Neuroprotective Activity of *Garcinia pedunculata* Roxb. *ex* Buch.-Ham. Fruit Extract Against Aluminium Chloride Induced Neurotoxicity in Mice

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**ABSTRACT**

Aluminium chloride (AlCl₃) is a known potent environmental neurotoxin causing progressive neurodegenerative changes in brain. The present study was aimed to evaluate the neuroprotective activity of aqueous extract of fruit rind of *Garcinia pedunculata* on chronic exposure of aluminium chloride induced neurotoxicity in Swiss albino mice. Albino mice were categorized into four different groups; Group 1—served as vehicle control, Group 2 mice were administered with AlCl₃ 40 mg/kg body weight i.p. for 45 consecutive days. Groups 3 & 4 mice were administered with AlCl₃ 40 mg/kg body weight i.p. for 45 consecutive days along with aqueous extract of fruits of *G. pedunculata* 200 & 400 mg/kg body weight. Chronic administration of AlCl₃ developed behavioral deficits, triggered lipid peroxidation and AChE activity and reduced levels of catalase, glutathione peroxidase and histological aberrations. Co administration of aqueous extract of *G. pedunculata* has attenuated the AlCl₃ induced alteration in the behavioral, lipid peroxidation, catalase, glutathione peroxidase, AChE and histological changes of brain tissue. Thus the results of the present study have demonstrated the protective role of aqueous extract of fruit rind of *G. pedunculata* against AlCl₃ induced neurotoxicity.

**Key words:** Acetylcholine esterase, Aluminium chloride, *Garcinia pedunculata*, Lipid peroxidation, Neurotoxicity.

**INTRODUCTION**

Aluminium chloride has been considered as an environmental factor that contributes neurodegenerative disorders. Aluminium particularly interact with enzymes and biomarkers related to Alzheimer's disease.¹ Occupational exposure of Aluminium can occur through diet by food processing & storage in aluminium vessels, cans and foil, drinking water, medicines such as antacids, vaccines and cosmetic agents etc. It has been reported that an average dietary intake of aluminium ranges from 3-12 mg/day. The population who are routinely exposed to the product may have higher chances of neurotoxicity.²,³ The repeated exposure of aluminium can cause severe oxidative stress and pathological alteration. Aluminium acts as a pro oxidant and on continuous exposure starts accumulation in cortex, hypothalamus and different parts of brain, where it induces the formation of reactive oxygen species (ROS) and result in oxidative damage.⁴ Aluminium also promotes amyloidosis β peptide formation and amyloidosis which leads to Alzheimer’s disease. Increased accumulation of AlCl₃ in brain will affect the slow and
fast axonal transports and cause neuro-inflammation and synaptic structural abnormality resulting in neurodegeneration. Aluminium mainly causes degeneration of cholinergic nerve terminals in cortical areas and cell depletion leads to severe learning deficits.5 Plants are considered as the great reservoir of structurally diverse molecules with therapeutic values and are useful in the treatment of various ailments. The fruits of *Garcinia pedunculata* belonging to family *Clusiaceae* are commonly known as 'Amlavethasa'. The pericarps of the fruits are extensively used in diet across the north eastern states of India. It has the following folklore claims such as rejuvenator, cardio tonic, asthma, obesity and arthritis.6,7 Phytochemical analyses their dried fruit rinds has confirmed the presence of (-) hydroxyl citric acid, benzophenones, pedunculol, garcinol and combogenn.8 The pharmacological studies have shown that the aqueous extract of the fruit rinds possesses anti-inflammatory, hepatoprotective,9 cardio-protective and *in vitro* antioxidant activities.10 The test drug has strong anti-inflammatory, anti-oxidant and cytoprotective properties and hence the present study was aimed to screen the neuroprotective activity of the aqueous extract of dried fruit rinds of *G. pedunculata* against aluminium chloride induced neurotoxicity.

