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Original Research Article

Moringa oleifera leaf extract ameliorates cisplatin evoked neurotoxicity in male wistar rats

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ABSTRACT

The study was intended to explore the potential role of Moringa oleifera leaf extract (MOLE) on cisplatin (CIS) induced neuronal damage in experimental male rats. In the current work, animals were divided into four groups, six animals in each group. Group-1 (Normal) administered with Distilled water 1ml p.o. Group-2 (CIS) was received cisplatin 5mg/kg, ip. single dose. Group-3 (CIS + MOLE 200mg/kg) was orally administrated MOLE 200mg/kg, dissolved in water for 14 days with a cisplatin 5 mg/kg, ip. single dose on 10^{th} day and Group-4 (CIS + MOLE 400mg/kg) was orally administrated MOLE 400mg/kg, dissolved in water for 14 days with a cisplatin 5mg/kg, ip. single dose on 10^{th} day. After 14 days of MOLE dosing, the rats were sacrificed after anesthetizing with ketamine. Brain of individual rat was excised and processed for evaluation of oxidative injury markers like malonyl dialdehyde (MDA), glutathione (GSH), superoxide dismutase (SOD), catalase and histopathological study. MOLE treated experimental groups explored a potential ($P<0.001$) protection against CIS induced neurotoxicity by elevating the GSH, SOD, catalase and suppressing MDA in the brain as well as amelioration of histopathological changes induced by CIS at different doses. Thus, investigational finding revealed that MOLE possess potential benefits against neurotoxicity induced by anti-cancer drug cisplatin by regulating oxidative stress markers and ameliorating neuronal death and alteration of microanatomy of rat brain.

1. Introduction

Cancer therapy has unavoidable and severe deleterious effects; there efforts to overcome such adverse reactions should be practiced to encourage cancer treatment with lesser adverse effects.[1] Cisplatin (CIS) is salutary antineoplastic drug preferred for various cancers therapy such as head and neck, esophageal, cervical, testicular, ovarian, bladder, and small cell lung cancer. CIS approach is hampered by major toxic effects like neurotoxicity, nephrotoxicity, ototoxicity and vomiting. Around 30% of patients, who received CIS treatment experienced neurotoxicity because CIS can cross the blood brain barrier and accumulate on repeated doses.[2]

Synthesis of reactive oxygen free radicals and nitrogenous compounds by oxidative stress injury and depletion of antioxidant defense mechanism are key factors in CIS induced toxicities.[2] Several researches evidenced that oxidative stress injury has a pivot role in brain and neuronal manifestations such as Alzheimer's disease, Parkinson's disease, mood disorders and neuronal oxidative stress. Many studies revealed that antioxidant treatment could enhance neuroprotection. To combat the deleterious effects evoked by CIS without affecting its anticancer activity lots of screening models were carried out in combination of CIS along with various free radical scavengers, enzyme inhibitors, thiol (SH^-) containing

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antioxidants and natural plants and their constituents with antioxidant property.[3–6]

Moringa oleifera belongs to family Moringaceae, is very rich in healthy antioxidants and bioactive compounds, high ascorbic acid levels, polyphenols, vitamins, phenolic acids, isothiocyanates, tannins, saponins and presence of flavonoids. Moringa oleifera leaf extract (MOLE) have been evaluated for pharmacological activities and have shown antimicrobial, antioxidant, testicular toxicity, anticarcinogenic, antiatherosclerosis, antifungal activity and improves organ function by acting as a regulator of oxidative stress and damage. Moringa oleifera has low toxicity and is evidenced safe for human consumption even at high doses.[7,8]

The current study was intended to assess the potential role of Moringa oleifera leaf extract (MOLE) on cisplatin (CIS) evoked neurotoxicity in male wistar rats.

2. Materials and Methods

2.1. Animals

Albino wistar Male rats (170-220g) were procured from animal house of Shree Devi College of pharmacy, Mangalore. They were acclimatized to controlled conditions of temperature (23 ± 2^0C), 30-70% humidity and 12 hr light-dark cycles. The animals were randomly divided into experimental and control groups and housed for each in sanitized polypropylene cages containing sterile paddy husk as bedding. Animals had free accessed to standard pellets as basal diet and water ad libitum. All the studies performed were approved by Institutional Animal Ethical Committee (IAEC), Shree Devi College of pharmacy (SDCP/IAEC/2019/01/08), Mangalore, Karnataka, asper guidelines of Committee for the Purpose of Control and Supervision of Experiments in Animals (CPCSEA), Government of India.

