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Hypericum perforatum extract demonstrates antioxidant properties against elevated rat brain oxidative status induced by amnestic dose of scopolamine

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Abstract

This study was designed to investigate if the impairment of learning and memory induced by acute administration of scopolamine (1.4 mg/kg ip) in rats is associated with altered brain oxidative stress status. The passive avoidance paradigm was used to assess retrieval memory of rats after scopolamine treatment. Following retrieval testing, biochemical assessments of malondialdehyde (MDA), glutathione peroxidase (GSHPx), glutathione (GSH), and superoxide dismutase (SOD) levels/activities as oxidative stress indices were performed. This study also investigated the effect of acute administration of *Hypericum perforatum* extract (4.0, 8.0, 12.0, and 25.0 mg/kg ip), containing flavonoids with documented antioxidant activity, on brain oxidative status of naïve rats treated with amnestic dose of scopolamine. Results showed that administration of 1.4 mg/kg of scopolamine impaired retrieval memory of rats and that such amnesia was associated with elevated MDA and reduced GSH brain levels. In naïve rats, which have not been exposed to conditioned fear, scopolamine administration also increased MDA and reduced GSH levels, although with an increase in brain GSHPx activity. Pretreatment of the animals with *Hypericum* extract (4, 8, and 12 mg/kg) resulted in an antioxidant effect through altering brain MDA, GSHPx, and/or GSH level/activity. Since oxidative stress is implicated in the pathophysiology of dementia, the findings of this study may substantiate the value of scopolamine-induced amnesia in rats as a valid animal model to screen for drugs with potential therapeutic benefit in dementia. Exposure of animals to conditioned fear may be suggested to impair the balance between the rate of lipid peroxidation and the activation of GSHPx as a compensatory antioxidant protective mechanism. It is also concluded that low doses of *Hypericum* extract, demonstrating antioxidant activity, may be of value for demented patients exhibiting elevated brain oxidative status. Since depression commonly coexists with dementia, *Hypericum* extract as a drug with documented antidepressant action may also be a better alternative than several other antidepressant medications that have not been evaluated to test their effect on brain oxidative status during amnesia.

Keywords: Dementia; Scopolamine; *Hypericum*; Antioxidant; Rat

1. Introduction

Partially reduced forms of oxygen are produced in the brain during cellular respiration and, at accelerated rates, during brain insults. This increase in production of free radicals has been reported to cause damage to cell membranes, enzymes, DNA, lipids, and proteins, impairing their function (Gu et al., 1998). Oxidative stress is a disparity between the rates of free radical production and elimination through endogenous antioxidant mechanisms such as the enzymes superoxide dismutase (SOD), glutathione peroxidase (GSHPx), and catalase, as well as the low molecular weight reductants alpha-tocopherol, glutathione (GSH), and ascorbate (Wilson, 1997). This imbalance is initiated by numerous factors including acidosis, transition metals, nitric oxide, dopamine, glutamate, amyloid beta-peptide, and uncouplers of mitochondrial electron transport. Lipid peroxidation is thought to be a prominent and especially deleterious form of neuronal oxidative injury damaging membranes and generating several secondary products, both from fission and endocyclization of oxygenated fatty acids that possess neurotoxic activity

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(Bassett and Montine, 2003). Increased level of malondialdehyde (MDA) as one of the reactive oxidative species (ROS) has been shown to be a reliable index of in vivo lipid peroxidation (Ilic et al., 1999). On the other hand, the tripeptide GSH as a redox regulator participates in the maintenance of oxidant homeostasis and the cellular detoxification of ROS in brain cells (Cruz et al., 2003). GSH depletion has been shown to affect mitochondrial function probably via selective inhibition of mitochondrial complex I activity (Bharath et al., 2002). Therefore, compromised GSH system in the brain has been considered as a relevant index of neuronal oxidative stress (Dringen and Hirrlinger, 2003). Enhanced expression/activity of the endogenous antioxidant enzymes SOD and GSHPx has also been commonly used as relevant indices of brain oxidative stress (Ilic et al., 1999; Maier and Chan, 2002).

