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RESEARCH ARTICLE

Dual Therapeutic Potential Of 2-(2-Nitrophenyl)-1,3-Thiazolidine-4-Carboxylic Acid Via Hepatoprotection and Iron Regulation in Rotenone Induced Sprague Dawley Male Rats

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ABSTRACT

Oxidative stress and iron accumulation are interconnected phenomena linking liver damage with Parkinson's disease (PD), making them critical therapeutic targets for treating hepatic disorders and mitigating PD symptoms. The present investigation was designed to assess the hepatoprotective capability, antioxidant potential, and iron-suppressing efficacy of 2-(2-nitrophenyl)-1,3-thiazolidine-4-carboxylic acid (2,2Np 1,3TZD 4-CA) in a rotenone-induced hepatotoxicity model of PD in rats. For this purpose, 42 male Sprague Dawley rats were divided into seven groups and treated with rotenone (2.5mg/kg) or 2,2Np 1,3TZD 4-CA at low (10mg/kg) and high (30mg/kg) doses, administered intraperitoneally once daily for 21 days. Serum alanine aminotransferase (ALT) levels were analyzed to assess liver enzymatic function. Antioxidant potential was evaluated using DPPH activity assays and reactive oxygen species (ROS) markers (SOD, CAT, MDA), while iron accumulation and histopathological changes in liver tissues were also assessed. The results demonstrated that 2,2Np 1,3TZD 4-CA significantly alleviated rotenone-induced elevations in serum ALT levels in a dose-dependent manner and markedly suppressed hepatic iron accumulation. Furthermore, the compound exhibited remarkable free radical scavenging activity, improved oxidative stress markers by increasing SOD and CAT levels while reducing MDA levels, and restored histological integrity in liver tissues damaged by rotenone. These findings highlight the potential of 2,2Np 1,3TZD 4-CA as a promising therapeutic agent for addressing hepatic dysfunction, iron dysregulation, and oxidative stress associated with PD.

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INTRODUCTION

Parkinson disease (PD) is the second most prevalent neurodegenerative condition worldwide, affecting over 1% of individuals aged 65 and older (Hassan *et al*., 2025). It is characterized by the progressive deposition of aggregated α-synuclein in the brain, a hallmark of its pathology (Albadawi *et al*., 2024). The disease is more common in males than females, and the number of cases is expected to double within the next 20 years, reaching an estimated 14 million by 2040 (Balakrishnan *et al*., 2021). This is a multifarious, multisystem neurodegenerative ailment classified into motor and non-motor symptoms (Bloem *et al*., 2021). The classic motor features of this disorder include bradykinesia, rest tremors, stiffness, walking abnormalities, balance confusion, and alterations in speech and swallowing (Uwishema *et al*., 2022). Non-motor symptoms can be characterized as olfactory complications, autonomic dysfunction like constipation and depression may occur before the appearance of motor symptoms (Aryal *et al*., 2020). Liver dysfunction is considered as comorbidities of PD (Balestrino and Schapira, 2020). Pathological features involve extrapyramidal pathways and multiple mechanisms includes alpha-synuclein metabolism, impaired dopamine, oxidative stress, mitochondrial dysfunction, cellular calcium imbalance, altered iron homeostasis and neuroinflammation. Risk factors include age, genetic factors and some environmental factors (Dorsey and Bloem, 2024; Ahmed *et al*., 2025). Currently used therapeutic efforts only facilitate

symptomatic relief but not proper treatment (Yasser *et al*., 2025). World of diagnosis of this ailment and treatment is still in need to be explored. The increasing prevalence of PD highlights the need to develop interventions aimed at slowing or halting its progression (Menozzi and Schapira, 2024).

Rotenone (ROT) is a commonly used insecticidal chemical used in agriculture. ROT is connected with numerous mechanisms like distorted process of calcium signaling, oxidative loss, mitochondrial dysfunction, α-synuclein deposition and cellular apoptosis and can cross blood brain barriers (Van Laar *et al*., 2023). ROT has been confirmed frequently to cause the damage of dopaminergic cells residing in the substantia nigra in vivo, which is representative feature of PD neuropathology in laboratory trials (Allam *et al*., 2025). ROT caused oxidative stress by producing ROS which triggered cytotoxicity in HepG2 cells and disturbed the pathways of p53, Bax/Bcl-2, and caspase-3 involved in mitochondria-mediated. Moreover, ROT remarkably boosted the hepatic index of experimental animals and also balanced the level of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in serum (Wang *et al*., 2022).

Iron dysregulation has emerged as a key pathological factor in both PD and liver diseases. Excess iron in the substantia nigra is strongly correlated with PD progression due to its role in promoting oxidative stress, ferroptosis, and α-synuclein aggregation (Wise *et al.*, 2022). Improved iron overload is impending remedial approach for PD. Although, iron is indispensable for existence of life, but extra iron deposition is noxious and seriously lethal. The liver acts as warehouse for iron storage hence it is predominantly vulnerable to damage from iron burden (Kouroumalis *et al*., 2023).

Hepatotoxicity has been reported as the mainly widespread pathological observations in people with iron burden (Arrizqiyani *et al*., 2025). Iron is deposited in both parenchymal cells and Kupffer cells of liver. This free iron is responsible for degeneration of ROS which results into oxidative stress as well as for diminishing of cellular stores of antioxidants (Gensluckner *et al*., 2024) and oxidative burden play vital role in hepatotoxicity (El Hanbally *et al.*, 2025 ; (Mohamed *et al.*, 2025; Shailabi *et al*., 2025). Patients suffering with hepatic encephalopathy associated with liver cirrhosis have been nominated for showing specific symptoms of motor dysfunction. Accordingly, maintenance of iron homeostasis is indispensable by making proper iron availability and by reducing buildup of surplus iron. Connection of liver fibrosis is connected with cognitive deficits in PD (Kouroumalis *et al*., 2023). Few published studies are available about the impact of liver health associated with pathways of PD (Zolin *et al*., 2024).

