MIRACLE ENZYMES SERRAPEPTASE AND NATTOKINASE MITIGATE NEUROINFLAMMATION AND APOPTOSIS ASSOCIATED WITH ALZHEIMER'S DISEASE IN EXPERIMENTAL MODEL

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ABSTRACT

Alzheimer's disease (AD) is a challenging neurodegenerative disorder in the elderly that is characterized by impairment of memory and eventually by disturbances in reasoning, planning, language and perception. The current study was planned to elucidate the protective role of the proteolytic enzymes, serrapeptase and nattokinase, in ameliorating neuroinflammation and apoptosis associated with AD induced in rats. Sixty adult male albino rats were enrolled in the present study and randomly classified into six groups; Group (1) set as control group, Group (2) AD induced group in which rats were orally administered with $AlCl_3$ (17 mg/ kg b.wt.) for 45 days and Groups from (3) to (6) were orally administered with $AlCl_3$ for 45 days and simultaneously supplemented with low and high doses of serrapeptase (10.800 U/ kg b.wt. and 21.600 U/ kg b.wt.) and nattokinase (360 FU/ kg b.wt. and 720 FU/ kg b.wt.) respectively. Brain cholinesterase activity, TGF-β , IL-6 , Bcl-2 and P53 levels were estimated as well as histological investigation of the brain tissue of the different studied groups was carried out. In comparison with the control group, $AlCl_3$ administration produced significant elevation in cholinesterase activity, TGF- β ,IL-6 and P 53 levels while it induced significant reduction in Bcl-2 level . Regarding the groups treated with either serrapeptase or nattokinase in different doses, our findings showed significant

decrease in cholinesterase activity, TGF- β ,IL-6 and P53 levels accompanied with significant increase in Bcl-2 level as compared to untreated AD induced group. These results are greatly supported by the histological findings. Thus, it could be concluded that serrapeptase and nattokinase may be considered as newly neuroprotective agents against inflammation and apoptosis characterizing AD through their proteolytic, anti-inflammatory and anti-apoptotic effects.

Key Words: Alzheimer's disease, Proteolytic enzymes, Inflammation, , Apoptosis, Rats.

INTRODUCTION

Alzheimer's disease (AD) is a type of dementia that causes problems with memory, thinking and behavior. This disease accounts for 50 to 80 percent of dementia cases. Symptoms of AD usually develop slowly and get worse over time, becoming severe enough to interfere with daily tasks. Neither AD etiology nor the onset of AD pathology is totally understood. The greatest known risk factor for AD is the increasing age as the majority of people with Alzheimer's are 65 and older. Multiple factors are also reported to influence AD onset such as the mutations in the β amyloid precursor protein (APP) and presenilins 1 and 2 (PS1 and PS2) that lead to the increased production of 42-residue amyloid β (Aβ42). Additional risk factors such as increasing cholesterol and homocysteine levels as well as chronic exposure to several minor metal ions such as aluminium, copper and iron are also linked to AD (**Bharathi et al., 2008**).

There are three major pathological features, namely the extracellular deposition of the amyloid β protein (Aβ), the formation of intraneuronal neurofibrillary tangles (NFTs) and selective neuronal loss predominantly observed in AD neurodegeneration (**Bharathi et al., 2008**).

Apart from the pathological hallmarks of the disease, AD brain exhibits constant evidence of ROS mediated injury (**Buizza *et al.,* 2012**). In addition, cerebral inflammation and systemic immunological alterations have a role in AD pathogenesis (**Salminen *et al*., 2009**). The inflammatory changes include activation of microglia and astrocytes and infiltrating inflammatory cells with increased levels of proinflammatory cytokines (**Galasko and Montine, 2010 and Fadel et al.,2013**).

Alzheimer's has no current cure and the present Alzheimer's treatments can temporarily slow the worsening of dementia symptoms and improve quality of life for those with Alzheimer's

and their caregivers. Today, there is a worldwide effort under way to find better ways to treat the disease, delay its onset and prevent it from developing.

