Morsy *et al. Beni-Suef Univ J Basic Appl Sci* (2024) 13:93
https://doi.org/10.1186/s43088-024-00526-5

Beni-Suef University Journal of Basic and Applied Sciences

RESEARCH

Open Access

Nano-Curcumin improves caffeine-induced cerebral alterations in male Wistar rats by modifying oxidative stress, inflammation, and COX-2/NF-κB/Nrf2 signaling

Moustafa M. Morsy[1], R. G. Ahmed[2*] and Mohammed Abdel-Gabbar[1]

Abstract

Background This research aims to determine the probable protective effect of nano-curcumin (N-CUR) on caffeine (1,3,7-trimethylxanthine)-induced neurotoxicity in cerebral rats.

Methods Twenty-four male Wistar rats were divided into three groups: control, caffeine (150 mg kg^{-1}), and caffeine (150 mg kg^{-1}) treated with N-CUR (300 mg kg^{-1}). All treatments were administrated by gavage every day for a month.

Results Administration of caffeine significantly elevated the levels of serum interleukins 6 (IL-6), tumor necrosis factor-alpha (TNF-α), vascular endothelial growth factor (VEGF), and cyclooxygenase2 (COX-2). Also, there was a significant increase in levels of cerebral malondialdehyde (MDA), significantly diminished glutathione (GSH), and superoxide dismutase (SOD) activity. Caffeine administration significantly downregulated the gene expression of nuclear factor erythroid 2-related factor 2 (Nrf2) and upregulated the expression of nuclear factor kappa-light-chain enhancer of activated B cells (NF-κB). Administration of N-CUR caused a significant amelioration in TNF-α and IL-6 levels and a significant rise in SOD activity, while it caused a significant downregulation in NF-κB mRNA expression. Additionally, N-CUR has exerted insignificant amelioration of COX-2 and MDA contents and Nrf2 mRNA expression compared to the caffeine-treated group.

Conclusion N-CUR may have a mild to moderate ameliorative effect on caffeine-induced apoptosis, oxidative stress, and inflammatory response in the cerebrum.

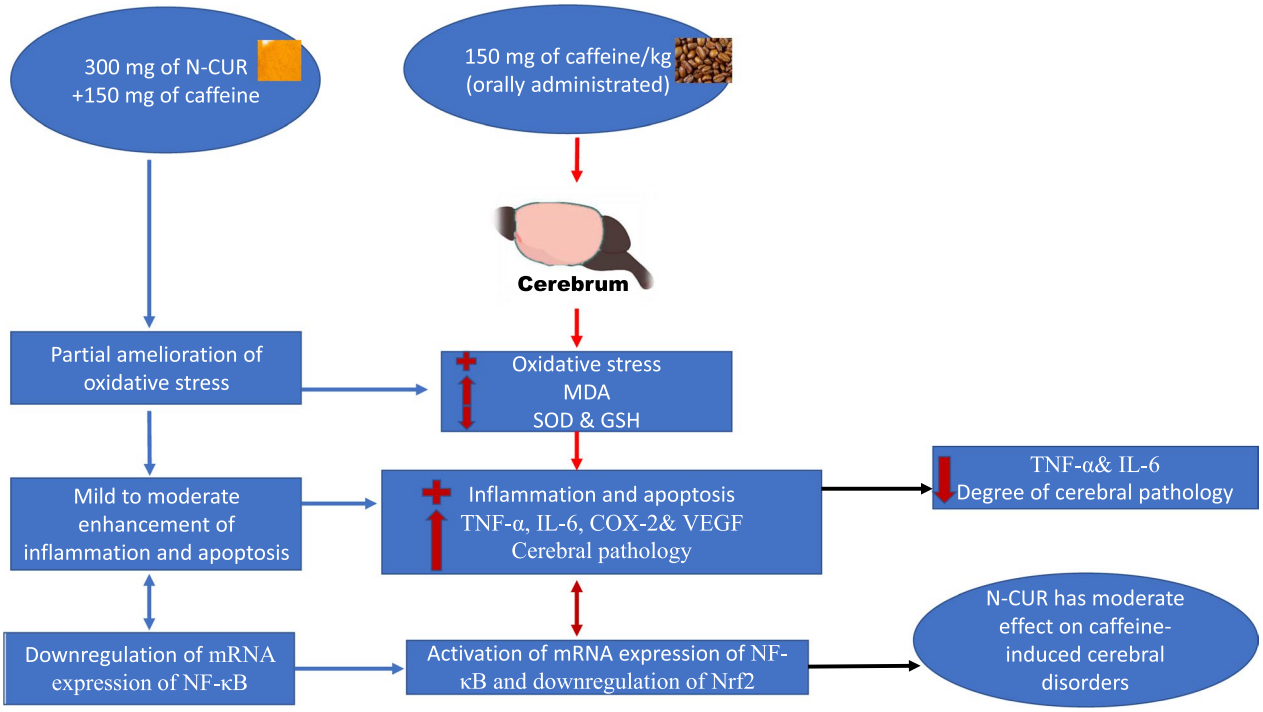
Highlights

- Caffeine induced oxidative stress, apoptosis, and inflammation in the cerebrum.
- Caffeine resulted in significantly increased COX-2 and VEGF levels.
- Caffeine increased the expression of NF-κB and inhibited Nrf2 in cerebrum.
- N-CUR may have a mild to moderate ameliorative effect on caffeine exposure.
- Humans could confine caffeine intake to evade any alterations in the cerebrum.

Keywords Caffeine, Nano-curcumin, Oxidative stress, Cerebrum, Rats

*Correspondence:
R. G. Ahmed
ahmedragab08@gmail.com
Full list of author information is available at the end of the article

Graphical abstract



1 Background

Caffeine (1,3,7-trimethylxanthine) is a methylxanthine central nervous system (CNS) stimulant [7, 35, 47, 95], the most widely consumed psychoactive drug [2, 67–69] and one of the most researched food components [50, 57], affecting the metabolic mechanisms [83]. Most people consume caffeine in drinks because it can be used in medical products such as analgesics, diuretics, muscle relaxants, and headache medications [45]. It is also used as a pain reliever in flu and cold treatments. Additionally, it has been discovered that Parkinsonism's motor symptoms and neuronal loss are slowed down by caffeine. [12]. Notably, moderate consumption of caffeine lowers mortality risk and metabolic disorders, and this action may be attributed to its ergogenic action [98]. Excessive caffeine consumption harms the body through significant toxic effects [88], including the symptoms of a colonic stimulant [58], anxiousness, delirium, headache, dehydration, insomnia, nervousness, hyperglycemia, and arrhythmia [66]. Also, the initiation of apoptosis and cell death [34], modulation of inflammatory actions [33], and low birth weight [82] were reported. High doses of caffeine intake may decrease neurogenesis in the cerebral cortex and subventricular zone (SVZ) [22] and elevate apoptosis across the cerebral hemisphere [10]. These pathological alterations might be attributed to oxidative stress (egress of malondialdehyde, MDA) [48]. Caffeine exposure decreases the levels of vascular endothelial growth factor (VEGF) [55], interleukin 1 beta (IL-1β) [56], IL-6 and tumor necrosis factor-alpha (TNF-α) [92]. In vitro, Chavez-Valdez et al. recorded that the addition of 10 mg caffeine/ml decreased the production of TNF-α [14]. Interestingly, caffeine can control cytokine production and immunity [33]. Its half-life is 5 or 6 h after digestion [33].

Curcumin (CUR, diferuloylmethane), turmeric's (Curcuma longa) active component, is a hydrophobic polyphenolic compound [5, 25] that is safe for both humans and animals and has chemo-preventive properties [6, 79]. Furthermore, CUR has positive effects in several clinical cases, like cancer in the esophagus, colon, skin, and breast [32, 71], liver injury [40], and experimental brain toxicity [61]. It is also used to cure a variety of mild to moderate human diseases in traditional medicine in Asia and Africa, such as liver, lungs, gastrointestinal, and muscular systems, as well as aches and pains abnormalities [41]. CUR is an effective anti-inflammatory, anticancer, and antioxidant and modulates several cellular signal transduction pathways [5, 49, 78]. Despite its medicinal benefits, CUR is difficult to dissolve in water and is

particularly sensitive to physiological pH fluctuations [44]. It also has a low absorption rate in the gastrointestinal system in acidic conditions [91]. As a result, methods for increasing the solubility of functional herbal components with low water solubility and protecting them until they get to their target site in the body are useful [91]. Some of the techniques that have been used for increasing the solubility of CUR include CUR liposomalization, CUR phospholipid complexing, CUR nanoformulation, and CUR nanoencapsulation [19].

Nano-curcumin (N-CUR) has the same activity as CUR but with higher stability and solubility [25]. N-CUR can profoundly enhance cell permeability and protection in vivo and in vitro [75]. Additionally, glutamate content in cerebrospinal fluid decreases because N-CUR enhances glutamate transporter-1 after subarachnoid hemorrhage and prevents microglia from being reactivated [96]. We used nanoparticles to elevate the bioavailability of CUR [15] and to prolong its retention time in the brain [87]. Additionally, Sarawi et al. stated that N-CUR's neuroprotection is more effective than curcumin's [61, 75]. It can reduce blood–brain barrier dysfunction following subarachnoid hemorrhage and prevent tight junction protein destruction [96]. N-CUR is more promising in dry eye syndrome [38] and fighting cancer [5]. Our study's objective was to assess the possible protective impact of N-CUR on caffeine-induced alterations in the cerebrum of male albino rats through histopathological, biochemical, and qRT-PCR studies.

