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IN SILICO ALPHA-AMYLASE ENZYME INHIBITION AND *IN VIVO* ATTENUATION OF HYPERGLYCEMIA AND OXIDATIVE STRESS BY THE N-ACETYL CYSTEINE (NAC) IN EXPERIMENTAL DIABETES

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Abstract: In the present study, we investigate the inhibition of the alpha-amylase enzyme and the antioxidant properties of N-acetyl Cysteine (NAC) in alloxan-induced experimental diabetes in rats. After one month of acclimatization in a room with controlled temperature (22±3 °C) and lighting (12-h light/dark cycle), rats divided into four groups of seven animals each: Group I: Control group (C); Group II: Untreated-diabetic group (UD) induced by injection of freshly dissolved alloxan in 1.0ml of sodium citrate buffer (0.1M, pH4.5) in the intraperitoneal way (i.p), at a rate of 150mg/kg body weight; Group III: Positive Control (PC+) treated orally (gavage) by N-acetyl-cysteine,150mg/kg/b.w; Group IV: Treated diabetic group (TD) by N-acetyl-cysteine (150mg /kg/b.w/day) orally along with the experimental protocol. Diabetes led to increased blood glucose beyond the baseline values. During diabetes situations, the total cholesterol, triglycerides, and malondialdehyde were significantly increased. On the contrary, the antioxidants enzymes activities (Catalase, Superoxide Dismutase, Glutathione Peroxidase, and Glutathione-S-Transferase) were decreased. The administration of NAC significantly improved the studied parameters. These results demonstrate that NAC ameliorates hyperglycemia, hyperlipidemia, and oxidative damage in the diabetes situations. In conclusion, the results obtained suggest that NAC supplementation reduces free radical generation, potentiates the antioxidant defense system, and attenuates intestinal glucose uptake.

Keywords: Experimental diabetes, N-acetyl Cysteine, Antioxidants enzymes, Alpha-amylase, Free radical.

INTRODUCTION

Diabetes mellitus is a universally prevalent disease recognized as a metabolic syndrome characterized by a disturbance of glucose homeostasis manifested by hyperglycemia repeatedly found in fasting basal conditions or evidenced by dynamic testing. The term "diabetes" referred to a variety of illnesses characterized by heavy urination, dehydration, and intense thirst (Oguntibeju and Oluwafemi,2019). There are two main types of diabetes: type 1 diabetes which is characterized by a complete insufficiency of insulin due to the autoimmune destruction of the beta (β) cells of the pancreas; Type 2 diabetes which is characterized by insulin deficiency and reduced sensitivity to this hormone (Abbas et *al.*, 2018). No population is immune to diabetes; it is present in 8.3% of the world's population. Each year, 1.3 million people are diagnosed with diabetes. This rapid increase in new cases of diabetes is of particular concern as the total number of people with diabetes is expected to increase from 415 million in 2015 to 642 million in 2040 (Ogurtsova et *al.*, 2017). Numerous studies have shown that diabetes mellitus is accompanied by increased production of free radicals by various mechanisms and a significant decrease of the enzymatic antioxidant capacity; superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and Glutathione-S-transferase (GST) (Jelodar et *al.*, 2020). The decrease of the enzymatic antioxidant capacity contributes to the appearance of oxidative stress in tissues, in particular the β cells that have limited defense mechanisms against free radicals (Abdel-Magied et al.,2018). The pathogenic role of oxidative stress on β cells causes their apoptosis, which may explain the decrease in pancreatic mass observed in people with diabetes (Adeyemi et *al.*, 2014). For this reason, the use of antioxidant compounds capable of scavenging free radicals and modulating oxidative stress seems to be a logical approach to tackling the damaging complications of diabetes. Hence the interest of our work, which aims at the physiological exploration of the antioxidant activities of N-acetyl-cysteine supplementation (NAC) in alloxan-induced diabetic rats. N-acetyl cysteine (NAC) is a synthetic derivative of cysteine, a non-essential amino acid

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synthesized in the body from methionine**.** N-acetyl cysteine can be administered orally or by intravenous infusion and can also inhale using a nebulizer (ref). In addition, it is giving to reduce poisoning with paracetamol (acetaminophen) (Tenório et *al.*, 2021). N-acetyl cysteine is an essential precursor of glutathione (GSH). Glutathione is the most abundant intracellular molecule that serves as a cofactor for several detoxifying enzymes.

