THE POTENTIAL PROTECTIVE AND CURATIVE EFFECTS OF ERYTHROPOIETIN ON DIABETIC MALE RATS

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ABSTRACT

Background: Erythropoietin (EPO) is a glycoprotein hormone member of the type 1 cytokine super family that is produced by renal cortical and outer medullary type 1 fibroblasts in response to tissue hypoxia. It has a hematopoietic function, but recent studies have shown that EPO has several non hematopoietic functions. EPO limits the destructive potential of tumor necrosis factor α and other proinflammatory cytokines in different tissues. **Objective:** Studying the possible protective and curative effects of EPO on diabetes in alloxan-induced diabetic rats. **Material and methods:** Fifty adult male albino rats of local strain between 7-8 weeks old and weighing 140-160 g were used. The rats were divided into 5 equal groups. Group I (control group), Group II (erythropoietin- treated group), Group III (diabetic group), Group IV (EPO- pretreated diabetic group), and group V (EPO-treated diabetic group). The body weight, food consumption, blood glucose level, serum tumor necrosis factor α (TNF-α) and serum interleukin 6 (IL-6) were determined for all groups. **Results:** Blood glucose level, serum tumor necrosis factor α (TNF-α) and serum interleukin 6 (IL-6) decreased in EPO-pretreated and treated diabetic groups when compared to the diabetic rats. These results were confirmed by the histopathological study which showed marked improvement of the destructive effect on pancreatic islet cells induced by alloxan especially when EPO treatment was given after two weeks of alloxan injection. **Conclusion:** EPO is a general tissue protective cytokine. It acts on glucose metabolism and increasing β cell mass. Thus, promotion of EPO signaling in β cells may be a novel therapeutic strategy for diabetes prevention and treatment.

Key words: Alloxan, Erythropoietin, anti-inflammatory activity, antidiabetic.

INTRODUCTION

Diabetes mellitus (DM) has become one of the most serious threats to global public health. It is the most common endocrinal disease in the world. According to the WHO (World Health Organization), it is expected to affect the lives of 380 million people by the year 2025. It is also estimated that 5 % of all deaths in the world are caused by diabetes; a number which will increase by

50% in the next 10 years *(Piya et al., 2010)*. At the time of clinical diagnosis, near 70% of β-cell mass is destroyed as a consequence of the auto-destruction that begins months or even years before the clinical diagnosis *(Si et al., 2011).* As there is no perfect cure for diabetes, one of the overarching goals in the treatment of all types of diabetes is the preservation and growth of β-cells.

Erythropoietin (EPO), is a 34kDa glycoprotein that was originally identified

because of its role in erythropoiesis. In the fetus, EPO is produced in the liver and, following the neonatal period, EPO is produced in the kidney and the liver. It is also considered as a cytokine with pleiotropic functions including erythropoiesis, modulation of inflammatory and immune responses, vasogenic and pro-angiogenic functions, and effects on brain development and repair *(Yvonne and Fernando, 2015)*.

EPO mediates preconditioning (ischemic tolerance) and specifically limits the destructive potential of tumor necrosis factor α and other proinflammatory cytokines in the brain, heart, kidney, and other tissues. As local production of EPO is generally suppressed following injury, administration of exogenous EPO has been a successful therapeutic approach in preclinical and clinical studies (*Brines and Cerami, 2006).*

The present study was planned to investigate the possible protective and curative effects of erythropoietin on diabetic male rats.

MATERIAL AND METHODS

Experimental animals:

Fifty adult male albino rats of local strain weighting 140- 160 gm were used in this study. They were obtained from Nile Pharmaceuticals Company. All rats were fed on well balanced diet (rat chow) and water *ad libitum* and allowed to adapt to the prevailing environments for 2 weeks prior to the beginning of the experiment in the laboratory of Physiology, Faculty of Medicine (Girls) AI-Azhar University. Rats were kept in cages (three rats per cage – 25 x 30 x 30

cm). All procedures were approved by the animal care committee.

