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ORIGINAL ARTICLE

POMEGRANATE JUICE TREATMENT REVERSES CARBON TETRACHLORIDE (CCL4)-INDUCED INCREASED ACETYLCHOLINESTERASE ACTIVITY AND CELL DEATH VIA SUPPRESSION OF OXIDATIVE STRESS IN RATS

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ABSTRACT

Background. Environmental pollution, including exposure to carbon tetrachloride (CCl4), poses serious health risks, particularly through oxidative stress, which may lead to neurodegenerative damage. Antioxidants, especially those found in natural products, show potential in mitigating these toxic effects. Pomegranate juice (PJ), rich in bioactive phytochemicals, has demonstrated antioxidant, anti-inflammatory, and neuroprotective properties.

Objective. This study aimed to investigate the protective effects of PJ on neurotoxicity induced by CCl4 in rats, assessing specific markers of oxidative stress, enzymatic activity, and apoptotic cell death.

Material and Methods. Twenty-eight male Wistar rats were divided into four groups: Control, CCl4, PJ, and CCl4+PJ. The CCl4 group received intraperitoneal injections of CCl4 (0.2 ml/100 g) twice weekly for six weeks, while the PJ group received PJ orally (4 ml/kg) daily for 30 days. The CCl4+PJ group received both treatments in sequence. Brain tissues were analysed for malondialdehyde (MDA), reduced glutathione (GSH), acetylcholinesterase (AChE), glutathione S-transferase (GST), glutathione reductase (GR), and carboxylesterase (CaE) activity. Apoptotic cell death was assessed using TUNEL staining.

Results. CCl4 exposure resulted in a marked increase in MDA levels and AChE activity in brain tissue ($p<0.05$), alongside a significant decrease in reduced GSH levels and GST activity ($p<0.05$). Treatment with PJ significantly lowered MDA levels and AChE activity in the CCl4+PJ group compared to the CCl4 group ($p<0.05$). However, GSH levels and GST activity showed no significant changes in the CCl4+PJ group. TUNEL staining indicated a reduction in apoptotic cells in the CCl4+PJ group versus the CCl4 group, suggesting reduced cellular damage with PJ treatment ($p<0.05$).

Conclusions. PJ demonstrates neuroprotective potential against CCl4-induced oxidative stress and neurotoxicity in rats by reducing oxidative markers and apoptosis. These findings suggest that PJ could serve as a natural protective agent against neurodegenerative risks associated with environmental pollutants like CCl4.

Keywords: *carbon tetrachloride, neurotoxicity, pomegranate juice, acetylcholinesterase, oxidative stress*

INTRODUCTION

Environmental pollution is one of the most important global problems that threatens humankind and health among with global warming [1]. According to a recent study, more than 9 million people died due to environmental pollution originated effects per year [2]. Contaminants that introduced to water, soil and air can effect to human body and causes irreversible damage to the organs [3]. Carbon tetrachloride (CCl4)

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which may be found in both ambient outdoor and indoor air is a dangerous chemical [4]. In ambient air, concentrations around 0.1 to 1 parts per billion (ppb) are generally considered safe due to regulation [5]. Toxic effects are unlikely at these low levels for the general population. exposure limit (PEL) for CCl4 in workplaces is 10 parts per million (ppm) over an 8-hour time-weighted average [5]. This is intended to prevent chronic toxicity, though acute exposure to 200 ppm or more can cause immediate health risks, including symptoms like dizziness, nausea, and damage to organs. The IDLH (immediately dangerous to life or health) level is 200 ppm, where even short exposures can lead to significant toxicity [6]. In laboratory settings, acute toxicity studies show that doses of CCl4 at 50-100 mg/kg body weight can cause significant liver and kidney damage in animal models [6]. In humans, ingestion of as little as a few millilitres of liquid CCl4 can be fatal, and high vapour concentrations (over 1,000 ppm) are similarly lethal if inhaled [6]. The major effects of CCl4 on health are hepatic and kidney damage and central nervous system (CNS) depression [4]. Despite its usage has been highly restricted since it was identified as a very harmful toxic substance, it is still possible for humans to be exposed to this substance [7]. CCI4 induce brain damage via increasing oxidative stress by free radical toxicities, inflammation, and neurotoxicity [8]. CCl4 has been shown to induces oxidative damage by the generation of free radicals and increases lipid peroxidation which are the main signs of oxidative stress [9]. The formation of free radicals increases in tissues exposed to CCl4 [9]. As a result of the depletion of antioxidant mechanisms in the cells, the pro-oxidant-antioxidant balance is disrupted and tissue damage occurs [10]. This complex tissues damage can cause neurodegeneration in CNS [11]. Antioxidants used against CCl4 toxicity may have a protective effect in this neurodegenerative process [12]. Numerous studies have investigated the use of specific antioxidant molecules for preservative or therapeutic purposes and promising data are available [12-14]. Although there are medical treatments against acute poisoning that caused by environmental pollution, it is important to avoid chronic toxic effects of CCl4 like pollutions by reducing the risk factors with natural products.

