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Evaluation of Caspase-3 mRNA Gene Expression Activity in Amyloid Beta-induced Alzheimer's Disease Rats

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Caspase-3 plays a role in cell death in experimental models of several acute and chronic neurodegenerative disorders, caspase-3 activation has been proposed as an early neurodegenerative event in the progression of Alzheimer disease (AD). Alzheimer disease (AD) is a progressive neurodegenerative disease of the central nervous system characterized by neuropathological deposition of amyloid beta (A β) peptides, with the function as an extracellular signal molecule for caspase-3 activation in AD. Amyloid beta (A β) peptide is widely believed to play a central and etiological role in Alzheimer disease (AD). The overproduction of A β in the brain is a primary cause of AD and various research activities are conducted for inhibition of A β generation has become a hot topic in AD research. Therefore, in this study tried to make AD modeling through induction using A β , it is expected to increase the activity of caspase-3 mRNA which is the early sign of Alzheimer's disease. Hence, the aim of this study was to evaluate caspase-3 mRNA levels in the wistar rat induced of A β . Twenty wistar rats were randomized into five groups K0: Without AD induction, K1: AD induction, K2: AD induction, K3: AD induction and K4: AD induction. Alzheimer's disease (AD) induction was performed by A β ₁₋₄₂ (0.2 μ g) injection at the intracerebroventricular area. The mRNA Caspase-3 level measurements were performed by RT-PCR. The data caspase-3 mRNA gene expression was statistically analyzed by one-way ANOVA followed by Tukey's test. Paired t-test analysis showed no significant differences of caspase-3 mRNA level before induction among 5 groups ($p > 0.05$). At 82 weeks post-induction, there was significant increased caspase mRNA in all groups except K0 ($p < 0.05$). Increased caspase-3 mRNA gene expression in amyloid beta-induced AD rats in all groups except K0 ($p < 0.05$).

Key words: Alzheimer's disease (AD), intracerebroventricular (ICV), caspase-3, amyloid beta (A β), wistar rat

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INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disorder that was characterized by senile plaques, neurofibrillary tangles and neuronal loss^{1,2}. Alzheimer's disease was a leading cause of dementia in the aging population³. Patients with AD experience symptoms including cognitive alterations, memory loss and behavioral changes^{4,5}. Pathologically, a frequent characteristic of these diseases was the accumulation and aggregation of abnormal or misfolded proteins⁶, as with amyloid- β (A β)^{6,7}. The disease manifests usually after the age of 65 years. The frequency of AD in this age group was 1-3% and doubles every 5 years^{6,9}, exceeding 40% in 85 years old group. It was estimated that the number of patients suffering from AD was 21-24 million and this number will double in next 15 years. The disease occurs approximately two times more often in female than male⁸. Alzheimer's disease (AD) was estimated to have cost the world \$604 billion in 2010 alone⁹. These costs are staggering, particularly in light of predictions that the worldwide number of AD cases, currently estimated at 36 million, will triple by 2050⁹. A common feature of AD is the accumulation of β -amyloid (A β), 39- to 43-amino acid peptides derived from the amyloid precursor protein (APP). The β -amyloid (A β) peptides aggregate to form fibrillar deposits that are the principal component of senile plaques². In AD it has been hypothesized that β -amyloid spreads in prion-like manner giving different pathological stages of β -amyloid deposition¹⁰. The importance of A β in the pathogenesis of AD was suggested by several findings. Notably, mutations in APP or presenilin, two proteins that are implicated in familial forms of AD, lead to an increase in the amyloidogenic form of A β ². Increased A β level was consistently observed in animal models of AD, it has been shown that a single dose A β ₁₋₄₂ injected into the wistar rat could induce increased of A β level¹¹. The neurodegenerative process in AD have recently been shown to be accompanied by alterations in neurogenesis¹², to understand neurodegenerative events it is worth understanding the state of neurogenesis. Neurogenesis is the process of generating new nerve cell, including neurons, astrocytes, glia and others. Neuroplasticity refers to the ability of the brain and the central nervous system to adapt to environmental changes, response to injury and to acquire novel information by modifying neural connectivity and function¹³. The pathologic brain features described in 1907 by Dr Alois Alzheimer-namely senile plaques and neurofibrillary tangles-still serve as the hallmarks for final diagnosis of the Alzheimer's disease. Landmark research since the 1980s has established that plaques are composed mainly of extracellular deposits of β -amyloid peptides, most of which contain 38-43 amino acids¹⁴. Several factors contribute to the progression of the Alzheimer's disease including amyloid beta (A β) accumulation, neurofibrillary tangle formation, cholinergic deficit, oxidative stress, neuroinflammation and apoptosis¹⁵. Apoptosis is a cell death program that is central to

cellular and tissue homeostasis and is involved in many physiological and pathological processes¹⁶. Apoptosis occurs normally during development and aging and as a homeostatic mechanism to maintain cell populations in tissues. Apoptosis also occurs as a defense mechanism such as in immune reactions when cells are damaged by disease or noxious agents¹⁷. Apoptosis is characterized morphologically by a series of events that include cytoplasmic shrinkage, chromatin condensation, nuclear and cellular fragmentation and the formation of apoptotic bodies. The role of apoptosis in normal physiology is as significant as that of its counterpart, mitosis. It demonstrates a complementary but opposite role to mitosis and cell proliferation in the regulation of various cell populations. It was estimated that to maintain homeostasis in the adult human body, around 10 billion cells are made each day just to balance those dying by apoptosis¹⁸. The number can increase significantly when there was increased apoptosis during normal development and aging or during disease¹⁹. There are two main apoptotic pathways: the extrinsic or death receptor pathway and the intrinsic or mitochondrial pathway. However, there was now evidence that the two pathways are linked and that molecules in one pathway can influence the other¹⁹. The intrinsic apoptotic signaling is most often induced by intracellular damage that leads to mitochondrial release of cytochrome c and the activation of intracellular cysteine proteases called caspases²⁰. Extrinsic apoptotic signaling was initiated by stimulation of plasma membrane death receptors that initiate apoptosis by activation of caspase-8 and subsequent apoptotic signaling can proceed through the mitochondrial pathway or independently of mitochondria by caspase-mediated direct activation of caspase-3²¹. Although, caspases are the main players involved in apoptosis, there are other molecules involved in the progression of the apoptotic cascade that are relevant to AD. The neuronal death in AD may result directly and/or indirectly from the triggering insults caused by A β toxicity, glutamate excitotoxicity, long-lasting oxidative stress, DNA damage and elevation of intracellular calcium levels. Thus, the mode of cell death in AD remains a matter of controversy and it was possible that both apoptotic and non-apoptotic cell death coexist in the brains of affected patients¹⁶. The evidence that A β accumulation was a determining factor in AD makes it important to determine the mechanism by which A β induces neuronal cell death. Recent studies have shown that in AD brains and in cultures of neurons exposed to A β , the dying cells display the characteristics of apoptosis²²⁻²⁴. β -amyloid first accumulates in the basal temporal and orbitofrontal neocortex, then spreads throughout the neocortex and then to the hippocampus, amygdala, diencephalon and basal ganglia. Then finally, in severe cases, this spreads to the mesencephalon, lower brainstem and cerebellar cortex^{10,25}, how this pathological spread interacts with cellular phenotypic networks remains to be elucidated. Neuronal death in a variety of neurodegenerative diseases, including Alzheimer's disease

