

# Stress Proteins and Glial Cell Functions During Chronic Aluminium Exposures: Protective Role of Curcumin

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**Abstract** Involved in the ongoing debate is the speculation that aluminium is somehow toxic for neurons. Glial cells cope up to protect neurons from this toxic insult by maintaining the glutathione homeostasis. Of late newer and newer roles of glial cells have been depicted. The present work looks into the other regulatory mechanisms that show the glial cells response to pro-oxidant effects of aluminium exposure. In the present investigation we have evaluated the inflammatory responses of the glial cells as well as HSP70-induction during aluminium exposure. Further, the protective role of curcumin is also evaluated. Aluminium was administered by oral gavage at a dose level of 100 mg/kg b.wt/day for a period of 8 weeks. Curcumin was administered i.p. at a dose of 50 mg/kg b.wt./day on alternate days. Enhanced gene and protein expression of HSP70 in the glial fractions of the aluminium exposed animals as compared to the corresponding neuronal population. Aluminium exposure resulted in a significant increase in the NF- $\kappa$ B and TNF- $\alpha$  expression suggesting inflammatory responses. In the conjunctive treatment group of aluminium and curcumin exposure marked reduction in the gene and protein expression of NF- $\kappa$ B and TNF- $\alpha$  was observed. This was further reflected in histopathological studies showing no evidence of inflammation in conjunctive group as compared to aluminium treatment. From the present study, it can be concluded that curcumin has a

potential anti-inflammatory action and can be exploited in other toxicological conditions also.

**Keywords** Inflammation · Heat shock proteins · Aluminium neurotoxicity · Cytokines

## Introduction

The brain contains a mixed population of cell types such as glial cells and neurons both of which are interdependent and important. Several studies have demonstrated that microglia and reactive astrocytes accumulate in the neighborhood of neurodegenerative sites in patients with Alzheimer's disease (AD), Parkinson's disease (PD), and acquired immunodeficiency syndrome, and brain injury following permanent, transient, or focal cerebral ischemia [1, 2]. In our previous study we have depicted that glial cells have more potential to combat oxidative stress caused due to aluminium neurotoxicity [3]. It has been suggested that the insult to neurons following microglial activation would be determined by a large number of local buffering system that could result in the inactivation of toxic microglial mediators such as heat shock proteins or the stress proteins [4, 5]. In the brains of patients with neurodegenerative disorders, however, stress proteins are expressed and frequently associated with protein aggregates, and glial cells are activated around degenerative regions [6]. It is thus envisaged that sustained microglial activation resulting in chronic inflammatory state may be a hallmark feature of neurodegenerative disorders and the agents that regulate the activities of these cell may represent new generation of therapeutics. Although the prospects for the development of neuroprotective agents for treatment of neurodegeneration are promissory at preclinical level, the translational studies ranging from basic research to clinical

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applications shows no convincing evidence in improving the overall outcome. This necessitates the exploration of novel therapeutic regimens, including herbal-based drugs for neuroprotection. Of late newer and newer roles of curcumin in neurodegeneration has been demonstrated especially with regards to Alzheimer's disease [7], tardive dyskinesia [8], major depression [9], epilepsy [10], and other related neurodegenerative and neuropsychiatric disorders. The mechanism of its neuroprotective action is not completely understood. However, it has been hypothesized to act mainly through its anti-inflammatory and antioxidant properties. Also, it is a potent inhibitor of reactive astrocyte expression and thus prevents cell death. The fact that curcumin antagonizes many steps in the inflammatory cascade prompted us to design the current study so as to evaluate its role in aluminium-induced neurotoxicity.

