The effectiveness of *Grewia hirsuta* (Vahl.) as a nootropic on scopolamine-induced cognitive deficit in young and aged mice

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Abstract
The dementia and loss of cognitive nature among humans are becoming epidemics and causing a socio-economic burden. Amnesia and cognitive behaviours are natural phenomena due to aging. The studies in young and aged mice were conducted in order to rule out the impact of aging. It has become necessary to find novel nootropic agents that are derived from natural resources. In synthetic drugs, we have a piracetam prototype nootropic. The natural products obtained from plant sources are safe and compatible with physiological conditions. Hence, we hope our scientific research may lead to novel nootropic agents with fewer side effects. Nootropic activity was assessed by elevated plus maze and T-maze activity of plant-based extracts with standard piracetam.

Keywords: Nootropic, scopolamine, piracetam, elevated plus maze, T-Maze, cognitive deficit.

Introduction
Grewia hirsuta Vahl. is used by the tribal population to treat heart disease, nerve problems, diabetes and inflammation.[8] The drug is only available at altitudes of up to 4,500 feet, similar to those found in the Himalayas. The plant parts are used for the treatment of nose and eye diseases, cholera, hydrophobia, kidney pain, piles, splenic enlargement, rheumatism and pain in joints and breasts.[3]

The phytochemical investigations revealed the abundance of flavonoids, phenolic acids and tannins. These compounds are scientifically proven to be antioxidants. Free radicals likely to liberate from biomolecules, which are the cause of neurobehavioral changes. The antioxidants are supposed to diminish the effect of free radicals.[4,6]

In this study, we made a qualitative assessment that gave clues about the neuroprotective actions of *Grewia hirsuta*.

Material and Methods
Animals: Swiss albino young mice 2–6 months weight 20–25 g aged mice 18–24 months 30–40 g. The mice of either sex were maintained on a pellet diet and used in the experiments. Protocol approval was obtained from the Institutional Animal Ethical Committee (approval number: 112/PO/Re/S/99/CPCSEA dated February 21, 2019) of S.E.T.'s College of Pharmacy, Dharwad.

Induction of Amnesia: Amnesia is induced by scopolamine in mice.[12]

Exteroceptive Behavioral Models
Elevated Plus-Maze Test (EPM): The EPM is used to evaluate the cognitive behaviour and learning capacity of animals.[13]

The animals were grouped as young and aged mice (n = 6).

Young group
Group 1: Control - Distilled water (10 ml/kg, p.o.)
Group 2: Negative control - Scopolamine (0.4 mg/kg, i.p.)
Group 3: Standard - Scopolamine (0.4 mg/kg, i.p.) + Piracetam (200 mg/kg, p.o.)
Group 4: Lower dose - Scopolamine (0.4 mg/kg, i.p.) + HEEGH (250 mg/kg, p.o.)
Group 5: Higher dose - Scopolamine (0.4 mg/kg, i.p.) + HEEGH (500 mg/kg, p.o.)
All mice were treated for 15 days, the transfer latency was recorded after 30 minutes of the last dose on the 15th day and again on the 16th day.
Aged group
Group 6: Control - Distilled water (10 ml/kg, p.o.)
Group 7: Standard - Piracetam (200 mg/kg, p.o.)
Group 8: Lower dose - HEEGH (250 mg/kg, p.o.)
Group 9: Higher dose - HEEGH (500 mg/kg, p.o.)
All mice were treated for 15 days, the transfer latency was recorded after 30 minutes of last dose on the 15th day and again on the 16th day.

T-Maze Spontaneous Alternation Test: The T-maze-alternation test is a simple method to evaluate spatial working memory in laboratory animals.[5] Albino mice of either sex were divided into nine groups of young and aged mice (n = 6).

Young group
Group 1: Control - Distilled water (10 ml/kg, p.o.)
Group 2: Negative- Distilled water (10 ml/kg, p.o.) + Scopolamine, (0.4 mg/kg, i.p.)
Group 3: Standard- Piracetam (200 mg/kg, p.o.) + Scopolamine (0.4 mg/kg, i.p)

Group 4: Lower dose - HEEGH (250 mg/kg, p.o.) + Scopolamine (0.4 mg/kg, i.p)
Group 5: Higher dose - HEEGH (500 mg/kg, p.o.) + Scopolamine (0.4 mg/kg, i.p)

The mice were treated for 7 days. Scopolamine was induced after 30 minutes of the last dose on the 7th day except in the control group. Spontaneous alternation behavior was recorded after 45 minutes of scopolamine administration.

