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# EFFICACY OF TAUROURSODEOXYCHOLIC ACID ON BIOCHEMICAL AND HISTOPATHOLOGICAL CHANGES IN 1-METHYL-4-PHENYL-1,2,3,6-TETRAHYDROPYRIDINE INDUCED PARKINSON'S DISEASE MICE MODEL

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**ABSTRACT**

**Objectives:** One of the main pathological features of Parkinson's disease (PD) is the loss of dopaminergic neurons in the nigrostriatal pathway. Pre-clinical research has shown that tauroursodeoxycholic acid (TUDCA) monotherapy can alleviate the neuropathological complications of PD. The present study aimed to compare the therapeutic benefits of combination TUDCA and syndopa therapy with those of TUDCA and syndopa monotherapy in a mouse model of PD induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP).

**Methods:** Male C57BL/6 mice received MPTP (30 mg/kg body weight/day, intraperitoneal, 5 days) to induce PD, followed by TUDCA (150 mg/kg body weight/day, intraperitoneal), syndopa (12 mg/kg body weight/day, oral), or both for 21 days. The midbrain reactive oxygen species (ROS), malondialdehyde (MDA), peroxynitrite, nitric oxide (NO), and urea contents, as well as the total antioxidant capacity (TAC), were measured. Midbrain and liver histopathology and histomorphometry assessed neuronal and hepatic damage.

**Results:** MPTP increased the ROS (2.41-fold, $p<0.01$), MDA (2.07-fold, $p<0.01$), peroxynitrite (1.62-fold, $p<0.01$), NO (2.23-fold, $p<0.01$), and urea (1.88-fold, $p<0.01$) contents and reduced the TAC (1.92-fold, $p<0.01$) compared with the control. Combination therapy reduced the ROS content by 2.33 fold, the MDA content by 2.12 fold, the urea levels by 1.83 fold, peroxynitrite levels by 1.59 fold, NO by 2.65 fold, and neuronal loss by 3.68 fold (all $p<0.05$ vs. the MPTP group), outperforming both TUDCA (1.66 fold reduction in the ROS content, $p>0.05$, NS) and syndopa (1.38 fold reduction in the ROS content, $p>0.05$, NS) monotherapy. The same trend followed for other parameters, such as urea, TAC, MDA, NO and peroxynitrite. Moreover, the histopathology and histomorphometric results confirmed that TUDCA monotherapy and combination therapy markedly attenuated MPTP-induced neuronal and liver damage, with the combination therapy showing superior efficacy.

**Conclusions:** TUDCA + syndopa offers synergistic neuroprotection and represents a novel therapeutic strategy for PD.

**Keywords:** Parkinson's disease, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, Tauroursodeoxycholic acid, Syndopa, Oxidative biomarkers, Histomorphometry, C57BL/6 mice.

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## INTRODUCTION

Parkinson's disease (PD) is a common degenerative disorder of the central nervous system that is characterized by the progressive impairment of voluntary motor control [1]. This condition involves the death of dopaminergic neurons, which leads to a decrease in dopamine [2,3] and thus disruption of neuronal communication and impaired motor functions [3]. The incidence and prevalence of PD are increasing markedly. Globally, the prevalence of PD cases was 11.77 million in 2021, and projections indicate the number of people living with PD will rise from 112% to 25.2 million worldwide by 2050 [4]. PD is now considered to be the second most prevalent neurological disorder [5,6].

Diagnosing PD is challenging because the symptoms manifest in different forms in different individuals and mimic other neurological disorders. The clinical symptoms of PD are divided into motor and non-motor [7]. The most common motor symptoms are tremors, dyskinesia, rigidity, and postural instability. The non-motor symptoms include depression, anxiety, sensory abnormalities, sleep difficulties, gastrointestinal problems, amnesia, and dementia [8]. Aggregation of α-synuclein in the form of Lewy bodies and neurites in various regions of the brain are the pathological hallmarks of PD [9]. The present standard therapy for PD involves the administration of dopamine precursors, such as levodopa and carbidopa, which relieve the symptoms but cannot cure the disease [10-12].

Oxidative stress, resulting from an imbalance between antioxidants and free radicals, is a major factor in the pathophysiology of PD and is particularly detrimental to dopaminergic neurons [11-14]. The CNS is particularly susceptible to oxidative damage due to its high oxygen demand: It consumes about 20% of the body's oxygen supply [11]. Moreover, it is vulnerable to lipid peroxidation due to its high content of polyunsaturated fatty acids and its relatively low levels of antioxidants [15-17]. Depletion of reduced glutathione (GSH) and the presence of malondialdehyde (MDA) are key indicators of increased lipid peroxidation and oxidative damage in dopaminergic neurons [18,19]. GSH can act alone or in coordination with other enzymes to scavenge hydroxyl radicals, superoxide radicals, and peroxynitrite [20,21].

Animal models have been crucial to better understand the biology of PD and to investigate novel therapies [22]. A widely used model involves the injection of mice with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which mimics the motor and non-motor symptoms of PD seen in

humans [23,24]. MPTP is a highly lipophilic substance that easily crosses the blood–brain barrier (BBB). In astrocytes, monoamine oxidase B (MAO-B) transforms MPTP into $MPDP^+$, which then spontaneously oxidizes to produce $MPP^+$ [25,26]. $MPP^+$ is taken up by dopamine transporters and accumulates in the mitochondria of dopaminergic neurons, where it disrupts respiratory chain complex I, thereby leading to oxidative stress and cell death [27,28]. This disruption leads to oxidative stress, including a reduction in adenosine triphosphate (ATP) synthesis and eventual neuronal death [25,29]. The neurotoxic effects of MPTP are dose-dependent, region-specific, and time-dependent [30], and can be protected against by an MAO-B inhibitor [31].

Tauroursodeoxycholic acid (TUDCA) is an endogenous bile acid that has been investigated for its therapeutic potential in neurodegenerative diseases, such as PD [32,33]. It has been shown to be neuroprotective as it prevented MPTP-induced dopaminergic cell death in a mouse model of PD [34-36,75]. According to Rosa *et al*. [38], TUDCA attenuated the MPTP-mediated decrease in dopaminergic fibers and ATP levels, mitochondrial dysfunction, and neuroinflammation. There was also a considerable reduction in foot dragging and gait improvement. In another study that used the MPTP mouse model, TUDCA inhibited the pro-inflammatory cytokine interleukin 1 beta as well as microglial markers and upregulated the anti-inflammatory protein annexin A1 [36]. TUDCA can alleviate a multitude of symptoms in various animal models of neurodegeneration by acting as a chemical chaperone [33,35,36]. It has also demonstrated protective effects against mitochondrial dysfunction and neuroinflammation caused by MPTP and has been shown to improve motor symptoms in a mouse model of PD [37].

All the published evidence stating that TUDCA exerts therapeutic effects in PD models through several mechanisms, and the mechanisms involved in the few, like how the free radicals, reactive oxygen species (ROS), antioxidants, urea, and histopathological changes were affected, were not significantly discussed. TUDCA has shown promise as a neuroprotective agent by ameliorating motor deficits and improving gait quality in such models, thereby addressing critical aspects of disease progression. Furthermore, TUDCA may protect healthy neurons and glial cells, preserving a greater number of dopaminergic neurons and thus potentially delaying disease progression and symptom onset alone. Moreover, researchers have studied TUDCA monotherapy alone, but it will be more beneficial if it can be used in combination therapy with the present drug of choices available to yield better results in the therapeutic progression and well-being, TUDCA, with other therapeutics, may exert more beneficial effects. In our study, we have used TUDCA in combination with Syndopa (which is the most effective drug of choice available in the PD treatment, but there are well-established consequences were reported with long-term use of syndopa alone which including aggravation of neurodegeneration). Hence, the combination therapies may be helpful in PD to overcome the limitations of individual drugs, to effectively reduce symptoms, and to circumvent the side effects by targeting multiple complications at once.

## METHODS

*o*-Phenylenediamine, a butanol-pyridine mixture, sodium dodecyl sulfate (SDS), and thiobarbituric acid (TBA) were procured from Thermo Fischer Scientific (Chennai, India). The Urea Assay Kit was purchased from Abcam ab83362 (Boston, MA, USA).

### Animals

Male C57BL/6 mice, 2–3 months old and weighing 30–40 g, were purchased from Mass Biotech (Chengalpattu, India). They were housed under a 12-h photoperiod with relative humidity of 30–70% and had access to sufficient food and water during the entire experiment. This research was approved by Saveetha Medical College's Institutional Animal Ethics Committee (IAEC Approval Number SU/CLAR/RD/34/2023), which adheres to the CCSEA guidelines and principles for the conduct of animal experimentation.

### Treatment groups

The mice were randomly divided into five groups of six mice (see the details below). The random number table method was employed for randomization. A specific non-sequential number was assigned to each mouse. Then, the numbers were picked randomly from a table to allocate each mouse to one of the experimental groups (described below). For blinding and to avoid sample bias, the group allocation was masked by randomly coding the samples with numbers. The tested doses of TUDCA (150 mg/kg body weight) [39] and syndopa (12 mg/kg body weight) [40] were selected based on previous studies. The five treatment groups were:

- Group 1: Control group, received saline (0.2 mL/mouse delivered intraperitoneally)
- Group 2: Administered MPTP (30 mg/kg body weight/day delivered intraperitoneally) dissolved in saline for 5 consecutive days
- Group 3: Administered MPTP as described for group 2, followed by TUDCA (150 mg/kg body weight/day delivered intraperitoneally) dissolved in phosphate-buffered saline (PBS) for 21 days
- Group 4: Administered MPTP as described for group 2, followed by syndopa (12 mg/kg body weight/day delivered orally) dissolved in water for 21 days
- Group 5: Administered MPTP as described for group 2, followed by TUDCA (150 mg/kg body weight/day delivered intraperitoneally) and syndopa (12 mg/kg body weight/day delivered orally) for 21 days after MPTP administration as described for group 2. The time interval between the administration of TUDCA and syndopa was 2 h.

