Neuroprotective Effects of Lantana camara in BPA Induced-cognitive Dysfunction and Oxidative Stress in Rats

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Authors’ contributions
This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT
The present study was aimed to screen neuroprotective effects of lantana camara in BPA induced-cognitive dysfunction and oxidative stress in rats. In methodology animals were divided into 5 groups. Group I serve as vehicle control group and was administered with 2 ml of normal saline. Group II was administered with BPA 50 µg/kg for 21 days. Group III and IV served as drug treated group and pre treated for 1 week with methanolic extract of L.camara (250 and 500 mg/kg bw/day orally). Group V serve as standard drug treated group and treated with piracetam 200mg/kg i.p. after the completion of dosing, rats were subjected to various test to analyze their behaviour performance and later sacrificed for further test. Animals were screened for elevated plus maze and Y-maze. Animals were sacrificed and evaluated the brain anti oxidant parameters like catalase (CAT), Estimation of lipid peroxidation (LPO), Estimation of superoxide dismutase (SOD), and Estimation of glutathione (GSH). All the Parameters of extract treated group animals have shown better results when compared with toxic and test groups. These findings provide a preliminary evidence for its potential as neuroprotective effect.

Keywords: Lantana camara; piracetam; elevated plus maze; Y-maze and anti oxidant; Bisphenol A.
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AABBREVIATIONS

CAT : Catalase
LPO : Lipid Per Oxidation
SOD : Superoxide Dismutase
MELC : Methanolic Extract of Lantana camara
BPA : Bisphenol A
NMDARs : N-Methyl-D-Aspartate-Receptors

1. INTRODUCTION

Neurodegeneration is a significant side consequence of ischemia and hypoxia, which can cause oxidative stress and neuro-inflammation, finally leading to neuropathy [1]. After acute toxicity or during chronic insult, neuroprotection aims to prevent neuronal degeneration, reduce damage, and promote recovery of a neural system. The estimate of neuroprotective activity is done using a variety of animal models [2]. Bisphenol A (BPA, 4x, 40-isopropyldiene-2-diphenol) is the polycarbonate plastics, epoxy resins, compact discs, dental sealants and thermal papers [3]. The pervasive prevalence of BPA in food and beverage packaging is the principal source of BPA exposure in the human population globally [4]. Oral, transdermal, and inhalation are the three different routes of BPA exposure [5]. The occurrence of frightening levels of conjugated BPA in urine, serum, umbilical cord fluid, and, most dangerously, breast milk has been documented in research [6,7]. As a result of its xenoestrogenic activity, BPA has been linked to a number of dangerous diseases, including cancer, thyroid abnormalities, cognitive impairments, and male infertility [8]. As a result, the surrounding environment has a significant impact on population health, and endocrine disrupting chemical exposure in the environment cannot be overlooked. The primary cause of cognitive decline is ageing, but there are a number of other variables that can contribute to it, including exposure to various chemicals, pesticides, head injury, and genetic predisposition [9]. BPA has been shown to pass the blood–brain barrier at varying doses, resulting in a variety of behavioural alterations linked to cognitive impairment, such as increased aggression, hyper-reactivity learning impairments, and increased drug dependency [10]. N-methyl-D-aspartate-receptors (NMDARs), a specific kind of ionotropic glutamate receptor found in the hippocampal region of the mid brain are crucial in controlling the synaptic plasticity and cognition [11]. The hippocampus is the main site for learning and memory. In the hippocampus, BPA has been shown to reduce the expression of NMDAR subunits as well as oestrogen receptor [12].

Because of the high likelihood of pharmacological side effects, allopathic substances have their own set of limitations [13]. Furthermore, the treatment is not always satisfactory. As a result, there is a pressing need to develop alternative treatments that are both safe and effective. Herbal preparations have been shown to have therapeutic potential in numerous research [14,15]. So the present study was designed to explore the neuroprotective properties of Lantana camara.

