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Bryostatin-1, a Marine Macrolide from *Bugula neritina*, demonstrates anti-diabetic efficacy and potential for managing Diabetes-associated complications

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ABSTRACT

BACKGROUND: Diabetes, a persistent degenerative metabolic condition associated with significant morbidity and mortality rates due to its complications, has prompted increased exploration into novel bioactive compounds for its treatment. This quest includes the investigation of metabolites derived from marine sources. Bryostatin 1 a cyclic macrolide originally isolated from a marine organism *Bugula neritina*. In recent years, bryostatins have garnered significant interest due to their diverse range of biological activities. It is a macro lyric lactone that activates protein kinase C and modulate cell signaling pathways. In light of the given background, the present study has aimed to investigate the anti-diabetic efficacy of Bryostatin 1 and its potential for the management of diabetes associated complications,

MATERIALS AND METHODS: The anti-diabetic efficacy of Bryostatin 1 was evaluated in *in vitro* conditions using α-amylase and α-glucosidase, carbohydrate hydrolyzing enzymes. Further, the mechanism of action was evaluated by Dipeptidyl peptidase-4 (DPP-IV) inhibitory activity. The effect of Bryostatin 1 on diabetes associated complications was assessed by evaluating its effect on advanced glycation end products (AGEs) and sorbitol accumulation.

RESULT: The results of the present study showed significant inhibition of carbohydrate hydrolyzing enzymes, α-amylase and α-glucosidase by Bryostatin 1 at varying concentrations which is compared with that of Standard acarbose. The DPP-IV activity was found to be markedly inhibited in a dose-dependent manner. The effect of Bryostatin 1 on AGEs formation and aldolase reductase was found to be significantly attenuated indicating its potential role in the management of diabetes associated complications viz., nephropathy and retinopathy.

CONCLUSION: The current study results sheds light on the potential of Bryostatin 1 as a novel therapeutic agent for diabetes mellitus and its associated complications. Nevertheless, additional *in vivo* investigations are necessary to confirm the safety and effectiveness of the compound, with a more comprehensive focus on uncovering its mechanism of action.

KEYWORDS: Bryostatin, Acarbose, Sitagliptin, Aminoguanidine, Metformin

INTRODUCTION

Diabetes mellitus is a persistent metabolic disorder characterized by either impaired insulin secretion or insulin resistance, resulting in a hyperglycemic state during fasting or postprandial periods [1]. Despite the availability of various synthetic anti-diabetic medications, the high costs, concerns about effectiveness, and adverse reactions have prompted researchers to actively explore alternative therapies that offer improved efficacy and fewer cost-related side effects. In many developing nations, there is a growing interest in alternative healthcare approaches, with individuals turning to treatments derived from natural sources, particularly marine resources, as a primary means of addressing their health needs [2, 3].

Numerous marine species with the potential to treat conditions like diabetes have been identified [4, 5]. In 1987, Cannell and colleagues conducted a screening of 500 freshwater and marine cyanobacteria to identify potential inhibitors of α-glucosidase and α-amylase using colorimetric assays. This research led to the discovery of 38 cyanobacteria species exhibiting promising properties as inhibitors of glycosidase enzymes [6]. Another study has documented enzyme inhibitors and bioactive compounds derived from marine actinomycetes, specifically *Streptomyces sp.* The subspecies *Streptomyces corchorusii*, known as rhodomarinus, demonstrated notable α-amylase inhibition. Furthermore, a different Streptomyces strain (Streptomyces sp.) obtained at a depth of around 100 meters from Otsuchi Bay in Iwate Prefecture was found to produce two novel compounds, Pyrostatins A and B, showing specific inhibitory activity against N-acetyl-glucosaminidase [7].

