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Der Pharmacia Sinica, 2011, 2 (4): 184-191

Der Pharmacia Sinica
ISSN: 0976-8688
CODEN (USA): PSHIBD

Ameliorative Effect of Curcumin against Excitotoxin induced brain damage in rats

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ABSTRACT

Curcumin (curcuma longa linn), the major constituent of turmeric is been used in food preparation as well as to treat inflammatory disorders. Excitotoxins, the ultimate brain layer, that have a bland taste, which stimulate the neuron brain cells and lead to exhaustion. The present study examined the ability of this compound to protect against neuronal brain damage of rats. The biochemical changes resulting from curcumin also correlated well with its ability to ameliorate the changes induced by excitotoxin. The beneficial effects of curcumin appear as a promising class of compounds for brain protection. The mitochondrial enzymes and histopathologiocal examination was carried out to measure the damage induced by excitotoxin. The coronal section of brain, in the presence and absence were compared microscopically to determine the toxin induced tissue damage.

Keywords: Excitotoxin, brain damage, curcumin, neuroprotective, mitochondrial enzymes.

INTRODUCTION

In the chemical age in which we live, we are surely live in an “exciting” time. If not careful, oursystem will be challenged by “poisons” even via food we intake. “Poisons” mentioned here mainly represent “excitotoxins” (ET) or excitatory amino acids. These ET are added to human foods and drinks in order to enhance the flavour and taste. ET, if ingested like this, stimulates brain cells so vigorously so that they die of exhaustion. The amino acids phenylalanine and aspartic acid are said to be excitatory amino acids. These excitotoxins (ET) cross the blood brain barrier and cause neurotoxicity [1] . Phenylalanine is said to be neurotoxic, since it can inhibit enzymes needed to synthesize the neurotransmitter and thus cause damage to the neurons [2].

Hypothalamic damage due to aspartic acid administration has also been reported [3]. In certain cases, damage to major organ like brain will also lead to liver injury secondarily affecting its function [4]

A variety of free radical scavenging antioxidants is found mostly in dietary sources [5]. The present study has been designed to observe the effect of these excitatory amino acids in rats and also to assess whether the curcumin (diferuloyl methane), an aromatic kitchen master ingredient, could affect the excitotoxin induced alterations in rat brain and liver. Curcumin and the other curcuminoids have been found to have potent properties such as hypolipidemic property [5], antitumor property [6,7] etc., and they have entered into Phase I clinical trials for cancer chemo prevention by the National Cancer Institute. It would be appropriate to consider the effect of this wonder drug in the case of organ injury due to dietary toxins.

MATERIALS AND METHODS

Pure bred, Wistar strain, albino rats weighing 150-200 gm, obtained from veppery, TANUVAS Chennai.. The animals are maintained at an ambient temperature of 25 ± 2 ^{0} C under at 12'hr light, 12 hr dark cycle Bihrnger [8] with food pellets (supplied by Lipton India Ltd. Bangalore), and clean drinking water. The animals were maintained and treated as per the CPCSEA guidelines. Curcumin was dissolved in distilled water and 80mg/kg, bodyweight was i.p injected daily for 15days followed by the induction of Excitotoxin 1g / kg bw for 10 days. (Oral administration) and the rats were sacrificed for histopathological and enzyme analysis.

Mitochondria from the tissues, brain and liver were isolated by the method of Johnson and Lardy [9].The selected tissue was disrupted by homogenization in ice-cold isotonic sucrose. Differential centrifugation was then employed to separate the mitochondria from organelles. The mitochondrial pellet was collected to perform protein and enzyme estimations.

Estimation of protein

The protein content was estimated by the method of Lowry et.al...[10] using alkaline copper reagent and folin's phenol reagent. The values obtained were used for calculating activities of enzymes.

Assay of Cytochrome C oxidase (Cox)

The activity of Cox was assayed by the method of Pearl *et.al*.[11]. The enzyme activity was expressed as change in absorbance (550nm) /min/mg of protein.

