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ORIGINAL PAPER

Dimethyl Fumarate Mitigates Tauopathy in Aβ-Induced Neuroblastoma SH-SY5Y Cells

Mithun Singh Rajput[1] · Nilesh Prakash Nirmal[2] · Devashish Rathore[1] · Rashmi Dahima[1]

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Abstract

Alzheimer's disease pathogenesis is measured by two key hallmarks viz extracellular senile plaques composed of insoluble amyloid beta (Aβ) and neurofibrillary tangles composed of hyperphosphorylated tau, resulting in microtubule destabilization, synaptic damage and neurodegeneration. Accumulation of Aβ is an introducing pathological incident in Alzheimer's disease; hence, the effect of dimethyl fumarate (DMF) on $A\beta_{1\text{-}42}$-induced alterations in phosphorylated tau, related protein kinases, fibrillogenesis and microtubule assembly in neuroblastoma SH-SY5Y cells was determined. DMF attenuated $A\beta_{1\text{-}42}$-induced neuronal apoptosis by down-regulating protein levels of Bcl-2/Bax, cleaved caspase-3 and caspase-9. $A\beta_{1\text{-}42}$-induced upsurge in tau phosphorylation at Ser396 and Thr231 epitopes was found to be declined by DMF pretreatment. The upregulated activity of glycogen synthase kinase-3 beta (GSK-3β) by $A\beta_{1\text{-}42}$ treatment was blocked by DMF pretreatment. PI3K substrate Akt (at Ser473) as well as Wnt dependent β-catenin and cyclin D1 activity was found to be upregulated by DMF pretreatment in $A\beta_{1\text{-}42}$ treated cells. ThT fluorescence and MTT assay showed that DMF reduces Aβ fibrillogenesis and inhibit related cytotoxicity. Also, DMF exerts a protective effect on $A\beta_{1\text{-}42}$-induced microtubule disassembly caused due to a reduction in polymerized β3-and α-tubulin. These results indicate that down-regulation of GSK-3β activity and subsequent activation of PI3K/Akt and Wnt/β-catenin signaling pathways are closely involved in the shielding effect of DMF against $A\beta_{1\text{-}42}$-induced tau hyperphosphorylation. Modulating cellular events related to $A\beta_{1\text{-}42}$-induced tau hyperphosphorylation, aggregation and microtubule stabilization offers new molecular insights into the defensive outcome of DMF towards appropriate management for Alzheimer's disease.

Keywords Dimethyl fumarate · Fibrillogenesis · GSK-3β · Microtubule assembly · Tau

Introduction

Alzheimer's disease pathogenesis is considered by two key hallmarks viz formation of extracellular senile plaques composed of insoluble amyloid beta (Aβ) peptides and intraneuronal deposit of neurofibrillary tangles of aggregated hyperphosphorylated tau [1]. Tau, a microtubule stabilizing protein, regulated by ample post-translational changes that include phosphorylation, n-glycosylation, acetylation, ubiquitination, truncation and many more [2]. Pathological hyperphosphorylation of tau consequences in its disassociation from microtubules and upsurge in aggregation and buildup as cytotoxic neurofibrillary tangles [3]. Numerous tau kinases have been studied, amongst them glycogen synthase kinase 3 beta (GSK-3β) is the foremost that phosphorylate tau together in vitro and in vivo and anticipated as a goal for therapeutic intervention [4]. An association between Aβ toxicity and tau pathology has been verified by experiments using neuronal cell cultures, wild and transgenic mouse models and Alzheimer's disease patients [5]. Considering the evidences, that alteration of tau by Aβ endorses generation of tangles in Alzheimer's disease, we hypothesize about the preventing strategy not merely by altering phosphorylated condition of tau, but also by obstructing Aβ

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✉ Mithun Singh Rajput
mithun.sgsits@gmail.com

[1] School of Pharmacy, Devi Ahilya Vishwavidyalaya, Takshashila Campus, Khandwa Road, Indore, M.P. 452001, India

[2] Institute of Nutrition, Mahidol University, 999 Phutthamonthon 4 Road, Salaya 73170, Nakhon Pathom, Thailand

aggregation and disrupting microtubule disassembly caused by Aβ.

During the progress of plaque formation, the spare amyloid remains in form of amyloid fibrils [6]. As soon as the fibrils are generated, they start to nurture by trapping metastable monomers, an autocatalytic procedure termed as fibrillation, consequently exert strong fatality on the cells [7]. As amyloid toxicity is associated with the active fibrillization process, attempts should be made to screen compounds that may inhibit Aβ fibrillogenesis and prevent related cytotoxicity. Concurrently, Aβ has been associated with withdrawal of synaptic links, which can be related to microtubule deregulation, way before major cytotoxic events occur [8]. Aβ cause a reduction in α-tubulin acetylation, signifying a microtubule instability in primary neuronal cultures [9]. These Aβ-induced toxicities may be prevented by a potent microtubule stabilizer.

Ample studies provide basis for our hypothesis that Aβ and induced tauopathy synergize to create a defining pathology in Alzheimer's disease, mitigation of such condition using novel therapeutic strategies are the focus of our current study. Regardless of enormous collection of natural and synthetic molecules against Alzheimer's disease, most of them failed in various phase of clinical trials [10]. Hence, there is a requirement to discover novel molecules against diverse targets of Alzheimer's disease.

Dimethyl fumarate (DMF) is a synthetic nuclear factor erythroid-2-related factor 2 (Nrf2) stimulator that alkylates cysteine residues on Keap-1, avert Nrf2 ubiquitination and endorse consequent activation of Nrf2 target genes, which effects on both antioxidant and inflammatory pathways [11]. DMF is evidenced to be a neuroprotective drug in plentiful studies in vitro and in vivo and confirmed rapid and persistent competence in clinical trials on patients with relapsing–remitting multiple sclerosis [12]. Campolo et al. (2018) confirms the neuroprotective effect of DMF against Aβ-induced cytotoxicity in neuronal cells demonstrating primary consequence of DMF treatment on tau phosphorylation [13]. Hence, we thought to extend the investigation by finding subsequent pathways related to tauopathy and other microtubule related abnormalities. Therefore, the present study has been undertaken to investigate the potential of DMF on downstream signaling targets of tau phosphorylation, Aβ fibrillogenesis and microtubule disassembly in neuroblastoma SH-SY5Y cells treated with $A\beta_{1\text{-}42}$.

Materials and Methods

SH-SY5Y Human Neuroblastoma Cell Culture

Procurement of SH-SY5Y human neuroblastoma cells was done from American Type Culture Collection (ATCC, Manassas, VA, USA) (RRID: CVCL_0019). The cells were full-fledged to monolayer in a culture medium comprising 45% Dulbecco's Minimal Essential Medium; 45% Ham's F12 modified with 2 mM L-glutamine and 1.0 mM sodium pyruvate; 10% foetal bovine serum and 100 U/ml penicillin/streptomycin. The cells were kept at 37 °C in a 5% CO_2/95% humidified air incubator for the specified time. Cells from passage 15 to 25 were used in the experiments. Cells were coated in 96-well plates and grown to 70–80% confluence for the experiments.

Preparation of Oligomeric and Fibrillar $A\beta_{1\text{-}42}$

To prepare oligomeric Aβ, lyophilized $A\beta_{1\text{-}42}$ was dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol at a concentration of 1 mM, stirred under a fume hood for 48 h. Hexafluoroisopropanol was removed under vacuum and the peptide film was stored desiccated at − 20 °C and used within 36 h [14]. Cell culture medium was utilized to dilute stock solution to attain 1 µM final concentration. To get fibrillar Aβ, 10 mM hydrochloric acid was used to dilute the peptide to a final concentration of 1 µM incubated for 24 h at 37 °C [14]. The excess hydrochloric acid was drained off using a Whatman filter paper and obtained fibrils were washed thrice with fresh culture media. The oligomeric and fibrillar forms of $A\beta_{1\text{-}42}$ have been used in the study after confirmation by Western blotting. Oligomeric and fibrillar forms were separately undergone Western blotting and matched with the standard blots obtained by Dahlgren et al. [14].

Cell Treatments

The SH-SY5Y cells were assigned to three separate experimental groups viz. control cells (without treatment), $A\beta_{1\text{-}42}$ stimulated cells and $A\beta_{1\text{-}42}$ + DMF treated cells. The later group of cells were pre-incubated with DMF (30 µM) (98%, Sigma-Aldrich Co., St. Louis, USA) for 2 h, thereafter stimulated with oligomeric $A\beta_{1\text{-}42}$ (1 µM) or $A\beta_{1\text{-}42}$ fibril (1 µM) for 24 h, for Western blotting and thioflavin-T (ThT) fluorometric assay and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay respectively. Dose of DMF were based on previous study indicating neuroprotective efficacy of DMF in Aβ-induced neuronal cells [13] and pilot studies conducted in our lab.

