CHAPTER 6

ANTIAMNESIC LIKE EFFECTS OF ETHANOLIC EXTRACT OF
H. hookerianum (EEHh) AND ITS GLYCOSIDIC FLAVONOID ENRICHED
EXTRACT (GFHh) IN SCOPOLAMINE INDUCED AMNESIA IN SWISS
ALBINO MICE

6.1. Objective

To analyze the antiamnesic effects of ethanolic extract of H. hookerianum (EEHh) and its glycosidic flavonoid enriched extract (GFHh) in scopolamine induced amnesia in Swiss Albino mice by behavioral and biochemical methods.

6.2. Introduction

Dementia is the syndrome of memory impairment which includes declined progressive effect in cognitive function due to neurodegeneration, which specifically affects older population by interrupting in their daily activities like memory, speaking skills and decision making. The most known type of dementia is Alzheimer’s disease (AD), which is a neurodegenerative disorder related to cognitive and behavioral impairments. Till date, the major or key cause of AD remains unclear, possibly it might be due to the β-amyloid (Aβ) tau protein and aggregation, reduced acetylcholine (ACH) level in the brain, and glutamatergic deficit which are the explored pathogenesis of AD (Kashani et al., 2008). Researchers have found that free radicals produced during oxidative stress and/or inflammatory processes as one of the pathological evidences in AD (Ferreira et al., 2006).

Acetylcholine (ACh) is a significant neurotransmitter connected to learning, memory and cognitive function. Scopolamine, a muscarinic receptor antagonist, interferes with memory in rodents and humans, particularly involved in the processes of learning acquisition and short-term memory (STM) (Jones et al., 1991; Jeong et al., 2008). Scopolamine significantly increases acetylcholinesterase
(AChE) and malondialdehyde (MDA) levels in the cortex and hippocampus (Sakurai et al., 1998; Jeong et al., 2008). Emerging evidences indicate that cerebral circulation plays an important role in learning and memory function in animals and humans (Wyper et al., 1993; Tota et al., 2010; Tota et al., 2011). Scopolamine has been shown to abolish cerebral blood flow (CBF) which was recovered by administration of physostigmine or tacrine suggesting the important role of cholinergic neurotransmission in regulation of CBF (Honer et al., 1988; Ogawa et al., 1994; Tsukada et al., 1997).

Augmentation of life style in human beings worldwide has resulted in proportionate increase in the number of patients suffering from senile dementia. Dementia in elderly individuals is usually recognized as Alzheimer’s disease (AD). AD persons exhibit deterioration in cognitive functions rendering them incapable to perform daily normal activities. However, some evidences suggested that AD can also afflict young individuals as early as 40 years of age (Sugimoto et al., 2002; Joshi et al., 2005). AD patient’s exhibited severe behavioral abnormalities such as irritability, aphasia, apraxia, agnosia and restlessness (Parle et al., 2004; Khachaturian, 1985).

There has been a potential rise in the number of patients suffering from dementia all over the world. Alzheimer’s disease is a genetically heterogenous neurodegenerative disorder, which is slow in onset but relentless in progress (Francis et al., 1999; Parle et al., 2004). Hebert et al (2003) reported that there are around 35 million patients suffering from Alzheimer’s disease all over the world, and United States of America alone has nearly 4.5 million patients. Due to high prevalence and severity of this disease, allopathic system of medicine is yet to provide a satisfactory antidote. Therefore neurobiologists are looking for new directions and alternative strategies for managing this disease among senior citizens worldwide. In India, AD patients are estimated to be less than 3.5 million (Shaji, 2005). These prevalence figures however, point out that the number of patients suffering from AD is considerably small in India when compared to USA.
Therefore, they motivated to explored the potential of certain antioxidants from Indian dishes responsible for this protection against AD (Joshi and Parle, 2007).

Currently, nootropic agents such as piracetam are used in the treatment of AD and they behave as acetylcholinesterase (AChE) inhibitors, which increase the availability of acetylcholine (ACh) at cholinergic synapses. However, the counterpart associated with these agents has limited their use. Since the cholinesterase inhibitors confer only modest benefits, additional non-cholinergic AD therapies are required as emergent. Multipotent and diverse targets are expected to act better than the single target aiming counterparts in the fight against AD (Kang et al., 2005) The impairment of memory in scopolamine induced mice is connected with altered status of brain oxidative stress (El-Sherbiny et al., 2003).

