



Effects of Lycopene on Paraoxonase and Adipokines Parameters in Streptozotocin - Induced Diabetic Rabbits.

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ABSTRACT

Background: Diabetes mellitus (DM) is a metabolic disease characterized by hyperglycaemia resulting from defects in insulin secretion, insulin action, or both, and occurs in almost all populations of the world a variable prevalence.

Objectives: The current study assessed the impact of lycopene on adipokines and paraoxonase (PON) in streptozotocin-induced diabetic rabbits.

Materials and Methods: A total of one hundred of rabbits (Albino Rabbits) of both sexes weighing between 1.5-2.1 kg were completely randomized in four groups (G) comprising 25 rabbits each. Diabetes was induced by a single intraperitoneal injection of streptozotocin (35 mg/kg body weight) feeding on a high fat regimen. Rabbits in G 3,4 which were administered 10 and 20 mg/kg respectively of lycopene for 4 weeks. The serum levels of leptin, adiponectin, ghrelin, resistin, and tumour necrosis factor – α (TNF- α) were measured by enzyme-linked immunosorbent assay (ELISA), while serum PON was measured by spectrophotometric method. SPSS computer software was used for data analysis.

Results: Results showed that lycopene administration at both doses significantly reduced the leptin, resistin, TNF- α , and glucose levels compared to diabetic control group G2. Consistent significant increases ($p < 0.05$) were noticed in the level of paraoxonase, adiponectin, and ghrelin in diabetic rabbits dealt with lycopene in comparison with diabetic rabbits of control group G2.

Conclusions: The high leptin, resistin, and TNF- α levels and oxidative damage whereas low linked with diabetes, were improved with the administration of lycopene.

المستخلص

المقدمة: يعد داء السكري أحد الاضطرابات الايضية الذي يتميز بانخفاض مستوى الجلوكوز في الدم نتيجة خلل اما في افراز الانسولين أو عمل الانزيم او كلاهما ويحدث داء السكري على مستويات مختلفة في معظم دول العالم.

المواد والمنهجية: اجريت هذه الدراسة لتقييم مدى فاعلية اللايكوبين على مستويات الأديبونكتين و الباروكسينيز في الارانب (نوع البابينو) التي احدث بها داء السكري بواسطة عقار الستريبتوزوسين. العدد الكلي لحيوانات المختبر كان 100 ومن كلا الجنسين تراوحت اوزانهم بين 1.5 - 2.1 كغم وتم توزيعهم عشوائيا الى اربعة مجموعات بواقع 25 حيوان لكل مجموعة. تم استحداث الإصابة بداء السكري في الحيوانات في مجموعات 2,3,4 عن طريق حقنهم بمادة الستريبتوزوسين داخل غشاء البريتون بجرعة مقدارها 35غم/كغم من وزن الجسم وتم تغذيتهم بغذاء عالي الدهون. الأرانب في مجموعات 3 و 4 فقط تم اضافة لايكوبين الى غذاءها 10 و 20 ملغم / كغم من وزن الجسم لمدة 4 أسابيع. تم قياس مستويات مصل

اللبتين، اديبونيكتين، جريلين، رزيستين ، و عامل نخر الورم نوع الفا بطريقة الأليزا، في حين تم قياس مصل الباروكسينيز (PON) بطريقة طيفية. وقد استخدم برنامج (SPSS) لتحليل البيانات.

النتائج: أشارت النتائج الى ان اعطاء لاكوبين و بتركيزين مختلفين قد قلل وبشكل ملحوظ احصائيا مستويات كل من اللبتين و رزيستين و العامل الورمي المسبب للنخر - نوع الفا في الدم وكذلك احدث انخفاض واضح وبمغزى احصائي لمستوى الجلوكوز بالدم مقارنة بعينة الحيوانات في المجموعة رقم 2 (المجموعة الضابطة المصابة بداء السكري). كما كان هناك زيادة مطردة ومستمرة في مستويات الباروكسينيز و اديبونيكتين و جريلين في الدم عند الحيوانات المصابة بداء السكري مقارنة بعينة الحيوانات في المجموعة الضابطة رقم 2.