**MATERIALS & METHODS**

**Chemicals**

Aluminum chloride hexahydrate (AlCl₃.6H₂O) (Thomas Baker Pvt. Chemicals Mumbai, India.) and all other chemicals and reagents used were of analytical grade.

**Plant material and extract preparation:**

Fruits of *G. pedunculata* were procured from Assam during April 2015, authenticated in Pharmacognosy laboratory at SDM Research Centre, Udupi, shade dried and powdered at SDM Pharmacy Udupi. 500 g was soaked in 2 L of cold distilled water for 24h, filtered and concentrated by evaporation on water bath until free from water.

**Experimental animals**

Swiss albino mice of either sex, (30 to 40g body weight) were obtained from animal house attached to the department of Pharmacology & Toxicology of SDM Centre for research in Ayurveda and Allied Sciences Udupi, India (temperature at 25 to 27°C, humidity of 50 to 55% and 12 h light and dark cycles). Animals were fed with commercial pellet diet and water *ad libitum*. Approval for the experimental protocol used for this study was made by the institutional animal ethical committee (No. is SDMCR/IAEC/SDM-03/25/08/2015).

**Acute oral toxicity test:** It was carried out as per OECD guidelines 425, using AOT software. The aqueous extract of fruit rinds of *G. pedunculata* (AFGP) was made into a suspension in 0.5% Carboxy methyl cellulose and dosed in the following order 175, 550, and 2000 mg/kg body weight. The animals were observed for 14 days for mortality. The LD₅₀ was determined by AOT 425 statpgm software.

**Experimental design:** Group 1 mice were treated with 0.5% Carboxy methyl cellulose orally for 45 consecutive days and considered as vehicle control. Group 2 (AlCl₃ control) were treated with 40 mg/kg AlCl₃ (pH 7) intraperitoneal for 45 consecutive days. Group 3 & 4 rats were co-administered with aqueous extract of dried fruit rinds of *G. pedunculata* (AFGP) 200 & 400 mg/kg body weight respectively an hour before AlCl₃ administration for 45 consecutive days. At the end of the experimental period mice were sacrificed by decapitation and sera and brain tissues were collected from each group. The brain tissues were immediately homogenized, centrifuged and the supernatant was stored at -20°C for 72 h and further used for biochemical analysis.

**Behavioral assessment**

**Tail suspension test:** The total duration of immobility induced by tail suspension was measured according to a standard method.12 Mice isolated both acoustically and visually, were suspended 50 cm above the floor by adhesive tape placed approximately 1 cm from the tip of the tail. Immobility time was recorded during a 6 min test.

**Forced Swimming Test:** An hour after the last dose of group specific drugs, individual mouse was put into a water filled (30 cm) glass cylinder measuring about 40 cm × 18 cm and observations were made for 6 minutes. First two minutes were not considered for recording the drug effect and were taken as stabilizing time. The limb movements and the effort of the mice to get out of the cylinder in the next 4 minutes was noted and subtracted later from total time (4 min) to find the time of immobility. This was considered as the index of depression.13

**Preparation of brain homogenate:** Brain was excised and cleaned with ice cold saline and stored in -20°C in freezer. Tissues were thawed and homogenized in phosphate buffer saline pH 7.4, centrifuged at 4°C and supernatant stored at -20°C. The homogenate was subjected to determination of catalase activity, glutathione peroxidase activity and lipid peroxidation.

**Determination of catalase activity:** 1 ml of brain tissue homogenate was mixed with 5 ml of phosphate buffer and 4 ml of 0.2 M H₂O₂ in phosphate buffer and time
was noted. Exactly after 180 seconds of adding $H_2O_2$, a set of 1ml of reaction mixture from the above was taken in 2 ml dichromate acetic acid. It was kept in boiling water bath for 10 minutes, cooled all the tubes under running tap water and finally noted the reading at 570 nm against reagent blank. Catalase activity in the tissue was expressed as $\mu$moles $H_2O_2$ consumed /mg protein /min.$^4$

**Determination of lipid peroxidation:** Lipid peroxidation activity was determined by measuring the content of the Thio-barbituric acid reactive substances (TBARs).$^{15}$ Level of lipid peroxidation was expressed as mmoles of MDA formed/g wet tissue.

