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Prophylactic Effect of *Hypericum Perforatum L.* Extract in Scopolamine Rat Model of Cognitive Dysfunction

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Abstract: The primary goal of the present study aimed to evaluate the prophylactic effect of *Hypericum Perforatum* L. extract on memory dysfunction induced by scopolamine in adult male albino rats (*Rattus norvegicus* Berkenout). Rats administered daily P.O *Hypericum Perforatum* L. extract (350 mg/kg) for two, four, eight weeks and the group of rats at the end of each interval intraperitoneally administered scopolamine (0.4 mg/kg). Scopolamine administration significantly increased the latency time in Morris Water Maze (MWM) task and significantly increased acetylcholinesterase (AChE) activity and significantly decreased noradrenaline, dopamine and serotonin levels in cortex and hippocampus and serum thyroid function (T3, T4 and TSH levels). In groups administered *Hypericum Perforatum L.* pre-scopolamine injection recorded significant decrease in the latency time in MWM, decrease in ACHE activity and significant increase in the monoamines in cortex and hippocampus and serum T3, T4 and TSH levels. The study highlights the prophylactic efficacy of the neuro-protective effect of *Hypericum Perforatum* L. extract against scopolamine-induced cognitive impairment.

Keywords: *Hypericum Perforatum L.,* cortex, hippocampus, acetylcholinesterase monoamines, thyroid.

INTRODUCTION

Memory is the ability of an individual to record sensory stimuli, events, information and retain them over short or long period of time when needed [1]. Cognition involves a large network of brain structures and cannot be understood in certain brain regions. There is interaction between the hippocampus and neocortex, the connection between hippocampus and striatum and coupling between the amygdale and striatum [2]. Alzheimer's disease is associated with multiple neurotransmitter system dysfunctions. The most well studied neuronal system dysfunction is the cholinergic system. Pathological changes have been reported to occur in glutamatergic, cholinergic, noradrenergic and serotonergic transmitter systems [3-5].

Hypericum Perforatum L. is a plant commonly known as St. John´s Wort which has been used as a medicinal herb since ancient times [6]. Hypericum species as *Hypericum perforatum* L. and *Hypericum hircinum*, have been reported to have sedative activity in relation to the CNS. *Hypericum perforatum L.* contains numerous pharmacologically active ingredients, including naphthodianthrones. The naphthodianthrones include hypericin, pseudohypericin [7-9]. For many years, the naphthodianthrone hypericin was considered as the main active constituent however, some studies explain the antidepressant activity of St. John´s Wort solely upon hypericin which is the basis for standardization of commercially available hypericum preparations [6, 10].

The present study aimed to evaluate the prophylactic effects of *Hypericum Perforatum* L. extract via oral administration on memory dysfunction induced by scopolamine in adult male albino rats *(Rattus norvegicus* Berkenhout). The evaluation recorded through behavioral assessment using Morris water maze test in addition to determination of the concentration of monoamine content (noradrenaline, dopamine and serotonin) and acetylcholinesterase activity in the cortex and hippocampus. Also the study aimed to ascertain the effect on thyroid function.

MATERIALS AND METHODS

Experimental Design

The experimental animals used in this study were adult male albino rats *(Rattus nirvegicus* Berkenhout) weighing 100±25 g and of nearly the same age obtained from the National Organization for Drug Control and Research, Egypt. The animals were allowed to free access to food and water *ad libitum* and were maintained on a 12h light/dark cycle regulated at 23°C room temperature throughout the time of the experiment. The handling and care of animals throughout the experimental period were strictly conducted in accordance with the internationally agreed guidelines.

Test Substances

- Scopolamine hydrobromide ($C_{17}H_{21}NO_4 \cdot HBr$) supplied as powder (assay ≥90%) from Sigma St. Louis, MO, USA.

