Neurotoxicology 74 (2019) 196–202

Contents lists available at ScienceDirect

Neurotoxicology

journal homepage: www.elsevier.com/locate/neuro

Full Length Article

Nesfatin-1 protects PC12 cells against high glucose-induced cytotoxicity via inhibiting oxidative stress, autophagy and apoptosis

Simin Nazarnezhad[a], Majid Rahmati[b], Asghar Shayannia[b], Zahra Abbasi[c], Majid Salehi[a], Mehdi Khaksari[d,*]

[a] Tissue Engineering and Stem Cells Research Center, Shahroud University of Medical Sciences, Shahroud, Iran
[b] Department of Medical Biotechnology, School of Medicine, Shahroud University of Medical Science, Shahrod, Iran
[c] Student Research Committee, School of Medicine, Shahroud University of Medical Sciences, Shahroud, Iran
[d] School of Medicine, Shahroud University of Medical Sciences, Shahroud, Iran

ARTICLE INFO

Keywords:
Nesfatin-1
High glucose neurotoxicity
Reactive oxygen species
Autophagy
Apoptosis

ABSTRACT

Objective: Diabetic neuropathy (DN) is the most common complication of diabetes mellitus. It is thought that neuronal cell death which is mainly due to reactive oxygen species (ROS) overproduction in the cells is responsible for most symptoms of this disorder. Nesfatin-1 has identified recently as a novel endogenous neuropeptide which recent studies have shown that it may have a protective effect. Therefore, we postulated that Nesfatin-1 might adequately prevent from high glucose-induced cell injury via inhibition of apoptotic, autophagy, and ROS responses.

Methods: In this study, PC12 cells were pretreated with different concentrations of Nesfatin-1 (1–100 ng/ml) and then co-treated with Nesfatin-1 and glucose (125 mM) for 48 h, and downstream pathways then were evaluated to investigate ROS, apoptosis, and autophagy.

Results: Results of this study showed that Nesfatin-1 can not only inhibit from intracellular ROS overproduction-induced by high glucose in PC12 cells ($p < 0.0001$) but also reduce the apoptotic cell death in PC12 cells following high glucose exposure by increasing cell viability and reducing apoptotic rates ($p < 0.05$). Furthermore, Nesfatin-1 decreased the LC3-II levels by western blotting ($p < 0.0001$), which showed a reduction in autophagy.

Conclusion: These results support the idea that Nasfatin-1can protect PC12 cells against high glucose-induced cell injury by inhibition of apoptosis, autophagy and ROS production and can be considered as a potential drug for treatment of diabetic neuropathy.

1. Introduction

Diabetes mellitus is a prevalent disease around the world, which includes 1–2% of the population and causes high mortality and morbidity (Gray et al., 2000). Islet cell transplantation for insulin-dependent diabetes mellitus: perspectives from the present and prospects for the future (Singh et al., 2014), affecting more than 50% of diabetic individuals and causes problems in the sensory, motor, and autonomic nerves that ultimately lead to amputation (Zimmet et al., 2001; Dewanjee et al., 2018; Brock et al., 2014). Although hyperglycemia, as the main symptom of diabetes, plays a pivotal role in the progression of DN, the precise mechanisms involved are still not well-known.

The potential pathways involved in the development of DN include the activation of the polyol pathway (Gabbay, 2004), hexose amine (Kolm-Litty et al., 1998), advanced glycation end products (AGEs) (Brownlee et al., 1988), diacylglycerol/ protein kinase C (PKC) (Greene et al., 1985), oxidative stress (Johansen et al., 2005), autophagy (Osman et al., 2015), and inflammation (Bishnoi et al., 2011). Evidence suggest that oxidative stress is involved in all of these pathways (Brownlee, 2005; Edwards et al., 2008). An increase in glucose levels results in damage to the mitochondrial membrane and the respiratory chain, and consequently, it leads to production of high amounts of reactive oxygen species (ROS) (Allen et al., 2005; Vincent et al., 2002). Excessive amounts of ROS accelerate the damage of lipids, proteins and nucleic acids, which ultimately end up in the neural apoptosis (Shakeel, 2015; Lee, 2012). The damaged mitochondria membrane leaks mitochondrial cytochrome C into the cytoplasm, which activates the caspase-3 protein and nucleus fragmentation as well as Caspase-3-

* Corresponding author.
E-mail address: khaksari417@yahoo.com (M. Khaksari).

https://doi.org/10.1016/j.neuro.2019.07.001
Received 11 June 2019; Received in revised form 6 July 2019; Accepted 10 July 2019
Available online 16 July 2019
0161-813X/

dependent apoptosis (i.e., the internal pathway of apoptosis) in neurons (Shen et al., 2017). In addition, pathways involved in diabetic neuropathy can directly or indirectly stimulate inflammatory mediators such as TNF-α that can exacerbate diabetic neuropathy along with oxidative stress (Sandireddy et al., 2014).

Nesfatin-1, an 82 amino acid peptide, was first identified in 2006 by Jiang et al. (Jiang et al., 2015) which is composed as a result of post-translational modifications from the NUCB2 molecule in the presence of prohormone convertase 1/3 (PC) (Szlachcic et al., 2013). Nesfatin-1 is a multifunctional EF-hand motif containing Ca^{2+}-binding protein that was initially identified as a satiety molecule in the hypothalamus (Su et al., 2010). Nesfatin-1 has different effects on the nervous system and has recently been reported to have anti-oxidant (Jiang et al., 2015), anti-inflammatory (Özsavcí et al., 2011), and anti-apoptotic (Tan et al., 2015) properties which could ameliorate the symptoms of diabetic neuropathy. Although its receptor has not identified yet, it is supposed to be a G-protein coupled receptor (GPCR) (Shen et al., 2017). It was also reported that nesfatin-1 peptide can penetrate the blood-brain barrier by a non-saturable mechanism, which provides the possible therapeutic permission for nesfatin-1 as a pharmacological transfer agent to the central nervous system(Pan et al., 2007).The PC12 or pheochromocytoma cell line originates from neural crest in rats and has been found to have neural properties. Hence, it is widely used to study a variety of neurological models, including diabetic neuropathy (Chen et al., 2016). These cells mimic from hyperglycemia effects for diabetic neuropathy induction and are widely used as an *in vitro* model for DN (Shahveisi et al., 2014; Zhao et al., 2013).