Aged groups
Group 6: Control - Distilled water (10 ml/kg, p.o.)
Group 7: Standard-Piracetam (200 mg/kg, p.o.)
Group 8: Lower dose - HEEGH (250 mg/kg, p.o.)
Group 9: Higher dose - HEEGH (500 mg/kg, p.o.)
All aged mice were treated for 7 days. On the last day of the treatment, the spontaneous alternation behavior was recorded after 45 minutes of scopolamine administration. [9,14]

The percentage of spontaneous alternation was calculated by using the following formula:

$$\text{Percentage alternation} = \frac{\text{Number of alternations}}{\text{(Total number of arm entries} - 2)} \times 100$$

Estimation of the acetylcholinesterase enzyme (AChE): The brain AChE activity was assessed by using the Ellman's method.[2,15]

AChE activity was calculated using the following formula:

$$R = \frac{\delta \text{ OD Volume of assay (5ml)}}{E} \times \text{mg of Protein}$$

where R = Rate of enzyme activity in "n" moles of substrate (Acetyl choline iodide) hydrolyzed per minute per mg of protein, δ OD = Change in absorbance per minute and E = Extinction coefficient ($1.36 \times 10^4\ M^{-1}cm^{-1}$).

Histopathological examination of the brain: The haemotoxylin and eosin-stained histopathological examinations of the hippocampus regions of CA1, CA2 and CA3 neurons were examined under 100 x and 400 x magnification.[1]

Statistical Analysis: One-way ANOVA with mean ± SEM between groups was analysed by Dunnet's test (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$) as compared to the control and negative control.

Results

Effect on transfer latency in young mice in EPM: Improvement in memory and learning was observed in young mice by comparing the effects of HEEGH (250 and 500 mg/kg) with piracetam (200 mg/kg) and control. The results indicated in table 1 indicate a statistically significant ($P<0.001$) improvement in learning and memory.

Effect on transfer latency in aged mice in EPM: Improvement in memory and learning was observed in aged mice by comparing the effects of HEEGH (250 and 500 mg/kg) with piracetam (200 mg/kg) and control. Results are shown in table 2 which indicate the statistically significant ($P<0.001$) improvement in learning and memory.

Effect on AChE activity in young mice: Scopolamine-induced amnesia was statistically significant at 500 mg/kg of HEEGH in comparison with piracetam (200 mg/kg) (Table 3).

Effect on AChE activity in aged mice: Scopolamine-induced amnesia was statistically significant at 500 mg/kg of HEEGH in comparison with piracetam (200 mg/kg) (Table 4).

Effect on Spontaneous Alteration Behavior in the T-Maze Test: Dose-dependent improvements in learning and memory were seen in the T-Maze test. There was a statistically significant increase in spontaneous alteration, duration of novel arm visits and average percentage alternation compared to the negative control (Table 5 and 6).

Estimation of the AChE in T-Maze test: The levels of AChE in young mice showed a dose-dependent effect.

Table 1
Effect of HEEGH on Transfer Latency in young mice

Group	Treatment	Transfer Latency (Sec) (15th day)	Transfer Latency (Sec) (16th day)
I	Control – Distilled Water	51.48±1.45	49.68±1.32
II	Negative control - Scopolamine	63.53±1.44	57.06±1.80
III	Standard - Piracetam+ Scopolamine	35.94±1.79***	33.74±1.71***
IV	Lower Dose - HEEGH+ Scopolamine	42.06±1.37	38.92±1.24
V	Higher Dose - HEEGH+ Scopolamine	38.62±1.79***	35.67±1.56***

Values are represented as mean ± standard error mean, (n=6), ***P<0.001 compared to control and negative control. One-way ANOVA followed by Dunnet's multiple comparison tests. HEEGH- Hydroethanolic extract of *Grewia hirsuta*

Table 2
Effect of HEEGH on Transfer Latency in aged mice

Group	Treatment	Transfer Latency (Sec) (15^{th} day)	Transfer Latency (Sec) (16^{th} day)
I	Control - Distilled Water	55.84±2.5	52.09±2.6
II	Standard - Piracetam	37.54±1.7***	33.42±1.5***
III	Lower Dose- HEEGH	47.06±1.87	42.56±1.86
IV	Higher Dose- HEEGH	43.74±1.86**	39.35±1.97**

Values are represented as mean ± standard error mean, (n=6), **p<0.01 compared to control. One-way ANOVA followed by Dunnet's multiple comparison tests. HEEGH- Hydroethanolic extract of *Grewia hirsuta*

Table 3
Effect of HEEGH on brain AChE activity in young mice

Group	Treatment	AChE (mM)
I	Control - Distilled Water	108.1±0.0014
II	Negative control - Scopolamine	160.4±0.0085
III	Standard – Piracetam +Scopolamine	115.6±0.0025***
IV	Lower Dose – HEEGH + Scopolamine	144.1±0.0042
V	Higher Dose – HEEGH + Scopolamine	125.8±0.0030**