Proteolytic enzymes work to aid the body in digesting proteins. Proteolytic enzymes are produced naturally in the pancreas but may also be found in certain foods. Supplements containing these enzymes may be used to address a variety of health concerns (**Fadel et al., 2013**).

Serrapeptase (SP) is one of the world's most exciting enzymes being studied in regard to its wide variety of clinical applications.Serrapeptase is a 50 kDa metallprotease that has gained wide acceptance in Asia and Europe as a potent analgesic and anti-inflammatory drug. Serrapeptase is being used to treat chronic inflammatory disease such as atherosclerosis, arthritis, bronchitis, fibrocystic breast disease and sinusitis (**Klein and Kullich, 2000**). Recent studies have even suggested the use of oral SP to aid in the treatment or prevention of viral disease, such as AIDS and hepatitis B and C (**Ruchir and Singhal, 2011**). Serrapeptase is an immunologically active enzyme and it can bind itself to the alpha 2 macroglobulin in our plasma where it is shielded from the immune system while retaining its enzymatic activity, and in this way it is transferred to the sites where it is needed in the body (**Mazzone et al., 1990**).

Nattokinase (NK) , an alkaline serine protease extracted from the traditional Japanese food "natto" (fermented soybean) is now widely used as a health-promoting over- the –counter medicine for reducing the risk of thrombosis due to its fibrinolytic activity.The thrombolytic activity of nattokinase has been found to be stronger than that of plasmin or elastase both *in vitro* and *in vivo* (**Chang *et al*., 2008**). In some aspects, NK is actually superior to conventional clot-dissolving drugs, as it has many benefits such as convenient oral administration, more efficient, prolonged effects and prevention of clot formation. Nattokinase has been demonstrated to have pH and temperature stability and so can be found in the gastrointestinal tract (**Zheng, *et al*., 2006**).

The goal of the current study   was to elucidate the protective role of serrapeptase and nattokinase in the amelioration of neuroinflammation and apoptosis associated with Alzheimer's disease induced in experimental animal model.

MATERIALS AND METHODS

Materials

Two proteolytic enzymes, serrapeptase (Doctors BEST serrapeptase 40,000 serratio units per veggie cap.) and nattokinase (Doctors BEST nattokinase 2,000 FUs per veggie cap) were purchased from a dietary supplement market in USA. The selected doses of the two proteolytic enzymes used in the present study were equivalent to the human recommended doses and converted to rat equivalent with the help of Paget equation (**Paget and Barnes, 1964**). Aluminum chloride (AlCl$_3$) was purchased from Sigma Co. (California,USA). The molecular weight of AlCl$_3$ is 133.34.

Adult male albino rats were purchased from the Animal House Breeding Colony of the National Research Centre, Cairo, Egypt. The rats were housed in polypropylene cages in an environmentally controlled clean air room with a temperature of 24±1°C, with an alternating 12 h light- dark cycle, and a relative humidity of 60±5%. The animals were subjected to standard diet and water *ad libitum.* All animals were accommodated with laboratory conditions for at least two weeks before the experiment and maintained under the same conditions all over the experimental period. All animals received human care and use according to the guidelines for Animal Experiments which were approved by the Institutional Animal Ethics Committee, National Research Centre, Egypt.

Experimental Design

Sixty male albino rats weighing 120-140 g were randomly classified into six groups, with ten animals in each group; Group (1) :set as control group ,Group (2) : Alzheimer's disease induced group (AD group) in which the rats were orally administered with AlCl$_3$ (17 mg / kg b.wt.) for 45 day (5 days / week) to induce Alzheimer's disease (**Fadl et al., 2013**); Groups from (3) to(6) were orally administered with AlCl$_3$ (17 mg / kg b.wt.) for 45 day (5 days / week) and simultaneously supplemented with the tested enzymes as follow: Group(3) and Group (4) [AD+ Sp] in which the rats were orally administered with water suspension of serrapeptase with low and high doses 10.800 U/kg.b.wt. and 21.600 U/kg b.wt. respectively. Group(5) and Group (6) [AD + Nk] in which the rats were orally administered with water suspension of nattokinase with low and high doses 360 FU/kg b.wt. and 720 FU/kg b.wt. respectively.

