Ameliorating Estimated Diabetic Stress Biomarkers Using Zinc or Chromium in Qassim Region

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Abstract— Among Saudi population, the role of zinc (Zn) and chromium (Cr) in diabetes complication is doubtful. Diabetes mellitus (DM) is closely associated with unbalanced trace elements and stress biomarkers that stem from uncontrolled hyperglycemia. The importance of these elements are less evident and subjected to long debate.

Self-administrated questionnaire of diabetic population in Qassim region - KSA was designed and the efficacy of Zn and Cr versus insulin as antioxidant and anti-stress in Alloxan (ALX)-induced diabetic rats was investigated. Sixty rats were divided into five groups: normal control, diabetic control by ALX and three treatment groups (ALX + Zn, ALX + Cr and ALX + Insulin). Cross-sectional survey revealed that 1160 subjects from Qassim region out of 1620 were suffered from DM (72.10%). Diabetic type 1 subjects were overall having higher prevalence of 52.86% compared to type11 subjects of 48.14%. The highest prevalence was observed in Buraidah city (44.25%). Alarming result of 85.11% of diabetic subjects that not used elements and vitamins. The prevalence of DM is shown to be increasing proportionally with age reaching 46.72% at age 30-50 years.

Serum levels of glucose, TGs and cholesterol and activity of ALT, AST, ALP were significantly increased in ALX group compared with control one. This was accompanied with reduction of C-peptide, insulin, antioxidant enzymes (SOD, GSH-PX, CAT) and anti-inflammatory cytokines (IL-4 and IL-5). The recorded drop in blood glucose levels for Zn, Cr and insulin treated groups were 10.195%, 9.06% and 55.86% respectively when compared to the initial level after diabetes induction. Treatments were significantly ameliorated serum liver enzymes, oxidative biomarkers and lipid profile. Inflammation induced by ALX was also mitigated via elevation of IL-4 and IL-5. Furthermore, insulin and C-peptide levels were alleviated and histopathological changes of treated rats were slightly to moderately recovered relative to the pancreatic sections from ALX group.

Diabetes is prevalent in study region, and this increase the need for urgent programs aiming at encouraging people to learn about stress biomarkers of diabetes and, to take actions to protect the health by element supplementation.

Key Words — *Questionnaire, Diabetes, Antioxidant, Zinc, Chromium, Qassim*

1 INTRODUCTION

Previous surveys have shown that, the prevalence of diabetes is increased strongly (30%) among the Saudi population and represents a major public health problem [1] [2]. The Kingdom of Saudi Arabia (KSA) is recently considered by the International Diabetes Federation to be among the top ten countries worldwide with high prevalence of diabetes in 2011 (16.2%) and 2030 (20.8%) [3]. According to the World health Organization, 22% (males) and 21.7% (females) of adults aged 25 and over in KSA had hyperglycemia [4]. Increasing evidence indicated a role for oxidative stress biomarkers in mediating diabetes-associated complications. Hyperglycemia induces the overproduction of oxygen free radicals and therefore increases the protein and lipid oxidation [5]. Islet β-cells are extremely affected by oxidative stress because of their reduced levels of endogenous antioxidants [6]. Oxidative stress biomarkers have been reported in the form of activities of antioxidants enzymes, such as superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) [7] and catalase (CAT) **[6].**

Diabetes is closely associated with unbalanced trace elements that stem from hyperglycemia. Among all the trace elements studied previously, zinc (Zn) and chromium (Cr) appear to be the most promising elements in the therapeutics of diabetes in particular diabetes-associated complications [8]. They are involved in insulin signal transduction, glucose metabolism, and cellular ant oxidative defense [9]. Cr is an essential nutrient and exerts anti-diabetic outcome through regulation of carbohydrate and lipid metabolism [10]. Insulin-like action of Zn has appeared both in-vitro and in-vivo by activating the insulin signaling cascade via Akt/ PKB, which indicated increase in cellular glucose uptake [11]. **[12] [13]** revealed that Zn ions have an important role in β-cell function, insulin effect, glucose homeostasis and the pathogenesis of diabetic complications.

Among Saudi population, the role of Zn and Cr in diabetes complication is doubtful. The role and importance of these elements are much less evident and subjected to long study. Therefore, the aim of the current study was to design self-administrated questionnaire of diabetic population of Qassim region and investigate the efficacy of Zn and Cr versus insulin as antioxidant and anti-stress in alloxan (ALX)-induced diabetic rats and to suggest their probable hypoglycemic mechanisms.