Values are represented as mean ± standard error mean, (n=6), ***P<0.001 compared to control and **p<0.01 compared to negative control. One-way ANOVA followed by Dunnet's multiple comparison tests. HEEGH- Hydroethanolic extract of *Grewia hirsuta*

Table 4
Effect of HEEGH on brain AChE activity in aged mice

Group	Treatment	AChE (mM)
I	Control - Distilled Water	128.2±0.002
II	Standard - Piracetam	84.54±0.003***
III	Lower Dose - HEEGH	116.8±0.004
IV	Higher Dose - HEEGH	98.14±0.003**

Values are represented as mean ± standard error mean, (n=6), **p<0.01 compared to control. One-way ANOVA followed by Dunnet's multiple comparison tests. HEEGH- Hydroethanolic extract of *Grewia hirsuta*

Table 5
Effect of HEEGH on spontaneous alteration behavior in young mice

Group	Treatment	Memory retrieval (Sec)			Percentage Alternation			Average Percentage Alternation
		60 min	120 min	240 min	60 min	120 min	240 min	
I	Control - Distilled Water	124.2± 4.00	104.6± 3.86	87.41± 3.90	43.63± 3.87	41.99± 3.63	40.12± 3.66	41.91
II	Negative control - Scopolamine	112.7± 5.51	95.09± 4.83	81.44± 4.17	32.77± 4.04	28.27± 3.74	24.73± 3.28	28.59
III	Standard - Piracetam + Scopolamine	142.8± 4.59***	149.3± 4.14***	154.6± 3.75***	51.72± 3.80	57.96± 3.37	62.39± 2.61	57.35
IV	Lower Dose - HEEGH + Scopolamine	122.6± 5.05	123.0± 4.10	127.8± 3.18	35.67± 3.92	36.53± 3.57	37.62± 3.42	36.60
V	Higher Dose - HEEGH + Scopolamine	137.2± 4.43**	140.2± 3.48**	145.7± 2.65**	46.78± 3.97	49.50± 3.46	52.14± 3.59	49.47

Values are represented as mean ± standard error mean, (n=6), ***P<0.001 compared to control and **p<0.01 compared to negative control. One-way ANOVA followed by Dunnet's multiple comparison tests. HEEGH- Hydroethanolic extract of *Grewia hirsuta*

Table 6
Effect on spontaneous alteration behavior in aged mice

Group	Treatment	Memory retrieval (Sec)			Percentage Alteration			Percentage Alteration
		60 min	120 min	240 min	60 min	120 min	240 min	
I	Control - Distilled Water	116.1± 3.41	101.3 ±3.26	88.15± 3.63	30.33± 3.17	28.66± 3.09	26.30± 3.11	28.43
II	Standard - Piracetam	128.6± 3.54***	136.7± 3.54***	143.1± 2.33	40.99± 2.54	42.66± 2.81	45.34± 1.73	42.10
III	Lower Dose - HEEGH	112.7± 4.62**	110.7± 4.62**	101.7± 3.35	35.06± 3.09	32.67± 3.37	28.00± 3.42	31.91
IV	Higher Dose - HEEGH	122.9± 4.24***	128.9± 4.24***	134.0± 3.25	33.10± 2.20	36.10± 2.79	39.24± 2.14	36.14

Values are represented as mean ± standard error mean, (n=6), ***P<0.001 compared to control. One-way ANOVA followed by Dunnet's multiple comparison tests. HEEGH- Hydroethanolic extract of *Grewia hirsuta*

Table 7
Effect of HEEGH on AChE activity in young mice

Group	Treatment	AChE (µ moles)
I	Control - Distilled Water	127.0±2.71
II	Negative control - Scopolamine	200.5±5.41
III	Standard – Piracetam + Scopolamine	136.5±2.66***
IV	Lower Dose – HEEGH + Scopolamine	177.2±3.81**
V	Higher Dose – HEEGH + Scopolamine	155.0±3.31***

Values are represented as mean ± standard error mean, (n=6), ***P<0.001 compared to control and **p<0.01 compared to negative control. One-way ANOVA followed by Dunnet's multiple comparison tests. HEEGH- Hydroethanolic extract of *Grewia hirsuta*

Table 8
Effect of HEEGH on whole brain AChE activity in aged mice

Group	Treatment	AChE (µmoles)
I	Normal control - Distilled Water	133.1±2.31
II	Standard - Piracetam	96.99±2.96***
III	Lower Dose- HEEGH	125.9±3.65
IV	Higher Dose- HEEGH	113.4±3.87**

The values are expressed as mean ± SEM, (n=6), ***P<0.001 compared to control; P<0.01 compared to negative control. One-way ANOVA followed by Dunnet's multiple comparison tests.

