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Repeated administration of curcumin attenuates the behavioral deficits induced by lead toxicity in rats

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Abstract: The present study was designed to investigate the protective effects of short term treatment of curcumin against psycho-behavioral abnormalities induced by lead exposure. Twenty four male Albino Wistar rats were divided into four groups namely vehicle control (VC) which received neutral oil orally and 0.9% saline intraperitoneally, curcumin (CUR) which received curcumin dissolved in neutral oil orally 100mg/ml/kg for 7 days, lead group (L) which received lead acetate intraperitoneally (ip) 100mg/ml/kg for 7 days and lead+curcumin (LC) which were co-administered with lead acetate and curcumin. To assess memory and cognition Morris water maze test (MWM) and Novel object recognition test (NOR) were performed. For the assessment of anxiety and depression like symptoms Elevated Plus maze (EPM) and Tail Suspension test (TST) were performed. Open Field test (OFT) was performed to monitor ambulatory activity. Exposure to lead significantly impaired memory and cognitive function and also induced anxiogenic and depression like symptoms in rats while co-administration of curcumin with lead attenuated lead induced behavioral deficits and recovered impairments in learning and memory suggesting preventive role of curcumin on brain against heavy metal induced toxicity.

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Introduction

Curcumin is a lipophilic molecule[1] and does not appear to be toxic even at high doses[1, 2]. It has been used extensively in ancient medicinal system and home remedies. Curcumin possesses a broad spectrum of biological actions including anti-inflammatory, anti-carcinogenic, anti-mutagenic, anti-coagulant, anti-diabetic, anti-bacterial, anti-ulcer, anti-venom, anti-fertility, anti-protozoal, anti-fibrotic, anti-apoptotic, anti-proliferative, anti-aging[1, 3, 4, 5]. Curcumin also provides protection against reactive oxygen and nitrogen species[6, 7]. Antioxidant effects of curcumin have been reported [4, 8, 9]. In addition to above biological activities curcumin has a major role in stress and depression like symptoms. Involvement of serotonergic system and 5-HT1A/1B and 5-HT2C receptors have also been reported in exerting antidepressant effects by curcumin[10, 11]. Neuroprotective effects of curcumin against diseases like Epilepsy and Alzheimer's have also been reported[12, 13]. Lead (Pb^{+2}) is a heavy metal and is widely used in number of technological processes as a result of which humans are exposed to it[14]. There many sources of lead contamination in environment for example lead-based paints, contaminated soil, water, air and a number of other lead-based products[15]. A number of actions have been taken in past to minimize the lead use but still a significant health hazard[14].

The basic mechanism responsible for lead toxicity is its ability to replace other cations like Ca^{+2} and Zn^{+2}in the cell[14, 16]. Lead poisoning cause cognitive deficits, motor impairment and is more dangerous in childhood, as brain is in the developmental stage[14, 17]. Glial and neuronal changes have been reported following lead poisoning[18]. It has also been reported that repeated exposure to lead affects brain serotonin metabolism and causes memory impairment[19]. On the basis of beneficial effects of curcumin the present study was designed to investigate the neuroprotective effects of curcumin on different behaviors following lead induced toxicity in rats.

Materials and Methods

Animals:

The study was performed on 24 male Albino wistar rats weighing 180-200gms. All animals were healthy and purchased from Aga Khan University. All animals were housed individually under a 12h light-dark cycle and controlled temperature (22$\pm$ 2^0C) with free access to cubes of standard rodent diet and tap water for at least 3-4 days before experimentation so that rodents could adapt themselves to the new environment. All experiments were conducted after approval from Local Animal Care Ethical Committee.

Drugs:

Lead acetate was purchased from Merck (Germany) and Curcumin was purchased from Sigma Chemicals (USA).

