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The protective role of *Calotropis procera Linn* against cisplatin-induced oxidative stress, insight into antioxidant effects

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Abstract: It has been hypothesized that inflammation and oxidative stress have a potential role in neurodegeneration. Cisplatin is a commonly used in different types of cancers but it is notorious for oxidative stress which leads to neurodegeneration. Therefore, the current study focused on the antioxidant effect of *Calotropis procera* during cisplatin treatment. The methanolic extract of *C. procera* was extracted using rotary evaporator. Memory impairment was evaluated by using Y-Maze and Morris water model. The antioxidant effect of *C. procera* extract was measured by assessing oxidative stress markers such as lipid peroxidation (LPO), reactive oxygen species (ROS), glutathione (GSH), and catalase (CAT) in the experimental groups. It has been found that *C. procera* extract showed a significant ($p<0.05$) effect on preventing memory dysfunction compared to a positive control (cisplatin). The effect of *C. procera* extract was observed in a dose-dependent manner. Similarly, the increase ($p<0.05$) in the level of oxidative stress marker was high compared to the positive control group in a dose-dependent manner. While the decrease in the level of malondialdehyde (MDA) was found in the *C. procera* extract treated groups dose-dependently. *C. procera* significantly reduces cisplatin-induced oxidative stress by restoring the level of antioxidant enzymes and reducing MDA levels. The therapeutic benefits are significant at a dose of 400 mg/kg.

Keywords: Brain cancer, *Calotropis procera*, cisplatin, neurodegeneration, oxidative stress makers,

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INTRODUCTION

Chemotherapy is commonly used to treat patients who have been diagnosed with cancer, either alone or in combination with other medicines (Vencappa *et al*. 2015). Neurotoxicity is a severe side effect of several chemotherapeutic drugs used to treat different diseases, including cancer. The frequency and severity (moderate to severe) of this toxicity may endanger patients' quality of life (Pellacani &Eleftheriou 2020). Platinum-derived chemotherapies are commonly used to treat various cancers (Zhang *et al*. 2010). Cisplatin (CDDP), one of the most commonly used platinum chemotherapies, has been widely recommended since it was approved by the US Food and Drug Administration in 1978 (Von Hoff &Rozencweig 1979). Cisplatin is a platinum medication from the very first generation that has been used to treat a variety of advanced and metastatic cancers over the past 40 years (Vencappa *et al*. 2015). Because of its anticancer properties, CIS is used to treat lung, breast, ovarian, testicular, and head and neck malignancies. Among chemotherapy medications, it is the most well-known cancer drug (Hanif &Hartinger 2018). However, CDDP's therapeutic utility is limited by adverse treatment reactions and vario us iatrogenic toxicities (Avan *et al*. 2015). One of the most dangerous side effects of CDDP treatment has been found as neurotoxicity (McWhinney *et al*. 2009). About 40% to 50% percent of patients who get CDDP therapy have moderate or severe neuropathy, which can lead to the chemotherapy being stopped or even terminated (Wolf *et al*. 2008). Many research has looked at CDDP's peripheral neuropathy, but few have looked at its central neurotoxicity (Almutairi *et al*. 2017, Owoeye *et al*. 2018). The neurotoxic action of CDDP has been attributed to a variety of mechanisms, including oxidative damage, activation of pro-inflammatory cytokines, and apoptosis (Jaggi & Singh 2012). Cankara *et al*, suggest that cisplatin causes oxidative stress and neuroinflammation and leads to neurodegeneration (Cankara & Günaydın 2021). Excess free radical production in the body causes oxidative stress, which can damage cell structure such as lipids, proteins, and DNA. Free radicals are formed in cells as a result of the redox process, which occurs when oxygen is used to generate energy. At lower quantities, ROS are useful to cellular responses and immunological function, but at greater levels, they are toxic to the system (Pham-Huy *et al*. 2008). Degeneration in chronic diseases such as rheumatoid arthritis, autoimmune disorders, cataracts, ageing, cancer, and cardiovascular and neurological diseases are all linked to an excess of free radicals

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(Kumar &Pandey 2012). Antioxidants, as free radical scavengers, reduce the damage produced by reactive oxygen species (ROS), enhancing immunological defense and lowering the risk of degenerative diseases (Pham-Huy *et al*. 2008). Studies suggest that catalases (CAT) and superoxide dismutase (SOD) are innate antioxidant systems that maintain endogenous hemostasis (Malik *et al*. 2020). It is observed that oxidative stress and inflammatory cytokines have been found to exacerbate cognitive dysfunction and memory loss (Ali *et al*. 2020).
The naturally occurring therapeutically useful chemicals that have a long history of usage as therapeutic agents for a variety of ailments (Hussain *et al*. 2018). Exploration of such compounds could give patients with a viable new treatment option. *Calotropis procera* (*C. procera*) is a tropical plant belong to Apocynaceae family that may be found all over the globe (Mali *et al*. 2019). It is recognized as an Ayurvedic plant since it's utilized in a variety of ancient medicines to treat a variety of ailments (Parihar & Balekar 2016). Antihelmintic, hepatoprotective, anticancer, antimicrobial, antioxidant, anti-inflammatory, antipyretic, analgesic, anti-angiogenic, antidiabetic, antifertility, and anticonvulsant activity have been discovered in all parts of this plant (roots, leaves, flowers, latex) (Mali *et al*. 2019).