A reduction in AChE concentration enhances acetylcholine, which is a marker of brain function like memory and cognitive properties (Table 7). It was observed in aged mice that the administration of piracetam at 200 mg/kg resulted in a significantly (P<0.001) decrease AChE (96.99±2.96) as compared to the control group (133.1±2.31). The activity of AChE after administration of HEEGH at the dose 500 mg/kg resulted in a significant (p<0.01) decreased AChE value (113.4±3.87) as compared to the control group. Piracetam and HEEGH have significant nootropic activity in old age induced amnesia in mice (Table 8).

Histopathological Study on the Hippocampus

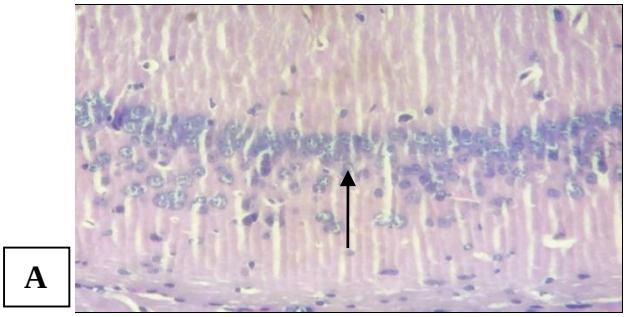
1. Effect of HEEGH on the hippocampus in young mice: Histopathological examination of the hippocampus of the control group showed an intact architecture consisting of the *Cornu ammonis* [CA] and dentate gyrus [Figure 1A]. Some cells have a pale nucleus [Figure 1B, Arrow]. The CA 3 showed a few layers of large pyramidal cells with a vesicular nucleus and abundant cytoplasm [Figure 1C Arrow]. Sections studied from the negative control group showed a loss of intact architecture [Figure 1D]. Plenty of degenerated shrunken cells with hyperchromatic nuclei are noted [Figure 1F Arrow]. In the section studied from the standard group, the hippocampus showed an intact architecture [Figure 1G Arrow].

The CA2 showed layers of small to medium pyramidal cells with vesicular nuclei and moderate cytoplasm without shrinkage or necrosis [Figure 1I Arrow]. In the section studied from higher dose-treated young mice, the hippocampus showed an intact architecture consisting of the *Cornu ammonis* [CA] and dentate gyrus [Figure 1M Arrow]. The CA 2 showed layers of small to medium pyramidal cells with vesicular nuclei and moderate. Some cells have a pale nucleus [Figure 1N Arrow].

Effect of HEEGH on the hippocampus of brain in aged mice: Histopathological examination of the hippocampus of the control-aged mice showed intact architecture consisting of the *Cornu ammonis* [CA] and dentate gyrus [Figure 2A Arrow]. Some cells have a pale nucleus [Figure 2B, Arrow]. The CA 3 showed a few layers of large pyramidal cells with a vesicular nucleus and abundant cytoplasm [Figure 2C Arrow]. In the section studied from standard-group-aged mice, the hippocampus showed an intact architecture consisting of the *Cornu ammonis* [CA] and dentate gyrus [Figure 2D Arrow]. Degenerated shrunken cells with hyperchromatic nuclei are noted [Figure 2F Arrow].

In the section studied from lower dose-treated aged mice, the hippocampus showed an intact architecture consisting of the *Cornu ammonis* [CA] and dentate gyrus [Figure 2G Arrow]. The CA 2 showed layers of small to medium pyramidal cells with vesicular nuclei and moderate cytoplasm without shrinkage or necrosis [Figure 2H Arrow]. In the section studied from higher dose-treated aged mice, the hippocampus showed an intact architecture consisting of the *Cornu ammonis* [CA] and dentate gyrus [Figure 2J]. The CA 2 showed layers of small to medium pyramidal cells with vesicular nuclei and moderate cytoplasm. Some cells have a pale nucleus [Figure 2K Arrow].

Control Group



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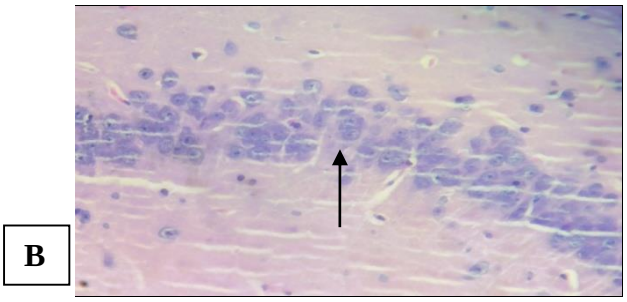