Protocol:

Animals were randomly divided into four groups, vehicle control (VC), curcumin (CUR), lead (L) and lead+curcumin (LC). Animals in the VC group were orally administered with neutral oil and injected (i.p) with saline. Animals in the CUR group were orally administered with curcumin at a dose of 100mg/ml/kg. Animals in the L group were injected with lead (i.p) at the dose of 100mg/ml/kg and animals in the LC group were orally administered with curcumin and i.p injected with lead. Doses of curcumin and lead acetate were selected on the basis of previously reported studies (Girish and Pradhan, 2012; Haider et al., 2005). All the drugs were administered for 7 days and then behavioral tests were performed.

Behavioral Tests

Plus Maze Test:

The Plus Maze test is used to assess anxiety-like behavior in laboratory animals[21]. The apparatus used in the present study consisted of two open arms (50x10 cm) crossed with two closed arms of the same dimensions with walls 40cm high. The arms were connected with a central square (5x5 cm) to give the apparatus a plus sign appearance. The maze was elevated 60cm above the floor. To monitor the activity, rats were individually placed in the central square facing an enclosed arm and the time spent in open arm by the rat was recorded.

Open Field Test:

The open field test is designed to measure behavioral responses such as locomotor activity and exploratory behavior[22]. The apparatus for open field test is a square (76 × 76 cm) with opaque walls 42 cm high. The floor is divided into 25 equal squares. To monitor the activity animals taken out from their home cages were placed in the central square of the open field (one at a time). Numbers of squares crossed with all four paws were counted for 5 min.

Tail Suspension Test:

The tail suspension test is most widely used to assess despair like behavior in rats and was developed by Steru et al (1985)[23]. The test is based on the fact that animals subjected to short term, inescapable stress of being suspended by their tail will develop an immobile posture. Method is essentially the same as described previously[22]. The test is usually quite short. Cut off time being 6 min, and the amount of time the rats spend immobile is recorded. In the present study the apparatus consisted of a wooden box painted gray (54 × 30 × 52 cm) with a hook in the center of the ceiling. Each rat was individually suspended by the tail from the hook with an adhesive tape.

Novel Object Recognition Test:

The experimental apparatus used for the object recognition task was an open field box (40x40x40 cm) made up of gray painted wood. The floor was covered with saw dust. The method was essentially the same as that described by Batool et al (2016)[24]. The objects to be discriminated were two similar transparent glasses filled with white cement (A1 and A2) and a metallic container of same size filled with white cement (new object, B). The test was comprised of three phases: 1) Habituation phase 2) Training phase and the 3) Test phase. On the 1st day, each rat was initially habituated to the open field box without any object for 15 minutes. On the 2nd day, each rat was placed in the open field for 15 minutes and allowed to freely explore two identical objects A1 and A2 (two glasses filled with white cement). On 3rd day, during the test phase, one the object used

during training session was replaced by a novel object (B) and animals were left to explore the objects until they had accumulated 30s of total object exploration time or for a maximum of 20 minutes.

Morris Water Maze Test:

Morris Water Maze (MWM) test is used to examine the effects on spatial memory. It was developed by Morris in 1981[25]. This is a well-known, conventional cognitive test which requires an animal to use spatial learning and memory to locate a hidden platform just below the surface of a circular pool of water and also to remember its location as in the previous trial. It is reported that the animal uses cues in order to locate the hidden platform. The maze used for rats is same as described by Haider et al (2011)[26]. It is a circular pool of water with a diameter of 45cm, height 37cm and depth of water is 12cm. The pool is a metal cylinder painted white on the inner surface and the escape platform is also made of metal cylinder with flat metallic top having a surface diameter of 8cm and is 2cm below the surface of water during water maze training. The pool is filled with water (23 ± 2ºC) and made opaque with milk in order to obscure the platform and to allow proficient tracking of the swim paths of the rats. In our experiment we have assessed the working (short-term) memory in terms of latency to locate the escape platform. The test is based upon 2 phases; the training phase and the test phase. Memory functions of rats were tested by noting down the retention latency. The cut off time was 2 minutes for each session. Initially the training session was performed during which each rat was placed into the water in such a way that their face was towards the wall of the tank. After placing 120 seconds were given each animal to find and mount onto the hidden platform, if the rat located the platform it was allowed to stay on it for 10 seconds. If it failed to locate the platform during the allocated time then it was guided gently onto the platform. Then test session was performed after 90 minutes in which time taken to reach the hidden platform was again recorded.