The pomegranate juice (PJ) is a natural product that is rich in various phytochemicals, exerting a wide range of biological activities such as anti-oxidative, anti-inflammatory, and anti-apoptotic properties [15]. These rich compounds play an important role in the oxidation of unsaturated fatty acids and detoxification of free radicals and makes PJ an efficient beneficial natural product for heath [16]. According to antioxidant capacity PJ has been safely used for centuries in both traditional and modern medicine [17]. The neuroprotective effect of pomegranate phytochemicals has been demonstrated in a few studies [18-20]. Regarding neurodegenerative diseases, a lot of in vivo studies on beneficial effects of pomegranate have been devoted. However, there is limited data about its neuroprotective potential against CCl4 induced toxicity.

In current study investigated the potential protective efficacy of PJ on neurotoxicity induced by CCl4 in rats. To better understand the neuroprotective effects of pomegranate juice (PJ) on neurotoxicity induced by carbon tetrachloride (CCl4), several key markers were assessed. Malondialdehyde (MDA), a well-known indicator of lipid peroxidation [21], and reduced glutathione (GSH), a vital antioxidant for neutralizing reactive oxygen species (ROS) and supporting cellular redox balance [22], were measured. Additionally, the activities of brain acetylcholinesterase (AChE), which is affected by CCl4 exposure [23], and brain glutathione S-transferase (GST), an enzyme crucial for detoxification by facilitating GSH conjugation to toxic compounds [22], were analysed. Glutathione reductase (GR), essential for recycling GSH from its oxidized form and maintaining cellular antioxidant capacity [24], as well as carboxylesterase (CaE), involved in ester metabolism and detoxification [25], were also evaluated. Together, these markers provide insight into the effects of CCl4 on oxidative stress, antioxidant defenses, and enzymatic activity impacted by neurotoxicity. Furthermore, we assessed apoptotic cell death to evaluate the potential protective effect of PJ.

MATERIAL AND METHODS

Preparation of pomegranate juice

Pomegranates freshly sourced from Adıyaman, Turkey, were carefully prepared. After washing and draining, they were halved, and their seeds along with the white pulp were shredded together using an electric blender. The resulting juice was then preserved in 1 ml quantities at -20°C until used.

Chemical composition of pomegranate juice

The chemical content of the PJ has been studied in the project 'My city is Adıyaman' as the following project number: TRC1/18/KBG/0.036. Project financial issues granted by Development of Urban Culture and Urban Awareness Financial Support Program. The PJ content determined as followed: phenolic acid 490.75 mg/kg, anthocyanin 137.1 mg/L, ellagic acid 175 mg/100 g, total flavonoids 63 mg/kg and total anti-oxidants 1530 mg/kg [26].

Animals

The study was conducted at the Adıyaman University Animal Experiment Centre. Ethical

approval was obtained from the Adıyaman University Animal Experiments Centre Ethics Committee under the protocol number 2022/18. Twenty-eight adult male Wistar albino rats weighing 200-250 g were used in the study. The rats were kept at 22±20°C room temperature in a 12-hr light 12-hr dark cycle with ad libitum access to chow and water.

Experimental design

The animals were randomly divided into four groups (n=7 for each group) as followed: Control group, CCl4 group, PJ group, CCl4+PJ group. Before all experimental procedures, the animals were housed in cages without any experimental applications for one week to allow for adaptation. Immediately after adaptation process experimental applications performed as followed:

Control group: The control group received the solvent of CCl4 twice a week for six weeks. Following this, they were administered saline (4 ml/kg) every other day for the next 30 days.

CCI4 group: CCI4 (Bayer, ≥99.9% purity) was prepared in 50% olive oil and injected intraperitoneally (i.p.) at a dose of 0.2 ml/100 g twice a week for 6 weeks [27].

PJ group: In this group, pomegranate juice was administered to the animals via an orogastric tube at a dose of 4 ml/kg per day for 30 days [28].

CCI4+PJ group: In this group, the animals were injected i.p. with CCI4 at a dose of 0.2 ml/100 grams twice a week for 6 weeks. For the following 30 days, PJ was administered to the animals via an orogastric tube at a dose of 4 ml/kg per day.

