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EVALUATION OF THE ANXIOLYTIC ACTIVITY OF CURCUMIN AGAINST LEAD INDUCED ANXIETY IN RATS

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ABSTRACT

Present research work was performed to evaluate the anxiolytic activity of curcumin against lead induced anxiety in rat. Anti-anxiety potentials of the curcumin were compared with diazepam. Lead poisoning induced severe behavioral abnormalities in experimental animals. Rats of either sex were divided into 12 groups of 6 animals in each group. Group were DMSO, Distilled Water, Saline water, Lead acetate 25mg/kg, Diazepam 2mg/kg, Curcumin 25mg/kg, Curcumin 50mg/kg, Diazepam 2mg/kg + lead acetate, Curcumin 25mg + lead acetate, Curcumin 50mg + lead acetate, Diazepam 2mg/kg + Curcumin 50mg and Diazepam 2mg/kg + Curcumin 50mg + lead acetate. All the test solutions were freshly prepared daily and administered to animals for 5 days by interaperitonial (i.p.) route. On first day, third day and fifth day, each animal was checked to anxiety by using elevated zero maze. Our result showed that Lead acetate induced more anxiety and fear-related behavior in rat. Curcumin 50mg/kg reduced and prevent the anxiety behavior, which induced by lead. Curcumin 50mg/kg is sufficient dose to give anxiolytic and neuroprotective activity against lead induced anxiety in rats. Curcumin is less potent than diazepam and Curcumin 50mg/kg with diazepam give synergetic anxiolytic activity against lead induced anxiety in rat.

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INTRODUCTION

Anxiety disorders can be considered as "intact" condition, which almost totally disturb the routine life of the person. It creates a condition of unexplained anticipatory fear and apprehension regarding the occurrence of even normal things in life. Anxiety is a state of excessive fear and characterized by motor sympathetic hyperactivity, apprehension and vigilance syndromes. The most common observation is an acute stress response characterized by a state of abnormal or exaggerated arousal or fear[1]. Anxiety states are controlled by both inhibitory and facilitatory mechanisms that either counter or favor anxiety states. The neurochemical and neuropeptide systems have been shown to have effects on distinct cortical and sub cortical brain areas that are relevant to the mediation of the symptoms associated with anxiety disorders[2].

Curcumin is a well known biologically active natural phytochemical phenolic compound found as a major component in turmeric, extracted from the rhizome of *Curcuma longa* L. (family Zingiberaceae). Curcumin shows a wide spectrum of pharmacological activities such as wound healing[3-4], anti-inflammatory[5], anti-arthritic[6], analgesic[7], anti-pyretic[8], anti-bacterial[9], anti-viral[10], anti-fungal[11], anti-allergic[12], anti-oxidant[13], neuroprotective[14], anti-depressant[15], cardio- protective and hypolipideamic activity[16], anti-cougulant[17], anti-ulcer[18], anti-diabetic[19], hepatoprotective[20], anti-cancer[21], anti-fertility[22] and anti-venom[23] activities. Curcumin give anxiolytic and memory retentive effect by increased the level of serotonin, norepinephrine and dopamine in various regions of the rat brain[24].

Epidemiological and experimental studies have provided consistent evidence that lead (Pb) is a well-known neurotoxic agent and a risk factor for neurologic and psychiatric disorders in humans. Recent studies have also demonstrated that exposure to environmental lead essentially affects a variety of neurotransmitter systems and causes a wide range of long-lasting adverse effects; especially in developing brains[25-27]. In some previous studies we showed that prenatal lead exposure strongly affects dopaminergic, serotoninergic and cholinergic systems in the rat's brain[28]. Chronic lead exposure has been shown to produce behavioral disturbances in animal models. These disturbances are associated with alterations in monoaminergic neurotransmission in the central nervous system (CNS), some of which have been attributed to serotonin (5-HT). The chronic effects of lead exposure on the serotoninergic system in the dorsal raphe nucleus (DRN) and the consequences of its toxicity affect the animal behavior. So, that lead exposure may possibly induce anxiety as a consequence of changes in neuronal 5-HT content in the DRN[29]. A study showed that prenatal lead exposure strongly affects dopaminergic, serotoninergic and cholinergic systems in the rat's brain[28].

