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Study on Social Isolation as a Risk Factor in Development of Alzheimer's Disease in Rats

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Abstract

Background: Alzheimer's disease (AD) is a neurodegenerative disease that leads to memory loss. It is characterized by deposition of Beta-amyloid peptides (Aβ), accumulation of neurofibrillary tangles and cell loss. Social isolation may exacerbate memory deficits. The risk of cognitive decline and the onset of AD may be lower by maintaining social connections and keeping mentally active. The relationship between frequent social activity and enhancing cognitive functions has been established.

Objective: Study the influence of complete social isolation for a long period on biochemical and histopathological changes as well as DNA fragmentation in the brain of normal rats. In addition, investigate the possible interaction between social isolation and development of AD using isolation-associated AD rat model.

Methods: Four groups of rats were used; 2 groups socialized and 2 isolated for four weeks. One of each socialized and isolated groups were served as control and the other served as AD groups and injected by $ALCl_3$ (70 mg/kg, IP) every day during four weeks of isolation or socialization. Isolated rats were housed individually in cages covered with black plastic while socialized rats were randomly paired and housed in transparent covered cages. Biochemical changes in the brain as acetyl cholinesterase (ACHE), Aβ, brain derived neurotrophic factor (BDNF), monoamins (Dopamine, Serotonin, Norepinephrine), inflammatory mediators (TNF-α, IL-1β), oxidative parameters (MDA, SOD, TAC) and DNA fragmentation were estimated for all groups. Histopathological changes in the brain were also evaluated.

Results: Complete social isolation for a long period resulted in brain neurological damage indicated by significant increase in Aβ, ACHE, MDA, TNF-α, IL-1β as well as decreases in SOD, TAC, BDNF, and monoamines and confirmed by histopathological changes in different brain regions. Brain neurological damage was more severe in isolation-associated AD than in socialized condition. Isolation also enhanced the DNA fragmentation induced by AD.

Conclusion: Complete social isolation for a long period induces brain neuronal degenerations. It represents a risk factor especially when associated with AD; it increases DNA fragmentation and enhances the severity of AD development. Thus, socialization is advised especially with AD to avoid worsen or deterioration of the disease.

Keywords: Alzheimer's disease; Social isolation; Neuronal degeneration; Socialization; Rats

Introduction

Alzheimer's disease (AD) represents the most important problem in aged population. It is the most common form of dementia and causes progressive loss of cognitive function together with behavioral dysfunction [1,2]. There is no effective treatment for preventing the neuronal death or memory impairment and cognitive decline characterizing this progressive neurodegenerative disease [3,4]. Indeed, the risk of AD development can be lowered by keeping mental activity and maintain strong social connections during aging. However, the underlying mechanisms of the relationship between frequent social activity and better cognitive function are still unclear [4-6].

Severe and/or chronic stress has negative impact on the brain structure as well as on learning and memory process [3,7,8]. Both AD and mental stress can impair cognitive function in animals and humans [9,10]. Mental stress can elevate excitatory amino acid and glucocorticoid (GC) levels, while there are sever age-associated loss of hippocampal neurons and reduction in the number of corticosteroid receptors in AD [10]. Progressive and sustained GC release can cause hippocampal atrophy, excitotoxicity and neurotoxicity [8,10]. Consequently, exposure to stress forms an additional deleterious effect on the brain of AD patients and can exacerbate AD-induced impairment of learning and memory. It is worthy to note that the concurrent incidence of AD and stress is increased with advancing age [11,12].

Social interaction is central to human well-being and can improve both mental and physical health; it can reduce risk of cognitive impairment and development of dementia [13-15]. Social isolation (SI) which means the absence or insufficient contact with others is harmful to both physical and mental development [16,17]. especially for elderly [18,19]. It represents the major source of mental or psychosocial stress and is associated with the increased prevalence of neurological diseases [20]. It also exacerbates the risk of morbidity and mortality as well as the onset of many neuropsychological disorders [20-23]. Moreover, it is considered as risk factor for age-related cognitive deterioration and dementia [24]. The influences of SI on the development of AD may be through the production of Aβ peptide and phosphorylation of tau [17,25]. Furthermore, SI increases oxidative stress and inflammatory reaction [26] while inhibits antiinflammatory responses [27], synaptic plasticity [28] and myelination [29]; all of these mentioned mechanisms

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are involved in the pathogenesis of AD. Additionally, AD patients are more likely to suffer from SI due to cognitive and emotional impairment especially at late stages where the loss of communication ability and the potential neglect by society [30,31]. Although SI may contribute to the onset of AD, better understanding of the internal interaction between them as well as the added effect of SI on the disease development and progression remains unclear [17]. Consequently; in order to establish an effective therapies or interventions to delay the progression of AD, it is necessary to determine whether SI exacerbates pathology of AD development and progression.

In the light of what was mentioned, the present work was designed to study the impact of SI for a long period on rat brain as regarding changes in biochemical, histopathological and DNA fragmentation, in addition to the possible interaction between social isolation and development of AD using isolation-associated AD rat model.