After the 30-day application process, the animals were euthanized by decapitation and the brain tissues were removed. The brain tissues were kept at −20°C for biochemical analysis.

Homogenization of brain tissue

Brain samples from all groups were homogenized by using a polytron homogenizer (Heidolph RZ 2021, Germany) in a chilled homogenization buffer (0.1 M, pH 7.4 in potassium phosphate buffer; containing 0.15M KCl, 1mM EDTA, 1mM DTT) at four times the total tissue weight (w/v). Following homogenization, the homogenates were transferred to Eppendorf tubes and centrifuged at 16.000g for 20 minutes at 4°C (Sigma Centrifuge Model 2–16K, Sigma, St. Louis, MO). Upon centrifugation, the supernatant fraction was collected, and enzyme activities along with other parameters were assessed. Each sample underwent three replicate absorbance readings, with a repetition of the reading if there was a correlation difference exceeding 10% between the values obtained for the same samples.

Determination of brain malondialdehyde (MDA) and reduced GSH levels

MDA and reduced GSH levels were assessed using a microplate reader spectrophotometer system (Thermo TM Varioskan Flash, Thermo Scientific). Brain MDA levels were determined by assessing the relative production of reactive substances of thiobarbituric acid [29]. Results presented as nmol/mg wet tissue weight. Reduced GSH activity was measured by its reaction with DTNB to form a compound absorbing at 412 nm [30]. Results expressed as nmol/mg wet weight tissue.

Determination of brain acetylcholinesterase (AChE) activity

Brain AChE activity was assessed according to the method described by Ellman et al [31] (some modifications has been approached to method [32]) utilizing a Thermo TMV arioskan Flash microplate reader spectrophotometer system from Thermo Scientific. In this process, acetylcholine iodide (ACTI) served as the substrate, and product formation was determined based on color change. To begin, 10 µl of supernatant was pipetted into microplate wells. Subsequently, the final solution, consisting of 200 µl (0.701 mM ACTI and 0.136 mM 5.5-dithiobis-2-nitro-benzoic acid (DTNB)) prepared in 0.1 M buffer (pH 8.0), was transferred into the microplate wells. Absorbance changes were recorded at 412 nm for 1 minute at 25°C. Specific AChE activity was calculated as nmol/min/mg protein. Total protein content in the tissue homogenates was determined using the Bradford method [33]. Following a 1:4 dilution, 5 µl of the diluted sample and 250 µl of Bradford solution were sequentially added to the microplate wells. This mixture was then incubated at room temperature in the dark for 15 minutes. Absorbance was measured at a wavelength of 595 nm, based on the resulting colour change.

Determination of brain glutathione s-transferase (GST) activity

To measure GST activity, a solution of 20 mM 1-chloro-2.4-dinitrobenzene (CDNB) was prepared first in 96% ethanol, and this served as the substrate. Reductive glutathione (0.002 M) was utilized as the cofactor in the reaction [34]. Briefly, 10 µl of supernatant, 100 µl of phosphate buffer (0.1 M, pH 6.5), 100 µl of the GSH mixture, and finally 10 µl of CDNB were transferred into the microplate wells. These were then inserted into the microplate reader system, and the absorbance change was recorded at 344 nm for 2 minutes at 25°C. Specific GST activity was computed as nmol/min/mg protein.

Determination of brain glutathione reductase (GR)

The analysis of GR activity was conducted using a modified method [35]. The assay solution comprised 50 mM Tris–HCl buffer (pH 8.0), 1 mM EDTA, 1 mM GSSG, and 0.1 mM NADPH. One enzyme unit was defined as the quantity that oxidizes 1 μmol NADPH per minute under the specified assay conditions.

Determination of brain carboxylesterase (CaE) activity

The previously described spectrophotometric methods were adapted for analysis of CaE activity using a microplate reader system [36, 37]. In the activity assay, p-nitrophenol acetate (PNPA) was dissolved in 26 mM 96% ethanol to serve as the substrate. A reaction solution comprising 5 ml of sample and 250 ml of 0.1 mM Trizma buffer (pH 7.4) was incubated for 3 minutes at 25°C. The reaction was initiated by adding 5 ml of the substrate to the reaction solution. Changes in absorbance were monitored at 405 nm for 2 minutes at 25°C. Specific CaE activity was calculated as nmol/min/mg protein.