The present study was undertaken to examine the anti-anxiety effect of curcumin against lead induced anxiety in rat by elevated zero maze apparatus and to find out neuroprotective activity of curcumin against lead induced neurotoxicity in rats. The curcumin effects were compared with anti-anxiety potentials of diazepam (classical anti-anxiety drug).

Preparation of curcumin extract:

The rhizomes of plant was collected, cleaned and dried under shade. The dried material powdered using a laboratory blender. About 200 gm of turmeric powder is extracted with 95% alcohol in a Soxhlet assembly until all the colouring matter was extracted. Alcoholic extract distilled off to a semi-solid brown coloured mass (about 75%). Then the crude extract was dissolved in 200 ml of benzene and extracted twice with equal volume of 0.1% Sodium hydroxide solution. The alkaline extracts combined and acidified with dilute hydrochloric acid. Obtained precipitate was allowed to settle for 15 minutes. After setting of precipitate, the extract concentrates by boiling on water bath and at the same time dissolving precipitate in boiling water. During this process of boiling, the resinous material was agglumate and form lumpy mass. Then the solution filtered in hot condition and filtrate was concentrated to very small volume and finally cooled to get curcumin (1.5%)[30].

MATERIALS AND METHOD

Animals: Albino rats (150-250 gm) were selected from the animal house of Department of Pharmacy, M.J.P Rohilkhand University, Bareilly. They were housed in group of 12 and fed on standard pellet diet and water ad libitum and kept in environmental controlled room at 25 ± 3 °C and 50 ± 20 % humidity with 12h light/dark cycle. The experimental protocol were approved by the Institutional Animal Ethical Committee and conducted according to the CPCSEA guideline on the use and Care of experimental animals.

Drug treatment and Experimental procedure:

All drug solution was freshly prepared before use. Diazepam (CALMPOSE, Mfd by RANBAXY) was diluted with distill water. Curcumin extract dissolved and diluted with dimethyl sulfoxide (DMSO). Lead acetate (Qualigens fine Chemicals) was dissolved and diluted with distill water. Rats of either sex were divided into 12 groups of 6 animals in each group as mentioned below. All the test solutions were freshly prepared daily and administered in animals for 5 days by interaperitonial (i.p.) route, 30 minutes prior to experimentation. Group I- DMSO 10ml/kg (Vehicle), Group II- Distilled Water 10ml/kg, Group III- Saline water 10ml/kg, Group IV- Lead acetate 25mg/kg, Group V- Diazepam 2mg/kg, Group VI- Curcumin 25mg/kg, Group VII- Curcumin 50mg/kg, Group VIII- Diazepam 2mg/kg + lead acetate25mg/kg, Group IX- Curcumin 25mg + lead acetate 25mg/kg, Group X- Curcumin 50mg + lead acetate 25mg/kg, Group XI- Diazepam 2mg/kg + Curcumin 50mg, Group XII- Diazepam 2mg/kg + Curcumin 50mg + lead acetate 25mg/kg. On first day, third day and fifth day, each animal of each group was checked for anxiety by using elevated zero maze.

Elevated zero maze:

All the animals were analyzed for anxiety levels by using elevated zero maze. This apparatus was standardized by Shepherd et al (1994)[31]. The elevated zero maze apparatus is used to a behavioral test of anxiety based on the naturalistic tendency of rodents to avoid open and elevated areas. It is similar to the more widely used elevated plus maze, except that the open and close arms are arranged circularly, thus eliminating the central area which removes ambiguity in interpretation of time spent in the central square of the traditional design. The maze is elevated (70 cm) and circular maze having outer diameter of 78 cm and inner diameter of 65 cm. The runway ring where the rat can explore is 6 cm width, which is divided into 4 quadrants, 2 opposing "open" quadrants without walls and 2 opposing "close" quadrants having 27 cm high walls. The open quadrants have a small lip (5 mm) to prevent the rat from falling off the maze (Fig .1). The rat was placed individually in the open arm facing towards the closed arm. The following parameters were noted for a period of five minutes: (a) Transfer Latency to enter the open arm. (b) Average time each animal spend in the open arm. (c) Total number of entries in the open arm (an open arm entry is defined as the entry when all the four paws of the animal are in the arm).

