EVALUATION OF LEARNING AND MEMORY ENHANCING ACTIVITY OF COCCINIA GRANDIS FRUITS IN RATS

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ABSTRACT

Objective: To assess the learning and memory enhancing activity of the Coccinia grandis fruits in rats using Elevated plus maze (EPM), Hebb-William maze (HWM) and Morris water maze (MWM) and to evaluate brain Acetylcholine esterase activity, lipid peroxidation, Superoxide dismutase, Catalase, Glutathione.

Materials and methods: Wistar rats (100-150 gm) of either sex, were divided into 5 groups (n=6). Group-I (control) animals received vehicle, Group-II animals received Diazepam (1 mg/kg i.p), Groups III, IV and V animals received Coccinia grandis-500 mg/kg p.o, 1000 mg/kg p.o and Piracetam (400 mg/kg i.p) respectively for 27 days, followed by Diazepam (1 mg/kg i.p) single dose on 19th and 27th day. Assessment of transfer latency (TL), time taken to reach reward chamber (TRC) and swim latency (SL) was done on 16th, 17th, 18th, 19th and 27th day using EPM, HWM and MWM respectively. Rats were sacrificed on 28th day, brain acetylcholine esterase activity, lipid peroxidation, superoxide dismutase, glutathione and catalase levels were estimated. The data was analyzed by one way ANOVA followed by Dunnett’s test. P ≤ 0.05 was considered significant.

Result: Coccinia grandis decreased TL, TRC and SL in comparison to Diazepam treated rats, decreased acetylcholine esterase activity and lipid peroxidation, and increased superoxide dismutase, glutathione and catalase in brain.

Conclusion: The Coccinia grandis enhanced learning and memory activity. This nootropic effect can be attributed to their antioxidant and neuroprotective property.

Keywords: Memory, EPM, HWM, MWM, Piracetam, Diazepam.

INTRODUCTION

Learning is the experience-dependent acquisition of knowledge and skills, whereas memory is the retention and retrieval of facts or events composed of experiences. Memory disorders can range from mild to severe and can be progressive (neurodegenerative disease) or immediate (brain injury). Almost all are linked with some damage to neuro-anatomical structures, either in part or full, which hinders acquisition (learning), consolidation (storage of labile stable memory), and retrieval (recall). Memory is an individual’s ability to encode, store, retain and subsequently recall information and past experiences in the brain of the individual. Memory gives an individual the capability to learn and adapt from previous experiences and the power of recalling the previously learned facts, skills and habits. Poor memory, slow recall and lower retention are common problems in today’s world. Memory declines mostly under stress and fatigue.

The brain is the centre of the nervous system which controls memory, thought, reason judgment, consciousness and emotion. Supporting the brain health
is vital for ensuring a successful regulation and coordination of body activities. There are a variety of nutritional supplements that are useful in preserving the health of brain. The natural system of medicine is exploring tremendous benefits from the herbs for brain function with includes improving memory, improving alertness, improving intelligence, improving mental performance etc.

Cognitive enhancers are drugs, supplements, nutraceuticals, and functional foods that enhance attentional control and memory. Nootropics are cognitive enhancers that are neuroprotective or extremely nontoxic.

Nootropics also referred to as smart drugs, memory enhancers, neuroenhancers, cognitive enhancers, and intelligence enhancers, are drugs, supplements, nutraceuticals, and functional foods that purportedly improve mental functions such as cognition, memory, intelligence, motivation, attention, and concentration.