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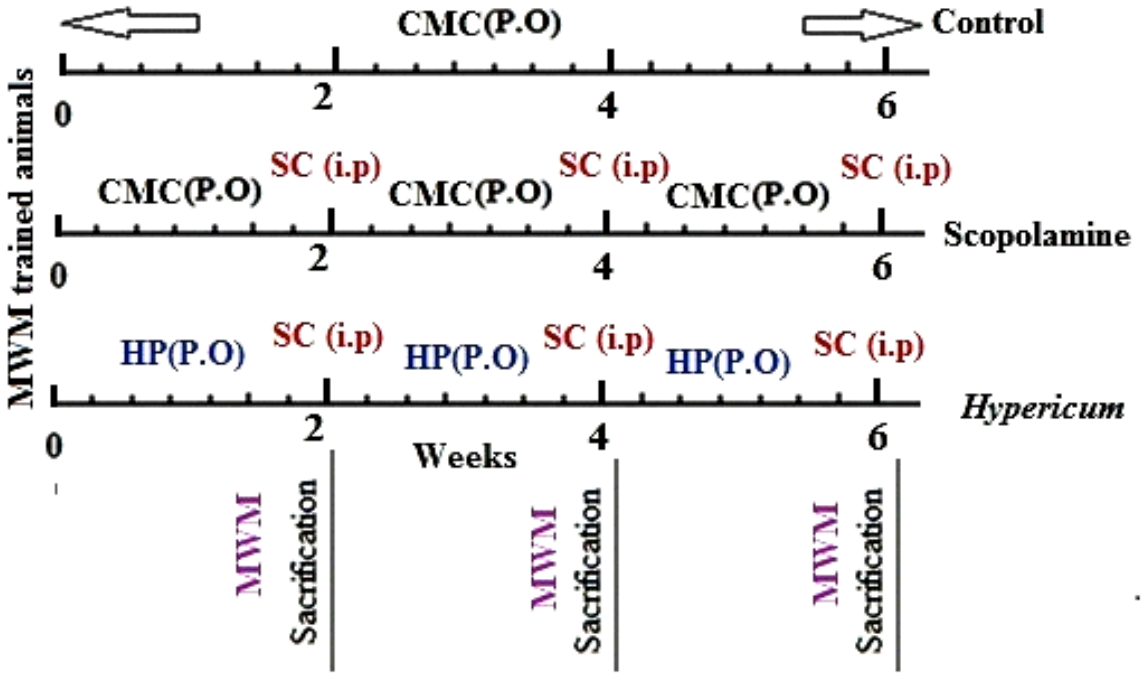

Fig. (1). Diagram of the experimental design.

- Standardized dry extract of Hypericum perforatum L. capsules (0.3% Hypericin) from Life Extension Products. USA. Catalog Number: 01396.

Training of Animals using Morris Water Maze

The experiment started by training using the Morris water maze (MWM) task which is one of the most extensively used tools in behavioral neuroscience to investigate spatial learning and memory [11]. In maze a black circular plastic pool (height 45 cm, diameter 120 cm) was filled with water and kept at 22–25°C. An escape platform (diameter 14x14 cm) was submerged 0.5–1 cm below the surface of the water in position. Pool was located in a room with varied distal room cues (such as posters, lamps), which are visible from the pool and can be used by rodents for spatial orientation. These extra-maze cues are kept constant throughout the testing period. The rats were given the training trials. On training trials, the rats were placed in the pool of water (placed in a different quadrant at each trial) and allowed to remain on the platform for ten seconds, and then returned to the home cage. The rats that did not find the platform within 60 seconds were placed on the platform for 10 seconds. All rats received eight training trials as two times per day for four consecutive days. During each trial session, the time taken to find the hidden platform (latency) was recorded.

