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Effects of beta-1,3-D glucan on systemic bortezomib treated rat pancreas

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Abstract

Bortezomib, selective inhibitor of the 26S proteasome, is used for treatment of some types of cancer and immunosuppressive therapies. B-1,3-(D)-glucan, a synthetic antioxidant is used complementary medical treatment for human. This study was conducted to investigate the effects of the antioxidant Beta-1,3-D glucan on rat pancreas treated with systemic bortezomib. In the study, 36 Sprague-Dawley adult male rats were divided into four groups: control (C), bortezomib (BZ), β-1, 3-D-glucan (BD) and bortezomib + β-1,3- (D) –glucan (BZ+BD). Each group was divided into two subgroups (48 or 72 hours), depending on the time of scarification. After experiments, immunohistochemical, stereological and histopathological changes in all rat pancreatic tissues were examined. It was determined increased degenerative, vacuolated serous acini cells and inflammatory cell infiltrations in the groups of BZ and BZ+BG. In immunohistochemical analysis, densities of insulin positive cells were decreased in the groups of BZ and BZ+BG. Furthermore, in stereological mean volume of serous acinus analysis, significantly increases were detected in the groups of BZ and BZ+BG ($p<0.05$). BZ treatment had the detrimental effects on pancreas tissues. Also, administration of BG was insufficient to prevent injury induced by BZ treatment in the pancreas tissues.

Keywords: bortezomib, beta glucan, pancreas, insulin, oxidative stress

1. Introduction

Proteasomes are multi-catalytic enzyme complexes that reduce oxidized and misfolded proteins (1, 2). The ubiquitin-proteasome pathway produced by proteasomes is responsible for misfolding and degradation of intracellular proteins that control processes such as cell proliferation, cell cycle, transcription, and apoptosis. Many studies showed that the proteasome system prevented the post-translational changes and accumulation of misfolded/oxidized protein induced by oxidative stress (2). The uncontrolled and rapid proliferation of cancer cells is a major problem. Inhibiting the proteasome system provides an accelerating effect on cell death (3). Using proteasome inhibitors, such as bortezomib, is one of the methods to prevent the uncontrolled and rapid proliferation of cancer cells. Bortezomib (BZ), a selective inhibitor of the proteasome, is a drug is used for the treatment of cancer and immunosuppressive therapies (2,4). 26S Proteasome inhibition may inhibit pro-apoptotic degradation dynamics, allowing the initiation of apoptosis in neoplastic cells by suppressing pro-apoptotic pathways. Many studies showed the therapeutic effects of BZ in cancer therapies. Although there are many studies reported that this drug is effective only in the treatment of target cancer cells (5), recent studies reported that BZ could cause changes in the intracellular peptide levels that might cause cell damage through many basic metabolic processes in other tissues (2, 6) and contribute to the biological and/or side effects of the drug (7) like neuropathy and myelosuppression (8-10). In some studies, it was revealed that oxidative stress might be responsible for a chemotherapeutic drug such as bortezomib-induced non-target tissue damage (11).

Oxidative stress is a state meaning an inequality in the balance between free radicals production and antioxidants. Inhibition of proteasome disrupts protein homeostasis that causes overproducing reactive oxygen species (ROS) (12). High levels of free radicals lead to damage to cellular proteins, membrane lipids, and nucleic acids (3). Moreover, this current state decreases different metabolic processes and induces cell deaths (1-3). Research has shown that diseases such as diabetes, pancreatitis, hepatitis C infection, and chronic kidney diseases are associated with increased oxidative stress to all the bimolecular (13-15). The elimination of ROS by protective

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mechanisms is mentioned as antioxidants (16).

Antioxidants are substances that prevent damage caused by unstable molecules known as ROS (3). They inhibit cell injury via interacting with and stabilizing free radicals (17). In many chemotherapeutic treatments to reduce non-target tissue damages, antioxidants are used clinically, and it has known that these antioxidants also have anti-tumor effects on cancer cells (18). Although initial studies showed that antioxidant substances might promote health, recent clinical trials showed that some synthetic antioxidant substances do not offer adequate protection against oxidative damage and may cause an increase in tissue damage (19). One of the examples of these antioxidants includes beta-glucans (BGs) (20). BGs are antioxidant supplements that join in the processes of metabolism, repair, and detoxification. Also, it has anti-tumor activity (21) and has also been used clinically in Japanese and Chinese medicine. In addition to neutralizing the pathological effects caused by oxidative stress, some studies have shown that the curative effect of this substance is insufficient (20, 22).