Benzothiazole can be categorized as a class of heterocyclic compounds which consists of sulfur and nitrogen hetero atoms. Thiazolidine motifs plays a connecting role in joining of organic synthesis and medicinal chemistry and force investigators to discover new drug candidates (Sahiba *et al*., 2020). Thiazolidine-4-carboxylic acid derivatives show beneficial role in reducing neuroinflammation, oxidative stress (Ehsanifar and Montazeri, 2022) and memory impairment (Naz *et al*., 2022). Thiazolidine-4-carboxylic acid derivatives have been proved for having antimicrobial, antiproliferative, anti-inflammatory, analgesic antiviral and iron chelating potential (Yücel *et al*., 2024).

2-(2-nitrophenyl)-1,3-thiazolidine-4-carboxylic acid (2,2Np 1,3TZD 4-CA) is a newly synthesized thiazolidine derivative with demonstrated antiviral, antioxidant, and hepatoprotective properties (Musaddiq *et al*., 2020). While preliminary studies highlight its ability to combat oxidative stress and enhance neurotransmission, its specific hepatoprotective and iron-suppressing effects in PD associated hepatic dysfunction remain unexplored. L-Cysteine (L-Cys), a core component of the thiazolidine scaffold, has also demonstrated significant antioxidant activity (Samad *et al*., 2023). These antioxidative effects primarily stem from the nitro group's electronegativity, enhancing its free-radical scavenging capacity (Sadowski and Kula, 2024). Although carboxylic acids alone do not directly scavenge free radicals, they enhance antioxidant activity indirectly through metal chelation and interactions with other antioxidants, making them critical in combating oxidative stress (Mahdy *et al*., 2017).

Literary evidences proved the iron involvement in ROT-induced PD rat model (Avci *et al*., 2022) and association of hepatotoxicity with ROT-induced PD mice model (Abdel-Salam *et al*., 2020). Hence current study revolves around these all inter-related events via modulating oxidative stress through 2,2Np 1,3TZD 4-CA. The novelty of this study lies in the comprehensive assessment of 2,2Np 1,3TZD 4-CA as a dual-function therapeutic agent targeting both systemic oxidative stress and hepatic iron dysregulation in a rotenone-induced PD model. By exploring its potential to mitigate hepatic damage, this research seeks to position 2,2Np 1,3TZD 4-CA as a promising candidate for integrated therapies addressing liver pathologies as comorbidities of PD.

MATERIALS AND METHODS

Ethics: Experiments were performed according to protocol sanctioned by the University Research Ethics Committee (UREC) of The Women University Multan via order NO: WUM/REC/24-22, Dated 08-07-2024.

Chemicals and reagents: Rotenone (Mackline, China), sunflower oil (Shifa Laboratories, Pakistan), dithio-bisnitrobenzoic acid (DTNB), hydrogen peroxide (H_2O_2), dimethyl sulfoxide (DMSO), nitroblue tetrazolium (NBT), thiobarbituric acid (TBA), and trichloroacetic acid (TCA). All reagents were of analytical grade and procured from Sigma Aldrich Co. (St. Louis, USA).

Experimental animals: Adult Sprague Dawley male rats (9 weeks old, 200±30g, n=42) were purchased from AK Traders (Registered) Multan and kept in animal house, at Department of Pharmacy, BZU, Multan. Rats were accommodated in polycarbonate cages (27cm in width, 40cm in length and 17.5cm in depth) with per cage three animals. Standard diet and water was accessible *ad libitum*. Room was maintained at 22±3°C temperatures, 50±5% humidity and light/dark rhythm that was continued at 14:10 (Sadiq *et al*., 2022).

Synthesis and characterization of 2,2Np 1,3TZD 4-CA: Synthesis and characterization of 2,2Np 1,3TZD 4-CA has been published in previous literature (Musaddiq *et al*., 2020).

Experimental design: Animals were distributed into seven (07) groups. ROT was dissolved in DMSO and then suspended in sunflower oil (Anjum *et al*., 2024) while 2,2Np 1,3TZD 4-CA was dissolved in DMSO (Demire Ozkay *et al*., 2017). All dilutions were freshly prepared and every rat was intraperitoneally administered with 0.2mL of respective treatment once a day for 21 days. Dose selection was adjusted according to previously published literature (Demire Ozkay *et al*., 2017). The experimental groups and treatments were as follows and has also been shown in Fig. 1:

- **Group I:** No treatment.
- **Group II:** Sunflower oil+DMSO (98%+2%) at a dose of 1mL/kg.
- **Group III:** ROT (2.5mg/kg body weight)
- **Group IV:** 2,2Np 1,3TZD 4-CA (10mg/kg).
- **Group V:** 2,2Np 1,3TZD 4-CA (30mg/kg).
- **Group VI:** 2,2Np 1,3TZD 4-CA (10mg/kg) with ROT (2.5mg/kg).
- **Group VII:** 2,2Np 1,3TZD 4-CA (30mg/kg) with ROT (2.5mg/kg).

Blood Sample collection: Blood samples were obtained via retro-orbital puncture using sterile capillaries. The samples were centrifuged at 3,000×g for 10 minutes which was stored at −80°C for subsequent analysis of ALT level (Taufani *et al*., 2024).

ALT test: ALT levels were measured using the ALT Assay Kit (Catalog No. MAK052, Sigma Aldrich).

Decapitation and tissue sample collection: Rats were sacrificed humanely under chloroform anaesthesia. Liver organs from all groups were excised immediately, washed with PBS (pH 7.4) and fixed in 10% formalin for histology and for biochemical analysis (Badria *et al*., 2015).

