NEUROPROTECTIVE ROLE OF QUERCETIN AGAINST NEUROTOXICITY INDUCED BY LEAD ACETATE IN MALE RATS

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ABSTRACT

Lead is known as the major environmental pollutant with intense toxic effects. The lead toxicity may lead to neurological and psychological illness including anxiety, depression and cognition deficits. Therefore, present study is designed to observe the behavioral outcomes of neuroprotective effects of quercetin on lead acetate induced neurotoxicity. The result presented significant neuroprotective effects of quercetin on anxiety and cognition impairment. Conclusively, the present study reveals the neuroprotective role of Quercetin against neurotoxicity induced by lead acetate in male rats

Key Words: Lead (Pb), Lead Acetate Toxicity, psychological deficits, cognitive deficits, Quercetin

INTRODUCTION

Lead is considered as a major cause of environmental pollution which has potent toxicological profile. Due to its highly resistive property and low melting, it is now commonly used in various industries and in paints. This extensive use of lead has made it immensely reactive in biological system (Fergusson 1990).The lead exposure possesses many irreversible hazardous manifestations. The exposure of lead to the biological system of animals occurs mainly by inhalation of aerosol by respiratory system and ingestion in soluble form by gastrointestinal tract where it can absorbed as soluble salt (acetate) causing high concentration of lead in blood. It gets transported from blood to different organs, Begins accumulating and causing broad range of biochemical, physiological and behavioral anomalies (Patra *et al*., 2011).

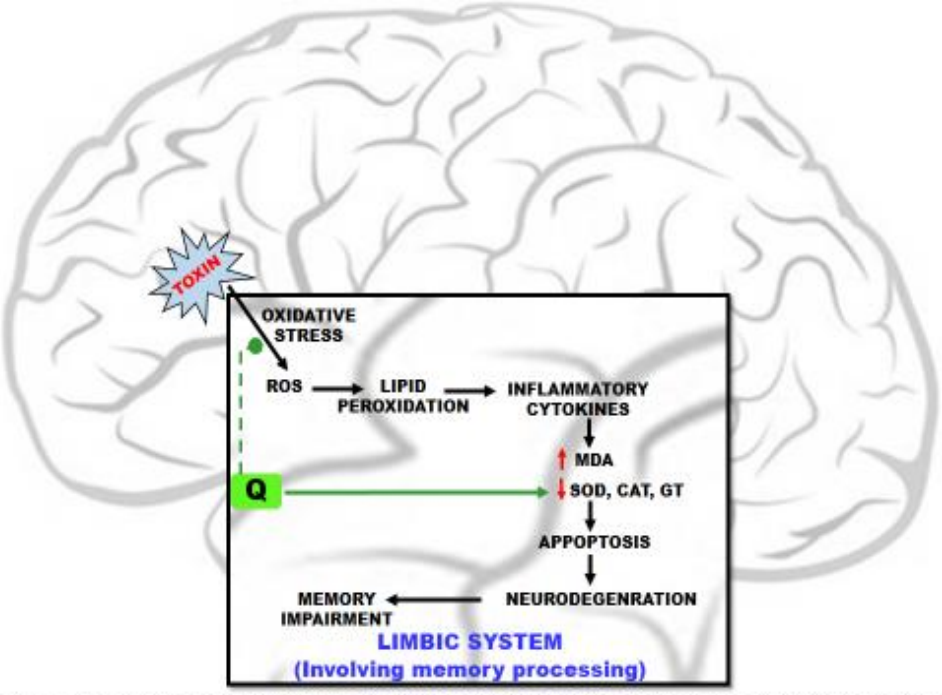


Fig.1.Influence of Reactive Oxygen Species on Brain.

Nervous system is one of the most sensitive system which is irreversibly affected by lead exposure. Lead acts as neuro-toxicant and disrupts the blood brain barrier and neurotransmission underlying the complex neuronal network (Surkan *et al.*, 2007). Exposure of lead in the brain can elicits altered metabolic cascade which initiates from oxidative stress induced ROS generation and terminates in the neurodegeneration (Fig. 1). It affects the brain functioning and tends to prone neurological disorders which is associated with many psychological abnormalities including behavioral impairments. The most common are psychiatric illness e.g. anxiety, depressive symptoms, memory and cognitive impairment. Many studies in animals have demonstrated the effect of toxins in the metabolic activities of brain and their impacts on animal behavior (Azab, 2014; Azoz *et al*., 2012).