Each mouse was housed for 7 days before treatment (for acclimatization), followed by 26 days of treatment, for a total of 33 days. The investigators were blinded to the group assignments during sample analysis to minimize bias.

### Cardiac perfusion, isolation of target organs, and preparation of midbrain homogenate

The animals were fasted overnight (for approximately 12 h) on the final day of the experiment. Then, they were anaesthetized with isoflurane inhalation and subjected to transcardial perfusion using normal saline (0.9% NaCl). The liver was carefully removed and immediately placed in 10% neutral buffered formalin. The skull of each mouse was opened, and the midbrain was carefully isolated on ice and then washed with ice-cold (PBS, pH 7.4). A portion of the midbrain was fixed in 10% neutral buffered formalin to examine histopathology, and the rest was preserved to examine biochemical markers.

The choice of homogenization method depends on the type of tissue and the purpose [41]. In this study, A 10% (w/v) homogenate was prepared by homogenizing one gram of midbrain tissue in 10 mL of ice-cold PBS (pH 7.0). The homogenate was centrifuged at 1000 rpm for 20 min at 4°C to produce the post-nuclear fraction. For the enzyme assays, the fraction was centrifuged again at 12,000 rpm for 60 min at 4°C.

### ROS assay

Antioxidants such as GSH can combat oxidative stress mediated by ROS. Apoptosis or necrosis can result when oxidative stress damages biomolecules such as proteins, DNA, and membrane lipids. Apoptosis is the primary cause of neuronal death in PD, underscoring the link between ROS generation and neurodegenerative diseases [42,43].

The ROS content was examined based on staining dihydroethidium (DHE), which is oxidized by ROS (especially superoxide radicals) to produce a red fluorescent product. In brief, midbrain tissue sections were stained with 2 μm DHE in the presence or absence of Tiron (10 mM, a non-enzymatic superoxide radical scavenger). At least five random fields from each section were examined with a fluorescence microscope (BX53, Olympus, Tokyo, Japan) with excitation at 485 nm and emission at 535 nm. At least three fields per replicate were photographed and analyzed with the Zen 3.1 Blue Edition software. The corrected total cell fluorescence (CTCF) was calculated, using the Image J software, based on

the formula: CTCF = Integrated density − (Area of selected field × Mean background fluorescence). The results are expressed as arbitrary units.

**The total antioxidant capacity (TAC) assay**

Given that oxidative stress is elevated in the PD brain [44,45], the TAC was determined to assess oxidative stress in the midbrain. It is based on the ability to prevent the formation of TBA reactive substances (TBARS) from sodium benzoate in the presence of free oxygen radicals from the Fenton reaction. Following the addition of the midbrain homogenate, a yellowish-brown color is produced; the antioxidants in the sample scavenge the oxidants in the process, providing a reliable estimate of TAC.

**Estimation of MDA**

Lipid peroxidation and DNA damage are increased in the substantia nigra of the brain in patients with PD [46]. The extent of lipid peroxidation is measured based on the TBARS assay, which measures MDA. For this assay, 1.5 mL of 20% acetic acid, 0.2 mL of 6% SDS, and 1.5 mL of TBA were added to 0.5 mL tissue homogenate. The volume was adjusted to 4.0 mL with distilled water, and then the sample was heated at 95°C for 60 min using a glass ball condenser. After cooling, 4.0 mL of a butanol-pyridine mixture was added to the reaction, and the tube was shaken. After centrifugation for 10 min at 4,000 rpm, the absorbance of the organic layer was measured at 532 nm using a spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan). Standards and blanks were run in parallel.

**Estimation of peroxynitrite**

Peroxynitrite may substantially induce lipid peroxidation and can directly damage DNA and proteins. According to Uttara *et al*. [47], lipid peroxidation has a role in the degeneration of neurons in both acute and chronic PD. Peroxynitrite in the midbrain was measured based on peroxynitrite-mediated oxidation of *o*-phenylenediamine, a colorless material, to produce a colored reaction [48]. With a limit of detection of $1.7 \times 10^{-7}$ mol/L (3σ), the increase in absorbance is linearly proportional to the peroxynitrite concentration for the range of $4.4 \times 10^{-7}$ to $8.0 \times 10^{-6}$ mol/L. In brief, 0.1 mL of tissue homogenate and 1.9 mL of 5 mM phenol in 5 M sodium phosphate buffer (pH 7.4) were added to a glass test tube. The solution was mixed and incubated for 2 h. Then, 15 µL of 0.1 M NaOH was added and the absorbance was measured at 412 nm.

**Estimation of nitric oxide (NO)**

Nitrosative stress can result from the overproduction of reactive nitrogen species (RNS), especially Nox, resulting in neuronal damage and death, apoptosis, and even neurodegeneration [49]. The sum of the nitrite and nitrate concentrations is frequently used to calculate the total NO concentration. The analyzed materials showed a color response with Griess's reagent (Sigma-Aldrich, Stainheim, Germany) when nitrate was reduced to nitrite in the presence of cadmium and then transformed to nitric acid. A spectrophotometer (UV-1800, Shimadzu) was used to measure the absorbance at 540 nm to assess the nitrite levels.

**Estimation of urea**

A significant metabolic abnormality in individuals with neurodegenerative diseases is elevated urea in the brain [50]. Thus, the urea content in the midbrain was estimated. To eliminate insoluble materials, tissue (20 mg) was homogenized in 100 µL of urea assay buffer and centrifuged at 15000 g for 10 min. Each well of a 96-well plate received 50 µL of the reaction mix containing urea assay buffer (42 µL), OxiRed probe (2 µL), enzyme mix (2 µL), developer (2 µL), and converter enzyme (2 µL), as well as 10 µL of the test sample or the urea standard. Fifty microliters of the sample control mixture were added to the sample control well. After thoroughly mixing, the reaction was incubated at 37°C for 60 min in the dark. The absorbance at 570 nm was with a microplate reader (DT-ER-007, Bio-Rad, Hercules, CA, USA).

**Midbrain and liver histology**

Midbrain and liver samples were fixed in 10% formalin, dehydrated, and embedded in paraffin wax. Sections (5–6 µm for the midbrain and 5–7 µm for the liver) were cut and stained with haematoxylin and eosin (H&E). The sections were viewed under a light microscope (LabomedCxl Binocular, Labo America, Fremont, CA, USA) at ×40 magnification, and photographs were taken.

**Midbrain histomorphometry**

The diameter and numerical density of neurons were calculated. Neuronal density (NV) was calculated using the formula NV = [NA/(A×(D+T))], where NA is the average number of cells, A is the area of the reticule, T is the section thickness, and D is the mean diameter of the cells. Three slides per animal/group were evaluated.

**Liver histomorphometry**

Ten hepatocytes were measured per image of the central vein or portal triad, totaling 100 hepatocytes per slide. The number of normal, necrotic, and bi-nucleated hepatocytes was determined.

**Statistical analysis**

The data showed a normal distribution (based on the Shapiro–Wilk test) and are expressed as the mean and standard error (SE). The data were analyzed by one-way analysis of variance (ANOVA). When the ANOVA result was significant, the t-test with Bonferroni's correction was used for multiple comparisons. A $p \leq 0.05$ was considered statistically significant. SigmaPlot 14.5 version (Systat Software Inc., San Jose, USA) was used for statistical analysis and to generate graphs.

## RESULTS

**Biochemical parameters in the midbrain**

The mean±SE values of ROS parameter for control, MPTP, TUDCA, syndopa, and TUDCA + syndopa groups were 2.067±0.433, 4.983±0.63, 3.0±0.147, 3.6±0.227, and 2.133±0.120, respectively. The concomitant values of urea content were 93.9±3.3, 176.1±17.7, 105.6±3.5, 121.4±6.4, and 95.9±2.1. The TAC values were observed to be 643.7±8.5, 334.7±54.9, 562.5±26.9, 485.3±11.9, and 605.3±4.7. The measured peroxynitrite levels were 4.11±0.064, 6.67±0.692, 4.32±0.107, 4.725±0.123, and 4.187±0.105. The values of MDA results were 0.897±0.024, 1.86±0.136, 1.093±0.024, 1.308±0.093, and 0.877±0.030. Finally, the values of NO content were 0.383±0.018, 0.855±0.098, 0.512±0.036, 0.645±0.037, and 0.323±0.030, respectively.

As shown in Fig. 1, the ROS, MDA, peroxynitrite, NO, and urea levels were significantly increased in the midbrain of the MPTP group compared with the control group ($p<0.05$). By contrast, the TAC was significantly decreased in the MPTP group compared with the control group ($p<0.05$). Treatment with TUDCA alone, syndopa alone, or TUDCA and syndopa together considerably ameliorated the changes caused by MPTP ($p<0.05$). Overall, the combination of TUDCA and syndopa therapy produced the best result, followed by TUDCA monotherapy and syndopa monotherapy.