The rats were divided into 5 equal groups. **Group I**: Rats were injected with normal saline 0.9 % (3.7ml /kg body weight) intra-peritoneally (i.p) 3 times /week for 6 weeks, and served as control group. **Group II:** Rats were injected with erythropoietin in the form of recombinant human erythropoietin (rHuEPO) intra-peritoneally at a dose of 180 u/kg three times weekly on alternating days for 6 weeks *(Katz et al., 2010).* **Group III:** Rats were subjected to induction of diabetes by alloxan monohydrate injection intra-peritoneally at a dose of 75 mg/kg body weight for 5 consecutive days *(Suresh and Das, 2001).* Development of diabetes in rats was confirmed by measuring blood glucose levels in blood samples taken from a tail vein. Rats with blood glucose levels > 200 mg/dL were considered to be diabetic. Diabetes mellitus was confirmed by the use of a digital glucometer (Accu-chek® Advantage, Roche Diagnostic, Germany). **Group IV:** Rats were injected with rHuEPO intra-peritoneally at dose of 180 u/kg three times per week for 6 weeks, then they were subjected to induction of diabetes by alloxan monohydrate injection intra-peritoneally at a dose of 75 mg/kg body weight for 5 consecutive days starting from the first day of rHuEPO treatment *(Choi et al., 2010).* **Group V:** Rats were subjected to induction of diabetes mellitus then, after two weeks, treated with recombinant human erythropoietin (rHuEPO) for one month at a dose of 180 u/kg three times weekly on alternating days.

Drugs and doses

1. Alloxan monohydrate was obtained from Algomhoria Chemical Company. Alloxan was dissolved in 0.9% normal saline to obtain 2% alloxan solution *(Macedo et al., 2005)* and injected intra-peritoneally immediately after preparation to overnight fasted animals at a dose of 75 mg/kg body weight for 5 consecutive days *(Suresh and Das, 2001).*

2. Erythropoietin (EPO) in the form of EPIAO, (rHuEPO, Epoetin alfa) was obtained from Egy Pharmex. Rats received rHuEPO i.p. 3 times weekly at a dose of 180 u/kg body weight *(Katz et al., 2010).*

Food consumption was measured daily for 6 weeks. The blood samples were collected from retro-orbital sinus by capillary tubes under light ether anesthesia *(Simmons and Brick, 1970)*. Blood was collected in a centrifuge tube and allowed to clot for an hour at room temperature, and then centrifuged at 3000 rpm for 15 minutes. Sera were separated and stored frozen until assayed. The separated sera were analyzed for estimation of serum tumor necrosis factor alpha (TNF-α) and serum interleukin 6 (IL-6). Finally, the rats were sacrificed and their abdominal cavities were opened for histopathological evaluation of pancreas.

Biochemical assay:

- Serum TNF-α level was determined by The Ray Bio Rat TNF-alpha ELIS (Enzyme-Linked Immunosorbent Assay) kit *(Broody et al., 1986 and Brenner et al., 1989).*

- Serum IL-6 activities were determined using the RayBio Rat IL-6 ELISA (Enzyme-Linked Immunosorbent Assay) kit *(Ferrari, et al., 2003 and Venihaki, et al., 2001).*

Histopathological study:

Half portion of pancreatic tissue including the tail part was fixed in 10% neutral formalin specimens were dehydrated and embedded in paraffin. Tissue sections of 5 ?m were prepared and stained by hematoxylin and eosin (H&E) to assess pancreatic tissue injury, Masson trichrome (MT) stain to confirm the presence of any fibrotic tissues and to assess the state of blood vessels in pancreatic tissue. Immune stain by anti-Ki 67 was used to assess the proliferative activity of pancreatic cells.

Morphometric assessment was done on H&E stained slides using the image analyzer optical micrometer (TS view), Pathology Department Faculty of Medicine (Girls), Al Azhar University. TS view was basically composed of operation system and hardware requirements. TS view was the software products designed in connection with digital microscope of the CMOS series and CCD with the functions of photo taking, image measuring and handling .The value was given by the system in Pixels (?m^2 as a unit).

Statistical analysis was done using statistic package for social science version 12 (SPSS, 12) for windows.