Various mechanisms by which nootropics acts are as follows:

1. Increasing circulation to the brain.
2. Providing precursors to neurotransmitters (chemical messengers in the brain).
3. Improving neuron function.
4. Preventing free radical and oxidative damage to brain cells.
5. Providing usable energy to the brain.5

*Coccinia grandis*, commonly known as ivy gourd and also called as baby water melon belongs to family Cucurbitaceae. *Coccinia grandis* is used by humans mostly as a food crop in several countries in Australia, Asia, Caribbean, and the southern United States, pacific Islands. It consists of β-amylin acetate, carotenoids, cucurbitacin, β-carotene, β-sitosterol, triterpinoinds, alkaloids, flavanoid glycosides. And is mainly used as antioxidant, antiabetic, larvicidal, analgesic, hepatoprotective, anti-inflammatory etc. The antioxidant activity is due to the reducing power ability; hydrogen peroxide scavenging potential.4 Extensive literature search revealed that *Coccinia grandis* possess learning and memory enhancing activity, but failed to get the scientific, documentary evidence. Hence, present study was taken up to investigate learning and memory enhancing activity of *Coccinia grandis*.

**MATERIALS AND METHODS**

**Plant material**

*Coccinia grandis* fruits were collected from Mandya, Karnataka in july- Aug 2015 and were identified and authenticated by Dr. Siddamalaiya at National Ayurveda Diabetics Research Institute, (RRCBI-4932) Bangalore 560011. The same was processed at Green chem Herbal extract and Formulations, Bangalore, India. And the ethanolic extract procured was used for the study. Preliminary, phytochemical analysis of the extract was carried out. The plant extract was suspended in distilled water and administered orally to rats.

**Chemicals and drugs**

Diazepam was taken from Calmposie injections Ranbaxy Laboratories Limited. Piracetam was taken from Neurocetam injections Micro Labs Limited, Bangalore.

**Experimental Animals**

Inbred, young Wistar rats (weighing around 100-150 g) were used in the current study. The animals were maintained under standard laboratory conditions of room temperature 24 ± 5˚ C, relative humidity 45-55% and natural day and night cycle. The animals had free access to food (standard rat pellet from Sri Venkateshvara traders, Bangalore), with water supplied *ad libitum*. All the experiments were conducted in compliance to ethical principles and guidelines provided by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Permission was taken from IAEC of Visveswarapura Institute of Pharmaceutical Sciences, Bangalore (CPCSEA 152/99, renewed for the three years i.e. 09-05-2012 to May 2015) before starting the experiments on animal.

**Experimental protocol**

Wistar albino rats were divided into five groups of six rats each for all the three models (EPM, HWM, MWM). Group-I animals served as control, received vehicle i.e, distilled water. Group-II, Group-III and IV animals, received diazepam 1 mg/kg i.p.5 ethanolic fruits extract of *Coccinia grandis*, 500 and 1000 mg/kg p.o. respectively. Group-V animals, received piracetam 400 mg/kg i.p.6 The rats of group III, IV and V received the respective treatment for 15 days, followed by training session on 16th, 17th, and 18th day. On 19th day, single dose of diazepam was administered to all the animals except group I animals, 30 min after the respective treatment. TL, TRC, and SL were assessed 45 min thereafter respectively. The respective treatments continued for one week and on 27thday, diazepam was administered to all the animals except group I animals, 30 min after the respective treatment. TL, TRC, and SL were assessed 45 min thereafter respectively.

**Elevated plus maze (EPM):** Elevated plus-maze was described as the tool for exterceptive behavioral model to evaluate memory in rats. On the first day each rat was placed at the end of an open arm, facing away from the central platform. Transfer latency (TL) is the time taken (in sec) by the animal to move from the open arm into any one of the enclosed arms with all its four legs. TL was recorded on the first day (training session) for each animal. The rat were allowed to explore the maze for another 10sec and then returned to its home cage. Retention of this learned-task (memory) was examined 24h after the first day trial and on 27th day of the treatment. Significant reduction in TL value of retention indicated improvement in memory.

