Advanced Pharmaceutical Bulletin

Accepted Manuscript (unedited)

The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form.

Research Article

How to cite this article:
Mohamed Elbakry MM, Ahmed E, Hammad A, Abo-Zaid O, Farrag M, Abdel-Hamid G. Modulatory Effect of Flavone on Brain Inflammation Induced by Radiation or Nicotine in Rats Flavone in Toxicant-Induced Brain Injury. Advanced Pharmaceutical Bulletin, doi: 10.34172/apb.47640

Modulatory Effect of Flavone on Brain Inflammation Induced by Radiation or Nicotine in Rats Flavone in Toxicant-Induced Brain Injury

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ARTICLE INFO

Keywords:
Flavones;
Neuroinflammation;
Nicotine;
Radiation;
SwissTargetPrediction

Article History:
Submitted: June 27, 2026
Revised: August 21, 2026
Accepted: October 17, 2026
ePublished: November 02, 2026

ABSTRACT

Purpose: Flavones have various beneficial health impacts, including neuroprotective activity, anti-inflammatory, antioxidant, and antimicrobial effects. Exposure to environmental toxicants like nicotine and radiation induces multi-organ toxicity and increases the risk of neurological problems. Therefore, this experimental study aimed to evaluate the anti-inflammatory and antioxidant qualities of flavone (F) against brain injury caused by IR or NIC in rats.

Methods: The potential targets of flavone were identified from the SwissTargetPrediction databases. The functional enrichment analyses were conducted using the KOBAS server. Sixty male Wistar rats were divided at random into 6 equal groups: normal rats; rats received flavone treatment (25 mg/kg b.wt./day) for 2 weeks; rats exposed to nicotine (1 mg/kg b.wt./day) for 3 weeks or radiation (3.5 Gy once/week for 2 weeks) with or without flavone treatment. Oxidative stress markers and calcium levels in the brain were evaluated besides histopathological examinations and grading. Additionally, the levels of caspase1, TLR4, phospho-Tau, cytochrome P450 (CYP2B1, CYP2E1) enzymes were evaluated using ELISA. Furthermore, the gene expression of brain NF-κB, NLRP3, BDNF and CDK5 were estimated.

Results: The results showed that flavone was associated with lower biochemical and histologic injury markers by decreasing CYP2B1 and CYP2E1 levels, oxidative stress, and calcium levels. Moreover, it mitigated neuroinflammation by suppressing the expression of TLR-4, NF-κB, NLRP3, and caspase-1, besides suppressing the CDK5 and Tau protein expression while increasing BDNF expression.

Conclusion: Collectively, flavone alleviated radiation- or nicotine-induced neuroinflammation and neurotoxicity, supporting further preclinical evaluation. However, short-term exposure limits definitive conclusions regarding flavone exact mode of action.

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Introduction

Continuous exposure to a variety of environmental toxins, including air pollutants, cigarette smoke, radiation, asbestos, and others, negatively affects brain functions and raises the risk for brain ageing disorders, brain damage, and neurodegenerative diseases.[1,2] Nicotine is the tobacco's most prevalent alkaloid. Previous studies have reported that besides being extremely addictive, it has major systemic negative effects.[3-5] The brain, lungs, heart, kidneys, and other organs are negatively impacted. It has also been demonstrated that brain circuits linked to negative outcomes for attention deficit hyperactivity disorder are impacted by susceptibility to nicotine addiction.[6-8] Additionally, it has been revealed that smoking increases the risk of neurological disorders like vascular dementia, stroke, Alzheimer's and small artery ischemic disease besides increasing the malfunction of vascular endothelial cells.[9] Moreover, it has been shown that nicotine from cigarettes activates nicotine receptors, which in turn trigger the acetylcholine-dependent release of nitric oxide from the vascular endothelium. This impairs blood-brain barrier (BBB) integrity, boosts reactive oxygen species (ROS) production, and activates pro-inflammatory signals causing loss of brain homeostasis.[10] Additionally, nicotine thickens blood, which reduces blood flow, and has a deleterious effect on the K/Na/Cl co-transporter that is found on the luminal surface of the BBB.[11,12]

Human radiation exposure, whether from natural sources, medical (X-rays, radiotherapy), occupational, or accidental, can have a serious harmful impact on human health, such as organ dysfunction, toxicity, and injury, in addition to suppressing the immune system because of the overproduction of ROS and oxidative stress resulting in oxidative damage of the cellular components and cell death.[13,14] Ionizing radiation exposure has been shown to cause detrimental health effects such as neuroinflammation, death of neurons, disruption of neurogenesis, and consequent cognitive decline.[15,16]

Regarding the mechanisms by which nicotine and ionizing radiation exert neurotoxicity and are consistent with the proposed modes of action, both toxicants generate ROS triggering oxidative stress. Sustained oxidative stress can cause mitochondrial dysfunction, cellular injury, and activation of inflammatory pathways leading to neuroinflammation.[17] This neuroinflammation triggers tau pathology and toxicity promoting neurodegeneration.[18]

Globally, natural products have proven essential in both preventing and treating human ailments. Recognizing the importance of traditional medicine, the World Health Organization has created standards, guidelines, and tactics for medicinal plants. New therapeutics can be developed from medicinal plants, and many contemporary medications are indirectly derived from plants.[19] Various plants, vegetables, and fruits contain phytochemicals called flavonoids, which may have applications in medical chemistry. Several health advantages are associated with flavonoids, such as their antiviral, anticancer, and antioxidant properties. In addition, they possess cardio and neuroprotective effects.[20,21] Flavones are a subtype of flavonoids that vary from other types of flavonoid families due to their lack of oxygenation at the third carbon and carrying a double bond connecting the carbon atoms two and three in addition to having a ketone group at the fourth carbon. They have become the subject of growing interest owing to their biological effects *in vitro* and *in vivo*.[22,23] Flavones are frequently found in various types of foods, such as orange, parsley, red peppers, chamomile, and mint, in addition to spices such as glucosides. Flavones have a wide range of medicinal, pharmacological, nutraceutical, and cosmetic applications because of their minimal toxicity, anti-tumor, anti-inflammatory, antibacterial, antioxidant, anti-diabetic and neuroprotection properties.[24,25]

Examining a common, dual-pathway defense against different environmental neurotoxins is what makes researching the modulatory effect of flavone on brain inflammation brought on by radiation or nicotine

compelling. Although each flavonoid has a proven neuroprotective effect, assessing flavone in particular focuses on overlapping mechanisms of oxidative stress and pro-inflammatory pathways brought on by these comparable insults in brain tissue. Moreover, bioinformatic analysis was performed to explore the novel targets for flavone and the mechanism by which it could improve neuronal damage. Therefore, the current study aimed to evaluate the neuroprotective effect of flavone against nicotine or gamma irradiation induced neuroinflammation in rats.

Materials and methods

Chemicals

Flavone (CAS No. 525-82-6; cat no. F2003; purity ≥99%) was purchased from Sigma Aldrich, USA. Nicotine (cat no. 54-11-5) was obtained from Scharlau, Spain.

Bioinformatic analysis

The chemical structure of flavone was queried in SwissTargetPrediction using PubChem CID 10680 against the Homo sapiens target database, accessed on November 3, 2024. To evaluate the robustness of the predicted targets, sensitivity analyses were performed using probability thresholds of ≥0.10 and ≥0.30. These thresholds yielded 25 and 19 predicted targets, respectively. KEGG PATHWAY and Gene Ontology (GO) enrichment analyses were performed using the KOBAS server for Homo sapiens. The corresponding human gene background was used as the enrichment reference. Statistical significance was evaluated using the hypergeometric/Fisher's exact test, followed by Benjamini–Hochberg FDR correction. Enriched terms with an adjusted $P < 0.05$ were considered significant. Closely related GO terms were grouped and interpreted according to shared biological functions to minimize redundancy and facilitate biological interpretation.