2 Methods

2.1 Chemicals

Caffeine anhydrous (1,3,7-trimethylxanthine) was purchased from SD Fine-India Company, and N-CUR powder was purchased from National Nanotech Company. Caffeine and N-CUR powder were stored at 2–4 °C. The purest chemicals and reagents were used in this experiment.

2.2 Experimental animals

Twenty-four adult Wistar rats (*Rattus norvegicus*) weighing 170–190 gm were received from the National Research Center. To exclude any concurrent infections and to adapt to the new circumstances, adult rats were kept in the department animal house for 14 days. All animals were sheltered in cages with six rats each at a normal room temperature and light/dark cycle. Standard pellet food and unlimited access to sterile water were provided for the animals. Each effort was made in order to decrease the use of animals and their pain.

Each animal used was conducted under a protocol that has been approved by BSU-IACUC with the permit number: 021–148.

2.3 Experimental design

Rats were assigned in a random way to three groups ($n=6$ rats per group), and the treatment was administered every day for a month as the following:

Group I (control group): Animals were used as vehicle controls that only received sterilized distilled water.

Group II (caffeine group): Animals were administered 150 mg caffeine/kg b.wt./day (dissolved in sterile D.W.) orally by gastric tubing.The dose was selected according to the publication of [7]. Notably, the mode of administration was different.

Group III (caffeine + N-CUR): Animals that administrated a daily dose of 150 mg caffeine were treated orally with N-CUR at a dose of 300 mg/kg b. wt/day [97].

2.4 Collecting blood and tissue samples

Anesthetized animals were euthanized soon after the thirty-day time frame was completed, and blood was extracted by ventricular puncturing. Clotting blood samples were centrifuged at 3000 rpm for 20 min (1006.2g) to obtain the serum. Then, this serum was stored at −20° C for further biochemical evaluation. Each rat's cerebrum was removed and separated into three sections. Using a Teflon homogenizer, the first portion was homogenized with 10% (w/v) cold phosphate-buffered saline (PBS), centrifuged at 1000 g for ten minutes, and we stored the pure supernatant at −20°C. For RNA isolation, the second fraction was stored in a frozen condition at −70 °C. The third cerebral part was maintained in 10% neutral buffered formalin (NBF).

2.5 Histological examination

Cerebral tissues were quickly fixed for one day in 10% NBF, then cleared in xylene, embedded in paraffin wax, blocked, serially sectioned, and stained with H&E stain. Zeiss binocular microscope with an ocular image acquisition software and Q Imaging digital camera was used to examine the slides.

2.6 ELISA assays

Serum (6 samples/6 rats/parameter/group) was used to determine the concentrations of the following:

2.6.1 Estimation of cyclooxgynase2 (COX-2)

COX-2 kit was purchased from Millipore ELISA Kit, USA, Cat No. CBA053. Rat anti-PTGS2/COX-2 antibodies were coated on the surface of ELISA microplates. Following the addition of samples or standards, each well received a sequential addition of an Avidin-Horseradish Peroxidase (HRP) mixture and a biotinylated detection antibody, especially for Rat PTGS2/COX-2. At a

wavelength of 450 nm, the optical density of PTGS2/COX-2 coupled with the biotinylated detection antibody was determined spectrophotometrically.

2.6.2 Estimation of interleukins IL-6 level

The IL-6 level was assayed by utilizing an ELISA kit purchased from MyBioSource (USA), Cat No. MBS355410. Rat anti-IL-6 antibodies were coated on the surface of ELISA microplates. Following the addition of samples or standards, each well received HRP conjugate and an IL-6 biotinylated specific antibody before being incubated. At a wavelength of 450 nm, the optical density of IL-6 coupled with the biotinylated detection antibody was determined spectrophotometrically.

2.6.3 Estimation of tumor necrosis factor-alpha (TNF-α)

The level of TNF-α was assayed by using a rat ELISA kit purchased from RayBio®, Cat No. ELR-TNF-α. Rat anti-TNF-α antibodies were coated on the ELISA microplate. Following the addition of samples or standards, each well received HRP conjugate, and TNF-α and HRP conjugate biotinylated specific antibody before being incubated. At a wavelength of 450 nm, the optical density of TNF-α coupled with the biotinylated detection antibody was quantified spectrophotometrically.

2.6.4 Estimation of vascular endothelial growth factor (VEGF)

VEGF kit was purchased from Millipore ELISA Kit, USA, Cat No. QIA52. Rat anti-VEGF antibodies were coated on ELISA microplates. Following adding samples or standards, each well received an HRP conjugate and biotinylated antibody that is specific for rat VEGF before being incubated. At a wavelength of 450 nm, the optical density of VEGF coupled with the biotinylated detection antibody was quantified spectrophotometrically.

2.7 Assays of antioxidants/prooxidants in the cerebrum

2.7.1 Estimation of reduced glutathione (GSH) content

The analysis was conducted using a commercial kit (Bio-diagnostic, Giza, Egypt; CAT # GR2511). In theory, the 5-thio-2-nitrobenzoic acid (TNB) yellow color results from the reduction of 5,5'-dithiobis (2-nitrobenzoic acid; DNTB) with GSH in the sample. The sample's GSH content was identified by estimating the decreased chromogen's absorbance at 405 nm.

2.7.2 Estimation of superoxide dismutase (SOD) activity

The test was conducted utilizing a commercial kit (Bio-diagnostic, Giza, Egypt; CAT # SD2521). In theory, nitro-blue tetrazolium was added to NADH, followed by the addition of phenazine methosulphate (PMS) prior to measurement. By estimating the increase in absorbance at 560 nm, the percentage of inhibition brought on by the presence of SOD in the sample could be measured.

2.7.3 Estimation of malondialdehyde (MDA) content

The analysis was conducted using a commercial kit (Bio-diagnostic, Giza, Egypt; CAT # MD2529). The malondialdehyde (MDA) in the sample interacted with thiobarbituric acid in an acidic medium to form a pink color of thiobarbituric acid reactive product that was detected at 534 nm.

2.8 RNA Isolation and qRT-PCR Examination

Cerebrum retained parts (6 specimens/6 rats/parameter/group) were sent to the Research Park (CURP) for further processing. Utilizing a Thermo Scientific RNA purification kit (**K0732**, Thermo Fisher Scientific, MA, USA), total RNA was isolated using the Trizol agent. Then, using MuLV reverse transcriptase and oligo-dT primers in accordance with the RevertAid First Strand cDNA Synthesis Thermo Scientific kit instructions (**K1622**, Thermo Fisher Scientific, Waltham, MA, USA), RNA was reverse transcribed into cDNA. The primer sets selected (Table 1) for the SYBR Green master mix analysis of the generated cDNA in a total volume of 10 µl. The PCR thermal cycles included firstly, denaturation for 10 min at 95 °C, followed by 40 cycles of denaturation for 15 s at 95 °C, annealing for 1 min at 60 °C, and extension at 60 °C for 60 s. Non-template and water controls were used to differentiate non-specific amplifications. Data from amplification were examined using $2^{-\Delta\Delta Ct}$ technique [51]. Notably, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and β-actin were used as reference genes to standardize all values.

2.9 Statistical analysis

SPSS version 26 for Windows (SPSS Inc., Chicago, IL, USA) was utilized to achieve the statistical analysis. The Least Significant Difference (LSD) test is then used to compare multiple groups to one another. Results were

Table 1 Primers used for real-time PCR analysis

Gene	Sequence 5'–3'
Nrf2	F: TTGTAGATGACCATGAGTCGC
	R: TGTCCTGCTGTATGCTGCTT
NF-κB	F: TCTCAGCTGCGACCCCG
	R: TGGGCTGCTCAATGATCTCC
β-Actin	F: TACAACCTTCTTGCAGCTCCT
	R: CCTTCTGACCCATACCCACC
GAPDH	F: AGGTCGGAGTCA ACGGATTTGGT
	R: CATGTGGGCCATGAGGTCCACCAC

provided as mean and standard error (SE), and one-way ANOVA was used for all statistical comparisons. P values less than 0.05 were regarded as significant, P values less than 0.01 as very significant, and P values greater than 0.05 as nonsignificant.

3 Results

3.1 Cerebral histopathology

Most of the cerebral sections treated with caffeine (group **II**) showed loss of cerebral cortical layers organization (Fig. 1B) compared to the control group (Fig. 1A). A marked extensive congestion, hemorrhage of cerebral blood vessels, neuronophagia, gliosis, and perivascular/perineuronal edema were observed. The molecular layer presented a considerable number of shrunken and vacuolated neurons. Many neurons showed pyknotic or karyolytic nuclei (Fig. 1B). On the other hand, N-CUR-treated rats (group **III**) revealed the previously mentioned cerebral neuropathology, but with less severity and extension. In other words, there was a mild to moderate ameliorative impact of N-CUR on caffeine-induced neuropathology in rats (Fig. 1C).

3.2 Cytokines and inflammatory mediators

Administration of caffeine caused a significant ($p < 0.05$; LSD) increase in COX-2 level (Fig. 2) and a highly noteworthy ($p < 0.01$; LSD) increase in IL-6 (Fig. 3), TNF-α (Fig. 4), and VEGF (Fig. 5); compared to the control one. The recorded percentage change was 16.3%, 19.4%, 27.3%, and 9.4%, respectively. Administration of N-CUR results in a nonsignificant ($p > 0.05$; LSD) improvement in COX-2 levels and a highly significant ($p < 0.01$; LSD) improvement in TNF-α and IL-6 levels. The recorded percentage changes were −7.4%, −21.5%, and −12.73%, respectively. On the contrary, N-CUR caused a highly significant ($p < 0.01$; LSD) elevation in VEGF levels; the recorded percentage change was 6.7% compared to the caffeine group.

