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Original Article

6-AF Evaluation of Neuroprotective Activity against Cd- Induced Oxidative Stress and Degenerative Brain Disease including PD in Mice

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Abstract

Background: The neurotoxicity caused by Cadmium (Cd) has been researched internationally. Since it has a wide range of unfavorable effects on people, it is believed to be one of the primary tissue-inducing target agents. Using adult male albino mice, the therapeutic potential of 6-AF to reduce memory impairment, neurodegeneration, and neuroinflammation caused by Cadmium Chloride ($CdCl_2$) was evaluated in the current study for the first time.

Methodology: The male adult mice were distributed into 4 sub-groups; Control, Cd treated (1 mg/kg thrice weeks), Cd (1 mg/kg 3 weeks) + 6-AF (30 mg/kg 3 a week for last 2 weeks) and 6-AF treated (30 mg/kg thrice a week for the last two weeks). After the initial seven-day Cdcl2 dosing cycle, the 6-Aminoflavone was administered interpretively intravenously for the following around 14 days (three per week). After receiving Cdcl2 injections for 30 days, behavior tests were conducted. Western blot analysis was performed after the hippocampus was extracted, and the results were then used to develop the X-rays.

Results: Our results demonstrate that 6-AF significantly enhanced behavior as assessed by the Y-maze and Morris Water Maze (MWM) and that this enhancement was followed by an inhibition of phospho C-Jun N Terminal Kinase (p-JNK) and its downstream signaling, including tumor necrosis factor-alpha (TNF-alpha), Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF-KB), and Poly (ADP-ribose In addition, 6-AF also reduced the expression of NRF-2 proteins in adult mice exposed to oxidative stress caused by cadmium chloride.

Conclusion: 6-AF is an effective neuroprotective drug in disorders causing neurodegeneration.

Keywords

$CdCl_2$, Neuro-inflammation, 6-AF, Phospho-JNK ,NRF-2,Parkonsion disease.

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Introduction

Neurodegeneration is a process where neurons gradually degenerate or die. This can lead to a decline in brain function and various neurological symptoms, including problems with movement, thinking, and behavior. At the same time, neurodegenerative diseases are conditions characterized by progressive damage to the neurons in the brain and nervous system[1].

Parkinson's disease (PD), a late-onset neurodegenerative disorder, is characterized by an extensive and progressive loss of dopaminergic neuronal cells in the substantia nigra compacta and the accumulation of Lewy bodies, which are intracellular inclusions comprised of -synuclein[2,3]. Parkinson's patients' post-mortem brain tests reveal lysosomal consumption and autophagosome accumulation, which may indicate impaired autophagic clearance. The proteasome, chaperone-mediated autophagy, and macro-autophagy can contaminate 4-synuclein[4,5]. Following the critical role of autophagy in -synuclein Lewy bodies, several investigations have demonstrated that pharmacological and genetic stimulation of autophagy reduces -synuclein aggregation and disease pathogenesis[6,7].

Several PD-related proteins, in addition to -synuclein, are directly involved in the autophagy pathway. The quality encodes the Lysosomal Trans Film ATPase protein[8]. PD-related mutations in ATP13A2 lead to defects in lysosomal acidification, which reduce autophagosome clearance and accumulate a-synuclein[9,10]. Other proteins identified with PD Loss-of-work modifications in prompted putative kinase-1, PTEN protein gene coding, and PARK2 are also known to cause autosomal-passive type PD and -synuclein[11-13]. The prodded putative kinase-1 gene genes for a threonine/serine protein kinase transmitted in the outer mitochondrial film[14]. These proteins regulate mitophagy, a process that results in mitochondrial damage.

