



Differential neuroprotective effect of curcuminoid formulations in aluminum chloride–induced Alzheimer’s disease

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Abstract

Alzheimer’s disease (AD) is a slowly progressive brain degenerative disorder which gradually impairs memory, thinking, and ability to perform easy routine tasks. This degenerative disorder mainly targets the elderly people and has imposed an endemic burden on society. Hence, there is a crucial need to investigate the efficacious herbal pharmacotherapies that can effectively mitigate and prevent the pathological hallmarks of AD. The current study aims to explore the potential efficacy of curcuminoid-rich extract (CRE) and its ternary complex (TC). Experimental rodents were administered with $AlCl_3$ (300 mg/kg) to induce AD and treated with rivastigmine, curcuminoid crude extract, CRE, and TC orally for three consecutive weeks. Neurobehavioral, biochemical, and histopathological studies were performed from the last week of the study period. The mRNA expression of different pathological biomarkers was estimated by RT-qPCR analysis. The results of the study suggested that CRE and TC significantly improved the behavioral, biochemical parameters and acetylcholinesterase inhibitory activity in treatment groups. Histological analysis was also carried out indicating that the neurodegenerative changes and neuronal loss were stabilized by CRE and TC supplementation. CRE and TC supplementation remarkably downregulated the interleukin-1 α , tumor necrosis factor- α , interleukin-1 β , acetylcholinesterase, and β -secretase pathological gene expression. Hence, it was concluded that CRE and TC may act as promising candidates in the prevention of AD via numerous underlying signaling pathways.

Keywords Aluminum chloride · Anti-Alzheimer activity · *Curcuma longa* · Curcuminoid-rich extract · Neuroinflammation

Introduction

Alzheimer’s disease (AD) is a debilitating progressive age-related neurodegenerative disease. This disorder is the most common cause of dementia and the sixth principal cause of

death around the world (Haque and Levey 2019). It is usually characterized by a cumulative episodic memory loss and loss of cognition, eventually leading to cause deficiencies in visuospatial and language skills, which are frequently followed by various behavioral disorders including aggressiveness, apathy, and depression (Sharma et al. 2021; Silva et al. 2019). The significant physio-pathological characteristics of AD are the deposition of extracellular β -amyloid peptide ($A\beta$) insoluble plaques and neurofibrillary tangles (NFTs) together with hyperphosphorylated tau proteins in the cytoplasm of brain tissues of AD patients (Hossain et al. 2019; Silva et al. 2019). According to an estimate, more than 6 million people aged above 65 years are suffering from AD in the USA. Hence, there is a crucial need to diagnose, prevent, and find out the essential therapeutic measures in order to control the consequences of this disastrous disease. The spatial and cognitive deficits were considered as initial clinical signs and multiple environmental factors are associated with AD pathology (Bhattacharjee et al. 2014; Mathiyazahan

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et al. 2015). It is documented that free radicals and excessive oxygen species are the main cause in the pathogenesis of AD (Kimura and Ohno 2009). Aluminum is the most abundant metal on earth crust and extensively reported to be neurotoxic to human beings and animals (Chiroma et al. 2019; Walton 2012). Aluminum can easily cross the blood–brain barrier (BBB) due to its high affinity for transferrin receptors and hence accumulate in the brain (Liaquat et al. 2019). Usually, aluminum accumulates in the most specific parts of the brain including the hippocampus and frontal cortex regions which are mainly concerned with perception and memory (Cheng et al. 2019). Aluminum exposure stimulated various neuronal and biochemical abnormalities which are directly associated with cognitive deformities linked to AD (Bondy 2014; Chauhdary et al. 2019).

Curcuma longa (turmeric) is a very familiar golden-yellow colored spice that belongs to the *Zingiberaceae* family (Hesari et al. 2019). It is documented that conventional medicines frequently used the powdered *Curcuma* as a curative agent, food additive, and coloring agent in Asian foods. Biologically, curcuminoids are active and are among the major constituents, comprising almost 2–9% of the *C. longa* constituents (Hesari et al. 2019; Priyadarsini 2014). Curcumin is the major representative of curcuminoids including curcumin (60–70%), demethoxycurcumin (DMC, 20–27%), and bisdemethoxycurcumin (BDMC, 10–15%) (Ahsan et al. 1999; Somparn et al. 2007). According to recent reports, extract of curcuminoids prepared by simple extractions using ethanol provided a higher yield of curcuminoid extracts, but because of the presence of non-volatile oils and oleoresins, their content levels are very low (Hatcher et al. 2008). Utilization of a pure natural composite is generally regarded as a restriction for industrial uses, as the purification procedure needs different steps and it is also time consuming and expensive (Li et al. 2009), hence we used curcuminoid-rich extract (CRE). CRE used in this study was prepared by using inexpensive microwave-assisted extraction (MAE) technique, a principle of the green extraction method as described previously (Lateh et al. 2019). CRE prepared by this method is cost-effective and comprised of a greater content of curcuminoids, i.e., 88% w/w. In the current investigation, we explored that CRE had a highly soluble ternary complex of CRE with hydroxypropyl- β -cyclodextrin and polyvinylpyrrolidone (Hatcher et al. 2008).

The study was planned to estimate the alterations in behavioral and biochemical parameters, oxidative stress, acetylcholinesterase levels, levels of proinflammatory cytokines, and mRNA expression of pathological genes caused by aluminum chloride administration. The main objective was to investigate the therapeutic potentials of CRE and TC in aluminum chloride induced AD rat model.

Materials and methods

Chemicals and reagents

Aluminum chloride (AlCl_3) (for the induction of AD), isoflurane (for induction and maintenance of anesthesia), rivastigmine as standard drug from Novartis Pharma (PVT) Ltd., phosphate buffer tablets, water for injection, TRIzol (Invitrogen), cDNA synthesis kit (WizScript), cyber green (SYBR green master mix of WizPure), primers (Thermo Fisher Scientific-US) for the synthesis of cDNA were procured. Curcuminoid extracts including CR (27% w/w curcuminoids), CRE (88% w/w curcuminoids), and TC (14% w/w curcuminoids) were obtained from Assoc. Prof. Pharkphoom Panichayupakaranant, Phytomedicines and Pharmaceutical Biotechnology Excellence Centre, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Thailand. Assay kits were used for the determination of proinflammatory cytokines and antioxidant enzymes (Elabscience).

Method of extract's preparation

CRE was prepared using a method as described previously (Lateh et al. 2019). Briefly, turmeric powder was extracted using ethanol by MAE technique. Crude ethanol extract was semipurified through a biodegradable resin (Diaion HP-20) column and eluted with hydro-ethanol to obtain CRE. In order to enhance the solubility and bioavailability of curcuminoids, its ternary complex (TC) was prepared using different ratios of hydroxyl propyl- β -cyclodextrin and polyvinyl pyrrolidone K30 with CRE (Lateh et al. 2022) and the curcuminoid contents in CRE (88% w/w) and TC (14% w/w) were analyzed by using HPLC.

Experimental animals

The healthy albino male Wistar rats (4 months of age) weighing 200–250 g were acquired from the animal house of Government College University, Faisalabad, Pakistan. All the rats were caged in groups separately in a well air-conditioned and ventilated animal house of University. All the rats were kept at a temperature of 25 ± 5 °C, 12 h dark and light cycle, and relative humidity of $55 \pm 10\%$ one week before the start of experimentation to acclimatize the animals. The animals were provided with unlimited access to standard rat pellet feed and water. All the procedures for animal experiments were performed under the approved laboratory animal bio-safety guidelines and the experimental study design was approved by the Institutional Review Board of Government

College University, Faisalabad, with an authorized Ref. No. (GCUF/ERC/2196).

Experimental induction of Alzheimer's disease

After acclimatization of all the rats for 2 weeks, they were fasted for 12 h and administered with AlCl_3 at a dose of 300 mg/kg (orally) to induce Alzheimer disease coupled with behavioral, molecular, and biochemical deficiencies for 21 days to obtain persistent deposition and accumulation of aluminum in the brain tissues of rats (Chiroma et al. 2018).

Experimental study design

All rats were alienated arbitrarily into six groups containing six rats in each group ($n = 6$). Group I was regarded as normal control group (NC) administered with normal saline (1 ml/kg). Group II was regarded as diseased group (AlCl_3) treated with aluminum chloride (300 mg/kg) per oral. Group III was regarded as standard group (STD) administered with AlCl_3 (300 mg/kg) + rivastigmine (1 mg/kg) per oral. Group IV was administered with aluminum chloride (300 mg/kg) + curcuminoids crude extract (30 mg/kg) per oral and considered as CR. Group V was regarded as CRE administered with AlCl_3 (300 mg/kg) + CRE (30 mg/kg) per oral. Group VI was regarded as TC administered with AlCl_3 (300 mg/kg) + ternary complex (30 mg/kg) per oral. All the doses were given for 3 weeks. After dose administration, neurobehavioral parameters were recorded on 19th and 20th day.

Neurobehavioral studies

Multiple neurobehavioral tasks such as wire hanging test, open field test, hole board test, elevated plus-maze test, and Morris water maze test were performed to investigate the behavioral abnormalities induced by aluminum chloride neurotoxicity.