Animals

Male Wistar rats (n=60) were purchased from the Nile Company for Pharmaceuticals & Chemical Industries S.A.E. (Egypt), weighing between 100 and 120 g. The rats were kept for 14 days as an acclimatization period in clean cages at a temperature of 22±2°C, and unrestricted access to water and a pellet diet.

Radiation facility

Utilizing Canadian gamma cell-40 (137 Cesium) at a dose of 3.5 Gy once weekly for two weeks (total exposure dose 7 Gy), rats were exposed to entire body γ-radiation at the National Center for Radiation Research and Technology (NCRRT), Egyptian Atomic Energy Authority according to Elsayed et al.[26]

Experimental design

Animals were divided into experimental groups at random to reduce experimental bias. To conceal the identity of drugs from the staff giving doses, drug formulations were made in coded bottles. Tissue samples along with data files were given random alphanumeric codes for histology and data analysis. Until all outcome evaluations were finished, investigators blinded to treatment allocations carried out histological scoring and statistical analytics.

The rats were distributed at random into six equal groups:

Control Group (C): Normal control rats that received food and water only.

Flavone Group (F): For two weeks, rats received daily oral gavages of flavone (dose: 25 mg/kg b.wt. suspended in 0.5% carboxymethyl cellulose) as stated by Elsayed et al.[26]

Nicotine Group (NIC): For three weeks, rats obtained daily 1 mg/kg b.wt. of nicotine intraperitoneally (i.p.) for three weeks as indicated by Okada et al.[27]

Nicotine + Flavone Group (NIC + F): Rats received NIC injections as demonstrated in the third group along with Flavone treatment as in group two, starting from the beginning of the second week of NIC injection.

Irradiated Group (IR): For two weeks, rats were irradiated entirely once with 3.5 Gy.

Irradiated + Flavone Group (IR + F): For two weeks, rats were irradiated as shown in group five in addition to two weeks of treatment with Flavone.

Collection of samples

Following the last dose of treatment; scarification of the rats was carried out utilizing 1.5g/kg b.wt. urethane i.p. injection following an overnight fast. Brain tissues were excised, washed with saline and divided into parts for biochemical investigations and histological analysis.

Evaluation of brain oxidative stress markers

The assessment of reduced glutathione content (GSH), glutathione peroxidase activity (GPx) and malondialdehyde (MDA) levels were carried out utilizing Bio-Diagnostic, Egypt analytical kits (cat no. GR-2510, GP-2524, MD-2528 respectively).

Estimation of brain tissue calcium level

The brain tissue calcium level was evaluated using a calcium assay kit obtained from Abcam, United Kingdom (cat no. ab102505).

Relative quantification of gene expression level via Realtime-PCR

For the gene expression level quantification of cyclin-dependent kinase 5 (CDK5), nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), brain-derived neurotrophic factor (BDNF) and nucleotide-binding domain like receptor protein 3 (NLRP3); RNeasy kit, RT-PCR kit and SYBR Green PCR Master Mix obtained from QIAGEN, Germany (cat no. 74004, 210212, 330513 respectively) were utilized. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene served as a housekeeping gene. The primer sequences utilized for qPCR analysis are listed in Table (1). Primer designs were optimized for high efficiency, and post-amplification melt-curve analysis was used to confirm single-product specificity. Across the different groups, the raw GAPDH CT values were highly consistent (variation < 0.5CT). Livak & Schmittgen[28] $2^{-\Delta\Delta CT}$ method was employed for the calculation of the target genes fold change. For validation all the samples were analyzed in triplicate.

Table 1. qPCR analysis primers

Target genes	Primers sequences
NF-κB	F: 5′- GGTTACGGGAGATGTGAAGATG-3′
	R: 5′- GTGGATGATGGCTAAGTGTAGG-3′
NLRP3	F: 5′- CTTCAGGCTGATCCAAGAGAAT-3′
	R: 5′- CAGTCTCCATCTGCTGCTTTA -3′
BDNF	F: 5′- CTCTTTCTGCTGGAGGAATACA-3′
	R: 5′- CTGTGACCCACTCGCTAATAC-3′
CDK5	F: 5′- GACCAGCTGAAGAGGATCTTT-3′
	R: 5′- GGTACATTGGGTAGGGCTTATAG-3′
GAPDH	F: 5′- ACTCCCATTCTTCCACCTTTG-3′
	R: 5′- CCCTGTTGCTGTAGCCATATT-3′

2.11. Determination of p-tau, TLT4 and CASP1 levels in brain tissue

ELISA kits acquired from MyBiosource, Inc., USA were employed for phosphorylated tau (p-tau), caspase 1 (CASP1) and Toll-like receptor 4 (TLR4) levels assessment (cat no. MBS765838, MBS705488, MBS029585 respectively).

Determination of CYP2B1, CYP2E1 levels in brain tissue

MyBiosource, Inc., USA ELISA kits were employed for the estimation of brain Cytochrome P450 2B1 (CYP2B1) and Cytochrome P450 2E1 (CYP2E1) levels (cat no. MBS724253 and MBS2023931 respectively).

Histopathological examination

Specimens of brain tissue were chopped off following fixation with ten percent isotonic formalin and dehydrated using increasing alcohol grades. Between Bregma coordinates -1.70 mm and -2.30 mm, brain tissues encompassing the cerebral cortex in addition to the dentate gyrus area of the dorsal hippocampus; slices of 4-6 µm thickness were cut off following xylene cleansing and paraffin embedding. Two representative sections were collected per animal using a methodical uniform random sample technique. Hematoxylin and eosin were employed to stain the acquired tissue sections for a blind histological analysis under an electric light microscope after they had been deparaffinized with xylol, as described by Downie.[29] For each location, morphological assessments were carried out at 400× magnification in five randomly chosen high-power fields per section. The accompanying histopathological grading scheme was used to assess brain lesions. Edema, vascular reaction, neuronal damage and microglial reaction were assessed and as indicated by Takahashi et al[30] the tissue and cell damage severity scoring system ranged from 0 to 3 (normal, mild, moderate and severe damage respectively). A single observer who was blind to the treatment groups examined every slide. The reliability of the intra-rater rating was confirmed by reevaluating 20% of the dataset in a second blindfolded session.

Statistical analysis

To find the sample size needed for a one-way analysis of variance (ANOVA) comparing six different experimental groups, a power analysis was conducted using G*Power software (version 3.1.9.7). The results obtained were found to be normally distributed using Shapiro-Wilk normality test and the data were analyzed by ANOVA followed by Tukey post hoc test utilizing SPSS software version 27. The data were represented as mean±SD and were considered significant at a value of $P < 0.05$.

Results

Functional Enrichment analysis

The sensitivity analysis showed that increasing the prediction probability threshold from ≥0.10 to ≥0.30 reduced the target set from 25 to 19 targets, excluding six lower-probability predictions. Despite this reduction, the major biological enrichment patterns were substantially maintained. At both thresholds, KEGG analysis identified pathways related to metabolic pathways, xenobiotic and drug metabolism by cytochrome P450, steroid hormone biosynthesis, retinol metabolism, arachidonic and linoleic acid metabolism, cAMP/cGMP and calcium signaling, and DNA repair (Figure 1 a). GO analysis similarly retained major functional themes involving oxidation–reduction/oxidoreductase activity, monooxygenase and P450-related functions, steroid and xenobiotic metabolism, DNA damage and repair, protein ADP-ribosylation, apoptosis, inflammatory responses, and oxidative-stress-related processes (Figure 1 b).

