https://doi.org/10.33451/florafauna.v31i2pp225-230
FLORA AND FAUNA ISSN 2456 - 9364 (Online)
2025 Vol. 31 No.2 PP 225-230 ISSN 0971 - 6920 (Print)

Therapeutic potential of caffeic acid against alterations induced by combined exposure to Aluminum and Beryllium

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Received **:** 11.09.2025; ***Accepted*** : 08.11.2025

How to cite : Behra P, Nirala SK, Bhadauria M. Therapeutic potential of caffeic acid against alterations induced by combined exposure to Aluminum and Beryllium. *Flora and Fauna* 2025. 31(2) : 225-230.

ABSTRACT

Metal contamination poses public health challenges around the world. This study evaluated therapeutic potential of caffeic acid against impairments induced by combined exposure to Aluminum and Beryllium in female albino rats. Rats were divided into five groups, group one served as control. Group two to five received combined dose of Aluminum nitrate (6.5 mg/kg, i.p.) and Beryllium nitrate (1 mg/kg, i.p.) daily for four weeks. Groups three to five received oral doses of caffeic acid at 10, 20 and 30 mg/kg, respectively for continuous two weeks of post- exposure.Behavioral performance was assessed 24 h after final administration of toxicants using elevated plus maze, T- maze and light and dark chamber. Rats exposed to toxicants showed pronounced anxiety-like behaviors and reduced time in dark compartment. Caffeic acid treatment significantly attenuated these effects in dose dependent manner, highlighted its neuroprotective roles against combined exposure to Aluminum and Berylliuminduced behavioral alterations.

Figure : 00 References : 30 Tables : 03
KEY WORDS : Aluminum, Beryllium, Caffeic acid, Neurotoxicity, Polyphenols.

Introduction

In the era of environmental pollution by toxic metals, much researches have focused on the toxicity of individual metals while real-world scenarios rarely involve exposure to a single metal. Living organisms are frequently exposed to mixtures of multiple metals simultaneously that create a complex interaction, which are not always predictable from single-metal exposure studies.Considerable studies have been conducted on heavy metals but comparatively less information is available on toxic effects after exposure to combination of light metals.

Aluminum is the third most abundant element in the Earth crust (~8%), widely used in electrical systems, leather processing, and water treatment and released naturally from soil, rocks and water under acidic conditions[20]. Aluminum induces oxidative stress, depletes antioxidant defense in vital organs,crosses blood-brain barrier, accumulates in neuronal tissues and disrupts calcium homeostasis[9]. It contributes neuroinflammation, protein aggregation and neurodegenerative disorders[15]. Beryllium is the lightest bivalent, low-densityhard metal with unique physical and chemical properties. Beryllium exposure generates reactive oxygen species leading to systemic toxicity[30].Beryllium exposure is linked to chronic lung disease (berylliosis), carcinogenesis, allergic, apoptosis, mutagenic action, neurotoxic effects and neuroinflammation disrupts blood brain barrier[23]. These effects are likely mediated by neuronal apoptosis, neurotransmitter imbalance and oxidative damage[25].

Caffeic acid(CA) is a plant derived hydroxy

TABLE-1: Therapeutic effect of caffeic acid on behavioral alterations

	Elevate Plus Maze			
Groups	**Total time spent in closed arm (Sec)**	**% time in closed arm**	**Total time spent in opened arm (Sec)**	**% time in opened arm**
Control	248.3± 13.7	82.76± 4.57	51.7± 2.85	17.23± 0.95
Al+Be	139.5± 7.71a	46.63± 2.57a	160.5± 8.87a	53.5± 2.95a
Al+Be+ CA 10	239.9± 13.2b	79.96± 4.42b	60± 3.31b	20± 1.10b
Al+Be+ CA 20	240.7± 13.3b	80.23± 4.43b	59.3± 3.27b	19.76± 1.09b
Al+Be+ CA 30	241.9± 13.4b	80.63± 4.45b	58.1± 3.21b	19.36± 1.07b
ANOVA	16.3^{Ω}	16.3^{Ω}	108^{Ω}	108^{Ω}

cinnamic acid that exhibits a diverse biological activity, including antioxidant, anti-inflammatory, antimicrobial, antiviral, anticancer, neuroprotective and metal chelating properties[17]. Its two phenolic hydroxyl (-OH) groups are responsible for its antioxidant activityto protect cells from oxidative damage. Carboxylic acid (-COOH) group contributes to slightly acidic medium that allows it to participate in chemical reactions involving proton exchange[29]. Caffeic acid improves memory learning and reduces anxiety and depression like behaviors by modulating neuro transmitters level, reducing neuro inflammation and preventing neuronal apoptosis[22].