Assay of succinate dehydrogenase (SDH*)*

The activity of succinate dehydrogenase was assayed according to the method of Slater and Bonner, [12]. Change in extinction at 455 nm was followed for period of 5 min in spectrophotometer at 30 sec intervals.

Assay of malate dehydrogenase (MDH)

The enzyme activity was assayed by the method of Mehler *et al*., [13]. The decrease in absorbance was read at 340nm. The activity of enzyme was express as µ moles of NADH oxidase /min/mg of protein.

Estimation of C- Reactive Protein (CRP)

CRP was estimated by using the turbidometric method of quantitative CRP [14]. The increase in turbidity (at 340nm) corresponds to the concentration of CRP in the test specimen. The values of CRP expressed as < 0.6 mg/dl are considered as negative.

Statistical analysis

The results were calculated by one way analysis of variance (ANOVA) using SPSS software package. Results were expressed as mean ± SD. The mean difference is significant at the 0.5 level.

RESULTS

Histological results evaluating the extent of damage induced by ET, in rat brain after 2-10 days of ET treatment were presented in Plate I and Plate II respectively and there wouldn't be any significant change after 2 days and 4 days of treatment (b and c of Plate I and II) and showing similar pattern as control histology (Plate I-a and Plate II- a). After 6^{th} day and 8^{th} day of treatment, tissue damage could be encountered (d and e of Plate I and II) based on John W.onley report. However, significant changes were observed after 10 days of treatment has evident from Plate I f and II- f. Hence, the latter period of 10 days of amino acid administration was selected, and used throughout for inducing damage and to assess the efficacy of curcumin.

The activities of the enzymes namely Cox, SDH and MDH in mitochondria of liver and brain of normal and experimental rats have been reported in Table 1 and Table 2 respectively. The enzymes Cox and MDH exhibited a significant decrease in activity in liver mitochondria as well as brain mitochondria of amino acid induced rats ($p<0.5$) when compared to control [15].

Mitochondria are important organelle for cellular oxidation reaction and are also the main source of reduced oxygen species in the cell. In the study, reduced activity of Cox was encountered in group 2 when compared ($p<0.5$) to group 1 (control), implying that the powerhouse of the cell is damaged on treatment with amino acids, there by altering the energy production, by affecting the electron transport chain. SDH is an important enzyme in TCA (Tri Carboxylic Acid cycle). It is only dehydrogenation in TCA that involves the direct transfer of H^+ from substrate to FAD (Flavin adenine dinucleotide) without NAD (Nicotinamide adenine dinucleotide). Normal activity of SDH may be ensured by the study supply of oxygen [16].

Table 1 Mitochondrial enzymes Cox, SDH, and MDH levels in the brain of control, ET induced and Curcumin pretreated rats. Values are expressed as mean ± SD

Parameters	**Cytochrome C Oxidase**	SDH	MDH
Group 1 (Control)	0.20 ± 0.12	1.80 ± .0.78	133.4 ± 1.81
Group 2 (ET)	0.18 ± 0.12	2.1 ± 0.2	109 ± 0.36
Group 3 a (80 mg)	0.19 ± .17*	1.75 ± 0.27*	137 ± 0.57*
Group 3 b (160)	0.26 ± .20*^{NS}	1.55 ± 0.12*^{NS}	114 ± 0.78*
Group 3 c (240)	0 .19 ± .11*	1.479 ±0 .12*	93 ± 0.49*
Group 3 d (320)	0.18 ± .12*^{NS}	1.126 ± 0.11*^{NS}	80 ± 0.68*^{NS}

n (no. of animals in a group)=6, the mean difference is significant at the 0.5 level.

