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Research Article

Differential effect of morphine on spatial learning and memory in rat model of streptozotocin-induced dementia of Alzheimer's type

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ABSTRACT

Introduction: Alzheimer's disease (AD) is characterized by the accumulation of intra and extracellular amyloid-β (Aβ) plaques, and hyperphosphorylated Tau protein and synaptic/neuronal loss. Certain experimental models support morphine can play a beneficial role against damage in the neuronal system. It has been shown that morphine application immediately after hypoxia decreases infarction volume in the rat brain of hypoxia–ischemia model.The aim of present study, was to investigate the therapeutic efficacy of morphine on learning and memory in streptozotocin (STZ) Rat Model of AD.

Methods: 60 male Wistar rats were divided to: control, vehicle and groups treated with STZ and STZ plus saline or morphine. For induction of AD, STZ (3 mg/kg, 10 µl/injection site) wereadministered bilaterally into lateral ventricles.Morphine (1, 2.5 and 5 mg/kg, i.p.) or saline (0.2ml), were injected daily, one week after operation for 10days before training. All rates were trained in the Morris water maze (MWM) 3 weeks after stereotaxic operation. On the training days, rats were given a daily session of 4 trials per day for 6 consecutive days, 24 h after the last acquisition session, a 'probe trial' was used to assess the spatial memory.

Results: The results indicated thati.c.v. injection of STZ significantly increased escape latency and Swimming distance to find the hidden platform in comparison with the control and saline groups ($P<0.05$). The amnesic effect of STZ was prevented in rats treated with Low doses of Morphine (1 and 2.5 mg/kg/day) whereas treatment with high doses of Morphine (5 mg/kg) led to further impairment of learning and memory in the STZ rat model of sporadic AD. So The latency time and Swimming distance to find the platform in the Morphine (2.5 mg/kg) group rats were significantly lower than those in the other STZ-induced AD groups ($P<0.05$) conversely, the percentage of time spent and distance swimming in the target quadrant in the STZ+Morphine (2.5 mg/kg) group rats were significantly higher than those in the other STZ-induced AD groups ($P<0.001$).

Conclusion: Our data show that Low doses of Morphine(1 and 2.5 mg/kg), facilitates, whereas higher doses of Morphine(5 mg/kg), impairs, learning and memory in STZ-induced rat model of sporadic AD.The results suggest that chronic treatment with Low doses of Morphine can play a beneficial role against damage in the neuronal system, which in turn reversed the impairment of spatial memory acquisition induced by i.c.v. injection of STZ.

INTRODUCTION

AD is characterized by the accumulation of intra and extracellular amyloid-β (Aβ) plaques, and hyperphosphorylated Tau protein and synaptic/neuronal loss (Price and Sisodia, 1998 , Li et al., 2007).Morphine has been used for a long time as an effective treatment for pain (Zhang et al., 2008).Although many studies have shown that chronic exposure to morphine can impair long-term potentiation, a form of plasticity that is considered as a possible basis for learning and memory in the brain and cause cognitive deficits(Spain and Newsom, 1991; Salmanzadeh et al., 2003; Bao et al., 2007),There are studies that show Low doses of morphine, can emorphineance long-term memory recognition (Zarrindast et al., 2013). Also several lines of

evidence suggest that morphine may be neuroprotective. In rat neonatal hypoxia–ischemia model, morphine application after hypoxia decreases infarct volume in the brain (Zhou et al., 1998). In zebrafish embryos, morphine,emorphineances neuron proliferation(Sanchez-Simon et al., 2010).