Animal Grouping

Fifty four adult trained male albino rats (*Rattus norvigicus* Berkenhout) were divided into 3 groups each of 18 rats treated for two, four and six weeks (n=6/ interval) as diagramed in Fig. (**1**):

1. First group (Control group) which received 1 ml/100 g b.wt./day of 0.5 g/100 ml carboxy-methyl cellulose sodium salt CMC.
2. Second group (Scopolamine group) (SC), treated with the CMC as in the previous group followed by a dose of scopolamine injected intraperitoneally (ip) (0.4 mg/kg) after the last dose at each time interval of the treatment according to Ali and Arafa [12].
3. Third group (*Hypericum perforatum* L. (HP) extract treated group), which was daily administered orally with *Hypericum perforatum* L. extract at a dose 350 mg/kg equivalent to human therapeutic dose according Reagan-Shaw *et al.* [13] followed by a dose of scopolamine injected intraperitoneally (ip) (0.4 mg/kg) after the last dose at each time interval of the treatment.

After 30 minutes of all treatments at every time interval, rats of all groups subjected to MWM task for four days (two trials/day) as previously mentioned then the rats were sacrificed by rapid decapitation. Brains were dissected out then quickly weighed and cleaned. The cortex and hippocampus were separated and each area was divided into two halves; the first half served for acetylcholinesterase activity assay determined by modified Ellman's method [14, 15]. The principle of the method is the measurement of the rate of production of thiocholine produced as a result of acetylthiocholine hydrolysis. The second half was used for the determination of the monoamines neurotransmitters contents by high pressure liquid chromatography system (HPLC) [16]. As regard to the blood sampling, whole blood collected from each animal into dry clean tubes and centrifuged at 4000 rpm to separate serum samples for the thyroid hormones assay. Total triiodothyronine (T3) and thyroxine (T4) levels were measured by radioimmunoassay (RIA) using commercial kits (Coat-A-Coat), while serum thyroid stimulating hormone (TSH) was measured by RIA using a specific rat TSH kit (supplied by Diagnostic Products Corporation DPC, Los Angeles, USA). Radioactivity was determined by the gamma-counter [17].

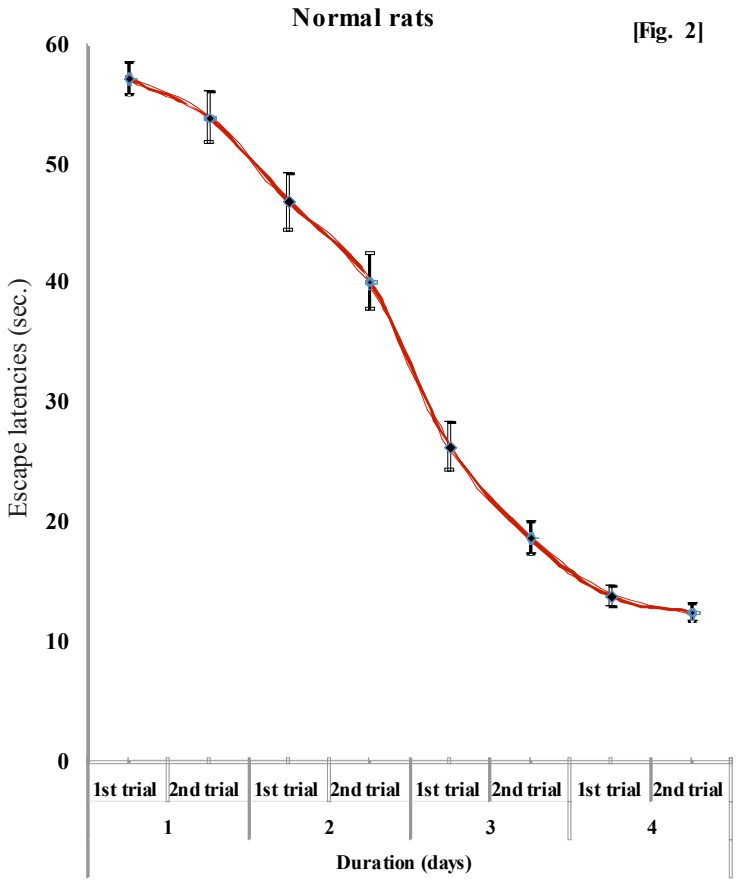

Fig (2). Escape Latency time (Sec) using Morris Water-maze task in normal rats. Data expressed as mean ± standard error**.**

STATISTICAL ANALYSIS

Reported values represent as means ± SE. Statistical analysis was evaluated by one-way ANOVA. Once a significant F test was obtained, LSD comparisons were performed to assess the significance of differences among various groups. Statistical Processor System Support "SPSS" for Windows software, Release 17.0 (SPSS, Chicago, IL) was used.