الاستنتاجات: النتائج اشارت الى ان المستويات العالية لكل من اللبتين والرزيستين وعامل نخر الورم نوع الفا في الدم وكذلك تلف الأنسجة والخلايا الحاصل بسبب الأكسدة والمرتبطين اساسا بشكل ضعيف بالميكانيكية المرضية لداء السكري كلها قد تحسنت بفعل اعطاء لاكوبين لحيوانات التجربة.

KEYWORDS: Diabetic rabbits, lycopene, paraoxonase, leptin, resistin and TNF- α .

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INTRODUCTION

Diabetes is a metabolic disorder of multiple etiologies characterized by hyperglycemia and hyperlipidemia. The patients suffer from diabetes experience various complications, such as atherosclerosis, diabetic nephropathy and neuropathy⁽¹⁾. Adipokines such as leptin, visfatin, resistin, apelin, omentin, sex steroids, and various growth factors are cell signaling proteins secreted by adipocytes. Adipokine dysregulation appears to play a central role in adiposity, metabolic syndrome, and obesity⁽²⁾.

Lycopene is the red pigment of ripe tomatoes, belongs to the family of carotenoids, that it is a symmetrical tetraterpene assembled from eight isoprene units, and consists of carbon and hydrogen. It is one of the most powerful antioxidants among dietary carotenoids found in foods especially tomato. It is transported in the blood by lipoproteins and found in most tissues. It has gained increased attention for its health giving properties⁽³⁾.

Aim of the study

The present study was planned to evaluate the effect of lycopene on blood glucose, PON, leptin, adiponectin, ghrelin, resistin and TNF- α , in streptozotocin -induced diabetic rabbits.

MATERIALS and METHODS

Experimental animals:

The experimental study was carried out on 100 adult albino rabbits weighing 1.5-2.1 kg, during the period from June 2015 to

May 2016. The animals were divided into 4 groups, each group consists of 25 animals:

Group 1 (G1): healthy control rabbits.

Group 2 (G2): diabetic rabbits .

Group 3 (G3): diabetic rabbits received lycopene 10 mg/kg.

Group 4 (G4): diabetic rabbits received lycopene 20 mg/kg.

All administrations were given orally once daily for four weeks.

Induction of diabetes mellitus:

Diabetes mellitus was induced with a high fat diet (HFD) and low-dose of STZ treatment as mentioned previously⁽⁴⁾. Briefly, the rabbits were fed with HFD for a period of 2 weeks and then injected with low dose of STZ . Seven days after STZ injection, the fasting blood glucose levels of all the rabbits were estimated; those having blood glucose levels ≥ 200 mg/ dl were considered diabetic and were selected for further experiments. These rabbits were continued on HFD until the end of the study.

Lycopene preparation

Tomato oleoresin was mixed with maize oil and stored at 48 C° for 24 hours, mixture was arosed for 20 min in a water-bath at 50 C°. Each milliliter of the solution contained 5mg of lycopene. Lycopene was stable in the mixture for 9 weeks at 20C°⁽⁵⁾. Thirty (30) mg lycopene in a gelatine was reconstituted in olive oil to appropriate working concentration⁽⁶⁾ .

Chemicals

Human leptin ELISA Kit, Human Adipone-ctin ELISA Kit, ghrelin ELISA Kit, resistin ELISA Kit and glucose kit.

