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PROTECTIVE EFFECT OF SELENIUM ON DIABETIC NEPHROPATHY IN WISTAR RATS

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ABSTRACT

The objective of the present paper is to study and evaluate the effect of selenium supplementation on diabetic nephropathy caused by reactive species generated following hyperglycemia.

This study conducted on thirty-five male albino Wistar rats divided into five groups : the first group serves as control (C), the second group was treated by intraperitoneal injection of selenium (sodium selenite) at 1.89 mg/kg / day, regarding the 3rd groups diabetes was induced by intraperitoneal injection of 150 mg/kg of alloxan (single dose) (DM). One of the diabetic groups was treated by intraperitoneal injection of insulin, 3 IU/100 g PC twice a day (DTins). Another diabetic group is treated with 1.89 mg/kg/day of sodium selenite intraperitoneally (DTSe), and this for 21 days (the two groups treated with selenium were previously treated by gavage of 2 mg/kg/day for 10 days by the same trace element).

The results showed in untreated diabetic rats, a drop in body weight, and an increase in blood glucose (13.87mmol/L ± 0.62), urea (10.58 mmol/l ± 0.59), creatinine (64 µmol/l ± 1.3), and phosphorus (1.8 mmol/l ± 0.2). A decrease in serum calcium (1.9 mmol/l±0.1), total protein (58g/l ± 5), albumin (32 g/l ± 3), insulin (0.64 µ UI/ml ± 0.06) and enzymatic activity of serum G6PDH (109.8 m UI/10GR ± 3). With a decrease in anti-oxidant defense, which results in a reduced renal tissue content in GSH (55 nmol/mg of proteins ± 2.2) and of GPx (0.198 µmol/mg of P ± 0.007) and catalase (14.64 µmol H_2O_2/mn/mg of P ± 0.8) activities. Meanwhile an increase activity of GSTs (0.791 nmol/mn/mg of P ± 0.03) and the concentration of TBARS (0.382 nmol/mg of P ± 0.02) were observed.

Sodium selenite induced the antioxidant defense of renal tissue, which protected it from the free radical damage that can be caused by the injection of Alloxan.

Keywords: Diabetes, Oxidative stress, Selenium, Nephropathy, Biochemical parameters, Alloxan

INTRODUCTION

Alloxan or (2,4,5,6-tetra-oxy-pyrimidine, 5,6-dioxyuracil) is a diabetogenic agent exerting a cytotoxic activity on β-cells. This molecule sets a redox cycle with superoxide radicals formation associated with high doses of calcium, it induces fast destruction of β-cells **(Szkudelski, 2001).** Alloxan is used in several animal species (Rat, Dog) to induce diabetes and its use can be coupled with streptozotocin, which is also cytotoxic **(Anderson and Stitt. 1993)**. Diabetes mellitus is characterized by a disorder of glucose regulation manifested by hyperglycemia leading to serious complications **(Rains and Jain, 2011)**.

