# CHAPTER IV

# RESULTS

## MTT cell viability

### 1. MTT cell viability of NGF-deprived PC12 cells treated by BM extract in normal-serum (NR) RPMI1640 medium

The effect of BM extract on viability of NGF-deprived PC12 cells cultured in normal-serum RPMI 1640 medium was determined by measuring the reducing of MTT to purple crystal formazan by living-cell mitochondrial enzyme. The more purple formazan crystal, the more cellular survival. Cells were treated with 0 (control), 50, 100, 150, 200, 250, and 300 μg/ mL BM extract for up to 7 days and measured for their viability daily. The experiment was performed in three-independent replications with triplication in an individual experiment. Their viabilities of each treatment in an individual experiment were calculated for their averages, which were further calculated again for mean and standard error (SE) of the same treatment from different independent experiments. The viability percentages of the BM treated cells were compared with the viability percentage of the control from the 1st day and analyzed by one-way ANOVA with $\alpha = 0.05$.

On the first day, BM extract at all concentrations (0-300 μg/ mL) displayed the cellular viabilities closely to that viability of the cells grown in the absolute normal-serum RPMI1640 medium (the control) (Table 4). On the second day, BM extract at all concentrations (0-300 μg/ mL) could enhance the cellular viability higher than that of the control on the 1$^{st}$ day, especially, at the 50 μg/ mL concentration. Later, BM extract at all concentrations could bring the cellular viabilities closely to that of the control on the 1$^{st}$ day again on the 3$^{rd}$ day. BM extract at 50-200 μg/ mL concentrations could still dose-dependently rise up the cellular higher than that of the control on the 1$^{st}$ day on the 4$^{th}$ day. At this time, BM extract at 150 and 200 μg/ mL concentrations could contribute to the significant increment (Figure. 9). Since the 5$^{th}$ day, BM at nearly all concentration caused the cellular viability closely to or higher than that of the control on the 1$^{st}$ day, except for the 300 μg/ mL concentration.

**2. MTT cell viability of NGF-deprived PC12 cells treated by BM extract in apoptotic-induced serum-free RPMI1640 medium**

NGF-deprived PC12 cells were brought up in the serum-free RPMI 1640 medium for apoptotic induction and cell viability was measured by MTT assay daily. The cells were treated with 0 (control), 50, 100, 150, 200, 250, and 300 μg/ mL BM extract for up to 7 days. The experiment was reproduced independently for six times with triplication in each individual experiment and their cellular viabilities were processed in the same way as those raised up in NR medium.

On the first day, BM extract at 50-200 μg/ mL concentration revealed the cellular viabilities higher than that of the cells brought up in absolute serum-free RPMI1640 medium (the control) (Table 5). Among BM extract at all seven concentration (0-300 μg/ mL), BM extract at 50 μg/ mL concentration could elicit the highest cellular viability on the 2$^{nd}$ and the 3$^{rd}$ days (Figure. 10). BM extract at 150-300 μg/ mL concentrations rose up the cell death higher than that of the control cells on these two days. The BM extract at 100 μg/ mL concentration could also enhance the cellular viability slightly higher than the viability of the cells brought up in absolute serum-free RPMI1640 medium on the 2$^{nd}$ day, but lower than that viability of those cells on the 3$^{rd}$ and 4$^{th}$ days (Figure. 10). Since the 5$^{th}$ day, BM extract at all six concentrations (50-300 μg/ mL) have diminished the cellular viabilities lower than that of the control on the first day.

**3. MTT cell viability of differentiated SH-SY5Y cells treated by BM extract in camptothecin apoptotic-induced condition**

The effect of BM extract on viability of differentiated SH-SY5Y cells was determined by measuring the reduction of MTT to purple formazan crystals by living-cell mitochondrial enzyme. The more purple formazan crystals appear, the more cell survive. Cells were treated with 0.025% DMSO (vehicle), 5 μM camptothecin, and BM extract at the 50 and 100 μg/ mL concentrations for 12 to 72 hours and measured for their viability daily. The experiment was performed in three-independent replications with triplication in an individual experiment. Their viabilities of each treatment in an individual experiment were calculated for their averages, which were further calculated again for mean and standard error (SE) of the same treatment from different independent experiments. The viability percentages of the BM treated cells

were compared with the viability percentage of the absolute DMSO-treated cells at 12 hours and analyzed by one-way ANOVA with $\alpha = 0.05$.

At 24-48 hours, DMSO attenuated the cellular viabilities of the differentiated SH-SY5Y cells lower than that viability of the cells treated by absolute 0.025% DMSO at 12 hours (the vehicle control) (Table 6). From 12 to 48 hours, 5 μM camptothecin could slightly decrease the cellular viability to lower than that of the vehicle control at 12 hours. BM extract at all concentration (50-250 μg/ mL) tended to have the same pattern of cellular viability as that pattern of the cells treated by absolute 5μM camptothecin, which displayed the cellular viability less than those of the vehicle cells at 12-48 hours (Figure 11).

**Immunoblotting**

**1. Immunoblotting of NGF-deprived PC12 treated by BM extract in normal-serum (NR) RPMI1640 medium**

To determine the effect of BM extract treatment on total Tau and dephosphorylated Tau-1, NGF-deprived PC12 cells treated with 0 (control), 50, and 100 μg/ mL BM extract for 2 days were immunoblotted with the phophorylation-independent total tau protein (Tau 5), dephosphorylated-dependent Tau (Tau-1), and house-keeping GAPDH protein antibodies in triplicate independent experiments. Tau 5 antibody recognizes all Tau and Tau-1 antibody recognizes only Tau which their $Ser^{195}$, $Ser^{198}$, $Ser^{199}$, $Ser^{202}$, and $Thr^{205}$ (numbering based on longest human brain isoforms) are not phosphorylated, and therefore exhibits decreased immunoreactivity when there is an increase in phosphorylation at Tau-1 epitope (Shelton and Johnson, 2001). Density of each protein band from the triplicate independent experiments was quantified by Scion Image program calculated for an average. The relative amount of total tau protein was normalized to its corresponding average of GAPDH protein. After that, the percentage of total tau protein was quantified by comparing each relative amount of total tau protein with the relative amount of total tau protein of the control. The final data were analyzed by one-way ANOVA with $\alpha = 0.05$.

Immunoblot showed that BM extract at both 50 and 100 μg/ mL concentrations could reduce the amount of total Tau expression in NGF-deprived PC12 cells, grown up in normal-serum RPMI1640 medium. However, BM extract at 100 μg/ mL

concentration could contribute to the dephosphorylated Tau (at Tau-1 site) increment in these cells (Figure. 12). Because of the grater increment in dephosphorylated Tau (at Tau-1 site) and more decrement in total Tau (Tau 5), the BM extract could dose-dependently accentuate Tau-1 immunoreactivity (Figure. 14). This might suggest that BM extract could attenuate Tau phosphoryltion (at Tau-1 site) in NGF-deprived PC12 cells, grown up in normal-serum RPMI1640 medium.

**2. Immunoblotting of serum-free apoptotic-induced NGF-deprived PC12 treated by BM extract**

Immunoblot showed that BM extract at both 50 and 100 μg/ mL concentrations could reduce the amount of total Tau expression in NGF-deprived PC12 cells, grown up in serum-free RPMI1640 medium. However, BM extract at 100 μg/ mL concentration could contribute to the dephosphorylated Tau (at Tau-1 site) increment in these cells (Figure. 13). The BM extract at 100 μg/ mL concentration higher increased both total Tau (Tau 5) and dephosphorylated Tau (at Tau-1 site) higher than BM extract at 50 μg/ mL concentration did. Thus, BM extract at 100 μg/ mL concentration could slightly improve Tau-1 immunoreactivity (Figure. 14). This might also suggest that BM extract could diminish Tau phosphoryltion (at Tau-1 site) in NGF-deprived PC12 cells, apoptotic-induced by serum-free RPMI1640 medium.

