Sciences & Technologie C – N°25, Juin (2007), pp.18-23.

OXIDATIVE STRESS IN STREPTOZOTOCIN-INDUCED EXPERIMENTAL DIABETES IN RATS IS ASSOCIATED WITH CHANGES OF ANTIOXIDANT STATUS OF HEART TISSUE.

Reçu le13-03-2005 – Accepté le 14-03-2007

K. OUALI [1]
F. TREA
ML TOUMI
M. BAIRI
P. SIAUD
M. GUELLATI

[1] Laboratoire Biologie Animale Appliquée. Département Biologie. Université Badji MOKHTAR. ANNABA. ALGERIE.

Résumé

L'objectif de notre travail est d'étudier chez le rat mâle Wistar les effets d'un stress oxydatif chronique diabétogène, durant une période de douze semaines sur l'évolution de certains marqueurs de présence de radicaux libres au niveau du tissu cardiaque. Nos résultats montrent une augmentation très significative de l'activité de la superoxide dismutase, glutathion peroxydase et de la glucose-6-phosphate déshydrogénase pendant toute la durée de l'expérimentation. L'activité de la catalase marqueur de la formation du peroxyde d'hydrogène, augmente initialement après la deuxième et la troisième semaine et à la douzième semaine de l'expérimentation. La teneur tissulaire en acide ascorbique, un des composés piégeur de radicaux libres, diminue significativement la 3éme et la 6éme semaine puis augmente à la 12ème semaine. Les niveaux des LPO, indicateurs de la peroxydation des lipides augmentent très significativement en fin d'expérimentation. Nos résultats suggèrent que l'hyperglycémie entraîne une augmentation des marqueurs de la présence de radicaux libres au niveau du tissu cardiaque et les mécanismes de défense de cet organe sont modérément efficaces.

Mots clés: *Streptozotocine; Antioxydant; hyperglycémie; Diabète ; cœur ; Rat.*

Abstract

The present study was designed to evaluate the oxidative stress-related parameters in streptozotocin-induced diabetes in rats. After 2, 3, 6 and 12 weeks of hyperglycaemia the enzymatic parameters were measured in heart tissue of diabetic and control groups. Superoxide dismutase, glutathione peroxidase and the glucose-6-phosphate dehydrogenase activities significantly increased at all time intervals. Catalase activity increased initially (after 2 and 3 weeks), decreased after 6 weeks and increased again at the 12th week of the experiment. Glutathione reductase activity increased initially (at 2nd week), decreased below control level after 3 weeks, and then increased again. Ascorbic acid concentration decreased after 3 and 6 weeks, and increased at the 6th and 12th weeks. The level of lipid peroxidation products was reduced after 2, 3 and 6 weeks of the experiment. After 12 weeks it was significantly elevated. These data suggest that chronic hyperglycaemia induces oxidative stress in the heart but the defense mechanisms in the heart tissue are fairly efficacious against oxidative injury.

Keywords: *Streptozotocin; Antioxidant; Hyperglycaemia; Diabetes; Heart; Rat.*

ملخص

الهدف من هذا العمل هو دراسة تأثير توتر تأكسدي الناتج عن ارتفاع مزمن في نسبة تركيز السكر في الدم خلال فترة اثنى عشر أسبوعا على تغير في نشاط بعض المؤشرات الأنزيمية الدالة على وجود الجذور الحرة على مستوى نسيج القلب. الذتائج المحصل عليها تبين ارتفاع معنوي لنشاط Cu-SOD و جليتاتيون بيروكسيداز و G6PD خلال كل فترات التجربة. نشاط Catalase مؤشر لتكوين H_2O_2 ارتفع في بداية التجربة (أسبوع أول وثالث و12). نسبة تركيز حمض أسكربيك في نسيج القلب الذي يعتبر من أحد المكونات المحولة للجذور الحرة تنقص معنويا خلال الأسبوع 3 و6 وترتفع بعد ذلك في الأسبوع 12 مستوى ال LPO الذي يعتبر كمؤشر لأكسدة الدهنيات يرفع معنويا في آخر التجربة. من خلال الذتائج المحصل عليها نستطيع القول أن ارتفاع نسبة السكر في الدم تؤدي الى ارتفاع المؤشرات الانزمية الدالة على وجود الجذور الحرة في النسيج القلبي (ضغط تأكسدي على مستوى هذا العضو) و آليات الدفاع الضد التوتر التأكسدي على مستوى هذا النسيج فعالة نسبيا .