Cell Extracts Preparation Comprising Polymeric Tubulin

The fraction of polymerized tubulin was extracted from SH-SY5Y cells according to the method discussed by Joshi and Cleveland (1989). Cells were gently washed twice with a microtubule stabilizing buffer containing 0.1 M n-morpholinoethanesulfonic acid (pH 6.75), 1.0 mM magnesium

sulphate, 2.0 mM EGTA and 4.0 M glycerol. Soluble proteins were extracted at 4 to 6 min at 37 °C in 500 µl of microtubule stabilizing buffer comprising Triton X-100 (0.1%). The soluble extract was removed and centrifuged for 2 min in order to pellet any cytoskeletal material dislodged from the culture dish during extraction. For analysis of polymerized tubulin, cells were extracted with a scraper in 200 µl of 25 mM Tris (pH 6.8) containing 0.5% sodium dodecyl sulphate; then, samples were frozen [15]. Concentration of polymerised tubulins was determined by bicinchonic acid assay as per Walker [16]. Extracted proteins (polymerized β3-and α-tubulin) were submitted to Western blotting, as described.

Western Blotting

SH-SY5Y cells were washed twice with ice-cold phosphate buffered saline, harvested and lysed by resuspending in lysis buffer containing Tris–HCl (20 mM, pH 7.5), NaF (10 mM), NaCl (150 µl), 1% Nonidet P-40 and protease inhibitor cocktail. After 40 min, cell lysates were centrifuged at 16,000×*g* for 15 min at 4 °C. The supernatants were collected, heated at 95 °C for 5 min and run for 12% sodium dodecyl sulphate–polyacrylamide gel electrophoresis. The protein bands were electrophoretically transferred to nitrocellulose membranes; then, the nitrocellulose membranes were washed with tris buffered saline for three times for 5 min each. The membrane was incubated with blocking solution containing 3% non-fat dry milk in 0.1% tris buffered saline with tween 20 (TBST) for 60 min and then washed three times for 5 min each with TBST. The membranes were then incubated in primary antibodies at 4 °C overnight with various antibodies as presented in Table 1. Then, the membranes were incubated with an HRP-conjugated anti-mouse antibody (1:10,000, Sigma-Aldrich Co., St. Louis, USA) for 90 min. After the incubation, the membranes were washed three times for 5 min each with TBST.

The blots were incubated for 1 min with the working solution of chemiluminescence substrate prepared as per manufacturer's instructions. The blots were removed from the working solution and placed in a clear sheet protector; the bubbles were removed by rolling with blot roller. Then the relative expression of protein bands was attained using enhanced chemiluminescence system (ThermoFisher Scientific, IL, USA) imaged with the Chemi-Doc XRS Plus System (Bio-Rad Labs Inc.) and quantitated by densitometry. Molecular weight standards (10–250 kD) were used to define molecular weight positions and as reference concentrations for each protein.

Thioflavin-T Fluorometric Assay

ThT (5 µM) was prepared in Tris–HCl buffer pH 7.4 and kept in a vial covered with aluminium foil to guard from the photo-oxidation. Tris–HCl buffer (200 µl, pH 7.4) added with 20 µl of ThT and $A\beta_{1\text{-}42}$ solubilization media were used as control. The black sterile 96 well microplate was incubated with $A\beta_{1\text{-}42}$ fibril (1 µM) and 5 µM ThT solution

Table 1 Antibodies used in Sect. "Western blotting" in the study

Antibody	Clone	Dilution	Supplier
Anti-cytochrome c	Polyclonal	1:1000	Bio-Rad Labs., Gurgaon, Haryana
Anti-Bcl-2 antibody	Monoclonal	1:1000	Bio-Rad Labs., Gurgaon, Haryana
Anti-Bax	Polyclonal	1:1000	Bio-Rad Labs., Gurgaon, Haryana
Cleaved anti-caspase-3	Polyclonal	1:1000	Sigma-Aldrich Co., St. Louis, USA
Cleaved anti-caspase-9	Polyclonal	1:1000	Sigma-Aldrich Co., St. Louis, USA
Anti-tau	Monoclonal	1:10,000	Sigma-Aldrich Co., St. Louis, USA
Anti-phospho tau (pSer396)	Polyclonal	1:1000	Sigma-Aldrich Co., St. Louis, USA
Anti-phospho tau (pThr231)	Polyclonal	1:1000	Sigma-Aldrich Co., St. Louis, USA
Anti-GSK-3β (Ser9)	Polyclonal	1:1000	Bio-Rad Labs., Gurgaon, Haryana
Anti-phospho-GSK-3β (Ser9)	Polyclonal	1:1000	Merck, Mumbai, India
Anti-phospho-p38 MAPK	Polyclonal	1:1000	ThermoFisher Scientific, MA, USA
Anti-phospho-JNK	Polyclonal	1:1000	ThermoFisher Scientific, MA, USA
Anti-Akt1	Monoclonal	1:1000	ThermoFisher Scientific, MA, USA
Anti-phospho-Akt (Ser473)	Polyclonal	1:1000	ThermoFisher Scientific, MA, USA
Anti-β-catenin	Polyclonal	1:1000	Bio-Rad Labs., Gurgaon, Haryana
Anti-cyclin D1	Polyclonal	1:1000	Bio-Rad Labs., Gurgaon, Haryana
Anti-β3-tubulin	Polyclonal	1:1000	Sigma-Aldrich Co., St. Louis, USA
Anti-α-tubulin	Monoclonal	1:4000	Sigma-Aldrich Co., St. Louis, USA
Anti-GAPDH	Monoclonal	1:1000	Sigma-Aldrich Co., St. Louis, USA
Anti-β-actin	Monoclonal	1:5000	Sigma-Aldrich Co., St. Louis, USA

with or without DMF (30 µM) for 2 h unshaken at the room temperature. The absorbance was measured at excitation 450 nm and emission 480 nm on fluorescence spectrophotometer (Hitachi). Sample fluorescence was determined by averaging the three readings and subtracting the fluorescence of a ThT blank [17].

MTT Assay

Different groups of cultured SH-SY5Y cells were pre-incubated with or without DMF (30 µM), for 2 h and stimulated with $A\beta_{1\text{-}42}$ fibril (1 µM) for 24 h in 96-well plates. Subsequently, culture medium was removed and cells were treated with 0.5 mg/ml of MTT and incubated at 37 °C for 4 h. Thereafter, the culture medium was removed and dimethyl sulfoxide was added to solubilize the reaction product formazan by shaking the plates for 10 min. The optical density at 570 nm was measured by means of a microplate reader. Cell viability percentage was calculated and expressed in relative to the value in the vehicle-treated control culture containing solubilization agent (10 mM hydrochloric acid) [14].

Statistical Analysis

Statistical analysis was accomplished by means of GraphPad Prism Version 7.0 for Windows. (GraphPad Software Inc., San Diego, CA). Communication of results was done as Mean ± S.E.M. Significance was assessed through two-ways or one-way analysis of co-variance (ANCOVA) trailed by Bonferroni or Tukey's multiple evaluation trials. Probability (p) values of less than 0.05 were considered as significantly different.

Results

DMF Amends $A\beta_{1\text{-}42}$-Induced Cell Apoptosis: Effect on Cytochrome c, Bcl-2, Bax, Caspase-3 and Caspase-9 Expression

There was a significant increase ($p < 0.001$) in release of cytochrome c by SH-SY5Y cells treated with oligomeric $A\beta_{1\text{-}42}$ in comparison to the control group which was significantly inhibited ($p < 0.001$) by DMF (30 µM) pretreatment [$F(2, 6) = 882.00$, $p < 0.0001$, $R^2 = 0.9966$] (Fig. 1a).

Figure 1b indicates a significant decrease ($p < 0.001$) in expression of protein ratio of Bcl-2/Bax in cells treated with oligomeric $A\beta_{1\text{-}42}$ in comparison to the control group, which was significantly ($p < 0.001$) reversed by DMF pretreatment [$F(2, 6) = 318.20$, $p < 0.0001$, $R^2 = 0.9907$].