RESULTS

1. Morris Water Maze Tests

Data in Fig. (**2**) showed the training trials of normal rats for four consecutive days (two trials per day). The latency time in seconds was required for rats to find the hidden platform in water maze task decreased significantly throughout the trial sessions as compared to the required time for the first trial in the first training day. Data in Fig. (**3**) showed that in *Hypericum* treated group exhibited significant decrease in the latency time from the 2nd trial of the 1st day till the end of the experimental trials as compared to the values recorded in SC group in treatment duration related manner.

2. Effect of HP Treatment on Cortex and Hippocampus AChE Activities

Table **1** showed that scopolamine administration significantly increased the AChE activity values in cortex and hippocampus from the corresponding control values at the 2, 4 and 8 weeks durations. In HP treated groups AChE activity decreased significantly as compared to SC group values in cortex and hippocampus after 2, 4 and 8 weeks. Meanwhile, HP groups AChE activities recorded significant increase as compared to the control value in both tissues throughout the experimental durations.

3. Effect of HP Treatment on Cortex and Hippocampus Monoamines Content

In cortex of SC group, the noradrenaline, dopamine and serotonin decreased significantly from the corresponding control at the 2, 4 and 8 weeks. In HP groups cortical monoamines noradrenaline dopamine and serotonin significantly increased from SC group values throughout the experimental duration. Noradrenaline, dopamine and serotonin levels in hippocampus of SC group decreased significantly from the corresponding control value at the three durations. Meanwhile, *Hypericum* treatments significantly increased levels of noradrenaline, dopamine and serotonin compared to the corresponding SC recorded values at the 2, 4 and 8 weeks Table **1**.

4. Effect of HP Treatment on Thyroid Hormones

Scopolamine administration significantly decreased the T4 values by -19.32%, -19.09% and -29.02%, T3 values by -16.19%, -8.91% and -16.07% and TSH values by -10.45%, -14.28%, -17.96% from the corresponding control values at the 2, 4 and 8 weeks duration, respectively. *Hypericum* treatment significantly increased from the SC group values