At the end of the experimental period, the animals were fasted overnight, sacrificed by decapitation under diethyl ether anesthesia. The whole brain of each rat was rapidly and

carefully dissected, thoroughly washed with isotonic saline and dried on filter paper. Then, each brain was sagitally divided into two portions; the first portion was weighed and homogenized immediately to give 10% (w/v) homogenate in ice cold medium containing 50 mM Tris-HCL and 300 mM sucrose, PH:7.4 (**Tsakiris** *et al.,* **2004**). The homogenate was centrifuged at 1800xg for 10 min. in cooling centrifuge at 4°C. The supernatant (10%) was stored at -80° till further analysis to assess cholinesterase, TGF- β, IL-6, Bcl-2 and P53. The second portion of the brain was immersed in formalin buffer (10%) for later histological examination.

METHODS

Brain cholinesterase activity was determined kinetically using kit purchased from Biostc Co., Egypt, according to the method of *Young (2000)*. While, brain TGF-β level was assayed by enzyme linked immunosorbent assay (ELISA) technique using kit purchased from DRG instrument GmbH, Germany, according to the method described by *Kropf et al. (1997)*. Brain IL-6 level was detected by ELISA technique using kit purchased from RayBiotech, Inc., USA, according to the method of *Bauer and Herrmann (1991)*. Meanwhile, brain Bcl-2 level was detected by ELISA technique using kit purchased from Bender Med Systems Co., Vienna, Europe, according to the method described by *Barbareschi et al. (1996)*. Brain P53 level was determined by ELISA technique using kit purchased from Diaclone a Tepnel Co, France according to the manufacturer's instructions. Quantitative estimation of total protein content in the brain was carried out according to the method of **Lowry et al. (1951)** to express the concentration of the different brain parameters per milligram protein.

For histological investigation ,brain sample taken from each rat in the different groups was fixed in 10% formalin buffer for twenty four hours. Washing was done in tap water, then serial dilutions of alcohol (methyl, ethyl and absolute ethyl) were used for dehydration. The specimens were cleared in xylene and embedded in paraffin at 56 degree in hot air oven for twenty four hours. Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns by slidge microtome. The obtained tissue sections were collected on glass slides ,deparaffinized and stained with hematoxylin and eosin stains **(Banchroft et al .,1996)**.Then, the slides were examined under the light microscope.

Statistical Analysis

The results were expressed as mean ± SE. Data were analyzed by one-way analysis of variance and were performed using the Statistical Package for the Social Science (SPSS)

program, version 11 followed by least significant difference to compare the significance between groups (**Armitage and Berry, 1987**). Difference was considered significance at P< 0.05.

RESULTS

The data in Table (1) illustrated the protective effect of serrapeptase and nattokinase on brain cholinesterase activity in rat model of Alzheimer's disease. The AD induced group showed significant elevation (P<0.05) in brain cholinesterase activity when compared with the control group. In contrast, the groups of rats that administered with low or high dose of serrapeptase (10.800 U/kg b.wt. and 21.600 U/kg b.wt**)** or nattokinase (360 FU/kg b.wt. and 720 FU/kg b. wt) during the induction of AD displayed significant reduction (P<0.05) in brain cholinesterase activity when compared with the AD induced group. Noteworthy, the simultaneous administration of high dose of nattokinase with AlCl$_3$ caused significant increase (P<0.05) in brain cholinesterase activity as compared with the groups of rats administered with low and high dose of serrapeptase as well as low dose of nattokinase.