Several clinical research findings implicated oxidative stress in the pathophysiology of dementia among other age-related neurodegenerative disorders (Cruz et al., 2003; Floyd, 1999). Impairment of learning and memory, as the most characteristic manifestation of dementia, could be induced chemically in experimental animals by administration of scopolamine, a cholinergic antagonist known to interfere with acetylcholine transmission in the central nervous system (Misane and Ogren, 2003). This experimental animal model of scopolamine-induced amnesia has been extensively used in research to screen for drugs with potential therapeutic value in dementia (Bejar et al., 1999; de Angelis and Furlan, 1995; Hiramatsu et al., 1998b; Mishima et al., 2003; Rubaj et al., 2003). Nevertheless, brain oxidative status in this experimental animal model of scopolamine-induced amnesia has yet to be evaluated. Therefore, this study aimed at investigating whether such impaired cognition due to scopolamine administration is associated with altered oxidative stress indices. A step-through passive avoidance paradigm was used to evaluate the effect of scopolamine (1.4 mg/kg ip) on memory function. Following retrieval testing, biochemical assessments of brain MDA, GSH, GSHPx, and SOD levels/activities, as oxidative stress indices, were conducted.

Hypericum perforatum extract contains flavonoids such as rutin, quercetin, and quercitrin, which demonstrated a free radical scavenging activity in a model of autooxidation of rat cerebral membranes (Saija et al., 1995). An antioxidant activity of quercetin was also demonstrated by inhibition of brain lipid peroxidation, as manifested by lowering MDA while elevating phospholipid contents in a rat model of endotoxemia (Abd El-Gawad and Khalifa, 2001). Therefore, *Hypericum* extract, with a potential antioxidant activity, may be of value in dementia among other disorders of senility in which free radical generation is implicated. In addition, since depression commonly coexists with dementia, there is need to test the effect of different antidepressant medications on elevated brain oxidative status during amnesia (Bassuk et al., 1998; Gallassi et al., 2001; Palsson et al., 1999). Nevertheless, studies investigating the effect of antidepressants, with potential antioxidant activity, on elevated brain oxidative status are lacking. *Hypericum perforatum* extract has been used as an antidepressant in folk medicine for over 2000 years (Maidment, 2000). Today, it is best known for its use in the treatment of mild to moderately severe depressive disorders (Barnes et al., 2001). *Hypericum* extract has been always referred to have a benign side-effect profile compared to tricyclic antidepressants and serotonin-specific reuptake inhibitors (Vitiello, 1999). There has not been a single fatal intoxication of the extract as a monotherapy reported in the literature (Kasper, 2001). *Hypericum* extract, as an efficacious antidepressant medication with a benign side effect profile together with a potential antioxidant activity, was therefore hypothesized to be a better alternative to other antidepressants for depressed demented elderly patients exhibiting elevated oxidative stress status. Therefore, this study also aimed at investigating the effect of *Hypericum* extract, in doses equivalent to the ones used clinically for depression, on brain MDA, GSH, GSHPx, and SOD levels/activities in naïve rats treated with amnestic dose of scopolamine.

2. Methods

2.1. Drugs and animals

Chinese *Hypericum perforatum* was grown in Hebei province and was harvested in August. The aboveground parts (leaves, flowers, and stem) were dried before extraction with 80% ethanol (vol/vol). The herb-to-extract ratio is 12:1 for a 100% native extract. The dried extract was obtained from China National Corporation of Traditional & Herbal Medicine, Beijing, China. The extract solutions were prepared according to Good Manufacturing Practice rules and the quality and identity of the constituents were checked by thin-layer chromatography. The naphthodianthrone content of the herb is reported to be 0.1–0.15% (wt/wt) [0.3% hypericin and 0.7% pseudohypericin]. The hyperforin content is 3% (wt/wt) whereas the flavonoid content is more than 20% (wt/wt).

Scopolamine hydrobromide (Winlab Laboratory Chemicals, Leicestershire, UK) and trichloroacetic acid (PS Park, UK) were used. Glutathione 1,13,3, tetramethoxypropane, 2-thiobarbituric acid, 5,5-dithiobis (2-nitrobenzoic acid), diethylenetriaminepentacetic acid, pyrogallol, SOD, and tris(hydroxymethyl) aminomethane, were all purchased from Sigma-Aldrich, Chemie, Germany. GSHPx test reagent kit (Ransel, Randox Laboratories, UK) was used. The rest of the chemicals and reagents used were of the highest commercial grade.