A-Descriptive statistics: Quantitative data were expressed by mean and standard error (S.E.) of mean.

B- Analytical statistics:

1. Comparing groups was done using ANOVA (analysis of variance) for

comparison of quantitative data of more than 2 groups, followed by post-hoc test.

2. The level of significance was taken at P value of < 0.05.

RESULTS

I- Effect of erythropoietin injection on body weight and food intake (Table 1):

There were significant differences on comparing all groups with each other in body weight and food intake respectively.

Recombinant human erythropoietin (rHuEPO) injection to rats of EPO-treated group for six weeks showed significant decrease of body weight (about 10.3%). EPO-pretreated diabetic group showed a significant decrease in body weight (about 11.13%). Also, rHuEPO injection to EPO-treated diabetic rats produced a significant decrease (about 22.68%) when all groups were compared to control group at the end of experimental period.

As regards to food intake, rHuEPO injection to rats showed a significant increase in food intake (about 14%), while, it showed a significant decrease in EPO- pretreated and treated diabetic rats (about 2.4% and 5.23% respectively) when compared to control group.

In comparison to diabetic group, rats pretreated with rHuEPO showed a significant increase in both body weight and food intake (about 23.14% and 13.4% respectively). Treatment of diabetic rats with rHuEPO injection produced also significant increase in body weight and food intake (about 7.14% and 10.22% respectively).

Table (1): Effect of erythropoietin injection on body weight and food intake.

Groups / Parameters	Group I (Control group)	Group II (EPO-treated group)	Group III (Diabetic group)	Group IV (EPO- pretreated diabetic group)	Group V (EPO-treated diabetic group)	ANOVA	
	Mean± S.E.M	Mean± S.E.M	Mean± S.E.M	Mean± S.E.M	Mean± S.E.M	F	P value
Body weight (g)	242.50 ± 2.26	217.50 ± 2.5	175 ± 1.66	215.50 ± 2.73	187.50 ± 2.14	5.13	P<0.05
Food intake (g)	63.58 ± 2.41	72.50 ± 3.1	54.66 ± 2.27	62 ± 2.29	60.25 ± 3.05	37.39	P<0.05

Data in this table showed that the statistical relations between all groups per each parameter were significant.

II- The effect of erythropoietin injection on blood glucose level (Table 2):

There were a significant differences in blood glucose level when comparing all groups with each other all over the period of the experiment.

Recombinant human erythropoietin (rHuEPO) injection to rats of erythropoietin-treated group for one week showed a significant decrease in blood glucose level (about 9%) and significant increase (about 3.6%) in erythropoietin-

pretreated diabetic group. Erythropoietin-treated diabetic rats also showed a significant increase in blood glucose level (about 21.3%) when compared to control rats.

Two weeks of recombinant human erythropoietin (rHuEPO) injection to rats of erythropoietin-treated group, showed a significant decrease in blood glucose level (about 15.4%). blood glucose level of erythropoietin- pretreated and treated diabetic groups showed significant increase (about 9.9% and 95.7% respectively) when compared to control rats.

Three weeks of recombinant human erythropoietin (rHuEPO) injection to rats to erythropoietin-treated group, showed a significant decrease in blood glucose level (about 19.7%) compared to control rats. Erythropoietin-pretreated and treated diabetic groups showed significant increases in blood glucose level (about 7.6% and 37.8 % respectively) when compared to control rats.

On the fourth week, rHuEPO injection to rats of erythropoietin-treated group produced a significant decrease in blood glucose level (about 25.8%). Blood glucose level of erythropoietin-pretreated and treated diabetic groups showed a significant increase (about 6.5% and 21% respectively) when compared to control rats.

Erythropoietin treated group for five weeks showed a significant decrease in blood glucose level (about 25.7 %) when compared to group I. Erythropoietin-pretreated and treated diabetic groups showed a significant increases in blood glucose level (about 7.2 % and 17.2 % respectively) when compared to control rats.