## Materials and Methods

### Experimental Animals

Healthy rats of Sprague–Dawley (SD) strain were produced from the central animal house of Panjab University, Chandigarh and were acclimatized in the departmental animal house for 1 month in plastic cages under hygienic conditions and were provided rat pelt feed and water ad libitum. The animals were divided into four groups of 10 animals each. Group I was served as control, group II was given a dose of, 100 mg aluminium/kg of body weight for a period of 8 weeks daily [11], group III was given a combined treatment of aluminium and curcumin, group IV was given curcumin alone for 2 months [12]. Curcumin was given at a dose of 50 mg/kg of body wt i.p. for a period of 2 months. Aluminium chloride was dissolved in drinking water. The rats were intubated orally with 1 ml of the specific dose. Weekly weight changes were recorded and the doses were adjusted accordingly. The rats were monitored for their health, general behaviour, and daily diet intake. The physical changes were minor and following combined administration no distinctive visual behavioural alterations were visualized. At the end of the experiment design, animals were sacrificed for glial cell isolation.

### Glial Cell Isolation

Glial cells were isolated by the method of Rani et al. [13] whereby the brain was digested by trypsin and dispersed tissue was then be allowed to filter through series of meshes and the two population of neuron tissue was separated using density gradient centrifugation using ficoll 400. The isolated fraction would then be used to study various parameters. The cellular fractions were identified

microscopically. The neuronal fraction mainly contained cell bodies and residues of dendrites whereas glial cells include astrocytes, microglial with some oligodendrocytes.

### HSP70 Gene Expression

mRNA expression analysis by RT-PCR was performed using QIAGEN one step RT-PCR kit (Germany). HSP-70 gene expression was done in both neurons and glial cells. Mouse  $\beta$ -actin was used as an internal control.

### Total RNA Isolation

Total RNA was isolated from the mid brain region using TRI-REAGENT (Mol. Res. Centre, Inc., Ohio, USA). To obtain RNA, following procedure was performed:

**Homogenization** 50 mg of brain from different treatment groups was homogenized in 0.5 ml TRI-REAGENT in 1.5 ml polystyrene microfuge tubes using hand homogenizer.

**Phase Separation** The samples were kept at room temperature for 5 min to permit the complete dissociation of nucleoprotein complexes. Then 0.1 ml chloroform was added and mixed vigorously for about 15 s. The homogenates were then kept at room temperature for 10 min followed by centrifugation at 12,000g for 15 min at 4°C. Following centrifugation three distinct layers, a lower phenol chloroform phase, interphase and an upper colourless aqueous phase were seen. The upper phase that is roughly 60% the volume of TRI-REAGENT contains RNA.

**RNA Precipitation** The aqueous phase was transferred to fresh tubes and then 250  $\mu$ l isopropanol was added to precipitate the RNA. The samples were kept at room temperature for 10 min and then centrifuged at 12,000g for 10 min at 4°C. RNA precipitate was seen as a small white pellet on the side of the tube.

**RNA Wash** The pellet was washed with 0.5 ml 75% ice-cold ethanol by centrifugation at 7,500g at 5 min at 4°C.

**RNA Solubilization** After removing the ethanol, the RNA pellet was briefly air-dried (not completely), and then dissolved in 10  $\mu$ l of diethyl pyrocarbonate (DEPC) treated water.

### Estimation of Purity and Concentration of RNA

**Purity** Purity of RNA was checked by determining the ratio of absorbance values at 260 and 280 nm. The final ratio for all the RNA preparations was approximately 1.6, which indicates a pure preparation.

**Concentration** Concentration of RNA was estimated by measuring the absorbance at 260 nm in spectrophotometer (UV-160A, Shimadzu) using  $A_{260} = 1 \cong 40 \mu\text{g/ml}$ .

### Agarose Gel Electrophoresis of RNA

Integrity and size distribution (quality) of isolated total RNA was checked by denaturing agarose-gel electrophoresis. 1.2% agarose gel (40 ml) was prepared in DEPC treated water in an Erlenmeyer flask using microwave oven, allowed to cool to 60°C and then 5 ml of 10× MOPS and 1.5 ml of formaldehyde were added and mixed well. The gel was poured into horizontal gel electrophoresis chamber with comb and allowed to stand at room temperature for 30 min to polymerize. Samples were prepared by mixing the following in microfuge tubes: RNA—4.5 µl (5 µg), 10× MOPS—1.0 µl, Formaldehyde—3.5 µl, Formamide—8.0 µl, Ethidium bromide (1 mg/ml)—1.0 µl. The samples were incubated at 65°C for 15 min and then chilled on ice. To all the samples, 1.5 µl of 10× RNA loading buffer was added and mixed well. 1× MOPS was used as running buffer and electrophoresis was carried out at 70 V. Finally, the bands were viewed and photographed using Gel Doc (BioRad, UK).

