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1. MBBS, M.Phil
Lecturer Anatomy
Liaquat University of Medical & Health Sciences, Jamshoro, Pakistan.
2. MBBS, M. Phil
Assistant Professor Anatomy
Liaquat University of Medical & Health Sciences, Jamshoro, Pakistan.
3. MBBS, DO; PhD
Lecturer Anatomy
Liaquat University of Medical & Health Sciences, Jamshoro, Pakistan.
4. MBBS, (Ph. D Scholar)
Lecturer Anatomy
Liaquat University of Medical & Health Sciences, Jamshoro, Pakistan.
5. MBBS, FCPS
Assistant Professor Gynaecology & Obstetrics
Liaquat University of Medical & Health Sciences, Jamshoro, Pakistan.
6. MBBS, Ph.D
Professor Anatomy
Liaquat University of Medical & Health Sciences, Jamshoro, Pakistan.

Correspondence Address:
Dr. Muhammad Yaqoob Shahani
Affiliation Department of Anatomy
Liaquat University of Medical & Health Sciences, Jamshoro, Pakistan.
doctor_shahani@hotmail.com

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POSSIBLE PROTECTION AGAINST CISPLATIN INDUCED BEHAVIORAL CHANGES BY DIETARY ANTIOXIDANTS IN ADULT ALBINO MICE.

Sameena Gul Memon[1], Pashmina Shaikh[2], Muhammad Yaqoob Shahani[3], Umbreen Bano[4], Shazia Rani[5], Samreen Memon[6]

ABSTRACT: Cisplatin (Cis) has been proved to be successful in treating cancers but it has several toxicities. Central nervous system toxicity is considered as one of its most common toxic effects. Vitamin E and Green tea are antioxidant with proven results on prevention of life threatening diseases. **Objectives:** To assess the preventive role of two antioxidants vitamin E and green tea against cisplatin induced neurobehavioral abnormalities in albino mice. **Study Design:** Experimental Study. **Setting:** Department of Anatomy, Liaquat University of Medical and Health Sciences (LUMHS) Jamshoro, in collaboration with Sindh Agriculture University TandoJam. **Period:** Dec 2015 to March 2016. **Material & Methods:** A total of 60 mice were grouped in to Group A, (control), Group B (Cisplatin), Group C (Cisplatin plus vitamin E) and Group D (cisplatin plus green tea). The weight, gross features and behavior of the animals was monitored before and in between drug administration. Behavioral studies were performed in quiet atmosphere, and included Pain stimulation test, heat Stimulation test, cold Stimulation test, Hearing, Object Recognition test. **Results:** A significant decrease in weight and body hair of the Cis treated animals was noticed as compared to control animals. Paw edema, mental orientation, object recognition, noise stimulation, heat and cold stimulation, time to move away from the stimulus were also significantly different to control. All features were improved with the addition of vitamin E and green tea. **Conclusion:** Toxic effects of cisplatin, on morphology and behavior of adult albino mice partially abrogated with antioxidant supplementation.

Key words: Behavior, Cisplatin, Green Tea, Morphology, Vitamin E.

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INTRODUCTION

Cancer is the second imperative reason of death in the world. The emotional and physical stress imposed by different types of cancers is even more distressing.[1] All over the world the prevalence of cancer is increasing and estimated 17 million new cases projected by 2020.[2]

Cisplatin (Cis-diamine dichloroplatinum (II), CDDP) a metallic platinum crystalline compound is an anti-neoplastic therapy used in the treatment of various organ cancers of the head, neck, lung, testis, ovary and breast.[3] However its use produces toxicities such as ototoxicity, gastro toxicity, bone marrow suppression and allergic reaction.[4] The neurological toxicities are one of the main side effects that constraints its clinical use and efficiency in cancer therapy.[5] The major damages due to its toxicity are believed to be caused by alterations in the antioxidant defense mechanism with resultant oxidative stress produced by generation of reactive oxygen species, including antioxidant enzymes and non enzymatic molecules and reduced glutathione levels.

Vitamin E is a potent antioxidant which may provide defense against grave diseases like heart diseases and cancer. One study showed that supplementing Vitamin E with cisplatin treatment during and three months after treatment with Cisplatin, reduced Cisplatin induced toxicities.[6] Further more protective effects of this vitamin was observed with radiation therapy where it was able to decrease cell death and was also able to improve healing process of tissue subsequent to

completion of radiotherapy.[7] Green tea considered as antioxidant due to its composition of tea polyphenols. These polyphenols proved to have ability to decrease the initiation and progression of tumor course in various animal models. Moreover, these have shown to inhibit cellular proliferation and incited apoptosis.[8] Besides antioxidative property green tea polyphenols have prooxidative activities under certain conditions and modulate phase II metabolic enzymes that can enhance the detoxification pathway of environmental toxicants and carcinogens.[9] This study was aimed to detect possible prevention brought about by green tea and vitamin E on cisplatin induced neuro-behavioral damage.