Oligomeric $A\beta_{1\text{-}42}$ treatment in cells caused a significant increase ($p < 0.001$) in protein levels of both cleaved

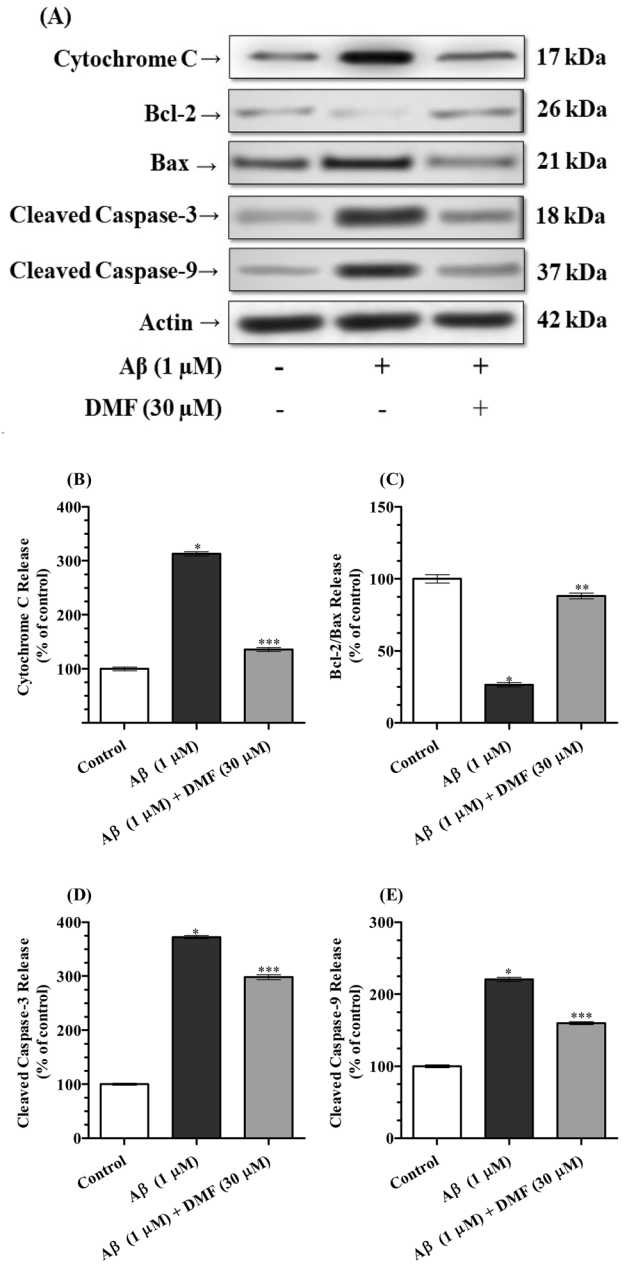

Fig. 1 DMF pretreatment regulated $A\beta_{1\text{-}42}$-induced changes in apoptotic markers in neuroblastoma SH-SY5Y cells. Cultured cells were pretreated with DMF (30 µM) for 2 h prior to the 24 h $A\beta_{1\text{-}42}$ (1 µM) treatment. Western blotting was performed to assess the expression of cytochrome c, Bcl-2, Bax, cleaved caspase-3 and cleaved caspase-9 using respective antibodies. The relative expression of protein bands was attained using ECL, imaged and quantitated by densitometry. $A\beta_{1\text{-}42}$ treatment caused increase in release of cytochrome c, cleaved caspase-3 and cleaved caspase-9 and decrease in expression of protein ratio of Bcl-2/Bax by SH-SY5Y cells in comparison to respective control groups. These effects were significantly inhibited by DMF pretreatment. The data indicating levels of all the mentioned proteins were normalized with respect to that of actin. **a** Western blots of cytochrome c, Bcl-2, Bax, cleaved caspase-3 and cleaved caspase-9; **b** expression of cytochrome c **c** expression of Bcl-2/Bax; **d** expression of cleaved caspase-3 and **e** expression of cleaved caspase-3. Results are expressed as Mean ± S.E.M.; (n = 3). Data was analyzed by one way repeat measure ANCOVA followed by Tukey's multiple comparison test. Significance: $^{*}p < 0.001$ compared with control group; $^{**}p < 0.01$ and $^{***}p < 0.001$ compared with $A\beta_{1\text{-}42}$ (1 µM) treated group. *Aβ* amyloid beta, *DMF* dimethyl fumarate

caspase-3 and cleaved caspase-9 respectively in comparison with separate controls. The expression of both the proteins was found to be significantly decreased ($p < 0.01$ and $p < 0.001$ respectively) in DMF pre-treated cells compared with respective $A\beta_{1\text{-}42}$ treatment group [$F(2, 6) = 1879.00$, $p < 0.0001$, $R^2 = 0.9984$ for caspase-3] [$F(2, 6) = 666.50$, $p < 0.0001$, $R^2 = 0.9955$ for caspase-9] (Fig. 1c, d).

DMF Reduces $A\beta_{1\text{-}42}$-Induced Tau Phosphorylation at Ser396 and Thr231 Sites

Oligomeric $A\beta_{1\text{-}42}$ treatment does not express any significant change in total tau protein [$F(2, 6) = 0.4614$, $p = 0.6511$, $R^2 = 0.1333$] (Fig. 2a, b); though, expression of phosphorylated tau protein at Ser396 and Thr231 sites was significantly increased ($p < 0.001$ for both) in SH-SY5Y cells when compared with respective controls. DMF treatment significantly reduced ($p < 0.001$ and $p < 0.01$ respectively) phosphorylation of tau at Ser396 and Thr231 sites in comparison with respective $A\beta_{1\text{-}42}$ treatment groups [$F(2, 6) = 60.64$,

