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The Effect of Leaf Extract of *Centella Asiatica* on Neurogenesis and Bdnf Level in Hippocampus Cell Culture in Young Mice

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Abstract: In many world, research of *centella asiatica (CA)* leaf extracts are used to treat neuronal disease with stem cell or cell line, but in the present study we try to make from hippocampus tissue culture. *Methods*: Young femalemice dowleyspraque types age of 2 weeks, as many as eighteen (18) tails each taken from its hippocampal tissue then cultured using a medium Roswell Park Memorial Institute (RPMI). Eighteen (18) culture of nerve cells exposed to the extract of *CA* at a concentration of 0.25 ug / ml (*CA A*) and eighteen (18) culture of nerve cells exposed to the extract of *CA* at a concentration of 0.50 ug / ml (*CA B*), where as eighteen (18) other nerve cell cultures not exposed (control). Then calculated the number of cells per field view and BDNF levels measured in the culture of the three populations. *Results*: (1) Anumber of larger nerve cells in cell cultures of hippocampal tissue of young rats were fed by *CA* extracts dose of 0.50 ug / ml compared to 0.25 ug / ml and the control group ($p < 0.05$); that new neurons plays an important role in sinaptogenesis circuit in order to improve the integration function of neurons and synapses that still exist between new and mature neurons in the hippocampus [1]. *Conclusion:* (2) Levels of BDNF *CA* dose of 0.50 ug / ml higher than 0.25 mg/ml *CA* doses but lower than in the controls ($p < 0.05$), that the effect of the extract *CA* neurogenesis does occur in particular BDNF through the expression of BDNF, the possibility of glutamate in *CA* extracts is not able to increase the level of GABA in the brain due to activation of GABA synapses on a young neuron is depolarization where the concentration of intracellular Cl ion is high because channel expression Cl exporter KCC2 which slowed (delayed) [2].

Keywords: *Centella Asiatica* (*CA*), Brain Derived Neurotrophic Factor (BDNF), Neurogenesis

1. Introduction

Delays in the development of the brain and nervous still become a health problem, estimated at 17% of the entire population [3]. It decreases cognitive/memory, basic pathology caused by the decline of synapses, neurons, neurotransmitters and neural networks [4], the structural change of neurons is cerebral cortex and hippocampus that effect activity-dependent change in synaptic strength and alter learning and memory performance, but also molecular substances [5], that is neurotrophic factor. Many studies suggest neurotrophic factor that Brain derived neurotrophic factor (BDNF) has a significant role in process of learning and memory, such as development of patterned connections, growth and complexity of dendrites in the cerebral cortex [6]. Brain derived neurotrophic factor (BDNF) is a member of neurotrophin family, including nerve growth factor (NGF), neurotrophin-3 (NT-3), NT-4, and NT-5 [7]. The health problem of the brain and nervous sytem, it can be avoided

by giving an adequate stimulus to trigger the process of neurogenesis and plasticity of synapses in the nervous system [8] that stimulus can be provided through food, vitamins, herbal remedies. In area of hippocampal neuron progenitor cells, there is a collection that can regenerate [4], it is related to the regeneration of intercellular signaling molecules and intracellular transduction pathways that regulate the growth of neurites, a new synapse and cell survival that occur throughout life. The important of signaling molecules including the neurotrophin factor (neurotrophin), neurotransmitter and hormone [8]. *Centellaasiatica* commonlyknown as gotu kola and belonging to the family mackinlayaceae is a special native to asia [9]. *Centellaasiatica (CA)* is herbal plants, growing in moist places in Asia countries, *centellaasiatica (CA)* is widely used as herbal plants in traditional medicines in many countries in Asia. Some important chemical constituents found in *CA* are triterpenoids and flavonoids [10]. Some studied highlighted Asiatic acid and asiaticoside, which are parts of triterpenoids properties in *CA,* that have function in wound healing, brain stimulating effects, treatments of hypertension, microangiopathy, gastric ulcer, and potent antioxidant and anticancer activity [11; 12]. Another chemical constituents found in *CA* are*madecassic acid* and *madecassosid* use traditional medicine in Indian ayurvedic from susrutasamhita India book, such as enhancement neuronal and brain cell function [13; 14]. *Centellaasiatica* herbal leaf has been observed in vivo in mice given the *CA* extract for 14 days can increase the ability of his memory 3-60 times than mice not given it [15]. *Centellaasiatica* contains the amino acid glutamate which is a precursor to the formation of gamma aminobutyric acid (GABA), which allegedly serves as an excitatory neurotransmitter that is growing which can increase BDNF expression through a mechanism of mitogen-activated protein kinase (MAPK) - AMPcyclic response element binding protein (CREB), a positive feedback circuit in hypothalamic neurons that are growing and growing [16; 17]. Brain derived neurotrophic factor (BDNF) is one of the important neurotrophins that plays a role in the survival, growth, development of neurons and synaptic plasticity [18; 19]. In the brain, BDNF is vital to learning, long term memory and higher thinking [20]. Research on fibroblast culture cell explained that the concentration *CA* extracts of 0.25 ug/ml has no effect [21].