In rat primary mesencephalic neuron morphine is found to be protective against 1-methyl-4-phenylpyridinium-induced dopaminergic neurotoxicity (Qian et al., 2007). In the CA1 regions of hippocampal slices morphine preconditioning reduced ischemia-induced celldeath (Zhao et al., 2006).In mouse hippocampal slices with oxygen-glucose deprivation, morphine preconditioning is reported toimprove neuronal cell survival rate through protein kinase C ε (PKCε)(Liu et al., 2008).In rat neuronal/glial cultures, morphine is reported to prevent cell death induced by HIV (Avdoshina et al., 2010).It has been shown that morphineandendomorphin can protect against intracellular amyloid β (iAβ) toxicity in human and rat primary neuronal cultures and in rat brains in vivo.

Morphine reverses the electrophysiological changes induced by iAβ. Morphine improves the spatial memory performance in rats infected by iAβ packaged virus and in APP/PS1 mice in Morris water maze tests (Cui et al., 2011)**.**Also in this study it has been shown that morphine protection is mediated through inducing estradiol release in hippocampal neurons, possibly by increasing P450 cytochrome aromatase activity. Released estradiol induces upregulation of heat shock protein 70 (Hsp70). Hsp70 protects against intracellular amyloid toxicity by rescuing proteasomal activity which is impaired by iAβ (Cui et al., 2011)**.**In the present study, the effects of morphine treatment upon impairment of spatial memory acquisition induced by icv injection of STZ have been investigated in rats.

MATERIALS AND METHODS

56 Adult male Wistar rats (Razi Institute, Karaj, Iran), weighing 200–300 g were used for Morris Water Maze apparatus. Animals were kept in an animal house with a 12/12-h light–dark cycle and controlled temperature (22 ± 2 °C). Animals had free access to food and tap water except during the limited periods of experiments.

Eight animals were used in each group; each animal was used once only and killed immediately after the experiment. Behavioral experiments were done during the light phase of the light/dark cycle (light on 07:00). Animals were divided into 7 experimental groups: control, vehicle (Saline), STZ, STZ+ saline and STZ+ morphine groups. Rats in the saline andmorphine groups received saline (0.2ml) ormorphine(1, 2.5 and 5 mg/kg, i.p.) for 10 days one week after i.c.v, injection of STZ (Zarrindast et al., 2006).All drugs were prepared immediately prior to use and given intra peritoneally (i.p.) in a volume of 0.1 ml per 100 g body weight of rats. For induction of AD, STZ (3 mg/kg, i.c.v, 10 µl each) was administered bilaterally into lateral ventricles. In the vehicle group saline (10 µl) was administered bilaterally into lateral ventricles.

Learning and memory performance of the rats was evaluated in the MWM starting 24 h after the last (18th day) saline and morphineinjection. All experiments were executed in accordance with the Guide for the Care and Use of Laboratory Animals (National Institute of Health Publication No. 80-23, revised 1996) and were approved by the Research and Ethics Committee of Qazvin University of Medical Sciences.Morphine waspurchased from TEMAD Company, (Tehran, Iran).and STZ was purchased from SIGMA-ALDRICH Company and anesthetic drugs (ketamine and xylazine) are products of Alfasan Company, Holland.Morphineand STZ,were dissolved in saline.

Induction of experimental dementia of AD by i.c.v. administration of STZ in rats

For induction of AD, Prior to surgery, rats were anesthetized with a combination of ketamine (100 mg/kg, ip) and xylazine (5 mg/kg, ip). The animal's head was fixed in the stereotaxic frame (stolting, USA).The scalp was cleaned with iodine

solution, and two holes were drilled in the skull bilaterally over the lateral ventricles. According to Paxinos and Watson's atlas(Paxinos 1997),the following coordinates were used for icv injection of STZ: 0.8 mm posterior to the bregma, 1.5 mm lateral to the sagittal suture, and 3.6 mm ventral from the surface of the brain with the tooth bar set at 0 mm. STZ was dissolved in normal saline shortly before use.