**Determination of Glutathione peroxidase:** Glutathione peroxidase was estimated by using a standard protocol$^{16}$ and the glutathione peroxidase activity was expressed as $\mu$moles glutathione utilized per mg protein per minute at 37$^o$C.

**Histopathology of brain:** Five brain samples from each group were used and ten slices per sample were examined for histopathological study. Immediately after the excision from mice, the brain tissue was transferred into 10% formalin. Sections of less than 5 $\mu$m thickness of brain tissue were prepared using microtome and stained with haematoxyline and eosin for microscopic observations.$^{17}$ All slides were then evaluated under light microscope (ZEISS Axio lab A1 India).

**Statistical analysis:** The obtained data were expressed as Mean ± SEM and analyzed by one way ANOVA, followed by Dunnet’s multiple comparison ‘t’ test using Graph Pad Prism 3. A p<0.05 were considered as statistically significant.

**RESULTS**

Acute oral toxicity study did not reveal any mortality in any dose up to 2,000 mg/kg of aqueous extract of fruits of the plant *G. pedunculata*. This indicates that LD$_{50}$ is much more than 2,000 mg/kg and hence 1/10$^6$ and 1/5$^6$ of the higher dose studied (200 mg/kg) dose was selected for the present neuro protective study.

**Effect of AFGP on AlCl$_3$-induced behavioral changes in mice:** In the tail suspension test, the duration of immobility time was significantly increased in AlCl$_3$ control as compared to vehicle control, whereas the co-administration of AFGP at both dose levels attenuated the immobility time but at higher dose level, observed significant reduction in immobility time as compared to AlCl$_3$ control (p<0.05) (Table 1).

In Behavioral despair test, the duration of freezing time was significantly increased in AlCl$_3$ administered group as comparison to vehicle control (p<0.01). Co administration of AlCl$_3$ has significantly attenuated the freezing time in both the dose levels of AFGP as compared to AlCl$_3$ control (p<0.01) (Table 2).

**Effect of AFGP on AChE:** AChE is a key enzyme involved in cholinergic neurotransmission and is a marker of extensive loss of cholinergic neurons in the brain. A significant increase (P<0.01) in the level of AChE was observed in the animals induced with AlCl$_3$. AFGP co administered with AlCl$_3$ attenuated AChE levels; however the observed decrease was non-significant in comparison to AlCl$_3$ control group (Table 3).

**Effect of AFGP on antioxidant parameters:** Aluminium chloride induced free radical mediated oxidative cell injury is one of the important manifestations to elevated lipid peroxidation. In the present study there is an increased lipid peroxidation in the Aluminium chloride administered group as compared to vehicle control (p<0.01). The AlCl$_3$ induced lipid peroxidation was significantly attenuated by co-administration of AFGP (p<0.01). Repeated administration of AlCl$_3$ caused marked oxidative stress, which led to decrease in the antioxidant enzymes activities such as catalase and glutathione peroxidase as comparison to control group mice; while AFGP co-administered with AlCl$_3$ has increased the activity of catalase and non-significant changes in glutathione peroxidase as compared to AlCl$_3$ control. (Table 4).

**Histopathological changes:** Chronic administration of aluminium chloride produced moderate intensity of neuro degeneration in different parts of the brain like fore brain, mid brain, hippocampus and cerebellum. In hippocampus, there is a decrease in the pyramidal cells population, cellular disorganization and distorted cells. These changes were significantly attenuated by the administration of AFGP at both the dose levels (Figure 1).

In AlCl$_3$ control mice forebrain and mid brain sections have shown edematous changes, cellular disorganization and cell distortion, whereas AFGP administered at higher dose level significantly prevented AlCl$_3$ induced changes (Figures. 2 and 3).

Repeated administration of AlCl$_3$ has caused micro cystic changes in the cellular layer of cerebellum and these changes were attenuated by AFGP administered at both the dose level (Figure 4).