The current results revealed significant increase (P< 0.05) in brain TGF-β and IL-6 levels in AD induced group as compared to the control group (Table 2). On the other hand, the groups of rats that administered with the low or high dose of serrapeptase or nattokinase during the induction of AD exhibited significant decrease (P<0.05) in brain TGF-β and IL-6 levels when compared with the AD induced group. It was observed that, the groups of rats administered with low or high dose of serrapeptase during AD induction show significant decrease (P<0.05) in brain TGF-β level in comparison with the group of rats administered with high dose of nattokinase.

In the view of the obtained results, AD induced group showed significant decrease (P<0.05) in brain Bcl-2 level accompanied with significant increase (P<0.05) in brain P53 level as compared to the control group (Table 3). In the contrary, the groups of rats administered with low or high dose of serrapeptase or nattokinase during the induction of AD showed significant increase (P<0.05) in brain Bcl-2 level associated with significant decrease (P<0.05) in brain P53 level when compared with the AD induced group. The groups of rats administered with low or high dose of nattokinase during AD induction showed significant decrease (P<0.05) in brain Bcl-2 level in concomitant with significant increase (P<0.05) in brain P53 level when compared with the groups of rats administered with low or high dose of serrapeptase.

Table (1): The protective effect of serrapeptase and nattokinase on brain cholinesterase activity in rat model of Alzheimer's disease. (Data are represented as Mean ± S.E)

Parameters / Groups	Cholinestrase (U/mg protein)
Control group	573.1 ± 25.7
AD group	918.7± 19.8[a]
AD +Serrapeptase (10.800 U/Kg b. wt.)	608.6 ± 15.6[b]
AD+Serrapeptase (21.600 U/Kg b. wt.)	606.0 ± 13.7[b]
AD + Nattokinase (360 FU/Kg b. wt.)	609.7 ±15.1[b]
AD + Nattokinase (720 FU/Kg b. wt.)	754.7 ± 12.9[bcde]

a: Significant change at P< 0.05 in comparison with the control group.
b: Significant change at P< 0.05 in comparison with the AD induced group.
c: Significant change at P< 0.05 in comparison with AD induced group protected with low dose of serrapeptase.
d: Significant change at P< 0.05 in comparison with AD induced group protected with high dose of serrapeptase.
e: Significant change at P< 0.05 in comparison with AD induced group protected with low dose of nattokinase.

Table (2): The protective effect of serrapeptase and nattokinase on brain TGF-β and IL-6 levels in rat model of Alzheimer's diseases. (Data are represented as Mean ± S.E)

Parameters / Groups	TGF-β (pg/mg protein)	IL-6 (pg/mg protein)
Control group	680.3 ± 8.6	26.6 ± 1.4
AD group	1022.3 ± 6.8[a]	35.9 ± 0.3[a]
AD+Serrapeptase (10.800 U/Kg b. wt.)	803.8 ± 17.1[b]	30.4 ± 0.7[b]
AD+Serrapeptase (21.600 U/Kg b. wt.)	766.8 ± 18.7[b]	29.5 ± 1.4[b]
AD+Nattokinase (360 FU/Kg b. wt.)	809.8 ± 4.8[bd]	32.0 ± 1.2[b]
AD+Nattokinase (720 FU/Kg b. wt.)	814.1 ± 22.9[bd]	32.6 ± 1.7

a: Significant change at P< 0.05 in comparison with the control group.
b: Significant change at P< 0.05 in comparison with the AD induced group.
d: Significant change at P< 0.05 in comparison with AD induced group protected with high dose of serrapeptase.

Table (3): The protective effect of serrapeptase and nattokinase on brain Bcl-2 and P53 levels in rat model of Alzheimer's diseases. (Data are represented as Mean ± S.E)

Parameters Groups	Bcl-2 (ng/mg protein)	P53 (U/mg protein)
Control group	0.113 ± 0.008	0.23 ± 0.01
AD group	0.051 ± 0.003^a	0.61 ± 0.01^a
AD+Serrapeptase (10.800 U/Kg b. wt.)	0.084 ± 0.006^b	0.37 ± 0.007^b
AD+Serrapeptase (21.600 U/Kg b. wt.)	0.089 ± 0.0005^b	0.35 ± 0.006^b
AD+Nattokinase (360 FU/Kg b. wt.)	0.070 ± 0.005^{bcd}	0.44 ± 0.02^{bcd}
AD+Nattokinase (720 FU/Kg b. wt.)	0.068 ± 0.002^{bcd}	0.47 ± 0.02^{bcd}

a: Significant change at P< 0.05 in comparison with the control group.