Previous evidences have strongly suggested that the involvement of oxidative stress within the forebrain cholinergic system is the main pathway for AD. The medicinal formulas with antioxidant effects might be beneficial for preserving brain cognitive function. Antioxidant enzymes are involved in the reduction of oxidative stress (Wilson, 1997) which is also seen in affected brain region of Alzheimer's patients. Moreover, the reduction in the level of intracellular oxidized protein under these conditions has been associated with the improvement of cognitive and/or psychomotor functions. Thus, an attempt has been made to find alternative therapeutic agents that could reduce the oxidative stress and promote a functional recovery in neurodegenerative disorders. So the present study aimed to investigate the antiamnesic like effects of EEHh and GFHh in scopolamine induced Swiss Albino mice.

6.3. Materials and Methods

6.3.1. (a) Preparation of plant extract

The procedure was previously described in Chapter: 3.3.6.

6.3.1. (b) Separation of Glycosidic Flavonoid enriched extract of *H. hookerianum* (GFHh) by acid hydrolysis method

The procedure was previously described in Chapter: 3.3.8.
6.3.2. Experimental animals

The procedure was previously described in Chapter: 4.3.2.

6.3.3. Experimental groups

For this purpose, Swiss Albino mice were divided into six groups (n=6), first group served as Control, second group is Scopolamine Induced (SI), while the third and fourth groups are Scopolamine Induced and treated with EEHh (200 and 400 mg/kg) respectively. Fifth group is Scopolamine Induced and treated with GFHh (100 mg/kg). Finally, sixth group treated with reference drug piracetam (Nootropic drug 100 mg/kg) which was administered intraperitoneally (i.p.) to the scopolamine induced groups.

6.3.3. 1. Y Maze Test (YMT)

Y-maze test is used to measure the exploratory and memory performance of mice (Fig.6.1). The mice were placed individually in a symmetrical Y– shaped runway made by wood (33cm×38cm×13cm) and painted with black for 6 min and percentage of alteration of arm entries were counted (Steinberg et al., 1961).

6.3.3. 2. Rectangular Maze Test (RMT)

Assessment of learning and memory can be effectively done by this method. The maze consists of completely enclosed rectangular wooden box with an entry and reward chamber appended at opposite ends (Fig.6.2). The box is partitioned with wooden slats white painted into blind passages leaving just twisting corridor leading from the entry to the reward chamber. Animals were trained prior to the experiment by familiarizing with the rectangular maze for a period of
10 min for 2 h. The transfer latency (time taken to reach the reward chamber) was recorded. For each animal, three readings were taken and the average was taken as learning score. Lower scores of assessment indicate efficient learning while higher scores indicate poor learning and cognitive impairment in animals (Indumathy et al., 2010).

6.3.3 3. Pole Climbing Test (PCT)

When an electrical stimulus is given to mice, it immediately tries to escape from the shock and try to reach near safe place (Fig.6.3). Before the treatment schedule, mice were trained. This pole climbing equipment is made with a dimension of 25x25x25 chamber, fixed with dimly light and sound attenuated box. A smooth stainless steel pole, 2.5 cm in diameter, is suspended by a counter balance weight through a hole in the upper centre of the chamber. A micro switch is activated when the pole is pulled down by 3 mm with weight greater than 200 gm. Twisted shock is delivered to the grid floor of the chamber. A response is recorded when a mice jumps on the pole and activates micro switch. The activation of light and speaker together is used as conditioned stimulus. Each animal was placed six times per day (Vogel et al., 2002).