The present study to investigation the neuroprotective effect of herbaceous cognitive enhancer, *centellaasiatica*on BDNF concentration, that is invitromethode using hippocampus nerve cell cultures is possible because we can see the development that occurs in nerve cells, which is why in this study used a dose of 0.25 ug/ml as the minimal dose and 0.50 ug/ml as maximum dose, given that the memory associated with the development of nerve cells, so it is expected that the *CA* extract may affect the development and survival of neurons and neuron precursor influences into mature neurons.

2. Material and Methods

The study was conducted on the physiology and integrated imunoendokrinologylab Faculty of Medicine, University of Indonesia

2.1. Materials and Tools

Materials: Spraquefemaledowley rat's hippocampal brain tissue age of 2 weeks, water soluble *CA* extract 6%, RPMI medium, natrium bicarbonate, fetal bovine srum (FBS), gentamycin, Fungizone, phosphate buffered saline (PBS), trypsin 0.025%, EDTA 0.01%, aquabidest sterile, BDNF assay kit (Chemicon), poly-L-Lysine 25 mg (sigma P-1524), antiseptic solution

Tools:

Laminar flow, analytical balance, stirrer magnetic, pH meter, filter disposable, scissors, tweezers, sterile Petri dish ø 10 cm and 35 cm, centrifuges, pasteur sterilepipette, sterile syringes, inverted phase contrast microscope, incubator, scalpel disposable, vortex mixer, polypropylene tubes, eppendorf tubes, microcentrifuge

2.2. Experimental Animal

Population and sample.

The research population is the healthy females Spraquedowley young mice aged 2 weeks (obtained from the Department of Histology medicine Faculty of Medicine, University of Indonesia). The samples were tissue hippocampal nerve culture cells which were exposed by *CA* extracts concentration of 0.25 ug/ml and 0.50 µg/ml.

2.3. How to Work

1. Selection of experimental animals: Samples were collected from the healthydowleyspraque mice 2 weeks old amounted to 18 heads
2. Preparation of RPMI stock: 500mgRPMI powder +500mlaquabidest sterile + 1 gram of $NaHCO_3$, adjust pH 7.4 and filtered with an 0.2 µm porous filter
3. Production of a complete RPMI medium: 40 ml RPMI stock + 10 ml FBS + 0.4 ml Fungizone + 0.4 ml gentamycin then filtered using 0.2 µm porous filter
4. Preparation of stock solutions extracts *CA* 2.5 ug/ml and 5 mg/ml: A stock solution extracts *CA* 2.5 ug/ml are made of 0.125 mg powder *CA* (from 6% extract powder form) +50 ml of sterile aquabidest then filtered porous filter of 0.2 µm. While the stock solution extracts *CA* 5 ug/ml are made from 250 mg powder *CA* (from 6% extract powder form) +50 ml of sterile aquabidest then filtered 0.2 µm porous filter
5. Preparation of *CA* extract + RPMI complete: 28 ml RPMI stock + 8 ml FBS (20%) + 0.4 ml Fungizone + 0.4 ml gentamycin + 4 ml of the *CA* extract solution concentration of 2.5 ug/ml which was made before, then filtered using 0.2 µmporous filter. Thus obtained a concentration of 0.25 ug/ml, according to this calculation method and apply to a concentration of 5 ug/ml

6. The insulation ofthe hippocampus nerve cells [22; 23]: Add Hippocampus culture cells in a petri dish + 1 drop of antiseptic solution, rinse the PBS and cut with a scalpel, add the centrifuge tube + 0.025% trypsin + 0, 01% 2 ml EDTA crushed mechanically, incubate at 37°C for 5 min + 0.5 ml FBS and centrifuged at 200 g for 3 minutes, remove supernatant and pellet is suspended with 2 ml of complete RPMI medium