Recombinant human erythropoietin (rHuEPO) injection to rats of erythropoietin-treated group for six weeks showed a significant decrease in blood glucose level (about 26.6%) when compared to control rats. Erythropoietin-pretreated and treated diabetic groups showed a significant increase in blood glucose level (about 4.6% and 10.6% respectively) when compared to control rats.

In comparison to diabetic group, the blood glucose level of erythropoietin-pretreated diabetic group at the end of 1st week of experiment showed a significant decrease (about 15%), while the blood glucose level of erythropoietin treated diabetic group showed insignificant decrease in blood glucose level (about 1.5%).

Recombinant human erythropoietin (rHuEPO) injection to rats of erythropoietin-pretreated diabetic group for two weeks showed a significant decrease in blood glucose level about (45.7%),whereas erythropoietin treated diabetic group, showed insignificant decrease (about 3.3 %) when compared to diabetic rats.

Recombinant human erythropoietin (rHuEPO) injection for three weeks to rats of erythropoietin- pretreated diabetic group showed a significant decrease in blood glucose level (about 48.3 %), while erythropoietin- treated diabetic group, showed a significant decrease in blood glucose level (about 33.8%) when compared to diabetic rats.

Four weeks of rHuEPO injection to rats of erythropoietin pretreated and treated diabetic groups, showed a significant decreases in blood glucose

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level (about 47.4% and 40.3% respectively) when compared to diabetic rats.

Recombinant human erythropoietin (rHuEPO) injection for five weeks to rats of erythropoietin- pretreated and treated diabetic groups, showed a significant decrease in blood glucose level (about

46.6 % and 41.6% respectively) when compared to diabetic rats.

Finally erythropoietin pretreated and treated diabetic groups showed significant decrease in blood glucose level (about 46.3 % and 43.2% respectively) when compared to diabetic rats.

Table (2): Effect of erythropoietin (EPO) injection on blood glucose level.

Groups / Duration	Group I (Control group)	Group II (EPO-treated group)	Group III (Diabetic group)	Group IV (EPO- pretreated diabetic group)	Group V EPO-treated diabetic group)	ANOVA	
	Mean± S.E.M	Mean± S.E.M	Mean± S.E.M	Mean± S.E.M	Mean± S.E.M	F	P value
1st week	122 ±2.3	111 ±1.52	150.3 ± 4.67	129.8 ± 1.56	148 ± 4.13	21.99	P<0.05
2nd week	120.8±2.37	102.10±2.18	244.6 ±7.52	132.80 ± .78	236.50 ± 6.83	231.06	P<0.05
3rd week	121.4±1.7	97.40 ± 1.8	253 ± 5.59	130.70 ±1.02	167.40 ±4.48	501.66	P<0.05
4th 4week	122.2± 1.9	90.60 ± 1.72	247.9 ±5.56	130.20 ±1.2	147.90 ±2.6	425.7	P<0.05
5th week	120.9±2.1	89.80 ±1.06	243 ±2.78	129.70± 1.3	141.80 ±1.08	974.89	P< 0.05
6th week	122.6±1.7	88.90 ±0.99	239.2 ±3.55	128.30±1.46	135.70 ±1.18	753.62	P< 0.05

Data in this table showed that the statistical relations between all groups per each parameter were significant.

III- The effect of erythropoietin injection on serum TNF-α and IL-6 (table 3):

There was a significant difference when comparing all groups with each other in serum TNF-α and IL-6 respectively.

Recombinant human erythropoietin (rHuEPO) injection to rats of erythropoietin-treated group for six weeks showed insignificant decrease in serum TNF-α level (about 0.22 %)**,** whereas rHuEPO injection to rats of erythropoietin-pretreated diabetic group showed insignificant increase (about 1.54%). rHuEPO injection to rats of

erythropoietin-treated diabetic group produced a significant increase (about 5.73 %) when all groups compared to control rats.

rHuEPO injection to rats of erythropoietin-treated group showed insignificant decrease in serum IL-6 (about 0.46%),while its injection to pretreated diabetic group showed insignificant increase (about 2. 92 %), and to erythropoietin-treated diabetic group showed a significant increase (about 8.58%) when all groups compared to control one.