**Midbrain and liver histology**

In the midbrain of the control group (Fig. 2a), there were neurons with normal nuclei and prominent nucleoli (black arrow) along with a few pigmented neurons (blue arrow). In the MPTP group (Fig. 2b, b1, and b2), there was an increase in pigmented neurons, the perivascular space (yellow arrow), cytoplasmic inflammation (black arrow), glial cells, structural damage, and pyknotic nuclei (brown arrow). In the TUDCA group (Fig. 2c, c1, and c2), there was less neurodegeneration and more normal nucleoli (orange arrowhead) compared with the MPTP group, vacuolation of neutrophils (green arrow), oligodendrocytes (blue arrowhead), and fewer apoptotic neurons in the substantia nigra (circle). In the syndopa group (Fig. 2d, d1, and d2), there were pigmented neurons (orange arrow), few glial cells (black arrow), and evidence of nuclear vacuolization (red broad arrow). Finally, in the midbrain of the combination TUDCA and syndopa group (Fig. 2e and e1), the neurons were the optimal size and had well-defined nuclei (black arrow) and pigmented neurons and microglia (triangle head), which indicates no cell damage, and an increased number of neurons. Taken together, TUDCA monotherapy and combination TUDCA and

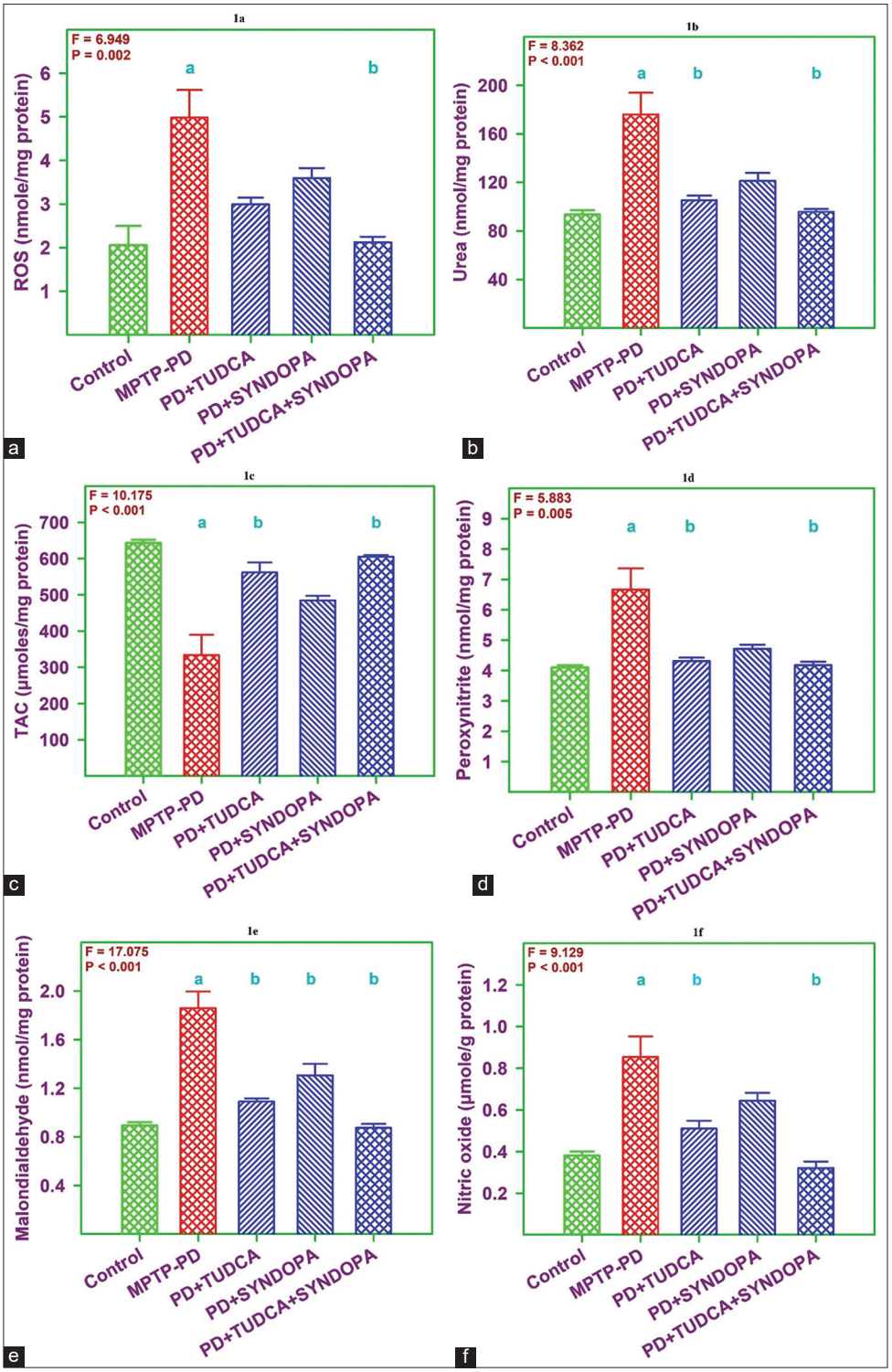


**Fig. 1: (a) The effect of TUDCA and syndopa on the midbrain ROS levels in MPTP-induced PD in mice. The MPTP group differed significantly from the combination TUDCA and syndopa group (p<0.05). (b) The effect of TUDCA and syndopa on the midbrain urea levels in MPTP-induced PD in mice. The MPTP group differed significantly compared with the TUDCA and combination TUDCA and syndopa groups (p<0.05). (c) The effect of TUDCA and syndopa on the midbrain TAC in MPTP-induced PD in mice. The MPTP group differed significantly compared with the TUDCA and combination TUDCA and syndopa groups (p<0.05). (d) The effect of TUDCA and syndopa on the midbrain peroxynitrite level in MPTP-induced PD in mice. The MPTP group differed significantly compared with the TUDCA and combination TUDCA and syndopa groups (p<0.05). (e) The effect of TUDCA and syndopa on the midbrain MDA level in MPTP-induced PD in mice. The MPTP group differed significantly compared with the TUDCA, syndopa, and combination TUDCA and syndopa groups (p<0.05). (f) The effect of TUDCA and syndopa on the midbrain nitric oxide level in MPTP-induced PD in mice. The MPTP group differed significantly compared with the TUDCA and combination TUDCA and syndopa groups (p<0.05). The graphs show the mean ± standard error of the mean (n = 5–6). The data were analyzed with one-way analysis of variance followed by t-tests with the Bonferroni correction for multiple comparisons. The F and p-values from the analysis of variance are indicated on each graph. Bars with the same lowercase letter do not differ significantly. MPTP: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, PD: Parkinson's disease, syndopa: Levodopa and carbidopa, TAC: Total antioxidant capacity, TUDCAL: Tauroursodeoxycholic acid**

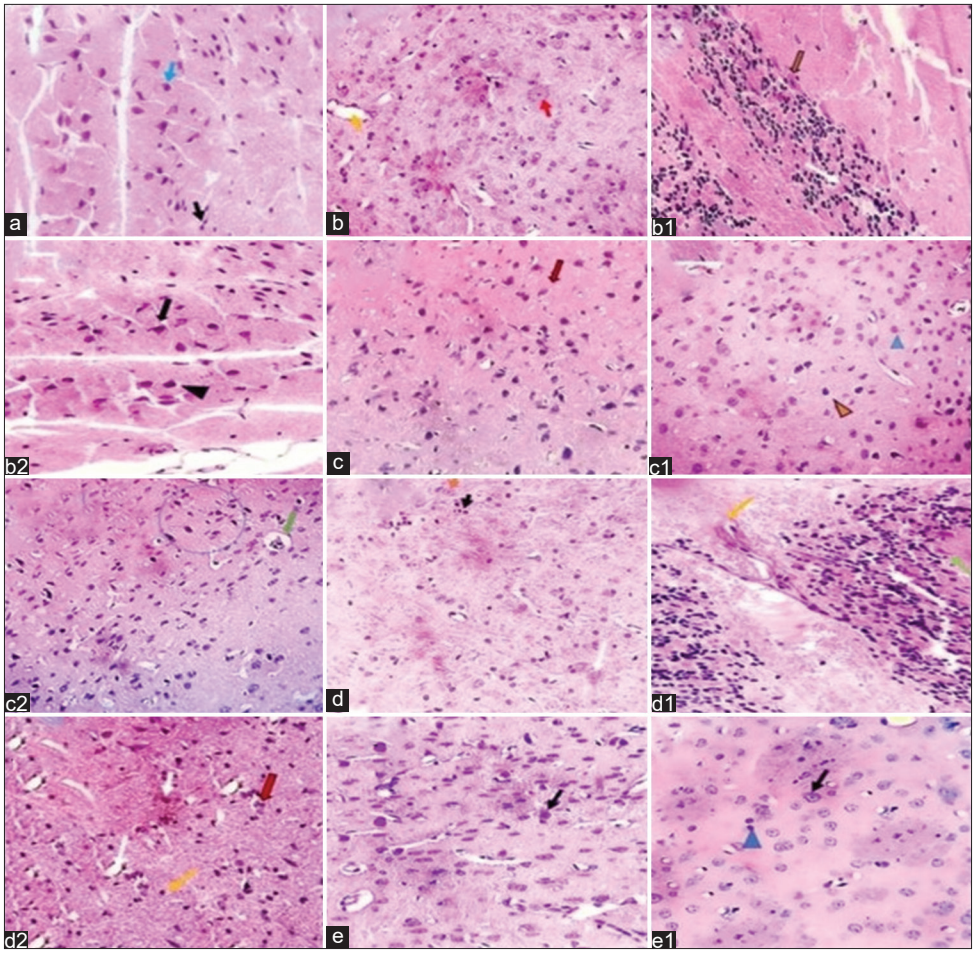


**Fig. 2: (a-e1) Micrographs of midbrain sections stained with haematoxylin and eosin (×40 magnification). The scale bar is 50 µm**

syndopa therapy drastically reduced the MPTP-induced neuronal abnormalities and neuronal death.