Bryostatins, a class of marine bioactive compounds initially derived from the bryozoan Bugula neritina, exhibit a wide range of pharmacological effects, including anti-cancer, anti-HIV, anti-Alzheimer's, anti-stroke, anti-multiple sclerosis, and anti-diabetic activities [8]. Bugula neritina yields substantial amounts of highly pure Bryostatin, reaching around 10,000 gallons [9]. Discovered by Pettit, Bryostatin was initially employed in cancer treatment due to its synergistic effects with oncolytic agents like Taxol. Its mechanism involves the inhibition of protein kinase C (PKC) isoenzymes and C1 domain proteins, which play crucial roles in regulating cellular processes such as proliferation, differentiation, motility, adhesion, inflammation, and apoptosis [10]. Bryostatin analogs, like Bryostatin-13, can serve as immunostimulants. Bryostatin 1, known for its high polyacetate activity, is a commonly used strain in research and applications.

Bryostatin 1 has demonstrated similarities to phorbol esters in specific biological contexts. In clinical trials, the marine compound was administered either through a bolus intravenous injection or continuous infusions [11]. Its application extended to HIV treatment when combined with highly active antiretroviral therapy (HAART). Additionally, Bryostatin 1 has been explored for its potential in preventing the progression of various central nervous system (CNS) diseases like multiple sclerosis and fragile X syndrome. Notably, the compound exhibited the ability to enhance the survival of human dermal fibroblasts under stressful conditions, suggesting anti-aging properties and promoting healthy skin [12, 13]. Despite extensive research highlighting its anti-cancer and neuroprotective attributes, there is a limited focus on Bryostatin 1's potential anti-

diabetic efficacy. Hence, the current study aims to assess the anti-diabetic effects of Bryostatin 1 using *in vitro* enzyme inhibitory assays. Furthermore, the research endeavors to investigate the impact of Bryostatin 1 on crucial enzymes associated with diabetes-related complications, including nephropathy and retinopathy.

MATERIALS AND METHODS

Chemicals and reagents

α–amylase, α-glucosidase (1U/ml) from *Saccharomyces cerevisiae*, Starch, p-nitrophenyl-α-D-glucopyranoside (p-NPG), DPP-IV, synthetic substrate Gly-Pro-p-nitroanilide, DL-glyceraldehyde, D-glucose, Fructose, lithium sulphate, NADPH, NADP, dimethyl sulphoxide (DMSO), sorbitol, bovine serum albumin, perchloric acid, ammonium sulphate, Tris-HCl, EDTA, sucrose and sorbitol dehydrogenase were procured from Sigma-aldrich, USA. Acarbose, Aminoguanidine hydrochloride, Metformin and Sitagliptin was purchased from TCI chemicals, India. All other chemicals, reagents and solvents used were of analytical grade and purchased from SRL chemicals, India.

In – vitro α- amylase inhibitory assay

The *in vitro* alpha amylase inhibition assay was carried out [14]. Briefly, 100µL of Bryostatin 1 (10-320µM) was mixed with 200µL of α-amylase enzyme (Hi media RM 638) and 100µL of 2mM phosphate buffer (pH-6.9). After a 20-minute incubation period, 100µL of 1% starch solution was added to the reaction mixture. Controls were also prepared, replacing the enzyme with a buffer. Following a 5-minute incubation, 500µL of Dinitro salicylic acid reagent was added to both the control and test samples. The mixture was then subjected to a 5-minute boiling water bath. The absorbance at 540 nm was measured using a spectrophotometer, and the alpha amylase inhibition percentage was determined using a specific formula.% inhibition = [(Control – Test)/Control] *100

Suitable reagent blank and inhibitor controls were simultaneously carried out.