Wire hanging test

This test was used to examine the perseverance and neuromuscular endurance of AD-induced rats. The apparatus required for the wire hanging task was composed of horizontal grids of stainless steel allocated on wooden walls (3 cm in width and 60 cm in length). Rats were handled gently by their tails and then placed on the wire until they firmly make their grip on the steel grid using their paws. They were hanged on a steel grid downward in the straight and erect position. Rats should have to hold on to the wire for up to 30 s. The fall down time was recorded from 30 s to 1.5 min (Chauhdary et al. 2019).

The open-field test

This test was designed primarily to investigate the exploration, fear, and impaired motor functions by evaluating the spontaneous movement of rats in an open area. This test is considered to be a fundamental test to examine the early pathological modifications in AD-induced rats (Naert et al. 2011). At the end of treatment duration, animals were placed in a box ($72 \times 72 \times 36$ cm). The floor was divided into 16 squares, each measuring 18×18 cm, using a black line. One wall of the box was of Plexiglas for visualization of the open area. The box was disinfected with 70% alcohol after performing each trial. The test lasted for 10 min to calculate the total distance covered and all lines crossed to examine the anxiety in AD induced rats.

Hole board test

A hole board test was conducted to observe the exploratory and anxiety behaviors by counting the number of head dipping of rats in the hole board apparatus. The apparatus used was composed of Plexiglas material of 30 cm high walls and (25×25 cm) dimensions. The board was categorized into 16 equally fractionated holes and these were 1.5 m high from the floor. Then, rats were left on the ground to let them explore the apparatus for 8–10 min. The distance covered by the rat in the central and peripheral regions and the head dipping count was recorded. One head dip was counted only when both eyes of rats have vanished in the hole (Tillerson and Miller 2003).

Elevated plus-maze test

This maze test was designed to assess the anxiety or fear-linked actions and to evaluate memory with exploration. For training on the first day, animals were kept gently at the open (unsafe) arm in the opposite direction of the platform and then transfer latency was measured. After 24 h, the exact method was performed again and transfer latency time was noted to analyze the memory, cognition, and learning impairment. Acquisition memory and retention memory were examined using this test on the 21st day. The equipment comprising of two crossed arms was used; two closed and the remaining two open arms. The animal was laid down on an open arm, in opposite direction to other arms. Acquisition session was defined as the time required by the animal to foot in the closed arm of apparatus in the very first session and was termed as first transfer latency of animal. The time to move out was settled to be 1.5 min and if a rat was not able to locate closed arm within the set time limit, the rat was again placed into one closed arm to let the rat further explore the elevated maze for 30 s. Retention session (second trial) was conducted 24 h after the very first trial and

transfer latency was recorded in the retention session (Naert et al. 2011).

Morris water maze test

This Morris water maze test was carried out to explore the task of spatial, and contiguous learning strategy. In neuropharmacology, this test is frequently used to estimate the neurocognitive deficits and very sensitive to detect early spatial disorientations in rodents. For this test, a test pool or plastic tub of 140 cm diameter, 60 cm depth was used and tap water was added to it (temperature of the water was maintained to 26 ± 1 °C). A platform of 10 cm lengths and 10 cm width was set at the midpoint approximately 1 cm. beneath the water surface. This test pool was required to be non-transparent so the addition of some milk powder was done to hide the platform and made it invisible to approach easily for rats during the trial. The rats were trained about the platform and training was done continuously for 6 days (3 trials every day). After training, rats were placed at four different starting points into the test pool and dropped to immense for about 1.5 min to test their ability to allocate the hidden platform. Each trial was ended when rats stayed on the platform for 20 s. The escape latency for each trial was recorded i.e., 150 s. Each trial was started and ended manually. The rats' movement from the hidden platform to four different directions was monitored very carefully (Vorhees and Williams 2006).

Preparation of brain tissue homogenate

After performing the behavioral studies, rats were sacrificed under light anesthesia using isoflurane via cervical dislocation. The brain of all experimental rats was isolated, rinsed with chilled normal saline to get rid of the blood and stored in a biomedical freezer at -21 °C. Brain tissues especially the hippocampus portion and cerebral cortex regions were preserved in 10% normal formalin buffer for histopathological analysis. Brain samples with 10% w/v were stored in phosphate buffer for biochemical estimation, then brain tissue homogenates were prepared using phosphate buffer with pH 7.4 in a tissue homogenizer and centrifuged at 900 rpm and 4 °C for 15 min. Then the supernatant obtained was used for further evaluation of the endogenous antioxidant enzymes and proinflammatory cytokines and RT-PCR analysis.

Estimation of oxidative stress and lipid peroxidation in brain

To evaluate the therapeutic impact of CRE and TC in $AlCl_3$ stimulated AD rats on oxidative stress and lipid peroxidation, we estimated the level of catalases (CAT) (catalog number E-BC-K106; Elabscience), superoxide dismutases (SOD) (catalog number E-BC-K020; Elabscience), glutathione peroxidases

(GPx) (catalog numbers E-BC-K096, E-EL-R2491; Elabscience), and lipid peroxidation (MDA) (catalog number E-EL-0060; Elabscience) in the brain tissue homogenates using ELISA kits according to the manufacturer's instructions.

Determination of acetylcholinesterase inhibitory activity

After observing the behavioral parameters, brain tissue homogenates were prepared by tissue homogenizer and mixed with 0.1 M phosphate buffer (2.6 ml) + 2,4 dithiobis nitrobenzoic acid (DTNB) 100 μ l, and acetylthiocholine iodide 20 μ l. The activity of acetylcholinesterase was calculated using the following formula:

$$R = 5.74 \times 10^{-4} \times A/CO$$

CO = concentration of tissue (mg/mL)

A = the change in absorbance/min

R = the rate of the substrate (moles) hydrolyzed min/gram of the brain tissue (Lakshmi et al. 2015).

Histopathological analysis of brain tissues

Animals were sacrificed in each group by cervical dislocation and isolated brain tissues were fixed in 10% neutral formalin buffer at 37 °C. The fixed tissues were then dehydrated in alcohol. After infiltration of the dehydrated tissues, these were embedded in paraffin wax. Transverse sections of paraffin embedded brain tissues of approximately 5 μ m thickness were incised with the aid of a microtome. The sliced sections were then mounted on a glass slide and staining is done by hematoxylin and eosin dye and covered with cover slips. Finally, the slides were observed under an electron microscope.

Estimation of CRE and TC effect on pro-inflammatory biomarkers in brain

The modulatory effect of CRE and TC in $AlCl_3$ stimulated AD rats on pro-inflammatory cytokines including interleukin-6 (IL-6) and TNF-alpha (TNF- α) were determined in the brain tissue homogenates using ELISA kits following the manufacturer's protocols.

Evaluation of AD-linked gene expression analysis

Brain tissues were isolated and washed with phosphate buffer. The total RNA was isolated by homogenizing the brain tissues using a polytron (VWR) device and treated with TRIzol reagent (Molecular Research Centre Inc., USA). Total RNA content was estimated by nano-drop at 260/280 nm wavelength and integrity was assured through gel green staining and agarose

gel electrophoresis. Single-stranded cDNA was assembled by using the Revert Aid First Strand cDNA kit (Thermo Scientific USA). The first-strand cDNA was additionally utilized in concomitance with a SYBER green PCR kit (Qiagen) and the wizScript primer assays for the analysis of TNF- α , IL-1 α , IL-1 β , AChE, and BACE-1 by real-time PCR. Quantitative PCR thermal cycling was carried out under the recommended conditions: 95 °C for 5 min, followed by 30–40 cycles, denaturation for 20 s at 95 °C, annealing for 30 s at 55–60 °C, and extension for 1 min at 72 °C by using real-time PCR machine (Bio-Rad). The size and sequence of the expected PCR product of both target genes and reference are mentioned in Table 1. Glyceraldehyde-3-phosphate dehydrogenase (GADPH) was utilized as an endogenous reference or housekeeping gene. The relative expression of all the target genes was measured by CT method using Real plex software against GADPH. Then products of real-time PCR were separated on agarose gel (2%) electrophoresis system.

Statistical analysis

Statistical analysis was performed by using post hoc test in GraphPad Prism version 5. The data comparison among all the groups was done by Tukey's post-test using one-way analysis of variance (ANOVA) and by Bonferroni post-test using two-way ANOVA. All the experimental procedures were carried out in triplicates. The statistically significant value was set up at $p < 0.05$ and all the data was expressed as mean \pm SEM; $n=6$.

Results

Neurobehavioral analysis

Effect of curcuminoid treatments on wire hang test

In the current study, it was examined that the hanging time was significantly decreased ($p < 0.05$) in AlCl₃ treated

groups compared to the NC (normal control), STD (standard group treated with rivastigmine), CR-30 (curcuminoids crude extract treated), CRE-30 (curcuminoid-enriched extract treated), and TC-30 (ternary complex treated) group (Fig. 1). The fall-off time was significantly expanded in NC, STD, CRE-30, and TC-30 treatment groups (i.e., approximately 60 min). Groups treated with CR-30 showed a significant decline ($p < 0.05$) in hanging time compared to NC, STD, CRE-30, and TC-30 groups. The hanging time of CRE-30 and TC-30 treated groups was non-significant to each other, revealing the protective effect of these treatments on neuromuscular coordination.