b: Significant change at P< 0.05 in comparison with the AD induced group.

c: Significant change at P< 0.05 in comparison with AD induced group protected with low dose of serrapeptase.

d: Significant change at P< 0.05 in comparison with AD induced group protected with high dose of serrapeptase.

Histopathological Results
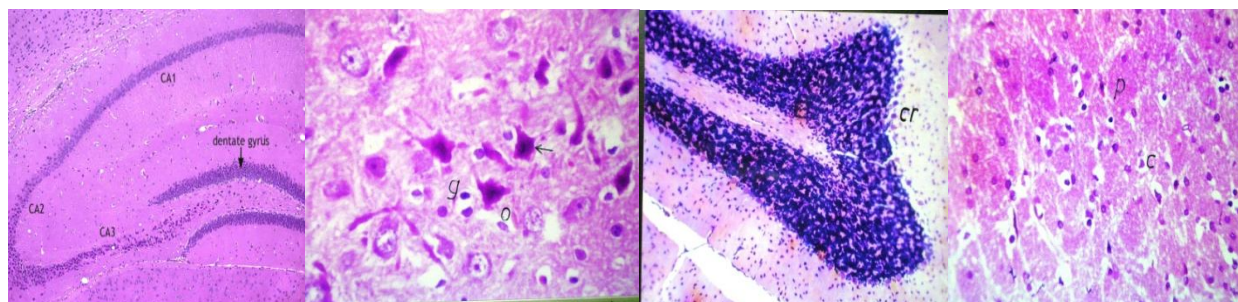

Fig (1)   Fig (2)   Fig (3)   Fig (4)

Fig (1) Microscopic examination of brain tissue sections of control rats showing the normal hippocampal architecture (H& E X40).

Fig (2): Microscopic examination of brain tissue sections of control rats showing the normal histological structure of the cerebellum (cr).(H&E X 40).

Fig (3) Microscopic examination of brain tissue section of AD rat model showed cerebral encephalomelacia (c) with plaques formation (p) (H& E X 40).

Fig (4) Microscopic examination of brain tissue section of AD rat model showed the neuronal degeneration (arrow) and oedema (o) with gliosis (g) (H& E X 400).
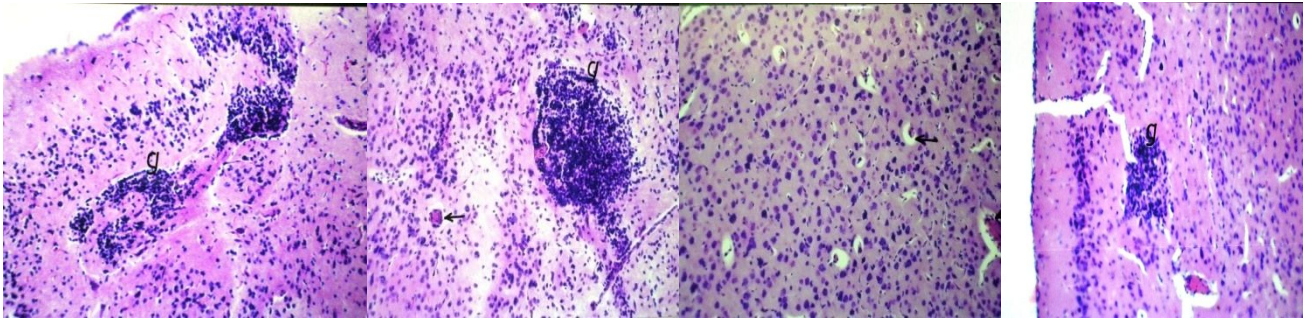

Fig (5)   Fig (6)   Fig (7)   Fig (8)

Fig (5)Microscopic examination of brain tissue sections of rat model of AD protected with low dose of serrapaptase showing focal gliosis in cerebral cortex (g). H&E (40).

