The Possible Protective Effect of Mefenamic Acid, Taurine, Soy-Phytoestrogen Extract Alone and in Combination in Experimental Alzheimer Disease.

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Abstract: Background: This study was conducted in a trial to reduce the possible common risk factors might progress the Alzheimer's disease (AD) through a combined treatment of mefenamic acid, a non steroidal anti-inflammatory drug, (NSAID), soy-phytoestrogen extract (plant soy bean) and taurine, (a major intracellular free ß-amino acid and potent endogenous antioxidant) in an animal model of scopolamine induced-AD. **Methods**: adult rats were daily supplemented with nutrients in 8 groups, 3 groups of adult male rats each supplemented individually with mefenamic acid (50mg/kg/B.wt), soy-phytoestrogen (20g/kg/B.wt) and taurine (500mg/kg/B.wt) in addition to a 4th one supplemented with a combined treatment of the aforementioned 3 nutrients along with i.p injection of scopolamine (1mg/kg/B.wt) for 5 weeks. To test the sex effect of phytoestrogen, another 2 groups of adult female rats were supplemented with soy-phytoestrogen and combined treatment. The effects of nutrients were compared against a +ve control group (treated with scopolamine only) and a –ve one (free diet). Morris water maze was initiated at 6th week. Rats were terminated for assays of levels of MDA (as end product of lipid peroxides), nitric oxides (NO, as total nitrate), energy metabolism (ATP), acetyl choline (Ach) content, excitatory (aspartic & glutamic acids) & inhibitory amino acids (glycine & γ-aminobuityric acid (GABA) in hippocampus and thalamus regions of animal brains. **Results** showed that: all the tested nutrients, in which the combined treatments have the powerful effect, were the effective prevents factors for decreasing prolonged escape latency and improving memory impairment. Mefenamic acid was significantly decreasing Ach and ameliorated oxidative stress. Soy-phytoestrogen extracts significantly increasing ATP levels and reversing oxidative defense system, while tau appear to be the effective factors for reversing scop-cholinergic and neurotoxicity as well as modulating excitatory and inhibitory amino acids. **Conclusion:** It is concluded that the combined treatment showed a powerful magnification effect in treated AD through its ability to reduce the most tested risk factors for developing AD. Soy-phytoestrogen supplementation was benefit for both female and male rats in reducing the risk of AD.
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Key Words: Alzheimer disease, scopolamine, mefenamic acid, taurine, soy-phytoestrogen, oxidative damage, Acetylcholine, ATP, excitatory and inhibitory amino acids, Estrogen deprivation.

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder, characterized by a progressive loss of cognitive, language, and behavioral functions (Albert 1996, Christopher, 2004). Dementia is one of the age-related mental problems and a characteristic symptom of Alzheimer's disease. Both oxidative damage and inflammation are elevated in brains of AD patients and are considered the most common risk factors for AD. Oxygen free radical the harmful by-product of oxidative metabolism, are known to cause organic damage to the living system, which may be responsible for the development of AD in the elderly (Jeong et al., 2008). Neuro-inflammation is a complex response to brain injury involving the activation of glia, release of inflammatory mediators within the brain, Prostaglandins (PGs), a class of lipid mediators which can have inflammatory actions and impair memory (Hein, 2009).

Acetylcholine esterase (AChEs), are the most extensively risk factor for the AD which decrease synaptic levels of available acetylcholine (ACh) and increasing its degradation, In animals, AChE inhibitors improve learning and memory, reverse scopolamine-induced amnesia, and produce hippocampal theta rhythm (Min et al., 2003).

Nonesteriodal anti-inflammatory drugs (NSAIDs) are widely-used therapeutic agents that have anti-inflammatory, analgesic and antipyretic activities. NSAIDs are involved in the suppression of prostaglandin synthesis by inhibiting cyclooxygenases, enzymes that catalyze the formation of prostaglandin precursors from arachidonic acid (Steven, 2008). In addition NSAIDs have an antioxidant effect and can directly scavenge oxygen radicals. So it was estimated that the neuroprotective effects of NSAIDs was through its anti-inflammatory actions and antioxidant effect.

Furthermore, NSAIDs found to have the ability for inactivation of AChE that induced by their radicals (Muraoka, and Miura 2009). In this study we investigated the therapeutic potential for Alzheimer's disease of mefenamic acid, a commonly used NSAID that is a cyclooxygenase-1 and 2, inhibitor with only moderate anti-inflammatory properties, antioxidant and anti-AChE properties.

Increased release of excitatory amino acids (EAAs) is considered as a common risk factor of AD. Researchers have proposed that over activation of glutamatergic transmission and excitotoxicity is involved in the pathogenesis of dementia of the Alzheimer type and other neurological disorders (Paula-Lima et al., 2005).

Taurine is a conditionally-essential amino acid which is not utilized in protein synthesis, but rather is found free or in simple peptides (Birdsall, 1998). Tau is found in high concentrations in the CNS and is essential for growth and survival of neurons (Whirley and Einat, 2008). Clinically, tau has been used with varying degrees of success in the treatment of a wide variety of conditions, including: cardiovascular diseases, hypercholesterolemia, seizure disorders, macular degeneration, Alzheimer's disease, hepatic disorders, alcoholism, and cystic fibrosis (Birdsall, 1998). Tau also rescues central neurons from the excitotoxicity induced by high concentrations of extracellular glutamate (Paula-Lima et al., 2005).

Estrogen deprivation is considered another risk factor for the development of AD. In clinical studies, it has been shown that estrogen replacement therapy in menopause is strongly correlated with a reduced risk of the development of AD. In vitro experiments, it was demonstrated that estradiol protects cells against the toxic effects of β-amyloid, the major component of plaques in brains of AD patients. Therefore, estrogens have become interesting candidates for a possible treatment of neurodegeneration (Roth, et al., 1999).

Phytoestrogens, plant-derived non-steroidal estrogens found in high abundance in most soy food products, can influence learning and memory and alter the expression of proteins involved in neural protection and inflammation in rats (Lephar**t** et al., 2002). It was revealed that visual spatial memory is enhanced in female rats (but inhibited in males) by dietary soy phytoestrogens a difference attributed to the hormonal influence of estrogen (Lund, et al., 2001). In this paper we compare the effect of soy phytoestrogens diet on both female and male rats in neural protection and reduce the risk of AD.