STZ (3 mg/kg, i.c.v 10 µl each) were administered bilaterally(Monisha et al 2001, Sodhi et al 2013, Tiwari et al 2009, Jessié et al 2014). The same surgical procedures were also used in the vehicle (saline) group, (10 µl/injection site). To determine that STZ was administered exactly into the cerebralventricles, some rat (30%) were injected with 5 µl of diluted potent blue dye and their brains were examined macroscopically after sectioning. After surgery, rats were housed individually, and three weeks later MWM test were performed to assess learning and memory.

Assessment of spatial learning and memory using the Morris water maze

To assess spatial learning and memory of animals, MWM tests were performed according to our previous work(Esmaeili et al 2016).Briefly, in MWM animal learns to escape to a hidden platform by swimming in circular water tank. This tank consisted of a large circular black colored pool of 150 cm diameter, 60 cm height, filled to a depth of 40 cm with water at 25±1 °C. A black colored round platform of 10 cm diameter was placed 1 cm below the surface of water in a constant position in the middle of the target quadrant (Q2) in the pool; the starting point was in the Q1 quadrant in all the trials. The rats could climb on the platform to escape from the necessity of swimming.

Only distal visual cues were available. The task was divided into two sessions: place learning and probe test. The rats were given a maximum time of 60 s (cut-off time) to find the hidden platform and were allowed to stay on it for 20 s. Once the animal found it, it was allowed to remain there for 20 s. If it did not find the platform after 60s, it was guided gently onto the platform and allowed to remain there for 20s.

The animals were given a daily session of 4 trials per day for 6 consecutive days. The numbers of quadrants crossed by the animals and path length (swimming paths) and latency time to reach the platform were recorded in each trial by water maze software. Twenty-four hours after the last acquisition session, a 'probe trial' was used to assess the spatial memory. During this trial, the platform was removed from the maze and the rat was allowed to search the pool for 60 s. The mean time spent by the animal in the target quadrant (Q2) searching for the hidden platform was measured and noted as an index of memory.

To assess whether any motivational factors interfered with the rat's ability to escape, 24h after probe test, a visible platform test was designed in which escape could be guided by proximal rather than distal spatial cues visible platform test (cue learning). During this trial, the platform was elevated above the water surface and extra maze cues were removed from the walls and the rats were allowed to swim freely for 60 s. The distance to platform (swim length), the escape latency and the number of quadrants crossed by the animals were measured. This test was aimed as measuring the visuo-motor abilities and the motivation of the animals.

STATISTICAL ANALYSES:

Data are expressed as mean ± SEM (standard error of mean). In order to compare the latency time and path length to reach the platform (distance) and values for the probe trial in MWM were assessed by one-way ANOVA followed by Tukey's test to detect statistical differences between the groups. P values less than 0.05 were considered to be statistically significant.

RESULTS AND DISCUSSION

Place learning

Figures 1 and 2 display place learning of different experimental groups in the MWM. As expected, the average escape latency (The latency time to find the hidden platform), escape distance (the

path length to find the platform) in searching for the hidden platform decreased with the increase in training days. In the control and vehicle groups there were shorter average escape latency and distance (Fig. 1, 2). The percentages of total time elapsed and distance swum in the target quadrant in the probe test were also relatively higher but there was no significant different between these two groups (Figs. 4).

An i.c.v. injection of 3mg/kg STZ, however, resulted in a significant decline in spatial learning, with longer latency and distance in searching for the underwater platform. These results indicate that STZ could significantly impair spatial learning and memory in rats. So the different between control and STZ/STZ+ saline groups on the all of training days were significant ($p < 0.05$). Our results also showed that treatment with low doses of morphine(1 and 2.5 mg/kg) protected spatial learning against impairment induced by STZ. As shown in Fig. 1- 2, treatment with low doses of morphine (1, 2.5 mg/kg) for 10 days, reversed the spatial learning and memory impairments induced by STZ.