TUNEL Analysis and apoptotic index

After embedding brain samples from each group into paraffin blocks, sections 5-6 μm thick were placed on poly-L-lysine-coated slides. Apoptotic cells were identified using the ApopTag Plus Peroxidase In Situ Apoptosis Detection Kit (Chemicon, cat. no.: S7101, USA) according to the manufacturer's instructions. The tissues were deparaffinized with xylene, passed through graded alcohol series, and washed with phosphate-buffered saline (PBS). Following this, the tissues were incubated for 10 minutes with 0.05% proteinase K, and then incubated for 5 minutes with 3% hydrogen peroxide to block endogenous peroxidase activity. After washing the tissues with PBS, they were incubated for 6 minutes with Equilibration Buffer and then incubated for 60 minutes at 37°C in a humid chamber with the working solution (70% Reaction Buffer + 30% TdT Enzyme). After a 10-minute incubation in Stop/Wash Buffer, the tissues were treated with Anti-Digoxigenin-Peroxidase for 30 minutes, and apoptotic cells were visualized using the Diaminobenzidine (DAB) substrate. The sections were counterstained with Harris hematoxylin and mounted using an appropriate medium. The images were examined and photographed using a Novel N-800M microscope [38].

To evaluate the TUNEL staining, nuclei stained blue with Harris hematoxylin were considered normal, while cells with brown nuclear staining were evaluated as apoptotic. In randomly selected areas at 10x magnification, at least 500 normal and apoptotic cells were counted in the sections. The apoptotic index (AI) was calculated by determining the ratio of apoptotic cells to the total number of cells (normal + apoptotic), and statistical analyses were performed based on this ratio [38].

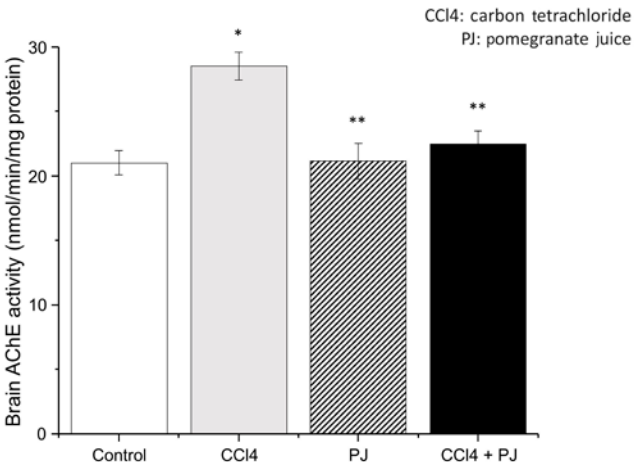
Statistical analysis

The results are presented as mean ± SEM. Group comparisons were conducted using one-way analysis of variance (ANOVA), followed by the Tukey–HSD test. Statistical significance was considered at $p<0.05$.

RESULTS

Brain AChE activity

The AChE activity significantly increased only in CCl4 group that compared to the control group (28.54±1.07 nmol/min/mg protein and 21.03±0.92 nmol/min/mg protein, respectively, $p<0.05$). The AChE activities of the PJ (21.15±1.41 nmol/min/mg protein) and CCl4+PJ (22.47±1.01 nmol/min/mg protein) groups were significantly lower that compared to CCl4 group ($p<0.05$). There was no significant difference neither in PJ nor in CCl4+PJ groups that compared to control group ($p>0.05$, see Figure 1).

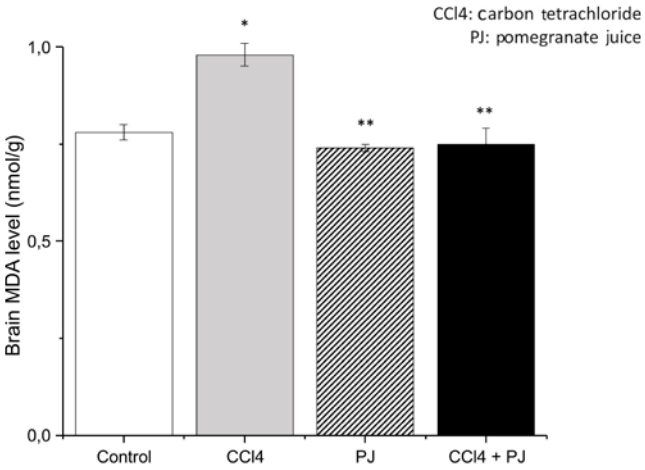


The data represent as mean ± SEM (n=7); * – $p<0.05$ compared to the control group; ** – $p<0.05$ compared to the CCl4 group