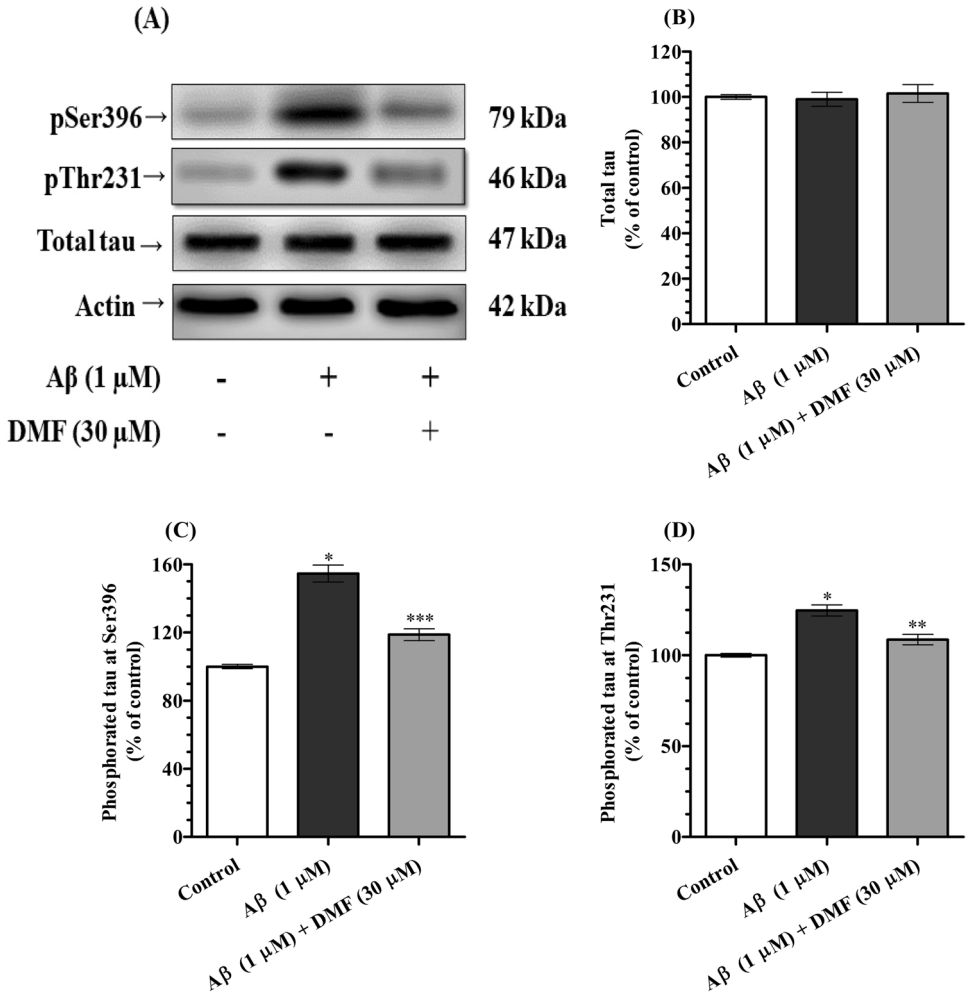

Fig. 2 Effect of DMF treatment on tau phosphorylation in oligomeric $A\beta_{1\text{-}42}$-induced neuroblastoma SH-SY5Y cells. As seen by Western blotting, $A\beta_{1\text{-}42}$ (1 μM) or DMF (30 μM) treatment does not cause any change in total tau levels in SH-SY5Y cells. Incubation with 1 μM of $A\beta_{1\text{-}42}$ significantly increased phosphorylated tau after 24 h of stimulation, which was protected by DMF (30 μM) pretreatment for 2 h. The levels of phosphorylated tau at both sites were normalized with respect to that of total tau. **a** Western blots with phosphorylated tau specific antibodies at Ser396 and Thr231; **b** total tau protein expression; **c** relative phosphorylated tau expression at Ser396 site and **d** Thr231 site. Results are expressed as Mean ± S.E.M.; (n = 3). The relative expression of protein bands in Sect. "Western blotting" was attained using ECL, imaged and quantitated by densitometry. Data was analyzed by one-way repeat measure ANCOVA followed by Tukey's multiple comparison test. Significance: $^{*}p < 0.001$ compared with control group; $^{**}p < 0.01$ and $^{***}p < 0.001$ compared with $A\beta_{1\text{-}42}$ (1 μM) treated group. *Aβ* amyloid beta, *DMF* dimethyl fumarate, *pSer396* phosphorylated tau at Ser396 site, *pThr231* phosphorylated tau at Thr231 site

$p = 0.0001$, $R^2 = 0.9529$ for pSer396] (Fig. 2a, c); and [$F(2, 6) = 25.70$, $p = 0.0011$, $R^2 = 0.8955$ for pThr231] (Fig. 2a, d).

DMF Decreases $A\beta_{1\text{-}42}$-Induced Tau Phosphorylation Via GSK-3β but not Through p38 MAPK and JNK Signaling

The effect of DMF on oligomeric $A\beta_{1\text{-}42}$-induced tau hyperphosphorylation was investigated on a possible signaling pathway; GSK-3β, a tau phosphorylation kinase recognized both in vivo and in vitro for converting normal tau to the paired helical filament state [18]. Treatment of SH-SY5Y cells with oligomeric $A\beta_{1\text{-}42}$ significantly increased ($p < 0.01$) expression levels of GSK-3β and reduced ($p < 0.001$) levels of GSK-3β phosphorylation at Ser9 (the inhibitory phosphorylated site) as compared with respective control groups. DMF treatment significantly blocked ($p < 0.01$) these oligomeric $A\beta_{1\text{-}42}$-induced phosphorylation effects on GSK-3β [$F(2, 6) = 20.60$, $p = 0.0021$, $R^2 = 0.8729$ for GSK-3β] and [$F(2, 6) = 25.41$, $p = 0.0012$, $R^2 = 0.8944$ for p-GSK-3β] (Fig. 3a, b).

Another kind of kinases that belong to microtubule associated protein kinase (MAPK) category, subcategorised as stress activated protein kinases viz p38 MAPK and JNK lead towards hyperphosphorylation of tau [19]. Hence, the effect of DMF on these kinases signaling on $A\beta_{1\text{-}42}$-induced tau

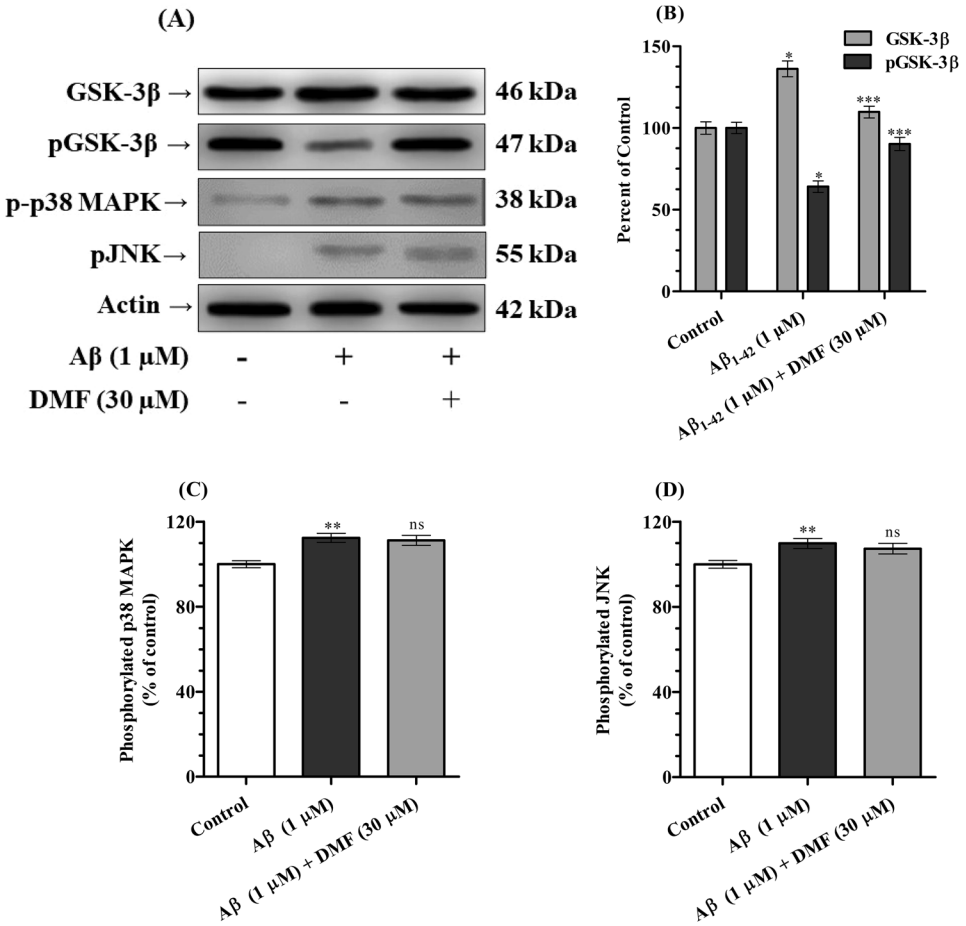

Fig. 3 Effect of DMF treatment on GSK-3β and MAPK expression in oligomeric $A\beta_{1\text{-}42}$-induced neuroblastoma SH-SY5Y cells. As evident from Western blotting using antibodies specifically to total GSK-3β or pGSK-3β, it was found that $A\beta_{1\text{-}42}$ (1 μM) increased GSK-3β levels and decreased pGSK-3β levels in SH-SY5Y cells after 24 h of stimulation, these effects of $A\beta_{1\text{-}42}$ were protected by DMF (30 μM) pretreatment for 2 h. The levels of p-p38 MAPK and p-JNK were increased by 24 h stimulation of $A\beta_{1\text{-}42}$ (1 μM), however DMF (30 μM) pretreatment does not cause any effect on these kinases. The level of pGSK-3β was normalized with respect to that of GSK-3β and p-p38-MAPK and p-JNK expression levels were normalized to actin. **a** Western blots of GSK-3β, pGSK-3β, p-p38 MAPK and p-JNK; **b** expression of GSK-3β and pGSK-3β **c** expression of p-p38 MAPK and **d** pJNK. Results are expressed as Mean ± S.E.M.; (n = 3). The relative expression of protein bands in Sect. "Western blotting" was attained using ECL, imaged and quantitated by densitometry. Data was analyzed by one-way repeat measure ANCOVA followed by Tukey's multiple comparison test. Significance: $^{*}p < 0.01$ and $^{**}p < 0.05$ compared with control group; $^{***}p < 0.01$ and $^{ns}p > 0.05$ compared with $A\beta_{1\text{-}42}$ (1 μM) treated group. *Aβ* amyloid beta, *DMF* dimethyl fumarate, *GSK-3β* glycogen synthase kinase 3 beta, *JNK* c-Jun N-terminal kinase, *pGSK-3β* phosphorylated GSK-3β, *pJNK* phosphorylated JNK, *p38 MAPK* p38 mitogen-activated protein kinase, *p-p38 MAPK* phosphorylated p38 MAPK

hyperphosphorylation was studied. The phosphorylation of p38 MAPK and JNK in the oligomeric $A\beta_{1-42}$ treated groups was found to be enhanced ($p < 0.05$ for both kinases) as compared with respective controls. Though, DMF treatment does not cause any significant changes ($p > 0.05$) in p-p38 MAPK and p-JNK in comparison with respective $A\beta_{1-42}$ treated groups [$F(2, 6) = 11.06$, $p = 0.097$, $R^2 = 0.7866$ for p-p38 MAPK] (Fig. 3a, c); and [$F(2, 6) = 5.1710$, $p = 0.0495$, $R^2 = 0.6328$ for p-JNK] (Fig. 3a, d).