2 MATERIALS AND METHODS

2.1 Cross-sectional survey (https://www.surveymonkey.com/)

Self-administrated questionnaire was designed consisted of 13 questions regarding infection, locality, age, types of diabetes, response treatment and changing the dose and type of insulin. The using of Zn and Cr also included. Data were collected during a cross-sectional survey included 1620 Saudi selected from five different areas of Qassim Region.

2.2 Laboratory experiments

Sixty male Wistar rats weighing between 180-200 g were used in this study. The animals were kept at a temperature ranged 23±2 °C, relative humidity of 65–80% and a light exposure of 12 h light: 12 h dark (lights on at 6:00). The animals were quarantined and acclimatized for 7 days prior to the initiation of the study. The animals were kept in groups of 12 and were given well balanced pellet diet and tap water *ad libitum*. The experimental protocol was duly approved by the Animal Ethics Committee of the faculty and met the Guidelines for Care and Use of Animals in Scientific Research.

2.3 Materials

Zinc supplementation was given by drinking water at 5.0 mg ZnSO4/kg daily for 4 weeks. After completely drinking of $ZnSO_4$ solution, rats were provided free access tap water [14]. The basal diet contained Zn (30.0 mg/kg) as standard diet. Chromium (Sigma, St. Louis, MO, USA) was applied daily at dose 1 mg/kg body weight and given to rats by gavage [15]. The animals were fasted for 12 h prior to the induction of diabetes. Alloxan (ALX- Sigma Chemicals, USA) was administered intraperitoneally (i.p.) at single dose of 120 mg/kg body weight [16]. All rats were given 5% glucose during the following 24 h in drinking water. Induction of diabetes was confirmed by measuring blood glucose level 3 days after ALX administration. Rats with blood glucose level above 200 mg/dl were considered to be diabetic and used for the studies.

2.4 Experimental design

Rats were divided into five groups (n=12), as follow: (G1) normal control rats not receive any treatment. (G2): control diabetic rats, receive ALX only. (G3): diabetic rats receive zinc solution daily for 28 days. (G4): diabetic rats receive chromium solution daily for 28 days. (G5): diabetic rats receive insulin daily for 28 days.

2.5 Sampling and necropsy

Blood samples were collected from inner canthus of the eye under mild anesthesia every week. The collected sera were stored in deep-freezer (-20°C) until used. At the end of the experiment, grouped rats were humanely anesthetized by diethyl ether, euthanized and sacrificed according to the standard necropsy procedures [17]. For routine paraffin wax histopathological examination, pancreatic specimens were taken fixed in 10% formal saline, processed and finally stained with Hematoxylin and Eosin stain (H&E) as described by **[18].**

2.6 Biochemical estimation

Serum was used for the determination of glucose level using SPECTRUM kits. The activity of the liver functions was determined by AST, ALT, ALP and TG using Linear Chemicals. S.L. Kits. Cholesterol was evaluated by HUMAN kits. Interleukin-4 (IL-4) and Interleukin-5 (IL-5) were assayed by ELISA kit (Cusabio Biotech Co., Ltd. Lot: 004152648 and 004152649). The antioxidant activity was determined by measurement of GSH-PX, SOD and CAT (Biodiagnostic kits, Cat. No. 2578, 2563 & 2552, respectively). Quantitative determination of rat C-peptide and insulin levels in serum were done by ELISA Kits (SE120040-1KT. Lot No. CPT4779 & SE120069-1KT. Lot No. INS4565, Sigma Aldrich)**.**

2.7 Statistical analysis

Obtained data were statistically analyzed by SPSS 19 version for Windows. All data were recorded on an individual basis. Data were expressed as means ± SE**.**

3 RESULTS

3.1 Cross-sectional survey

One thousand and hundred sixty-eight subjects, out of 1620 were called to have DM (72.10%). Comparing different areas of Qassim region revealed the highest prevalence of DM is observed in the Buraidah city (44.25%), while the Al-Muznib area has the lowest prevalence of 9.83 %. Half number of subjects had type-1 diabetes with the treatment of insulin. Alarming result of 85.11% was the percent of diseased subjects that not used Zn, Cr, Se and vitamins. Diabetic type 1 subjects were overall having higher prevalence of 52.86% compared to type11 subjects of 48.14%.The prevalence of DM is shown to be increasing proportionally with age reaching 46.72% at age 30-50 years.