Effect of curcuminoid treatments on open field test

In the present study, open field test was performed to investigate either the anxiety, impaired locomotion, or exploration behaviors in rats due to administration of AlCl₃ (Fig. 2). It was examined that the time spent by rats at the periphery was significantly higher ($p < 0.05$) in comparison to the time spent by rats at the center of wooden box among all groups. AlCl₃ and CR-30 treated groups showed the significant decline in ($p < 0.05$) the exploratory behavior and locomotion compared to NC, STD, CRE-30, and TC-30 treatment groups (Fig. 2). In comparison, CRE-30, TC-30 treatment groups, and STD group showed significant higher number of total lines crossed and more locomotion at the periphery of chamber per 10 min when compared with each other. The open field test results indicated that there were marked differences for both total counts of lines crossed (a) termed as spontaneous locomotor and number of rearings (b) termed as exploratory activities between the AlCl₃, STD, CR-30, CRE-30, and TC-30 treatment groups. CRE-30 and TC-30 showed non-significant results when compared with each other in the case of both the number of lines crossed and the count of rearings.

Table 1 The primers used in the current study along with the expected protein size of qRT-PCR products

Genes	Primers	Primer sequence (5'–3')	Accession no.	Expected size (bp)
TNF- α	Forward	GGAGGGAGAACAGCAACTCC	NM_019204.2	178
	Reverse	TCTGCCAGTTCCACATCTCG		
IL-1 α	Forward	CCTCGTCCTAAGTCACTCGC	NM_017019.1	158
	Reverse	GGCTGGTTCCACTAGGCTTT		
IL-1 β	Forward	GACTTCACCATGGAACCCGT	NM_031512.2	105
	Reverse	GGAGACTGCCCATTTCTCGAC		
AChE	Forward	TCCCTCAACATCAGGCTCA	NM_172009.1	200
	Reverse	TCCCTCAACATCAGGCTCA		
BACE-1	Forward	CCAACCTTCGTTTGCCCAAG	NM_019204.2	104
	Reverse	GCGGAAGGACTGATTGGTGA		
GADPH	Forward	GGAGTCCCCATCCCAACTCA	XM_017592435.1	173
	Reverse	GCCATAACCCCCACAACAC		

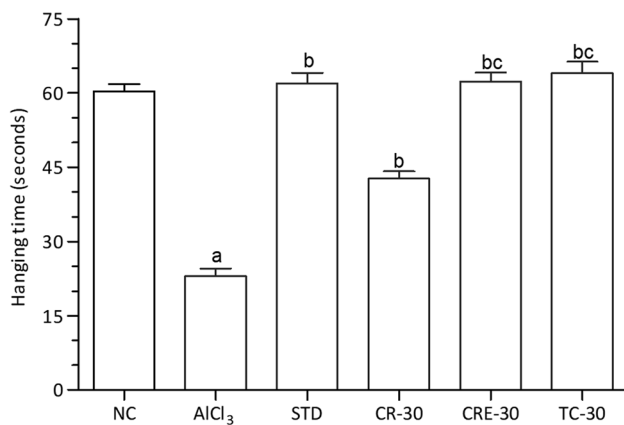


Fig. 1 Bar diagram representing the effects of CRE-30 and TC-30 treatment on wire hanging test on AlCl₃ induced AD rats. The test results were obtained by Tukey's post test using one-way ANOVA and the level of significance was set at $p < 0.05$. Each error bar expresses mean \pm SEM; $n=6$. ^aWhen compared with NC group, ^bwhen compared with AlCl₃ group, ^cwhen compared with CR-30 group. NC = normal control, STD = standard group, AlCl₃ = aluminum chloride disease control, CR-30 = curcuminoids crude extract, CRE-30 = curcuminoid-rich extract, and TC-30 = complex of curcuminoid-rich extract

Effect of curcuminoid treatment on hole board test

In hole board test, it was explored that the counts of head dipping were significantly decreased ($p < 0.05$) in the AlCl₃, and CR-30 groups when compared to the NC, CRE-30, and TC-30 treatment groups (Fig. 3). AlCl₃ induced depression-like behavior and impaired locomotion and exploratory abilities of rats in the disease control group

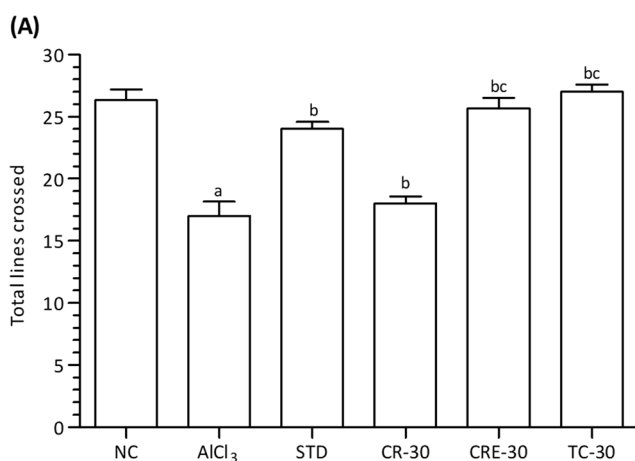


Fig. 2 The effects of CRE-30 and TC-30 treatment groups on the open-field test in AlCl₃ triggered AD rats. **a** Total lines crossed. **b** Number of rearings. The test results were estimated by Tukey's post test using one-way ANOVA and the level of significance was set at $p < 0.05$. Each error bar expresses mean \pm SEM; $n=6$. ^aWhen compared

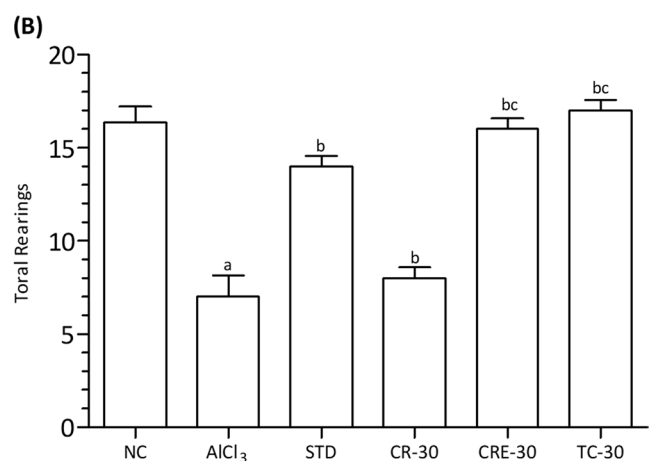
of this study. CRE-30 and TC-30 showed non-significant results when compared with each other. CR-30 group also showed a difference of $p < 0.05$ and TC-30 treatment group shows $p < 0.05$ when compared with STD group. In NC, STD, CRE-30, and TC-30 treatment groups, head dipping count was significantly mitigated in a dose-dependent way demonstrating their protective effects against anxiety and depleted exploratory behaviors (Fig. 3).

Effect of curcuminoid treatment on elevated plus maze

The effects of CRE-30 and TC-30 treatment groups on the execution of cognitive memory acquisition and memory retention in elevated plus maze in AlCl₃ induced AD rats are given in Fig. 4. The outcomes of transfer latency test indicated that STD, CRE-30, and TC-30 treatment groups in contrast to NC group rats were non-significant ($p > 0.05$). While the transfer latency was significantly decreased in AlCl₃ induced group ($p < 0.05$) compared to NC group, STD, CRE-30, TC-30, and CR-30 showing their protective effects against AD pathological biomarkers.

Effect of curcuminoid treatment on Morris water maze test

The outcomes of Morris water maze test revealed that after training trial of rats, the escape latency time (s) was significantly elevated ($p < 0.05$) in AlCl₃ and CR-30 compared to normal control groups (Fig. 5). However, STD, CRE-30, and TC-30 treatment groups showed that time of escape latency was decreased and all these groups exhibited non-significant results when comparison is done among these



with NC group, ^bwhen compared with AlCl₃ group, ^cwhen compared with CR-30 group. NC = normal control, STD = standard group, AlCl₃ = aluminum chloride disease control, CR-30 = curcuminoids crude extract, CRE-30 = curcuminoid-rich extract, and TC-30 = complex of curcuminoid-rich extract