MATERIAL & METHODS

This experimental study was conducted at the department of Anatomy, Liaquat University of Medical and Health Sciences (LUMHS) Jamshoro, in collaboration with Sindh Agriculture University TandoJam for a period of six months (December 2015 to March 2016) after approval from ethical review committee of Liaquat University of Medical & Health Science. 60 adult mice of 8 to 16 weeks' age with average weight of 220-280 gm (female and male) were included in this study. Mice less than 8 weeks' age, not feeding properly and Pregnant were excluded.

Animals were housed in stainless steel cages about 4-6 per cage in a temperature controlled room (22± 2^0C) and humidity (55%±5%), and a 12-h light/dark cycle. The cages were equipped with feed containers and plastic drinkers. Standard National guidelines for the care and use of these animals were followed. The animals were allowed free access to food and tap water. The researchers performed this study were remained blind to the treatment being administered. Pre-approved proforma was used to collect and document all data during research.

The chemicals used in this were Cisplatin, Vitamin E capsules and Green Tea. All chemicals and tea were purchased from local market.

- Cisplatin was purchased in powered form and was dissolved in distilled water.
- Vitamin E was purchased in capsule form. The capsules were dissolved in the mouse feeding.
- Green tea was cooked in drinking water and was given once cooled down.

The mice were divided in to four groups each comprised of 15 animals. They were labeled as Control group (Group A), Cisplatin treated animals (Group B), Cisplatin plus vitamin E (Group C), cisplatin plus green tea (Group D).

In group A or control group mice were given distilled water orally along with normal diet for six weeks. In group B, low dose cisplatin (1mg/kg/day) was given through intraperitoneal infusion (I.P). Mice were given the drug for five days followed by five days of rest up till the period of six weeks. In Group C, in addition to low dose of intraperitoneal (I.P) Cisplatin (1mg/kg/day), Vit E D-L-α tocopherol (30mg/ml) was given orally in the diet. In Group D, in addition to low dose of intraperitoneal (I.P) Cisplatin (1mg/kg/day), green tea (1gm/L/day) was given in drinking water. All the animals were allowed free access to food and water till the end of the experiment. The weight and other gross features and behavior of the animals was monitored and recorded before administration of the drugs and in between the doses for whole duration of six weeks.

The animals were weighed before and after administration of the drugs using an electronic weight measuring machine as shown in the diagram. The readings were noted in grams.

After measuring the weight of the animals, mice were examined for any gross abnormality. A scoring criterion was made to examine different parameters, as shown in Table-I.

In order to examine the effects of cisplatin on central nervous system and any protection brought about by antioxidants, behavioral studies were performed. All the experiments were observed by at least two observers and were conducted in quiet atmosphere.

Pain Stimulation Test was performed to examine

the pain sensations in the animals. The hind and front paws of animals of all groups were paws pricked with the help of insulin syringe needle and effects were observed. If the animals withdraw their paws immediately the pain was counted as instant otherwise not instant. All readings were taken after every five days of rest.

Heat Stimulation Test was performed to examine the thermal sensations in the animals. Animals were kept on hot gels with constant heat measured with the help of a thermometer. The time was noted for the mice to be moved away from the heat pad. The mouse was thrown on the hot pack gel and the time was assessed till the mouse moved away from the hot pack gel. All readings were taken after every five days of rest.

Cold Stimulation Test was performed to examine the cold sensations in the animals. Animals were kept on cold gels with constant temperature measured with the help of a thermometer. The time was noted for the mice to be moved away from the cold pad. The mouse was thrown on the cold pack gel and the time was assessed till the mouse moved away from the cold pack gel. All readings were taken after every five days of rest.

Hearing sensations of all animals was assessed by making noise through clapping or whistling near the ears of the animals. Animal's response to noise was assessed and noted. All readings were taken after every five days of rest.

Higher behavior was examined by assessing the confusion level of the animals. First animals were made habitual of different objects such as their feeding bowls and water bottles, in their cages and after treatment the objects were moved to different places other than the original place. They were observed to recognize the objects and results were assessed.