الكلمات المفتاحية: *ستراتبوزوتوسين, مضاد التاكسد, داء السكري, القلب, فأر .*

Myocardial infarction and stroke constitute major causes of death in patients with diabetes mellitus. Several hypotheses have been put forth to explain the adverse effects of hyperglycemia on the vasculature. These include the activation of the polyol pathway, nonenzymatic glycation redox potential alterations, as well as stimulation-increased production of reactive oxygen species in response to the activation of the diacylglycerol (DAG) protein kinase C (PKC) pathway [29, 30].

It has been shown that elevated extra- and intracellular glucose concentrations result in oxidative stress, which is defined as an imbalance between prooxidants and antioxidants. Several mechanisms seem to be involved in the genesis of this oxidative stress, which has been reported both in experimental diabetes in animals and in diabetic patients: glucose autooxidation, protein glycation and formation of advanced glycation endproducts, and the polyol pathway [29, 20].

On the other hand, oxidative stress is involved in the origin of type1 diabetes, especially *via* apoptosis of pancreatic β-cells, as well as insulin resistance in type 2 diabetes [3].

There is also evidence that elevation in glucose concentration may depress natural antioxidant defense agents such as vitamin C or glutathione [8, 22]. Recent experimental findings suggest that overproduction of reactive oxygen and nitrogen species, lowered antioxidant defense and alterations of enzymatic pathways in humans with poorly controlled diabetes mellitus can contribute to endothelial, vascular and neurovascular dysfunction [9,10,25].

Over the past decade, there has been substantial interest in oxidative stress and its potential role in diabetogenesis, development of diabetic complications, atherosclerosis and associated cardiovascular disease. Cardiovascular disease is the leading cause of mortality in patients with diabetes. Myocardiac infarction and stroke constitute the cause of death in as many as 80% of subjects with type 2 diabetes [6].

Several studies in human and animal models have shown alterations in the antioxidant status in diabetes. Especially, disturbances in the defense system in various tissues from animals with experimental diabetes are reported. So far, a number of experiments have been performed to evaluate changes in the antioxidative system of the diabetic heart [13,2,4]. The activity of superoxide dismutase (SOD) increased in diabetic myocardium during seven weeks of the disease [11,27, 23]. Matkovics *et al.* [18] reported that the SOD activity in the heart was midly elevated 48 h after alloxan treatment. The activity of catalase (CAT) was markedly elevated in diabetic myocardium during 32weeks [23] and after 48 h of diabetes [18]. Similar results were obtained by Tatsuki *et al.* [26] and by Kakkar *et al.* [11]. On the other hand, Yadav *et al.* [28] reported that CAT activity in the diabetic heart was not affected.Total glutathione (GSH) level was slightly increased in the heart tissue at the 15th week of diabetes [17]. Kakkar *et al.* [11] stated that glutathione peroxidase (GSH-Px) activity in the heart increased significantly during 6 weeks of the experiment. Parinandi *et al.*[21] reported an increase in the level of GSH and GSH-Px activity. On the other hand, Yadav *et al.* [28] reported that GSH content and glutathione reductase (GSSG-R) activity in the heart tissue were markedly lowered. In the study of Doi *et al.*[4] the GSH level was also significantly reduced. Sun *et al.*[24] stated that ascorbic acid concentration of diabetic hearts decreased significantly after 8 weeks of the experiment. Parinandi *et al.*[21] did not observe an intensification of lipid peroxidation (LPO) in the diabetic heart. In other experiment the level of LPO products significantly increased 2 weeks after the initiation of diabetes, after 7 weeks it returned to the level of the control [26]. An increase of lipid peroxidation was also observed in the cardiac muscle tissue during 32 weeks of diabetes [23].