Overall hypometabolism may be partially caused cognitive impairment in AD. Indeed, AD is characterized by an early region-specific decline in glucose utilization and by mitochondrial dysfunction, which have deleterious consequences for neurons through increased production of reactive oxygen species (ROS), ATP depletion and activation of cell death processes (Ferreira et al., 2010). It is suggested that mitochondrial dysfunction may play a key role in Alzheimer's disease (AD) of the post-menopausal female, and may serve as the target for endogenous estrogen and exogenous phytoestrogen.

Based on these observations, the current experiment designed to determine the benefits and to assess an additional reduction in the possible factors of AD, through, a combined treatment of mefenamic acid, tau and soy-phytoestrogen.

2. MATERIALS AND METHODS

Animals

Male and female albino rats weighing 170±10g were used. The animals were brought from laboratory animal breeding of National Organization of Drug Control and Research (NODCAR), Giza, Egypt. They were kept under strictly hygienic conditions. They were put on a standard basal diet and allowed free access to drinking water.

Materials

-Scopolamine hydropromide and tau were purchased from Sigma Co. USA.

-Soy phytoestrogen were purchased from Egyptian market.

-Mefenamic acid was purchased from Egyptian market pharmacy.

Tau, mefenamic acid were freshly prepared before administration dissolved in water and given orally, while scopolamine was dissolved in saline and injected intrapretonially but soy phytoestrogen was given mixed in diet.

Rats were classified into 8 equal groups each comprises 8 rats and treated daily for 5 weeks, as follow:

G1, -ve control group (C), fed on basal diet and injected with saline.

G2, +ve control group (Scop), injected *i.p* by 1 mg/kg/B.wt of scop-HBr.

G3, Mefenamic treated group (M), orally administrated 50 mg/kg/B.wt (equivalent to human daily dose) of mefenamic acid along with *i.p.* injection of 1 mg/ kg /B.wt of scop-HBr.

G4, Taurine treated group (Tau), orally administrated 500mg/kg/B.wt of tau along with *i.p.* injection of 1 mg/ kg / B.wt of scop-HBr.

G5, Male Soy phytoestrogen treated group (Phy-m.), group of male rats received a diet contain 20 g/kg/B.wt of Soy phytoestrogen extract along with *i.p.* injection of 1 mg/kg/B.wt of scop-HBr.

G6, Female Soy phytoestrogen treated group (Phy-f), group of female rats received a diet contain 20

g/kg/B.wt of Soy phytoestrogen extract along with *i.p.* injection of 1 mg/kg /B.wt of scop-HBr.

G7, Male Combined treated group (Comb-m), male rats administrated a combined treatment as in G3+4+5

G8., Female Combined treated group (Comb-f), female rats administrated a combined treatment as in G3+4+6.

At the end of the treatment schedule, rats were sacrificed; brain tissues were removed and were homogenates in 2 different areas (hippocampus and thalamus regions) in iced 70% methanol. Blood samples were collected then separated serum and supernatant of homogenates tissues were processed for the biochemical analysis included: oxidative stress **(**MDA, NO, as total nitrate), ATP, ACh concentration, excitatory & inhibitory amino acids, all were determined by HPLC methods of Karatepe (2004)., Everett et al, (1995), Zhang et al., (2000)., Jones and Stutte (1985)**,** Heinrikson and Meredith (1984) respectively.

Morris Water Maze Tank

The water maze consisted of a white circular galvanized tank (its size was 150 cm diameter and 60 cm height) filled with opaque tap water made by adding dry milk powder to water at the temperature of 27 C. Four locations around the edge of the pool were defined as start points, and these divided the pool into four equal quadrants. A circular escape platform 15 cm in diameter was placed 2 cm below the surface of the water in the middle of one of the four quadrants of the pool. A video camera suspended from the bracket above the middle of the tank permitted the observer to monitor the animal's behavior on a monitor. Animals were tested on three daily trials, each trial separated by 2 min, for three consecutive days. Animals were placed into the tank, facing the wall of the pool, and were allowed to circumnavigate the pool in search of the escape platform for a maximum of 90 s. On each day, the start points used for each trial varied in a pseudorandom sequence such that no two trials on the same day commenced from the same start point. The time (latency) to reach the escape platform was recorded, and the animals were permitted 30 s to rest on the platform before removal from the tank. If an animal failed to locate the platform within 90 s, it was guided to the platform by the experimenter, placed on it for 30 s and assigned a latency score of 90 s for that trial. A single probe trail was done on the final test days in which the platform was removed and animals were allowed to swim freely for 90 s. The number of times the animals spent in where the platform had been located was recorded (Guangqin et al., 2009).

Statistical analysis: Data are expressed as mean ± S.E. of 8 rats values. One-way ANOVA tests were applied to study the relationship between the different variables. $P<0.05$ was considered significant.

3. **RESULTS**

Learning and memory impairment:

From the first learning session day, it was observed that all nutrients showed a shorter latency time than Scop-treated group ($P<0.05$, figure1). The potency effect of the nutrient in a descending order showing that: combined treatment (♂ or ♀) have more improving effect > than individually phy-treatment > then Tau & M treatment. To confirm whether memory impairment, shown in scop-treated rats, was attenuated by the different nutrients, we performed a probe test and recorded average latencies in zone without platform. Both combined treatment stayed significantly ($p<0.05$ figure.2) longer in that zone more than individually treated nutrients as compared with Scop-treated group.

On contrast, rats treated with all nutrients for 5 weeks reversed amnesia induced by scop, oral administration of 500mg/kg/B.wt of tau, 10 mg/kg/B.wt of mefenamic acid and 20 g/kg/B.wt of soy phytoestrogen extract can attenuated ($P<0.05$) the learning and memory impairment induced by scop-treatment.