(a) Side view **(b) Top view**

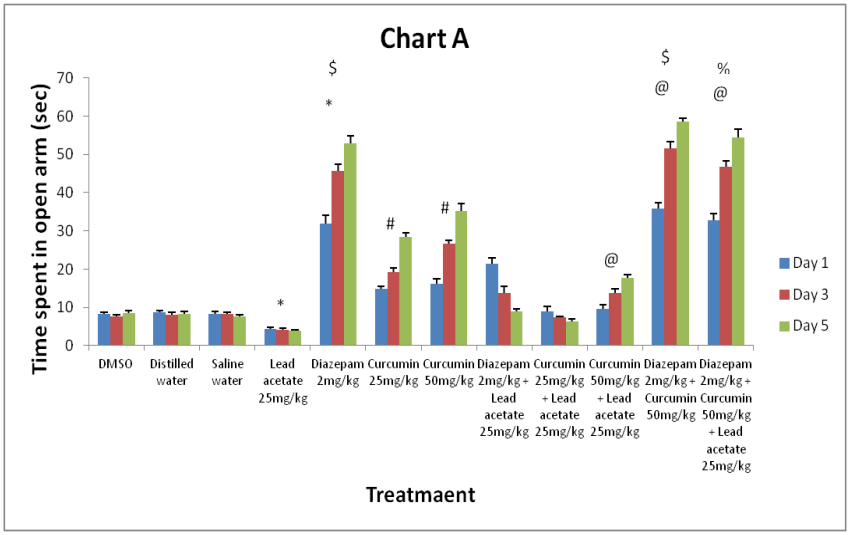
Fig .1 Elevated zero maze.

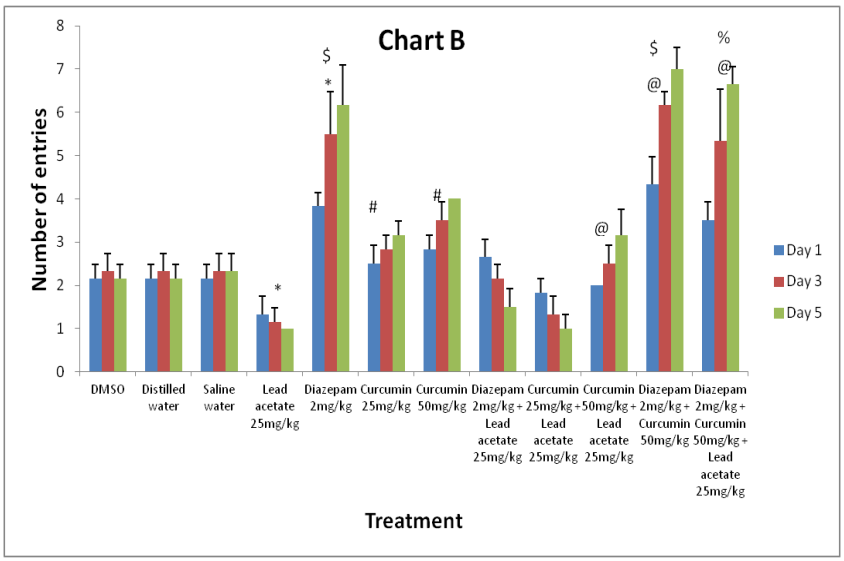
***Statistical Analysis*:**

All results were expressed as mean ± SEM. Data was analyzed using one-way ANOVA followed by Dennett's test and Student t-test. $P < 0.05$ was considered to be statistically significant.