Quercetin is a natural constituent and metabolite of plant. It is a polyphenol compound with authentic pharmacological activities. It presentsin vegetables and fruits like onion and apple (Manach *et al.*, 2004). Many studies reported its impact on biological system including anti-microbial (María José *et al.*, 2013) anti-inflammatory (Cho *et al.*, 2006), antioxidant (D'Andrea, 2015) and anticancer (Kumar *et al.*, 2003). The most described property of quercetin is its anti-oxidant, radical scavenging property appearsduring the oxidative stress. There are many ROS generation and inflammatory processes which lead to neurodegeneration. Quercetin has potential to mitigate the toxin induced as well as age dependent inflammatory neurodegeneration, as can easily cross the blood brain barrier and exert its protective effects on neuronal network. It can reduce the ROS induced lipid peroxidation which is a consequence of lead toxicity. Many studies demonstrate its pharmacological effects in the manifestation of oxidative stress (Hu *et al*., 2015).

Therefore, present study was designed to observe the pharmacological effects of quercetin on anxiety and memory of lead acetate inducedneurotoxicity.

METHODOLOGY

Male albino wistar rats randomly divided into two groups as Control and Test animals. Test animals were treated with lead acetate and after the induction of neurotoxicity they were further divided into 4 subgroups i.e. 1. Control Saline 2. Control Quercetin 3. Test Saline 4. Test Quercetin. All animals were treated according to their respective groups. Quercetin was given orally 100mg/kg/day. This phase was continued for two weeks. During this experimental phase behavioral assessments were done. Food intake, ambulatory activity, anxiolytic activity (Open Field) and cognitive activity (Novel object recognition and Morris water test) were monitored as described in diagram (Fig. 2).

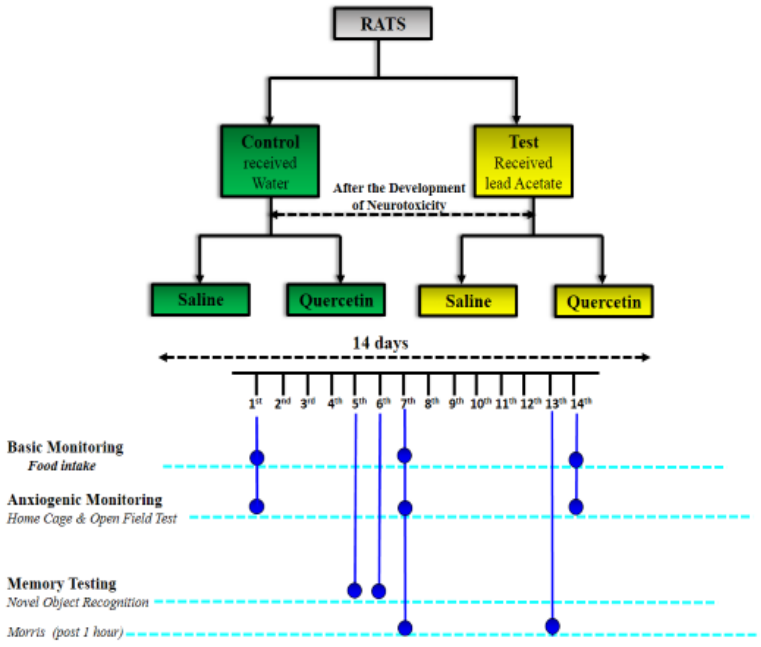


Fig. 2. Sketch of Experimental Protocol.

Behavioral Monitoring

1. Food intake

To study the effects of quercetin on food intake, a measured amount of food was placed in individual cages and the amount of food left in the Hooper of cage was measured after 24 hours of 1st 7th and 14th treatment.

2. Activity Box

The effects of quercetin on locomotor activity in familiar environment were monitored in activity box as described in previous study (Rafiq and Farhan 2015). All animals were individually placed in the center of activity box for 15 minutes before testing, after habituation the number of cage crossing was recorded for 10 minutes.

3. Open Field Test

To monitor the effects of quercetin on lead neurotoxicity in novel environment the Open Field apparatus was used as described earlier (Rafiq and Farhan, 2015). Test and control group were separately placed in center of the field and monitored with the cut off time 5 minutes. The number of square crossed with four paw was recorded.

4. Novel object recognition test

This test is used for the assessment of cognitive ability of animals; it can also be used for the assessment of anxiety and attention in animals. The rodent exposed to unfamiliar environment with unfamiliar object to access their natural tendency to make evaluation of stimulus. The apparatus consist of a wooden box (40 x 40 x 40 cm) with sawdust covered floor. The test comprises on three stages i.e. habituation, training and test stages. In first stage, each animal allowed to explore the area of NOR for 15 minutes. In second stage, two identical objects were placed and each animal allowed to explore the objects. In third stage, a new object was replaced by one of the two identical objects. The sniffing time and number were observed with the cutoff time 5 minutes.