3.2 Laboratory experiments

The level of glucose was significantly increased in ALX group as compared to control one. While, levels were showed a significant decrease in zinc ($p<0.05$) and insulin ($p<0.001$) treated groups in comparison with ALX group (Table 2). The recorded drop in blood glucose levels for Zn, Cr and insulin treated groups were 10.195%, 9.06% and 55.86% respectively when compared to the initial level after diabetes induction. ALX group showed a significant higher cholesterol ($p<0.05$) and TGs ($p<0.01$) level as compared to the control one. The overall means of cholesterol ($p<0.01$) and TGs ($p<0.05$) were showed a significant drop in Zn and Cr treated groups in comparison with ALX group (Table 3). Serum activities of ALT, ALP ($p>0.05$) and AST ($p<0.05$) were elevated in ALX group as compared to control one of the present study (Table 4). The overall means of ALT and ALP were showed marked decrease in insulin treated groups in comparison with ALX untreated group ($p<0.05$). ALX group was recorded a significant ($p<0.05$) lower GSH-PX, SOD and CAT activities as compared to the control rats (Table 5). In contrast, the overall means of GSH -PX were showed a remarkable raise in Cr, Zn ($p>0.05$) and insulin ($p<0.05$) treated groups in comparison with ALX untreated group. In addition, the SOD and CAT were illus-

trated notable elevation in Cr ($p<0.01$& $p<0.05$), Zn and Insulin ($p>0.05$) treated group in comparison with ALX untreated group (Table 5). The analysis of IL-4 and IL-5 levels revealed a considerable fall ($p<0.05$) in ALX group as compared to control one. In contrast, the IL-4 (overall means) and IL-5 (at 28th day) were showed a significant increase ($p<0.05$) in Cr, Zn, and insulin treated groups in comparison with ALX untreated one (Table 6). In general, levels of insulin ($p<0.05$) and C-peptide ($p<0.01$) estimation revealed a significant decrease in ALX group as compared to control one (Table 7). In contrast, the overall means of insulin were showed increase in Zn, Cr (non-significant) and insulin ($p<0.05$) treated groups in comparison with ALX diabetic untreated group. Moreover, the overall means of C-peptide were showed a significant raise in Zn ($p<0.05$) and insulin ($p<0.001$) treated groups relative to ALX untreated one (Table 7).

The histopathological features of pancreatic sections from control rats were noted normal well-defined encapsulated Langerhans islets distributed within the acinar portion without any degenerative or necrotic changes (Fig.1 A). ALX group were showed sever pathological changes (pyknosis, karryorexis, karryolysis and vaculation- Fig.1B). While treated rats sections were noted a variable slight to moderate pathological response (Fig.1C, D, E) relative to the micro-image of pancreatic sections from ALX untreated group.

4 DISCUSSION

The data obtained from our survey are representative of a major health problem evolving in Qassim region, KSA. Clearly, a substantially higher prevalence of DM was observed as one thousand and hundred sixty-eight subjects, out of 1620 were called to have DM (72.10%). The data obtained from this community based study report an overall prevalence of DM was observed in the Buraidah city (44.25%), while the Al-Mozneb area has the lowest prevalence of 9.83 %. Alarming result of 85.11% was the percent of diseased subjects that not used Zn, Cr, Se and vitamins. More emphasis should be made on the role of primary healthcare by using Zn and Cr, in the management and promoting public awareness of DM. A large prospective trial is recommended to aware people to the importance of Zn and Cr in the ameliorating diabetes complication.

The blood glucose levels of the ALX group continued to increase significantly throughout the experimental period. ALX is selectively toxic to pancreatic β cells because it accumulates in it through uptake via the GLUT2 glucose transporter [19]. In contrast, the overall means of glucose were showed a significant decrease in Zn and insulin treated groups in comparison with ALX group. The recorded drop in blood glucose levels for Zn, Cr and insulin treated groups were 10.195%, 9.06% and 55.86% respectively when compared to the initial level after diabetes induction.