In the liver of the control group (Fig. 3a and a1), hepatocytes appeared normal (blue arrowhead) with well-preserved cytoplasm, prominent nuclei, and normal architecture (black arrow). In the MPTP group (Fig. 3b, b1, b2, and b3), there was severe thinning of hepatic cords due to hepatocellular atrophy (blue arrow), severe structural damage characterized by necrosis (black arrow mark) around the central vein, inflammatory cell infiltration (yellow arrow), and slight diffuse cytoplasmic hepatocellular vacuolation (long arrow). In the TUDCA group (Fig. 3c and c1), there was hepatocyte regeneration, along with cytoplasmic hepatocellular vacuolation (arrowheads) and occasional focal widening of the perivenous centrilobular spaces. In the syndopa group (Fig. 3d, d1, and d2), there was some necrosis (small arrow), less pronounced loss of centrilobular hepatocytes, and less inflammation adjacent to the central veins (arrowheads). Finally, in the combination TUDCA and syndopa group (Fig. 3e, e1, and e2), the hepatocytes were normal with no inflammation (arrowhead) and occasional cytoplasmic focal widening of the perivenous centrilobular spaces (red arrow mark). These results demonstrate that MPTP causes liver damage in mice; this damage can be ameliorated by TUDCA monotherapy and combination TUDCA and syndopa therapy. However, syndopa monotherapy exerted less protection against liver damage.

**Histomorphometric analysis of the midbrain and liver**

Fig. 5 shows the histomorphometric analysis of the liver. The MPTP group again showed notable changes compared with the control group: A reduction in normal hepatocytes (3.7±0.8 vs. 22.0±2.0, p<0.001), an increase in necrotic hepatocytes (18.7±2.6 vs. 0.7±0.6, p<0.001), and a small reduction in binucleated hepatocytes (1.5±0.5 vs. 2.3±0.6, p>0.05, NS). Normal hepatocytes were restored to the control level with TUDCA monotherapy (17.9±1.9, p<0.001 compared with the MPTP group) and TUDCA + syndopa combination therapy (17.7±3.0, p <0.001 compared with the MPTP group). Syndopa monotherapy had a less pronounced effect (11.3±1.5, p <0.001 compared with the MPTP group).

The necrotic hepatocytes were considerably reduced with TUDCA monotherapy (5.8±1.7, p<0.001 compared with the MPTP group) and TUDCA + syndopa combination therapy (2.3±0.6, p<0.001 compared with the MPTP group). However, syndopa monotherapy also manifested a reasonable effect (11.0±1.4, p <0.001 compared with the MPTP group).

Compared with the control group, the binucleated hepatocytes were found to be higher with TUDCA monotherapy (3.8±1.0, p<0.01 compared with the MPTP group) and TUDCA + syndopa combination therapy (5.0±1.0, p<0.001 compared with the MPTP group). The effect of syndopa monotherapy was not significant (3.0±0.8, p>0.05 compared with MPTP), and the number of binucleated hepatocytes were little higher than the control group.

Based on these results, the syndopa monotherapy is the least efficacious, TUDCA monotherapy shows intermediate efficacy, and TUDCA + syndopa combination therapy has the highest efficacy in ameliorated MPTP-induced histomorphometric changes.

## DISCUSSION

The major neuropathological sequelae that drive the progression of PD are oxidative stress and neuroinflammation in the brain. These phenomena lead to the degeneration and death of dopaminergic

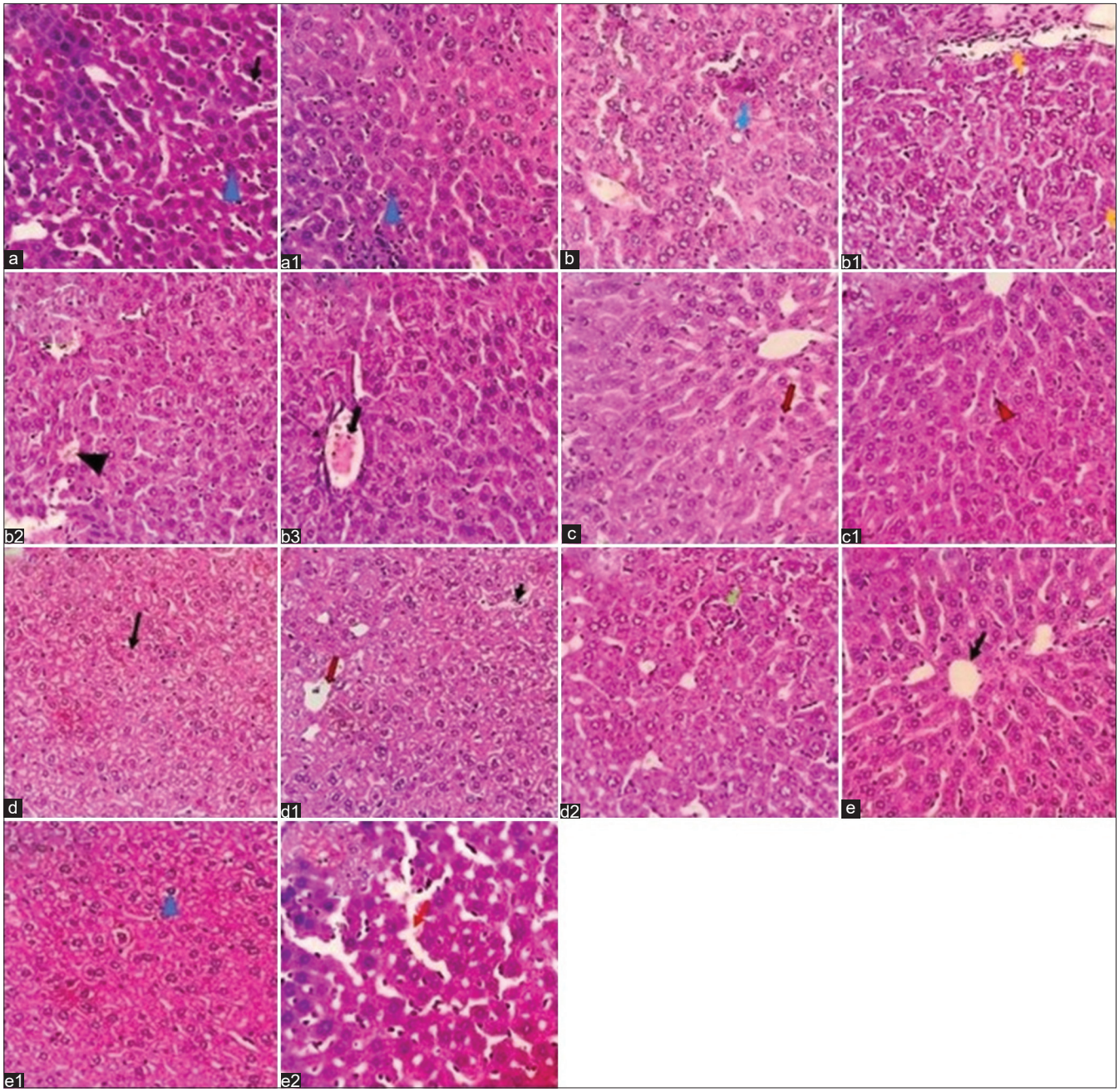


**Fig. 3: (a-e2) Micrographs of liver sections stained with haematoxylin and eosin (×40 magnification). The scale bar is 25 µm**

neurons [51,52]. ROS is considered a by-product of MPTP toxicity, and its production is markedly elevated in the brains of patients with PD. Antioxidant therapy is essential for those who are at high risk of PD when they have elevated oxidative stress in their brains [44]. Based on findings from animal models of PD and humans with PD, there is notable lipid peroxidation in the midbrain, denoted by elevated MDA and a decrease in the TAC [53-56]. Moreover, the substantia nigra shows lipid peroxidation and DNA damage [44]. Peroxynitrite may substantially induce lipid membrane peroxidation and can directly damage DNA and proteins. This injury has a role in neuronal degeneration in both acute and chronic degenerative PD [46]. Accumulating evidence indicates that defective antioxidant signaling, including GSH and its associated molecules, is involved in the progression of PD [57]. In general, $MPP^+$ triggers astrocyte apoptosis, thereby elevating the ROS content and the ratio of oxidized GSH (GSSG) to GSH [58]. Moreover, GSH depletion in the substantia nigra of patients with PD correlates negatively with motor activity and functions [59].