5. Morris water maze test

Morris water maze test is widely used for memory testing. The apparatus is consisted of a white circular pool containing diameter 90 cm and depth 60. The apparatus was filled with water at the depth of 30 cm. The pool was made opaque by adding non-fat powder milk and divided into four quadrants. A hidden platform with 15 cm x 15 cm was placed in one quadrant 1cm below the water surface. For the spatial memory testing, spatial cues about the rooms were placed with water filled Morris maze in a room with quite environment. The apparatus was located in the way that animal can't see the experimenter. The test was based on two phases: trial and test phase. In trial phase, each animal was placed in center of pool. They were allowed to explore the quadrants and hidden platform. When an animal explored the hidden platform the trial was stopped but, if the animals failed to explore the platform within 2 minutes they were gently guided toward it and allowed to stay for 20 seconds so that they observe the position and room cues about the position of platform. In test phase, animals were placed in the center and allowed to find the hidden platform with the cut off time 2 minutes.

Statistical Analysis

Results are presented as mean ± SD. Variation between individual mean values of different groups were analyzed by three way ANOVA (analysis of variance) of SPSS (Version 17). Comparison between mean value of different groups were analyzed by Newman-Kuel test $p<0.05$ were considered as significant.

RESULTS

Figure 1 shows the effects of quercetin on food intake of normal and lead acetate treated rats. Analyzed data by three-way ANOVA showed that the effect of days ($F=12.216$; $df= 2, 21$; $p<0.05$), lead acetate ($F= 77.217$; $df= 1, 21$; $p<0.05$) and interaction between these factors ($F= 24.414$; $df=2, 21$; $p<0.05$) were significant whereas, the effects of quercetin was shown ($F= 14.901$; $df= 1, 21$) non-significant. Post-hoc analysis by Newman-Keuls showed test showed that administration of quercetin modulates food intake in both normal and lead treated rats after one week administration as compared to saline treated animals. However, more significant results were shown in lead treated rats than normal rats, where the increased food intake was measured from 1st day to 14th day administration.

Figure 2 shows the effects of quercetin in both normal and lead treated rat on Locomotor Activity. Data analyzed by three-way ANOVA presents the significant effect of days ($F= 185.190$; $df= 2, 21$; $p<0.01$), lead acetate ($F= 235.689$; $df= 1, 21$; $p<0.05$), quercetin ($F= 334.026$; $df= 1, 21$; $p<0.01$) and interaction between these factor ($F= 116.641$, $df= 2, 21$; $p<0.01$). Post-hoc analysis by Newman-Keuls test showed that administration of quercetin significantly improved the locomotor activity not only in normal rat but lead treated rats as well after one week of administration as compared to their saline controls. The effects quercetin doses on locomotor are also significant when compared to 1st day of administration in both normal and lead treated rats. However normal rats with quercetin doses always showed higher activity than lead treated respective test groups.

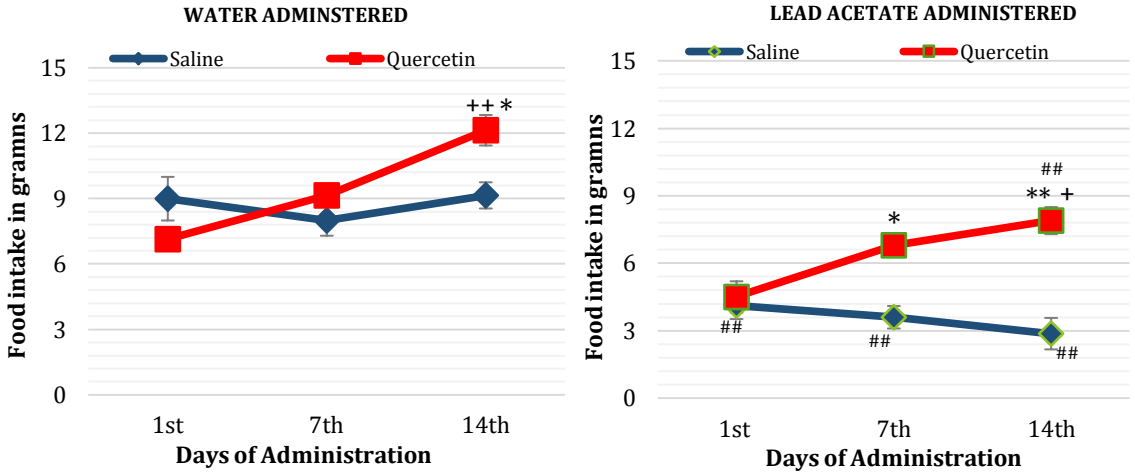


Fig. 1. Effects of Quercetin on Food Intake.