Lantana camara is a species of flowering plan within the verbena family (Verbenaceae), native to the American tropics. Other common names of L. camara include Big-sage, wild-sage, red-sage, white-sage, tick berry, West Indian lantana, and umbelanterna. L. camara was probably introduced before 19th century. Currently L. camara is distribute throughout India where there is a moderate to high summer rainfall and well-drained sloping sites. L. camara is a well known medicinal plant in traditional medicinal system and recent scientific studies have emphasized the possible use of L. camara in modern medicine.

2. MATERIALS AND METHODS

2.1 Plant Collection and Authentication

Lantana Camara Leaves were obtained from the local places of Tirupati, AP. Lantana Camara Leaves plants was authenticated by Dr. K. Madhava Chetty, M.Sc., M.Ed., M.Phil., Ph.D., PG DPD., Assistant Professor, Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh.

2.2 Extraction by Maceration

Fresh Lantana Camara Leaves were washed with water to remove contaminants such as dirt and other impurities before being dried in the shade. To get a homogeneous, coarse powder, the dried leaves were pulverised and sieved. One kilogramme of powdered plant material was weighed, immersed in Methanol, and macerated for seven days with intermittent stirring. On the eighth day, the solvent was filtered through a muslin cloth and evaporated at 40°C in a rotary evaporator. To eliminate any remaining methanol, the extract was placed in a desiccator.
The dried Methanolic extract of *Lantana Camara* (ME-LC) was packed in an air-tight bottle and put in a dry place for further studies.

### 2.3 Qualitative Evaluation of Phytoconstituents

The ME-LC were screened for the presence of various phytoconstituents like carbohydrates, flavonoids, polyphenolic compounds, saponins, tannins, triterpenoids, etc. [16-19]

### 2.4 Experimental Animals

Healthy, male Albino-Wistar rats with average weight of 150-200gms were used for this study. Animals have been provided with 24-hour access with water and standard nutritional pellets, prior to and during the treatment. They were acclimatized under a time period of one week under approved laboratory environment, i.e., 25°C±1°C temperature, 45-55% RH and also free access to food and water, after which they have been employed in the experiment.

### 2.5 Acute Toxicity Studies

Literature survey found that acute toxicity studies were done on ME-LC according to OECD Guideline 420, Fixed Dose Procedure and it was found to be safe up to 5000mg/Kg in animals.

### 2.6 Experimental Protocol

Animals were divided into 5 groups. Each group contain six animals (n=6). Group I served as vehicle control was administered with 2ml of normal saline. Group II was administered with BPA 50 µg/kg (P.O) for 21 days. Group III and IV served as Test group and pre treated for 1 week with ME-LC (250 and 500 mg/kg bw/day orally). Group V served as standard drug treated group and treated with piracetam 200mg/kg i.p. after the completion of dosing, rats were subjected to various test to analyze their behaviour performance and later sacrificed for further test.

### 2.7 Elevated Plus Maze Method

Elevated plus maze EPM comprises of ‘+’ shaped apparatus uplifted above the ground with two enclosed and open arm. The rats are placed at the junction of the four arms and rats are allowed to explore all the four arms of the maze. The time spend in the enclosed and open arms are recorded. The anxiety is examined in rats by the proportion time spend in the enclosed arm. Reduction in the anxiety is examined by the proportion of time in the open arms of the maze. It is expressed as- time in open arms/total time in open or closed arms entries into open arms/total entries into open or closed arms was observed.

### 2.8 Y-maze Spontaneous Alternation Test

The maze involves ‘Y’ shaped apparatus with 120° angle from each other. The rats were placed at the junction of the three arms and are allowed to explore for 5 minute to all the three arms of the maze. The rats with good memory working prefer to explore the less visited arm than the previously explored arm. The number of entries and alterations are recorded and percentages of alteration are calculated.

### 2.9 In vivo Anti oxidant Studies

#### 2.9.1 Estimation of catalase (CAT)

To the tissue homogenate, equal amount of Tris HCL, 15% TCA and 0.375% TBA are added. Boil the tubes in the water bath maintaining the temperature at 90 - 100°C for 15 minutes. The tubes is removed from water bath and cooled at room temperature. Later, they are centrifuged at 2000 rpm for 10 minute. Absorbance was measured at 532 nm by using UV-Spectrophotometer.