Compared to the STZ/STZ+ saline groups, the average escape latency (Fig. 1B) and average escape distance (Fig. 2B) in searching for the hidden platform significantly decreased in the morphine (1, 2.5 mg/kg) plus STZ groups. Although the differences between the control with STZ/STZ+ saline groups on all of the training days were significant, such differences were not observed between the control and morphine (2.5 mg/kg) plus STZ group.

On the other hand treatment with high doses of morphine (5 mg/kg) led to further impairment of spatial learning and memory in the STZ rat model of sporadic AD. The escape latency and distance and the number of crossed quadrants in searching for the hidden platform in the STZ+ morphine (5 mg/kg) group rats similar to STZ/STZ+ saline groups were significantly higher than the control groups. Also, our results show that swimming speed of all groups of rats increased in the consecutive training days. But there were no significant difference between experimental groups indicating both STZ and morphine treatment had no effect on the motor activity of rats.

Probe test

In the probe test, control and vehicle group's rats spent most time and swum most in the target quadrant indicating memory consolidation were took place well in this group. However, in the STZ/STZ+ saline groups, (Fig. 4) time spent and swimming distance in the target quadrant were significantly less than those in control and vehicle groups. On the other hand, in the STZ + morphine treatment (1 and 2.5mg/kg) groups, time spent and swimming distance in the target quadrant were close to those in control group rats. The percentage of time spent and distance swimming in the target quadrant in the STZ plus morphine (2.5mg/kg) groups rats similar to control group rats were significantly higher than those in the other STZ-induced AD groups.

The percentage of total time elapsed and the distance swum in the target quadrant decreased in the morphine (5 mg/kg) plus STZ group, compared to the control group. Low doses of morphine (1 and 2.5mg/kg) increased time spent and swimming distance in the target quadrant in probe test whereas high doses of morphine has opposite effects.

Indicating that low doses of morphine treatment attenuated STZ- induced impairment in memory consolidation.

However, morphine treatment had no effect on the swimming speed.