2. Diazepam was taken from Calmposie injections Ranbaxy Laboratories Limited. Piracetam was taken from Neurocetam injections Micro Labs Limited, Bangalore.
3. Inbred, young Wistar rats (weighing around 100-150 g) were used in the current study. The animals were maintained under standard laboratory conditions of room temperature 24 ± 5˚ C, relative humidity 45-55% and natural day and night cycle. The animals had free access to food (standard rat pellet from Sri Venkateshvara traders, Bangalore), with water supplied *ad libitum*. All the experiments were conducted in compliance to ethical principles and guidelines provided by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Permission was taken from IAEC of Visveswarapura Institute of Pharmaceutical Sciences, Bangalore (CPCSEA 152/99, renewed for the three years i.e. 09-05-2012 to May 2015) before starting the experiments on animal.
4. Wistar albino rats were divided into five groups of six rats each for all the three models (EPM, HWM, MWM). Group-I animals served as control, received vehicle i.e, distilled water. Group-II, Group-III and IV animals, received diazepam 1 mg/kg i.p.5 ethanolic fruits extract of *Coccinia grandis*, 500 and 1000 mg/kg p.o. respectively. Group-V animals, received piracetam 400 mg/kg i.p.6 The rats of group III, IV and V received the respective treatment for 15 days, followed by training session on 16th, 17th, and 18th day. On 19th day, single dose of diazepam was administered to all the animals except group I animals, 30 min after the respective treatment. TL, TRC, and SL were assessed 45 min thereafter respectively. The respective treatments continued for one week and on 27thday, diazepam was administered to all the animals except group I animals, 30 min after the respective treatment. TL, TRC, and SL were assessed 45 min thereafter respectively.
5. Elevated plus maze (EPM): Elevated plus-maze was described as the tool for exterceptive behavioral model to evaluate memory in rats. On the first day each rat was placed at the end of an open arm, facing away from the central platform. Transfer latency (TL) is the time taken (in sec) by the animal to move from the open arm into any one of the enclosed arms with all its four legs. TL was recorded on the first day (training session) for each animal. The rat were allowed to explore the maze for another 10sec and then returned to its home cage. Retention of this learned-task (memory) was examined 24h after the first day trial and on 27th day of the treatment. Significant reduction in TL value of retention indicated improvement in memory.
Hebb-Williams maze (HWM): Hebb-Williams maze was taken as the tool for incentive-based exteroceptive behavioral model, useful for measuring spatial working memory of rats. In this model changes in time taken by the animal to reach reward chamber from start box (TRC) were taken as learning and memory enhancing activity. The rat was placed in the animal chamber or start box and the door were opened to facilitate the entry of the animal into the next chamber. The door of the start box was closed immediately after the animal moves into the next chamber to prevent back-entry. The time taken by the animal to reach the reward chamber from the start box was recorded on the first day (training session) for each animal. Each animal was allowed to explore the maze for 3 min with all the doors opened before returning to its home cage. Retention of this learned task (memory) was examined 24 h after the first day trial and on the 27th day of the treatment.

Morris water maze (MWM): A task was developed where rats learn to swim in a water tank to find an escape platform hidden under the water (Morris 1984). As there are no proximal cues to mark the position of the platform, the ability to locate it efficiently will depend on the use of a configuration of the cues outside the tank. The water maze consists of a circular tank with 100 cm diameter and a wall 20 cm above the water level. A circular platform (9 cm diameter, covered with white linen material for grip) is hidden 2 cm below the water level. The water was made opaque using titanium dioxide suspension and is kept at about 23°C during the experiment. Training takes place on three consecutive days, with the rats receiving 4 consecutive trials per day with an inter-trial interval of 6–10 min. Each trial was started from one of four assigned polar positions with a different sequence each day. The latency to find the platform was measured as the time of placement of the rat in the water to the time it finds the platform. If the animal fails to find the platform in any trial within 3 min it is placed on it for 10 s. 

Biochemical Estimation

Sampling of brain tissue: The animals were sacrificed after the treatment i.e., on 28th day; whole brain was carefully removed from the skull. For preparation of brain homogenate, the fresh whole brain was weighed and transferred to a glass homogenizer and homogenized in an ice bath after adding 10 volumes of 0.9% w/v sodium chloride solution. The homogenate was centrifuged at 3000 rpm for 10 min and the resultant cloudy supernatant liquid was used for estimation of brain acetylcholinesterase activity, lipid peroxidation, superoxide dismutase, catalase and glutathione activity.