2.2. Chemicals

Cisplatin was procured from Sigma Aldrich, Bangalore and other chemicals used were of analytical grade and purchased from standard companies. Biochemical kits were procured from Robonik India Pvt Ltd, Mumbai.

2.3. Experimental design

Moringa oleifera leaves were extracted by Soxhlet apparatus by using distilled water and methanol as solvents. After acclimatization for one week, the male rats were randomly categorized into four equal groups in separate polypropylene cages with six rats each. Group I was normal & received distilled water 1ml p.o. Group II was used as control and received single i.p. dose of CIS (5mg/kg) on 10^{th} day, after successive administration of distilled water (1 ml, orally). Group III & IV were used as test groups with low and high dose (received MOLE 200mg/kg p.o. and 400mg/kg p.o. respectively) for 14 days along with CIS (5 mg/kg, i.p) on 10^{th} day.[9,10]

After 5days of administration of CIS, animals were sacrificed by anesthetizing with ketamine. To evaluate neurotoxicity brain of the animals were excised and behavioral changes also noted, washed in cold saline, blotted and subjected for biochemical and histological studies. Samples of brain were divided into two parts; first half portion was used for evaluation of antioxidant parameters and other half portion used for histopathological investigation. The brain was homogenized using a mortar and pestle in 0.25M sucrose solution, and then centrifuged at 10,000 rpm for 15 min. The supernatants of the centrifuged tissues were preferred for evaluations of malonyl dialdehyde (MDA), reduced glutathione GSH, Catalase and super oxide dismutase (SOD) by colorimetric method.

The lipid peroxidation was assessed by evaluating MDA using the thiobarbituric acid method. The supernatant (600μL) was mixed with 200μL 1.2% thiobarbituric acid and the mixture was heated at 98°C for 30 min. After cooling to normal room temperature, the absorbance of the supernatant was measured at the wavelength 532 nm ($\in$ = 1.56×105 M^{-1} cm^{-1}). The values were expressed as micromoles MDA per gram of wet tissue (μmol/g tissue).[11]

The brain GSH content was measured by using Ellman's technique. About 3ml of supernatant was mixed with 2ml phosphate buffer (pH 7) & 5ml of distilled water. Later, 0.02ml of DTNB solution was transferred to 3ml of above mixture & absorbance was immediately noted at 412 nm. The level of reduced glutathione was determined by using absorption co-efficient, $\in = 13.6 \times 10^4$ M^1cm^{-1}.[12]

Catalase activity was estimated by the Aebi method (1974). 0.1 ml of supernatant was transferred to cuvette containing 1.9 ml of 50mM phosphate buffer (pH 77.0). Reaction was initiated by the inclusion of 1ml of freshly prepared 30mM H_2O_2. The rate of decomposition of H_2O_2 was determined spectrophotometrically from variation in absorbance at 240nm and the activity of catalase was expressed as units/mg protein.[13]

SOD activity in the brain tissue was measured by the Kakkar et al. method (1984). SOD activity in the tissues was analyzed based on the ability of the enzyme to inhibit nitroblue tetrazolium (NBT) reduction by superoxide. The results were expressed as U/mg protein.[14]

2.4. Histopathological study

The isolated portion of brain of experimental animal from the respective groups was taken and stored in 10% formal saline and subjected with paraffin wax. For histopathological feature examination, 5μm sections were stained with hematoxylin and eosin for observing under light microscope.[15]

2.5. Statistical analysis

The data obtained by the various parameters was statistically evaluated by one way Analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison tests using Graph Pad prism software. The mean values ±SEM were calculated for each parameter.

3. Results

The initial phytochemical screening of MOLE was performed and evidenced the presence of flavonoids, anthraquinone, terpenoids, cardiac glycoside, alkaloids, steroids, saponins and tannins.

3.1. Study of antioxidant biomarkers

In our study, a remarkable decline (P>0.001) in MDA level was identified in MOLE pre-received male wistar rats with both higher dose and low dose group compared to CIS treated group as marked elevation in MDA level was noted in CIS treated animals. MOLE pre-treated experimental animals of higher & lower dose groups were explored significant higher (P>0.001) in content of reduced glutathione compared to CIS treated animals where as significant lower in GSH level was observed in CIS treated rats compared to normal group. There was significant increase (P>0.001) in SOD and catalase units were observed in MOLE pre-treated rats of higher & lower dose groups compared to CIS treated group. But in contrast, compared to normal group animals a remarkable depletion (P>0.001) in SOD and Catalase were found in CIS treated rats (Table 1).