Weight of liver: The relative liver weight was measured (Adane *et al*., 2023).

Homogenization of liver tissues: Liver tissues were homogenized in PBS (pH 7.4). The homogenates were centrifuged at 30,000×g for 30 minutes at 4°C (Badria *et al*., 2015).

DPPH activity assay: DPPH activity assay of liver tissue homogenates was done by the method of Damgaard *et al*. (2014).

Biochemical analysis: SOD activity was measured by method of Marklund and Marklund (1974), CAT activity was evaluated by adopting method of Aebi (1984) and MDA content was noted by method of Nair and Turner (1984).

Iron analysis: Liver tissues were digested with nitric acid and perchloric acid and analyzed using an atomic absorption spectrophotometer (Analytik-Jena NovAA, 400P, Germany) (Xiong *et al*., 2012).

Histopathological analysis: The histopathological studies were performed by using a standard protocol (Sari *et al*., 2025). Liver tissues were fixed in 10% neutral buffered formalin solutions, followed by washing with water and dehydration with ascending grades of ethanol. Later the specimens were cleared with xylene, embedded in melted paraffin wax. 3–5µm thick sections were cut by using a rotary microtome. The sections were stained with hematoxylin and eosin (H&E) and photomicrographs were

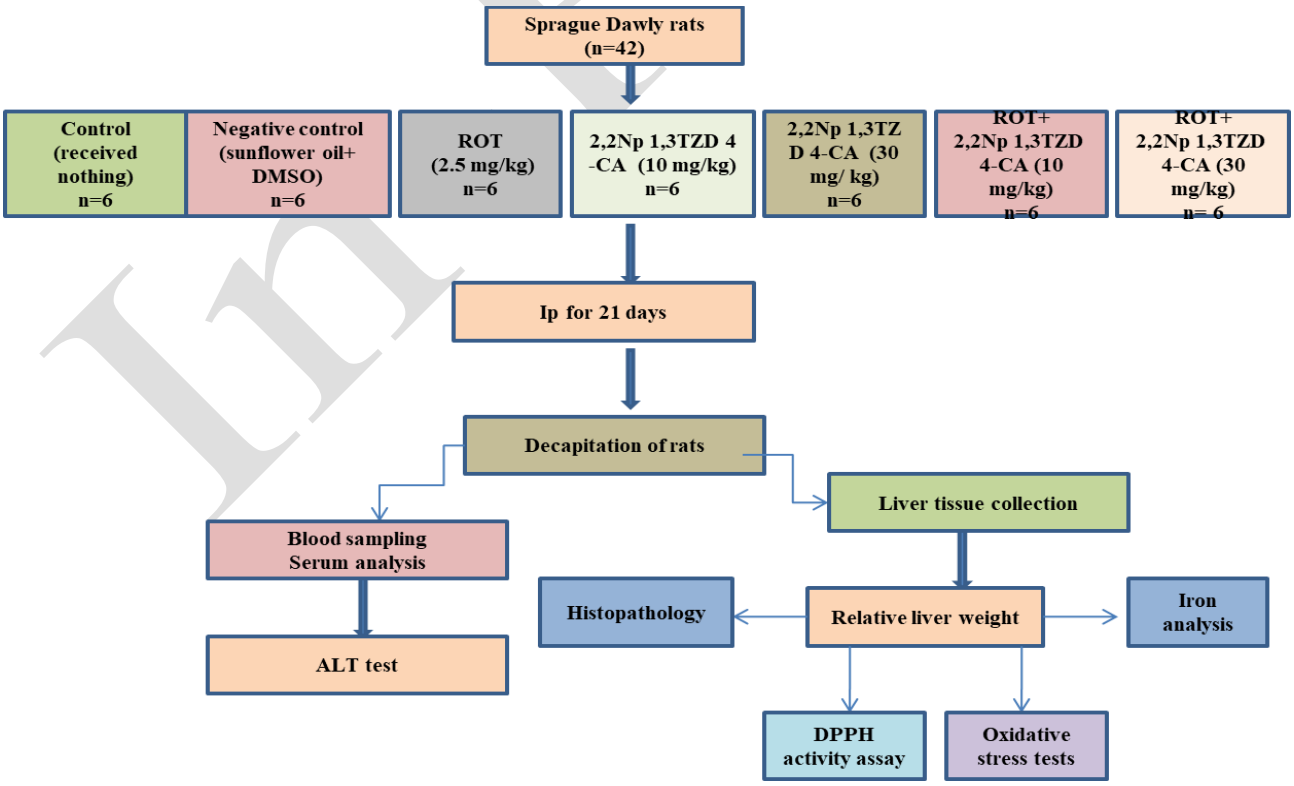

Fig. 1: Schematic representation of experimental study plan.

captured by an OPTIKA (Italy) microscope at 10 and 40X magnifications. Hepatocyte counts were analyzed through ImageJ software (Version 1.53p, USA).

Statistical analysis: Statistical analysis was performed with one way ANOVA by using GraphPad Prism software (Version 10.4.1, USA).

RESULTS

Treatment with ROT increased the relative liver weight to 5.423±0.213% (P<0.001) as compared to the control group (4.13±0.22%). However, treatment with 2,2Np 1,3TZD 4-CA (30mg) prevented the ROT-induced increase, reducing the relative liver weight to 3.85±0.13% (P<0.001). In contrast, 2,2Np 1,3TZD 4-CA (10mg) produced a non-significant effect (4.33±0.09%, P=0.0697) (Fig. 2).