### Primer Designing and Synthesis

Optimal primer pairs were designed using the software “Gene Runner” or their sequence were obtained from literature and were got synthesized from Sigma-Aldrich (USA). Lengths of the primers chosen were ~20 bp. The sequences for the forward and reverse primers were procured from Sigma Aldrich (Forward: -ATG AAG GAG ATC GCC GAG G Reverse: AGG TCG AAG AT G AGC ACG TTG) [14].

### Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)

One step—RT-PCR kit was used (QIAGEN Inc., Germany) in which cDNA synthesis and PCR are carried out sequentially in the same tube (Labware Scientific Inc., USA).

**Procedure** RT-PCR was performed according to the manufactures instructions. 3 µg RNA was used for each reaction. Firstly, a master mix was prepared as follows: 5× QIAGEN RT-PCR buffer—10 µl, dNTP mix—2 µl, QIAGEN one step RT-PCR enzyme mix—2 µl, RNase inhibitor—1 µl. The master mix was mixed thoroughly and 15 µl of it was added to each PCR tube. To this 5 µl of each sense and antisense gene specific primers (from 10 µM stock) were added. Then 3 µg template RNA was added and final volume was adjusted to 50 µl with RNAase free water provided in the kit. All the reactions were carried out on ice. The PCR tubes were gently vortexed and centrifuged in order to settle all the components at the

bottom. The PCR tubes were placed in a thermal cycler (Techmne Inc., UK), which was programmed as follows:

#### Thermal cycler conditions

	Temperature	Duration
Reverse transcription	50°C	50 min
Initial PCR activation	95°C	15 min
3 step cycle		
Denaturation	94°C	45 s
Annealing	Variable	45 s
Extension	72°C	1 min
No of cycles 35 <sup>a</sup>		
Final extension	72°C	10 min
Final hold	10°C	10 min

<sup>a</sup> In initial experiments, RT-PCR was performed by using different number of cycles-20, 25, 30 and 35. It was found that the PCR products were progressively amplified till 35 cycles and hence 35 amplification cycles were performed

The PCR products were analysed on agarose gel electrophoresis.

### Agarose Gel Electrophoresis for PCR Products

30 ml of 1.5% agarose was prepared in 1× TAE buffer in an Erlenmeyer flask in a microwave oven. After cooling to approximately 60°C, ethidium bromide was added to a final concentration of 0.5 µg/ml. The gel was poured into horizontal plastic tray with comb and left undisturbed for 30 min to allow it to polymerize. Three µl of the PCR product was mixed with 1 µl DNA gel loading buffer and the samples were loaded in separate wells. 1× TAE was used as the running buffer. Electrophoresis was carried out at 70 V. The bands were visualized and photographed on Gel Doc (BioRad, UK).

### Densitometric Analysis of Bands

Densitometric analysis of bands was done by using the Image J software (NIH). The cycle time (Ct) values genes first normalized with  $\beta$ -actin of the same sample, and then the relative differences between control and treatment groups were calculated and expressed as relative change.

### Protein Expression

ELISA was carried out to quantitate the levels of NF- $\kappa$ B, and TNF- $\alpha$  in various treatment groups under study. Titrating the different concentration of antigens and antibodies standardized the assay. Ten percent (w/v) tissue homogenates were prepared in 50 mM Tris-HCl (pH 7.4) under ice-cold conditions. The homogenates