Materials and Methods

Animals

Forty male Sprague Dawley rats, weighing 250-280 g and obtained from The Nile Co. for Pharmaceuticals and Chemical Industries, Cairo, Egypt were used. Animals were housed in stainless-steel cages, at a temperature of 25 ± 1°C. Isolated rats were housed individually in cages covered with black plastic for four weeks, while socialized rats were randomly paired and housed in transparent covered cages. Animals were kept under adequate environmental conditions. They were kept on standard diet pellets and water was given ad-libitum. The work was conducted in accordance with the ethical guidelines of Faculty of Pharmacy, Al-Azhar University, Egypt.

Drugs and chemicals

Aluminum chloride - hydrated ($ALCl_3.6H_2O$), was purchased from Sigma Chemical Co. (St. Louis, MO, USA). It was freshly dissolved in distilled water. All other chemicals and solvents were of the highest grade-commercially available.

Experimental design

Forty rats were equally divided into 4 groups (10 rats/each), rats were classified and IP injected every day during four weeks as follows:

Control socialized group

Rats received normal saline (1 ml/kg), randomly paired and housed in transparent covered cages.

AD socialized group

Rats received $ALCl_3$ (70 mg/kg), randomly paired and housed in transparent covered cages.

Control isolated group

Rats received normal saline (1 ml/kg), housed individually in cages covered with black plastic.

AD isolated group

Rats received $ALCl_3$ (70 mg/kg), housed individually in cages covered with black plastic. At the end of the four weeks; rats were sacrificed, the brain tissues were dissected and washed with ice-cold saline. For all groups, brain tissues were either subjected for analysis immediately or kept frozen till the time of analysis at -80°C. They were homogenized in saline, the homogenates were used to assess β-amyloid (Aβ) content, acetylcholine esterase (ACHE) activity and brain derived neurotrophic factor (BDNF). Oxidative stress markers {malondialdehyde (MDA), superoxide dismutase (SOD), total antioxidant capacity (TAC)}, inflammatory mediators {tumer necrosis factor-alpha (TNF-α), Interleukin 1β (IL-1β)}, brain monoamins {Dopamine (DA), Norepinephrine (NE), Serotonin (5-HT)} as well as DNA fragmentation were also estimated for all groups. In additions, specimens from the brain tissue from different groups were taken for histopathological examination.

Biochemical parameters

Determination of Aβ content: It was assessed in brain tissue homogenate by using ELISA kit supplied by (MyBioSource, Inc., SanDiego, USA, Product Number MBS702915), according to the manufacturer's instructions.

Determination of ACHE activity: It was carried out in brain tissue homogenate using commercially available test kit supplied by Sigma-Aldrich Co. (St. Louis, MO, USA), Product Number MAK119, according to the method of [32].

Determination of BDNF: It was assessed in brain tissue homogenate by using ELISA Kit supplied by (MyBioSource, Inc., SanDiego, USA, Product Number MBS494147), according to the method of [33].

Assessment of oxidative stress markers (MDA, SOD, TAC): Lipid peroxidation was determined in brain tissue homogenate by estimating the level of thiobarbituric acid reactive substances (TBARS) measured as MDA [34]. SOD activity was achieved relying on the ability of the enzyme to inhibit the phenazine methosulphate mediated reduction of nitroblue tetrazolium dye [35]. The increase in absorbance at 560 nm for 5 min is measured. Finally, determination of TAC was assessed by the reaction of antioxidants with a defined amount of exogenously provide H_2O_2. The residual H_2O_2 was determined colourimetrically by an enzymatic reaction which involves the conversion of 3, 5-dichloro-2-hydroxybenzene sulphonate to a colored product [36].

Brain inflammatory mediators (IL-1β, TNF-α): Determination of TNF-α was done in brain tissue homogenate by using ELISA Kit, Product Number (RTA00, SRTA00, PRTA00) and according to the method of [37], determination of IL-1β was performed in brain tissue homogenate by using ELISA Kit supplied by RayBiotech, Inc., USA, Product Number (ELR-IL1b) according to the manufacturer's instructions.

Assessment of neurochemical parameters (DA, NE, 5-HT): Rats were sacrificed rapidly by decapitation with minimum disturbance to avoid any changes in the concentrations of brain monoamines that may occur within few minutes [38]. Fluorometric assay of serotonins, norepinephrine and dopamine were determined in rat's brain according to the method of [39].

DNA fragmentation

Apoptosis and DNA fragmentation was detected in brain tissue sections using the kit supplied by Qiagen (Hilden, Germany). To detect DNA fragmentation, 10 µg of each DNA was electrophoretically fractionated on 1.5% agarose gel, stained with 0.5 µg/mL ethidium bromide solution and destained with deionized water. Finally, the DNA in the gel was visualized and photographed under UV light [40].

Histopathological examination of the brain

For histopathological examinations, brain specimens were prepared and stained for light microscopy [41]. They were fixed in 10%

formalin for 24 h and then washed with tap water. Serial dilutions of alcohol (methyl, ethyl and absolute ethyl) were used for dehydration. Specimens were cleared in xylene embedded in paraffin at 56°C in hot air oven for 24 h. Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns thickness by microtome. The obtained tissue sections were collected on glass slides and deparaffinized. All sections were stained with Hematoxylin & Eosin stain for the routine histological examination.