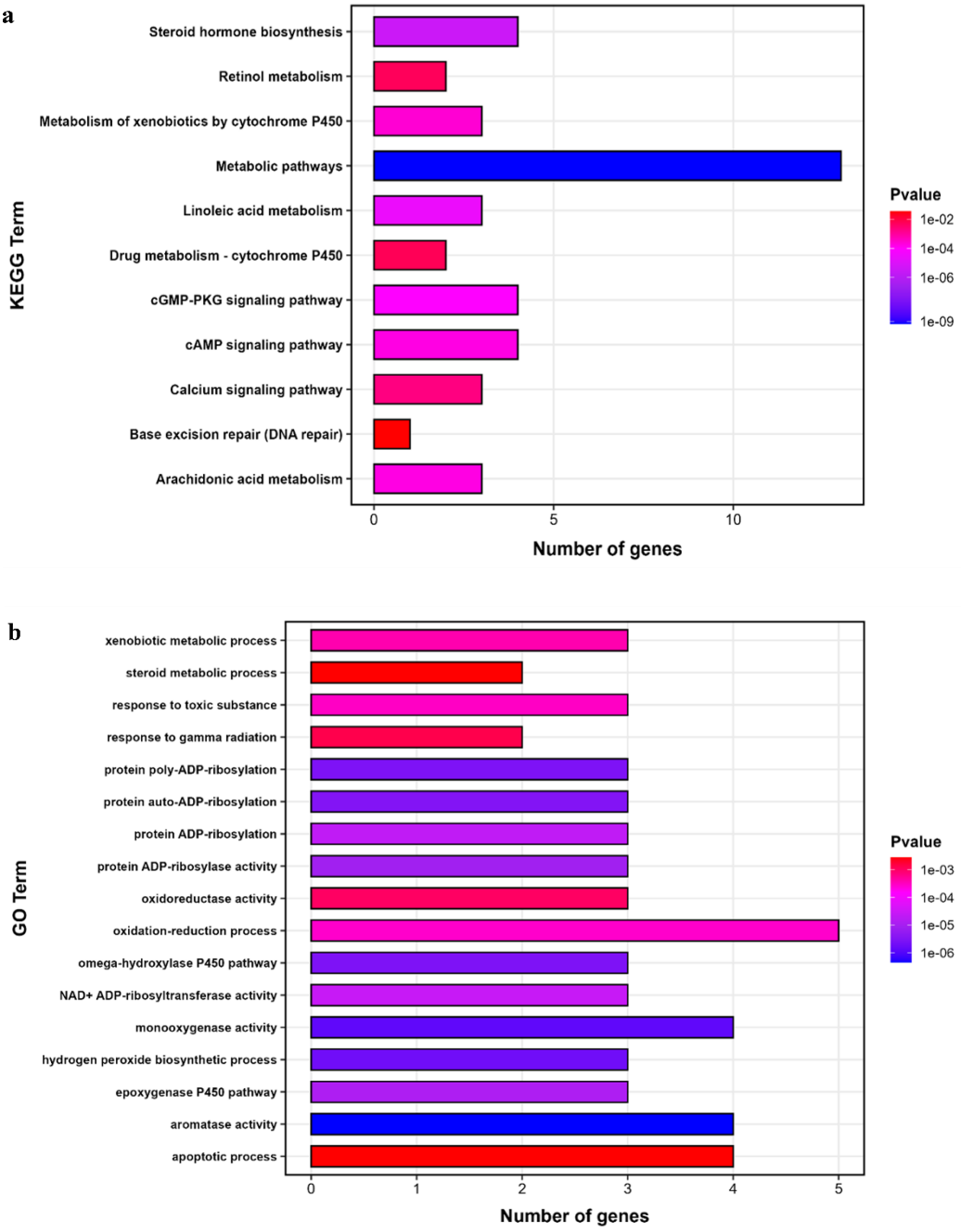


Figure 1. (**a**): KEGG (Koto Encyclopedia of Genes and Genomes) analysis for flavone targets. Bar charts show the KEGG pathways of flavone target genes. (**b**): GO (Gene ontology) analysis for flavone targets. Bar charts show the GO terms for flavone target genes.

Flavone influence on brain CYP2B1 and CYP2E1 levels

The data presented in Figure (2) depicted the levels of brain CYP2B1 and CYP2E1 in the different groups. Intoxication with nicotine or exposure to γ-IR substantially elevated ($P \leq 0.001$) the levels of CYP2B1 and CYP2E1 relative to control rats. Meanwhile, oral gavage with flavone significantly reduced ($P \leq 0.001$) the concentrations of CYP2B1 and CYP2E1 in comparison with NIC and IR untreated rats.

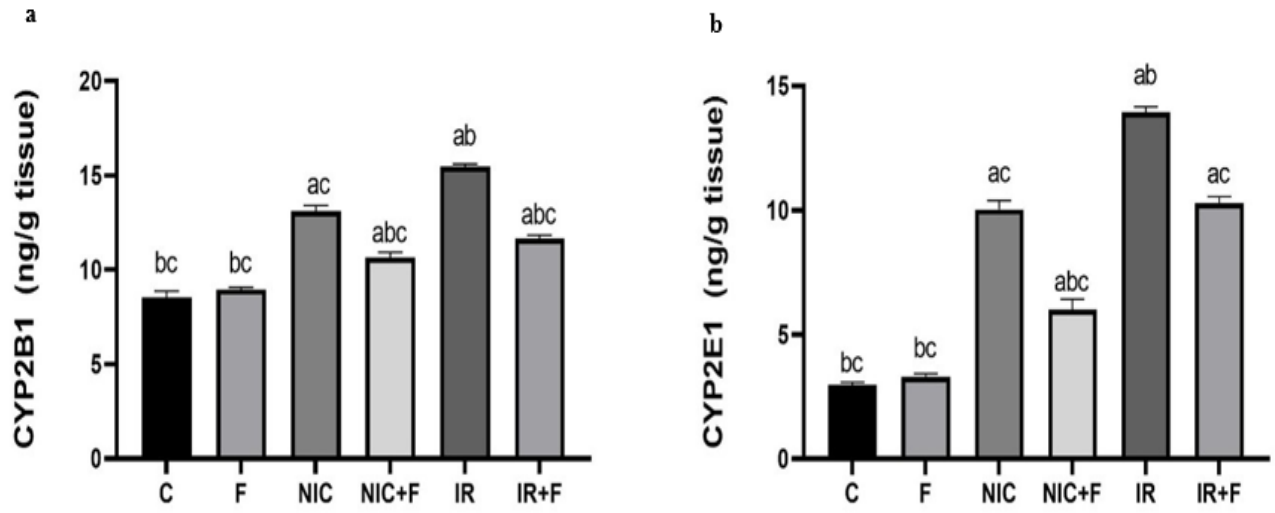


Figure 2. Levels of (**a**) CYP2B1= Cytochrome P450 2B1 and (**b**) CYP2E1= Cytochrome P450 2E1 proteins in brain tissue in the different groups. Values were expressed as Means±SD (N=10 rats per group). Data are significantly different: a: significance ($P < 0.05$) *vs.* control group, b: significance ($P < 0.05$) vs. NIC group and c: significance ($P < 0.05$) vs. IR group. C: control, F: flavone, NIC: nicotine and IR: radiation. C: control, F: Flavone, NIC: nicotine, NIC+F: nicotine + Flavone, IR: irradiated and IR+F: irradiated + Flavone.