The purpose of this study is to investigate the effects of BGs on systemic BZ-treated rat pancreas. In addition to our knowledge, this is the first study on the effects of BGs on BZ-treated pancreas tissue by immunohistochemical and stereological methods.

2. Materials and Methods

2.1. Animals

36 adult 12 weeks male Sprague-Dawley rats weighing between 230 g and 250 g were used in this study. The rats were housed in a light-controlled room with a constant temperature and fed standard laboratory water and chow. Ethics committee approval of this experiment was given by Aksaray University Institutional Animal Care and Use Committee. (2021/3-8). Chemicals Bortezomib (Velcade®) as a lyophilized powder (Velcade; Janssen-Cilag, Beerse, Belgium) dissolved in a solution of sterile saline at final concentration and β-1,3-D-glucan (SigmaAldrich (Steinheim, Germany) were used. All the other chemicals were purchased from Sigma-Aldrich (Germany) for laboratory experimentation.

2.2. Experimental procedure

For this study, the rats were divided into four groups: Control (sham), β-1,3-D-glucan, bortezomib, and bortezomib + β-1,3-D-glucan. Each group was divided into two subgroups according to the time they would be sacrificed (48 and 72 hours after drug administration) (n=6). The rats in the bortezomib and bortezomib + β-1,3-D-glucan group were injected only with 0.2 mg/kg bortezomib subcutaneously (sc) on the first day of the study, and no further treatment was applied to these rats in the following days. Rats in the bortezomib + β-1,3-D-glucan group were injected with bortezomib. Then, 75 mg/kg of beta-glucan was injected intraperitoneally until the end of the experiment. Rats in the β-1,3-D-glucan group were injected daily with 75 mg/kg β1,3-D-glucan intraperitoneally until the day they were sacrificed.

2.3. Histopathological procedures

Pancreas tissues were fixed in 10% neutral-buffered formalin solution for 72 h. The specimens were dehydrated in graded alcohol series, cleared with xylene, and embedded in paraffin wax. 4-μm thick histological sections were obtained using a Leica RM2125RT microtome (Leica Microsystems, Wetzlar, Germany). Sections were mounted onto glass slides. Samples were evaluated under a light microscope (Leica DM) after being stained with Hematoxylin & Eosin (H&E) and Mallory's triple modified by Crossman.

2.4. Immunohistochemical procedures

Pancreas tissues were examined by immunohistochemistry using the streptavidin-biotin peroxidase method (DAKO-Universal LSAB Kit-K0690) and 3,3'-diaminobenzidine tetrahydrochloride (DAB, SIGMA-D5905). Sections were held in xylene series and a decreasing series of ethanol. Then, they were incubated with 3 % H2O2 for blocking endogenous peroxidase activity and with normal bovine serum for blocking nonspecific binding sites of antibodies. Later primary antibody (anti-insulin IgG -dilution: 1/150-Polyclonal Guinea Pig Anti-Swine Insulin, DAKO-A0564) for beta cells and biotinylated secondary antibody (DAKO-Universal LSAB Kit-K0690) were respectively used for 30 min. Subsequently, specimens were incubated with streptavidin-Horseradish Peroxidase (DAKO Universal LSAB Kit-K0690), and binding sites of antibody were made visible with DAB and rinsed with PBS. Harry's hematoxylin was used to stain nuclei. After the samples were dehydrated with gradually increasing alcohol series, they were cleaned in xylene series and covered using entellan.

2.5. Semi-quantitative analysis for histopathological and immunohistochemical changes

Histological and immunohistochemical assessments and scores were done semi-quantitatively using light microscopy on sections from each animal. The 100 square micrometer area was determined by means of a micrometer slide (almost in 1 mm2) for the X 10magnification. The endocrine and exocrine parts of specimens were counted in 5 randomly selected microscopic areas at X 10 objective in each section, and the arithmetic mean was scored semi-quantitatively. Insulin-positive cells in immunohistochemical assessment, Intracytoplasmic vacuolization, PMNL infiltration, and degenerative cells in the histopathological assessment were scored. The scoring was reported as follows: none = –, mild = +, moderate = ++, severe = +++ (Table 2).