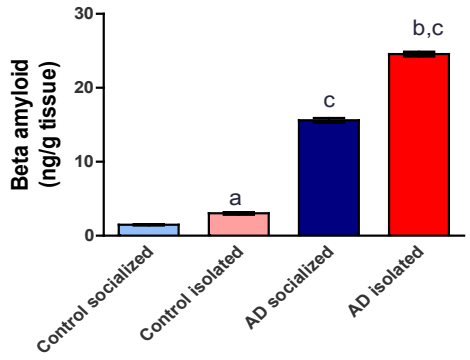
Statistical analysis

Data are expressed as mean ± SEM and multiple comparisons were performed using one-way ANOVA followed by Tukey Kramer as a post hoc test. All statistical analysis and graphs were performed using GraphPad Prism (ISI®, USA) software (version 5).

Results

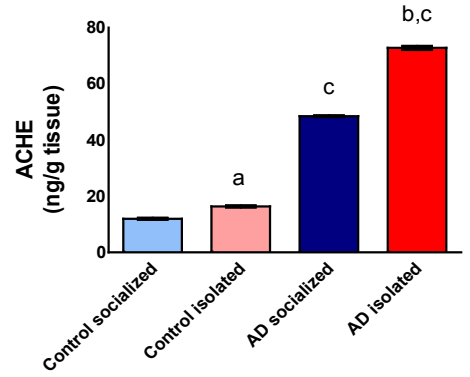
Brain β-amyloid (Aβ) content

As illustrated in (Figure 1), social isolation for a long period resulted in brain neurological damage indicated by significant elevation in the brain Aβ content to 204.52% as compared to corresponding control socialized group. Also, AD isolated group showed a significant elevation in the brain Aβ content to 157.5% as compared to corresponding AD socialized group. Additionally, AD socialized and isolated groups showed significant elevation in brain Aβ content to 1051.9% and 810.09% with respect to their corresponding control groups respectively.



Data expressed as Mean ± SEM (n=10).
Significant difference at p<0.05 between:
a: Control isolated and socialized.
b: AD isolated and socialized.
c: AD and the corresponding control either socialized or isolated.

Figure 1: Effect of social isolation on brain β-amyloid (Aβ) content in normal and Alzheimer's disease rat model.



Data expressed as Mean ± SEM (n=10).
Significant difference at p<0.05 between:
a: Control isolated and socialized.
b: AD isolated and socialized.
c: AD and the corresponding control either socialized or isolated.

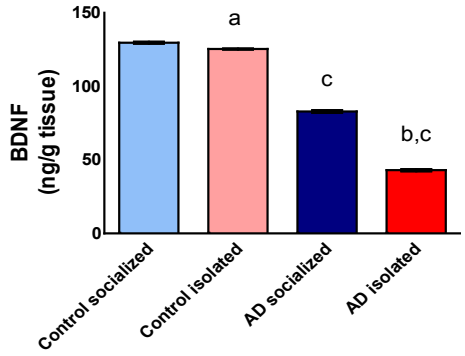
Figure 2: Effect of social isolation on brain acetylcholine esterase (ACHE) activity in normal and Alzheimer's disease rat model.

Brain Acetylcholine Esterase (ACHE) activity

As shown in Figure 2, social isolation for a long period induced brain neurological degeneration as indicated by significant elevation in the brain ACHE activity to 136.82% as compared to corresponding control socialized group. Also, AD isolated group showed a significant elevation in the brain ACHE activity to 150.1% as compared to corresponding AD socialized group. Additionally, AD socialized and isolated groups showed significant elevation in brain ACHE activity to 405.9% and 445.26% with respect to their corresponding control groups respectively.

Brain Derived Neurotrophic Factor (BDNF)

As illustrated in (Figure 3), social isolation for a long period resulted in brain neurological damage indicated by a significant reduction in the brain BDNF to 96.7% as compared to corresponding control socialized group. Also, AD isolated group showed a significant reduction in the brain BDNF to 51.9% as compared to corresponding AD socialized group. Additionally, AD socialized and isolated groups showed significant reduction in brain BDNF to 63.9% and 34.3% with respect to their corresponding control groups respectively.



Data expressed as Mean ± SEM (n = 10).
Significant difference at p<0.05 between:
a: Control isolated and socialized.
b: AD isolated and socialized.
c: AD and the corresponding control either socialized or isolated.

Figure 3: Effect of social isolation on brain derived neurotrophic factor (BDNF) in normal and Alzheimer's disease rat model.