Similarly, the methanolic extract's antioxidant capability was assessed by their ability to scavenge the stable 1, 1-diphenyl-2-picryl hydrazyl (DPPH) free radical. The IC_{50} of *C. procera's* methanol extract was 110.25 g/ml, suggesting the plant's significant antioxidant activity. The aqueous extract, on the other hand, demonstrated moderate antioxidant action (Yesmin *et al*. 2008). In cell culture tests, the methanolic extract of DL, as well as its fraction 8, caused substantial cell death in Huh-7 (hepatoma cells) and COS-1 (non-hepatoma cells), but not in AML12 (non-transformed hepatocytes cells) (Choedon *et al*. 2006, Smit *et al*. 1995). This plant is a precious gift to humanity because of its cytotoxic compounds and many other helpful features (Mascolo *et al*. 1988). There are currently just a few treatments available for cisplatin induce neurodegeneration. Therefore, it is of considerable relevance to explore new therapeutic drugs that may help in either avoiding or treating the cisplatin induce neurodegeneration. Therefore, the current study is focused on the effect of *C. procera* to rescue the neurodegeneration caused by cisplatin through evaluation of its antioxidant effect in the mice models.

MATERIALS AND METHODS

Chemicals

Cisplatin (Pharmedic laboratories (Pvt) Ltd, Lahore, Pakistan), Sodium carbonate (SLCL1511), Sodium bicarbonate (MKCL4235), Sodium Phosphate dibasic dihydrate (BCCC4108), Potassium phosphate dibasic (SLCJ0423), Hydrogen peroxide solution(10F20013), Iron (111) chloride hexahydrate (SLCF2050), Ascorbic acid (BCCC1421), Thiobarbituric acid (BCBK7728V) were obtained from Sigma Aldrich. Potassium di-hydrogen phosphate anhydrous (BMP-132-X1) was obtained from BIO-M. Trichloroacetic acid (2515) was obtained from SMART-LAB. Nitrobenzoic acid (00169-2019050901) was obtained from CHEM-IMPEX INT.

Animals

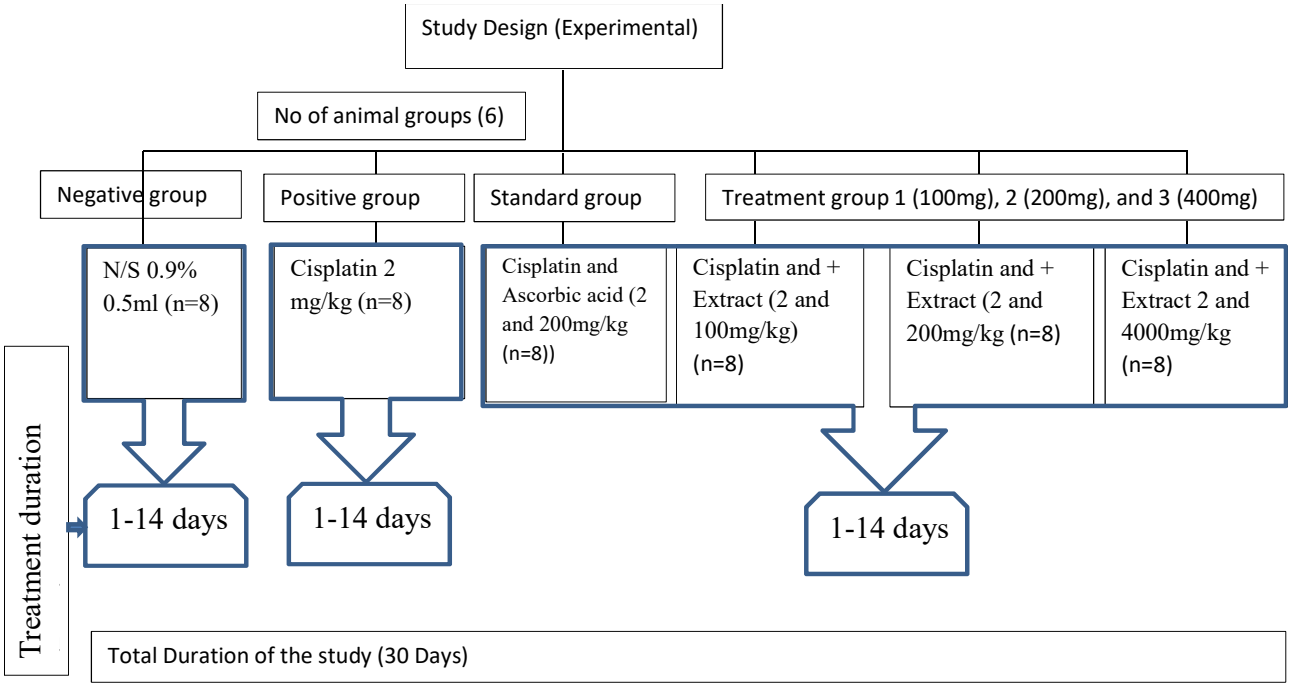
Adult male white albino mice (n=8) weighing 25–30 g was housed in a cage at the animal house of Khyber medical university (KMU) under laboratory animal ethics (temperature: 22°C; humidity: 50%–10%) during a 12 h light/dark cycle with free access to water and food. The mice were handled in accordance with the Khyber Medical University's approved ethics protocols in Peshawar.