**DISCUSSION**

Aluminium on repeated exposure is experimentally documented neurotoxin and able to cross the blood brain barrier. It is implicated in the pathological changes in Alzheimer’s disease and other neurodegenerative disorders. It is considered as an important environmental factor to wide range of neurodegenerative disorders.$^{18}$
Correlation between AlCl₃ accumulation and progressive oxidative damage on the brain tissues have been already reported, it can induce lipid peroxidation and alter physiological and biochemical characteristics of biological system. Several natural products such as curcumin, quercetin, naringin and catechins have potential neuroprotective activities against AlCl₃ induced neurotoxicity. The fruit rinds of Garcinia pedunculata contains (-) hydroxyl citric acid, benzophenones, pedunculol, garcinol and combogenphyto-constituents and major contributors to the antioxidant activity of G. pedunculata. Current findings have shown that chronic administration of AlCl₃ leads to increased levels of lipid peroxidation due to elevated concentration of MDA and decreased catalase and glutathione peroxidase anti-oxidant enzymes in the AlCl₃ administered control group. From the earlier studies it has been documented that the AlCl₃ has no direct effect on lipid peroxidation. On repeated exposure it gets accumulated in the brain cells and accelerates iron mediated oxidative stress and cellular damage in acidic and neutral environment leading to neurotoxicity.

Normally brain cells have nonenzymatic and enzymatic mediators to counteract reactive oxygen species mediated oxidative stress. Nonenzymatic molecules are glutathione, thioredoxin and thiol containing molecules, whereas...
enzyme mediators include superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px). SOD is the first defensive enzyme against superoxide and it converts superoxide anions to hydrogen peroxide and oxygen. The generated hydrogen peroxide should be efficiently neutralized by CAT and GSH-Px. Thus these enzymes protect the cells from damaging aggressive hydroxyl radicals. In the present study there is a significant attenuation of lipid peroxidation and elevated levels of catalase and glutathione peroxidase enzymes in the *G. pedunculata* co-administered with AlCl<sub>3</sub> and attenuated the oxidative stress induced by AlCl<sub>3</sub>. Acetylcholine esterase is an important enzyme in the cholinergic system involved in the metabolism of acetylcholine neurotransmitter. It has been reported that the increase in the acetylcholine esterase level increased metabolism of acetylcholine leads to oxidative stress causes neurobehavioral changes especially memory and cognitive failure. However, recently it has been shown that circulating AChE activity reflects inflammatory response, since acetylcholine suppresses inflammation. Based on this premises donepezil an AChE inhibitor is being investigated for neuro protective activity. In the present study significant elevation in serum AChE activity was observed in aluminium chloride administered group in comparison to the control. This elevation was found to be attenuated in test drug treated groups. This observation can be considered as an additional evidence for the neuroprotective activity indicating the role of *G. pedunculata* in the regulation of cholinergic function.

Repeated administration of AlCl<sub>3</sub> has caused microcytic changes in the cellular layer of cerebellum. In forebrain and mid brain sections that there were edematous changes, cellular disorganization and cell distortion in few mice. In hippocampus, there was a decrease in the pyramidal cells population, cellular disorganization and distorted cells. Thus chronic administration of AlCl<sub>3</sub> produced moderate intensity of neurodegeneration in different parts of the brain like fore brain, mid brain, hippocampus and changes in cerebellum in few rats. These changes were significantly attenuated by the administration of AFGP at both dose levels. These results demonstrated that the AFGP significantly prevented brain damage and improved functional outcome.
The observed protection by AFGP treatment may be due to its antioxidant and cytoprotective property.

CONCLUSION

On the basis of the present study it can be concluded that the aqueous extract of *Garcinia pedunculata*, exhibited significant neuroprotection against AlCl₃ induced neurotoxicity in mice according to histological, behavioral and biochemical analysis. The anti-oxidant effect and AChE activity of the test drug may also contribute to the observed neuroprotection.

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