Figure 1. Effects of CCl4 and PJ on the AChE activity in male rat brain

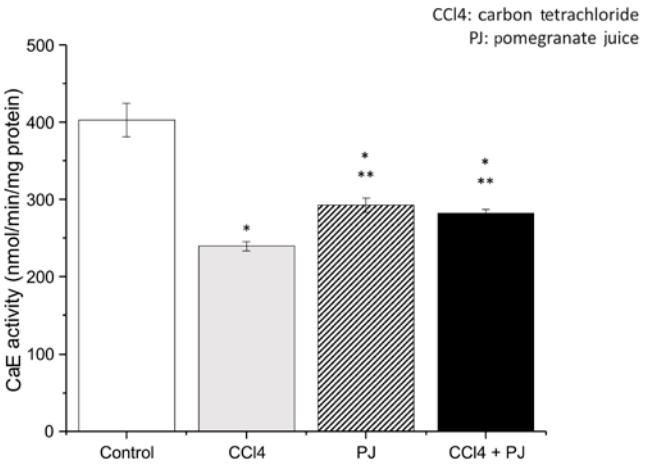
Brain MDA levels

The brain MDA concentration of the CCl4 group was significantly higher than the control group (0.98±0.03 nmol/g and 0.78±0.02 nmol/g, respectively, $p<0.05$). There was no significant difference in the PJ (0.74±0.01 nmol/g) and CCl4+PJ group (0.75±0.04 nmol/g) that compared to the control group. In comparison to the CCl4 group, the brain MDA levels were lower in the PJ and CCl4+PJ groups ($p<0.05$, see Figure 2).



The data represent as mean ± SEM (n=7); * – p<0.05 compared to the control group; ** – p<0.05 compared to the CCl4 group

Figure 2. Effects of CCl4 and PJ on the MDA level in male rat brain

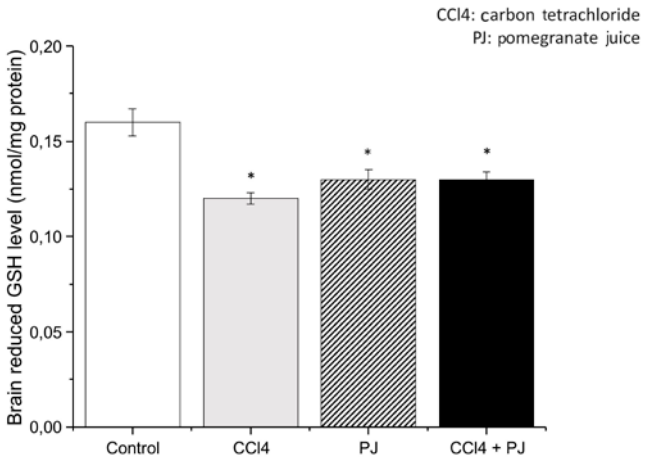


The data represent as mean ± SEM (n=7); * – p<0.05 compared to the control group; ** – p<0.05 compared to the CCl4 group

Figure 4. Effects of CCl4 and PJ on the CaE levels in male rat brain

Brain reduced GSH levels

The brain reduced GSH levels were significantly lower in the CCl4, PJ and CCl4+PJ groups that compared to the control group (0.12±0.003 nmol/g, 0.13±0.005 nmol/g and 0.13±0.004 nmol/g respectively, p<0.05). In comparison to CCl4 group, there was no significant difference in both PJ and CCl4+PJ groups (p>0.05, see Figure 3).



The data represent as mean ± SEM (n=7); * – p<0.05 compared to the control group

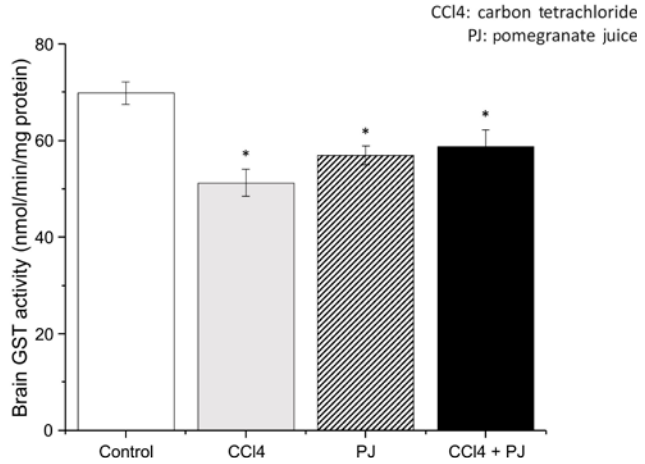
Figure 3. Effects of CCl4 and PJ on the reduced GSH level in male rat brain

Brain CaE activity

Brain CaE activity decreased in the CCl4, PJ and CCl4+PJ groups that compared to the control group (239.56±6.16 nmol/g, 292.40±9.64 nmol/g, 281.85±5.24 nmol/g and 402.93±21.53 nmol/g, respectively, p<0.05). However, the CaE activity was higher in both PJ and CCl4+PJ groups than in the CCl4 group (p<0.005, see Figure 4).

