12): Correlation between PPAR-α & TG

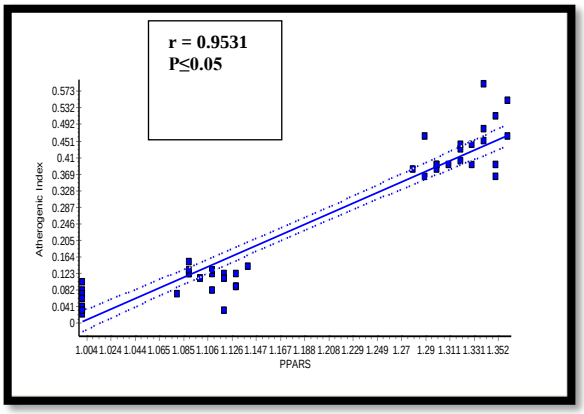

Figure (3-13): Correlation between PPAR-α & atherogenic index

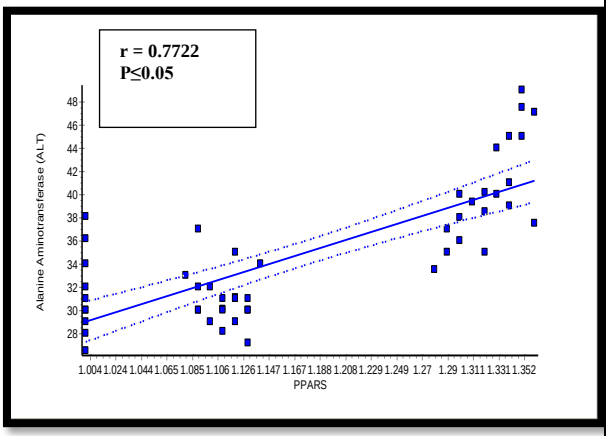

Figure (3-14): Correlation between PPAR-α & serum ALT

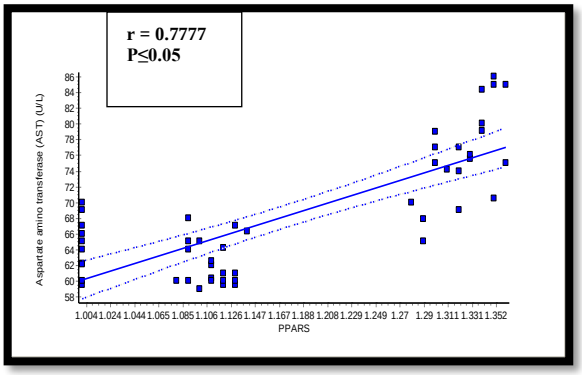

Figure (3-15): Correlation between PPAR-α & serum AST

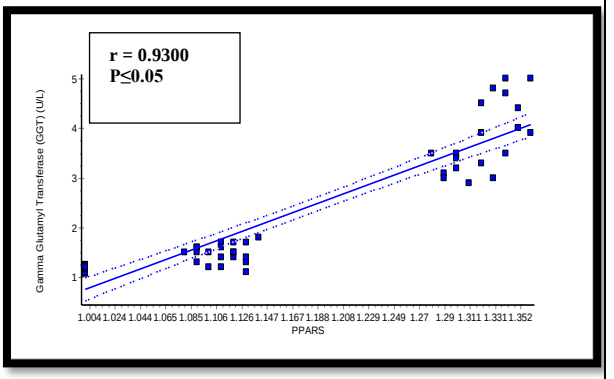

Figure (3-16): Correlation between PPAR-α & serum GGT

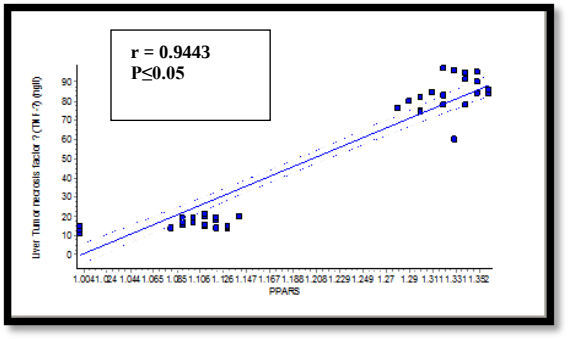

Figure (3-17): Correlation between PPAR-α & liver (TNF-α)

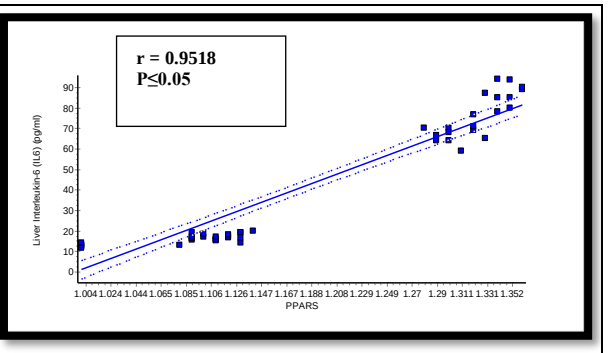

Figure (3-18): Correlation between PPAR-α & liver (IL-6)