Assay of acetylcholinesterase activity in the brain: The method of AChE activity estimation is popularly known as Ellman’s method named after George Ellman who developed this method in 1961. The esterase activity is measured by providing an artificial substrate, acetylthiocholine (ATC). Thiocholine released because of the cleavage of ATC by AChE is allowed to react with the -SH reagent 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB), which is reduced to thionitrobenzoic acid, a yellow coloured anion with an absorption maximum at 412 nm. The extinction coefficient of the thionitrobenzoic acid is 1.36 × 10−4 molar/centimeter. The concentration of thionitrobenzoic acid detected using a UV spectrophotometer is then taken as a direct estimate of the AChE activity.

Free radical scavenging potential

LPO: Ohkawa et al., (1979) method was used to determine lipid peroxidation in tissue homogenate, spectrophotometrically. Here one molecule of malondialdehyde (MDA) reacts with two molecules of 2-thiobarbituric acid (TBA) at pH 3.5 .The pink chromogen was measured spectrophotometrically at 532 nm with extinction coefficient of 156 mM−1 cm−1.

SOD: Superoxide dismutase activity in brain homogenate was determined spectrophotometrically. In superoxide dismutase assay, ions are generated from the
conversion of xanthine tauric acid and hydrogen peroxide in presence of xanthine oxidase (XOD), these ions converts NBT to NBTdiformazan.10

Catalase: Catalase activity was determined spectrophotometrically by the method of Aebi et al (1984). Catalase catalyzes the decomposition of hydrogen peroxide according to the above equation. The decrease in absorbance at 240 nm was followed for one min. 10

Glutathione assay: Ellman (1959) was used to measure the Glutathione in brain tissue homogenate. SH group of glutathione reduce 5, 5-dithiobis-(2-nitrobenzoic acid) (DTNB) to form 2-nitro-S-mercaptobenzoic acid per mole of glutathione. The reduction product is measured spectrophotometrically at 412 nm using the extinction coefficient of 13.6 mM$^{-1}$ cm$^{-1}$.10

Statistical analysis: All the values are expressed as mean ± SEM. The data was analyzed by one-way ANOVA, followed by Dunnett’s test. P ≤ 0.05 was considered significant.

RESULTS

Preliminary phytochemical analysis of the Coccinia grandis fruits confirms presence of carbohydrates: reducing sugars, saponins, steroids/terpenes, alkaloids, flavonoids, cardinolides and terpinoids.

EPM: Effect of Coccinia grandis on transfer latency (TL).

As shown in Table 1, diazepam increases the time taken by rat to reach the closed arms as compared to control group, (i.e. induces amnesia). Pretreatment with Coccinia grandis (500 & 1000 mg/kg) for 27 days, resulted in decrease in TL, in a dose dependent manner, which was comparable with Piracetam (400 mg/kg).

Coccinia grandis decreased TL significantly (P<0.01) on day 27th as compared to day 16, 17, 18 and 19th.