Hypericum extract was dissolved in 0.3 ml dimethylsulfoxide (DMSO) and completed to the final volume with saline (NaCl 0.9%) to give DMSO concentration of 3% (vol/vol). Scopolamine was dissolved in saline. All drugs or vehicles were administered intraperitoneally in a volume of

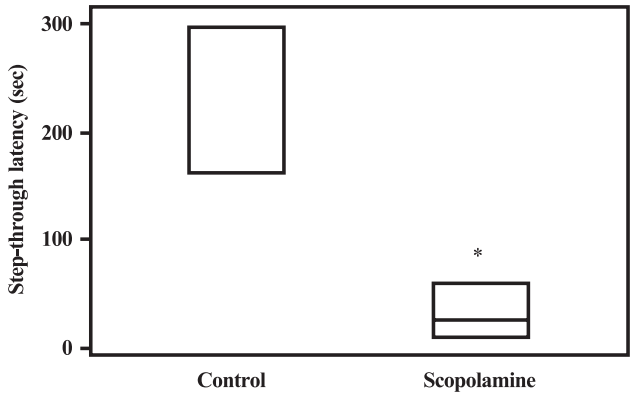

Fig. 1. Effect of scopolamine (1.4 mg/kg ip) on retrieval memory of a step-through passive avoidance task in rats. Scopolamine was dissolved in saline and administered 30 min before the test session. Ten animals were used in each group. The step-through latency values are shown as median (horizontal bar), 25th and 75th percentile (vertical column). * Significantly different from control group ($P < .001$) (Mann–Whitney U test).

1 ml/kg body weight. Control animals received respective vehicle injections, and they were run concurrently with drug-treated groups.

Male albino rats of Wistar strain weighing 200–250g were used. They were kept in a temperature of 23–25 °C with alternating 12-h light and dark cycles and allowed free access to food and water. On the day of the experiment, animals were brought to the experimental room and allowed to habituate to the environmental conditions for approximately 60 min before the beginning of the experiment. Handling and experimentation were conducted in accordance with the international ethical guidelines concerning the care and use of laboratory animals and the experimental protocol was approved by Ain Shams University College of Pharmacy Review Committee for the Use of Animal Subjects.

2.2. Apparatus for Experiment 1

A step-through passive avoidance apparatus was used (UGO BASILE, Italy). It consisted of a Plexiglas box divided into two compartments. One compartment is white and illuminated by a light fixture, featuring a 24-V, 10-W bulb, fastened to the compartment lid. The second compartment is dark and made of black Perspex panels. The two compartments are separated by an automatically operated sliding door. The apparatus included a steel-rod grid floor, which consisted of 40 parallel bars (0.3 cm in diameter, set 1.2 cm apart). The bars of the dark compartment floor are wired to a constant current high-precision eight-pole scrambling circuit located in the controller.

2.3. Procedure for Experiment 1

Habituation session: Only one habituation session was performed in which each animal was first gently placed in the dark compartment for 5 min and returned to its home cage for another 5 min. The animals were then gently placed in the light compartment and the latency to enter the dark compartment with all four feet was measured in seconds. Animals with a step-though latency that was longer than 20 s in the habituation session went through the previous habituation procedures several times, with 5 min between trials, until they enter the dark compartment in less than 20 s (Wright et al., 1996). Animals entering the dark compartment in less than 4 s were noted to be hyperactive and therefore were excluded from the experiment. Such excluded animals were replaced by other naïve ones. The habituation session was performed on these naïve animals to reach an equal number of animals (10) with latencies between 4 and 20 s in each group.

Training session: Training session followed the habituation session, where each rat was trained by gently placing it in the light compartment and when the animal stepped through the dark compartment putting all its paws on the grid floor, the door automatically closed and electric shock (2 mA) was delivered for 3 s. The electrical shock was delivered only during this training session. The animal was then returned to its home cage.

Test session: Twenty-four hours after training, each rat was introduced to the light compartment and the latency to step-through to the dark compartment was recorded as a passive avoidance behavior indicating memory level. Electrical shock was not delivered during this test session. An upper cutoff time of 300 s was set and all tests were run between 10:00 and 15:00 h.

Two groups of rats (10 animals each) went through both the habituation and training sessions. Twenty-four hours after training, one group of animals was injected with scopolamine (1.4 mg/kg ip) 30 min before retrieval testing. The second group of rats was injected with saline 30 min before retrieval testing. Step-through latencies were recorded during the test session. After retrieval testing,

t1.1 Table 1
t1.2 Effect of scopolamine (1.4 mg/kg ip) on brain MDA, GSH, GSHPx, and SOD levels/activities in rats

t1.3 Treatment	MDA (nmol/g wet weight)	GSH (μmol/g wet weight)	GSHPx (μmol/min/g wet weight)	SOD (U/g wet weight)
t1.4 Vehicle	55.01 ± 6.05	2.17 ± 0.09	2.29 ± 0.11	1.58 ± 0.09
t1.5 Scopolamine	111.13 ± 9.88***	0.68 ± 0.07***	2.92 ± 0.14	1.45 ± 0.11

t1.6 After retrieval testing, animals were decapitated and biochemical assessment was performed. Values represent the mean ± S.E. Data were analyzed using a two-tailed Student's t test.
t1.7 *** $P < .001$, significantly different from normal rats.