RESULT

Control group (Saline water, Distilled water and DMSO administrated group) give no significant difference in animal (rat) behavior during zero maze anxiety test. Lead acetate (25mg/kg) treated group was significantly decreasing time spent in open arm with decreasing number of entry into open arm and increasing transfer latency period to enter open arm of zero maze as compared to control (distilled treated) group, during trail conducted on day 1, day 3 and day 5. Diazepam (2mg/kg) treated group was significantly increasing time spent in open arm with increasing number of entry into open arm and decreasing transfer latency period to enter open arm of zero maze as compared to control (distilled water) treated group, during trail conducted on day 1, day 3 and day 5. Curcumin (25mg/kg) treated group was significantly increasing time spent in open arm with increasing number of entry into open arm and decreasing transfer latency period to enter open arm of zero maze as compared control (DMSO) treated group, during trail conducted on day 1, day 3 and day 5. Curcumin (50mg/kg) treated group was significantly increasing time spent in open arm with increasing number of entry into open arm of zero maze and decreasing transfer latency period to enter open arm as compared to control (DMSO) treated group, during trail conducted on day 1, day 3 and day 5. Diazepam (2mg/kg) + lead acetate (25mg/kg) treated group have no significant difference in time spent in open arm, number of entry into open arm and transfer latency period to enter open arm of zero maze as compared to lead acetate (25mg/kg) treated group, during trail conducted on day 1, day 3 and day 5. Curcumin (25mg/kg) + lead acetate (25mg/kg) treated group was no significant difference in time spent in open arm, number of entry into open arm and transfer latency period to enter open arm of zero maze as compared to lead acetate (25mg/kg) treated group, during trail conducted on day 1, day 3 and day 5. Curcumin (50mg/kg) + lead acetate (25mg/kg) treated group was significantly increasing time spent in open arm with increasing number of entry into open arm and decreasing transfer latency period to enter open arm of zero maze as compared to lead acetate (25mg/kg) treated group, during trail conducted on day 1, day 3 and day 5. Curcumin (50mg/kg) + diazepam (2mg/kg) + lead acetate (25mg/kg) treated group was significantly increasing more time spent in open arm with increasing more number of entry into open arm and more decreasing transfer latency period to enter open arm of zero maze as compared to curcumin (50mg/kg) + lead acetate (25mg/kg) treated group, during trail conducted on day 1, day 3 and day 5. Diazepam (2mg/kg) treated group was significantly increasing more time spent in open arm with increasing more number of entry into open arm and more decreasing transfer latency period to enter open arm of zero maze as compared to curcumin (50mg/kg) treated group, during trail conducted on day 1, day 3 and day 5 (Fig .2 Chart A, B and C).





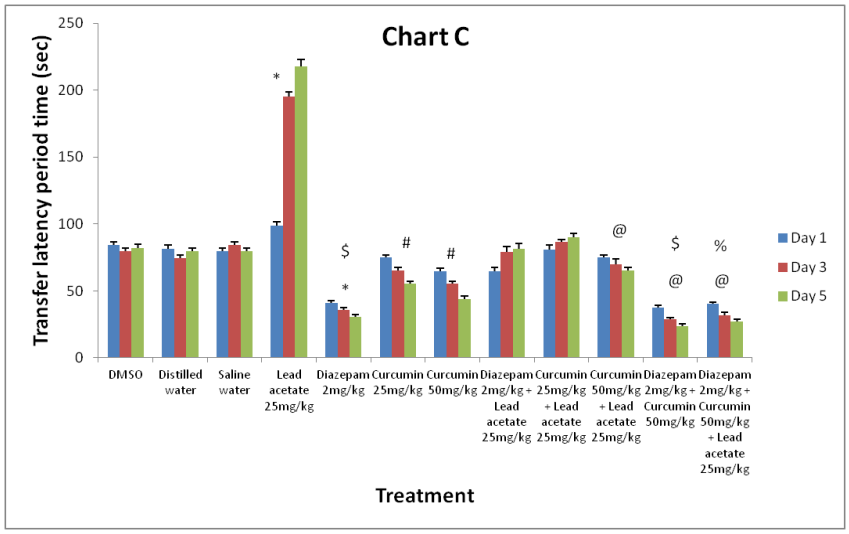


Fig .2 Effect of different treatment on- (Chart A) time spent in open arm; (Chart B) number of entries in open arm; (Chart C) Latency to enter in open arm at elevated zero maze [Values are expressed as mean ± SEM. *$P \leq 0.05$ as compared to distilled water group, #$P \leq 0.05$ as compared to DMSO group, @$P \leq 0.05$ as compared to lead acetate group, $$P \leq 0.05$ as compared to curcumin 50mg/kg group, %$P \leq 0.05$ as compared to curcumin 50mg/kg + lead acetate].