7. nerveculturecells [24; 222; 23]: Nerve cells in RPMI complete are cultured in petridish covered with cover glass and poly-L-Lysine, an incubate at 37°C for 96 hours (4 days)

8. Adding*CA* extract: On day 6 of each cell cultures were exposed by *CA* extracts 0.25 ug/ml and 0.50 pg/ml for 48 hours (2 days), then take the culturecell medium and add a sterile eppendorf tube of 2.5 ml, 200 g centrifuges for 1 minute then take the supernatant add 2 ml polypropylene tubes and store at -20°C to measure the levels of BDNF done.

2.4. Examine the Level of BDNF (BDNF Assay Kit. Cat. No. CYT306 Chemicon, CA)

Create a standard solution of 500, 250, 125, 62.5, 31.25, 15.63, 7.82, 0.0 pg/ml. The sample solution and controls of 100 mL add into wells + 100 mL Biotinylated anti BDNF dilute, cover and incubate at room temperature for 2 hours, open the cover and aspirate the entire contents of the wells and rinse 250 mL wash solution (4x), and + streptavidin-HRP into each well, cover and and incubate at room temperature for 1 hour + TMB substrate solution incubate at room temperature at 2.0 OD maximum for 15 mins, the absorbance by spectrophotometry at a wavelength of 450 nm.

2.5. Staining Cells and Counting the Number of Cells

Performed on pellet, nerve cell cultures were washed by PBS (2-3x) + Glucose 5% ± 1-2 ml let it stand for 1 minute and discard the liquid, give ± 1-2 ml of buffer formalin let it stand for 1 minute and discard the liquid. Soak Giemsa 1-2 ml up to 20 ml for 60 minutes, rinse and pat to dry, then cover the surface of the glass contained in the culture dish take the paste on the surface of the glass object, then do the calculation of the number of cells per field view on phase contrast microscope (100x magnification), the number of nerve cells are counted in five areas of the visual field and then the calculation results are summed and averaged.

3. Results

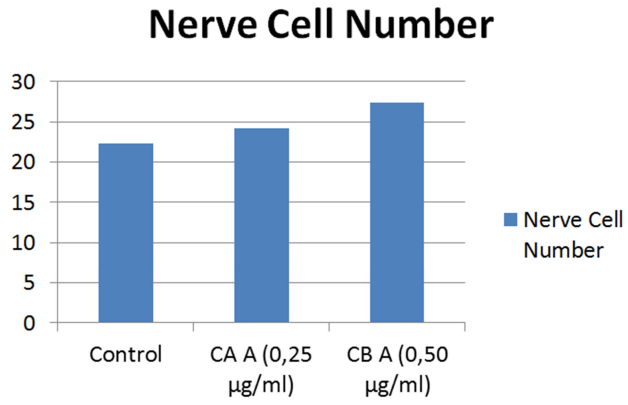
3.1. Number of Nerve Cell

The number of nerve cells per field view in the treatment group and the control group after 48 hours incubationcan be seen in the following table 1 below:

Table 1. *The number of nerve cells per field view of the control group and the treatment after 48 hours incubation.*

No. Samples	Control	*CA* A (0,25 µg/ml)	*CA* A (0,50 µg/ml)
1	21	28	25
2	20	30	28
3	22	27	31
4	31	28	33
5	20	22	27
6	22	19	24
7	17	21	25
8	21	23	20
9	24	19	30
10	23	26	31
11	19	19	23
12	23	25	28
13	21	25	31
14	25	23	28
15	22	28	31
16	27	27	30
17	26	22	21
18	20	25	28
Total	18	18	18
r^2	22,24	24,28	27,44
SD	3,28	3,44	3,73

In Table 1 above shows that the number of nerve after exposure to extract *CA* A and B for 48 hours and without exposure (control) can result in the number of nerve cells in 18 samples, namely: the average mean control by 22.44, *CA* A 24.28 and *CA* B 27.44. After Anova obtained F count 9.466 with a significance 0,0001 greater than F table, and then test the Post Hoc Test A probability value 0,265 cells and B cells 0,001, which means 0.001 <0.005, then the treatment of B cells have a significant mean difference with control. Thus looked seen an increase in the average number of cells per field view of between the control group, *CA* A and B, where the number of living cells in cultured nerve cells that extract *CA* B (0.50 ug / ml) was higher compared with *CA*A (0.25 ug / ml) and control (no treatment), differences in the three groups was statistically different (p <0.005), the results are shown in Figure 1 below, where it dominates the chart *CA* B is higher than *CA*A and *CA* A higher than controls