**3. Immunoblotting of camptothecin apoptotic-induced differentiated SH-SY5Y cells, treated by BM extract**

To determine the effect of BM extract treatment on total Tau and dephosphorylated Tau-1, differentiated SH-SY5Y cells grown with only RPMI1640 medium, 0.025% DMSO (vehicle), 5 μM camptothecin, and BM extract at the 50 and 100 μg/ mL concentrations for 12 to 72 hours were immunoblotted with the phophorylation-independent total tau protein (Tau 5), dephosphorylated-dependent Tau (Tau-1), and house-keeping GAPDH protein antibodies in triplicate independent experiments. Tau 5 antibody recognizes all Tau and Tau-1 antibody recognizes only Tau which their $Ser^{195}$, $Ser^{198}$, $Ser^{199}$, $Ser^{202}$, and $Thr^{205}$ (numbering based on longest human brain isoforms) are not phosphorylated, and therefore exhibits decreased immunoreactivity when there is an increase in phosphorylation at Tau-1 epitope (Shelton and Johnson, 2001). Density of each protein band from the triplicate independent experiments was quantified by Scion Image program calculated for an

average. The relative amount of total tau protein was normalized to its corresponding average of GAPDH protein. After that, the percentage of total tau protein was quantified by comparing each relative amount of total tau protein with the relative amount of total tau protein of the control. The final data were analyzed by one-way ANOVA with $\alpha = 0.05$.

At 12 hours of the experiment, 0.025% DMSO (vehicle) and 5 μM camptothecin could slightly reduce both the amount of total Tau (Tau 5) and the amount of dephosphorylated Tau (at Tau-1 site) expression, compared to the absolute RPMI1649 medium. BM extract at both 50 and 100 μg/ mL concentrations could be able to ebb down the amount of total Tau expression lower than the 0.025% DMSO could. On the other hand, BM extract at both concentration, could rise up the amount of dephosphorylated Tau (at Tau-1 site) expression greater than the 0.025% DMSO could, especially for BM extract at 100 μg/ mL concentration (Figure. 15). Therefore, BM extract could vivid dose-dependently enhance up Tau-1 immunoreactivity in differentiated SH-SY5Y cells, apoptotic-induced by camptothecin for 12 hours (Figure16).

At 24 hour of the experiment, 0.025% DMSO (vehicle) and 5 μM camptothecin could enhance up both the amount of total Tau (Tau 5) and the amount of dephosphorylated Tau (at Tau-1 site) expression, compared to the absolute RPMI1649 medium. BM extract at 50 μg/ mL concentration could enhance up only the amount of total Tau expression, compared to the absolute 0.025% DMSO. However, extract at 100 μg/ mL concentration could increase up both the amount of total Tau and dephosphorylated Tau expression, compared to the absolute 0.025% DMSO, with the greater extent in the amount of dephosphorylated Tau expression (Figure. 17). BM extract could have impacts on the reduction of Tau-1 immunoreactivity in differentiated SH-SY5Y cells, apoptotic-induced by camptothecin for 24 hours (Figure. 18).

At 48 hour of the experiment, 0.025% DMSO (vehicle) could slightly reduce both the amount of total Tau (Tau 5) and the amount of dephosphorylated Tau (at Tau-1 site) expression, compared to the absolute RPMI1649 medium. The absolute 5 μM camptothecin could enhance up the amount of total Tau expression but ebb down the amount of dephosphorylated Tau (at Tau-1 site) expression, compared to the

absolute RPMI1649 medium. BM extract at both 50 and 100 μg/ mL concentrations could be able to diminish the amount of total Tau expression lower than the 0.025% DMSO could (Figure. 19). Thus, BM extract could again dose-dependently accentuate up Tau-1 immunoreactivity in differentiated SH-SY5Y cells, apoptotic-induced by camptothecin for 48 hours (Figure. 20). However, this increment of Tau-1 immunoreactivity at 48 hours of the experiment was at little extent than that increment at 12 hours of the experiment.

**Determination of gene expression**

Tau cDNA content in the SH-SY5Y cultured cells brought up with the low-serum RPMI 1640 medium only (the control cells), 0.025% DMSO, 5 μM camptothecin, and BM extract at the 50 and 100 μg/ mL concentrations for 12 to 72 hours was evaluated by PCR analysis, using primers specific designed to amplify region covering the Exon 10 of the longest isoform of the tau mRNAs. Two PCR products were predicted, the 254 bp fragment deriving from 4R isofroms (expressing Exon 10) of the tau mRNAs and the other 200 bp fragment deriving from 3R isofroms (excluding Exon 10) of the tau mRNAs. The density of each PCR band product was quantified. The relative density of each PCR band product was determined by normalizing with the density of PCR band product of each corresponding GAPDH cDNA to determine the mRNA expression of the *tau* gene.

As shown in Figure. 22 A (upper panel), no any PCR band product was detected from each condition of 12 and 24 hour culturing cells (lane 1 to lane 10). When the cells were cultured for 48 hours (Figure. 22 B, upper panel), the 3R isofroms of tau mRNAs were expressed from in the SH-SY5Y cultured cells brought up with the absolute low-serum RPMI 1640 medium (lane 1) and 0.025% DMSO (vehicle) (lane 2) but the 4R isofroms of tau mRNAs were expressed from in the SH-SY5Y cultured cells brought up BM extract at the 50 μg/ mL concentrations (lane 4). At this time the 4R isoforms were revealed with the highest expression. When the cells were cultured for 72 hours (Figure. 22 B, upper panel), all cultured cells expressed 3R isofroms of tau mRNAs (lane 6, 7, 9, and 10) except for the cells brought up with 5μM camptothecin which displayed not any PCR band product. At this time, high tau

mRNA expression was detected from in the SH-SY5Y cultured cells brought up with the absolute low-serum RPMI 1640 medium (lane 6) and 0.025% DMSO (lane 7).

**Table 4 MTT viability percentage of NGF-deprived PC12 cells, treated by BM extract for 7 days in normal-serum RPMI1640 (NR) medium.** The viability percentage were compared to that of the cells treated with no any BM extract on the 1st day The more purple formazan crystal appear, the more MTT viability. Each group n =3-9. All data represented Mean±SEM and were analyzed by statistic one-way ANOVA with $\alpha = 0.05$. $^{a}P < 0.05$

| Days | BM extract concentration (µg/ mL | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 | 50 | 100 | 150 | 200 | 250 | 300 |
| 1 | 100.00±17.28 | 98.93±11.89 | 91.54±5.68 | 103.34±12.18 | 118.87±27.18 | 96.86±17.20 | 94.78±22.59 |
| 2 | 123.00±9.76 | 141.51±12.38 | 131.76±8.19 | 126.43±4.36 | 132.47±16.32 | 129.20±7.09 | 101.98±9.03 |
| 3 | 106.01±9.96 | 110.97±11.08 | 101.55±2.56 | 101.83±9.54 | 113.02±10.14 | 100.15±6.56 | 69.36±2.09 |
| 4 | 105.50±10.27 | 132.75±9.19 | 156.04±4.48 | 171.92±9.80[a] | 162.83±18.65[a] | 150.54±13.25 | 51.16±11.82 |
| 5 | 66.15±0.29 | 69.83±5.53 | 86.36±14.16 | 73.45±1.95 | 86.40±3.70 | 85.96±5.21 | 36.66±1.92 |
| 6 | 96.30±18.20 | 114.49±21.22 | 121.08±20.43 | 126.52±24.01 | 149.54±13.88 | 123.23±1.33 | 44.88±4.56 |
| 7 | 83.40±10.03 | 103.03±21.54 | 105.12±19.90 | 79.33±19.37 | 86.40±3.70 | 85.96±5.21 | 36.66±1.92 |