Instruments

ELISA system, Centrifuge, Spectropho-tometer, Water bath, Incubator, Hot plate, Vortex maxi-mix, pH meter, Balance, Oven, and Automatic micropipettes. At the end of experiment, 5 ml of blood samples were collected by ear vein of the rabbits, and 1 ml of the sera prepared through centrifuging at 2500 rpm for 15 minutes, then stored at freeze until assayed. Measurement of fasting serum glucose concentration was carried out on all the samples by the glucose oxidase method to make sure that the participating subjects were non-diabetic. Quantitative analysis of leptin, adiponectin, ghrelin, and resistin serum levels were measured by enzyme-linked immunosorbent assay (ELISA) with a commercially available kit (mediagnost, Germany) according to the manufacturer's instruction after the serum samples were thawed at room temperature. PON 1 activity was determined by spectrophotometric assay of Therry *et al.*⁽⁷⁾ in which phenyl acetate was used as a substrate.

STATISTICAL ANALYSIS

All values are presented as mean \pm standard deviation. All groups were compared by one-way analyses of variance (ANOVA) and multiple comparisons were done with Duncan test in SPSS (version 20) to determine the differences in all parameters.

RESULTES

The results in figures arranged as following: C: healthy control (G1), D.C: dia-betic control (G2), D-L: diabetic rabbits received lycopene (G3): rabbits receive lycopene 10 mg/kg BW & 20 mg/kg BW. Results indicated that STZ administration increased glucose level from (100 \pm 2.37 mg/dL) in C to (283.40 \pm 7.19a mg/dL) in D. C. Treatment of diabetic animals with the graded doses of lycopene (10 and 20 mg/kg -body weight) decreased the level of blood glucose considerably ($P < 0.05$) in D-L

progressively to (247.00 \pm 5.41b mg/dL), (246.00 \pm 8.79b mg/dL) respectively, after 4 week compared with (G2) D.C (Figure 1). Results showed that the serum leptin levels were (10.18 \pm 0.52 pg/mL) in normal control and increased to (38.04 \pm 1.40 pg/mL) in the diabetic rabbits following streptozotocin treatment. Following oral administration of lycopene (10 and 20 mg/kg body weight), the levels of leptin was significantly ($P < 0.05$) lowered to (30.50 \pm 1.72 pg/mL) and (25.04 \pm 0.89 pg/mL) respectively in diabetic animals when compared to G2 (Figure 2).

The adiponectin level in normal control rabbits G1 was (7.90 \pm 0.36 μ g/mL) and significantly lowered in G2 to (4.85 \pm 0.30 μ g/mL). On treatment with the graded doses of lycopene (10 and 20 mg/kg body weight) there was an increase in adiponectin level to (5.24 \pm 0.21 μ g/mL) and (6.03 \pm 0.37 μ g/mL) when compared with diabetic untreated rabbits (Figure 3).

The resistin level of diabetic rabbits were significantly higher (7.94 \pm 0.34 ng/ml) ($P < 0.05$) than those of the normal control rabbits (4.18 \pm 0.17 ng/ml). After 4 weeks of the lycopene extract treatment with doses of 10 and 20 mg kg⁻¹ lycopene, resistin level were significantly decreased respectively to (7.10 \pm 0.37 ng/ml) and (6.51 \pm 0.42 ng/ml) as compared to diabetic control rabbits (Figure 4).

The ghrelin level revealed a significantly lowered concentration in G2 (8.38 \pm 0.38 mol/L) when compared with normal control rabbits that recorded (17.21 \pm 0.49 mol/L). On treatment with the graded doses of lycopene (10 and 20 mg/kg body weight) there was increase ghrelin level to (12.09 \pm 0.52 mol/L) and (11.40 \pm 0.49 mol/L) when compared with diabetic untreated rabbits (Figure 5).

The TNF- α level of untreated diabetic rabbits were significantly higher (38.42 \pm 0.52, pg/ml) ($P < 0.05$) than those of the normal control rabbits (20.08 \pm 0.51 pg/ml). After 4 weeks of the lycopene extract treatment with doses of 10 and 20

mg kg⁻¹ lycopene, TNF- α level were significantly decreased respectively to (32.04 \pm 1.08, pg/ml) and (34.03 \pm 0.93 pg/ml) when compared to diabetic control rabbits(Figure 6).