From Schinder AF, Gage FH (2004)

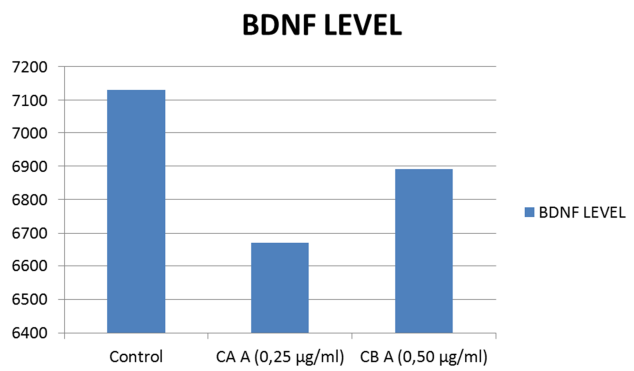
Figure 1. *The average number of nerve cells per field view of the control group and the treatment after 48 hours incubation.*

3.2. BDNF Level

Table 2. *Levels of BDNF (pg/ml) in the culture medium after 48 hours incubation.*

Sampel	Kontrol	*CA* A (0,25 µg/ml)	*CA* A (0,50 µg/ml)
1	6525	6000	8251
2	6014	5926	8251
3	6677	8090	6781
4	8090	6525	7295
5	6984	7925	10780
6	6830	6830	5926
7	5853	7452	6150
8	6525	6074	6374
9	7452	5926	5633
10	6830	6074	6984
11	6525	6223	5633
12	6677	7769	6302
13	6525	5633	6077
14	6984	6677	6374
15	7769	5488	5780
16	8742	7534	7929
17	9576	7769	7531
18	7769	6155	6004
Total	18	18	18
r^2	7130,39	6670,56	6891,94
SD	951,68	861,45	1299,44

In Table 2 above can be seen the frequency and distribution the results of measurements of the levels of BDNF in the culture medium of nerve cells after exposure extracts *CA* A and *CA* B for 48 hours and without exposure (control). can be seen the frequency and distribution in Table 2 above, the result is the number of nerve cells in 18 samples, namely: the mean control 7130,39 and *CA* B 6891,94 and *CA* A 6670.56. On the measurement of BDNF after the exposure we got the result that there are increased levels of BDNF from *CA* A to group *CA*B but the two levels lower than the control. After Anova obtained F count 0.991 with a significance of 0.378 is smaller than F table, it can be deduced that the average levels of BDNF in the third test sample there are no significant differences, the results are shown in Figure 2 below, in which the image appears it is clear that the control group looks higher than the graphed line *CA* B and *CA*B is higher than *CA* A



From Elfving B, P. H Plougman and G Wegener (2010)

Figure 2. *BDNF Levelafter 48 hours incubation.*

4. Discussion

In this research, we used on pellet from nerve cell cultures for staining cells and counting the number of cells by giemsastaining. That level BDNF, we used the sample solution and controls from nerve cell cultures. To measure BDNF concentration using ELISA method because according to previous studiesnerve cell cultures BDNF is not affected by handling of cell cultures, its absorbancies are identical with those from cell cultures to determine BDNF, and its storage is more stable that has been postulated for more than 12 months at -20°C [25]. Therefore, we measured BDNF level in nerve cell cultures in order to discover its level in hippocampus.

