*Available online*www.ijpras.com

International Journal of Pharmaceutical Research & Allied Sciences, 2017, 6(2):61-69

Research Article

ISSN : 2277-3657
CODEN(USA) : IJPRPM

Attenuating impacts of chromium and nano resveratrol against hyperglycemia induced oxidative stress in diabetic rats

***Widad M. Al-Bishri*[1*]**

[1]*Biochemistry Department, Faculty of Science-Al Faisaliah, King Abdulaziz University, Jeddah, KSA*
[*]*Corresponding authors: Widad M. Al-Bishri*
Email: walbishry@kau.edu.sa

ABSTRACT

Oxidative stress is a deleterious impact, plays a key role in diabetic complications. The current work was designed to assess the hypoglycemic action of either nanoresveratrol (nano- Resv) or chromium picolinate (CrPic) and their effects in attenuating hyperglycemic stress induced in diabetic rats. Induction of diabetes in rats was performed by injection of intraperitoneal single dose of streptozotocin (STZ) (40mg/Kg body weight). Nano- Resv (20mg/Kg b.w.) or CrPic (80µg/Kg b.w.) were administered orally to diabetic rats for thirty successive days. The results demonstrated that both nano-Resv and CrPic markedly down regulated the serum glucose content and increased the level of serum insulin in diabetic animals. The study also showed that treatment by both agents, significantly modulated the increase in the serum oxidative stress biomarker, nitric oxide (NO), and the decreases in the serum antioxidant markers, including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione S transferase (GST) and reduced glutathione GSH). Conclusion: The current study proved that both nano- Resv and CrPic have a potential impact in modulating hyperglycemia-induced oxidative stress and may candidate as useful drugs in controlling diabetes related oxidative stress.

Key words: *oxidative stress, type 2 diabetes, nanoresveratrol, insulin, antioxidant*

INTRODUCTION

Diabetes mellitus is a systemic disorder in metabolism and one of the diseases that lead to morbidity and mortality. The disease affects carbohydrate metabolism and is characterized by either a lack of insulin production or insensitivity of receptors to insulin, resulting in hyperglycemia. In 2010, approximately 230 million people had diabetes all over the world and the number of diabetic patients is expected to be elevated in 2025 to 333 million **(1)**.

Hyperglycemia, resulting from alteration of blood glucose level, is well known as the principle cause of complications related to diabetes **(2)**. High glucose level was reported to induce tissue damage through nonenzymatic interaction between diabetic glucose and different proteins to produce advanced glycosylated end product (AGE) **(3)**. Generation of AGE impairs cellular functions by affecting the function of proteins or by generation of oxygen radicals (**4)**. Diabetic oxidative stress, resulting from the overproduction of oxidative radicals and lipid peroxidation parallel with decreases in the capacity of neutralizing defense systems, plays a fundamental role in diabetic deleterious impacts (**4).**

Although the current available therapies provide successful glycemic management, but do a little in preventing diabetic complications. In addition, the treatment with these agents are associated with dangerous effects **(5).** There

are some investigations have been addressed the beneficial role of micronutrients, such as polyphenolic compounds and chromium, in the management of diabetes.

Resveratrol (3,5,4'-trihydroxy-trans-stilbene), is a natural plant polyphenolic compound, found in many plants such as peanuts, red grapes, root of *Polygonum cuspidatum* and berries **(6).** This compound has many therapeutic benefits including anti-inflammatory, antioxidant, antiaging, anti-carcinogenic, anti-platelet aggregation, cardio-protective, neuro-protective, cartilage-protective and insulin sensitizer **(7).** However, pharmacokinetic properties of resveratrol demonstrated that it has rapid degradation, poor solubility and extensive metabolism, resulting in poor oral bioavailability **(8).** So using resveratrol with nano scale is a new strategy to enhance the therapeutic potential ability of this compound **(9).** It has been reported that the concentration of nanoresveratrol ((nano- Resv) in different tissues (kidney, brain and liver) of experimental animals is higher by more than two-folds than free resveratrol (**10).** In addition, nano-Resv has been reported to have higher safety in gastrointestinal tract than free resveratrol (**10).** In Comparison to free resveratrol, nano- Resv can suppress reactive species generation and increase antioxidant defense capacity in experimental animal model **(11).** Lee et al. **(11**) reported that nano- Resv is more effective than free resveratrol in reducing oxidative stress and inflammatory cytokine generation in rat livers intoxicated with CCl4.