In comparison to the diabetic group, RHuEPO injection to erythropoietin-

pretreated and treated diabetic groups produced a significant decrease in serum TNF-α (about 26.11 % and 23.06 % respectively), while serum IL-6 showed a significant decrease (about 32.63 % and 28.93% respectively).

Table (3): Effect of erythropoietin injection on serum TNF-α and IL-6.

Groups / Parameters	Group I (Control group) Mean± S.E.M	Group II (EPO treated group) Mean± S.E.M	Group III (Diabetic group) Mean± S.E.M	Group IV (EPO pretreated diabetic group) Mean± S.E.M	Group V (EPO treated diabetic group) Mean± S.E.M	ANOVA F	ANOVA P value
Serum TNF-α (Pg/ml)	34.85 ±0.17	34.77±0.26	47.90±0.50	35.39±0.22	36.85± 0.20	349.14	P<0.05
Serum IL- 6 (Pg/ml)	15.02 ±0.21	14.95 ±0.08	22.95 ±0.60	15.46±0.15	16.31 ±0.13	118.01	P<0.05

Data in this table showed that the statistical relations between all groups per each parameter were significant.

Histopathological results

Microscopic examination of all cases of diabetic group showed variable sized pancreatic islets with reduction of its cellular components than normal cases. Some pancreatic islets were very small, cord-like and difficult to be distinguished from the surrounding exocrine cells with which they merged (figure 1). **Ki 67** immune staining showed no proliferate activity (figure 2). Morphometric analysis showed that:-

1. The mean number of pancreatic islets was (2.6± 0.19).

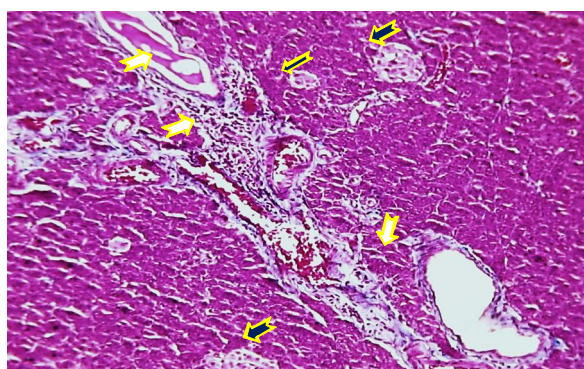2. The mean average of pancreatic islets area was (90.2?m±10.72).

Figure (1): Diabetic case showing decrease number and size of pancreatic islets, perivascular cellular infiltration by mononuclear cells in exocrine pancreatic tissue, and dilated pancreatic ducts (H&E X125)
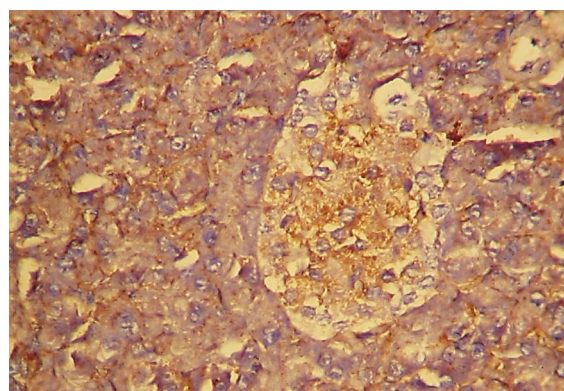

Figure (2): Diabetic case showing no proliferative activity by Ki 67 immunostaining (x 400).

Morphometric analysis of EPO-pretreated diabetic group showed that (figure 3):

1. The mean number of pancreatic islets was 3.7± 0.21

2. The mean average of pancreatic islets area was 168.55 ?m ± 13.8.

Figure (3): EPO pretreated diabetic case with normal vascularity pattern of pancreatic islets (MT X400).