Based on recent accumulating evidence [60,61], including from our group [14,62], urea accumulation in the brain is one of the detrimental events in PD progression, although the cause for urea accumulation in PD is still not clear. Indeed, a significant metabolic abnormality in individuals with neurodegenerative diseases is elevated urea in the brain [50], which can result in the overproduction of RNS, especially NOx, leading to nitrosative stress, neuronal damage, and eventual neuronal death through apoptosis [49]. Consistently, there are elevated ROS and RNS levels in the midbrain and substantia nigra of PD models [63]. RNS-mediated events in PD affect axo-dendritic functions, leading to a reduction in dendritic spines, mitochondrial dysfunction, such as impaired respiration in dopaminergic neurons, and defective dopamine homeostasis [63]. These abnormalities result from the breakdown of dopamine by mitochondrial MAO, which produces the metabolite 3,4-dihydroxyphenylacetic acid (DOPAC). DOPAC interacts with NO to disrupt mitochondrial respiration [64] and to decrease the GSH content [65]. Moreover, NO and peroxynitrite serum levels in PD also correlate

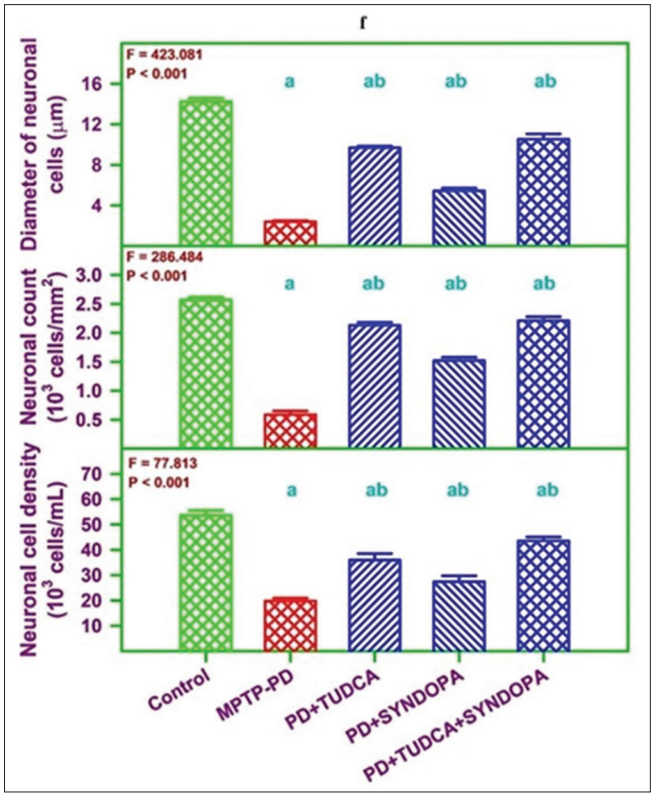


**Fig. 4: The effects of TUDCA and syndopa on MPTP-induced PD changes in brain histomorphometry (neuronal diameter, count, and density). The data are presented as the mean ± standard error (n = 5-6). The data were analysed with one-way analysis of variance followed by t-tests with the Bonferroni correction for multiple comparisons (p < 0.01 was considered significant). For each panel, the F and p values from the analysis of variance are indicated, and bars with the same lowercase letter do no differ significantly. MPTP = 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; PD = Parkinson's disease; syndopa = levodopa and carbidopa; TUDCA = tauroursodeoxycholic acid. It shows the histomorphometric analysis of the midbrain. The MPTP group showed significant changes compared with the control groups: a drastic decrease in the neuronal count (0.602 ± 0.118 × 103 cells/mm2 vs. 2.577 ± 0.0666 × $10^3$ cells/$mm^2$, p < 0.001), the neuronal cell diameter (2.438 ± 0.166 µm vs. 14.267 ± 0.569 µm, p < 0.001), and neuronal density (20.243 ± 1.720 cells × 103 cells/mL vs. 53.933 ± 2.775 × $10^3$ cells/mL, p < 0.01), reflecting MPTP-induced neurotoxicity. Compared with the MPTP group, TUDCA monotherapy significantly increased the neuronal count to 2.140 ± 0.0816 × $10^3$ cells/$mm^2$, the neuronal diameter to 9.725 ± 0.261 µm and the neuronal density to 36.367 ± 4.289 × 103 cells/mL (p < 0.01). TUDCA + syndopa also significantly increased the neuronal count (2.220 ± 0.101 × $10^3$ cells/$mm^2$), the neuronal diameter (10.567 ± 0.862 µm), and the neuronal density (43.933 ± 1.909 × 103 cells/mL) compared with the MPTP group (p < 0.001). Syndopa monotherapy led to a moderate improvement in the neuronal count (1.532 ± 0.0946 × $10^3$ cells/$mm^2$), the neuronal diameter (5.480 ± 0.474 µm), and the neuronal density (27.850 ± 3.740 × 103 cells/mL) (p < 0.001).**

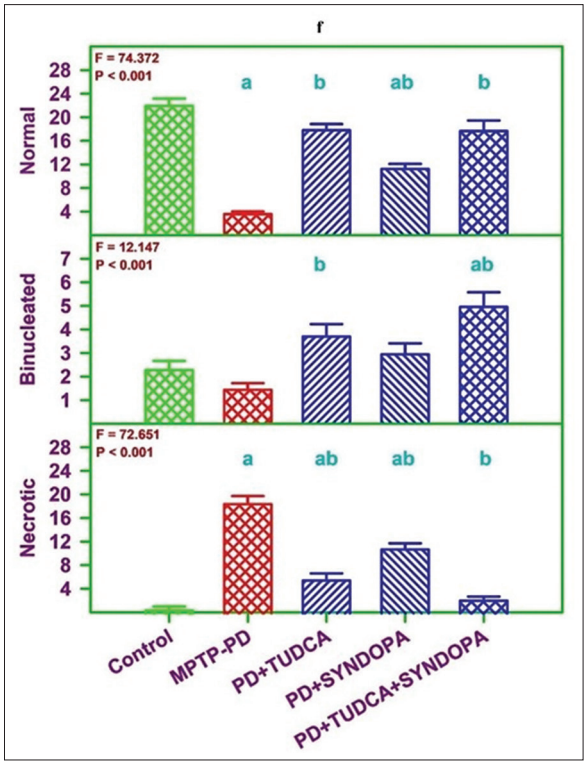


**Fig. 5: The effects of TUDCA and syndopa on MPTP-induced PD on liver histomorphometry (the number of normal, binucleated, and necrotic cells). The data are presented as the mean±standard error of the mean (n=5–6). The data were analyzed with one-way analysis of variance followed by t-tests with the Bonferroni correction for multiple comparisons (p<0.01 was considered significant). For each panel, the F and p-values from the analysis of variance are indicated, and bars with the same lowercase letter do no differ significantly. MPTP: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, PD: Parkinson's disease, syndopa: Levodopa and carbidopa, TUDCA: Tauroursodeoxycholic acid**

with the oxidative stress and the severity of PD [66]. Peroxynitrite inactivates tyrosine hydroxylase through sulfhydryl oxidation [67]. In addition, nitration of tyrosine residues induced by peroxynitrite triggers oxidative stress in patients with PD [68].

TUDCA is a natural compound that easily crosses the BBB and has no known adverse effects [38,69,70]. It has antioxidant, anti-inflammatory, and anti-apoptotic activities that together account for its neuroprotective properties in different models of neurodegenerative diseases, including PD [32-38,71-73,75-77]. It likely exerts these functions by binding efficiently to the plasma membrane and stabilizing mitochondria [76]. TUDCA has been shown to exert anti-apoptotic activity and to ameliorate $MPP^+$-induced mitochondrial dysfunction and to promote mitochondrial turnover. It prevents MPTP-mediated ROS formation in the midbrain and striatal areas [32,75]. However, in the cortical regions of MPTP-treated mice, short- or long-term TUDCA treatment could not inhibit ROS based on DCF-DA measurements [38]. Our results from the midbrain differ from the findings reported by Moreira *et al*. [75], who also examined the effects of TUDCA in the MPTP-induced mouse model of PD. Importantly, the combination of TUDCA and syndopa therapy had a better protective effect than TUDCA or syndopa monotherapy based on its ability to reduce the ROS, MDA, NO, peroxynitrite, and urea contents in the midbrain. The benefits of TUDCA would likely also extend to other neurogenerative diseases.

TUDCA renders neuroprotection against MPTP-induced neurotoxicity in the mouse brain cortex through the upregulation of antioxidant enzymes (glutathione peroxidase 1 and haem oxygenase 1), Parkin, AMP-activated protein kinase, and the anti-inflammatory protein ANXA1. It also reduces the MPTP-mediated loss of ATP and inhibits the inflammatory markers connected with microgliosis and astrogliosis to stimulate neuroprotection [37]. The neuroprotective mechanism of TUDCA in the MPTP-induced PD model involves inhibition of motor impairment, dopaminergic fibre loss, ATP loss, mitochondrial damage, brain inflammation, and chaperone activity [32,37,38,74].

TUDCA attenuates ROS levels and alters mitochondrial biogenesis and neurogenesis by activating the nuclear factor erythroid 2-related factor 2 signaling [75,77]. In mice with spinal cord injury, TUDCA administration led to a striking reduction of inflammation, apoptosis, and oxidative stress to improve axon regeneration and remyelination in the affected spinal cord region [73]. We found that TUDCA markedly inhibited ROS and RNS, which implicates its significant role in the redox balance and antioxidant signaling mechanisms.

Histopathological techniques, such as H&E staining of brain sections, have clarified the anatomical and structural abnormalities of PD [14], including one of the pathological hallmarks of PD: Accumulation of Lewy bodies, which contain alpha-synuclein. Of note, the administration of acute or sub-chronic doses of MPTP to mice does not lead to the formation of Lewy bodies [24]. Nevertheless, we used H&E staining to examine the anatomical structure of the midbrain and liver, specially to determine whether the tested drugs could reverse MPTP-induced toxicity. Based on hepatocellular morphometry, we found that TUDCA and syndopa ameliorated the toxic effects of MPTP. TUDCA was able to protect hepatocytes from MPTP-induced toxicity when administered alone or combined with syndopa. Researchers have stated that variations in the hepatic disposition of MPTP may influence the extent of neurotoxicity [78]. Mitochondrial dysfunction has been implicated in hepatotoxicity induced by another neurotoxin, alpha-amanitin [79]; it involves mitochondrial ROS production, loss of the mitochondrial membrane potential, and hepatocyte apoptosis. TUDCA-mediated hepatoprotective effects have been reported for different hepatic diseases and toxic agents [80,81]. A recent study involving a $CCl_4$-induced model of hepatotoxicity suggests that TUDCA protects the liver and enhances liver regeneration by reducing fibrosis through GATA3 activation [82]. In addition, LPS-induced liver injury, inflammation, and apoptosis were ameliorated by TUDCA in mice by altering hepatic tumor necrosis factor-$\alpha$, interleukin-1$\beta$, caspase-1, and caspase-11 mRNA expression, hepatic caspase-3 and CAD protein expression levels and serum aspartate transaminase and alanine transaminase levels [83]. Although TUDCA protects against MPTP-mediated hepatotoxicity, the mechanisms require further investigation. Drugs that efficiently cross the BBB can be useful for PD treatment [84].