Chromium is an essential trace element for human nutrition. This micronutrient has a fundamental impact on glucose metabolism and insulin function **(12).** Cr ion is an essential constituent of glucose tolerance factor, that has an important role in lowering blood glucose levels in experimental animals (**13).** Cr also is a part of low molecular weight chromium binding substance (LMWCr, known as chromodulin), which has an activating effect on the insulin signal through interacting with insulin-activated insulin receptors, causing promotion of tyrosine kinase activity, and hence , increasing glucose uptake by muscle and adipose cells and convert it into triglycerides (**14).** Some authors reported that supplementation with Cr could improve glucose level in subjects consuming diets with low-chromium (**15).** Very small amounts from Cr are required to maintain glucose homeostasis in vivo **(16).** Cr supplementation in diabetic patients can modulate both insulin and glucose metabolism **(14)**.

The present study is designed to assess the hypoglycemic action of either nano- Resv or CrPic or their possible therapeutic beneficial impacts in neutralizing oxidative stress in diabetic rats as a consequence of diabetic complications.

MATERIAL AND METHODS

Chemicals

Nano-Resv was purchased as nanoemulsion (< 100nm) from Life Enhancement, Petaluma, California, USA. CrPic was obtained from General Nutrition Centers (GNC). STZ was bought from Sigma Company (St. Louis, MO, USA). Kits used for the estimation of some biomarkers were obtained from Biogamma, Stanbio, West Germany.

Animals

Forty male Wistar albino rats (160-200 g) were utilizing for this study. The rats were purchased from Animal Care Center, King Abdulaziz University. The rats were lived under standard conditions (12 h light/12 h dark cycle, at 20–22 °C and 50% humidity) in special cages. The animals were provided with rat chow with standard constituents and free access to faucet water for seven days for acclimatization. Animal handling was carried out according to the guidelines obtained from the Experimental Animal Laboratory and approved by the Animal Care and Use Committee of the King Abdulaziz University, Faculty of Science.

Induction of Type 2 diabetes

For induction of diabetes (type 2), thirty rats were selected randomly and each one was administered with a single intraperitoneal dose (40mg/Kg b.w.) of streptozotocin (STZ, Sigma, USA) **(17).** STZ was dissolved in a citrate buffer (50 mM , pH 4.5) before administration and a similar amount of vehicle (0.90% NaCl) was injected in the intact control. After STZ injection, animals were provided with 5% glucose solution to drink overnight to overcome

hypoglycemia induced by the drug **(18).** After ten days, hyperglycemic rats (blood glucose level more than 250 mg/dl) were chosen as diabetic **(19).**

Experimental design

The rats were divided into four groups, each of 10 rats:

Group 1: non-diabetic control rats

Group2: diabetic rats

Group 3 : diabetic rats treated with nano- Resv (20 mg/kg b. w.) **(20).**

Group 4: diabetic rats treated with CrPic (80µg/Kg b. w.) **(21)**

Nano- Resv and CrPic were administered orally to rats for 30 successive days commenced 2 weeks post induction of diabetes. Nano- Resv was found as nanoemulsion (< 100nm) and diluted by olive oil before administration. CrPic was suspended in Tween 80 (1%) before administration. 30 days after administration of the two agents, the rats were fasted (12-14 hours) overnight, the blood samples were gathered from the experimental rat groups into clean tubes for clotting and separation of serum. Different biomarkers were measured in serum.