Table 1: Effect of Coccinia grandis fruits on transfer latency (TL) in Diazepam induced rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>TL on 1$^{st}$ day (sec)</th>
<th>TL on 2$^{nd}$ day (sec)</th>
<th>TL on 16$^{th}$ day (sec)</th>
<th>TL on 17$^{th}$ day (sec)</th>
<th>TL on 18$^{th}$ day (sec)</th>
<th>TL on 19$^{th}$ day (sec)</th>
<th>TL on 27$^{th}$ day (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>57.17±1.0</td>
<td>55.17±1.6</td>
<td>55.67±1.0</td>
<td>48.67±0.7</td>
<td>39.17±1.0</td>
<td>38.67±1.9</td>
<td>26.5±0.9</td>
</tr>
<tr>
<td>Diazepam 1mg/kg i.p.</td>
<td>58±1.3</td>
<td>52.67±1.4</td>
<td>53.17±1.0</td>
<td>48.5±1.1</td>
<td>41.33±0.6</td>
<td>51.5±0.8</td>
<td>53.17±2.2</td>
</tr>
<tr>
<td>LCG 500mg/kg body wt, PO.</td>
<td>58.67±0.6</td>
<td>57.5±0.7</td>
<td>53.83±1.3</td>
<td>48.17±0.9</td>
<td>29.33±0.8</td>
<td>28.5±1.0</td>
<td>19.8±1.0</td>
</tr>
<tr>
<td>HCG 1000mg/kg body wt, PO.</td>
<td>58.5±0.7</td>
<td>56.17±1.7</td>
<td>53.5±1.4</td>
<td>43.17±2.0*</td>
<td>26.33±1.3**</td>
<td>26.17±1.1**</td>
<td>18.33±0.9**</td>
</tr>
<tr>
<td>Piracetem 400mg/kg i.p.</td>
<td>59.17±1.4</td>
<td>54.33±1.5</td>
<td>52.17±0.8</td>
<td>46.17±1.1</td>
<td>25.17±1.6**</td>
<td>24.17±1.5*</td>
<td>14.5±0.7**</td>
</tr>
</tbody>
</table>

n=6. Values are expressed as mean ± SEM, one way ANOVA followed by Dunnett’s test. *P<0.05, **P<0.01 v/s diazepam control.

HWM: Effect of Coccinia grandis on time taken to reach reward chamber (TRC).

As shown in Table 2, diazepam increases the TRC as compared to control group, (i.e. induces amnesia). Pretreatment with Coccinia grandis, for 27 days, resulted in decrease in TRC, in a dose dependent manner, which was comparable with Piracetam (400 mg/kg).

Coccinia grandis decreased TRC significantly (P<0.01) on day 27th as compared to day 16, 17, 18 and 19th.

Table 2: Effect of Coccinia grandis fruits on transfer latency (TRC) in Diazepam induced rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>TRC on 1$^{st}$ day (sec)</th>
<th>TRC on 2$^{nd}$ day (sec)</th>
<th>TRC on 16$^{th}$ day (sec)</th>
<th>TRC on 17$^{th}$ day (sec)</th>
<th>TRC on 18$^{th}$ day (sec)</th>
<th>TRC on 19$^{th}$ day (sec)</th>
<th>TRC on 27$^{th}$ day (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>157.5±1.8</td>
<td>134.3±1.7</td>
<td>140.2±2.5</td>
<td>113.7±2.3</td>
<td>96.67±1.6</td>
<td>74.33±1.7</td>
<td>53.67±1.7</td>
</tr>
<tr>
<td>Diazepam 1mg/kg i.p.</td>
<td>159.2±1.7</td>
<td>136.7±1.7</td>
<td>141.7±1.9</td>
<td>137.8±2.6</td>
<td>129.7±1.7</td>
<td>123.7±2.3</td>
<td>125.7±1.9</td>
</tr>
<tr>
<td>LCG 500mg/kg body wt, PO.</td>
<td>159.5±1.3</td>
<td>134±1.4</td>
<td>132.7±1.1</td>
<td>95.33±1.9</td>
<td>79±2.4</td>
<td>61.17±1.6</td>
<td>30.33±2.2</td>
</tr>
<tr>
<td>HCG 1000mg/kg body wt, PO.</td>
<td>160±0.7</td>
<td>129.3±1.4</td>
<td>132.3±0.9</td>
<td>82.67±1.6*</td>
<td>71.17±1.1**</td>
<td>49.33±1.3**</td>
<td>20.83±1.7**</td>
</tr>
<tr>
<td>Piracetem 400mg/kg i.p.</td>
<td>160±1.7</td>
<td>127±1.4</td>
<td>125.8±0.7</td>
<td>77.67±2.0*</td>
<td>63.17±1.1**</td>
<td>41.33±1.4**</td>
<td>14.83±1.3**</td>
</tr>
</tbody>
</table>

n=6. Values are expressed as mean ± SEM, one way ANOVA followed by Dunnett’s test. *P<0.05, **P<0.01 v/s diazepam control.
MWM: Effect of *Coccinia grandis* on Swim latency (SL).

