EFFECT OF GALANOLACTONE ON LEARNING AND MEMORY: A STUDY ON ROLE OF SEROTONIN.

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Summary

Galanolactone is a diterpenoid possessing anti-5HT effect which can be related to antagonism of $5HT_3$ receptors. $5HT_3$ receptor antagonists improve basal performance in rhodent and primate tests of cognition and inhibit the cognitive impairments. This study is undertaken to investigate the effect of galanolactone (200mg/kg) on both working and long term memory in mice. Spontaneous alteration task paradigm using Y-maze, transfer latency on elevated plus maze, estimation of brain acetylcholinoesterase activity, passive avoidance paradigm and transfer latency on rectangular maze were used as models. Piracetam 200mg/kg p.o. was used as standard. Galanolactone (200mg/kg p.o.) significantly reversed scopolamine induced reduction in spontaneous alteration in behaviour (SAB) and step down latency (SDL) and increase in transfer latency, acetylcholinoesterase (AChE) activity , stepdown error(SDE) and time spent in shock zone (TSZ). Central cholinergic system plays an important role in learning and memory. $5HT_3$ receptor mediates inhibition of acetyl choline release in cortical tissue. Galanolactone is a $5HT_3$ receptor antagonist. So, it might increase the release of acetylcholine to act as memory enhancer. Galanolactone is found to be effective in improving both spatial working memory and long term memory.

Key words: amnesia, 5-HT_3 receptor, scopolamine, transfer latency, AChE, passive avoidance, SAB

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Introduction

The ethanolic extract of *Zingiber officinale* (100mg/kg) significantly improves learning and memory in young mice and also reverses the amnesia induced by diazepam (1mg/kg i.p) and scopolamine (0.4mg/kg i.p). Its memory enhancing activity is attributed to antioxidant and acetylcholine inhibition property [1].

Galanolactone is a diterpenoid isolatated from acetone extract of *Z. oficinale*. It has been reported that acetone extract of ginger and its fraction have anti-5HT effects which can be related to antagonism of $5HT_3$ receptors. The analysis of fraction 2-3 of acetone extract of Z. Officinale (SiO_2 column chromatography, solvent:n-hexane:ethylacetate 30:1 to 10:1) indicated that galanolactone is one of the active constituents [2]

5-HT_3 receptor antagonists like Ondansetron potently improves basal performance in rhodent and primate tests of cognition [3,4] and inhibits the cognitive impairments caused by cholinergic deficits and glutamatergic hypofunction [5]-

So the present study is undertaken to investigate the effect of galanolactone on learning and memory in mice. Both working memory and long term memory were evaluated.

Material and Method

Animals:

Swiss albino mice of either sex (25-30g) procured from Animal house of School of Pharmaceutical sciences, Siksha O Anusandhan University (Regd No. 117/c/08/CPCSEA) were used. They were acclimatized to the laboratory conditions from one week before studies.The animals had free access to food and water and maintained under 12:12hr light and dark cycles.

All experiments were carried out during day time from 9.00 to 14.00 hrs, after due approval from institutional animal ethical committee.

Drug:

Galanolactone:

Ginger (*Zinger officinale*) was purchased from the local market and root were coarsely cut and soaked in three times the volume of acetone for two days. The filtrate was concentrated under reduced pressure below 40^0C, dried and kept in desiccators. Fractionation of the acetone extract was done as per method given by Huang et al., using SiO_2 column chromatography and n-hexane: ethylacetate (30:1 to 10:1) as solvent. Fraction-2 was further separated by silica gel column chromatography using n-hexane: ethylacetate (10:1 to 3:1) as solvent to obtain fractions 2.1 to 2.4. Fraction 2, 3 was further purified by silica gel chromatography with 50% methanol as solvent. The chief ingredient of fraction 2, 3 is reported to be galanolactone (Huang et al., 1991).

A pilot study is initially conducted to select dose of Galanolactone. Galanolactone 200mg/kg was administered 1hr prior to each observation. Control group were administered saline (0.9%w/v NaCl) 2ml/kg body weight. All studies were done for 7 days and drugs were administered between 10-12AM every day. All observations were made on day 8 after 1hr of galanolactone administration.

Piracetam: Piracetam (Nootropil syrup, UCB) was given per oral dose of 200mg/kg prior to each experiment.

Scopolamine:

Scopolamine hydrobromide (Sigma Aldrich, USA) was used in a dose of 0.4 mg/kg i.p.