Flavone's impact on brain tissue oxidative stress indicators

Data in Table (2) revealed a remarkable decrease ($P \leq 0.001$) in GSH level and GPx activity coupled with a marked elevation in brain tissue MDA level ($P \leq 0.001$) in NIC or IR groups contrasted to normal rats. Alternatively, NIC or IR rats subjected to flavone treatment demonstrated a notable amelioration ($P \leq 0.001$) in the oxidative stress indicators versus untreated animals.

Table 2. Effect of flavone on brain GSH (reduce glutathione) level, GPx (glutathione peroxidase) activity, and MDA (malondialdehyde) levels in the different groups

Groups / **Parameters**	**C**	**F**	**NIC**	**NIC+F**	**IR**	**IR+F**
GSH	20.01±	21.48±	7.21±	9.56±	10.83±	14.71±
(mg/g)	0.77^{bc}	0.49^{abc}	0.54^{ac}	0.27^{abc}	0.59^{ab}	0.63^{abc}
GPx	27.19±	27.64± 0.41^{bc}	10.15±	14.75±	12.9±	19.3±
(mU/ml)	0.89^{bc}		0.58^{ac}	0.69^{abc}	0.67^{ab}	0.85^{abc}
MDA	55.48±	58.04±	118.97±	95.58±	97.04±	78.82±
(nmol/g)	1.08^{bc}	1.04^{abc}	0.88^{ac}	0.53^{abc}	0.35^{ab}	0.7^{abc}

Values were expressed as Means±SD (N=10 rats per group). Data are significantly different; a: significance ($P < 0.05$) *vs.* control group, b: significance ($P < 0.05$) vs. NIC group, and c: significance ($P < 0.05$) vs. IR group. C: control, F: flavone, NIC: nicotine and IR: radiation. C: control, F: Flavone, NIC: nicotine, NIC+F: nicotine + Flavone, IR: irradiated and IR+F: irradiated + Flavone.

Flavone influence on brain tissue calcium level

As shown in Figure (3), brain calcium level was considerably increased in rats intoxicated with NIC or exposed to IR ($P \leq 0.001$) in comparison with the control group. On the contrary, flavone treatment significantly diminished the calcium level in either NIC or IR rats ($P \leq 0.001$) compared to their relative untreated rats.

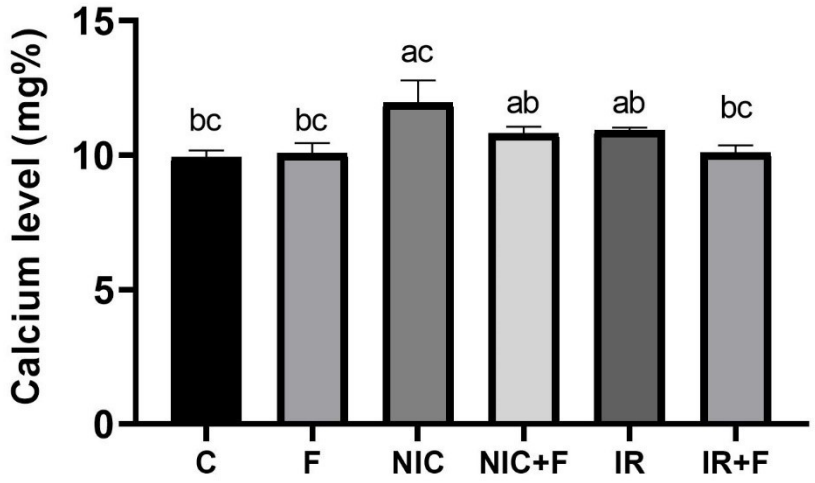


Figure 3. Level of brain calcium in the different groups. Values were expressed as Means±SD (N=10 rats per group). Data are significantly different; a: significance ($P < 0.05$) vs. control group, b: significance ($P < 0.05$) vs. NIC group and c: significance ($P < 0.05$) vs. IR group. C: control, F: flavone, NIC: nicotine and IR: radiation. C: control, F: Flavone, NIC: nicotine, NIC+F: nicotine + Flavone, IR: irradiated and IR+F: irradiated + Flavone.

Flavone effect on brain inflammatory mediators

The current results in Figure (4) elicited a significant increase in the levels of the TLR4 as well as the caspase-1 protein in NIC or IR groups. On the contrary, flavone notably reduced the protein levels of the TLRP4 and caspase-1 in comparison with untreated rats. Additionally, considerable upregulation ($P \leq 0.001$) in the gene expression of NF-κB and NLRP3 in both NIC and IR groups was illustrated in contrast to healthy rats. However, treatment with flavone remarkably suppressed the gene expression of NF-κB and NLRP3 in comparison with untreated rats.