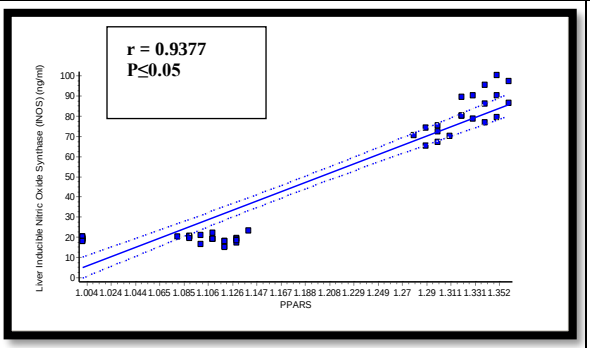

Figure (3-19): Correlation between PPAR-α & liver (iNOS)

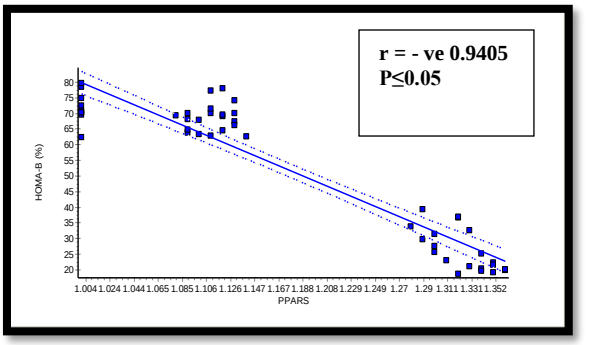

Figure (3-20): Correlation between PPAR-α & B- Cell function

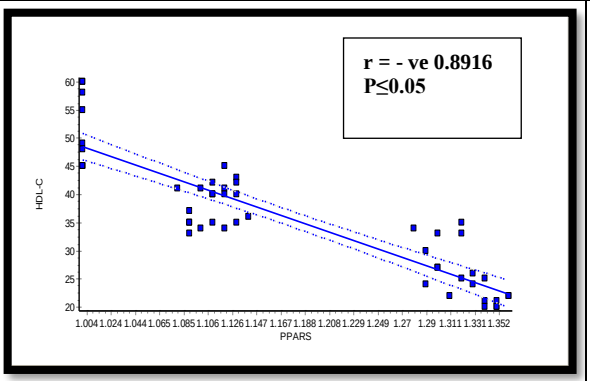

Figure (3-21): Correlation between PPAR-α & HDL-

Fig-(3): Correlation between PPAR-α &all parameter (the body weight gain, final rat length, BMI, AC/TC, lee index, fasting blood glucose, serum insulin, HbA1c, HOMA-IR, total cholesterol, LDL-C, serum TG, atherogenic index, ALT, AST, TNF-α, IL6, iNOSB- Cell function and HDL-C in all groups. r denotes correlation coefficient Vs PPAR-α relative gene expression. P≤0.05 denotes statistically significant.

Discussion

T2DM is a disease marked by oxidative damage, inflammation, and insulin resistance. Insulin function is hampered by saturated fatty acids, and prior research shown that eating a high-fat, low-carbohydrate diet without exercising control leads to insulin resistance. Diabetes and its consequences, such as oxidative stress, gastroparesis, neuropathy, retinopathy, and diabetic nephropathy, are all linked to insulin resistance. *(Dang et al., 2019).* Intra-peritoneal injections of adropin resulted in substantial reductions in serum levels of blood glucose, insulin, HbAc percent, and HOMA-IR index, as well as a significant increase in B-cell activity, as compared to groups II and III. Group (IV) and (V) had a substantial rise in serum blood glucose and HbAc percent compared to the control group, but no change in serum insulin, HOMA-IR index, or -cell function. Other research backed up the findings *(Costa et al., 2016, Akcilar et al., 2016, and Gao et al., 2015).*

Higher glucose tolerance as a consequence of higher glucose utilization, as seen by a lower HOMA-IR index and enhanced B-cell activity, might be linked to adropin's capacity to lower blood glucose levels. Furthermore, the increase in glucose intake (PDH) may be explained by the direct stimulatory impact of glucose through the pyruvate dehydrogenase enzyme activation *(Jacas et al., 2014).* These results seem to be compatible with another studies forming T2DM in rat model *(Galicia-Garcia et al., 2020 and Carvalho et al., 2018).*