Fig (6)) Microscopic examination of brain tissue sections of rat model of AD protected with low dose of serrapaptase showing focal gliosis (g) and congestion in cerebral blood capillaries of the cerebrum. H&E (40)

Fig (7) Microscopic examination of brain tissue sections of rat model AD protected with low dose of nattokinase showing capillary congestion with perivascular oedema in the cerebrum. H&E(40)

Fig (8) Microscopic examination of brain tissue sections of rat model of AD protected with low dose of nattokinase showing focal gliosis near to central cortex (g) .H&E (40)
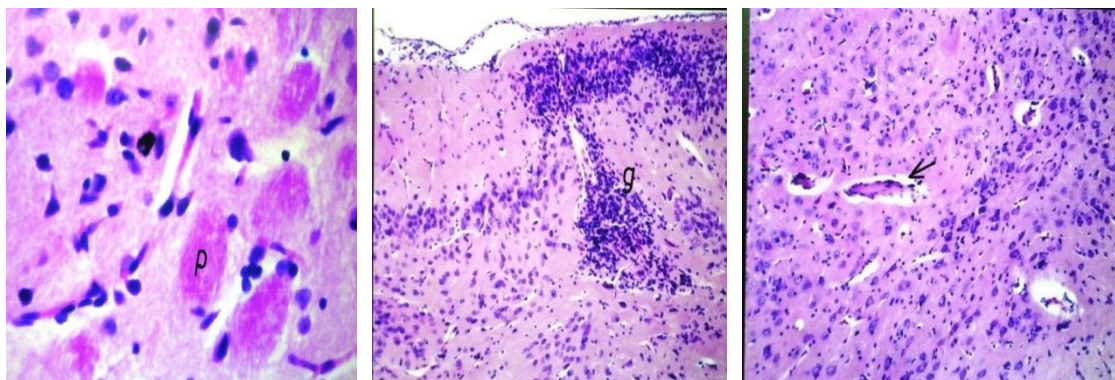

Fig (9)   Fig (10)   Fig (11)

Fig (9): Microscopic examination of brain tissue sections of rat model of AD protected with high dose of serrapaptase showing plaque formation (P) in the striatum. H&E (40)

Fig (10): Microscopic examination of brain tissue sections of rat model of AD protected with high dose of nattokinase showing focal gliosis in cebral cortex (g). H&E (40)

Fig (11): Microscopic examination of brain tissue sections of rat model of AD protected with high dose of nattokinase showing capillary congestion with perivascular oedema in the striatum. H&E (40)

DISCUSSION

For a quarter of a century, the pathogenesis of Alzheimer's disease has been linked to the deficiency in the brain neurotransmitter acetylcholine (**Tabet, 2006**). Acetylcholine is a neurotransmitter (a brain chemical) that helps with memory and thinking. Alzheimer's disease breaks down acetylcholine. And people who have Alzheimer's disease make less of this chemical over time. These two things result in the gradual loss of memory and thinking skills (**Tabet et al., 2006**).

The present study revealed that, $AlCl_3$ administration shows significant elevation in brain cholinesterase (AChE) activity. Acetylcholiesterase (AChE) itself has been implicated in the pathogenesis of Alzheimer's disease. In particular, it appears that AChE may directly interact with amyloid-beta in manner that increases the deposition of this peptide into insoluble plaques. This finding is in agreement with that of **Zhang et al. (2009) and Fadl et al. (2013)**. Aluminium is known to increase the brain Aβ burden in experimental animals and this might be due to direct influence upon Aβ anabolism or indirect effects upon Aβ catabolism (**Clauberg and Joshi, 1993**). Aβ has been found to induce elevation in AChE activity through the production of hydrogen peroxide (H_2O_2) as a consequent induction of lipid peroxidation in the neuronal membranes (**Melo et al., 2003**). Hydrogen peroxide acts as a modulator in the activity of functionally important proteins, receptors and enzymes (**Kamster and Segal, 2004**).