Modulation of GSK-3β Mediated PI3K/Akt and Wnt/β-Catenin Signaling Pathways by DMF Employs Shielding Effect Against $A\beta_{1-42}$-Induced Toxicity

The upsurge in GSK-3β is brought through inhibition of PI3K/Akt signaling pathway. Moreover, Wnt/β-catenin signaling employs a shielding effect on neurons in contradiction of Aβ-induced toxicity and GSK-3β is known to employ an adverse effect on Wnt signaling pathway, that results in decreased transcription and expression of β-catenin and the target gene cyclin D1 [20]. Hence, we studied whether DMF exerts any effect on GSK-3β mediated PI3K/Akt and Wnt/β-catenin signaling pathways.

As evident from Fig. 4a, oligomeric $A\beta_{1-42}$ treatment caused a significant reduction in the phosphorylated Akt protein at the Ser473 site ($p < 0.001$), which was significantly blocked ($p < 0.01$) by DMF pretreatment in $A\beta_{1-42}$-treated cells [$F(2, 6) = 58.88$, $p = 0.0001$, $R^2 = 0.9515$]. The amount of total Akt protein expression in all treatment groups kept unchanged in SH-SY5Y cells. The expression of β-catenin as well as cyclin D1 was significantly reduced ($p < 0.01$ for both) in oligomeric $A\beta_{1-42}$-treated cells, which was significantly blocked ($p < 0.05$ for β-catenin and $p < 0.01$ for cyclin D1) by DMF treatment [$F(2, 6) = 23.85$, $p = 0.0014$, $R^2 = 0.8883$ for β-catenin] [$F(2, 6) = 20.14$, $p = 0.0022$, $R^2 = 0.8703$ for cyclin D1] (Fig. 4b). It was observed that

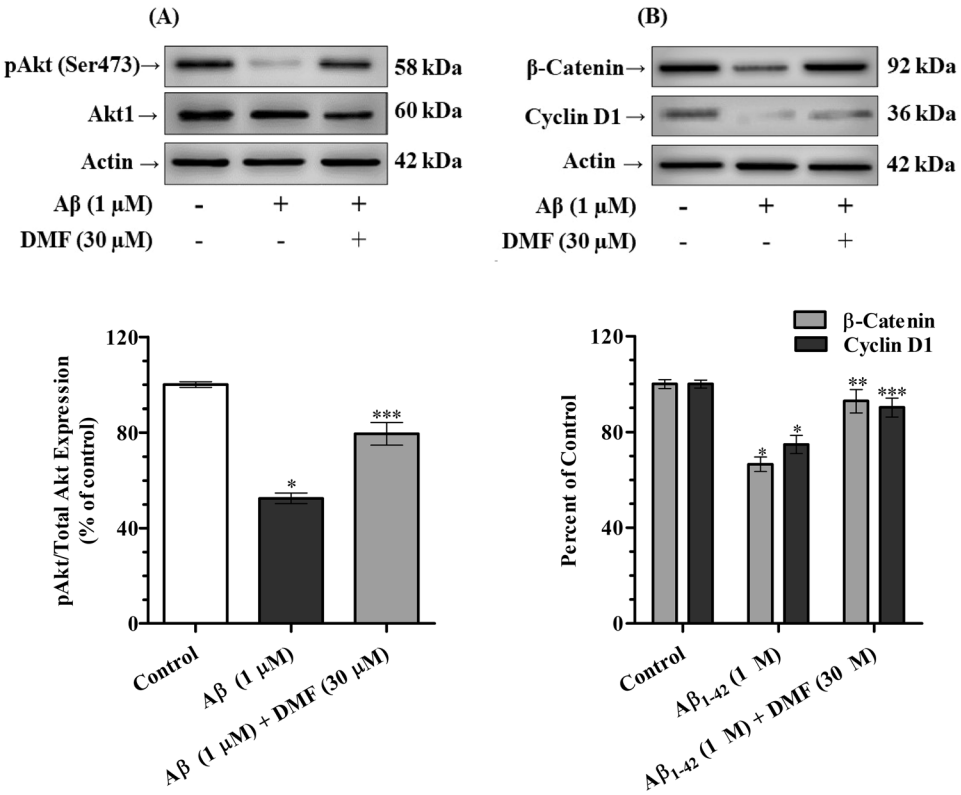

Fig. 4 Influence of DMF on **a** GSK-3β mediated PI3K/Akt and **b** Wnt/β-catenin signaling pathways in SH-SY5Y cells treated with oligomeric $A\beta_{1-42}$. SH-SY5Y cells were pretreated with DMF (30 μM) for 2 h prior to the 24 h $A\beta_{1-42}$ (1 μM) treatment. Western blotting was performed to test the expression of p-Akt at Ser473 site, along with total Akt1 expression as a control using respective antibodies. DMF pretreatment protected lowering of expression ratio of these kinase brought by $A\beta_{1-42}$ treatment. Effect of DMF (30 μM) pretreatment for 2 h on Wnt/β-catenin pathway was studied using Western blotting in the 24 h $A\beta_{1-42}$ (1 μM) treated SH-SY5Y cells using β-catenin and cyclin D1 antibodies and data was normalized with respect to that of actin. β-catenin and cyclin D1 expression levels were lowered by $A\beta_{1-42}$ treatment and protected by DMF pretretment. The relative expression of protein bands in Sect. "Western blotting" was attained using ECL, imaged and quantitated by densitometry. Results are expressed as Mean ± S.E.M.; (n = 3). Data was analyzed by one-way repeat measure ANCOVA followed by Tukey's multiple comparison test. Significance: $^{*}p < 0.01$ when compared with control group; $^{**}p < 0.05$ and $^{***}p < 0.01$ when compared with $A\beta_{1-42}$ (1 μM) treated group. *Aβ* amyloid beta, *DMF* dimethyl fumarate

DMF treatment exerts shielding effect against oligomeric $A\beta_{1\text{-}42}$-induced toxicity by interfering with both the PI3K/Akt and Wnt/β-catenin signaling pathways.

DMF Prevents $A\beta_{1\text{-}42}$-Induced Microtubule Disassembly, Aβ Fibrillogenesis and Related Cytotoxicity

Hyperphosphorylated tau bears the capacity to modify cytoskeletal dynamics in neurons [7]. Hence, it was thought worth to evaluate the effect of DMF on the concentration of the neuronal marker, β3-tubulin and microtubule assembly following Aβ exposure. Western blotting revealed that exposure of SH-SY5Y cells to oligomeric $A\beta_{1\text{-}42}$ significantly ($p<0.01$) decreased total protein levels of cytoskeletal β3-tubulin, which was significantly prevented ($p<0.01$) by DMF pretreatment [$F(2, 6)=23.75$, $p=0.0014$, $R^2=0.8878$] (Fig. 5a, b).

Moreover, with the intention to examine whether the protective effect of DMF on oligomeric $A\beta_{1\text{-}42}$-induced reduction in neuronal specific β3-tubulin was related to lessening in polymerized tubulin (β3-and α-tubulin), the fraction of polymerized tubulin was extracted from SH-SY5Y cells. The levels of polymerized β3-and α-tubulin were found to be significantly reduced ($p<0.01$ for both) by $A\beta_{1\text{-}42}$ treatment as comparison with control. Preincubation with DMF