MATERIALS AND METHODS

Animals

After one month of acclimatization in a room with controlled temperature (22±3 °C) and lighting (12-h light/dark cycle), rats were randomly divided into four groups of seven animals each: Group I: Control (C); Group II: Untreated-diabetic group (UD) induced by injection of freshly dissolved alloxan in 1.0ml of sodium citrate buffer (0.1M, pH4.5) in intraperitoneal way (i.p), at a rate of 150mg/kg body weight (Gamage et *al.*, 2014); Group III: Positive Control (PC^{+}) treated orally (gavage) by (N-acetyl-cysteine ,150mg/kg/b.w); Group IV: Treated diabetic group (TD) by N-acetyl-cysteine (150mg /kg/b.w/day) orally along with the experimental protocol. Every seven days, the blood withdrawn from the tail-vain of the rats in order to determine the fasting blood glucose using Accu-Chek® glucometer along with the experimental protocol. After 30 days, the rats were sacrificed under anesthesia. Blood was collected and centrifuged to obtain the serum. The liver and the kidney were immediately removed, weighed, and stored at -2**0°C** in the freezer for the subsequent preparation of the homogenate. The experimental procedures were carried out according to the National Institute of Health Guidelines for Animal Care and approved by the Ethics Committee of the University.

Liver and Kidney homogenate preparation

Liver and Kidney homogenate was obtained according to (Adeyemi et *al.*, 2014). Briefly, the liver and the kidney of the groups under study were crushed, and homogenised in TBS (50mM Tris, 150mM NaCl, pH 7.4), the cell suspension was centrifuged using an automatic refrigerated centrifuge (DCS®-16RTV, Argentina) (9000 rpm, 4°C, 15 min). The obtained supernatant was aliquoted and then stored at -20°C.

Biochemical assays and analysis

Biochemical parameters were evaluated by a spectrophotometric way according to appropriate standardized procedures, using commercially available kits from Spinreact® (Girona, Spain) such as Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Albumin, Creatinine, and Urea. Plasma lipid profile was also estimated by the measurement of the total cholesterol (TC) and Triglycerides (TG), using the above-mentioned commercial kit (Spinreact®).

Lipid peroxidation level determination

The lipid peroxidation (LPO) level in the kidney and the liver homogenate is estimated by Malondialdehyde (MDA), the final lipid peroxidation product. The measurement is carried out according to Buege and Aust, (1978). First, a quantity of 375µl of the TCA-BHT solution (TCA 20% and BHT 1%) added to 375µl of the homogenate and 150µl of TBS (Tris 50mM, 15Mm NaCl, pH = 7.4). The mixture is well vortexed and then centrifuged at 1000 rpm for 10 minutes. Then, 400µl of the supernatant is taken and added to 80µl of HCl (0.6M) and 320µl of the Tris-TBA buffer (26mM and 120mM, respectively). This last mixture incubated at 80°C for 10 minutes.

Reduced glutathione determination

Reduced glutathione (GSH) level in kidney and liver homogenate was estimated using a colorimetric essay mentioned by Ellman (1959). In brief, 0.8 ml of kidney or liver supernatant added to 0.2ml of 0.25% sulphosalicylic acid; then, tubes were centrifuged at 2500xg for 15min using an automatic refrigerated centrifuge (DCS-16RTV, Argentina). The resulting supernatant (0.5 ml) was mixed with 0.025 ml of (0.01M) DTNB and 1ml phosphate buffer (0.1M, pH 7.4). In the end, absorbance at 412 nm recorded. The amount of GSH expressed as Nano-moles of GSH/mg protein.

Antioxidant enzymes activity measurement

The glutathione peroxidase (GPx; E.C 1.11.1.9) activity measured according to the procedure of Flohe and Gunzler (1984). The superoxide dismutase (SOD; E.C 1.15.1.1) activity assayed by the method of Fridovich (1987). Catalase activity (CAT; E.C.1.11.1.6) in the liver and the kidney homogenate measured according to the method described by Aebi (1984), While Glutathione-S-transferase (GST; E.C 2.5.1.18) activity calculated according to the method described by Habig et *al.* (1974).