animals were decapitated and skulls were split on ice and salt mixture. The whole brain of each animal was separated, weighed, and homogenized in ice-cold saline solution using an ice-cold Teflon homogenizer (Glas-Col Terre Haute, USA) for 1 min to make a 20% homogenate. Estimation of MDA content in 0.5 ml of brain homogenate was performed using the thiobarbituric-acid-reactive substances (TBARS) assay of Uchiyama and Mihara (1978). For assessment of GSH content, brain homogenate (0.25 ml) was mixed with 0.25 ml precipitating solution, the mixture was centrifuged at 2000 rpm for 5 min and then GSH content was determined according to Ellman (1959). GSHPx activity was assessed according to Paglia and Valentine (1967). Another 1 ml of brain homogenate was centrifuged at 8500 rpm at 2 °C for 10 min and then assayed for SOD activity according to Marklund and Marklund (1974).

2.4. Procedure for Experiment 2

Four groups of naïve animals ($n = 6$) received different dose levels of *Hypericum* extract (4.0, 8.0, 12.0, and 25.0 mg/kg ip) 60 min and scopolamine (1.4 mg/kg ip) 30 min before decapitation. Two more groups ($n = 6$) served as their amnestic and normal controls receiving scopolamine or saline with respective solvent injections, and they were run concurrently with drug-treated groups. Tissue sampling and biochemical assessment of brain MDA, GSH, GSHPx, and SOD in the six groups of rats were accomplished as in Experiment 1.