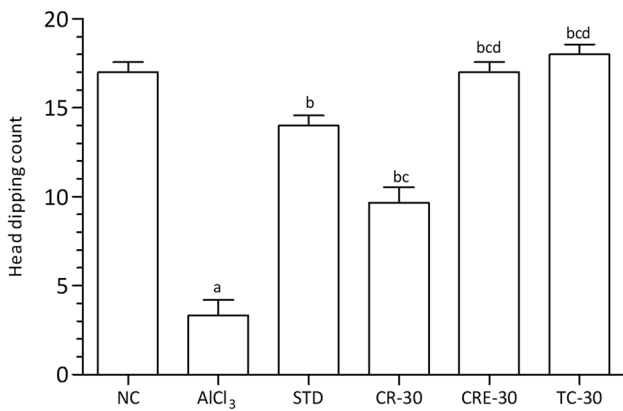


Fig. 3 Bar diagram representing the effects of treatment on hole board test in AlCl₃ induced AD rats. The test results were estimated by Tukey’s post test using one-way ANOVA and the level of significance was set at $p < 0.05$. Each error bar expresses mean \pm SEM; $n=6$. ^aWhen compared with NC group, ^bwhen compared with AlCl₃ group, ^cwhen compared with STD group, ^dwhen compared with CR-30 group. NC = normal control, STD = standard group, AlCl₃ = aluminum chloride disease control, CR-30 = curcuminoids crude extract, CRE-30 = curcuminoid-rich extract, and TC-30 = complex of curcuminoid-rich extract

groups. Visual behavioral evaluation suggested that rats in AlCl₃ induced diseased group were moved slowly toward the platform and thigmotaxis behavior (it showed the time spent at surroundings of test pool as an index of anxiety and

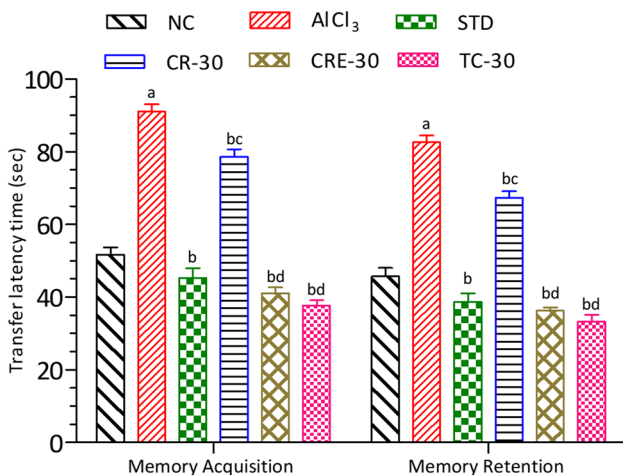


Fig. 4 Effects of treatment on the execution of cognitive memory acquisition and memory retention in elevated plus maze in AlCl₃ induced AD rats. The test results were estimated at the end of treatment by Tukey’s post test using one-way ANOVA and the level of significance was set at $p < 0.05$. Each error bar expresses mean \pm SEM; $n=6$. ^aWhen compared with NC group, ^bwhen compared with AlCl₃ group, ^cwhen compared with STD group, ^dwhen compared with CR-30 group. NC = normal control, STD = standard group, AlCl₃ = aluminum chloride disease control, CR-30 = curcuminoids crude extract, CRE-30 = curcuminoid-rich extract, and TC-30 = complex of curcuminoid-rich extract

platform searching strategy) was much higher in contrast to NC, STD, CRE-30, and TC-30 treatment groups. CR-30 showed non-significant results when compared with disease control group.

Biochemical analysis

Estimation of malondialdehyde (MDA) level

Administration of AlCl₃ (300 mg/kg) in rats caused a significant elevation in MDA level in brain ($p < 0.05$) versus NC, STD, CRE-30, and TC-30 groups (Fig. 6). The NC group showed a normal level of MDA which was 0.344 ± 0.023 nmol/mg. In comparison, STD, CRE-30, and TC-30 showed non-significant ($p > 0.05$) results as compared to NC group. Similarly, CRE-30 and TC-30 groups also showed non-significant ($p > 0.05$) results versus the STD-treated group. CRE-30 and TC-30 groups showed a significant decline ($p < 0.05$) in MDA levels when compared with CR-30 group. There were non-significant differences between CRE-30 and TC-30 treatment groups revealing that all these treatment groups have a protective impact on oxidative damage due to lipid peroxidation.

Catalase (CAT)

Administration of AlCl₃ caused a significant decrease in CAT level in brain ($p < 0.001$) when compared with NC, STD,

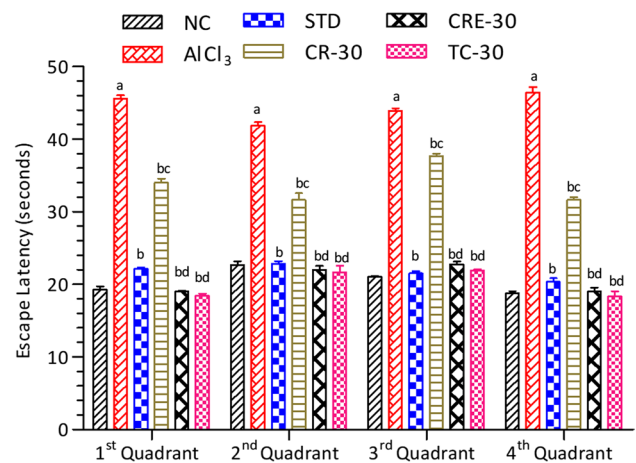


Fig. 5 Effects of treatment on escape latency time in Morris water maze test in AlCl₃ induced AD rats. The test results were estimated at the end of treatment by Tukey’s post test using one-way ANOVA and the level of significance was set at $p < 0.05$. Each error bar expresses mean \pm SEM; $n=6$. ^aWhen compared with NC group, ^bwhen compared with AlCl₃ group, ^cwhen compared with STD group, ^dwhen compared with CR-30 group. NC = normal control, STD = standard group, AlCl₃ = aluminum chloride disease control, CR-30 = curcuminoids crude extract, CRE-30 = curcuminoid-rich extract, and TC-30 = complex of curcuminoid-rich extract

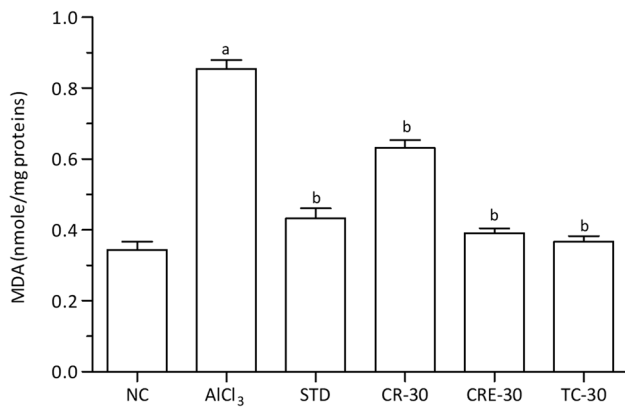


Fig. 6 Bar diagram representing the effects of CRE-30 and TC-30 treatment groups on lipid peroxidation test in AlCl₃ induced AD rats. The level of MDA was estimated at the end of treatment by using Tukey's post test using one-way ANOVA and the level of significance was set at $p < 0.05$. Error bar expresses mean \pm SEM; $n=6$. ^aWhen compared with NC group, ^bwhen compared with AlCl₃ group. NC = normal control, STD = standard group, AlCl₃ = aluminum chloride disease control, CR-30 = curcuminoids crude extract, CRE-30 = curcuminoid-rich extract, and TC-30 = complex of curcuminoid-rich extract

CR-30, CRE-30, and TC-30 groups (Fig. 7). The NC group showed the normal level of CAT which was 0.85 ± 0.04 IU/ μ l. In comparison, STD, CRE-10, CRE-30, TC-10, and TC-30 showed non-significant ($p > 0.05$) results as compared to NC group. Similarly, CRE-30 and TC-30 also showed non-significant ($p > 0.05$) results when compared with STD group. CRE-30 and TC-30 treatment groups represented a remarkable increase ($p < 0.05$) in CAT level in contrast to CR-30. Moreover, CRE-30 and TC-30 treatment groups showed non-significant difference compared to each other.

Superoxide dismutase (SOD)

The SOD level was remarkably decreased ($p < 0.05$) in brains of AlCl₃ treated rats when compared with NC, STD, CR-30, CRE-30, and TC-30 groups (Fig. 8). The NC group showed the normal level of SOD which was 0.85 ± 0.04 IU/ μ l. CR-30 treatment group rats represented a marked reduction ($p < 0.05$) in the SOD levels within brain as compared to CRE-10 and TC-30 groups and a difference of $p < 0.05$ versus STD-treated rats. In contrast, STD, CRE-30, and TC-30 showed non-significant ($p > 0.05$) results as compared to NC group. Similarly, CRE-30 and TC-30 also showed non-significant ($p > 0.05$) results compared to the STD group.