The mean of serum PON decreased significantly ($P < 0.05$) in streptozotocin-induced diabetic rabbits

(151.00 \pm 7.49 μ mol/L) in comparison with control healthy group (225.00 \pm 4.01 μ mol/L). After oral administration of lycopene, the PON level (10 and 20 mg/kg body weight) increase (240.00 \pm 6.20 μ mol/L) and (228.00 \pm 3.99 μ mol/L) respectively as compared to the diabetic control group (Figure 7).

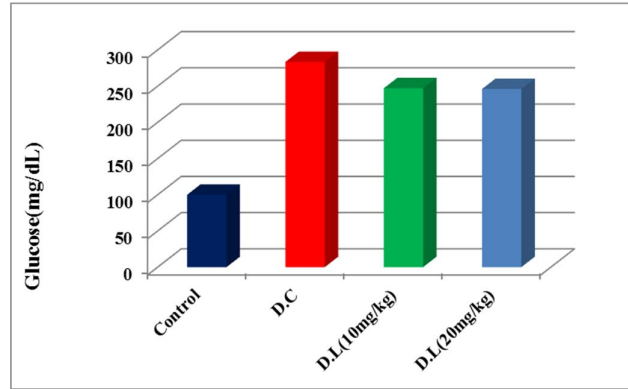


Figure.1: Effect of Lycopene on serum glucose levels in diabetic rabbits.
C : healthy control, D.C: diabetic control, D-L: diabetic rabbits received lycopene

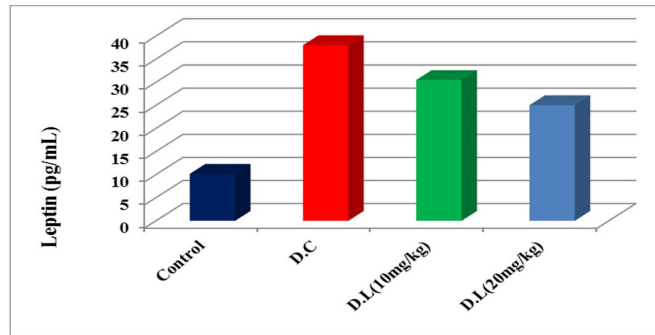


Figure.2: Effect of Lycopene on serum leptin levels in diabetic rabbits.

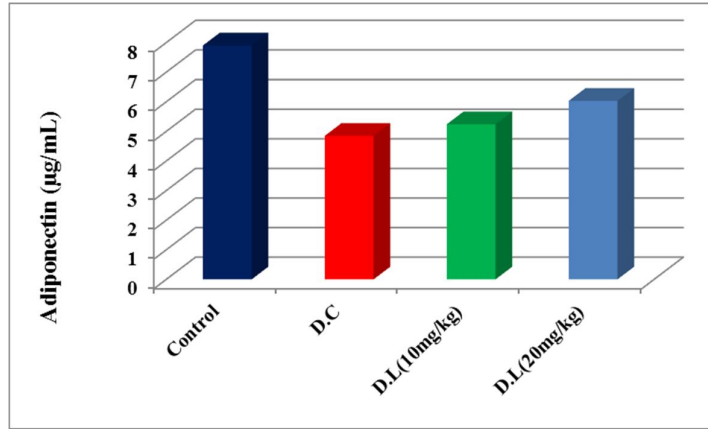


Figure.3: Effect of Lycopene on serum adiponectin levels in diabetic rabbits.