Values are means ± SD (n=6) as monitored on next day of 1st 7th and 14thadministration. Significant differences by Newman-Keuls test: *p<0.05, **p<0.01 from respective saline injected controls, +p<0.05, ++p<0.01 from respective similarly administrated animals of 1st day, ## p<0.01 from water treated animals following three-way ANOVA (repeated measures design)

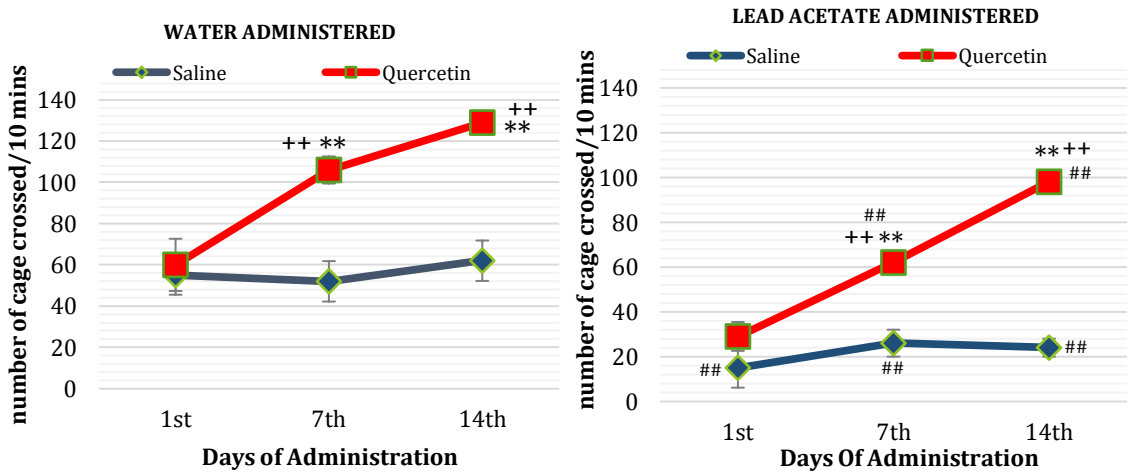
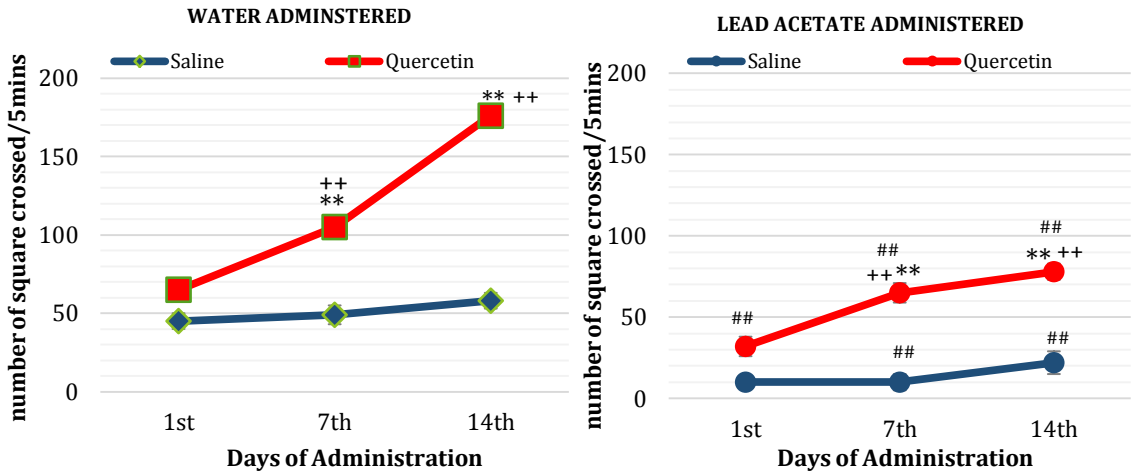


Fig. 2. Effects of Quercetin on Locomotor Activity.

Values are means ± SD (n=6) as monitored on next day of 1st 7th and 14thadministration. Significant differences by Newman-Keuls test: **p<0.01 from respective saline injected controls, ++p<0.01 from respective similarly administrated animals of 1st day, ##p<0.01 from water treated animals following three-way ANOVA (repeated measures design)



Fig, 3. Effects of Quercetin in Open Field.