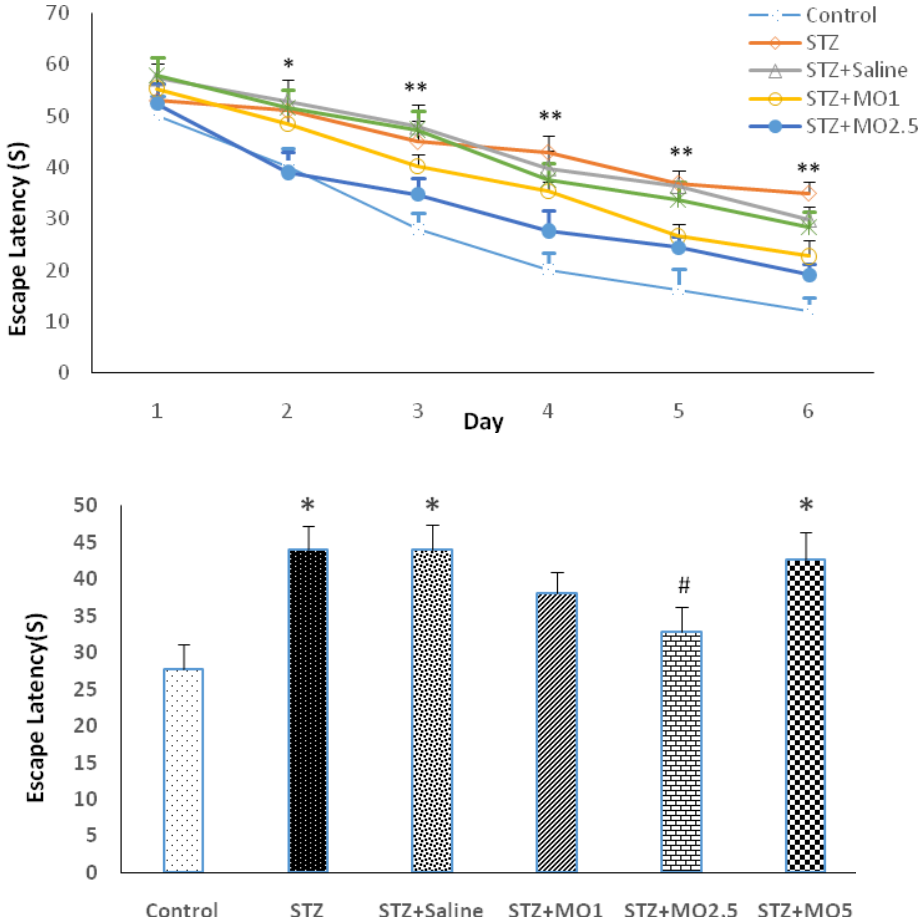
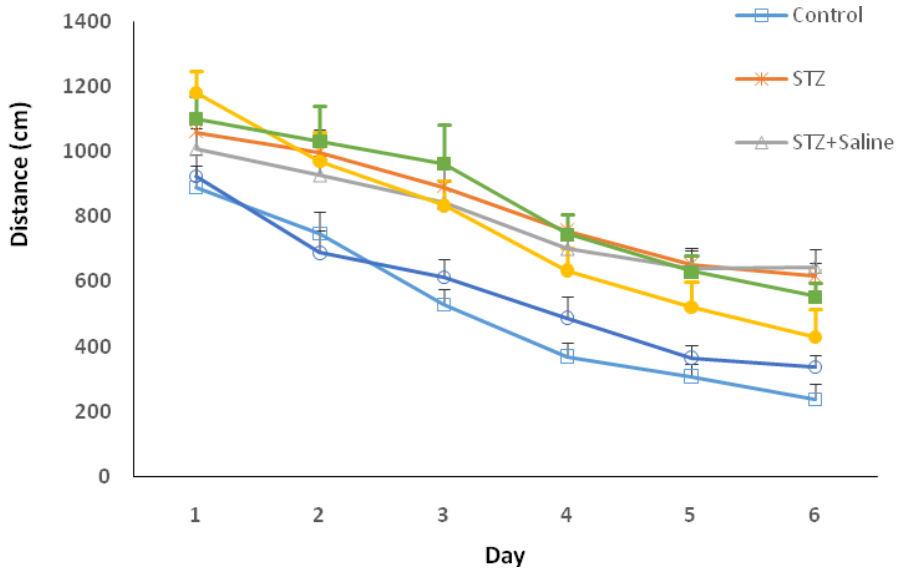


Fig.1. Place learning. The upper panel shows the escape latency (the latency time to find the hidden platform) of the experimental groups during successive training days (four sessions per day). The lower panel shows the escape latency in all of the 6 training days. *p<0.05; **p<0.001; relative to the control group, # p<0.05, relative to the STZ group, one-way repeated measure of ANOVA followed by the Tukey Post Hoc Test.



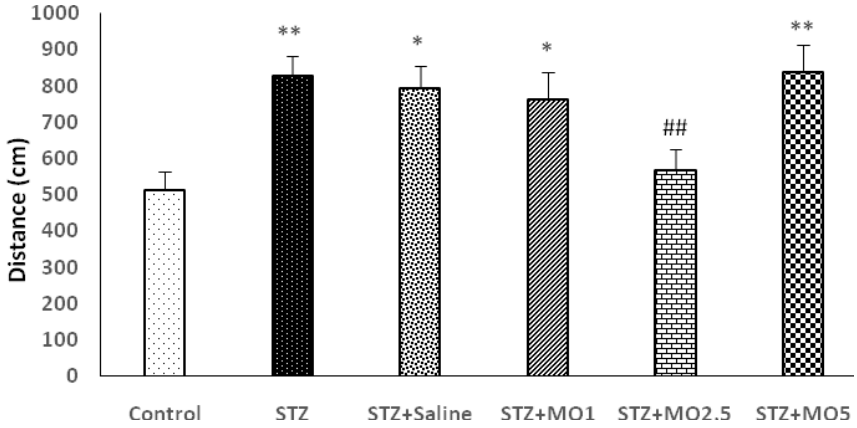


Fig. 2. Place learning. The upper panel shows the distance (the path length to find the platform) of the experimental groups during successive training days. The lower panel shows the distance in all of the 6 training days. *p<0.05; **p<0.001; relative to the control group, # p<0.05, relative to the STZ group, one-way repeated measure of ANOVA followed by the Tukey Post Hoc Test.