2.6. Stereological procedures and analysis

Sections were placed under the microscope, and serous acini were projected onto the monitor at 40X magnification via the camera. Each section was analyzed by systematic sampling.

The mean serous acinus volume was evaluated using the software Stereo Investigator (Microbrightfield) with the "nucleator method" described by Gundersen (23).

2.7. Statistical analysis

The statistical analysis was performed using SPSS (IBM SPSS

Statistics 18.0, IBM Corporation, Somers, NY, USA). Groups were compared using one-way analysis of variance (ANOVA) followed by an LSD test. (p-value less than 0.05 was considered significant ($p<0.05$). Values were also expressed as means ± standard deviation. [abc] the footnote letters in the same column indicate significant differences between groups.

3. Results

3.1. Histopathological examinations

Histopathological analysis of H&E and Mallory's triple modified by Crossman staining of pancreas tissues revealed that the control group's sections were seen as normal histologic structure. The experimental group's histologic analysis revealed that the quantity and distribution of the zymogen granules were altered when compared to the control group. BG group was similar to the control group than the other experimental groups except for a few polymorphonuclear leukocyte (PMNL) infiltrations and a few cellular degenerations, and the acinar cells showed either central distribution like the control group. BZ 48 h group sections showed a little vacuolization in acinar cells. Increased cellular degeneration and PMNL infiltration were more pronounced than the control and BG group in BZ 48 h group. BZ 72h group sections were near to BZ 48 h group except for vacuolization of the striated duct cells. In the sections of the group BZ 48h+BG, in addition to the other experimental groups, it was seen some capillaries were dilated and congested. BZ 72h+BG results showed the vacuolation and degeneration of the acinar cells were more intense than the other experimental groups. Furthermore, there was interstitial edema in this group. In all groups, collagen depositions were nearly similar. (Fig. 1) (Table 1).