Statistical analysis

The statistical evaluation was performed by One-way anova while post-hoc analysis was done by Tukey's test using SPSS version 20. Results are expressed as the mean ± SD; p value < 0.05 was considered significant.

Results

Effect of curcumin and lead on cognition:

Data analysis by one-way anova revealed a significant [F (3, 20)= 17.06, P<0.01)] of drugs on cognition in NOR. Post-hoc analysis showed significant reduction in cognitive abilities in lead (P<0.01) treated and lead+curcumin (P<0.05) treated rats as compared to vehicle controls, however there was a significant increase (P<0.05) in cognition in lead+curcumin treated rats as compared to only lead treated rats. (Fig. 1)

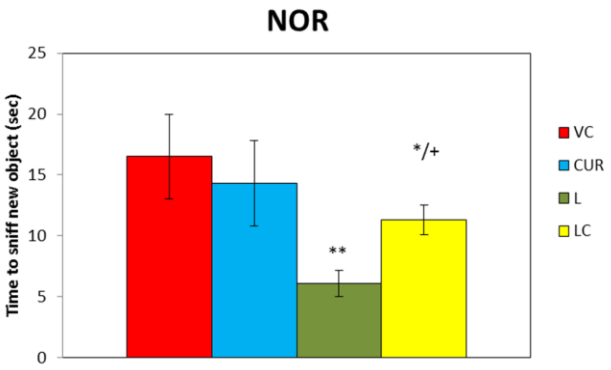

Figure 1: Effect of curcumin and lead on cognition. Data represented as mean +SD; n= 6 rats per group. * p<0.05; ** p<0.01 versus vehicle control and +p<0.05; ++p<0.01 versus lead group.

Effect of curcumin and lead on short-term memory (STM):

Data analysis by one-way anova revealed a significant [F (3, 20)= 10.38, P<0.01)] of drugs on short term memory in MWM. Post-hoc analysis showed a significant (P<0.01) increase in escape latency in lead treated rats as compared to vehicle controls while this increase in escape latency was significantly attenuated (p<0.05) by curcumin in LC group when compared to lead treated rats only. (Fig. 2)

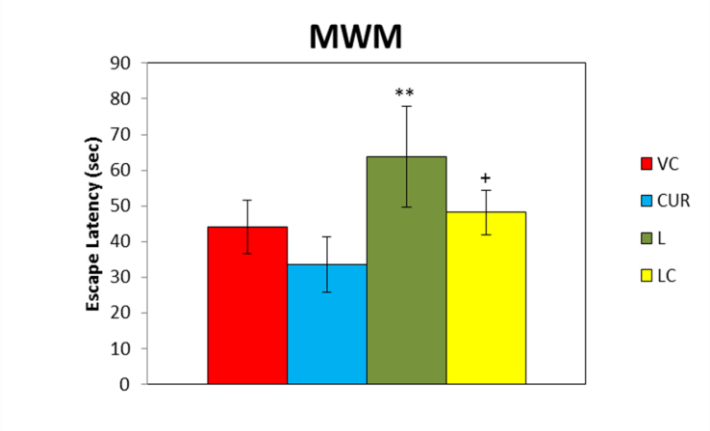

Figure 2: Effect of curcumin and lead on memory. Data represented as mean +SD; n= 6 rats per group. * p<0.05; ** p<0.01 versus vehicle control and +p<0.05; ++p<0.01 versus lead group.