There is increasing evidence that neurotoxicity in AD is mediated by inflammatory processes (**McGeer et al., 2006; Yu and Chung, 2007**) and one contributing factor inherent to AD is the presence of glial cells that are activated by Aβ to produce pro-inflammatory cytokines like IL-1 _, IL-6, TNF-α , TGF-β, interferon $α$, and IL-2 (**Mraka and Griffinb, 2005**). The obtained results revealed significant increase in brain TGF-β of rats administered with $AlCl_3$ as compared to control one. TGF-β is present in senile plaques and is overexpressed in AD brain compared with controls. The study of **Luedecking (2000)** demonstrated that TGF- β1, upregulated in Alzheimer patients, drives astrocytic overexpression of the mRNA encoding

for the amyloid precursor protein. The data obtained from the study of **Van der Wal (1993)** on transgenic mice support the involvement of TGF-β1 in AD pathology—for example, overproduction of TGF-β1 in transgenic mice induces an AD like cerebrovascular degeneration and the immunoreactive astrocytes for TGF- β are present in early Aβ deposits.

The present results revealed a significant elevation in brain IL-6 in AlCl$_3$ administered group which is in agreement with **Weaver et al. (2002), Combarros et al. (2009) and Cojocaru et al. (2011)**. Raised levels of IL-6 have been associated with various conditions considered to be risk factors for dementia and/or AD. IL-6 was found not only in plaques, but also around the bodies of isocortical neurons, only in AD. A growing body of evidence suggested that the dysregulation of IL-6 contributes to the development of AD (**Combarros et al., 2009).** Furthermore, **Cojocaru et al. (2011**) found a significant elevation of IL-6 secretion levels in both mild and moderately severe AD patients. There is a growing body of evidence which supports the hypothesis of immune regulation and autoimmunity or inflammatory processes as vital mechanisms in the pathogenesis of the disease. The immunological disturbances seem to be a common feature of all patients with Alzheimer's disease in the form of local, brain tissue inflammatory reactions and autoaggressive processes directed against the brain cells **(Cojocaru et al., 2011).**

Other main cause of neurodegeneration in Alzheimer's disease is the increased level of oxidative stress. β-amyloid causes neurotoxicity through production of hydrogen peroxide. Aβ reportedly causes apoptotic neuronal death accompanied with DNA fragmentation. DNA-end labeling technique suggests that some neurons die by apoptosis. Numerous apoptosis regulating factors have been identified; these are represented in Bcl-2 family, apoptotic protease activating factors, caspase family, P53 and nuclear factor- kB (**Kitamura et al., 1999**).

The present results showed a significant decrease in brain Bcl-2 and an increase in brain P-53 levels in AlCl$_3$ administered group as compared to the control one. **Bharathi et al. (2008)** concluded that Al causes neurotoxicity in multifaceted way by modulating (i) Inhibition of DNA repair enzymes, (ii) Enhancement of ROS production, (iii) Decreasing the activity of antioxidant enzymes, and (iv) Alterations in NF-kB, p53 and JNK pathways. Al also binds to Zn finger domains of transcription factors, thereby decreasing RNA polymerase activity and upregulating micro-RNA. All these events lead to genomic instability and cell death. Our results could be explained as P53 has a critical regulatory function at the G1-cell cycle

checkpoint and in apoptosis caused by exposure to DNA-damaging substances. It has been reported that P53 protein induces the upregulation of pro-apoptotic Bax and the downregulation of anti-apoptotic Bcl-2 in the hippocampal neurons (**Kitamura et al., 1999**). Degradation of Aβ is a major target in the treatment of AD (**Barron, 2009**). Regarding the protective effect of serrapeptase and nattokinase on AD-like pathology, due to $AlCl_3$ administration, our hypothesis suggests that serrapeptase, as a proteolytic enzyme, can break down a number of different proteins within the body including Aβ (**Fadl et al., 2013**), and also nattokinase enzyme may help to prevent AD through its ability to dissolve amyloid fibrils, which build up as plaque in the brain and lead to brain cell damage. (**Barron,2009**).