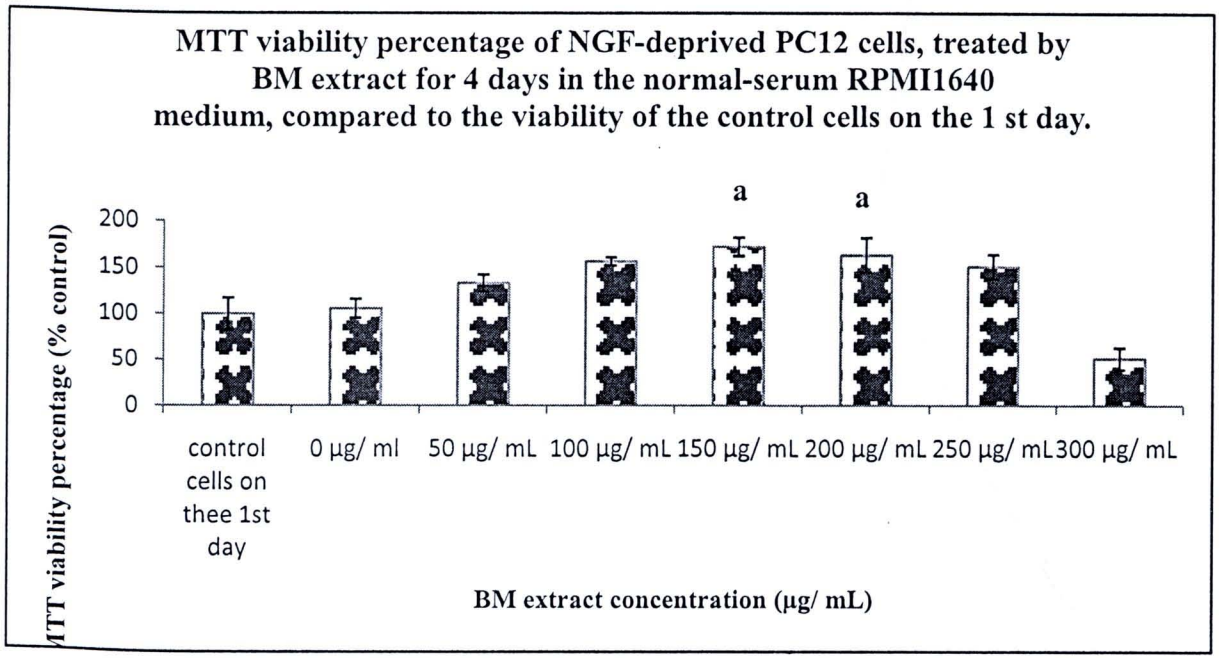


**Figure 9 MTT viability percentage of NGF-deprived PC12 cells, treated by BM extract for 4 days in normal-serum RPMI1640 (NR) medium.** The viability percentage were compared to that of the cells treated with no any BM extract on the 1st day. Each group n =3-9. Each bar represents Mean±SEM. All data were analyzed by one-way ANOVA with $\alpha = 0.05$. $^{a}P < 0.05$

**Table 5 MTT viability percentage of NGF-deprived PC12 cells, treated by *Bacopa monnieri* for 7 days in apoptotic-induced serum-free RPMI1640 medium.** The viability percentages were compared to that of the cells treated with no any BM extract on the 1st day. The more purple formazan crystal, the more MTT viability. Each group n =3-18. All data represented Mean±SEM and were analyzed by statistic one-way ANOVA with $\alpha = 0.05$.

| Days | BM extract concentration (µg/ mL | | | | | | |
|---|---|---|---|---|---|---|---|
| | **0** | **50** | **100** | **150** | **200** | **250** | **300** |
| 1 | 100.00±8.00 | 126.03±7.58 | 124.55±6.49 | 116.20±14.59 | 122.10±15.45 | 99.44±9.54 | 40.03±9.37 |
| 2 | 115.29±9.91 | 130.50±11.63 | 108.16±9.66 | 44.76±6.50 | 22.61±3.79 | 21.66±3.56 | 20.27±3.39 |
| 3 | 104.87±8.42 | 132.60±14.73 | 77.19±7.94 | 27.52±6.99 | 14.71±1.85 | 14.69±1.58 | 15.45±1.51 |
| 4 | 145.49±16.62 | 101.20±16.88 | 24.99±2.50 | 14.39±1.00 | 13.83±1.39 | 13.80±1.37 | 15.44±1.29 |
| 5 | 76.74±9.25 | 40.45±8.55 | 16.74±2.06 | 18.31±4.35 | 14.53±0.70 | 15.09±1.07 | 16.67±0.79 |
| 6 | 76.98±6.89 | 56.04±18.22 | 18.86±1.77 | 16.73±1.48 | 17.73±1.48 | 18.19±1.53 | 18.39±1.54 |
| 7 | 63.34±7.98 | 26.48±5.90 | 15.50±1.85 | 14.29±0.76 | 13.39±0.90 | 14.34±0.64 | 15.77±0.60 |

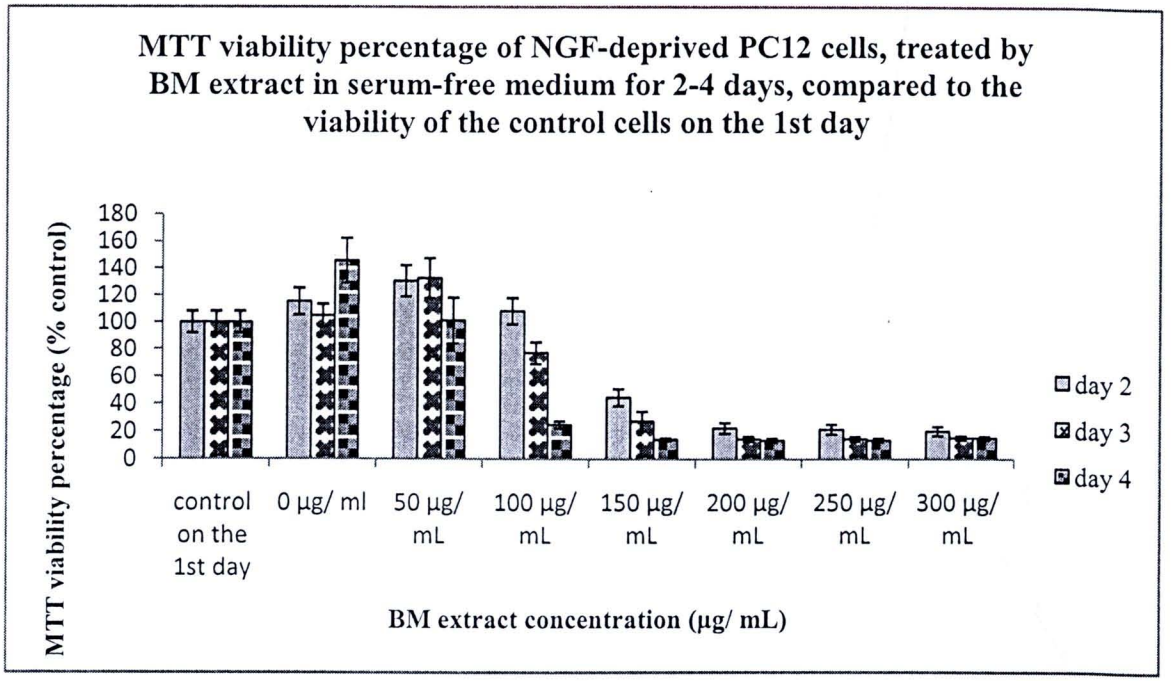


**Figure 10 MTT viability percentage of NGF-deprived PC12 cells, treated by *Bacopa monnieri* for 2-3 days in apoptotic-induced serum-free RPMI1640 medium.** The viability percentages were compared to that of the cells treated with no any BM extract on the 1st day. Each bar represented Mean±SEM, n =3-18. All data were analyzed by statistic one-way ANOVA with $\alpha = 0.05$.