Effect on Cognition dysfunction:

Spontaneous alteration task paradism using Y-maze:

The spatial working memory was measured through the spontaneous alteration of behaviour in Y-maze (INCO). The Y-maze consists of three identical removal sun mica lined chambers arranged in Y-shape connected to the central chamber. Each arm has a working dimension of approx. 30 ×15× 15 cm with rat presence indicator and hinged top. Each mouse is placed in the central chamber and allowed to move freely through the maze during a 8-minute session. The mouse trend to explore the maze systematically, entering each arm in turn. When mouse enters one arm the rat presence indicator glows. The series of arm entries was recorded. Alteration is defined as the number of successive entries in to the three arms on overlapping triplet sets. The percentage alteration was calculated as the ratio of actual to possible alterations (defined as the total no. of arm entries minus two), multiplied by 100 [6].

Transfer latency on elevated plus maze:

The spatial long term memory was assessed by using elevated plus maze. The elevated plus maze consisted of two open arms (50×10×40 cm) with an open roof. The maze was elevated to a height of 50cm from the floor. Transfer latency (TL) was used as an index of learning and memory. TL is the time in which animal moves from the open arm to the closed arm. Animal not entering the closed arm within 180 sec were assigned the transfer latency of 180s.The animals were trained 24hr prior to testing [7].

Estimation of brain acetyl cholinesterase (AChE) activity:

Ellmen *et al* method was used to estimate whole brain AChE within one hour of sampling in all mice. Brain was weighted and homogenized in 0.1M phosphate buffer (P^H8.0) at a concentration of 5mg tissue/ml of buffer. AChE activity was determined spectrophotometrically at 412nm with 0.01M dithio-bis-nitrobenzoic acid and 0.075M acetylthiocholine iodide as substrate at 25^0C [8]

Passive avoidance paradism:

This method uses the principle of negative reinforcement to examine the long term memory. Step down latency (SDL) was recorded and used as the index to measure the passive avoidance paradigm in a Esho memory evaluator. SDL is the time taken in seconds by the mouse to step down from wooden platform to grid floor with all the four paws on the grid floor [9-11].

Learning, memory and reasoning evaluation using Hebb's William maze (Rectangular maze):

Rectangular maze (INCO) is used for studying learning, memory and reasoning in animals. The clever the rat, the more quickly it is able to make use of past experience and therefore more quickly it learns its way out in the maze. The rectangular maze is divided into chamber A, in which the rat is placed and has a sliding door that is opened to allow the rat to enter the maze; chamber C. The maze, animal has to explore and chamber B, at the other end of maze in which the reward is kept.

An electrical system provides indication when the rat is placed in chamber A, when it leaves it to enter the maze i.e. chamber C and when enters chamber B, thus enabling the reaction time to be noted without observing the animal. A four digit timer records the time taken by animal in exploring the maze [12].

Results

Effect on spontaneous alteration behaviour (SAB) using Y-maze:

The effect of Galanolactone, Piracetam on SAB in normal and scopolamine treated animals is given in Table 1. Scopolamine (0.4mg/kg i.p) significantly ($p<0.05$) reduced the spontaneous alteration behaviour (SAB) in mice as compared to control. Both Piracetam (200mg/kg p.o) and galanolactone (200mg/kg p.o) significantly ($p<0.05$) improved the scopolamine induced reduction in SAB.

Table 1: Effect of galanolactone 200mg/kg on spontaneous alteration behaviour in mice using a Y-maze.

Group	Treatment	Dose	% alteration(3 out of 3)
I	Control	2ml/kg i.p	75.83± 2.13
II	Piracetam	200 mg/kg p.o	72.50 ± 1.78
III	galanolactone	200 mg/kg p.o	71.8± 2.03
IV	Scopolamine	0.4mg/kg i.p	50.66±1.76*
V	Scopolamine + Piracetam	0.4mg/kg i.p 200 mg/kg p.o.	67.66± 0.91*
VI	Scopolamine+ galanolactone	0.4 mg/kg i.p 200 mg/kg p.o	65.33±2.75*
$F_{(5,30)}$			22.08*

*P<0.05. One way ANOVA followed by Dunnet's t-test. Gr II, III, IV are compared with Gr I (Control) and Gr V, VI are compared with Gr IV (Scopolamine)

Effect on transfer latency using Elevated plus maze:

The effect of Galanolactone, Piracetam on transfer latency in normal and scopolamine treated animals is given in Table 2. Piracetam (200mg/kg p.o.) significantly ($p<0.05$) reduced the transfer latency as compared to control but this reduction is not significant in case of galanolactone. Scopolamine as compared to control significantly increased the transfer latency which was significantly reduced by both Piracetam (200mg/kg p.o) and galanolactone (200mg/kg p.o).