(AD), has been associated with deregulated caspase activation²⁶. Caspases are a family of cysteine proteases that cleave their substrates after aspartic residues. They are usually synthesized as inactive zymogens that are proteolytically cleaved into subunits at the onset of apoptosis and function as active caspases after reconstitution to molecular heterodimers²⁷. Caspases are divided into long prodomain caspases (caspases-2, -8, -9 and -10), which are initiators of apoptosis and short prodomain caspases (caspases-3, -6, -7 and -14), which are generally termed the effectors of apoptosis. However, some caspases, including caspase-3 (Casp3) and caspase-6 (Casp6), appear to function as both initiators and effectors. Aberrant activation of caspases has been implicated in several neurodegenerative diseases, such as AD, HD, spinocerebellar ataxias and amyotrophic lateral sclerosis²⁸. Apoptotic injury during Alzheimer's disease may require caspase-mediated pathways. A strong body of evidence supports the premise that caspase activation is involved in the pathological process of Alzheimer's disease. The elevation of caspase genes including caspases 1, 2, 3, 5, 6, 7, 8 and 9 has been observed in human postmortem brains from Alzheimer's disease patients²⁷. However, several lines of evidence suggest that the role of caspases in AD may involve more than just action as cellular executioners driven by upstream disease processes. Caspase-mediated cleavage of β -amyloid precursor protein (APP) has been reported¹⁹ and has caspase activation by amyloid- β peptide³⁰. In one murine AD model, caspase activation associated with disease onset occurred earlier than the induction of neuronal apoptosis³¹. Similarly, caspase activation has been noted before the development of neurofibrillary tangles of Tau in the brain of tau transgenic mice³². In the brains of Alzheimer's patients, single neurons with DNA fragmentation have been shown to contain cytoplasmic immunoreactivity for active caspase 3, implying that apoptotic injury results during Alzheimer's disease. Caspase-3 acts as a caspase executor in the apoptosis process³³. In addition, activation of caspase 3 was found to occur in the parahippocampal gyrus in brains from patients with mild forms of Alzheimer's disease. Caspase 3 immunoreactivity was also co-localized with paired helical filaments in neurons, suggesting that caspase 3 activation may contribute to the formation of neurofibrillary tangles²⁷. Increased levels caspase-3 and A β were found in neurodegenerative diseases, including Alzheimer's Disease (AD)²⁶. Previous studies found that expression levels of Bcl-2 family proteins, such as Bax, Bak, Bad, Bcl-2, Bim, Bcl-2_v and Bcl-x are altered in the vulnerable neurons in AD³⁴. That in cortical neurons exposed to A β , activated c-Jun N-terminal kinase (JNK) is required for the phosphorylation and activation of the c-Jun transcription factor, which in turn stimulates the transcription of several key target genes, including the death inducer Fas ligand. The binding of Fas ligand to its receptor Fas then induces a cascade of events that lead to caspase

activation and ultimately cell death. By analyzing the effects of mutations in each of the components of the JNK-c-Jun-Fas ligand-Fas pathway, we demonstrate that this pathway plays a critical role in mediating A β -induced death of cultured neurons. The possibility that the JNK pathway may also contribute to A β -dependent death in AD patients³⁵.

In this study, caspase-3 mRNA gene expression in an AD rat model induced by injection of A β for 6 weeks was evaluated. The purpose of this study was to evaluate the effect of the caspase-3 mRNA gene expression in the wistar rats model of Alzheimer's disease, with the hypothesis that A β ₁₋₄₂ 0.2 μ g injected in ICV for 6 weeks can increase the expression of the caspase-3 mRNA gene in wistar rats.

MATERIALS AND METHODS

Experimental procedures were carried out in the Molecular Microbiology and Immunology Laboratory, Faculty of Medicine Hasanuddin University Makassar Indonesia. This research was an experimental study *in vivo* pre- and post-design that was conducted in the period from February to July, 2016. The experiment has been approved by the Medical and Health Research Ethics Committee Faculty of Medicine Hasanuddin University Makassar Indonesia (Number: 391/H4.8.4.5.31/PP36-KOMTIK/2016).

Animals

Animal preparation: Twenty wistar rats (2.5-3 months, 150-250 g) were selected from central animal house (Faculty of Medicine Hasanuddin University Makassar Indonesia) for this study. Animals were randomly divided into 5 groups (= 4 in each group): T₀ control (K0) and treatment groups (K1, K2, K3 and K4). They were kept in the animal house (The Molecular Microbiology and Immunology Laboratory, Faculty of Medicine Hasanuddin University Makassar Indonesia) for 1 week for proper acclimatization before starting the experiment under controlled condition of illumination (12 h light/12 h darkness) and temperature 23 \pm 2°C. They were housed under ideal laboratory conditions, maintained on standard pellet diet and water *ad libitum* throughout the experimental period. All procedures were in accordance with the internationally accepted guideline for experimental animal use and care of laboratory animals of the Molecular Microbiology and Immunology laboratory, Faculty of Medicine Hasanuddin University Makassar.

Experimental design: In this study, total number of 20 animals were divided into following 5 groups having 4 rats in each group: K0 = No induction AD model, K1 = AD induction model, K2 = AD induction model, K3 = AD induction model and K4 = AD induction model. Caspase-3 mRNA expression was assessed before AD induction and 6 weeks after AD induction.

Animal model: In this study advance preliminary research was done to develop an animal model of AD. To develop an AD model, author used a dose of A β ₁₋₄₂ of 0.2 μ g injected in ICV and observed the mice for 6 weeks. A β levels were measured using the SAA Mouse Elisa Kit. The AD animal model was generated using A β ₁₋₄₂ peptide from Abcam, code number.Ab120959 (Cambridge, MA, USA)¹¹, is a reference modeling AD protocol. The formation of amyloid plaques was detected at weeks 4-6 post-injection of A β in the area of the neocortex³⁶.

Blood collection: Blood samples were collected at two time points: day 0 before A β injection (baseline) and last was the day 42 (week: 6, changes). Blood was taken from the tail vein using a 0.1 mL hematocrit needle. The samples were centrifuged and was kept in a sterile tube at -20°C until analyses.