Biochemical serum analysis

Estimation of glycemic markers

Serum fasting glucose level (biomarker of hyperglycemia) was estimated using an automatic biochemical analyzer (ci16200, Abbott, USA). Insulin level was measured by utilizing rat insulin direct sandwich enzyme linked immunosorbent assay (ELISA) kit

Estimation of NO in serum

NO concentration was determined using Griess reagent (N-1-naphthylethylenediamine dihydrochloride and sulfanilamide) in an acidic medium **(22).**

Estimation of antioxidant biomarkers in serum

The reduced form of glutathione (GSH) was assessed **(23)** based on its interaction with 5,5'-dithiobis (2-nitrobenzoic acid) to produce 5-thio-2-nitrobenzoic acid with a yellow color. Catalase (CAT) was assessed spectrophotometrically by monitoring the decomposition rate of H_2O_2 at 240 nm **(24).** SOD was estimated by a spectrophotometer based on the inhibition of a superoxide-driven. The reaction was monitored by the repression in the rate of NADH oxidation at 340 nm (**25).** GPx activity was estimated according to Flohé and Günzler **(26).** Thiol groups (−SH) was measured according to Ellman's method **(27).** GST activity was assayed spectrophotometrically by measuring the conjugation rate between GSH and 1-Chloro-2,4-dinitrobenzene (CDNB) (**28).**

RESULTS

The effects of nano- Resv and CrPic on different biochemical markers are shown in Table 1. The results demonstrated that induction of diabetes in rats resulted in an obvious increase in the serum glucose content parallel with a reduction in the insulin concentration with respect to control rats ($P \leq 0.001$). Oral intake of either nano- Resv or CrPic to diabetic rats for thirty consecutive days, markedly ameliorated the alteration in these diabetic biomarkers. Treatment with CrPic was more effective in modulating the glucose and insulin levels compared with nano- Resv.

The data of the current work also showed a marked elevation in the serum oxidative stress biomarker, NO, in diabetic rats with respect to diabetic untreated ones. Ingestion of nano- Resv or CrPic to diabetic rats, significantly down modulated the increase in this biomarker. CrPic was more beneficial in reducing the level of this marker in

relation to nano- Resv ($P \leq 0.05$). In addition, the present results showed that reduction in the serum enzymatic antioxidant activities , including SOD, CAT GPX and GST, as well as in the level of non- enzymatic antioxidant, GSH, in diabetic rats in relation to control ones ($P \leq 0.001$). Supplementation of diabetic rats with either nano- Resv or CrPic, significantly up-regulated the deviations in these antioxidants to near normal levels.

Table 1: Effect of nano- Resv and CrPic on the serum levels of different biomarkers in diabetic groups

Parameters	Control	diabetes	Diab + nano-Resv	Diab + CrPic
Glucose (mg/dL)	85.45± 5.6	354.76±10.56	130.56±7.34	120.34±2.7
Insulin µU/ml	12.45±0.56	7.7±0.35	9.45±0.47	10.15±0.57
NO (µmole/L)	139.23±4.9	225.4±8.6 a	160.4±5.8b**$	143.9±2.9**
SOD (U/ dL)	40.6±1.9	27.5±2.3 a	35.4±1.6c*$	40.3±2.5**
CAT (U/ dL)	28.3±2.5	16.6±1.4 a	25.4±1.5**	25.9±2.4**
GPX (U/ dL)	36.12±3.2	25.8±3.4b	35.2±2.4*	34.4±1.5*
GST (U/ dL)	147.4±10.5	108.5±9.9a	140.9±9.3*	145.4±4.9*
GSH (nmole/ml)	115.6±4.9	84.7±4.1 a	108.4±2.5**	112.2±3.6**

Data are presented as mean ± S.D. from 10 rats, a $P \leq 0.001$, b $P \leq 0.01$, c $P \leq 0.05$ compared with the control group, * $P \leq 0.01$, ** $P \leq 0.001$ compared with diabetic group, \$$P \leq 0.01$ compared with CrPic group using ANOVA followed by Bonferroni as a post-ANOVA test

DISCUSSION

Oxidative stress is a key role in diabetic pathogenesis and its complications. Hyperglycemia promotes overproduction of reactive species with a concomitant depletion in antioxidant defense mechanism in diabetic subjects and experimental animals **(29) (30).** So, the strategy of treatment should focus on therapeutic agents with multidimensional impacts.