α – glucosidase inhibitory activity

The assessment of α-glucosidase enzyme inhibition activity was performed following the method outlined by Shruthi et al. (2011) [15] with slight modifications. The reaction mixture included 50µL of 0.1M phosphate buffer (pH 7.0), 25µL of 0.5mM 4-nitrophenyl α-D-glucopyranoside (dissolved in 0.1M phosphate buffer, pH 7.0), 10µL of varying concentrations of Bryostatin (110-320µM), and 25µL of α-glucosidase solution (1mg/mL stock solution in 0.01M phosphate buffer, pH 7.0, diluted to 0.1Unit/mL with pH 7.0 before the assay). The reaction mixture was incubated at 37°C for 30 minutes, and the reaction was arrested by adding 100µL of 0.2M sodium carbonate solution. The enzymatic hydrolysis of the substrate was recorded by measuring the amount of p-nitrophenol released in the reaction mixture at 410 nm using a microplate reader. Blanks were prepared to correct for background absorbance, replacing enzymes with buffers. Controls were also kept for reference by substituting plant extracts with solvent alone. Acarbose served as the positive control. All experiments were performed in triplicates (n=3).

In vitro DPP-IV inhibition assay [16]

Different sets of tubes each in triplicate were used for DPP-IV control; DPP-IV + different concentrations of sitagliptin (10-320nM), DPP-IV+Bryostatin 1(10-320µM). Briefly, the sitagliptin phosphate monohydrate and Bryostatin 1 were dissolved in dimethyl sulphoxide (DMSO) and diluted with tris buffer (pH 8.0, 50mM) to achieve the highest concentration. From the stock concentration of extract and standard, the working dilutions of 10, 20, 40, 80, 160, 320µg/ml and 10, 20, 40, 80, 160, 320nM were prepared. The assay was conducted using DPP-IV enzyme diluted with Tris buffer (0.05 U/ml) and pipette 50µl into clear microplate wells. Subsequently, 10µl of tris buffer or sitagliptin phosphate monohydrate or Bryostatin 1 was also added and incubated at 37^{o}C for 20 min. Finally, 50µl of (0.20mM prepared in tris buffer) chromogenic substrate Gly-Pro P-nitroanilide (Sigma–Aldrich, USA) was added into each tube. The hydrolysis of the substrate was monitored at 405 nm wavelength using a Microplate plate reader from Synergy Biotek, USA. Activity was expressed as ΔA_{405} nm/min. The experiment was performed in triplicate and compared with negative control (enzymatic solution without inhibition), while standard DPP-IV inhibitor drug sitagliptin employed as positive control.

Advanced Glycation end product (AGE) assay [17]

AGEs, or advanced glycation end products, result from the non-enzymatic glycosylation of proteins, leading to increased vascular permeability in both micro and macro vascular structures through binding to specific macrophage receptors. The effect of Bryostatin 1 on AGEs formation was assessed at concentrations ranging from 10 to 320 µM. The AGE reaction mixture comprised 1 mg/mL bovine serum albumin in 50 mM sodium phosphate buffer (pH 7.4), 0.02% sodium benzoate, 0.2 M fructose, and 0.2 M glucose. Different concentrations of Bryostatin 1 (10-320 µM) were added to a 2.75 mL of the reaction mixture, with amino guanidine serving as the positive control. Following an incubation period at 37°C for 3 days, the fluorescence intensity of the reaction was measured at excitation and emission wavelengths of 350nm and 450nm, respectively, using a Biotek Synergy multi-mode reader in the USA. The percentage activity was then calculated relative to the solvent control.

Determination of Aldose Reductase Inhibition [18]

In a cuvette, a mixture was prepared by combining 531µL of 0.1 M potassium buffer (pH 7.0), 90µL of NADPH solution (1.6 mM in potassium buffer), 90µL of recombinant human aldose reductase (AR) (6.5U/mg) from Sigma, USA (SRP6371-100UG), 90µL of 4 M ammonium sulfate solution in potassium buffer, and 90µL of DL-glyceraldehyde (25 mM in potassium buffer). To this mixture, 9µL of Bryostatin 1 at various concentrations (10-320µM) was added. The spectrophotometric assessment of AR activity was conducted by measuring the reduction in NADPH absorbance at 340 nm over a 3-minute period using a Biotek Synergy H4 multi-mode reader, USA. Metformin served as the positive control. The inhibition of AR (%) was calculated using the following equation: (1 − (△A sample/min) – (△A blank/min)/(△A control/min) − (△A blank/min)) × 100%, where △A sample/min is the decrease in absorbance over 3 min with reaction solution, test sample, and substrate, and △A control/min without the test sample.