4. Histopathological Observation

A normal architecture of brain tissues was evidenced in the normal group showing nerve cells and neuroglia cells. CIS (5mg/kg) control group showed shrunken; congestion and oedema with cells of brain tissues was noted. MOLE (200mg/kg) + CIS (5mg/kg) were effective in normalizing the CIS induced damage with numerous nerve cells and less degeneration. MOLE (400mg/kg) + CIS (5mg/kg) brings the neurons in normal shape and size of nerve cells and neuroglia.

5. Discussion

In our study, we demonstrated the possible protective role of Moringa oleifera leaf extract (MOLE) on neurotoxicity induced by CIS in wistar rats.

CIS is a commonly preferred antineoplastic agent but its deleterious effects, its usage has been limited in cancer treatment at therapeutic doses. CIS administration leads to lipid peroxidation by elevating oxygen free radicals and supressing antioxidant synthesis, finally lead to an extensive tissue damage. Different mechanisms were demonstrated for the CIS neuronal damage in that imbalance with

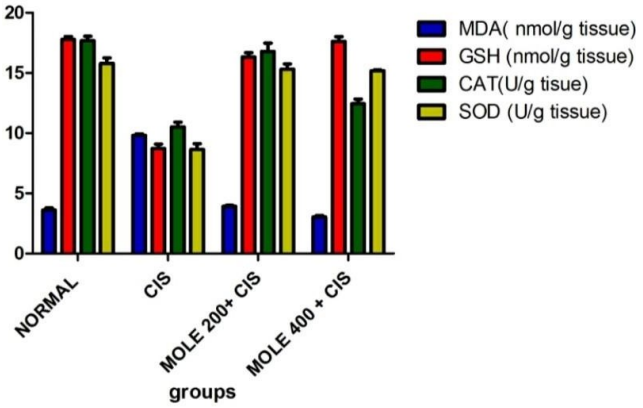

Fig. 1: Effect of MOLE on antioxidant parameters in CIS induced neurotoxicity in rats.

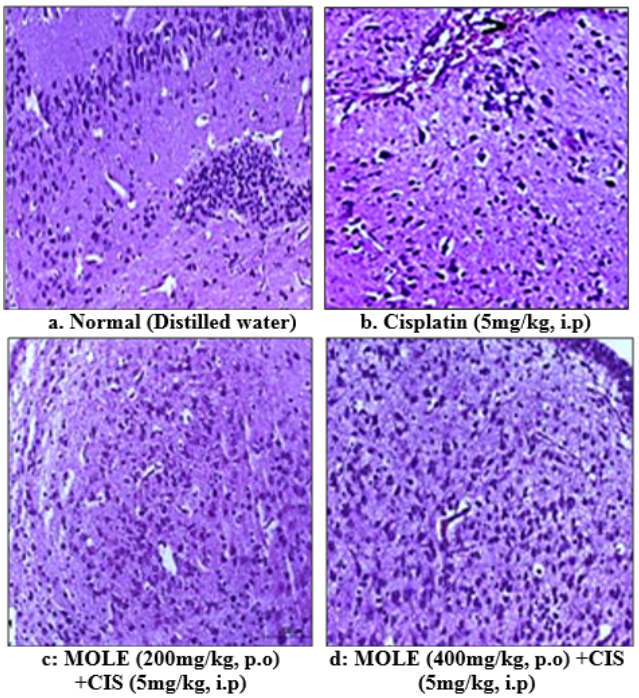

Fig. 2: Histological architecture of brain tissues in different experimental groups (H and E, X400). H and E = Hematoxylin and Eosin; **a:** Normal group (A normal architecture of brain tissues was maintained in the normal group showing nerve cells and neuroglia cells.); **b:** Cisplatin (5mg/kg) control group (showed shrunken, degeneration, congestion and oedema with cells of brain tissues); **c:** MOLE (400mg/kg) + CIS (5mg/kg) (mild CIS induced damage with numerous nerve cells and less degeneration); **d:** MOLE (400mg/kg) + CIS (5mg/kg) (No degeneration, brings the neurons in normal shape and size of nerve cells and neuroglia.