The current study demonstrated that oral ingestion of either nano- Resv or CrPic (20 mg/kg body weight and 80 µg/ Kg b. w. respectively) to diabetic rats for thirty consecutive days, significantly down-regulated the serum glucose level and up-regulated insulin concentration. These results indicate the beneficial hypoglycemic impact of these two agents. Similar results have been obtained by earlier studies. Some authors have documented the improving action of Resv on glycemic markers (glucose and insulin) in diabetic rats **(31).** Also, previous reports demonstrated that administration of Cr daily for thirty-two weeks at doses of 10 and 1 mg/kg to diabetic rats could improve the glucose tolerance and cellular insulin sensitivity (32) (30). A clinical study showed that Cr at doses up to 400 µg/kg, can modulate glycemic biomarkers, but these doses are not sufficient to normalize these biomarkers **(33).** Another clinical investigation revealed that a daily dose of Cr at 1 mg/kg body weight for 4 months had a marked impact in reducing glycated hemoglobin concentrations in type 2 diabetic patients (**34).** The hypoglycemic action of nono- Resv may be related its ability to increase the release of insulin by suppressing potassium channels in β cells of pancreas (**35).** Also some studies revealed that Resv could improve insulin sensitivity in obese animals (**36).** Resv can conjugate with the insulin receptor and induce phosphorylation of insulin receptor substrates (IRS -1 and IRS-2) which play an important role in transmitting signals from insulin to intracellular metabolic pathways, including phosphatidylinositol 3-kinase (PI3K). PI3K has a principle role in the function of insulin, through the activation of protein kinase B (PK-B) . PI3K and PK-B have an important role in translocation of glucose transporter 4 (GLUT4) **(37).** Some authors have confirmed that Resv administration to diabetic rats, increases the expression of insulin signaling cascade biomarkers in the liver through increasing IRS -1 and PI3K levels **(31).** Also, Cr has been reported to exert hypoglycemic effects through many mechanisms. It has

been shown that Cr has the ability to increase glucose uptake by adipose tissue through increasing GLUT4 trafficking at the plasma membrane **(38),** stimulate insulin binding, increase number of insulin receptor and β-cell sensitivity **(16)** and regulate glucose metabolism (**39).** It was found that alteration in glucose tolerance, hyperglycemia and glucosuria are contributed to lacking in Cr **(40).**

It is well known that diabetic hyperglycemia has a major role in the induction of oxidative stress. An imbalance between the antioxidant defense systems and the generation of reactive oxygen species in favor of oxygen species, contributes to diabetic pathogenesis **(41).** Data generated in the present investigation demonstrated augmentation of oxidative stress in diabetic rats with concomitant impairment in the antioxidant defense systems, as observed by a marked elevation in the oxidative stress biomarker, NO and reduction in the free-radical neutralizing antioxidants, including SOD, CAT, GPx, GST and GSH. Increased free radical generation and alterations in antioxidant defense systems in liver, kidney and other organs have been also documented in diabetes (42) (43) (44) (30). Also, a depletion in antioxidant capacity in the serum of diabetic patients has been investigated (**45).** Beside, some authors suggested that hyperglycemia increases free radical production and down-regulates gene expression of antioxidant enzymes **(45).**