Computational procedure

Lately, virtual screening of compound libraries has become a standard technology in modern drug discovery pipelines (Kitchen et *al.*, 2004). In our study, to perform *in-silico* specific site docking, we used a powerful bioinformatics tool; Molecular Operating Environment software (MOE V15.10)

Methods and Reagents

The structure of our ligand (NAC, PubChem CID 12035) was downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov) and subjected to energy minimization and hydrogen bonds optimization before the beginning of the docking study, then saved as an MDP file in the docking database.

Molecular Docking study

The complete genome of the Alpha-Amylase retrieved from the PDB database. Whit PDB ID: 4GQR. The downloaded structure prepared before docking as fellow: First, we visualized the PDB file in MOE software and removed the non-desired sequence, including inhibitors and water molecules. Next, we optimized hydrogen bond structures and added atoms in missing loops or side chains to prepare the protonated 3D structure. Finally, we fix the potential of the protein receptors, and we saved our files in a MOE file format after energy minimization of the system using the

Amber 10: EHT force field to achieve a stable and optimized geometrical structure for performing the docking study. To select the pockets of our receptors, we use the site finder option of the MOE tool. The ligand pocket was chosen and saved as a MOE file ready for docking. Virtual screening carried out using the MOE software tool, the best mode of ligand/protein interaction was identified based on the binding energy. The ligand was designated based on the computed binding scores. Table 6 summarizes the obtained results of the docking study.

Statistical analysis

Data were expressed as Mean± standard error (SE). The difference between each peers group was calculated using "student-test" (Microsoft® Office Excel® 2016), and the difference between all studied groups was assessed by One-Way ANOVA using (Origin® Pro15). p values<0.05 are considered statistically significant, and those of p values< 0.01 are considered highly significant.

RESULTS

Effect of treatment on blood glucose levels

Fig. 1 summarizes the effect of treatment with NAC on blood glucose in all groups under consideration. In this study, we observed a significant increase in blood glucose levels in diabetic rats compared to normal rats. In contrast, a significant decrease in blood glucose level was recorded in the diabetic group treated with NAC compared with the untreated ones. No change in blood glucose level was observed between the control group and the positive control group.

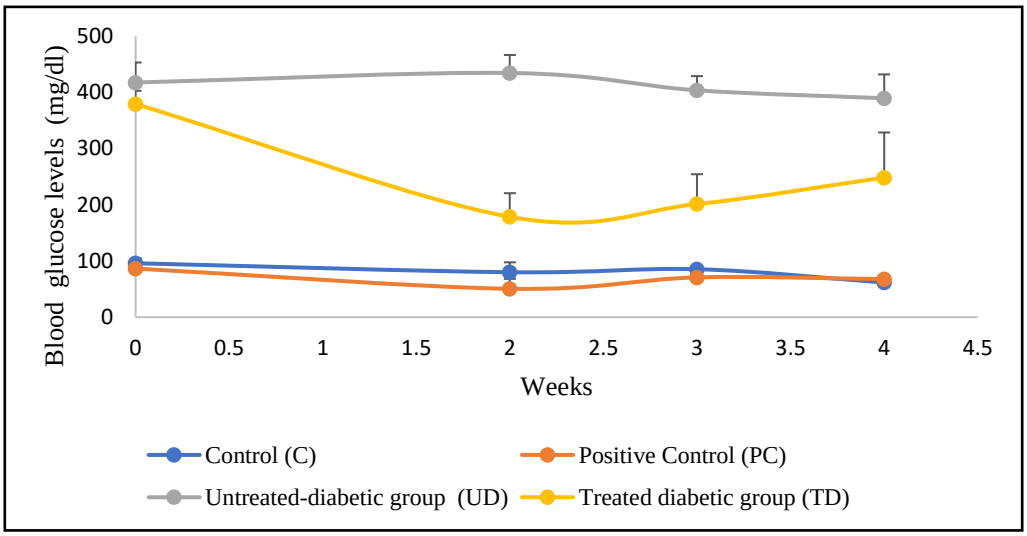

Fig. 1. Effect of treatment by N-acetyle cysteine (150mg/kg/dw) on blood glucose levels.