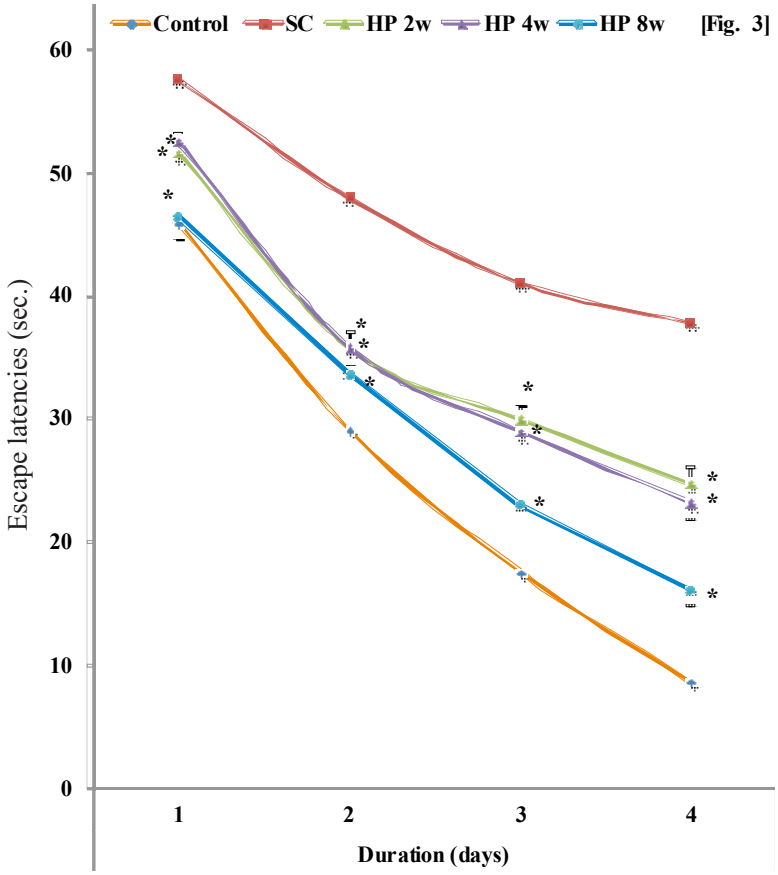

Fig (3). Effect of *Hypericum perforatum* extract (HP) (350mg/kg) treatment for 2, 4 and 8 weeks in rats pre scopolamine (SC) injection on the Escape Latency time (Sec) using Morris Water-maze task. Data expressed as mean ± standard error. (*) indicate P< 0.05 as compared to SC group within the same day of trial.

Table 1. Effect of *Hypericum* Extract (HP) Daily Administration on Scopolamine (SC) Induced Changes in Cortex and Hippocampus Acetylcholinesterase (µmole SH/min/g) and Monoamines Levels (µg/g)

Tissue	Item	Duration / Groups	2 Weeks	4 Weeks	8 Weeks
Cortex	AChE	C	9.40±0.23	9.44±0.19	9.88±0.20
		SC	14.90±0.43*	14.43±0.43*	15.04±0.53*
		HP	12.95±0.59*a	11.40±0.42*a	11.47±0.37*a
	Nor	C	188.68±6.46	204.35±4.46	196.81±7.53
		SC	90.44±3.98*	116.50±4.88*	113.44±4.53*
		HP	156.23±4.19*a	171.95±6.86*a	167.20±6.82*a
	Dop	C	266.48±9.98	248.41±4.46	254.74±8.01
		SC	154.17±4.52*	156.62±4.29*	167.97±4.24*
		HP	193.00±7.12*a	204.00±7.14*a	226.50±4.38*a
	Sero	C	528.83±11.23	515.10±7.79	526.50±6.35
		SC	364.50±10.15*	324.08±5.49*	315.67±5.10*
		HP	430.83±7.83*a	421.50±12.07*a	426.67±7.37*a

Table 1. contd….

Tissue	Item	Duration / Groups	2 Weeks	4 Weeks	8 Weeks
Hippocampus	AChE	C	11.51±0.27	10.84±0.34	10.93±0.39
		SC	17.04±0.51*	16.28±0.55*	16.07±0.40*
		HP	13.91±0.25*a	14.02±0.41*a	12.58±0.33*a
	Nor	C	424.99±15.92	532.26±7.92	471.61±15.25
		SC	240.44±8.07*	311.00±10.30*	311.06±11.47*
		HP	352.19±12.12*a	428.36±10.95*a	412.97±12.51*a
	Dop	C	423.32±6.28	448.01±13.07	438.50±9.31
		SC	234.00±5.69*	287.50±9.08*	268.00±8.27*
		HP	299.50±9.09*a	384.91±10.24*a	386.50±7.98*a
	Sero	C	481.67±9.89	507.50±7.23	493.17±11.33
		SC	308.41±6.57*	329.50±9.10*	309.67±6.83*
		HP	378.58±8.67*a	421.33±9.31*a	434.00±11.54*a

Data expressed as mean ± standard error, (n=6). One Way analysis performed between groups. Multiple range Duncan test with significance level 0.05. superscript (*) indicate Significant as compared to control, superscript (a) Significant as compared to scopolamine(SC) treatment within the same column. (HP) represented the Hypericum treated group.