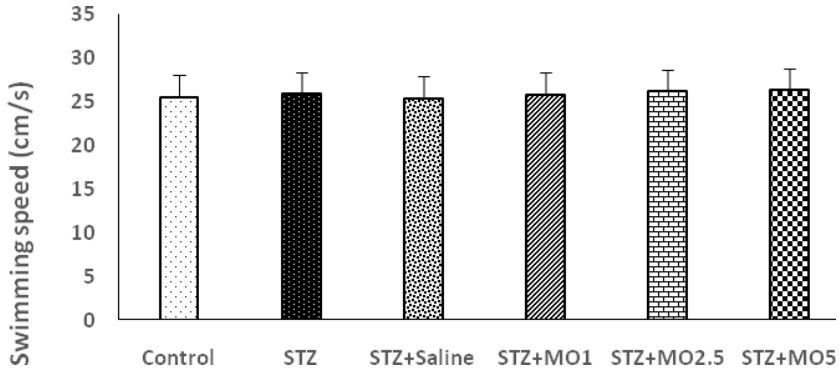


Fig 3. Place learning. The panel shows the animals' swimming speed in the experimental groups during successive training days. There was no significant difference between experimental groups.

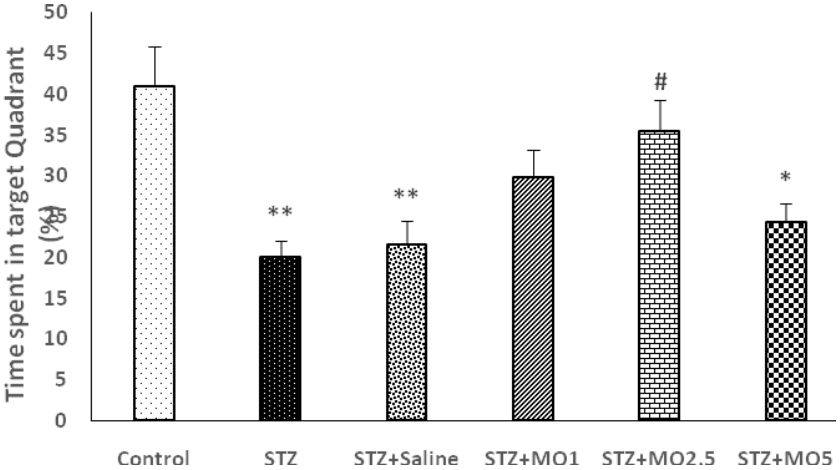


Fig. 4. Probe test. The panel shows the percentage of time spent in the target quadrant during only one day in the experimental groups. Each value represents the mean± SEM of the percentage of time spent in the target quadrant. *p<0.05; ***p*<0.001; relative to the control group, one-way repeated measure of ANOVA followed by the Tukey Post Hoc Test.