Treatment and administration

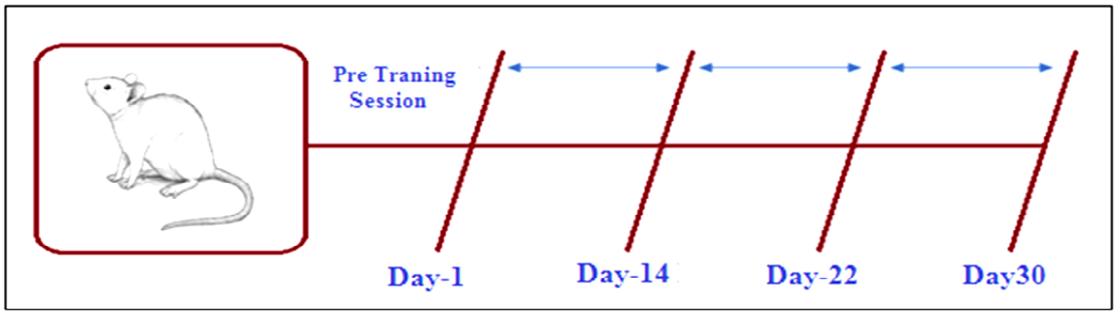
Mice were given a seven-day acclimatization period before being randomly assigned into six experimental groups (table 1). Group 1 was injected with 0.5ml of 0.9 percent regular saline injections once daily throughout the experimental study, Group 2 was injected with Cisplatin 2 mg/kg , Group 3 was injected with Cisplatin and Ascorbic acid (2mg/kg+200mg/kg) , Group 4 was injected with Cisplatin and methanolic plant extract (2 mg/kg +100mg/kg), Group 5 was injected with Cisplatin plus methanolic plant extract (2 mg/kg + 200 mg/kg), Group 6 was injected with Cisplatin plus methanolic plant extract (2 mg/kg +400mg/kg) (table 1). Cisplatin was administered intra-peritoneally for eleven consecutive days and Methanolic plant extract was given for 13 days per orally through feeding tubes one hour before cisplatin, then on 14th day behavioral studies was started (Fig. 1). All the behavioral studies were carried out during the day time. All animals were euthanized using a CO_2 chamber at the completion of the behavioral analysis, and the brain tissue was removed and frozen at -80°C. Overall, three mice died during the experiment, two from the Cis group and one from the Cis + plant extract group, which was not included in the study. The mice given saline and ascorbic acid survived the entire experiment.

Y-Maze Test

The Y-Maze is comprised of three arms that form a Y shape (30* 15* 15 cm). The mice were placed in the center of the maze and were allowed to roam (up to 8 min). Mice entering the opposite arm were referred to as spontaneous alternation, while mice entering the adjacent arm set were referred to as a total number of arm entries. The percentage (percent) of alternation behavior was calculated using the number of successful entries into three distinct arms/total number of arm entries - 2 *100. In a larger percentage of participants, alternate activity was linked to improved spatial working memory, and vice versa.



Sketch 1: Study design showing six groups treated with cisplatin and/or Calotropis procera extract (100, 200, 400 mg/kg) over a 14-day period in a 30-day experimental timeline.