Brain GST activity

Brain GST activity were significantly lower in the CCl4, PJ and CCl4+PJ groups that compared to the control group (51.24±2.78 nmol/g, 56.94±1.98 nmol/g, 58.81±3.41 nmol/g and 69.81±2.28 nmol/g respectively, p<0.05). In comparison to CCl4 group, there was no significant difference in both PJ and CCl4+PJ groups (p>0.05, see Figure 5).

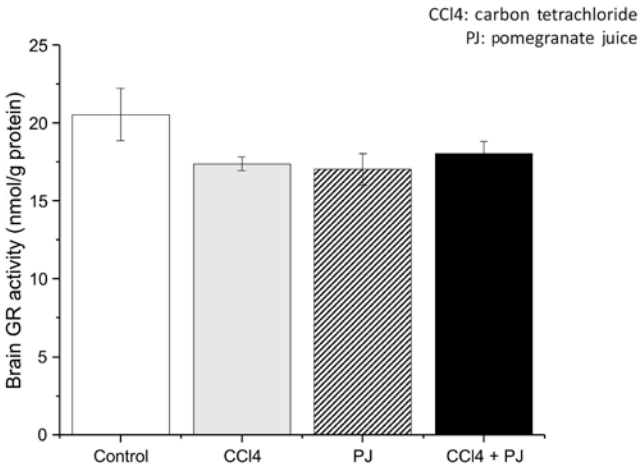


The data represent as mean ± SEM (n=7); * – p<0.05 compared to the control group

Figure 5. Effects of CCl4 and PJ on the GST levels in male rat brain

Brain GR activity

The brain GR activity was not significantly higher or lower in the CCl4, PJ and CCl4+PJ groups that compared to the control group (17.38±0.46 nmol/g, 17.01±1.02 nmol/g, 18.04±0.80 nmol/g and 20.52±1.67 nmol/g respectively, p>0.05). In comparison to CCl4 group, there was no significant difference in both PJ and CCl4+PJ groups (p>0.05, see Figure 6).