Effect of treatment on body weight and relative mass of liver and the kidneys

Variations of body mass and relative mass of the liver and the kidneys of all the rats under treatments are presented in (Table 1). During the present study, there was a gradual increase in body weight of the control rats (+ 24.6 g) as well as the control group treated with NAC (+ 24.3 g) and a reduction in weight gain (- 59.18 g) of the untreated diabetic rats. On the other hand, a significant gain in body weight is recorded in diabetic rats treated by NAC compared to those of untreated diabetic rats. In addition, a significant decrease in the mass of the pancreas and liver wright were recorded in the group of diabetic rats compared to the control group; however, there was a significant increase of kidney weight of untreated diabetic group in comparison with control ones, a significant increase of absolute liver wright and the absolute weight of the pancreas were observed in diabetic rats treated by the NAC in compared to untreated diabetic group.

Table 1.

Effects of oral treatment with N-acetyl-cysteine (150 mg/kg BW / day / for 30 days) on the body weight, the absolute and relative weight of the liver of the kidneys and the pancreas of the control and treated rats.

	Treatment			
Parameters	**Control**	**Untreated-diabetic group**	**Positive control**	**Treated diabetic group**
Initial weight (g)	256,25± 4,65	259,62 ± 6,87	262,5 ± 9,45	262,75 ± 8,12
Final weight (g)	280.8± 10.26	200.4 ± 07,54***	286,4 ± 11.75$^{\#\#\#}$	238.7 ± 10.43 $^{\#\#}$
Weight gain (g)	+24,6	-59,18	+24,3	-24,56
%	+8,57%	-29,50%	+8,39%	-10,08%
Absolute kidney weight (g)	1.87 ± 0.30	2.17 ± 0.34***	1.86 ± 0.21$^{\#\#\#}$	1.92 ± 0.43$^{\#\#}$
Relative kidney weight (%)	0.64±0.022	1.03±0.039***	0.63±0.013$^{\#\#\#}$	0.77±0.034$^{**\#\#\#}$

Absolute liver weight (g)	8.38 ± 0.75	5.81 ± 0.70***	8.05 ± 0.46###	7.55 ± 0.48##
Relative liver weight (%)	2.98 ± 0.081	2.90 ± 0.063	2.82 ± 0.034	2.96 ± 0.03
Absolute weight of pancreas (g)	1.511 ± 0.321	1.265 ± 0.315***	1.824 ± 0.286###	1.328 ± 0.208*#
Relative weight of pancreas (%)	0.521 ± 0.046	0.601 ± 0.031**	0.619 ± 0.026**##	0.534 ± 0.023##

Significant difference compared to the control group: * p <0.05, ** p <0.01, *** p <0.001
Significant difference comparing to the diabetic group: ### p <0.001

OXIDATIVE STRESS PARAMETERS STUDY

Effects of treatment by the NAC on the antioxidant status of the liver and the kidneys

Variations of oxidative stress parameters (GPx, Catalase, SOD, GST, MDA, and GSH) on the liver and the kidney of treated and control rats are shown in tables below. The variations are expressed in (mean ± SEM), the percentages of decreasing and increasing are indicated in those same tables.

Malondialdehyde (MDA)

According to the results obtained (Table 2), a very highly significant increase (+ 95.97%, $P \leq 0.001$) is observed in the level of MDA in the liver of diabetic rats in comparison with the control group. Similarly, there is also a significant increase (+ 84.85%, $P \leq 0.01$) in the level of MDA in the kidneys of diabetic rats compared to the control group. Treatment with NAC results in a significant drop of MDA levels in the liver (-98.68%, $P \leq 0.05$) and in the kidneys (-96.26%, $P \leq 0.05$) in treated diabetic rats compared to untreated diabetic rats. No significant variation was recorded when comparing the control group with the control group treated with NAC.