DISCUSSION

The opioid system plays an important role in memory processes. Morphine mimics endogenous opioids by acting on opioid receptor in brain to regulate memory. However, the effects of morphine on spatial memory acquisition are controversial. Low doses of morphine, comparable with endogenous brain concentrations, enhanced long-term memory recognition; while high doses did the reverse. In this regardZarrindast et al., 2013 have showed that social memory formation appears to be impaired in presence of morphine at analgesic doses (mg kg-1). In contrast, opposite effect was induced on memory formation by morphine at low (µg kg-1) doses. Several studies have reported facilitation of memory retention by opioid antagonists such as naloxone, whereas the systemic administration of morphine at analgesic doses impairs memory processes (Izquierdo, 1980). In the present study, we investigated the effects of morphine treatment on impairment of spatial memory acquisition induced by i.c.v injection of STZ (3 mg/kg, 10 µl/injection site) in adult male rats. The results indicated that bilateral pre-training,i.c.v injection of STZ impaired acquisition of spatial memory on the training and test day. The amnesic effect of STZ was decreased in rats treated daily with morphine (1 and 2.5mg/kg, ip) for 10 days. The results suggest that sub-chronic morphine treatment at low doses may be have protective effect against STZ-induced neurotoxicity which in turn reversed the impairment of spatial learning and memory acquisition induced by STZ.In agreement with our result several lines of evidence suggest that morphine may be haveneuroprotective effects against damage in the neuronal system. For example (Zarrindast et al., 2011) have shown that morphine administration for 3 day could attenuate deficits in memory consolidation produced by post training intra dorsal hippocampus (intra-CA1) administration of the non-selective cannabinoid CB1/CB2 receptor agonist, WIN55, 212-2. Likewise Farahmandfar et al 2013 have shown that bilateral pre-training intra-CA1 infusions of WIN55,212 impaired acquisition of spatial memory and this amnesic effect of WIN55, 212-2 was prevented with morphine. Also it has been shown that chronic pretreatment with morphine could decrease memoryimpairment by peripheral (Zarrindast and Rezayof, 2004) andcentral (ventral tegmental area) (Zarrindast et al., 2005a) administrationsof morphine or histamine (Zarrindast et al., 2006c).Morphine treatment can enhance conditioned place preferencewhen injected in the ventral pallidum (Roohbakhsh et al., 2007).Several reports have addressed the neuroprotective effect of morphineagainst damage in the neuronal system. In this regard it has been shown that morphine, endomorphin-1 and endomorphin-2 can protect against iAβ toxicity in human and rat primary neuronal cultures and in rat brains in vivo. Furthermore Morphine can improves the spatial memory performance in rats infected by iAβ packaged virus. Also it has been shown that Morphine protection is mediated through inducing estradiol release in hippocampal neurons, possibly by increasing P450 cytochrome aromatase activity. Released estradiol induces up regulation of heat shock protein 70 (Hsp70). Hsp70 protects against intracellular amyloid toxicity by rescuing proteasomal activity which is impaired by iAβ (Cui et al., 2011). Our results suggest that Low doses of Morphine (1 and 2.5 mg/kg), facilitates, whereas higher doses of Morphine (5 mg/kg), impairs, learning and memory in STZ-induced rat model of sporadic AD.Morphine is indicated to modify cell death/survival and play ant apoptotic roles in neurons of the CNS. However, themechanisms of morphine protection are not clear yet. In this regard previous studies have shown that Activation of PKC epsilon isoform is important in mediating the Preconditioning protection against necrosis and apoptosis induced by ischemia.Morphine pretreatment dose-dependently increased neuronal cell survival after oxygen and glucose deprivation. It has be shown that morphine preconditioning-induced ischemia

tolerance is mediatedthrough novel PKCє (nPKCє) isoform and NMDA receptors becausemorphine protection is greatly reduced by ev1–2 and MK-801as nPKCєand NMDA receptor-specific antagonists or by blockage of mem- brane translocations of nPKCє and NMDA receptors (Liu et al., 2001; Fanjun et al., 2006).It has been shown that the extent of PKCs translocation to the cell membrane during ischemia correlates well with the extent of cell injury. It has be shown that morphine pretreatment inhibited nPKCe membrane translocation during the period of reperfusion, in mouse hippocampal slices and administration of ev1–2 stopped the inhibition of nPKCe translocation, which suggested that nPKCє might be involved in the neuroprotection of morphine (Fanjun et al., 2006). The results suggested that moderate activation of NMDA receptors or nPKCє might be involved in morphine pretreatment–induced neuroprotectionalthough other study has suggested that neuroprotection effect of morphine is mediated via nitrogen oxide release.

