Evaluation of The Neuroprotective Activity of 6-AF Mitigates Cd-Induced Oxidative Stress and Neurodegeneration in Mice

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Abstract

Neurotoxicity brought on by cd has been studied globally. It is thought to be one of the main tissues-inducing target agents since it has a wide variety of negative effects on people. The therapeutic potential of 6-AF to lessen memory impairment, neurodegeneration, and neuroinflammation caused by Cdcl2 was assessed in the current investigation for the first time in adult male albino mice. Our findings show that 6-AF significantly improved behaviour as measured by the Y-maze and Morris Water Maze (MWM), and that this improvement was followed by an inhibition of Phospho C-Jun N Terminal Kinase (p-JNK) and its downstream signaling, including, tumour necrosis factor-alpha (TNF-alpha) and Poly (ADP-ribose polymerase-1 (PARP-1) (NF-KB), proteins in the mice brain homogenates detected through western blotting. Additionally, NRF-2 proteins were likewise downregulated by 6-AF in adult mice exposed to Cd induced oxidative stress. In conclusion, 6-AF is a strong neuroprotective agent in neuro-degenerative diseases.

Keywords: $Cdcl_2$, Neuro-inflammation,6-AF, Phospho-JNK, NRF-2.

1.1 Alzheimer's Disease

Alzheimer's Disease is a progressive neurodegenerative condition characterized by the presence of intra-neuronal fibrillary tangles and extra-cellular crumbling plaques. On a very fundamental level, the plaques are composed of amyloid-(A) peptides, which are produced by the proteolytic cleavage of the precursor amyloid protein using processes involving - and -secretases. Tau, a protein associated to microtubules, is found phosphorylated in neurofibrillary tangles. [2-4] These views suggest that auto phagosome deserts may be a general feature of AD pathogenesis. The most well-known cause of familial autosomal-predominant Alzheimer's Disease is alterations in Presenilin1 (PS1), a component of the c-secretase complex required for the production of Amyloid-(A). Since they include both amyloid precursor protein and its handling chemicals, the accumulated autophagic vacuoles can serve as a key intracellular source of pathogenic Amyloid-, increasing amyloid-age. [5] These results suggest that excessive auto phagosome accumulation and amyloid- (A testimony) deposition in Alzheimer's Disease brains occur from a synergistic interaction between defective lysosomal proteolysis and suboptimal auto phagosome transport. Additionally, auto-phagy is activated under the control of solvent tau and destroyed neuro-fibrillary tangles' intracellular latitude. Tau freedom is delayed and tau accumulation occurs as a result of 3-MA and chloroquine's inhibition of autophagy. [6] Rapamycin, on the other hand, increases tau pathology and reverses mice's psychological deficits when autophagy is activated. Model for Alzheimer's disease. [7,8] Hereditary reduction of Beclin-1 results in an increase of Amyloid- statement and neurodegeneration in a transgenic mouse model of Alzheimer's disease that transmits human (APP). Alternately, lenti-viral organization of Beclin-1 in mice that are transgenic for the amyloid precursor protein essentially reduces the disease caused by amyloid. [9,10].

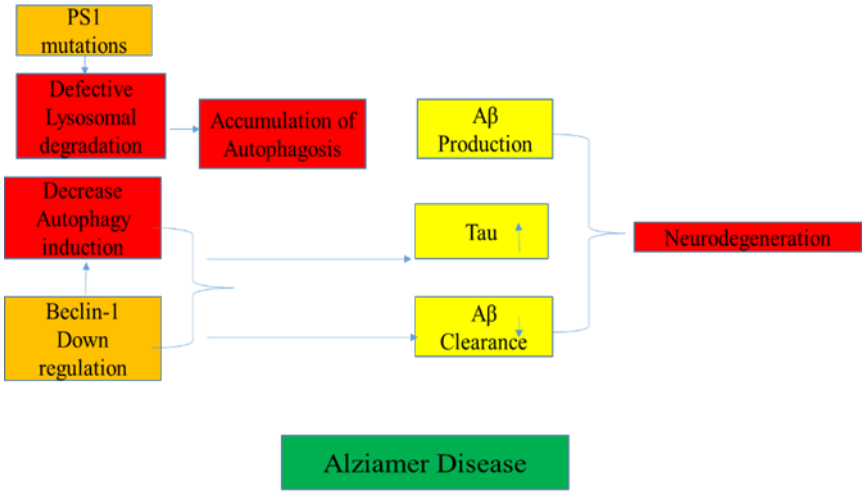

Fig. 1.1 In Alziamer disease, (PS1) alterations prevent the auto-phagic vacuoles' ability to move freely within the lysosomes, which causes an increase in an age. Additionally, the Beclin-1 downregulation guideline reduces the initiation of autophagy. (Zhang et al., 2016) [1]

1.2 Cadmium

Cadmium is a frequent industrial and environmental pollutant and contaminant that is primarily released by the burning of fossil fuels, metal refining from municipal trash, and smoking of cigarettes. [11] Through natural sources of frequent, anthropogenic sources, it is spread in different ecological regions. Natural causes include mining, forest fires, volcanic eruptions, and so forth. Anthropogenic sources include tobacco smoke, metals purification, burning fossil fuels, burning rubbish, and burning fossil fuels. [12]