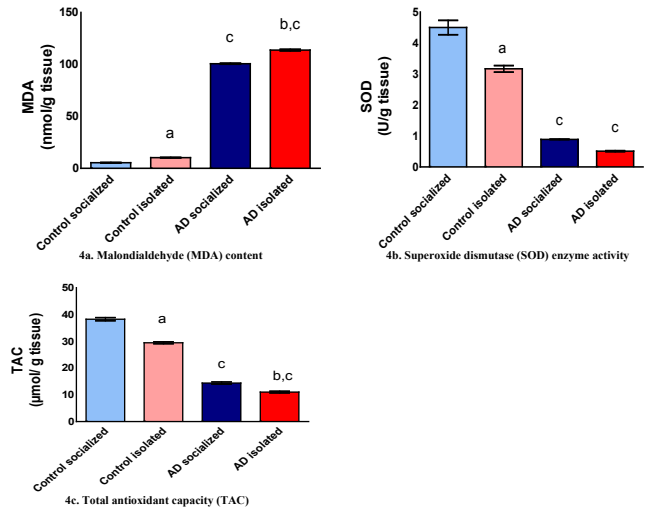
Brain Oxidative Stress Markers (MDA, SOD, TAC)

As shown in Figures 4a, 4b and 4c, social isolation for a long period resulted in brain neurological degeneration as indicated by a significant elevation in brain MDA content to 188.4% as compared to the corresponding control socialized group. Also, AD isolated group showed a significant elevation in the brain MDA content to 113.0% as compared to corresponding AD socialized group. Additionally, AD socialized and isolated groups showed also significant elevation in brain MDA content to 1842% and 1104.9% with respect to their corresponding control groups respectively. Moreover, social isolation induced significant

reduction in brain SOD and TAC to 70.4% and 77.03% respectively as compared to the corresponding control socialized group. Also, AD isolated group showed a significant reduction in brain TAC to 76.6% as compared to the corresponding AD socialized group. Additionally, AD socialized and isolated groups showed significant reduction in brain SOD activity to 19.8% and 16.1% as well as in brain TAC to 37.6% and 37.4% respectively with respect to their corresponding control groups.

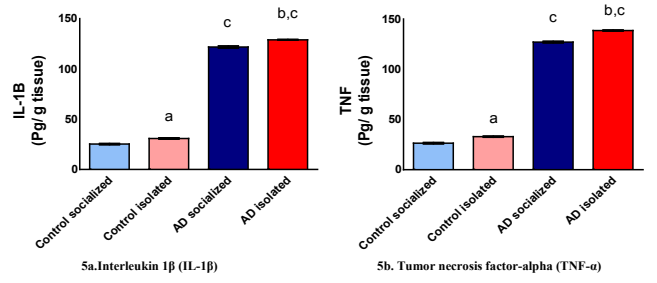
Brain Inflammatory Mediators (IL-1β, TNF-α)

As illustrated in Figures 5a and 5b, social isolation for a long period induced brain neurological degeneration indicated by significant elevation in brain IL-1β and TNF-α to 122.2% and 124.21% respectively as compared to corresponding control socialized group. Also, AD isolated group showed significant elevation in brain IL-1β and TNF-α to 105.9% and 109.04% respectively as compared to corresponding AD socialized group. Additionally, AD socialized and isolated groups showed significant elevation in brain IL-1β to 482.4% and 418.2% as well as in TNF-α to 480.2% and 421.5% respectively with respect to their corresponding control groups.



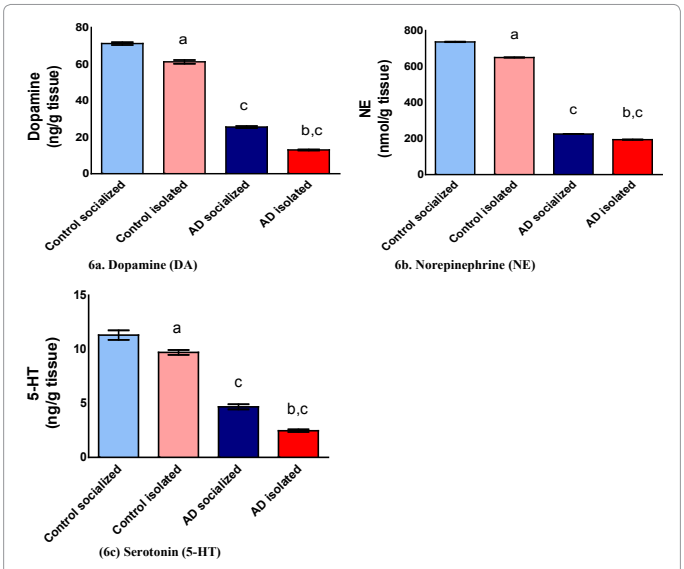
Data expressed as Mean ± SEM (n=10).
Significant difference at p<0.05 between:
a: Control isolated and socialized.
b: AD isolated and socialized.
c: AD and the corresponding control either socialized or isolated.

Figure 4: (a, b, c) Effect of social isolation on brain oxidative stress markers (MDA, SOD, TAC) in normal and Alzheimer's disease rat model.



Data expressed as Mean ± SEM. (n=10)
Significant difference at p<0.05 between:
a: Control isolated and socialized.
b: AD isolated and socialized.
c: AD and the corresponding control either socialized or isolated.

Figure 5: (a, b): Effect of social isolation on brain inflammatory mediators (IL-1β, TNF-α) in normal and Alzheimer's disease rat model.



Data expressed as Mean ± SEM (n=10).
Significant difference at p < 0.05 between:
a: Control isolated and socialized.
b: AD isolated and socialized.
c: AD and the corresponding control either socialized or isolated.

Figure 6: (a, b, c) Effect of social isolation on brain monoamines (DA, NE, 5-HT) in normal and Alzheimer's disease rat model.