were then centrifuged at 10,000 rpm for 30 min. The supernatant (post mitochondrial fraction, PMF) thus obtained was quantitated for protein by Lowry method and collected for ELISA. 2.5 µg protein was loaded onto ELISA strip wells in 100 µl carbonate buffer and kept overnight at 4°C, in a moist chamber. Flicked the plate to remove the unbound antigen and the wells were blocked with 1% BSA in 0.1 M PBS for 1 h at 37°C. The plate was then flicked and washed thrice with PBS containing 0.05% (v/v) Tween-20. Wells were then incubated with primary antibody diluted in PBS containing 0.05% Tween-20 and 1% BSA and kept for 2 h at 37°C. Plate was again washed thrice in the same manner and incubated with peroxidase labelled anti-rabbit IgG diluted in PBS containing 0.05% Tween-20 and 1% BSA (1:500) and kept for 2 h at 37°C. The plate was again washed thrice as above and one last washing was given with distilled water since Tween acts as an inhibitor of the substrate i.e. ABTS [2,2-azino-di-(3-ethylbenzothiozolinisulphonic acid)]. The substrate was then added to each well and the plate was kept in dark for 30 min after which the colour developed was read at 405 nm.

Proteins were estimated in the samples by the method of Lowry et al. [15]. The method is based on colour reactions of amino acids namely tryptophan and tyrosine with the folin phenol reagent. To 10 µl of sample from each treatment group, 3 ml of 50:1 mixture of 2% sodium carbonate in 0.1 N NaOH and 0.5% CuSO<sub>4</sub>/1% Na-K tartrate in distilled water was added. The tubes were incubated for 10 min at room temperature. Then 300 µl of 1 N Folin's phenol reagent was added to each tube, mixed and again incubated for 30 min at room temperature. The optical density was then measured at 620 nm on spectrophotometer (UV-160A, Shimadzu). Bovine serum albumin (BSA) was used as standard (10–100 µg).

#### Nitric Oxide Estimation

Nitric oxide synthase activity was determined in terms of nitric oxide (NO) production. The estimation was carried out by the method of [16]. NOS convert L-arginine to L-citrulline and NO, which then reacts with oxygen to yield nitrite. Nitrite, thus formed reacts with the Griess reagent to form a purple azo dye, which can be read at 540 nm. To 0.100 ml of tissue homogenates, 0.100 ml of Griess reagent was added into the wells of ELISA plate. The ELISA plate was then incubated in dark at 37°C for 30 min. The pink colour so obtained was read at 540 nm on ELISA plate reader. The amount of nitrite produced was determined by a standard curve prepared by using sodium nitrite. Results were expressed as *nM of nitrite/g tissue*.

#### Histopathological Studies

Peritoneum was opened and perfusion with chilled normal saline followed by formalin was carried out. Small sections of cerebrum and cerebellum from each of the normal control and treated animals were taken, washed with ice-cold 0.9% NaCl and fixed in the buffered formalin for about 24–48 h. After fixation, tissues were processed carefully for embedding in paraffin wax (58–60°C) after subjecting them to different ascending grades of alcohols according to the standard technique [17].

Paraffin sections with 5–7 microns thickness were cut, dewaxed in xylene, downgraded (hydrated) decreasing grades of alcohol and brought to water. They were stained haematoxylin for approx. Twenty seconds, rinsed in ammonia water till the appearance of blue colour, again washed with water, treated with acid water (if over stained), upgraded (dehydrated) in alcohol till 70%, stained with 1% alcoholic eosin for 30 s, differentiated with 90% alcohol, washed with absolute alcohol and were cleared in xylene and finally mounted in DPX. Solochrome was used to stain myelin [18].

#### Statistical Analysis

For analyzing the data, one way analysis of variance followed by Newman Keul's test was performed using the statistical software package "SPSS v 11 for windows". The post hoc comparison for means from different treatment groups were made by the method of least significant difference (LSD), results corresponding to a *P* value of 0.05 or less was considered statistically significant.

#### Results

While prolonged exposure to conditions of extreme stress is harmful and can lead to cell death, induction of HSP synthesis can result in stress tolerance and cytoprotection against stress-induced molecular damage.