6.3.3. 4. Novel Object Recognition (NOR) test

The NOR task is conducted in an open type arena with two different kinds of objects. Both objects were generally consistent in height and volume, but are different in shape and appearance. In first trail, the mice were allowed to explore an empty arena. After twenty-four
hours, the mice were exposed to the familiar arena with two identical objects placed at an equal distance and after that the mice were allowed to explore the open field with familiar object and a novel one to test long-term recognition memory capability (Fig.6.4). The time spent in exploring each object and the discrimination index percentages are recorded. This test is useful for assessing impaired cognitive ability in mice and evaluating novel chemical entities for their effect on cognition (Dhingra et al., 2004)

6.3.4. Antioxidant assays

6.3.4.1. Estimation of enzymic antioxidants

The activities of enzymic antioxidants Superoxide Dismutase-SOD (Kakkar et al., 1984), Catalase-CAT (Sinha, 1972), Glutathione Peroxidase –GPx (Rotruck, 1973), Glutathione-S-Transferase – GST (Habig et al., 1974) were studied using standard methods. The protein concentrations present in the tissues were determined by Lowry's method.

6.3.4.2. Estimation of lipid peroxidation

Lipid peroxidation (LPO) of brain tissue homogenate was estimated spectrophotometrically by measuring thiobarbituric acid reactive substances (TBARS) (Ohkawa et al., 1979).

6.3.4.3. Estimation of non-enzymic antioxidants

Reduced glutathione content-GSH (Ellman, 1959), levels of vitamin C (Omaye et al., 1994) and vitamin E (Desai, 1984) were studied using standard methods.

6.3.5 Estimation of brain neurotransmitters

6.3.5.1. Estimation of Acetylcholine esterase

Brain AChE activity was measured using Elman method (1961).
6.4. Statistical analysis

All the data were expressed as mean ± S.D. The results were analyzed with the help of analysis of variance (ANOVA) followed by Bonferroni’s test (multiple comparison). For all the experiments, first the comparisons were made between the control and scopolamine induced group and also between scopolamine induced and treated groups. Statistical difference were considered significant when the 'p' value was <0.005.

6.5. Results

6.5.1. Effect of EEHh and GFHh on behavioral analysis

6.5.1.1. Effect of EEHh and GFHh on Y-Maze Test in scopolamine-treated mice

Fig.6.5. Effect of EEHh and GFHh on Y-maze test in scopolamine- treated mice

Data are expressed as mean ± SD. a p < 0.001 compared with control and scopolamine induced group and # p<0.001 when compared with scopolamine induced and treatment groups using one- way ANOVA followed by Bonferroni’s test as a post- ANOVA test
In Y maze test, % of alteration has been examined in experimental rodents. The scopolamine induced group showed increased % of alteration which indicated the cognitive impairment of mice when compared with control group (p<0.001). In EEHh (200 and 400 mg/kg), GFHh (100 mg/kg) and piracetam (100 mg/kg) treated groups, significantly decreased % of alteration (p<0.001) was observed when compared with scopolamine induced group (Fig.6.5).

6.5.1.2. Effect of EEHh and GFHh on Rectangular Maze Test (RMT) in scopolamine-treated mice

In this test, scopolamine induced group showed high learning scores when compared with control group (p<0.001). However, EEHh (200 and 400 mg/kg) showed reduced learning scores (p<0.01) when compared with scopolamine induced group. As expected, GFHh (100 mg/kg) treated group produced significant reduction in learning scores (Fig. 6.6) when compared with scopolamine induced group (p<0.001).

![Graph showing effects of EEHh and GFHh on Rectangular Maze Test (RMT) in scopolamine-treated mice](image)

**Fig.6.6.** Effect of EEHh and GFHh on Rectangular Maze Test (RMT) in scopolamine-treated mice

Data are expressed as mean ± SD. a p < 0.001 compared with control and scopolamine induced group and # p<0.001 when compared with scopolamine induced and treatment groups using one-way ANOVA followed by Bonferroni’s test as a post-ANOVA test.
6.5.1.3. Effect of EEHh and GFHh on Pole Climbing Test in scopolamine- treated mice