**Table 6 The MTT viability percentage of differentiated SH-SY5Y cells, grown up in only low-serum RPMI 1640 medium for 12 to 72 hours.** The viability percentages were compared to that of the viability of these cells at 12 hours and the MTT viability percentage of the cells, treated by only 0.025% DMSO (vehicle), 5 μM camptothecin, and BM extract at the 50 to 250 μg/ mL concentrations for 12 to 72 hours, compared to the viability of the vehicle-treated cells at 12 hours (a). Each group n = 3. All data represented by Mean ± SEM and analyzed by statistic one-way ANOVA with $\alpha = 0.05$. [a,] $P < 0.05$.

| Time | RPMI 1640 | 0.025% DMSO | 5 μM mptothecin | BM extract concentration (μg/ mL) 50 | 100 | 150 | 200 | 250 |
|---|---|---|---|---|---|---|---|---|
| **12** | 100.00±5.85 | 100±7.90 | 93.55±8.17 | 92.11±8.42 | 93.25±8.67 | 88.06±7.44 | 87.96±8.18 | 88.88±5.55 |
| **24** | 74.65±5.95 | 79.94±5.46 | 65.14±2.95[a] | 62.11±3.90[a] | 63.86±2.95[a] | 65.13±2.50[a] | 64.25±2.00[a] | 62.20±2.98[a] |
| **48** | 86.97±1.49 | 79.66±2.20 | 79.39±4.56 | 76.55±2.81 | 82.24±6.43 | 80.64±2.97 | 79.10±5.71 | 78.62±5.90 |

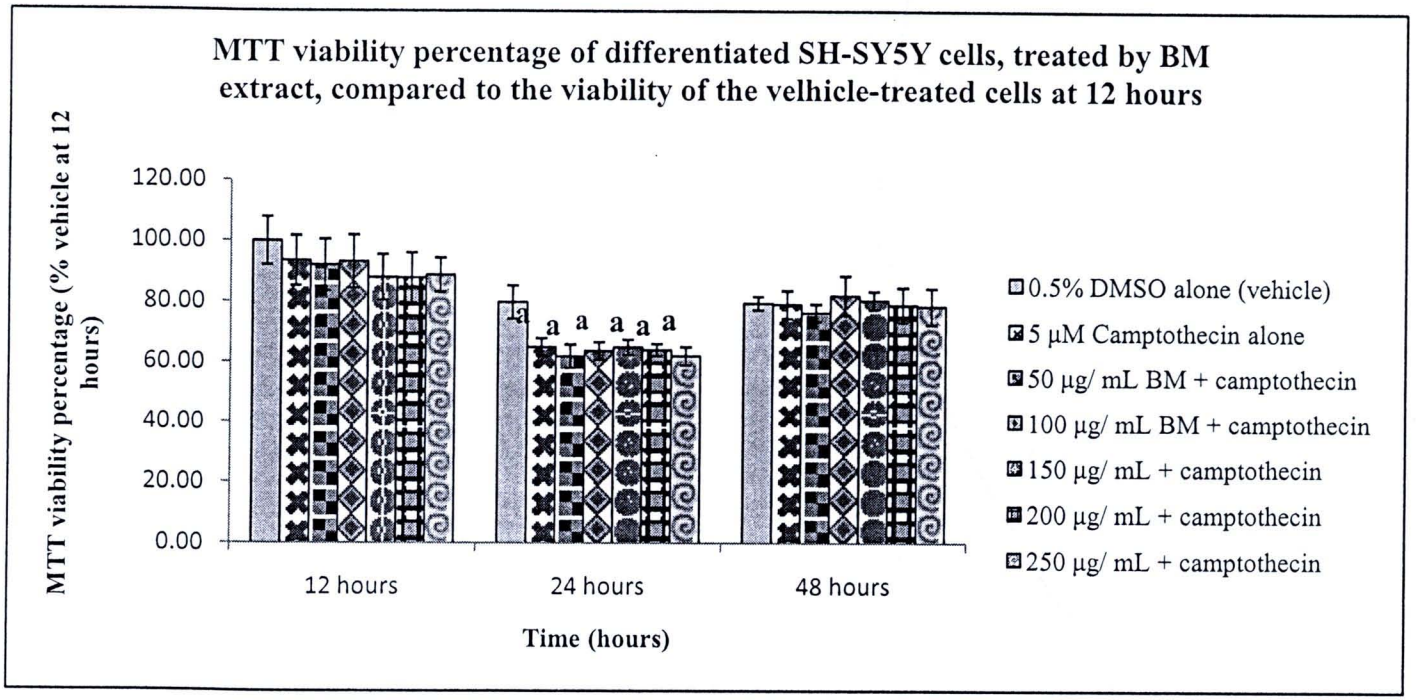


**Figure 11 The MTT viability percentage of the cells, treated by only 0.025% DMSO (vehicle), 5 µM camptothecin, and BM extract at the 50 to 250 µg/ mL concentrations for 12 to 48 hours.** The viability percentages were compared to that of the vehicle-treated cells at 12 hours (a). Each group n = 3. All data represented by Mean ± SEM and analyzed by statistic one-way ANOVA with $\alpha = 0.05$. $^{a}P < 0.05$.

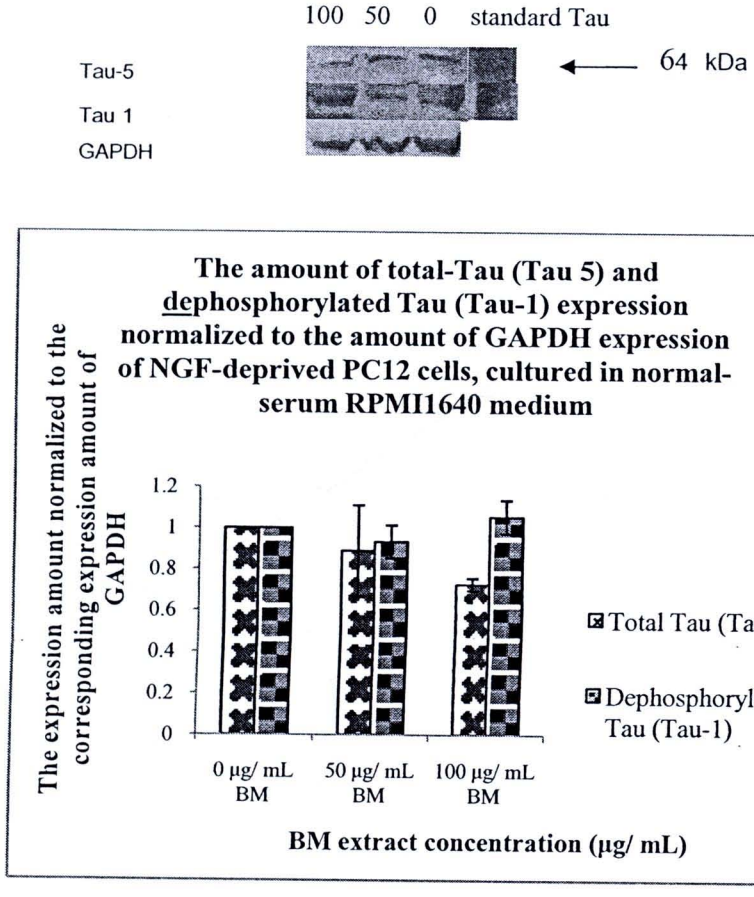


**Figure 12 The relative amount of total tau (Tau 5) and dephosphorylated Tau (Tau-1) expression of NGF-deprived PC12 cells in normal-serum RPMI1640, treated with BM extract for 2 days.** Each group n =3. All data represented by Mean ± SEM