DISCUSSION

In the present study, the anxiolytic activity of curcumin against lead induced anxiety in rats were studied by using elevated zero maze apparatus. Elevated zero maze apparatus was standardized by Shepherd et al (1994)[31]. The elevated zero maze apparatus is used to a behavioral test of anxiety based on the naturalistic tendency of rodents to avoid open and elevated areas. It is similar to the more widely used elevated plus maze, except that the open and close arms are arranged circularly, thus eliminating the central area which removes ambiguity in interpretation of time spent in the central square of the traditional design (Elevated plus maze). When the animal (rat) spent more time in open arm of zero maze with increase number of entry into open arm and decrease the transfer latency to enter in open arm, its indicate anxiolytic behavior of the rat. When the animal (rat) spent less time in open arm of zero maze with decrease number of entry into open arm and increase the transfer latency to enter in open arm, its indicate anxiety behavior of the rat[32]. From the obtained results, the following salient finding may be possible - Control group (saline water, distilled water and DMSO administrated group) give no significant difference in animal (rat) behavior during zero maze anxiety test. These result shows that all control group were not effect behavior of animal. Lead acetate (25mg/kg) treated group was significantly increasing anxiety behavior of rats as compared to control (distilled water) treated group, during, zero maze anxiety test. This result shows that Lead acetate induced more anxiety in rat. This result further supported that Lead (pb) exposure rat showed to increased anxiety and fear-related behavior in both elevated plus maze and light dark box tests, due to Lead acetate intoxication increased the level of lipid peroxidation in brain, decreased brain glutamate oxaloacetate transaminase activities and increased glutamate pyruvate transaminase[33]. Chronic lead exposure has been shown to produce behavioral disturbances in animal models. These disturbances are associated with alterations in monoaminergic neurotransmission in the central nervous system (CNS), some of which have been attributed to serotonin (5-HT). The chronic effects of lead exposure on the serotoninergic system in the dorsal raphe nucleus and the consequences of its toxicity affect the animal behavior. So, that lead exposure may possibly induce anxiety as a consequence of changes in neuronal 5-HT content in the dorsal raphe nucleus[29]. Lead pass through the blood-brain barrier is due in large part to its ability to substitute for calcium ions. Within the brain, lead-induced damage in the prefrontal cerebral cortex, hippocampus, and cerebellum can lead to a variety of neurological disorders, such as brain damage, mental retardation, behavioral problems, nerve damage, and possibly Alzheimer's disease, Parkinson's disease, and schizophrenia[34]. Diazepam (2mg/kg) treated group was significantly decreasing anxiety behavior of rats as compared to control (distilled water) treated group, during zero maze anxiety test. Diazepam (2mg/kg) + lead acetate (25mg/kg) treated group have no significant difference in anxiety behavior of rats as compared to lead acetate treated group. This result showed that lead acetate decreases the anxiolytic activity of diazepam. This result further support that neurotoxic actions of lead include apoptosis (programmed cell death), excitotoxicity affecting neurotransmitter storage and release, and altering neurotransmitter receptors, mitochondria, second messengers, cerebrovascular endothelial cells, and both astroglia and oligodendroglia. Symptoms can appear immediately after exposure or may be delayed and include loss of memory, vision, cognitive and behavioral problems, and brain damage/mental retardation[34]. Lead has toxic effect through intrinsic and extrinsic induction of apoptotic pathway with prominent effect on brain tissue even at low dose[35]. Curcumin (25mg/kg) treated group was significantly decreasing anxiety behavior of rats as compared control (DMSO) treated group. Curcumin (25mg/kg) + lead acetate (25mg/kg) treated group have no significant difference in anxiety behavior of rats as compared to lead aceatae treated group.