The two mechanisms of glucose regulation (insulin secretion and action) in diabetes are associated with oxidative stress **(Bloch-Damti, and Bashan, 2005, Rains and Jain, 2011, Osasenaga, 2018)**. Moreover, are controlled by a molecular pathway known as the insulin-signaling cascade. Glucokinase initiates the process of insulin secretion by the beta cells of the pancreatic islets **(Schuit *et al.*, 2001)**. It detects and captures glucose in the pancreas, and phosphorylates it to glucose-6-phosphate by triggering the pathway for glycolysis and the formation of ATP. A high concentration of intracellular ATP closes the K + channel (KATP) **(Alekseev *et al.*, 2010, Flagg *et al.*, 2010)**, and simultaneously promotes the influx of sodium (Na +), leading to a disturbance of the Na + / K + ratio. This causes depolarization of the membrane and subsequent opening of the T-type calcium (Ca2 +) and sodium (Na +) channels **(Osasenaga, 2018)**. Intracellular Ca^{2+} promotes the fusion of the secretory granules containing insulin with the plasma membrane and the subsequent release of insulin into the circulation and then transported to peripheral tissues where it binds to its receptor and exerts its glucose mobilizing action. In cells of the body **(Stewart *et al.*, 2015)**. In addition, the insulin-receptor complex activates a cascade of events characterized by series of phosphorylation and protein coupling **(Saini, 2010)**. It has been noted that oxidative stress induced by hyperglycemia activates decoupling protein 2 (UCP-2) and thus decreases the ATP / ADP ratio, thereby inhibiting insulin secretion **(Holley *et al.*, 2015)**. In addition, oxidative damage to beta cells in pancreatic islets by reactive species generated by hyperglycemia influences the quantity and quality of insulin secreted by these cells **(Newsholme *et al.*, 2007).** Oxidative stress alters the translocation of insulin-stimulated glucose transporter 4 (GLUT4) and protein kinase B activity in adipocytes. Oxidative stress-stimulated interactions between the PI3-kinase-dependent signaling pathway and p38 MAPK activation **(Rashid *et al.*, 2017; Mariana and Joilson, 2018)**. These pathways contribute to the stimulation of GLUT 4 for glucose transport **(Leto and Saltiel, 2012)** and the utilization of glucose for the synthesis of proteins, lipids and glycogen in target tissues **(Withers *et al.*, 1998)**. This oxidative stress deactivates by dephosphorylation of these pathways ($P_{13}K$ and MAPK), by stimulating the activities of phosphatases such as protein tyrosine phosphatase 1B (PTP1B) and tyrosine protein phosphatase containing SH_2 (SHO_2), thus stopping the action of insulin **(Langlais *et al.*, 2011)**. **Dokken *et al.* (2008)**, reported that inhibition of the enzyme glycogen synthase kinase-3 (GSK-3beta) induced by oxidative stress leading to a decrease or even cessation of glycogen synthesis.

Various natural antioxidants have shown their therapeutic effects in different disease models **(Banerjee *et al.*, 2018; Chowdhury *et al.*, 2016b; Ghosh *et al.*, 2015; Sadhukhan *et al.*, 2016; Saha *et al.*, 2016 ; Sinha *et al.*, 2015)**. Polyphenols, found in various natural compounds, exhibit medicinal properties against a wide range of diseases **(Ghosh *et al.*, 2017)**. Selenium is a trace element that plays an essential role in the elimination of reactive species generated during diseases and in the development of several pathologies including cancer, neurogenic diseases, cardiovascular disorders, diabetes and infectious diseases **(Rayman, 2012; Avery and mann 2018; Olivia *et al.*, 2019).**

In addition to its cofactor function like all trace elements, selenium is able to bind covalently to the amino acid; cysteine by forming selenocysteine, which will then be incorporated into a group of essential proteins called selenoproteins **(Vindry *et al.*, 2018)**.

Josef and Peter (2018), reported that decreased dietary intake of selenium causes a significant decrease in glutathione (GSH) in the liver and kidneys. In addition, the deficiency or lack of glutathione (GSH) causes severe pathogenic nephropathy **(Burk *et al.*, 1995, Josef and Peter, 2018)**.
The objective of our study is to study and evaluate the effect of selenium supplementation on diabetic nephropathy caused by reactive species generated following hyperglycemia.

MATERIALS AND METHODS

Animal treatment

Our tested animals were male albino Wistar rats of the *Rattus norvegicus*, aged from 04 to 05 weeks of average weighing between 200 and 220 g, were obtained from the Pasteur Institute (Algeria). These rats were subjected to an adaptation period of 2 weeks, under the conditions of the animal house; at a temperature of 23° C ± 2 ° and a natural photoperiod (12 h light:12 h dark) with a minimum relative humidity of 40%. They were fed with an energetically balanced concentrate (ONAB Elharrouche Skikda - Algeria), and water were available ad libitum. Thirty five animals were divided into five equal groups (7 each). The first served as control; the second received an oral pretreatment with selenium at 2 mg/kg/day for 10 days, then intraperitoneally at 1.89 mg/kg/day for 21 days **(Berg *et al.*, 1995),** the three remaining groups underwent an injection of Alloxan (150 mg/kg) to induce diabetes **(Rotruck *et al.*, 1973)**. After confirmation of diabetes by measurement blood glucose from the tail of rats, the fourth group was treated twice a day with insulin Actrapid (Novo Nordisk) at a dose 3 IU/L for 21 days **(Suthagar *et al.*, 2009).** The fifth group was treated with selenium before (oral 2 mg/kg/day for 10 days) and after the induction of diabetes (intraperitoneal route 1.89 mg/kg/day) for 21 days, at the end of the experiment, decapitation was used to sacrifice the animals. With recovery of the blood and organs (pancreas and kidneys) for the study of biochemical parameters and histological sections.