Table 1: Effect of MOLE on and antioxidants in CIS-induced neurotoxicity

Treatment	MDA (nmol/g tissue)	GSH (mmol/g tissue)	CAT (U/g tissue)	SOD (U/g tissue)
Normal	3.61±0.189	17.77±0.247	17.68±0.368	15.79±0.448
CIS	9.81±0.109$^{###}$	8.73±0.356$^{###}$	10.52±0.389$^{###}$	8.65±0.468$^{###}$
MOLE200+CIS	3.93±0.081$^{***###}$	16.33±0.342$^{***###}$	16.8±0.681$^{***###}$	15.31±4.288$^{***###}$
MOLE400+CIS	3.05±0.104$^{***###}$	17.62±0.388$^{***###}$	12.46±0.371$^{***###}$	15.19±0.067$^{***###}$

n=6, values are expressed in mean ± SEM, one-way ANOVA followed by Tukey-Kramer multiple comparison test. **P<0.01, ***P<0.001 when compared to control and ##P<0.01, ###P<0.001 compared to normal.

antioxidant defense is the prime mechanisms in CIS and other anticancer drugs toxicities. The oxidative injury modifies the cell architecture and function, and deplete the antioxidant mechanisms which lead to destruction of DNA. Combination of antineoplastic agent and another protective agent is one of the potent approaches to augment adverse effects evoked by cancer drugs. Agents from natural origin play a key role in the prevention and treatment of neurodegenerative / neurological disorders. Anti-inflammatory and antioxidant potential of isolated plants have been demonstrated beneficial effects against neuritis, anxiety and spasm.[10,16]

Many literature studies explored that the recurrence of oxidative stress, lipid peroxidation and mitochondrial dysfunction in CIS-evoked neurotoxicity. The administration of CIS causes cytotoxicity by generating ROS, lead to an elevation in MDA and fall in the activity of antioxidant defense enzymes like SOD, catalase, as well as a change in the concentrations of nonenzymatic components of GSH that prevent or protect against LPO in the brain tissues. It is accepted that both correlate to oxidative stress and can cause an imbalance in the synthesis of oxygen derived free radicals and the organism′s antioxidant potential.[17,18]

Our research work demonstrated that neuronal SOD, catalase activities as well as GSH level significantly decreased in the CIS-treated animals compared to the normal group. These observations support the hypothesis that the mechanism of neurotoxicity in CIS-treated animals is related to depletion of antioxidant defense system. Treatment with MOLE (400 mg/kg bodyweight, orally) after, before or concurrently with CIS-treatment prevents the depletion of brain antioxidants.

The decrease in Super oxide dismutase level on CIS administer might be due to the depletion of copper and zinc, which are much needed for enzyme activity. CIS has been demonstrated to induce the loss of copper and zinc in the brain. The decreased SOD activity is insufficient to scavenge the superoxide anion produced during the normal metabolic process. The superoxide anion can cause initiation and progression of LPO.[19]

The reduced glutathione content is also found to decline after CIS injection. This resulted in the decreased ability of the brain to scavenge toxic H_2O_2 and lipid peroxides. Due to CIS injection, platinum sulfhydryl group complexes are formed and taken up by neuronal cells and stabilized by intracellular GSH for several hours. During intracellular GSH depletion, the complexes undergo rapid transformation to reactive metabolites.[20] Restoration of neuronal SOD and GSH activities by MOLE reveal that the extract is capable of protecting the enzymes even 5 days before or after CIS injection. GSH depletion can markedly raise the toxicity CIS. The elevated GSH content render protection, which is evident from the MOLE plus CIS-treated rats.

Moringa oleifera leaves contains of natural sources of polyphenols and amino acids that are potential to have antioxidant activity. The leaves are recommended as a supplement because of rich nutrients such as nitrile compounds, mustard oil glycosides, benzyl glycosides, phenolic glycosides, and flavonoids.[21]

The leaf extract demonstrated potent antioxidant activity in a concentration dependent manner. The extract depicted higher percentage inhibition against DPPH, ABTS and nitric oxide radicals which were comparable with reference standard antioxidants (vitamin C and BHT). MOLE increased the antioxidant activity of GSH, SOD and catalase. Lipid peroxidation was significantly reduced by MOLE at both doses. MOLE is a potent cofactor, ameliorate the neurodegeneration in the brains of rats evoked by CIS. So, histopathological investigation revealed that inhibition of neurodegeneration in the MOLE treated rats compared with of the CIS treated rats.[16,22]

6. Conclusion

In our study, cisplatin administration results in neuronal damage through lipid peroxidation and oxidative stress noted by changes in biomarkers, lipid peroxidation (MDA), antioxidant enzymes (GSH, catalase and SOD) and neurodegeneration confirmed by histopathological studies. Prophylactic treatment of Moringa oleifera leaf extract (200 mg/kg and 400 mg/kg) explored neuroprotection on cisplatin evoked neuronal injury. The efficacy of the study illustrates that MOLE may potentiates its neuroprotective effects by modulation of LPO and enhancing antioxidant enzymes. These findings validate the hypothesis that medicinal plants rich in antioxidants are potential neuroprotective agents.

7. Conflict of Interest

None.

8. Source of Funding

None.

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