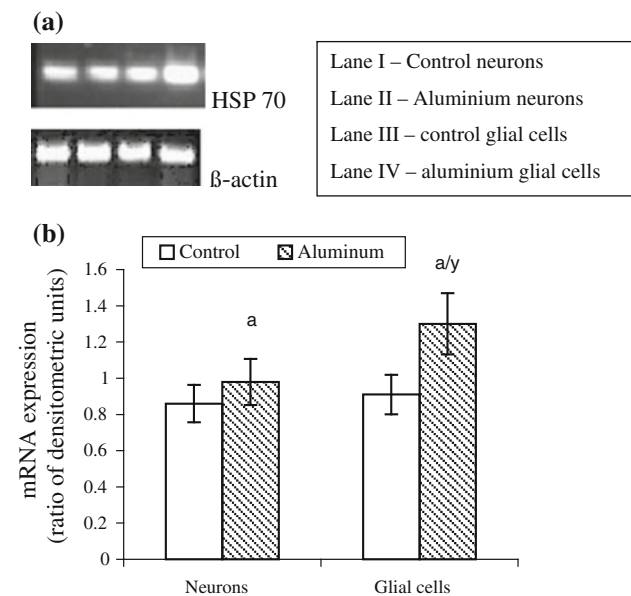
#### HSP70 Expression

There are several mechanisms of aluminium neurotoxicity. The various oxidative stress insults are usually accompanied by compensatory, protective and regenerative mechanism that could counteract the oxidative stress. One such mechanism is induction of stress proteins. They have been shown to be involved in the pathogenesis of several neurodegenerative disorders such as AD, PD. Therefore, in the present experiment the induction of HSP70 has been asserted. The gene and protein expression of HSP70 was evaluated in both neuron and glial cell population using

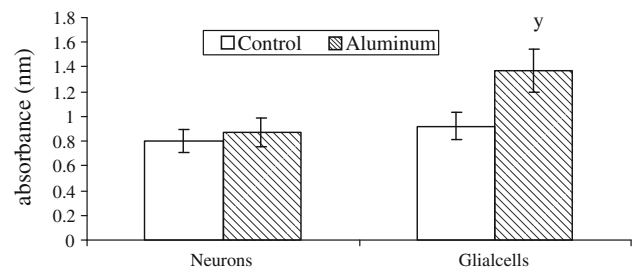
RT-PCR (Fig. 1) and ELISA (Fig. 2), respectively. There was an enhanced gene ( $P \leq 0.01$ ) and protein expression ( $P \leq 0.01$ ) of HSP70 in the glial fractions of the aluminium exposed animals.

NF- $\kappa$ B and TNF- $\alpha$  Gene Expression

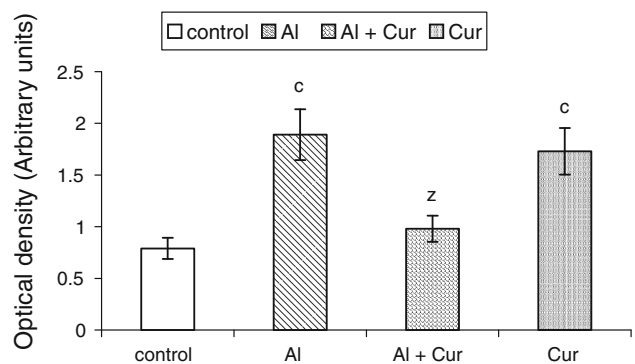
Glial cell activation is another mechanism to combat environmental stress/oxidative stress. Physical and psychological stress elevates plasma levels of several cytokines (i.e. TNF- $\alpha$ , IL-1). The physiological significance of this elevation is not known. One of these cytokines TNF- $\alpha$ , is rapidly produced in the brain in response to tissue injury. TNF- $\alpha$  is a marker of inflammatory attack or a major cleanup operation following infection or injury. It is released by microglial and when released in larger amount it helps to trigger an immunological cascade that can kill or wound every neuron in vicinity. Therefore, in the present study immune response could also be evident from enhanced protein expression of cytokines involved in inflammation. The TNF- $\alpha$  levels were increased significantly ( $P \leq 0.001$ ) following aluminium exposure as shown in Fig. 4. Co-administration of curcumin inhibited aluminium-induced increase in levels of TNF- $\alpha$ . TNF- $\alpha$  and several other stimuli are known to induce the activation