Microscopic examination of EPO-treated diabetic group showed increased number of pancreatic islets due to
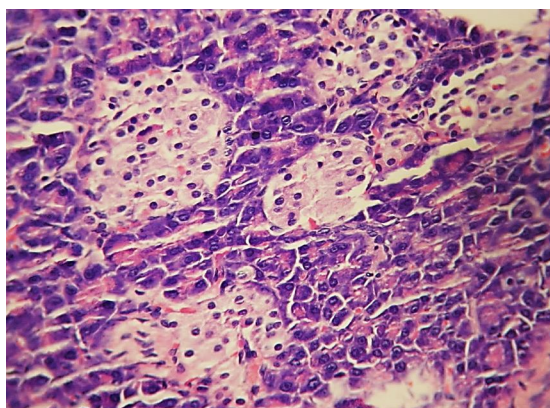

Figure (4): EPO treated diabetic case showing increased number of regenerating pancreatic islets showing regenerating buddings (H&E x 125).

hyperplasia. There were great variabilities in size of the pancreatic islets, as compared to diabetic and normal groups. Some islets showed compensatory hypertrophy with hyper- cellularity and hyper-chromatic nuclei and with ill-defined borders and cellular budding which may be a sign of regeneration as shown in (figure 4).

The exocrine pancreatic tissue was also nearly normal. **Ki 67** immune staining showed high proliferative activity as shown by brown coloration of the proliferative nuclei (figure 5).

Morphometric analysis showed that:

1. The mean number of pancreatic islets was 4.05± 0.23

2. The mean average of pancreatic islets area was 177.4 ?m ±21.6.

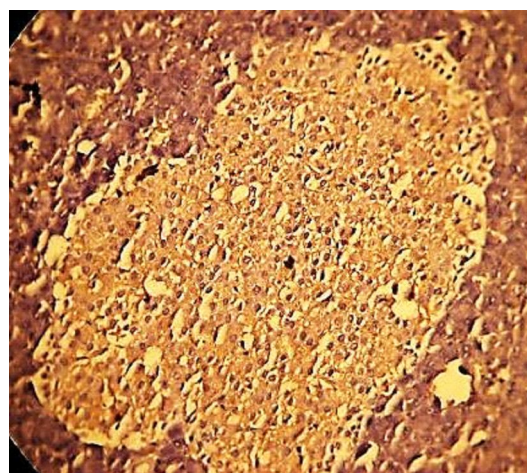**Figure (5):** EPO treated diabetic case showing high proliferative activity of cells in pancreatic islet by Ki 67 immunostaining (X400).

DISCUSSION

Diabetes mellitus is possibly the world's fastest growing metabolic disorder. It is one of the most important health problems worldwide, indicating high prevalence and mortality *(Hassan*

and Emam, 2012). One of the determinants in the development of diabetes is inadequate mass of the beta cells of islet of the pancreas either absolute, or relative with declining in beta cell functions *(Triplitt, 2007).*

The body weight is a sensitive indicator that reflects the state of health of experimental animals and decrease in body weight correlates with defects in body metabolism that is due to toxicity (*Bhatia and Khera, 2013)*. The present study showed significant decrease in the mean body weight after injection of alloxan monohydrate. These results were in agreement with *Hassan & Emam (2012) and Ojo et al. (2012)* who found that alloxan injection to normal rats induced marked reduction in body weight and contributed this reduction to hyperglycemia. Alloxan causes alkylation of DNA which produces hyperglycemia and necrotic lesions. This increase in the blood sugar results in lack of sugar in the cells, forcing the cells to use amino acids and fatty acids as a source of energy which eventually leads to the reduction of proteins and fats in the body which causes body weight loss. *Al-Attar and Zari (2010)* reported that, in diabetes mellitus, deranged glucagon-mediated regulation of cyclic adenosine monophosphate (AMP) formation in insulin deficiency leads to accelerated proteolysis. Since structural and tissue proteins contribute to 30 to 40% of total body weight, the excessive breakdown of tissue proteins due to diminished insulin response as well as the unavailability of carbohydrate for energy metabolism in diabetes mellitus results in decreased body weight.

The present study showed significant reduction of food intake after alloxan injection. This significant decrease was due to the specific necrosis of the pancreatic islets in alloxan-induced diabetic rats which affects the metabolism of glucose in the rats *(Adeyi et al., 2012)*.