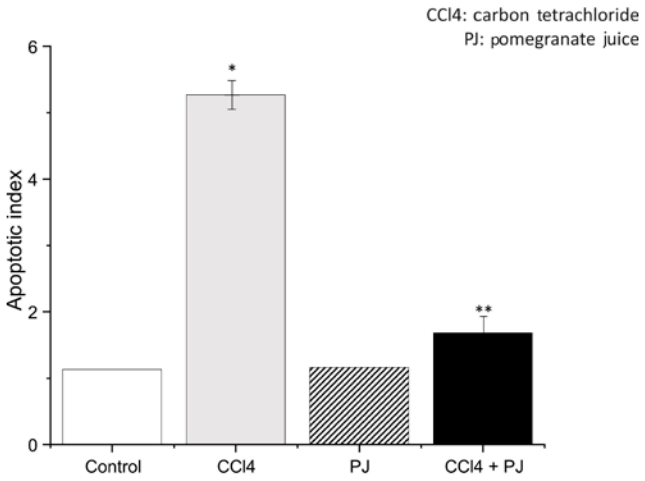


The data represent as mean ± SEM (n=7); p>0.05 compared to the CCl4 group

Figure 6. Effects of CCl4 and PJ on the GR activity in male rat brain

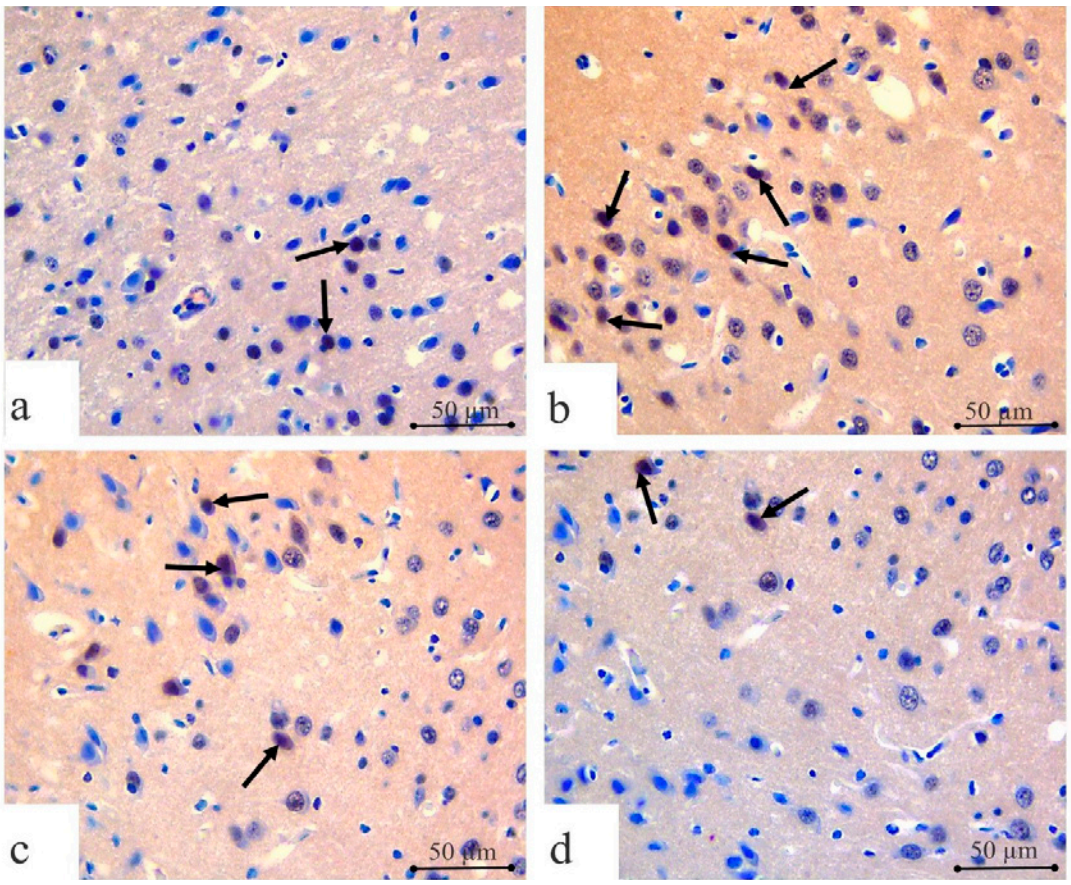
TUNEL findings

Upon examining TUNEL staining under a light microscope to identify apoptotic cells, no statistically significant difference in TUNEL positivity was observed in the PJ group (Figure 8d) compared to the control group (Figure 8a) (p=0.637). However, a statistically significant increase in TUNEL positivity was observed in the CCl4 group (Figure 8b) compared to the control group (p=0.0001). In contrast, a statistically significant decrease in TUNEL positivity was observed in the CCl4+PJ group (Figure 8c) compared to the CCl4 group (Figure 8b) (see Figure 7).



The data represent as mean ± SEM (n=7); * – p<0.05 compared to the control group; ** – p<0.05 compared to the CCl4 group

Figure 7. Effects of CCl4 and PJ on the apoptotic activity in male rat brain



a – control, b – CCl4, c – PJ, d – CCl4+PJ; apoptotic cells are indicated with black arrows; DAB chromogen, Mayer's haematoxylin, scale bar: 50 µm

Figure 8. Photomicrographs of TUNEL staining in the cortical region of rat brains from all groups