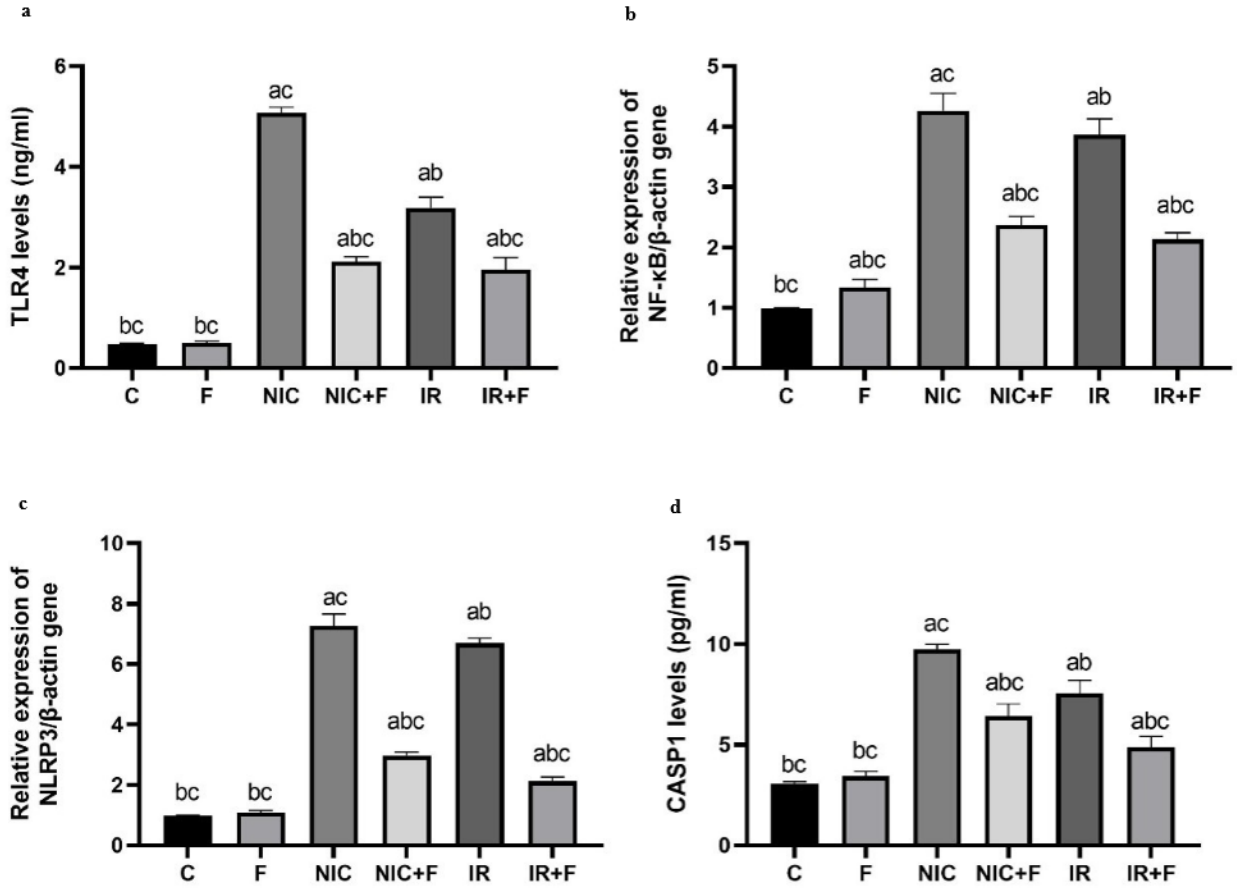


Figure 4. Flavone impact on brain inflammatory mediators; (**a**): TLR4=Toll-like receptor 4, (**b**): NF-κB= nuclear factor kappa-light-chain-enhancer of activated B cells, (**c**): NLRP3=nucleotide-binding domain like receptor protein 3 and (**d**): CASP1=Caspase-1 in different groups. Values were expressed as Means±SD (N=10 rats per group). Data are significantly different; a: significance ($P < 0.05$) vs. control group, b: significance ($P < 0.05$) vs. NIC group and c: significance ($P < 0.05$) vs. IR group. C: control, F: Flavone, NIC: nicotine, NIC+F: nicotine + Flavone, IR: irradiated and IR+F: irradiated + Flavone.

Impact of Flavone effect on neurotoxicity markers CDK5, p-Tau and BDNF

Figure (5) revealed a substantial overexpression of CDK5 gene coupled with pronounced increment of Tau protein in the brain tissues of rats intoxicated with nicotine or exposed to IR than the control group. Meanwhile, the brain BDNF gene expression was downregulated significantly in both NIC and IR groups as opposed to healthy animals. Conversely, NIC or IR rats administrated flavone showed a notable improvement in the measured indicators manifested by significant suppression of the CDK5 gene expression and Tau protein content as well as considerable upregulation of the BDNF expression ($P \leq 0.001$) as opposed to untreated groups towards healthy animals.

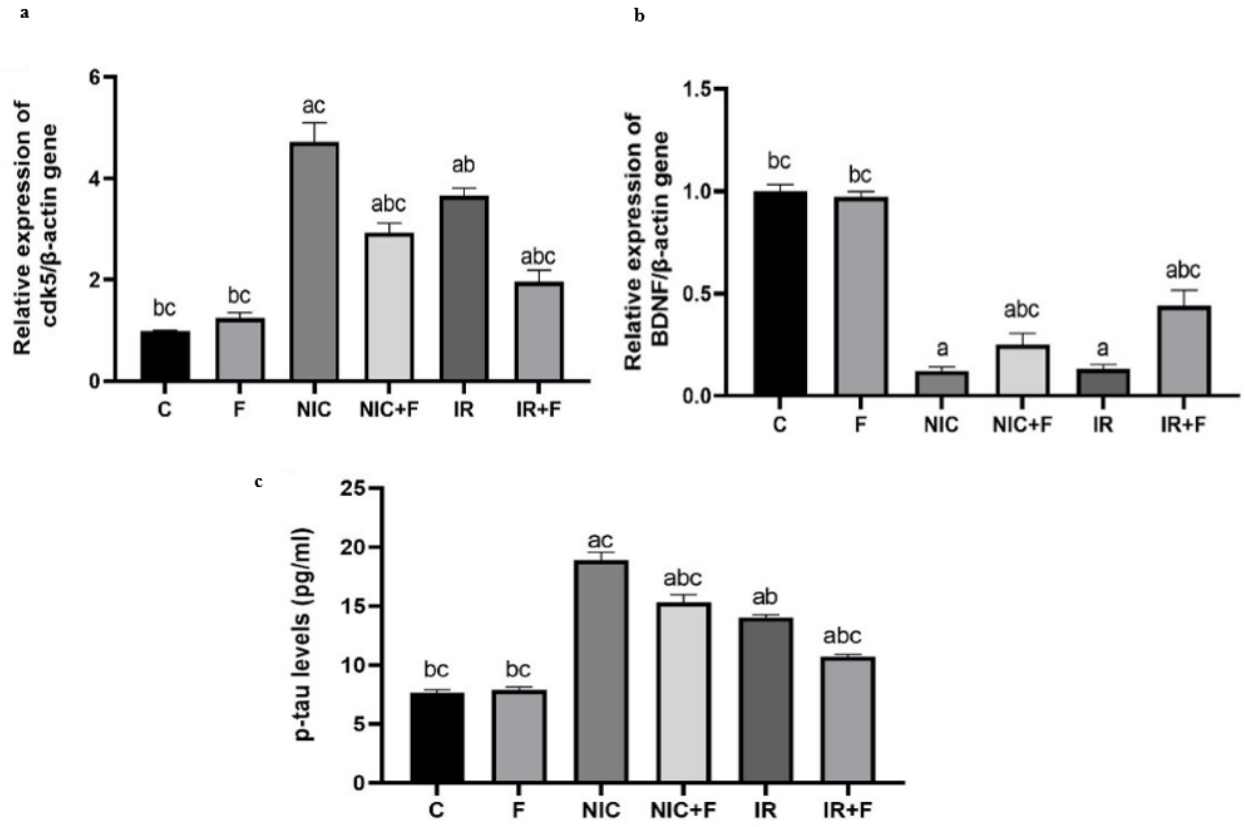


Figure 5. Relative quantification of brain (**a**) CDK5= cyclin-dependent kinase 5, (**b**) BDNF= brain-derived neurotrophic factor genes and (**c**) levels of p-tau= phosphorylated tau protein in the different groups. Values were expressed as Means±SD (N=10 rats per group). Data are significantly different; a: significance ($P < 0.05$) *vs*. control group, b: significance ($P < 0.05$) vs. NIC group and c: significance ($P < 0.05$) vs. IR group. C: control, F: Flavone, NIC: nicotine, NIC+F: nicotine + Flavone, IR: irradiated and IR+F: irradiated + Flavone.

Histopathological studies

Cerebral cortex

Histopathological examination of control and flavone groups cerebral cortex displayed a normal histological anatomy (score=0), with small-sized blood capillaries positioned between layers of nerve cells stacking without any sharp borders. The nerve cells had rounded or oval nuclei with noticeable nucleoli encircled by scanty basophilic cytoplasm (**Figure 6 a & b**). Conversely, localized gliosis and nuclear pyknosis were observed in NIC group cerebral cortex as an indication of moderate neurological degeneration. A little quantity of neurons displayed apoptosis which appeared as dense eosinophilic bodies. Additionally, mild microglial and vascular reactions and edema were observed (**Figure 6 c**). Meanwhile, NIC group treated with flavone cerebral cortex demonstrated a substantial enhancement as compared to the NIC group, which seemed to have mild neurons vacuolar degeneration. A few nerve cells looked undersized and heavily stained. Several glial cells and neuronophagia were detected (**Figure 6 d**). For the IR group, the cerebral cortex revealed moderate neural degenerative changes, glial cell-encircled nuclear pyknosis, neuronophagia, as well as severe vascular reactions. Also, some apoptotic neurons were observed in conjunction with cerebral blood capillary perivascular edema (**Figure 6 e**). However, cerebral cortex tissue section of the IR rats treated with flavone revealed localized gliosis as well as mild neurons swelling. A few nerve cells were shrunk and darkly stained within the cellular haloes zone (**Figure 6 f**).