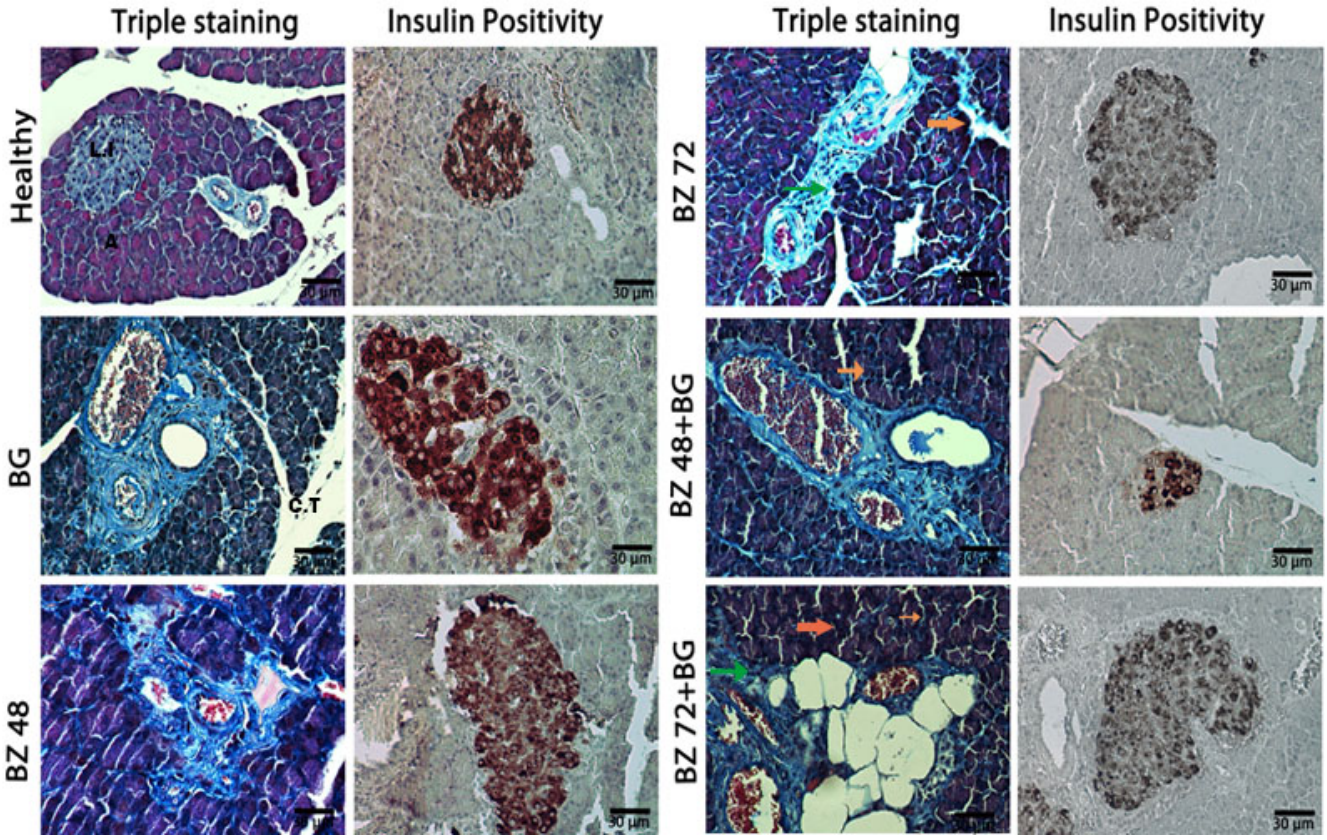

Fig. 1. Serous acinus (A), connective tissue (C.T), Langerhans islands (L.I). Degenerative acinar cells (thick orange arrow), vacuolization (thin orange arrow), interstitial edema (green arrow)

Table 1. The assessments of the mean serous acinus area of all groups. [abc] the footnote letters in the same column indicate significant differences between groups

Groups	Mean serous acinus area	Mean amylase level
C group	1001.66±110.06[a]	1730±153.84
BG group	913.77±68.66[a]	1901±410.54
BZ 48h group	1015.54±140.12[b]	2058±389.97
BZ 72h group	1100.46±72.48[b]	2139±83.18
BZ 48h+BG group	1025.82±62.73[c]	1487±121.19
BZ 72+BG group	1130.64±97.36[c]	1274±22.80

3.2. Immunohistochemical examination

Immunohistochemical analysis of positive beta cells revealed that BG groups results were close to the control group. Insulin (+) results of cells in BZ 48h and BZ 48h+BG groups were found to be decreased compared to control and BG groups. BZ 72h and BZ 72h+BG groups have the least insulin (+) cells compared to other groups (Fig. 1) (Table 1).

3.3. Stereological examinations

Stereological examinations showed that BG group results were nearly similar to the control group. BZ 48h and BZ 48h+BG groups have increased mean serious acinus area and significant differences when compared to control and BG groups. BZ 72h and BZ 72h+ BG group mean serous acinus area results were

higher than BZ 48h and BZ 48h+ BG groups and have significant differences from the other groups. (Table 2).

Table 2. Semi-quantitative analysis of histopathological and Immunohistochemical assessments

Groups	Insulin	Intracytoplasmic vacuolization	PMNL infiltration	Degenerative cells
C	+++	-	-	-
BG	+++	-	+	+
BZ 48h	++	+	+	++
BZ 72h	+	++	++	++
BZ 48h+BG	++	++	++	++
BZ 72h+BG	+	+++	+++	+++

4. Discussion

The ubiquitin-proteasome pathway is responsible and very important for the eukaryotic intracellular protein turnover. This function is crucial to many cellular processes, comprising cell growth, activation, differentiation, and signaling. The proteasome inhibitors are used for inhibition of these pathways in cancer therapies (24). Although they are effective in preventing cancer cell proliferation, they can be harmful to some healthy tissues via mediated oxidative stress (2, 6, 12). The antioxidants have protective effects on damaged tissues mediated oxidative stress, and the positive effects of glucans have been reported to modulation of immune function and antioxidant effects (22, 25). Contrary to this information, insufficient or adverse effects of BGs have been reported recently (20, 22, 26) Tsiapali et al. (26) observed an increase in free radical levels in BG treated groups and reported the modulatory effects of BGs could not be sufficient in inflammatory components comprising tissue culture and/or disease models. In this study, the effects of BZ, which is a selective inhibitor of 26S proteasome, and B-1,3-(D)-glucan (BG), a synthetic antioxidant, on rat pancreatic tissues were examined according to dose and time.