As shown in Table 3, diazepam increases SL compared to control group, (i.e. induces amnesia). Pretreatment with *Coccinia grandis*, for 27 days, resulted in decrease in SL, in a dose dependent manner, which was comparable with Piracetam (400 mg/kg). *Coccinia grandis* decreased SL significantly (P<0.01) on day 27th as compared to day 16, 17, 18 and 19th.

Table 3: Effect of *Coccinia grandis* on Swim latency (SL) in diazepam induced rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>SL on 1&lt;sup&gt;st&lt;/sup&gt; day (sec)</th>
<th>SL on 2&lt;sup&gt;nd&lt;/sup&gt; day (sec)</th>
<th>SL on 16&lt;sup&gt;th&lt;/sup&gt; day (sec)</th>
<th>SL on 17&lt;sup&gt;th&lt;/sup&gt; day (sec)</th>
<th>SL on 18&lt;sup&gt;th&lt;/sup&gt; day (sec)</th>
<th>SL on 19&lt;sup&gt;th&lt;/sup&gt; day (sec)</th>
<th>SL on 27&lt;sup&gt;th&lt;/sup&gt; day (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>160.7±1.3</td>
<td>175.7±2.8</td>
<td>176.2±2.5</td>
<td>154.7±1.6</td>
<td>134.7±1.5</td>
<td>113.8±2.1</td>
<td>100.5±1.6</td>
</tr>
<tr>
<td>Diazepam 1mg/kg i.p.</td>
<td>160.7±1.9</td>
<td>175.1±1.8</td>
<td>173.3±2.1</td>
<td>158±1.4</td>
<td>135.3±2.0</td>
<td>164.7±2.3</td>
<td>172.7±2.8</td>
</tr>
<tr>
<td>LCG 500mg/kg body wt.</td>
<td>160±2.0</td>
<td>175.3±3.7</td>
<td>166.3±2.1</td>
<td>154.7±2.1</td>
<td>130.7±2.7</td>
<td>116.5±2.4</td>
<td>75.83±1.7**</td>
</tr>
<tr>
<td>HCG 1000mg/kg body wt.</td>
<td>160.2±2.7</td>
<td>172.2±1.6</td>
<td>151.8±1.9</td>
<td>125.2±2.1*</td>
<td>107±2.3**</td>
<td>67.17±2.4**</td>
<td>51.5±1.6**</td>
</tr>
<tr>
<td>Piracetem 400mg/kg i.p.</td>
<td>162.5±4.3</td>
<td>171.7±1.8</td>
<td>148.3±1.8</td>
<td>110±2.1*</td>
<td>83±1.8**</td>
<td>61.33±1.8**</td>
<td>36.17±1.6**</td>
</tr>
</tbody>
</table>

As shown in “Fig. 4”, diazepam increases the acetylcholine esterase activity compared to control group, (i.e. induces amnesia). Pretreatment with *Coccinia grandis* for 27 days, resulted in a significant (P<0.01) decrease in acetylcholine esterase activity in a dose dependent manner, which was comparable with Piracetam (400 mg/kg).

**Figure 4: Effect of Coccinia grandis on Acetylcholine esterase activity using diazepam induced rats,**

As shown in Table 4, diazepam increases LPO activity and decreases the SOD, Catalase and Glutathione activity. Pretreatment with *Coccinia grandis* for 27 days, resulted in, significant (P<0.01) decrease in LPO and increase in SOD, Catalase, Glutathione activity, in a dose dependent manner, which was comparable with Piracetam (400 mg/kg).

Effect of *Coccinia grandis* free radical scavenging potential.