DISCUSSION

Although the brain is quite sheltered by many anatomic and physiological component, it is particularly susceptible to extensive oxidative damage due to its high lipid content, elevated energy demands, and limited antioxidant defence system [39]. ROS, generated under toxic conditions such as CCl4 exposure, increase neuronal vulnerability by causing oxidative alterations, ultimately contributing to the development of neurodegenerative diseases [40]. The maintenance of ROS levels and the regulation of metal overload are governed by cellular defence mechanisms, intracellular signalling pathways, and the physiological roles of antioxidants within the brain [39]. Numerous antioxidants, including polyphenols and vitamins, play crucial roles in ROS regulation, metal ion chelation, and cellular signalling to defend against oxidative stress in the brain [41]. AChE, a prominent enzyme in brain tissue, plays a significant role in the pathogenesis of neurodegenerative diseases. It can influence various processes including the inflammatory response, apoptosis, oxidative stress, and aggregation of pathological proteins [42]. Many studies have demonstrated that toxins and heavy metals typically lead to a reduction in AChE activity [43, 44]. In our study, CCl4 exposure led to a significant increase in AChE activity in the brain tissue, diverging from findings where toxins and heavy metals typically suppress AChE [44]. This unexpected increase may be attributed to CCl4's unique toxic mechanisms, which can induce an inflammatory response, apoptosis, and aggregation of pathological proteins through AChE modulation. Our findings align with studies on neurotoxic exposures like aluminium and cadmium, which similarly induce AChE activity in the brain by triggering stress-response pathways [45]. PJ treatment, known for its polyphenol content, showed a reversing effect on this increase. Polyphenol-rich antioxidants like resveratrol, curcumin, and N-acetylcysteine have similarly been shown to stabilize or even reduce AChE activity after toxic exposure [46], suggesting PJ's potential to counteract neurotoxic effects mediated by AChE. This finding represents the novel aspect of our study. MDA, final products of polyunsaturated fatty acids peroxidation in the cells. An increase in free radicals causes overproduction of MDA and level is commonly indicator of oxidative stress and the antioxidant status [47]. It is known that pollutants and heavy metals causes increase of MDA levels. Lipid peroxidation, as measured by MDA levels, was significantly elevated in the CCl4 group, which aligns with the established increase in oxidative stress markers following exposure to toxins. Antioxidants such as vitamin E, melatonin, and curcumin have demonstrated efficacy in reducing MDA levels in comparable models, reflecting their capacity to counteract oxidative lipid damage [48-50]. The reduction in MDA levels with PJ treatment supports its lipid-protective properties, yet PJ alone did not alter MDA levels, implying its antioxidative role is particularly relevant in mitigating CCl4-induced stress rather than as a standalone effect. Reduced GSH levels, a key marker of antioxidant defence, were significantly decreased in the CCl4-treated group, consistent with the depletion of cellular antioxidants under toxic stress. Interestingly, PJ treatment alone also reduced GSH levels, which might reflect the pro-oxidant tendencies of certain flavonoids when administered chronically. Similar findings have been observed with antioxidants like quercetin and epigallocatechin gallate (EGCG), which can exhibit pro-oxidant effects depending on their concentration and duration of exposure [51]. This dual role of PJ and other polyphenols suggests that, while protective in acute toxic stress, these compounds may modulate redox balance differently under extended treatment. GST, like reduced GSH, is also on the frontline of cellular defence against both acute and chronic oxidative stress toxicity [52]. A reduction was observed with CCl4 exposure, aligning with its known vulnerability to toxic insults. PJ treatment alone also reduced GST activity, again potentially due to the pro-oxidant properties of flavonoids in high doses or chronic exposure. Antioxidants like selenium and silymarin, which have been studied in CCl4 models, similarly demonstrate dose-dependent impacts on GST, either boosting or reducing its activity depending on treatment conditions [53, 54]. This suggests that the dose and duration of antioxidant treatment are critical in modulating GST activity, and further studies could explore optimal dosing regimens for PJ to balance its antioxidative and pro-oxidant effects.GR in an important component of cells that plays an essential role in the defence system against ROS by regenerating the glutathione [55]. Unlike GST, GR activity did not change significantly across groups, suggesting that the treatment period of our study may have been insufficient to alter this enzyme's activity, which plays a crucial role in glutathione regeneration. In some studies, low-dose CCl4 and moderate doses of antioxidants such as coenzyme Q10 show limited effects on GR [56], yet antioxidants in higher doses or different compounds, like alpha-lipoic acid, have demonstrated a significant impact on GR activity [57]. This highlights that PJ's effect on GR might also be dose-dependent, warranting further investigation to understand its role in modulating cellular redox balance at varying concentrations. CaE activity, involved in metabolizing endogenous and exogenous esters, was significantly reduced by CCl4, reflecting its susceptibility to oxidative damage [25].

PJ treatment in both CCl4 and standalone groups enhanced CaE activity, indicating a protective effect. This is consistent with antioxidants like melatonin and alpha-lipoic acid, which have been shown to maintain or even restore CaE activity following oxidative stress in CCl4 and similar models [58]. PJ's ability to enhance CaE activity suggests a protective role in maintaining esterase function, which is essential for the detoxification of environmental toxins and preservation of neural homeostasis. In TUNEL staining analysis, we observed a significant increase in apoptotic cells in the CCl4 group, while PJ treatment notably reduced apoptosis, indicating a neuroprotective effect. Studies with antioxidants like curcumin, EGCG, and selenium show similar anti-apoptotic effects in the context of CCl4-induced oxidative stress [59], supporting the potential of PJ to protect against cell death pathways. The reduction in TUNEL positivity in the PJ-treated group suggests that PJ's polyphenols may help stabilize cellular environments and mitigate the impact of ROS-induced apoptosis.

CONCLUSIONS

In conclusion, our findings underscore the potential of PJ as a neuroprotective agent in CCl4-induced oxidative stress, with similarities to antioxidants. The dual effects observed, particularly the time dependent modulation of antioxidant enzymes and pro-oxidant properties of PJ, highlight the importance of optimizing treatment duration and dosing for effective neuroprotection. Further studies are needed to compare PJ's effects with these antioxidants across different dosages and durations to further elucidate its therapeutic potential in oxidative stress and neuroprotection.

Conflict of interest

The authors declare that they have no conflicts of interest concerning this article.

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