Zinc ions was previously showed to have insulin-like action both in-vitro and in-vivo by activating the insulin signaling cascade via Akt/ PKB, which indicated by increase in cellular glucose uptake [11]. **[12] [13]** revealed that Zn had important role in function of β-cell, insulin activity, glucose homeostasis and the pathogenesis of diabetic complications. A tendency to ameliorate blood glucose was observed when zinc ions were administered orally [20]**; [21].** Activity of these ions on insulin was strongly related to lipophilicity of the ligands, indicating that the ion has to reach intracellular space [22].

ALX - induced diabetic rats showed a significant higher cholesterol and TGs levels as compared to the control one. Diabetes is often linked with abnormal lipid metabolism and is considered as a major factor for the development of cardiovascular complication [23]. The overall means of cholesterol and TGs were showed a significant drop in Zn and Cr treated group in comparison with ALX untreated group. Generally, treatment with Cr significantly reduce TGs level in relation to ALX group. **[24]** demonstrates supplementation of Cr lowered total cholesterol and TGs levels in diabetic rats. This suggests that improvements in blood cholesterol and TGs could be due to reduced glucose levels in Cr supplemented diabetic rats.

Serum ALT, AST and ALP activities were found to be significantly increased in ALX group as compared to control one. The result give an indication on the hepatotoxic effect of ALX [25]. The results of **[26]** [27] revealed that changes in blood liver enzymes and the morphological and ultrastructural lesions found in the livers of animals were closely correlated to DM-induced stress in liver cells. This amelioration in hepatic function might be due to increased activity and mRNA levels of araginase as previously recorded by **[28].** Increase in the levels of ALP in diabetic rats was reported by **[29].** The overall means of ALT were showed a significant decrease in insulin treated groups in comparison with ALX untreated group. In addition, the ALP levels were found to be significantly decreased with the treatment with Cr, Zn and insulin relative to ALX group. Previous report suggested that Cr supplementation lower the blood levels of ALT and AST in diabetic rats [30].

ALX rats showed a significant lower GSH-PX, SOD and CAT activities as compared to the non-diabetic control rats throughout the experimental period. Islet β-cells are highly sensitive to oxidative stress because of their reduced levels of endogenous antioxidant enzymes [31]. Moreover, GPx activity is decreased in type 1 diabetes patients in experimentally induced diabetic rats, as well as patients [32]. Also, diabetes is associated with a decrease in SOD activity in most animal studies [33]**;[34].** Previously, abnormal catalase gene have been suggested to contribute to the increased risk of diabetes [35]. This was also associated with increased H_2O_2 levels and dysfunctional signaling of insulin receptor [36]. Treatment with Cr, Zn and insulin significantly increase GSH, and SOD content when compared to the ALX group. Also, the CAT activity changes in ALX diabetic rats treated with Zn, Cr and insulin were recorded in the current study.

The importance of anti-inflammatory cytokines in protecting β-cells is still under question although there is evidence that the production of these cytokines may be reduced in type 1 diabetes. The analysis of IL-4 and IL-5 levels revealed a significant decrease in ALX group as compared to control one. The treatment with Cr, Zn, and insulin showed a significant elevation in IL-4 levels whereas, IL-5 levels were found to be significantly increase at 28th day in treated groups in comparison with ALX untreated one. Th2 cells (including IL-4, and IL-5) was protect rodents against diabetes progression. Hence,

several studies have revealed that treatment of mice model of type 1diabetes with IL-4 delays the onset and reduces the incidence of spontaneous diabetes [37]. Pancreatic expression of IL-4, moreover, completely prevents diabetes in mice [38].

[39] reported that measurement of serum C-peptide provides an accurate marker of residual β-cell function and insulin secretion in patients with DM. Insulin and C-Peptide levels revealed a significant decrease in ALX group as compared to control one throughout experimental period. The data enforces the previous study of **[40]** who reported that diabetic rats showed significant reduction in the levels of C-Peptide and insulin. This might be due to the destruction of the pancreatic -cells and thereby induces hyperglycemia. **[41] ; [42]** indicated that a single dose of ALX to adult male albino rats was suitable to induce hypoinsulinemia state. On the other hand, the treatment with Zn and insulin showed a significant elevation in insulin and C-peptide concentration when compared to the ALX untreated rats at the same day of the current study. This data are in agreement with previous work of [11]. who reported that zinc ions activate the insulin signaling cascade and increase cellular glucose uptake. Also, **[12] [13]** revealed that Zn plays an important role in β-cell function, insulin activity, glucose transport and the pathogenesis of diabetic complications.