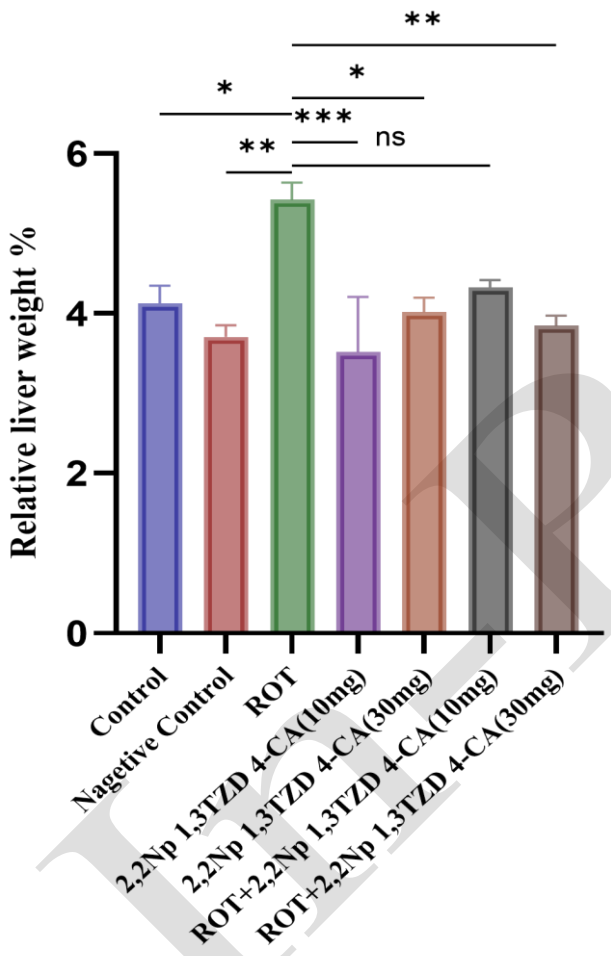

Fig. 2: Effect of 2,2Np 1,3TZD 4-CA on relative liver weight. All values are presented as mean ± SEM. Correlation among different treatment groups was done using one way ANOVA followed by Dunnett test. P values (P<0.001) were expressed as significant.

Effect of treatment on ALT test: Serum biochemical parameter was measured as classical indicators of liver function and varied noticeably F (6, 14) = 4.253, P=0.0120. The exposure of ROT significantly raised the level of serum ALT to 35.67±0.88U/L as compared to the control (30.33±0.88U/L) (P<0.05). The treatment with 2,2Np 1,3TZD 4-CA (30mg) significantly lowered the concentrations of ALT to 28.67±0.33U/L (P<0.05), while ALT levels were non-significantly changed (31.67±0.881U/L) as presented in Fig. 3.

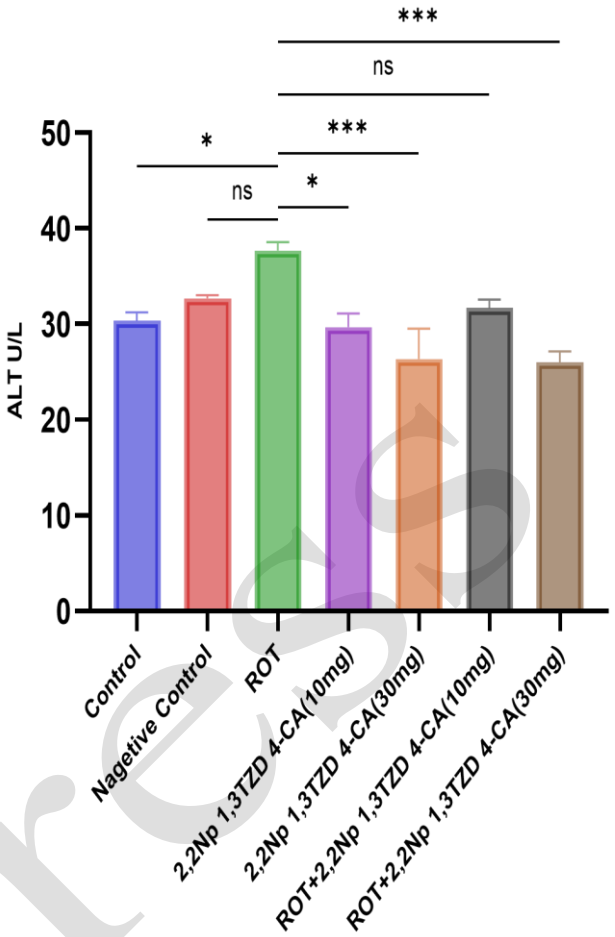

Fig. 3: Effect of 2,2Np 1,3TZD 4-CA on ALT in serum of experimental rats. All values are presented as mean ± SEM. Correlation among different treatment groups was done using one way ANOVA followed by Dunnett test. P values (P<0.001) were expressed as significant.

Effect of treatment on DPPH activity assay: To assess the antioxidant profile, free radical scavenging activity (%) was measured, which varied notably among groups (F (6, 14) = 18.66, P<0.0001). ROT-treated group exhibited very low DPPH activity of 43±0.83% as compared to the control group (7.02 ± 1.85%) unexpected non significantly but as statistically bar indicated that control group have higher free radical scavenging activity as compared to ROT treated group. The results showed that 2Np 1,3TZD 4-CA is an efficient antioxidant candidate at both doses, with scavenging activities of 23.2±1.95% (P<0.05) and 30.16±5.77% (P<0.01) at 10 and 30 mg, respectively. The treatment with 2,2Np 1,3TZD 4-CA, 30 mg restored ROT-reduced free radical scavenging activity to 52.97± 9.56% (P<0.0001) (Fig. 4).

Effect of treatment on ROS test: The SOD activity changed notably among all groups 14=8173, P<0.0001). The treatment with ROT reduced SOD activity to 0.37±0.0011nmol/mg of liver tissue (P<0.0001) in comparison to the control group (0.38±0.0005nmol/mg). Treatment with 2,2Np 1,3TZD 4-CA (30mg/kg) noticeably restored SOD activity to 0.58±0.001nmol/mg (P<0.0001) as shown in Fig. 5A.

