Original Research Article

NEUROPROTECTIVE EFFECTS OF *LANTANA CAMARA* IN BPA INDUCED-COGNITIVE DYSFUNCTION AND OXIDATIVE STRESS IN RATS

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.

1. INTRODUCTION

Neurodegeneration is the serious side effect of acute injuries or chronic irregular incidents of ischemia and hypoxia, which can generate oxidative stress and neuro-inflammation, eventually leading to neuropathy [1]. Neuroprotection intends to prevent the neuronal degeneration, and to minimize the damage and maximize the recovery of a neural system after acute toxicity or during chronic insult. There are various animal models used for the estimation of neuroprotective activity [2].

Bisphenol A (BPA, 4zx, 40-isopropylidene-2-diphenol) is the polycarbonate plastics, epoxy resins, compact discs, dental sealants and thermal papers [3]. The primary source of BPA exposure in human population, worldwide is due to its ubiquitous presence in food and beverage packaging [4]. The different routes of BPA exposure are oral, Transdermal and inhalation [5]. The studies have reported the presence of alarming levels of conjugated BPA in urine, serum, umbilical cord fluid and, most hazardously in breast milk [6, 7]. BPA has been identified as a causative agent for various perilous disorders such as cancer, thyroid deformities, cognitive impairments and male infertility as a result of its xenoestrogenic activity [8]. The surrounding environment has a major impact on health of the population consequently; the environmental exposure of endocrine disrupting chemicals cannot be ignored. The primary factor for cognitive impairment is ageing but there are various associated factors including the exposure of various toxins, pesticides, head injury, along with genetic predisposition [9]. BPA has been reported to cross the blood–brain barrier in different concentrations leading to various behavioral changes associated with cognitive impairment along with increased aggression, hyper-reactivity learning deficits, and increased drug dependency [10]. N-methyld-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. BPA has been reported to decrease the expression of the subunits of NMDAR along with estrogen receptor in the hippocampus [12].

Allopathic compounds have their own limitations due to the high propensity of drug side effects [13]. Also, the treatment is not satisfactory every time. Therefore, there is a need to discover alternative treatments, which can be safe enough and retain optimum efficacy. Many studies have revealed the medicinal potential of herbal products [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 leaves of *Lantana Camara Leaves* washed with water to get rid of contaminants like dirt and other impurities and were shade-dried. These dried leaves were ground and sieved to get a uniform, coarse powder. Powdered plant material was weighed (1Kg) and is immersed in Methanol and kept for maceration for a period of 7 days with occasional stirring. On the 8^{th} day, the solvent was filtered by pressing with a muslin cloth and was evaporated in a rotary evaporator at 40^{o}C. The resultant extract was put in a desiccator to remove any methanol left in it. The dried Methanolic extract of *Lantana Camara* (MELC) was packed in an air-tight bottle and put in a dry place for further studies.

2.3 Qualitative evaluation of Phytoconstituents

The MELC 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., $25^{o}C \pm 1^{o}C$ temperature, 45-55% RH and also free access to food and water, after which they have been employed in the experiment.

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

2.5 Acute Toxicity Studies

Literature survey found that acute toxicity studies were done on MELC according to OECD Guideline 420, Fixed Dose Procedure and it was found to be safe up to 5000mg/Kg in animals [20].

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 for 21 days. Group III and IV served as Test group and pre treated for 1 week with MELC (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

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)

Tissue homogenate was mixed with 1.95 ml of phosphate buffer, later mixed with 1ml of hydrogen peroxide; Absorbance was measured at 240 nm at 0 and 1 minute 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-Spectrometer.

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 P^H 7.5 is added. To that 0.6mM of DTNB is added. They are further incubated at room temperature (25°C) for 10 minutes. Absorbances 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*)*.

Table 1: Effect of MELC on Elevated plus maze

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

The Elevated plus maze test is based on a premise where the exposure to an EPM evoked an approach –avoidance conflict that was considerably stronger than that evoked by the exposure to an enclosed arm. The decrease in aversion to the open arm is the result of an anxiolytic effect, expressed by the increased time spent and entries in the open arm. The MELC increased the time spent and percent entries in the open arm, with percent decreased in the closed arm. Results were showed in **Table 1**.