Table 2: Effect of galanolactone 200mg/kg on transfer latency in mice using a plus-maze.

Group	Treatment	Dose	Transfer latency(in sec) (Plus maze)
I	Control	2ml/kg i.p	5.83 ± 0.60
II	Piracetam	200 mg/kg p.o	3.67 ± 0.33*
III	galanolactone	200 mg/kg p.o	4.16± 0.30
IV	Scopolamine	0.4mg/kg i.p	15.33 ± 1.65*
V	Scopolamine + Piracetam	0.4mg/kg i.p 200 mg/kg p.o.	4.5± 0.42*
VI	Scopolamine+ galanolactone	0.4mg/kg i.p 200 mg/kg p.o	6.50±0.42*
$F_{(5,30)}$			56.21*

*P<0.05. One way ANOVA followed by Dunnet's t-test.Gr II, III, IV are compared with Gr I(Control) and Gr V, VI are compared with Gr IV(Scopolamine)

Effect of transfer latency using Rectangular maze:

The effect of Galanolactone, Piracetam on transfer latency in normal and scopolamine treated animals is given in Table 3. Scopolamine (0.4mg/kg i.p) significantly increased the transfer latency as compared to control in a rectangular maze. This was significantly ($p<0.05$) reduced by both Piracetam (200mg/kg p.o) and galanolactone (200mg/kg p.o).

Table 3: Effect of galanolactone 200mg/kg on transfer latency in mice using a Rectangular maze.

Group	Treatment	Dose	Transfer latency (in sec) (Rectangular maze)
I	Control	2ml/kg i.p	74.50 ± 2.68
II	Piracetam	200 mg/kg p.o	67.16 ± 3.51
III	galanolactone	200 mg/kg p.o	68.50 ± 2.56
IV	Scopolamine	0.4mg/kg i.p	119.16±4.36 *
V	Scopolamine + Piracetam	0.4mg/kg i.p 200 mg/kg p.o.	79.16 ± 4.36 *
VI	Scopolamine+ Galanolactone	0.4mg/kg i.p 200 mg/kg p.o	84.33±3.52*
$F_{(5,30)}$			30.54*

*P<0.05. One way ANOVA followed by Dunnet's t-test.Gr II, III, IV are compared with Gr I (Control) and Gr V, VI are compared with Gr IV (Scopolamine)

Effect of Acetylcholine esterase (AChE) activity:

The acetylcholine esterase activity was significantly increased by scopolamine (0.4mg/kg i.p) as compared to control (Table 4). The increase in AChE activity by scopolamine was significantly reduced by both Piracetam (200mg/kg p.o) and galanolactone (200mg/kg p.o).

Table 4: Effect of galanolactone 200mg/kg on AChE activity in mice.

Group	Treatment	Dose	AChE activity(μmoles)
I	Control	2ml/kg i.p	132.50± 11.67
II	Piracetam	200 mg/kg p.o	118.33± 10.38
III	galanolactone	200 mg/kg p.o	127.5± 9.46
IV	Scopolamine	0.4mg/kg i.p	253.33 17.11*
V	Scopolamine + Piracetam	0.4mg/kg i.p 200 mg/kg p.o	136.66 ± 14.47*
VI	Scopolamine+ galanolactone	0.4mg/kg i.p 200 mg/kg p.o	143.33±9.63*
$F_{(5,30)}$			16.40*

*P<0.05. One way ANOVA followed by Dunnet's t-test. Gr II, III, IV are compared with Gr I (Control) and Gr V, VI are compared with Gr IV (Scopolamine)

Effect of SDL, SDE and TSZ:

Both Piracetam (200mg/kg p.o) and galanolactone (200mg/kg p.o) significantly reversed the decrese in SDL and increase in SDE and TSZ induced by scopolamine (0.4mg/kg i.p). The results are given in Table 5.

Table 5: Effect of galanolactone 200mg/kg on step down latency (SDL), step down error (SDE) and time spent by animal in shock zone (TSZ) in mice using passive shock avoidance paradism..