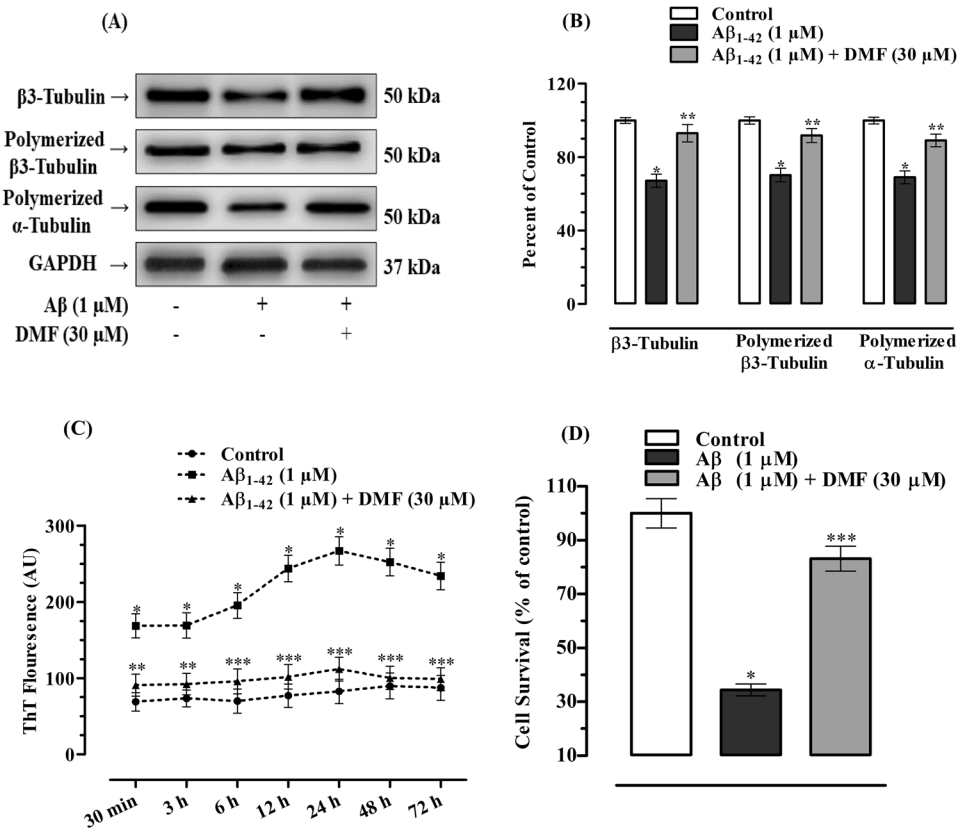

Fig. 5 Effect of DMF treatment on **a**, **b** microtubule disassembly in SH-SY5Y cells treated by oligomeric $A\beta_{1\text{-}42}$; **c** Aβ fibrillogenesis and **d** related cytotoxicity in SH-SY5Y cells treated by fibrillar $A\beta_{1\text{-}42}$. Cells were preincubated with DMF (30 μM) for 2 h and then treated with $A\beta_{1\text{-}24}$ (1 μM) for 24 h. Western blotting was performed and β3-tubulin protein levels were expressed in arbitrary units relative to GAPDH. $A\beta_{1\text{-}42}$ treatment decreased levels of β3-tubulin protein which was protected by DMF pretreatment. Polymerized tubulin fraction was extracted and Western blotting was performed for polymerized β3-tubulin or polymerized α-tubulin using respective antibodies and the data was normalized in respect to that of GAPDH. $A\beta_{1\text{-}42}$ treatment decreased levels of polymerized tubulin proteins which was protected by DMF pretreatment. Separate group of cells were treated in the similar manner and ThT fluorescence intensity was measured and expressed in relation to the control group at various time intervals. $A\beta_{1\text{-}42}$ treatment significantly enhanced ThT fluorescence which was protected by DMF pretetment. For MTT assay cultured SH-SY5Y cells were preincubated with DMF (30 μM) for 2 h and stimulated with $A\beta_{1\text{-}42}$ fibril (1 μM) for 24 h in 96-well plates. The optical density was measured and cell viability percentage was calculated and expressed in relative to the value in the vehicle-treated control group. $A\beta_{1\text{-}42}$ treatment decreased cell viability which was defended by DMF pretreatment. The relative expression of protein bands in Sect. "Western blotting" was attained using ECL, imaged and quantitated by densitometry. Results are expressed as Mean ± S.E.M.; ($n=3$). Data was analyzed by two-ways or one-way repeat measure ANCOVA followed by Bonferroni or Tukey's multiple comparison test. Significance: $^{*}p<0.01$ when compared with control group; $^{**}p<0.01$ and $^{***}p<0.001$ when compared with $A\beta_{1\text{-}42}$ (1 μM) treated group. *Aβ* amyloid beta, *DMF* dimethyl fumarate

significantly prevented ($p < 0.01$ for both) the decrease in polymerised β3-and α-tubulin level brought by oligomeric $A\beta_{1\text{-}42}$ [$F(2, 6) = 22.34$, $p = 0.0017$, $R^2 = 0.8816$ for polymerized β3-tubulin] and [$F(2, 6) = 26.74$, $p = 0.0010$, $R^2 = 0.8991$ for polymerized α-tubulin] (Fig. 5a, b).

$A\beta_{1\text{-}42}$ fibril were incubated in the presence and absence of DMF and fibrillogenesis was measured with the fluorescence after incubation with ThT solution. Formation of aggregates were significantly enhanced ($p < 0.001$) in $A\beta_{1\text{-}42}$ fibril treated group as compared to control cells. Contrarily, formation of Aβ aggregates were suppressed significantly ($p < 0.001$) in the presence of DMF [$F(2, 42) = 159.82$, $p < 0.0001$ (the column factor)] and time effect [$F(6, 42) = 3,81$, $p = 0.0040$ (the row factor)] (Fig. 5c) (Online Resource 1).

As evident from MTT assay, there was a significant decrease ($p < 0.001$) in survival of cells treated with fibrillar $A\beta_{1\text{-}42}$ in comparison with control cells. Pretreatment with DMF (30 μM) significantly diminished ($p < 0.001$) the effect of fibrillar $A\beta_{1\text{-}42}$ on cell death [$F(2, 6) = 62.75$, $p < 0.0001$, $R^2 = 0.9544$] (Fig. 5d).

Discussion

In comparison with other forms, principally oligomeric Aβ persuaded neuronal cell death, preventing synaptic function and plasticity [21]; hence, in present study shielding role of DMF on oligomeric $A\beta_{1\text{-}42}$-induced cytotoxicity of neuroblastoma pertaining to tau hyperphosphorylation and subsequent microtubule distortion, has been investigated.

The part of apoptosis in commencement and development of Alzheimer's disease has been finely recognized in vitro, in transgenic animal models and human patients of Alzheimer's disease [22]. In mammalian cells, apoptosis is chiefly mediated by mitochondria, the inner and outer membrane permeability of mitochondria is regulated by Bcl-2 and Bax expression, controlling the release of proteins from intermembrane space. Outcome of the mitochondrial proteins in cytosol triggers caspase proteases that shred the cells and signal effective phagocytosis of cell corpses [23]. Mitochondrial membrane permeability is preserved by an anti-apoptotic protein Bcl-2, therefore steadying mitochondrial integrity, weakening the release of cytochrome c and avoiding apoptosis. On the other hand, a pro-apoptotic protein, Bax expression consequences just reverse to that of Bcl-2, leading towards release of cytochrome c followed by activation of caspase-9, then caspase-3, in due course leading to cellular apoptosis [24]. Aβ persuades apoptosis by means of varying expression of the Bcl-2 and Bax genes [25]. DMF caused to reverse oligomeric $A\beta_{1\text{-}42}$-induced expression of Bcl-2/Bax, thus inhibiting the activity of cleaved caspase-9 and cleaved caspase-3 and release of cytochrome c, suggesting the shielding efficacy of DMF via inhibition of mitochondria dependent cellular apoptosis.

Aβ associated tau hyperphosphorylation (at Ser396 and Thr231) leads to its disturbed functioning in axonal transport, resulting in gathering of toxic neurofibrillary tangles [5]. Hence, in a zest to find therapeutic line for AD, it was supposed to cumulatively target these two proteins and found that DMF pretreatment vitally dropped oligomeric Aβ associated tau hyperphosphorylation.

Our study demonstrated that oligomeric $A\beta_{1\text{-}42}$ causes tau hyperphosphorylation largely through stimulating of GSK-3β, on the other hand DMF impedes GSK-3β bustle by enhancing p-GSK-3β (Ser9) expression, which constrains tau hyperphosphorylation in oligomeric Aβ-treated SH-SY5Y cells. As Aβ is known to cause cellular oxidative distress and DMF lowers Aβ-induced oxidative impairment [13], it is fascinating whether stimulation of GSK-3β brought up by Aβ is facilitated by oxidative stress. NF-κB is known to be positively regulated by GSK-3β via the degradation of IκB, a central inhibitor of NF-κB [26] and DMF is known to inhibit NF-κB pathway [13], Hence it can be postulated that DMF may regulates GSK-3β phosphorylation by affecting oxidative stress mediated by NF-κB signaling.