**Fig. 1** **a** HSP70 mRNA expression in neurons and glial cells by RT-PCR. **b** Densitometric analysis of HSP70 mRNA expression in neurons and glial cells after 2 months of aluminium exposure. Height of *histogram bar* represents mean values with *error bars* showing SD values of 6 animals each. <sup>a</sup> $P \leq 0.05$ , <sup>b</sup> $P \leq 0.01$ , <sup>c</sup> $P \leq 0.001$  by Newman–Keuls test when the values are compared with normal control group. <sup>y</sup> $P \leq 0.01$ , <sup>z</sup> $P \leq 0.001$  by Newman–Keuls test when values of Al + curcumin treated group are compared with Al treated group



**Fig. 2** Graphical representation of protein expression of HSP70 Elisa in neurons and glial cells after 2 months of aluminium exposure. Height of *histogram bar* represents mean values with *error bars* showing SD values of 6–8 animals each. <sup>a</sup> $P \leq 0.05$ , <sup>b</sup> $P \leq 0.01$ , <sup>c</sup> $P \leq 0.001$  by Newman–Keuls test when the values are compared with normal control group. <sup>y</sup> $P \leq 0.01$ , <sup>z</sup> $P \leq 0.001$  by Newman–Keuls test when values of Al + curcumin treated group are compared with Al treated group

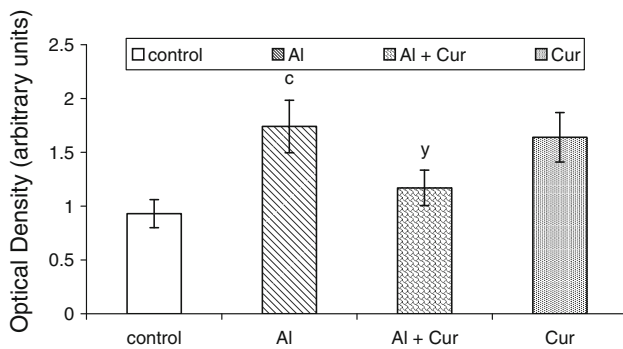


**Fig. 3** Protein expression of NF- $\kappa$ B by ELISA in mid brain region of rat after 2 months of aluminium treatment. Height of *histogram bar* represents mean values with *error bars* showing SD values of 6–8 animals each. <sup>a</sup> $P \leq 0.05$ , <sup>b</sup> $P \leq 0.01$ , <sup>c</sup> $P \leq 0.001$  by Newman–Keuls test when the values are compared with normal control group. <sup>y</sup> $P \leq 0.01$ , <sup>z</sup> $P \leq 0.001$  by Newman–Keuls test when values of Al + curcumin treated group are compared with Al treated group

of NF- $\kappa$ B by promoting the degradation of I $\kappa$ B and nuclear translocation of dimer released from the I $\kappa$ B. In the present observations, aluminium exposure resulted in a significant increase in the NF- $\kappa$ B expression as shown in Fig. 3. Co-administration of curcumin significantly inhibited NF- $\kappa$ B p65 unit ( $P \leq 0.001$ ) levels in the nuclear fraction of rat brain homogenate. Also

Nitric Oxide Levels

NO has diverse yet versatile biological functions such as neurotransmitter release, neurotransmitter reuptake, neurodevelopment, synaptic plasticity, and regulation of gene expression. NOS is found to be coexisted with NFT bearing neurons and thus signifies the importance of nitric oxide in Alzheimer’s disease pathology where aluminium-induced neurotoxicity is also one of the causative factor. Following aluminium exposures nitrite levels were found to be