We decided to examine the long-term effect of the diabetogenic streptozotocin by determining the parameters of the antioxidative status in diabetic heart at various stages of development of the disease (2, 3, 6 and 12 weeks).We examined the enzymatic antioxidants SOD, CAT, GSH-Px, GSSG-R, glucose-6 phosphate déshydrogénase G6PD and non-enzymatic parameter vitamin C. Lipid peroxidation was also monitored at each stage of the experiment.

MATERIALS AND METHODS

Experimental animals

The institutions guide lines for the care and use of laboratory animals was observed. The animals used were adult male Wistar rats weighing 250-300g. The animals were obtained from Pasteur Institute of Algeria. They were group housed in polyacrylic cages with not more than 4 animals per cage and maintained under standard laboratory conditions with natural dark and light cycle. The rats were fed standard diet and were given water *ad libitum*. Diabetes was induced by a single intraperitoneally injection of streptozotocin (50 mg/kg, Sigma Chemical, St. Louis, USA) in citrate buffer (0.05 mol/L, pH 4.5). Control animals were injected with same volume of citrate buffer. Seven days after streptozotocin administration serum glucose concentration was measured by glucose oxidase method [34]. All streptozotocin-treated animals in which glucose concentration in the serum was lower than 11mmol/l were excluded from the experiment. Glucose concentration and body mass were monitored weekly and at the time of sacrifice (after 2, 3, 6 and 12 weeks of disease).

The animals were anaesthetized with pentobarbital sodium (60 mg/kg), the heart of each animal was removed, washed with ice-cold physiological saline solution (Nacl 9‰), dried and processed for biochemical measurements. Homogenates were prepared on ice in the ratio 500 mg tissue for 4 ml of phosphate buffer, pH 7.5, containing 1 mmol/l Na_2EDTA. For each sample 10 µl of 500 mmol/l butylated hydroxytoluene in acetonitrile was added to prevent formation of new peroxides during the assay. The homogenates were centrifuged at 20 000 x ***g*** for 15 min at 4°C and frozen at -70°C until analysis. Proteins were determined in diluted aliquots of the homogenates by the method of Lowry [15].

Chemical substances

All chemical substances were obtained form Sigma Chemical, St. Louis, USA and Sigma-Aldrich Germany. Citrate buffer 0.05mol /l pH 4,5 : 26,7 ml of A: 21,01g citric acid dissolved in 1000 ml of distilled water and 23,2 ml of solution B: 29,41g sodium citrate dissolved in 1000 ml of distilled water.

Phosphate buffer pH 7.5: NaH2PO4 10mmol/l, EDTA 1mmol/l et saccharose 250 mmol/l.

Samples analysis

The activities of of CuZn superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione reductase (GR) were evaluated in homogenates using the spectrophotometric method (Bioxytech kit, SOD- 525, GPx- 340 and GR-340, Oxis Rechaerch, USA). Catalase (EC 1.11.1.9) activity was assayed by the decrease in absorbance of hydrogen peroxide at 240 nm as described by Aebi [1].The G6PD activity was measured according to the procedure of Löhr and Waller [16], NADP was reduced by

G6PD in presence of a glucose-6-phosphate, the the production of NADPH was proportionally as a formation of phosphor- gluconate acid, which measured at 340 nm. Ascorbic acid concentration was assayed according to Kyaw [14], using a simple acid phosphotungstate procedure. LPO products were determined using the method based on the reaction with *N*-methyl-2-phenylindole to yield a stable chromophore with maximal absorption at 586 nm. The method was used to assay malondialdehyde together with 4-hydroxyalkenals (MDA + 4-HNE) **Esterbaur et al 1991**.