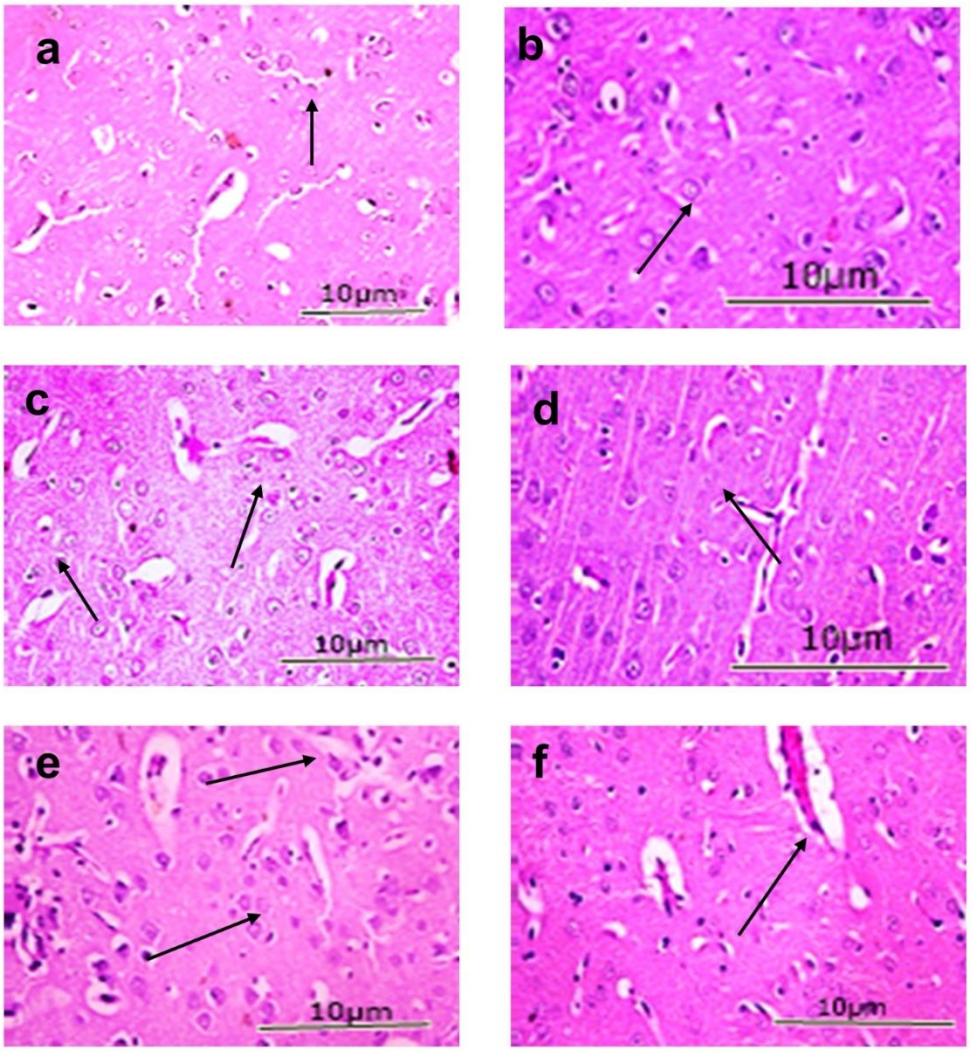


Figure 6. Histological findings of brain cerebral cortex in the different groups (Hematoxylin and Eosin; 400x magnification). (**a**) C group demonstrated a typical histological arrangement made up of many layers of neurons arranged without distinct sharp borders along with tiny blood capillaries (arrow). (**b**) F group indicated normal cerebral cortex histology (arrow). (**c**) NIC group cerebral cortex illustrated some apoptotic neurons in addition to cerebral blood capillaries perivascular edema (arrow). (**d**) Cerebral cortex of NIC+F group showed localized gliosis and a mild neural swelling (arrow). (**e**) IR group cerebral cortex represented a moderate degree of degeneration of neurons, with localized gliosis and nuclear pyknosis (arrow). (**f**) IR+F rats cerebral cortex illustrated mild nerve cells vacuolar degeneration (arrow). C: control, F: Flavone, NIC: nicotine, NIC+F: nicotine + Flavone, IR: irradiated and IR+F: irradiated + Flavone.

Hippocampus

The dentate gyrus area of the control and Flavone groups hippocampus section displayed a typical histological morphology made up of compacted granular cells with darkened nuclei, grade (0). Pyramidal cells and glial cells were visible in the molecular layer. Three layers were identified by high-power analysis of the hippocampus: polymorphic, pyramidal, and molecular layers (**Figure 7 a & b**). Meanwhile, a disorganization of molecular cell layers besides reduced large pyramidal cells size were observed in NIC group hippocampus dentate gyrus area (**Figure 7 c**). Conversely, treatment with flavone (NIC+F group) resulted in normal arrangement of the granular cell layers, darkened nuclei along with decreased large pyramidal cells size (**Figure 7 d)**. The histological section of the hippocampal dentate gyrus area of the irradiated rats revealed cellular disorganization with nuclear pyknosis and moderate degeneration of some molecular cell layers besides mild reactions and edema (**Figure 7 e**). However, the histological section of the dentate gyrus region of the hippocampus in irradiated rats subjected to flavone treatment (IR+F) indicated large pyramidal cells shrank in size, disarray within cells organization and

darkened nuclei. Additionally, granular cell layers displayed a typical pattern (**Figure 7 f**). The accompanying histopathological grading scheme was summarized in Table (3).