RT-PCR: Extraction of total RNA qualitative RT-PCR using the method of Boom³⁷, with stages: DNA extraction, PCR mixing and dilution. In the extraction of DNA, the materials used are diatoms, L6 (lysis buffer), L2 (washing buffer) and TE (elution buffer), Diatoms after the vortex and then inserted as much as 40 μ into the sample containing L6 then shaken for 15 sec and in the rotator for 15 sec and centrifuge 13,000 rpm for 15 sec, then wash with L2 (1 mL) to 2 times, L2 (1 mL) to 2 times, ethanol (1 mL) to 2 times and the last wash acetone (500 μ) as much as 1 times, then enter the heater block for 20 sec, add 79 μ IS EDTA (TE) 100 μ L, incubation for 10 min, centrifuge 13,000 rpm for 3 min, take the supernatant and place it into a mini tube and cover and keep it at -20°C. The reverse transcriptase polymerase chain reaction (RT-PCR) was carried out to determine⁶⁴ the levels of caspase-3 mRNA gene expression. The RT-PCR was performed with the following primers, caspase-3 forward: 5'-AGCTTCTTCAGAGGCGA CTA-3' and reverse: 5'-GGACACAATACACGGGA⁵³ T-3³⁸ and glycerol-dehyde-3-Phosphate dehydrogenase (GAPDH) forward: 5'-CTCAAGATTGTCAGCAATGC-3' and reverse⁷⁸: 5'-CAGGATGCCCTTTAGTGGGC-3'³⁹, reactions were performed⁵² using the One-Step RT-PCR kit (Macrogen, Korea). GAPDH was used as an internal control. Primers for RT-PCR were designed using Oligo software (Bio-Rad CFX Manager). All samples in both the treatment and control in homogenized and a total RNA sample in the presented in the form of curves. Reactions were visualized by agarose gel electrophoresis analysis. Rate threshold cycle in the calculation of the standard software (Applied Biosystems). The level of expression extraction according to the instructions of the protocol (Ambion, Austin, TX). Making PCR mix in all using solution are shown in Table 1, for caspase-3 multiplied by 80 due to triplicate, for one run: K0 (12 tube)+K1 (12 tubes) = 24 tubes, triplicate made into 72 tubes, rounded to 80, so on for K2, K3, K4. Then take 5.2 μ L caspase-3 into each well (each well requires a 5.2 μ L PCR mix). Also distribute

Table 1: Stages of PCR mix manufacturing

77 PCR	Caspase-3 (\times 80)	GAPDH (\times 30)
Master mix = 2.5 μ L	200 μ L	75 μ L
Free water = 2.5 μ L ⁶³	200 μ L	75 μ L
Primer Caspase-3 F= 0.1 μ L	8 μ L	3 μ L
Primer Caspase-3 R = 0.1 μ L	8 μ L	3 μ L
Template = 5 μ L		

GAPDH (as standard) into the wells, Each well needs a 5.2 μ L PCR mix. Here fill the samples in the wells each containing the PCR mix, caspase-3 and GAPDH, wait for 1.5 h. Then turn on the PCR tool and make a layout, then input the sample that has been idle for 1.5 h into the wells, each enter as much as 5.2 μ L, neither with the standard (GAPDH). Next was dilution: 20 μ RNA free water inserted into new tube (tubes 1, 2, 3, 4, 5 and 6), then the DNA template of all samples take as much as 20 μ and diluted with 20 μ which had been diluted and so on up to the 6 tube. Then enter 5 μ to the wells that have been made earlier, close and insert into the⁴⁵ PCR machine, perform an initial denaturation stage of 96°C for 3 min, next 95°C for 30 sec and next annealing 55°C for 30 sec with volume contents 5+5 = 10 μ for 40 times.

Figure 1 shows schematic of the research process. Twenty wistar rats were divided into 5 groups: K0 = No induction AD model, K1 = AD induction model, K2 = AD induction model, K3 = AD induction model and K4=AD induction model. Caspase-3 mRNA expression was assessed before AD induction and 6 weeks after AD induction.

Statistical analysis: The data caspase-3 mRNA gene expression were⁷⁶ statistically analyzed and the significance calculated using one-way ANOVA (Bonferroni³⁷ followed by Tukey's test. All numerical values were expressed as Mean \pm SD and the value of p<0.05 was considered as statistically significant.

RESULTS

A total of 20 wistar rats mean age 2.5-3 months, with 150-250 mg kg⁻¹ body weight were included in the analysis. The mean, standard deviation and range of study variables presented in Table 2. The animals are grouped into groups induced by A β (K1, K2, K3 and K4) and those not induced by A β (K0), then continuous variable⁶² between the groups are compared. In Table 2 it shown that the mean level of⁷⁵ caspase-3 mRNA expression was significantly different p<0.05. Caspase-3 mRNA expression was as⁵¹ sed before A β induction and 6 weeks after A β induction. In this study, we examined the effects evaluation of caspase-3 mRNA gene expression in single doses of A β injection. Amyloid beta (A β) used in this study: peptide from Abcam⁷⁴ code number.Ab120959 (Cambridge, MA, USA). The levels of caspase-3 mRNA at various time points throughout measurements were performed by RT-PCR and the analysis was evaluated. The caspase-3 mRNA expression levels in all observation groups are shown in Table 2.

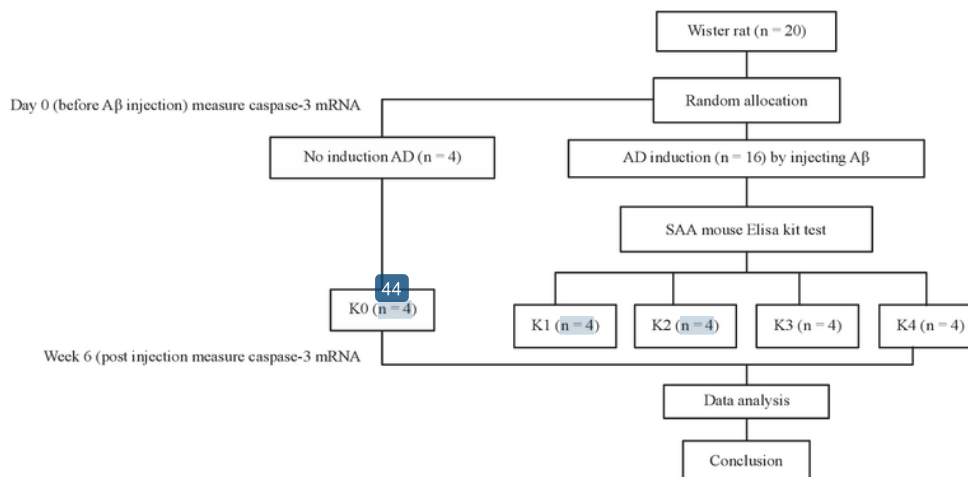


Fig. 1: Outline research process

Table 2: Caspase-3 mRNA expression in each group

Variable	Observation time			0-6 p*
	Group	Before induction (baseline week 0)	6 weeks after induction (weeks 6)	
Caspase-3 mRNA expression mean (SD)	K0 (n = 4)	7.68 (0.25)	7.66 (0.23)	0.900
	K1 (n = 4)	7.65 (0.07)	12.69 (0.16)	<0.001
	K2 (n = 4)	7.54 (0.38)	12.49 (0.07)	<0.001
	K3 (n = 4)	7.63 (0.07)	12.73 (0.15)	<0.001
	K4 (n = 4)	7.68 (0.10)	12.53 (0.04)	<0.001

*p<0.05

Single-dose induction effect of 0.2 µg Aβ given by injection in the ICV region of wistar rat: The caspase-3 mRNA level at various time points increased in the Aβ-induced group compared with the non-induced Aβ, Aβ induction is given for 6 weeks (Table 2).