3.3 Cerebral antioxidant/oxidative markers

Caffeine administration resulted in significant changes in the antioxidant/oxidative markers (GSH, SOD, and MDA) in the cerebrum in contrast to the control one, as shown in Fig. 6A–C. The percentage of changes was −11.5%, −28.0%, and 60.68%, respectively. Administration of N-CUR induced a profound ($p < 0.05$; −14.2%) reduction in the GSH level, a highly significant ($p < 0.01$; 45.2%) increase in the SOD level, and mitigated a noteworthy ($p > 0.05$; −1.3%) decrease in the MDA level as compared to the caffeine group.

3.4 Cerebral Nrf2 and NF-κB gene expression

The caffeine-treated group exhibited a highly substantial ($p < 0.01$; LSD) downregulation of gene expression of Nrf2 (Fig. 7) and upregulation of gene expression of NF-κB (Fig. 8) in contrast to the control group. Furthermore, administration of N-CUR induced a nonsignificant ($p > 0.05$; LSD) upregulation in Nrf2 gene expression and a highly significant ($p < 0.01$; LSD) downregulation of NF-κB expression as compared to the caffeine group.

4 Discussion

Due to the fact that excessive doses of caffeine may have harmful effects on the brain [13, 69, 85], and N-CUR has a positive impact on improving a wide range of diseases, including neurological disorders [31], the current study aims to examine the protective effect of N-CUR (300 mg kg^{-1}) on caffeine (150 mg kg^{-1})-induced alterations in the cerebrum of male albino rats, focusing on biochemical, histopathological, and qRT-PCR assays. In general, caffeine can affect the cerebrum due to its stimulatory effect on the hypothalamic-pituitary axis and CNS [54] and its insulinotropic effect, which results in energy loss and an increase in intracellular calcium concentration [20].

The primary observation of the current study is that there was a loss in the organization of the cerebral cortical layers, significant cerebral blood vessel congestion and hemorrhages, neuronophagia, gliosis, perivascular/pericellular edema, and numerous vacuolations in the molecular layer. Consuming high concentrations of caffeine may induce cellular apoptosis [53]. These pathological lesions are consistent with the unbalanced egress of reactive oxygen species (ROS) in the caffeinated group. Caffeine may enhance LPO, which may encourage the formation of free radicals and cause a rise in oxidative stress [77]. The production of ROS is linked to aging and several chronic diseases [39]. However, the half-life of ROS is so short that its concentration cannot be measured directly. Thus, we have measured changes in MDA, GSH, and SOD levels, which are signs of oxidative stress [1]. The current study reported that exposure of male rats daily to 150 mg of caffeine for one month resulted in a profound elevation in cerebral MDA. Leelarungrayub et al. reported that consuming caffeinated coffee resulted in increased MDA levels as compared to consuming decaffeinated coffee [48]. Also, Metro et al. displayed that consuming foods with high concentrations of caffeine caused an increase in intramuscular fat oxidation [59]. In parallel, a reduction in cerebral GSH content was recorded in the caffeinated group. This reduction in GSH may be induced by the depletion of nicotinamide adenine

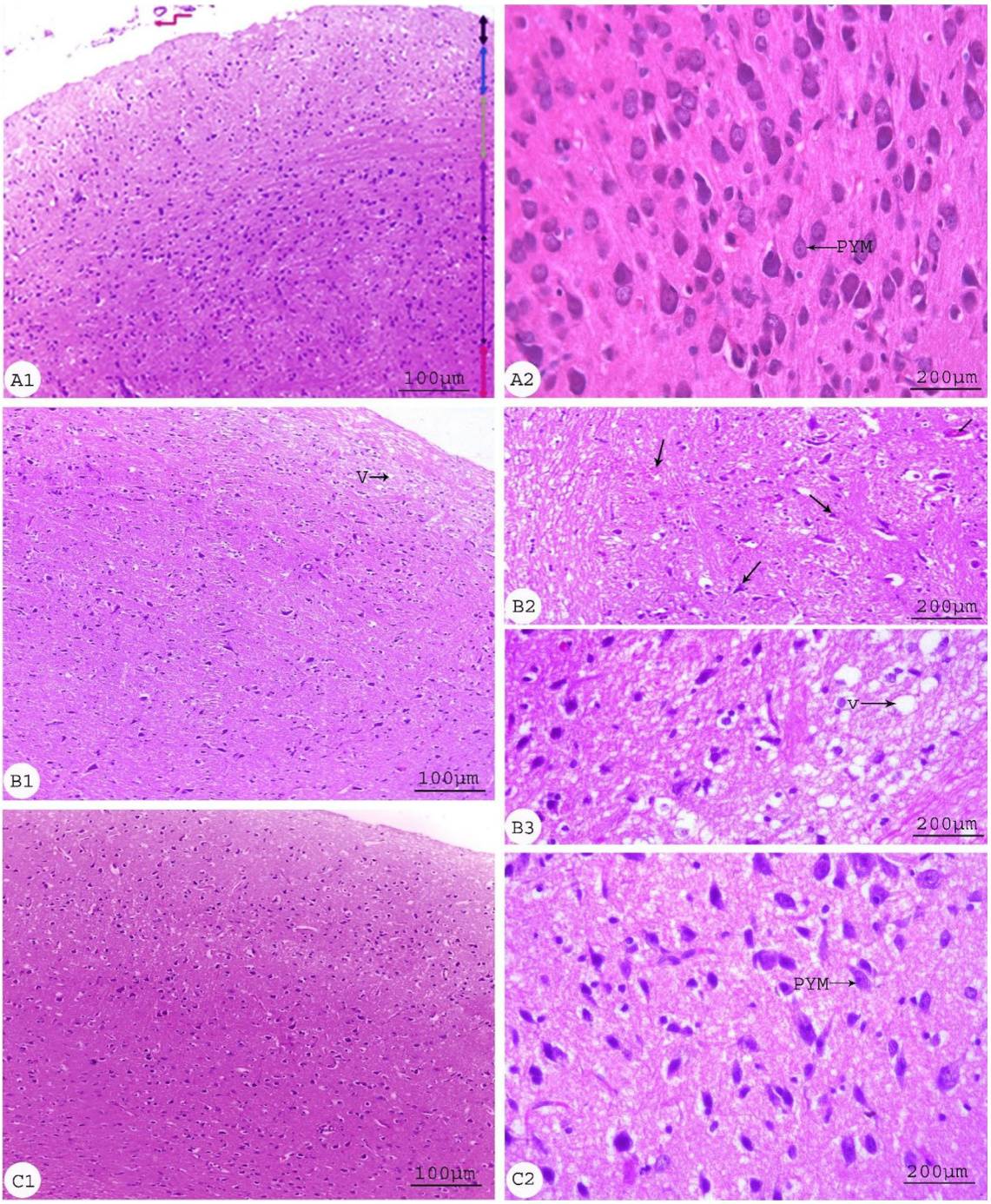


Fig. 1 Representative photomicrographs of rat cerebral cortices sections stained with Hematoxylin–Eosin stain (magnification 100 X): (**A**) control rats (group **I**) revealed the regular arrangement of six layers of the cerebral cortex, named from the outer to the inner into; outer molecular (plexiform) layer (black arrow) covered with pia matter (bent arrow), external granular layer (blue arrow), pyramidal cell (PYM), internal granular layer (violet arrow), internal pyramidal layer (thin black arrow), and lastly the polymorphic cell layer (orange arrow). (**B**) The caffeine group (group **II**) showed congestion (thin black arrow) and hemorrhages (thick black arrow) of cerebral blood vessels, neuronphagia, gliosis, and perivascular/perineuronal edema (thick black arrow). The molecular layer presented a considerable number of shrunken and vacuolations (**V**). Many neurons showed pyknotic (thick black arrow) or karyolytic nuclei. (**C**) Rats treated with N-CUR presented a mild to moderate ameliorative effect of caffeine-induced neuropathology (necrobiotic and vascular changes)