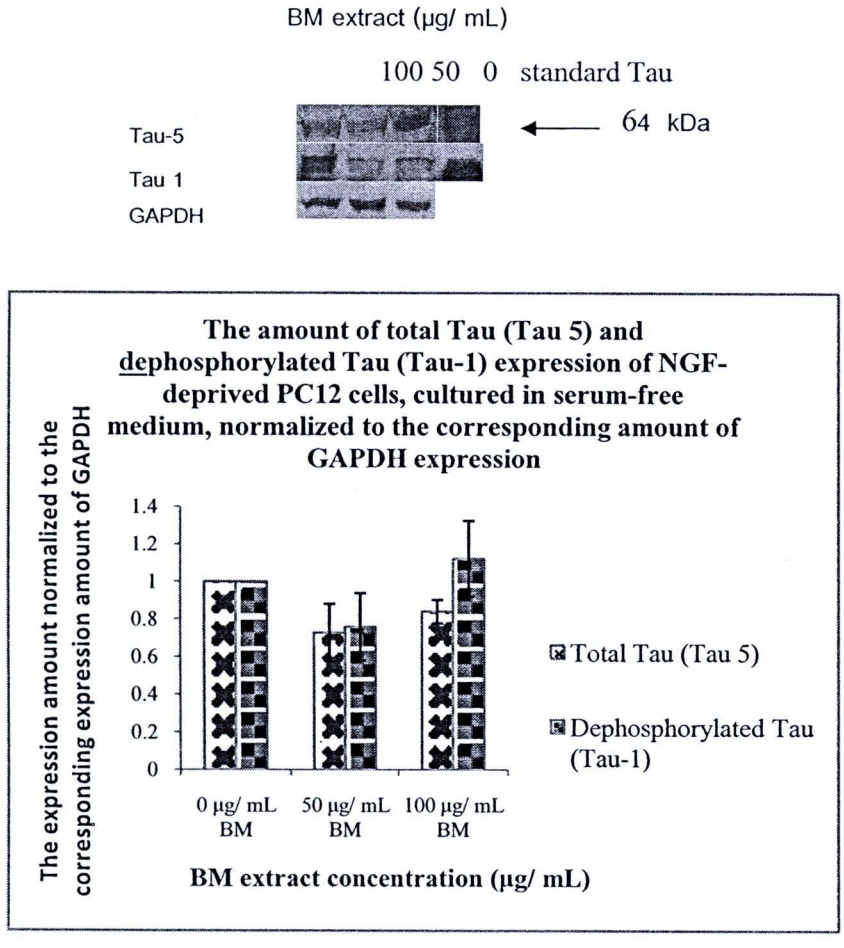


**Figure 13 The relative amount of total tau (Tau 5) and dephosphorylated Tau (Tau-1) expression of NGF-deprived PC12 cells in serum-free RPMI1640, treated with BM extract for 2 days.** Each group n =3. All data represented by Mean ± SEM

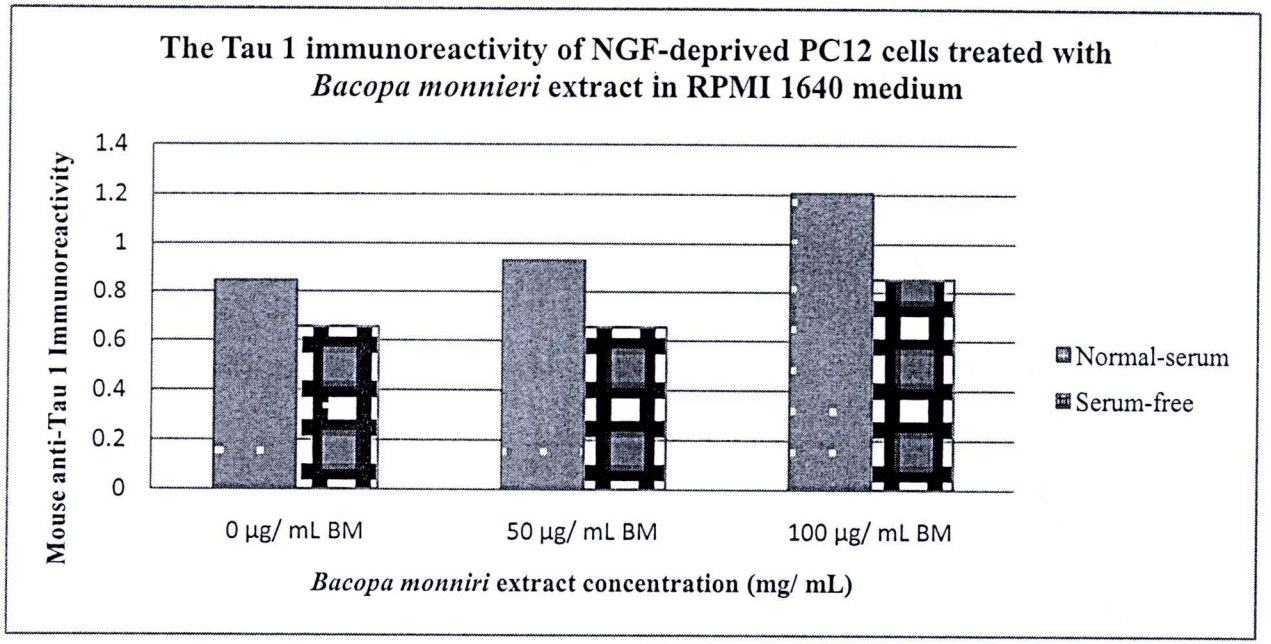


**Figure 14 Tau-1 immunoreactivity of total Tau expression of NGF-deprived PC12 cells treated with BM extract in RPMI1640 medium for 48 hours.** Each group n =3. All data were represented Mean±SD and were analyzed by statistic one-way ANOVA with $\alpha = 0.05$ and *P*-value = 0.269.

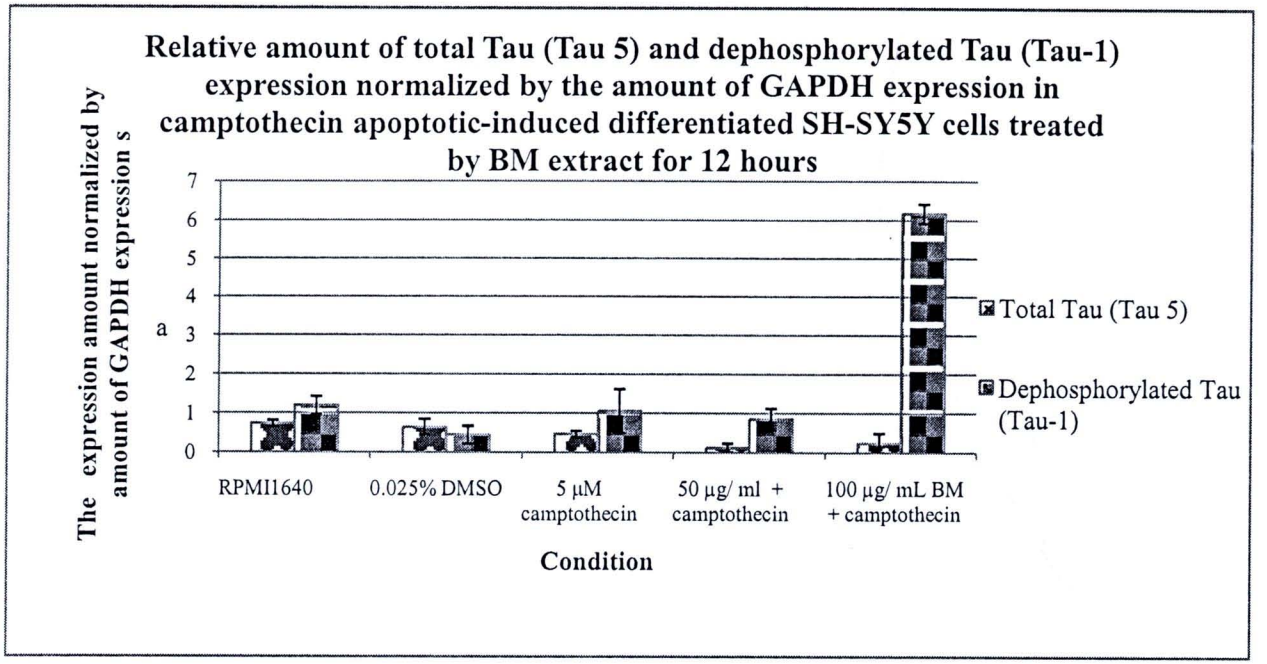


**Figure 15 The relative amount of total Tau (Tau 5) and dephosphorylated Tau (Tau-1) expression of differentiated SH-SY5Y cells at 12 hours.** The cells were grown up in only low-serum RPMI 1640 medium, 0.025% DMSO (vehicle), 5 µM camptothecin, and BM extract at the 50 and 100 µg/ mL concentrations for 12 hours. Each group n =3. All data represented Mean±SD and were analyzed by statistic one-way ANOVA at $\alpha = 0.05$.