Since oxidative stress, apoptosis, and inflammation are the major pathways which lead to the progression of DN, and currently, there is no effective treatment for this disorder, it is essential to search a promising treatment that can suppress symptoms and complications associated with the pathogenesis of the disease.

2. Materials and methods

2.1. Materials

RPMI-1640 medium and fetal bovine serum (FBS) were purchased from Gibco (Paisley, UK). Penicillin/streptomycin and phosphate-buffered saline (PBS) were obtained from DNA biotech. 3-(4,5-Dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT), Nesfatin-1, Fluorometric Intracellular ROS Kit (Catalog Number MAK143), and autophagy assay kit (Catalog Number MAK138) were all purchased from Sigma-Aldrich (USA). Primary antibodies against LC3-II (microtubule-associated protein 1A/1B-light chain) and β-actin and horseradish peroxidase-conjugated (m-IgGκ BP-HRP) secondary antibody were purchased from Santa Cruz Biotechnology (Santa Cruz, UK, USA). Apoptosis detection kit was obtained from Invitrogen (USA).

2.2. Cell culture

The PC12 cell line was purchased from Bou'ali institute of Mashhad (Mashhad, Iran). Cells were maintained in RPMI-1640 medium containing 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (as complete media) at 37 °C and 5% CO_2 incubator. The medium was changed every 2 days and cells were subcultured when they were about 70–75% confluent.

2.3. Nesfatin-1 treatment

PC12 cells were prepared with different concentrations of Nesfatin-1 including 1, 5, 10, 50, and 100 ng/ml. We found that 5 ng/ml was the most protective concentration. PC12 cells were pretreated with 5 ng/ml Nesfatin-1 in culture medium for about 1 h, and then complete media containing high glucose (125 mM) was added to the desired groups as indicated in Fig. 1.

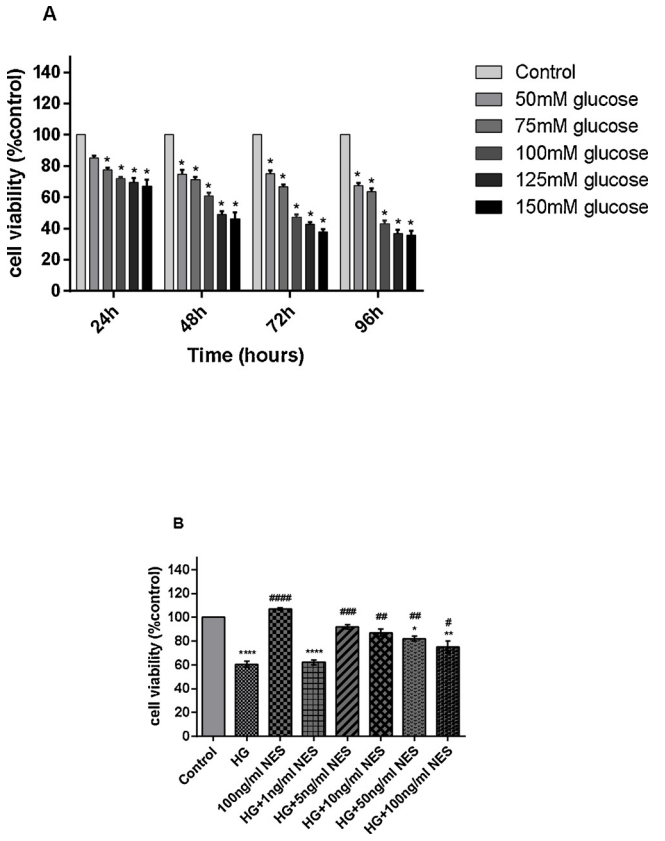


Fig. 1. (A) Effect of HG administration for 24, 48, 72, and 96 h on neuronal PC12 cell viability. (B) Effect of NES (Nesfatin-1) on HG-induced cell toxicity in PC12 cells for 48 h. Control, cells were incubated with control medium; HG, cells were treated with HG (125 mM) medium; 100 ng/ml NES, cells were treated with Nesfatin-1 (100 ng/ml) in control medium; HG + NES, cells were pretreated with different concentrations of Nesfatin-1 (1, 5, 10, 50, and 100 ng/ml) for 1 h and then treated with HG (125 mM) medium. Cell viability was determined by MTT assay. HG administration decreased cell viability to 50%. Nesfatin-1 could increase cell viability. (*) $p < 0.05$ compared with control and (#) $p < 0.05$ compared with HG, (**) $p < 0.01$ compared with control and (##) $p < 0.01$ compared with HG, (###) $p < 0.001$ compared with HG, and (****) $p < 0.0001$ compared with control and (####) $p < 0.0001$ compared with HG.

2.4. MTT assay

Cell viability and the most cytoprotective concentration of Nesfatin-1 was determined by MTT assay. In brief, PC12 cells were plated in 96-well culture plates at a density of 10^4 cells/well. After 24 h, the cells were incubated with different concentrations of glucose (25 mM as normal glucose (Shahveisi et al., 2014; Chen et al., 2016) and 50, 75, 100, 125, and 150 mM as high glucose concentrations) for different time points (24, 48, 72, and 96 h). To exclude the role of osmotic toxicity for the HG condition, we refer to Koshimura (Koshimura et al., 2002) et al. and Tie et al. (Tie et al., 2008) who showed that neurotoxicity of glucose is not related to osmolarity in PC12 cells. After desired time of incubation with Nesfatin-1 and high glucose, 10 µl/well MTT (final concentration of 0.5 mg/ml of MTT in medium) was added and cells were incubated for 3 h at 37 °C incubator. After that, the medium was removed carefully. The cells and dye crystals then were solubilized by adding 100 µl DMSO. The absorbance of each well was detected at 570 nm and 690 nm using a microplate reader and cell viability was determined based on the control group (Fig. 1A).