Statistical analysis

The reported data are the means of measurements and their S.E.M. values. For statistical evaluation the ANOVA two ways test followed by the Student's *t*-test were applied using the Minitab 1.4 (Minitab Inc) software.

RESULTS

The initial mean value for serum glucose concentration was 6.39mmol/l. This level remained unchanged in the control groups but increased progressively in the diabetic animals. At the 2nd week the mean glucose concentration was 22.0 mmol/l ($P< 0.01$), at the 3rd and 6th weeks 32.3 and 30.1mmol/l ($P< 0.001$) respectively. At the 12th week of the experiment the mean value for glucose concentration was 23.3mmol/l ($P< 0.01$). There was a progressive and significant increase in body mass of control rats. The diabetic animals showed a significantly reduction in body mass, which attained 171.80 compared to control 283.48 at the 12th week (table 1). All other results are summarized in table 2 and 3.

Table 1: Body weight and blood glucose in control and diabetic rats

Duration of glucose DM (weeks)	Body weight g		Blood mol/l	
	C	**D**	**C**	**D**
2	246.64 ± 20.16	197.52 ± 24.12[b]	6.29.8 ± 1.2	22.0 ± 0.8[b]
3	275.51 ± 34.40	179.78 ± 30.42[a]	6.91 ± 0.9	32.3 ± 1.5[a]
6	253.14 ± 20.41	175.74 ± 32.20[a]	5.96.8 ± 0.7	30.1 ± 0.4[a]
12	283.48 ± 30.35	171.80 ± 36.50[a]	6.32 ± 1.4	23.3 ± 0.9[b]

Values are the mean ± S.E.M.; n = 6 in all groups. [a] Significantly different from control $P < 0.001$; [b] Significantly different from control $P< 0.01$.

Within the diabetic groups, Cu,Zn-SOD and G6PD activities were significantly increased at all time intervals as compared to respective control. There was a significant increase in CAT activity at the 2nd, 3rd and 12th weeks of disease. At the 6th week of experiment the activity of the enzyme was reduced below the level of the control group.

At the same time, GSH-Px activity was significantly increased. GSSG-R activity in the diabetic heart increased at the 2nd week, decreased at the 3rd and 6th weeks, then again increased above the level of the respective control group.

At the 2nd and 3rd weeks of experiment, we observed a significant decrease in the level of ascorbic acid. At the 6th and 12th weeks the concentrations of ascorbic acid were significantly higher than in the respective control groups. We found a decrease in the level of LPO products (estimated as MDA + 4-HNE) at the 2nd, 3rd and 6th weeks of diabetes. After 12 weeks of the disease the level of LPO products was significantly higher in diabetic animals than in the control group.

DISCUSSION

In our investigations, Cu,Zn-SOD and G6PD activity increased in the heart of diabetic animals probably to increase dismutation of superoxide anions as a compensatory activation mechanism. Similar results were obtained in other studies [11, 27, 18, 23]. G6PD is the predominant player in NADH regulation and can play important role in an organism's overall redox status and subsequent ability to handle oxidative stress [32]. The augmentations of G6PD activity in heart of diabetic animals contribute in formation of NADH which is required in order to convert oxidized glutathione (GSSG) into GSH [33]. In addition **kirkman et al [33]**. shown a protective effect of NADH on catalase activity.

The increase of CAT activity at the 2nd, 3rd and 12th weeks of our experiment and in other tests [11, 26, 18, 23] suggests a compensatory response to oxidative stress due to an increase in endogenous H_2O_2 production.

A significant increase in the level GSH-Px activity and a less significant one in the GSSG-R activity during the experiment confirm an efficacious defense of the diabetic heart against oxidative stress. The data obtained were similar to those presented by other authors [11, 17, 21].