Reduced Glutathione (GSH)

The obtained results show that diabetic rats have disturbances redox status in the liver and kidney; indicated by altered levels of glutathione (GSH); thus, a statistically significant drop in the level of GSH is recorded in the diabetic rats in comparison with control ones (-91.99% and -95,23%, in the liver and the kidney respectively $p \leq 0.001$). On the other hand, the antioxidant properties of NAC are revealed by an improvement of the GSH level in the liver and the kidney of treated diabetic rats compared to the untreated diabetic rats. Indeed, the antioxidant benefit of this molecule leads to a significant increase in hepatic (+ 91.36%) and kidney (+ 93.3%) of GSH levels. No significant variation was recorded in the GSH level when comparing the control group with the control group treated with NAC (Table 2).

Table 2.

Effects of oral treatment with N-acetyl-cysteine (150 mg/kg BW / day / for 30 days) on the level of MDA and GSH in control and treated rats.

	Parameters	Treatment			
		Control	Untreated diabetic group	Positive control	Treated diabetic group
Liver	MDA (nmol/mg prot)	2,05± 0,54	4,53± 0,52***	2,30± 0,56###	2,77± 0,16###*
	%	—	+95,97	—	-98,68
	GSH (nmol GSH/mg prot)	14,63± 0,7	9,89± 0,5***	14,51± 0,4###	14,61± 0,03###
	%	—	-91,99	—	+91.36
Kidney	MDA (nmol/mg prot)	11,03± 0,36	15,55± 0,62***	8,65± 0,36###	8,38± 0,16###**
	%	—	+84.85	—	-96,26
	GSH (nmol GSH/mg prot)	6,82± 0,7	4,77± 0,5***	6,55± 0,4###	6,32± 0,03###
	%	—	-95,23	—	+93.3

Significant difference compared to the control group: * p <0.05, ** p <0.01, *** p <0.001 Significant difference comparing to the diabetic group: ### p <0.001

Glutathione peroxidase (GPx)

The obtained results show that diabetic rats have redox status disturbances in the liver and kidney, indicated by altered Glutathione peroxidase levels (GPx). Thus a statistically significant drop in the level of Glutathione peroxidase (GPx) is recorded in the liver and the kidney of diabetic rats in comparison with control rats (-26.31% and -27.27% respectively, $P \leq 0.001$). On the other hand, the antioxidant properties of NAC are revealed by an improvement of the Glutathione peroxidase (GPx) activity in the liver and the kidney of treated diabetic rats compared to the untreated ones. Indeed, the antioxidant benefit of this molecule leads to a significant increase of Glutathione peroxidase (GPx) activity in both of hepatic (+ 64.28 %) and the kidney (+ 23.3%). No significant variation was recorded in the Glutathione peroxidase (GPx) activity when comparing the control group with the control group treated with NAC (Table 3).

Glutathione - S- transferase (GST)

According to the obtained results, the GST activity is significantly decreased in the liver (-31.25%, $p \leq 0.05$) and the kidneys (-56.25%, $p \leq 0.01$) of diabetic rats compare d to the control group. However, no significant difference in the GST activity was noted in control rats treated with NAC compared to untreated rats. In contrast, there was a significant increase in GST activity in diabetic rats treated with NAC compared to untreated diabetic rats in all organs understudies (Table3).

Table 3.

Effects of oral treatment with N-acetyl-cysteine (150 mg/kg BW / day / for 30 days) on the GPx and GST activity of control and treated rats.

	Parameters	Treatment			
		Control	Untreated diabetic group	Positive control	Treated diabetic group
	GPx (mol GSH/mg prot)	0,19 ± 0,02	0,14± 0,01***	0,22± 0,02###	0,23± 0,01**###
	%	–	-26,31	–	+64,28
Liver	GST (nmol CDNB/min/mg prot)	0,08 ± 0,01	0,055±0,01***	0,10±0,011###	0,10±0.021**###
	%	–	-31,25	–	+32.3
	GPx (mol GSH/mg prot)	0,11 ± 0,01	0,08± 0,01***	0,10± 0,02###	0,097± 0,01**###
	%	–	-27,27	–	+21,25
Kidney	GST (nmol CDNB/min/mg prot)	0,1 ±0,01	0,05±0,01***	0,09±0,011###	0,07±0.021**###
	%	–	-56,25	–	+32.3

Significant difference compared to the control group: * p <0.05, ** p <0.01, *** p <0.001
Significant difference comparing to the diabetic group: ### p <0.001

Catalase (CAT)

The obtained results show a significant loss ($p < 0.05$) of catalase activity in diabetic rats compared to control ones. The significant decrease in catalase activity in diabetic rats observed in our results indicates the loss of antioxidant power in the liver and kidneys. On the other hand, the treatment of diabetic rats with NAC results in a significant improvement in catalase activity compared with untreated rats in the liver and the kidneys (Table4).