It is proposed that morphine-induced nitrogen oxide release mediates neuroprotection in a human neuroblastoma cell line against intracellular oxidative stress and neuroinflammationand extracellular Aβ toxicity (Rambhia et al., 2005). In this regard, other mechanisms have been suggested, including: Protection of morphine against peroxynitrite induced apoptosis in primary rat neonatal astrocytes is mediated by phosphoinositide 3-kinase (Kim et al., 2001). Downregulation of BACE-1(beta-site amyloid precursor protein cleaving enzyme 1) and upregulation of BACE-2 has been shown to decrease beta-amyloid peptide levels. It has been shown that Morphinecan down regulates the expression of BACE-1 and up regulates the expression of BACE-2 in human neuroblastoma HTB-11 cells. Because in this study when HTB-11 cells were treated with NO synthase inhibitor (L-NAME), the neuroprotectioneffects of morphine were blocked. (Pak et al., 2005). Based on the above evidences and our data, we thought that the changes in the nitrogen oxide release and in the activity of nPKCє and NMDA receptors might be involved in the neuroprotection induced by morphine treatment.On the other hand our result show that higher doses of Morphine (5 mg/kg), impairs, learning and memory in STZ-induced rat model of sporadic AD. Memory is often considered to be a process that has several stages including acquisition, consolidation, and retrieval. In this regard many reports have demonstrated that pre- or post-training administrations of moderate doses of morphine (5 – 10 mg/kg) impairs learning and memory of behavioral tasks (Izquierdo,1979, 1980, Bruins Slot, 1999, Ahmadi, 2007 Jafari, 2006 Rezayof 2006Zarrindast,2013). Several studies have reported facilitation of memory retention by opioid antagonists such as naloxone, whereas the systemic administration of morphine at analgesic doses impairs memory processes (Izquierdo, 1980). There is some evidence that shown chronic exposure to morphine can impair long-term potentiation (LTP), and cause cognitive deficits (Bliss and Collingridge, 1993) (Spain and Newsom, 1991),it has be shown that chronic exposure to morphine leads to the impairment of hippocampal CA1 LTP (Pu et al., 2002; Salmanzadeh et al., 2003; Bao et al., 2007)and induces cognitive deficits(Pu et al., 2002; MiladiGorji et al., 2008).Accumulating evidence has demonstrated that chronic exposure to morphine diminishes hippocampal CA1 LTP (Pu et al., 2002; Salmanzadeh et al., 2003) and leads to the impairment of acquisition (Spain and Newsom, 1991; Li et al., 2001) or retention (Alaei et al., 2006; MiladiGorji et al., 2008) of spatial memory via accumulation of extracellular adenosine (Gang. et al., 2010).In conclusion, these results demonstrated that Low doses of Morphine (1 and 2.5 mg/kg), facilitates, whereas higher doses of Morphine (5 mg/kg), impairs, learning and memory in STZ-induced rat model of sporadic AD.It seems that morphine's route of administration and the doses used are critical elements defining the morphine effect on learning

and memory. Morphine treatment administration may either disrupt or facilitate spatial memory, in STZ-induced rat model of sporadic AD, depending on the dose, The bimodal effects of morphine previously shown in Social memory and pain.(Bianchi, 2013;Jacquet and Lajtha, 1973;Kayser et al., 1987 ; Crain and Shen, 2001) Biphasic dose–response relationships have been demonstrated in animals following administration of morphine (Jacquet and Lajtha, 1973). Beyond the analgesic effect exerted by morphine at analgesic doses, acute thermal hyperalgesia has been demonstrated by different researchers after morphine administration to animals at extremely low doses (Kayser et al., 1987; Crain and Shen, 2001).

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