Organ preparation

The kidneys was weighed and washed with physiological water, and one stored at -20° C for the assay of biochemical parameters.

Homogenate preparation

Renal tissue (1g) was homogenized in 2 ml of phosphate buffer (pH=7.4), the supernatant obtained after centrifuging the homogenate at 10000 rpm for 15 minutes at 4° C was used for the determination of oxidative stress parameters (Glutathione, GPx, GSTs, catalase) and a marker for lipid peroxidation (MDA) (**Messarah *et al.*, 2012; Djeffal *et al.*, 2012)**.

Blood biochemical parameters assay

The serum concentrations of glucose, total proteins, albumin, urea, creatinine, calcium, phosphorus were estimated using the COBAS INTEGRA® 400 Plus analyzer and the commercial kits Spinréact reference (41011, 1001291, MX1001020, 1001329, 1001115, MX1001065, 1001155), respectively **(Kaplan. 1984; Koller and Kaplan. 1984; Fossati *et al.*, 1983**; **Farell and Kaplan. 1984**). Glucose 6-phosphate dehydrogenase activity was determined in erythrocytes by following the production of NADPH at 340 nm and at 37° C using the Rx Monza analyzer **(Lohr and Waller. 1974)**, and then the serum concentration of insulin was determined by electro chimiluminescence method **(Sapin. 2003)**.

Tissue biochemical parameters

The content of reduced glutathione (GSH) in kidney tissue was estimated according to the method described by **Weekbecker and Cory (1988)**. GPx activity was assessed using the **Flohe and Gunzler method (1984);** 0.3ml of supernatant was added to 0.7ml of the reactive mixture (0.3 ml of phosphate buffer pH7, 0.2ml of GSH (2mM), 0.1 ml of sodium azide (10 mM), 0.1 ml of (H_2O_2), incubation for 10 min, addition of 1 ml of 1% TCA to stop the reaction. The mixture is put in the ice for 30 min and after centrifugation for 10 min at 3000 rpm, 0.48ml of supernatant are placed in a tank is added with 2.2ml of phosphate buffer (0.32 M + 0.32 ml of DTNB at 1mM). The optical density was measured at 412 nm within 5 min. The measurement of GSTs activity was determined according to the **Habig *et al* (1974)**.
The catalase activity (CAT) is measured at 240 nm using the method of **Aebi (1984)**, using a UV/visible spectrophotometer and following the disproportionation of hydrogen peroxide (H_2O_2) for 1 min at an interval of 15 seconds, reacting in 780 µl (100 mM) of phosphate buffer pH 7.4, 200 µl HO (500mM) on 20 µl of the homogenate. Results were expressed in µmoles of HO per minute and per mg of protein. The concentration of MDA is determined according to the method of **Esterbauer *et al* (1991).** Kidney tissue Protein content in was determined by the Bradford method **Bradford (1976)**, using bovine serum albumin as a standard.

Statistic study

The results were represented as Mean ± SD, All statistical tests were performed using Prism GraphPad *(Software Inc. 2019)* with a significance level $p < 0.05$.

RESULTS

Effects of treatments on body weight and relative weight of kidneys

In measuring this parameter, we wanted to know what is selenium to have an effect on the body weight evolution of healthy subjects and diabetic subjects (fig. 1A). No significant difference in the relative weight of the kidneys was observed between the 05 treatment lots (fig. 1B).