Effect of curcumin and lead on immobility:

Data analysis by one-way anova revealed a significant [F (3,20)= 37.73, P<0.01)] of drugs on depression like behavior in TST. Immobility time was significantly (p<0.01) decreased in curcumin treated rats as compared to vehicle controls while immobility was significantly increased (P<0.01) in lead treated rats as compared to vehicle controls. Co-administration of curcumin with lead significantly (p<0.05, P<0.01) attenuated lead induced increase in escape latency as compared to vehicle controls and lead treated rats respectively. (Fig.3)

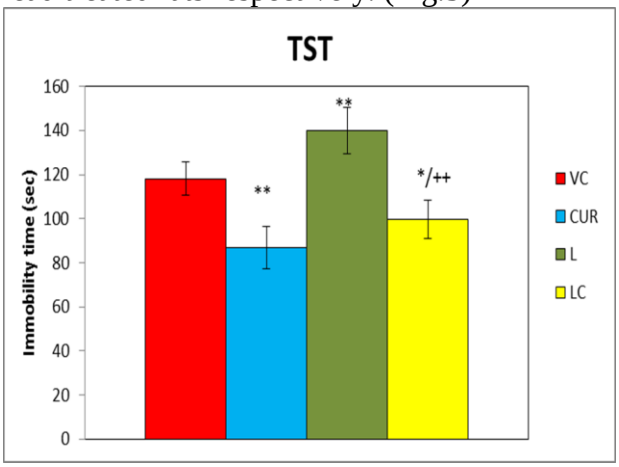

Figure 3: Effect of curcumin and lead on immobility. Data represented as mean +SD; n= 6 rats per group. * p<0.05; ** p<0.01 versus vehicle control and +p<0.05; ++p<0.01 versus lead group.

Effect of curcumin and lead on anxiety like behavior:

Data analysis by one-way anova revealed a significant [F (3,20)= 25.74, P<0.01)] of drugs on anxiety like behavior in EPM. Post-hoc analysis showed a significant decrease (P<0.01) in time spent in open arm in rats treated with lead as compared to vehicle controls while rats co-treated with lead and curcumin showed a significant increase (P<0.05) in time spent in open arm as compared to only lead treated rats. (Fig. 4)

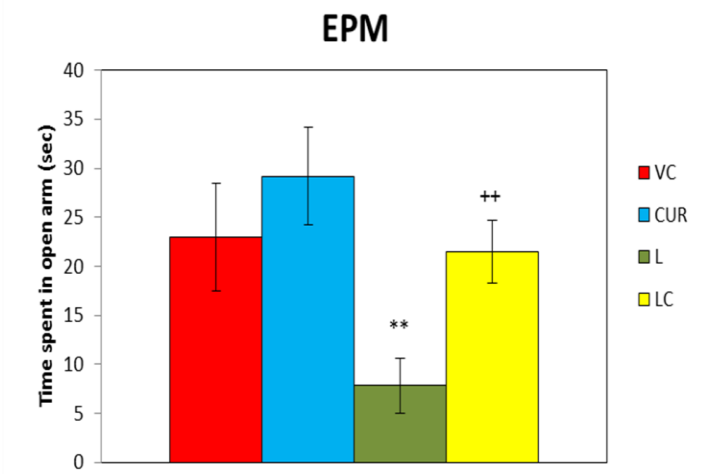

Figure 4: Effect of curcumin and lead on anxiety like behavior. Data represented as mean +SD; n= 6 rats per group. * p<0.05; ** p<0.01 versus vehicle control and +p<0.05; ++p<0.01 versus lead group.

Effect of curcumin and lead on ambulatory activity:

Data analysis by one-way anova revealed a significant [F (3,20)= 15.52, P<0.01)] of drugs on ambulatory activity in OFT. Post-hoc analysis showed significant (P<0.01) reduction in number of squares crossed/5min in lead exposed rats as compared to vehicle controls. However co-administration of curcumin with lead significantly increased (P<0.05) number of squares crossed/5min as compared to lead treated rats. (Fig. 5)