Ascorbic acid is a major antioxidant that is essential for the scavenging of toxic free radicals in both blood and tissues. The disturbances in ascorbic acid metabolism in diabetes are therefore of great interest and might be important in the pathogenesis of some diabetic complications [12, 19]. The active transport of ascorbic acid appears to be decreased by hyperglycemia and insulin deficiency. Hyperglycaemia has also been shown to inhibit the uptake of dehydroascorbic acid, the oxidized species of vitamin C [2]. In the diabetic heart we observed a decrease in the level of ascorbic acid after 3 weeks and then an increase up to the 12th week of the experiment. In a previous paper, the decreased level of ascorbic acid was found in the heart after 8 weeks of the experiment [24]. Because of an impaired balance between the generation of free radicals and antioxidant defense systems some lipid peroxidation products or degradation products may be formed [8].

Since polyunsaturated fatty acid peroxides generate malondialdehyde and 4-hydroxyalkenals, measurement of MDA and 4-HNE may be used as an indicator of lipid peroxidation. In the present work we used a method based

on the reaction of MDA and 4-HNE with a chromogenic reagent to form a stable product with maximal absorption at 586 nm. The applied method is more specific than the most frequently used thiobarbituric acid (TBA) test because several other compounds give color products having absorption at the maximum of the TBA–MDA complex [7].

In our experiment, the heart lipid peroxidation did not increase during 6th weeks of the disease, probably due to the elevated activities of SOD and GSH-Px and to the decrease level of GSH. Similar results were reported by Parinandi *et al.*[21] and Tatsuki *et al.*[26]. Opposite results were obtained by other authors [11, 24, 13, 23].

In summary, we report significant differences between the diabetic and control groups. However, mechanisms of the defense in the heart were fairly efficacious against oxidative stress under diabetic conditions. This was demonstrated by the high levels of antioxidant compounds and the relatively low concentration of LPO products, especially during the first 12 weeks of diabetes. The tissue levels of the mentioned compounds seemed to depend on the stage of diabetes.

Table 2: Antioxidant: parameters in the heart tissue of the control (C) and diabetic (D) rats.

Duration of DM (Weeks)	CAT (µmol H_2O_2 / min mg protein)		SOD (U/mg protein)		GSH-Px (mU/mg protein)		GSSG-R (mU/mg protein)		G6PD (U/mg protein	
	C	**D**	**C**	**D**	**C**	**D**	**C**	**D**	**C**	**D**
2	84.8± 2.0	103.7± 0.9[a]	1.8± 0.2	5.2± 0.2[a]	30.7± 0.4	42.5± 0.8[a]	14.1± 0.2	14.6± 0.1[d]	198.1± 8.6	216.8± 8.7[n]
3	86.7± 1.4	108.9± 1.6[a]	1.9± 0.2	6.5± 0.4[a]	30.6± 0.4	69.0± 0.5[a]	14.1± 0.2	12.8± 0.3[d]	201.9± 12.8	256.4± 15.9[b]
6	85.5± 1.8	63.5± 1.2[a]	1.8± 0.2	5.1± 0.3[a]	30.4± 0.3	54.9± 0.8[a]	14.2± 0.1	9.7± 0.2[a]	202.8± 10.9	259.1 10.1[b]
12	89.6± 1.6	105.4± 1.8[a]	1.6± 0.1	3.9± 0.2[b]	30.3± 0.4	61.0± 1.2[a]	14.2± 0.1	14.8± 0.2[n]	203.8± 11.6	295.7 19.1[C]

Values are the mean ± S.E.M.; n = 6 in all groups. [a] Significantly different from control at $P < 0.001$; [b] significantly different from control at $P < 0.005$; [c] Significantly different from control at $P < 0.01$; [n] not significantly different.

Table 3 : Ascorbic acid and LPO products concentration in the heart tissue of the control (C) and diabetic (D) rats.