2.5. Measurement of ROS

To investigate oxidative stress in high glucose-induced toxicity in PC12 cells, we used a Fluorometric Intracellular ROS kit (Sigma-Aldrich, USA) according to the manufacturer's instrument. In brief, cells at the density of 10^4 cells/well were seeded in 96-well black plates with clear bottom. After 24 h, cells were pretreated with Nesfatin-1 and master reaction mix and incubated in 37 °C for 1 h. Then, 20 µl/well of 11x high glucose (125 mM) was added and incubated for 48 h at 37 °C. Finally, the fluorescence intensity at λ_{ex} = 490 nm and λ_{em} = 520 nm was measured by a microplate reader.

2.6. Annexin V and propidium iodide staining

Apoptosis was detected using an Annexin V fluorescein isothiocyanate (FITC)/propidium iodide (PI) apoptosis detection kit according to the manufacturer's instruction. Briefly, the cells were seeded in 6-well culture plates at the density of 1.5×10^5 cells/well. After treatment, cells were harvested and washed twice with phosphate buffer saline (PBS) and then resuspended in binding buffer followed by addition of Annexin V-FITC to microtubes and incubated for 10–15 minutes in the dark. Cells were washed by binding buffer and PI was added to microtubes. Then, cells were analyzed using flow cytometry. Ultimately, percentage of apoptotic [Annexin V-FITC (+)/PI (+)] and necrotic [Annexin V (−)/PI(+)] cells were determined based on the control cells [Annexin V(−)/PI(−)].

2.7. Determination of autophagy

Autophagy was measured using an Autophagy Assay Kit (Sigma-Aldrich, USA) according to the manufacturer's instrument. In brief, PC12 cells were seeded in 96-well black plates with clear bottom and pretreated with Nesfatin-1 followed by incubation in high glucose medium for 48 h in 37 °C. After that, the autophagosome detection reagent was added to each well and incubated at 37 °C for 15 min. The cells then were washed by the wash buffer for four times, and the fluorescence intensity was measured at λ_{ex} = 360 nm and λ_{em} = 520 nm.

2.8. Western blotting

PC12 cells were harvested and lysed using a cocktail of lysis buffer and a protease inhibitor. The concentration of total protein was quantified using the BCA assay. Eventually, 25 µg of the protein of each group was separated using SDS-PAGE and electrotransferred onto PVDF membrane. After blocking with 3% BSA, membranes were incubated overnight with primary antibodies LC3-II (1:500) and β-actin (1:1000) at 4 °C. After washing, membranes were incubated with an HRP-conjugated secondary antibody (1:5000) for 2 h at room temperature. The blots then were detected using the ECL detection kit (Santa Cruz, UK). The intensities of the bands were normalized to the actin band using Image J software (National Institutes of Health, Bethesda, MD).

2.9. Statistical analysis

All the data were presented as mean ± standard error of the mean and statistical evaluation of the data was performed by one-way analysis of variance (ANOVA). A value of $p < 0.05$ was considered to indicate a statistically significant difference. For statistical analysis, GraphPad Prism™ 6.07 was used (GraphPad Software; San Diego, CA). Asterisks indicate statistical differences between the treatment and control condition (****$p < 0.0001$, ***$p < 0.001$, **$p < 0.01$, and *$p < 0.05$), # shows statistical differences between the treatment and HG condition (####$p < 0.0001$, ###$p < 0.001$, ##$p < 0.01$, and #$p < 0.05$).

3. Results

3.1. Protective effect of Nesfatin-1 on high glucose-induced neuropathy in PC12 cells

To investigate the impact of high glucose on cell viability, PC12 cells were incubated with 25 mM glucose as normal glucose, and 50, 75, 100, 125, and 150 mM glucose as high glucose for 24, 48, 72, and 96 h as mentioned above. The results of the MTT assay showed a reduction in cell viability up to 50% after 48 h incubation with 125 mM glucose ($p < 0.05$) compared with control (25 mM) cells (Fig. 1A). Hence, 125 mM glucose was chosen for the following experiments.

To investigate whether Nesfatin-1 could antagonize HG-induced cytotoxicity, PC12 cells were pretreated with a corresponding concentration of Nesfatin-1 (1–100 ng/ml) for 1 h, followed by exposure to 125 mM glucose (as high glucose) for 48 h. As shown in Fig. 1B, cell viability increased significantly with 5 ng/ml Nesfatin-1 pretreatment ($p < 0.0001$). Besides, 100 ng/ml Nesfatin-1 did not show any cytotoxicity to PC12 cells (Fig. 1B). These results implied that 5 ng/ml Nesfatin-1 could protect PC12 cells from high glucose-induced cytotoxicity (Fig. 1B). Thus, 5 ng/ml Nesfatin-1 was chosen for further experiments.

3.2. Antioxidant effect of Nesfatin-1 on high glucose-induced ROS generation

After 48 h incubation of PC12 cells with 125 mM glucose (as high glucose), the fluorescence intensity increased significantly ($p < 0.001$). As shown in Fig. 2, pretreatment of the cells with Nesfatin-1 markedly reduced the oxidative stress generated by high glucose ($p < 0.0001$).