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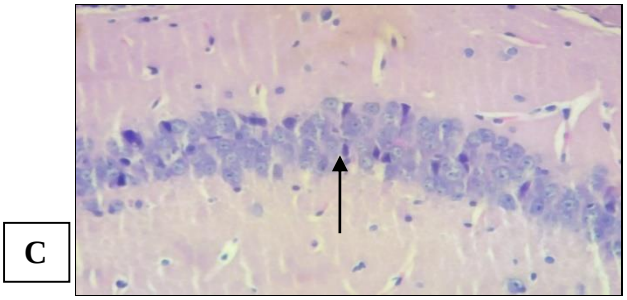

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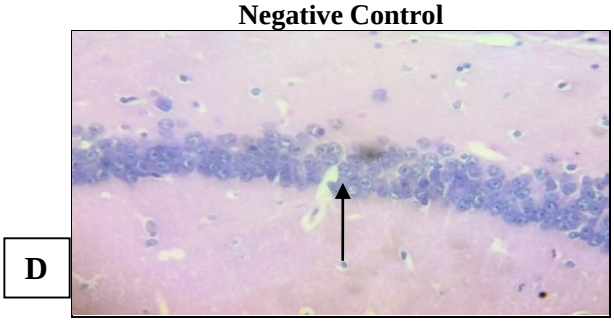
Negative Control



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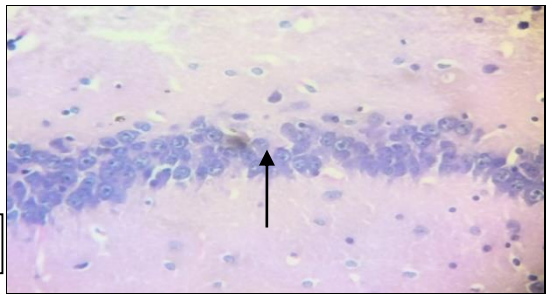

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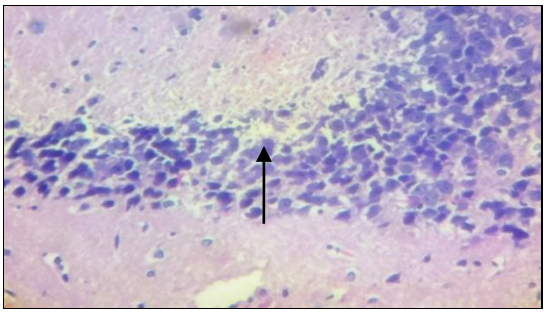

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Standard Group

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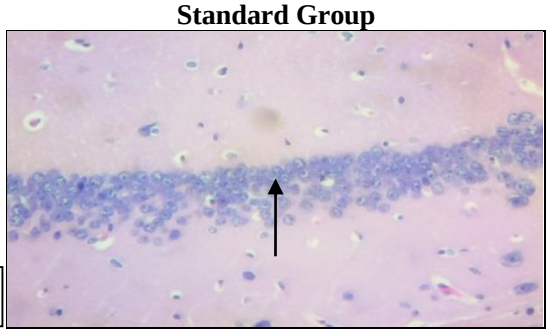

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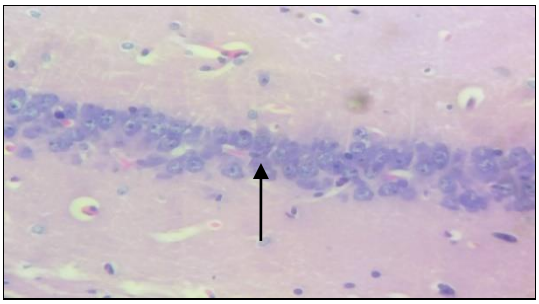

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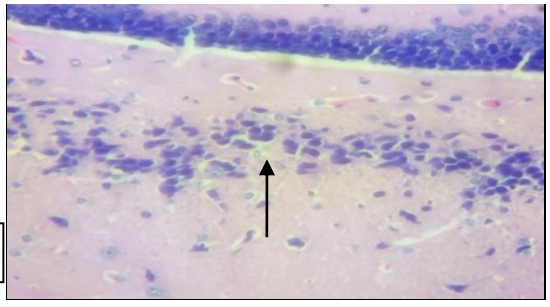

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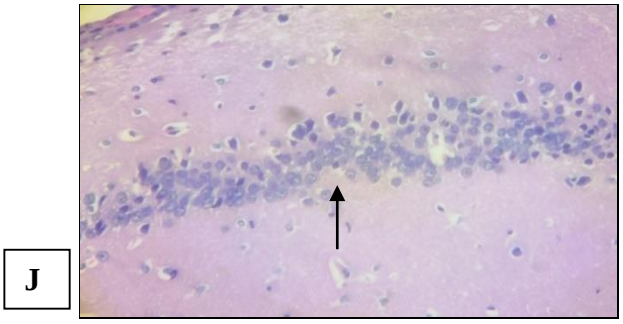
Lower Dose - HEEGH (250 mg/kg)



