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RESEARCH ARTICLE

IN VITRO EVALUATION OF THE EFFECT OF GLUTATHIONE ON CASPASE SYSTEM AND OXIDATIVE DNA DAMAGE IN HIGH GLUCOSE CONDITION

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ABSTRACT

This study was planned to investigate the effects of glutathione, known to be a strong antioxidant, on caspase-dependent apoptosis and oxidative DNA damage in the kidney cells (BHK-21) exposed to high glucose. BHK-21 cell line was cultivated by regular passages *in vitro* conditions. Study groups were set up as control, study and its combinations groups ((Glucose; (285 mM, HG), glutathione (250 μM)). After 24 hours of incubation, trypsinized cells were disrupted by freeze-thaw method and analyte was prepared. Caspase 3, 8, 9, M30 and 8-OHdG (oxidative DNA damage marker) levels were determined using commercial ELISA kits. M30, caspase 3, 8 and 9 which are the parameters of apoptosis, were found highest in the HG group. In GSH-treated groups, these parameters decreased slightly. There was no significant difference in the levels of 8-OHdG which is the indicator of oxidative DNA damage.

Keywords: High glucose, Glutathione, Apoptosis, DNA damage, *in vitro*, Kidney cells

1. INTRODUCTION

Diabetes mellitus leads to acute metabolic complications and chronic vascular, renal, retinal, and neuropathic disorders, via high glucose condition. Increased free radicals, induced by diabetes, affect lipids, proteins and nucleic acids, leading to structural, functional and genetic cellular changes. Enzymatic and non-enzymatic antioxidant defense systems are responsible for the prevention from the harmful effects of free radicals in the organism [1-3].

Use of antioxidants by chronic diabetes patients is recommended to alleviate the increased effects of oxidative stress. GSH, an antioxidant agent, contains a thiol group and widely found in almost all mammals against oxidative stress. By reacting with free radicals and peroxides, it protects the cells against oxidative damage [4-6].

Apoptosis is the process where the cells that are no longer needed in the organism are activated by the stimulation of intracellular signaling systems and self-destruct. Caspases are cysteine-protease (CASPASE) group enzymes that play an important role during apoptosis. The cells undergo apoptosis due to the oxidative DNA damage caused by diabetes as well [7-9].

In cellular DNA damage, cells often undergo apoptosis. Cell destruction by apoptosis plays a role in the pathogenesis of many important diseases. Oxidative stress caused by various factors also plays an important role in the onset of apoptosis [7-9].

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The present study was planned to investigate the effects of glutathione administration, known to possess antioxidant properties, on caspase-dependent apoptosis in baby hamster kidney (BHK-21) cell line with high glucose induced hyperglycemic conditions.

2. MATERIAL AND METHOD

2.1. Cell Culture

BHK-21 (Mesocricetus auratus, hamster, Syrian golden, ATCC, CCL-10) cell line was used as the cell material in the study. BHK-21 cells in RPMI-1640 (Capricorn) containing 5% FBS (Capricorn), 10% horse serum (Capricorn), 1% L-Glutamine (Capricorn), 1% penicillin / streptomycin (Capricorn) and 5% CO_2 was incubated at its growth culture in 95% humidity and 37°C.

2.2. Cytotoxicity (MTT Cell Viability) Test

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) cell viability tests were conducted to determine the GSH proliferation concentration and the half maximum inhibition concentrations (IC_{50}) for glucose the results of the test were summarized below (Table 1).

Table 1. Study Groups

Groups	Application
Control	22 mM glucose
High Glucose (HG)	285 mM glucose
GSH	250 µM
GSH+HG	250 µM GSH+285 mM glucose

2.3. Preparation of the Cellular Lysate

The study values (GSH: 250 µM and glucose: 285 mM) for the cell samples that would be used to determine the biochemical parameters were identified, and then, 350 µl suspension that included the medium was implanted in 12-well plates at 5×10^5 cells per well. Suspended cells were prepared after following 24 h of incubation, the cells treated with trypsin were removed by the freeze/thaw cycle (-80°C for 25 min and 37°C for 10 min) method and analyzed.

2.4. Biochemical Analysis

In the obtained cell culture lysate, the caspase 3, 8 and 9 activities and M30 (YH Biosearch Laboratory, Shangai China) and 8-OHdG (ADI-EKS-350; Enzo Life Science) content were determined with kit procedure.

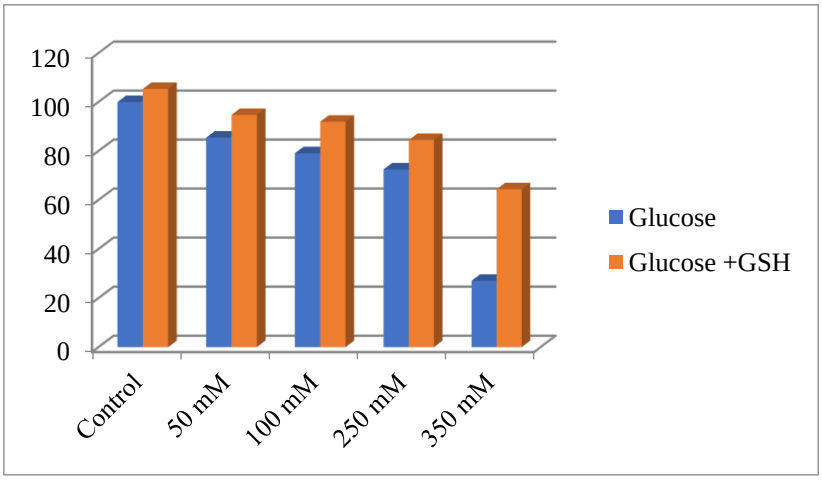
2.5. Statistical Analysis

Descriptive statistics for the scrutinized properties were expressed with mean, standard deviation, minimum and maximum values. For these properties, ANOVA tests were conducted to determine whether there was a difference between the groups (SPSS 22.0). Statistical significance level was accepted as $p<0.05\%$.