Group	Treatment	Dose	SDL (in sec)	SDE(in sec)	TSZ(in sec)
I	Control	2ml/kg i.p	3.17±0.54	7.66±0.33	134.16±3.69
II	Piracetam	200 mg/kg p.o	3.67±0.42	7.16±0.48	126.83±2.71
III	galanolactone	200 mg/kg p.o	2.83±0.40	7.33±0.33	130.83±4.72
IV	Scopolamine	0.4mg/kg i.p	1.33 ±0.40 *	9.83 ±0.60*	235.83±5.35*
V	Scopolamine + Piracetam	0.4mg/kg i.p 200 mg/kg p.o.	4.16±0.03 *	7.55±0.42 *	143.3±4.77*
VI	Scopolamine+ galanolactone	0.4 mg/kg i.p 200 mg/kg p.o	3.83±0.48 *	7.83±0.30 *	153.33±6.00*
$F_{(5,30)}$			6.20 *	5.30 *	80.52*

*P<0.05. One way ANOVA followed by Dunnet's t-test.Gr II, III, IV are compared with Gr I (Control) and Gr V, VI are compared with Gr IV(Scopolamine)

Discussion

The present study aims at evaluating the memory enhancing effect of Galanolactone. Both spatial long term memory and working memory were evaluated. Model like Y-maze, rectangular maze, elevated plus maze, passive shock avoidance paradigm and AchE activity were used. Working memory allows animals to remember information that is useful for subsequent sessions. It is a form of short term memory with limited capacity and an extremely rapid decay. It's importance is more depictive of memory disorder in the Alzheimer's dementia [13]

Scopolamine, a muscarinic receptor antagonist induces amnesia in various animal models and is a widely cited model for human dementia. Scopolamine is used in models of passive avoidance task, spatial memory deficits, and working memory impairment in radial arm maze [17, 18, 19].

Spatial orientation and memory performance in the Y-maze is at least partly dependant on the hippocampus a brain area often affected by aging [14, 15, 16]. Galanolactone improves SAB in Y-maze. So, it may have action on hippocampus.

Passive avoidance response (PAR) is extensively used for screening of drugs affecting learning and memory. The test involves training of rhodents to avoid electric shock (punishment) by curbing a normal behaviour. Then animals are again treated for retention of such learning. Parameter like step down latency (SDL), step down error (SDE) and time spent by animal in shock zone (TSZ) were evaluated. Scopolamine significantly reduced SDL and increased SDE and TSL. Galanolactone (200mg/kg, p.o) significantly ($p<0.05$) reverse the amnesia produced by scopolamine [20-22]

Galanolactone reduced the transfer latency in elevated plus maze. The elevated plus maze served as the exteroceptive behavioural model to evaluate memory in rats. It measures spatial long term memory. Acquisition and retention of memory can be evaluated. A short latency period to reach enclosed arm indicates improved retention compared to significantly low latencies.

The Hebb's William maze or rectangular maze is an incentive-based exteroceptive behavioural model useful for measuring the natural working memory of rats [23-25]. Galanolactone significantly reduced the transfer latency in rectangular maze. So, it may be improving the working memory.

Central cholinergic system plays an important role in learning and memory. *Z. officinale* is a potential anti-cholinesterase agent. In addition to that its anti-inflammatory, antioxidant and neuroprotective effect also contribute towards nootropic activity of *Z. officinale*. In our study Galanolactone decreases AChE activity.

Galanolactone, a diterpenoid isolated from *Z. officinale* shows antagonism of 5-HT_3 receptors. Several studies describe that 5-HT_3 receptor antagonist can act as cognitive enhancer [1,2].

Recently, a growing body of research has focused on participation of serotonin (5-HT) in the neurochemical mechanism of cognition and especially of learning and memory. Various studies have demonstrated that $5HT_3$ receptor antagonists inhibit impairments in cognition caused by cholinergic deficits [26]$5HT_3$ receptor antagonism facilitates the cognitive performance through induction of long term potentiation, possibly through acetylcholine release [27,28]. Antagonists at $5HT_3$ receptors have demonstrated to reverse scopolamine

induced impaired task in passive avoidance paradigm and morris water maze[29,30]. In another study, ondansetron but not tropisetron reversed the memory deficits due to scopolamine treatment in the step through passive avoidance task. Contrariwise, spatial navigation impairments induced by scopolamine successfully antagonized by tropisetron but not ondansetron in morris water maze. There may be subtype of $5HT_3$ receptors and $5HT_3$ receptor antagonists might also bind differently to the receptor to display different effects on cognition[31].

The release of cerebral acetylcholine from terminals in cerebral cortex has been shown to be regulated by 5-hydroxy triptamine (5-HT). There is evidence that 5-HT_3 receptors mediate inhibition of acetylcholine release in cortical tissue[32]. So, galanolactone being a 5-HT_3 receptor antagonist might be increasing the release of ACh.

Conclusion

Galanolactone is found to be effective not only in improving spatial working memory but also long term memory. This memory enhancing effect of galanolactone may be attributed to its $5HT_3$ antagonistic activity which in turn might be increasing the release of acetylcholine in cerebral cortex.

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