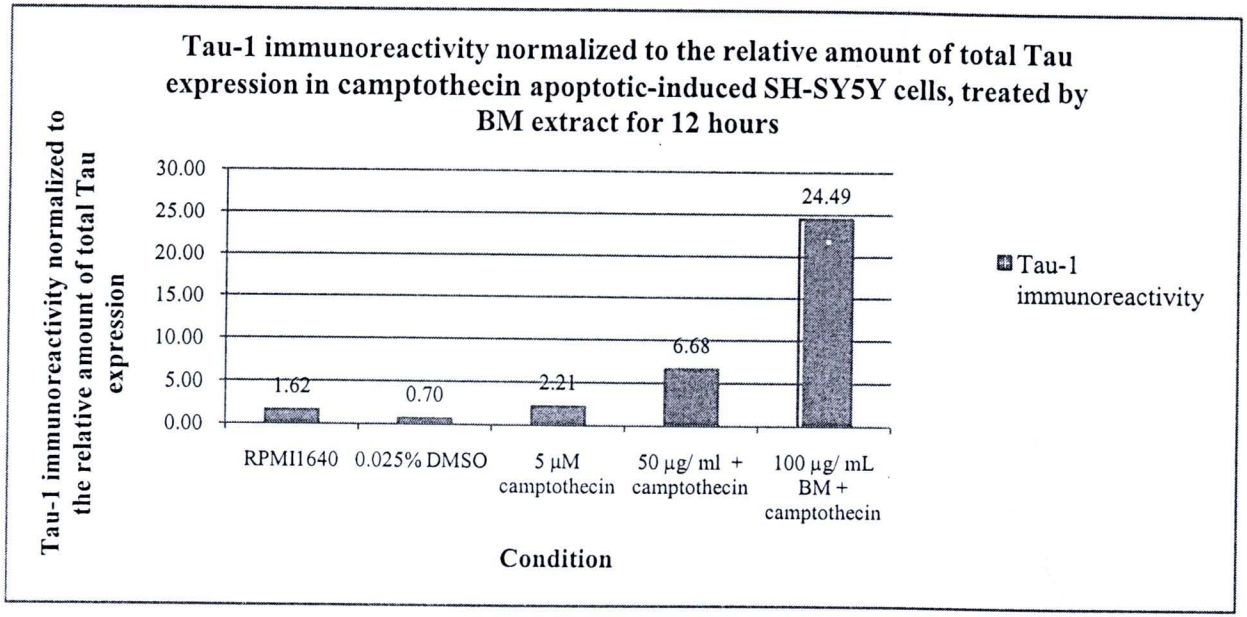


**Figure 16 Tau-1 immunoreactivity normalized to the relative amount of total Tau expression of differentiated SH-SY5Y cells.** The cells were grown up in only low-serum RPMI 1640 medium, 0.025% DMSO (vehicle), 5 µM camptothecin, and BM extract at the 50 and 100 µg/ mL concentrations for 12 hours. Each group n =3. All data represented Mean±SD and were analyzed by statistic one-way ANOVA at $\alpha = 0.05$.

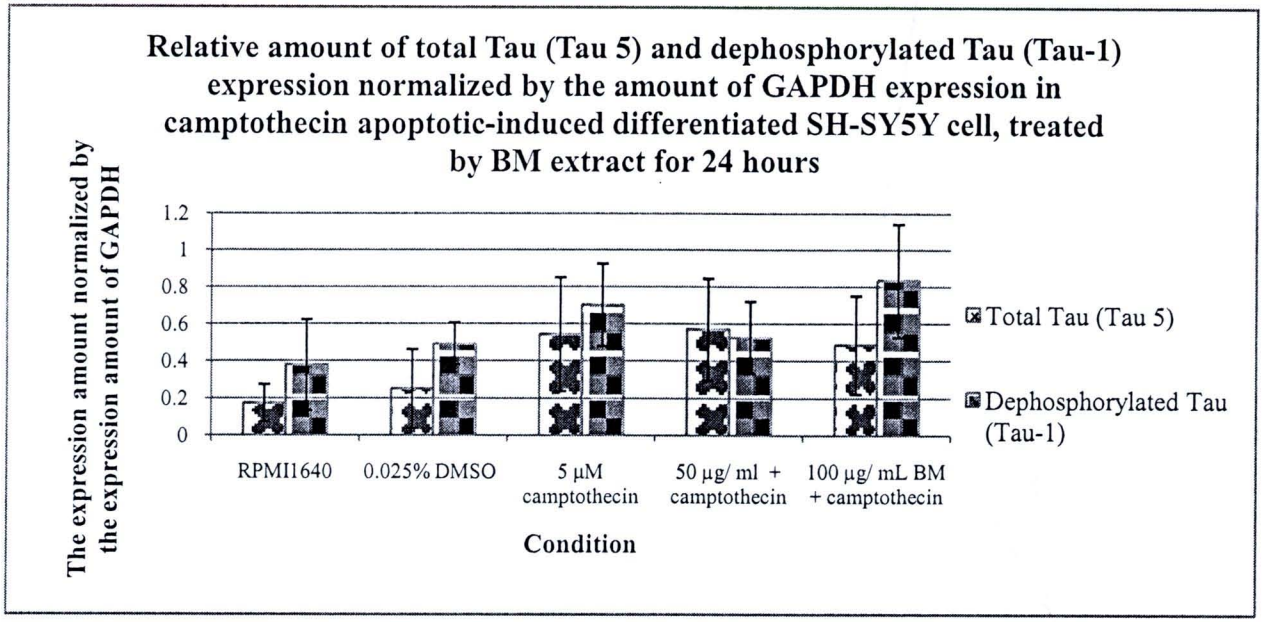


**Figure 17 The relative amount of total Tau (Tau 5) and dephosphorylated Tau (Tau-1) expression of differentiated SH-SY5Y cells at 24 hours.** The cells were grown up in only low-serum RPMI 1640 medium, 0.025% DMSO (vehicle), 5 µM camptothecin, and BM extract at the 50 and 100 µg/ mL concentrations for 24 hours. Each group n =3. All data represented Mean±SD and were analyzed by statistic one-way ANOVA at $\alpha = 0.05$.