In the present study, injection of recombinant human erythropoietin (rHuEPO) to erythropoietin-treated rats, erythropoietin-pretreated and treated diabetic rats showed significant decrease in the mean body weight and significant increase in amount of food intake. These results were in line with *Katz et al. (2010)*.

Our present study also showed that the administration of rHuEPO either before alloxan injection or after its injection showed significant increase in the mean body weight. *Gulam et al. (2011)* explained this improvement in the body weight of the erythropoietin-treated diabetic animals to the better glycemic control as a result of the restoration of normal metabolism in muscle cells.

Recombinant human erythropoietin (rHuEPO) injected to erythropoietin-pretreated diabetic rats showed significant increase in amount of food intake, whereas its injection to erythropoietin-treated diabetic rats showed non significant decrease in amount of food intake when compared to diabetic rats. These findings may be explained by increased energy consumption or expenditure due to reduced blood glucose levels associated with EPO injection.

The present study showed that alloxan injection induced marked increase in blood glucose level. *Etuk & Muhammed (2010) and Adeyi et al. (2012)* attribute this increase in glucose levels to the diabetogenic action of alloxan which is mediated by reactive oxygen species, with a simultaneous massive increase in cytosolic calcium concentration, leading to a rapid destruction of pancreatic β-cells which reduces the synthesis and the release of insulin. Histopathological

findings of this study revealed marked reduction in the size of cellular components of pancreatic islets. There were also variable degrees of degeneration extending from vaculation to necrosis and appearance of apoptotic cells with eosinophilic cytoplasm and condensed nuclei. *Adeyemi et al. (2010),* found a significant reduction in the numerical density of islets (number of islet/pancreas), islet area, islet diameter, numerical density of β-cells, volume of islets, and volume of β-cells in the diabetic group of rats.

Recombinant human erythropoietin (rHuEPO) in erythropoietin- pretreated diabetic group and in erythropoietin-treated diabetic group produced a significant decrease in serum glucose level. *Montel-Hagen et al. (2009)* reported lowered blood glucose associated with exposure to high EPO levels which may result from an increase in the erythrocyte counts and their consequent uptake of glucose. *Choi et al. (2010)* found decrease in blood glucose levels after the recombinant human erythro-poietin (rHuEPO) treatment to the beneficial effects of rHuEPO on the β-cells. On the other hand, *Katz et al. (2010)* explained the decrease in blood glucose level after erythropoietin injection to increasing sensitivity to insulin.

These data were supported by the histopathological results of our study which showed that the recombinant human erythropoietin (rHuEPO) treatment led to increase number of pancreatic islets due to hyperplasia. Some islets also showed compensatory hypertrophy with hypercellularty and increased vasculature with branched capillaries. There was an increase in Ki67-positive β-cells treatment with recombinant human erythropoietin (rHuEPO). Even in non-diabetic condition, which enhanced islet angiogenesis. rHuEPO treatment may have anti-apoptotic, proliferative, and angiogenic effects on the pancreatic islets (*Choi et al., 2010*) .

In the present study, we noticed elevated level of serum TNF-alpha and IL-6 of diabetic rats. These results were in agreement with *Pennathur and Heinecke (2007)* who demonstrated that diabetic rats had elevated blood levels of TNF-alpha and IL-6. *Mirza et al. (2012)* indicated that diabetes was strongly associated with elevated levels of IL-6 and TNF-α. The proinflammatory cytokines TNF-alpha and IL-6 play an important role in the pathogenesis of insulin-development diabetes mellitus *(Alexandrak et al., 2008)*, while TNF-alpha is also involved in promoting insulin resistance, development or progression of IDDM *(Shbaklo et al., 2003).*

Igarashi et al. (1999) and Yamakawa et al. (1999) found that high glucose has been shown to activate p38 MAPK (Mitogen Activated Protein kinase) which regulate the production of inflammatory cytokines such as TNF-alpha and IL-6. **Sridevi et al. (2005)** reported that, under high glucose, monocytes secrete increased amounts of IL-6 via upregulation of protein kinase (PKC-alpha- and B), P38 MAPK and nuclear factor ~ B (NF~B) activity leading to increased IL-6 transcription and release.