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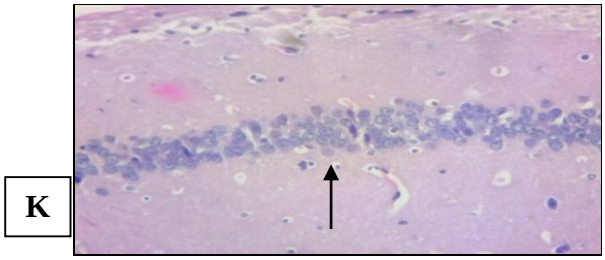

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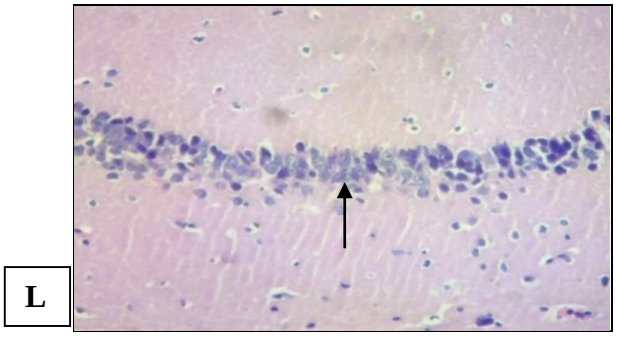

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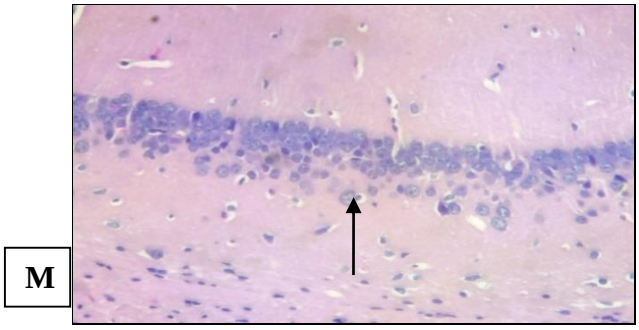
Higher Dose- HEEGH (500 mg/kg)



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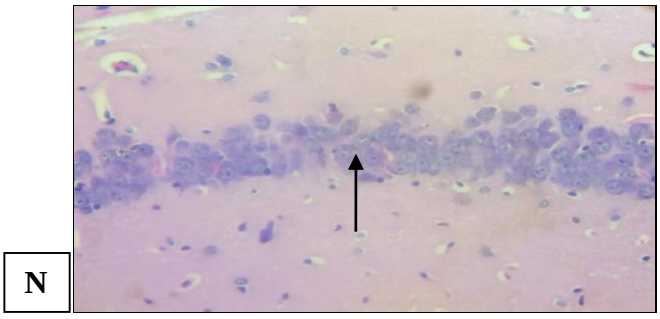

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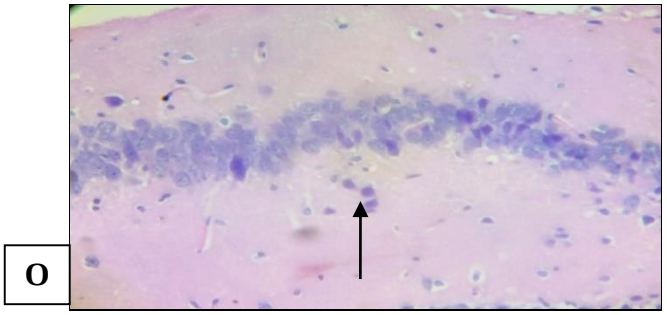
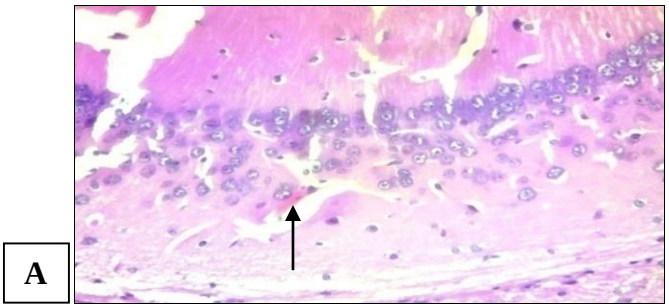


CA3

Figure 1: Section of the hippocampus of young mice (100 x and 400 x [H and E])