Glutathione peroxidase (GPx)

The GPx level was remarkably decreased ($p < 0.05$) in brains of AlCl₃ treated rats compared to NC, STD, CR-30, CRE-30, and TC-30 groups (Fig. 9). The NC group showed

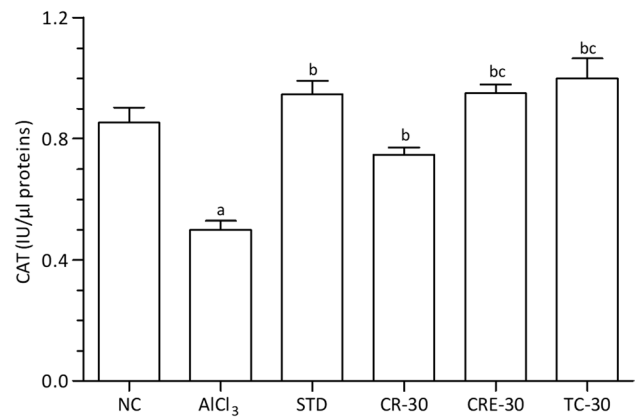


Fig. 7 Bar diagram representing the effects of CR-30, CRE-30, and TC-30 treatment groups on oxidative stress in AlCl₃ induced AD rats. The level of catalase (CAT) was estimated at the end of treatment by Tukey's post test using one-way ANOVA and the level of significance was set at $p < 0.05$. Error expresses mean \pm SEM; $n=6$. ^aWhen compared with NC group, ^bwhen compared with AlCl₃ group, ^cwhen compared with CR-30 group. NC = normal control, STD = standard group, AlCl₃ = aluminum chloride disease control, CR-30 = curcuminoids crude extract, CRE-30 = curcuminoid-rich extract, and TC-30 = complex of curcuminoid-rich extract

a normal level of GPx which was 9.47 ± 0.31 U/mg. CR-30 treatment group rats represented a marked reduction ($p < 0.05$) in the GPx levels within brain versus NC, CRE-30, and TC-30 groups and STD-treated rats, whereas STD, CRE-30, and TC-30 showed non-significant ($p > 0.05$) results as compared to the NC group. Similarly, CRE-30 and TC-30 also showed non-significant ($p > 0.05$) results when compared with the STD group.

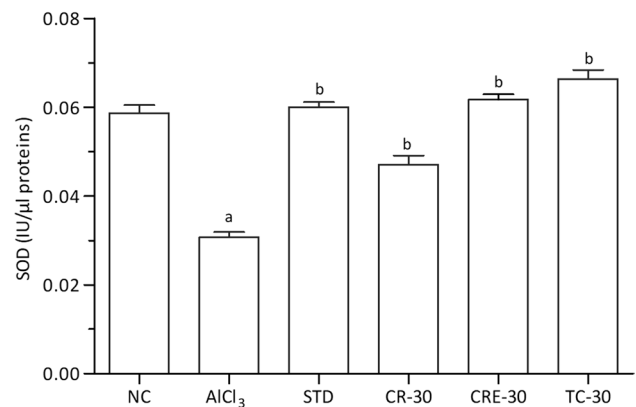


Fig. 8 Bar diagram representing the effects of treatment on SOD levels in AlCl₃ induced AD rats. The level of SOD was estimated at the end of treatment by Tukey's post test using one-way ANOVA and the level of significance was set at $p < 0.05$. Error bar expresses mean \pm SEM; $n=6$. ^aWhen compared with NC group, ^bwhen compared with AlCl₃ group. NC = normal control, STD = standard group, AlCl₃ = aluminum chloride disease control, CR-30 = curcuminoids crude extract, CRE-30 = curcuminoid-rich extract, and TC-30 = complex of curcuminoid-rich extract

Acetylcholinesterase inhibitory activity

Acetylcholine is the chief neuro-regulator in the brain region responsible for the modulation of cognitive and motor functions. The level of acetylcholine is diminished by the choline acetyltransferases degeneration resulting in cognitive inadequacies in AD. CRE and TC have a protective effect on the acetylcholine level due to their AChE inhibitory action, which causes the breakdown of acetylcholine. In the current study, we explored the changes in AChE activity in brains of AlCl_3 induced rats after administration of CR, CRE, and TC presented in Fig. 10. In the NC group, the concentration of AChE was found to be $2.8 \pm 0.173 \mu\text{mol}/\text{mg}$. When the rats were administered with AlCl_3 , the level of AChE was enhanced remarkably ($p < 0.05$) in cerebellum region in contrast to NC, STD, CR-30, CRE-30, and TC-30 groups. However, there was a significant increase in AChE levels in CR-30 treated group ($p < 0.05$) versus the NC and STD treated group. Similarly, chronic administration of CRE-30 and TC-30 ($p < 0.05$) significantly prevented the AChE inhibitory activity in the brain compared to the CR-30 treated group. The concentration of AChE was also significantly decreased in TC-10 ($p < 0.05$) compared to CR-30 and TC-30 ($p < 0.05$) compared to CRE-30.

Effect of curcuminoid treatments on inflammatory biomarkers TNF- α and IL-6

The IL-6 level was remarkably decreased ($p < 0.05$) in brains of AlCl_3 treated rats in contrast to NC, STD, CRE-30,

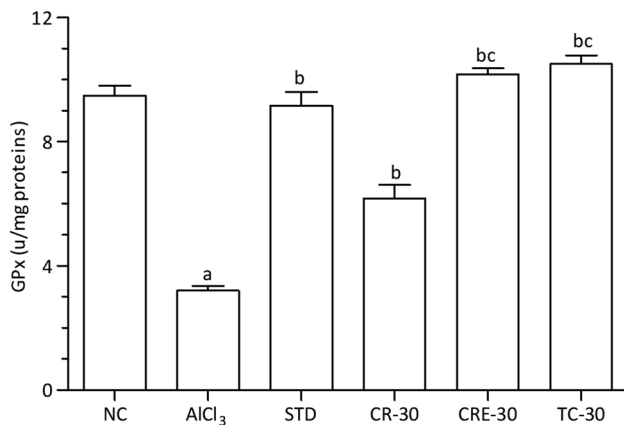


Fig. 9 Bar diagram representing the effects of CRE-30 and TC-30 treatment groups on GPx in AlCl_3 induced AD rats. The test results were estimated at the end of treatment by Tukey's post test using one-way ANOVA, and the level of significance was set at $p < 0.05$. Each error bar represents mean \pm SEM; $n=6$. ^aWhen compared with NC group, ^bwhen compared with AlCl_3 group, ^cwhen compared with CR-30 group. NC = normal control, STD = standard group, AlCl_3 = aluminum chloride disease control, CR-30 = curcuminoids crude extract, CRE-30 = curcuminoid-rich extract, and TC-30 = complex of curcuminoid-rich extract

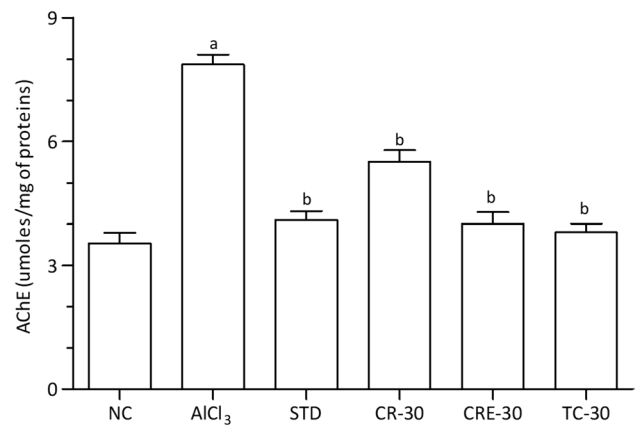


Fig. 10 Bar diagram representing the effects of CRE and TC treatment on AChE in AlCl_3 induced AD rats. The AChE level was estimated at the end of treatment by Tukey's post test using one-way ANOVA, and the level of significance was set at $p < 0.05$. Error bar expresses mean \pm SEM; $n=6$. ^aWhen compared with NC group, ^bwhen compared with AlCl_3 group. NC = normal control, STD = standard group, AlCl_3 = aluminum chloride disease control, CR-30 = curcuminoids crude extract, CRE-30 = curcuminoid-rich extract, and TC-30 = complex of curcuminoid-rich extract

and TC-30 groups (Fig. 11). The NC group showed a normal level of IL-6 which was $45.00 \pm 2.88 \text{ pg}/\text{mg}$. CR-30 treatment group rats represented a marked reduction ($p < 0.05$) in the SOD levels within brain versus CRE-30 and TC-30 groups. In comparison, STD, CRE-30, and TC-30 showed non-significant ($p > 0.05$) results as compared to NC group. Similarly, CRE-30 and TC-30 also showed non-significant ($p > 0.05$) results when compared with the STD group.

TNF- α level was remarkably decreased ($p < 0.05$) in brain lysates from rats cotreated with AlCl_3 and STD or TC compared to those treated with AlCl_3 alone or AlCl_3 and CR (Fig. 10). The NC group showed the normal level of TNF- α which was $104.33 \pm 03.48 \text{ pg}/\text{ml}$. CR-30 treatment group rats represented a marked reduction ($p < 0.05$) in the SOD levels within brain in contrast to CRE-30 and TC-30 group rats, whereas STD, CRE-30, and TC-30 showed non-significant ($p > 0.05$) results as compared to NC group. Similarly, CRE-30 and TC-30 also showed non-significant ($p > 0.05$) results when compared with STD group.

Effect of curcuminoid treatments on mRNA expression of pathological biomarkers of neurodegeneration

After the accomplishment of a 21 days experimental study plan in healthy Wistar rats, mRNA expression of various pathological biomarkers of AD including TNF- α , IL-1 α , IL-1 β , AChE, and BACE-1 was evaluated (Fig. 12). It was observed that TNF- α expression was significantly increased ($p < 0.05$) in AlCl_3 diseased group versus NC group (3.00 ± 0.58 -fold).