Table 2 shows that caspase-3 mRNA expression before Aβ induction had no significant differences (p>0.05) between the 5 groups. After 6 weeks post Aβ induction there were significant increased (p<0.05) in all groups except K0 group. The mean increased level of caspase-3 mRNA expression in each group was (5.04) in K1, (4.95) in K2, (5.10) in K3 and (4.86) in K4. There were no significant differences in Caspase-3 mRNA expression increased (p>0.05) between induction groups.

Paired t-test and one-way ANOVA (Bonferroni) showed changes in caspase-3 mRNA expression after 6 weeks post-induction Aβ, no significant differences in caspase-3 mRNA expression among all 5 groups before induction observed (p>0.05). At 6 weeks post-induction Aβ, there was a significant increase (p<0.05) in caspase-3 mRNA expression in all groups except the K0 group. There were no significant differences in the increase in caspase-3 mRNA expression among induction groups (p>0.05). 6 weeks after induction Aβ, it was detected increased caspase-3 mRNA

expression in the induction Aβ groups, while no changes occurred in the non-induction Aβ group (K0).

DISCUSSION

Alzheimer disease (AD) is a progressive neurodegenerative disorder and the most common cause of senile dementia and is characterized by progressive dementia accompanied by personality changes, psychosis and language problems. Neuropathology is characterized mainly by extracellular senile plaques, consisting primarily of β-amyloid (Aβ) and intracellular neurofibrillary tangles, with hyperphosphorylated microtubule associated protein tau as a main constituent⁴⁰. The accumulation of Aβ in the brains of AD patients has been implicated as a cause of the neuronal loss that occurs in Alzheimer's disease. However, the mechanisms by which Aβ induces neuronal death are not well understood. In the present study, showed the evaluation of caspase-3 mRNA expression of Aβ-induced AD rats. This study results showed that detected an increase of caspase-3 mRNA expression at 6 weeks after Aβ induction, which was similar to the result presented by Cetin *et al.*⁴¹. Their research found high caspase-3 activity levels in hippocampus, temporal and parietal cortex in aged

mice injected with A β ₁₋₄₂⁵⁶. Amyloid β (A β) has been proposed as the main factor in the AD pathophysiology mechanism and the major component of senile plaques, was considered to play a central role in neuronal cell death (apoptosis), has received the most attention⁴³. This has been demonstrated in neurons of human and rodent brains. In patients suffering from neurodegenerative disorders such as AD, there was a prominent increase in the neuronal RNA damage when compared to normal aging people⁴⁴. The formation of extracellular plaques is described by the amyloid cascade (A β -protein) theory of plaque causing pathology^{6,45}. Plaques which arise when the amyloid precursor protein (β -APP) was cleaved by the beta-amyloid cleaving enzyme (BACE) to result the A β -42 type aggregations^{46,47}, because of its high lipid content and high oxygen consumption, the brain is particularly susceptible to oxidative stress. Several mechanisms have been proposed to explain A β neurotoxicity, production of reactive oxygen species such as hydrogen peroxide, nitric oxide, superoxide, highly reactive hydroxyl radicals and nitric oxide (NO), excitotoxicity with intracellular calcium accumulation, decreased membrane fluidity, energy depletion, alteration of the cytoskeleton, inflammatory processes and alteration of metal homeostasis⁴⁸⁻⁵⁰. All of these events converge into similar pathways of synaptic disruption, necrosis or apoptosis, leading to progressive loss of specific neuronal cell populations. The accumulation of A β in the brains of AD patients has been implicated as a cause of the neuronal loss that occurs in Alzheimer's disease, however, the mechanisms by which A β induces neuronal death are not well understood⁵¹. Amyloid β (A β) peptide contributes to the neurodegeneration in Alzheimer's disease (AD) and operates through activation of an apoptotic pathway. Apoptotic signal is driven by a family of cysteine proteases called caspases. The β -amyloid precursor protein (APP) is directly and efficiently cleaved by caspases during apoptosis, resulting in elevated β -amyloid peptide formation⁵². Changed expression of proteins linked to apoptosis, such as Bcl-2, Bax, p53 and Fas was reported in AD brains^{53,54}. Caspases are a family of intracellular cysteine-aspartic proteases that are not only essential for triggering programmed cell death, but have also been shown to play key roles in non-apoptotic pathways, such as differentiation and proliferation of diverse cell types, axon guidance and synaptic activity and plasticity²⁸. It is known that apoptosis with the participation of caspases e.g., caspase-3 may be involved in pathogenesis of AD^{55,56}. Another result suggested that A β could function as an extracellular signal molecule for caspase-3 activation in AD⁵⁷. These observations indicate the presence of a pathway from A β , as an *in vivo* trigger signal, to neuronal cell death through APP and caspase-3. It is also likely that other pathways mediated by APP derived fragments⁵⁸. Play roles in A β -induced toxicity themselves and/or together with APP. Whether A β activates caspase-3 directly or via APP and/or other proteins, our findings suggest that caspase-3 is a key molecule in

A β -induced neuronal loss. Although *in vivo* model suggests that a relation between apoptosis and A β may exist, whether apoptosis occurs in AD brain remains unclear⁵⁹. These results suggest that A β could function as an extracellular signal molecule for caspase-3 activation in AD. The yet unknown relationship between a soluble A β oligomer and amyloid burden as a sequestering reservoir remains to be studied. However, it is likely that A β derived from APP functions as a key suicide molecule through caspase-3 in AD and that genetic factors such as mutations of APP, presenilin-1, or presenilin-2 enhance this suicide cycle⁵⁷ and A β induces the activation of c-Jun in a JNK-dependent manner. JNK3 appears to promote apoptosis by phosphorylating and activating the transcription factor c-Jun.

CONCLUSION

The study concluded that although the molecular mechanism of damage to neurons in AD is not fully understood, but the beta-amyloid (A β) peptide is a biochemical toxicological effect in contributing to the onset of neurodegeneration in Alzheimer's disease (AD), it was seen after post-injection induction of A β in intracerebroventricularly (ICV) area for 6 weeks, i.e., the increment of caspase-3 mRNA significantly in all groups except K0 which was not induced. It is likely to operate through the activation of the apoptotic pathway. It is possible that A β activates caspase-3 directly or via APP and/or other proteins, that A β could function as an extracellular signal molecule for caspase-3 activation in AD. Amyloid β (A β) derived from APP functions as a key suicide molecule through caspase-3 in AD. The evidence that A β induces the activation of c-Jun in a JNK-dependent manner. The c-Jun N-terminal kinase-3 (JNK3) appears to promote apoptosis by phosphorylating and activating the transcription factor c-Jun. However, it is suggested that more research on the effects of A β should be undertaken in AD using different experimental models.