Control Group



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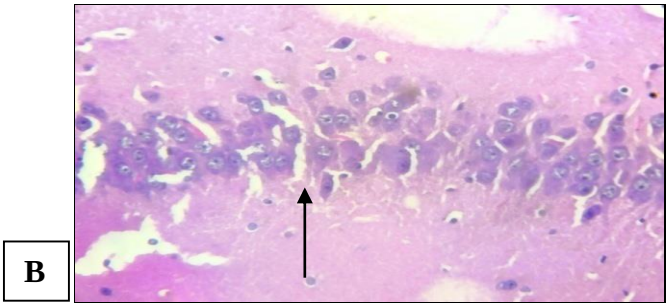

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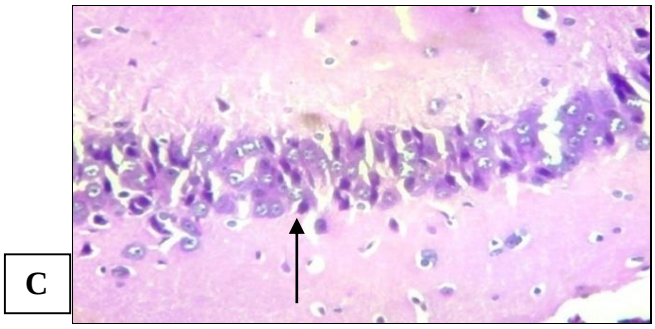

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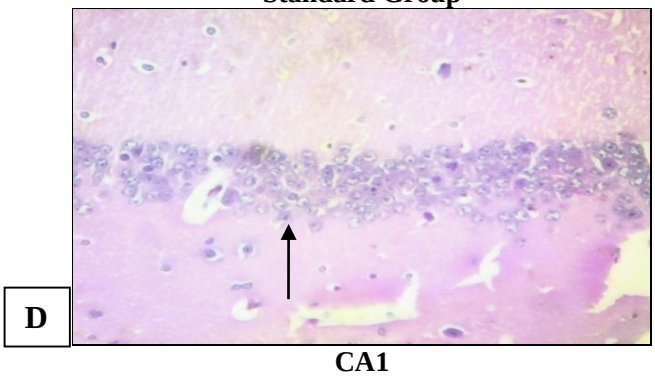
Standard Group



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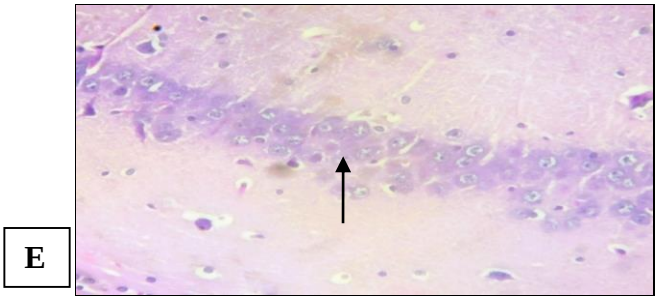

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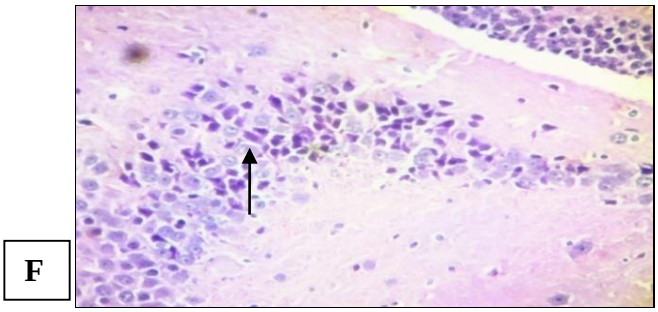

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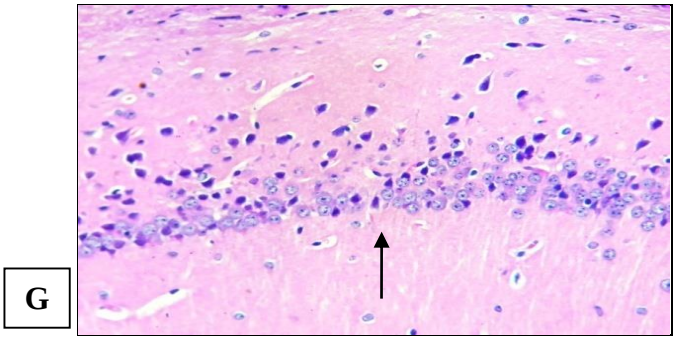
Lower Dose - HEEGH