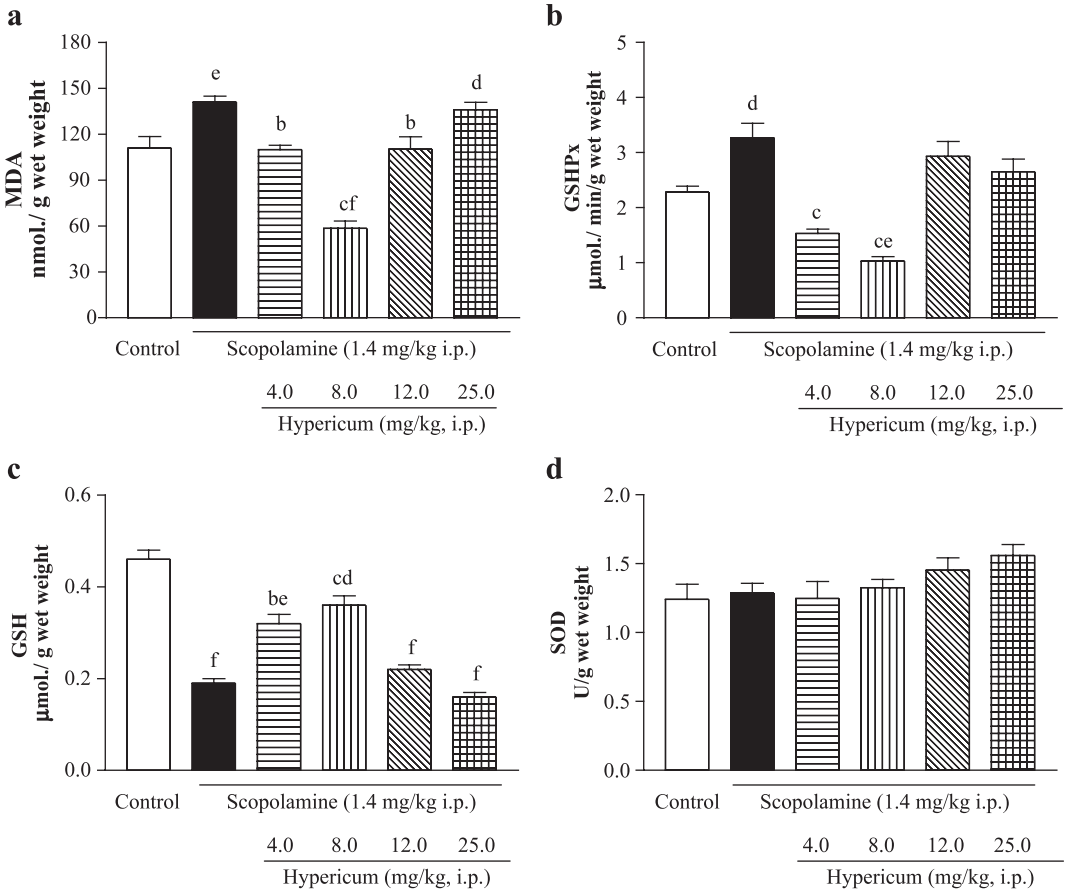

Fig. 2. (a) Effect of *Hypericum* extract on MDA level in rat brain. *Hypericum* extract (4.0, 8.0, 12.0, and 25.0 mg/kg ip) was administered 30 min before scopolamine injection. Six naïve animals were used in each group. Values represent the mean ± S.E. Data were analyzed using one-way ANOVA followed by Tukey HSD test. Significantly different from scopolamine-treated group: $^{b}(P < .01)$, $^{c}(P < .001)$. Significantly different from normal rats: $^{d}(P < .05)$, $^{e}(P < .01)$, $^{f}(P < .001)$. (b) Effect of *Hypericum* extract on GSHPx activity in rat brain. *Hypericum* extract (4.0, 8.0, 12.0, and 25.0 mg/kg ip) was administered 30 min before scopolamine injection. Six animals were used in each group. Values represent the mean ± S.E. Data were analyzed using one-way ANOVA followed by Tukey HSD test. Significantly different from scopolamine-treated group: $^{c}(P < .001)$. Significantly different from normal rats: $^{d}(P < .05)$, $^{e}(P < .01)$. (c) Effect of *Hypericum* extract on GSH level in rat brain. *Hypericum* extract (4.0, 8.0, 12.0, and 25.0 mg/kg ip) was administered 30 min before scopolamine injection. Six animals were used in each group. Values represent the mean ± S.E. Data were analyzed using one-way ANOVA followed by Tukey HSD test. Significantly different from scopolamine-treated group: $^{b}(P < .01)$, $^{c}(P < .001)$. Significantly different from normal rats: $^{d}(P < .05)$, $^{e}(P < .01)$, $^{f}(P < .001)$. (d) Effect of *Hypericum* extract on SOD level in rat brain. *Hypericum* extract (4.0, 8.0, 12.0, and 25.0 mg/kg ip) was administered 30 min before scopolamine injection. Six animals were used in each group. Values represent the mean ± S.E. Data were analyzed using one-way ANOVA followed by Tukey HSD test.

2.5. Statistical analysis for Experiments 1 and 2

The step-through latencies were analyzed by nonparametric analysis of Mann–Whitney U test. Comparisons between means of biochemical data of animals exposed to conditioned fear were analyzed by a two-tailed Student's t test. One-way analysis of variance (ANOVA) followed by Tukey HSD test was used for statistical evaluation of differences in oxidative stress indices in naïve animals. Probability values of less than .05 were considered statistically significant. All statistical analyses were performed using SPSS statistical software package version 8.0.

3. Results

3.1. Experiment 1

Mann–Whitney U test showed that scopolamine administration (1.4 mg/kg) before retrieval testing resulted in shorter latency to step-through during the test session compared to the control group ($U=2.0$, $P<.001$) (Fig. 1). Student's t test showed that in animals exposed to conditioned fear such amnestic dose of scopolamine caused about twofold increase in brain MDA level while reducing GSH level by 69%. Nevertheless, scopolamine administration did not affect either brain GSHPx or SOD activities (Table 1).

3.2. Experiment 2

One-way ANOVA showed an overall significant effect of drug treatment on brain MDA, GSHPx, and GSH levels/activity [$F(5,30)=26.79$, $P<.001$; $F(5,30)=20.03$, $P<.001$; and $F(5,30)=29.92$, $P<.001$, respectively]. On the other hand, one-way ANOVA revealed that drug treatment did not affect brain SOD level [$F(5,30)=1.86$, $P=.13$]. The Tukey HSD post hoc test showed that treatment of naïve animals with scopolamine (1.4 mg/kg) elevated brain MDA and GSHPx level/activity by 27% and 43%, respectively, while reducing GSH level by 59%. Pretreatment of the animals with 4 mg/kg of *Hypericum* extract 30 min before scopolamine injection reduced the elevated level/activity of brain MDA and GSHPx by 22% and 53%, respectively, while elevating the reduced brain GSH level by 68%. Similarly, treatment of the animals with 8 mg/kg of *Hypericum* extract reduced the elevated level/activity of brain MDA and GSHPx by 59% and 69%, respectively. The same dose of the extract caused about twofold increase in reduced brain GSH level. Pretreatment of the animals with 12 mg/kg of *Hypericum* extract also reduced the elevated level of brain MDA by 22%, approaching normal levels though without changing altered GSH or GSHPx level/activity due to scopolamine administration. On the other hand, pretreatment of the animals with 25 mg/kg of *Hypericum* extract did not affect brain MDA, GSHPx, or GSH levels/activities. It is worth mentioning that SOD activity was not altered due to administration of scopolamine or different levels of *Hypericum* extract (Fig. 2a–d).