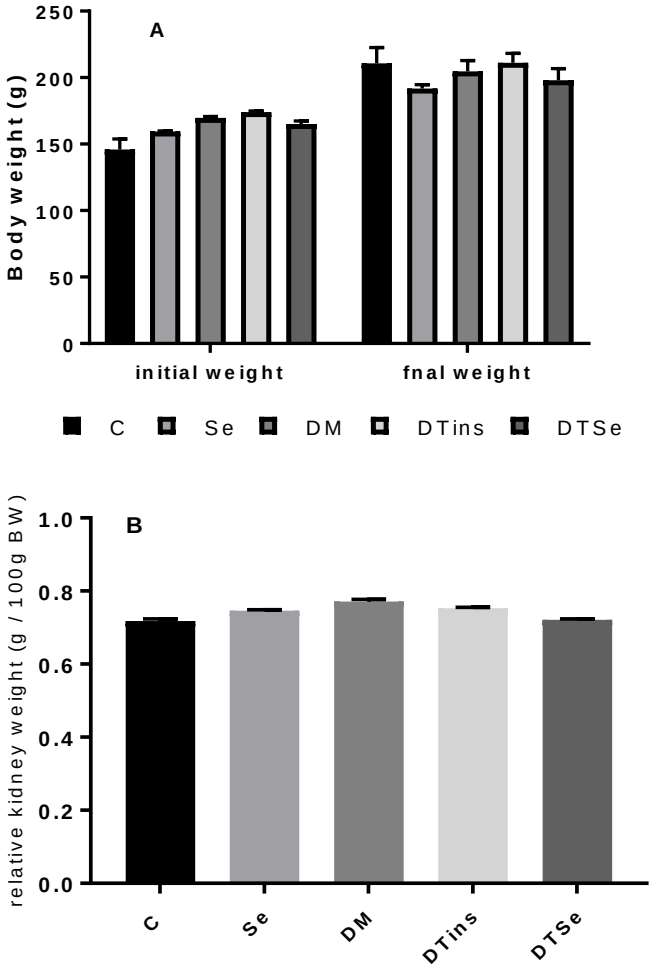


Figure 1 Study of the evolution of **(A)** body weight, and **(B)** relative weight of kidneys in control and treated groups after 21 days of treatment.

Effects of treatments on plasma biochemical parameters

Blood glucose, insulin levels and G6PDH activity

Analysis of the results showed that untreated diabetic rats exhibited a significant decreased serum insulin concentration by 73%, G6PDH activity by 23% and an increased glucose by 75% compared to controls. Whereas insulin treated diabetic rats showed an increase of insulin and blood G6PDH by 69% and 15% respectively, with a decrease in blood glucose by 67% compared to untreated diabetic rats. Similarly the selenium supplementation in diabetic rats increased insulin and G6PDH activity by 66% and 20% respectively and decreased blood sugar by 43% compared to untreated diabetic rats (Table 1).

Blood urea, creatinine, calcium, phosphor, total proteins and albumin

Our results show a very highly significant increase in serum concentrations of urea, creatinine, phosphorus with significant decreases in total protein, albumin and calcium concentrations in untreated diabetic rats compared to control rats, whereas the treatment of diabetic rats by insulin and selenium significantly normalize its parameters compared to the group of untreated diabetic rats (Table 1).