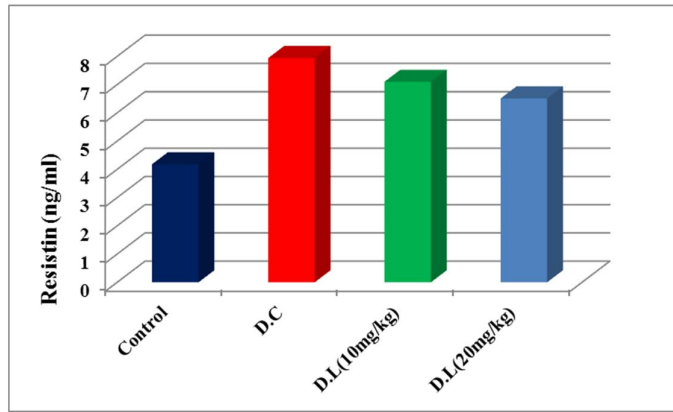


Figure.4: Effect of Lycopene on serum resistin levels in diabetic rabbits.

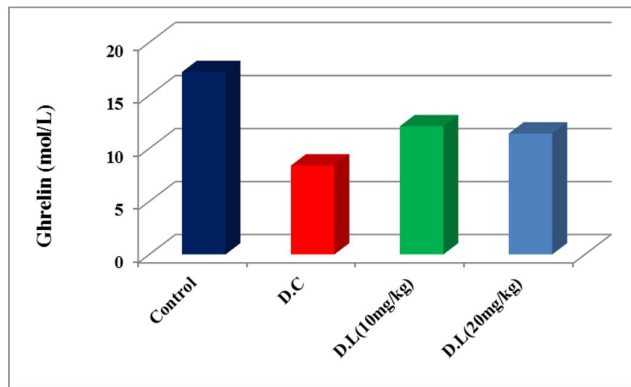


Figure.5: Effect of Lycopene on serum ghrelin levels in diabetic rabbits.

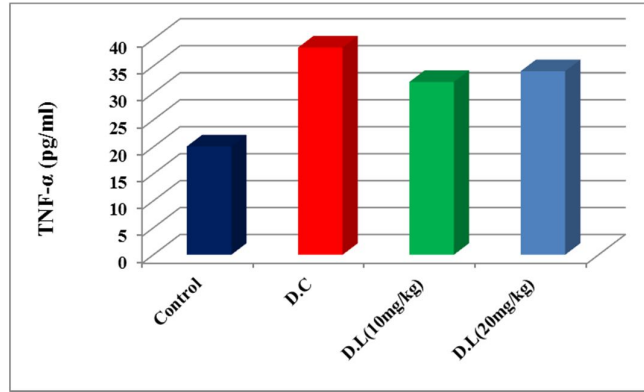


Figure 6: Effect of Lycopene on serum TNF-α levels in diabetic rabbits.

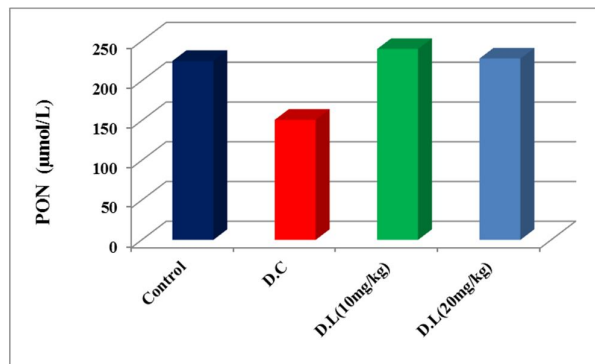


Figure 7: Effect of Lycopene on serum PON levels in diabetic rabbits.

DISCUSSION

The administration of streptozotocin induced diabetic rabbits which were confirmed by elevated glucose levels. This finding is in agreement with the report⁽⁸⁻¹⁰⁾ stating that STZ encourages diabetes being in the picture of hyperglycaemic non-ketotic diabetes mellitus in the models of animal⁽¹¹⁾.