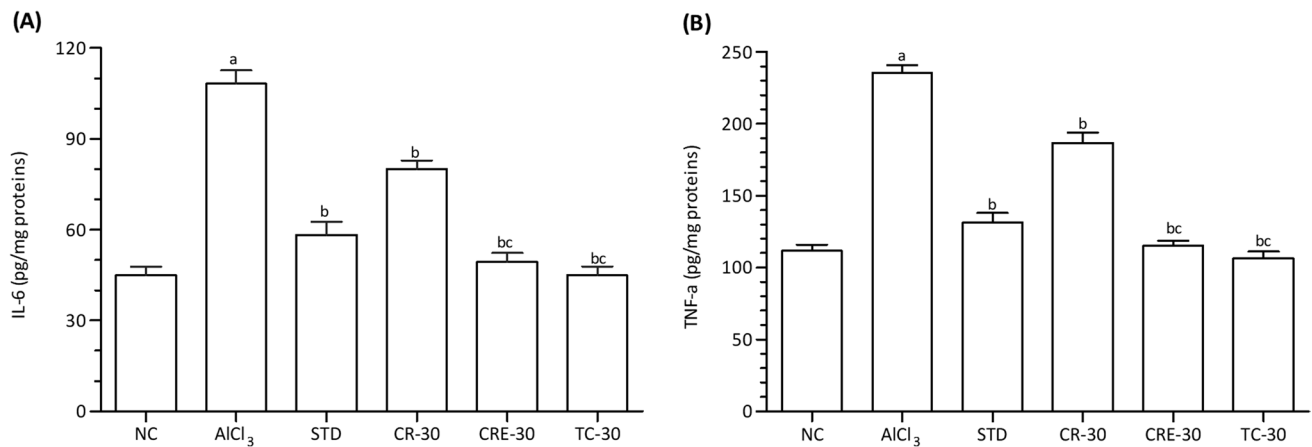


Fig. 11 Bar diagram representing the effects of CRE-30 and TC-30 treatment groups on proinflammatory cytokines in AlCl₃ induced AD rats. The level of IL-6 and TNF-α was estimated by Tukey's post test using one-way ANOVA, and the level of significance was set at $p < 0.05$. Error bar expresses mean \pm SEM; $n=6$. ^aWhen compared

with NC group, ^bwhen compared with AlCl₃ group. NC = normal control, STD = standard group, AlCl₃ = aluminum chloride disease control, CR-30 = curcuminoids crude extract, CRE-30 = curcuminoid-rich extract, and TC-30 = complex of curcuminoid-rich extract

However, the level of TNF-α was considerably alleviated ($p < 0.05$) in STD group (1.53 ± 0.12), CRE-30 (1.40 ± 0.11 -fold), and TC-30 (1.09 ± 0.048 -fold), in comparison to diseased control group. The mRNA expression of IL-1α was notably upregulated ($p < 0.05$) in AlCl₃ model group in comparison to NC group (2.76 ± 0.14 -fold). Nevertheless, the expression of IL-1α was notably downregulated ($p < 0.05$) in STD group (1.41 ± 0.094), CRE-30 (1.27 ± 0.085 -fold), and TC-30 (1.13 ± 0.031 -fold) in contrast to diseased control. A remarkable proliferation

($p < 0.05$) in mRNA expression of IL-1β was observed in AlCl₃ diseased group versus NC group (2.93 ± 0.17 -fold) and STD group (1.40 ± 0.17 -fold). However, mRNA expression level of IL-1β after treatment with TC-30 was incredibly improved (1.16 ± 0.120 -fold) compared to diseased control. A notable decrease ($p < 0.05$) in expression level of IL-1β was observed in CR-30 and CRE-30 (1.53 ± 0.18 -fold) group versus AlCl₃ diseased group. It was noticed that AChE mRNA expression was incredibly upregulated ($p < 0.05$) in AlCl₃ induced group

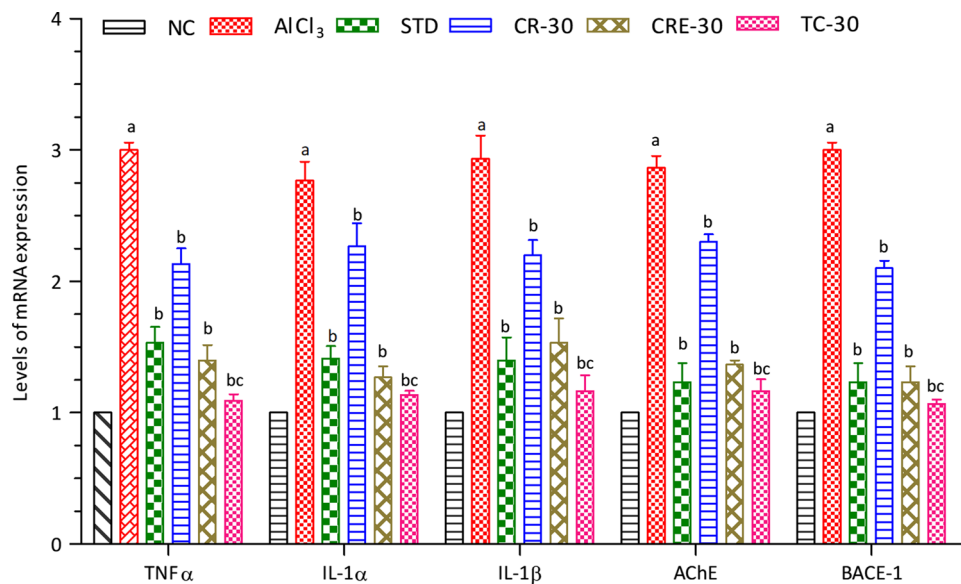


Fig. 12 Analysis of TNF-α, IL-1α, IL-1β, AChE, and BACE-1 expressions by RT-qPCR after treatment of AD rats with curcuminoid extracts and ternary complex. The results were analyzed by using two-way ANOVA followed by Bonferroni post test and the level of significance was set at $p < 0.05$. Data expressed as mean \pm SEM; $n =$

6. ^aWhen compared with control group, ^bwhen compared with disease group, ^cwhen compared with CR-30 group. NC = normal control, STD = standard group, AlCl₃ = aluminum chloride disease control, CR-30 = curcuminoids crude extract, CRE-30 = curcuminoid-rich extract, and TC-30 = complex of curcuminoid-rich extract

compared to the NC group (2.86 ± 0.08 -fold). Yet, the increased level of AChE was found to be alleviated ($p < 0.05$) in TC-30 (1.36 ± 0.08 -fold) and CRE-30 (1.53 ± 0.03 -fold) in contrast to the diseased group. On the other hand, STN group (1.23 ± 0.14 -fold) indicated a significant decrease in AChE expression ($p < 0.05$) versus disease control. The elevated expression of AChE was moderately recovered ($p < 0.05$) in CR-30 (2.30 ± 0.05 -fold) compared to the AlCl_3 model. The mRNA expression of β -secretase (BACE-1) showed remarkable proliferation ($p < 0.05$) in AlCl_3 diseased control group versus NC group (3.00 ± 0.05 -fold) and standard group. Notably, downregulation ($p < 0.05$) of BACE-1 was obtained in CRE-30 (1.23 ± 0.12 -fold) and TC-30 (1.06 ± 0.06 -fold) versus the AlCl_3 diseased group. This gene expression was mitigated in CR-30 (2.10 ± 0.05) compared to the AlCl_3 model. Treatment with curcuminoid extracts including CR-30, CRE-30, and TC-30 demonstrated a notable decline in the pathological indicators of AD in a dose-dependent manner showing that treatment with curcuminoid extracts helps to reduce the ferocity of AD in albino Wistar rats.

Histopathological analysis

The changes in the brain function after the induction of AlCl_3 in experimental rats were correlated with histopathological examination in brain section (Fig. 13). NC group indicated the normal tissue architecture and salutary framework of frontal cortex (H&E normal brain tissue and intact structure of neurons were observed in the NC group). In AlCl_3 treated rats, the obvious signs of neurodegeneration including darkened and shrunken neurons, neurofibrillary tangles, pigmentation, vacuolated cytoplasm, and deposition of amyloid plaques were observed. In the STD group, improved normal tissue architecture of cerebral cortex was seen. In CR-30 treatment groups, vascular spaces among neurons appeared to be narrowed, neuronal loss and neurofibrillary tangles were slightly improved, while shrunken and dark neuronal cells evidenced by the presence of spaces and also narrow vasculature were examined in the CR-30 treatment group. In CRE and its TC treatment group, neurofibrillary tangles, neuroinflammation and neuronal loss, pigmentation, and narrow vascular spaces were improved clearly (Fig. 13). Significantly less neuronal loss, and alterations due to neurodegeneration and vacuolated cytoplasm were seen to be recovered in the CRE-30 treatment group. TC treatment group appreciably preserves the gross pathological aberrations, neuronal pigmentation, neuroinflammation, and loss of neurons.. TC-30 treatment groups widen the narrowed spaces around pyramidal cells of frontal cortex and greatly recovered the salutary framework of brain tissue by preventing neuronal loss, pigmentation, and neurofibrillary tangles in AlCl_3 induced AD rat model.