To conclude, our findings underscore that oxidative stress is a primary pathogenic factor in PD, leading to the degeneration of dopaminergic neurons. The observed increase in MDA and decrease in the TAC in the MPTP group align with the literature, which has confirmed lipid peroxidation and oxidative damage in animal models of PD. The elevated ROS, peroxynitrite, and NO in the midbrain also correlate with previous research [51-53]. Our study demonstrated that both TUDCA monotherapy and the combination of TUDCA and syndopa therapy effectively ameliorated the MPTP-induced biochemical and histopathological changes. While syndopa offers only symptomatic relief, our findings indicate that combining it with TUDCA could represent a comprehensive approach to rescue the underlying oxidative stress, free radical complications, and neuronal cell death associated with PD. While TUDCA monotherapy also considerably ameliorated the oxidative stress to protect midbrain neurons, the synergistic effects of the combination therapy were more beneficial. Our results suggest that combining TUDCA with syndopa provides a more robust neuroprotective effect than either drug alone.

**Limitations and future directions**

Although this study has provided valuable insights into the combined effects of TUDCA and syndopa, it has also some limitations. The MPTP model does not mimic all the symptoms manifested in patients with PD. Our sample size is small; a larger number of samples is required to draw more robust conclusions. We did not assess striatal dopamine levels and complex I activity. However, we have explored tyrosine hydroxylase immunoreactivity in our recent publication [85]. Thus, our study has limited mechanistic depth, and future studies should examine these parameters to provide a more detailed mechanistic understanding of the neuroprotective effects. We did not include PD-specific functional or behavioral assays, such as the rotarod or pole test. Based on an earlier publication [38], behavioral parameters, such as the rotarod and pole tests are not able to confirm whether a drug leads to a functional improvement of motor deficits in the MPTP mouse model. Indeed, the authors reported that MPTP did not affect rotarod or balance beam test performance, suggesting that the balance of these animals was preserved after MPTP administration. However, the authors did show that TUDCA had positive effects on swimming latency, gait quality, and foot dragging in MPTP-treated mice. Finally, we did not examine the mechanism by which TUDCA counteracts MPTP-mediated hepatotoxicity. Future studies are needed to explore the precise mechanisms.

## CONCLUSION

The findings of the current study results suggest the therapeutic potential of TUDCA monotherapy and TUDCA-Syndopa combined therapy in ameliorating the toxicity of MPTP through various biochemical parameters, histopathological and histomorphometric studies. However, the current findings could pave way for an elaborate preclinical investigations and molecular pathways to facilitate TUDCA-Syndopa treatment in translational research.

## ETHICS APPROVAL

The Saveetha Medical College Institutional Animal Ethics Committee approved the research proposal (IAEC Approval Number SU/CLAR/RD/34/2023). The animal experimental protocol adhered to the principles and guidelines of CCSEA.

## AUTHOR CONTRIBUTION

Senthilkumar Sivanesan contributed to the design and conceptualization of the study. Mahalakshmi Rajan, Senthilkumar Sivanesan, and Nivetha Rajendran performed the experiments. N Ashok Vardhan revised the manuscript. All authors have read and approved the final version of the manuscript.

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None.

## COMPETING INTERESTS

The authors disclose no conflicts of interest.

## REFERENCES

1. Lotankar S, Prabhavalkar KS, Bhatt LK. Biomarkers for Parkinson's disease: Recent advancement. Neurosci Bull. 2017 Oct;33(5):585-97. doi: 10.1007/s12264-017-0183-5, PMID 28936761
2. Braak H, Braak E. Pathoanatomy of Parkinson's disease. J Neurol. 2000 Apr;247 Suppl 2:II3-10. doi: 10.1007/PL00007758, PMID 10991663
3. Singhal B, Lalkaka J, Sankhla C. Epidemiology and treatment of Parkinson's disease in India. Parkinsonism Relat Disord. 2003 Aug;9 Suppl 2:S105-9. doi: 10.1016/S1353-8020(03)00024-5, PMID 12915075
4. Su D, Cui Y, He C, Yin P, Bai R, Zhu J, *et al*. Projections for prevalence of Parkinson's disease and its driving factors in 195 countries and territories to 2050: Modelling study of Global Burden of Disease Study