#### 2.9.2 Estimation of lipid peroxidation (LPO)

To the tissue homogenate, equal amount of Tris HCL, 15% TCA and 0.375% TBA are added. Boil the tubes in the water bath maintaining the temperature at 90 - 100°C for 15 minutes. The tubes is removed from water bath and cooled at room temperature. Later, they are centrifuged at 2000 rpm for 10 minute. Absorbance was measured at 532 nm by using UV-Spectrophotometer.

#### 2.9.3 Estimation of superoxide dismutase (SOD)

To the tissue homogenate, tris HCL is mixed. Later pyrogallol is added just before the measurement. Change in absorbance was recorded at 325 nm by using UV-Spectrometer.

#### 2.9.4 Estimation of glutathione (GSH)

To the tissue homogenate, amount of phosphate buffer solution P7.5 is added. To that 0.6mM of DTNB is added. They are further incubated at room temperature (25°C) for 10 minutes. Absorbanes are measured at 412 nm by using UV-Spectrometer.

### 3. RESULTS

The preliminary phytochemical screening showed the presence of various phyto-
constituents like flavonoids, phenolic compounds, triterpenoids, tannins, and saponins, in Methanolic extract of *Lantana Camara* (MELC).

The Elevated Plus Maze (EPM) test is based on the assumption that exposure to an EPM elicited a much higher approach–avoidance conflict than exposure to an enclosed arm. Anxiolytic effects cause a decrease in aversion to the open arm, as seen by increased time spent and entries in the open arm. In the open arm, the MELC increased time spent and % entries, but in the closed arm, percent fell. Table 1 summarised the findings.

After a single administration of MELC, there was an increase in the percentage of spontaneous alternation in animals treated with the high dose of the plant extract, when compared to control group, suggesting effects on short-term memory. This increase in the percentage of spontaneous alternations was significant. Results were showed in Table 2.

### 3.1 In vivo Antioxidant Studies

The significant values of LPO levels of normal, disease, standard, MELC 250 mg/Kg and MELC 500 mg/Kg were found to be 2.853±0.0897, 5.233±0.2124, 2.965±0.1684, 3.303±0.0892, 3.590±0.0885 respectively on Day 29. There is a significant decrease in LPO levels of animals treated with MELC 250 mg/Kg and 500 mg/Kg compared to disease control. Results were depicted in Fig. 1.

The significant values of GSH levels of normal, disease, standard, MELC 250 mg/Kg and MELC 500 mg/Kg were found to be 4.227±0.1054, 2.907±0.1336, 3.610±0.0823, 3.550±0.1029, 3.410±0.0823 respectively on Day 29. There is a significant increase in GSH levels of animals treated with MELC 250 mg/Kg and 500 mg/Kg compared to disease control. Results were depicted in Fig. 2.

The significant values of CAT levels of normal, disease, standard, MELC 250 mg/Kg and MELC 500 mg/Kg were found to be 0.720±0.0429, 0.450±0.0341, 0.637±0.0262, 0.598±0.0302, 0.603±0.0512 respectively on Day 29. There is a significant increase in CAT levels of animals treated with MELC 250 mg/Kg and 500 mg/Kg compared to disease control. Results were depicted in Fig. 3.

### 4. DISCUSSION

The effect of BPA 50µg/kg bw/day and in combination with MELC (250 mg/kg bw/day and 500 mg/kg bw/day) was investigated on the cognition of Rats. Here we hypothesized to explore the neurotoxic effects of BPA on cognitive impairment and memory dysfunction and its alleviation using MELC. BPA intoxication was found to severely damage the main site for learning and memory, i.e., hippocampus.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Treatment</th>
<th>Time spent in closed arm in sec</th>
<th>Time spent in open arm in sec</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal control</td>
<td>221.6±8.52</td>
<td>10.0±4.01</td>
</tr>
<tr>
<td>2</td>
<td>Disease control BPA (50 µg/kg)</td>
<td>146.6±19.22**</td>
<td>53.3±13.34**</td>
</tr>
<tr>
<td>3</td>
<td>MELC 250 mg/Kg</td>
<td>156.6±19.22**</td>
<td>63.3±13.34**</td>
</tr>
<tr>
<td>4</td>
<td>MELC 500 mg/Kg</td>
<td>187.5±18.03**</td>
<td>80.0±12.69**</td>
</tr>
<tr>
<td>5</td>
<td>Standard control Piracetam (200 mg/kg)</td>
<td>187.8±16.3**</td>
<td>48.7±3.9**</td>
</tr>
</tbody>
</table>