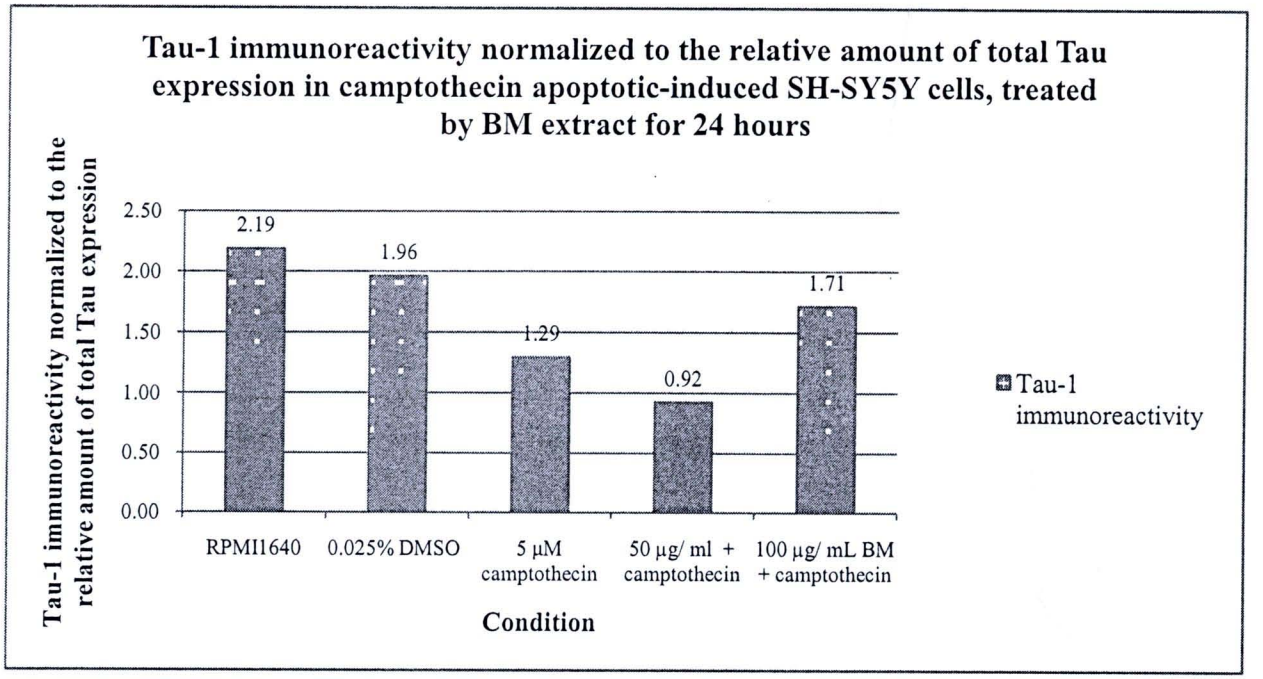


**Figure 18 Tau-1 immunoreactivity normalized to the relative amount of total Tau expression of differentiated SH-SY5Y cells at 24 hours.** The cells were grown up in only low-serum RPMI 1640 medium, 0.025% DMSO (vehicle), 5 µM camptothecin, and BM extract at the 50 and 100 µg/ mL concentrations for 24 hours. Each group n =3. All data represented Mean±SD and were analyzed by statistic one-way ANOVA at $\alpha = 0.05$

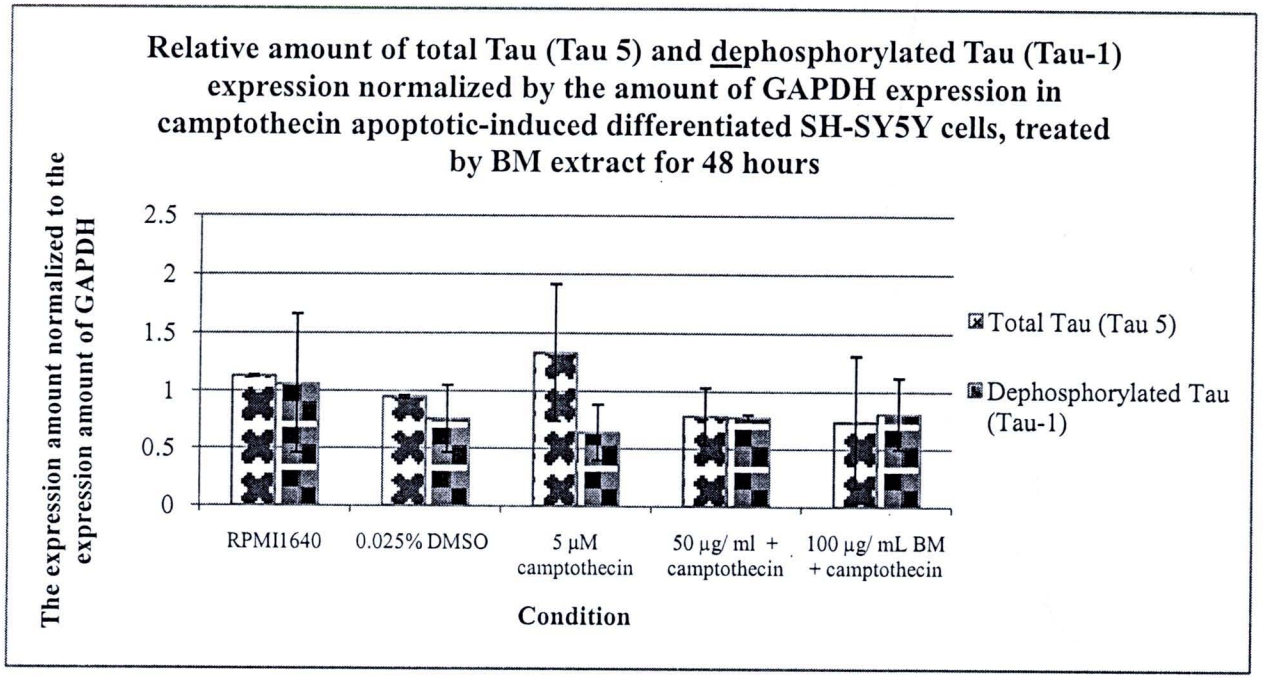


**Figure 19 The relative amount of total Tau (Tau 5) and dephosphorylated Tau (Tau-1) expression of differentiated SH-SY5Y cells at 48 hours.** The cells were grown up in only low-serum RPMI 1640 medium, 0.025% DMSO (vehicle), 5 μM camptothecin, and BM extract at the 50 and 100 μg/ mL concentrations for 48 hours. Each group n =3. All data represented Mean±SD and were analyzed by statistic one-way ANOVA at $\alpha = 0.05$.

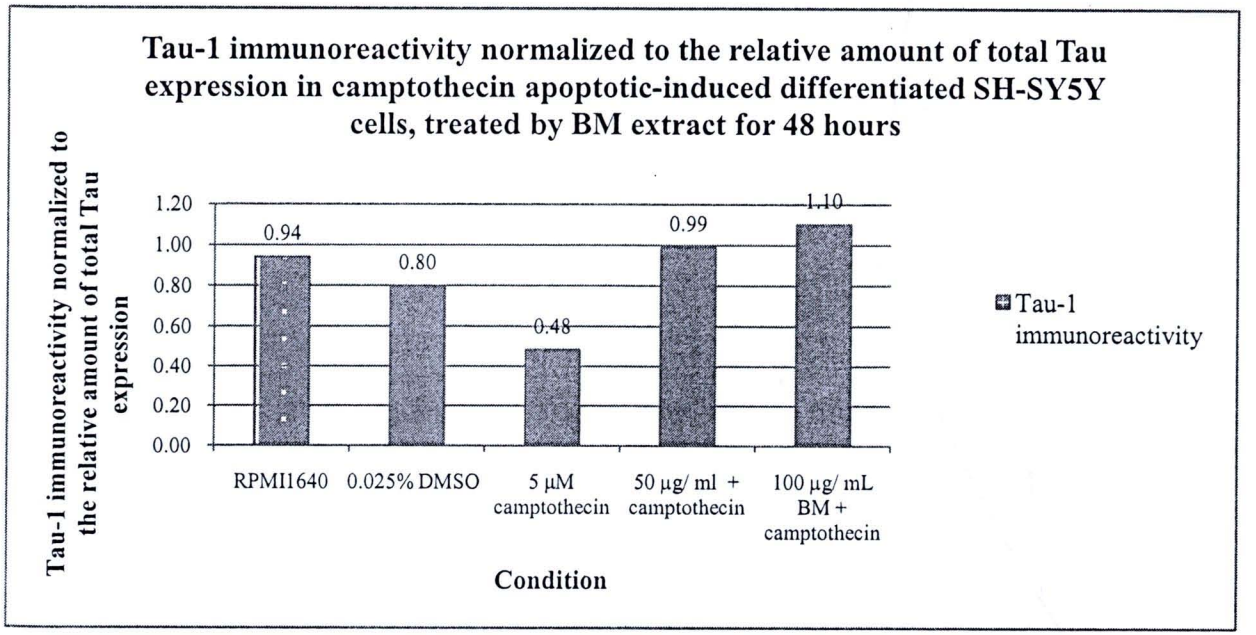


**Figure 20 Tau-1 immunoreactivity normalized to the relative amount of total Tau expression of differentiated SH-SY5Y cells at 48 hours.** The cells were grown up in only low-serum RPMI 1640 medium, 0.025% DMSO (vehicle), 5 µM camptothecin, and BM extract at the 50 and 100 µg/ mL concentrations for 48 hours. Each group n =3. All data represented Mean±SD and were analyzed by statistic one-way ANOVA at $\alpha = 0.05$