SIGNIFICANCE STATEMENTS

This study discovered the role of the expression activity of the Caspase-3 mRNA gene in A β -induced Alzheimer's disease (AD) rats, where it is known that caspase-3 acts as a caspase executor in the apoptosis process. Increased level caspase-3 and A β were found in neurodegenerative disease including AD. That can be beneficial for developing animal modeling AD, so as to facilitate the researchers for the exploration of material and drug discovery trials for AD. This study will help the researcher to uncover the critical areas of the occurrence of AD earlier, that many researchers were not able to explore. Thus a new theory on induction of A β given to wistar rats by injection in the ICV area for 6 weeks, may increase the expression activity of the caspase-3 mRNA gene.

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REFERENCES

1. Yankner, B.A., 1996. Mechanisms of neuronal degeneration in Alzheimer's disease. *Neuron*, 16: 921-932.
2. Selkoe, D.J., 1999. Translating cell biology into therapeutic advances in Alzheimer's disease. *Nature*, 399: A23-A31.
3. Ashford, J.W., 2004. APOE genotype effects on Alzheimer's disease onset and epidemiology. *J. Mol. Neurosci.*, 23: 157-165.
4. Katzman, R., 1986. Alzheimer's disease. *N. Engl. J. Med.*, 314: 964-973.
5. Budson, A.E. and B.H. Price, 2005. Memory dysfunction. *N. Engl. J. Med.*, 352: 692-699.
6. Hardy, J.A. and G.A. Higgins, 1992. Alzheimer's disease: The amyloid cascade hypothesis. *Science*, 256: 184-185.
7. Karran, E., M. Mercken and B. De Strooper, 2011. The amyloid cascade hypothesis for Alzheimer's disease: An appraisal for the development of therapeutics. *Nat. Rev. Drug Discov.*, 10: 698-712.
8. Reitz, C., C. Brayne and R. Mayeux, 2011. Epidemiology of Alzheimer disease. *Nat. Rev. Neurol.*, 7: 137-152.
9. Wimo, A., L. Jonsson, A. Gustavsson, D. McDaid and K. Ersek *et al.*, 2011. The economic impact of dementia in Europe in 2008-cost estimates from the Eurocode project. *Int. J. Geriatric Psychiatry*, 26: 825-832.
10. Goedert, M., 2015. Alzheimer's and Parkinson's diseases: The prion concept in relation to assembled A β , tau and α -synuclein. *Science*, Vol. 349. 10.1126/science.1255555.
11. Kamelia, B., H. Miko, M.B. Karo, S. Pertiwi, C. Kaelan, A.I. Andi and H. Mochammad, 2016. Beta amyloid peptide role in animal modeling trial of Alzheimer's disease. *Int. J. Sci.: Basic Applied Res.*, 28: 90-99.
12. Boekhoorn, K., M. Joels and P.J. Lucassen, 2006. Increased proliferation reflects glial and vascular-associated changes, but not neurogenesis in the presenile Alzheimer hippocampus. *Neurobiol. Dis.*, 24: 1-14.
13. Kamelia, E., H. Miko, M.B. Karo and M. Hatta, 2016. Neurogenesis and brain-derived neurotrophic factor levels in herbal therapy. *Int. J. Res. Med. Sci.*, 4: 4654-4658.
14. Glenner, G.G. and C.W. Wong, 1988. Alzheimer's disease: Initial report of the purification and characterization of a novel cerebrovascular amyloid protein. *Alzheimer Dis. Assoc. Disorders*, Vol. 2.
15. Syad, A.N. and K.P. Devi, 2014. Botanicals: A potential source of new therapies for Alzheimer's disease. *Evolution*, Vol. 17. 10.2147/BTAT.S33554.
16. Lee, J.H., Y.H. Cheon, R.S. Woo, D.Y. Song, C. Moon and T.K. Baik, 2012. Evidence of early involvement of apoptosis inducing factor-induced neuronal death in Alzheimer brain. *Anat. Cell Biol.*, 45: 26-37.
17. Norbury, C.J. and I.D. Hickson, 2001. Cellular responses to DNA damage. *Annu. Rev. Pharmacol. Toxicol.*, 41: 367-401.
18. Renehan, A.G., C. Booth and C.S. Potten, 2001. What is apoptosis and why is it important? *Br. Med. J.*, Vol. 322, 10.1136/bmj.322.7301.1536.
19. Igney, F.H. and P.H. Krammer, 2002. Death and anti-death: Tumour resistance to apoptosis. *Nat. Rev. Cancer*, 2: 277-288.
20. Earnshaw, W.C., L.M. Martins and S.H. Kaufmann, 1999. Mammalian caspases: Structure, activation, substrates and functions during apoptosis. *Annu. Rev. Biochem.*, 68: 383-424.
21. Ashkenazi, A. and V.M. Dixit, 1998. Death receptors: Signaling and modulation. *Science*, 281: 1305-1308.
22. Anderson, A.J., J.H. Su and C.W. Cotman, 1996. DNA damage and apoptosis in Alzheimer's disease: Colocalization with c-Jun immunoreactivity, relationship to brain area and effect of postmortem delay. *J. Neurosci.*, 16: 1710-1719.
23. Estus, S., H.M. Tucker, C. van Rooyen, S. Wright, E.F. Brigham, M. Wogulis and R.E. Rydel, 1997. Aggregated amyloid- β protein induces cortical neuronal apoptosis and concomitant "apoptotic" pattern of gene induction. *J. Neurosci.*, 17: 7736-7745.
24. Stadelmann, C., T.L. Deckwerth, A. Srinivasan, C. Bancher, W. Bruck, K. Jellinger and H. Lassmann, 1999. Activation of caspase-3 in single neurons and autophagic granules of granulovacuolar degeneration in Alzheimer's disease: Evidence for apoptotic cell death. *Am. J. Pathol.*, 155: 1459-1466.
25. Thal, D.R., U. Rub, M. Orantes and H. Braak, 2002. Phases of A β -deposition in the human brain and its relevance for the development of AD. *Neurology*, 58: 1791-1800.
26. Rohn, T.T. and E. Head, 2009. Caspases as therapeutic targets in Alzheimer's disease: Is it time to "cut" to the chase? *Int. J. Clin. Exp. Pathol.*, 2: 108-118.