Values are means ± SD (n=6) as monitored on next day of 1st 7th and 14thadministration. Significant differences by Newman-Keuls test: **p<0.01 from respective saline injected controls, ++p<0.01 from respective similarly administrated animals of 1st day, ##p<0.01 from water treated animals following three-way ANOVA (repeated measures design)

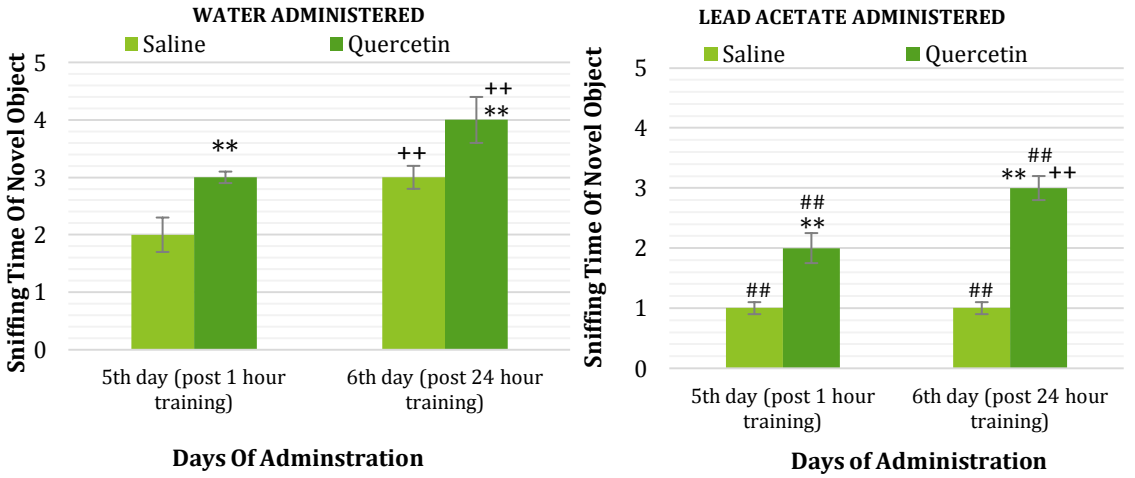


Fig. 4. Effects of Quercetin in NOR.

Values are means ± SD (n=6) as monitored on next day of 1st 7th and 14thadministration. Significant differences by Newman-Keuls test: *p<0.05, **p<0.01 from respective saline injected controls, +p<0.05, ++p<0.01 from respective similarly administrated animals of 1st day, ##p<0.05 #p<0.01 from water treated animals following three-way ANOVA (repeated measures design)

Figure 3 shows the effects of quercetin on lead acetate neurotoxicity in novel environment of open Filed (Square crossed). Data analyzed by three-way ANOVA showed the significant effects of days (F= 128.656; df= 2, 21; p<0.05), lead (F= 348.284; df= 1, 21; p<0.01), quercetin (F= 471.647; df= 1, 21; p<0.01) and interaction between all factors (F= 86.467, df= 2, 21; p<0.05). Post-hoc analysis by Newman-Keuls test showed that administration of quercetin increased the number of square crossed in open field arena after one week as compared to their saline treated animals, there was significant increase in number of square crossing from 1st to 14th administration in the same group. However this increased activity was more prominent in normal rats with quercetin doses as compared to their lead treated rats.

Figure 4 shows the effects of quercetin in normal and lead treated rats on Novel Object Recognition test (Sniffing time). Data analyzed by three-way ANOVA showed the significant effect of days (F= 1614.873; df= 1, 21; p<0.01), lead (F= 1466.379; df= 1, 21; p<0.01), quercetin (F= 887.069; df= 1, 21; p<0.01) and interaction between

these factors (F=677.848, df= 1, 21; p<0.01). Post-hoc analysis by Newman-Keuls test showed that quercetin doses significantly increased the recognition, as the sniffing time of novel object was increased in both normal and lead treated quercetin administered rats as compared to their saline treated animals. Quercetin doses in both normal and lead treated rats increased sniffing from 5th to 6th administration. However normal rats with quercetin have more recognition improvement than their respective lead treated controls.