The increase in NO and the decreases in the above mentioned antioxidants in diabetic rats may ascribe so that hyperglycemia can promote non-enzymatic protein glycation and glucose oxidation, leading to the generation of oxygen species which have a pivotal role in complications of diabetes **(46) (47).** NO generation exerts deleterious impacts on different body organs, causing tissue damage **(48).** This pathogenesis of NO is increased by interacting with superoxide radical forming peroxynitrite (ONOO̅), a powerful secondary toxic oxidizing agent, which can oxidize cellular structure, causing lipid peroxidation **(48),** the proximal cause of cellular membrane damage and cell death (**49).** The inhibition of reactive species neutralizing enzymes (SOD, CAT and GPX), harm the enzymatic defense against superoxide and hydrogen peroxide mediated tissue damage. SOD catalyzes the transformation of the superoxide (O2−) radical into either molecular oxygen (O2) or H_2O_2 **(50)**. CAT and GPx neutralize hydrogen peroxide, which has damaging impacts on lipids, RNA and DNA, into water and oxygen. GST catalyzes the conjugation of GSH to different electrophilic substances (such as carcinogens and environmental toxins) for the detoxification **(51).** The marked reduction in GST activity and GSH levels in diabetic rats may indicate the alteration of second line of antioxidant defense. This may cause tissue damage by influencing GSH functions, including scavenging of oxygen radicals, detoxification of xenobiotics and repair pathways. So, alterations in the levels of antioxidant during diabetes may make the cells of different tissues prone to oxidative stress and hence cell injury.

Supplementation of either nano- Resv or Cr Pic to diabetic rats, significantly modulated the alterations in the serum oxidative stress marker (NO) and antioxidant biomarkers. This result may give a clue to the beneficial antioxidant capabilities of the used agents. Similarly, previous studies have been reported that Resv has anti-oxidant action and can prevent oxidative stress in diabetic animals (**52) (31).** Also, some investigations reported that Resv could restore the glutathione system in cardiac of diabetic animals to normal levels **(53) (54)**. On the other hand, the same authors revealed that Resv administration inhibits NO production and iNOS protein expression in cultured rat astroglioma C6 cells treated with beta-amyloid as well as in osteoarthritic animals exposed to monosodium iodoacetate **(53) (54)**. A recent study has been documented the powerful activity of Resv in preventing protein glycation that has the major role in free radical generation **(55).** Also, previous investigations have been reported that Cr can attenuate diabetic oxidative stress and improve antioxidant capacity in cell culture and experimental animals **(56) (57) (30).** The current modulating effect of nano- Resv and Cr Pic on oxidative stress and antioxidant biomarkers may be related to their gluco-regulatory impact as documented in the present study.

CONCLUSION

The current investigation demonstrated that nano- Resv and CrPic therapy to diabetic rats have a potential glycemic control as well as they have beneficial impacts in attenuating hyperglycemia induced oxidative stress and alterations in antioxidant defense mechanism. These results may support the use of both agents in management of hyperglycemia induced oxidative stress in diabetes

REFERENCES

1- NCD Risk Factor Collaboration (NCD-RisC). Worldwide trends in diabetes since 1980: a pooled analysis of 751 population-based studies with 4.4 million participants. Lancet 2016; http://dx.doi.org/10.1016/S0140-6736(16)00618-8

2-Brownlee, M. (2001). Biochemistry and molecular cell biology of diabetic complications. Nature 414, 813-820.

3- Lapolla, A., Traldi, P., Fedele, C. (2005). Importance of measuring product of non-enzymatic glycation of proteins. Clinical Biochemistry 38:103-115.

4- Maritim, A.C., Sanders, R.A., Watkins, J.B. (2003). Diabetes, oxidative stress, and antioxidants: a review. Journal of Biochemical and Molecular Toxicology. 17 (1): 24–38.

5- Berger, S. (1985). Incidence of severe side effects during therapy with sulphonylurea and biguanides. Hormone and Metabolic Research 17:111-115.

6- Tomé-Carneiro, J., Larrosa, M., González-Sarrías, A., Tomás-Barberán, F.A., García-Conesa, M.T., Espín, J.C. (2013). Resveratrol and clinical trials: the crossroad fromin vitro studies to human evidence. Current Pharmaceutical Design 19 : 6064–6093

7- Delmas, D., Aires, V., Limagne, E., Dutartre, P., Mazue, F., Ghiringhelli, F., Latruffe, N. (2011). Transport, stability, and biological activity of resveratrol. Annals of the New York Academy of Sciences 1215:48–59.