RESULTS

The Group B mice exposed to 1mg/kg/day over the period of six weeks in divided doses with five days of continuous exposure followed by five days of resting period. During that period, the mice exhibit gross abnormalities in morphological features. A table for morphological scoring was developed based on the certain variables (Table-I). The scoring was done according to the criteria in Table-I. Different morphological features were analyzed as Mean±SEM. Statistical analysis showed no difference in weight of animals before start of the drug dose. However gross difference in the mean weight of group B cisplatin treated mice (1.571±0.297 and control group A mouse (3±0) $p < 0.05$ was observed over the period of exposure. Although group C exhibit weight loss over the time but is not significantly different to group A $p > 0.05$ during initial phases but overall the weight was less than control (1.914±0.359). Results also show mean weight of Group B and D (1.471±0.340) are comparable and significantly different to that of Group A as shown in Table-II.

Similarly, Statistical analysis showed no difference in hair loss of animals before start of the drug dose. However gross difference in the hair loss of group B cisplatin treated mice (2±0.365) and control group A mouse (3±0) was observed over the period of exposure $p<0.05$. Group C animals were observed to have no hair loss and were comparable to control group (2.800±0.224). Animals in group D (2.333±0.333) show less hair loss as compared to group B but were not significantly different to it as shown in Figure-1.

Regarding Paw edema statistical analysis showed no difference before start of the drug dose. However gross difference in group B cisplatin treated mice (1.833±0.307) and control group A mouse (3±0) was observed over the period of exposure $p< 0.05$. Group C (2.333±0.211) and D animals (2.333±0.211) were observed to have less paw edema as compared to group B animals as shown in Table-III.

Statistical analysis showed animals in all groups were mentally active before the administration of experimental drugs. However, the level of mental orientation decreased as the time advanced in group B animals (2.000±0.365) $p<0.05$. While Group A animals were remained active throughout the time period (3±0). Although group C (2.500±0.224) and D (2.500±0.224) exhibit some confusion but not significantly different to

group A as shown in Table-IV.

Statistical analysis showed animals in all groups had instant response to pain stimuli before the administration of experimental drugs. However, the level of response decreased as the time advanced in group B animals (1.833±0.307). While Group A animals were remained active throughout the time period (3±0). However, group C (2.500±0.224) and D (2.333±0.211) exhibit relatively instant response and were not significantly different to group A as shown in Figure-2.

Statistical analysis showed animals in all groups responded to noise before the administration of experimental drugs. However, group B animals (1.833±0.401) were insensitive to noise as compared to group A (3±0) after third dose $p < 0.05$. However, administration of antioxidants in group C (2.167±0.307) and D (2.333±0.333) reversed the effects of cisplatin and responded well to noise as shown in Table-V.

Statistical analysis showed animals in all groups were able to recognize the object before the administration of experimental drugs. However, Group A (3±0) animals were able to recognize throughout duration of study while their ability to recognize deceased significantly in group B (1.833±0.307) $p< 0.05$. In Group C (2.500±0.224) and D (2.500±0.224) ability to recognize was better as compared to group B animals and were comparable to control $p> 0.05$ as indicated in Figure-3.

Morphological Features	1	2	3
hair loss	Gross hair loss (hair loss on front and back)	Moderate hair loss (hair only present on the back)	No Hair Loss
Paw edema	Edema of both front and hind paw	Edema of either hind or front paw	No edema
weight reduction	More than 50 % weight loss	Less than 50% weight loss	No weight loss
Mental orientation	Extremely confused/no response to stimulus	Less active/ late response to stimulus	Active
Respond to noise	No response to stimulus	Late response to stimulus	response to stimulus
Object recognition	No recognition	Recognize objects slowly	Recognize objects quickly

Table-I. Morphological scoring system based on gross features of the mice.

	Control	Cisplatin Only	Cisplatin + Vitamin E	Cisplatin + Green Tea
Mean	3	1.314	1.914	1.471
Std. Deviation	0	0.786	0.951	0.899
Std. Error	0	0.297	0.359	0.340

Table-II. Mean weight of animals based on the scoring criteria in table-I.

	Control	Cisplatin Only	Cisplatin + Vitamin E	Cisplatin + Green Tea
Mean	3	1.833	2.333	2.333
Std. Deviation	0	0.753	0.516	0.516
Std. Error	0	0.307	0.211	0.211

Table-III. Mean Paw edema of animals based on the scoring criteria in table.

	Control	Cisplatin Only	Cisplatin + Vitamin E	Cisplatin + Green Tea
Mean	3	2.000	2.500	2.500
Std. Deviation	0	0.894	0.548	0.548
Std. Error	0	0.365	0.224	0.224

Table-IV: Mean of mental orientation of animals based on the scoring criteria in table-I.