Day 1- 14 ⟶ Dosing

Day 15- 22 ⟶ Behavioral Analysis (Y maze test and WMM)

Day 23- 30 ⟶ Removal of hippocampal area of the brain after mice scarification

Onward Day 30 ⟶ Oxidative stress markers analysis

Fig. 1: Scheme of treatment allocation and duration across six experimental groups

Water-Maze Test

The Morris water model (MWM) is a simple approach that normally takes six days to complete, with the main benefit of distinguishing between spatial (hidden platform) and non-spatial (visible platform) circumstances. A round water tank filled with coloured water was utilized for the MWM test, with the temperature maintained at 25°C and a submerged platform put in one quadrant of the device. The mice were taught to reach the platform during training days. The platform was withdrawn when the training was completed, and the mice were allowed to access the platform. The probe test measured the time it took to reach the platform, swimming speed, time spent in the target quadrant and platform crossings. A video capturing device was used to capture the data. Throughout the learning trials, the platform remained in the same location.

Table 1: Dosimetry and duration of treatment

Group No.	No. of Mice	Treatment	Route	Day
1	8	Normal saline 0.9% 0.5 mL	Intraperitoneal (I.p)	Day 1 to Day 14
2	8	Cisplatin 2 mg/kg	Intraperitoneal (I.p)	Day 1 to Day 11
3	8	Cisplatin 2 mg/kg + Ascorbic acid 100 mg/kg	Intraperitoneal (I.p)	Day 1 to day 11 + Day 1 to Day 14
4	8	Cisplatin 2 mg/kg + *Calotropis procera* extract 100 mg/kg	Intraperitoneal (I.p) + *C. procera* extract orally	Day 1 to Day 11+ Day 1 to Day 14
5	8	Cisplatin 2 mg/kg + *C. procera* extract 200 mg/kg	Intraperitoneal (I.p) + *C. procera* extract orally	Day 1 to Day 11+ Day 1 to Day 14
6	8	Cisplatin 2 mg/kg+ *C. procera* extract 400 mg/kg	Intraperitoneal (I.p) + *C. procera* extract orally	Day 1 to Day 11+ Day 1 to Day 14