Statistical analysis

Data were analyzed using Graphpad prism (version 7.0). The results were expressed as Mean±SEM and the IC_{50} values were obtained from the linear regression plots. Two-way ANOVA was used to assess differences between means at $p<0.001$ level of significance. The means were compared with standards groups using the Holm-Sidak Test.

RESULTS

α-amylase and α-glucosidase inhibitory activity of Bryostatin 1

Bryostatin 1 was assessed for its impact on carbohydrate-hydrolyzing enzymes, specifically α-amylase and α-glucosidase, across a range of concentrations (10-320 μM). The inhibitory effects were compared to the standard acarbose. The findings revealed that Bryostatin 1 effectively inhibited α-amylase, with lowest inhibitory percentage of 4.42% observed at 10μM. A maximum inhibition of 71.08% was observed by Bryostatin 1 at 320μM. Standard acarbose at concentration between 10-32μM exhibited inhibitory activity of 8.38% to 84.39%.

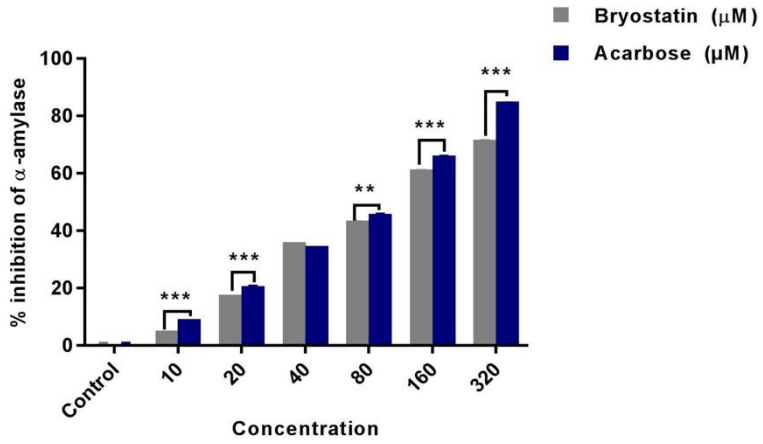


Figure 1: Effect of Bryostatin 1 on α-amylase enzyme activity. Bar graph represent % inhibition of α-amylase enzyme by different concentrations of Bryostatin 1 (10-320μM) and Standard Acarbose (10-320μM). Data expressed as Mean±SEM (n=3). Two-way ANOVA was used to assess differences between means of Bryostatin 1 at ***p<0.001, **p<0.01 and *p<0.05 level of significance Vs Standard Acarbose.

α-glucosidase inhibitory activity of Bryostatin 1

Figure 2 illustrates the inhibitory effect of Bryostatin 1 against α-glucosidase activity. The inhibitory activity was observed at the range of 16.67% to 77.67%, at concentrations of 10-320μM. These inhibitory activities were compared with the inhibitory effects of Standard Acarbose. The results showed that Bryostatin 1 at 10μM exhibited 16.67% of inhibition against α-glucosidase while Acarbose at 10μM exhibited 18.55% inhibition. Maximum inhibition was achieved at 320μM with an inhibitory percentage of 77.67% for Bryostatin 1. The inhibitory effect of Bryostatin 1 against α-glucosidase was found to be increasing with increase in concentration.