8- Almeida, L., Vaz-da-Silva, M., Falcao, A., Soares, E., Costa, R., Loureiro, A.I., Fernandes-Lopes, C., Rocha, J.F., Nunes, T., Wright, L., et al (2009). Pharmacokinetic and safety profile of trans-resveratrol in a rising multiple-dose study in healthy volunteers. Molecular Nutrition & Food Research. 53: S7–S15.

9-Summerlin, N., Soo, E., Thakur, S., Qu, Z., Jambhrunkar, S., Popat, A. (2015). Resveratrol nanoformulations: Challenges and opportunities. International Journal of Pharmaceutics 479:282–290.

10- Frozza, R.L., Bernardi, A., Paese, K., Hoppe, J.B., da Silva, T., Battastini, A.M., et al (2010). Characterization of trans-resveratrol-loaded lipid-core nanocapsules and tissue distribution studies in rats. Journal of Biomedical Nanotechnology 6:694–703.

11- Lee ,C.W., Yen, F.L., Huang, H.W., Wu, T.H., Ko ,H.H., Tzeng, W.S., Lin, C.C. (2012). Resveratrol nanoparticle system improves dissolution properties and enhances the hepatoprotective effect of resveratrol through antioxidant and anti-inflammatory pathways. Journal of Agricultural and Food Chemistry 60:4662–71.

12- Vincent, J.B. (2010). Chromium: celebrating 50 years as an essential element? Dalton Transactions 39(16):3787–94.

13- Tuman, R.W., Doisy, R.J. (1977) Metabolic effects of the glucose tolerance factor (GTF) in normal and genetically diabetic mice. Diabetes 26:820e6.

14- Cefalu, W.T., Hu, F.B. (2004) Role of chromium in human health and in diabetes. Diabetes Care 27(11):2741–51.

15- Anderson, R.A., Polansky, M., Bryden, N., Canary, J. (1991). Supplemental chromium effects on glucose, insulin, glucagon, and urinary chromium losses in subjects consuming controlled low-chromium diets. American Journal of Clinical Nutrition 54:909–16.

16- Anderson, R.A. (2000). Chromium in the prevention and control of diabetes. Diabetes & Metabolism 26(1):22–7.

17- Milani, E., Nikfar, S., Khorasani, R., Zamani, M.J., Abdollahi, M. (2005). Reduction of diabetes-induced oxidative stress by phosphodiestrase inhibitors in rats. Comparative Biochemistry Physiology 140 C: 251-255.

18- Bhandari, U., Kanojia, R., Pillai, K.K. (2005). Effect of ethanolic extract of Zingiber officinale on dyslipidaemia in diabetic rats. Journal of Ethnopharmacology 97: 227-230.

19- Samarghandian, S., Hadjzadeh, M., Amin Nya, F., Davoodi, S. (2012). Antihyperglycemic and antihyperlipidemic effects of guar gum on streptozotocin-induced diabetes in male rats. Pharmacognosy Magazine 8: 65–72.

20- Singh, G., Pai, R.S., Pandit , V. (2014). In vivo pharmacokinetic applicability of a simple and validated HPLC method for orally administered trans-resveratrol loaded polymeric nanoparticles to rats. Journal of Pharmaceutical Investigation 44: 69-78

21- Al-Masri, S. A. (2013). Synergistic Effect of Cichorium and Chromium Supplementation on Diabetic Rats. Middle-East Journal of Scientific Research 13 (3): 347-353,

22- Moshage, H., Kok, B., Huizenga, J.R., Jansen, P.L. (1995). Nitrite and nitrate determination in plasma: a critical evaluation. Clinical Chemistry 41: 892-896.

23- Bentler, E., Duran, O., Mikus, K.B. (1963). Improved method for determination of blood glutathione. Journal of Laboratory and Clinical Medicine 61,882.

24- Aebi, H. (1984). Catalase in vitro. Methods in Enzymology 105: 121–6.

25- Paoletti, F., Aldinucci, D., Mocali, A., Caparrini, A. (1986). A sensitive spectrophotometric method for the determination of superoxide dismutase activity in tissue extracts. Analytical Biochemistry 154 : 536-541.