Brain neurochemical parameters

As shown in Figures 6a, 6b and 6c, social isolation for a long period resulted in brain neurological changes as indicated by the significant reduction in brain DA, NE and 5-HT to 85.9%, 88.23% and 85.84% respectively as compared to their corresponding control socialized group. Also, AD isolated group showed significant reduction in brain DA, NE and 5-HT to 50.9%, 86.33% and 52.8% respectively as compared to corresponding AD socialized group. Additionally, AD socialized and isolated groups showed significant reduction in brain DA to 35.9%, 21.24% and in NE to 30.6%, 29.9% as well as in 5-HT to 41.4%, 25.4% respectively with respect to their corresponding control groups.

DNA fragmentation

By agarose gel electrophoresis, DNA isolated from control socialized brain tissues did not show any DNA fragmentation (Figure 7, Lane c). However, control isolated as well as AD either socialized or isolated groups (Figure 7, Lanes 1-3) showed characteristic DNA fragmentation or laddering as which was found in the model (M) laddering shape.

Histopathological alterations in the brain

Histopathological alterations in the brain specimens from different treated groups are shown in Figures 8A-8D and Table 1. Brain specimens from control socialized rats showed normal histological structure of hippocampus. On the other hand, brain specimens of control isolated and AD socialized groups showed focal nuclear pyknosis as well as degeneration in the neuronal cells of cerebral cortex associated with atrophy in some neurons of the substantia nigra but no histopathological alteration in the hippocampus as well as in the striatum. However, brain specimens of AD isolated group showed marked pathological changes indicated by nuclear necrosis and degeneration in cerebral cortex associated with focal gliosis. It is worthy to note that, hippocampus as well as neurons of the fascia dentate, striatum and substantia nigra in isolation-associated AD rat model showed nuclear pyknosis and

degeneration with congestion in the blood vessels. Consequently, it is clear that the severity of brain neurological damage induced by social isolation was more pronounced in AD rats.

Discussion

The impact of Aluminum (AL) on neural tissues is well known [42] It has been implicated in the etiology of AD; excessive AL intake leads to accumulation of Aβ in the brain and over expression of β-amyloid precursor protein (APP) [43]. The neurotoxicity of Aβ is strongly related to oxidative stress which plays an effective role in the pathogenesis of AD [44]. The generation of reactive oxygen species (ROS) causes damage of neuronal membrane as well as lipids, proteins and nucleic acids [45]. Chronic stress has been proposed as a risk factor in AD progression [46].

Results of the present study showed that SI for a long period resulted in brain neurological damage indicated by significant elevation in the brain Aβ content as compared to control socialized group. These findings are in agreement with [46] but the exact mechanism of how

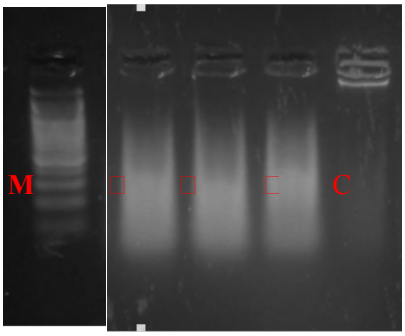


Figure 7: Effect of social isolation on DNA fragmentation (DNA ladder) in normal and Alzheimer's disease rat model. (Lane M: DNA Marker with 100 bp; Lane 1: Control isolated; Lane 2: AD socialized; Lane 3: AD isolated; Lane C: Control socialized).

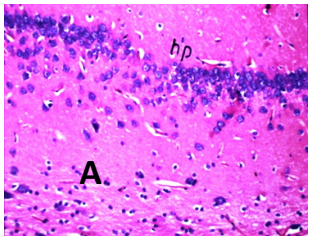


Figure 8A: Representative photomicrograph (magnification 40 X) of brain section stained by Hematoxylin–Eosin: Section taken from brain of control socialized group showed normal histological structure of the hippocampus (hp).

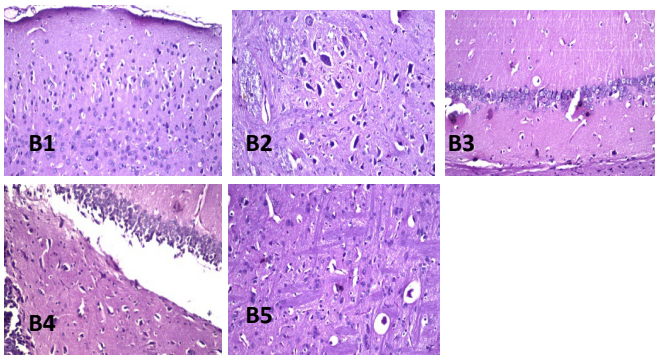


Figure 8B: (B1-B5) Representative photomicrographs (magnification 40 X) of brain sections stained by Hematoxylin–Eosin: Sections taken from brain of control isolated group showed focal nuclear pyknosis and degeneration in the neuronal cells of cerebral cortex (B1), atrophy in some neurons of the substantia nigra (B2) but no histopathological alteration in the hippocampus (B3, B4) as well as in the striatum (B5).