3.3. Anti-apoptotic and anti-necrotic effects of Nesfatin-1 against high glucose-induced apoptosis

The results of the MTT assay revealed that pretreatment with 5 ng/ml Nesfatin-1 increased cell viability. So, we chose and examined the anti-apoptotic effect of 5 ng/ml Nesfatin-1 on high glucose-induced apoptosis in PC12 cells. We found that pretreatment with 5 ng/ml Nesfatin-1 prevented from high glucose-induced cytotoxicity and increased cell viability. As shown in Fig. 3, apoptotic cells generation by high glucose incubation was also attenuated after pretreatment with

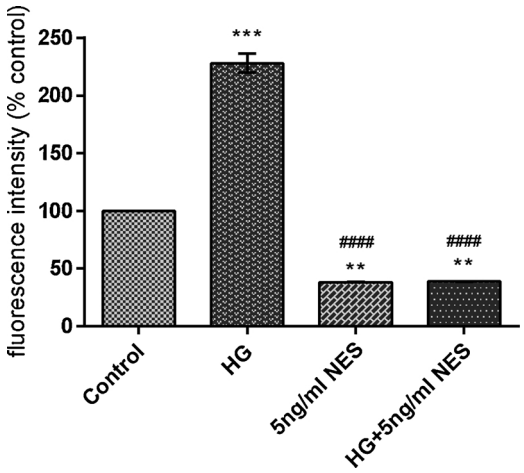
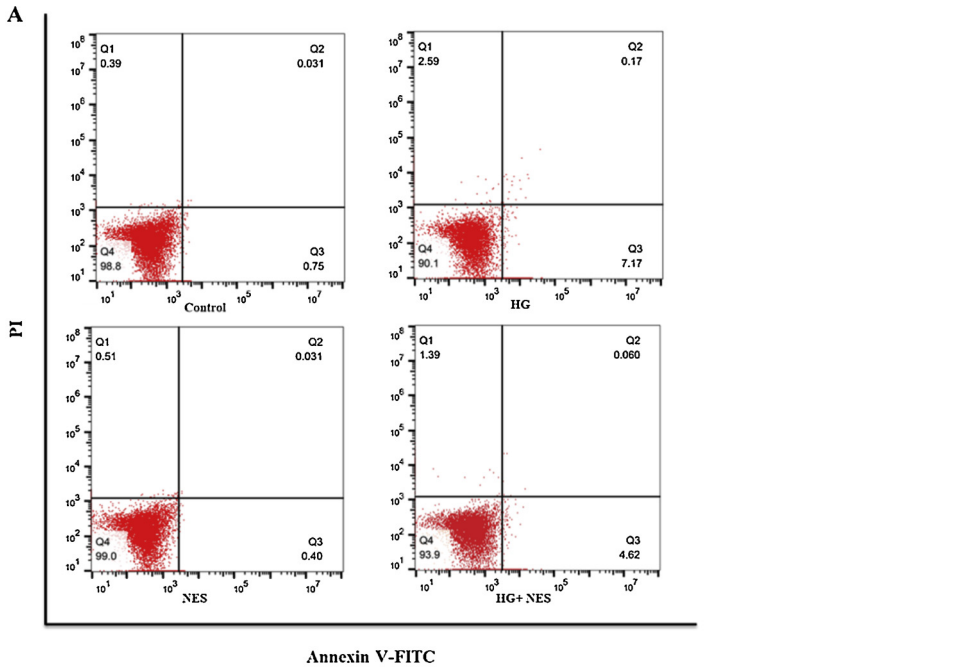


Fig. 2. Effect of Nesfatin-1 on HG-induced oxidative stress in neuronal PC12 cells. PC12 cells were pretreated with 5 ng/ml Nesfatin-1 and fluorescent ROS reagent for 1 h, followed by exposure to HG for 48 h. Fluorescence intensity is expressed according to control percentage. (**) $p < 0.01$ compared with control, (***) $p < 0.001$ compared with control, and (###) $p < 0.001$ compared with HG.



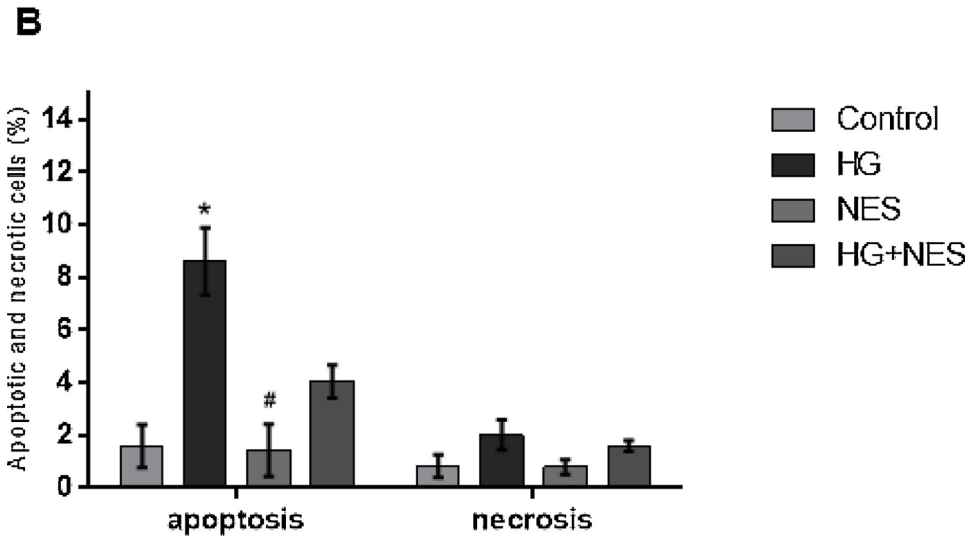


Fig. 3. Effect of Nesfatin-1 on HG-induced apoptosis in PC12 cells. Cells were pretreated with 5 ng/ml Nesfatin-1 for 1 h, followed by exposure to 125 mM glucose for 48 h. (A) The cells were stained with FITC-Annexin V/PI and analyzed by flow cytometry to determine the population of cells in early and late apoptosis. (B) Quantitative data for the percentage of apoptotic and necrotic cells. (*) $p < 0.05$ compared with control and (#) $p < 0.01$ compared with HG.