2021. BMJ. 2025 Mar 5;388(8458):e080952. doi: 10.1136/bmj-2024-080952, PMID 40044233
5. Chen ZJ, Liang CY, Yang LQ, Ren SM, Xia YM, Cui L, *et al*. Association of Parkinson's disease with microbes and microbiological therapy. Front Cell Infect Microbiol. 2021 Mar 8; 11:619354.
6. Bisaglia M. Mediterranean diet and Parkinson's disease. Int J Mol Sci. 2022 Dec 20;24(1):42. doi: 10.3390/ijms24010042, PMID 36613486
7. Garcia-Ruiz PJ, Chaudhuri KR, Martinez-Martin P. Non-motor symptoms of Parkinson's disease A review…from the past. J Neurol Sci. 2014 Mar 15;338(1-2):30-3. doi: 10.1016/j.jns.2014.01.002, PMID 24433931
8. Srinivasan E, Chandrasekhar G, Chandrasekar P, Anbarasu K, Vickram AS, Karunakaran R, *et al*. Alpha-synuclein aggregation in Parkinson's disease. Front Med (Lausanne). 2021;8:736978. doi: 10.3389/fmed.2021.736978, PMID 34733860
9. Kwon DK, Kwatra M, Wang J, Ko HS. Levodopa-induced dyskinesia in Parkinson's disease: Pathogenesis and emerging treatment strategies. Cells. 2022 Nov 23;11(23):3736. doi: 10.3390/cells11233736, PMID 36496996
10. Kispotta S, Das D, Prusty SK. A recent update on drugs and alternative approaches for Parkinsonism. Neuropeptides. 2024 Apr;104:102415. doi: 10.1016/j.npep.2024.102415, PMID 38402775
11. Johnson WM, Wilson-Delfosse AL, Mieyal JJ. Dysregulation of glutathione homeostasis in neurodegenerative diseases. Nutrients. 2012 Oct 9;4(10):1399-440.
12. Pandey N, Karthik VP, Selva P, Hazeena P. A network pharmacology-based drug repurposing study of levetiracetam uncovers its interaction with multi-drug targets in Parkinson's disease. Int J Appl Pharm. 2024;16(6):69-78. doi: 10.22159/ijap.2024v16i6.51887
13. Sanitha M, Vengatachalam G. A review of oxidative stress induced Parkinsonism and the potentials of antioxidants in treating Parkinson's diseases. Rev Electron Vet. 2024;25(1):1591-8. doi: 10.69980/redvet.v25i1.908
14. Ramachandra VH, Sivanesan S, Koppal A, Anandakumar S, Howell MD, Sukumar E, *et al*. Embelin and levodopa combination therapy for improved Parkinson's disease treatment. Transl Neurosci. 2022 Jun 29;13(1):145-62. doi: 10.1515/tnsci-2022-0224, PMID 35855085
15. Shichiri M. The role of lipid peroxidation in neurological disorders. J Clin Biochem Nutr. 2014 May;54(3):151-60. doi: 10.3164/jcbn.14-10, PMID 24895477
16. Naudí A, Cabré R, Dominguez-Gonzalez M, Ayala V, Jové M, Mota-Martorell N, *et al*. Region-specific vulnerability to lipid peroxidation and evidence of neuronal mechanisms for polyunsaturated fatty acid biosynthesis in the healthy adult human central nervous system. Biochim Biophys Acta Mol Cell Biol Lipids. 2017 May;1862(5):485-95. doi: 10.1016/j.bbalip.2017.02.001, PMID 28185952
17. Nam TG. Lipid peroxidation and its toxicological implications. Toxicol Res. 2011 Mar; 27(1):1-6. doi: 10.5487/TR.2011.27.1.001
18. Ayala A, Muñoz MF, Argüelles S. Lipid peroxidation: Production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxid Med Cell Longev. 2014;2014:360438. doi: 10.1155/2014/360438
19. Akyazı O, Korkmaz D, Cevher SC. Experimental Parkinson models and green chemistry approach. Behav Brain Res. 2024 Aug 5;471:115092. doi: 10.1016/j.bbr.2024.115092, PMID 38844056
20. Dringen R. Metabolism and functions of glutathione in brain. Prog Neurobiol. 2000 Dec;62(6):649-71. doi: 10.1016/S0301-0082(99)00060-X
21. Smeyne M, Smeyne RJ. Glutathione metabolism and Parkinson's disease. Free Radic Biol Med. 2013 Sep;62:13-25. doi: 10.1016/j.freeradbiomed.2013.05.001, PMID 23665395
22. Dovonou A, Bolduc C, Soto Linan V, Gora C, Peralta MR, Lévesque M. Animal models of Parkinson's disease: Bridging the gap between disease hallmarks and research questions. Transl Neurodegener. 2023;12(1):36. doi: 10.1186/s40035-023-00368-8, PMID 37468944
23. Chen SJ, Li G, Zhang Y, Guan YL, Li XJ, Liu SH, *et al*. Comparison and evaluation of MPTP-induced subacute and chronic models of Parkinson's disease in mice. Zhongguo ZuzhiGongcheng Yanjiu. 2022;26(8):1247-52.
24. Meredith GE, Rademacher DJ. MPTP mouse models of Parkinson's disease: An update. J Parkinsons Dis. 2011;1(1):19-33. doi: 10.3233/JPD-2011-11023, PMID 23275799
25. Schildknecht S, Pape R, Meiser J, Karreman C, Strittmatter T, Odermatt M, *et al*. Preferential extracellular generation of the active parkinsonian toxin MPP+ by transporter-independent export of the intermediate MPDP+. Antioxid Redox Signal. 2015 Nov 1;23(13):1001-16. doi: 10.1089/ars.2015.6297, PMID 26413876
26. Mallajosyula JK, Kaur D, Chinta SJ, Rajagopalan S, Rane A, Nicholls DG, *et al.* MAO-B elevation in mouse brain astrocytes results in Parkinson's pathology. PLoS One. 2008 Feb 20;3(2):e1616. doi: 10.1371/journal.pone.0001616, PMID 18286173
27. Chen Y, Ni YY, Liu J, Lu JW, Wang F, Wu XL, *et al*. Dopamine receptor 3 might be an essential molecule in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced neurotoxicity. BMC Neurosci. 2013;14:76. doi: 10.1186/1471-2202-14-76, PMID 23902361
28. Subramaniam SR, Chesselet MF. Mitochondrial dysfunction and oxidative stress in Parkinson's disease. Prog Neurobiol. 2013 Jul-Aug;106-107:17-32. doi: 10.1016/j.pneurobio.2013.04.004, PMID 23643800
29. Kung HC, Lin KJ, Kung CT, Lin TK. Oxidative stress, mitochondrial dysfunction, and neuroprotection of polyphenols with respect to resveratrol in Parkinson's disease. Biomedicines. 2021 Jul 30;9(8):918.
30. Hwang DJ, Kwon KC, Song HK, Kim KS, Jung YS, Hwang DY, *et al*. Comparative analysis of dose-dependent neurotoxic response to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine in C57BL/6 N mice derived from three different sources. Lab Anim Res. 2019;35:10. doi: 10.1186/s42826-019-0012-2, PMID 32257898
31. Kupsch A, Hauser RA, Jankovic J, Rascol O, Treuer H, Schwarz J, *et al*. Monoamine oxidase-inhibition and MPTP-induced neurotoxicity in the non-human primate: Comparison of rasagiline (TVP 1012) with selegiline. J Neural Transm (Vienna). 2001;108(7):755-65. doi: 10.1007/s007020170063
32. Castro-Caldas M, Carvalho AN, Rodrigues E, Henderson CJ, Wolf CR, Rodrigues CM, *et al*. Tauroursodeoxycholic acid prevents MPTP-induced dopaminergic cell death in a mouse model of Parkinson's disease. Mol Neurobiol. 2012;46(2):475-86. doi: 10.1007/s12035-012-8295-4, PMID 22773138
33. Ramalho RM, Viana RJ, Low WC, Steer CJ, Rodrigues CM. Bile acids and apoptosis modulation: An emerging role in experimental Alzheimer's disease. Trends Mol Med. 2008 Feb;14(2):54-62. doi: 10.1016/j.molmed.2007.12.001
34. Lo AC, Callaerts-Vegh Z, Nunes AF, Rodrigues CM, D'Hooge R. Tauroursodeoxycholic acid (TUDCA) supplementation prevents cognitive impairment and amyloid deposition in APP/PS1 mice. Neurobiol Dis. 2013 Feb;50:21-9. doi: 10.1016/j.nbd.2012.09.003, PMID 22974733
35. Khalaf K, Tornese P, Cocco A, Albanese A. Tauroursodeoxycholic acid: A potential therapeutic tool in neurodegenerative diseases. Transl Neurodegener. 2022 Jun 4;11(1):33. doi: 10.1186/s40035-022-00307-z, PMID 35659112
36. Kusaczuk M. Tauroursodeoxycholate-bile acid with chaperoning activity: Molecular and cellular effects and therapeutic perspectives. Cells. 2019 Nov 20;8(12):1471. doi: 10.3390/cells8121471, PMID 31757001
37. Mendes MO, Rosa AI, Carvalho AN, Nunes MJ, Dionísio P, Rodrigues E, *et al*. Neurotoxic effects of MPTP on mouse cerebral cortex: Modulation of neuroinflammation as a neuroprotective strategy. Mol Cell Neurosci. 2019;96:1-9. doi: 10.1016/j.mcn.2019.01.003, PMID 30771505
38. Rosa AI, Duarte-Silva S, Silva-Fernandes A, Nunes MJ, Carvalho AN, Rodrigues E, *et al*. Tauroursodeoxycholic acid improves motor symptoms in a mouse model of Parkinson's disease. Mol Neurobiol. 2018 Dec;55(12):9139-55. doi: 10.1007/s12035-018-1062-4, PMID 29651747
39. Zhang Z, Chen J, Chen F, Yu D, Li R, Lv C, *et al.* Tauroursodeoxycholic acid alleviates secondary injury in the spinal cord via up-regulation of CIBZ gene. Cell Stress Chaperones. 2018 Jul;23(4):551-60. doi: 10.1007/s12192-017-0862-1, PMID 29151236
40. Carvalho MM, Campos FL, Marques M, Soares-Cunha C, Kokras N, Dalla C, *et al*. Effect of levodopa on reward and impulsivity in a rat model of Parkinson's disease. Front Behav Neurosci. 2017;11:145. doi: 10.3389/fnbeh.2017.00145, PMID 28848409
41. Graham JM. Homogenization of mammalian tissues. ScientificWorldJournal. 2002;2:1626-9. doi: 10.1100/tsw.2002.849, PMID 12806150
42. Rao R. Oxidative stress-induced disruption of epithelial and endothelial tight junctions. Front Biosci. 2008 May 1;13:7210-26. doi: 10.2741/3223, PMID 18508729
43. Üremiş N, Üremiş MM. Oxidative/nitrosative stress, apoptosis, and redox signaling: Key players in neurodegenerative diseases. J Biochem Mol Toxicol. 2025;39(1):e70133. doi: 10.1002/jbt.70133, PMID 39799559