Cadmium (Cd) is a common industrial pollutant and environmental contaminant released mainly by burning fossil fuels, refining metal from municipal waste, and smoking cigarettes15. When Cd enters the atmosphere and a living thing, it has various adverse effects related to Cd damage[15]. Human exposure to Cd damages and affects body organs like the liver, bone, testicles, kidney, and cerebrum and can result in cancer, tumors, etc[16,17]. Due to its ability to pass the (BBB), Cd can cause damage to nerve cells and the cerebrum. It also results in a defective nervous system that impairs vascular functioning, neurological disorders in the brain, and learning difficulties in addition to PD[18].
Additionally, Cd increased the generation of free radicals, which led to oxidative stress and protein and phospholipid degradation (DNA)[19]. Additionally, it is claimed that Cd is to blame for developing neurodegenerative disorders like AD17. Cadmium also replaces copper and iron in the protein's structure[20]. These unbound metals result in oxidative stress through Fenton chemistry processes, which causes cancer to develop in various organ systems and systems of a living organism. Consequently, cadmium is regarded as a class of human carcinogen[s21].

In contrast, Polyphenols called flavonoids are found in foods including fruits, vegetables, herbs, tea, wine, and other organic and natural items. They stand out for having a wide-ranging understanding of that medication. Flavonoids work as breast cancer chemotherapeutic agents. The function of 6-Amino Flavone in cancer chemoprevention. 6-AF works in a variety of ways, including by inactivating carcinogens.

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The 6-Aminoflavones have a strong anti-breast cancer effect. In cancer, the 6-AF are incredibly active. Studies have also shown that 6-AF's antioxidant activities are vigorous; as a result, 6-Aminoflavone was used in the current investigation to test its effectiveness against reactive radicals in a mouse model [22-24].

The above evidence suggests that heavy metals like cadmium severely harm the health of humans, and it is one of the leading causes of neurological disorders, including PD, AD, and HD. Therefore, this study aims to determine the remedial action to lessen and defeat this harmful disease by investigating the brain damage and poisoning effects of cadmium chloride in mice. Moreover, we also investigate how 6-AF activity reduces Cd-induced neuroinflammation, and we further hypothesize to identify a treatment for the oxidative stress brought on by Cd toxicity.

Methodology

Mice weighing between 26-30g were collected for this experimental study. These mice were bought from the Peshawar Veterinary Research Institute. These mice were kept in a 23–24 degrees Celsius chamber, with 12-hour cycles of light and 12-hour cycles of darkness. The (NMRC), associated with the chemistry department, SUIT Peshawar, conducted this experimental study. The committee of the Centre oversaw carried out the research activities.

The male adult mice were distributed into 4 sub-groups as given under

1. Control (Normal) mice. Cd treated (1 mg/kg thrice weeks).
2. Cd (1 mg/kg 3 weeks) + 6-AF (30 mg/kg 3 a week for last 2 weeks).
3. 6-AF treated (30 mg/kg thrice a week for the last two weeks).

All animals were handled with extreme care. Adult albino mice received Cd injections for three weeks. After the initial seven-day Cdcl2 dosing cycle, the 6-Aminoflavone will be administered interpretively intravenously for the following around 14 days (three per week). After receiving Cdcl2 injections for 30 days, behavior tests were conducted. 6-AF, indicated in the study's instructions, contributed to the successful outcome of the neuroprotective agent drug. 6-Amino Flavone repaired adult albino mice's memory impairment caused by cadmium.

The Y-Maze test was also conducted. A 120-degree angle is formed by the three arms of the Y-maze, which measure 50 by 10 by 20 cm3 (LxWxH). Mice were given a 10-minute window to become used to their new surroundings. The mouse was then kept in the maze's center for 8 minutes while it was free to explore. Track was kept on the mice's total arm entries and consecutive triplet counts, and the percentage of alternations was calculated using the formula [successive triplet sets/total arm entries 2] times 100. Working memory performance in the spatial domain was positively linked with the percentage of alternations.

$$\% \text{ Spontaneous Alternation} = \frac{\text{Successive Triplet}}{\text{Total Number of Arm Entries-2}} \times 100$$

Morris Water Maze (MWM) was used to examine the hippocampus region's role in long-term spatial learning. For the first three days, the mice were trained twice daily. The mice's 60-second escape delay in locating the submerged platform was then observed. If the mice could not locate the platform on their own, they were manually guided there and made to stay there for 10 seconds. Up until day 5, this procedure was followed, and each day's data (seconds) for the three experimental groups were separate. The

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mice were given two days to recuperate before being subjected to a probe test.