Therefore, this investigation focused on the evaluation of therapeutic potential of caffeic acid against behavioral alterations induced by combined exposure to Aluminum and Beryllium.

Materials and Methods

Experimental animals and chemicals

Wistar female rats (160±10 g) were procured from All India Institute of Medical Science (AIIMS), New Delhi, India. Animals were maintained in polypropylene cages with provision of access to animal feed pellets and water *ad libitum*. The experimental study was approved by the Institutional Animal Ethics Committee (IAEC).All the animals were procured from recognized standard chemical dealers.

Experimental design

Thirty animals were randomly assigned into five groups having six animals in every group.

Group I: Control. **Group II:**$Al(NO_3)_3$ (6.5 mg/kg; i.p.) + $Be(NO_3)_2$(1 mg/kg; i.p.) for four weeks.**Group III:**$Al(NO_3)_3$ + $Be(NO_3)_2$(as in group II)+ CA 10 mg/kg (p.o., simultaneously for two weeks after two weeks exposure to toxicants). **Group IV:**$Al(NO_3)_3$ + $Be(NO_3)_2$ (as in group II)+ CA 20 mg/kg(p.o., simultaneously for two weeks after two weeks exposure to toxicants). **Group V:**$Al(NO_3)_3$ + $Be(NO_3)_2$ (as in group II)+ CA 30 mg/kg(p.o., simultaneously for two weeks after two weeks exposure to toxicants).

Behavioral studies

Elevated plus maze : The experimental device consisted of an elevated plus maze (40 cm above the floor) that consists of two opened and two enclosed arms, an open roof arranged in such a manner that two open arms were opposite to each other (four arms were 30 cm long and 5 cm wide). Rats were individually placed at the center of maze facinga closed arm. An entry into an arm was registered when animal placed all four paws on it. The parameters included number of entries and the percent time spent in closed and opened arms were recorded during a 300 second observation period. Data were expressed as the total time spent in closed arm (Sec), % time in closed arm and total time spent in opened arm (Sec), % time in opened arms[4].

T- maze : The T- maze was conducted over two phases. In phase one, animals were habituated to maize by allowing free exploration for 5 minutes with all arms accessible. In phase two, each animal underwent six trials; Trial phase: one goal arm was blocked, and the animal was placed in the start arm. Animal was allowed

TABLE-2 : Therapeutic effect of caffeic acid on behavioral alterations

	T- Maze	
Groups	**Correct alteration**	**% correct alteration**
Control	4± 0.22	66.6± 3.68
Al+Be	1.5± 0.08a	24.9± 1.37a
Al+Be+ CA 10	4± 0.22b	66.6± 3.68b
Al+Be+ CA 20	4± 0.22b	66.6± 3.68b
Al+Be+ CA 30	4± 0.22b	66.6± 3.68b
ANOVA	37.04^{Ω}	37.2^{Ω}

to explore opened goal arm and remained there for 20 seconds before being returned to its home cage. Choice latency: After a 30-second interval, both arms were opened, and the animal was placed again in the start arm. The animal's choice of arm was recorded, and entry into the arm opposite to the one visited during the trial phase was considered a correct alternation[8].

Light and dark chambers : Light and dark two distinct chambers, a dark chamber (20x 30x 35 cm) painted black and bright chamber painted white and brightly illuminated with white light sources. By observing the time duration of an animal which spent light-dark area in light and dark chamber, one can predict the anxiety or depression status of that animals[1].

Statistical analysis

The results were subjected to one way analysis of variance (ANOVA) followed by Tukey's *HSD post hoc* analysis at a significance level of 5%. All data were expressed as mean ± standard error. All statistical analyses were performed by using SPSS statistical software package(SPSS Inc, Chicago, IL).

Results

Elevated plus maze : Elevated plus maze test demonstrated significant behavior alteration among the group. Combined exposure to Aluminum and Beryllium group exhibited a drastic reduction in time spent in opened arm as well as % time in open were increased significantly as compared to control group as well as significantly decreased time spent and % time spent in closed arm. Different concentration of caffeic acid at 10, 20 and 30 mg/kg treatment reversed these effects in dose dependent manner. Group 10, 20 and 30 mg/kg showed significant restoration. *Tukey's* HSD *post hoc* test revealed that 30 mg/kg dose had maximum therapeutic efficacy (Table-1).