In this study, diabetic rabbits had significantly ($p < 0.05$) increased serum leptin and decreased serum adiponectin levels as compared to normal control rabbits. Lycopene extract treatment was able to decrease the circulating levels of leptin and increase adiponectin in diabetic rabbits. Kusakabe *et al*⁽¹²⁾ observed beneficial effects of leptin on glycaemic and lipid control in a mouse model of diabetes with increased adiposity induced by STZ and a high-fat diet. In a study with a rabbit model mimicking human diabetes (STZ/HFD), authors showed that continuous

leptin infusion improved lipid and glucose metabolism and reduced food intake with enhancement of insulin sensitivity, accompanied by an increase of alpha-2 AMPK activity in skeletal muscle, leptin also reduced hepatic triacylglycerol content. In pair-feeding experiments authors showed that the independently of the food intake reduction, leptin improved glucose and lipid metabolism⁽¹²⁾.

Resistin, a new adipokine belongs to the cysteine-rich proteins family, It increases blood glucose and insulin levels and impairs hypoglycaemic response to insulin infusion⁽¹³⁾. The present study has shown the ability of lycopene to decrease serum resistin, which consistent with that of Kushiya *et al*⁽¹⁴⁾, which has been shown to view anti-inflammatory effects and less inflammation in adipose tissue^(15,16) by the lycopene administration.

Ghrelin is a 28 amino acid peptide hormone, it motivates growth hormone secretion, appetite, food intake, weight gain, gastric emptying, and controls the energy balance^(11,13). Our result showed that administration of lycopene can significantly increase serum ghrelin level in STZ - induced rabbits, the exact mechanism of lycopene affecting ghrelin levels remains to be determined.

Tumor necrosis factor- α interferes with insulin signaling in a variety of non-insulin producing cells, essentially inducing a state of insulin resistance⁽¹⁷⁾. In the present study, we showed that lycopene extract administration was able to reduce serum TNF in diabetic rabbits, due to immunomodulatory properties of lycopene and could be related to their potential anti-diabetic activity. This result is disagree with the report of Pierineet *al.*, and Markovits^(18,19).

Paraoxonase level dropped in group 2 in STZ induced diabetic rabbits, and elevated again by lycopene administration. PON is an antioxidant, HDL bound enzyme. It prevents low density lipoprotein (LDL) oxidation and therefore consumption of lycopene decreases cholesterol and LDL-C concentration. There are several mechanisms of adipokines influencing PON1 activity, leptin as a hydrophobic peptide be linked to HDL and inhibit directly the PON1 enzyme, and leptin enhances oxidative inflammatory cytokines and other acute phase proteins which have diminishing effect on hepatic function⁽¹³⁾.

Conclusion: It is concluded that using lycopene should be considered in the treatment of diabetic complications and hyperglycemia. Lycopene supplementation can be beneficial for humans in order to reduce the harmful effects of diabetes, such as increased serum leptin, resistin, TNF- α and blood glucose levels.

REFERENCES

1. Abul Barkat M. and Mohd. M.(2013): Comparative study of *Catharanthu-*

roseus extract and extract loaded chitosan nanoparticles in alloxan induced diabetic rats. *International Journal of Biomedical Research*;04(12), 670-678.

2. Tilg H, Moschen A.(2008): Role of adiponectin and PBEF/visfatin as regulators of inflammation: involvement in obesity-associated diseases. *Clin Sci (Lond)*, 114:275–288.
3. Eze Ejike Daniel, Aliyu Mohammed, Yusuf Tanko, Abubakar Ahmed, Moses Dele Adams, Denen Atsukwei.(2015): Effects of Lycopene on Thyroid Profile in Streptozotocin-Induced Diabetic Wistar Rats. *European Journal of Biotechnology and Bioscience*; 3 (1): 21-28.
4. Srinivasan K, Viswanad B, Asrat L, Kaul CL, Ramarao P. (2005): Combination of high-fat diet-fed and low-dose streptozotocin-treated rat: a model for type 2 diabetes and pharmacological screening. *Pharmacol Res.* 52:313–320.
5. Renatade Azevedo Melo Luvizotto, Andre F. Nascimento, Erika Imaizumi, Damiana T. Pierine, Sandro J. Conde, Camila R. Correa, Kyung-Jin Yeum and Ana Lucia A. Ferreira. (2013): Lycopene supplementation modulates plasma concentrations and epididymal adipose tissue mRNA of leptin, resistin and IL-6 in diet-induced obese rats. *British Journal of Nutrition*. 1 - 7.
6. Ogundeji T, Ayo JO, Aluwong T, Mohammed A. (2013): Behavioural and haematological studies on effects of lycopene in Wistar rats subjected to psychological stress. *Journal of Neuroscience and Behavioral Health*;5(2):30-35.
7. Therry D, Gean D.(1998): Decrease of serum PON 1 activity in renal failure. *J Am. Soc of Nephrol*; 2082-88.
8. Kolb H, Kroneke D.(1993). IDDM: lessons from the low dose Stereptozotocin model in mice. *Diabetes Rev*; 1:116- 26