Furthermore, adropin controlled glucose and lipid metabolism in skeletal muscle. It stimulated enzymes that boosted glucose oxidation while decreasing fat oxidation (PDH) *(Jacas et al., 2014).* Adropin boosted PDH activity in muscle via a number of methods. Adropin inhibited pyruvate dehydrogenase kinase-4 (PDK4) expression by causing (PGC-1) down-regulation. PDK4 is in charge of lowering the activity of PDH *(Thapa et al., 2019).*

A high-fat diet increases mitochondrial fatty acid uptake in muscle, causing the -oxidative pathway to become overburdened *(Muoio, 2014).* Many adaptive metabolic pathways were activated as a result of the overload, including an increase in the capacity of the tri-carboxylic acid (TCA) and -oxidation cycle. Furthermore, increased oxidation may exceed the capacity of the TCA cycle, resulting in an accumulation of partially oxidized fatty acid products *(Miao et al., 2015).*The increased levels of incomplete oxidation products in mitochondria created a metabolic milieu that stimulated the conversion of free CoA to acetyl-CoA (the oxidation end product), resulting in a decrease in the CoA/acetyl-CoA ratio and a decrease in PDH activity *(Partridge et al., 2014).*

In terms of fat metabolism, adropin decreased fat oxidation by lowering the gene expression of carnitine-palmytoil-transferase-1B (Cptb), which led to CPT-1b suppression. Adropin also decreased the expression of cluster of differentiation (Cd36), which is essential for fatty acid absorption by muscle *(Stein et al., 2016).*
A model of dyslipidemia in the rats was done by supplying them with diets rich in cholesterol and saturated fats. Our findings indicated that obese group (II) and obese diabetic group (III) showed significant increase in serum levels of TC, LDL-C, TG and lee index & significant decrease in serum HDL-C as compared to

control group, which was consistent with previous reports of *(Zheng et al., 2017, Kuloglu and Aydin, 2014).*

In comparison to group II and III, adropin administration to obese rats in group (IV) and obese diabetic rats in group (V) resulted in large decreases in blood levels of TC, LDL-C, TG and lee index as well as a significant increase in serum HDL-C. However, group IV and V experienced substantial increases in serum TC, LDL-C, and significant reductions in serum HDL-C compared to the control group, but no significant changes in serum TG. The capacity of adropin to inhibit (HMG-COA) reductase activity may explain the decrease in blood levels of TC, LDL-C, and TG, as well as the increase in serum HDL-C. It might also be because it stimulates the production of several enzymes involved in cholesterol manufacture, such as lipoprotein lipase *(Tian et al., 2015).*

In this research, the relative gene expression of (PPAR) was shown to be significantly higher in the obese (II) and obese diabetic (III) groups as compared to the control group. This finding was consistent with *(Pawlak et al., 2015),* who found that feeding a high-fat diet to wild-type mice resulted in increased PPAR expression in the liver and PPAR target genes involved in fatty acid oxidation, and that this was a protective or adaptive response by PPAR.

Adropin administration resulted in a considerable reduction in PPAR relative gene expression in groups (IV) and (V) compared to groups (II) and (III), although it was still considerably higher than the control group. Adropin may have reduced PPAR relative gene expression by inhibiting (PGC-1), which was one of the (PPAR-) co-activators, and adropin influenced its signalling pathways *(Cheng et al., 2016).* One of the PPAR co-activators was (PGC-1). The PGC-1 co-activator was thought to be a crucial regulator of mitochondrial metabolism. It was previously revealed that adropin's metabolic effect was mediated by signal pathways involving the activities of (PGC-1) and Sirtuin-1 (SIRT) *(Miao et al., 2015).* Adropin inhibited target genes of PGC-1 by reducing the activity of SIRT deacetylase, which resulted in hyper-acetylation (meaning inhibition) of PGC-1, which involved (PDK4), which lowered (PDH) and promoted (Cptb) activity *(Gao et al., 2015).*

There were significant positive correlations between PPAR-α relative gene expression and the body weight gain, final rat length, BMI, AC/TC, lee index, fasting blood glucose, serum insulin, HbA1c, HOMA-IR, total cholesterol, LDL-C, serum TG, atherogenic index, ALT, AST, TNF-α, IL6 and iNOS in all groups. While there were negative correlations between PPAR-α relative gene expression and B-Cell function and HDL-C in all groups.