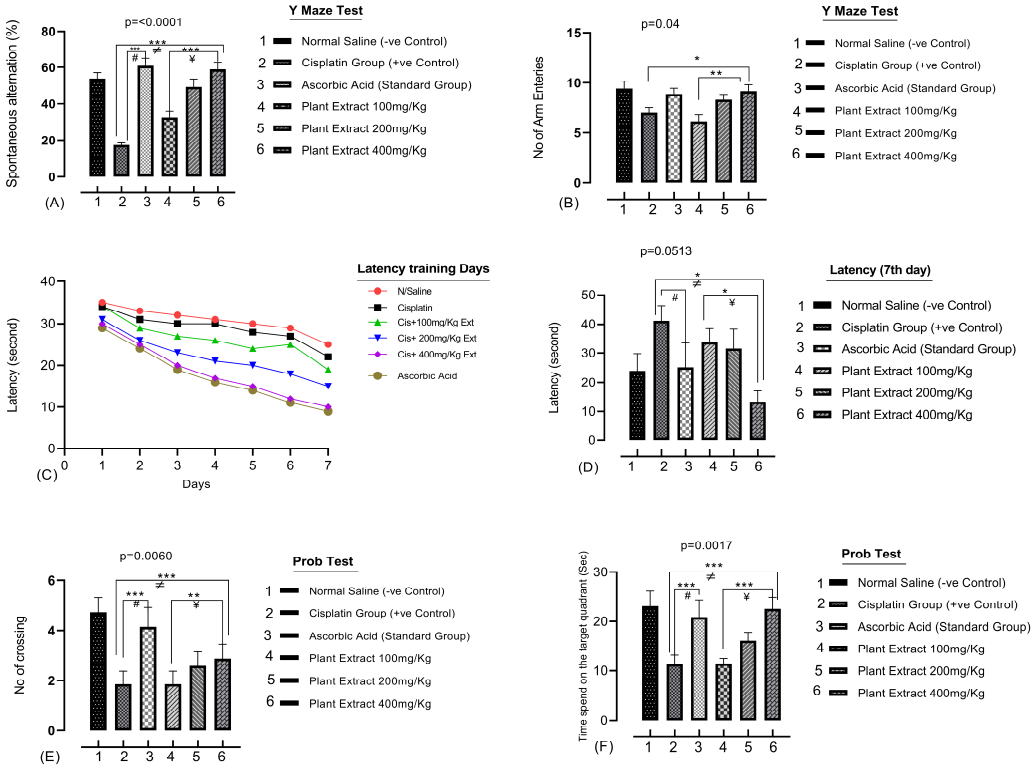


Fig. 2: A. Effect of *Calotropis procera* extract at the dose of (100 mg/kg, 200 mg/kg, and 400 mg/kg) on spontaneous alternation (%) in cisplatin-induced neurodegenerative mice model. B. Effect of *Calotropis procera* extract at the dose of (100 mg/kg, 200 mg/kg, and 400 mg/kg) on the number of total arm entries in the cisplatin-induced neurodegenerative mice model. C. Effect of *Calotropis procera* extract (100 mg/kg, 200 mg/g, 400 mg/kg) on latencies during training days in cisplatin-induced neurodegenerative mice model. D. Effect of *Calotropis procera* extract (100 mg/kg, 200 mg/g, 400 mg/kg) on latencies during the 7th day in cisplatin-induced neurodegenerative mice model. E. Effect of *Calotropis procera* (100 mg/kg, 200 mg/kg, 400 mg/kg) on the number of No of crossings in the cisplatin-induced neurodegenerative model. F. Effect of *Calotropis procera* (100 mg/kg, 200 mg/kg, 400 mg/kg) on time spent in the target quadrant in the cisplatin-induced neurodegenerative model. A one-way ANOVA was used to compare the means of each group, followed by a post hoc Bonferroni test. Three independent studies present the data as the mean ± SEM. with eight mice per group. # (hash), denoting a significant difference from Cis + Ascorbic acid injected mice, *(asterisk), denotes a significant difference from the cisplatin group. ¥ is an indication of the difference among *C. Procera* fruit extract group (100,200 and 400 mg/kg). Significance: # p≤ 0.05, *p ≤ 0.05

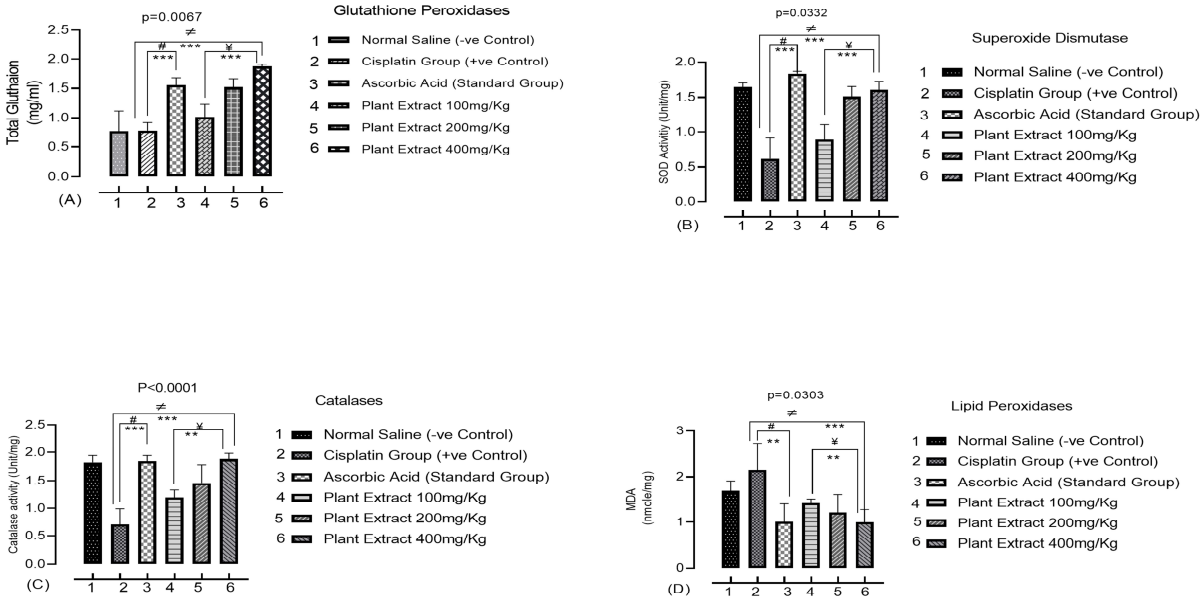


Fig. 3: **A.** Effect of *Calotropis procera* (100, 200, and 400 mg/kg) on the activities of total glutathione peroxidase in the brain of mice. B. Effect of *C. procera* (100, 200, and 400 mg/kg) on the activities of superoxide dismutase in the brain of mice. C. Effect of *C. procera* (100, 200, and 400 mg/kg on the activities of catalases in the brain of mice. D. Effect of *C. procera* (100, 200, and 400 mg/kg) on the activities of lipid peroxidases in the brain of mice. A one-way ANOVA was used to compare the means of each group, followed by a post hoc Bonferroni test. The data are presented as the mean ± SEM with eight mice per group for three independent studies. # (hash), denoting a significant difference from Cis + Ascorbic acid injected mice, *(asterisk), denotes a significant difference from the cisplatin group. ¥ is an indication of the difference among *C. procera* fruit extract group (100,200 and 400 mg/kg). Significance: # p≤ 0.05, *p ≤ 0.05.