4. Discussion

In this study, administration of scopolamine (1.4 mg/kg ip) 30 min before retrieval testing induced amnesia reflected by reducing step-through latencies of animals beyond their normal controls. The resultant impairment of memory function after administration of 1.4 mg/kg of scopolamine substantiate previous research finding of induced amnesia in rats intraperitoneally injected with the same dose of scopolamine (Wanibuchi et al., 1994). In animals exposed to conditioned fear, scopolamine treatment (1.4 mg/kg) elevated brain MDA while reducing GSH levels. Nevertheless, this change in oxidative stress indices was not accompanied by altering either GSHPx or SOD brain activities. Elevation of brain oxidative status of amnestic rats resembled the clinical situation where considerable studies reported the incidence of oxidative stress and membrane lipid peroxidation in demented patients (Palmer, 1999). More specifically, the entire brain of patients with Alzheimer's disease (AD) was shown to be subjected to an oxidative challenge (Balazs and Leon, 1994). In addition, the overall peroxidation activity in brains of AD patients was significantly elevated compared to normal subjects (Marcus et al., 1998). Such peroxidation process and the overproduction of free radicals may lead to consumption of detoxifying endogenous antioxidants such as GSH. The effect of oxidative stress in AD on brain GSH level though is rarely investigated. One study showed that AD patients with the epsilon4 allele of apolipoprotein E gene (APOE) displayed lower hippocampal GSH concentration (Ramassamy et al., 2000). Another study reported a decrease in GSH level in substantia innominata and in the cingulate cortex of AD patients (Gu et al., 1998). The above-mentioned oxidative stress consequences may be counteracted by certain enzymatic systems and antioxidants like GSHPx, and SOD, which may eliminate peroxides (Clausen, 1984). Nevertheless, compensatory increase in brain antioxidant enzymes may not occur as a result of the degenerative process in AD (Kish et al., 1986). The activity of brain GSHPx was indeed found to be the same in AD and control samples (Marcus et al., 1998). Similarly, controversial reports exist regarding the activity of SOD in brains of AD patients reflecting specific localized changes in the activity of this enzyme (Chen et al., 1994).

Treatment of naïve animals with scopolamine (1.4 mg/kg) elevated brain MDA and GSHPx level/activity while reducing GSH levels. Nevertheless, brain SOD activity was not altered after treatment of naïve rats with scopolamine. Exposure of animals to conditioned fear during the passive avoidance procedure may be responsible for the difference in response of amnestic animals versus naïve ones with respect to GSHPx activity. It is suggested that conditioned

fear may have resulted in impairing the balance between lipid peroxidation and compensatory activation of GSHPx as an antioxidant protection mechanism. Nevertheless, amnestic dose of scopolamine was reliably demonstrated in this study to elevate rat brain oxidative status through affecting both MDA and GSH levels. This association of oxidative stress with amnesia could be substantiated by the findings of other studies. Intracerebroventricular injection of colchicine and streptozotocin caused impairment of learning and memory with an associated increase in oxidative stress in rat brain (Veerendra Kumar and Gupta, 2002; Sharma and Gupta, 2001). Furthermore, exposure to ozone caused memory impairment accompanied by enhanced rat brain lipid peroxidation levels (Rivas-Arancibia et al., 2000). In this study, the resultant effect of scopolamine on oxidative stress indices may not be fully interpreted since the role of cholinergic neurotransmission in mediation of brain oxidative stress is yet to be defined. Brain oxidative stress was reported following intraventricular administration of ethylcholine aziridinium (AF64A), a toxic analogue of choline that disrupts high-affinity choline transport producing a persistent presynaptic cholinergic hypofunction with the induction of amnesia (Hashimoto et al., 1991; Gulyaeva et al., 1996). Moreover, a relatively low dose of carbachol reported to stimulate presynaptic muscarinic autoreceptors was shown to increase the generation of ROS in neuroblastoma cells (Naarala et al., 1997; Hiramatsu et al., 1998a). Furthermore, decreased numbers of [^{3}H]*N*-methylscopolamine binding sites, observed in the presence of high concentrations of H_2O_2 as an inducer of lipid peroxidation in rat cerebral cortex membranes, was accompanied by a decrease of TBARS levels (Kvaltinova et al., 1993). On the other hand, atropine and scopolamine did not affect rat brain synaptosomes in which a prooxidant activity was produced by treating rats with high lethal doses of armine (Zimakov et al., 1983). Yet, from another perspective, cholinergic nerve stimulation in basal forebrain and hippocampus was reported be excitotoxic, causing tonic–clonic convulsions due to the release of glutamate mediated through the production of ROS (Savolainen et al., 1994). Therefore, the role of cholinergic neurotransmission in mediation of brain oxidative stress has yet to be clarified.