Table 2: Effect of MELC on Y-Maze apparatus

S. No	Treatment	No. of Entries
1	**Normal control**	13.5±2.86

2	**Disease control BPA (50µg/kg)**	5.00±0.65*
3	**MELC 250mg/Kg**	3.83±0.30*
4	**MELC 500mg/Kg**	2.00±0.68*
5	**Standard control Piracetam (200mg/kg)**	1.66±0.42*

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

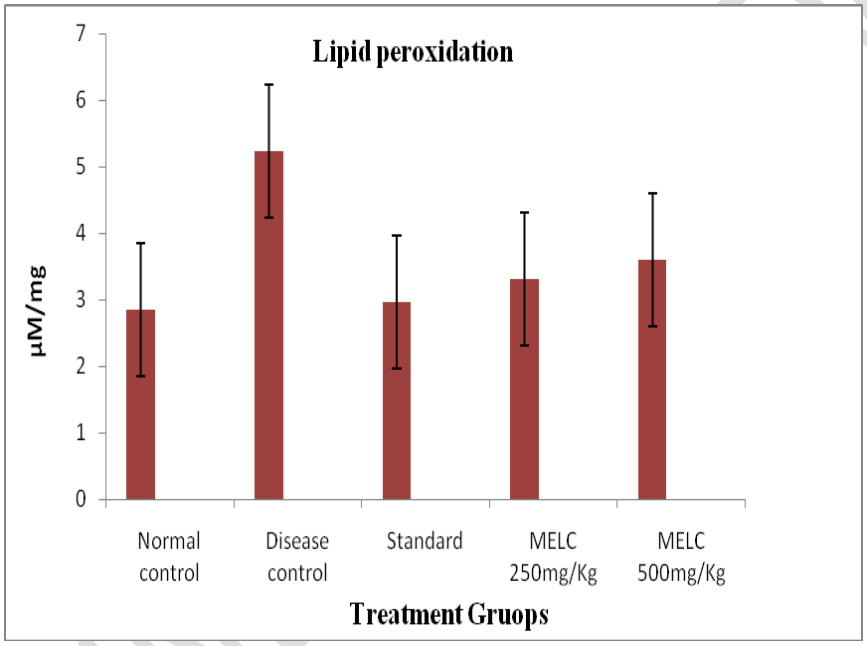

Fig. 1: Effect of MELC on Lipid peroxidation

The significant values of LPO levels of normal, disease, standard, MELC 250mg/Kg and MELC 500mg/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 250mg/Kg and 500mg/Kg compared to disease control. Results were depicted in **Fig. 1**.

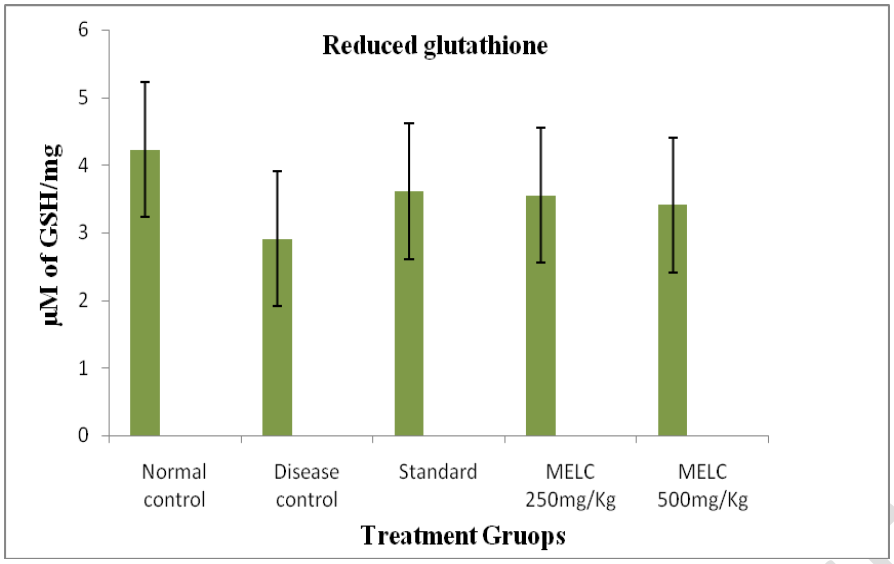

Fig. 2: Effect of MELC on Reduced glutathione

The significant values of GSH levels of normal, disease, standard, MELC 250mg/Kg and MELC 500mg/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 250mg/Kg and 500mg/Kg compared to disease control. Results were depicted in **Fig. 2.**