T-maze : T-maze exhibited significant decline in correct alteration behavior and percentage correct alteration indicating impaired spatial memory and cognitive functions in toxicity group. Caffeic acid treatment at 10, 20 and 30 mg/kg doses significantly restored performance towards control. Tukey's HSD *Post hoc* test revealed significant recovery at all three doses of caffeic acid for both correct alteration and % of correct alteration. These results suggested that caffeic acid markedly ameliorated aluminum and beryllium induced memory impairments (Table-2).

Light and dark chambers

Combined exposure to Aluminum and Beryllium reduced the time spent in the dark and correspondingly increased time spent in light suggesting heightened anxiety like behavior. Caffeic acid treatment led to a dose dependent improvement. Caffeic acid at 20 and 30 mg/kg dose showed significant improvement as compared to lower dose of caffeic acid 10 mg/kg. The ANOVA revealed significant differences in total time and percentage time spent in light, while time spent in dark and its percentage did not differ significantly. These findings suggested that caffeic acid effectively mitigated anxiety like behavior induced after combined exposure to Aluminum and Beryllium (Table-3).

Discussion

Metal toxicity is a major concern in occupational and environmental health problems. Aluminum and Beryllium are widely used as light metals; however, their

TABLE-3 : Therapeutic effect of caffeic acid on behavioral alterations

Light and Dark Chamber				
Groups	**Total time spent in dark (Sec)**	**% time in dark**	**Total time spent in light (Sec)**	**% time in Light**
Control	260± 14.4	86.66± 4.79	39.9± 2.20	13.3± 0.73
Al+Be	239± 13.2	79.93± 4.41	60.2± 3.32a	20.06± 1.10a
Al+Be+CA 10	240± 13.3	80.13± 4.42	59.6± 3.29a	19.86± 1.09a
Al+Be+CA 20	252± 13.9	84.26± 4.65	47.2± 2.60bc	15.7± 0.86bc
Al+Be+CA 30	256± 14.1	85.36± 471	43.9± 2.42bc	14.63± 0.80bc
ANOVA	0.54	0.53	13.0^{Ω}	13.0^{Ω}

exposure poses significant health risks. Neurotoxicity associated with these metals is well documented, involving oxidative stress, neuronal damage and cognitive impairment[26]. Both direct and indirect exposures to their salts impose adverse effect on the behavior and cognition, including attention, memory, learning capacity *etc*. Pathogenesis in neuro degeneration is linked to oxidative stress, which disrupts antioxidant defence and promotes neuronal cell death[3,20]. Plant derived molecules have ability to control reactive oxygen/nitrogen species, which may be utilized to treat memory impairments and cognitive problems. Caffeic acid has wide spectrum of pharmacological activities[17]. To mitigate toxic effect of Aluminum and Beryllium, caffeic acid was evaluated due to its strong antioxidant and metal chelating properties, which helped in reducing oxidative damage and metal induced toxicity[2].

Exposure to Aluminum nitrate and Beryllium nitrate resulted neurotoxicity in experimental animals as indicated by inability of rats to maintain equilibrium for 5 min in each trial on elevated plus maze[13]. Number of entries in opened and closed arms reflected the safety of closed arm. Reduction in % time spent, entry in opened arm and increased defections indicated high level of fear or anxiety. Anxiolytic xenobiotics enhances the number of entries and time spent in opened arms. Combination of toxicants decreased the number of entries and % time spent in closed arms showing anxiety effects. Improvement may be attributed to antioxidant of caffeic acid reduced neuronal damage and enhanced cognitive functions[9].

The T-maze is well established behavioral tool, used to asses spatial learning and memory in rats. Exposure to Aluminum and Beryllium impaired the spatial learning by increased latency and reduced alternative behavior[23]. This suggested neurotoxic effects, likely linked to oxidative stress. However, different doses of caffeic acid improved performance, indicating its neuroprotective role through antioxidant and metal chelating actions[5].

In light dark chamber, bright light acts as environment stress that minimizes the explorative behavior of rats. Reduction in the time spent, rearing behavior and number of entries in the light chamber was regarded as markers of anxiety[21]. Combined exposure to Aluminum and Beryllium decreased time spent, rearing behavior and number of entries in the light chamber, which confirmed anxiogenic effects after exposure to Aluminum and Beryllium[6].

On the basis of results, it may be concluded that all the three doses of caffeic acid *i.e.*, 10, 20 and 30 mg/kg recovered from behavioral alterations on a dose dependent manner and confirmed its therapeutic potential against behavioral alterations induced by combined exposure to Aluminum and Beryllium.

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