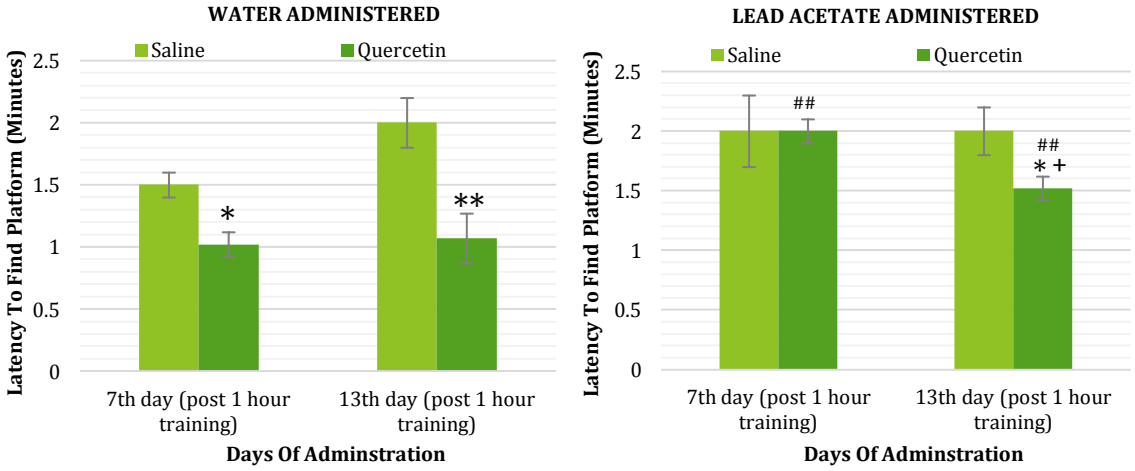


Fig. 5. Effects of Quercetin in Morris Water Maze (Latency).
Values are means ± SD (n=6) as monitored on next day of 1st 7th and 14thadministration. Significant differences by Newman-Keuls test: *p<0.05, from respective saline injected controls, +p<0.05 from respective similarly administrated animals of 1st day, ##p<0.01from water treated animals following three-way ANOVA (repeated measures design)

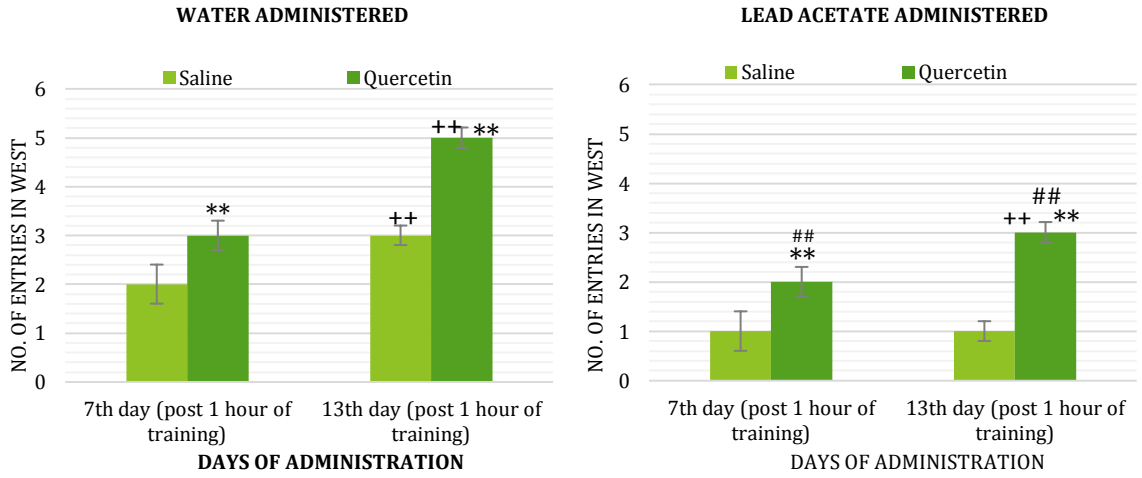


Fig. 6. Effects of Quercetin in Morris Water Maze (Entries).
Values are means ± SD (n=6) as monitored on next day of 1st 7th and 14thadministration. Significant differences by Newman-Keuls test: **p<0.01 from respective saline injected controls, ++p<0.01 from respective similarly administrated animals of 1st day, ##p<0.01 from water treated animals following three-way ANOVA (repeated measures design)

Figure 5 shows the effects of quercetin on normal and Lead Acetate treated rats in Morris Water Maze (latency to find platform post 1 hour of training). Data analyzed by three-way ANOVA showed the effect of days (F= 1.792; df= 1, 21), Lead Acetate (F= 5.313; df= 1, 21), quercetin (F= 21.362; df= 1, 21) and interaction between all of these (F=3.745, df= 1, 21) were non-significant. Post-hoc analysis by Newman-Keuls test showed that quercetin administration decreased the latency time to find platform as compared to saline group of normal and lead treated animals.

Figure 6 shows the effects of quercetin on both normal and lead Acetate treated rats in Morris Water Maze on number of entries in west quadrant post 1 hour of training. Data analyzed by three-way ANOVA showed the significant effect of days ($F= 252$; $df= 1, 21$; $p<0.01$) lead ($F= 232.377$; $df= 1, 21$; $p<0.05$), quercetin ($F= 239.069$; $df= 1, 21$; $p<0.05$) and interaction between all of these ($F=126$; $df= 1, 21$; $p<0.05$). Post-hoc analysis by Newman-Keuls test showed that quercetin administration quercetin doses significantly increased the spatial memory, as the number of entries in west quadrant were increased in both normal and lead treated groups as compared to their respective saline groups. The doses of quercetin were also shown to increase activity from 1st to 8th day of administration of same group. However normal rats with quercetin have more improvement in memory than their respective lead treated controls.