Mean number cell of cells nerve cell cultures for all treatment and control groups are shown in table 1. Research that has been conducted shows that the higher number of nerve cells in the hippocampus tissue cell cultures which were exposed on young rats by *CA* extract concentration of B (0.50 ug/ml), an increase in the mean number of cells hereindicate that cells undergoingfewer neuroptosis and it is possibly that*CA* extract contains a factor that can inhibit neuroptosis so that cells survive, and the possibility can induce neurogenesis through the mechanism of processing, storage, and/or recalling a memory that overall form a circuit to sustain sinaptogenesis in order to improve the functioning of the integration of new neurons or changes resulting synapse which still exist established between new and mature neurons in the hippocampal circuit [1]. Cell treated with *CA* had significantly longer primary and secondary neuritis as well as a higher number of neurit per cell compared to control cells and then induces differentiation of neural precursor cells (NPCs) into neural cells and this may be mechanism underlying the neuroprotective effect of *CA* [26]. Mean nerve cell cultures BDNF concentration for all treatment and control groups are shown in table 2. For a slightly lower levels of BDNF in young rats' hippocampal tissue cell cultures which were exposed by*CA* extracts compared to the control. Thus the purpose of the study is the effect of giving the *CA* extract on neurogenesis especially in BDNF levels did not occur through the expression of BDNF, besides the possibility of glutamate in the *CA* extracts can not increase the level of GABA in the brain due to activation of GABA synapses on a young neuron depolarization where the concentration of Cl^- ionintracellular is high because the Cl^- exporter KCC2 expression channel which performing slowdown (delayed) [2]. Expression of BDNF was also enhanced by *CA* in PC12 cell [26]. BDNF and its receptor TRkB play important role during stress injury. Reduction of BDNF mRNA and protein expression were observed in the CA3 and dentate gyrus of the hippocampus after repeated immobilization stress (26), together with increased expression of TrkBmRNA [28]. BDNF- TrkB signaling and NMDA receptors are very important for spatial memory formation, contribution of BDNF inducing TrkB/Phosphatidil Inositol 3-Kinase (P13-K) signaling pathway is critical for spatial learning in the radial arm maze [29]. *Centellaasiatica*

contain large amount of pentacyclictriterpenoid, that triterpenoid regulates neurogenesis, for example, oleanolic acid which is a pentacyclic acid triterpenoid is abundantly herb plants and induces differentiation of NSCs to neuron via a Nkx-2,5 dependent mechanism [30]. Centellaasiatica was found to be able to increased serum BDNF level and maintain its increasing level at certain dose. An active component of *CA* that has known as a cognitive enhancer is asiaticoside. Asiaticoside is also reported function as adementia treating agent [31].

References

[1] Schinder AF, Gage FH. 2004. A hypothesis about the role of adult neurogenesis in hippocampal function. Int Union physiolSci/Am physiolSoc 2004; 19: 253-261.

[2] Ben Ary Y. 2002. Excitatory actions of GABA during development: the nature of the nurture. Nature review neurosci.

[3] Duman RS. 2002. Mood and anxiety disorder. J Am Acad child Adolesc Psychiatry. 41 (6): 745-748.

[4] Snyder JS, Kee N, and Wojtowics. 2001. Effects of adult neurogenesis on synaptic plasticity in the rat dentate gyrus. J Neurophysiology; 85 (6): 2420-2431.

[5] Morris, R. G. M., E. I. Moser, G. Riedel, S. J. Martin, J. Sandhin, M. Day, and C. O'Carroll. 2003. Elements of a neurobiological theory of the hippocampus the role of activity dependent synaptic plasticity in memory. Philosophical transactions of the royal society of London. Series B, Biological sciences, vol. 358 (1432): 773-86.

[6] Yamada K, M. mizuno, and T. Nabeshima. 2002. Role for brain derived neurotrophic factor in learning and memory. Life sciences 70 (7) (January 4): 735-44.

[7] Leibrock J, F. lottspeich, A. Hohn, M. Hofer, B. Hengerer, P. Masia-kowski, H. Thoenen, and Y-A. Barde. 1989. Molecular cloning and expression of brain derived neurotrophic factor. Nature, vol. 341: 149-52.

[8] Matson MP, Chan SL, and Duan W. 2002. Modification of brain aging and neurodegenerative disorder by genes, diet and behavior. Physiol rev; 82: 637-672.

[9] Kashmira J, Gohil JAP, Anuradha K, Gajjar. 2010. Pharmacological review on centellaasiatica: A potential herbal cure-all, Indian J Pharm Sci. Vol. 72: pp 546-556.

[10] Zheng C, and L. Qin. 2007. Chemicalcomponents of centellaasiatica and their bioactivities. Journal of Chinese integrative medicine, Vol. 5: 348-351.

[11] Pitella F, R. C. Dutra, D. D. Junior, M. T. P. Lopes, and N. R. Barbosa. 2009. Antioxidant and Cytotoxic activities of centellaasiatica (L) Urb. International journal of molecular sciences 10 (9) (September): 3713-21.