MDA and NO content:

The result of this study showed that scop-treated rats significantly increases ($p<0.05$) the levels of MDA (figure 3), while the level of NO was significantly ($P<0.05$) decreased in the neural cells of hippocampus and thalamic regions of rats brain (figure 4). Treatment with mefenamic acid, tau, soy-phytoestrogen and their combined forms significantly ($P<0.05$) reduced the ROS generation (by decreasing levels of MDA and increasing level of NO) induced by scop-treatment. This effect was potent with tau administration.

Acetyl choline content:

Acetyl choline concentration of hippocampus and thalamus regions of brain was markedly declined ($P < 0.05$) after i.p injection (1mg/kg) of scop (figure 5). Treatment with all tested nutrients significantly increased Ach concentration ($P<0.05$, figure 5), indicating the counteracting actions of these nutrients on the cholinergic system.

Excitatory and inhibitory amino acids content: The data in figure (6&7), showed that i.p injection of scop (1mg/kg/B.wt) significantly increases ($P<0.05$) the level of excitatory amino acids, (aspartic & glutamic acids) in hippocampus and thalamus regions of rat brains, while the level of glycine & γ-aminobuityric

acid (GABA) was decreased. Data in figure (6&7) also showed that administration of tau could decrease ($P<0.05$) the level of excitatory amino acids (aspartic & glutamic acids) & increase the level of inhibitory amino acids (glycine & γ-aminobuityric acid (GABA) in hippocampus and thalamus regions of animal brains. On other hand the mefenamic acid and soy-phytoestrogen treatment failed to ameliorated these levels ($P>0.05$).

ATP content:

The presented study showed that after scop-injection, mitochondrial ATP content of the hippocampus was significantly decreased ($P<0.05$), figure (8). The data also revealed that only treatment with soy phytoestrogen diet, could recovered ($P<0.05$) mitochondrial function and the ability of ATP synthesis was enhanced.

4. DESCUSSION

*The effect of tested nutrients on learning and memory impairment in scop-induced AD***:**

Scopolamine hydrobromide (1mg kg^{-1} *i.p*) decreased time of latency on first day after training, indicating impairment of memory and amnesia. In agreement with the study of Aaro et al., (2007), they evaluated an impairment of both learning and memory by scop (0.4 mg/kg *i.p.*) in rats, this effect was supported by the recent study of Saraf et al., (2011), who studied the attenuation effect of *Bacopa monniera* against scop-induced impairment of spatial memory in mice. On contrast, rats treated with all nutrients for 5 weeks reversed amnesia induced by scop. This result confirmed the previous studies of Joo et al., (2005) in which they estimated that mefenamic acid improves learning and memory impairment in an AB1-42-infuced Alzheimer disease rat model. The improvement effect of soy-phytoestrogen diet on learning and memory impairment induced by scop treatment was in agreement with the study of Sarkaki, et al., (2008) they showed that soy meal diet (with or without isoflavone) in ovariectomized rats with Alzheimer**'s** disease caused improvement of performance across 18 trials of Acquisition. They suggest that soy meal is a potential alternative to estrogen in the prevention and treatment of Alzheimer's disease. Memory performance can be restored in senescent female rats by estradiol, even at an advanced age and after long-term hormone deficiency. Consistent with these behavioral findings, cholinergic pathways involved in memory processes can still be activated by estrogen in the brain of aged female rats (24 months). Data depicted in figure (1) revealed that there is no deference between male and female rats by dietary soy phytoestrogens on learning and memory test, in contrast with the study of Lund et al., (2004), it was revealed that visual spatial memory is enhanced in female rats (but inhibited in males) by dietary soy phytoestrogens a difference attributed to the hormonal influence of estrogen. On other hand, Zhao and Brinton (2007) revealed that development of an effective phytoestrogen formulation would benefit both women and man to improve neurological health and reduce the risk of Alzheimer's disease. The data implicated in figure (1) showed that oral administration of 500 mg of tau can attenuated the learning and memory impairment induced by scop-treatment. These results were in agreement with study of Guangqin, et al., (2009), they showed that in prophylactic supplementation, the combined nutrients (methionine, taurine, zinc, ascorbic acid and glycine) were the effective preventive factors for decreasing prolonged escape latency against lead-induced learning and memory impairment in rats, in which the effect of tau been related to their antioxidant potency through attenuating the alteration in SOD, NOS activities and NO levels induced by lead neurotoxicity. In addition, the previous results of Ye et al., (2005) showed that tau effectively improves learning-memory ability in model mice induced by scop injection. The mechanism relates with the improvement of M-cholinergic receptors of brain.

The effect of tested nutrients on MDA and NO content in scop-induced AD:

Many clinical studies have reported strong evidence that oxidative stress is involved in the pathogenesis of Alzheimer's disease. The oxygen-free radicals are implicated in the process of age related decline in the cognitive performance may be responsible for the development of Alzheimer's disease in elderly persons (Jeong et al., 2008). Kanwall et al., (2010) reported that memory impairment in the scop-induced animal model is associated with the increased oxidative stress within rat brain. Moreover, strong evidence supporting the involvement of oxidative damage in neurodegenerative disease has been suggested by various clinical studies, in accordance with the recent study of Vandana et al., (2011) they revealed that the learning and memory was impaired by administration of scop (1 mg/kg, i.p.) in mice which is associated with altered brain oxidative status. Nitric oxide (NO) is considered as an intracellular messenger in the CNS, and the NO/sGC/cGMP signal transduction system is considered to be important for modulating synaptic transmission and plasticity in brain regions such as the hippocampus, cerebral cortex, and cerebellum. Thus compounds that inhibit NO synthase, the key synthesizing enzyme, may inhibit cognition, while NO donors may facilitate it (Pitsikas,