The histopathological features of diabetic rat sections were showed sever pathological changes, the results agree with **[41] ; [42]** who indicated that a single dose of ALX to adult male albino rats was suitable to induce histological changes of the islets of Langerhans characterized appearance. While treated diabetic rats sections were noted a variable slight to moderate pathological response relative of pancreatic features of control rats. **[43]** concluded that, zinc sulfate as a therapeutic agent has a protective effect on the pancreatic cells of diabetic rats.

CONCLUSION The findings of the current study show that Zn and Cr treatments demonstrate a protective effect in the ALX model of diabetes by modulation of oxidative and stress biomarkers. Diabetes is prevalent in study region, and this increase the need for urgent programs aiming at encouraging people to learn about stress biomarkers of diabetes and, to take actions to protect the health by element supplementation. This supplementation may help to control diabetes and prevent diabetes-related oxidative injuries, but require further study.

5 REFERENCES

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Table 1: Cross-sectional survey of the study population by diabetes in Qassim region.

Q	Items	%	No.	Q	Items	%	No.
(Q1) Infection	Infected	72.10	1168	(Q7) Response of treatment	Yes	72.5	328
	Non infected	27.90	452		No	27.5	90
	Total		1620		Total		328
(Q2) Locality	Buraidah	44.25	517				
	Unaizah	16.40	191	(Q8) Changing the dose at each visit to the doctor	Yes	70.36	231
	Al-Muznib	9.83	115		no	29.64	97
	Al-Bukairyah	13.94	163		Total		328
	Ar Rass	15.57	182	(Q9) Modulation of dose	Increase	63.94	148
	Total		1168		Non increased	36.06	83
(Q3) Age	>10	0.81	10		Total		231
	10-30	20.50	240	(Q10) Changing the type of insulin	Yes	30.32	70
	30-50	46.72	545		No	69.68	161
	≤50	31.96	373		Total		231
	Total		1168				
(Q4) Type	Type-1	52.86	617	(Q11) Using Zn, Cr, Se and vitamins	Yes	14.89	92
	Type-2	47.14	551		No	85.11	525
	Total		1168		Total		617
(Q5) Type of treatment	Insulin	53.27	328	(Q12) Complications	Heart problems	22.95	269
	Diet	14.75	92		Kidney problems	26.22	306
	Other	31.97	197		Others	50.81	593
	Total		617		Total		1168
(Q6) Kind of treatment	Injection	79.13	260	(Q13) Multiplication after diabetes	Yes	58.98	689
	Tablets	18.12	59		No	41.01	479
	Other	2.75	9		Total		1168
	Total		328				

Table 2: Table 2: Glucose changes (mg/dl and %) in ALX group treated with Zn, Cr and insulin.

Variables/ Grouping	Day 3	Day 7	Day 14	Day 21	Day 28	Changes in level (mg/dl)	Changes in level %
Control	118.47 ±7.82	123.48 ±12.82	112.13 ±6.45	127.38 ±9.08	111.13 ±8.40	-11.35 ±2.04	10.00 ±1.04
ALX	358.28 ±10.285	354.92 ±12.36[c]	387.54 ±17.13[c]	418.43 ±11.30[c]	365.64 ±10.65[c]	+19.62 ±2.24	5.05 ±0.54
ALX +Cr	344.65 ±12.665	288.19 ±13.10	316.56 ±22.22	366.84 ±16.23	306.82 ±14.63	-36.37 ±2.33	10.20 ±1.26
ALX +Zn	319.73 ±11.150	285.52 ±12.19*	365.32 ±14.28	303.25 ±13.86	287.43 ±18.88	-27.1 ±2.48	9.06 ±1.43
ALX+ Insulin	348.74 ±10.56	189.65 ±9.11*	149.86 ±10.31***	166.81 ±8.68***	174.89 ±9.16***	-196.76 ±6.80	55.86 ±2.88

Mean ± standard error (SE)

(a, b,c) Values of the diabetic groups were differs significantly from the value of control group within the same day at P<0.05,P<0.01 and P<0.001 respectively.