27. Chong, Z.Z., F. Li and K. Maiese, 2005. Stress in the brain: Novel cellular mechanisms of injury linked to Alzheimer's disease. *Brain Res. Rev.*, 49: 1-21.
28. Uribe, V., B.K. Wong, R.K. Graham, C.L. Cusack and N.H. Skotte *et al.*, 2012. Rescue from excitotoxicity and axonal degeneration accompanied by age-dependent behavioral and neuroanatomical alterations in caspase-6-deficient mice. *Hum. Mol. Genet.*, 21: 1954-1967.
29. Rohn, T.T., E. Head, J.H. Su, A.J. Anderson, B.A. Bahr, C.W. Cotman and D.H. Cribbs, 2001. Correlation between caspase activation and neurofibrillary tangle formation in Alzheimer's disease. *Am. J. Pathol.*, 158: 189-198.
30. O'Brien, R.J. and P.C. Wong, 2011. Amyloid precursor protein processing and Alzheimer's disease. *Ann. Rev. Neurosci.*, 34: 185-204.
31. D'amelio, M., V. Cavallucci, S. Middei, C. Marchetti and S. Pacioni *et al.*, 2011. Caspase-3 triggers early synaptic dysfunction in a mouse model of Alzheimer's disease. *Nat. Neurosci.*, 14: 69-76.
32. De Calignon, A., L.M. Fox, R. Pitstick, G.A. Carlson, B.J. Bacskai, T.L. Spire-Jones and B.T. Hyman, 2010. Caspase activation precedes and leads to tangles. *Nature*, 464: 1201-1204.
33. Wolf, B.B., M. Schuler, F. Echeverri and D.R. Green, 1999. Caspase-3 is the primary activator of apoptotic DNA fragmentation via DNA fragmentation factor-45/inhibitor of caspase-activated DNase inactivation. *J. Biol. Chem.*, 274: 30651-30656.
34. Vargas, T., C. Ugalde, C. Spuch, D. Antequera and M.J. Moran *et al.*, 2010. A β accumulation in choroid plexus is associated with mitochondrial-induced apoptosis. *Neurobiol. Aging*, 31: 1569-1581.
35. Morishima, Y., Y. Gotoh, J. Zieg, T. Barrett and H. Takano *et al.*, 2001. β -Amyloid induces neuronal apoptosis via a mechanism that involves the c-Jun N-terminal kinase pathway and the induction of Fas ligand. *J. Neurosci.*, 21: 7551-7560.
36. Jahrling, N., K. Becker, B.M. Wegenast-Braun, S.A. Grathwohl, M. Jucker and H.U. Dodt, 2015. Cerebral β -amyloidosis in mice investigated by ultramicroscopy. *Plos One*, Vol. 10. 10.1371/journal.pone.0125418.
37. Hatta, M. and H.L. Smits, 2007. Detection of *Salmonella typhi* by nested polymerase chain reaction in blood, urine and stool samples. *Am. J. Trop. Med. Hyg.*, 76: 139-143.
38. Cai, Y., J. Li, S. Yang, P. Li, X. Zhang and H. Liu, 2012. CIBZ, a novel BTB domain-containing protein, is involved in mouse spinal cord injury via mitochondrial pathway independent of p53 gene. *Plos One*, Vol. 7. 10.1371/journal.pone.0033156.
39. Zetterstrom, C.K., M.L. Strand and O. Soder, 2006. The high mobility group box chromosomal protein 1 is expressed in the human and rat testis where it may function as an antibacterial factor. *Hum. Reprod.*, 21: 2801-2809.
40. Keck, S., R. Nitsch, T. Grune and O. Ullrich, 2003. Proteasome inhibition by paired helical filament tau in brains of patients with Alzheimer's disease. *J. Neurochem.*, 85: 115-122.
41. Cetin, F., N. Yazihan, S. Dincer and G. Akbulut, 2013. The effect of intracerebroventricular injection of beta amyloid peptide (1-42) on caspase-3 activity, lipid peroxidation, nitric oxide and NOS expression in young adult and aged rat brain. *Turk. Neurosurg*, 23: 144-150.
42. Vauzour, D., 2012. Dietary polyphenols as modulators of brain functions: Biological actions and molecular mechanisms underpinning their beneficial effects. *Oxid. Med. Cell. Longev.* 10.1155/2012/914273.
43. Mattson, M.P., 2006. Neuronal life-and-death signaling, apoptosis and neurodegenerative disorders. *Antioxid. Redox Signal.*, 8: 1997-2006.
44. Nunomura, A., P.I. Moreira, R.J. Castellani, H.G. Lee, X. Zhu, M.A. Smith and G. Perry, 2012. Oxidative damage to RNA in aging and neurodegenerative disorders. *Neurotox. Res.*, 22: 231-248.
45. Van Dam, D. and P.P. de Deyn, 2006. Drug discovery in dementia: The role of rodent models. *Nat. Rev. Drug Discov.*, 5: 956-970.
46. Oddo, S., A. Caccamo, M. Kitazawa, B.P. Tseng and F.M. LaFerla, 2003. Amyloid deposition precedes tangle formation in a triple transgenic model of Alzheimer's disease. *Neurobiol. Aging*, 24: 1063-1070.
47. Oddo, S., A. Caccamo, J.D. Shepherd, M.P. Murphy and T.E. Golde *et al.*, 2003. Triple-transgenic model of Alzheimer's disease with plaques and tangles: Intracellular A β and synaptic dysfunction. *Neuron*, 39: 409-421.
48. Hardy, J. and D.J. Selkoe, 2002. The amyloid hypothesis of Alzheimer's disease: Progress and problems on the road to therapeutics. *Science*, 297: 353-356.
49. Bush, A.I., 2003. The metallobiology of Alzheimer's disease. *Trends Neurosci.*, 26: 207-214.
50. Barnham, K.J., F. Haeflner, G.D. Ciccotosto, C.C. Curtain and D. Tew *et al.*, 2004. Tyrosine gated electron transfer is key to the toxic mechanism of Alzheimer's disease β -amyloid. *FASEB J.*, 18: 1427-1429.
51. Carrillo-Mora, P., R. Luna and L. Colin-Barenque, 2014. Amyloid beta: Multiple mechanisms of toxicity and only some protective effects? *Oxidat. Med. Cell. Longevity*, 10.1155/2014/795375.
52. Marin, N., B. Romero, F. Bosch-Morell, M. Llansola, V. Felipo, J. Roma and F.J. Romero, 2000. β -Amyloid-induced activation of Caspase-3 in primary cultures of rat neurons. *Mech. Ageing Dev.*, 119: 63-67.
53. Kitamura, Y., S. Shimohama, W. Kamoshima, T. Ota and Y. Matsuoka *et al.*, 1998. Alteration of proteins regulating apoptosis, Bcl-2, Bcl-x, Bax, Bak, Bad, ICH-1 and CPP32, in Alzheimer's disease. *Brain Res.*, 780: 260-269.