2009). The present data showed that 1mg/kg injection of the anti-musccarinic cholinergic receptor, scop could inhibit NO synthase. These results indicate that NO is involved in spatial recognition memory by acting with the cholinergic system on spatial memory. Furthermore, the present results were supported with the recent study of Javadi-Paydar et al., (2011) they showed that the improvement of atorvastatin against scop-induced impairment in memory acquisition was through acting as a donor of NO that ameliorated the spatial recognition memory, through its acting on the cholinergic system. The drugs with antioxidant effects and inducer to NO might be beneficial for preserving brain function. Augmentation of endogenous antioxidants by therapeutic substances has recently evoked scientific interest due to their significant improvement in the endogenous defense against oxidative stress (Guangqin et al., 2009). Nutritional supplements should also include the key antioxidant vitamins C and E, as well as β-carotene and the mineral micronutrients found in the oxygen radical-detoxifying enzymes glutathione peroxidase and superoxide dismutase. This result were in agreement with the study of Joo et al., (2005), their vitro results suggest that mefenamic acid exerts a neuroprotective effect against Aβ1–42 treatment or Swe-APP or APP-CTs expression through the inhibitory effects of mefenamic acid on ROS. Moreover, the data of Miquel et al., (2006), revealed that administration of synergistic combinations of some antioxidants in which included soy may help to prevent antioxidant deficiency with resulting protection of mitochondria against premature oxidative damage with loss of ATP synthesis and specialized cellular functions. In addition Shi et al., (2008) support the previous concept of Miquel et al., (2006) that soy phytoestrogen diet may help to prevent antioxidant deficiency, offer protection to mitochondria and preserve ATP levels via enhanced oxidative phosphorylation and lowered oxidative load. Also tau may act as direct and indirect antioxidants to scavenge free radicals such as $O_{1,2}$ and OH thus ameliorating NOS activities and increased NO levels decreased by scop in the hippocampus and thalamic region. Results showed that tau supplementation, decreasing prolonged escape latency, and increasing NO levels in the hippocampus and thalamic region. The impairment in learning and memory one possible mechanism of scop-neurotoxicity may be interference with NO production. The antioxidants activity of tau was revealed in many previous studies, they first evident the potential effect of tau against N-methyl-D-aspartate (NMDA) receptor overactivation and points to the inhibition of the NMDA receptor-mediated NO synthesis as a possible mechanism of its neuroprotective action (Wojciech et al, 2003).The actions of tau as an inhibitory neurotransmitter, neuromodulator, and antioxidant were evoked by Rosemberga, et al., (2010) for studying its potential protective role against ethanol-mediated neurotoxicity. In this study, they investigated that acute tau co-treatment or pretreatment (1 h) prevent ethanol-induced changes in oxidative stress parameters in zebrafish brain. This study support the previous study of Guangqin, et al (2009), they suggested that tau could ameliorate learning and memory impairment induced by lead through reversing lead-induced decrease in activities of SOD, NOS and levels of NO.

*The effect of tested nutrients on ACh content in scop-induced AD***:**

Scopolamine-induced amnesia has been likened to early symptoms of Alzheimer's disease and is reversed by AChE inhibitors in rats as well as in subhuman, AChE release from hippocampus and thalamus regions of brain and ACh concentration are the central event in AD. In fact, among the multiple transmitter deficits that have been described in AD, is an early and severe degeneration of forebrain cholinergic system, as revealed by the correlation observed between the cholinergic pathology and dementia (Geula and Mesulam 1994). Furthermore, Alreja et al., (2000), suggesting that scop can be observed in vitro in a brain slice preparation, originates locally within the medial septum/diagonal band of Broca which is critical for learning and memory. Therefore, the enhancement of brain cholinergic transmission in AD remains a major goal for many putative therapeutic agents that are in use or under development (Kanwall et al., 2010). Treatment with all tested nutrients significantly increased Ach concentration indicating the counteracting actions of these nutrients on the cholinergic system. The present study suggests that mefenamic acid is a potential anti-cholinesterase. As reported with the study of Muraoka and Miura (2009) indicating that the protective effect of NSAIDs on Alzheimer's disease seems to occur through increasing the concentration of ACh by inactivation of ChE induced by NSAIDs radicals and that ChE may be inactivated through modification of tyrosine residues by mefenamic radicals. The present study revealed that soy-phytoestrogen showed an anti-cholinesterase action against scop-treatment. Other studies have provided evidence that the ability of estradiol to enhance cholinergic and cognitive functions declines with age in rats (Savonenko and Markowska (2003). Schumacher et al., (2007) demonstrated that, estrogen replacement can reduce memory deficits induced by the muscarinic receptor antagonist scop in young and in middle-aged female rats with irregular cyclicity

(12–13 months of age), but not at a more advanced age characterized by consistent estrus (20 months of age). Hence central cholinergic system plays an important role in learning and memory. In human or animal experiments, it has been found that phytoestrogen can block acetylcholine degradation, reduce the release of lactate dehydrogenase and increase the levels of mRNAs of brain-derived neurotrophic factor, choline acetyltransferase and nerve growth factor in the frontal cortex and hippocampus Therefore, phytoestrogens could be a promising substitute for estradiol to prevent AD of post-menopausal females. The data also showed that tau can significantly raise the level of ACh. Tau is an essential amino acid needed to increase the level of ACh in the hippocampus, which is a part of the brain for storing neurotransmitters. Adding tau increases brain levels of ACh, the most critical neurotransmitter involved in memory. As ACh levels fall, memory fails (Larry, 2007). Also it was evident that tau effectively improves learning-memory ability in model mice induced by scop-injection through the improvement of M-cholinergic receptors of brain (Ye et al., 2005). Furthermore, it was showed that ethanol exposure (1% in volume) during 1 h increased AChE activity, whereas the co treatment with 400 mg·L−1 tau prevented this enhancement. A similar protective effect of 150 and 400 mg·L−1 tau was also observed when the animals were pretreated with this amino acid (Rosemberga, et al., 2010).