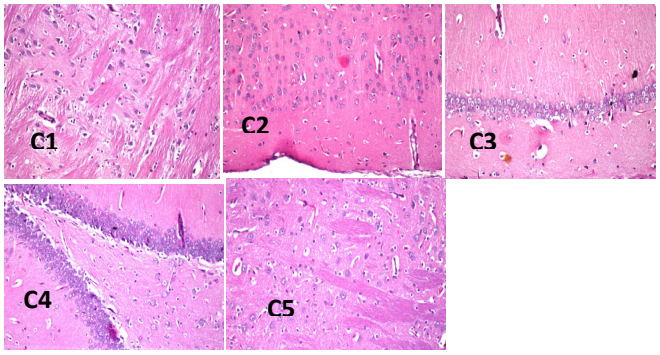


Figure 8C: (C1-C5) Representative photomicrographs (magnification 40 X) of brain sections stained by Hematoxylin–Eosin: Section taken from brain of AD socialized group showed normal histological structure in the striatum (C1) while showed nuclear pyknosis and degeneration in the neurons of cerebral cortex (C2) and hippocampus (C3, C4). Atrophy was observed in some neurons of the substantia nigra (C5).

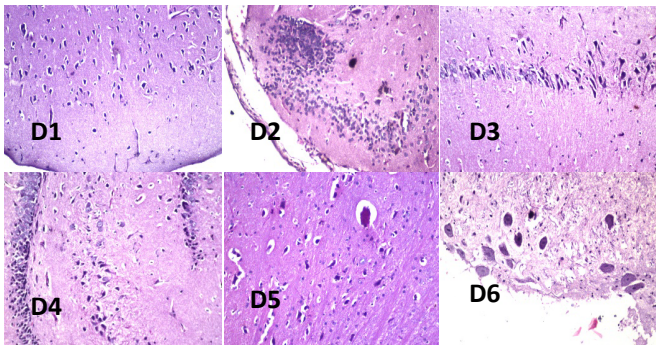


Figure 8D: (D1-D6) Representative photomicrographs (magnification 40 X) of brain sections stained by Hematoxylin–Eosin: Section taken from brain of AD isolated group showed nuclear necrosis and degeneration in cerebral cortex (D1) associated with focal gliosis (D2). Hippocampus as well as neurons of the fascia dentate, striatum and substantia nigra showed nuclear pyknosis and degeneration with congestion in the blood vessels (D3, D4, D5, D6).

Histopathological alterations	Control socialized	Control isolated	AD socialized	AD isolated
Degeneration and pyknosis in hippocampus neurons	-	-	-	+++
Eosinophillic plaque formation in striatum	-	-	-	+++
Gliosis	-	-	-	+++
Focal nuclear pyknosis and degeneration in neuronal of cerebral cortex	-	+	++	+++
Atrophy in some neurons of the Substantia nigra	-	+	+	+++
+++ Severe ++ Moderate + Mild - Nil				

Table 1: Effect of social isolation on the severity of brain histopathological alterations in normal and Alzheimer's disease rat model.

SI led to Aβ increase is not clear. Social isolation leads to oxidative stress [20,47] which in turn can stimulate β- and γ-secretase activity [25,48] resulting in Aβ elevation and cognition decline. Social isolation can also aggravate the inflammatory processes [49,50]. In the present study, Aβ content was elevated in the brain of both socialized and isolated rats but isolated rats showed significant increase in Aβ content than socialized one. Several lines of evidence suggest that oxidative stress has been proposed to facilitate Aβ secretion [51]. Accumulation of Aβ in cellular compartments interferes with normal cell function [52] and promotes cellular changes. It is suggested that Aβ plays a major role in AD development and progression [53] Additionally, the correlation between the beginning of cognition decline with Aβ levels and stress promotes APP processing along the amyloidogenic pathway has been established [46,54] and resulting in the exacerbation of AD-like neuropathology [11,25].

Brain aging is characterized by memory deficits and cognitive decline that could be the result of oxidative stress and impaired cholinergic function. Studies have been also highlight that various stresses as SI can lead to cholinergic dysfunction [55]. In the brain, the cholinergic neurotransmission system plays an essential role in learning and memory. It is terminated by acetylcholine hydrolysis via ACHE; this enzyme is important in maintaining the normal function of the nervous system and participates in the underlying processes of AD [56,57]. The activity of ACHE has been shown to be elevated in brain of aluminum- treated animals [58]. The elevation of ACHE activity may be due to neurotoxic effect on the plasma membrane which caused by increased lipid peroxidation. Changes in plasma membranes may influence the integrity and the functions of cholinergic system. Consequently, lipid membrane is a decisive factor in affecting ACHE and results in learning and memory deficits [57,59]. It is worthy to note that ACHE inhibitors are used for the symptomatic treatment of patients with AD. The present study examined the effect of social isolation on brain cholinergic functions since ACHE activities influence cognitive performance [60,61]. The results of the present study showed that social isolation for a long period induced significant elevation in the brain ACHE activity as compared to control socialized group which was evidenced to be paralleled to impairment on learning and memory as mentioned above. It is well known that individuals with more social engagement have a reduced rate of cognitive decline with aging [62]. Moreover, the rate of memory decline effectively doubles with SI [63]. On the contrary to the present study, a previous study did not show the change in ACHE between socialized and isolation-reared mice [64].