As shown in Table 4, diazepam increases LPO activity and decreases the SOD, Catalase and Glutathione activity. Pretreatment with *Coccinia grandis*, for 27 days, resulted in, significant (P<0.01) decrease in LPO and increase in SOD, Catalase, Glutathione activity, in a dose dependent manner, which was comparable with Piracetam (400 mg/kg).
DISCUSSION

Memory is the ability of an individual to record sensory stimuli, events, information etc, retain them over a short or long period of time and recall the same at later date when needed. Learning is the process of acquiring knowledge about the world and memory could be considered as the retention of the acquired knowledge, which can be recalled as and when needed.13

Dementia is generally defined as the loss of intellectual abilities. In dementia, memory capacity to solve problems of day-to-day living, performance of learned motor, social skills and control of emotions are primarily affected.12

Herbal drugs have shown the promising effect in the treatment of memory loss.13 Nootropics popularly referred as smart drugs, boost human cognition ability. Nootropic agents such as piracetam, pramicacetam, amiracetam and choline esterase inhibitors like donepezil are being used to improve memory. However, the resulting adverse effects associated with these agents makes it necessary explore the utility of traditional medicine in the treatment of various cognition disorders.14 The present study was undertaken to evaluate the learning and memory enhancing activity of Coccinia grandis in amnestic rats.

In the present study, diazepam induced amnesia in rats. Coccinia grandis 500 and 1000 mg/kg body wt, p.o were effective in reverting amnesia when memory retention was evaluated in exceroceptive behavior model of Elevated plus maze, Hebb’s William maze and Morris water maze. Learning and memory enhancing activity of test drugs were effective dose dependently. In our study we found that Coccinia grandis reduced transfer latency, time taken to reach reward chamber and swim latency in diazepam induced amnesic rats using EPM, HWM, MWM, reduction in acetylcholine esterase activity, reduction in LPO level and increase in SOD, GSH, catalase level, in a dose dependent manner respectively. This can be interpreted as the learning and memory enhancing activity of Coccinia grandis, which may be due to its antioxidant properties. Coccinia grandis contains flavanoids, saponins, cardenolides15 which shows antioxidant activity according to earlier studies. This antioxidant activity of Coccinia grandis may be responsible for enhancing, learning and memory in rats.

Diazepam enhances the GABA in the brain which decreases the neuronal activity.16,17 The animal models have been extensively used in research to screen drugs with potential therapeutic value in dementia. The Elevated plus maze, Hebb’s William maze and Morris water maze were used in order to deduce the effectiveness of test drugs. It was found that dose 500mg/kg and 1000 mg/kg body wt for Coccinia grandis administered orally for 27 days attenuated the diazepam induced learning and memory from amnesia. Test drugs also prevented diazepam induced memory impairment when evaluated in all the three models. The Elevated plus maze, Hebb’s William maze and Morris water maze, investigated spatial learning and memory. It is especially sensitive to impair cholinergic hippocampal function, hence suggesting attenuation of diazepam induced spatial learning and memory deficit in rats by Coccinia grandis.

Acetylcholine in cholinergic nerve system considered as the most important neurotransmitter involved in the regulation of cognitive functions.5 The regulation of memory function depends upon the level of neurotransmitters such as acetylcholine, serotonin, catecholamine, GABA and glutamate. The inhibitory effects of choline esterase results in memory enhancement by increasing the acetylcholine level and decreasing dopamine level.18 Cholinergic neuronal loss in hippocampal area is the major feature of Alzheimer’s disease and enhancement of central cholinergic activity by use of anticholinesterase is presently the mainstay of the pharmacotherapy of dementia in AD. In the present study, The role of central cholinergic system is fairly well established and its deficiency being implicated in memory deficits. Diazepam, a GABA mimetic agent induces memory impairment and the subsequent inhibition of GABA-B receptor has been found to facilitate acetylcholine in the brain.18 In the present study, with the administration of Coccinia grandis extracts for 27 days, The metabolism of some neurosteroids was inhibited, which are responsible for enhancement of GABA activity in brain against diazepam induced amnesia. This facilitates cholinergic transmission and improving memory of rats and