The effect of tested nutrients on the excitatory and inhibitory amino acids content in scop-induced AD:

Excitotoxicity (the neuronal damage caused by overstimulation of excitatory receptors) has been implicated in several neurological disorders, including Alzheimer's disease (AD). Glutamate, the major excitatory neurotransmitter in the CNS, the overstimulation of glutamate receptors has been clearly implicated in the neuronal injury observed in several neurodegenerative disorders (Louzada et al., 2004). Possible involvement of GABA receptor systems in scop-induced memory deficits was investigated in many previous studies. They extended support to the cholinergic concept in cognitive performance and provided an evidence for the influence of GABAergic (particularly GABA) modulation in scop-induced learning and memory deficits in mice (Sharma and Kulkarni, 1993). Also the reversal of scop-induced amnesia by the GABA receptor antagonist was studied by Mauro and Alberto (1993), they investigated that after scop-injection disturbance in GABA receptor systems was occurred, lead to dementia. And this effect was reversed after administration of GABA receptor antagonist. On contrast, scop induced an increment in the level of excitatory amino acids, glutamate and aspartate. Interaction of cholinergic and glutamatergic inputs in influencing learning behavior is a topic of great interest for many studies. Barber and Haggarty (2010) postulated that activation of NMDA receptors by glutamate is particularly important in the initial stages of memory consolidation. In addition, acetylcholine receptor activation has been shown to be a necessary component of memory formation for this task. These results indicate a relationship between glutamate and ACh in memory formation, In addition, the study of Mahmoodi et al., (2011) showed that administration of a nonselective muscarinic acetylcholine antagonist scop (1 and 2 microg/rat) and NMDA receptor antagonist, MK-801 (0.75 and 1microg/rat) immediately after training, impaired consolidation of inhibitory avoidance (IA) memory. They concluded that muscarinic ACh and NMDA glutamate receptors are involved in the mechanism(s) underlying consolidation and retrieval of the IA memory. Administration of tau could decrease the level of excitatory amino acids (aspartic & glutamic acids) & increase the level of inhibitory amino acids (glycine & γ-aminobuityric acid (GABA) in hippocampus and thalamus regions of animal brains. Tau rescues central neurons from the excitotoxicity induced by high concentrations of extracellular glutamate. louzada et al., (2004) evident that the protective effect of tau is not mediated by interaction with glutamate receptors, as demonstrated by binding studies using radio labeled glutamate receptor ligands, but through the activation of GABA receptors which blocked by picrotoxin, an antagonist of GABA receptors. These results suggest that activation of GABA receptors decreases neuronal vulnerability to excitotoxic damage and that GABAergic transmission may represent a promising target for the treatment of AD and other neurological disorders in which excitotoxicity plays a relevant role. In addition, the previous study was supported by the study of Paula-Lima et al., (2005) they showed that tau should be investigated as a novel therapeutic tool in the treatment of AD and of other neurological disorders in which excitotoxicity plays a relevant role.

The effect of tested nutrients on the ATP content, in scop-induced AD:

Altered brain energy metabolism is an early and prominent feature of AD. Increasing evidence suggests that mitochondrial dysfunction is directly responsible for the pathogenesis of energy deficit in AD. Mitochondria are the 'power house of the cell' in which the metabolites are converted into ATP through oxidative phosphorylation, mitochondrial dysfunction led to decreased ATP production.

Therefore, mitochondrial dysfunction may be directly responsible for dysregulation of energy metabolism. The lowered antioxidant functions induced by scop-injection resulted in oxidative stress and lipid peroxidation by which mitochondrial membrane lipid peroxidation can further destroy the mitochondrial membrane and impair the functions of respiratory enzymes. Additionally, increase in oxygen free radicals and the damage of respiratory chain complexes lead to reduced cellular ATP concentration (Shi et al., 2008). After using soy phytoestrogen diet mitochondrial function recovered and the ability of ATP synthesis was enhanced. Soy phytoestrogen diet may help to prevent antioxidant deficiency, offer protection to mitochondria against premature oxidative damage and preserve ATP levels via enhanced oxidative phosphorylation and reduced ATPase activity followed by increased mitochondrial respiration via lowered oxidative load, increased transcript that lead to enhanced mitochondrial respiratory chain activity, subsequently increasing the ATP content (Shi et al., 2008). Furthermore, Xu et al., (2008) revealed that ATP content of hippocampal CA1 region after rat ovariectomy declined significantly and estrogen & phytoestrogen could reverse these alterations. In addition, the recent study of Vanize et al., (2011) revealed that phytoestrogen displays strong neuroprotective properties, preserve energy metabolism in rat striatum. They showed that rats subjected to ovariectomy presented a significant increase in energy parameters, Na+,K+-ATPase, succinate dehydrogenase and complex II activities. Treatment with isoflavones-rich soy diet was able to reverse the increase of Na+,K+-ATPase activity. Taken together, these results revealed that soy phytoestrogen may have a protective role against the neurodegeneration after scop-injection via protecting mitochondrial structure and functions.

Hence a combined form of mefenamic acid, tau and soy-phytoestrogen, posses the major anti risk factors including., neuroprotective, memory improving activities, neuromodulator, acetyl cholinesterase inhibitor, NO-donor, hormonal (estrogen) donor, antioxidant, anti-inflammatory, anti-excitotoxicity, mitochondrial function improvement and ATP production) may be of enormous use in delaying the onset and reducing the severity of Alzheimer's disease.

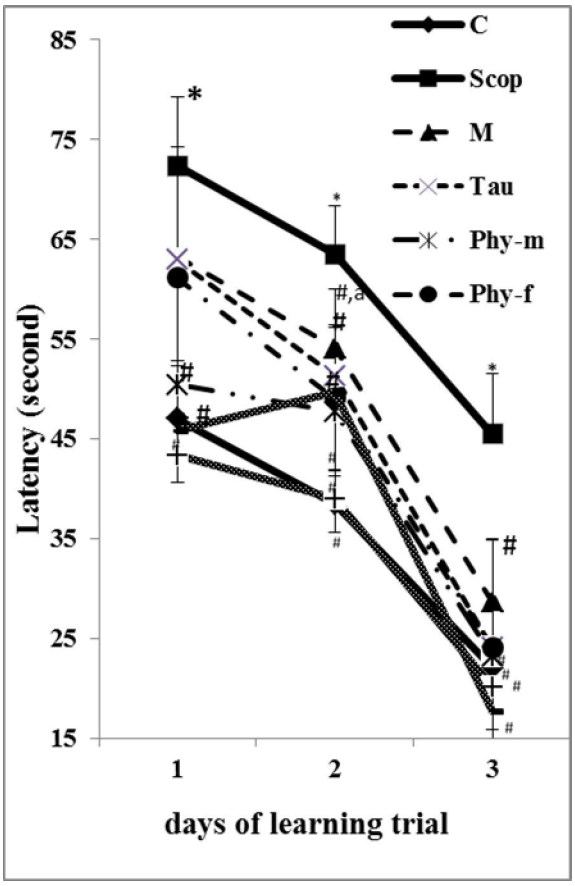

Figure (1): The effect of mefenamic acid, taurine, soy phytoestrogen and their combined treatment on: learning impairment in a scopolamine-induced AD of ♂ & ♀ rats after 5 weeks of treatment.
Data expressed as mean values of 8 rats ± SE.
Significant difference *v.s.* C: *P<0.05
Significant difference *v.s.* Scop: #P<0.05.