Oxidative stress markers assay

Glutathione Peroxidases

The procedure created by Ellman (121), which was detailed by Beutler *et al* (Beutler *et al*. 1963) is commonly used to determine GSH levels. It is based on the Ellman reagent's (DTNB) capacity to react with substances that have sulfhydryl groups, resulting in the formation of mixed disulfide (GS-TNB) and 2-nitro-5-thiobenzoic acid (TNB). The spectrophotometric absorbance of the anion (TNB_2^-) at 412 nm is used to determine the latter's amounts (Czeczot *et al*. 2006).

Superoxide Dismutase

Antioxidant enzyme known as superoxide dismutase regulates the amount of reactive oxygen species (ROS) by catalyzing the conversion of superoxide to hydrogen peroxide and molecular oxygen (Halliwell &Gutteridge 1986). 3 mL of EDTA-sodium carbonate buffer 0.5 M was added to the brain tissue sample (pH 10.2). The activity of the reaction was evaluated (at 480 nm for 4 min) after epinephrine of 100 mL (30 M in 0.1 M HCl) was added to it. The amount of an enzyme that is suppressed by 50% of the rate at which epinephrine is oxidized was used to define a unit of superoxide dismutase (Zakria *et al*. 2021).

Catalases

The body has the antioxidant enzyme catalase, which breaks down hydrogen peroxide into water and oxygen (Djordjević 2004). By measuring the decline in ultraviolet absorbance of a hydrogen peroxide solution at 240 nm over a period of time, a quantitative spectrophotometric approach devised by Sizer and Beers (Beers &Sizer 1952) and reported by Aebi or Nelson and Kiesow (Nelson & Kiesow 1972) monitors the breakdown of hydrogen peroxide catalyzed by catalase (Bernardes *et al*. 2015).

Lipid Peroxidases

The thiobarbituric acid reactive substances (TBARSs) were used to determine the amounts of lipid peroxidation in mice brain homogenate (Draper &Hadley 1990). In a nutshell, the samples were combined with 10% trichloroacetic acid (1 mL) and 0.67% thiobarbituric acid (1 mL) in a ratio of 1:10. The absorbance at 535 nm was used to determine thiobarbituric-acid-reacting compounds after centrifugation (800 g; 5 min). The results were represented as malondialdehyde (MDA) nmol/g (Pires *et al*. 2014).

Ethical Approval

The animals were handled in accordance with the Khyber Medical University's approved ethics protocols at Peshawar, Pakistan.

STATISTICAL ANALYSIS

Data are presented as means ±SEM for dependent continuous variables of the experiments (8 mice in each group for oxidative stress), and (8 mice per group) for behavioral analysis. With the help of Graph Pad Prism, statistical analysis was completed. According to the experimental plan, we employed One-way ANOVA followed by a post hoc Bonferroni test, was performed. Three to four times each for every experiment repeated. Statistical significance was considered as $p<0.05$.

RESULTS

Effects of C. procera on the Cisplatin-induced behavioral impairments and neurodegeneration

Cisplatin-treated mice show a significant behavioral impairment in the positive control group as evidenced by the lower percentage of alternation in the Y-maze ($p< 0.05$) as compared to the animal treated with Cisplatin +Ascorbic acid standard control group as shown in Fig. 2A, In the standard group, a significant improvement in alternation (%) were observed which were comparable to the group treated with *C. procera* extract at the dose of 400 mg/kg (Fig. 2A). However, as compared to mice treated with *C. procera* extract, at a dose of 400 mg/kg showed a substantial-high improvement ($p<0.05$) in short term memory compared to 100 and 200 mg/kg dose (Fig 2A). It shows that the effects of the *C. procera* extract were dose-dependent, as they became significant at 400 mg/kg but remained non-significant at 100 mg/kg.

The effects of *C. procera* on the number of entrances into the open and closed arms after 8 minutes of Y plus maze exploring is shown in (Fig. 2B). Cisplatin treated group shows fewer arm entries. In animals given ascorbic acid treatment, a high number of entries were seen for the frequency of arm entries, which is comparable to the group treated with *C. procera* extract at the dose of 400 mg/kg ($p<0.05$) (Fig. 2B). There was no difference between the arm entries between the group treated with normal saline (NS) and standard group.