When Cd enters the atmosphere and a living thing, it has a variety of negative effects that are related to Cd damage. [13] Human exposure to Cd damages and affecting body organs like the liver, bone, testicles, kidney, and cerebrum and can result in cancer, tumours, etc. [14-19] Due to its ability to pass the blood brain barrier Cd can cause damage to nerve cells and the cerebrum. Additionally, it results in a defective nervous system that impairs vascular functioning, neurological disorders in the brain, and learning difficulties in addition to PD. [15,16] Additionally, Cd increased the generation of free radicals, which led to oxidative stress and protein and phospholipid degradation (DNA) [16,17] Additionally, it is claimed that Cd is to blame for the development of neurodegenerative conditions like AD. [18,19]

Direct production of free radicals is not possible with cd. On the other hand, it can indirectly produce a number of free radicals, including nitric oxide and hydroxyl superoxide. [20] The creation of hydrogen peroxide, which in turn can generate a large number of free radicals, has also been demonstrated by multiple research. [21] Cd also takes the place of iron and copper in the protein's structure. [22,23]

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. [25-26].

1.3 Flavonoids

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. It is important to keep in mind that these combinations are not dangerous when ingested as part of a typical eating regimen. Flavonoids work as breast cancer chemotherapeutic agents. The inactivation of experts who cause malignant growth, the cessation of the cell cycle, and the choice of apoptosis are fundamental to the antitumor development of flavonoids. Three, six-membered cyclic rings make up the total of the flavonode's critical classes: A ring attached to a heterocyclic C-ring that is connected to a B-ring by a single C-C bond. (Fig. 1.3).

The function of 6-AF in cancer chemoprevention. 6-AF works in a variety of ways, including by inactivating carcinogens. 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 strong; as a result, 6-Aminoflavone was used in the current investigation to test its effectiveness against reactive radicals in a mouse model [27,28]

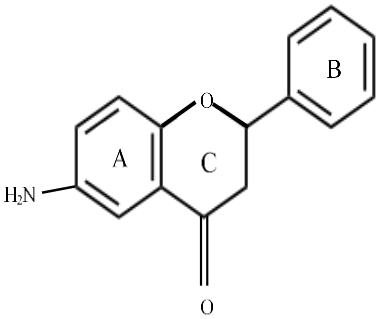

Fig.1.3 Chemical Structure of 6-AF

1.4 Classes of Flavonoids

Flavonoid's includes, catechin's chalcone's flavonols, anthocyanin's, flavanol's flavanonol's and flavone's, flavanone's, Neoflavonoids, Anthocyanins, iso flavonoids (Fig. 1.4). [27-34] These sub-groups are:

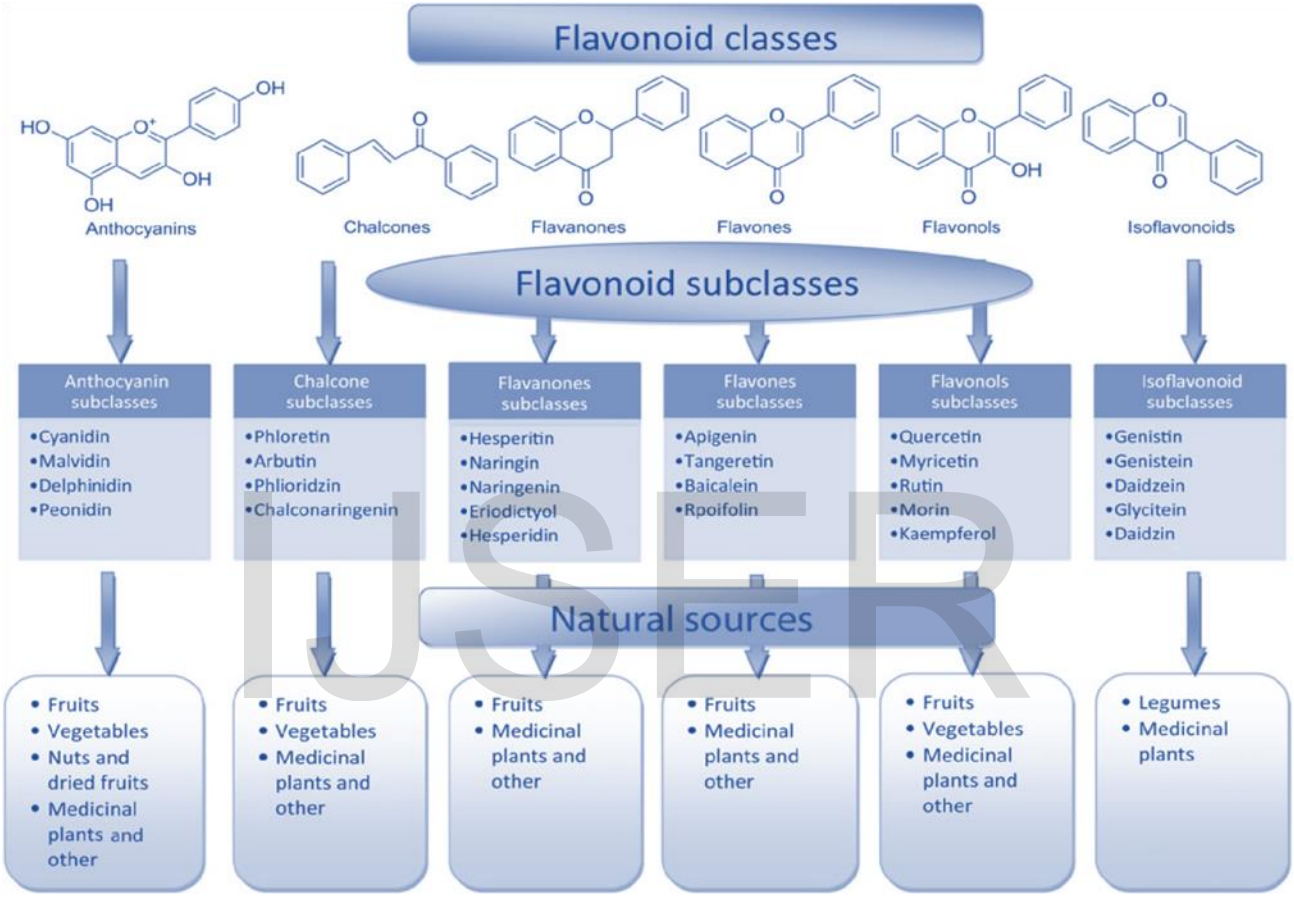

Figure 1.4 a Flavonoid Classes and Sub Classes

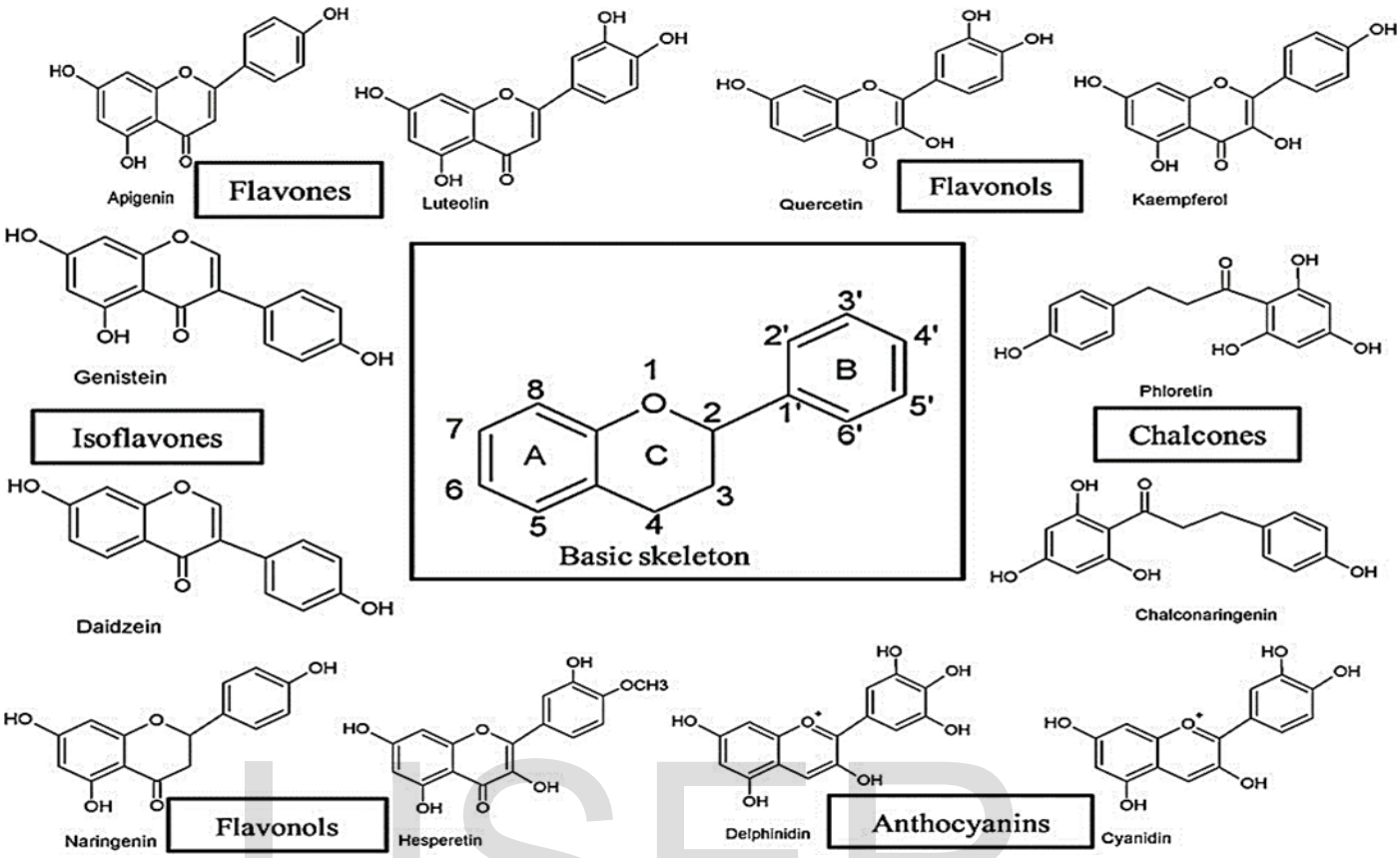

Fig. 1.5 b Basic frame structure of flavonoids and their classes. [27]

1.6 Problem Statement

The health of humans is severely harmed by heavy metals like cadmium. One of the main causes of neurological disorders including (PD) (AD) (HD) is cd. To determine the remedial action to lessen and defeat this harmful disease, proper and ongoing research is required.