In pole climbing test, scopolamine induced group took more time to climb the pole when compared with control group (p<0.001) in all tested alternative days (1, 3, 5, 7, 9). On 3\textsuperscript{rd} day, EEHh (200 mg/kg) took more time to reach the pole which is non significant when compared with scopolamine induced group. But it produced significant values i.e., took less time to reach the pole (p<0.001). The other treated groups EEHh (400 mg/kg), GFHh (100 mg/kg) and piracetam (100 mg/kg) took less time to reach the pole using climbing apparatus when compared with scopolamine induced group (p<0.001) in all alternative days (Fig. 6.7).
6.5.1.4. Effect of EEHh and GFHh on Novel Object Recognition Test in scopolamine treated mice

Fig. 6.8. Effect of EEHh and GFHh on Novel Object Recognition (NOR) test in scopolamine treated mice

Data are expressed as mean ± SD. a p < 0.001 compared with control and scopolamine induced group and # p<0.001 when compared with scopolamine induced and treatment groups using one-way ANOVA followed by Bonferroni’s test as a post-ANOVA test

During the training period, all the groups spent similar time in exploring two different objects. But during experimental period, the scopolamine induced group spent only less time with new object when compared with control group (p<0.001). The administration of EEHh (200 and 400 mg/kg), GFHh (100 mg/kg) and piracetam (100 mg/kg) increased the exploration of mice with the new object when compared with scopolamine induced group (p<0.001) (Fig.6.8).
6.5.2. Evaluation of *in vivo* antioxidant potential of EEHh and GFHh

6.5.2.1. Effect of EEHh and GFHh on enzymic antioxidants in brain of scopolamine treated mice

Data are expressed as mean ± SD. a $p < 0.001$ compared with control and scopolamine induced group and # $p < 0.001$, @ $p < 0.05$ and $p < 0.01$, when compared with scopolamine induced and treatment groups using one-way ANOVA followed by Bonferroni’s test as a post-ANOVA test.

Fig. 6.9. Effect of EEHh and GFHh on enzymic antioxidants (A) Superoxide Dismutase (B) Catalase (C) Glutathione peroxidase and (D) Glutathione -S- Transferase in brain of scopolamine treated mice.
The effect of EEHh, GFHh, and piracetam on enzymic antioxidants (SOD, CAT, GPx and GST) activities is shown in Fig 6.9. (A, B, C and D) respectively. Biochemical analysis showed significant decrease in SOD, CAT, GPx and GST activities in scopolamine induced group when compared with control group (p<0.001).

Treatment of animals with 200 mg/kg of EEHh resulted in increased activities of all enzymes SOD, CAT (p<0.001), GPX and GST (p<0.05) when compared with control group. The other treated groups, EEHh (400 mg/kg), GFHh (100 mg/kg) and piracetam (100 mg/kg) showed significant increase in all enzyme activities (p<0.001) when compared with scopolamine induced group. Interestingly, GFHh (100 mg/kg) treated group showed more improved SOD, CAT, GPx and GST activities than EEHh (400 mg/kg) treated group. The GFHh treatment remarkably improved antioxidant defense in scopolamine induced mice which is similar to standard drug piracetam.

6.5.2.2. Effect of EEHh and GFHh on non-enzymic antioxidants in brain of scopolamine treated mice

The non-enzymic antioxidant levels of lipid peroxidation, GSH, Vitamin C and E are depicted in Fig. (6.10. (A), (B), (C) and (D).

6.5.2.3. Effect of EEHh and GFHh on LPO in brain of scopolamine treated mice

Biochemical analysis of LPO indicated that the level of MDA was significantly increased (p < 0.001) in scopolamine induced group as the result of increased free radical generation, as compared with the control group. While administration of EEHh (200 and 400 mg/kg), GFHh (100 mg/kg) and imipiramine (10mg/kg) significantly (p < 0.001) lowered the level of MDA indicated the decreased LPO level, when compared with the scopolamine induced group (Fig.6.10.(A)).
Fig. 6.10. Effect of EEHh and GFHh on enzymic antioxidants- (A) Lipid peroxidation, (B) Reduced Glutathione, (C) Vitamin C and (D) Vitamin E in brain of scopolamine-treated mice

Data are expressed as mean ± SD. a p < 0.001 compared with control and scopolamine induced group and # p<0.001 when compared with scopolamine induced and treatment groups using one-way ANOVA followed by Bonferroni’s test as a post-ANOVA test

Reduced glutathione (GSH) and vitamins (C and E) are non-enzymic antioxidants which are also involved in defense mechanisms. The effect of EEHh, GFHh and piracetam on non-enzymic antioxidants is depicted in Fig 6.10. (B, C and
D). Biochemical analysis indicated decreased GSH content, vitamin C and E levels in scopolamine induced group when compared with control group (p<0.001).