(*) (**)(***)Values of the treated groups were differs significantly from the value of diabetic group within the same day at P- <0.05 , P < 0.01 and P < 0.001 respectively

Table 3: Cholesterol (mg/dl) and TGs (mg/dl) changes in ALX group treated with Zn, Cr and insulin.

Variables/ Grouping		Day 7	Day 14	Day 21	Day 28	Overall mean
Cholesterol	Control	116.82±7.07	120.91±4.30	126.82±8.16	111.10±5.23	119.14±6.19
	ALX	118.96±11.21	128.46±4.53	113.20±9.46	142.62±7.12[c]	125.81±8.08
	ALX+Cr	127.27±8.70	106.09±6.49	127.01±4.29	112.73±7.89	118.28±6.84
	ALX+Zn	85.92±8.37	78.57±6.21*	67.34±9.63**	52.61±5.67***	71.12±7.47**
	ALX +Insulin	96.36±8.47	94.92±8.28	110.33±7.87	92.93±9.83	98.63±8.61
TGs	Control	67.12±7.10	64.58±3.52	60.80±4.28	69.32±4.86	65.45±4.94
	ALX	87.21±9.12	71.78±4.45	74.95±3.83	86.22[a]±5.47	80.04[a]±4.12
	ALX+Cr	82.94±7.07	73.29±8.16	68.18±8.11	63.23±3.45*	71.91±9.20
	ALX+Zn	71.06±4.06	61.02±5.36	55.38±7.62*	84.30±7.00	67.94±5.76*
	ALX +Insulin	65.31±8.83	88.55±6.85	76.15±10.49	96.31±10.81	81.58±8.25

Mean ± standard error (SE)

(a, b,c) Values of the diabetic groups were differs significantly from the value of control group within the same day at P<0.05,P<0.01 and P<0.001 respectively.

(*) (**)(***)Values of the treated groups were differs significantly from the value of diabetic group within the same day at P- <0.05 , P < 0.01 and P < 0.001 respectively

Table 4: ALT (U/dl), AST (U/dl) and ALP (U/dl) activity changes in ALX group treated with Zn, Cr and insulin.

Variables/ Grouping		Day 7	Day 14	Day 21	Day 28	Overall mean
ALT	Control	37.4±6.67	35.68±6.27	31.33±5.90	39.94±4.34	36.10±5.79
	ALX	39.53±4.12	47.18±4.41	51.81±5.78 [b]	41.75±2.33	45.07±4.16
	ALX+Cr	43.88±5.22	38.79±2.10	38.71±3.67	37.77±8.32	39.79±4.83
	ALX+Zn	35.51±3.60	43.70±3.49	42.26±5.59	40.57±6.73	40.51±4.85
	ALX +Insulin	30.96±3.30	38.24±4.30	33.56±4.82*	31.87±3.51*	33.66±3. 09*
AST	Control	26.75±2.71	23.89±2.14	26.50±1.20	21.25±2.04	24.60±2.02
	ALX	32.02±4.70	24.04±2.35	38.90[b]±3.18	36.40[c]±3.81	32.84[a]±3.11
	ALX+Cr	28.13±2.79	33.28±3.73	32.97±3.58	28.74±4.90	30.78±3.75
	ALX+Zn	37.30±2.29	24.05±3.83	38.21±3.95	28.80±3.51	32.09±3.40
	ALX +Insulin	28.54±1.01	33.59±2.40	34.05±1.54	26.88±1.65*	30.77±2.40
ALP	Control	54.98±8.61	60.30±8.67	64.10±8.33	61.90±7.32	60.54±8.23
	ALX	64.23±6.31	58.69±9.89	81.18[a]±4.08	79.61[a]±6.03	70.93±6.58
	ALX+Cr	55.17±9.26	58.03±10.03	60.15±10.23*	59.52±8.34*	58.22±9.47
	ALX+Zn	63.45±9.56	66.69±7.34	60.12±10.39	60.04±4.57*	62.57±7.96
	ALX +Insulin	63.47±6.61	54.18±5.36	59.37±8.44**	45.79±7.84**	55.70±7.06*

Table 5: Glutathione peroxidase (GSH -PX -U/ml), superoxide dismutase (SOD- U/ml) and catalase (CAT - U/ml) activity changes in ALX group treated with Zn, Cr and insulin.