In the immunohistochemical analysis, immune-positive beta cells densities were decreased in the BZ and especially in the BZ+BG groups. Several studies (27, 28) reported that proteasome inhibition by bortezomib might cause hyperglycemia. Tsiapali et al. (26) determined an increase in free radical levels in BG treated groups, and many studies (29-31) showed that oxidative stress played a major role in the pathogenesis of type 1 and 2 diabetes. In our study BG alone showed no effect on insulin levels. On the contrary, the lower insulin levels of BZ+BG groups can be attributed to the effects of BZ.

According to histopathological evaluation, the BG group was similar to the control group. Degenerative changes, intracytoplasmic vacuolizations, and PMNL infiltrations were observed in the acini and duct cells of the BZ-treated groups. It was determined that BG treatments did not reduce the pathological changes in pancreatic tissue after BZ application.

In recent years in some case reports, it was reported BZ induced acute pancreatitis (32, 33), and BZ treatment can cause morphological alterations like intracytoplasmic vacuolization (34-36) and degenerations (37) on the cellular level, and in some researches (38) it was suggested that there could be an increase in ROS levels in BG treated groups and combined BG therapies with some drugs can cause activation of immune responses and inflammation.

In stereological examinations, the mean acinus volume of BG group sections was near to the control group. But there were increases in acinus volumes of BZ groups and more in BZ + BG groups. It has known BZ may cause hyperglycemia. The pancreas exocrine and parotid gland are only serous glands. Parlak et al. (39) detected that diabetes caused vacuoluzations in the parotid gland. Tsiapali et al. (26) determined in BG treated groups an increase in free radical levels.

ROS stimulated various hypertrophic transcription factors, myocardial growth-related signals, cellular dysfunction and matrix remodeling (40). In our study, it was suggested that BG application may be protective against degenerations and hypertrophied acinus cells. This inference is consistent with our result of decreased amylase levels. In addition, it was thought that increased amylase levels in BZ groups might be related to changes in molecular level, not morphological level.

In conclusion, the study results revealed that treatment with BZ causes pancreatic damage, and administration of BG have insufficient effects against BZ-related degenerations. The related molecular mechanism of BZ toxicity on pancreas tissue requested further investigation.

Conflict of interest

None to declare.

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Authors' contributions

Concept: N. A., SNP., Design: N. A., SNP., Data Collection and Processing: N. A., SNP., Analysis and Interpretation: N. A., SNP., Literature Search: N. A., SNP., Writing: N. A., SNP.

References

1. Dasmahapatra G, Patel H, Dent P, Fisher RI, Friedberg J, Grant S. Non-Hematologic Toxicity of Bortezomib in Multiple Myeloma: The Neuromuscular and Cardiovascular Adverse Effects. Cancers (Basel). 2020;12(9):2540.
2. Otoda T, Takamura T, Misu H, Ota T, Murata S, Hayashi H. et al. Proteasome Dysfunction Mediates ObesityInduced Endoplasmic Reticulum Stress and Insulin Resistance in the Liver. Diabetes. 2013;62.3:811-824.
3. Yang X. Proteasome inhibitor bortezomi-induced the apoptosis of laryngeal squamous cell carcinoma Hep-2 cell line via disrupting redox equilibrium. Biomedicine & Pharmacotherapy

2012;66:607-611.

4. Stein ML, Groll M. Applied techniques for mining natural proteasome inhibitors. Biochimica et Biophysica Acta (BBA)-Molecular Cell Research 2014;1843(1),26-38.

5. Dai Y, Guo X, Yang C. Caponigro F. Effect of bortezomib on proliferation and apoptosis of myeloma cells by activating Wnt/beta-catenin signaling pathway. Oncol Lett. 2020;20(2):1295-1299.

6. Yu S, Zheng L, Li Y, Li C, Ma C, Yu Y, et al. Causal co-expression method with module analysis to screen drugs with specific target. Gene. 2013; 518.1:145-151.

