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RESEARCH ARTICLE

Evaluation of some stress indicators and their relation with leptin injection in experimentally induced diabetic rats.

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Abstract

The aim of this study was to investigate the effects of exogenous leptin administration on some stress indicators in streptozotocin (STZ)-induced diabetes rats.In this study wistar albino rats were divided into two main groups (first was 5 day, and second was 10 day), each one subdivided into four groups (5 rat per subgroup): 1. Control, 2. Leptin (10 µg/kg leptin, sc. daily), 3. Diabetes, (induced by single injection of streptoztocin (65 mg/kg bw)) 4. Leptin + diabetes. At the end of the experiments the blood collected by cardiac puncture under anesthesia. Plasma catalase, glutathione, malondialdehyde, and IL-6 levels were measured. Our results showed that diabetes decrease glutathione and catalase levels and increase MDA and IL-6 levels, while leptin treatment reverse these changes.

Introduction:-

Diabetes is a chronic metabolic disorder and a major worldwide health problem. It is characterized by absolute or relative deficiencies in insulin secretion and/or insulin action associated with chronic hyperglycemia and disturbances of carbohydrate, lipid, and protein metabolisms.The effects of diabetes mellitus include long term damage, dysfunction and failure of various organs [1].

Oxidative stress results from an imbalance between the generations of oxygen derived radicals and the organism s antioxidant potential. The free radical-mediated peroxidation of membrane lipids increases membrane fluidity and permeability with loss of its integrity [2]. The process of lipid peroxidation is one of the oxidative conversion of polyunsaturated fatty acids to products known as malondialdehyde (MDA), MDA is a highly toxic molecule and its secondary products such as thiobarbutiric acid reactive substance (TBARS), are commonly used to evaluate lipid peroxidation [3]. Glutathione (GSH) is a major non-enzymatic component of the cellular antioxidant system, playing an important role in the antioxidation of ROS and free radicals [4]. Various studies have shown that both insulin dependent (Type 1) and insulin non-dependent diabetes (Type 2) are associated with increased formation of free radicals and decrease in antioxidant potential [5]. Tissue damage induced by oxidative stress has also been implicated in the pathogenesis of diabetic complications [6]. Various plasma protein and lipids were reported to be oxidatively modified both in experimental and clinical studies [7,8]. Streptozotocin (STZ)-diabetic animals, often used as a study model of Type 1 diabetes, are characterized not only by decrease insulin levels and hyperglycemia, but also by decrease in circulating leptin levels [9]. Leptin is predominantly expressed by adipocytes, and its plasma levels correlate well with the body fat mass [10]. It was previously suggested that this decrease in leptin contribute to hyperphagia in diabetic rats [11]. Administration of leptin results in partial correction of food intake, as well as normalization of postabsorbtive plasma glucose levels in STZ-induced diabetes [12] .

There are no studies on the role of leptin on oxidative stress in STZ-induced diabetes. The aim of our present study was to investigate the role of exogenous human leptin on plasma catalase, GSH, MDA, and IL-6 levels of non-diabetic and STZ-induced diabetic rats.

Material and Methods:-

The following experiments were use of laboratory animals, 40 male Wistar albino rats weighing 200±20 g were used in this study. They were fed a standard laboratory diet and tap water ad libitum and kept in a room with controlled temperature (22 ± 1o C), and a 12:12-h light-dark cycle. They were divided into two main groups (first was 5 day, and second was 10 day), each one subdivided into four groups (5 rat per subgroup) and allowed free access to standard diet and water ad libitum.

1. Control group; physiological saline solution, PS, sc. daily.
2. Leptin group ; 10 μg/kg, recombinant human leptin, sc. daily.
3. Diabetes group, a single ip injection of STZ at a dose of 65 mg/kg bw.
4. Leptin + diabetes group; leptin injections (10 μg/kg sc. daily).

Diabetes was induced in overnight fasted rats by a single intraperitoneal injection of STZ (SIGMA Chemicals, USA) at adose of 65 mg/kg body weight freshly dissolved in 0.1 mol/L citrate buffer, pH 4.5 .The animals with fasting blood glucose values more than 250 mg/dl after 72 h of STZ injection were considered diabetic and included in the study one week after induction of diabetes, rats began treatment protocol of leptin injections [13]

Serum Biochemical parameters:

In serum, catalase and IL-6 levels were measured by enzyme immunoassay (ELISA) kit (ABO, **Switzerland**), Reduced glutathione was measured as described by [14]. The level of serum MDA was determined by a modified procedure described by [15].

Results:-

Plasma catalase levels are shown in table 1, Plasma catalase levels were significantly higher ($p<0.05$) in the leptin groups than the control groups. Plasma catalase levels were significantly lower in the diabetes group comparing all the other three groups ($p<0.05$). Leptin administration (10**μ**g/kg s.c. for 5 and 10 days) to leptin+diabetes groups led to increase in plasma catalase levels as compared to the diabetes groups ($p<0.05$).A similar result was obtained between the 5 and 10 days experiments, and the difference were not statistically significant except in leptin groups.

Table 1: Catalase levels in the plasma of control, leptin, diabetes, and leptin+diabetes groups.

Groups	Catalase level (U/L)	
	5 Day	10 Day
Control	18.40±0.42b	18.40±0.51b
Leptin	*24.00±0.82a	29.60±0.96a
Diabetes	13.40±0.51d	11.40±0.50e
Diabetes + leptin	15.80±0.5cd	14.60±0.30c
LSD	2.68	2.97

Each value represents the mean ± S.D. of five animals per group.
Star (*) represents significant difference between (5 and 10 day) groups.