In the present study, it was noticed lowered level serum TNF-alpha and IL-6 after rHuEPO injection to erythropoietin-pretreated and erythropoietin-treated

diabetic rats. These results possibly may be attributed to anti-apoptotic and anti-inflammatory actions of erythropoietin (EPO) which was confirmed by the histopathological changes of pancreatic islets which showed cells with hyper-chromatic nuclei and cellular budding which is a sign of regeneration

Erythropoietin (EPO) mediates a number of additional effects related to the resolution of damage and recovery from injury *(Santhanam et al., 2008)*. Further, EPO also triggers production of tissue-specific growth factors *(Viviani et al., 2005)*. *Kilic et al. (2005)* suggested that EPO activates factors that inhibit apoptosis, decrease inflammation and increase angiogenesis.

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خلفيــة البحـث: يعد هرمـون الإريثروبيـوتين واحدا مـن أهم الهرمونـات التـي تفرز مـن قشـرة الكلي بالإضافة إلى خلايا الفيروبلاست الموجودة في الجزء العلوي من خلايا النخاع الكلوي نتيجة لتعرضها للحرمـان مـن الأكسـجين. ولهذا الهرمـون دور معروف فـي تكوين خلايا الـدم، وقد أثبتت الدراسـات الحديثة أن لهذا الهرمون العديد من الوظائف الأخرى.

الهدف من البحث: إختبـار تأثير إعطاء الإريثروبويتين علـى نماذج من النوع الأول من داء السكري بإستخدام الجرعة المناسبة من آلالوكسان حيث أنة يقوم بتدمير إنتقائي لخلايا البنكرياس (نوع بيتا).

مـواد وطرق البحث: تم تنفيذ الدراسـة الحاليـة علـى 50 من ذكور الفئران البيضـاء تم تقسيمهم إلى خمس مجموعات متساوية على النحو التالي :

المجموعة الأولى : (المجموعة الضابطة) : حيث تم حقن الفئران بمحلول ملحي

المجموعـة الثانيــة: (مجموعـة ضـابطة معالجـة بـالإريثروبويتين) : تـم حقن هذه المجموعـة بعقار الإريثروبويتين داخل الغشاء البريتونى .

المجموعة الثالثة (مجموعة السكري) : تم تعريض الفئران لمرض السكري عن طريق حقن الوكسان مونوهيدرات داخل الغشاء البريتونى.

المجموعة الرابعة: (مجموعة إرثروبويتين سابقة التجهيز): تم حقن الفئران في هذه المجموعة بعقار الإريثروبويتين داخل الغشـاء البريتونى ثم تعريض الفئران للإصابة بمرض السكري عن طريق حقن آلوكسان مونوهيدرات داخل الغشاء البريتونى.

المجموعة الخامسـة :(مجموعة معالجـة بالإريثروبويتين): تم تعريض الفئران لمرض السكري ثم بعد أسبوعين تم حقنهم بعقار الإريثروبويتين.

وفى نهايـة التجربـة تـم تحديـد كميـة الطعـام التي تناولهـا الفئـران و تغيـرات وزن الجسـم و مسـتويات الجلوكـوز في الـدم و مستوي عامـل نخـر الـورم ألفا، مستوي الإنترلوكين-6 بالإضـافة إلـي الفحـص المجهري لأنسجة البنكرياس.

النتائـج: تتلخص نتائـج البحث الحالي في أنه نقل مستويات كـلا من الجلوكوز و عامـل نخـر الورم ألفا، والإنترلوكين-6 في دم فئران مجموعتي الإيثروبيوتين سابقة التجهيز والمعالجة بالإريثروبيوتين بالمقارنة بمجموعـة السـكري. وقـد تأكـدت هـذه النتائـج بـالفحص النسـيجي لبنكريـاس الفئـران المصـابة بمـرض السكري والذي أظهر درجات متفاوتة من تنكس خلايا جزر البنكرياس.

الاستنتاج: العلاج بعقار الإريثروبيوتين أدى إلى تحسين التأثير المدمرالناجم عن حقن الالوكسان في نهاية التجربة.