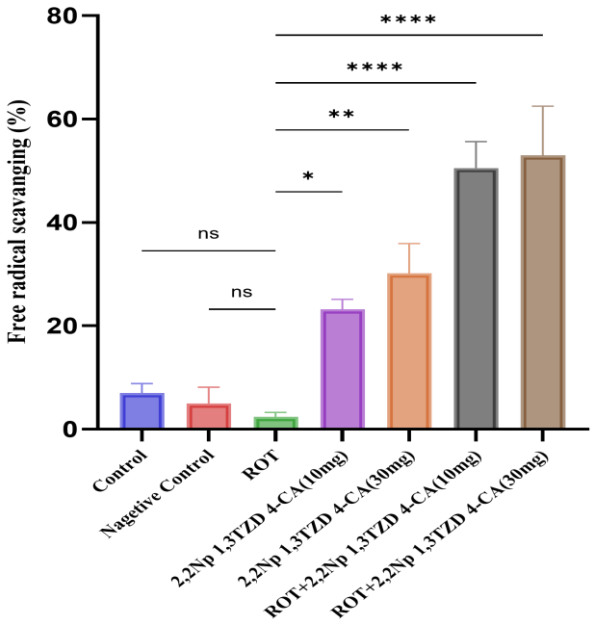

Fig. 4: Effect of 2,2Np 1,3TZD 4-CA on free radical scavenging activity in liver tissues. All values are presented as mean ± SEM. Correlation among different treatment groups was done using one way ANOVA followed by Dunnett test. P values (P<0.001) were expressed as significant.

The CAT activity was significantly different among all groups (F (6, 14) = 80.99, P<0.0001). The treatment with ROT reduced the CAT activity to 1.118±0.0018nmol/mL (P<0.0001) as compared to healthy control (0.677±0.0005nmol/mL). Treatment with 2,2Np 1,3TZD 4-CA (30 mg) restored ROT-reduced activity of CAT to 1.035±0.0351nmol/mL (P= 0.0336) as presented in Fig. 5B.

The MDA levels varied notably among all groups (F (6, 14) = 13.52, P<0.0001). The treatment with ROT caused an elevation in MDA levels to 0.39±0.0012nmol/g of liver tissues as compared to control group 0.53±0.0012nmol/g (P<0.0001). The treatment with 2,2Np 1,3TZD 4-CA dose-dependently restored MDA levels, as 0.48±0.0362nmol/g (P=0.0023) was noted in animals treated with 30 mg/kg while 0.41±0.0023nmol/g (P=0.9010) was noted in animals receiving 10 mg/kg (Fig. 5C).

Effect of treatment on iron accumulation in liver: Iron concentration in liver tissues varied notably among differently-treated groups (F (6, 14) = 7.910, P=0.0007). Total iron level was significantly increased to 6.08±0.49ppm in the ROT-treated group in comparison to the healthy control group2.74±0.30ppm (P<0.001). The treatment with 2,2Np 1,3TZD 4-CA (10 mg) restored the