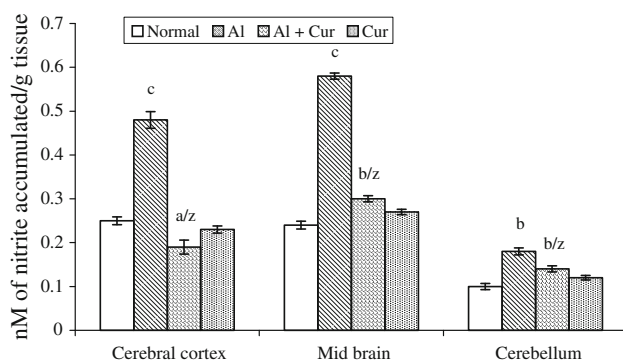


**Fig. 4** Protein expression of TNF-alpha by ELISA in mid brain region of rat after 2 months of aluminium treatment. Height of *histogram bar* represents mean values with *error bars* showing SD values of 6–8 animals each. <sup>a</sup> $P \leq 0.05$ , <sup>b</sup> $P \leq 0.01$ , <sup>c</sup> $P \leq 0.001$  by Newman–Keuls test when the values are compared with normal control group. <sup>y</sup> $P \leq 0.01$ , <sup>z</sup> $P \leq 0.001$  by Newman–Keuls test when values of Al + curcumin treated group are compared with Al treated group

significantly increased in all the three regions of brain [cerebral cortex ( $P \leq 0.001$ ), mid brain ( $P \leq 0.001$ ) and cerebellum ( $P \leq 0.01$ )] as shown in Fig. 5. With curcumin supplementation, significant decrease ( $P \leq 0.001$ ) in the enzyme activity was observed when compared to Al treated animals though values were still high in mid brain region in comparison to normal control.

#### Histopathology Studies

Paraffin embedded sections were evaluated so as to localize and characterise the lesions induced by aluminium and Curcumin exposures. Figure 6 shows the representative photographs of a section of cerebral cortex in the experimental groups stained with H&E. the photomicrographs



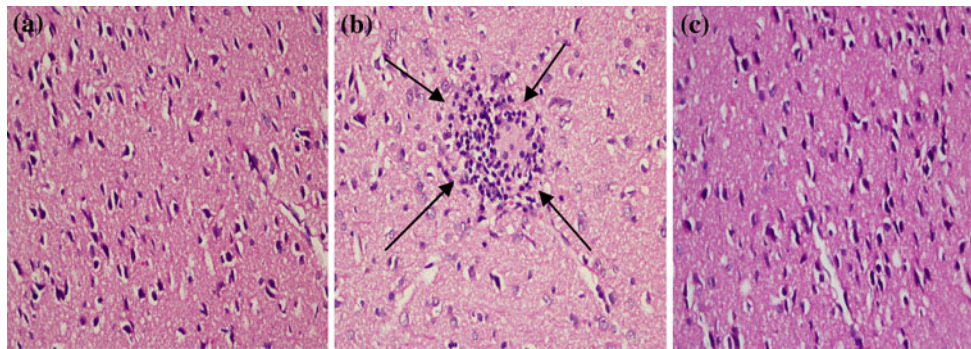
**Fig. 5** Effect of curcumin treatment on a Nitric oxide in rats subjected to Aluminium treatment. Height of *histogram bar* represents mean values with *error bars* showing SD values of 6–8 animals each. <sup>a</sup> $P \leq 0.05$ , <sup>b</sup> $P \leq 0.01$ , <sup>c</sup> $P \leq 0.001$  by Newman–Keuls test when the values are compared with normal control group. <sup>y</sup> $P \leq 0.01$ , <sup>z</sup> $P \leq 0.001$  by Newman–Keuls test when values of Al + curcumin treated group are compared with Al treated group

showed significant increase in the focal inflammatory response after aluminium treatment as compared to normal control where no such evidence of inflammation could be seen. In contrast no evidences of inflammation could also be seen in-group receiving curcumin treatment along with aluminium.

#### Discussion

The present work is the extension of our previous work showing the protective role of glial cells against aluminium neurotoxicity by maintaining glutathione homeostasis and also by the up regulation of anti-oxidative enzymes [3]. The current study demonstrates an enhanced gene and protein expression of HSP70 in the glial fractions of the aluminium-exposed animals as compared to neurons. In addition it can be observed that neurons and glia differ in their response towards stress suggesting that neurons have a limited ability to respond to stress as compared to glia. The low level expression of HSP70 after stress could be attributed to the absence of heat shock transcription factor-1 (HSF1) in neurons [19]. The heat shock response contributes to establish a cytoprotective state, a phenomenon which has been involved in a variety of disturbances and injuries, including stroke, AD, PD, polyglutamine diseases [20–23]. Several studies on transfection of cultured astrocytes with the gene for HSP70 have demonstrated protection from ischemia and glucose deprivation. Kakimura et al. [24] have suggested that HSPs, which may leak extracellularly from dying neurons, affect the surrounding microglia, which in turn contribute to other neuroprotection mechanisms such as phagocytic digestion of amyloid plaques by microglia, which further mediates microglial activation.