1.7 Aim and Goals

The study's objectives were as follows:

1. To investigate the brain damage and poisoning effects of cadmium chloride in mice
2. To research how 6-AF activity reduces Cd-induced neuroinflammation
3. To identify a treatment for the oxidative stress brought on by Cd toxicity.

2.1 List of Chemicals

Tetramethylethylenediamine (TEMED), Methanol,1x Tris buffer saline, Cdcl2 and 6-AF was purchased from Sigma Aldrich, Phosphate buffer saline (PBS), Acrylamide, Skimmed milk,

Ammonium persulfate (APS), Protein assay dye reagent, Bio-Rad solution, ECL (Enhanced chemiluminescence) (Bio-Rad), Tween (1x TBST) Tissue protein extraction reagent, Sodium dodecyl sulfate (SDS).

2.2 List for Instruments

Orbital Shaker (Insta Bioanalytik Pte. Ltd. Singapore), Trans blot (Bio-Rad Germany), Electric Balance , Pipettes Cages, Homogenizer ,UV Spectrometer , Centrifuge, Electrophoresis (Eppendrof USA), ,Incubator (MRC England),Vortex Mixer.

2.3 Animals Housing

For the experimental work, five male mice weighing 26–30g were collected. These mice were bought from the Peshawar Veterinary Research Institute. These mice were kept in a chamber that was 23–24 degrees Celsius, with 12-hour cycles of light and 12-hour cycles of darkness. Water and food were given to them. The (NMRC), associated with the chemistry department, SUIT Peshawar, conducted experimental studies. The committee of the Centre oversaw and carried out the research activities.

Fig. 2.3 Animals Housing

Materials and Methods

i. Experimental Groups

The male mice will be divided into four sub-groups as given under.

i. Control (Normal) mice. Cadmium Chloride treated (1 mg/kg three wks).
ii. Cadmium Chloride (1 mg/kg 3 weeks) + 6-AF (30 mg/kg thrice a wks for last two wks).
iii. 6-Aminoflavone treated (30 mg/kg thrice a week for the last two weeks).

ii. Drug Treatment

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 the exams and exercises were completed, all of the trial mice were slaughtered.

2.4 Test Behavioural

After receiving Cdcl2 injections for 30 days, behaviour tests were conducted. 6-AF, which was indicated in the study's instructions, contributed to the neuroprotective agent drug's successful outcome. Adult albino mice's memory impairment caused by Cdcl2 was repaired by 6-AF.

2.5 Y-maze Test

Y-Maze: As reported, the Y-Maze test was completed [35]. 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 each time 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. Software was used to keep track of the mice's total arm entries and consecutive triplet counts, and to calculate the percentage of alternations 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.

$$\% \text{ Spontaneous Alternation} = \frac{\text{Successive Triplet}}{\text{Total No. of Arm Entries-2}} \times 100$$

Fig. 2.5 Y-Maze Test Apparatus

2.6 Morris Water Test

Water Maze Test by Morris. A test known as the Morris Water Maze was used to look into the hippocampus region's role in long-term spatial learning. The MWM testing apparatus is described in full in a recent study [36,37]. For the 1st three days, the mice were trained twice daily. The mice's 60 second escape delay to locate the submerged platform was then observed. If the mice were unable to 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 various experimental groups are separate. The mice were given two days to recuperate before being subjected to a probe test.

Fig. 2.7 Morris water test Tank

Wester Blotting Analysis

The mice were slaughtered for the Western blot analysis as previously reported [36,37] after the course of therapy. The mice brains were rapidly recovered in their whole, and the hippocampus was carefully separated and immersed in a 1:1 RNA-to-PBS solution on ice. Then, the hippocampal component was homogenized in a T-PER (Thermo Scientific) solution for tissue extraction protein reagent. In order to determine the protein concentration, Bio-Rad protein assay tests were performed to measure the absorbance at 595 nm. Every sample's proteins were normalised to 30 g/group, and an electrophoresis using a SDS PAGE gel 12–15% was carried out.

The proteins were then transferred to PVDF membrane using a semi-dry transblott method Santa Cruz, CA, USA) (Bio Rad) (Santa Cruz Biotechnology. The sandwich was placed, in the transblot machine.This conditions applied for the operation of trans blot machine were1.6, Ampere 5 Volts, Watt 10 and 45 minutes. Different primary antibodies were used, including mouse-derived monoclonal antibodies (actin, PARP-1 , NF-kB, anti-TNF, caspase-3 and PJNK followed by secondary antibodies that were HRP-conjugated against mice from Santa Cruz, CA, USA. After that, the X-rays were developed with the results.