Animals treated with EEHh (200 and 400 mg/kg), GFHh (100 mg/kg) and piracetam (100 mg/kg) showed significant increase in all non-enzymic antioxidants, GSH (p<0.001), vitamin C and E (p<0.05) when compared with scopolamine induced group. Among the extracts, GFHh treatment remarkably improved the non-enzymic antioxidants in scopolamine induced mice which is similar to standard drug piracetam.

6.5.3. Effect of EEHh and GFHh on brain acetyl choline esterase in scopolamine induced Swiss Albino mice

6.5.3.1. Effect of EEHh and GFHh on brain acetylcholine esterase in scopolamine treated mice

As shown in figure. 6.11, scopolamine induced group showed increased concentration of acetylcholine esterase (p<0.001) when compared with control group. But significantly decreased acetylcholine esterase activity was observed in treated groups, 200 and 400 mg/kg dose of EEHh, 100 mg/kg of GFHh and 100 mg/kg of piracetam when compared with scopolamine induced group (p<0.001).

![Graph showing effect of EEHh and GFHh on brain Acetylcholine esterase in scopolamine treated mice]

**Fig. 6.11. Effect of EEHh and GFHh on brain Acetylcholine esterase in scopolamine- treated mice**

Data are expressed as mean ± SD. *p < 0.001 compared with control and scopolamine induced group and # p<0.001 when compared with scopolamine induced and treatment groups using one- way ANOVA followed by Bonferroni’s test as a post-ANOVA test.
6.6. Discussion

Age, oxidative stress, free radicals, and inflammation play key role in the development of memory and cognitive impairment which thereby leads to mental conditions like dementia, schizophrenia, and Alzheimer's disease. In the recent days, plants possessing ethnopharmacological properties are used for mental ailments. Nootropics are used as memory enhancers, smart drugs, neuroenhancers, and cognitive enhancers. They may act by improving the availability of neurotransmitters, enzymes, hormones, antioxidants, brain’s oxygen supply and consumption, or by stimulating nerve cell growth. *Bacopa monnieri* (Singh et al., 1997), *Acorus calamus* (Girish Achliya et al., 2004), *Emblica officinalis* (Vasudevan et al., 2007), *Withania somnifera* (Kulkarni et al., 2003), *Evolvulus alsinoides* (Nahata et al., 2010) and *Centella asiatica* (Singh et al., 2012) etc., are well established memory enhancing drugs. With this background information, this set of experiment were carried out to find the nootropic activity of ethanolic extract of *H.hookerianum* (EEHh) and its glycosidic flavonoid enriched extract of *H.hookerianum* (GFHh) in scopolamine induced amnesia in Swiss Albino mice by behavioral and biochemical analysis of brain antioxidants and neurotransmitters. In this study, effects of extracts were compared with the standard drug diazepam.

Analysis of scopolamine induced amnesia studies are widely used as a basic model for anti-Alzheimer and anti-dementia drugs (Abhinav et al., 2010). Central cholinergic system impairment negatively affects learning and memory in a variety of paradigms. Scopolamine, a non-selective muscarinic cholinergic antagonist, is a well known centrally acting cholinergic probe, which interferes with memory and cognitive function in human and experimental rodents (Kanwall et al., 2010). Moreover, scopolamine disrupts acquisition of new tasks and performance on previously learned tasks (Aigner and Mishkin, 2001).

Currently, there has been an increased appreciation in the role of inflammation in the pathogenesis of cognitive impairment. Epidemiologically long-term usage of NSAID (Non Steroidal Anti Inflammatory Drugs) in neurological
disorders confirmed the risk of side effects. Till date, the molecular mechanisms by which NSAIDs intervenes in the pathological mechanisms underlying cognitive impairment and neuronal damage is not clearly understood (Stewart et al., 1997; In’t Veld et al., 2001).