54. Nishimura, T., H. Akiyama, S. Yonehara, H. Kondo and K. Ikeda *et al.*, 1995. Fas antigen expression in brains of patients with Alzheimer-type dementia. *Brain Res.*, 695: 137-145.
55. Salvesen, G.S. and V.M. Dixit, 1997. Caspases: Intracellular signaling by proteolysis. *Cell*, 91: 443-446.
56. Krantic, S., N. Mechawar, S. Reix and R. Quirion, 2005. Molecular basis of programmed cell death involved in neurodegeneration. *Trends Neurosci.*, 28: 670-676.
57. Takuma, H., T. Tomiyama, K. Kuida and H. Mori, 2004. Amyloid beta peptide-induced cerebral neuronal loss is mediated by caspase-3 *in vivo*. *J. Neuropathol. Exp. Neurol.*, 63: 255-261.
58. Lu, D.C., S. Rabizadeh, S. Chandra, R.F. Shayya and L.M. Ellerby *et al.*, 2000. A second cytotoxic proteolytic peptide derived from amyloid β -protein precursor. *Nat. Med.*, 6: 397-404.
59. Perry, G., A. Nunomura and M.A. Smith, 1998. A suicide note from Alzheimer disease neurons? *Nat. Med.*, 4: 897-898.

ORIGINALITY REPORT

%**48**

SIMILARITY INDEX

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INTERNET SOURCES

%**41**

PUBLICATIONS

%**26**

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PRIMARY SOURCES

1	www.intechopen.com Internet Source	% 8
2	arthritis.pure.elsevier.com Internet Source	% 2
3	drrajivdesaimd.com Internet Source	% 2
4	Yoshiyuki Morishima, Yukiko Gotoh, Janine Zieg, Tamera Barrett et al. " β -Amyloid Induces Neuronal Apoptosis Via a Mechanism that Involves the c-Jun N-Terminal Kinase Pathway and the Induction of Fas Ligand", The Journal of Neuroscience, 2001 Publication	% 2
5	onlinelibrary.wiley.com Internet Source	% 2
6	Iraj Jafari Anarkooli. "Evaluation of Bcl-2 Family Gene Expression and Caspase-3 Activity in Hippocampus STZ-Induced Diabetic Rats", Experimental Diabetes Research, 2008 Publication	% 2
7	pdfs.semanticscholar.org	

8 www.scilit.net
Internet Source

% 1

9 Samiha M. Abd El Dayem, Hanaa H. Ahmed, Fateheya Metwally, Fatma M. Aly Foda, Aziza B. Shalby, Asmaa M.A. Zaazaa. "Alpha-chymotrypcin ameliorates neuroinflammation and apoptosis characterizing Alzheimer's disease-induced in ovariectomized rats", *Experimental and Toxicologic Pathology*, 2013
Publication

% 1

10 cshperspectives.cshlp.org
Internet Source

% 1

11 jaoa.org
Internet Source

% 1

12 Mari@?n, N.. "@b-Amyloid-induced activation of Caspase-3 in primary cultures of rat neurons", *Mechanisms of Ageing and Development*, 20001020
Publication

% 1

13 preview-alzres.biomedcentral.com
Internet Source

% 1

14 www.j3.jstage.jst.go.jp
Internet Source

% 1

15 www.gssiweb-de.com
Internet Source

% 1

16

www.research.manchester.ac.uk

Internet Source

% 1

17

tpx.sagepub.com

Internet Source

% 1

18

Kudo, T, T Katayama, K Imaizumi, D Kanayama, J "Hitomi," M Okochi, M Tohyama, and M Takeda. "Alzheimer pathology and endoplasmic reticulum stress", Recent Progress in Alzheimer s and Parkinson s Diseases, 2005.

Publication

% 1

19

"Pathophysiological Aspects of Proteases", Springer Nature, 2017

Publication

% 1

20

Saeid Ghavami, Shahla Shojaei, Behzad Yeganeh, Sudharsana R. Ande et al. "Autophagy and apoptosis dysfunction in neurodegenerative disorders", Progress in Neurobiology, 2014

Publication

% 1

21

Khanam, Hena, Abad Ali, Mohd Asif, and Shamsuzzaman. "Neurodegenerative diseases linked to misfolded proteins and their therapeutic approaches: A review", European Journal of Medicinal Chemistry, 2016.

Publication

% 1

22

Zhao Zhong Chong, Faqi Li, Kenneth Maiese. "Stress in the brain: novel cellular

% 1

mechanisms of injury linked to Alzheimer's disease", Brain Research Reviews, 2005

Publication

23

Parul Tripathi, Suresh K Yadav, Somali Sanyal, Aditi Singh. "Polymorphism of Two Genes and Oral Lesion Risk in North Indian Population", International Journal of Cancer Research, 2017

Publication

% 1

24

www.transduction-meeting.lu

Internet Source

% 1

25

pubs.acs.org

Internet Source

<% 1

26

jurnal.ugm.ac.id

Internet Source

<% 1

27

www.dovepress.com

Internet Source

<% 1

28

biotm.cis.udel.edu

Internet Source

<% 1

29

"Abstracts of papers presented at the thirty-first Annual Meeting of the Japanese Teratology Society", Teratology, 12/1991

Publication

<% 1

30

www.cnnjournal.org

Internet Source

<% 1

31

Ferihan Cetin. "Chapter 4 Role of Oxidative Stress in A β Animal Model of Alzheimer's

<% 1

Disease: Vicious Circle of Apoptosis, Nitric Oxide and Age", InTech, 2013

Publication

32

Submitted to Indian Institute of Science
Education and Research

Student Paper

<% 1

33

hmg.oxfordjournals.org

Internet Source

<% 1

34

www.depkes.go.id

Internet Source

<% 1

35

mcb.asm.org

Internet Source

<% 1

36

www.eurekaselect.com

Internet Source

<% 1

37

iovs.arvojournals.org

Internet Source

<% 1

38

www.hindawi.com

Internet Source

<% 1

39

Natalia Louneva, Julia W. Cohen, Li-Ying Han,
Konrad Talbot et al. "Caspase-3 Is Enriched in
Postsynaptic Densities and Increased in
Alzheimer's Disease", The American Journal
of Pathology, 2008

Publication

<% 1

40

Monika Egerbacher. "Loss of Homeostatic
Tension Induces Apoptosis in Tendon Cells:
An In Vitro Study", Clinical Orthopaedics and

<% 1

Related Research, 07/2008

Publication

41

Submitted to University of Nicosia

Student Paper

<% 1

42

Bo Ma, Xiangbao Meng, Jing Wang, Jing Sun, Xiaoyu Ren, Meng Qin, Jie Sun, Guibo Sun, Xiaobo Sun. "Notoginsenoside R1 attenuates amyloid- β -induced damage in neurons by inhibiting reactive oxygen species and modulating MAPK activation", International Immunopharmacology, 2014

Publication

<% 1

43

"A MINI REVIEW OF INDONESIAN MEDICINAL PLANTS FOR VULVOVAGINAL CANDIDIASIS", Rasayan Journal of Chemistry, 2017