26- Flohé, L., Günzler, W.A. (1984). Assays of glutathione peroxidase. Methods in Enzymology. 105:114–21.

27- Ellman, G.L. (1959). Tissue sulfhydryl groups. Archives of Biochemistry and Biophysics 82:70–7.

28- Vontas, J, G, Enayati, A.A., Small, G.J., Hemingway, J. (2000). A Simple Biochemical Assay for Glutathione S-Transferase Activity and its possible field application for screening glutathione S-transferase-based Insecticide Resistance. Pesticide Biochemistry and Physiology 68: 184–192

29- Maxwell, S.R., Thomason, H., Sandler, D., Leguen,,C., Baxter, M.A., Thorpe, G.H., Jones, A.F., Barnett, A.H. (1997). Antioxidant status in patients with uncomplicated insulin-dependent and noninsulin-dependent diabetes mellitus. European Journal of Clinical Investigation 27:484–90.

30- Sundaram, B., Aggarwal, A., Sandhir, R. (2013). Chromium picolinate attenuates hyperglycemia-induced oxidative stress in streptozotocin-induced diabetic rats. Journal of Trace Elements in Medicine and Biology . 27(2):117-21.

31- Sadi, G., Pektaş, M.B., Koca, H.B., Tosun, M., Koca, T. (2015). Resveratrol improves hepatic insulin signaling and reduces the inflammatory response in streptozotocin-induced diabetes. Gene 570(2):213-20.

32- Abdourahman, A., Edwards, J.G. (2008). Chromium supplementation improves glucose tolerance in diabetic Goto-Kakizaki rats. IUBMB Life 60:541–8.

33- Anderson, R.A. (1998). Chromium, glucose intolerance and diabetes. Journal of the American College of Nutrition 17:548–55.

34- Anderson, R.A., Cheng, N., Bryden, N.A., Polansky, M.M., Chi, J., Feng, J. (1997). Elevated intakes of supplemental chromium improve glucose and insulin variables in individuals with type 2 diabetes. Diabetes 46:1786–91

35- Chen, W.P., Chi, T.C., Chuang, L.M., Su, M.J. (2007). Resveratrol enhances insulin secretion by blocking K(ATP) and K(V) channels of beta cells. European Journal of Pharmacology 568 : 269–277.

36- Hong, H.J., Kang,W., Kim, D.G., Lee, D.H., Lee, Y., Han, C.-H. (2014). Effects of resveratrol on the insulin signaling pathway of obese mice. Journal of Veterinary Science 15: 179–185.

37- Lizcano, J.M., Alessi, D.R. (2002). The insulin signalling pathway. Current Biology 12, R236–R238.

38- Chen ,G., Liu ,P., Pattar, G.R., Tackett, L., Bhonagiri, P., Strawbridge, A.B., Elmendorf, J.S. (2006). Chromium activates glucose transporter 4 trafficking and enhances insulin-stimulated glucose transport in 3T3-L1 adipocytes via a cholesterol-dependent mechanism. Molecular Endocrinology 20:857–70.

39- Sundaram, B., Singh, K., Sandhir, R. (2012). Ameliorating effect of chromium administration on hepatic glucose metabolism in streptozotocin-induced experimental diabetes. Biofactors 38:59–68.

40- De Flora, S. (2000). Thre shold mechanisms and site specificity in chromium (VI) carcinogenesis. Carcinogenesis 2000 21:533–41.

41- Jin, L., Xue, H.Y., Jin, L.J., Li, S.Y., Xu, Y.P. (2008). Antioxidant and pancreas-protective effect of aucubin on rats with streptozotocin-induced diabetes. European Journal of Pharmacology 582: 162–167.

42- Opara, E.C. (2002) Oxidative stress, micronutrients, diabetes mellitus and its complications. Journal of the Royal Society for the Promotion of Health 122:28–34.

43- Lee, Y.T., Hsu, C.C., Lin, M.H., Liu, K.S., Yin, M.C. (2005). Histidine and carnosine delay diabetic deterioration in mice and protect human low density lipoprotein against oxidation and glycation. European Journal of Pharmacology 513:145-150.