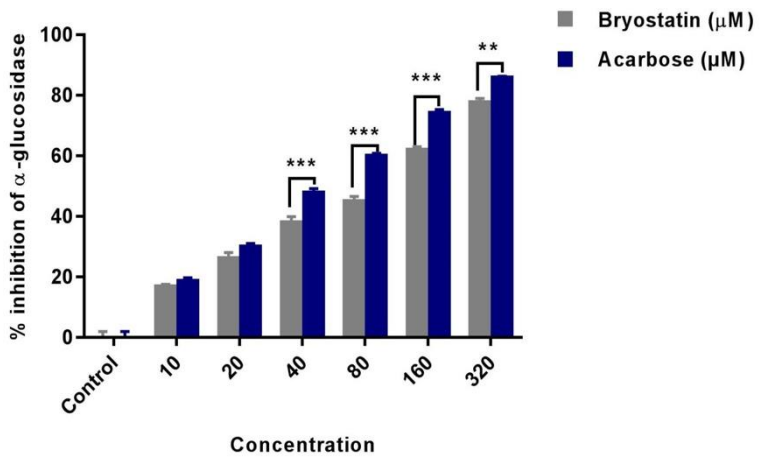


Figure 2: Effect of Bryostatin 1 on α-glucosidase enzyme activity. Bar graph represent % inhibition of α-glucosidase enzyme by different concentrations of Bryostatin 1 (10-320μM) and Standard Acarbose (10-320μM). Data expressed as Mean±SEM (n=3). Two-way ANOVA was used to assess differences between means of Bryostatin 1 at ***p<0.001, **p<0.01 and *p<0.05 level of significance Vs Standard Acarbose.

DPP-IV inhibitory activity of Bryostatin 1

Inhibiting DPP-4 is a promising strategy in the development of antidiabetic drugs as it enhances the lifespan of GLP-1 (Glucagon-like peptide 1) [19]. *In vitro* DPP-IV inhibitory activity was carried out for Bryostatin 1. The inhibitory activity was expressed in terms of optical density at 450nm/min and was compared with Standard Sitagliptin. The results of the present study exhibited significant inhibition of DPP-IV activity by Bryostatin 1. A maximum inhibition was observed at 320μM with lowest absorbance value of 0.019±0.001 whereas standard sitagliptin exhibited absorbance of 0.004±0.001 at the same concentration.

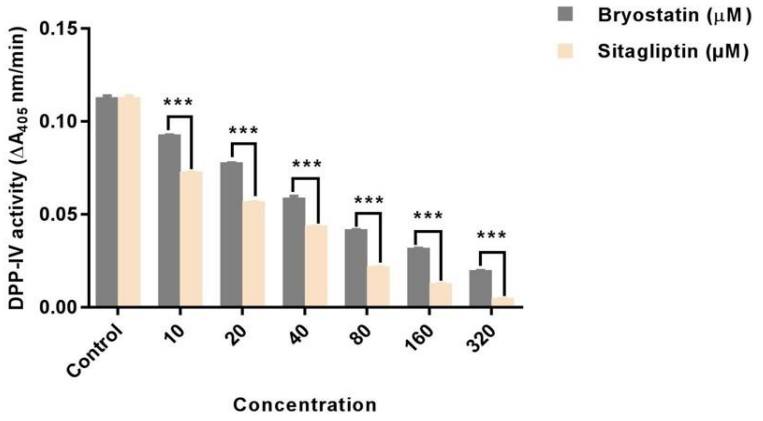


Figure 3: Effect of Bryostatin 1 on DPP-IV enzyme activity. Bar graph represent optical density/absorbance of DPP-IV activity at 450nm/min by different concentrations of

Bryostatin 1 (10-320µM) and Standard Sitagliptin (10-320µM). Data expressed as Mean±SEM (n=3). Two-way ANOVA was used to assess differences between means of Bryostatin 1 at ***p<0.001, **p<0.01 and *p<0.05 level of significance Vs Standard Sitagliptin.