Another kind of kinases that belong to MAPK category, subcategorised as stress activated protein kinases viz p38 MAPK and JNK lead towards hyperphosphorylation of tau [18, 27]. Aβ is known to induce MAPK signaling [28]; hence, the effect of DMF on these kinases signaling was studied and found that DMF treatment does not cause any significant changes in p-p38 MAPK and p-JNK. Our results are found to be in accordance with the study by Peng et al. (2012), where it was found that in an in vitro model of bone marrow-derived dendritic cells, DMF modulates inflammatory conditions via NF-κB signaling but do not influence p-p38 or p-JNK activity [29]. Our studies suggest the shielding effect of DMF through involvement of GSK-3β but not p38 MAPK and JNK signaling in oligomeric $A\beta_{1\text{-}42}$-treated SH-SY5Y cells.

An added mechanism negatively regulated by GSK-3β, accountable for incidence and progression of AD is Wnt/β-catenin signaling [30]. The Wnt ligands are identified to stimulate two pathways: β-catenin reliant canonical signaling pathway (Wnt/β-catenin) and non-canonical signaling pathways (Wnt/PCP and Wnt/Ca^{2+}) [30]. The non-canonical signaling is known to ultimately activate JNK, regulating cytoskeleton reorganization [31]. Hence, we did not screen the effect of DMF on non-canonical signaling pathways as DMF does not cause any effect on JNK, previously shown in our study (Fig. 3). The expression of β-catenin and the target gene cyclin D1 is reduced by GSK-3β via phosphorylation and ubiquitination, hindering normal cell growth and differentiation, causing neuron death [20]. The present study suggested that DMF activates β-catenin and cyclin D1

in the Wnt/β-catenin signaling pathway to inhibit GSK-3β activity and exert cytoprotective effects against oligomeric $A\beta_{1-42}$-induced toxicity may be by affecting expression of Wnt target genes.

PI3K/Akt signaling pathway plays a fundamental part in the GSK-3β-mediated tau protein hyperphosphorylation and neuronal survival [32]. As, exposure to Aβ is known to influence PI3K/Akt signaling, thus causing alteration in AD brains [33], the involvement of PI3K/Akt/GSK-3β pathway on oligomeric Aβ-induced tau phosphorylation and protective effect of DMF was investigated in this study. DMF found to inhibit dephosphorylation of Akt at Ser473 and proved that Akt mediates the protective efficacy of DMF against oligomeric Aβ-induced activation of GSK-3β and tau phosphorylation. We speculated that DMF at first may trigger PI3K by membrane receptor, resulting in production of PIP3 and PIP2. These two phospholipids function in numerous vital cellular processes, like plasma membrane-cytoskeleton linkages, membrane trafficking, cell adhesion and motility, second messenger signaling and regulation of proteins involved in phospholipid metabolism. These phosphoinositides direct two chief self-regulating signaling cascades. PIP3 is the effector of multiple downstream targets of the PI3K pathway. Stimulation of PI3K by growth factor activation of cells outcomes in PIP3 synthesis caused by phosphorylation of PIP2. PIP2 is the precursor of the second messengers in cellular signaling- Ca^{2+}-mobilizing messenger IP3 and the protein kinase C activator diacylglycerol. Subsequent interaction of these phospholipids with Akt cause a conformational change, further consequential inhibition of GSK-3β. It's a well-meaning point of note that stimulatory effect of DMF on PI3K/Akt signaling pathway has been formerly specified in the cell model of systemic sclerosis dermal fibrosis [34] and was consistent with our results.

Cuadrado et al. (2018) demonstrated that DMF moderates quite a few mechanisms of brain protection, crucial in tauopathy. For the first time the investigators confirmed that pharmacological treatment with DMF provides a double mechanism of activation of NRF2 that might be used to treat tau-related neurodegeneration. Cuadrado et al. (2018) found that DMF induces the NRF2 transcriptional through a mechanism that involves KEAP1 but also PI3K/AKT/GSK-3-dependent pathways; also, DMF modulates GSK-3β activity in mouse hippocampi. This study reveals neuroprotective effects of DMF beyond disruption of the KEAP1/NRF2 axis by inhibiting GSK3 in a mouse model of tauopathy [35]. This study by Cuadrado et al. (2018) provided us a basis that DMF has a potency to mitigate tauopathy by various mechanisms, hence we investigated the potential of DMF on $A\beta_{1-42}$-induced tau phosphorylation and found that down-regulation of GSK-3β activity and subsequent activation of PI3K/Akt and Wnt/β-catenin signaling pathways are closely involved in the shielding effect of DMF against $A\beta_{1-42}$-induced tau hyperphosphorylation.

Microtubule destabilization triggered by Aβ was previously evidenced in neuronal cell cultures, animal models and human brains [36]. Our results indicated that DMF pretreatment ameliorated oligomeric $A\beta_{1-42}$-induced reduction in polymerized β3- and α-tubulin levels suggesting its preventive role on microtubule disassembly. The insinuation of ATP binding to microtubules is well acknowledged as it arouses polymerization [37]. Protective role of DMF on oligomeric Aβ-induced reduction in tubulin polymerization may be associated with its shielding effect on mitochondrial dysfunction and thus ATP stabilization. Indeed, protective role of DMF on an early lessening in MTT reduction (Fig. 5d) is indicative of enhanced activity of mitochondrial and cellular dehydrogenases accountable for preserving high proportions of $NADH/NAD^+$, crucial for ATP generation [38].

It has been established that the oligomeric arrangement of Aβ is comparatively neurotoxic than other aggregation forms of Aβ and proposed that the toxicity is linked to the vigorous fibrillization progression: almost certainly the cells are affected by some metastable particles forming during the fibrillization [18]. ThT is a benzothiazole salt obtained by the methylation of dehydrothiotoluidine with methyl alcohol with aid of hydrochloric acid and both in vitro and in vivo it is extensively utilized to quantify and visualize the presence of misfolded protein aggregates [39]. When ThT interacts to β-sheet rich assemblies, like those in amyloid aggregates, the dye exhibits enhanced fluorescence and a characteristic red shift of its emission spectrum [39]. In our experiments, as indicated by ThT assay, $A\beta_{1-42}$ added in a predominantly oligomeric form, induced fibrillization and DMF pretreatment found to block the process. The present study indicates that the ThT fluorescence in DMF treated group was significantly decreased ($p < 0.001$) by 58% when compared to $A\beta_{1-42}$ fibril (1 μM) treated group indicating that DMF has an influence in the aggregation of Aβ, may be by disturbing Aβ changing into a β-sheet configuration. Our study implies that DMF may redirects amyloidogenic molecules into off-pathway aggregation. The most likely reason is that soluble Aβ is thought to undergo a conformational change to high β-sheet content, rendering it prone to aggregate into soluble oligomers and larger insoluble high-molecular-weight assemblies and DMF treatment affected these dynamics. Considering the results on the inhibition of aggregate binding, suggest that the presence of DMF (an electrophilic compound) acting through covalent inhibitory mechanisms [40], may interact with the hydrophobic cluster of the Aβ fibril, thus protecting aggregation. Also, Aβ aggregates affect membrane integrity leading to an elevation of intracellular free Ca^{2+} levels and ROS, two key events in the pathogenesis of amyloidosis [41]. Finally, it cannot

be excluded that the previously reported antioxidant power of DMF [42] and intracellular Ca^{2+} altering property [43] could also play protective roles. In fact, they could inhibit Aβ aggregation by protecting some amino acids, especially Tyr10 and His13, against oxidation [44] a modification important for Aβ fibrillization.

In conclusion, DMF could be a potential therapeutic compound to mitigate Aβ-induced pathology through reduction in tau hyperphosphorylation via GSK-3β mediated PI3K/Akt and Wnt/β-catenin signaling pathways, protecting amyloid fibrillogenesis and microtubule assembly distortion caused via lessening in polymerized β3- and α-tubulin. By means of DMF, controlling tauopathy may be a suitable approach towards finding cure of neurotoxic events leading to pathology of AD.

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Author Contributions Conceptualization: MSR; Methodology: MSR, DR; Formal analysis and investigation: MSR, NPN; Writing—original draft preparation: MSR; Writing—review and editing: MSR, NPN, RD; Funding acquisition: MSR; Resources: MSR, DR, RD; Supervision: NPN, RD.

Compliance with Ethical Standards

Conflict of interest We declare that we do not have any conflict of interest.