7. Zajączkowska R, Kocot-Kępska M, Leppert W, Wrzosek A, Mika J, Wordliczek J. Mechanisms of Chemotherapy-Induced Peripheral Neuropathy. Int J Mol Sci. 2019;22;20(6):1451.

8. Gelman JS, Sironi J, Berezniuk I, Dasgupta S, Castro LM, Gozzo FC, Ferro ES, Fricker LD. Alterations of the intracellular peptidome in response to the proteasome inhibitor bortezomib. PLoS One. 2013;8(1):e53263.

9. Schmidt N, Alloway RR, Walsh RC, Sadaka B, Shields AR, Girnita AL, et al. Prospective evaluation of the toxicity profile of proteasome inhibitor-based therapy in renal transplant candidates and recipients. Transplantation. 2012 Aug 27;94(4):352-61.

10. Mateos MV. How to maintain patients on long-term therapy: understanding the profile and kinetics of adverse events. Leukemia research. 2012;36 Suppl 1:S35-43.

11. Narayanan S, Cai CY, Assaraf YG, Guo HQ, Cui Q, Wei L, et al. Targeting the ubiquitin-proteasome pathway to overcome anti-cancer drug resistance. Drug Resist Updat. 2020;48:100663.

12. Weniger MA, Rizzatti EG, Pérez-Galán P, Liu D, Wang Q, Munson PJ, et al. Treatment-induced oxidative stress and cellular antioxidant capacity determine response to bortezomib in mantle cell lymphoma. Clinical Cancer Research, 2011;17;5101-5112.

13. Gonzalez A, Huerta-Salgado C, Orozco-Aguilar J, Aguirre F, Tacchi F, Simon F, et al. Role of Oxidative Stress in Hepatic and Extrahepatic Dysfunctions during Nonalcoholic Fatty Liver Disease (NAFLD). Oxid Med Cell Longev. 2020;19:1617805.

14. Yaribeygi H, Sathyapalan T, Atkin SL, Sahebkar A. Molecular Mechanisms Linking Oxidative Stress and Diabetes Mellitus. Oxid Med Cell Longev. 2020;9;8609213.

15. Leung PS, Chan YC. Role of oxidative stress in pancreatic inflammation. Antioxidants & redox signaling. 2009; 11: 135-165.

16. Reuter S, Gupta SC, Chaturvedi MM, Aggarwal BB. Oxidative stress, inflammation, and cancer: how are they linked? Free Radic Biol Med. 2010 Dec 1;49(11):1603-16.

17. Styskal J, Van Remmen H, Richardson A, Salmon AB. Oxidative stress and diabetes: what can we learn about insulin resistance from antioxidant mutant mouse models? Free Radic Biol Med. 2012 Jan 1;52(1):46-58.

18. Zhang P, Li T, Wu X, Nice EC, Huang C, Zhang Y. Oxidative stress and diabetes: antioxidative strategies. Front Med, 2020;14(5):583-600.

19. Poljsak B, Milisav I. The neglected significance of "antioxidative stress". Oxidative medicine and cellular longevity. 2012;2012:480895.

20. Jurczyńska E, Saczko J, Kulbacka J, Kawa-Rygielska J, Błazewicz J. Beta-glukan, jako naturalny antykarcynogen [Beta-glucan as a natural anticancer agent]. Pol Merkur Lekarski. 2012 Oct;33(196):217-20. Polish.

21. Akaras N, Abuc OO, Koc K, Bal T, Geyikoglu F, Atilay H, Erol HS, Yigit S, Gul M. (1 3)-beta-d-glucan enhances the toxicity induced by Bortezomib in rat testis. J Food Biochem. 2020;44(3):13155.

22. Ozdemir S, Toplan S, Tanriverdi G, Sunamak O. The effects of beta-glucan on iron levels and lipid peroxidation in intra-abdominal sepsis in rats. General physiology and biophysics. 2011; 30:138-144.

23. Albayrak Y, Halici Z, Odabasoglu F, Unal D, Keles ON, Malkoc I, et al. The effects of testosterone on intestinal ischemia/reperfusion in rats. Journal of Investigative Surgery 2011; 24: 283-291.

