

Antioxidant Role of *Clitoria ternatea* extract against Aluminum-induced Oxidative Stress in Hippocampus of albino Rats

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Abstract— The present investigation aims to know the exact mechanism of *Clitoria ternatea* (CT) extract as protective and therapeutic agent against neurodegenerative disorders such as Alzheimer's disease induced by Aluminum maltolate (AIM) in the hippocampus of albino rats. Animals were divided into four groups, six for each, control, AIM treated group, CT administered group and AIM plus CT treated group for one month. After experimental period, the activities of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) in hippocampus were assayed. Thiobarbituric acid reactive substance (TBA-RS) levels were measured as a marker of lipid peroxidation. The results demonstrated that AIM could induce TBA-RS levels and decrease SOD, CAT and GPx activities in hippocampus. However, treatment with *C. ternatea* significantly attenuated the lipid peroxidation process and restored the levels of antioxidant enzymes. These findings strongly implicate that CT has potential to protect hippocampus from oxidative damage resulting from AIM toxicity.

Index Terms— Aluminum maltolate, Alzheimer's disease Catalase, *Clitoria ternatea*, Glutathione peroxidase, Thiobarbituric acid reactive substance and Superoxide dismutase.

1 INTRODUCTION

Brain plays a key role in individual's developmental and behavioral outcomes. Hippocampus is mainly concerned with cognitive functions like learning and long-term memory potentiating activity (Andreas *et al.*, 2013). Various epidemiological investigations reported that the brain is more susceptible to oxidative damage induced by metal toxicity compared to other organs, due to high oxygen consumption through multiple mechanisms (Andersen *et al.*, 2004, Jankord *et al.*, 2008, Zafir *et al.*, 2009). Aluminum is a potential environmental neurotoxicant, it's increased bioavailability leads to oral ingestions into the body via diet, drinking water, vaccines, antacid exposures (Tripathi *et al.*, 2011) causes adverse effects on various organs and play a significant role in degeneration of nerve cells of human brain. Al is the secondary aggregating factor in the pathogenesis of Alzheimer's disease (AD) (Bharati *et al.*, 2008).

Increased Al concentrations can induce high oxidation process and free radical generation leads to molecular damage to neuronal pathways, neurotransmission alterations, bring on neuropathogenesis, ageing and death in Alzheimer's disease (Sohel and Forster 2007). Aluminum acts as a pro-oxidant and promotes formation of β -amyloid plaques in Alzheimer's disease (Bharati *et al.*, 2008). Many extensive evidences suggested aluminum exposure causes learning and memory impairment in hippocampus brain regions (Liang *et al.*, 1986).

Unfortunately, a great number of pharmacologically active molecules are not able to exert their activity *in vivo* owing to the CNS blood brain barriers, for this reason the conventional variety of plant compounds is becoming a promising future direction towards prevention and treatment of a broad range of neurological diseases.

Traditionally *Clitoria ternatea* (L.) has been used as memory enhancer (Sethiya *et al.*, 2009), gaining more attention in neuroscience, it is a perennial twining herb, commonly known as butterfly pea. Pharmacological studies have confirmed that *Clitoria ternatea* exhibit a extensive range of active biochemical constituents are found in different parts of the plant, are tannins, resins, starch, taraxerol, taraxerone, alkaloids, flavonoids, saponins, proteins and anthocyanins, Which are responsible for its various pharmacological activities such as nootropic, anxiolytic, anticonvulsant, sedative, anti-pyretic, anti-inflammatory, anti-diabetic, anti-oxidative, anti-stress (Jain *et al.*, 2003), immunomodulatory, larvicidal, proteolytic, antihelmintic, diuretic, anti-microbial and memory enhancing activity (Giurgea *et al.*, 1973). Previous studies on hippocampus of rat had demonstrated that CT root extract has enhanced memory (Sethiya *et al.*, 2009; Rai *et al.*, 2002; Mukherjee *et al.*, 2007). The mechanism behind the neuroprotective action is still incomprehensible, consequently *Clitoria ternatea* leaf extract has potential to enhance the levels of enzymatic and non-enzymatic antioxidants (Zheng *et al.*, 2001).

Hence the present study is designed to investigate the therapeutic potential of *Clitoria ternatea* leaf extract against AIM induced neurotoxicity in hippocampus of albino rat. modify the header or footer on subsequent pages.

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2 MATERIALS AND METHODS

2.1 Preparation of *Clitoria ternatea* Leaf Extract

The *Clitoria ternatea* plants were obtained from surrounding areas of Tirupati, Andhra Pradesh, India. The plants were thoroughly washed with double distilled water, leaves were separated and dried under shade dust-free condition for one week at room temperature. Then leaves were ground to fine powder. Finally powder was extracted with 60% of methanol. The mixed solution was left on constant magnetic stirring at room temperature for 72-hr. The extract was filtered and dried using vacuum desiccators, the powder was stored at 4°C for further experiments.