5 ng/ml Nesfatin-1 ($p < 0.05$).

3.4. Effect of Nesfatin-1 on autophagy in high glucose-induced cytotoxic PC12 cells

As autophagy is mentioned to be increased in diabetic neuropathy (Dewanjee et al., 2018), we measured the protein level of LC3-II, an autophagy marker protein (Barth et al., 2010; Hansen and Johansen, 2011). The protein levels of LC3-II were increased in high glucose-treated cells (125 mM glucose) compared with control cells (25 mM glucose) ($p < 0.0001$), and this was reversed by Nesfatin-1 treatment ($p < 0.0001$). To confirm this claim, we used an autophagy assay kit. As indicated in Fig. 4, autophagosome formation increased in high glucose-treated PC12 cells ($p < 0.05$) compared with control cells, while pretreatment of these cells with Nesfatin-1 diminished the fluorescent intensity of autophagosomes ($p < 0.05$).

4. Discussion

In the present study, we demonstrated that Nesfatin-1 protects PC12 cells against high glucose-induced cell injury, and further elucidated that the cytoprotective effects of Nesfatin-1 was mediated through attenuation of ROS overproduction, which then inhibited apoptosis induced by mitochondrial dysfunction.

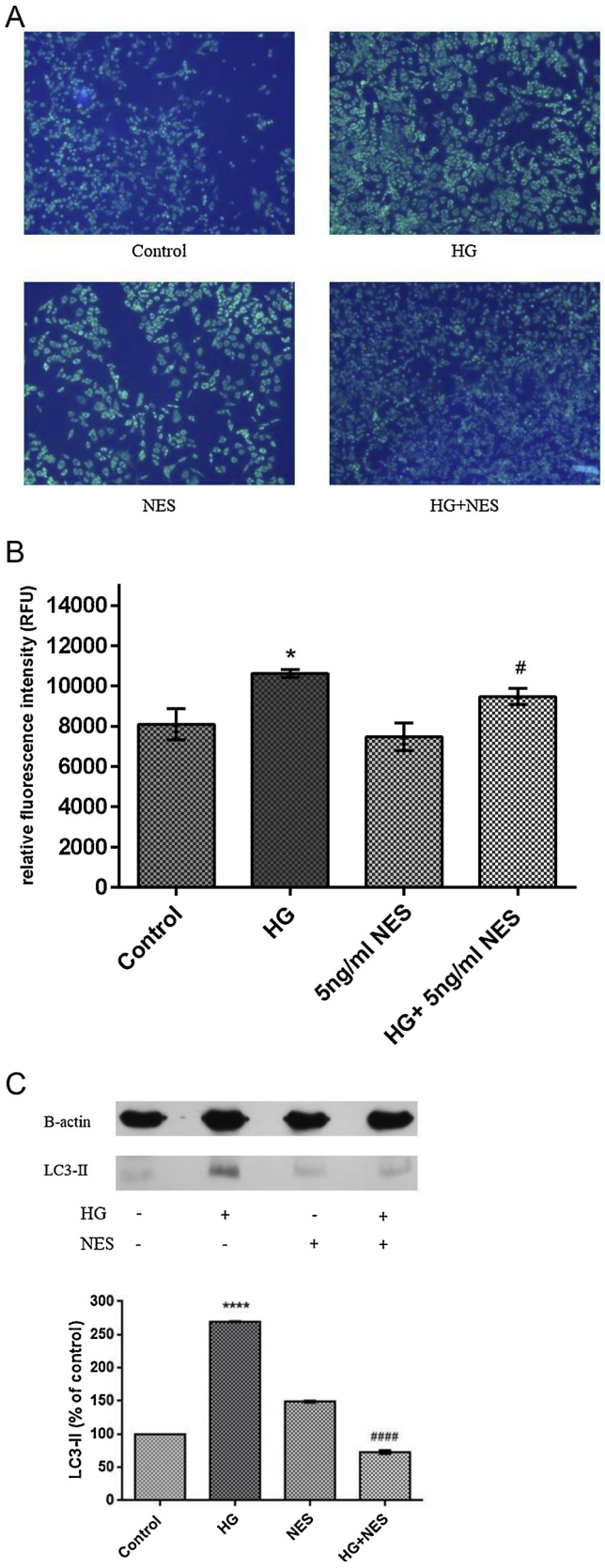


Fig. 4. Effect of Nesfatin-1 on HG-induced autophagy in PC12 cells. (A) Fluorescence microphotographs. Control, cells were treated with control medium; HG, cells were treated with high glucose (125 mM) medium; NES, cells were treated with Nesfatin-1 (5 ng/ml); and HG + NES; cells were pre-treated with Nesfatin-1 followed by exposure to high glucose medium. The fluorescence intensity was increased in the HG group. (B) The fluorescent intensity of autophagosomes of different groups. Relative fluorescent unit (RFU). (C) Protein levels of LC3-II were determined by western blot. HG administration enhanced autophagy by increasing the LC3-II expression, while pretreatment with Nesfatin-1 reversed this effect. β-actin was used as a positive control. The bands were quantified by Image J software. (*) $p < 0.05$ compared with control, (#) $p < 0.05$ compared with HG, (****) $p < 0.0001$ compared with control, and (####) $p < 0.0001$ compared with HG.

It is well-known that hyperglycemia plays a critical role in the progression and development of diabetic neuropathy and causes damages to neurons (Shahveisi et al., 2014; Katagi et al., 2014). Although the numerous studies have been done in this field, the precise underlying mechanism(s) of diabetic neuropathy have not been clarified.