** p<0.5- Group 1 - Control Vs Group 2 - ET induced, Group 2 - ET induced Vs Group 3a (80mg), 3c (240mg) – Curcumin pretreated, and NS Group 3 b (160mg)-d (320mg) Curcumin pretreated-Vs. Group 1-Control*

HISTOPATHOLOGICAL CHANGES ENCOUNTERED IN RAT BRAIN ADMINISTERED ASPARTATE AND PHEYLALANINE (ET) AT 1g/KG OF BODY WEIGHT FOR DIFFERENT NO. OF DAYS

Plate I

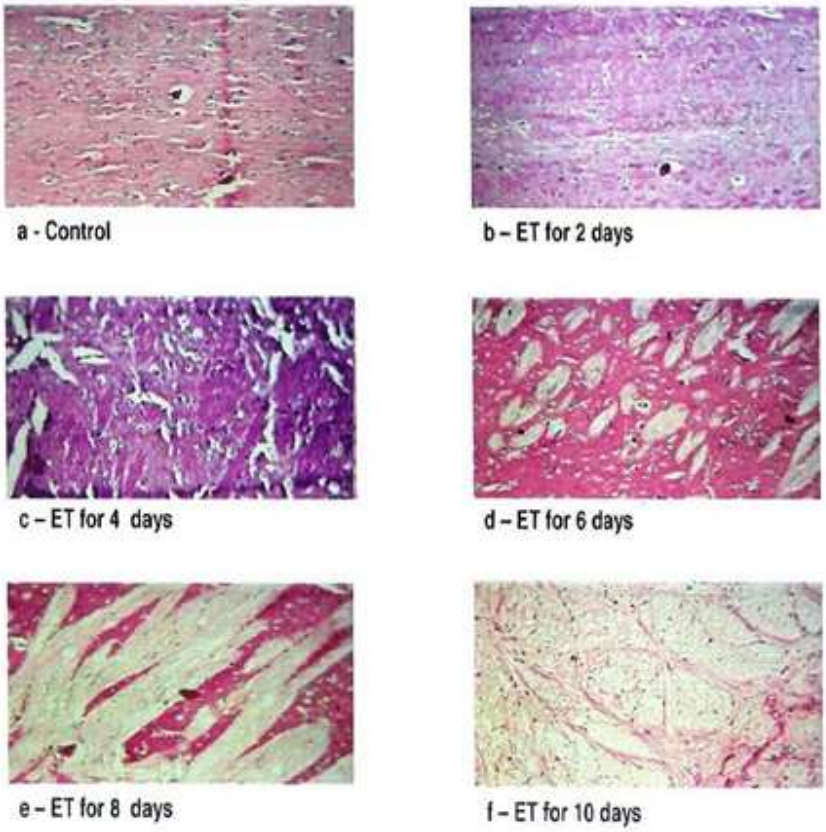


Table 2 Mitochondrial enzymes Cox, SDH, and MDH levels in the liver of control, ET induced and Curcumin pretreated rats. Values are expressed as mean ± SD

Parameters	**Cytochrome C Oxidase**	SDH	MDH
Group 1 (Control)	0.30 ±0.18	1.70 ± 0.23	131.28 ± 1.3
Group 2 (ET)	0.11 ± 0.09	2.24 ± 0.34	106.33 ± 0.33
Group 3 a (80 mg)	0.31 ± 0.28*	1.65 ±0.22*	135.5± 0.22*
Group 3 b (160)	0.27 ± 0.5	1.48 ±0.10*	121.6 ± 0.26*
Group 3 c (240)	0.26 ± 0.17*^{NS}	1. 43 ±0.30*	116.3 ± 0.32*^{NS}
Group 3 d (320)	0.23 ± 0.9*^{NS}	1.49 ± 0.34*^{NS}	149.3 ± 0.65*^{NS}