[12] Krishnamurthy, G. Rajanikant, M. C. Senut, D. Zemke, J. Min, B. Frenkel, E. J. greenberg, S. W. Yu. 2010. Asiatic acid, a pentacyclictriterpene from centellaasiatica is neuroprotective in a mouse model of focal cerebral ischemia. Journal of neuroscience research 87 (11): 2541-2550.

[13] Winarto WP and Surbakti M. 2003. Kasiat-dan manfaat tanaman pegagan. Cetakanke 1. Agromediapustaka. Jakarta 2003.

[14] Inamdar PK, et al. Determination of biologically active constituents in centellaasiatica. Journal of chromatography 1996; 742: 127-130.

[15] Nalini K, Arroor AR, KaranthKS, and Rao A. 1992. Effect of centellaasiatica fresh leaf aqueous extract on learning and memory and biogenic amine turnover in albino rats. Fitoterapia LXIII: 232-7.

[16] Centella Herba. 1998. WHO monographs on selected medicinal plants.

[17] Obrietan K, Gao XB, and Anthony N. 2002. Excitatory actions of GABA increase BDNF expression via a MAPK-CREB dependent mechanism a positive feedback circuit in developing neurons; 88: 1005-1015.

[18] Gibbs RB. 1998. Level of trk-b and BDNF mRNA, but not NGF mRNA, fluctuate across the estrous cycle and increase in response to acute hormone replacement. Brain Res; 787: 259-268.

[19] Yamada MK. 2002. Brain Derived Neurotrophic Factor promotes the maturation of GABAergic mechanism in cultured hippocampal neuron. J. Neurosc; 22: 7580-7585.

[20] Beckinschtein P, Cammarota M, Katche C, Clipczuk L, Rossato JI, Goldin A, Izquierdo I, Medina JH. 2008. BDNF is essential to promote persistence of long-term memory storage, ProcNatclAcadSci USA, Vol 105; pp 2711-2716.

[21] The European agency for the evaluation of medicinal products. 1998. Committee for veterinary medicinal products centellaasiaticaeextractum. Summary report veterinary medicines evaluation unit EMEA/MRL/494/98-final.

[22] Freshney RI. 2000. Culture of animal cell: a manual ofbasic technique. 4th ed. New York: Wiley-Liss.

[23] Morgan SL, Darling DC. 1993. Animal cell culture. Oxford: BIOS Scientific Publisher.

[24] Subakir SB. 2003. Cell culture, option program international class. Faculty of Medicine University of Indonesia: 1-10.

[25] Elfving B, P. H Plougman and G Wegener. 2010. Detection of Brain derived neurotrophic factor (BDNF) in rat blood and brain preparation using Elisa: Pitfalls and solutions. Journal of neuroscience methods 187 (1) (March 15): 73-7.

[26] Haeun M, Jin Tae Hong, MiiHee Park. 2015. Centellaasiatica enhances neurogenesis and protects neuronal cells against H_2O_2 induced oxidative injury. Journal of biomedical research vol. 16. Pp 121-128. DOI: http://dx.doi.org/10.12729/Jbr.2015.16.3.121.

[27] GilvaloisL, Marmigere F, F Rage, G. Ixart, S Arancibia and L. Tapia-Arancibia. 2001. Immobilization stress rapidly and differentially modulates BDNF and TrkB mRNA expression in the Pituitary gland of adult malerats. Neuroendocrinology. Sep; 74 (3): 148-59.

[28] Nibuya M, M. Takahashi, D. S. Russell, and R. S. Duman. 1999. Repeated stress increases catalytic TrkB mRNA in rat hippocampus. Neurosci Lett. May 267 (2): 81-4.

[29] Mizuno M, K. Yamada, N. Takei, M. H. Tran, J. He, A. Nakajima, H. Nawa, and T. Nabeshima. 2003. Phosphatidylinositol 3-kinase: a Molecule mediating BDNF-dependent spatial memory formation. Molecular Psychiatry 8 (2) (March): 217-24.

[30] Ning Y, Huang J, Kalionis B, Bian Q, Dong J, Wu J, Tai X, Xia S, Shen Z. 2015. Oleanolic acid induces differentiation of neural stem cells to neurons: an involvement of transcription factor Nkx-2,5. Stem cells int. Vol. 2015. pp 672312-672323.

[31] Kumar A, A. Prakash, and S. Dogra. 2011. Centellaasiatica attenuates D-galaktose induced cognitive impairment, oxidative strss and mitochondrial dysfunction in mice. International kournal of alzheimer's disease, vol. 2011, p. 347569.