### Table 2. Effect of MELC on Y-Maze apparatus

<table>
<thead>
<tr>
<th>S. No</th>
<th>Treatment</th>
<th>No. of Entries</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal control</td>
<td>13.5±2.86</td>
</tr>
<tr>
<td>2</td>
<td>Disease control BPA (50 µg/kg)</td>
<td>5.00±0.65*</td>
</tr>
<tr>
<td>3</td>
<td>MELC 250 mg/Kg</td>
<td>3.83±0.30*</td>
</tr>
<tr>
<td>4</td>
<td>MELC 500 mg/Kg</td>
<td>2.00±0.68*</td>
</tr>
<tr>
<td>5</td>
<td>Standard control Piracetam (200 mg/kg)</td>
<td>1.66±0.42*</td>
</tr>
</tbody>
</table>
Fig. 1. Effect of MELC on Lipid peroxidation

Fig. 2. Effect of MELC on reduced glutathione

Fig. 3. Effect of MELC on Catalase
Cognitive impairment was seen to be linked with the hippocampal down-regulation of NMDA receptor [20,21]. In this context, our findings showed that BPA poisoning causes NMDA receptor expression to decrease. MELC treatment, on the other hand, can restore NMDAR levels in BPA-intoxicated rats. Furthermore, the MELC extract improved learning and memory in rats that had been exposed to BPA. The pathogenesis of several diseases such as ageing, atherosclerosis, diabetes, cancer, and other degenerative conditions has been linked to oxidative stress [22]. Endogenous antioxidant enzymes, such as catalase, SOD, and other compounds, are effective at scavenging the increased ROS produced in the brain and other organs [23]. Thus, the involvement of oxidative stress in BPA induced cognitive impairment was assessed using various parameters like Catalase, SOD and LPO in the brain. In comparison to control rats, BPA intoxicated rats had higher levels of MDA, which resulted in higher levels of LPO. This finding was consistent with previous research, which found that BPA exposure causes an increase in ROS formation in the brain, as well as a decrease in endogenous antioxidants in the liver and epididymal sperm [24-26]. Furthermore, Aydogan et al. [27] claimed that BPA exposure resulted in an increase in MDA levels in the brain. In this study, BPA-intoxicated rats showed considerable impairment in learning and memory when compared to control rats. As a result, this data suggests that prolonged oxidative stress is one of the likely causes of cognitive impairment in the BPA-affected rat group. Antioxidants help to slow the onset of degenerative illnesses by reducing oxidative stress. Researchers have suggested the alteration in the redox potential in experimental animals upon contamination with the environmental insults [28]. Several studies have suggested that MELC has neuroprotective effects in the brain due to its antioxidant nature [29,30]. Te MELC in our study was seen to significantly reduce the level of LPO and increase the activities of SOD and Catalase through its free radical scavenging activity. As a result, MELC serves as an antioxidant, regulating the levels of endogenous antioxidants, which are typically decreased as a result of increased oxidative stress. It has also been shown to protect against the oxidative damage and memory impairment caused by BPA.

5. CONCLUSION

Dementia and cognitive impairment have been shown to have a significant impact on the elderly's quality of life and life duration, and are the most common symptoms of neurodegenerative illnesses. Furthermore, oxidative stress is connected to cognitive impairment and plays an important role in the pathophysiology of these disorders. BPA is a powerful endocrine disruptor that has been linked to cognitive impairment. The present study deals with the neuroprotective activity of Lantana camara against BPA-induced oxidative stress and memory impairment in mice. Therefore, our study put-forward Lantana camara as a potent drug candidate for BPA-induced cognitive impairment.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Institutional Animal Ethical Committee (IAEC) has given its approval to the experimental protocol with ethical clearance No: CPCSEA/1657/IAEC/CMRCP/COL-20/78.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

6. REFERENCES