Discussion

Oxidative stress and inflammation are one of the predominant factors causing numerous neurodegenerative diseases (Butterfield et al. 2007; Cheignon et al. 2018; Salter and Stevens 2017; Sultana and Butterfield 2008). Several in vitro, in vivo, and clinical studies demonstrated that curcuminoids hold numerous biological activities including antitumor, antioxidant, anti-acidogenic, neuroprotective, anti-arthritis, anti-inflammatory, and radioprotective (Amalraj et al. 2017; Kabir et al. 2021a; Kabir et al. 2021b). The study was planned to estimate the neurobehavioral alterations induced by oral administration of AlCl_3 mitigated by plant-oriented treatment. The spatial and cognitive deficits were considered as initial clinical signs with severe health-related threats by aluminum in AD (Bhattacharjee et al. 2014; Mathiyazhan et al. 2015). Usually, aluminum accretes in the most fundamental parts of the brain including the hippocampus and frontal cortex regions which are chiefly concerned with perception and memory (Cheng et al. 2019). Aluminum exposure stimulated various neuronal and biochemical abnormalities which were directly associated with cognitive deformities and that is a corresponding outcome mostly observed in cases of AD (Bondy 2014; Walton 2012; Wang et al. 2014a). In the current investigation, supplementation of CRE and TC appreciably improved the behavioral aberrations including deficits in locomotion, spatial memory, and task strategy in contrast to CR in AD rats induced by AlCl_3 , indicating the neuroprotective and beneficial properties of CRE and TC against AD. Regarding the perseverance and neuromuscular strength of AlCl_3 induced AD rats in wire hanging test, our findings suggested that treatment with CRE and TC significantly ameliorate neuromuscular endurance in contrast to the CR treatment group which was greatly reduced by administration of AlCl_3 , although CRE and TC greatly enhance the locomotor strength. In AlCl_3 treated group, the outcomes for open-field behavioral test showed a decline in spontaneous locomotion and exploration similar to a former study (Justin Thenmozhi et al. 2016; Thenmozhi et al. 2015). All these behavioral changes were remarkably subsided by treatment with CRE and TC. This might be due to the enhanced release and solubility of curcuminoids in the ternary cyclodextrin complex, whereas curcuminoids present in the aqueous form increased the curcuminoid uptake into the cell (Tan et al. 2012). Current exploration suggested that exposure to AlCl_3 induce neurobehavioral abnormalities by causing changes in curiosity, neophilia, and exploratory abilities in rats similar to previous related studies (Abuelezz et al. 2021; Zerrouki et al. 2016). CRE, TC, and rivastigmine (standard) significantly enhanced the curiosity and exploratory ability of AD rats in hole board apparatus. These locomotor deficiencies and behavioral changes are intensifying

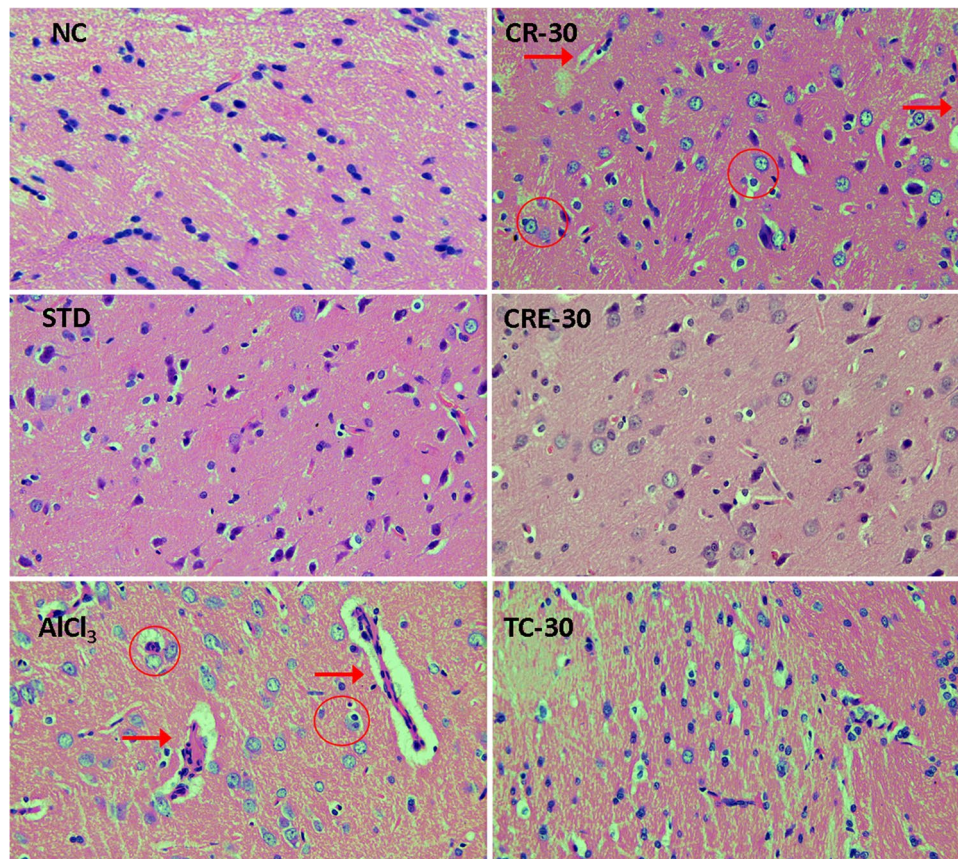


Fig. 13 The effect of curcuminoid-rich extract and its ternary complex on AlCl_3 triggered histopathological alterations in hematoxylin and eosin-stained frontal cortex of experimental rats, where normal control (NC) is showing the normal architecture of brain tissues. Standard group showing improved normal tissue architecture of cerebral cortex. Aluminum chloride disease control (AlCl_3) showing obvious signs of neurodegeneration including darkened and shrunken neurons, plaques (red circles), neurofibrillary tangles (red arrows), pigmentation, and vacuolated cytoplasm. Curcuminoids crude extract

(CR-30) showing narrowed vascular spaces among neurons, neuronal loss, plaques (red circles), and neurofibrillary tangles (red arrows). Curcuminoid-rich extract (CRE-30) showing less neuronal loss and alterations due to neurodegeneration and vacuolated cytoplasm. Ternary complex (TC-30) widens the narrowed spaces around pyramidal cells of frontal cortex and greatly recovered the salutary framework of brain tissue by preventing neuronal loss, pigmentation, and neurofibrillary tangles

the consequences of oxidative damage and compromised neurotransmission of acetylcholine instigated via AlCl_3 administration. Both CRE and TC treatment ameliorated the locomotion impairments and cognitive deficits revealing their neuroprotective action in AD.

The elevated plus-maze test is considered as a simple test for the estimation of learning and memorizing behavior. As the experimental animals are capable of remembering the structure of the enclosed and open arms, they try to escape the open (unsafe) arm on the second session more rapidly. It is easy to determine the learning stimulated by fear, which regulated the transfer latency process in this behavioral test. The outcomes of elevated plus-maze indicated the enhancement in memory as shown by the transfer latency decline in retention trial in comparison to acquisition trial. In the same way, elevated plus-maze test performance in AlCl_3 administered rats was significantly impaired. CR-treated group

showed less minute recovery of the memory and learning performance whereas CRE and its TC showed improvement in cognitive abilities.

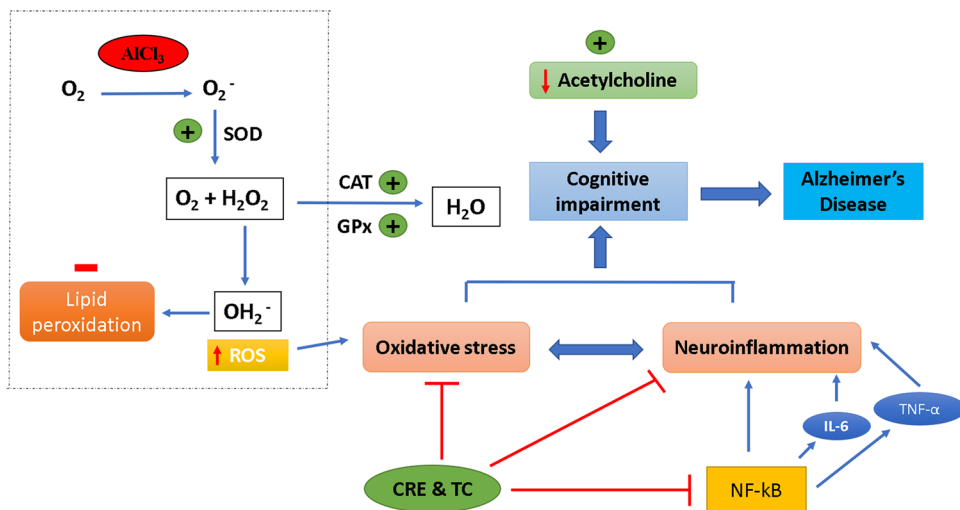
In addition, deficits in spatial and contiguous learning estimated by the Morris water maze test have also been demonstrated in AD rodent models (D’Hooge and De Deyn 2001). The inferences of the Morris water maze test of increased escape latency time in AlCl_3 induced groups are compatible with previous work (Galeano et al. 2014; Petrasek et al. 2018). Hence, it was among one of the study’s objectives to determine the predictable protective impact of CR, CRE, and TC on spatial learning in the AlCl_3 treated AD rats’ model. Similarly, a pronounced decrease in the latency duration of normal control group in contrast to AlCl_3 induced group was observed. Surprisingly, treatment of AlCl_3 administered rats with 30 mg/kg of CRE and TC resulted in a remarkably shorter latency time for rats to

approach the platform. This is because rats usually despise swimming, they tend to avoid contact with the water, which was achieved by approaching the platform used in the test. The decline in escape latencies estimated in animals of CEE-30 and TC-30 treatment groups suggested that the treatment effectively ameliorates the deficits in spatial memory caused by AlCl_3 . Hence, observing the outcomes of the Morris water maze test, it is indicated that both CRE and its TC may have a protective impact on cognitive and spatial learning deficits. It has also been documented in many previous studies that curcumin (the most vital constituent of curcuminoids) enhances memory and learning ability and provides neuroprotective impact (Haider et al. 2015; Noorafshan et al. 2013; Rui et al. 2008).