Results and Discussion

3.1 6-AF Inhibited Phospho-JNK Activation in Cdcl2 Induced Adult Mice Brain

Cdcl2 is a well-familiar agent to induce phospho-JNK activation [38] In the current study, we have injected Cadmium chloride intraperitoneally for thrice wks to mice. The outcomes show that Cadmium induced a high expression of phospho-JNK in the brain homogenates of mice. On the other hand we also injected 6-Amino flavone for the two weeks after cd induce phospho-JNK activation to know its inhibitory ability of phospho-JNK proteins as shown in (figure 3.1). Our outcomes revealed that 6- AF reversed the expression of phospho-JNK in the brain of albino mice.

One of the most prevalent neurodegenerative disease mediators is JNK. Our findings are consistent with earlier research because Chen et al. (2018) [39] also showed that cadmium can cause the phosphorylation of the JNK protein in mouse brain tissue. According to [40], the celastrol neuroprotective pharmacological agent Cd caused neuron cell death by phosphorylating JNK and targeting the PTEN-Akt/mammalian target of Rapamycin network. According to our findings, celastrol may protect against Cd-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 SP600125 is highly efficient for slowing disease progression by reducing multiple pathological features in Traumatic Brain Injury mice brains and regulating cognitive dysfunction [41].

It has been crystal clear here that are work is better and similar with rest of research and articles.But the 6-AF is potentially an authentic and applicable drug. It also has the ability to be evaluated against the work of other researchers. The goal of the current investigation was to examine how 6-

AF protects against oxidative stress caused by cadmium in adult mice as well as its neuroprotective effects.

[42] Our outcomes that Rapamycin ameliorates may have, combat in preventing Cd-induced oxidative stress and neurodegeneration diseases.

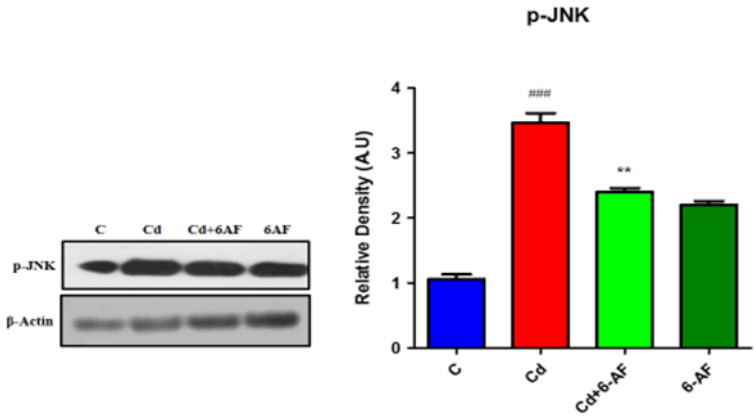

Fig. 3.1 6-AF inhibited p-JNK activation in $Cdcl_2$ Induced adult Mice brain.

Fig.3.1 shown are the immunoblots of phospho-JNK and β-actin along with its histogram for all the 4 experimental groups (n=4/group). β-actin was used as a standard. Image J software was used to quantify protein bands. The bands density were expressed in arbitrary units (A.Us) as the mean ± S.E.M. # significantly different from control and cadmium chloride treated mice $Cdcl_2$ +6-AF is different from control mice, and 6-Aminoflavone vs control mice respectively, **## $p < 0.01$.

3.2 6-AF Inhibited Tumor Necrosis Factor Neuroinflammation in $Cdcl_2$ Induced Mice

Cd was administered intraperitoneally to the male, mice for a wks three to induced neuroinflammation and neurodegeneration in adult albino mice and then treated with 6-AF for two weeks. We have have injecte cadmium chloride for three weeks and upregulated signal activation in the mice brain. We have injected another neuroprotective drug 6-AF for last two week and downregulated signal activation in adult albino mice as shown in (figure 3.2).

Adult albino mice were later slaughtered, and the brains were preserved. Western blotting was performed on the homogenates of the mice's brains. To gauge the degree of inflammation caused by Cdcl2 in mice, many inflammation-related markers were found. The current findings demonstrate that in the brain homogenates of mice, cadmium chloride caused a very high

production of Tumor Necrosis Factor - protein. On the other hand, we also administered 6- Amino Flavone for two weeks to determine its capacity to suppress Tumor Necrosis Factor -proteins. According to the study's most recent findings, 6-AF dramatically altered the expression of TNF- in the brains of albino mice. One of the frequent mediators of neuroinflammation is TNF-α [43].

When compared to mice that had been exposed to Cd, immunohistochemical investigation showed this, according to earlier studies. Mice that had been pre-treated with (SME) showed a decrease in the amount of Tumor Necrosis Factor - appearing as a protein. Our findings are consistent with those of who also showed that Cd can cause the TNF protein to be expressed in the mouse brain.[43].

Protocatechuic acid was shown to protect against Cd-induced inflammation by reducing proinflammatory cytokines like TNF- and interleukin-1, according to the study.[44].