Table 4: Effect of Coccinia grandis free radical scavenging potential in diazepam induced rats using Elevated plus maze.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Lipid peroxidation in moles/gm of tissue</th>
<th>Glutathione in moles/gm of protein</th>
<th>Superoxide dismutase in moles/gm of protein</th>
<th>Catalase in U/mg of protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>31.54±1.14</td>
<td>6.22±0.02</td>
<td>0.58±0.026</td>
<td>0.60±0.056</td>
</tr>
<tr>
<td>Diazepam 1mg/kg i.p.</td>
<td>51.98±2.34</td>
<td>4.42±0.34</td>
<td>0.30±0.020</td>
<td>0.42±0.031</td>
</tr>
<tr>
<td>LCG 500mg/kg body wt, PO</td>
<td>45.13±2.09</td>
<td>5.00±0.30</td>
<td>0.35±0.051</td>
<td>0.47±0.056</td>
</tr>
<tr>
<td>HCG 1000mg/kg body wt, PO</td>
<td>34.98±1.74</td>
<td>5.98±0.03</td>
<td>0.58±0.032</td>
<td>0.59±0.024</td>
</tr>
<tr>
<td>Piracetam 400mg/kg i.p.</td>
<td>33.08±1.27</td>
<td>6.41±0.06</td>
<td>0.62±0.053</td>
<td>0.67±0.051</td>
</tr>
</tbody>
</table>

n=6. Values are expressed as mean ± SEM, one way ANOVA followed by Dunnett’s test. *P<0.05, **P<0.01 v/s diazepam control.
increasing the availability of acetylcholine in brain. This also diminishes the neurosteroid metabolism to subsequent inhibition of GABA-B receptors, which leads to indirect stimulation of cholinergic system. Hence, a combination of neuroprotective, anticholinesterase and nootropic effects exhibited by *Coccinia grandis* may all be eventually responsible for the memory improving effect observed in the present study.

Age, oxidative stress, harmful free radicals and inflammation are the key components in the development of memory impairment, including the conditions such as dementia, schizophrenia and Alzheimer’s disease. The oxidative stress, generation of free radicals, harmful byproducts of oxidative metabolism are known to cause organic damage to the living system. It is hypothesized that increasing antioxidant levels in the organism might retard or reverse the damaging effects of free radicals on neurons. Oxidative stress in brain generates oxygen radicals like superoxide anion, hydroxyl radical, and hydrogen peroxide, which act on polyunsaturated fatty acids in brain. The major antioxidant and oxidative free radical scavenging enzymes like peroxidase, glutathione, SOD and catalase play an important role to reduce oxidative stress in brain.

In the present study after diazepam (27th day) treatment rats showed a significant increase in the brain levels of lipid peroxidation. At the same time there was a significant reduction in levels of glutathione, a tripeptide found in all cells, which reacts with free radicals to protect cells from superoxide radical, hydroxyl radical and singlet oxygen. Pre-treatment of *Coccinia grandis* reduced the lipid peroxidation levels and increased GSH content in brain after diazepam treatment. Diazepam reduced the SOD activity in brain. SOD is the only enzyme that uses the superoxide anions as the substrate and produces hydrogen peroxide as a metabolite. Super oxide anion is more toxic than H₂O₂ and has to be removed. Pre-treatment with *Coccinia grandis* significantly prevented the reduction of SOD activity in brain. The results also suggest that the *Coccinia grandis* induced oxidative stress by reducing lipid peroxidation and increasing the endogenous antioxidant enzymes in brain. Thus the present study demonstrates that *Coccinia grandis* has potential therapeutic effects on improving the anti-amnesic activity in rats through inhibiting lipid peroxidation, augmenting endogenous antioxidant enzymes and decreasing acetylcholinesterase (AChE) activity in brain.

**CONCLUSION**

Thus it is concluded that *Coccinia grandis* improves learning and memory. This may be due to antioxidant property present in it, which may be attributed to Flavonoids, tannins and polyphenols.

**ACKNOWLEDGEMENT**

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**REFERENCES**