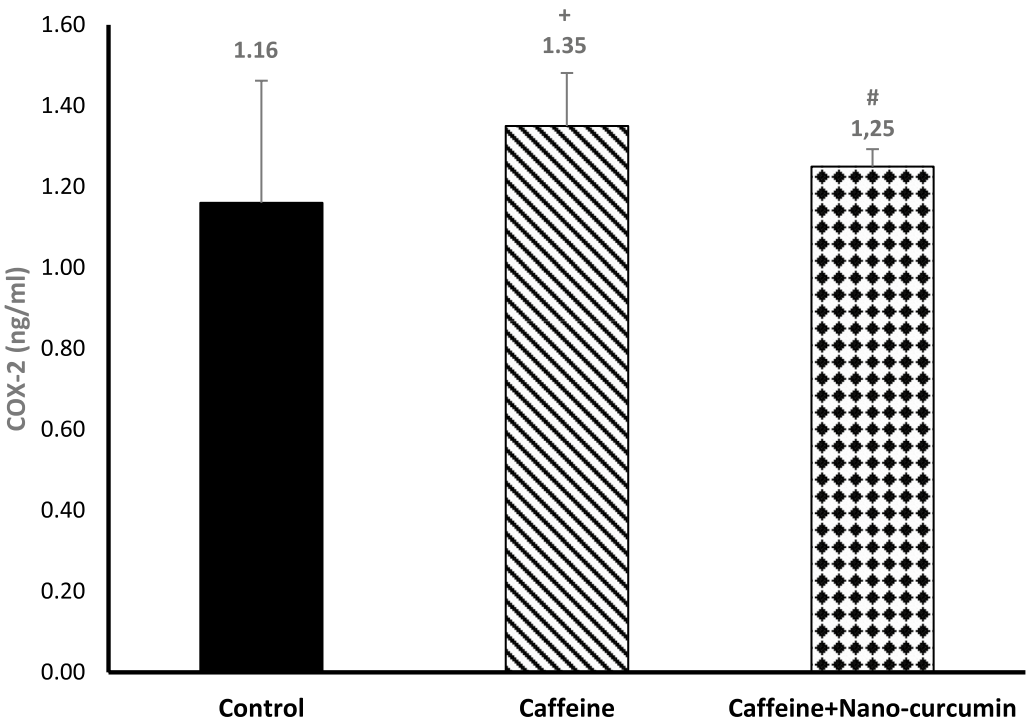


Fig. 2 COX-2 in control, caffeine, and caffeine treated with N-CUR. (+) significantly changed in contrast to the control group, and (#) nonsignificantly changed in contrast to the caffeine group

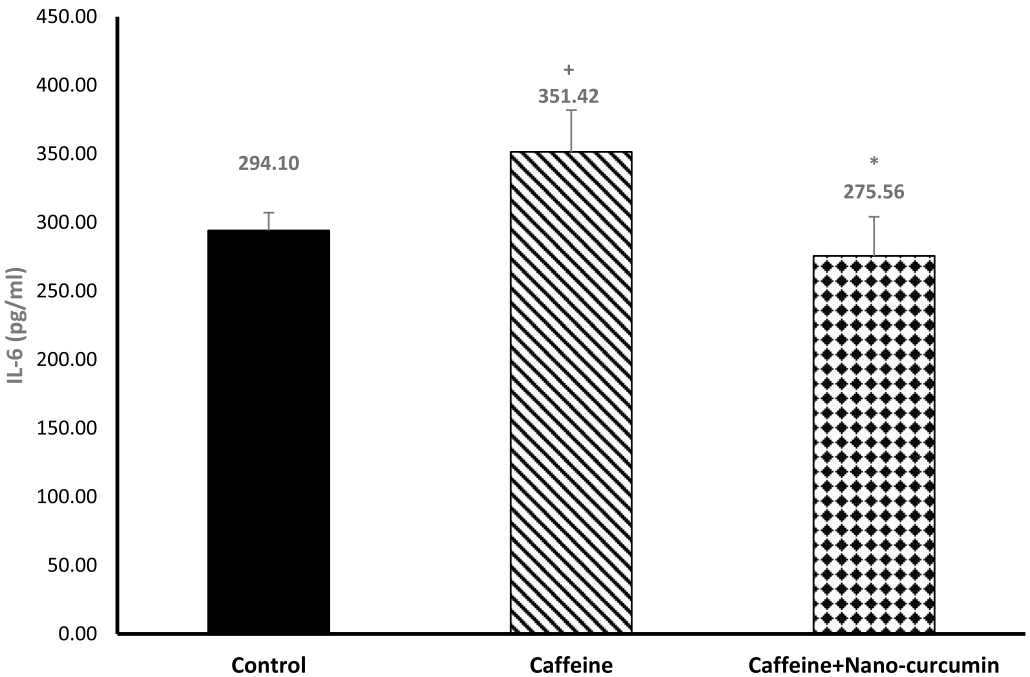


Fig. 3 IL-6 in control, caffeine, and caffeine treated with N-CUR. (+) significantly changed in contrast to the control group, and (*) significantly changed in contrast to the caffeine group

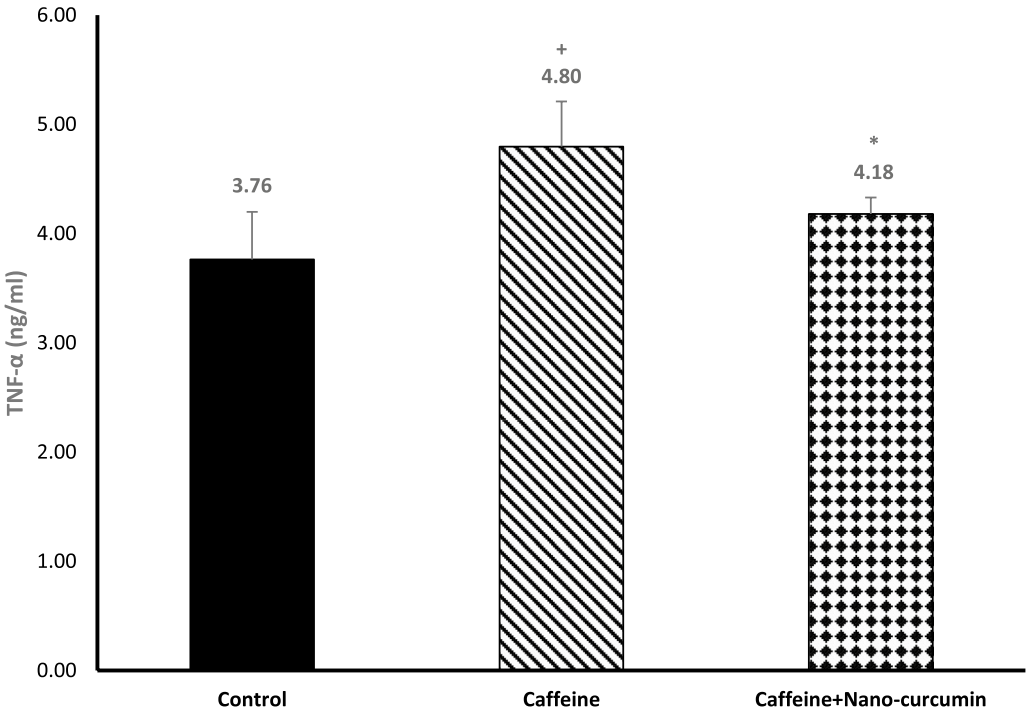


Fig. 4 TNF-α in control, caffeine, and caffeine treated with N-CUR. (+) significantly changed in contrast to the control group, and (*) significantly changed in contrast to the caffeine group

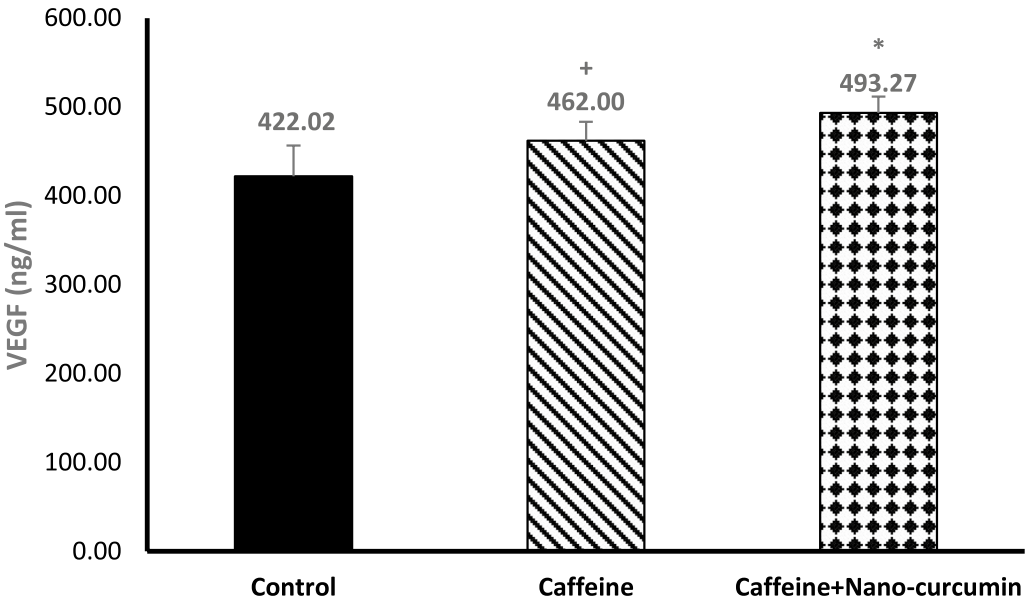


Fig. 5 VEGF in control, caffeine, and caffeine treated with N-CUR. (+) significantly changed in contrast to the control group, and (*) significantly changed in contrast to the caffeine group

dinucleotide phosphate (NADPH) or by raised GSH consumption during the non-enzymatic elimination of oxygen radicals [28]. Depletion of intracellular GSH significantly increases the generation of ROS in the mitochondria and results in depolarization of the mitochondrial membrane [52]. Alternatively, a reduction in cerebral SOD activity was recorded in the caffeinated group. Cruz et al. concluded that caffeine caused