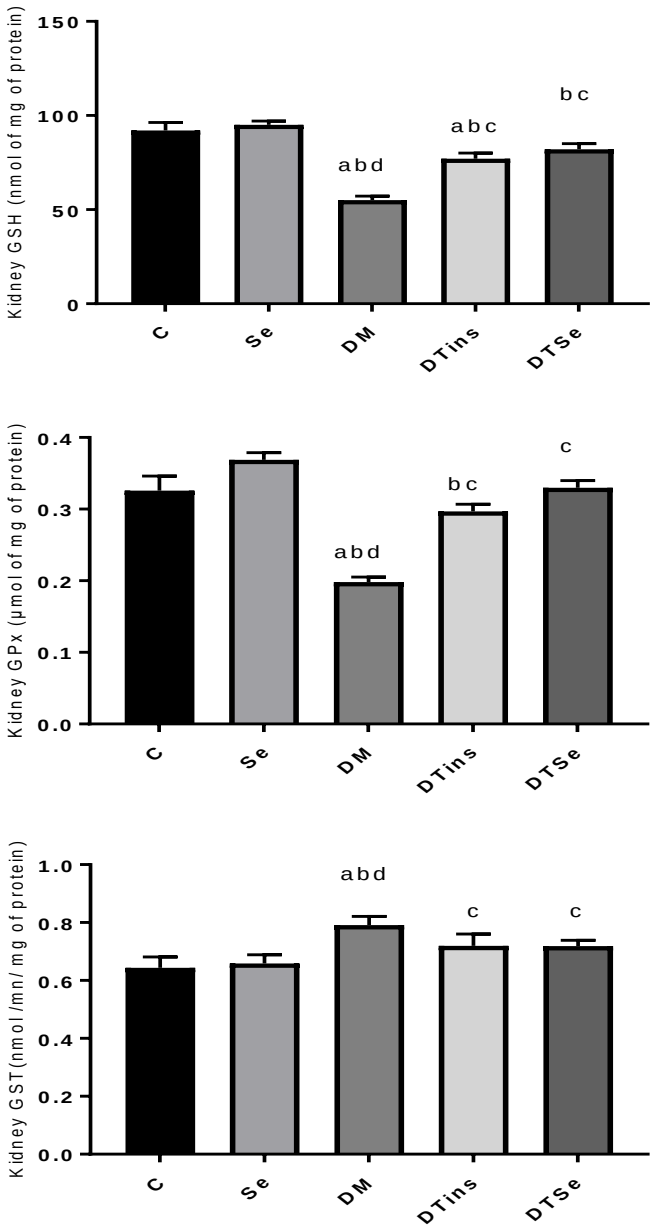
Table 1 Blood glucose, insulin, urea, creatinine, total protein, albumin, calcium, phosphorus and glucose-6-phosphate dehydrogenase enzyme activity in control and treated groups after 21 days of treatment.

	Experimental groups				
Parameters & treatments	**C (n=7)**	**Se (n=7)**	**DM (n=7)**	**DTIns (n=7)**	**DTSe (n=7)**
Glucose (mmol/l)	3.5±0.11	3.44±0.11	13.87 ±0.62^{abd}	4.49 ± 0.24^{abc}	8.04 ± 0.4^{abcd}
Insulin (µ UI/ml)	2.34 ± 0.048	2.41 ± 0.04	0.64± 0.06^{abd}	2.06 ± 0.06^{abc}	1.90 ± 0.038^{abcd}
G6PDH (m UI/10GR)	141.9±2	143.5±1	109.8 ± 3^{abd}	130.4 ± 2.2^{abc}	136 ± 1.3^{abcd}
Urea (mmol/l)	6.81 ±0.2	6.69 ± 0.2	10.58 ± 0.59^{ab}	9.74 ± 0.59^{ab}	7.91 ± 0.2^{abcd}
Créatinine (µmol/l)	53 ± 1	52 ± 1.1	64 ± 1.3^{ab}	64 ± 1.8^{ab}	61 ± 1.1^{ab}
Calcium (mmol/l)	2.3 ± 0.3	2.4 ± 0.3	1.9 ±0.1^{a*}	2.0 ± 0.2	2.0 ± 0.4
Phosphorus (mmol/l)	1.3 ±0.2	1.2 ±0.2	1.8 ± 0.2^{a*}	1.4 ± 0.3	1.4 ±0.1
Total proteins (g/l)	64 ± 5	64 ± 4	58 ± 5^{a*}	64 ± 4	63 ± 6
Albumin (g/l)	36 ± 4	34 ±2	32 ± 3^{a*}	32 ± 4	33± 3

*a: comparison with the C group (p≤0.05), b: comparison with the Se group (p≤0.05), c: comparison with the DM group (p≤0.05), *** (p≤0.001)*.

Effects of treatments on Kidney tissue parameters

The induction of diabetes in rats via an alloxan treatment resulted in a significant decrease in GSH concentration by 40 %, GPx activity by 39 %, catalase activity by 43%, and an increase in GST activity and TBARS content by 18 and by 34 % in kidney tissue respectively compared to control group (fig. 3).