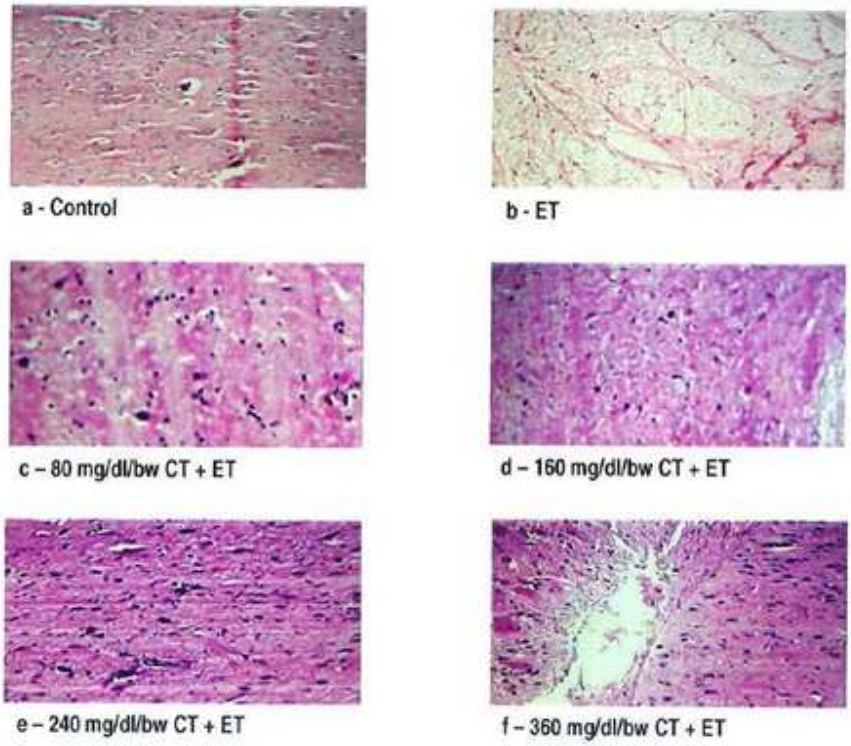
*n (no. of animals in a group)=6, *p<0.5 Group 1- Control Vs Group 2-ET induced, Group 2- ET induced Vs Group 3 a (80mg), 3b (160mg)- Curcumin Pretreated , NS Group 3 c(240mg),3 d (360mg) Vs Group 1-Control. In the present study (Table 1 and 2), ET treated rats registered elevated activity of SDH when compared to control (p<0.5). MDH which catalyze the reduction of oxaloacetate to L-malate and generation of NADH in ET rats showed a reduction in activity when compared to control (p<0.5).*

When the activities of Cox, SDH and MDH were studied in CT rats at the dosage level of 240mg, 360mg and 420 mg/kg bw, moderate difference was encountered and no near normal activities found when compared to control. Hence it is considered that at the dosage level of 80 mg/kg body wt could be suggested as optimal one against experimental brain injury by ET in rats.

Since near normal picture could be encountered in CT rats at the dosage level of 80 mg/kg bw, CRP test was done in serum of rats exposed to that dosage and also done in serum of rats exposed to 160 mg/kg bw. CRP is produced in liver for the detection of low-grade injury and inflammation and is an acute phase protein (APP). Hepatic production increase up to a 1000 fold directly after any trauma, ischemia, infection or necrosis. CRP is known to bind to the plasma membrane and to small nuclear ribonucleic protein particles in the exposed nuclei of damaged cells [17]. After such binding, human CRP activates the complement pathway [18].

HISTOPATHOLOGICAL CHANGES ENCOUNTERED IN RAT BRAIN PRETREATED CURCUMIN (15 DAYS) FOLLOWED BY ET ADMINISTERATION

Plate II



DISCUSSION

Plate I and II exhibit histological pattern of control, ET treated and CT (Curcumin pretreated) rats, in brain. Plate I a and II a shows normal architecture of control brain. Both Plate I-b and Plate II-b (ET treated brain), present derangement of cells and damaged regions of brain, when compared to control. Plate I c – f and Plate II c-f exhibit tissue architecture on exposure to curcumin (c-80, d-160, e-240, f-320 mg/kg bw) showing near normal picture. The result suggests the protective nature of the drug against the injury [19].