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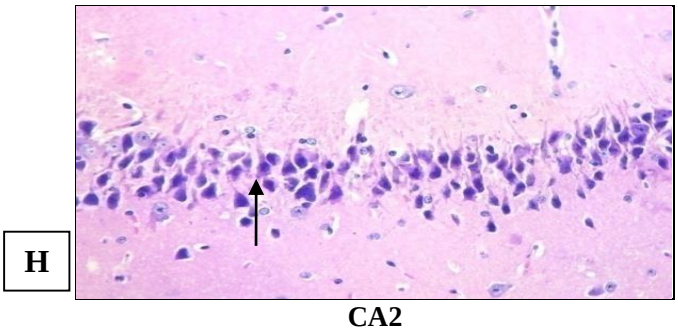
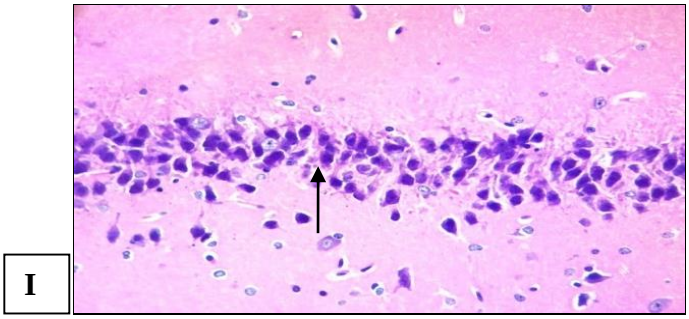
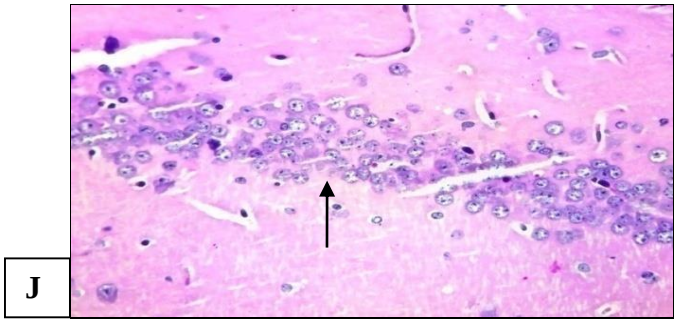


CA2



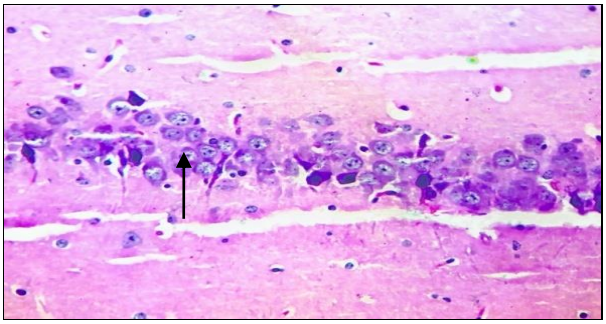
CA3

Higher Dose- HEEGH



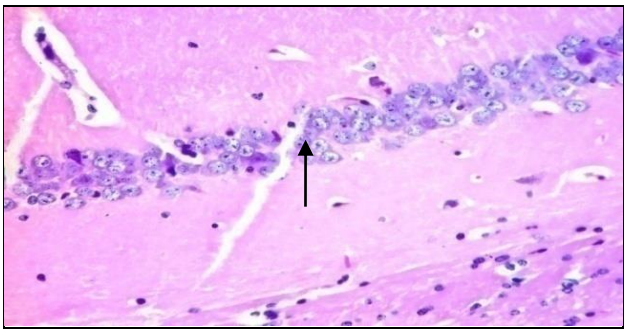
J

CA1



K

CA2



L

CA3

Figure 2: Section of the hippocampus of aged mice (100 x and 400 x [H and E])

Discussion

There are very few drugs, natural or synthetic, for the treatment of amnesia that is age-related and chemically induced. Amnesia can be assessed in animal models like the elevated plus maze, T-maze, Y-maze, Morris water maze test etc.[7] Nootropic drugs are smart drugs that are known to enhance cognitive properties, memory and learning. There are very few nootropic agents available for the treatment of amnesia. Information on the mechanisms of amnesia and nootropic drugs is in the discovery phase.[10] Animal models for nootropic activity include indirect activity and it is necessary to establish a mechanism of amnesia and the nootropic activity of drugs.

Current information assesses parametric approach for which correlation needs to be established. The treatment of amnesia is empirical and has to depend upon observational models.[11] The HEEGH which is obtained from *Grewia hirsuta,* is rich in phenolic acids, flavonoids and tannins. All these compounds are known to act as antioxidants and free radical scavenging might be the cause of their neuroprotective properties.

Conclusion

The extract of *Grewia hirsuta,* which is rich in phenolic compounds, is a potential antioxidant agent. The antioxidant nature of the plant is going to be neuroprotective against oxidative damage caused by ageing, stress and a sedentary lifestyle. In this study, we established the nootropic activity of *Grewia hirsuta* in animal experimental models.

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