Effect of Bryostatin 1 on advanced glycation endproducts (AGEs)

The effect of Bryostatin 1 was studied against AGEs product formation (Figure 4). Different concentrations of the compound (10-320µM) was assessed for its potential against AGE product and was compared with Standard aminoguanidine. The compound Bryostatin 1 exhibited 74.39% inhibition at a concentration of 320µM while aminoguanidine exhibited 87.88% inhibition at the same concentration.

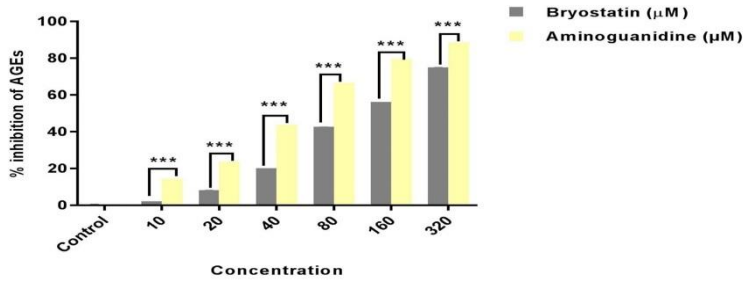
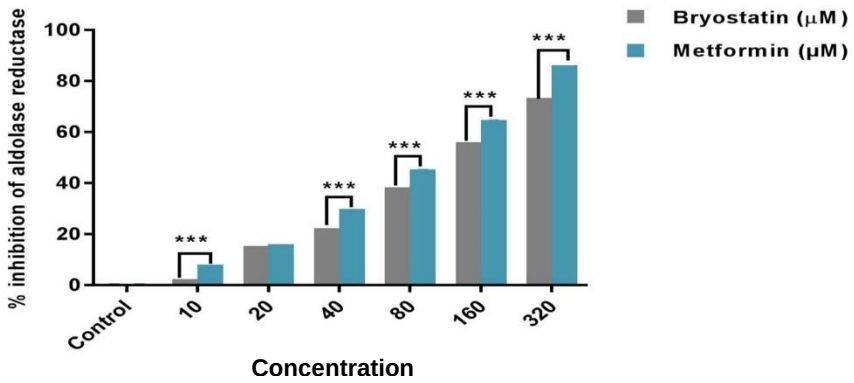


Figure 4: Effect of Bryostatin 1 on AGE product formation. Bar graph represent % inhibition of AGEs formation by different concentrations of Bryostatin 1 (10-320µM) and Standard aminoguanidine (10-320µM). Data expressed as Mean±SEM (n=3). Two-way ANOVA was used to assess differences between means of Bryostatin 1 at ***p<0.001, **p<0.01 and *p<0.05 level of significance Vs Standard aminoguanidine.

Inhibitory effect of Bryostatin 1 on Aldolase reductase

The inhibitory effect of Bryostatin 1 against aldolase reductase enzyme was evaluated and compared with Standard Metformin. Significant inhibition of aldolase reductase was observed at all the tested concentrations of Bryostatin 1. The lowest inhibition of 1.54% was observed at 10µM of Bryostatin while Metformin exhibited 7.22% inhibition at similar concentration. The maximum inhibition of 72.62% and 85.57% was achieved at maximum concentration of 320µM for Bryostatin 1 and for Metformin respectively.



Discussion

In this study, the effects of the marine macrolide compound Bryostatin 1 were evaluated against diabetic enzymes such as α-amylase, α-glucosidase, and DPP-IV, as well as key targets involved in diabetes-related complications, including AGEs and aldolase reductase. These enzymes are known to be elevated under diabetic conditions. The results of the study demonstrated that Bryostatin 1 effectively and significantly inhibited these enzymes, with its effects compared to those of standard drugs.