From the first learning session day, it was observed that all nutrients showed a shorter latency time than Scop-treated group (one-way ANOVA-P<0.05). The potency effect of the nutrient in a descending order showing that: combined treatment (♂ or ♀) have more improving effect > than individually phy-treatment > then Tau & M treatment (^{a}P<0.05 *v.s.* M-treatment). Also the data revealed that there is no deference between male and female rats by dietary soy phytoestrogens on learning and memory test.

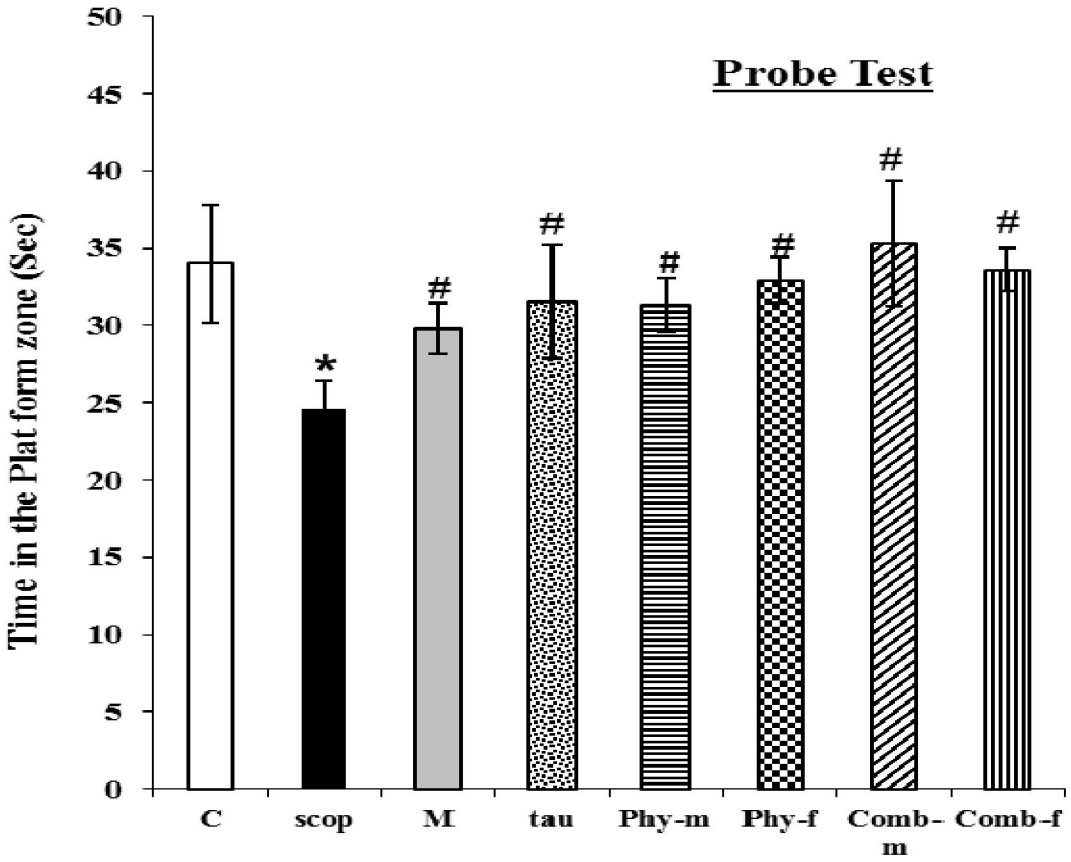

Figure (2): The effect of mefenamic acid, taurine, soy phytoestrogen and their combined treatment on memory impairment in a scopolamine-induced AD of ♂ & ♀ rats after 5 weeks of treatment.
Data expressed as mean values of 8 rats ± SE.
Significant difference *v.s.* C: *P<0.05
Significant difference Scop: #P<0.05.
Probe test was performed and recorded average latencies in zone without platform to show memory impairment in scop-treated rats. The different nutrients attenuated memory impairment induced by scop. Both combined treatment stayed longer in that zone more than individually treated nutrients *v.s.* Scop treatement (One-way ANOVA-P<0.05).