Pretreatment of the animals with 4, 8, and 12 mg/kg of *Hypericum* extract 30 min before scopolamine injection resulted in an antioxidant activity through affecting brain MDA, GSHPx, and/or GSH level/activity. On the other hand, pretreatment of the animals with 25 mg/kg of the extract did not affect levels/activities of any of the measured oxidative stress indices. These results suggest that low doses of *Hypericum* extract demonstrate antioxidant properties by protecting rat brain from elevated oxidative status due to administration of scopolamine. The findings of this study substantiate the findings of several studies reporting an antioxidant activity for low doses of *Hypericum perforatum* extract. In a mouse model exhibiting chronic fatigue syndrome, *Hypericum* extract (10 mg/kg po) significantly reduced elevated lipid peroxidation and restored GSH levels decreased due to chronic swimming (Singh et al., 2002). In addition, low concentrations of *Hypericum* extract were reported to have antioxidant properties when such activity was evaluated in vitro on both human placental vein tissues and a cell-free system (Hunt et al., 2001). This was demonstrated when low concentrations of commercially available formulations of *Hypericum* extract dissolved in alkaline solution (1:2.5, 1:5, 1:7.5, 1:10; 1:20) showed a dose-related inverse relationship of superoxide inhibition with the most dilute concentration causing the largest inhibition of free radical generation. Furthermore, different standardized extracts of *Hypericum perforatum* demonstrated a free radical scavenging activity since they prevented a colored reaction produced by the horseradish peroxidase catalyzed formation of hydroxyl free radicals from hydrogen peroxide (Sloley et al., 2000). Such free radical scavenging capacity was found to correlate with the content of several flavonoids including quercetin and hyperoside. In another study, ialibinone E, hyperguinone B, and hyperforin, as phloroglucinol derivatives isolated from *Hypericum* species, demonstrated a strong reduction in oxidative burst of polymorphonuclear cells after stimulation with *N*-formyl-methionyl-leucyl-phenylalanine (Heilmann et al., 2003). The same study also revealed a potent activity for ialibinone E against the production of oxygen radicals in an H_2O_2/horseradish peroxidase system. Ialibinone E was also found be significantly active as a superoxide scavenger at lower micromolar concentrations in a cytochrome *c* assay. It was therefore hypothesized that the activity of both ialibinone E and hyperguinone B in the different cellular and enzymatic assays is most likely caused by different and perhaps specific mechanisms and cannot be explained by a radical scavenger activity alone. Indeed, it was reported that the mechanism of protection from oxidative insults by flavonoids is highly specific for each compound (Ishige et al., 2001). Three distinct mechanisms of protection were found and included increasing intracellular GSH, directly lowering levels of ROS, and preventing the influx of Ca^{2+} despite high levels of ROS.

In this study, the resultant elevation in brain oxidative status after administration of amnestic dose of scopolamine may further substantiate the value of scopolamine-induced amnesia as an animal model to test for drugs with potential therapeutic benefit in dementia. In addition, *Hypericum* extract, as an antioxidant medication may be of value for demented elderly patients with elevated brain oxidative status. Since depression commonly coexists with dementia, *Hypericum* extract as an antidepressant medication with added advantage of preventing oxidative stress could be a better alternative for depressed demented patients. This study may also provide more evidence that *Hypericum perforatum* extract, as an antioxidant medication, can be a novel type of antidepressant with memory-enhancing properties. Herbal compounds demonstrating antioxidant activity have been reported to ameliorate disturbed cognitive func-