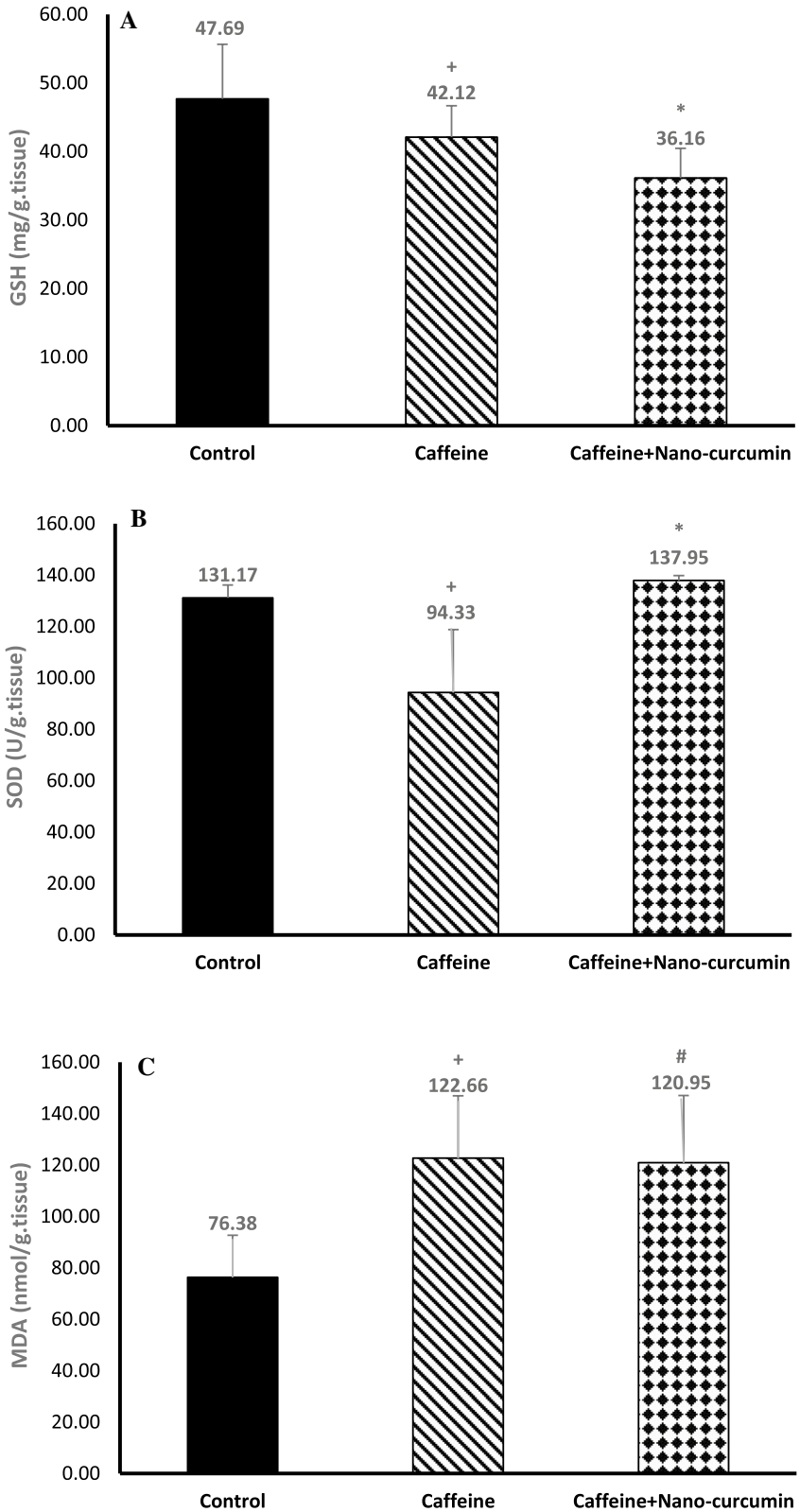


Fig. 6 **A**: Cerebral GSH in control, caffeine, and caffeine treated with N-CUR; **B**: Cerebral SOD in control, caffeine, and caffeine treated with N-CUR; and **C**: Cerebral MDA in control, caffeine, and caffeine treated with N-CUR. (+) significantly changed in contrast to the control group; (*) significantly changed in contrast to the caffeine group; and (#) nonsignificantly changed as compared to the caffeine group

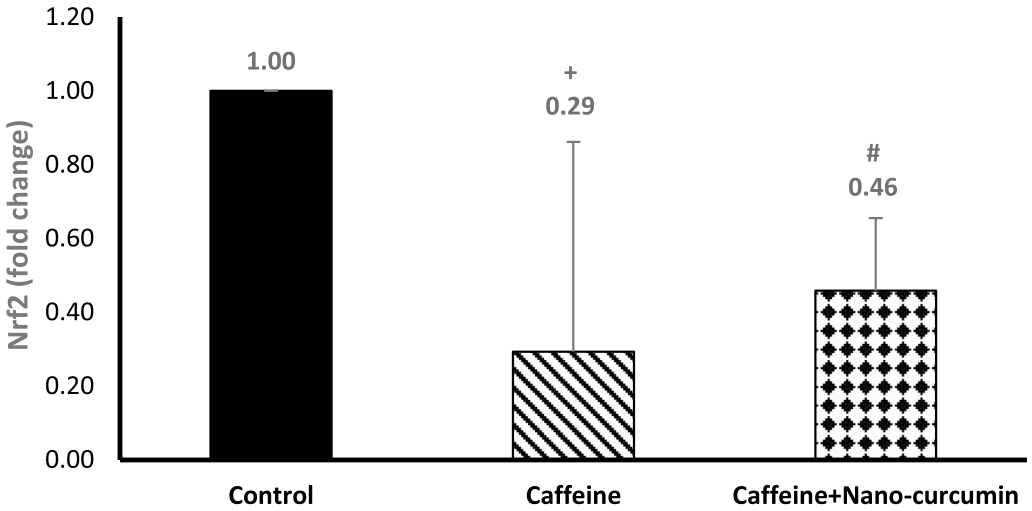


Fig. 7 Cerebral Nrf2 expression in control, caffeine, and caffeine treated with N-CUR. (+) significantly changed in contrast to the control group, and (#) nonsignificantly changed in contrast to the caffeine group

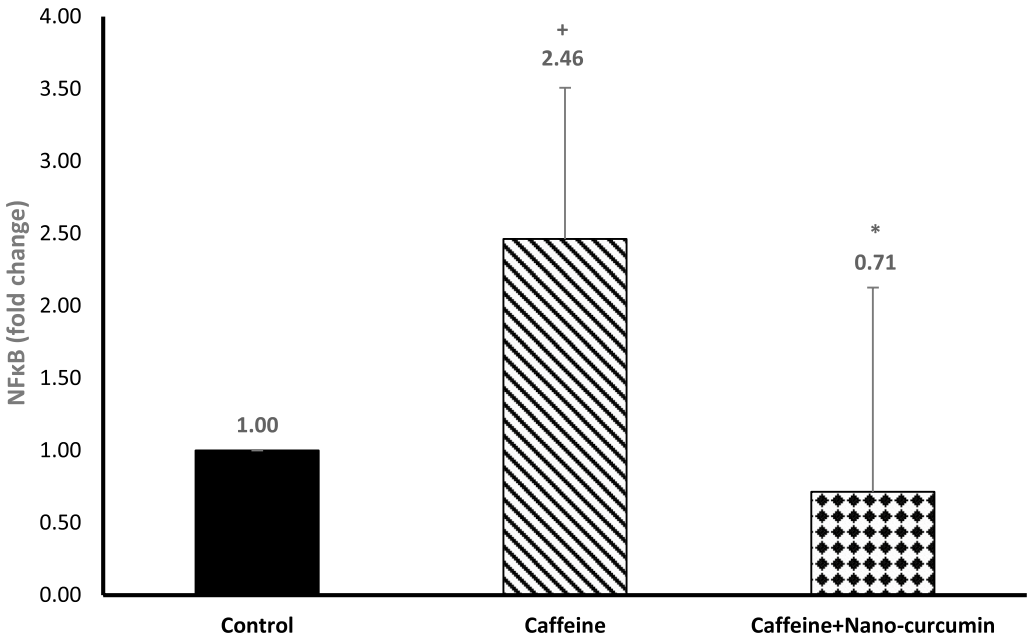


Fig. 8 Cerebral NF-κB expression in control, caffeine, and caffeine treated with N-CUR. (+) significantly changed in contrast to the control group, and (*) significantly changed in contrast to the caffeine group

an oxidative state in rats and decreased GSH content and SOD activity, respectively [17]. This disturbance, which might be attributed to consuming caffeine during an acute inflammatory response, might cause tissue damage because it suppresses adenosine 2a receptor (A2aR)-mediated adenosine signaling, which is an essential tissue-protective mechanism [64]. Generally, caffeine binds to adenosine receptors and blocks the neurotransmitter's function [93].

These pathological changes and oxidative stress states were mild to moderately ameliorated by N-CUR (300 mg kg^{-1}). This result was supported by a nonsignificant decrease in the level of MDA and an elevation in SOD activity, but failed to increase the GSH content. This partial ameliorative effect of N-CUR might be caused by CUR's antioxidant and anti-inflammatory impacts on a variety of molecular targets, which have therapeutic impacts on various chronic inflammatory diseases [23] by

inhibiting ROS generation [9]. Biological investigations revealed that CUR might be considered a potent antioxidant that stops cellular proliferation by liberating active free thiol groups at target sites [4]. CUR's antioxidant properties may result from the following interactions: free radicals scavenging by oxygen quenching, inhibition of cytochrome P450, or chelating oxidative properties of metal ions [70]. According to a study, CUR inhibits the oxidation of Fe^{2+} in the Fenton reaction, which prevents the production of hydroxyl radical and superoxide anion [21]. Thus, N-CUR may revoke the oxidative imbalance and mitigate the cerebral histopathology induced by caffeine.