Duration of DM (weeks)	ascorbic acid nmol/g tissue		LPO products (nmol/g tissue)	
	C	**D**	**C**	**D**
2	240 ± 7	221 ± 3[c]	36.8 ± 0.3	24.3 ± 0.8[a]
3	239 ± 6	207 ± 2[a]	36.9 ± 0.3	16.2 ± 1.5[a]
6	246 ± 5	298 ± 4[a]	36.8 ± 0.3	17.4 ± 0.4[a]
12	242 ± 6	267 ± 5[d]	37.1 ± 0.5	61.4 ± 0.9[a]

Values are the mean ± S.E.M.; n = 6 in all groups. [a] Significantly different from control at $P < 0.001$; [b] significantly different from control at $P < 0.005$; [c] Significantly different from control at $P < 0.01$.

REFERENCES

[1]- Aebi H. Catalase *in vitro*. *Methods Enzymol.* **105** (1984) 121–6.

[2]- Akkus I, Kalak S., Vural H., Caglayan O., Menekse E., Can G., Durmus B. Leukocyte lipid peroxidation, superoxide dismutase, glutathione peroxidase and serum and leukocyte vitamin C levels of patients with type II diabetes mellitus. *ClinChim Acta.* **244** (1996) 221–7.

[3]- Bonnefont-Rousselot D., Bastard J.P., Delattre J. Consequences of the diabetic status on the oxidant/antioxidant balance. *Diabetes Metab.* **26** (2000) 163–76.

[4]- Doi K., Sawada F., Toda G., Yamachika S., Seto S., Urata Y., Sakata N., Tanigchi N., Kondo T., Yano K.Alteration of antioxidants during the progression of heart disease in streptozotocin-induced diabetic rats. *Free Radic Res.* **34** (2001) 251–61.

[5]- Ellman G.L. Tissue sulfhydryl groups. *Arch Biochem. Biophys.* **82** (1959) 70–7.

[6]- Giugliano D., Ceriello A. Oxidative stress and diabetic vascular complications. *Diabetes Care.* **19** (1996) 257–67.

[7]- Inouye M., Hashimoto H., Mio T., Sumino K. Levels of lipid peroxidation product and glycated hemoglobin A1c in the erythrocytes of diabetic patients. *Clin Chim Acta.* **276** (1998) 163–72.

[8]- Inouye M., Mio T., Sumino K. Link between glycation and lipooxidation in red blood cells in diabetes. *Clin Chim Acta.* **285** (1999) 35–44.

[9]- Jakus V. The role of free radicals, oxidative stress and antioxidant systems in diabetic vascular disease. *Bratisl Lek Listy.* **101** (2000) 541–51.

[10]- Julius D, Militante J.B., Lombardini B., Schaffer S. W.The role of taurine in the pathogenesis of the cardiomyopathy of insulin-dependent diabetes mellitus. Cardiovascular Research. **46** (2000) 393–402.

[11]- Kakkar R., Mantha S.V., Kalra J., Prasad K.Time course study of oxidative stress in aorta and heart of diabetic rat.*Clin Sci.* **91** (1996) 441–8.

[12]- Kinalski M., Sledzeiwski A., Telejko B., Zarzycki W., Kinalska I. Antioxidant therapy and streptozotocin-induced diabetes in pregnant rats. Acta Diabetol. **36** (1999) 113-117.

[13]- Kowluru R.A., Engerman R.L., Kern T.S. Diabetes-induced metabolic abnormalities in myocardium: effect of antioxidant therapy. *Free Radic Res.* **32** (2000) 67–74.

[14]- Kyaw A. A simple colorimetric method for ascorbic acid determination in blood plasma. *Clin Chim Acta.* **86** (1978)153–67.

[15]- Lowry O.H., Rosebrough N.J.; Farr A.L..; Randall R.J. Protein measurement with Folin phenol reagent. *J Biol Chem.***193** (1951) 265–7.

[16]- Löhr G.W., Waller H.D. Glucose-6-phosphate dehydrogenase, in: methods of enzymatic analysis, ed. By Bergmayer, H.U. Acad. Press, Newyork (1965) 744-751.