DISCUSSION

Lead is considered as neurotoxic substance, as it is major constituent of environmental pollutant nowadays, the prevalence of its toxicity is increasing day by day. The most possible mechanism of toxicity is the stimulation of oxidative stress and initiates the process of lipid peroxidation which tends to start cellular damages and neurodegeneration. The increasing levels of lead can induce the psychiatric syndromes i.e. anxiety, depression and cognitive deficits etc. Quercetin is a natural polyphenolic compound with the range of pharmaceutical properties, one of the most dominant is antioxidative property which traps the free radical that can produce by oxidative stress during the normal metabolism of cells. Therefore, the present study intended to observe the role of quercetin on behavioral level in normal rats and lead acetate treated rats. The results from this study present the neuroprotective profile of quercetin.

Our results shows the effects quercetin on behavioral level. Result from Figure 1 and 2 shows the effects of quercetin on normal and lead acetate treated rats on food intake and ambulatory activity, respectively. The graphical diagram presents that the quercetin modulates the food intake as well as increased the ambulatory activity in both normal and lead acetate treated rats as compared to their respective saline treated control animal, it shows that quercetin increase the energy expenditure and regulates the metabolic activities, that might be due to the positive effects of quercetin on adipose tissue and leptin secretion which increase the energy expenditure (Beckmann *et al*., 2014; Haleagrahara *et al*., 2013).

Figure 3 present the quercetin effects on anxiety and its anxiolytic effects on normal and lead treated rats as compared to their respective control. The possible mechanism of action behind this anxiolytic activity might be the regulation of catecholaminergic and serotonergic neurotransmission which is involved in emotional and behavioral regulations (Alyu *et al.,* 2015: Kanwaljit and Kaur, 2003). Figure 4 presents the NOR results in which the quercetin is shown to increase the recognition of novel object in NOR after one and 24 h of training period, as the quercetin administered to both normal and lead treated animals shows more time spent in sniffing novel object than familiar one therefore, quercetin is shown to increase the cognition in animals after 5th and 6th administration. Figure 5 and 6also present the results of the effects of quercetin on memory and cognition on Morris water maze (Latency to find platform and number of entries in west). The graphical figures are the evidences of cognitive improvements after the administration of quercetin, as it decrease the time of finding platform after one hour training, it also shows the number of entries in west compartment than normal rats as well as their lead treated control animals. The overall result of NOR and Morris water maze tests shows the cognitive enhancement effects of quercetin that might be due to its neuroprotective effects as it reduces the process of oxidative stress and lipid peroxidation(Boots *et al*., 2003: Nabavi *et al.,* 2012: Preedy *et al*., 2014)and regulates the neurotransmission in limbic system (Pu *et al.,* 2007. Nassiri-Asl *et al.,* 2013) involved memory processing.

CONCLUSION

Lead toxicity is more common among all of the metal poisoning due to its indiscriminate uses. It infers many metabolic pathways but the most sensitive system is neurological system. As the lead can expose to the brain via blood brain barrier, it can immensely disrupt the normal cellular mechanisms. The consequences of these neurological disruption can appear in behavioral deficits. Our study demonstrates the amelioration of lead toxicity by quercetin. There are many research reports which support the present study and the pharmaceutical effects of quercetin. Our results suggest the positive impact of quercetin on lead toxicity as well as normal individuals.

REFERENCES

Alyu, F., UmideDemirOzkay and Yusuf Ozturk (2015). Antidepressant-like effects of quercetin in mice: evidence for the involvement of monoaminergic mechanisms. *Bulletin of Clinical Psychopharmacology.* 25(Suppl. 1):S113-S4.

Azab, E. A. (2014). Hepatoprotective effect of sesame oil against lead induced liver damage in albino mice: histological and biochemical studies. *Am. J. Bioscience.* 2(6): 1-11.

Azoz, H. A. and R.M. Raafat (2012). Effect of lead toxicity on cytogenisity, biochemical constituents and tissue residue with protective role of activated charcoal and casein in male rats. *Aust. J. Basic Appl. Sci.* 6(7): 497-509.

Beckmann, D.V., F.B. Carvalho, C.M. Mazzanti, R.P. Dos Santos, A.O. Andrades, G.Aiello, A. Rippilinger, D.L. Graça, F.H. Abdalla, L.S. Oliveira, J.M. Gutierres, M.R. Schetinger and A. Mazzanti (2014). Neuroprotective role of quercetin in locomotor activities and cholinergic neurotransmission in rats experimentally demyelinated with ethidium bromide. *Life Sci.* 2:79-87.