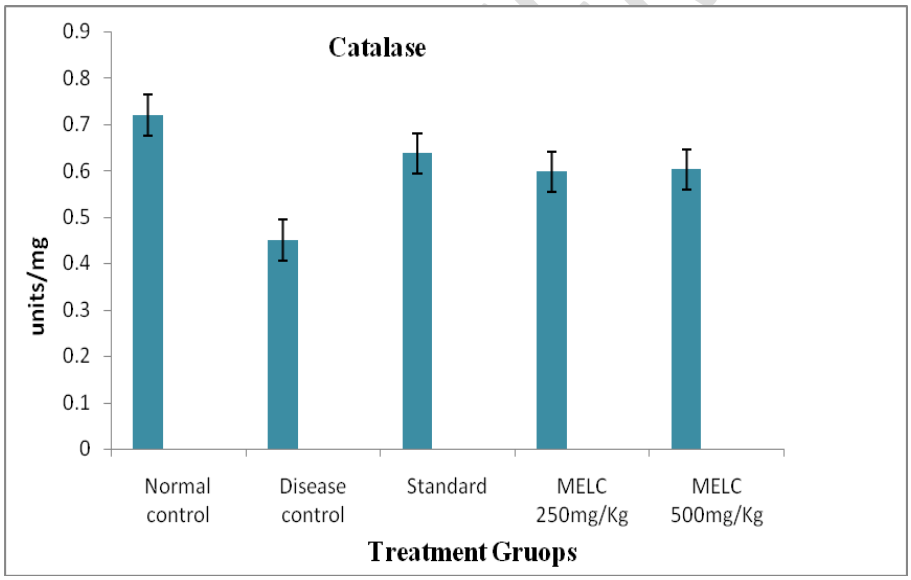

Fig. 3: Effect of MELC on Catalase

The significant values of CAT levels of normal, disease, standard, MELC 250mg/Kg and MELC 500mg/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 100mg/Kg and 200mg/Kg compared to disease control. Results were depicted in **Fig. 3**.

4. DISCUSSION

Te 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.

Cognitive impairment was seen to be linked with the hippocampal down-regulation of NMDA receptor [21, 22]. In this regard, our result also demonstrated that BPA intoxication leads to decreased expression of NMDA receptor. Whereas, MELC administration is capable of restoring the level of NMDAR in BPA intoxicated rats. Moreover, the MELC extract was also effective in enhancing the learning and memory in BPA intoxicated Rats. Oxidative stress has been closely associated with the pathophysiology of various diseases such as aging, atherosclerosis, diabetes, cancer and other degenerative disorders [23]. Te endogenous anti-oxidant enzymes like catalase, SOD and other molecules are efficient in scavenging the amplified ROS generated in brain and other tissues [24]. Thus, the involvement of oxidative stress in BPA induced cognitive impairment was assessed using various parameters like Catalase, SOD and LPO in the brain. Increased level of MDA, which subsequently marks increased LPO level, was observed in the BPA intoxicated rats as compared to control. This result was in accordance with various studies which demonstrated that on the exposure of BPA, there is an increased generation of ROS in the brain, decreased endogenous antioxidants in the liver and the epididymal sperm [25-27]. Furthermore, Aydogan et al. [28] also suggested that enhanced level of MDA in the brain was observed upon exposure to BPA. In the present study, significant impairment in learning and memory was observed in BPA intoxicated rats n comparison to the control. Hence, this fact can be put forward that sustained oxidative stress is one of the possible phenomena responsible for cognitive impairment in BPA administered rat group. Antioxidants play a major role in preventing the progression of degenerative diseases by alleviating the oxidative stress. Researchers have suggested the alteration in the redox potential in experimental animals upon contamination with the environmental insults [29]. Various reports have suggested the neuroprotective effects of MELC through its antioxidant nature in the brain [30, 31]. 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. Thus, MELC acts as a potent anti-oxidant, regulating the level of endogenous antioxidants, which are usually depleted as a result of aggravated oxidative stress. Furthermore, it has been seen to provide protection against oxidative damage and memory impairment induced by BPA.

5. CONCLUSION

Dementia and cognitive impairment has been seen to severely affect the quality of life and life span of elderly and are the primary symptoms of neurodegenerative disorders. Moreover, oxidative stress plays an essential role in the pathogenesis of these diseases and is linked to cognitive impairment. BPA is a potent endocrine disruptor and is seen to induce the cognitive impairment. Te 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 potent drug candidate for BPA-induced cognitive impairment

Abbreviations

CAT: Catalase, **LPO:** lipid per oxidation, **SOD:** superoxide dismutase,

MELC: Methanolic extract of *Lantana Camara,* BPA: Bisphenol A, **NMDARs**: N-methyld-aspartate-receptors.

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