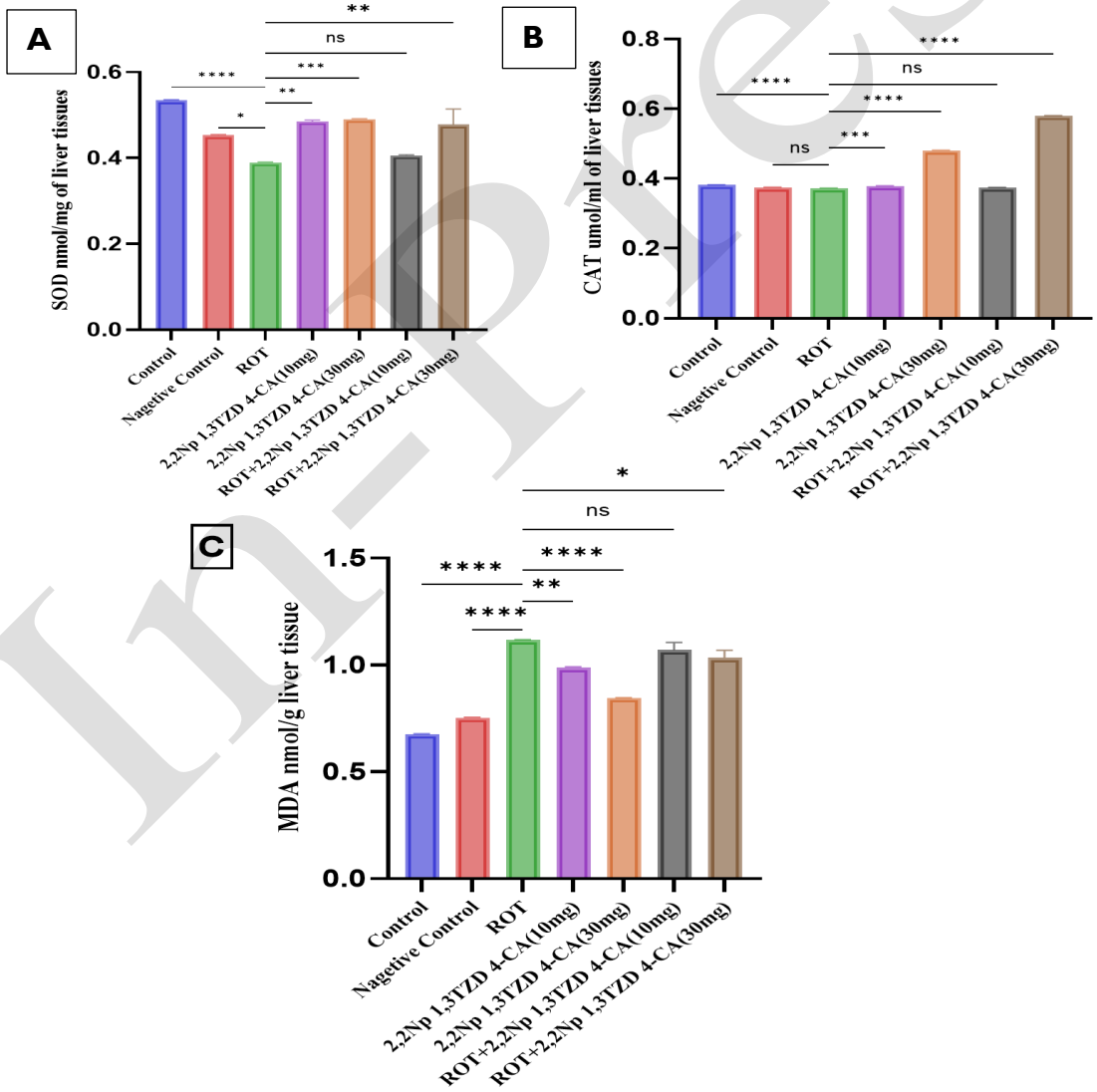

Fig. 5: Effect of 2,2Np 1,3TZD 4-CA on oxidative stress (A) SOD (B) CAT (C) MDA. All values are presented as mean ± SEM. Correlation among different treatment groups was done using one way ANOVA followed by Dunnett test. P values (P<0.001) were expressed as significant.

total iron level to 4.31±0.33ppm (P<0.05) and a further reduction to 3.42±0.21ppm (P<0.01) in animals treated with 30 mg/kg (Fig. 6).

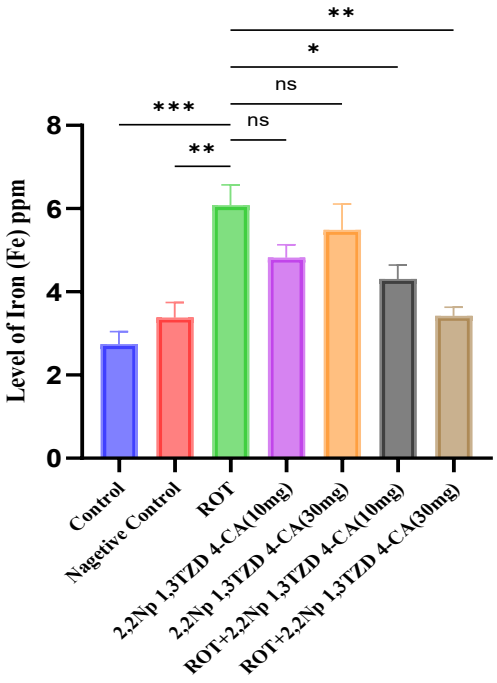

Fig. 6: Effect of 2,2Np 1,3TZD 4-CA on level of total iron accumulation in liver tissues. All values are presented as mean ± SEM. Correlation among different treatment groups was done using one way ANOVA followed by Dunnett test. P-values (P<0.001) were expressed as significant.

Effect of treatment on histopathology of liver tissues: Histological observations of liver tissues sections captured from control group displayed normal hepatic framework with normal central vein and properly radiating cord of hepatocytes as shown in Fig. 7(A). Liver tissues sections from negative control group represented histological changes little bit different from control group as hepatocytes were found with irregular arrangement with congested central vein and visibly dilated sinusoids as depicted in Fig. 7(B). Animal injected with ROT showed degeneration of hepatocytes, congested and filled central vein and sinusoids with spaces as demonstrated in Fig. 7(C). Liver tissues sections obtained from 2,2Np 1,3TZD 4-CA (10mg) treated group showed nearly normal heaptocytes architecture but with dilated sinusoids and smaller but normal central vein as shown in Fig. 7(D). Liver slides observed from 2,2Np 1,3TZD 4-CA (30mg) administrated rats showed normal hepatocytes, proper central vein and sinusoids likewise observed in control group as depicted in Fig. 7(E). Tissue sections taken from ROT and 2,2Np 1,3TZD 4-CA (10mg) showed less repairing of ROT damaged hepatocytes and congested and infiltered central vein as shown in Fig. 7(F). Histological explanation of liver sections captured from ROT and 2,2Np 1,3TZD 4-CA (10mg) treated group showed comparatively repairing of ROT destroyed hepatocytes and infiltered central vein and dilated sinusoids as displayed in Fig. 7(G). Cell count was calculated from every slide of all liver tissues sections and cells were counted in specific area (400 l and 100 h) with the Image J software showed significant cell numbers in 2,2Np 1,3TZD 4-CA (30 mg) treated group 60±2.65 cell count/400um (P<0.001) as