Mitochondrial membrane has a special affinity towards oxygen and respiration process involves the transport of electrons via cytochromes to molecular oxygen [20]. From the tables 1 and 2 it is evident that, when the activity of Cox of CT animal is compared to that of amino acid treated

animal, the activity was found to be increased (p<0.5, group 3 a in brain and 3a in liver *vs* Gp 2; NS *vs* control) which would lead to normal electron transport chain, based on both antioxidant and anti-inflammatory properties of curcumin, the mode of action of curcumin, and its therapeutic usage against different pathological conditions [21].

When the drug treated group (CT) is compared to amino acid treated animal, the SDH activity was found to be decreased. (p<0.5, group 3 a in brain and 3a in liver *vs* group 2) showing in near normal activity in group[NS, group 3a (brain) and group 3a (liver) *vs* control]. Reduced activity of mitochondrial Cox and increased activity of SDH have been reported in conditions with improper utilization of glucose and hormonal disturbances [22]. Hence the present observation of similar picture, on ET treatment might be linked to ET induced hormonal disturbances and this suggests differential response of mitochondrial enzymes to ET. The activities of Cox, which is encoded by mitochondrial DNA and SDH which is encoded by nuclear DNA, registered near normal activities (p<0.5, group 3 a and *vs* control) and comparatively reduced activity (p<0.5) when compared to ET rats (group 3 a-e *vs* group 2) suggesting protective action of curcumin against sensitivity to ET[23].

Similarly From Table 1 and 2, it could be followed that the activities of MDH of brain and liver mitochondria in ET group rats showed reduced activity if compared to control rats (p<0.5). When the CT group (group 3 a of brain and liver) is compared to the ET group, the activity of MDH was found to be increased (p<0.5) and it was NS when compared to control. Improper utilization of cerebral glucose, oxygen and impaired production of NADH were said to be associated with neurodegeneration and hence reduction in activities of enzymes such as MDH [24]. The neurodegeneration of group 2 rats was evident from histological studies (Fig 3 and 4 b) and this might be responsible for observed result in group 2. The near normal activities on curcumin treatment against suggest its action against degeneration of the cells which is also evident from Fig 3 and Fig 4 c-f). Hepatoprotective effects have been associated with plant extracts rich in antioxidants, since curcumin is also an effective antioxidant, it can reduce the deleterious effect [25].

Table 3 Levels of CRP in serum of Control, ET induced and Curcumin Pretreated animals

Groups	Observation
Group 1(control)	Negative (-ve)
Group 2(ET)	Positive (+ve)
Group 3 a (CT-80 mg/kg bw)	Negative (-ve)
Group 3 b (CT-160 mg/kg bw)	Negative (-ve)

Positive and negative results based on concentration of <0.6mg/dl

When the serum of control, CT rats (80, 160 mg/kg bw) and that of ET rats were subjected to CRP analysis, control and CT rats (group 3 a and group 3b) registered negative CRP, while ET showed positive value (Table 3). It has been reported that following inflammation, low grade injury or trauma, there will be production of CRP [26]. The present observation of positive value in ET induced injury; coincide well with the above reports and also with the report showing induction of APP after brain injury[4]. The result observed in CT rats could be supported by a report showing reduced inflammatory response by curcumin [27]. This negative CRP test in CT, suggest the efficacy of curcumin in minimizing effect of injury induced by ET.

CONCLUSION

The present study suggest that ET (phenyl alanine and aspartate) treatment , result in brain injury as well as liver injury after ten days of administration at the dosage level of 1g/kg bw and the damage brought about by ET could be counteracted by curcumin pretreatment (best at 80mg/kg bw). Thus the study attempts to address the adverse effect of dietary ET excitotoxins, as used in foods today, may produce blood elevations high enough to cause damage to the nervous system, damage which is not detectable at the time of occurrence but which may give rise to subtle disturbances in neuroendocrine and also the importance of curcumin which will be helpful in counteracting such effects.

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