Results of the present study showed that $ALCl_3$ significantly increased ACHE activity of both socialized and isolated rats, but isolated rats showed more pronounced increase than socialized one. These findings are in agreement with previous results stated that AL exposure increased ACHE activity and leads to pathological deterioration related to the etiology of AD [65,66] These results could be attributed to the ability of AL to alter the blood brain barrier and produce changes in the cholinergic neurotransmission [67]. Besides the fact that AL is a cholinotoxin, its neurotoxicity could be attributed to an additional mechanisms as induction of oxidative stress or disarrangement of the cell membrane caused by the associated increase in lipid peroxidation [42,68].

It is known that the onset and severity of AD symptoms can vary dramatically among patients even with similar plaque burden [20,69]. Moreover, individuals with larger social networks have greater cognitive function [70]. It is suggested that larger brains have more neural matter that can be lost before manifestation of clinical symptoms of aging [71,72]. Indeed, social engagement has been linked to larger brain volumes [73] and individuals who engage in affiliative interaction are less liable to develop AD and dementia. It is possible that SI exacerbates the oxidative stress-mediated damage; reduce the available brain reserve, increases inflammation and allowing clinical symptoms of neuropathology to manifest at earlier stages [74,75].

On the other hand, BDNF is a key protein in maintenance as well as survival of neurons [76,77] and influences learning and memory [78]. Brain of patients with AD exhibits low expression of BDNF [79,80]. In the current study, we examined the effect of SI on neurogenesis, it was found that neurogenesis was significantly reduced in isolated group as compared to socialized one. It is quite evident that neurogenesis can improve brain function in AD [16,81]. In this study, BDNF levels were decreased in the isolated group. Decreased BDNF levels have been linked to faster cognitive decline and poor memory performance in AD [82]. These findings are in agreement with other studies which showed that SI resulting in a significant reduction in BDNF levels in the hippocampus when compared to paired housed rats [49,83,84]. Furthermore, social isolation can cause apoptosis of hippocampal cells and memory decline [85]. Thus, preventing social isolation can improve neurogenesis, inhibit memory deterioration and reduce cognitive deficits in AD patients. On the contrary to the present study, BDNF has been implicated in the pathophysiology of anxiety disorders [86,87] and there is augmentation in the expression of BDNF in cerebral cortex of mice after social isolation [88,89].

Results of the present study showed that injection of $ALCl_3$ decrease BDNF in both socialized and isolated rats but isolated rats showed more significant decrease than socialized rats. These findings are in agreement with other results [90,91] The BDNF has been shown to be decreased in the hippocampus of AD patients and it is suggested that a loss of BDNF may contribute to the progressive atrophy of neurons in AD. It is also possible that social isolation may reduce the protection of the brain against AD-related damage as mentioned before [92,93].

In the current study, it has been demonstrated that SI increased oxidative damage in the brain via enhanced lipid peroxidation measured as MDA accompanied with depletion of endogenous antioxidants SOD and TAC level in the brain tissue as compared to control socialized group. These findings are in agreement with other results that linked the short and long term SI with the generation of oxidative stress in the brain [94,95]. Moreover, reactive oxygen species are implicated in the pathogenesis of CNS damage; there are lines of evidence indicating the underlying pathological consequences of stress in the tissues by enhancement of lipid peroxidation [96]. Additionally, previous study suggested that chronic treatment with the antioxidant helped to reverse SI- induced oxidative damage, thus supporting the idea that SI influences AD pathology [20,54].

Results of the present study also showed that $ALCl_3$ increased MDA and decreased SOD and TAC in socialized rats. However, isolated rats showed a significant increase in MDA and decrease in TAC as compared to socialized rats. Oxidative stress and synaptic damage are known to be important factors in the pathogenesis of AD and are contributed to Aβ generation and neurofibrillary tangles formation [53,97,98]. The production of ROS can be indirectly evaluated by analyzing MDA. It is well known that oxidative stress and cognitive dysfunction are strongly linked and antioxidants that modulate ROS are considered to play a major role in improving learning and memory deficits, moreover SOD can prevent diseases linked to oxidative stress. As previously mentioned, AL alters physiological and biochemical behavior of the living organism and implicated in the increased brain MDA level [99]. It has been

demonstrated that AL exposure increases ROS production in different brain areas [66]. It also causes impairment of the antioxidant defense system that may lead to oxidative [58,68] and attacks almost all cell components including membrane lipids producing lipid peroxidation [67,100]. Thus, oxidative stress could be one of the main contributing factors for AL-induced CNS disorders [66,101]. The obtained data revealed also a significant inhibition in the activities of SOD and TAC in the brain tissue of socialized rats and of TAC of isolated rats treated with $ALCl_3$. These findings are in harmony with the study of [102] as regarding lower SOD with AL exposure which may attribute to the altered conformation of SOD molecule as a result of AL-SOD complex formation. Also, AL-intoxicated rats showed a decrease in brain TAC. Long term exposure to oxidative stress due to AL exposure leads to exhaustion of antioxidative enzymes [66]. In addition, Aβ can induce oxidative stress with increased production of hydrogen peroxide and lipid peroxides in neurons. Finally, oxidative stress plays an important role in the development and progression of AD [51].