Oxidative stress is a crucial factor that could affect the pathological progression and insistence of AD (Wang et al. 2014b; Zhao and Zhao 2013). Pieces of evidence indicated that oxidative damage plays a critical pathogenic role in persistent inflammatory conditions. The impairments caused by the oxidative stress such as oxidized proteins, lipid peroxidation (MDA), and glycated products usually result in the degradation of neurons, frequently documented in brain diseases (Grewal et al. 2021; Popa-Wagner et al. 2013). Aluminum produces oxidative damage by producing reactive oxygen species (ROS) and by diminishing the level of lipid peroxidation and proteins in frontal cortex and hippocampus regions. The free radicals and ROS levels were increased due to a decline in first-line defense antioxidant enzymes including SOD, CAT, and GPx levels resulting in oxidative damage in AlCl_3 induced AD rat model. Cognitive impairments and oxidative stress are strongly associated with one another, as increased accumulation of free radicals causing oxidative insult to biological molecules subsequently results in cognitive deficits and neuronal damage (Bertram and Tanzi 2008). Several studies have mentioned the strong antioxidant potential of curcuminoids and their ability to prevent the oxidative damage and related cognition deficits (Hatcher et al. 2008; Tokaç et al. 2013). The findings of the current investigation are consistent with the results of the previous studies, as administration of AlCl_3 triggered pathological modifications in the disease control group, caused a marked increase in lipid peroxidation, and decreased the first-line defense antioxidant enzymes. However, CRE and TC supplementation markedly improved the status of antioxidant enzymes particularly CAT, SOD, and GPx. Current findings manifested the reduced tendency of rivastigmine as an antioxidant compared to CRE and its TC. Thus, revealing that CRE and its TC decreased the AlCl_3 triggered oxidative stress in the brain of experimental rats. AChE is the fundamental enzyme peculiarly produced in the muscles, neurons, and hematopoietic cells, and also moderately present on muscles and neurons' surface extracellularly. According to a study, AChE enzyme inhibitors hold an important spot

for imparting the symptomatic treatment among different classes of drug (Arya et al. 2021; Zetterberg 2006). Administration of AlCl_3 triggered pathological modifications in the diseased group, caused a marked increase in AChE level, and caused a notable reduction in all treatment groups. However, CRE and its TC supplementation improved the status of AChE in the brain regions, thus revealing that CRE and its TC decreased the AlCl_3 triggered reduction in AChE levels in the brain of experimental rats more efficiently contrary to CR (30 mg/kg) treated group. It is illustrated from previous data that besides oxidative stress, neuroinflammation serves as an important earliest event and plays a vital role in the pathogenesis of AD including amyloid accumulation, cholinergic malfunction, neural injuries, and necrosis. In the present study, AlCl_3 supplementation appreciably increased the pro-inflammatory cytokines such as IL-6 and TNF- α status in the brain tissue homogenates of AlCl_3 administered AD rats. Moreover, treatment with CR (30 mg/kg) showed no significant improvement in the status of IL-6 and TNF- α in brain. TNF- α and IL-6 both were considered as principal pro-inflammatory cytokines in the central nervous system and involved in the progression of pathological events related to learning and memory deficits in the AD. Curcumin has been reported to improve the viability of microglial cells and prevent their activation and involvement in blockage of extracellular p38 kinase signaling pathway and signal-regulated kinase 2 (ERK1/2) and also preventing the production of TNF- α and IL-6 (Salehi et al. 2020; Shi et al. 2015). Findings of the present study suggested that the supplementation of CRE and its TC was noticeably diminished the proinflammatory chemicals including IL-6 and TNF- α in the AlCl_3 administered AD rats in contrast to CR (30 mg/kg). This clearly explained the neuroinflammatory potentials of CRE and TC. Curcuminoids have been documented to inhibit $\text{A}\beta$ production by downregulating the expression of β site amyloid precursor proteins cleaving enzyme-1 (BACE-1) (Thenmozhi et al. 2015), an enzyme that breaks the APP to $\text{A}\beta$. The rats treated with curcumin had ameliorated memory responses and spatial behaviors and had been relieved from the degradation of synaptic sites (Zheng et al. 2017). Correspondingly, according to an *in vitro* study, curcumin has also been recognized as a potent inhibitor of BACE-1 (Di Martino et al. 2016). Curcuminoid mixture is considered to decrease the harmful effects of $\text{A}\beta$ in cortical neurons and anticipate the phagocytosis of $\text{A}\beta$ plaques. It also prevents neurotoxicity induced by $\text{A}\beta$ by decreasing reactive oxygen species (ROS) levels. It is explored from the current investigation that the supplementation of CRE and its TC noticeably downregulated the BACE-1 expression in the AlCl_3 administered AD rats in contrast to CR (30 mg/kg). In comparison, levels of BACE-1 were greatly increased in the AlCl_3 administered diseased group. This clearly explained the neuroprotective potentials of CRE and TC.

Fig. 14 Graphical representation of the possible signal pathway mechanism by which CRE and TC attenuate Alzheimer's disease



After discovering the prominent neuroinflammation in patients of AD, it was revealed that the elevations in concentrations of IL-1 played a key role in AD progression and pathogenesis. IL-1 β has been observed to be involved in enhancing the processes of acute neuroinflammation in vivo. Exposure of IL-1 β in brain of the mouse model elicits rapid, robust stimulation of both microglia and astrocytes (Shaftel et al. 2008). The findings of the current investigation suggested that the supplementation of CRE and its TC noticeably downregulated the modulation of IL-1 and IL-1 β in the AICl₃ administered AD rats in contrast to CR (30 mg/kg), whereas the mRNA expression levels of IL-1 α and IL-1 β increased in the AICl₃ administered diseased group. This clearly explained the neuroprotective potentials of CRE and TC. It was precisely summarized in several experiments that the increased levels of IL-1 α and IL-1 β ultimately result in spatial, cognitive, and memory deficits via the neurodegeneration process in AD. Histopathological investigation of present research conducted revealed that normal control and standard groups preserved the intact architecture of neurons but AICl₃ indicates gross pathological signs of neurodegeneration. TC significantly improved the neuronal damage, pigmentation, neurofibrillary tangles, and neuroinflammation triggered by AICl₃ compared to CRE. This impact can be distinctly ascribed by the synergy indicated by the components possessing anti-oxidant and anti-inflammatory characteristics in the curcuminoid extract including CR, CRE, and its TC, hence manifesting a piece of evidence for the utilization of the enriched extract as a promising alternative treatment option for CR, which is not available commercially and is comparatively simple and cheaper in contrast to expensive purification process. The signal pathway mechanism indicating the mode of action of CRE and TC is represented in Fig. 14.

Conclusion

From the current research work, it is concluded that CRE and its TC hold the potential to alleviate Alzheimer's disease pathological hallmarks, as shown by improvement in behavioral parameters, attenuation of oxidative stress, and modulatory effect on AChE and pro-inflammatory cytokines. Furthermore, the present study also showed the elevated potential of TC in contrast to CRE itself. All these results were supported by mRNA expression concentrations of IL-1 α , TNF- α , IL-1 β , AChE, and BACE-1 genes involved in Alzheimer's disease. However, levels of these genes varied with dose and time favorable effects were obtained even during the treatment duration. Hence, CRE and its cyclodextrin complex exhibited similar effects like pure curcuminoids for industrial practices mainly utilizing simple, inexpensive, and environment-friendly procedures, and these should be suggested as promising treatment options against AD.

Author contribution AS: literature search, data curation, experimental analysis, investigation, writing—original draft, and validation. KR: investigation, conceptualization, writing—final draft, and editing. MSHA: project administration, supervision, conceptualization, methodology, writing—original draft preparation, and editing. MA and ZC: data curation. PP and MAS prepared and standardized the extracts.

Data availability All data generated and/or analyzed during this study are included in this published article.

Declarations

Ethical approval This study was ethically approved from the Institutional Review Board (GCUF/ERC/2196) of Government College University Faisalabad (GCUF).

Conflict of interest The authors declare no competing interests.

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