Table 2. Effect of *Hypericum* Extract (HP) Daily Administration on Scopolamine (SC) Induced Changes in Serum Thyroid Hormones Contents

Item	Duration / Groups	2 Weeks	4 Weeks	8 Weeks
T4 (µg/dl)	C	5.02±0.14	5.55±0.22	5.72±0.29
	SC	4.05±0.13*	4.49±0.15*	4.06±0.26*
	HP	4.85±0.22a	5.31±0.11a	5.47±0.13a
T3 (ng/dl)	C	1.05±0.06	1.01±0.06	1.12±0.03
	SC	0.88±0.03*	0.92±0.04	0.94±0.05*
	HP	1.05±0.06a	1.03±0.07	1.05±0.04
TSH (ng/ml)	C	2.01±0.06	1.68±0.12	2.06±0.06
	SC	1.80±0.04*	1.44±0.03*	1.69±0.05*
	HP	1.97±0.05	1.60±0.06	2.04±0.05a

Data expressed as mean ± standard error, (n=6). One Way analysis performed between groups. Multiple range Duncan test with significance level 0.05. superscript (*) indicate Significant as compared to control, superscript (a) Significant as compared to scopolamine(SC) treatment within the same column. (HP) represented the Hypericum treated group.

the T4 levels recording 19.78%, 18.20% and 34.76% after 2, 4 and 8 weeks, respectively. T3 and TSH values increased by 19.04%, 11.55% and11.65% and 9.23%, 11.38% and 21.25% from the corresponding SC recorded values at the 2, 4 and 8 weeks duration, respectively. Treatment with HP induced significant increase in T3 after two weeks and TSH level only at the end of the experiment as compared to SC group (Table **2**).

DISCUSSION

Scopolamine pretrial administration caused memory impairment in the behavioral tests which has been reported to be associated with dysfunction in central cholinergic system which plays an important role in the learning and memory [18, 19]. The amnestic action produced in animals by scopolamine, has been widely used as a gold standard model in screening of potential cognition-enhancing drugs [20] which may be due to the probable action at the brain multiple receptor subtypes [21]. In rats, both hippocampus and prefrontal cortex appear to be required for working memory tasks, albeit through processing of different informational components [22]. It was proposed that prefrontal cortex lesions might alter neural activity in the hippocampus, a region implicated in memory processing. Indicating that prefrontal cortex normally modulates spatial responses in the hippocampus [23]. Previously scopolamine has been shown to increase extracellular concentrations of

acetylcholine in frontal cortex and hippocampus [24]. In addition, it increased stress in the brain [25, 26]. Imbalance between cholinergic–serotonergic systems may be responsible for the cognitive impairment associated to Alzheimer's disease (AD) in cortex [27].

Hypericum perforatum was found to prevent the stressful assaults in hippocampus and prefrontal cortex on working memory [28]. Trofimiuk *et al*. [29] reported that chronic administration of *Hypericum perforatum L.* (350 mg/kg for 21 days), potently and significantly improved the processing of spatial information in the aged rats using MWM task which in pipeline with our study. Previously Trofimiuk *et al*. [30] discussed that the herb not only prevented stress- and corticosterone-induced memory impairments, but it significantly improved recognition memory. Memory enhancing properties in rodents were observed only after acute or short-term *Hypericum* administration [31, 32]. With regard to the antidepressant effects of Hypericum perforatum, hypericin as originally thought as one of the major constituents responsible for antidepressant activity [33]. Hypericin is a highly lipophilic molecule with a rigid planar configuration. It has been recently shown that hypericin can preferentially incorporate and partition into ordered raft domains of membrane systems [34]. Ion channels are embedded in the lipid environment of the plasma membrane. They can be modulated by dynamic alterations in the microenvironment of the membrane [35-37]. It has been shown that lipid soluble molecules can regulate many K^+ channels, such as Ca^{2+}-dependent BKCa channels [38] and voltage-dependent K^+ channels [39]. Wang *et al.* [40] study found that extracellularly applied hypericin increased action potential duration in hippocampal neurons and that effect of hypericin might be explained by its modulation of voltage-gated K^+ currents. Sauviat *et al*. [41] work showed that hypericin prolonged action potential duration in cardiac myocytes. Hence, small increase in action potential duration can lead to a dramatic increase in synaptic efficiency [42, 43]. Several early in vitro experiments with Hypericum perforatum focused on pathways that alter monoamine neurotransmission in the CNS. Initial reports suggested that inhibition of monoamine oxidase (MAO)-the enzyme that is responsible for the catabolism of biogenic amines is the main mechanism of antidepressant action of Hypericum extract. Based on the work of Suzuki *et al.* [44] hypericin was considered to be an inhibitor of both MAO type A and type B. The mechanism of Hypericum perforatum is believed to involve inhibition of serotonin reuptake, much like the conventional SSRIs antidepressants [45]. Hypericin has shown significant antidepressant effects in behavioral models of major depression in rodents [46]. Due to its SSRIs mechanism of action, Hypericum perforatum could be attributed to the significant improvements in serum levels of T4, TSH in irradiated rats. Besides its anti-stressful [47] and anti-inflammatory properties [48], Hypericum perforatum has a powerful antioxidant activity by normalizing serum total antioxidant capacity as well as Malondialdehyde level [49, 50]. Also *Hypericum perforatum* had the ability for protecting neurons from nitric oxide (NO) oxidative stress-induced cell death which occurs in glucose deprivation (GD)-induced neurotoxicity [51].