This result showed that curcumin (25mg/kg) was decrease anxiety behavior of animal in general condition, but curcumin (25mg/kg) is not sufficient dose, which not give anxiolytic activity against lead induced anxiety in rats. This result further supports that Curcumin produce significant anti-anxiety like effect in stressed mice and curcumin significantly decreased plasma nitrite levels in stressed mice[36]. Curcumin (50mg/kg) treated group was significantly decreasing anxiety behavior of rats as compared to control (DMSO) treated group. Curcumin (50mg/kg) + lead acetate (25mg/kg) treated group was significantly decreasing anxiety behavior of rats as compared to lead acetate treated group. This result showed that Curcumin (50mg/kg) dose give anxiolytic activity against lead induced anxiety in rat. This result further supported that anxiolytic effect of curcumin against lead induced anxiety in rats and this may possibly result from modulation of central neuronal monoaminergic neurotransmission, especially serotonin, which has shown a significant reduction of the immunoreactivity within the dorsal raphe nucleus[37]. Curcumin give anxiolytic and memory retentive effect by increased the level of serotonin, norepinephrine and dopamine in various regions of the rat brain[24]. Curcumin (50mg/kg) + diazepam (2mg/kg) treated group and curcumin (50mg/kg) + diazepam (2mg/kg) + lead acetate (25mg/kg) treated group were not significant difference in the anxiolytic behavior of rats. This result showed that Curcumin (50mg/kg) is significant dose to give neuroprotective and anxiolytic activity against lead induced anxiety in rat. This result supported that curcumin have neuroprotective activity against lead induced neurotoxicity in rats. Curcumin cause decrease in lipid peroxidation with concomitant decrease in lead levels in all the brain regions[38]. Curcumin, a well-established dietary antioxidant, is capable of playing a major role against lead induced neurotoxicity and has neuroprotective properties[39]. Curcumin (50mg/kg) + diazepam (2mg/kg) + lead acetate (25mg/kg) treated group was significantly decreasing anxiety behavior of rats as compared to curcumin (50mg/kg) + lead acetate (25mg/kg) treated group. Diazepam (2mg/kg) treated group was significantly decreasing anxiety behavior as compared to curcumin (50mg/kg) treated group. This result showed that curcumin is less potent anxiolytic agent than diazepam in general condition and curcumin (50mg/kg) + diazepam (2mg/kg) are given synergetic anxiolytic activity against lead induced anxiety in rat.

On the basis of above discussion the following salient finding may be summarized - Lead acetate induced more anxiety behavior in rat, when compare with control (distilled water) group on zero maze anxiety tests. Diazepam, a benzodiazepine give anxiolytic (anti-anxiety) activity in rat, when compare with control (distilled water) group on zero maze anxiety test. Diazepam not gives neuroprotective and anxiolytic activity against lead induced anxiety in rats, when compare between lead acetate (25mg/kg) and diazepam (2mg/kg) + lead acetate (25mg/kg) treated group on zero maze anxiety tests. Curcumin (25mg/kg) give anxiolytic (anti-anxiety) activity in rat, when compare with control (DMSO) group on zero maze anxiety tests. Curcumin (25mg/kg) is not a significant dose to give neuroprotective and anxiolytic activity against lead induced anxiety in rats, when compare between lead

acetate and Curcumin 25mg/kg + lead acetate treated group on zero maze anxiety test. Curcumin (50mg/kg) give anxiolytic (anti-anxiety) activity in rat, when compare with control (DMSO) group on zero maze anxiety tests. Curcumin (50mg/kg) is less potent anxiolytic agent as compared to diazepam, when compare with diazepam treated group on zero maze anxiety test. Curcumin (50mg/kg) is a significant dose to give neuroprotective activity and anxiolytic activity against lead induced anxiety in rats, when compare between lead acetate (25mg/kg) and curcumin (50mg/kg) + lead acetate (25mg/kg) treated group on zero maze anxiety test. Curcumin (50mg/kg) + diazepam (2mg/kg) give synergetic anxiolytic activity against lead induced anxiety in rat, when compare between curcumin (50mg/kg) + lead acetate (25mg/kg) and curcumin (50mg/kg) + diazepam (2mg/kg) + lead acetate (25mg/kg) treated group on zero maze anxiety test.

CONCLUSION

It may be concluded that, Curcumin (50mg/kg) is give anxiolytic effect might be due to its neuroprotective activity against lead induced anxiety in rats. Curcumin is less potent than diazepam, and Curcumin (50mg/kg) with diazepam give synergetic anxiolytic activity against lead induced anxiety in rat.

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