2.2 Animal

Male wistar strain rats of 3-months age group, weighing 150 ± 200 g procured from an authorized vendors (Sri Venkateswara Traders, Bengaluru, India), were used in the study. Rats were acclimatized in the lab for one week to adopt the laboratory conditions. The animals were randomized in to four groups, each group contains six animals housed in a polypropylene cages (47x34x20cm) containing sterile paddy husk as bedding and maintained at 22-25°C regulated temperature, with a light/dark cycle (12h/12h). The rats were fed with standard rat chow (Sai Durga Feed and Foods, India) and water *ad libitum*

2.3 Experimental Design

Experimental protocols were approved by the institutional ethical committee (CPCSEA Registration no.: 1677/PO/a/12/CPCSEA-3). The 3-months aged animals were equally randomized to four groups and six animals in each:

Group-I : Control: administered with (0.9%) saline solution

Group-II : AIM treated rats: AIM was dissolved in (0.9% saline solution and administered orally for 30 days.

Group-III : CT treated rats: CT leaf extract was administered orally for 30 days.

Group-IV : AIM+CT treated rats: AIM & CT were administered simultaneously for 30 days.

2.4. Tissue Collection and Preparation of Tissue Homogenates

After the 30 days of the experimental period rats of each group were sacrificed by cervical dislocation and the brain tissues were immediately isolated, the discrete brain hippocampus was dissected on an ice cold glass plate and collected in ice cold medium and homogenate was prepared.

2.5. Determination of Lipid Peroxidation

Thiobarbituric acid reactive substances (TBA-RS) an index of lipid peroxidation was estimated in hippocampus as described by Okhawa *et al* (1979). The amount of TBA-RS was determined spectrometrically by UV-Vis spectrophotometer at 532 nm and the values were expressed as nano moles of TBA-RS per mg protein/hr.

2.6 Measurement of Antioxidant Enzyme Activities

Superoxide dismutase activity was estimated in tissue homogenates according to the method of Misra and Fridovich, (1972). The activity of the enzyme was expressed as units/mg protein. Catalase (CAT) activity was assayed spectrophotometrically using the slight modification method of Aebi (1984). One unit of catalase activity is equal to the μ moles of H_2O_2 degraded / mg protein/ min. Glutathione peroxidase activity (GPx) was measured by the method of Flohe and Gunzler 1984. The absorbance was measured at 340nm. GPx activity is expressed as μ moles of GSH oxidized / mg protein / min. Protein concentration in all brain tissue homogenates was estimated by the standard protocol of Lowry *et al.* (1951). Bovine serum albumin (BSA) was used as standard, and the color developed was read at 660 nm. The amount of total proteins present in the sample was measured by using bovine albumin standard and the values are expressed as mg/g wet weight of the tissue.

2.7 Histopathology

Histopathological evaluation of brain regions was performed in both, control and treated groups (Humason 1972). They were then sectioned, stained with hematoxylin-eosin and examined under an optical light microscope.

2.8 Statistical Analysis

Experimental data were summarized as Mean \pm S.D. groups were analyzed by using one-way ANOVA followed by Dunnett test (compare all vs. control). The statistically significant ($p < 0.0001$).

3 RESULTS

3.1 Lipid Peroxidation

The administration of AIM for 30-days induced alterations in the both biochemical parameters as well as the histopathology in hippocampus of rat. The results obtained in the present study revealed that the level of TBA-RS was found to be significantly increased by AIM treatment respectively as compared to the control. The Fig-2 explains about AIM enhanced the levels of TBA-RS in hippocampus brain regions of rats compared to control and *Clitoria ternatea* treated rats. Whereas CT only administered group showed the TBA-RS levels similar to control. And in both AIM and CT administered group, it was clearly observed that the TBA-RS levels were somewhat decreased compared to AIM only administered group.

3.2. Enzymatic Antioxidant Status Activated by *Clitoria ternatea* Treatment

By the results, it was confirmed that AIM showed inhibiting activity in the level of enzymatic antioxidants such as SOD, CAT and GPx and CT administered group showed normal activity of these enzyme as in control in both CT and AIM administered group it was confirmed the activity of these enzymes was enhanced compared to AIM.

In the present study AIM reduced the antioxidant enzymes

SOD, GPx and CAT in the hippocampus region and it was fascinatingly restored by CT leaf extract, which was clearly observed in AIM+CT administered group. It is worthy to note that CT did not affect the enzymatic antioxidant levels due to its natural radical scavenging activity, whereas we found AIM as a pro-oxidant showed a decreased activity of SOD, GPx and CAT in hippocampus brain region when compared to that of control group.