Western blot analysis was performed, and the hippocampus was extracted and placed in an ice-cold 1:1 RNA-to-PBS solution. T-PER (Thermo Scientific) solution for tissue extraction protein reagent was used to homogenize the hippocampus component. A semi-dry trans-blot technique transferred the proteins to the PVDF membrane (Bio-Rad). They were followed by secondary antibodies that were HRP-conjugated against mice from Santa Cruz, California, USA. The results were then used to develop the X-rays.

Result

The results show that Cadmium chloride induced a significantly high expression of p-JNK in the brain homogenates of mice. On the other hand, we also injected 6-Amino flavone for two weeks after cd induces p-JNK activation to know its inhibitory ability of p-JNK proteins, as shown in figure 1. Our outcomes revealed that 6- Amino Flavone significantly reversed the expression of phospho-JNK in the brain of albino mice.

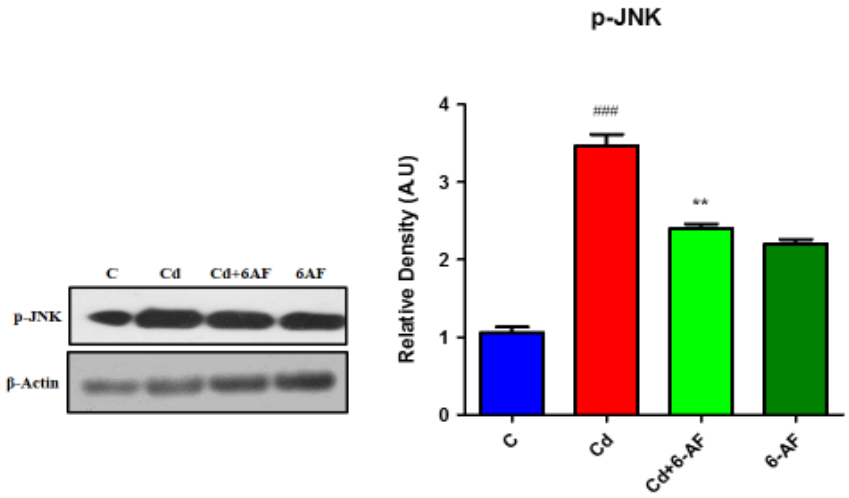

Figure 1: 6-AF inhibited phospho-JNK activation in Cdcl2 Induced adult albino Mice brain.

Downregulated signal activation in adult albino mice is observed when injected with another neuroprotective drug 6-AF, for the last two weeks. This suggests that 6-Amino Flavone dramatically altered the expression of TNF expression in the brains of albino mice. One of the frequent mediators of neuroinflammation is TNF-α.

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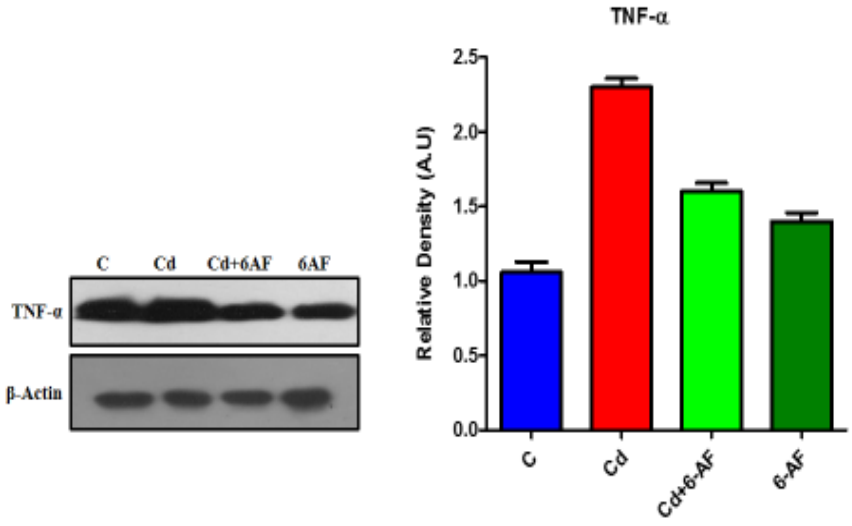