24. Brignole C, Marimpietri D, Pastorino F, Nico B, Di Paolo D, Cioni M, et al. Effect of bortezomib on human neuroblastoma cell growth, apoptosis, and angiogenesis. Journal of the National Cancer Institute 2006;98:1142-1157.

25. Patchen ML, D'alesandro MM, Brook I, Blakely WF, MacVittie TJ. Glucan: mechanisms involved in its" radioprotective" effect. Journal of leukocyte biology. 1987;42:95-105.

26. Tsiapali E, Whaley S, Kalbfleisch J, Ensley HE, Browder IW, Williams DL. Glucans exhibit weak antioxidant activity, but stimulate macrophage free radical activity. Free Radical Biology and Medicine. 2001;30:393-402.

27. Richardson PG. Assessment of Proteasome Inhibition for Extending Remissions I. Bortezomib or high-dose dexamethasone for relapsed multiple myeloma. N Engl J Med. 2005; 352: 2487-2498.

28. Gomez-Abuin G, Winquist E, Stadler WM, Pond G, Degendorfer P, Wright J, et al. A phase II study of PS-341 (Bortezomib) in advanced or metastatic urothelial cancer. A trial of the Princess Margaret Hospital and University of Chicago phase II consortia. Investigational new drugs. 2007;25:181-185.

29. Zhang P, Li T, Wu X, Nice EC, Huang C, Zhang Y. Oxidative stress and diabetes: antioxidative strategies. Front Med. 2020;14(5):583-600.

30. Pang L, Lian X, Liu H, Zhang Y, Li Q, Cai Y, et al. Understanding Diabetic Neuropathy: Focus on Oxidative Stress. Oxid Med Cell Longev. 2020;9524635.

31. Luc K, Schramm-Luc A, Guzik TJ, Mikolajczyk TP. Oxidative stress and inflammatory markers in prediabetes and diabetes. J Physiol Pharmacol. 2019;70(6):26402.

32. Elouni B, Salem CB, Zamy M, Sakhri J, Bouraoui K, Biour M. Bortezomib-induced acute pancreatitis. Journal of the Pancreas 2010;11:275-276.

33. Badalov N, Baradarian R, Iswara K, Li J, Steinberg W. Tenner S. Drug-induced acute pancreatitis: an evidencebased review. Clinical Gastroenterology and Hepatology. 2007;5:648-661.

34. Cavaletti G, Gilardini A, Canta A, Rigamonti L, Rodriguez-Menendez V, Ceresa C, et al. Bortezomib-induced peripheral neurotoxicity: a neurophysiological and pathological study in the rat. Experimental neurology. 2007;204:317-325.

35. Ding W-X, Ni H-M, Yin X-M. Absence of Bax switched MG132-induced apoptosis to non-apoptotic cell death that could be suppressed by transcriptional or translational inhibition. Apoptosis. 2007;12:2233-2244.

36. Carozzi VA, Renn CL, Bardini M, Fazio G, Chiorazzi A, Meregalli C, et al. Bortezomib-induced painful peripheral neuropathy: an electrophysiological, behavioral, morphological and mechanistic study in the mouse. PLoS One. 2013;8:e72995.

37. Takamatsu H, Yamashita T, Kotani T, Sawazaki A, Okumura H, Nakao S, et al. Ischemic heart disease associated with bortezomib treatment combined with dexamethasone in a patient with multiple

myeloma. International journal of hematology. 2010;91:903-906.

38. Bagni M, Romano N, Finoia MG, Abelli L, Scapigliati G, Tiscar PG, et al. Short-and long-term effects of a dietary yeast β-glucan (Macrogard) and alginic acid (Ergosan) preparation on immune response in sea bass. Fish & Shellfish Immunology. 2005;18:311-325.

39. Parlak SN, Tatar A, Keles ON, Selli J, Can I, Unal B. Effects of menopause and diabetes on the rat parotid glands: A histopathological and stereological study. Int J Med Sci Public Health. 2014;3:749.

40. Sabri A, Hughie HH, Lucchesi PA. Regulation of hypertrophic and apoptotic signaling pathways by reactive oxygen species in cardiac myocytes. Antioxidants and Redox Signaling. 2003;5:731-740.