References

1. Bloom GS (2014) Amyloid-beta and tau: the trigger and bullet in Alzheimer disease pathogenesis. JAMA Neurol 71:505–508
2. Mietelska-Porowska A, Wasik U, Goras M, Filipek A, Niewiadomska G (2014) Tau protein modifications and interactions: their role in function and dysfunction. Int J Mol Sci 15:4671–4713
3. Iqbal K, Liu F, Gong CX (2015) Tau and neurodegenerative disease: the story so far. Nat Rev Neurol 12:15–27
4. Medina M, Garrido JJ, Wandosell FG (2011) Modulation of GSK-3 as a therapeutic strategy on tau pathologies. Front Mol Neurosci 4:24
5. Pascoal TA, Mathotaarachchi S, Shin M et al (2017) Synergistic interaction between amyloid and tau predicts the progression to dementia. Alzheimer's Dement 13:644–653
6. Rambaran RN, Serpell LC (2008) Amyloid fibrils: abnormal protein assembly. Prion 2:112–117
7. Krishtal J, Bragina O, Metsla K, Palumaa P, Tougu V (2017) *In situ* fibrillizing amyloid-$beta_{1-42}$ induces neurite degeneration and apoptosis of differentiated SH-SY5Y cells. PLoS ONE 12:e0186636
8. Parihar MS, Brewer GJ (2010) Amyloid-β as a modulator of synaptic plasticity. J Alzheimer's Dis 22:741–763
9. Mota SI, Ferreira IL, Pereira C, Oliveira CR, Rego AC (2012) Amyloid-beta peptide 1–42 causes microtubule deregulation through N-methyl-D-aspartate receptors in mature hippocampal cultures. Cur Alzheimer Res 9:844–856
10. Yaari R, Hake A (2015) Alzheimer's disease clinical trials: past failures future opportunities. Clin Invest 5:297–309
11. Majkutewicz I, Kurowska E, Podlacha M et al (2018) Age-dependent effects of dimethyl fumarate on cognitive and neuropathological features in the streptozotocin-induced rat model of Alzheimer's disease. Brain Res 1686:19–33
12. Kappos L, Giovannoni G, Gold R et al (2015) Time course of clinical and neuroradiological effects of delayed-release dimethyl fumarate in multiple sclerosis. Eur J Neurol 22:664–671
13. Campolo M, Casili G, Lanza M et al (2018) Multiple mechanisms of dimethyl fumarate in amyloid β-induced neurotoxicity in human neuronal cells. J Cell Mol Med 22:1081–1094
14. Dahlgren KN, Manelli AM, Stine WB Jr et al (2002) Oligomeric and fibrillar species of amyloid-beta peptides differentially affect neuronal viability. J Biol Chem 277:32046–32053
15. Joshi HC, Cleveland DW (1989) Differential utilization of beta-tubulin isotypes in differentiating neurites. J Cell Biol 109:663–673
16. Walker JM (1996) The bicinchoninic acid (BCA) assay for protein quantitation. In: Walker JM (ed) The protein protocols handbook. Springer protocols Handbooks. Humana Press, Totowa
17. Omar SH, Scott CJ, Hamlin AS, Obied HK (2019) Olive biophenols reduces Alzheimer's pathology in SH-SY5Y cells and APPswe mice. Int J Mol Sci 20:125
18. Sun ZK, Yang HQ, Pan J et al (2008) Protective effects of erythropoietin on tau phosphorylation induced by β-amyloid. J Neurosci Res 86:3018–3027
19. Cao M, Liu F, Ji F et al (2013) Effect of c-Jun N-terminal kinase (JNK)/p38 mitogen-activated protein kinase (p38 MAPK) in morphine-induced tau protein hyperphosphorylation. Behav Brain Res 237:249–255
20. Engel T, Hernandez F, Avila J, Lucas JJ (2006) Full reversal of Alzheimer's disease-like phenotype in a mouse model with conditional overexpression of glycogen synthase kinase-3. J Neurosci 26:5083–5090
21. Li S, Jin M, Koeglsperger T et al (2011) Soluble Abeta oligomers inhibit long-term potentiation through a mechanism involving excessive activation of extrasynaptic NR2B containing NMDA receptors. J Neurosci 31:6627–6638
22. Eleanor D, Wisniewski T (2017) Alzheimer's disease: experimental models and reality. Acta Neuropathol 133:155–175
23. Jang JH, Surh YJ (2004) Bcl-2 protects against Abeta (25–35)-induced oxidative PC12 cell death by potentiation of antioxidant capacity. Biochem Biophys Res Commun 320:880–886
24. Wang C, Youle RJ (2009) The role of mitochondria in apoptosis. Annu Rev Genet 43:95–118
25. Yao M, Nguyen TV, Pike CJ (2005) Beta-amyloid-induced neuronal apoptosis involves c-Jun N-terminal kinase-dependent downregulation of Bcl-w. J Neurosci 25:1149–1158
26. Du Q, Geller DA (2010) Cross-regulation between Wnt and NF-κB signaling pathways. Immunopathol Dis Therap 1:155–181
27. Du Y, Du Y, Zhang Y et al (2019) MKP-1 reduces Aβ generation and alleviates cognitive impairments in Alzheimer's disease models. Sig Transduct Target Ther 4:58
28. Jin Y, Fan Y, Yan EZ (2006) Effects of sodium ferulate on amyloid-beta-induced MKK3/MKK6-p38 MAPK-Hsp27 signal pathway and apoptosis in rat hippocampus. Acta Pharmacol Sin 27:1309–1316
29. Peng H, Guerau-de-Arellano M, Mehta VB et al (2012) Dimethyl fumarate inhibits dendritic cell maturation *via* nuclear factor κB (NF-κB) and extracellular signal-regulated kinase 1 and 2 (ERK1/2) and mitogen stress-activated kinase 1 (MSK1) signaling. J Biol Chem 287:28017–28026

30. Silva-Alvarez C, Arrazola MS, Godoy JA, Ordenes D, Inestrosa NC (2013) Canonical Wnt signaling protects hippocampal neurons from Aβ oligomers: role of non-canonical Wnt-5a/$Ca^{(2+)}$ in mitochondrial dynamics. Front Cell Neurosci 7:97
31. Arrazola MS, Silva-Alvare C, Inestrosa NC (2015) How the Wnt signaling pathway protects from neurodegeneration: the mitochondrial scenario. Front Cell Neurosci 9:166
32. Kisoh K, Hayashi H, Itoh T et al (2017) Involvement of GSK-3β phosphorylation through PI3-K/Akt in cerebral ischemia-induced neurogenesis in rats. Mol Neurobiol 54:7917–7927
33. Xian XF, Qing-Qiu M, Justin CY et al (2014) Isorhynchophylline treatment improves the amyloid-β-induced cognitive impairment in rats *via* inhibition of neuronal apoptosis and tau protein hyperphosphorylation. J Alzheimer's Dis 39:331–346
34. Toyama T, Looney AP, Baker BM et al (2018) Therapeutic targeting of TAZ and YAP by dimethyl fumarate in systemic sclerosis fibrosis. J Invest Dermatol 138:78–88
35. Cuadrado A, Kugler S, Lastres-Becker I (2018) Pharmacological targeting of GSK-3 and NRF2 provides neuroprotection in a preclinical model of tauopathy. Redox Biol 14:522–534
36. Henriques AG, Vieira SI, daCruz E et al (2010) Abeta promotes Alzheimer's disease-like cytoskeleton abnormalities with consequences to APP processing in neurons. J Neurochem 113:761–771
37. Zabrecky JR, Cole RD (1982) Effect of ATP on the kinetics of microtubule assembly. J Biol Chem 257:4633–4638
38. Marshall KE, Marchante R, Xue WF, Serpell LC (2014) The relationship between amyloid structure and cytotoxicity. Prion 8:192–196
39. Wolfe LS, Calabrese MF, Nath A, Blaho DV, Miranker AD, Xiong Y (2010) Protein-induced photophysical changes to the amyloid indicator dye thioflavin T. PNAS USA 107:16863–16868
40. Kees F (2013) Dimethyl fumarate: a Janus-faced substance? Expert Opin Pharmacother 14:1559–1567
41. Demuro A, Parker I (2013) Cytotoxicity of intracellular Aβ42 amyloid oligomers involves Ca^{2+} release from the endoplasmic reticulum by stimulated production of inositol trisphosphate. J Neurosci 33:3824–3833
42. Huang H, Taraboletti A, Shriver LP (2015) Dimethyl fumarate modulates antioxidant and lipid metabolism in oligodendrocytes. Redox Biol 5:169–175
43. Herrmann AK, Wullner V, Moos S et al (2019) Dimethyl fumarate alters intracellular Ca^{2+} handling in immune cells by redox-mediated pleiotropic effects. Free Radic Biol Med 141:338–347
44. Enache TA, Oliveira-Brett AM (2017) Alzheimer's disease amyloid beta peptides *in vitro* electrochemical oxidation. Bioelectrochemistry 114:13–23

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