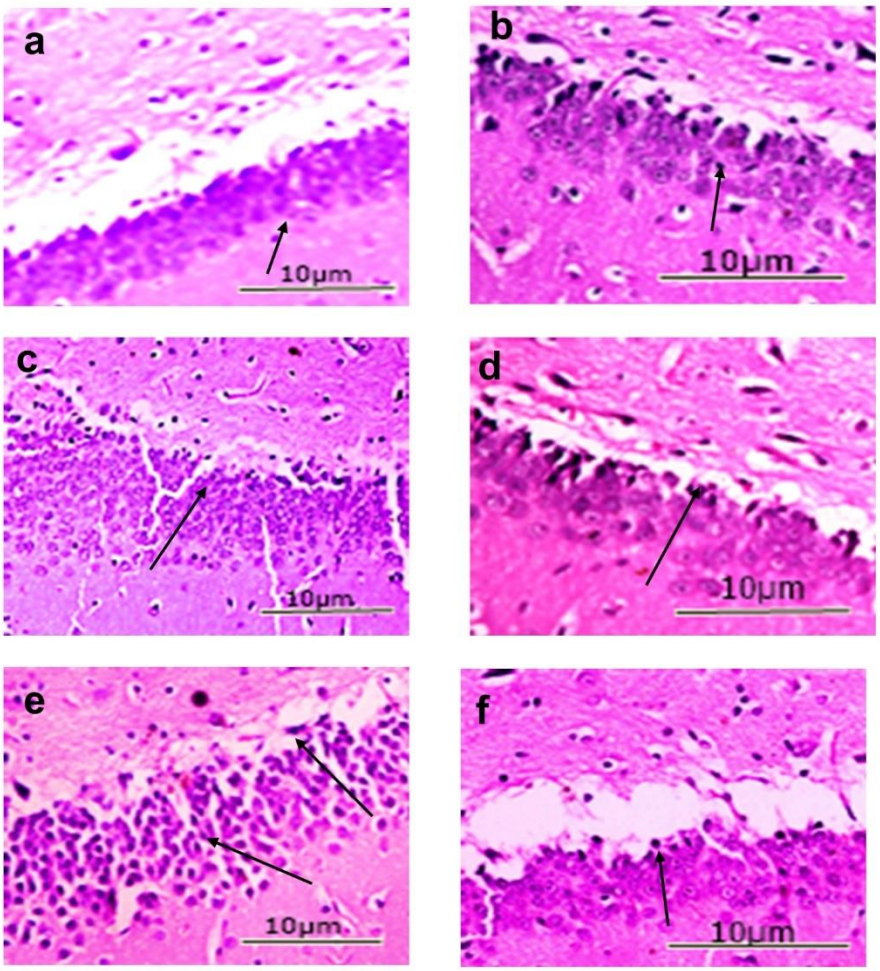


Figure 7. Histopathological findings of hippocampus in the different groups (Hematoxylin and Eosin; 400x magnification). (**a & b**) C and F rats hippocampal dentate gyrus region demonstrated regular histological architecture, which consisted of compact granular cells without any significant pathological changes (arrow). (**c**) NIC group hippocampal dentate gyrus region showed disorganization of molecular cell layers besides large pyramidal cells size shrinkage (arrow). (**d**) NIC+F rats revealed large pyramidal cells size reduction (arrow). (**e**) Hippocampal of the dentate gyrus region in IR rats illustrated nuclear pyknosis and degeneration in a few molecular cell layers (arrow). (**f**) IR+F group hippocampal dentate gyrus area depicted decrease in the large pyramidal cells size (arrow). C: control, F: Flavone, NIC: nicotine, NIC+F: nicotine + Flavone, IR: irradiated and IR+F: irradiated + Flavone.

Table 3. Histopathological grading and scoring of brain injury

Groups	Neuronal damage		Microglial reaction		Vascular reaction		Edema	
	Cortex	Hippocampus	Cortex	Hippocampus	Cortex	Hippocampus	Cortex	Hippocampus
C	0	0	0	0	0	0	0	0
F	0	0	0	0	0	0	0	0
NIC	2	1	1	0	1	0	1	1
NIC+ F	1	1	1	0	1	1	1	0
IR	2	2	1	1	3	1	2	1
IR+F	1	1	1	0	0	0	1	0

C: control, F: Flavone, NIC: nicotine, NIC+F: nicotine + Flavone, IR: irradiated and IR+F: irradiated + Flavone.

Discussion

The present research demonstrated that exposure to toxicants such as γ-IR and nicotine (NIC) promoted neurotoxicity. Brain cytochrome P450 (CYP) enzymes regulate various neurological responses, including behavioral, biotransformation response, and neurotoxicity, besides their role in drug metabolism of either endogenous compounds or drugs and xenobiotics.[31,32] Rat brain and neural tissue have a significant amount of CYP2E1,[33] and a very low expression of CYP2B, which is responsible for the metabolism of nicotine to cotinine.[34]

The current investigation data indicated a significant boost in both CYP2B1 and CYP2E1 following exposure to γ-IR or NIC in rats' brains, indicating the harmful impact of both toxins on the brain and is in alignment with former findings that showed both radiation and NIC resulted in the elevation of both CYP2E1 and CYP2B1.[35,36] Various studies showed that treatment with nicotine provoked cerebral CYP2E1 activity,[37] as well as the brain expression of CYP2B.[38] Additionally, it was found that exposure of rats to γ-radiation or X-rays elevated the mRNA and protein levels of some cytochrome P450, such as CYP2B1 and CYP2E1.[39,40]

Notably, it was reported that increased expression of CYP2E1 remarkably intensified neurotoxicity and cellular damage by generating ROS and provoking oxidative stress as well as inflammation in the brain.[41] Consistently, Khaled et al[42] reported oxidative damage after nicotine administration due to cytochrome P-450 stimulation and excessive generation of ROS. Subsequently, the resulting ROS interacts with the cellular biomolecules (lipid, protein and DNA) of the brain mediating lipid peroxidation, protein modifications and DNA damage leading to brain oxidative damage and cellular toxicity.[43,44] Additionally, it was revealed that nicotine-mediated oxidative stress significantly elevated lipid peroxidation in the brain of rats,[45] leading to long-term neurochemical and behavioral alteration and promoting neurodegenerative diseases.[46,47] Furthermore, IR exposure promoted ROS generation through radiolysis of water and molecular ionization which augmented brain oxidative stress.[48,49]

However, the current bioinformatic analysis explored that flavone has a crucial role in regulating cytochrome P450, NF-kappa B signaling, and oxidative stress. Additionally, flavone administration resulted in a marked amelioration in brain oxidative markers because of its powerful antioxidant capacity and ability to scavenge free radicals because of the ketonic group besides the double bond across carbon two and three improves different free radicals scavenging, including reactive nitrosative and oxidative species. Antioxidant and pro-oxidant enzyme levels within cells can be altered by flavones. Additionally, they can stop the activity of enzymes that produce free radicals, like nicotinamide adenine dinucleotide phosphate oxidase and inducible nitric oxide synthase.[50-52] Flavone treatment distinctly diminished CYP2B1 and CYP2E1 levels, which are attributed to its powerful antioxidant/anti-inflammatory potential, thus, alleviating oxidative damage, maintaining the integrity of the BBB, and lessening brain injury in agreement with agrees with earlier findings.[44,53,54]

Numerous neural functions depend on maintaining mitochondrial calcium (Ca^{2+}) homeostasis.[55-57] Excessive levels of ROS-dysregulated calcium signaling and homeostasis leading to Ca^{2+} content overload.[58] Brain calcium was considerably elevated in the present research following γ-IR or intoxication with NIC in rats. Wu et al[59] revealed that X-ray irradiation enhances extracellular Ca^{2+} influx into cells, leading to Ca^{2+} overload mediated by the Orai3 Ca^{2+} channel through store-operated Ca^{2+} entry in rat brain microvascular endothelial cells. Additionally, it was reported that nicotine augmented intracellular Ca^{2+} levels by increasing the influx of extracellular Ca^{2+}, mainly through L-type voltage-gated channels in a fashion dependent on increasing the calcium permeability of the nicotinic acetylcholine receptor and its alpha-4-beta-2 subtype,[60,61] in addition to its release from the endoplasmic reticulum.[62]

Interestingly, altered Ca^{2+} signaling impeded astrocyte function and triggered neurotoxic inflammation.[63] Mitochondrial damage after Ca^{2+} overload augments ROS production which in turn activates NLRP3.[64,65] According to Mekala et al[66] Ca^{2+} entry into the cytosol, promotes various pro-apoptotic signaling pathways and activates caspase-1 and the assembly of inflammasomes. The current study data revealed that γ-IR or NIC mediated a remarkable overexpression of the TLR4 protein, NF-κB, NLRP3 mRNA, as well as caspase-1 protein levels in the brain tissues. Our findings correspond with earlier research demonstrating TLR-4, NLRP3, caspase-1 and NF-κB overexpression in brain disorders in animal models.[67,68] Moreover, a growing body of evidence has indicated that γ-IR and NIC provoke neuroinflammation.[69,70] Lei et al[71] indicated that TLR4 production is upregulated after brain injury which in turn activates its downstream factor NF-κB. Moreover, the activated NF-kB triggers NLRP3 inflammasome, and proinflammatory mediators,[72] with subsequent activation of Caspase-1.[73]