Superoxide dismutase (SOD)

From the results obtained in this study, it is noted that diabetic rats presented a significant decrease ($p > 0.05$) of SOD activity in the liver and a very highly significant decrease ($p > 0.001$) in the kidneys. On the other hand, rats treated by NAC show a significant improvement of the activity of this key enzyme which is strongly implicated in the antioxidant fight on the liver and the kidneys. No difference was recorded when comparing the control group with the control group treated with NAC (Table4).

Table 4.

Effects of oral treatment with N-acetyl-cysteine (150 mg/kg BW / day / for 30 days) on the CAT activity and the SOD of the control and treated rats.

	Parameters	Treatment			
		Control	Untreated diabetic group	Positive control	Treated diabetic group
	SOD (UI/mg prot)	103,28± 9,15	43,58± 4,68**	106,22 ± 5.72##	123,61 ± 5,26##
	%	–	-58.8	–	+65,04
Liver	CAT (µmol H_2O_2/min/mg prot)	67,78± 3,41	34,07± 2.36***	60.06± 2.65**###	46,67±5.01*###
	%	–	-49,73	–	+26,08
	SOD (UI/mg prot)	117,47± 7, 19	85,83± 5,78**	123,05 ± 6.26##	118,50 ± 2,97##
	%	–	-27,35	–	+27,69
Kidney	CAT (µmol H_2O_2/min/mg prot)	32.50,78± 2,16	20.45± 3,4***	37,23± 3.95**###	30,9±3,68*###
	%	–	-37,5	–	+33,34

Significant difference compared to the control group: * p <0.05, ** p <0.01, *** p <0.001
Significant difference comparing to the diabetic group: ### p <0.001

Effects of treatment on some biochemical parameters

Table 5 summarizes the results of biochemical parameters. As we can see, the diabetic rats have a high level of serum creatinine and urea in comparison with the normal ones, in addition, the levels of triglycerides and cholesterol are significantly increased in the diabetic rats. On the contrary, the daily treated rats with the NAC show a normal rate of triglycerides and cholesterol.

The activity of AST and ALT enzymes is highly increased in diabetic rats, which is a sign of hepatic cirrhosis and inflammation, on the contrary of diabetic rats treated by NAC, which present near-normal rates in the activities of the above-mentioned enzymes.

Table 5.

Effects of oral treatment with N-acetyl-cysteine (150 mg/kg BW / day / for 30 days) on some biochemical parameters in the control and treated rats

	Treatment			
Parameters	**Control**	**Untreated diabetic group**	**Positive control**	**Treated diabetic group**
Albumin (g/l)	37,5 ± 1,96	38,17 ± 2,39	38,26 ± 1,158	32,9 ± 2,255
Creatinine (mg/dl)	04,6 ± 0,54	05 ± 0,96***	04 ± 0.56###	08,84 ± 0,57##
Urea (mg/dl)	0,41 ± 0,06	0,64 ± 0,19**	0,44 ± 0,02	0,46 ± 0,57##
Triglyceride (g/l)	0.51 ± 0.078	1.99 ± 0.44***	0.67 ± 0.17###	0.60 ± 0.03##
AST (IU/l)	43.4 ± 12.08	649.5 ± 1.56***	39.2 ± 05.30###	147 ± 12.14**###
ALT (IU/l)	55 ± 3.24	366.47 ± 12.36***	44.72 ± 3.122###	125.5 ± 08.48##
Cholesterol(g/l)	0.50 ± 0.054	0.897 ± 0.15 ***	0.49 ± 0.06###	0.59 ± 0.11**##

Significant difference compared to the control group: * p <0.05, ** p <0.01, *** p <0.001
Significant difference comparing to the diabetic group: ### p <0.001