In the present study, a significantly elevated production of TNF-α and IL-6 (pro-inflammatory mediators) was observed in the caffeinated group. Overproduction of TNF-α and IL-6 as an inflammatory response might cause several pathological consequences [81]. However, these cytokines have multifunctional actions for immunity, angiogenesis, apoptosis, and inflammation [29, 63, 65, 73]. In disagreement with the present data, the IL-6 and TNF-α levels were decreased after exposure of KK-A(y) mice to caffeine [92]. This diminution could be ascribed to the inhibition action of caffeine on the inflammatory pathways or the phosphorylated STAT1 mechanism [36]. On the contrary, administration of N-CUR herein exhibited anti-inflammatory and chemo-preventive effects where the levels of TNF-α and IL-6 were substantially ameliorated. Several publications have presented the therapeutic effects of CUR on these cytokines. Ghandadi and Sahebkar reported that CUR was used as a potential treatment for pathological values involving IL-6 [27]. Aggarwal et al. stated that CUR could block the action and production of TNF-α [3]. Shehzad et al. revealed that CUR reduced neuroinflammation by lowering IL-1β and TNF-α [80]. Thus, the administration of N-CUR attenuated the neuroinflammatory state induced by caffeine.

The present research revealed that exposure of rats to 150 mg of caffeine resulted in significantly increased COX-2 and VEGF levels. In agreement, Han et al. reported that caffeine promoted the initiation of apoptosis through COX-2 pathways [30]. Notably, the expression of COX-2 is noticeably upregulated in astrocytes and microglia during the neuroinflammatory response [24]. A neuroinflammatory response is caused by activated neuronal COX-2 and microglial cells, which produce and express IL-6 and TNF-α [84]. The stimulation of COX-2 has been related to an increment in VEGF production and angiogenesis [86]. The alteration in growth markers might be attributed to the fact that caffeine can diminish the activity of phosphatidylinositol-3-kinase (PI3K)/protein kinase B (AKT) and induce the cAMP-dependent protein kinase (PKA) [16, 62]. Administration of N-CUR (300 mg kg^{-1}) resulted in a nonsignificant amelioration of COX-2 and VEGF levels. This alteration could be elucidated by the considerable oxidative stress and inflammation related to caffeine administration. However, other studies found that CUR inhibits the activity of COX-2 [60] and reduces VEGF levels [74].

The current study also followed the mRNA expression of two vital transcription factors, which are responsible for the transient response to cellular stresses. Also, nuclear factor erythroid 2-related factor 2 (Nrf2) can be targeted to provide cell protection [18] via the regulation of antioxidant genes and suppression of oxidative stress [76]. Nuclear factor κB (NF-κB) contributes to harmful cell impact [11]. These factors may regulate cellular reactions to inflammation and oxidative stress. Inhibition of Nrf2 expression and stimulation of NF-κB expression were observed in the caffeinated group. This result largely agrees with the data of Kang et al. [42]. Similarly, Ito et al. stated that DNA synthesis and the cell cycle could both be inhibited by caffeine [37]. Cell death initiation by caffeine was facilitated by NF-κB [42], Cox2 [89], mitogen-activated protein kinase, and extracellular signal-regulated kinases [72] pathways. These changes might reflect the degree of degeneration in the cerebral cortex, inflammation, and oxidative stress and might be a sign of neural disorders. Although N-CUR (300 mg kg^{-1}) administration had a nonsignificant effect on Nrf2 expression, NF-κB was significantly downregulated. In cell lines, CUR may exert effective results via the regulation of Nrf2 [26]. CUR can activate the Nrf2 signaling pathway in response to stress and decrease electrophiles and ROS levels [26]. Activation of NF-κB produces several mediators that jointly cause neuroinflammation [53]. In the current data, N-CUR inhibited pro-inflammatory cytokines and NF-κB in the cerebrum of the caffeinated group. The inflammatory reaction noticed in caffeine-treated rats might be due to elevated ROS production [46]. ROS production can lead to the stimulation of NF-κB followed by the release of many inflammatory mediators [43]. In agreement, Wang et al. stated that NF-κB inhibition might be a key mechanism underlying CUR's anti-inflammatory effects [90]. CUR exhibits the capability to inhibit NF-κB in the treatment of several illnesses, involving ulcerative colitis [90], acute kidney injury [99], and lipopolysaccharide (LPS)-induced neuroinflammation [94]. Collectively, the bioavailability and neuroprotective efficiency of CUR could be attributed to piperine alkaloids [8]. In general, Li et al. (2023) stated that CUR could enhance neuroprotective mechanisms by controlling neurogenesis.

5 Conclusion

These data suggest new findings on the moderate ameliorative effect of N-CUR on cerebral disorders induced by a high dose of caffeine administration. N-CUR diminished cell death, oxidative stress, and inflammation in the cerebrum of caffeine-administered rats as it resulted in a significant improvement in TNF-α and IL-6 levels and a significant elevation in SOD activity; furthermore, it significantly downregulated NF-κB mRNA expression. These data recommend that humans should limit caffeine intake to avoid any adverse problems. Additional investigations into human health should be required.

Abbreviations

A2aR	Adenosine A2 receptors
AKT	Protein kinase B
COX-2	Cyclooxygenase-2
CNS	Central nervous system
CSF	Cerebrospinal fluid
CUR	Curcumin
DNTB	5, 5: -Dithiobis (2-nitrobenzoic acid)
ERK	Extracellular signal-regulated kinase
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GSH	Reduced glutathione
HRP	Avidin-horseradish peroxidase
H_2O_2	Hydrogen peroxide
IL-6	Interleukins -6
LPS	Lipopolysaccharide
LSD	Least significant degree
MAPK	Mitogen-activated protein kinase
MDA	Malondialdehyde
NADPH	Nicotinamide adenine dinucleotide phosphate
NF-κB	Nuclear factor kappa B
Nrf2	Nuclear factor erythroid 2-related factor 2
NBF	Neutral buffered formalin
NBT	Nitro-blue tetrazolium
N-CUR	Nano-curcumin
PBS	Phosphate-buffered saline
PD	Parkinson's disease
PI3K	Phosphatidylinositol-3-kinase
PKA	Protein kinase A
PMS	Phenazine methosulphate
PYM	Pyramidal cell
ROS	Reactive oxygen species
qRT-PCR	Quantitative reverse transcription polymerase
SE	Standard error
SOD	Superoxide dismutase
SPSS	Statistical package for the social sciences
SVZ	Subventricular zone
TBA	Thiobarbituric acid
TNF-α	Tumor necrosis factor
VEGF	Vascular endothelial growth factor
V	Vacuoles

Acknowledgements
The authors thank the Department of Biochemistry and Zoology members, Faculty of Science, Beni-Suef University, for providing the lab facilities for the present research work.

Author contributions
Moustafa M. Morsy[1] contributed to formal analysis, methodology, resources, software, writing – original draft preparation, and Ahmed R.G.[2] and Mohammed Abdel-Gabbar[1] contributed to conceptualization, formal analysis, methodology, project administration, resources, software, supervision, visualization, writing – review & editing.

Funding
No funding was obtained for this study.

Availability of data and materials
All data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval and consent to participate
All animals used were conducted under a protocol that has been approved by BSU-IACUC with the permit number: 021–148.

Consent of publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
[1]Biochemistry Department, Faculty of Science, Beni-Suef University, Beni-Suef, Egypt. [2]Zoology Department, Faculty of Science, Beni-Suef University, Beni-Suef, Egypt.

Received: 1 March 2024 Accepted: 11 July 2024
Published online: 23 September 2024

References

1. Abdel-Gabbar M, Esmail M, Kandeil M et al (2020) The ameliorative effect of atorvastatin on serum testosterone and testicular oxidant/antioxidant system of HFD-fed male albino rats. F1000Research 9:1300
2. Adekeye AO, Fafure AA, Jeje-Pius ST et al (2021) Caffeine abrogates oxidative stress imbalance: Its implication on lateral geniculate nucleus and visual cortex following hyaluronic acid exposure. J Chem Neuroanat 117:101996
3. Aggarwal BB, Gupta SC, Sung B (2013) Curcumin: an orally bioavailable blocker of TNF and other pro-inflammatory biomarkers. Br J Pharmacol 169(8):1672–1692
4. Aggarwal BB, Deb L, Prasad S (2014) Curcumin differs from tetrahydrocurcumin for molecular targets, signaling pathways and cellular responses. Molecules (Basel, Switzerland) 20(1):185–205
5. Ahmad I, Ahmad S, Ahmad A et al (2024) Curcumin, its derivatives, and their nanoformulations: Revolutionizing cancer treatment. Cell Biochem Funct 42(1):e3911. https://doi.org/10.1002/cbf.3911
6. Ahmad M, Akhter S, Mohsin N et al (2014) Transformation of curcumin from food additive to multifunctional medicine: nanotechnology bridging the gap. Curr Drug Discov Technol 11(3):197–213
7. Ahmed RG (2019) Gestational caffeine exposure acts as a fetal thyroid-Cytokine disruptor by activating caspase 3/BAX/Bcl-2/Cox2/NF-κB at ED 20. Toxicol Res 8(2):196–205
8. Balasubramanian K (2016) Quantum chemical insights into Alzheimer's disease: Curcumin's chelation with Cu(II), Zn(II), and Pd(II) as a mechanism for its prevention. Int J Quantum Chem 116(14):1107–1119
9. Biswas SK, McClure D, Jimenez LA et al (2005) Curcumin induces glutathione biosynthesis and inhibits NF-kappaB activation and interleukin-8 release in alveolar epithelial cells: mechanism of free radical scavenging activity. Antioxid Redox Signal 7(1–2):32–41
10. Black AM, Pandya S, Clark D et al (2008) Effect of caffeine and morphine on the developing pre-mature brain. Brain Res 1219:136–142
11. Buelna-Chontal M, Zazueta C (2013) Redox activation of Nrf2 & NF-κB: A double end sword? Cell Signal 25(12):2548–2557
12. Cappelletti S, Daria P, Sani G et al (2014) Caffeine: cognitive and physical performance enhancer or psychoactive drug? Curr Neuropharmacol 13(1):71–88
13. Chavez L, Bancalari E (2022) Caffeine: some of the evidence behind its use and abuse in the preterm infant. Neonatology 119(4):428–432