In behavioral test, Y Maze test was used to assess the short-term memory by spontaneous alternation in experimental mice (Foyet et al., 2011). The test relies on the innate tendency of experimental mice to explore a new circumstances (Yusuf et al., 2009). Usually mice tend to make 20-25 entries in a five min trial on the Y maze task, as exhibited by the control group and increased % of alteration specifically indicates the memory impairment due to a characteristic amnestic agent like scopolamine. (Ahmad et al., 2011). This behavior was confirmed in scopolamine induced (SI) group because of the amnesic effects of scopolamine, resulting in transient memory impairment. In EEHh, GFHh and piracetam treated groups; significant reduction in % of alteration was observed due to the spatial recognition of memory and nootropic effect. The results of the current study are similar to the studies carried out by Ahmad et al (2011), Suba et al (2002) and Beppe et al (2014).

In Rectangular Maze test (RMT), the learning scores (time in seconds) obtained by each group are presented in figure (6.6). The learning scores obtained by scopolamine induced groups were higher which led to indirect poor memory retention of mice. But the treated EEHh, GFHh and piracetam groups showed less learning score indicating better and efficient learning of mice. These findings were consistent with the results of Saxena et al (2013).

Pole climbing test is mainly used for testing the learning ability of experimental animals like mice. In this test, mild current shock was given to experimental groups and during this period of latency time taken to climb the pole was recorded. The values were checked on 1st, 3rd, 5th, 7th, 9th day and scopolamine induced group took more time whereas plant extracts and standard drug piracetam treated groups took less time to climb the pole in this apparatus. The results are in
accordance with Koppula and Chou (2012) who reported the memory improved activity of Coriandrum sativum Linn in PCT of scopolamine induced mice.

Novel Object Recognition (NOR) test is widely used to confirm the working memory task. In NOR study, scopolamine induced group spent very less time with novel object due to the memory impairment. The memory performance was restored by the treatment of EEHh (200 and 400 mg/kg), GFHh (100 mg/kg) which indicated that the mice spent more time with novel objects. The current study results are similar to works of Vandana et al (2011) where they used Hibiscus rosa sinensis extracts for cognitive improvement. Similarly, the flavonoid enriched extracts of Cynomorium songaricum (parasitic root plant) improved memory in NOR (Yoo et al., 2014).

After the treatment schedule, mice were sacrificed and neuroantioxidants of enzymatic, non-enzymatic and vitamin activities were analyzed using spectrophotometric methods. Previous clinical studies have reported that oxidative stress is involved in the pathogenesis of memory impairment (Sano et al., 1997; Jeong et al., 2008). The oxygen-free radicals are also involved in the process of age related decline in cognitive performance which may be responsible for the development of Alzheimer’s disease in elderly persons (Querfurth and LaFerla, 2010). It has been reported that memory impairment in scopolamine-induced animal model of dementia is associated with the increased oxidative stress in rat brain and they were treated with H.perforatum (El-Sherbiny et al., 2003). Recently, Hritcu et al (2012) reported that, inhalation of lavender essential oils improved spatial memory deficits in scopolamine-induced animal model of dementia. Overall the finding of in vivo antioxidant studies implies the neuroprotective mechanism of H.hookerianum (EEHh and GFHh) extracts against oxidative and free radical injuries occurring in dementia.

Oxidative stress is mainly reduced by antioxidant enzymes like superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase (CAT) and glutathione-S-transferase (GST). The antioxidant enzyme activities are found to be
reduced in the affected brain region of amnesia patients. Additionally, reduction in the level of intracellular oxidized protein has been associated with cognitive improvement and/or psychomotor effects (El-Sherbiny et al., 2003). Antioxidants are mainly involved in the necrotic and apoptotic process of neurons which may be beneficial in aging and neurodegenerative disorders (Zhou et al., 2008).