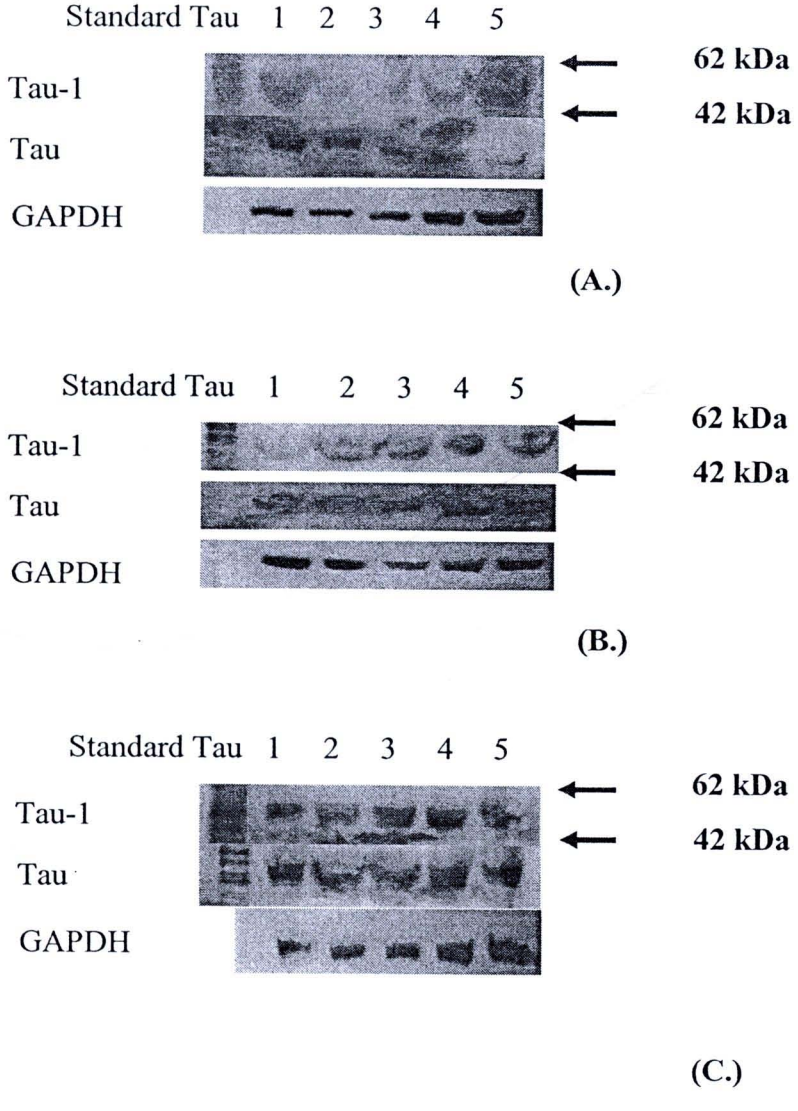


**Figure 21 Single-selected immunoblottogram of differentiated SH-SY5Y cells at 12 hours** (A), 24 hours (B), and 48 hours (C). The cells were grown up in absolute low-serum RPMI 1640 medium (lane 1), 0.025% DMSO (vehicle) (lane 2), 5 μM camptothecin (lane 3), and BM extract at the 50 (lane 4) and 100 (lane 5) μg/ mL concentrations.

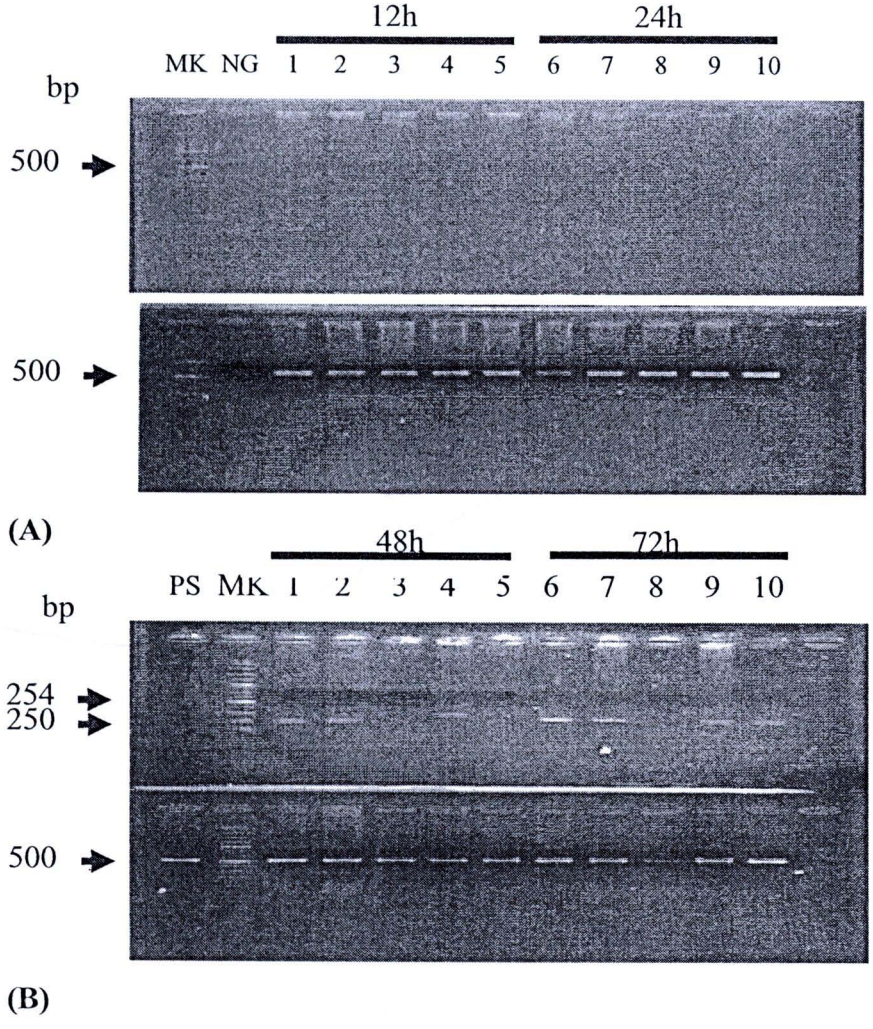


**Figure 22 Tau PCR band products (upper panels of A and B) and GAPDH PCR band products (lower panels of A and B) of differentiated SH-SY5Y cells at 12 and 24 hours (A) and 48 and 72 hours (B).** The cells were grown up in (left to right) absolute low-serum RPMI 1640 medium (lane 1, and 6), 0.025% DMSO (lane 2 and 7), 5 μM camptothecin (lane 3 and 8), and BM extract at the 50 (lane 4 and 9) and 100 (lane 5 and 10) μg/ mL concentrations for MK = Molecular weight marker, NG = Negative control, PS = Positive control