Results of the present study also showed that SI for a long period resulted in brain neurological damage indicated by a significant elevation in inflammatory mediators (IL-1β, TNF-α) in the brain as compared to control socialized group. These findings are in agreement with [26] who found that SI increases oxidative stress and inflammatory reaction. Also, [49] reported that rats subjected to SI showed elevate IL-1ß protein levels in the hippocampus. Furthermore, inflammatory markers associated with isolation can be increased in AD patients [103] together with increased the rate of cognitive decline [104]. Moreover, impaired inflammatory control and unregulated inflammation are highly linked to AD pathogenesis [103,105]. Also, IL-1 plays an important role in the process of neuroinflammation and in the pathogenesis of AD through its inhibition on other inflammatory factors such as TNF-α and IL-1β [106]. In addition, TNF-α is one of the major proinflammatory response regulators in the brain [107]. Previous study findings showed that TNF-α could increase the neurotoxicity and resulted in cellular damage [108]. It is worthy to note that, cognitive decline in can be improved by TNF-α inhibition [109]. This would explain the increase in IL1β and TNF-α observed in the AL treated rats in present work.

Results of the present study also showed that $ALCl_3$ significantly elevate inflammatory mediators (IL-1β, TNF-α) in the brain of isolated rats more than socialized rats. It has been speculated that this inflammatory response associated with the presence of neuritic plaques is secondary to accumulation of Aβ and is involved in neuronal damage and in the progression of AD [110]. Aggregation of Aβ can activate microglia and induces the production of different factors as nitric oxide (NO), ROS, chemokines and proinflammatory cytokines (TNF-α, IL-1β) that promote neuronal death [111,112]. On the other hand, stress and inflammatory mediators enhance the production of APP and restricts the generation of its soluble fraction which provides neuronal protection [113,114].

Long period of SI also resulted in brain neurological changes as indicated by a significant reduction in brain monoamins (DA, NE, 5-HT) as compared to control socialized group. These findings are in agreement with other studies [20,115] which stated that SI decreases noradrenergic and serotonergic neurons in the brain. Frontal cortex and hippocampus of animals have been damaged by SI leading to abnormal function of neurotransmission [116,117]. Additionally, previous studies have reported that SI elicits a variety of behavioral abnormalities which may be attributed to deficiency in the brain neurotransmitters as NE, 5-HT or DA [118-120]. It is worthy to mention that higher 5-HT levels were observed in socialized rats; this finding is consistent with the postulation that increased utilization of 5-HT during SI cannot be compensated by an increase in its synthesis, thus leading to depressive-like behaviors [121,122]. It is well known that, 5-HT together with BDNF can facilitate the maintenance and the formation of synapses in CNS [121]. In addition, 5-HT increases BDNF expression while BDNF ensures neuron survival of 5-HT [122,123]. On the other hand, socialization improves BDNF and induces higher levels of 5-HT [122,124]. Previous reports demonstrated that isolated animals showed NE depletion which could account for the observed depressive-like behaviors [122,125]. Additionally, SI in rats can alter dopamine concentrations in the cortex and in other brain regions [126,127].

Results of the present study also showed that injection of $ALCl_3$ to both socialized and isolated rats significantly decrease brain monoamins (DA, NE, 5-HT) but isolated rats showed more pronounced reduction than socialized rats. These findings are in agreement with other studies [128,129], they found that these neurotransmitters are significantly decline with AD. It is also confirmed that, AL neurotoxicity are linked, to deficiencies of these neurotransmitters. It is well known that, altered production of neurotransmitters produces severe neurological illness [130].

Finally, the occurrence of DNA fragmentation is demonstrated by gel electrophoresis. Labeled DNA isolated from control isolated, AD socialized and isolated groups induced characteristic DNA fragmentation which is characteristic of apoptotic cell degeneration, these findings are in agreement with the opinion of [131] In addition, histopathological examinations have confirmed the biochemical results and showed that brain specimens of control isolated and AD socialized groups showed focal nuclear pyknosis as well as degeneration in the neuronal cells of cerebral cortex associated with atrophy in some neurons of the substantia nigra but no histopathological alteration in the hippocampus as well as in the striatum. Additionally, brain specimens of AD isolated group showed marked pathological changes as indicated by nuclear necrosis and degeneration in cerebral cortex associated with focal gliosis. Finally, Hippocampus, neurons of the fascia dentate, striatum and substantia nigra showed nuclear pyknosis and degeneration with congestion in the blood vessels. These findings confirm the other measured biochemical parameters and are in harmony with other studies concerning some of these measurements [17,100,132].

Conclusion

Social isolation for a long period causes severe brain neurological degenerations as indicated by the biochemical and the histopathological changes as well as DNA fragmentation; these degenerations are more pronounced in isolation-associated AD rat model than socialized ones. Accordingly, SI can be identified as a risk factor in AD development. Consequently, socialization is advised especially with AD to avoid severe progression of the disease.

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