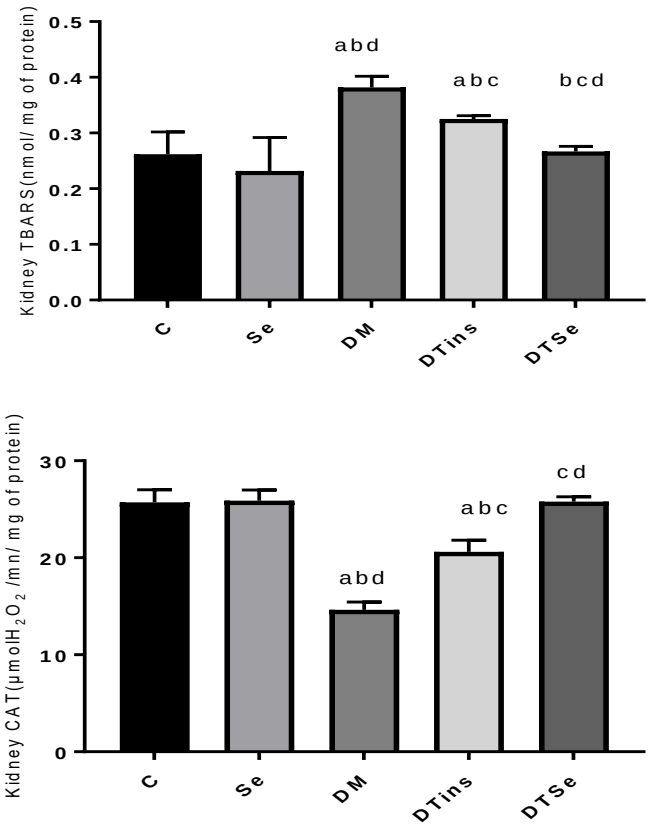


Figure 2 Variation in reduced glutathione (GSH), TBARS content, enzymatic activities of glutathione peroxidase (GPx), Glutathione-S-transferase (GST), Catalase (CAT), of kidney tissue in the control group and treated groups after 21 days of treatment.

DISCUSSION

The variations of the body weight and the relative weight of the kidneys of the animals subjected to different treatments were presented in figure 1. During this study, it was observed that the body weight of the control group and of the group treated with sodium selenite was gradually increased throughout the study, which in agreement with **Messarah *et al.*, 2012**. On the contrary, in untreated diabetic rats (DM) and diabetic rats treated with selenium (DM-Se), the results revealed a significant decrease in body weight, which is in agreement with the result obtained by **Murat and Belma, (2006).**

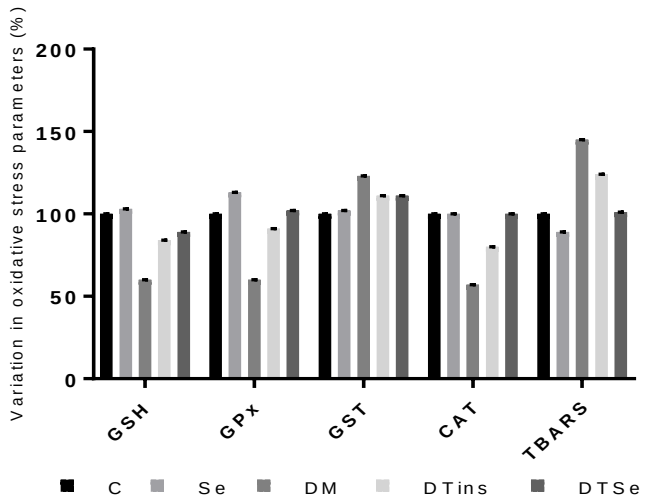


Figure 3 Percentage change in oxidative stress parameters (GSH, GPx, GST, CAT, TBARS), of kidney tissue in the control group and treated groups after 21 days of treatment.

This effect may be caused by a lack of insulin, which allows structural proteins to break down and increase body weight **(Vats *et al*., 2004).** While the study of the relative weight of the kidneys did not show any significant variation between the rats treated with alloxan and the control rats.