The Morris water maze test results showed that mice given *C. procera* had better short-term memory compared to the mice used as the positive control (Fig. 2D). In the WMM, training session, the mean escape latency of control and test mice was comparable, the mean escape latency of NS treated group and the cisplatin-treated group was increase but it was decrease in the standard ascorbic acid group and a group treated with plant extract at the dose of 400 mg/kg as shown in (fig 2D). Then on the 7th day of training session, the cisplatin-administered animals still had poor platform quadrant memory in the maze and longer escape latencies as compared to the standard ascorbic acid group as shown in fig. (fig. 2D) ($p<0.05$). When compared to the Cisplatin-treated group, the administration of *C. procera* plant extract resulted in shorter escape latencies as they took less time to reach the hidden platform shown in Fig. (Fig. 2D). Test mice that receive plant extract at the dose of 400 mg/kg showed a significant difference in escape latencies as compared to the mice which receive plant extract at the dose of 100 mg/kg ($p<0.05$) (Fig. 2D).

In WMM, when the platform was removed in the probing trial, the animals' capacity for long-term memory was assessed by counting the number of times they entered the quadrant of the platform and how long they stayed, the number of crossings was considerably decreased in cisplatin administered animals as compared to the standard ascorbic acid group and normal saline-treated group ($p<0.05$) but was comparable to the group treated with *C. procera* plant extract as shown in Fig. 2E, however, there was significant difference shown between the group treated with plant extract at the dose of 100 mg/kg and 400 mg/kg ($p<0.05$) (Fig 2E). The length of stays was considerably shorter in the group treated with cisplatin as compared to the standard ascorbic acid control group ($p<0.05$), indicating that they had less memory of that platform as shown in (Fig 2F). The extract-treated group mainly at the dose of 400 mg/kg was comparable to the standard control group. The normal saline-treated group also spend more time on the targeted area, this indicates that the test extract significantly overcame these symptoms of memory impairment because animals given 400 mg/kg of *C. procera* made more crossings and stayed longer in the quadrant where the platform was previously located.

Effect of Methanolic extract of C. procera on the Level of Glutathione Peroxidases

Glutathione peroxidase is an endogenous defense enzyme that protects against the harmful effects of peroxides. All treated groups' animal samples had significantly different Glutathione peroxidase levels. When compared to the standard control animals, the cisplatin-treated animals had considerably lower levels of glutathione peroxidases as shown in Figure 3A. In contrast to mice treated with cisplatin, the increased level of glutathione peroxidases was seen in all *C. procera* extract-treated groups (100, 200, and 400 mg/kg) (Fig. 3A).

Effect of Methanolic extract of C. procera on the Level of Superoxide Dismutase

Superoxide dimutase levels significantly varied across animals in all groups, according to the results of this biochemical analysis. Comparing the affected animals to the control groups, the treatment of cisplatin resulted in significantly lower levels of this protective enzyme as compared to the normal saline group and standard control group as shown in fig. 3B ($p<0.05$). When compared to the animal treated with 100 mg/kg extract, the animals treated at the doses of 200 and 400 mg/kg of *C. procera*

showed enhanced levels of superoxide dismutase, demonstrating a considerable reversal of this degeneration ($p<0.05$) (fig. 3B).

Effect of methanolic extract of C. procera on the level of catalases

Comparing the Cisplatin group to the standard control group, a substantial decrease in the Catalase level was found in the cisplatin-treated group. In contrast to the Cisplatin group, plant extract treatment significantly raised CAT levels in extract treated group in a dose-dependent manner as shown in (fig 3C), but the level of Catalase in a group administered with the of 400 mg/kg of plant extract was high as compared to the group administered with 100 mg/kg of plant extract ($p<0.05$).

Effect of Methanolic extract of C. procera on the Level of Lipid Peroxidases

Thiobarbituric acid reaction substances (TBARS) analysis was used to quantify the MDA concentrations. MDA concentrations over threshold in brain homogenates are associated with enhanced oxidative stress in neuronal tissues. According to our findings, animals from all groups experienced significant MDA level fluctuations as shown in fig. 3D ($p< 0.05$). This shows that administering *C. procera* concurrently with cisplatin can reduce the effect of cisplatin on hippocampus MDA levels (fig. 3D).

DISCUSSION

In the current study, the antioxidant activity of *C. procera* was investigated by examining its impact on cognition and an oxidative stress marker in a cisplatin induced neurodegenerative model. Using the Y-maze, which is used to assess drugs used in learning and memory. The Morris water maze was employed to evaluate hippocampal-dependent spatial learning ability. In addition, oxidative stress enzymes assay from mice brain homogenate were examined. Results showed that increasing the amount of antioxidant enzyme improved cognition. The MWM test and Y maze test results of the current study revealed that mice treated with *C. procera* extract shown improved spatial memory and cognition, while mice treated with cisplatin displayed impaired spatial and cognitive memory.

The Y maze test measures sustained cognition, and the capacity of mice to explore less frequently visited arms, known as increasing alternation, was raised in all *C. procera* extract-treated mice but decreased in cisplatin-treated mice. The mice treated with cisplatin showed increased escape latency in MWM, whereas the animals treated with *C. procera* extract showed decreased escape latency in MWM. Results of oxidative stress assays shows that the level of antioxidant enzymes were raised in all *C. procera* extract-treated mice but decrease in cisplatin treated mice.