According to earlier research, these findings revealed that tumor necrosis factor and trail augment Cd-mediated death cell by altering the patterns of p53 expression.[45]

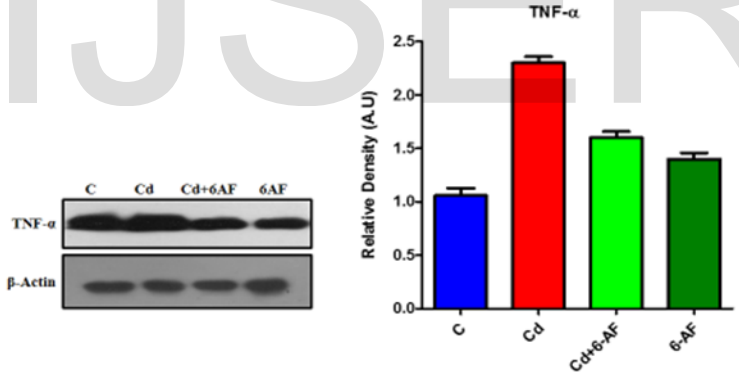

Fig. 3.2 6-AF inhibited Tumor necrosis factor Neuroinflammation in Cdcl2 Induced Mice

Fig. 3.2 The immunoblots of TNF- and -actin, as well as their histograms, are displayed for each of the four experimental groups (n=4/group). Actin served as the benchmark. Protein bands were measured with Image J software. The bands density was reported as the mean S.E.M. # significantly different between control and Cdcl2 treated mice for the bands density in arbitrary units (A.Us). Cdcl2 +6-Aminoflavone differs from control mice, and 6-AF differs from control mice, with **## p 0.01 for each comparison.

3.3 6-AF Inhibited NF-κB And Induced By Cadmium Chloride in Adult Mice Brain

It is well known that Cd can cause the activation of the NF-B signal (Khan et al., 2019) [46] To determine whether cadmium chloride can cause NF-B signal activation, mice were given Cd treatment for three weeks before having their brain homogenates put onto gel for western blotting. 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 4.3). The brains of all the mice were kept after their sacrifice.The brain of mice homogenates and subjected to western blotting. To measure the extent of inflammation neuron creation by $CdCl_2$ in adult albino mice different biomarkers of inflammatory were detected.

The previous finding shown that, following cadmium chloride-induced neurodegeneration and neuroinflammation in adult albino mice, NF-B becomes active in the mouse brain homogenate.

Previous research indicated higher amounts of inflammatory cytokines, including NF-B and -Actin proteins, which were also significantly found. Notably, research on (Nrf-2) silencing and (NF-B) has shown that, correspondingly, Cadcl2 can trigger the NF-B in the brain of albino male mice (Khan et al., 2019).[47]

Additionally, caffeine greatly lessened the effects of Cd on synaptic dysfunction, learning deficits, and neuronal death. Caffeine induces neuroprotection via Nrf-2- and (NF-B) dependent pathways, respectively, in the BV-2 cell lines and HT-22 according to studies of nuclear factor-B (NF-B) inhibition and nuclear factor-2 erythroid-2 (Nrf-2) gene silencing (Khan et al., 2019) [47]. Activation of the redox-sensitive transcription factors NF-B and activator protein-1 (AP-1) and increased expression of oxidative stress-related genes like metal transport protein-1 heme oxygenase-1, metallothionein, glutathione S-transferase pi and were found to be additional signs of Cd-induced oxidative stress in microglia-enriched cultures by Yan et al., 2007 . to summarise, gel-shift assays and real-time PCR revealed that cadmium is harmful to neuron-glia cultures, activating the redox-sensitive transcription factors NF-B and activator protein-1 as well as increased expression of oxidative stress-related genes like glutathione S-transferase pi, metallothionein, metal transport protein-1 heme oxygenase-1were further evidence of Cd-induced oxidative stress.

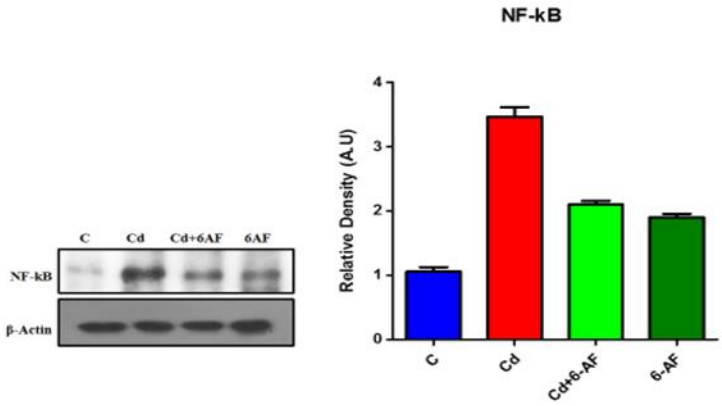

Fig. 3.3 6-AF inhibited NF-κB and instigated by Cd in adult male mice brain

Fig.3.3 Shown are the immunoblots of β-actin and NF-κB along with its histogram for all the 4 experimental groups (n=4/group). β-actin was used as a standard. software image J was used to quantify protein bands. The bands density were expressed in arbitrary units as the mean ± S.E.M. # significantly different from control and Cd treated mice Cd+6-Aminoflavone is different from control mice, and 6-AF vs control mice respectively respectively, **## $p < 0.01$.