A significant increase in glucose with a decrease in insulin was observed in the untreated diabetic (DM) group compared to the control and treated groups. Hyperglycemia associated with hypoinsulinemia is a sign or marker of type I diabetes. **(Chowdhury *et al*., 2016a; Sekiou *et al*., 2018).** The pretreatment of the rats with selenium before the injection of Alloxane protected the pancreatic islands of the reactive species recorded by a glycemia significantly lower than that of the untreated diabetic rats, Granted by the results obtained by: **Osasenaga, 2018; Josef and Peter, 2018)**.

The decrease in enzymatic activity of G6PDH in untreated diabetics compared to diabetic controls treated with insulin and selenium is due to the state of oxidative stress caused by prolonged hyperglycemia as well as peroxidation of lipid. The modification of proteins and biomolecules by lipid peroxidation products plays a major role in the pathogenesis associated with radical damage **(Luke *et al*., 1993; Aouacheri *et al*., 2014).** MDA, is a product of lipid peroxidation that can react with biological macromolecules and more particularly proteins which causes their modification, the example of the formation of 4-HNE-Lysine adduct of G6PDH which the origin of the inactivation of this enzyme **(Esterbauer *et al*., 1991; Luke *et al*., 1993; Uchida and Stadtman. 1992; Palsamy, and Subramanian, 2011).**

The decrease in insulin concentration in untreated diabetic rats is due to the destruction of β-cells of Langerhan islands following the injection of alloxan, which is in agreement with (**Ankur and Shahjad,. 2012; Lenzen, 2008; Chowdhury *et al*., 2016a)**. Increases in serum urea, creatinine, and phosphorus with decreased total protein, albumin and calcium mean a sign of renal dysfunction. Such as hyperglycemia and proteins modified by hyperglycemia such as amadori products and AGEs (products of advanced glycation), play a key role in the occurrence of diabetic nephropathy and renal failure **(Wolf, 2005; Palsamy, and Subramanian, 2011; Aouacheri *et al*., 2014).**

The study of antioxidant defense parameters in renal tissue shows a significant decrease in the reduced content of tissue glutathione with a decrease in the activities of GPx, catalase and an increase in the activity of GST. In a state of hyperglycemia, glucose is used through polyols and is transformed into sorbitol this reaction consumes the NADPH essential for the regeneration of GSH molecules by glutathione reductase. Moreover the presence of free radicals decreases the concentration of GSH which represents the first scavenger of free radicals due to the presence of the SH group of cysteine **(Ghosh *et al*., 2015; Ghosh *et al*., 2018).** Since GSH is a cofactor of the GPX enzyme, its decrease in renal tissue is responsible for the decrease in GPx activity. NADPH is a cofactor of catalase and its decrease leads to a decrease in the activity of this enzyme. During our research, we recorded a decrease in the enzymatic activity of G6PDH in untreated diabetic rats; this enzyme catalyzes the first reaction of the pentose phosphate pathway generating the NADPH molecules. So that the decrease in the activity of G6PDH leads to a decrease in the activity of catalase **(Figueroa *et al*., 2008; Srivastava *et al*., 2005 ; Gleissner *et al*., 2008 ; Chowdhury *et al*., 2016a)**.

The increase in the activity of the GST enzyme is a physiological response to compensate for the alterations that are due to free radicals (generated during diabetes which in agreement with many workings **(Kade et al., 2008 ; Xi-qun et al., 2004 ; Nahla et al., 2006 ; Barbosa et al., 2006 ; Nuray and Belma. 2005; Avery, 2018; Olivia *et al*., 2019)**.

CONCLUSION

Based on the present study, we conclude that supplementation of selenium in mineral form provides protection of cells against reactive species generated by Alloxane by regulating plasma insulin levels, blood glucose, G6PDH, urea, creatinine, calcium and phosphorus. Thus, the study provides detailed insight into the intracellular redox balance of kidney cells.

Conflicts of interest/competing interests: The authors declare that they have no conflict of interest.

Ethics approval: All of the experimental protocols were approved and performed by the ethics committee of Pasteur Institute of Algeria (PIA) under the ethical code: No.2503/3.2016.

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