Publication

<% 1

44

F. Leveille. "Suppression of the Intrinsic Apoptosis Pathway by Synaptic Activity", Journal of Neuroscience, 02/17/2010

Publication

<% 1

45

baadalsg.inflibnet.ac.in

Internet Source

<% 1

46

journal.unair.ac.id

Internet Source

<% 1

47

Xian Zhang, Timothy Y. Huang, Joel Yancey, Hong Luo, Yun-wu Zhang. "Role of Rab GTPases in Alzheimer's Disease", ACS

<% 1

Chemical Neuroscience, 2018

Publication

48

www.jem.org

Internet Source

<% 1

49

Chauhan, V.. "Oxidative stress in Alzheimer's disease", Pathophysiology, 200608

Publication

<% 1

50

lansbury.bwh.harvard.edu

Internet Source

<% 1

51

www.citeulike.org

Internet Source

<% 1

52

Koichi Inoue. "Brain-type creatine kinase activates neuron-specific K⁺-Cl⁻ co-transporter KCC2", Journal of Neurochemistry, 1/2006

Publication

<% 1

53

spandidos-publications.com

Internet Source

<% 1

54

Carlos Fernández-Moriano, Elena González-Burgos, M. Pilar Gómez-Serranillos. "Mitochondria-Targeted Protective Compounds in Parkinson's and Alzheimer's Diseases", Oxidative Medicine and Cellular Longevity, 2015

Publication

<% 1

55

med.unhas.ac.id

Internet Source

<% 1

56	ro.ecu.edu.au Internet Source	<% 1
57	www.turkishneurosurgery.org.tr Internet Source	<% 1
58	www.hgh-frag-176-191.com Internet Source	<% 1
59	Tenn, C.C.. "VX-induced cell death involves activation of caspase-3 in cultured rat cortical neurons", Neuroscience Letters, 20070501 Publication	<% 1
60	X. D. Zhou. "Enhanced Expression of Metallothionein Isoform 3 Protein in Tumor Heterotransplants Derived from As+3- and Cd+2-Transformed Human Urothelial Cells", Toxicological Sciences, 06/28/2006 Publication	<% 1
61	hangtuah.ac.id Internet Source	<% 1
62	Ren-Huan Xu, Jing Liu, Xian-Wen Chen, Feng Xu, Qing Xie, Hong Yu, Qing Guo, Xia-Qiu Zhou, You-Xin Jin. "Ribozyme-mediated inhibition of caspase-3 activity reduces apoptosis induced by 6-hydroxydopamine in PC12 cells", Brain Research, 2001 Publication	<% 1
63	Submitted to University of Edinburgh Student Paper	<% 1

- 64 Su-Jin Kim, Jae-Young Um, Sung-Hoon Kim, Seung-Heon Hong. "Protective Effect of Rosmarinic Acid is Through Regulation of Inflammatory Cytokine in Cadmium-Induced Ototoxicity", The American Journal of Chinese Medicine, 2013
Publication <% 1
-
- 65 "Neuronal Alterations and Antioxidant Status of Lipopolysaccharide Induced Neuronal damage in Mice: Efficacy of Three Medicinal Plants", Journal of Applied Pharmaceutical Science, 2017
Publication <% 1
-
- 66 media.neliti.com
Internet Source <% 1
-
- 67 pure.uva.nl
Internet Source <% 1
-
- 68 ikee.lib.auth.gr
Internet Source <% 1
-
- 69 www.omicsonline.org
Internet Source <% 1
-
- 70 Beom S. Jeon. "Activation of Caspase-3 in Developmental Models of Programmed Cell Death in Neurons of the Substantia Nigra", Journal of Neurochemistry, 7/1999
Publication <% 1
-
- 71 scholarcommons.usf.edu
Internet Source <% 1

72

Deligezer, U.. "Circulating fragmented nucleosomal DNA and caspase-3 mRNA in patients with lymphoma and myeloma", *Experimental and Molecular Pathology*, 200602

Publication

<% 1

73

journals.plos.org

Internet Source

<% 1

74

D. Kobayashi, N. Watanabe, N. Yamauchi. "Heat-induced apoptosis via caspase-3 activation in tumour cells carrying mutant p53", *International Journal of Hyperthermia*, 11/1/2000

Publication

<% 1

75

Wnimunk Oriyanhan, Kazuhiro Yamazaki, Senri Miwa, Kiyooki Takaba, Tadashi Ikeda, Masashi Komeda. "Taurine prevents myocardial ischemia/reperfusion-induced oxidative stress and apoptosis in prolonged hypothermic rat heart preservation", *Heart and Vessels*, 2005

Publication

<% 1

76

Hyung-Mun Yun, Peng Jin, Jin-Yi Han, Moon-Soon Lee, Sang-Bae Han, Ki-Wan Oh, Sung-Han Hong, Eun-Yong Jung, Jin Tae Hong. "Acceleration of the Development of Alzheimer's Disease in Amyloid Beta-Infused Peroxiredoxin 6 Overexpression Transgenic Mice", *Molecular Neurobiology*, 2013

<% 1

77

Submitted to Middlesex University

Student Paper

<% 1

78

Gui-Hua Liang, Gui-Hua Liang, Zhe Jin, Gui-Hua Liang, Zhe Jin, Mats Ulfendahl, Leif Järlebark. "Molecular analyses of KCNQ1–5 potassium channel mRNAs in rat and guinea pig inner ears: expression, cloning, and alternative splicing", Acta Oto-Laryngologica, 2009

Publication

<% 1

79

Haeffner, F.. "Model studies of cholesterol and ascorbate oxidation by copper complexes: Relevance to Alzheimer's disease @b-amyloid metallochemistry", Journal of Inorganic Biochemistry, 200512

Publication

<% 1

80

link.springer.com

Internet Source

<% 1

81

biblioteca.universia.net

Internet Source

<% 1

82

Yin, Hai-Yan, Jian-Rui Wei, Rui Zhang, Xiao-Ling Ye, You-Feng Zhu, and Wei-Jian Li. "Effect of Glutamine on Caspase-3 mRNA and Protein Expression in the Myocardium of Rats With Sepsis :", The American Journal of the Medical Sciences, 2014.

Publication

<% 1

83 "The 9th International Child Neurology Congress The 7th Asian and Oceanian Congress of Child Neurology", Brain and Development, 2002 <% 1
Publication

84 "TUESDAY SESSION", Journal of Neurochemistry, 8/2007 <% 1
Publication

85 Submitted to St. Lawrence University <% 1
Student Paper

86 Submitted to University of Melbourne <% 1
Student Paper

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Student Paper

88 D. R. McIlwain, T. Berger, T. W. Mak. "Caspase Functions in Cell Death and Disease", Cold Spring Harbor Perspectives in Biology, 2013 <% 1
Publication

89 Submitted to Higher Education Commission Pakistan <% 1
Student Paper