3.4 6-AF Inhibited Caspase-3 Proteins in Cdcl2 Administered Mice

A well-known and harmful environmental contaminant is cd. First, we gave adult albino mice Cd injections for three weeks. The outcome demonstrates that in the adult albino mouse brain, the Caspase-3 signal is up-regulated and activated by Cd. Additionally, we have been inducing 6-AF in adult mice for the past two weeks. According to (figure 4.4), the 6-AF decreased Caspase-3 down-regulated expression in the brain of albino mice. In advance, we killed every mouse, took their brains, homogenised them, and then submitted them to western blotting. Image J software was used to measure the bands.

Kanter et al., 2016 [48]showed that In mice treated with Cd, the caspase-3 immune positivity increased in dying neurons. Quercetin treatment in particular reduced the immunological reactivity of dying neurons. The results of the current study demonstrate that quercetin treatment improves the morphology of mice's neurodegenerating hippocampus following Cd exposure.

According to Chong et al., 2017 .'s study [49] -synuclein causes death cell in a dopaminergic neuronal model of PD by increasing oxidative stress, increasing Cadmium uptake, changing caspase-9 and caspase-3 activation, and decreasing the neuroprotective effect of Akt. This is in response to acute Cadmium exposure.

Dong et al., 2015 [50] showed that By triggering protein kinase B (PKB), also known as Akt phosphorylation, downregulating the amount of caspase-3, and improving the neuronal cell endurance mice up to 24 hours, proanthocyanidins therapy in refined mice hippocampus neurons showed potential Cd-instigated excitotoxicity. According to most research, proanthocyanidins play a critical role in neuroprotection mitigates Cadmium-induced oxidative neurotoxicity in mice's brains by influencing the beginning of cell reinforcement status, AChE/Akt phosphorylation , and other processes (lipid peroxidation) controlling layer damage apoptotic protein caspase-3.

[51] previous result showed that In the brains of Cd-treated mice, the anti-apoptotic marker B-cell lymphoma 2 (Bcl2) was lowered while the apoptotic indicators caspase 3 and caspase-9) Associated X Protein (Bax), Bcl2 cytochrome C, and the proteolytic and membrane-bound enzymes, Mg2+-ATPase, Na+ K+-ATPase and Ca2+-ATPase were Additionally, Cadmium treatment markedly reduced the I, II, III, and IV mitochondrial (ETC) complexes in mouse brain tissue.

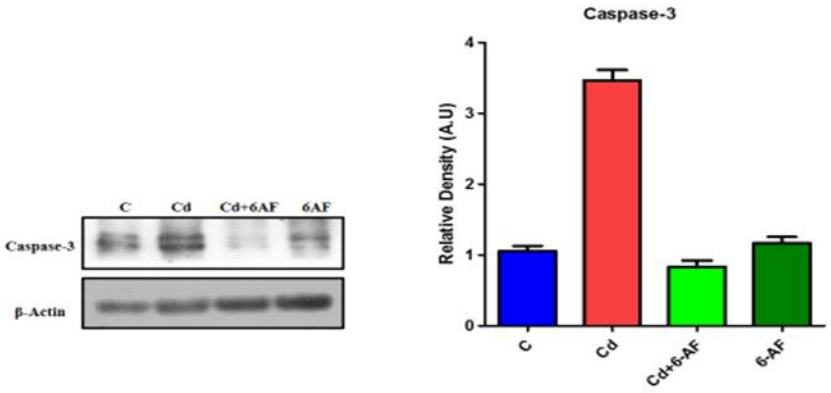

Fig. 3.4 6-AF inhibited Caspase-3 proteins in Cdcl2 Administered Mice

Fig. 3.4 shown are the immunoblots of Caspase-3 proteins and β-actin along with its histogram for all the 4 experimental groups (n=4/group). β-actin was used as a standard. software image J was used to quantify protein bands. The bands density were expressed in arbitrary units as the mean ±

S.E.M. # significantly different from control and Cd treated mice Cd+6-AF is different from control mice, and 6-Aminoflavone vs control mice respectively **## $p < 0$.

3.5 6-AF Inhibited PARP-1 Proteins Expression in Cd Mice

El-kott et al. (2020) [52] reported in earlier literature that Cd causes PARP-1 protein up-regulation. In order to determine if the PARP-1 protein was expressed in the brain homogenate of the experimental mice, we performed the western blotting approach. As shown in our results, 6-AF dramatically reduced the expression of the PARP-1 protein (figure 4.5). In the beginning stages of the experiment, we gave adult albino mice three weeks of interpretive Cd injections. According to our findings, Cd raised the PARP-1 signal in the adult mouse brain. As a result, we treated adult albino mice with the helpful medication 6-AF whose expression of the PARP-1 signal was downregulated. All of the animals were slaughtered, and the homogenised brains were then processed using western blotting. We consequently used the helpful medication 6-AF on adult albino mice whose PARP-1 signal expression was downregulated. After all the mice were killed, the homogenate of their brains was tested using western blotting.