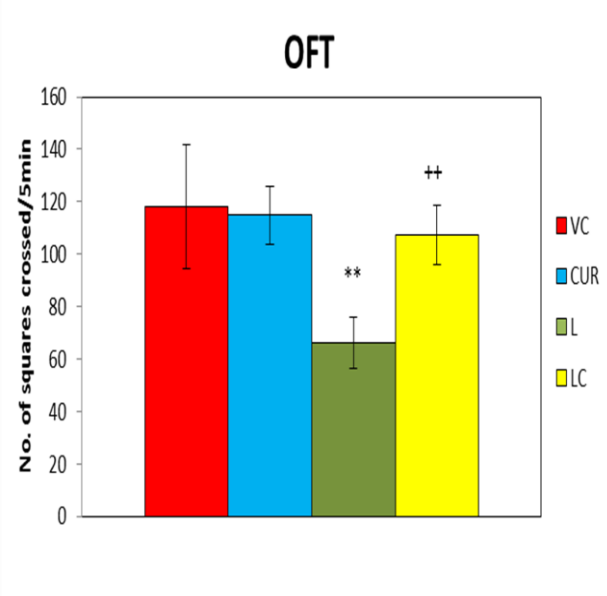

Figure 5: Effect of curcumin and lead on ambulatory activity. Data represented as mean +SD; n= 6 rats per group. * p<0.05; ** p<0.01 versus vehicle control and +p<0.05; ++p<0.01 versus lead group

Discussion

Lead is a toxic metal without any essential biological function. It is a critical environmental pollutant that is accumulated in soils, especially in urban systems, creating a public and environmental health concern[27]. Due to its use in a number of products of daily life humans are highly exposed to it. Lead is a neurotoxin and cause damage to the central nervous system[28] and also affects the synthesis and release of various neurotransmitters[29,30].

In the present study subcutaneous administration of lead for 7 days severely affects the time spent in open arm in plus maze test indicating anxiogenic effect of lead. The adverse effect of lead on rat behavior is in agreement with the findings of other researchers[31,32,33]. However co-administration of curcumin with lead significantly attenuated lead induced anxiety in rats showing its neuroprotective and anxiolytic effect against heavy metals induced changes. Our study also showed that administration of lead resulted in increased immobility and despair indicating depressogenic effect of lead. Previously it has been reported that exposure to lead results in alterations in complex behaviors of brain[32]. It has been reported that repeated exposure to lead affects brain serotonin metabolism and causes memory impairment[19,34]. In the present study treatment with curcumin significantly attenuated depressogenic effect of lead evident by decreased immobility in forced swim test. This decrease in immobility reflects the anti-depressogenic property of curcumin against neurotoxic metal and this effect of curcumin may be due to restoration of lead induced decrease in 5-HT metabolism. Increased 5-HT levels following curcumin administration have been reported earlier[35]. Involvement of curcumin in stress and depression like symptoms have also been reported previously[10]. Lead poisoning is a possible factor in severe behavioral deficits, vulnerability of brain damage, mental impairment and cognitive impairments[14,36]. Reduction of 2-4 IQ points has been reported with every increase of 1µg/dl in blood lead levels in the range of 5-35µg/dl[14,37]. The present study is also in agreement with the previously reported studies as exposure to lead significantly impaired performance in both memory tests. In Morris water maze test time taken to reach the platform in lead exposed rats was significantly increased reflecting impairment in memory whereas co-administration of curcumin with lead reversed the lead induced impairment in memory. In the present study a 62% decrease in cognition was observed in lead exposed rats whereas rats co-administered with lead and curcumin exhibited only 31% decrease in cognition demonstrating attenuation of memory deficit by curcumin as compared to vehicle controls. Compared to lead exposed rats an increase of 83% in cognition was observed in rats co-administered with lead and curcumin indicating the neuroprotective role of curcumin against memory deficits induced by lead. Improved memory function and cognition following curcumin administration in lead exposed rats may be attributed to the scavenging and chelating property of curcumin as reported previously[38]. The results of the present study therefore suggests a neuroprotective role of curcumin against lead induced behavioral deficits and toxicity.

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