9. Mohammed A, Tanko Y, Okasha MA, Magaji RA, Yaro AH. (2007). Effects of aqueous leaves extract of *Ocimum gratissimum* on blood glucose levels of streptozotocin induced diabetic wistar rats. *African Journal of Biotechnology*; 6:2087-2090.
10. Entedhar R, Sarhat , Ayhan R., Mohammed Sh.(2015). Effect of Glibenclamide and Tomato lycopene extract on some biochemical parameters in serum of alloxan Induced diabetic rabbits. *Kirkuk University Journal /Scientific Studies*. 10, (3), ;140-154.
11. Mohamed WS, Hassanien MA, Sayed Abo khosheim KEL.(2014). Role of Ghrelin, Leptin and Insulin Resistance in Development of Metabolic Syndrome in Obese Patients. *Endocrinol Metab Synd*.3:122.
12. Kusakabe T, Tanioka H, Ebihara K, Hirata M, Miyamoto L, Miyanaga F, Hige H, Aotani D, Fujisawa T, Masuzaki H, Hosoda K, Nakao K. (2009). Beneficial effects of leptin on glycaemic and lipid control in a mouse model of type 2 diabetes with increased adiposity induced by streptozotocin and a high-fat diet. *Diabetologia*;52(4):675-83.
13. Ebtessam A AL-Suhaimi, and Adeeb Shehzad .(2013) Leptin, resistin and visfatin: the missing link between endocrine metabolic disorders and immunity. *European Journal of Medical Research* .18:12
14. Kushiyama A., Shojima N., Ogihara T., Inukai K., Sakoda H., Fujishiro M. (2005): Resistin-like molecule beta activates MAPKs, suppresses insulin signaling in hepatocytes, and induces diabetes, hyperlipidemia, and fatty liver in transgenic mice on a high fat diet. *J.Biol.Chem.*, 280:42016–42025.
15. Marcotorchino J, Romier B, Gouranton E, *et al.* (2012). Lycopene attenuates LPS-induced TNF- α secretion in macrophages and inflammatory markers in adipocytes exposed to macrophage-conditioned media. *Mol Nutr Food Res* 56, 725–732.
16. Gouranton E, Thabuis C, Riollet C, *et al.* (2011). Lycopene inhibits proinflammatory cytokine and chemokine expression in adipose tissue. *J Nutr Biochem* 22, 642–648.
17. Del Aguila, L. F.; Claffey, K. P. and Kirwan, J. P.(1999): TNF- α impairs insulin signaling and insulin stimulation of glucose uptake in C2C12 muscle cells. *Am. J. Physiol.*, 276: 849-855.
18. Pierine, DT, Navarro MEL, Minatel IO, Luvizotto RAM, Nascimento AF, Ferreira ALA ,Yeum K-J and Corrêa CR. (2014). Lycopene supplementation reduces TNF- α via RAGE in the kidney of obese rats. *Nutrition & Diabetes*. 1 – 6.
19. Markovits N, Ben Amotz A, Levy Y.(2009). The effect of tomato-derived lycopene on low carotenoids and enhanced systemic inflammation and oxidation in severe obesity. *Isr Med Assoc J*. 11: 598–601.