Insulin resistance and inflammation, all of which are linked to lipid metabolism, were linked to NAFLD development and progression, as well as fibrosis and cirrhosis. Multiple cytokines, including TNF- and IL-6, have been implicated in the pathophysiology of (NASH) disease *(Wasilewska et al., 2018).* TNF-, IL-6, and iNOS levels in the liver tissue of obese (II) and obese diabetics (III) were considerably greater than in the control group, suggesting that hyperlipidemia triggered the up-regulation of pro-inflammatory cytokines and caused liver tissue damage. This was supported by *(Ding et al., 2014 and Kunkel et al., 2012).* Our results were comparable to those of Safhi et al., (2018), who discovered that type-

2 diabetic rats had increased TNF- and IL-6 levels *(Kataoka et al., (2014), and Wang et al., (2011)* also discovered that iNOS mRNA expression was increased in hyperlipidemic rats.

Treatment with adropin resulted in substantial reductions in liver TNF-, IL-6, and iNOS in groups (IV) and (V), respectively, as compared to groups (II) and (III). Our findings resembled those of *(Kuloglu and Aydin., 2014)*, who found that following adropin therapy, elevated production of TNF-, IL-6, and iNOS mRNA in hyperlipidemic rats was decreased, indicating that adropin may reduce inflammation. The reduction in inflammatory indicators following adropin therapy was thought to be due to its direct stimulatory action on TNF-, IL-6, and iNOS mRNA, as well as its inhibitory effect on fat storage, which reduced macrophage infiltration, which was the major source of these inflammatory cytokines *(Jung and Choi, 2014).*

The result of this work revealed that rats in groups II and III acquired a large amount of weight over time, as well as a significant increase in BMI, AC/TC ratio, and Lee index, indicating obesity, as compared to the control group. Surprisingly, adropin therapy lowered body weight, BMI, AC/TC ratio, and lee index in groups IV and V, compared to groups II and III. They did, however, exhibit a considerable increase when compared to the control group. This is supported by other studies *(Ganesh et al., 2012 and Miao et al., 2015).*

The decrement of body weight can be explained partly by the effect of adropin on food intake, and partly due to the ability of adropin to decrease expression of lipogenic genes in adipose tissue with subsequent decrease of body fat deposition. Also, it can be explained by change in energy homeostasis *(Stein et al., 2016)*. Severe fatty degeneration in the hepatocytes termed non-alcoholic fatty liver disease could results from hyperlipidemia, and 20–30% of the cases might be converted to non-alcoholic steato-hepatitis (NASH) and seriously led to liver fibrosis *(Mirtschink et al., 2018).* STZ caused a major elevation in liver enzymes levels. The increment of liver enzymes by STZ was mainly due to damage of the liver that was caused by the oxidative stress produced from hyperglycemia. Furthermore, in diabetes mellitus increased reactive oxygen radical levels caused damage to lipid membrane specially the construction of poly unsaturated fatty acids and led to serious abnormality in metabolism of lipid and its peroxidation and caused damage of multiple organs involving the liver *(Haas et al., 2016).*

In this work both obese group (II) and obese diabetic group (III) showed significant increase in serum levels of ALT, AST, and GGT in comparison to control group. These findings matched with *(Kitade et al., 2017 and Gharibi et al., 2016)* who reported that the levels of serum enzyme elevated in HFD animal models treated with STZ. After administration of adropin, group (IV) and (V) showed significant decrease in serum levels of ALT, AST, and GGT as compared to group (II) and (III) respectively. However GGT in obese and obese diabetic group still showed significant increase as compared to control group, but the serum levels of ALT, AST showed insignificant change when compared to control group. The improvement of liver enzymes after administration of adropin could be attributed to its direct antioxidant effect and its stimulatory effect on the function of mitochondria. Also, it could be explained by its counteracting effect on lipotoxicity

produced by free fatty acids when their number exceeded the storage capacity of the adipose tissue *(Shiferaw et al., 2020).* Hepatic lipase was one of its target regulatory proteins that hydrolyzed TG in chylomicron and VLDL-C to be used by the tissue in the form of mono-acyl-glycerol and free fatty acid, and also (HDL) with phospholipids and apo-lipoproteins for the process of reverse cholesterol transport *(Cave et al., 2016).*

Conclusion

These findings highlight that adropin had an important anti-hyperlipidemic and anti-diabetic effect, through improving the insulin sensitivity, enhancing glucose metabolism, suppressing the liver enzyme markers, the cytokines of inflammation, the serum lipid and PPARα relative gene expression in high fat diet plus STZ induced type-2 diabetic model and might be a potential target for treatment of diabetes and hyperlipidemia.

Recommendation

At the end, it is suggested that more research work is needed for:

- Better understanding of adropin physiology and its role in regulation of fuel selection.
- Determining the involved mechanisms of adropin in the regulation of vascular function, that is also of interest in terms of maintaining the vascular health and in potentially contributing to metabolic control.
- Providing more relations between adropin and the expression of PPAR-α gene that give further promise for the continued investigation of this peptide in the therapeutic context of diabetes and metabolic dysfunction.

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