The exact chemical process by which cisplatin causes neurotoxicity is unknown, but numerous toxic substances of all kinds are released and cumulatively, change cognitive decline. Additionally, these cognitive deficits in animal models are consistent with evidence from humans, highlighting the possibility that animal research could serve as a mimic to shed some light on the pathophysiology of chemotherapy (Seigers &Fardell 2011). Chemotherapeutic agents, particularly cisplatin, frequently have neurodegenerative side effects. It's critical to understand the mechanisms underlying cisplatin neurotoxicity to develop an efficient supplementary therapy. Although various explanations have been put forth, oxidative damage appears to be the fundamental mechanism behind cisplatin neurotoxicity.

Pederson *et al*. (2006) show that chemotherapeutics, particularly cisplatin, has an impact on patients' psychological health both during and months after treatment, and that this psychological discomfort may in turn compromise cognitive function (Santos *et al*. 2020). In a research by Schagen *et al*. (2008), testicular cancer patients receiving cisplatin-based chemotherapy (etoposide, cisplatin and bleomycin) were found to frequently experience cognitive problems (Santos *et al*. 2020). Our research showed that cisplatin treatment in mice had an impact on their capacity for memory and learning. In the current study, memory and learning in mice were assessed using the Y maze and the MWM tests. According to the results of the current study, the cisplatin-treated mice group had impaired spatial and cognitive memory. Y maze test results shows, sustained cognition, and the mice's capacity to switch between arms were reduced in diseased animals but increased in all extract-treated groups, and in WMM, the disease group treated with cisplatin showed a decrease in mean escape latency but the cotreatment with extract dramatically improved the impaired memory function by reducing escape latency, increasing the number of platform crossings and increasing total time spent in the target quadrant during the probing test. These outcomes aligned with those of earlier studies done by Oz *et al*, and Abdelkader *et al*, 2017 which demonstrate that mice in the y-maze and MWM tests displayed impairments with learning and memory due to treatment with cisplatin (Abdelkader *et al*. 2017, Oz *et al*. 2015). Our findings demonstrate that methanolic extract of *C. procera* enhanced cognitive function in mice with cognitive impairment brought on by cisplatin. All these parameters significantly improved after being treated with *C. procera* methanolic extract. The results of the present study are similar to the prior work done by Prosper T. Kinda *et al*, (2021) which shows that an extract from the root bark of *C. procera* protects against the behavioral and oxidative disturbances induced by scopolamine in mice (KINDA *et al*. 2021).

Jaggi AS *et al*, study shows that Cisplatin had a potent neurotoxic effect on the mice's brains, as seen by the altered oxidative/antioxidative status (Jaggi &Singh 2012). Syad *et al*, study has shown that oxidative stress is one of the early steps in the pathophysiology of memory impairment, and also other reports suggested that it plays a crucial role in some illnesses, including dementia (Syad *et al*. 2013). To resist oxidative stress, cells require both enzymatic and nonenzymatic antioxidants. Mueen Ahmed *et al*, 2003, 2004 study demonstrated that the latex of *C. procera* has antioxidant and free radical scavenging properties comparable to those of vitamin C, a common antioxidant (Kk *et al*. 2003). The study by Teugwa *et al*, found that the hydro ethanol and methanol extracts of S. oleraceus whole plant demonstrated much lower levels of oxidative stress indicators, higher levels of polyphenols, and free radical-scavenging action (Teugwa *et al*. 2013). Also some reports shows that *C. procera* strengthens the cellular antioxidant defense mechanism by restoring GSH, SOD and Catalase synthesis (Samuni *et al*. 2013).

CONCLUSION

Considering all the above-mentioned factors discussed, it can be concluded from the study that *C. procera* (methanolic extract), significantly reduces cisplatin induced oxidative stress by restoring the level of antioxidant enzymes (SOD, Catalases, Glutathione peroxidases) and reduced MDA level significantly. At a dose of 400mg/kg, the therapeutic benefits are significant. Therefore, the methanolic extract of *C. procera* could be considered a source for potential future naturally occurring antioxidant chemicals in the treatment of cisplatin induce neurodegeneration.

Limitations of the Study

As the limitation of the present study, we can mention the Lack of analysis of AchE, TNF-, IL-1, and IL-6 gene expression using PCR and protein levels using Western blot.

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Consent to Participate

The study does not involve humans, so there are no human subjects requiring consent.

Consent to Publish

All authors have read and agreed for the content to be published in the journal 'Pakistan Journal of Pharmaceutical Sciences'.

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