14. Chavez-Valdez R, Wills-Karp M, Ahlawat R et al (2009) Caffeine modulates TNF-alpha production by cord blood monocytes: the role of adenosine receptors. Pediatr Res 65(2):203–208
15. Cheng KK, Yeung CF, Ho SW et al (2013) Highly stabilized curcumin nanoparticles tested in an in vitro blood-brain barrier model and in alzheimer's disease Tg2576 Mice. AAPS J 15(2):324–336
16. Conde SV, Nunes da Silva T, Gonzalez C et al (2012) Chronic caffeine intake decreases circulating catecholamines and prevents diet-induced insulin resistance and hypertension in rats. Br J Nutr 107(1):86–95
17. Cruz D, Almeida Â, Calisto V et al (2016) Caffeine impacts in the clam Ruditapes philippinarum: Alterations on energy reserves, metabolic activity and oxidative stress biomarkers. Chemosphere 160:95–103
18. Cuadrado A, Pajares M, Benito C et al (2020) Can Activation of NRF2 Be a Strategy against COVID-19? Trends Pharmacol Sci 41(9):598–610
19. Cui J, Yu B, Zhao Y et al (2009) Enhancement of oral absorption of curcumin by self-microemulsifying drug delivery systems. Int J Pharm 371(1–2):148–155
20. da Silva LA, Wouk J, Weber VMR et al (2017) Mechanisms and biological effects of Caffeine on substrate metabolism homeostasis: a systematic review. J Appl Pharm Sci 7(6):215–221
21. Dairam A, Fogel R, Daya S et al (2008) Antioxidant and iron-binding properties of curcumin, capsaicin, and S-allylcysteine reduce oxidative stress in rat brain homogenate. J Agric Food Chem 56(9):3350–3356
22. Desfrere L, Olivier P, Schwendimann L et al (2007) Transient inhibition of astrocytogenesis in developing mouse brain following postnatal caffeine exposure. Pediatric Res 62(5):604–609
23. Dulbecco P, Savarino V (2013) Therapeutic potential of curcumin in digestive diseases. World J Gastroenterol : WJG 19(48):9256
24. Font-Nieves M, Sans-Fons MG, Gorina R et al (2012) Induction of COX-2 enzyme and down-regulation of COX-1 expression by lipopolysaccharide (LPS) control prostaglandin E 2 production in astrocytes. J Biol Chem 287(9):6454–6468
25. Gera M, Sharma N, Ghosh M et al (2017) Nanoformulations of curcumin: an emerging paradigm for improved remedial application. Oncotarget 8(39):66680–66698
26. Ghafouri-Fard S, Shoorei H, Bahroudi Z et al (2022) Nrf2-related therapeutic effects of curcumin in different disorders. Biomolecules 12(1):82
27. Ghandadi M, Sahebkar A (2016) Curcumin: an effective inhibitor of interleukin-6. Curr Pharm Des 23(6):921–931
28. Gumieniczek A (2005) Effects of repaglinide on oxidative stress in tissues of diabetic rabbits. Diabetes Res Clin Pract 68(2):89–95
29. Ham B, Fernandez MC, D'Costa Z et al (2016) The diverse roles of the TNF axis in cancer progression and metastasis. Trends Cancer Res 11(1):1
30. Han W, Ming M, He YY (2011) Caffeine promotes ultraviolet B-induced apoptosis in human keratinocytes without complete DNA repair. J Biol Chem 286(26):22825
31. Hassanizadeh S, Shojaei M, Bagherniya M et al (2023) Effect of nanocurcumin on various diseases: a comprehensive review of clinical trials. BioFactors 49(3):512–533
32. Hesari A, Azizian M, Sheikhi A et al (2019) Chemopreventive and therapeutic potential of curcumin in esophageal cancer: current and future status. Int J Cancer 144(6):1215–1226
33. Horrigan LA, Kelly JP, Connor TJ (2006) Immunomodulatory effects of caffeine: friend or foe? Pharmacol Ther 111(3):877–892
34. Huang J, Zhou S, Ping J et al (2012) Role of p53-dependent placental apoptosis in the reproductive and developmental toxicities of caffeine in rodents. Clin Exper Pharmacol Physiol 39(4):357–363
35. Ingegnoli F, Cavalli S, Giudice L et al (2022) Caffeine and rheumatoid arthritis: A complicated relationship. Autoimmun Rev 21(7):103117
36. Iris M, Tsou P-S, Sawalha AH (2018) Caffeine inhibits STAT1 signaling and downregulates inflammatory pathways involved in autoimmunity. Clin Immunol 192:68–77
37. Ito K, Nakazato T, Miyakawa Y et al (2003) Caffeine induces G2/M arrest and apoptosis via a novel p53-dependent pathway in NB4 promyelocytic leukemia cells. J Cell Physiol 196(2):276
38. Jahromy MH, Qomi M, Fazelipour M et al (2024) Evaluation of curcumin-based ophthalmic nano-emulsion on atropine-induced dry eye in mice. Heliyon 10(7):e29009. https://doi.org/10.1016/j.heliyon.2024.e29009
39. Jakubczyk K, Dec K, Kałduńska J et al (2020) Reactive oxygen species - sources, functions, oxidative damage. Polski merkuriusz lekarski : organ Polskiego Towarzystwa Lekarskiego 48(284):124–127
40. Jiang Z, Liu L, Su H, et al (2024) Curcumin and analogues in mitigating liver injury and disease consequences: From molecular mechanisms to clinical perspectives. Phytomedicine 123:155234. https://doi.org/10.1016/j.phymed.2023.155234
41. Jurenka JS (2009) Anti-inflammatory properties of curcumin, a major constituent of Curcuma longa: a review of preclinical and clinical research. Altern Med Rev : J Clin Ther 14(2):141–153
42. Kang CH, Jayasooriya RGPT, Dilshara MG et al (2012) Caffeine suppresses lipopolysaccharide-stimulated BV2 microglial cells by suppressing Akt-mediated NF-κB activation and ERK phosphorylation. Food Chem Toxicol 50(12):4270
43. Kastl L, Sauer SW, Ruppert T et al (2014) TNF-α mediates mitochondrial uncoupling and enhances ROS-dependent cell migration via NF-κB activation in liver cells. FEBS Lett 588(1):175
44. Katiyar S, Srivastava N, Choudhury AR et al. (2024). Microbial fermentation-based synthesis of nano-curcumin suggesting the role of pullulan in nano-formulation. Int J Biol Macromol. 265(Pt 2):131088. https://doi.org/10.1016/j.ijbiomac.2024.131088
45. Koblihová E, Lukšan O, Mrázová I et al (2015) Hepatocyte transplantation attenuates the course of acute liver failure induced by thioacetamide in lewis rats. Physiol Res 64(5):689
46. Korbecki J, Bobiński R, Dutka M (2019) Self-regulation of the inflammatory response by peroxisome proliferator-activated receptors. Inflamm Res 68(6):443–458
47. Kuo-Esser L, Chen R, Lawson K et al (2024) Early-life caffeine exposure induces morphological changes and altered physiology in Caenorhabditiselegans. Biochem Biophys Res Commun 690:149240. https://doi.org/10.1016/j.bbrc.2023.149240
48. Leelarungrayub D, Sallepan M, Charoenwattana S (2011) Effects of acute caffeinated coffee consumption on energy utilization related to glucose and lipid oxidation from short submaximal treadmill exercise in sedentary men. Nutr Metab Insights 4:NMIS8299
49. Li L, Cheng J, Ji Y et al (2023) High-throughput sequencing and bioinformatics analysis reveals the neurogenesis key targets of curcumin action in mouse brain with MCAO. Combinatorial Chem High Throughput Screen. https://doi.org/10.2174/1386207325666220803090112
50. Lijing W, Sujie K, Linxi W et al (2022) Altered caffeine metabolism is associated with recurrent hypoglycemia in type 2 diabetes mellitus: A UPLC–MS-based untargeted metabolomics study. Front Endocrinol. https://doi.org/10.3389/fendo.2022.843556
51. Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25(4):402–408
52. Lohan SB, Vitt K, Scholz P et al (2018) ROS production and glutathione response in keratinocytes after application of β-carotene and VIS/NIR irradiation. Chemico-Biol Interact 280:1–7
53. Lu PZ, Lai CY, Wen-Hsiung C (2008) Caffeine induces cell death via activation of apoptotic signal and inactivation of survival signal in human osteoblasts. Int J Mol Sci 9(5):698
54. Lyte JM (2018) Eating for 3.8 × 1013: examining the impact of diet and nutrition on the microbiota-gut-brain axis through the lens of microbial endocrinology. Front Endocrinol. https://doi.org/10.3389/fendo.2018.00796
55. Ma ZL, Wang G, Lu WH et al (2016) Investigating the effect of excess caffeine exposure on placental angiogenesis using chicken 'functional' placental blood vessel network. J Appl Toxicol 36(2):285–295
56. Machado-Filho JA, Correia AO, Montenegro ABA et al (2014) Caffeine neuroprotective effects on 6-OHDA-lesioned rats are mediated by several factors, including pro-inflammatory cytokines and histone deacetylase inhibitions. Behav Brain Res 264:116–125
57. Magno MS, Utheim TP, Morthen MK et al (2023) The relationship between caffeine intake and dry eye disease. Cornea 42(2):186
58. Mehta V, Hopson P, Irastorza L et al (2023) Effect of caffeine on colonic manometry in children. J Pediat Gastroenterol Nutr 76(1):20–24
59. Metro D, Cernaro V, Santoro D et al (2017) Beneficial effects of oral pure caffeine on oxidative stress. J Clin Transl Endocrinol 10:22
60. Mirzaei M, Harismah K, Soleimani M et al (2021) Inhibitory effects of curcumin on aldose reductase and cyclooxygenase-2 enzymes. J Biomol Struct Dyn 39(17):6424–6430
61. Mohammadi M, Tajedini SS, Sadeghian Z et al (2024) Molecular, biochemical and histological assessment in paraquat induced neurotoxicity:

restoring role of nano-curcumin in an experimental study. Acta Neurol Taiwan 33(3):90–102
62. Nakabayashi H, Hashimoto T, Ashida H et al (2008) Inhibitory effects of caffeine and its metabolites on intracellular lipid accumulation in murine 3T3-L1 adipocytes. BioFactors 34(4):293–302
63. Norata D, Peri M, Giammalva GR et al (2023) Immunological aspects of von hippel-lindau disease: a focus on neuro-oncology and myasthenia gravis. Diagnostics (Basel, Switzerland) 13(1):144
64. Ohta A, Lukashev D, Jackson EK et al (2007) 1,3,7-Trimethylxanthine (Caffeine) may exacerbate acute inflammatory liver injury by weakening the physiological immunosuppressive mechanism. J Immunol 179(11):7431
65. Park J, Zhao Y, Zhang F et al (2023) IL-6/STAT3 axis dictates the PNPLA3-mediated susceptibility to non-alcoholic fatty liver disease. J Hepatol 78(1):45
66. Pelchovitz DJ, Goldberger JJ (2011) Caffeine and cardiac arrhythmias: a review of the evidence. Am J Med 124(4):284–289
67. Penna DBS, Costa SG, Santos-Rodrigues AD et al (2024) The association of caffeine and nandrolone decanoate modulates aversive memory and nociception in rats. Brain Res. 148937. https://doi.org/10.1016/j.brainres.2024.148937
68. Quiquempoix M, Drogou C, Erblang M et al (2023) Relationship between habitual caffeine consumption, attentional performance, and individual alpha frequency during total sleep deprivation. Int J Environ Res Public Health 20(6):4971
69. Rosen RL, Ramasubramani RS, Benowitz NL et al (2023) Caffeine levels and dietary intake in smokers with schizophrenia and bipolar disorder. Psychiatry Res 319:114989
70. Rukkumani R, Aruna K, Varma PS et al (2004) Comparative effects of curcumin and an analog of curcumin on alcohol and PUFA induced oxidative stress. J Pharm Pharm Sci 7(2):274–283
71. Sa G, Das T (2008) Anti cancer effects of curcumin: cycle of life and death. Cell Div 3:1
72. Saiki S, Sasazawa Y, Imamichi Y et al (2011) Caffeine induces apoptosis by enhancement of autophagy via PI3K/Akt/mTOR/p70S6K inhibition. Autophagy 7(2):176–187
73. Sakthivel KM, Guruvayoorappan C (2013) Acacia ferruginea inhibits tumor progression by regulating inflammatory mediators-(TNF-a, iNOS, COX-2, IL-1β, IL-6, IFN-γ, IL-2, GM-CSF) and pro-angiogenic growth factor- VEGF. Asian Pac J Cancer Prev 14(6):3909–3919
74. Santos C, Costa J, Santos J et al (2010) Caffeine intake and dementia: systematic review and meta-analysis. J Alzheimer's Dis 20:187–204
75. Sarawi WS, Alhusaini AM, Fadda LM et al (2021) Curcumin and nano-curcumin mitigate copper neurotoxicity by modulating oxidative stress, inflammation, and akt/gsk-3β signaling. Molecules 26(18):5591
76. Satta S, Mahmoud AM, Wilkinson FL et al (2017) The role of Nrf2 in cardiovascular function and disease. Oxid Med Cell Longev 2017:1–18
77. Schmitt GC, Arbo MD, Lorensi AL et al (2016) Gender differences in biochemical markers and oxidative stress of rats after 28 days oral exposure to a mixture used for weight loss containing p-synephrine, ephedrine, salicin, and caffeine. Braz J Pharm Sci 52(1):59–68
78. Shanmugam MK, Kannaiyan R, Sethi G (2011) Targeting cell signaling and apoptotic pathways by dietary agents: Role in the prevention and treatment of cancer. Nutr Cancer 63(2):161–173
79. Sharma RA, Gescher AJ, Steward WP (2005) Curcumin: the story so far. Eur J Cancer 41(13):1955–1968
80. Shehzad A, Rehman G, Lee YS (2013) Curcumin in inflammatory diseases. BioFactors (Oxford, England) 39(1):69–77
81. Simopoulos AP (2002) Omega-3 fatty acids in inflammation and autoimmune diseases. J Am College Nutr 21(6):495
82. Soltani S, Salari-Moghaddam A, Saneei P et al (2023) Maternal caffeine consumption during pregnancy and risk of low birth weight: a dose-response meta-analysis of cohort studies. Crit Rev Food Sci Nutr 63(2):224–233
83. Souza LL, Moura EG, Lisboa PC (2024) Can mothers consume caffeine? The issue of early life exposure and metabolic changes in offspring. Toxicol Lett. 393:96–106. https://doi.org/10.1016/j.toxlet.2024.02.005
84. Stark DT, Bazan NG (2011) Synaptic and extrasynaptic nmda receptors differentially modulate neuronal cyclooxygenase-2 function, lipid peroxidation, and neuroprotection. J Neurosci 31(39):13710
85. Taalat M, Aly E, Mohamed E et al (2018) Caffeine and nifedipine effect on cataract induced by selenite in rats. J Arab Soc Med Res 13(1):32
86. Toomey DP, Murphy JF, Conlon KC (2009) COX-2, VEGF and tumour angiogenesis. Surg : J Royal Colleges Surg Edinburgh Ireland 7(3):174–180
87. Tsai Y, Chien C, Lin L et al (2011) Curcumin and its nano-formulation: the kinetics of tissue distribution and blood–brain barrier penetration. Int J Pharm. https://doi.org/10.1016/j.ijpharm.2011.06.030
88. Tsvetkova DD (2015) Investigation of some pharmacological effects of caffeine and taurine in food supplements. Int J Nutr Food Sci 4(1):18
89. Ullah F, Ali T, Ullah N et al (2015) Caffeine prevents d-galactose-induced cognitive deficits, oxidative stress, neuroinflammation and neurodegeneration in the adult rat brain. Neurochem Int. https://doi.org/10.1016/j.neuint.2015.07.001
90. Wang Y, Tang Q, Duan P et al (2018) Curcumin as a therapeutic agent for blocking NF-κB activation in ulcerative colitis. Immunopharmacol Immunotoxicol 40(6):476–482
91. Yallapu MM, Jaggi M, Chauhan SC (2012) Curcumin nanoformulations: a future nanomedicine for cancer. Drug Discov Today 17(1–2):71–80
92. Yamauchi R, Kobayashi M, Matsuda Y et al (2010) Coffee and caffeine ameliorate hyperglycemia, fatty liver, and inflammatory adipocytokine expression in spontaneously diabetic KK-Ay mice. J Agric Food Chem 58(9):5597–5603
93. Yinka OS, Oladele OJ, Olanrewaju AJ (2017) Excessive oral intake caffeine altered cerebral cortex histoarchitecture and cell morphologies in adult mice. Anat J Africa 6(1):856
94. Zhang J, Zheng Y, Luo Y et al (2019) Curcumin inhibits LPS-induced neuroinflammation by promoting microglial M2 polarization via TREM2/TLR4/ NF-κB pathways in BV2 cells. Mol Immunol 116:29–37
95. Zhang RC, Madan CR (2021) How does caffeine influence memory? Drug, experimental, and demographic factors. Neurosci Biobehav Rev 131:525–538
96. Zhang Z, Jiang M, Fang J et al (2017) Enhanced therapeutic potential of nano-curcumin against subarachnoid hemorrhage-induced blood-brain barrier disruption through inhibition of inflammatory response and oxidative stress. Mol Neurobiol 54(1):1–14
97. Zhao J, Zhao Y, Zheng W et al (2008) Neuroprotective effect of curcumin on transient focal cerebral ischemia in rats. Brain Res 1229:224–232
98. Zheng J, Zhu X, Xu G, Wang X, Cao M, Zhu S, Zhou Y (2023) Relationship between caffeine intake and thyroid function: results from NHANES 2007–2012. Nutr J 22(1):36
99. Zhu H, Xinjun W, Xiaoxiao W et al (2020) Curcumin attenuates inflammation and cell apoptosis through regulating NF-κB and JAK2/STAT3 signaling pathway against acute kidney injury. Cell Cycle. https://doi.org/10.1080/15384101.2020.1784599

Publisher's Note