Superoxide dismutase (SOD) and catalase (CAT) augmentation in hippocampus and striatum is an important activity of antioxidants (Singh et al., 2009). In this study, SOD, CAT, GPx and GST activities were reduced in the scopolamine induced group, whereas the treated groups (EEHh, GFHh and piracetam) restored the values of all enzymatic activities. The lipid peroxidation level was increased and reduced values of non-enzymatic antioxidants of GSH, vitamin C and E were found in scopolamine induced group. Expected beneficial reduced levels of LPO and increased GSH were obtained with treatment of EEHh, GFHh and piracetam. In addition, vitamin C and E levels were also increased in treated groups.

Clinical studies have also reported that there is an increase in oxidative stress and membrane lipid peroxidation in dementia patients (Francis and Palmer, 1999). Preclinical study of intra cerebroventricular injections of colchicine and streptozotocin have been reported to cause impairment of learning and memory with an associated increase in oxidative stress in experimental rodents (Sharma et al., 2005). Here, the EEHh and GFHh extract significantly reversed the changes in GSH and LPO induced by scopolamine. The maximal effect was observed at GFHh and piracetam group and also normalized the GSH and LPO levels. The findings of the present study substantiate the findings of several other studies reporting the antioxidant activity of H. perforatum (Radulovic et al., 2007; Sagratini et al., 2008) which is the major species of Hypericum groups.

Vitamin C or L-ascorbic acid is an essential nutrient for humans and certain other animal species, in which it functions as a vitamin. Ascorbate is involved in anti-oxidant defense mechanism since it protects the body against oxidative stress
by acting as a free radical scavenger to scavenge the unpaired electrons (Padayatty et al., 2003). It is active within the cells as well as in the plasma and also regenerates α-tocopherol (Vitamin E) from the tocopheroxy radical (Wang and Quinn, 1999). Scopolamine induced group showed reduced levels of vitamin C and E, whereas the treated groups EEHh, GFHh and piracetam showed increased vitamin C and E in mice brain.

The major mechanism of memory impairment is due to neurotoxins and induction of mild anaemic hypoxia. This leads to increased generation of oxidative free radical which impairs ACh synthesis in the cholinergic neurons. Further, increased free radical generation also disrupts the activity of antioxidant enzymes which protect the cholinergic neurons (Parihar and Hemnani, 2004) and these events lead to severe cholinergic neuronal dysfunction with scopolamine. Both EEHh and GFHh showed that the anti-amnestic effect may also involved in the antioxidant activity. The plant extracts such as *Ginkgo biloba* (Christen, 2000; Sastre et al., 2000), *Centella Asiatica* (Veerendra and Gupta, 2002) and *Withania somnifera* (Bhatacharya et al., 1996) are reported to have memory enhancing effects by virtue of their antioxidant property.

There are extensive evidences linking the central cholinergic system to memory (Sun et al., 2007). Previous research studies using *Centella asiatica*, *Glycyrrhiza glabara*, *Zingiber officinale*, *Daucus carota* have displayed a link between the memory improving effect and AchE inhibitory activity (Vasudevan and Parle, 2007). Hence, it is possible that, memory enhancing activity of *H.hookerianum* extract is also mediated by AchE inhibition. The standard drug piracetam also significantly inhibited the rise of brain AchE activity.

According to Proestos et al (2006), the hydroxyl benzoic acid derivatives of flavonoids such as luteolin and quercetin are responsible for antioxidant activity. The memory improving activity of EEHh and GFHh may be attributed to its antioxidant, neuroprotective and memory enhancing activity suggesting that both the extracts might have chemical constituents which possess nootropic activity. The
above behavioral and biochemical results suggest that EEHh and GFHh ameliorate short term memory loss as well as decreasing acetylcholine esterase activity in brain while elevate both enzymic and non-enzymic antioxidants. The observed beneficial effects of EEHh and GFHh may be attributed to its diversified chemical flavonoids. The overall antiamnensic like activity was found to be higher in GFHh when compared to EEHh.
Graphical summary - Antiamnesic like activity of EEHh and GFHh in scopolamine induced Swiss Albino mice

Swiss Albino mice

Scopolamine induction

Treatment with EEHh and GFHh

Oxidative stress

Neuro protection by EEHh and GFHh

**YMT**
- Decreased arm alteration

**RMT**
- Increase in learning scores

**PCT**
- Increased latency in pole climbing

**NOR**
- Spent less time with new object

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