Plasma GSH levels are shown in table 2, Plasma GSH levels were significantly higher ($p<0.05$) in the leptin groups than the control groups. Plasma GSH levels were (non significantly in 5 day group, and significantly ($p<0.05$) in 10 day group) lower in the diabetes groups than the control groups. Leptin administration (10**μ**g/kg s.c. for 5 and 10 days) to leptin+diabetes groups led to increase in plasma GSH levels (non significantly in 5 day group, and significantly ($p<0.05$) in 10 day group) as compared to the control and diabetes groups. A similar result was obtained between the 5 and 10 days experiments, and the difference were not statistically significant except in diabetes and diabetes+leptin groups.

Table 2:GSH levels in the plasma of control, leptin, diabetes, and leptin+diabetes groups.

Groups	GSH level (μmol/L)	
	5 Day	10 Day
Control	3.26±0.24b	3.39±0.23b
Leptin	4.18±0.29a	4.48±0.25a
Diabetes	*3.05±0.25b	2.37±0.34c
Diabetes + leptin	*3.36±0.28b	4.16±0.21a
LSD	0.605	0.559

Each value represents the mean ± S.D. of five animals per group.
Star (*) represents significant difference between (5 and 10 day) groups

Plasma MDA levels are shown in table 3, Plasma MDA levels were non significantly lower in the leptin groups than the control groups. Plasma MDA levels were significantly increase in the diabetes groups than the control groups. Leptin administration (10**μ**g/kg s.c. for 5 and 10 days) to leptin+diabetes groups led to significantly ($p<0.05$) decrease in plasma MDA as compared to the control and diabetes groups. A similar result was obtained between the 5 and 10 days experiments, and the difference were not statistically significant except in diabetes groups.

Table 3:MDA levels in the plasma of control, leptin, diabetes, and leptin+diabetes groups.

Groups	MDA level (μmol/L)	
	5 Day	10 Day
Control	1.322±0.35d	1.22±0.17d
Leptin	1.042±0.18d	1.006±0.13d
Diabetes	*3.67±0.11a	4.40±0.19a
Diabetes + leptin	2.49±0.12c	2.38±0.11c
LSD	0.493	0.496

Each value represents the mean ± S.D. of five animals per group.
Star(*) represents significant difference between (5 and 10 day) groups

Plasma IL-6 levels are shown in table 4, Plasma IL-6 levels were non significantly higher in the leptin groups than the control groups. Plasma IL-6 levels were significantly increase ($p<0.05$) in the diabetes groups than the control groups. Leptin administration (10**μ**g/kg s.c. for 5 and 10 days) to leptin+diabetes groups led to non significantly decrease in plasma IL-6 as compared to the diabetes groups. A similar result was obtained between the 5 and 10 days experiments, and the difference were not statistically significant among all groups.

Table 4:IL-6 levels in the plasma of control, leptin, diabetes, and leptin+diabetes groups.

Groups	IL-6 level (pg/ml)	
	5 Day	10 Day
Control	4.85±0.37c	4.76±0.41c
Leptin	5.37±0.38cb	5.63±0.35c
Diabetes	7.54±0.47ab	7.88±0.41ab
Diabetes + leptin	6.90±0.66b	6.60±0.51bc
LSD	1.824	1.35

Each value represents the mean ± S.D. of five animals per group.
Star(*) represents significant difference between (5 and 10 day) groups

Discussion:-

In the present study diabetes mellitus, as a metabolic stress, causes decrease in catalase and GSH levels and increase in MDA level, this finding agreement with [16].The elevation of mean concentration of MDA in STZ-injected group may be due to the hypoinsulinemia that increases the activity of fatty acyl coenzyme -A -oxidase, which initiates β-oxidation of fatty acids, resulting in lipid peroxidation [17].

Type 1 and 2 Diabetes associated with increased formation of free radicals and a decrease in the level of antioxidants as increased oxidative stress in diabetes cases for several reasons, including that the case of hyperglycemia arise ROS, which in turn caused lipid peroxidation and destroying cell membranes through self-

oxidation which produces free radicals [5]. The treatment of Streptozotocin-Diabetic Rats with leptin increases GSH level, note that the level did not show any differences in the treatment of diabetes and normal rats with leptin from the normal level [1].

The levels of enzymatic antioxidants including catalase was low in ob mice, and increased in level at treatment with leptin as leptin causing a significant increase in the effectiveness of GSH in both obese and thin mice , also leptin reduced MDA level in rats exposed to oxidative stress [18] and this mention too by [19] who indicate that the treatment with leptin reduces the level of MDA in any dose used. Results showed a significant rise in the IL-6 level in diabetic group compared with control, This is consistent with previous studies [20,21]. As it found that high blood sugar increases the inflammatory signs such as tumor necrosis factor and interleukin -6 [22] It is known that diabetes is a strong relationship with oxidative stress output of free radicals and ROS that work as a intercellular second messenger receives many of signals such as gene expression of proinflammatory cytokines, including IL-6 [23]. Diabetes+leptin groups showed a low level of IL-6 compared with diabetes groups, because leptin increases the level of antioxidants and the recent reduces the immune response through inhibit oxidative stress [24].

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