The assessment of anti-diabetic potential involves the examination of key enzymes in the glucose metabolism pathway, including α-glucosidase, α-amylase, glucose-6-phosphatase, dipeptidyl peptidase 4, glucose transporter 4 (Glut4), glycogen synthase kinase-3β, hexokinase, aldose reductase, and N-acetyl-glycosaminidase. These enzymes play a crucial role in addressing diabetic effects in both animal models and patients [20]. α-amylase and α-glucosidase are primarily responsible for breaking down and digesting carbohydrates [21, 22]. By limiting glucose absorption and inhibiting carbohydrate hydrolases, particularly in the gastrointestinal tract, a reduction or delay in post-prandial hyperglycemia can be achieved [21]. Earlier studies have shown significant inhibition of α-amylase and α-glucosidase by marine organisms like cyanobacteria, actinomycetes, and marine fungi [23]. Additionally, marine resources such as microalgae enriched with carotenoids like astaxanthin, lutein, and macroalgae (seaweeds) enriched with bioactive compounds such as polyphenols, polysaccharides, and minerals have been reported for their potent anti-diabetic effects [4, 24]. Consistent with these previous findings, the marine macrolide bryostatin 1 has also shown significant inhibition of carbohydrate-hydrolyzing enzymes, suggesting that the compound may either reduce or delay post-prandial hyperglycemia, thereby limiting glucose absorption.

Another frequently studied approach in diabetes research involves investigating the potential of inhibiting dipeptidyl peptidase 4 (DPP-IV). DPP-IV inhibitors work by reducing glucagon hormone levels, resulting in a decrease in blood glucose levels. Furthermore, their main mechanism involves increasing incretin hormones, specifically GLP-1 and GIP, which then inhibit glucagon release and ultimately stimulate the heightened secretion of insulin [25]. A prior study demonstrated that a spineless marine cuttlefish, Sepiella inermis, containing two novel chromenyl derivatives, significantly inhibited DPP-IV enzyme activity [26]. In a recent investigation, Unnikrishnan and colleagues (2022) [27] extensively explored the potential anti-diabetic effects of extracts from Ulva reticulata, an edible seaweed. They highlighted the significant inhibitory effects of *Ulva reticulata* on amylase, glucosidase, and DPP-IV, as well as its antioxidant potential (DPPH) in vitro. Supporting these findings, the substantial inhibition of DPP-IV activity by Bryostatin 1 suggests that the compound may demonstrate anti-diabetic efficacy by influencing incretin hormones and the associated cascade of events.

Diabetic retinopathy, a common complication arising from diabetes, is characterized by damage to the retina, leading to a rapid deterioration of vision and the potential risk of blindness. Key areas of research in diabetic retinopathy center around the formation and buildup of advanced glycation end-products (AGEs) and elevated levels of specific proteins and enzymes like aldolase

reductase [28, 29]. Additionally, these complications stem from increased diacylglycerol-Protein kinase C (DAG-PKC) levels, oxidative stress, and an augmented presence of glycation [30]. Bryostatin 1, known as a Protein Kinase C (PKC) regulator, plays a crucial role. It can either stimulate or suppress PKCs, influencing the progression of cell cycles. The ultimate action of PKC depends on the duration of exposure to Bryostatin 1; short-term interaction leads to autophosphorylation and PKC activation, while prolonged interaction results in PKC inhibition [31, 32]. This recent study has demonstrated significant inhibition against both AGEs and aldolase reductase, suggesting that the compound could be effective in managing diabetes-related complications. The underlying mechanism for this effect may involve the modulation of the PKC pathway.

CONCLUSION

In conclusion, the findings from this study on the anti-diabetic effect of Bryostatin 1 present a promising avenue for managing complications associated with diabetes. However, it's crucial to acknowledge certain limitations within this study. First and foremost, while the results showcase significant inhibition against key diabetic enzymes, further research is needed to explore the long-term effects and potential side effects of Bryostatin 1. Continued research and clinical trials will be essential to validate its safety, efficacy, and broader applicability in managing diabetes-related complications.

CONFLICT OF INTEREST

There is no conflict of interest.

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