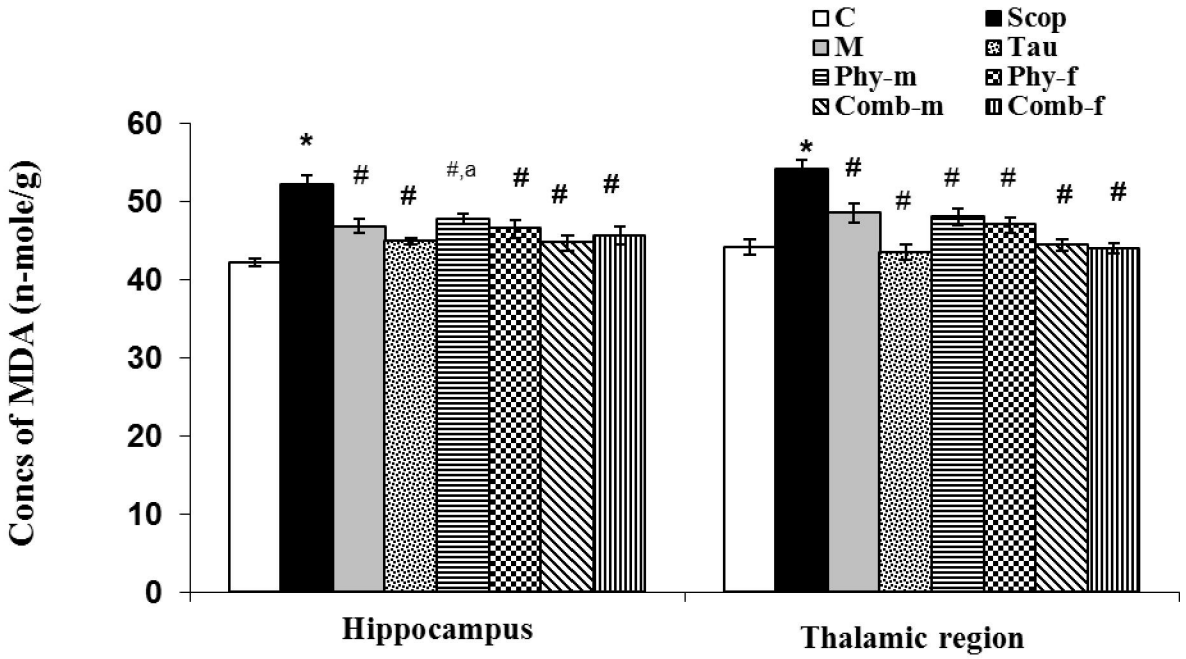

Figure (3): The effect of mefenamic acid, taurine, soy phytoestrogen and their combined treatment on the level of MDA concentration (nmole/g) in hippocampus and thalamic region of ♂ & ♀ brain rats after 5 weeks of treatment. Data expressed as mean values of 8 rats ± SE.
Significant different *v.s* C: $^{*}P<0.05$, *v.s* Scop: $^{\#}P<0.05$, *v.s* Comb-m: $^{a}P<0.05$.

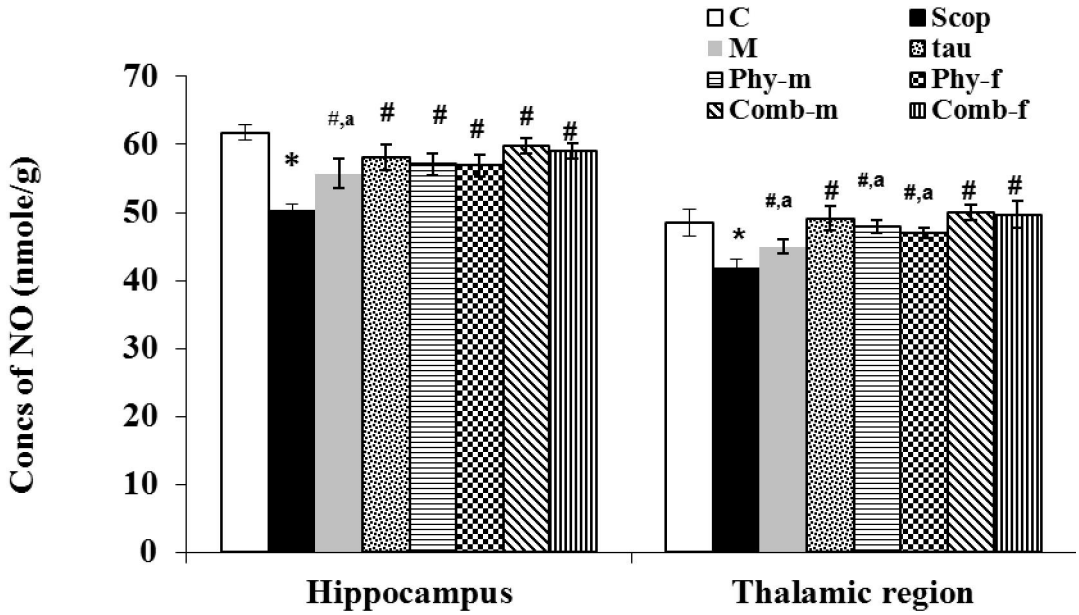

Figure (4): The effect of mefenamic acid, taurine, soy phytoestrogen and their combined treatment on the level of NO concentration (nmole/g) in hippocampus and thalamic region of ♂ & ♀ brain rats after 5 weeks of treatment. Data expressed as mean values of 8 rats ± SE.
Significant different *v.s* C: $^{*}P<0.05$, *v.s* Scop: $^{\#}P<0.05$, *v.s* Comb-m: $^{a}P<0.05$.

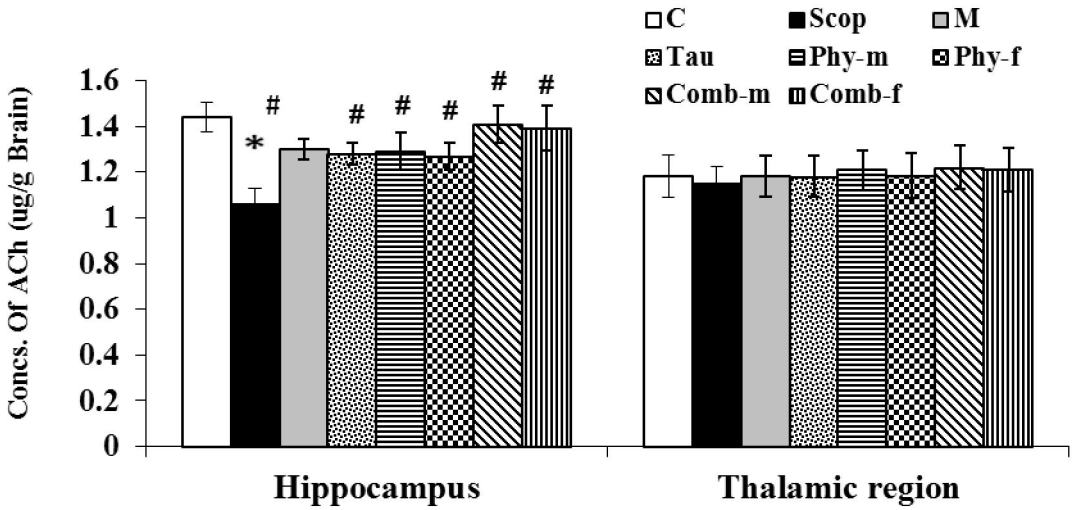

Figure (5): The effect of mefenamic acid, taurine, soy phytoestrogen and their combined treatment on the level of ACh concentration (µg/g) in hippocampus and thalamic region of ♂ & ♀ brain rats after 5 weeks of treatment.
Data expressed as mean values of 8 rats ± SE.
Significant different *v.s* C: $^{*}P<0.05$, *v.s* Scop: $^{\#}P<0.05$, *v.s* Comb-m: $^{a}P<0.05$.