Figure 2: 6-AF inhibited Tumor necrosis factor TNF-α Neuroinflammation in Cdcl2 Induced Mice

Figure 3 shows the immunoblots of NF-κB and β-actin along with their histogram for all 4 experimental groups (n=4/group). Western analysis reveals that cadmium chloride dramatically raises NF-B expression in the brain of adult albino mice. After two weeks of 6-Amino Flavone injection, the brains of adult albino mice showed decreased NF-B expression.

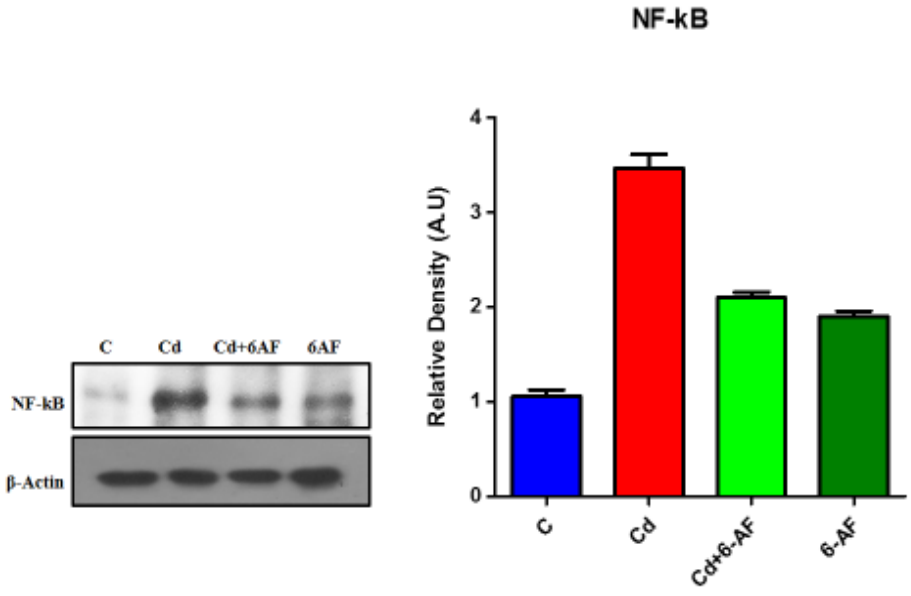

Figure 3: 6-AF inhibited NF-κB and instigated by Cd in adult male mice brains.

The outcome demonstrates that in the adult albino mouse brain, the Caspase-3 signal is up-regulated and activated by Cd. Figure 4 shows the 6-AF decreased Caspase-3 down-regulated

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expression in the brain of albino mice. This demonstrates that quercetin treatment improves the morphology of mice's neurodegenerating hippocampus following Cd exposure.