Although the precise neuroprotective signaling pathways of Nesfatin-1 on dopaminergic neurons have not been clarified, several studies have shown its anti-oxidant (Jiang et al., 2015; Kolgazi et al., 2015), anti-inflammatory (Solmaz et al., 2016), and anti-apoptotic (Shen et al., 2017; Özsavcí et al., 2011) effects in different cell lines and animal models. Using MTT assay, our data showed that Nesfatin-1 effectively protected PC12 cells from high glucose-induced cell death. The results of our study suggest that Nesfatin-1 could protect cholinergic cells against hyperglycemia-induced diabetic neuropathy. According to available evidence, elevated levels of glucose result in reactive oxygen species (ROS) and free radicals generation (Kumar et al., 2017). Hyperglycemia-induced oxidative-nitrosative stress induces inflammation and neurodegeneration via augmented tuberous sclerosis complex-2 (TSC-2) activation in neuronal cells (Sharifi et al., 2007). It is well documented that the induction of oxidative stress is one of the primary mechanisms for the development of diabetic neuropathy and other neurodegenerative disorders. It is also clearly apparent that oxidative stress leads to apoptosis initiation in many cell lines (Bournival et al., 2012). Therefore, a substance that can suppress oxidative stress caused by high levels of glucose and consequently prevent the cell death, can act as a potential drug to reduce the symptoms and underlying mechanism(s) of these disorders.

Hong Jiang et al. (2015) reported that Nesfatin-1 attenuated ROS generation and accumulation via regulating mitochondrial dysfunction by restoring the mitochondrial respiratory chain complex I activity as well as the mitochondrial transmembrane potential. They also demonstrated that Nesfatin-1 could protect against rotenone neurotoxicity by inhibiting the activation of caspase-3 (Tan et al., 2015). Another study showed that Nesfatin-1 protects dopaminergic neurons against MPP + /MPTP-induced neurotoxicity through the C-Raf–ERK1/2-dependent anti-apoptotic pathway (Shen et al., 2017). Nesfatin-1 has been reported to inhibit active caspase-3 expression and it has decreased the Bax/Bcl-2 ratio against renal ischemia/reperfusion injury in rats which are involved in the intrinsic pathway of apoptosis (Jiang et al., 2015). Consistent with our findings, Nesfatin-1 could rescue PC12 cells from high glucose-induced cell toxicity.

Based on *in vivo* studies on the diabetic mouse, intravenous administration of Nesfatin-1 had anti-hyperglycemic effects that its function was dose-, time-, and insulin-dependent (Su et al., 2010). Indeed, Nesfatin-1 interact with signaling pathways of insulin so that Nesfatin-1 administration led to increased insulin secretion from pancreatic β-cells (Anık et al., 2014). Nesfatin-1 is an insulinotropic peptide that effects on pancreatic β-cells and increases insulin secretion and function. Therefore, it plays a critical role in glucose metabolism (Gonzalez et al., 2011b).

Recent evidence showed the anti-inflammatory properties of Nesfatin-1 by downregulation of different inflammatory cytokines, including nuclear factor kappa-B (NF-κB), tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6) after traumatic brain injury in rats (Tang et al., 2012). However, it has been mentioned that

Nesfatin-1 has apoptotic effects in adrenocortical cells (Ramanjaneya et al., 2015). In this study, we investigated the anti-oxidant and anti-apoptotic effects of Nesfatin-1 on cells with high glucose-induced cytotoxicity for the first time. We indicated that HG promoted ROS generation markedly, while it was reversed by pretreatment with Nesfatin-1.

Autophagy is a cellular process that destroys old and damaged proteins and organelles to recycle their products for further use. It also degrades apoptotic cells. So, it protects cells against stressors. On the other hand, if the damage to the cell is too high, autophagy stimulates another kind of cell death which is called type 2 programmed cell death. Hence, autophagy is a cellular process that can cause both cell survival and death, depending on cellular condition and environment. Microtubule-associated protein1 light chain3 (MAP-LC3) is a commonly used marker for autophagy. LC3-II is the leading product resulting from post-translational modifications of pro-LC3 protein which becomes lapidated and tightly bound to autophagosomal membranes (Katagi et al., 2014). There is contradictory evidence to determine the role of autophagy in diabetic neuropathy. For example, in a study using human SH-SY5Y cells and serum of patients with diabetic neuropathy, the expression of LC3-II increased significantly (Gonzalez et al., 2011a), while Another study reported decreased autophagosome formation by reduced LC3-II and Beclin-1 expression in hyperglycemic condition (Yerra and Kumar, 2017). We found that pretreatment of PC12 cells with Nesfatin-1 prevented from increased autophagy by high glucose.

Our results revealed that high glucose increased the formation of autophagosomes through the immunofluorescence reaction, as well as increased expression of LC3-II in PC12 cells, while their pretreatment with Nesfatin-1 prevented from this increase. We also found that pretreatment of PC12 cells with 5 ng/ml Nesfatin-1 for one hour, followed by exposure to HG (125 mM glucose) for 48 h prevented from the increased oxidative stress and apoptosis.

Since the receptor of Nesfatin-1 has not been identified yet, the precise underlying mechanisms of its neuroprotective effects on dopaminergic cells have not been determined. However, based on previous studies it is evident that Nesfatin-1 prevented increased apoptosis in neurotoxic cells by inhibiting the ROS generation (Shen et al., 2017). Our results showed that elevated levels of glucose led to increased ROS generation in PC12 cells while pretreatment of these cells with 5 ng/ml Nesfatin-1 for one hour reduced ROS generation.

5. Conclusion

In conclusion, we demonstrated a protective effect of Nesfatin-1 on high glucose-induced cell injury in PC12 cells. Pretreatment with Nesfatin-1 prevented from oxidative stress, apoptosis, and autophagy in high glucose-treated PC12 cells. These results suggest a therapeutic potential of Nesfatin-1 against high glucose-induced cell death. However, further studies are required to investigate the precise mechanisms and functions of Nesfatin-1 on the nervous system.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The present study was supported by Shahroud University of Medical Sciences as an MSc Thesis, Shahroud, Iran [Grant No. 9686].