Furthermore, it has also been indicated that NIC increases ROS production stimulating TLR4, Nf- κB, TNF-α and Il-8,[74-76] and TLR4 overexpression promoted NLRP3 inflammasome activation aggravating the neuro-inflammatory response and causing brain damage.[77,78] In response to neuronal cell damage induced by radiation, the BBB integrity was impaired allowing the passage of systemic inflammatory cells to the brain[15] besides, the activation of the microglia, astrocytes, and endothelial cells accompanied by a release of inflammatory mediators and the modification of inflammatory-related transcription factors (NF-κB) together with the production of ROS thus, promoting the neuroinflammatory response cascade.[79-81] Furthermore, former investigations exhibited the overexpression of TLR4 in irradiated animals.[82,83] Additionally, irradiation increased CASP1 levels inferred its role in neurodegeneration, BBB damage, brain injury and its inhibition aids in repairing processes.[84-87]

Activation of astrocytes and microglia, overexpression of NLRP3, ROS surpluses and other toxic agents besides disturbed BBB integrity and release of inflammatory mediators, altogether contribute to neurodegeneration.[88-90] Moreover, impaired Ca^{2+} influx triggers mitochondrial dysfunctions, energy deficiency and generation of ROS,[50] contributing to neuronal cell death leading to brain damage and neurodegeneration,[91,92] manifested by aggravating tau pathology.[93,94] The neurotoxic stress orchestrated with increased Ca^{2+} triggers aberrant activation of the CDK5 through its binding with its activator p25 with subsequent hyperphosphorylation of various downstream substrates aggravating the neurological toxicity. Sun et al[95] indicated a considerable upregulation in the CDK5 expression was observed in the IR group confirming impaired neurogenesis, neurotoxicity and neurodegeneration. Collectively, this abnormal activation of the CDK5 and the upstream regulatory kinase of tau, leads to hyperphosphorylation of tau.[96-98]

The current results align with Salem et al[67] who declared that the overexpression of the TLR4/ NF-κB/NLRP3 inflammasome was associated with a pathological downregulation of the neuroprotective factor BDNF. According to Ebrahim et al[99] radiation lowers BDNF levels in rats. Furthermore, repeated exposure to nicotine in rats reduced brain BDNF expression at the protein and mRNA levels significantly.[100]

Coinciding with the current research histopathological results, Elgayar et al[101] demonstrated that the administration of nicotine caused neuronal apoptosis in the temporal cortices of rats and reduced the number of dendritic spines and the size of perikria in the prefrontal cortex. Additionally, nicotine resulted in a reduction of cell layer thickness and cellular elements in the cerebellum, along with neurodegenerative changes in the hippocampus and striatum. Furthermore, rats exposed to γ-irradiation experienced significant neural degeneration in the cerebral cortex with pyknosis and loss of the nucleus and condensation of the cytoplasm, apoptosis of neuronal cells, along with substantial vacuolation in the granular cell layers linked to neuronophagia in the hippocampus, as well as perivascular edema and cerebral blood vessels obstruction.[16,102]

Meanwhile flavone administration showed a notable amelioration against γ-IR and NIC deleterious impact on brain. These observations show that flavones might serve as a potential neuroprotective agent, and its neuroprotective impact may be explained by its anti-inflammatory and antioxidant abilities that can enhance the endogenous pathway of neuroplasticity to acquire central nervous system functions and protect neurons and glial cells from injury caused by neurotoxins.[56,58]

Flavone treatment manifested a notable reduction in brain Ca^{2+} levels. It was demonstrated that some flavones along with other flavonoids regulate neural processes by balancing Ca^{2+} homeostasis by decreasing Ca^{2+} influx via plasma membrane Ca^{2+}-ATPase[103] as well as voltage-gated calcium channels[104] thereby modulating Ca^{2+}-regulated biological functions such as lowered ATP generation, the production of free radicals and apoptosis in addition to attenuating neuroinflammation and hindering the neurotoxicity of hyperphosphorylated tau protein.

Based on its anti-inflammatory properties, flavone either blockades the assembly of inflammasomes mediated by signaling molecules, TLR4/NF-κB/NLRP3 or suppresses the production of NLRP3 inflammasome-related components like NLRP3, IL-1β/ 18, and caspase-1.[105,106] Concurrently, inhibiting TLR4/NF-κB and NLRP3 inflammasome switched microglia from M1 to homeostatic M2 displaying a neuroprotective potential against neurotoxicity[107,108] by depleting the p-Tau level and preventing their accumulation into neurofibrillary tangles by suppressing activity of Tau kinases.[93,109] Additionally, flavone modulated tau phosphorylation and reduced tau aggregation by encouraging Aβ peptide clearance.[110,111]

Various studies reported that restraining CDK5 activity ameliorated neuronal functions and hampered inflammation and tau hyperphosphorylation.[98,112] Moreover, it was found that as a flavonoid, quercetin reduced the NLRP3 inflammasome's activation[113] in addition to hindering abnormal tau levels via inhibiting the Ca^{2+}-calpain-p25-CDK5 pathway.[114] Filho et al[115] showed that chrysin increased the brain levels of BDNF in stressed mice. Consequently, flavones are gaining much attention as therapeutically multifunctional cytoprotective agents since they may simultaneously affect several brain targets.[116,117]

Even though the current research highlights the promising effect of Flavone in modulating neuroinflammation, some limitations arise such as the duration of the study was short, further in-depth assessments are required to explore and fully understand the exact mechanisms underlying the neuroprotective effects of flavone in chronic exposure models. Additionally, thorough profiling and physiological safety monitoring will be required to determine Flavone's therapeutic index. The experimental design also lacked a standard reference positive control; including a benchmark drug might assist in directly evaluating comparative potency. Moreover, bioinformatics results could provide a basis for upcoming bioactivity and target-validation tests.

Conclusion

In summary, flavone demonstrated significant protective effects against nicotine- or radiation-triggered neurotoxicity, perhaps by systematically reducing the detrimental effects that oxidative stress causes to the brain and ameliorating the brain architecture. Moreover, it modulated the inflammatory pathways indicators (TLR4/NF-κB and NLRP3), diminished CDK5 expression and tau aggregations alongside with BDNF gene expression level improvement. Those preceding observations were further substantiated by histological examination. Overall, it can be concluded that flavone is an auspicious neuroprotective agent against environmental toxins inducing brain injury. These results warrant further mechanistic studies and translational evaluation in chronic exposure models.

Ethical Statement

All procedures were conducted as per ARRIVE guidelines. Moreover, the Research Ethics Committee of the National Center for Radiation Research and Technology, Egyptian Atomic Energy Authority, which is set up and run in accordance with the CIOMS and ICLAS international guiding principles for biomedical research involving animals, approved every procedure used in this study. Animal ethics approval (REC-NCRRT, P/52A/24) was obtained prior to animal recruitment and start of experiments. Every effort was made to alleviate the suffering of animals. Animal welfare and health were evaluated daily throughout the experiment by keeping an eye on physical characteristics. Shielded containment and calibrated dose monitoring were used to manage radiation exposure in accordance with established biosafety and radiation safety procedures. Pre-established humane endpoints such as extreme lethargy or signs of acute radiation sickness were defined to prompt early intervention or euthanasia. The animals were humanely put to death at the end of the experiment utilizing urethane i.p. injection (1.5 g/kg b.wt.) to guaranty total anesthesia before tissue harvesting.

Authors' Contribution

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Consent for publication

Not applicable

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or non-profit sectors.

Acknowledgements

We thank Prof. Dr. Ahmed Osman (professor of Pathology, Faculty of Veterinary Medicine, Cairo University) for his assistance in the histopathological assessment.

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