44- Liu, Z., Li, J., Zeng, Z., Liu, M., Wang, M. (2008). The antidiabetic effects of cysteinyl metformin, a newly synthesized agent, in alloxan- and streptozocin-induced diabetic rats. Chemico-Biological Interactions 173: 68–75.

45- Patel, H., Chen, J., Das, K.C., Kavdia, M. (2013). Hyperglycemia induces differential change in oxidative stress at gene expression and functional levels in HUVEC and HMVEC. Cardiovascular Diabetology 12 (1): 142–146.

46-Szkudelski, T. (2001) The mechanism of action of alloxan and streptozotocin in B cells of the rat pancreas. Physiological Research 50: 536–546.

47- Sudnikovich, E.J., Maksimchik, Y.Z., Zabrodskay, S.V., Kubyshin, V.L., Lapshin, E.A., Bryszewsk, M., Reiter, R.J., Zavodnik, I.B., Elena, J.U. Sudnikovich, E.J., Maksimchik, Y.Z., Zabrodskay, S.V., Kubyshin, V.L., Lapshin, E.A., Bryszewsk, M., Reiter, R.J., Zavodnik, I.B. (2007). Melatonin attenuates metabolic disorders due to streptozotocin-induced diabetes in rats. Eurpean Journal of Pharmacolology 569:180–187.

48- Mohamed, A.A., Abo-Amou, D.E., Shehata, M.A., El-Ashery, N.E. (2001). Glutathione peroxidase and nitric oxide in patients with chronic liver diseases. Egyptian Journal of Schistosomiasis and Infectious Endemic Diseases 23: 27-46.

49- Freeman, B.A., Crapo, J.D. (1982). Biology of disease: free radicals and tissue injury. Laboratory Investigation 47: 412-426.

50- Tiwari, B. K., Pandey, K.B., Abidi, A. B. (2013) Syed Ibrahim Rizvi SI 2013. Markers of oxidative stress during diabetes mellitus. Journal of Biomarkers ID 378790: 1-8.

51- Hayes, J.D., Flanagan, J.U., Jowsey, I.R. (2005) Glutathione transferases. Annual Review of Pharmacology and Toxicology 45:51–88.

52- Chang, C.C., Chang, C.Y., Huang, J.P., Hung, L.M. (2012). Effect of resveratrol on oxidative and inflammatory stress in liver and spleen of streptozotocin-induced type 1 diabetic rats. Chinese Journal of Physiology 55: 192–201.

53- Kim ,Y.A., Lim, S.Y., Rhee, S.H., Park, K.Y., Kim, C.H., Choi, B.T., Lee ,S.J., Park, Y.M., Choi ,Y.H. (2006). Resveratrol inhibits inducible nitric oxide synthase and cyclooxygenase-2 expression in beta-amyloid-treated C6 glioma cells. International Journal of Molecular Medicine 17(6):1069-75.

54-Wang, Z.M. , Chen, Y.C., Wang, D.P.(2016). Resveratrol, a natural antioxidant, protects monosodium iodoacetate-induced osteoarthritic pain in rats. Biomedicine & Pharmacotherapy 83:763-770.

55- Shen, Y., Xu, Z., Sheng, Z.(2017). Ability of resveratrol to inhibit advanced glycation end product formation and carbohydrate-hydrolyzing enzyme activity, and to conjugate methylglyoxal. Food Chemistry 216:153-60.

56- Ueno, S., Sus, N., Furukaw, Y., Aikawa, K., Itagaki, I. , Komiyama, T., Takashima, Y. (1988) Effect of chromium on lipid peroxidation in isolated rat hepatocytes. Nihon Juigaku Zasshi 50:45–52.

57- Shinde Urmil, A., Sharma, G., Xu Yan, J., Dhalla Naranjan, S., Goyal Ramesh, K. (2004) Anti-diabetic activity and mechanism of action of chromium chloride. Experimental and Clinical Endocrinology & Diabetes 112:248–52.