[17]- Mak D.H., Ip S.P, Poon M., Ko K.M. Alterations in tissue glutathione antioxidant system in streptozotocin-induced diabetic rats. *Mol Cell Biochem.* **162** (1996) 153–8.

[18]- Matkovics B., Sasvari M., Kotorman M., Varga I.S., Hai D.Q., Varga C . Further prove on oxidative stress in alloxan diabetic rat tissues. *Acta Physiol Hung* **85** (1997/1998) 183–92.

[19]- Opoka-Winiarska V. Impaired metabolism and effects of vitamin supplementation on diabetes. *Diabetol Pol.* **7** (2001) 208–11.

[20]- Melvin R., Suresh C. Islet redox stress: the manifold toxicities of insulin resistance, metabolic syndrome and amylin derived islet amyloid in type 2 diabetes mellitus.JOP. J Pancreas **3** (4) (2002) 86-108.

[21]- Parinandi N.L, Thompson E., Schmid H.H.O. Diabetic heart and kidney exhibit increased resistance to lipid peroxidation. *Biochim Biophys Acta.* **1047** (1990) 63–9.

[22]- Pèrez C., Canal J.R., Torres M.D. Experimental diabetes treated with ficus carica extract : effect on oxidative stress parameters. Acta Diabetol.**40** (2003) 3-8.

[23]- Stefek M., Sotnikova R., Okruhlicova L., Volkovova K., Kucharska J., Gajdosik A., Gajdosikova A., Michalova D., Hozova R., Tribulova N., Gvozdjakova A.. Effect of dietary supplementation with the pyridoindole antioxidant stobadine on antioxidant state and ultrastructure of diabetic rat myocardium. *Acta Diabetol.* **37** (2000)111–7.

[24]- Sun F., Iwaguchi K., Shudo R., Nagaki Y., Tanaka K., Ikeda K;, Tokumaru S., Kojo S. Change in tissue concentrations of lipid hydroperoxides, vitamin C and vitamin E in rats with streptozotocin-induced diabetes. *Clin Sci.* **96** (1999)185–90.

[25]- Sydow K., Münzel T. Diabetes mellitus, oxidative stress and endothelial dysfunction. International Congress Series 1253 (2003) 125– 138.

[26]- R. Tatsuki, K. Satoh, A. Yamamoto, K. Hoshi, K. Ichihara. Lipid peroxidation in the pancreas and other organs in streptozotocin diabetic rats. *Jpn J Pharmacol.* **75** (1997) 267–73.

[27]- Yadav P, Sarkar S., Bhatnagar D. Lipid peroxidation and antioxidant enzymes in erythrocytes and tissues in aged diabetic rats. *Indian J Exp Biol* **35** (1997a) 389–92.

[28]- Yadav P., Sarkar S., Bhatnagar D. Action of *Capparis decidua* against alloxan-induced oxidative stress and diabetes in rat tissues. *Pharmacol Res.* **36** (1997b) 221–8.

[29]- West I.C. Radicals and oxidative stress in diabetes. *Diabete Med.* **17** (2000) 171–80.

[30]- Ishii H., Koya D., King J.L.Protein kinase C activation and its role in the development of vascular Complications in diabetes mellitus, J. Mol. Med. **76** (1) (1998) 21–31.

[31]- Esterbauer H., Schaur R.J., Zollner H. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. Free radical Biology and medicine. 11, (1991), 81-128.

[32]- Wajcman H., Galactéros F.Le déficit en glucose-6 phosphate déshydrogénase : protection
contre le paludisme et risque d'accidents hémolytiques. C.R.Biologie. 327 (2004) 711–720.

[33]- Kirkman H.N., Rolfo M., Ferrari A.M., Gaetani G.F. Mechanisms of protection of catalase by NADPH, J. Biol. Chem. 274, (1999), 13908–13914.

[34]- Trinder P. Determination of glucose in blood using glucose oxidase with alternative oxygen acceptor. Ann. Clin. Biochem. 6 (24),(1969).