The ACh concentration in thalamic area was not affected by scop-injection while the hippocampus area was significantly affected (P<0.05).

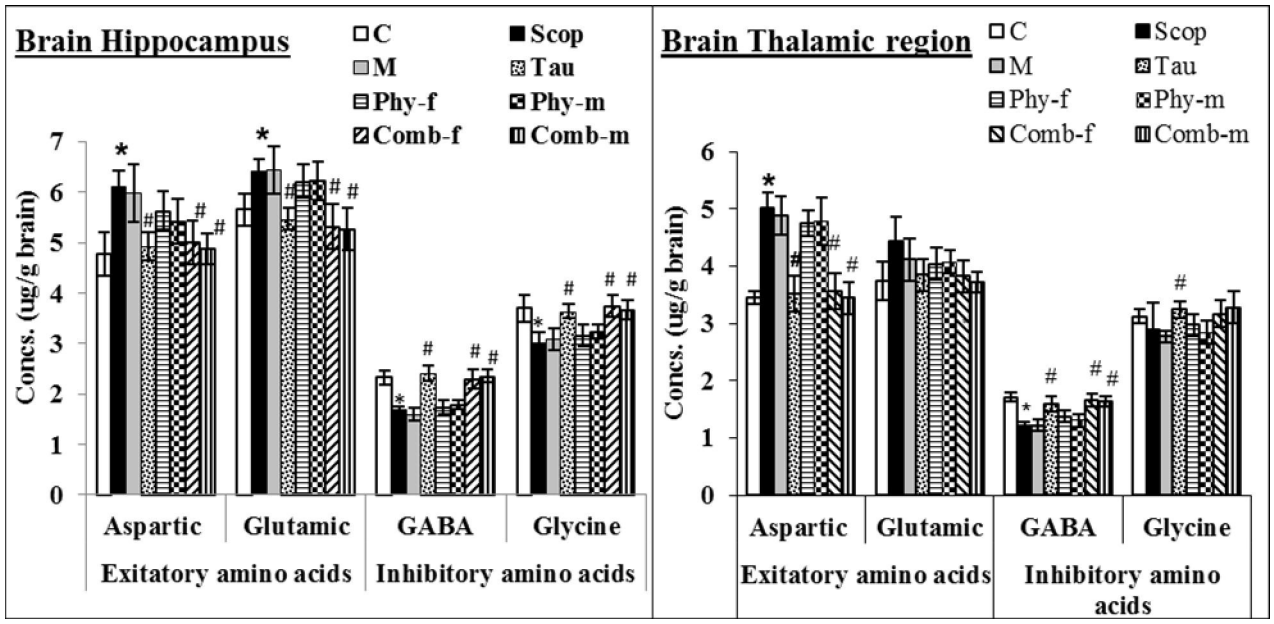

Figure (6, 7): The effect of mefenamic acid, taurine, soy phytoestrogen and their combined treatment on the level of excitatory and inhibitory amino acids concentration (µg/g) in hippocampus (figure 6) and thalamic region (figure7) of ♂ & ♀ brain rats after 5 weeks of treatment.
Data expressed as mean values of 8 rats ± SE.
Significant different *v.s* C: $^{*}P<0.05$, *v.s* Scop: $^{\#}P<0.05$.

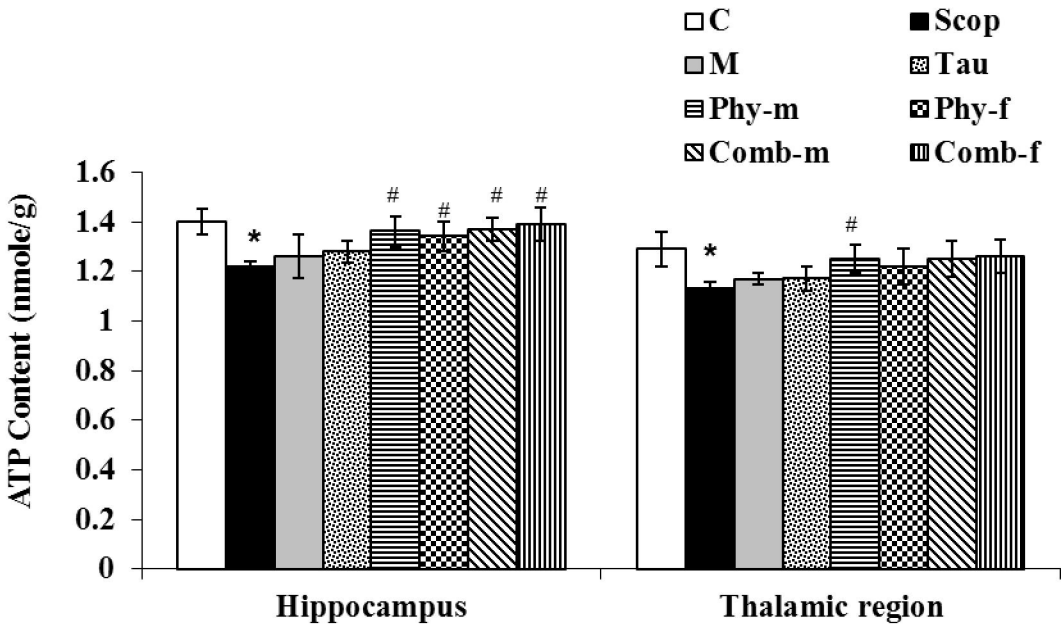

Figure (8): The effect of mefenamic acid, taurine, soy phytoestrogen and their combined treatment on the level of ATP content (nmole/g) in hippocampus and thalamic region of ♂ & ♀ brain rats after 5 weeks of treatment. Data expressed as mean values of 8 rats ± SE.
Significant different *v.s* C: $^{*}P<0.05$, *v.s* Scop: $^{\#}P<0.05$.

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