tion in both humans and animals (Howes and Houghton, 2003). Indeed, low doses of *Hypericum* extract were recently reported to enhance cognitive function of experimental animals in several studies (Khalifa, 2001; Klusa et al., 2001; Kumar et al., 2000). In addition, higher doses of *Hypericum* extract (100 and 200 mg/kg), administered once daily for three consecutive days, were effective in antagonizing scopolamine-induced deficit of passive avoidance retention in rats (Kumar et al., 2000). Moreover, in a passive avoidance response test in mice, a single oral dose (1.25 mg/kg) of hyperforin improved memory acquisition and consolidation (Klusa et al., 2001). The same dose of pure hyperforin, and not the same dose administered in 25 mg/kg of the whole extract, was also effective in reversing scopolamine-induced amnesia of passive avoidance task in mice. Hyperforin, by virtue of its inhibition of monoamine uptake (Chatterjee et al., 1998), could be involved in the antiamnestic properties of *Hypericum* extract. Indeed, pharmacological manipulations with several monoaminergic receptor blockers revealed the possible involvement of adrenergic and serotonergic 5-HT1A receptors in the facilitatory effect of *Hypericum* extract on retrieval memory of passive avoidance conditioning in mice (Khalifa, 2001). In addition, hyperforin was reported to inhibit synaptosomal uptake of glutamate (Di Carlo et al., 2001). This property may also explain its antiamnestic activity since antagonists of NMDA-type of glutamate receptors were reported to impair memory indicating a desirable cognitive effect for enhancing glutamatergic neurotransmission (Ohno and Watanabe, 1996). Moreover, hyperforin was shown to act as an antioxidant where it demonstrated a strong reduction in oxidative burst of polymorphonuclear cells after stimulation with *N*-formyl-methionyl-leucyl-phenylalanine (Heilmann et al., 2003). Such antioxidant activity of hyperforin may be involved in its effect on cognitive function. The reported antioxidant activity of flavonoids may also contribute to the nootropic activity of *Hypericum* extract. This could particularly be demonstrated with chronic administration of higher doses of the extract (50 mg/kg). Oral daily administration of 50 mg/kg/day of *Hypericum* extract for seven consecutive days enhanced the acquisition of conditioned avoidance response in rats from Day 2 onward until Day 7 (Klusa et al., 2001). This dose of the extract also had a positive effect on memory consolidation since such memory of learned conditioned avoidance response was largely retained even after 9 days without further treatment or training. Importantly, retention of this learned behavior at day 17 was almost complete in animals treated with 50 mg/kg/day of *Hypericum* extract, which was strikingly different than the response of animals treated with 2.5 mg/kg/day of hyperforin sodium salt corresponding to the hyperforin dose administered in 50 mg/kg/day dose of the extract. Therefore, flavonoids, by virtue of their antioxidant properties, may have potentiated the effect of hyperforin as a nootropic medication through enhancing the retention of learned responses for long period of time. Flavonoids may also be relevant to the memory-facilitating properties of the extract since they were suggested to inhibit monoamine oxidase enzyme (MAO), therefore augmenting monoaminergic neurotransmission that was reported to positively affect cognition (Rosenzweig et al., 1998). Inhibition of MAO was reported to be more evident with *Hypericum* extract fractions containing higher concentrations of flavonoids (Bladt and Wagner, 1994).

In this study, the resultant effect of *Hypericum* extract on MDA, GSH, and GSHPx as oxidative stress indices may be hypothesized to be involved in the reported effect of the drug on cognition. Several studies showed that modulation of these parameters by psychotropic compounds was directly related to their effect on cognitive function. The aqueous extract of *Celastrus paniculatus* seeds (200 and 300 mg/kg for 14 days) was shown to have cognitive-enhancing properties paralleled with a significant decrease in MDA and a simultaneous increase in GSH brain levels (Kumar and Gupta, 2002). The maintenance of normal GSH level was also reported to be important for acquisition of spatial memory since GSH unavailability induced failures in hippocampal synaptic plasticity mechanisms that were related to spatial memory deficits (Cruz et al., 2003). In addition, in aged memory-impaired rats, nerve growth factor treatment was documented to attenuate the increase in hippocampal and cortical GSHPx activity elevated as compensatory responses to cope with the oxidative damage that has been accumulated by the aged brain (Cruz et al., 2003; Cruz-Aguado et al., 1998).

In conclusion, *Hypericum* extract, as an antidepressant and memory-facilitating medication with antioxidant properties, could be a better alternative for depressed demented elderly patients. Future studies should be directed toward testing the effect of other antidepressant medications on brain oxidative status during amnesia. Determination of the effect of such medications on the levels/activities of oxidative stress indices in hippocampal and cortical structures involved in mediation of learning and memory processes would be of interest to neuroscientists. Studies are also needed to investigate the role of cholinergic neurotransmission in mediation of brain oxidative stress. Whether such mediation is dependent on the dose of cholinergic drugs used, their neuronal site of action, and/or on different brain areas involved should be researched. Future research should also attempt to investigate the relationship between conditioned fear and neuronal compensatory antioxidant mechanisms. More studies should also examine if *Hypericum* extract before scopolamine administration alters the amnesia pattern of conditioned animals.

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