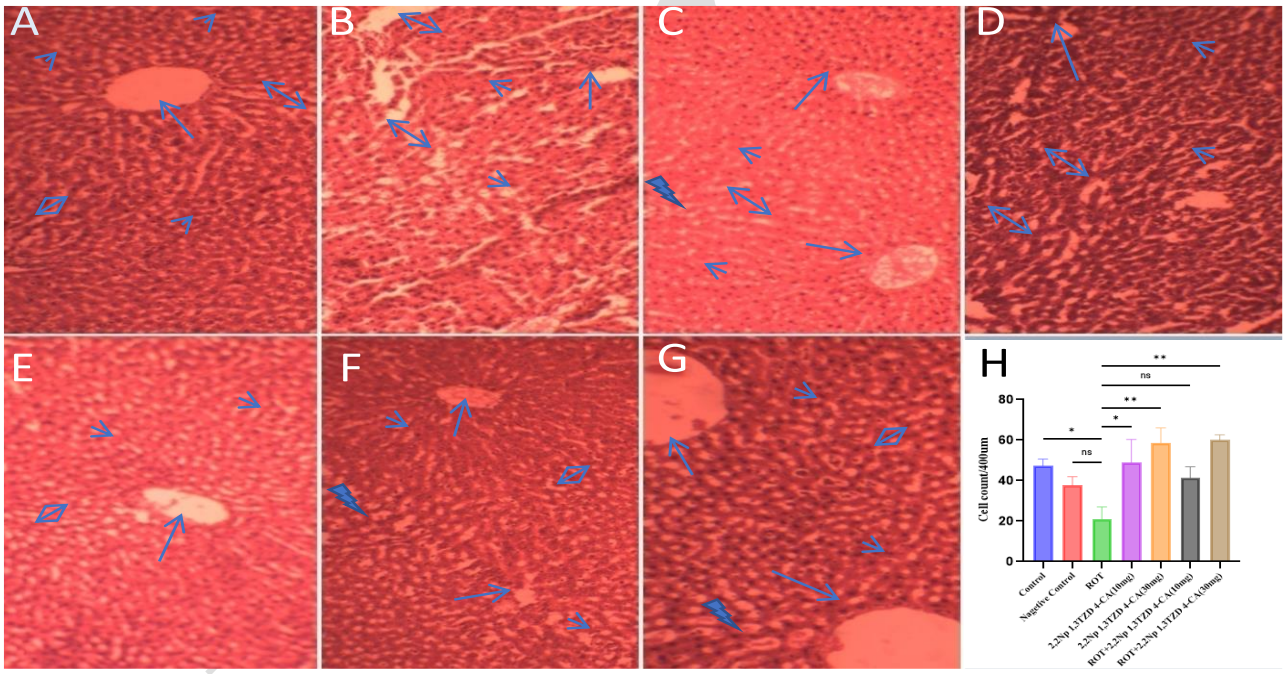

Fig. 7: (A) Photomicrograph of liver tissues of control group showing normal hepatocytes architecture (arrow head), proper central vein (arrow) and sinusoids (double head arrow) (H &E 100x). (B) Photomicrograph of DMSO and sunflower oil treated liver tissues of rats showed hepatocytes (arrow head), central vein (arrow) and sinusoids (double head arrow) with nearly appropriate structure (H &E 100X). (C) Photomicrograph showing liver tissues from ROT treated rats depicting degenerated hepatocytes (lightning bolt), congested central vein (arrow) with irregularly arranged sinusoid (double head arrow), large inter cellular spaces (H &E 100X). (D) Photomicrograph of liver tissues taken by rats injected with 2,2Np 1,3TZD 4-CA (10mg) explained almost normal hepatocytes (arrow head), dilated sinusoids (double headed arrow) and smaller central vein (arrow) (H &E 100X). (E) Photomicrograph of rat liver sections from animal group received 2,2Np 1,3TZD 4-CA (30mg) displayed hepatocytes (arrow head) with no destruction, proper central vein (arrow) and sinusoids (double headed arrow) with some spaces (H &E 100x). (F) Photomicrograph of liver tissues excised from rat group facilitated with ROT and 2,2Np 1,3TZD 4-CA (10mg) showed necrosis of hepatocytes but also normal hepatocytes (arrow head), congested central vein (arrow) and sinusoids (double headed arrow) in irregular arrangements and spaces (H &E 100X). (G) Photomicrograph of rat liver sections from ROT and 2,2Np 1,3TZD 4-CA (30mg) injected group showed normal hepatocytes (arrow head) with less necrosis, central vein (arrow) congested and normal, some sinusoids (double headed arrow) are dilated some are with proper shape (H &E 100X). (H) Graph showing cell count in different groups taken with J image software (version 1.53p, NIH, USA).

compared to control group 47.33±3.28 cell count/400um (P<0.001) and ROT injected group 21±6.03 cell count/400um (P<0.001).

DISCUSSION

PD is the most widespread neurodegenerative movement issue. According to the statistics analyzed here, Pakistan has shown an upsurge in PD during the last ten years (Jamali *et al*., 2024). ROT executes its mechanism of poisoning in such a way that it creates oxidative stress that lead to damages organelles of hepatocytes like mitochondria and endoplasmic reticulum and finally resulting loss or weakness in their function and death of hepatocytes (Wang *et al*., 2022). Literary evidence proved that ROT also affect non-neuronal organs also like liver (Allam *et al*., 2025). Oxidative damage is responsible for rotenone-induced hepatic toxicity. In accordance with our investigations ROT has been affirmed a reason of liver damage (Jiang *et al*., 2017). The most vital mechanism of iron-induced liver damage is the newly described ferroptosis which is pathological hallmarks of PD (Wu *et al*., 2021 ; Arrizqiyani *et al*., 2025). Implicating with neuronal cell death, iron can be regarded as extrapyramidal element due to showing itself as a marvelous therapeutic agent and novel diagnostic indicator hence badly involved in PD which is also an extrapyramidal disease (Foley *et al*., 2022). Now a days, liver damage or failure is very common health issue. Liver diseases gain much attention in recent years and are considered one of the leading global health problems in developing countries. Liver diseases affect more than 2 billion people worldwide. Liver, the largest solid organ of the body, is the center of metabolism and detoxification and highly vulnerable to oxidative stress (Banerjee *et al*., 2023).