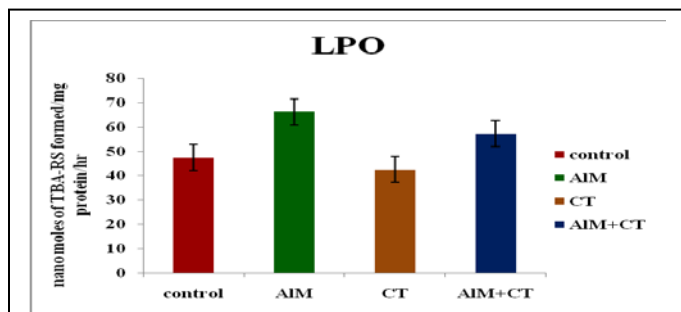


Fig.1 Effect of CT on Thiobarbituric acid reactive substance (TBARS) levels in hippocampus tissue of rats exposed to AIM. Each column represents the mean \pm S.D. (n=6). Statistical analysis was performed by one-way ANOVA, followed by dunnett test. Significant compared all vs. to control ($p < 0.0001$).

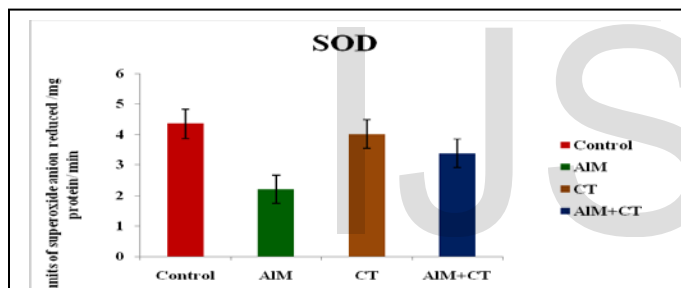


Fig: 2 Effect of CT on Superoxide dismutase (SOD) levels in rat hippocampus exposed to AIM. Each column represents the mean \pm S.D. (n=6). Statistical analysis was performed by one-way ANOVA, followed by dunnett test. Significant compared all vs. to control ($p < 0.0001$).

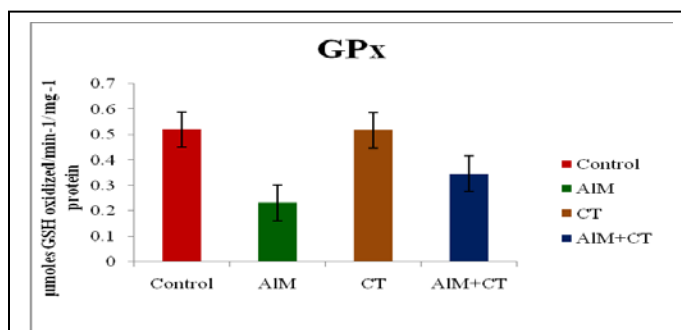


Fig: 3 Effect of CT on glutathione peroxidase (Gpx) levels in rat hippocampus exposed to AIM. Each column represents the mean \pm S.D. (n=6). Statistical analysis was performed by one-way ANOVA, followed by dunnett test. Significant compared all vs. to control ($p < 0.0001$).

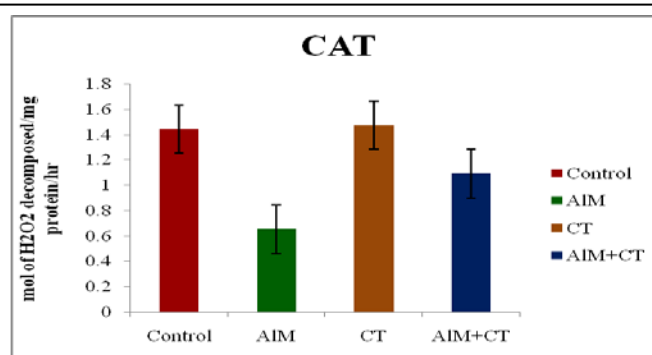


Fig: 4 Effect of CT on Catalase activity (CAT) levels in rat hippocampus exposed to AIM. Each column represents the mean \pm S.D. (n=6). Statistical analysis was performed by one-way ANOVA, followed by dunnett test. Significant compared all vs. to control ($p < 0.0001$).

3.3 Histopathology of Hippocampus

In the current study histopathological changes were observed in all groups of hippocampus of rats. In both control and CT administered hippocampus it was observed the normal pyramidal neuron of neuro fibrillary network histo architecture (Fig-5A & 5C). AIM administered rats showed different morphological degenerative changes in pyramidal neurons (DGPYNEU), structural damage in neuro fibrillary network (DGNFN) and vacuolated neuro mesh work (Fig-5B). Whereas these degenerative changes were reduced by CT (Fig-5D).