3. RESULTS

The control group was accepted as 100% vital and the results obtained after 24 hours in the MTT % viability test was performed for GSH in BHK-21 cell line and the beneficial proliferation dose (250 µM) was determined. MTT test was repeated on various doses of glucose and glucose + GSH (250 µM) administered BHK-21 cell line, and summarized in Figure 1. According to this test, it was determined that cellular viability increased in all glucose doses, after addition of GSH.

Figure1. MTT results of groups



To evaluate of the effect of GSH on M30, caspase 3, 8, 9 enzymes and 8-OHdG levels of high glucose condition were calculated. The study concentrations were prepared according to MTT test, as GSH beneficial proliferation dose (250 µM), and IC50 high glucose dose (285 mM) (Table 1).

The results obtained for the groups after the analysis of biochemical parameters are presented in Table 2.

Table 2. Study group M30, caspase 3, 8, 9 enzymes and 8-OHdG levels

Parameters	Control (S±SD)	HG (S±SD)	GSH (S±SD)	GSH+HG (S±SD)
M30 (IU/L)	122.396±7.299a	199.044±3.383b	112.505±14.358a	128.837±0.614a
Caspase 3 (ng/ml)	1.781±0.0536a	3.980±0.229b	1.993±0.049a	2.957±0.371c
Caspase 8 (ng/ml)	6.056±0.575a	11.169±1.878b	6.193±0.274a	8.492±0.215c
Caspase 9 (ng/ml)	6.169±0.334a	11.966±1.222b	6.012±0.371a	8.381±0.253c
8-OHdG (ng/ml)	2.067±0.669	1.994±0.840	1.532±0.312	1.521±0.332

The differences between the groups denoted with different letters are significant (p≤005)

It was observed that M30 levels were significantly higher in the high glucose treated group when compared to the control and GSH groups (p≤005) (Table 2). Caspase 3, 8 and 9 activities were higher in the high glucose group when compared to all other groups (P ≤005), and there was no significant difference between control and GSH groups, and although there was a significant decrease in the GSH + HG group when compared to the HG group (P ≤005), it was still significantly high when compared to the control (P ≤005) (Table 2). 8-OHdG levels were not change statistically.

4. DISCUSSIONS

Anti-diabetic preparations are commonly used in the treatment of diabetes. Furthermore, the uses of antioxidant agents as complementary and therapeutic agents against diabetic oxidative stress are recommended [10-12].

Glutathione (GSH), a significant antioxidant defense system, assists other antioxidants to fulfill their functions [13,14]. Previous studies demonstrated that administration of GSH in various doses and routes plays an important role in the prevention of diabetic complications [11,15].

Uncontrolled long-term hyperglycemia leads to microvascular/macrovascular complications. One of the most important complications is nephropathy. Combined administration of GSH with vitamins E and C was found to be more beneficial and these elements strengthen each other's action [16].

In the present study, the MTT results obtained with different doses of glucose and combined GSH (250 μM) administration to the cell line were analyzed. It was determined that cellular viability increased with each dose in combined administration of GSH, and cell viability levels of GHS+HG group were closer to the control group level. Similarly, Yur et al. [12] reported protective effects of GSH in a study where the effects of GSH on high glucose administration in the kidney cell line.

In some studies, it has been reported that the intracellular GSH level plays an important role in the initiation or maintenance of cellular apoptosis. High oxidative stress, insufficient antioxidant and glutathione are seen in diabetics. Advanced glycation end products (AGE) induce hydrogen peroxide and superoxide production due to GSH reduction and lead to oxidative stress [17,18].

M30 immunoreactivity is confined to the cytoplasm of apoptotic epithelial cells and expressed during early apoptosis [19]. In the present study, it was observed that M30 caspase 3, 8 and 9 enzyme levels significantly increased in the high glucose group when compared to the control and GSH groups and apoptosis was activated. A significant decrease was found in M30, caspase 3, 8, and 9 enzyme levels in the high glucose plus GSH (GSH+HG) group. This suggested that glutathione supported the inhibition of high glucose-induced apoptosis. However, it was concluded that the fact that it was still significantly higher when compared to the control was due to the GSH and especially the administration duration.

Assessment of 8-OHdG levels as a marker of oxidative DNA oxidation is important in monitoring the oxidative DNA damage, as commonly observed in diabetes, cardiovascular diseases and cancer [20]. Several researchers demonstrated that oxidative stress leads to DNA damage in experimental diabetes and in vitro studies [2,21]. It was suggested that preventive GSH administration against diabetes-induced renal failure and neuropathy suppressed the excretion of 8-OHdG in urine [22].

In the present study, it was observed that 8-OHdG levels were lower in GSH-treated groups, but the differences were not statistically significant. The use of GSH against diabetes induced nephropathy is considered beneficial against *in vivo* renal and neural complications. In a study where the potential benefits of GSH against diabetic renal failure and neuropathy were investigated, it was reported that GSH was effective on the reduction of urinary 8-OHdG levels [22].

In the current study, when analyzed with MTT results, it was observed that cellular death was induced by apoptosis. It is considered that the fact that there was no significant difference between the control and the GSH group could be regarded as a sign of the reliability of GSH administration. Thus, it was observed that GSH administration led to a significant decrease in high glucose induced apoptosis and did not result in a significant change in oxidative DNA damage. It was concluded that the effect of oxidative damage on high glucose induced apoptosis mechanism may be limited to the present doses and study duration.

CONFLICT INTEREST

The authors declare that there is no conflict interests any one or any firm.

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