Variables/ Grouping		Day 7	Day 14	Day 21	Day 28	Overall mean
GSH - PX	Control	6.01±0.38	8.93±1.15	7.07±2.32	9.88±1.23	7.97±0.67
	ALX	5.56±0.91	5.00±1.21	4.31±1.71[a]	5.07±2.41[a]	4.98±0.96 [a]
	ALX+Cr	5.49±0.95	5.46±1.19	7.06±1.24*	7.34±2.05	6.34±1.36
	ALX+Zn	5.39±0.95	6.26±1.16	6.78±1.43	8.98±1.83*	6.85±1.34
	ALX + Insulin	5.68±1.03	6.16±1.09	7.41±1.24*	8.48±1.45*	7.98±1.16*
SOD	Control	127.54±15.27	126.39±13.26	126.41±15.97	122.32±14.44	125.66±14.73
	ALX	75.72±12.48 [a]	88.72[a]±14.81	101.04±14.82	83.37±8.45 [a]	87.21[a]±7.64
	ALX+Cr	137.28±14.41*	179.46±11.72**	168.77±14.14*	112.14±14.87	149.41±13.79**
	ALX+Zn	128.42±14.50	126.26±9.50	119.50±8.32	133.75±13.38*	126.98±11.42
	ALX + Insulin	118.36±13.21	106.59±9.66	117.63±11.31	102.18±15.93	117.41±13.79
CAT	Control	117.40±10.10	126.11±9.23	122.71±8.48	127.46±8.83	123.42±7.16
	ALX	91.94±13.92	95.17±13.11	92.41±11.24	104.71±12.23	96.06[a]±6.62
	ALX+Cr	121.09±10.63	120.41±9.92	118.84±9.69	129.59±7.63	122.48±9.47*
	ALX+Zn	119.28±12.59	119.17±11.87	118.43±8.73	119.85±9.95	119.18±10.79
	ALX + Insulin	93.98±5.26	98.25±9.49	95.99±9.58	99.50±7.02	96.93±5.84

Mean ± standard error (SE)

(a, b,c) Values of the diabetic groups were differs significantly from the value of control group within the same day at P<0.05,P<0.01 and P<0.001 respectively.

(*) (**)(***)Values of the treated groups were differs significantly from the value of diabetic group within the same day at P- <0.05 , P < 0.01 and P < 0.001 respectively

Table 6: Interleukin-4 (IL-4 pg/ml) and interleukin-5 (IL-5 pg/ml) changes in ALX group treated with Zn, Cr and insulin.

Variables/ Grouping		Day 7	Day 14	Day 21	Day 28	Overall mean
IL-4	Control	16.73±0.53	15.27±0.32	15.36±0.33	16.81±0.48	16.04±0.42
	ALX	13.15±0.61 [b]	14.17±0.55	14.37±0.46	14.17±0.54 [a]	13.96±0.54 [a]
	ALX+Cr	16.31±0.47*	15.03±0.44	16.89±0.50*	15.82±0.25	16.01±0.41*
	ALX+Zn	17.33±0.25**	16.79±0.60*	16.61±0.39	15.71±0.64	16.61±0.47*
	ALX + Insulin	14.38±0.80	16.98±0.38*	14.40±0.57	16.08±0.52	15.46±0.57*
IL-5	Control	16.73±0.53	14.92±0.34	14.48±0.23	14.46±0.23	15.15±0.33
	ALX	13.12±0.62 [a]	13.45±0.61	14.47±0.29	12.37±0.58 [a]	13.16±0.32 [a]
	ALX+Cr	16.09±0.456*	14.13±0.28	13.84±0.285	14.55±0.29*	14.65±0.32
	ALX+Zn	14.39±0.29	14.26±0.19	14.28±0.25	14.45±0.35*	14.35±0.27
	ALX + Insulin	15.17±0.31*	14.00±0.24	14.39±0.39	14.79±0.30*	14.59±0.21*

Mean ± standard error (SE)

(a, b,c) Values of the diabetic groups were differs significantly from the value of control group within the same day at P<0.05,P<0.01 and P<0.001 respectively.

(*) (**)(***)Values of the treated groups were differs significantly from the value of diabetic group within the same day at P- <0.05 , P < 0.01 and P < 0.001 respectively

Table 7: Insulin (uU/ml) and C-peptide (ng/ml) changes in ALX group treated with Zn, Cr and insulin.