44. Bej E, Cesare P, Volpe AR, D'Angelo M, Castelli V. Oxidative stress and neurodegeneration: Insights and therapeutic strategies for Parkinson's disease. Neurol Int. 2024;16(3):502-17. doi: 10.3390/neurolint16030037, PMID 38804477
45. Koracevic D, Koracevic G, Djordjevic V, Andrejevic S, Cosic V. Method for the measurement of antioxidant activity in human fluids. J Clin Pathol. 2001 May;54(5):356-61. doi: 10.1136/jcp.54.5.356, PMID 11328833
46. Rajasankar S, Manivasagam T, Surendran S. Ashwagandha leaf extract: A potential agent in treating oxidative damage and physiological abnormalities seen in a mouse model of Parkinson's disease. Neurosci Lett. 2009;454(1):11-5. doi: 10.1016/j.neulet.2009.02.044, PMID 19429045
47. Uttara B, Singh AV, Zamboni P, Mahajan RT. Oxidative stress and neurodegenerative diseases: A review of upstream and downstream antioxidant therapeutic options. Curr Neuropharmacol. 2009 Mar;7(1):65-74. doi: 10.2174/157015909787602823, PMID 19721819
48. Otulak-Kozieł K, Kozieł E, Treder K, Rusin P. Role of peroxynitrite in the pathogenesis of Parkinson's disease and its fluorescence imaging-based detection. Int J Mol Sci. 2024;25(10):5256. doi: 10.3390/ijms25105256, PMID 38791293
49. Olufunmilayo EO, Gerke-Duncan MB, Holsinger RM. Oxidative stress and antioxidants in neurodegenerative disorders. Antioxidants (Basel). 2023 Feb 18;12(2):517. doi: 10.3390/antiox12020517, PMID 36830075
50. Patassini S, Begley P, Reid SJ, Xu J, Church SJ, Curtis M, *et al.* Identification of elevated urea as a severe, ubiquitous metabolic defect in the brain of patients with Huntington's disease. Biochem Biophys Res Commun. 2015 Dec 4;468(1-2):161-6. doi: 10.1016/j.bbrc.2015.10.140, PMID 26522227
51. Sheikh M, Saiyyad A, Jirvankar PS. Exosomes as mediators of neuroinflammation in Parkinson's disease: A review. Int J Appl Pharm. 2025;17(3):80-92. doi: 10.22159/ijap.2025v17i3.53756
52. Wu DC, Teismann P, Tieu K, Vila M, Jackson-Lewis V, Ischiropoulos H, *et al*. NADPH oxidase mediates oxidative stress in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine model of Parkinson's disease. Proc Natl Acad Sci U S A. 2003 May 13;100(10):6145-50. doi: 10.1073/pnas.0937239100, PMID 12721370
53. Khan Z, Ali SA. Oxidative stress-related biomarkers in Parkinson's disease: A systematic review and meta-analysis. Iran J Neurol. 2018 Jul 6;17(3):137-44. doi: 10.18502/ijnl.v17i3.373, PMID 30886681
54. Mbiydzenyuy NE, Ninsiima HI, Valladares MB, Pieme CA. Zinc and linoleic acid pre-treatment attenuates biochemical and histological changes in the midbrain of rats with rotenone-induced Parkinsonism. BMC Neurosci. 2018 May 9;19(1):29. doi: 10.1186/s12868-018-0429-9, PMID 29739324
55. Fu Y, He Y, Phan K, Bhatia S, Pickford R, Wu P, *et al*. Increased unsaturated lipids underlie lipid peroxidation in synucleinopathy brain. Acta Neuropathol Commun. 2022 Nov 14;10(1):165. doi: 10.1186/s40478-022-01469-7, PMID 36376990
56. Sarbishegi M, Charkhat Gorgich EA. The effects of celecoxib on rotenone-induced rat model of Parkinson's disease: Suppression of neuroinflammation and oxidative stress-mediated apoptosis. Gene Cell Tissue. 2019 May 6;6(2):e92178. doi: 10.5812/gct.92178
57. Asanuma M, Miyazaki I. Glutathione and related molecules in Parkinsonism. Int J Mol Sci. 2021;22(16):8689. doi: 10.3390/ijms22168689, PMID 34445395
58. Chuang JI, Chen TH. Effect of melatonin on temporal changes of reactive oxygen species and glutathione after MPP(+) treatment in human astrocytoma U373MG cells. J Pineal Res. 2004;36(2):117-25. doi: 10.1046/j.1600-079X.2003.00107.x, PMID 14962063
59. Shukla D, Goel A, Mandal PK, Joon S, Punjabi K, Arora Y, *et al*. Glutathione depletion and concomitant elevation of susceptibility in patients with Parkinson's disease: State-of-the-art MR spectroscopy and neuropsychological study. ACS Chem Neurosci. 2023 Dec 20;14(24):4383-94. doi: 10.1021/acschemneuro.3c00717, PMID 38050970
60. Zhang S, Wan G, Qiu Y, Zhang M, Deng H, Wang Q, *et al*. Urea cycle dysregulation drives metabolic stress and neurodegeneration in Parkinson's disease. NPJ Parkinsons Dis. 2025;11(1):237. doi: 10.1038/s41531-025-01099-5, PMID 40790122
61. Scholefield M, Church SJ, Xu J, Patassini S, Roncaroli F, Hooper NM, *et al*. Severe and regionally widespread increases in tissue urea in the human brain represent a novel finding of pathogenic potential in Parkinson's disease dementia. Front Mol Neurosci. 2021;14:711396. doi: 10.3389/fnmol.2021.711396, PMID 34751215
62. Kavuri S, Sivanesan S, Howell MD, Vijayaraghavan R, Rajadas J. Studies on Parkinson's-disease-linked genes, brain urea levels and histopathology in rotenone-induced Parkinson's disease rat model. World J Neurosci. 2020;10(4):216-34. doi: 10.4236/wjns.2020.104021
63. Stykel MG, Ryan SD. Nitrosative stress in Parkinson's disease. NPJ Parkinsons Dis. 2022 Aug 11;8(1):104. doi: 10.1038/s41531-022-00370-3, PMID 35953517
64. Nunes C, Almeida L, Laranjinha J. Synergistic inhibition of respiration in brain mitochondria by nitric oxide and dihydroxyphenylacetic acid (DOPAC). Implications for Parkinson's disease. Neurochem Int. 2005 Aug;47(3):173-82. doi: 10.1016/j.neuint.2005.03.005, PMID 15893407
65. Nunes C, Barbosa RM, Almeida L, Laranjinha J. Nitric oxide and DOPAC-induced cell death: from GSH depletion to mitochondrial energy crisis. Mol Cell Neurosci. 2011 Sep;48(1):94-103. doi: 10.1016/j.mcn.2011.06.009, PMID 21708261
66. Kouti L, Noroozian M, Akhondzadeh S, Abdollahi M, Javadi MR, Faramarzi MA, *et al*. Nitric oxide and peroxynitrite serum levels in Parkinson's disease: Correlation of oxidative stress and the severity of the disease. Eur Rev Med Pharmacol Sci. 2013 Apr;17(7):964-70. PMID 23640445
67. Anjo SI, He Z, Hussain Z, Farooq A, McIntyre A, Laughton CA, *et al*. Protein oxidative modifications in neurodegenerative diseases: from advances in detection and modelling to their use as disease biomarkers. Antioxidants (Basel). 2024;13(6):681. doi: 10.3390/antiox13060681, PMID 38929122
68. Kavya R, Saluja R, Singh S, Dikshit M. Nitric oxide synthase regulation and diversity: Implications in Parkinson's disease. Nitric Oxide. 2006 Dec;15(4):280-94. doi: 10.1016/j.niox.2006.07.003, PMID 16934505
69. Parry GJ, Rodrigues CM, Aranha MM, Hilbert SJ, Davey C, Kelkar P, *et al.* Safety, tolerability, and cerebrospinal fluid penetration of ursodeoxycholic acid in patients with amyotrophic lateral sclerosis. Clin Neuropharmacol. 2010 Jan-Feb;33(1):17-21. doi: 10.1097/WNF.0b013e3181c47569, PMID 19935406
70. Mertens KL, Kalsbeek A, Soeters MR, Eggink HM. Bile acid signaling pathways from the enterohepatic circulation to the central nervous system. Front Neurosci. 2017;11:617. doi: 10.3389/fnins.2017.00617, PMID 29163019
71. Chiang JY, Ferrell JM. Bile acids as metabolic regulators and nutrient sensors. Annu Rev Nutr. 2019 Aug 21;39:175-200. doi: 10.1146/annurev-nutr-082018-124344, PMID 31018107
72. Keene CD, Rodrigues CM, Eich T, Chhabra MS, Steer CJ, Low WC. Tauroursodeoxycholic acid, a bile acid, is neuroprotective in a transgenic animal model of Huntington's disease. Proc Natl Acad Sci U S A. 2002 Aug 6;99(16):10671-6. doi: 10.1073/pnas.162362299, PMID 12149470
73. Hou Y, Luan J, Huang T, Deng T, Li X, Xiao Z, *et al.* Tauroursodeoxycholic acid alleviates secondary injury in spinal cord injury mice by reducing oxidative stress, apoptosis, and inflammatory response. J Neuroinflammation. 2021 Sep 20;18(1):216. doi: 10.1186/s12974-021-02248-2, PMID 34544428
74. Nutt JG. Continuous dopaminergic stimulation: is it the answer to the motor complications of Levodopa? Mov Disord 2007;22:1-9. doi:10.1002/mds.21060.
75. Moreira S, Fonseca I, Nunes MJ, Rosa A, Lemos L, Rodrigues E, *et al*. Nrf2 activation by tauroursodeoxycholic acid in experimental models of Parkinson's disease. Exp Neurol. 2017 Sep;295:77-87. doi: 10.1016/j.expneurol.2017.05.009, PMID 28552716
76. Sabat MJ, Wiśniewska-Becker AM, Markiewicz M, Marzec KM, Dybas J, Furso J, *et al*. Tauroursodeoxycholic acid (TUDCA)-lipid interactions and antioxidant properties of TUDCA studied in model of photoreceptor membranes. Membranes (Basel). 2021 Apr 29;11(5):327. doi: 10.3390/membranes11050327, PMID 33946822
77. Soares R, Ribeiro FF, Xapelli S, Genebra T, Ribeiro MF, Sebastião AM, *et al.* Tauroursodeoxycholic acid enhances mitochondrial biogenesis, neural stem cell pool, and early neurogenesis in adult rats. Mol Neurobiol. 2018 May;55(5):3725-38. doi: 10.1007/s12035-017-0592-5, PMID 28534273
78. Yang MC, McLean AJ, Le Couteur DG. Cell membrane transport of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in the liver and systemic bioavailability. Biochem Biophys Res Commun. 2001 Nov 23;289(1):130-6. doi: 10.1006/bbrc.2001.5954, PMID 11708789
79. Chen X, Shao B, Yu C, Yao Q, Ma P, Li H, *et al.* Energy disorders caused by mitochondrial dysfunction contribute to α-amatoxin-induced liver

function damage and liver failure. Toxicol Lett. 2021 Jan 1;336:68-79. doi: 10.1016/j.toxlet.2020.10.003, PMID 33098907

80. Henzel K, Thorborg C, Hofmann M, Zimmer G, Leuschner U. Toxicity of ethanol and acetaldehyde in hepatocytes treated with ursodeoxycholic or tauroursodeoxycholic acid. Biochim Biophys Acta. 2004 Feb 2;1644(1):37-45. doi: 10.1016/j.bbamcr.2003.10.017, PMID 14741743
81. Portincasa P, Palmieri V, Doronzo F, Vendemiale G, Altomare E, Sabbà C, *et al*. Effect of tauroursodeoxycholic acid on serum liver enzymes and dyspeptic symptoms in patients with chronic active hepatitis. Curr Ther Res. 1993;53(5):521-32. doi: 10.1016/S0011-393X(05)80659-9
82. Bai C, Song X, Yan J, Xu J, Zhou Y, Sun Z, *et al*. Tauroursodeoxycholic acid induces liver regeneration and alleviates fibrosis through GATA3 activation. Biomedicines. 2025 Apr 9;13(4):910. doi: 10.3390/biomedicines13040910, PMID 40299532
83. Lebeaupin C, Proics E, de Bieville CH, Rousseau D, Bonnafous S, Patouraux S, *et al*. ER stress induces NLRP3 inflammasome activation and hepatocyte death. Cell Death Dis. 2015 Sep 10;6(9):e1879. doi: 10.1038/cddis.2015.248, PMID 26355342
84. Vasanthi C, Sureshkumar R. Neuroprotection by resveratrol: A review on brain delivery strategies for Alzheimer's and Parkinson's disease. J Appl Pharm Sci. 2022;12(7):1-17.
85. Rajan M, Sivanesan S, Ramachandran K, Goyal P, Palanivelu P, Ghosh A, *et al*. TUDCA combined with Syndopa protects the midbrain and gut from MPTP toxicity in a Parkinson's disease mouse model: Immunohistochemical evidence. Biomol Biomed. 2025 Aug 4;25(12):2776-88. doi: 10.17305/bb.2025.12519, PMID 40763215