Boots, A.W., N. Kubben, G.R.M.M. Haenen and A. Bast (2003). Oxidized quercetin reacts with thiols rather than with ascorbate: Implication for quercetin supplementation. *BiochemBiophys Res Commun*., 308: 560-565.

Cho, J.Y., S.C. Park, T.W. Kim, K.S. Kim, J.C. Song, S.K. Kim, H.M. Lee, H.J. Sung, H.J. Park, Y.B. Song, E.S. Yoo, C.H. Lee and M.H. Rhee (2006). Radical scavenging and anti-inflammatory activity of extracts from *Opuntia humifusa Raf. J Pharm Pharmacol*. 58: 113-9.

D'Andrea, G. (2015). Quercetin: A flavonol with multifaceted therapeutic applications. *Fitoterapia.* 106: 256–271.

Fergusson, J.E. (1990). The Heavy Elements: Chemistry, Environmental Impact and Health Effects. 1st ed. Oxford: Pergamon Press. 211–212.

Haleagrahara, N., C. J. Siew and K. Ponnusamy (2013). Effect of quercetin and desferrioxamine on 6-hydroxydopamine (6-OHDA) induced neurotoxicity in striatum of rats. *The Journal of Toxicological Sciences*, 38(1): 25-33.

Hu, J., Q. Yu, F. Zhao, J. Ji, Z. Jiang, X. Chen, P. Gao, Y. Ren, S. Shao, L. Zhang and M. Yan (2015). Protection of Quercetin against Triptolide-induced apoptosis by suppressing oxidative stress in rat Leydig cells. *ChemBiol Interact*. 240: 8-46.

Kanwaljit, M. A. and C. I. Kaur (2003). Antidepressant Activity of Quercetin, a Bioflavonoid, in Streptozotocin-Induced Diabetic Mice. *Journal of Medicinal food.* 6(4):391-5.

Kumar, P. I., S.V. Rana, N. Samanta and H.C. Goel (2003). Enhancement of radiation-induced apoptosis by Podophyllumhexandrum. *J Pharm Pharmacol.* 55: 1267-73.

Manach, C., Augustin Scalbert, Christine Morand, Christian Rémésy and Liliana Jiménez (2004). Polyphenols: food sources and bioavailability. *The American Journal of Clinical Nutrition.*79(5): 727-747.

María José Rodríguez-Vaquero, Pedro AredesAredes-Fernández and María Cristina Manca de (2013). Phenolic Compounds from Wine as Natural Preservatives of Fish Meat. *Food Technol. Biotechnol*., 51 (3) 376–382.

Nabavi, S.D., S.F. Nabavi, S. Eslami and A.H. Moghaddam (2012). In vivo protective effects of quercetin against sodium fluoride induced oxidative stress in the hepatic tissue. *Food Chem*. 132:931-935.

Nassiri-Asl, M., S. Moghbelinejad, E. Abbasi, F. Yonesi, M.R. Haghighi, M. Lotfizadeh and P. Bazahang(2013). Effects of quercetin on oxidative stress and memory retrieval in kindled rats. *Epilepsy Behav*. 28(2):151-5.

Patra, R.C., A.K. Rautray and D. Swarup (2011). Oxidative stress in lead and cadmium toxicity and its amelioration. *Vet Med Int.* 1-9.

Preedy, V., M.T. Sung, Y.C. Chen and C.W. Chi (2014). Quercetin's Potential to prevent and Inhibit Oxidative stress Induced Liver. Cancer: *Oxidative Stress and Dietary Antioxidants.* Academic press, London. pp 231-239.

Pu, F., K. Mishima, K. Irie, K. Motohashi, Y. Tanaka, K. Orito, T. Egawa, Y. Kitamura, N. Egashira, K. Iwasaki and M. Fujiwara (2007). Neuroprotective effects of quercetin and rutin on spatial memory impairment in an 8-arm radial maze task and neuronal death induced by repeated cerebral ischemia in rats. *Journal of Pharmacological Sciences*104 (4):329–334.

Rafiq, H. and M. Farhan (2015). High Fat Diet-Induced Obesity Stimulate Depressive Behavior in Rats. *Current Psychopharmacology.* 4(2):125 – 132.

Surkan, P.J., A. Zhang, F. Trachtenberg, D.B. Daniel, S. McKinlay and D.C. Bellinger (2007). Neuropsychological function in children with blood lead levels < 10 microg/dL. *Neurotoxicology*, 8(6): 1170-7.

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