It is known that glial cells produce pro-inflammatory and neurotoxic factors which may also act as autocrine and paracrine mediators that induce glial proliferation. One such mediator is nitric oxide. Following aluminium exposures nitrite levels were found to be significantly increased in all the three regions of brain [cerebral cortex ( $P \leq 0.001$ ), mid brain ( $P \leq 0.001$ ) and cerebellum ( $P \leq 0.01$ )] (Fig. 6). Increase in HSP70 protein expression was also found after treatment of cells with the NO generating compound sodium nitroprusside (SNP), thus suggesting a role for NO in inducing HSP70 proteins. The molecular mechanisms regulating the NO-induced activation of a heat shock signal seems to result from cellular oxidant/antioxidant balance, regulated by the glutathione status and the antioxidant enzymes [25–27]. With curcumin supplementation, significant decrease in the NO activity was observed when compared to Al treated animals though values were still high in mid brain region in comparison to



**Fig. 6** Sections of rat brain showing lesions of inflammation **a** Control. **b** Aluminium. **c** Curcumin + Al. Arrows showing lesions of inflammation

normal controls. Acute and chronic exposure to aluminium stimulates nitric oxide synthase activity probably due to the augmentation of the activity of this enzyme present in glial cells, whose contribution to the total activity is around 70% under resting conditions [28]. NOS is found to be coexisted with NFT bearing neurons and thus signifies the importance of nitric oxide in Alzheimer's disease pathology where aluminium-induced neurotoxicity is also one of the causative factor [29].

Further, studies by Kitamura and Nomura [6] have suggested, that extracellular HSPs induce the activation of NF- $\kappa$ B and p38 MAP Kinase which in turn contributes to the production of certain cytokines. In addition, certain stress activated protein kinases or other upstream signaling enzymes can also activate the redox sensitive transcription factors such as NF- $\kappa$ B, AP-1 and NRF-2 thereby up regulating the synthesis of early response genes to tolerate or survive the subsequent oxidative injury. In our present study too there is an increased expression of TNF- $\alpha$  in mid brain region after aluminium treatment an observation similar to this, TNF- $\alpha$  levels are increased in the CNS after damage due to traumatic injury, ischemia, infections or diseases that involve brain degeneration, playing an important role in the adaptive response to these conditions indicating the inflammatory response [30]. Several authors have also shown the increased inflammatory response in experimental models of aluminium [31]. In the current study the histological picture of the cerebral cortex region of the aluminium treated group also showed significant increase in the focal inflammatory response as observed by the accumulation of neutrophils. However, in the curcumin exposed animals the cerebral region displayed no evidences of inflammatory responses. This is further supported by the reduced expression of TNF- $\alpha$  after curcumin treatment in aluminium fed animals. Singh and Aggarwal [32] have reported that activation of transcription factor NF- $\kappa$ B is suppressed by curcumin. It has a protective effect on cisplatin-induced experimental nephrotoxicity, and this effect is attributed to its direct anti-inflammatory action

[33]. Weber et al. [34] have demonstrated that the anti-inflammatory activity of curcumin is mediated by limiting the activity of transcription factors, co activators of activator protein-1, including p300 histone acetyltransferase [35]. Curcumin is known to inhibit the activation of transcription factor NF- $\kappa$ B as well as AP-1 [36]. Based on the screening studies of curcumin and their analogs on the panomic cell lines 29, suggested inhibition of TNF- $\alpha$ -induced activation of the transcription factor NF- $\kappa$ B [35]. It is suggested that the phenolic ring methoxy groups, but not the C=C double bonds of the dienone of curcumin, mediate anti-inflammatory effects in vivo [36]. Therefore, the present study is a clear evidence of protective role of curcumin in the management of inflammation occurring due to environmental stress.

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