Results of the molecular docking study

The binding energies obtained from the docking (MOE) of the active site of 4GQR were presented in Table 6. **The N-acetyl cysteine** exhibited a lowest binding energy to 4GQR (Binding energy to 4GQR = -42.24 kcal/mol). As shown in Fig. 2 NAC was well fitted into the active pocket of **Alpha-Amylase (**4GQR). **N-acetyl cysteine** formed hydrogen bond with ASP-197 (H-donor, Distance 3.40Å, binding energy -2.5 kcal/mol), and hydrogen bond acceptor with ARG-195 and HIS-299 (binding energy -5.7 kcal/mol, and -0.7 kcal/mol respectively). Furthermore, **N-acetyl cysteine** was found to be interacting with ARG -195 via ionic interaction (binding energy -4.3 kcal/mol). **N-acetyl cysteine** sulfhydryl group will be responsible for the formation of Van der Waals interactions.

Table 6.

The hydrogen bond energy of the N-acetyl cysteine interaction with Alpha-Amylase **(**4GQR) active site

Ligand	Receptor	Interaction	Distance (Å)	E (kcal/mol)
N 7	OD1 ASP 197 (A)	H-donor	3.40	-2.5
O 17	NH2 ARG 195 (A)	H-acceptor	3.02	-5.7
O 18	CE1 HIS 299 (A)	H-acceptor	3.29	-0.7
O 17	NH2 ARG 195 (A)	Ionic	3.02	-4.3

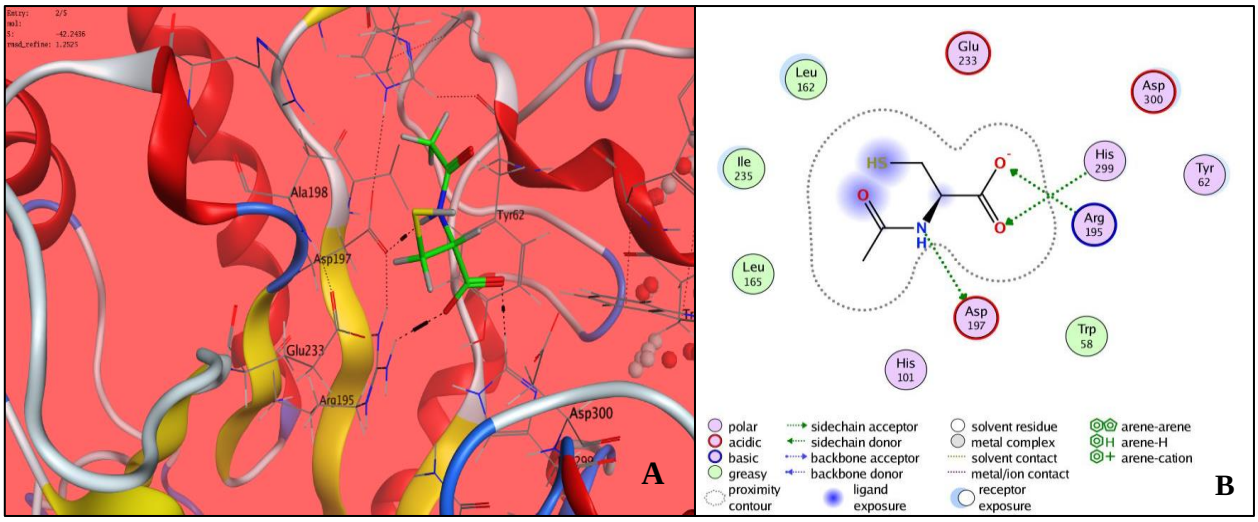

Fig. 2. Representation of docked ligand-protein complex: **(A)** animation pose of N-acetyl cysteine within the cavity of Alpha-Amylase **(**4GQR), **(B)** 2D interaction of NAC with amino acid residues of Alpha-Amylase. (Generated using *Molecular Operating Environment* software).