Thyroid hormones (including T3 and T4) are recognized as metabolic hormone modulating metabolic pathway through changes in protein, lipid, carbohydrate metabolism [52]. In brain, thyroid hormones affect mitochondrial respiratory enzyme activity [53] and acetate metabolism [54]. There is evidence that the decreased thyroid hormone levels observed in aging are due to lower thyroid-stimulating hormone (TSH) and T3 concentrations, and that lower TSH concentrations may be linked to an impaired pituitary activity. Previous studies have shown that blood concentrations of free thyroxin and TSH decrease during adult life [55]. Thyroid hormone deficiency during fetal and neonatal period in rats produce reduced synaptic connectivity, decreased myelination and alterations in level of neurotransmitters [56, 57]. Also thyroid hormone deficiency reduces the number and distribution of dendritic spines in the auditory cortex [58] and also in pyramidal cells of the visual cortex [59, 60]. Neurologically, hypothyroidism has been associated with memory impairment. Hypothyroidism has been considered a reversible cause of secondary dementia in the elderly [61, 62] and the thyroid function tool has been adopted as a valuable tool in the evaluation of dementia. Therefore, thyroid dysfunction was postulated as a risk factor for Alzheimer disease. Furthermore, the hippocampus which plays an important role in memory and learning and is a major site of Alzheimer's disease pathology, exhibits reductions in thyroid hormone mRNAs in Alzheimer's disease patients [52]. This finding may relate to general cell loss, and thus reduction in cells containing thyroid hormones, which is prevalent in this region in Alzheimer's disease. Neuropathology of Alzheimer's disease involved reductions of cholinergic cell bodies and Ach level in basal forebrain and hippocampus formation [3]. Antidepressants could regulate thyroid hormones [63]. Previous study suggests a probable association between Hypericum perforatum and elevated TSH levels [64]. There is a very close association between thyroid hormones and acetylcholine and cholinergic function which persists throughout life. These effects appear to be isolated to specific cholinergic nuclei and their pathways, notably the basal forebrain and hippocampus [56]. It was investigated that thyroxin treatment enhanced the ability of rats administered scopolamine to learn a spatial memory task as it diminished the scopolamine-induced hyperactivity by augmenting cholinergic function which enhances the cognitive performance [52].

In conclusion, the memory-enhancing ability of *Hypericum perforatum* L. extract may result from its effect on the acetylcholinesterase activity in cortex and hippocampus, and through the increment of the brain level of monoamines. The active ingredient Hypericin of Hypericum perforatum extract initiates multiple actions in cortex and hippocampus interacting with the neuronal circuit through the effect on neurotransmitters levels to maintain homeostasis on the neuronal function may add to its beneficial effects on the memory dysfunction.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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Declared none.

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