In the view of the obtained results, the significant inhibition of brain AchE activity due to co-treatment with serrapeptase or nattokinase during $AlCl_3$ supplementation for 45 days, could be explained by the antiamyloidogenic effect of these two enzymes (**Fadl et al., 2013**). This property enables serrapeptase and nattokinase to counteract Aβ deposition with consequent inhibition to AChE activity. Moreover, serrapeptase and nattokinase have been reported to have antioxidant potential (**Fadl et al., 2013**) and free radical scavenging activity (**Davies, 1986**), which aid the enzymes to reduce ROS accumulation in the brain including H_2O_2 which plays a crtical role in activating AChE. By these ways, both serrapeptase and nattokinase could ameliorate AChE activity in the brain.

Co-treatment with either serrapeptase or nattokinase during AlCl3 administration for 45 days resulted in significant depletion in brain TGF-β and IL-6 levels. The proteolytic activity of serrapeptase and nattokinase is well established and their capability to dissolve Aβ in the brain has been previously reported (**Fadl et al., 2013**). The dissoluation of Aβ by serrapeptase and nattokinase leads to the the prevention of pro-inflammatory cytokines production by glial cells. As the stimulation of glial cells to secrete pro-inflammatory cytokines is promoted by the presence of Aβ (**Mraka and Griffinb, 2005**). Thus, these enzymes represent good anti-inflammatory agents that capable to counteract inflammation through the cessation of the production of the pro-inflammatory cytokines such as TGF-β and IL-6 in the brain.

Co-treatment with serrapeptase or nattokinase plus $AlCl_3$ for 45days in the present study resulted in significant increase Bcl-2 associated with significant decrease in P53 in the brain tissue. This effect could be attributed to ability of serrapeptase and nattokinase to digest Aβ accumulated in the brain tissue according to **Fadl et al. (2013)** demonstration. This property

leads to amelioration of neuronal death and restoration of brain Bcl-2 level. Additionally, the antioxidant activity of both serrapeptase and nattokinase (**Davies, 1986**) plays an important role in correcting brain levels of Bcl-2 and P53. As these enzymes have the ability to prevent ROS accumulation in the brain through their free radical scavenging effects, which have a major contribution in the neuronal death. Thus, serrapeptase and nattokinase could modulate neuronal apotosis *via* restoration of the mitochondrial membrane permeability and in turn mitochondrial function that finally lead to the preservation of Bcl-2 and suppression of P53 brain levels.

The biochemical findings in the present study were well documented by our histological results which showed the formation of cerebral encephalomelacia with plaques formation, neuronal degeneration and oedema with gliosis in $AlCl_3$ administered group. This indicted that this is a good model for AD-like pathology. These findings are merging with the previous studies (**Kawahara et al., 2001, and Zhang et al., 2003**) which provide a direct evidence to support viewpoint that Al may be a potential contributing factor in the formation of neurofibrillary tangles and cognitive deficits in Alzheimer's disease.

Regarding the peotective effect of serrapeptase and nattokinase, it is clear from the pathological investigation the disappearance of most of the amyloid plaques. This effect could be attributed to the effect of serrapeptase and nattokinase as antiamyloidogenic agents *via* promoting the activity of α-secretase-like action (**Fadl et al., 2013**).

In conclusion, the present study provides a strong evidence that serrapeptase and nattokinase have potent protective effect against AD in AD like pathology model. The neuroprotective effect of serrapeptase and nattokinase might be attributed to their property as proteolytic enzymes capable to degrade Aβ as well as their anti-inflammatory and anti-apoptotic potentials. Serrapeptase and nattokinase could be considered as excellent addition to Alzheimer's disease prevention programs.

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