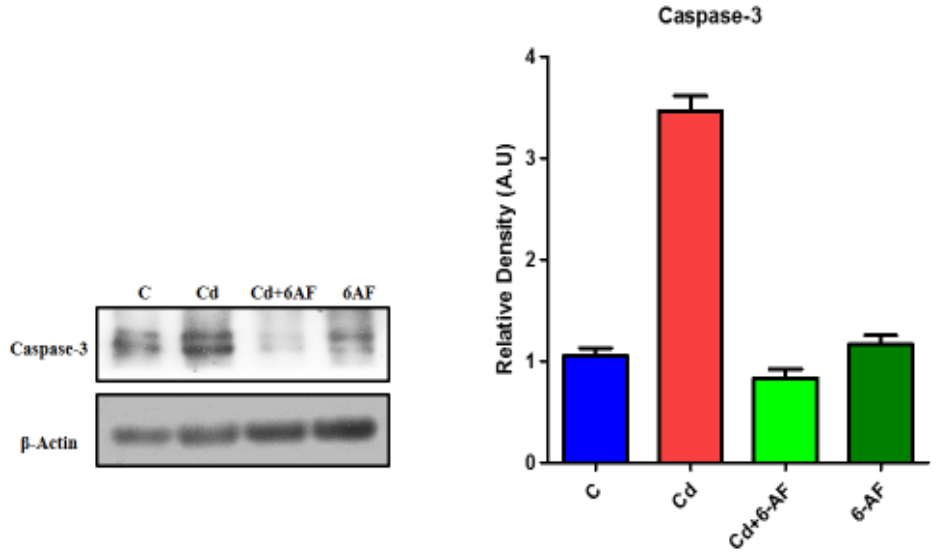

Figure 4: 6-AF inhibited Caspase-3 proteins in Cdcl2 Administered Mice

As shown in our results, 6-AF diagrammatically reduced the expression of the PARP-1 protein (Figure 5). This suggests that Caspase-3, Caspase-9, and PARP-1 protein levels decreased in response to Cd, and the levels of cleaved Caspase-3, Caspase-9, Caspase-8, and FasL proteins rose dose-dependently. While N-acetylcysteine successfully prevented these changes.

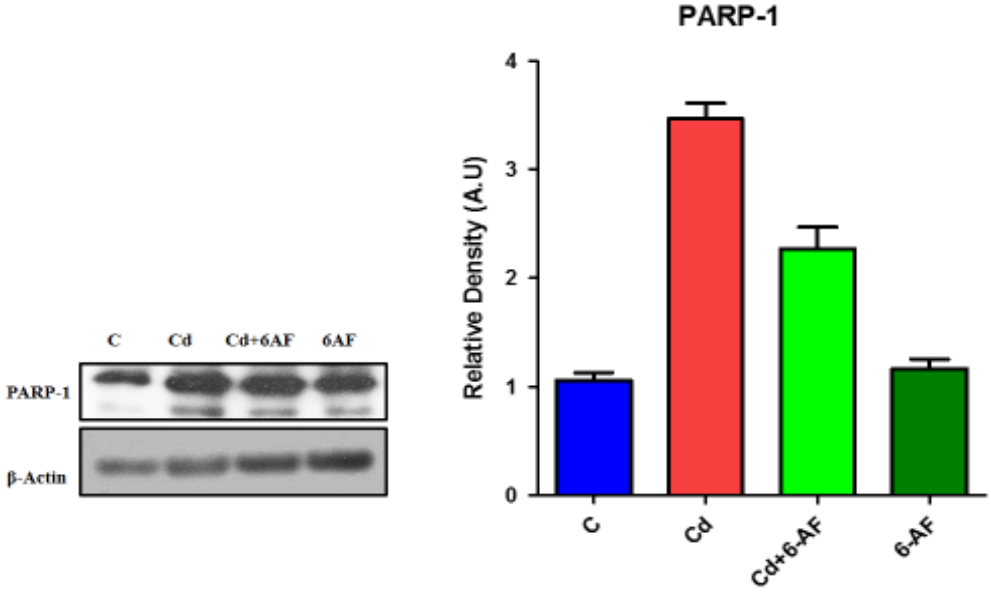

Figure 5: 6-AF inhibited PARP-1 proteins expression in Cd Mice

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Discussion

Cdcl2 is a well-familiar agent to induce phospho-JNK activation[15]. In a recent study, we injected CdCl2 intraperitoneally for three weeks into adult albino mice. And aims to determine the remedial action to lessen and defeat harmful neurological disorders. Studies suggest that Cdcl2 has been shown to be a hazardous metal that enters the body directly and harms cells. Cd can throw off oxidative equilibrium. In mice treated with Cd, it results in a loss of motor function and damages the DNA in cells and the structural integrity of the cerebellum[25].