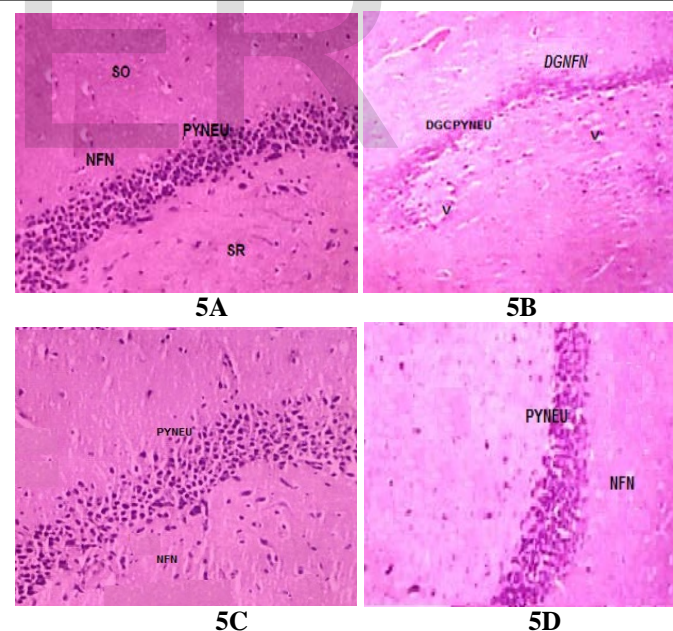


Fig: 5 Control and Treated hippocampus

Fig: 5A (Control) & **5C** (CT)-showing the normal pyramidal neurons (PYNEU), neuro fibrillary network (NFN).

Fig: 5B AIM treated hippocampus showed the morphological degenerative changes in neuro fibrillary network (DGNFN).

Fig: 5D AIM+CT showed administered group very much reduced degenerative changes were observed.

DISCUSSION

Hippocampus plays a major role in learning and memory, it is involved in potentiation of memory and is particularly sensitive to Al induced neurotoxicity. Aluminium with maltolate combination used as a suitable compound for neurotoxicology studies. The results demonstrated that AIM significantly increased the TBA-RS levels as markers of lipid peroxidation in the hippocampus of rats. This is because aluminum bound to the polar head groups of membrane phospholipids and induce changes in membrane lipids which facilitates lipid peroxidation, Such results are in harmony with those obtained by Deloncle *et al* 1999.

It is evident that high concentrations of Al affects enzyme levels in brain regions that may relevant to oxidative stress contribute increased ROS production causes neurodegenerative diseases. Aluminum intoxicated animals, showed decreased levels of enzymatic antioxidants, This is due to rise in LPO in aluminium treated rats associated decrease in the activity of some antioxidant enzymes involved in the detoxification of ROS, namely SOD, CAT as well as the level of GPx in the hippocampus compared with the control, due to the pro-oxidant effect of Al. These findings are in agreement with previous studies of Savory *et al* 2003. SOD and CAT which function as blockers of free radical processes, as a result of higher intracellular concentrations of aluminium significant decrease in the activities of these enzymes in brain could be related to a reduced synthesis of the enzyme proteins (Albendea *et al.*, 2007). Although previous studies have suggested that excess Al accumulates in the hippocampus, has reported elevated oxidative stress conditions may causes neuronal damage was established (Sushma *et al.*, 2014). *Clitoria ternatea* leaf extract has been significantly prevented the increase in oxidative stress marker i.e TBA-RS levels due to antioxidant properties of CT constituents that protect cellular membranes integrity from Al-induced oxidative damage and induced the biological antioxidant system. The decreased antioxidant enzymes SOD, GPx and CAT by AIM toxicity enhanced by CT in ALM+CT administered group. This may be because of antioxidants present in the CT play an significant role in preventing free radical causing neuronal damage.

Histopathological studies revealed morphological changes in neuro fibrillary network (NFN) and neurodegenerative changes in pyramidal cells (PYNEU) in AIM administered group rat hippocampus compared to control animals. These changes are due to elevated LPO levels in the hippocampus are also participate in free radical- induced oxidative structural loss in neurons by aluminium (Dua and Gill 2001). Lipid peroxidation of biological membranes affect in membrane potential, causes loss of membrane fluidity (Dua and Gill 2001, Yousef 2004, Albendea *et al.*, 2007, Gomez *et al.*, 2005, Flora 2003). CT treated group showed normal architecture of pyramidal neuron layer formation of neuro fibrillary network and enzyme activities as in control. According to the results, it is evident that CT does not show any toxic effect in rat hippocampus and it may be counteracting the Al toxicity. Further investigations are needed to find out the mechanism involved in therapeutic

effect of CT against Al.

CONCLUSION

From the results, it is concluded that CT possess neuroprotective properties by decreasing oxidative stress and increasing antioxidant enzymes against AIM induced neurotoxicity in hippocampus of albino rat.

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