DISCUSSION

During diabetes, chronic hyperglycemia causes an overproduction of reactive oxygen species (ROS), which leads to the onset of oxidative stress. It is by now generally accepted that oxidative stress is a very aggressive molecular factor concerning biological tissues and tends to develop lesions and damage the functions of various organs, including kidneys, liver, pancreas. Despite the development of several antidiabetic therapies, patients with diabetes mellitus end up developing complications such as renal failure, microangiopathy, and macroangiopathy (Avogaro, and Fadini 2019). We speculate that this might be due to oxidative stress, which constitutes the main reason for this study. The assumption of this research work was that oxidative stress mediates resistance to those antidiabetic treatments. We hypothesized that natural antioxidant compounds scavenge free radicals and modulate oxidative stress, which increases the efficiency of diabetes treatment and may improve responses to currently available therapies. From the short review above, the main findings objective of this study emerges: First, induction of experimental diabetes associated with oxidative stress induced by alloxan injection. Second, develop a therapy with N-acetyl Cysteine (NAC). The intraperitoneal injection of alloxan causes the β-cells destruction, which results in a disorder of carbohydrate metabolism regulation because of the decline in insulin secretion. Hyperglycemia is a pro-oxidative agent that induces an increased generation of reactive oxygen species (ROS) Rehman and Akash, (2017). Overproduction of free radicals in diabetes mellitus situations is cytotoxic. Reactive Oxygen Species increases the processes of lipid peroxidation in biological systems, which increases the tissue lesions (Juan et *al.*, 2021). Using spectrophotometric-based assays, we measured the activities of key enzymes involved in the antioxidant defense systems, including glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase (CAT), and Glutathione-S-transferase (GST) in all groups of this study. From the obtained results, we recorded a significant activity decrease of all those enzymes in the diabetic group compared to the controls ones, which is consistent with what has been recorded in previous studies. Sugumar et *al.* (2016) also noted a lessening activity of antioxidant enzymes in streptozotocin-induced diabetic rats. Antioxidant enzymatic activity dysfunction may be due to the reactive oxygen species highly generated in oxidative stress situations which, cause proteins oxidation (Estévez, and Xiong 2019). On the contrary, in the NAC treated diabetic rats, we observe a significant increase ($p \leq 0.001$) of antioxidant enzymes activity. The enhancement of the enzymatic activity may be due to the antioxidant properties of NAC. This last (NAC) scavenges free radicals and allowing the maintenance of the antioxidants/free radicals ratio in a near-normal manner.

ALT and AST are diagnostic biomarkers for liver damage because they release into blood circulation after liver cell damage (Ramaiah, 2007). In this study, the elevated serum levels of ALT and AST in diabetic rats were the result of leakage of damaged cells. NAC significantly improved these perturbations and did not significantly affect these parameters in the control group. This positive effect on ALT and AST could explain the protective role of NAC in the liver. In this study, disturbances in the lipid profile significantly improved in the diabetic group treated with NAC. Several studies have shown that NAC improves lipid profile with a decrease in TC and TG levels (El-Lakkany et *al.*, 2016). In this research, the obtained result of the molecular docking study shows potent inhibition of alpha-amylase enzyme by NAC. Alpha-amylase (EC 3.2.1.1) is a digestive enzyme classified as glycosidase (an enzyme that hydrolyzes polysaccharides). The main role of alpha-amylase is the hydrolysis of starch present in the diet, generating glucose, maltose, and dextrin. Potent inhibition of alpha-amylase enzyme by NAC can explain the significant decrease in blood glucose levels recorded in the diabetic group treated by NAC.

CONCLUSION

The results obtained in this study suggest that NAC supplementation during diabetes situations reduces free radical generation, potentiates the antioxidant defense system, and attenuates blood glucose levels. We conclude that the use of the NAC is substantial in the field of investigation of a potent complementary drug for eliminating oxidative stress in diabetes situations.

AUTHORS CONTRIBUTIONS

Conceptualization: Sekiou Omar and Mahfoud Messarah; methodology Sekiou Omar; data collection Sekiou Omar; data validation, Mahfoud Messarah and Sekiou Omar; data processing Sekiou Omar, Aissa Benselhoub Wahida Kherfane, and Zihad Bouslama; writing—original draft preparation, Sekiou Omar, Aissa Benselhoub and Honcharova Olena; writing—review and editing, Sekiou Omar, Mahieddine Boumendjel and Mahfoud Messarah.

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CONFLICT OF INTEREST

The authors report no conflict of interest.

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