One of the most prevalent neurodegenerative disease mediators is JNK. Our findings are consistent with earlier research because Chen et al. also showed that cadmium could cause the phosphorylation of the JNK protein in mouse brain tissue. According to a study, the celastrol neuroprotective pharmacological agent Cd caused neuron cell death by phosphorylating JNK and targeting the PTEN-Akt/mammalian target of the Rapamycin network[15]. According to our findings, cholesterol may protect against Cadmium-induced neurodegenerative diseases. The researcher showed that active p-JNK is critically involved in disease development after Traumatic Brain Injury and that inhibition of p-JNK with SP6001[25] is highly efficient for slowing disease progression by reducing multiple pathological features in Traumatic Brain Injury mice brains and regulating cognitive dysfunction[26].

Cadmium Chloride was administered intraperitoneally to the male mice for a week thrice to induce neurodegeneration and neuroinflammation in adult albino mice and then treated with 6-Amino flavone for two weeks. When compared to mice exposed to Cd, immunohistochemical investigation shows that in earlier studies, mice that had been pre-treated with (SME) showed a decrease in the amount of TNF- appearing as a protein. Our findings are consistent with those of Elkhadragy et al., who also suggest that Cd can cause the tumor necrosis factor protein to be expressed in the mouse brain[27].

Our findings reveal that cadmium chloride dramatically raises NF-B expression in the brain of adult albino mice. Previous research indicated higher amounts of inflammatory cytokines, including NF-B and -Actin proteins, were also significantly found. Notably, research on (Nrf-2) silencing and (NF-B) has shown that Cadcl2 can trigger the NF-B in the brain of albino male mice[28]. Studies of nuclear factor-B (NF-B) inhibition and nuclear factor-2 erythroid-2 (Nrf-2) gene silencing demonstrate that caffeine produces neuroprotection via Nrf-2- and (NF-B) dependent pathways, respectively, in the HT-22 and BV-2 cell lines[28].

It is also demonstrated that quercetin treatment improves the morphology of mice's neurodegenerating hippocampus following Cd exposure. According to Chong et al., synuclein causes cell death in a dopaminergic neuronal model of Parkinson's disease by increasing oxidative stress, increasing Cd uptake, changing caspase-9 and caspase-3 activation, and decreasing the neuroprotective effect of Akt. This is in response to acute Cadmium exposure[29]. According to previous research, oxidative stress, mitochondrial damage, and Cd-induced cell death are all caused by parthanatos and the MAPK signaling pathway. JNK1/2 and p38 are implicated in parthanatos, which also synergistically affect apoptosis when paired with oxidative stress[30].

Even though the current study focused on 6-AF's neuroprotective and memory-improving effects in mice exposed to Cd. The

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only concentration of 6-AF employed as a neuroprotective agent in this investigation. However, it is advised to use alternative concentrations of 6-AF in future research on animal models of neurodegenerative disorders. It is also advised to thoroughly investigate the 6-AF in vitro model of cell culture to better understand its usefulness as a medication.

Conclusion

According to this study, 6-AF is a neuroprotective drug that reduces Cd-induced toxicity in an animal model. It has been demonstrated that 6-AF works in vivo to reverse the memory deficits caused by Cd while lowering neuroinflammation and being a natural, safe, and accessible therapeutic agent. This work also demonstrated the molecular mechanism by which 6-AF in Cd causes neurological disease.

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