Variables/ Grouping		Day 7	Day 14	Day 21	Day 28	Overall mean
Insulin	Control	16.34±1.32	13.67±0.84	16.47±1.04	14.25±1.04	14.57±2.01
	ALX	11.46±1.72 [a]	9.05±1.254 [a]	8.87±0.97 [c]	8.33±1.01 [b]	9.83±1.16 [a]
	ALX+Cr	8.13±1.04	9.28±1.11	11.08±0.94	12.34±1.54	11.32±1.28
	ALX+Zn	11.32±1.11	9.65±2.05	13.54*±1.52	11.44±1.23	11.34±1.05
	ALX + Insulin	13.65±1.25*	14.51±2.39*	14.53±1.53*	16.34±1.61**	14.40±2.34*
C-peptide	Control	6.15±0.43	7.26±0.60	6.24±0.45	6.31±0.71	6.43±0.63
	ALX	2.15±0.27 [c]	2.46±0.19 [c]	2.24±0. 03 [c]	2.35±0.37 [c]	2.34±0.24 [c]
	ALX+Cr	2.08±0.11	2.06±0.11	3.74±0.22	2.34±0.12	2.32±0.29
	ALX+Zn	11.23±2.11	12.21±1.12	14.87±1.95*	13.77±2.34*	13.09±1.10*
	ALX + Insulin	3.18±0.65	5.15±0.65**	6.05±0.61***	5.65±0.54***	6.36±0.87***

Mean ± standard error (SE)

(a, b,c) Values of the diabetic groups were differs significantly from the value of control group within the same day at P<0.05,P<0.01 and P<0.001 respectively.

(*) (**)(***)Values of the treated groups were differs significantly from the value of diabetic group within the same day at P- <0.05 , P < 0.01 and P < 0.001 respectively

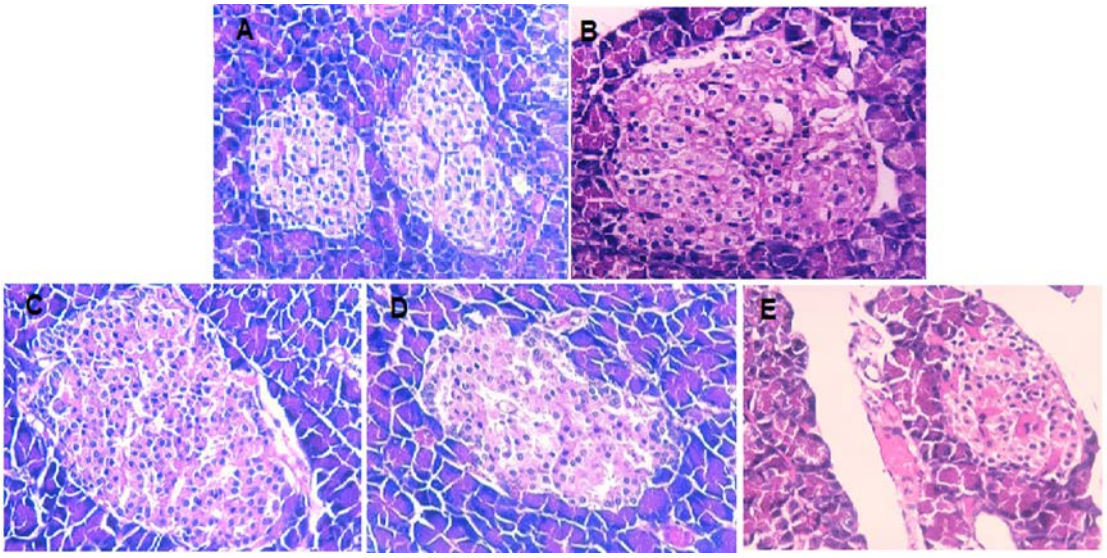

Fig. (1): Histopathology of pancreatic sections (H&E.400X Magn.).

A- control rats noted normal well defined encapsulated Langerhans islets distributed within the acinar portion

B- ALX- diabetic rat section showed sever pathological changes (Pyknosis and vaculation)

C- D-E: ALX-plus treated diabetic rat section (Zn, Cr and insulin) were noted a variable slight to moderate pathological response.