References

Allen, D.A., Yaqoob, M.M., Harwood, S.M., 2005. Mechanisms of high glucose-induced apoptosis and its relationship to diabetic complications. J. Nutr. Biochem. 16, 705–713.
Anık, A., Çatlı, G., Abacı, A., Küme, T., Bober, E., 2014. Fasting and postprandial levels of a novel anorexigenic peptide nesfatin in childhood obesity. J. Pediatr. Endocrinol. Metab. 27, 623–628.
Barth, S., Glick, D., Macleod, K.F., 2010. Autophagy: assays and artifacts. J. Pathol. 221, 117–124.
Bishnoi, M., Bosgraaf, C.A., Abooj, M., Zhong, L., Premkumar, L.S., 2011. Streptozotocin-induced early thermal hyperalgesia is independent of glycemic state of rats: role of transient receptor potential vanilloid 1 (TRPV1) and inflammatory mediators. Mol. Pain 7, 52.
Bournival, J., Francoeur, M.-A., Renaud, J., Martinoli, M.-G., 2012. Quercetin and sesamin protect neuronal PC12 cells from high-glucose-induced oxidation, nitrosative stress, and apoptosis. Rejuvenation Res. 15, 322–333.
Brock, C., Søfteland, E., Frøkjær, J., Drewes, A.M., Nielsen, L., 2014. Associations between sensorimotor, autonomic and central neuropathies in diabetes mellitus. J. Diabetes Metab. 5.
Brownlee, M., 2005. The pathobiology of diabetic complications: a unifying mechanism. Diabetes 54, 1615–1625.
Brownlee, M., Cerami, A., Vlassara, H., 1988. Advanced glycosylation end products in tissue and the biochemical basis of diabetic complications. N. Engl. J. Med. 318, 1315–1321.
Chen, M., Zheng, H., Wei, T., Wang, D., Xia, H., Zhao, L., Ji, J., Gao, H., 2016. High glucose-induced PC12 cell death by increasing glutamate production and decreasing methyl group metabolism. Biomed Res. Int. 2016.
Dewanjee, S., Das, S., Das, A.K., Bhattacharjee, N., Dihingia, A., Dua, T.K., Kalita, J., Manna, P., 2018. Molecular mechanism of diabetic neuropathy and its pharmacotherapeutic targets. Eur. J. Pharmacol. 833, 472–523.
Edwards, J.L., Vincent, A.M., Cheng, H.T., Feldman, E.L., 2008. Diabetic neuropathy: mechanisms to management. Pharmacol. Ther. 120, 1–34.
Gabbay, K.H., 2004. Aldose reductase inhibition in the treatment of diabetic neuropathy: where are we in 2004? Curr. Diab. Rep. 4, 405–408.
Gonzalez, C.D., Lee, M.-S., Marchetti, P., Pietropaolo, M., Towns, R., Vaccaro, M.I., Watada, H., Wiley, J.W., 2011a. The emerging role of autophagy in the pathophysiology of diabetes mellitus. Autophagy 7, 2–11.
Gonzalez, R., Reingold, B.K., Gao, X., Gaidhu, M.P., Tsushima, R.G., Unniappan, S., 2011b. Nesfatin-1 exerts a direct, glucose-dependent insulinotropic action on mouse islet β-and MIN6 cells. J. Endocrinol. 208, R9–R16.
Gray, D.W., Titus, N., Badet, L., 2000. Islet cell transplantation for insulin-dependent diabetes mellitus: perspectives from the present and prospects for the future. Expert Rev. Mol. Med. 2, 1–28.
Greene, D.A., Lattimer, S., Ulbrecht, J., Carroll, P., 1985. Glucose-induced alterations in nerve metabolism: current perspective on the pathogenesis of diabetic neuropathy and future directions for research and therapy. Diabetes Care 8, 290–299.
Hansen, T.E., Johansen, T., 2011. Following autophagy step by step. BMC Biol. 9, 39.
Jiang, G., Wang, M., Wang, L., Chen, H., Chen, Z., Guo, J., Weng, X., Liu, X., 2015. The Protective effect of nesfatin-1 against renal ischemia–reperfusion injury in rats. Ren. Fail. 37, 882–889.
Johansen, J.S., Harris, A.K., Rychly, D.J., Ergul, A., 2005. Oxidative Stress and the use of antioxidants in diabetes: linking basic science to clinical practice. Cardiovasc. Diabetol. 4, 5.
Katagi, M., Terashima, T., Okano, J., Urabe, H., Nakae, Y., Ogawa, N., Udagawa, J., Maegawa, H., Matsumura, K., Chan, L., 2014. Hyperglycemia induces Abnormal Gene Expression in hematopoietic stem cells and their progeny in diabetic neuropathy. FEBS Lett. 588, 1080–1086.
Kolgazi, M., Cantali-Ozturk, C., Deniz, R., Ozdemir-Kumral, Z.N., Yuksel, M., Sirvanci, S., Yeğen, B.C., 2015. Nesfatin-1 alleviates gastric damage via direct antioxidant mechanisms. J. Surg. Res. 193, 111–118.
Kolm-Litty, V., Sauer, U., Nerlich, A., Lehmann, R., Schleicher, E., 1998. High glucose-induced transforming growth factor beta1 production is mediated by the hexosamine pathway in porcine glomerular mesangial cells. J. Clin. Invest. 101, 160–169.
Koshimura, K., Tanaka, J., Murakami, Y., Kato, Y., 2002. Involvement of nitric oxide in glucose toxicity on differentiated PC12 cells: prevention of glucose toxicity by tetrahydrobiopterin, a cofactor for nitric oxide synthase. Neurosci. Res. 43, 31–38.
Kumar, P., Raman, T., Swain, M.M., Mishra, R., Pal, A., 2017. Hyperglycemia-induced oxidative-nitrosative stress induces inflammation and neurodegeneration via augmented tuberous sclerosis complex-2 (TSC-2) activation in neuronal cells. Mol. Neurobiol. 54, 238–254.
Lee, K.W.K., 2012. High Glucose-Induced Ros Production is Mediated by C-Src in Mesangial Cells.
Osman, A.A., Dahlin, L.B., Thomsen, N.O., Mohseni, S., 2015. Autophagy in the posterior interosseous nerve of patients with type 1 and type 2 diabetes mellitus: an ultrastructural study. Diabetologia 58, 625–632.
Özsavcí, D., Erşahin, M., Şener, A., Özakpinar, Ö.B., Toklu, H.Z., Akakín, D., Şener, G., Yeğen, B.Ç., 2011. The novel function of nesfatin-1 as an anti-inflammatory and antiapoptotic peptide in subarachnoid hemorrhage–induced oxidative brain damage in rats. Neurosurgery 68, 1699–1708.
Pan, W., Hsuchou, H., Kastin, A.J., 2007. Nesfatin-1 crosses the blood–brain barrier without saturation. Peptides 28, 2223–2228.
Ramanjaneya, M., Tan, B.K., Rucinski, M., Kawan, M., Hu, J., Kaur, J., Patel, V.H., Malendowicz, L.K., Komarowska, H., Lehnert, H., 2015. Nesfatin-1 inhibits proliferation and enhances apoptosis of human adrenocortical H295R cells. J. Endocrinol. 226, 1–11.
Sandireddy, R., Yerra, V.G., Areti, A., Komirishetty, P., Kumar, A., 2014. Neuroinflammation and oxidative stress in diabetic neuropathy: futuristic strategies based on these targets. Int. J. Endocrinol. 2014.
Shahveisi, K., Mousavi, S.H., Hosseini, M., Rad, A.K., Jalali, S.A., Rajaei, Z., Sadeghnia,