[52] ultimately demonstrated that Kaempferol decreases Cadmium-induced memory deficits as well as oxidative stress, inflammation, and apoptosis in the hippocampus decreasing PARP1 activity and by increasing SIRT1 activity .

According to previous research, oxidative stress, mitochondrial damage, and Cd-induced cell death are all caused by parthanatos and the MAPK signalling pathway. JNK1/2 and p38 are implicated in parthanatos, which also has a synergistic effect on apoptosis when paired with oxidative stress.; Luo et al. 2017 [53]The previous findings showed that whereas Caspase-9, Caspase-9, Caspase-3 and PARP-1 protein levels decreased in response to Cd, the levels of cleaved Caspase-3, Caspase-8, and FasL proteins rose dose-dependently. N-acetylcysteine successfully prevented these changes. N-acetylcysteine served as a protective factor against Cd damage while Cd produced apoptosis in BRL 3A cells via the mitochondrial and FasL pathways (Liu et al., 2016)[54].

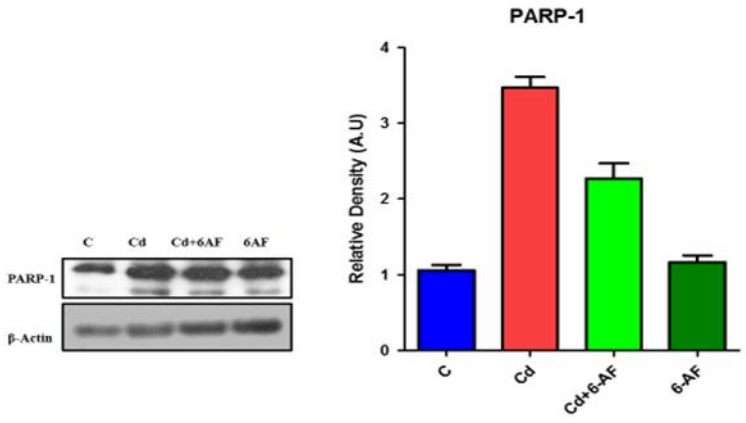

Fig. 3.5 6-AF inhibited PARP-1 proteins expression in Cd Mice

Fig. 3.5 shown are the immunoblots of PARP-1 proteins and β-actin along with its histogram for all the 4 experimental groups (n=4/group). β-actin was used as a standard. software Image J was used to quantify protein bands. The bands density were expressed in arbitrary units as the mean ± S.E.M. # significantly different from control and Cd treated mice Cd +6-Aminoflavone is different from control mice, and 6-AF vs control mice respectively, **## $p < 0.01$.

3.6 6-AF Enhance Memory And Behavioural in Cdcl2 Induced Memory Impaired Mice

3.6.1 Y-Maze Test

Y-maze test was done for recognition of the short term memory. Now this behavior Y-maze test the %age of spontaneously repetition was, considered for the three distribution experimentally groups, over and done with its Y-maze formula. It was set up from the Y-maze test, that the %age of spontaneous repetition of the while the Cd mice exhibited a very low, Normal mice was very high, %age of spontaneous, changes. Entertainingly, the third group of mice receive 6-AF now combined with Cdcrevealed considerably higher percentage repetition against only Cd treated mice (Figure 3.6.1). (A) The bar chart depicting, the % age, of spontaneous, changes during test Y-maze. The recorded data given is as a mean ±, S.E.M.,#pointedly unlike from control and *significantly different from Cd-treated mice, respectively; **## $P < 0.01$.

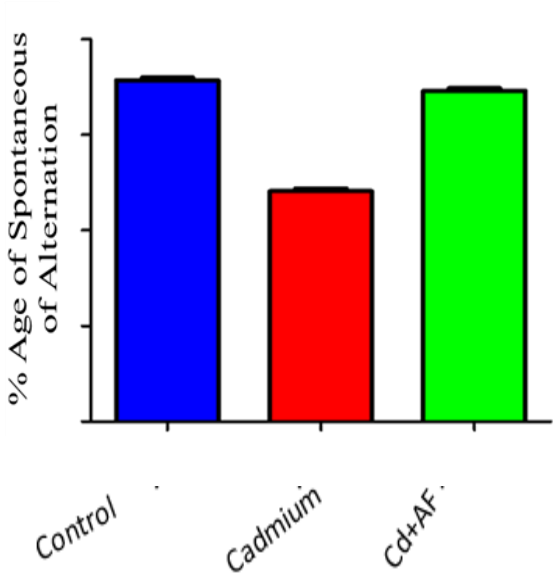

Fig. 3.6.1 6-AF Enhance Memory and Behavioural in Cd Memory Impaired Induced Mice

4. Conclusions

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 also lowering neuroinflammation and being a natural, safe, and accessible therapeutic agent. Additionally, this work demonstrated the molecular mechanism by which 6-AF in Cd causes neurological disease.

5. Recommendations

Despite the fact that the current study focused on 6-AF's neuroprotective and memory-improving effects in mice exposed to Cd. The 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. Additionally, it is advised to thoroughly investigate the 6-AF in vitro model of cell culture to better understand its usefulness as a medication.

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