Benzothiazole is the most active lead molecule in the field of drug discovery for its wide range of pharmacological activity (Mahapatra and Hazra, 2023). Thiazolidine-4-carboxylic acid derivatives have remarkable moiety of having hepatoprotective capability, antioxidant potential and mineral chelationreservation (Sahiba *et al*., 2020). Several thiazolidine derivatives have been proved as antioxidants via reduced glutathione creation and lipid peroxidation (Ham *et al*., 2020). Numerous studies have confirmed that these compounds exhibit potent free radical scavenging properties reservation (Naz *et al*., 2022). This might be of reason that thiazolidine-4-Carboxylic Acid derivatives have phenolic moiety in their structure (Payaz *et al.*, 2019; Ehsanifar and Montazeri, 2022). 2-Substituted thiazolidine-4-carboxylic acids behaving as prodrugs of L-cysteine have been proved as suitable candidate for protection against acetaminophen triggered hepatotoxicity in mice model (Madhavi and Devi, 2025). There is scarce literature available showing hepatoprotective activity of thiazolidine-4-carboxylic acids in ROT induced PD rat model.

When liver become damaged or inflamed, ALT is revealed in blood and increases the ALT level. So, in our experiment ROT group have increased ALT level which indicate that liver become damaged by the effect of ROT. This was certainly due to the oxidative stress caused by ROT which was responsible for destruction of hepatocytes. Thaizolidine derivatives have been proved for restoring liver damage by inhibiting the elevated ALT level in serum in rats (Akree *et al*., 2024).

In present investigations, ROT treated group show decrease weight of animal and relative liver index also. In accordance with our results a study proved that relative liver weight rise up indicates liver damage (Adane *et al*., 2023). For measuring antioxidant profile, DPPH radical scavenging assay is simple, easy, reliable, low cost and extremely valuable approach. Even though, there is scarcity of literature related to implementation of DPPH activity assay on biological samples like animal tissues or organs, this method can be a highly versatile and appropriate choice for detecting antioxidant activity (Daamgard *et al*., 2014). In our findings, our selected drug showed remarkable significant radical scavenging activity in liver tissues. This might be reasoning that thaizolidine derivatives have been reported as having capabilities of marvelous radical scavenging activity in accordance with our results by using DPPH protocol in liver dysfunction.

Oxidative stress is linked with the pathophysiology of several degenerative human being diseases, including PD (Ham *et al*., 2020) and liver disorders also (El Hanbally *et al.*, 2025; (Mohamed *et al.*, 2025). Diverse vulnerability of brain areas to oxidative stress, make a payment to Parkinsonism under the conditions of inadequate liver clearance capability (Li et al., 2024). In confirmation of our results elevation of total iron level due to ROT was reported in PD rat model (Avcı *et al*., 2023).

Present study concluded that most of liver issues related to PD can be preventable and that we can help to create a world where PD is increasingly rare. Present findings purpose that thiozolidine derivative may open a new window in therapeutically improving hepatic iron accumulation and establishing the body endogenous antioxidant defense mechanism. In this project, we estimate the efficacy of thiozolidine derivative to lessen hepatic abnormalities, iron burden, antioxidant stress and to gain insight into the underlying mechanisms in relation with PD.

There are many literature evidences from histological point of view that proved that ROT is famous for causing hepatocytes destruction, vacuolar degeneration, irregularity in sinusoids shapes and central vein distortion and infiltration. Accordingly histological changes observed indicating ROT has damaged the hepatocytes architecture as compared to control which is in line with our results (Abdel-Salam *et al*., 2020).

Currently, there is urgent desire for framing unique disease modifying therapies because dopaminergic treatment present symptomatic relief but these are unable to control disease progression (Lastovetskyi *et al*., 2024). Present research revealed that oxidative stress is badly concerned in rotenone-induced hepato-toxicity and supply confirmation for resulting due to ROT. For the meantime, injection of 2,2Np 1,3TZD 4-CA resulted in substantial hepato-protection via strategies that focused decreased cell death and oxidative stress. Furthermore, there is need for clinical trials at high level for authentication of the effectiveness and safety of these treatments (Lastovetskyi *et al.*, 2024). Future recommendations of this project involves that this synthetic compound may be therapeutic approach towards others neurodegenerative diseases like PD and other synthetic compounds related to this derivative may be used for therapeutic and neuroprotective studies.

Limitations: Several limitations need to be considered to support the therapeutic potential of 2,2Np 1,3TZD 4-CA and advance its development for clinical applications. Limited treatment period (restricts understanding of the chronic effects), more extensive dose-response study covering a wider range of concentrations, lack of positive control group, specific molecular mechanisms or molecular docking, biomarkers related to ferroptosis and systemic inflammation.

Conclusions: The findings of this study highlight the hepatoprotective, antioxidant and iron-suppressing potential of the novel compound 2,2Np 1,3TZD 4-CA in a ROT induced rat model of PD. The compound demonstrated significant efficacy in reducing serum ALT levels, improving oxidative stress markers (SOD activity and CAT activity), lowering malondialdehyde (MDA) content, and exhibiting high free radical scavenging activity. Moreover, 2,2Np 1,3TZD 4-CA effectively mitigated total iron accumulation and restored histological architecture in liver tissues damaged by ROT. These results indicate that this compound not only possesses remarkable hepatoprotective effects but also addresses the pathological mechanisms such as oxidative stress and iron dysregulation in PD-associated liver dysfunction. In conclusion, 2,2Np 1,3TZD 4-CA holds substantial promise as a therapeutic candidate for the treatment of liver damage and iron overload in neurodegenerative conditions like PD.

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Authors' contributions: S.K made a significant contribution to the work reported in the conception, study design, execution, acquisition of data and experimentation. S.P., S.K supervised, technically and critically reviewed all aspects of study. M.F analyzed and interpreted the data. M.M.A and S.M performed their role in revising or critically reviewing the article. Moreover, all authors made a noticeable involvement giving final approval of the version to be published; agreeing on the journal to which the article has been submitted; and confirming to be accountable for all aspects of the work. All authors have read and agreed to the published version of the manuscript.

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