H.R., Hadjzadeh, M.-A.-R., 2014. The role of local renin-angiotensin system on high glucose-induced cell toxicity, apoptosis and reactive oxygen species production in PC12 cells. Iran. J. Basic Med. Sci. 17, 613.
Shakeel, M., 2015. Recent Advances in understanding the role of oxidative stress in diabetic neuropathy. Diabetes Metab. Syndr. Clin. Res. Rev. 9, 373–378.
Sharifi, A.M., Mousavi, S.H., Farhadi, M., Larijani, B., 2007. Study of high glucose-induced apoptosis in PC12 cells: role of bax protein. J. Pharmacol. Sci. 104, 258–262.
Shen, X.-L., Song, N., Du, X.-X., Li, Y., Xie, J.-X., Jiang, H., 2017. Nesfatin-1 protects dopaminergic neurons against MPP+/MPTP-induced neurotoxicity through the C-Raf–ERK1/2-dependent anti-apoptotic pathway. Sci. Rep. 7, 40961.
Singh, R., Kishore, L., Kaur, N., 2014. Diabetic peripheral neuropathy: current perspective and future directions. Pharmacol. Res. 80, 21–35.
Solmaz, A., Bahadır, E., Gülçiçek, O.B., Yiğitbaş, H., Çelik, A., Karagöz, A., Özsavcı, D., Şirvancı, S., Yeğen, B.Ç., 2016. Nesfatin-1 improves oxidative skin injury in normoglycemic or hyperglycemic rats. Peptides 78, 1–10.
Su, Y., Zhang, J., Tang, Y., Bi, F., Liu, J.-N., 2010. The novel function of nesfatin-1: anti-hyperglycemia. Biochem. Biophys. Res. Commun. 391, 1039–1042.
Szlachcic, A., Sliwowski, Z., Krzysiek-Maczka, G., Majka, J., Surmiak, M., Pajdo, R., Drozdowicz, D., Konturek, S.J., Brzozowski, T., 2013. New satiety hormone nesfatin-1 protects gastric mucosa against stress-induced injury: mechanistic roles of prostaglandins, nitric oxide, sensory nerves and vanilloid receptors. Peptides 49, 9–20.
Tan, Z., Xu, H., Shen, X., Jiang, H., 2015. Nesfatin-1 antagonized rotenone-induced neurotoxicity in MES23. 5 dopaminergic cells. Peptides 69, 109–114.
Tang, C.-H., Fu, X.-J., Xu, X.-L., Wei, X.-J., Pan, H.-S., 2012. The anti-inflammatory and anti-apoptotic effects of nesfatin-1 in the traumatic rat brain. Peptides 36, 39–45.
Tie, L., Xu, Y., Lin, Y.-H., Yao, X.-H., Wu, H.-L., Li, Y.-H., Shen, Z.-F., Yu, H.-M., Li, X.-J., 2008. Down-regulation of brain-pancreas relative protein in diabetic rats and by high glucose in PC12 cells: prevention by calpain inhibitors. J. Pharmacol. Sci. 106, 28–37.
Vincent, A.M., Brownlee, M., Russell, J.W., 2002. Oxidative Stress and Programmed Cell Death in Diabetic Neuropathy.
Yerra, V.G., Kumar, A., 2017. Adenosine monophosphate-activated protein kinase abates hyperglycaemia-induced neuronal injury in experimental models of diabetic neuropathy: Effects on mitochondrial biogenesis, autophagy and neuroinflammation. Mol. Neurobiol. 54, 2301–2312.
Zhao, S., Zhang, L., Xu, Z., Chen, W., 2013. Neurotoxic effects of iron overload under high glucose concentration. Neural Regen. Res. 8, 3423.
Zimmet, P., Alberti, K., Shaw, J., 2001. Global and societal implications of the diabetes epidemic. Nature 414, 782.