	Control	Cisplatin Only	Cisplatin + Vitamin E	Cisplatin + Green Tea
Mean	2.833	1.833	2.167	2.333
Std. Deviation	0.408	0.983	0.753	0.816
Std. Error	0.167	0.401	0.307	0.333

Table-V. Mean of response of noise stimulus of animals based on the scoring criteria in table-I.

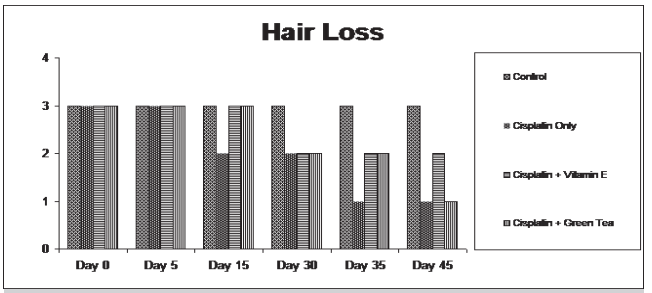

Figure-1. Mean Hair loss of animals based on the scoring criteria in Table-I

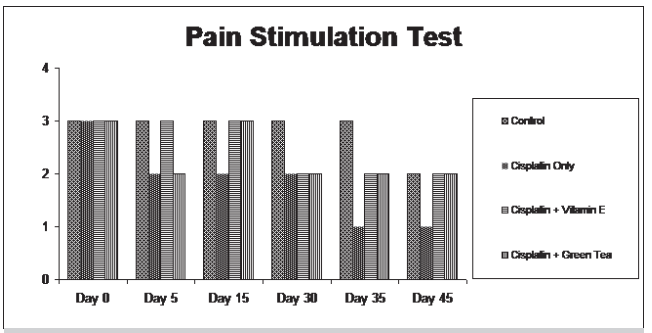

Figure-2. Mean of response to pain stimulus of animals based on the scoring criteria in table 1

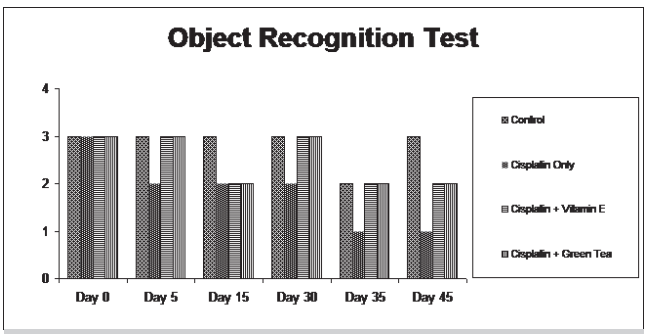

Figure-3. Mean of ability to recognize objects of animals based on the scoring criteria in table 1

DISCUSSION

Cisplatin is widely used to treat various solid tumours of the body due to its promising results. Its deleterious side effects on body including renal and neural toxicity make its use limited.[10,11] Due to this fact various approaches have been tried to prevent its side effects. This study was conducted to assess side effects of cisplatin on physical status of the body, and behavior of the mouse models. Furthermore, preventive effects of antioxidants against cisplatin induced injury were also examined and compared. The mouse has been used as laboratory animal since long time due to the fact that it is easily available, relatively cheap and easy to handle. Apart from this, the genome of mouse resembles closely with human genetic framework.[12,13]

Morphological study results showed that administration of cisplatin in mice produced significant effects. A morphological scoring system was developed to examine different gross abnormalities in different groups of animals used in this study. Control mice, showed no or minimal changes in the weight over the entire study period. In contrast, the weight of the cisplatin treated group started to decrease significantly, the other two groups also showed some weight loss but was not significant. This indicates some protection provided by antioxidants. Vitamin E showed better improvement as compared to green tea, this may be due to the fact that green tea was given in drinking water and was not compliable to mice to some extent. Previous studies conducted on different animal models on cisplatin, also elucidated weight reduction with its administration.[14,15] The significant reduction in the weight of the experimental animals may be due to less food intake and more catabolic activity of the body. Cisplatin treated animals had obvious hair loss throughout body. However, the animals treated with vitamin E showed less hair loss, which was seen on front of the body only. This indicates the ability of antioxidants to reduce hair loss if given as supplement during chemotherapy.

Apart from other side effects, Platinium based chemotherapy has life compromising neuropathic effects.[16] These effects are dose dependant and may limit the dose and discontinuation of the treatment regimens. The overall impact of this neuropathic side effect is on patient survival and quality of life. In this study cisplatin treated mice

show peripheral neuropathic effects such as increased pain threshold, heat hyperalgesia and less response to cold stimulus. Similar results were observed by Vencappa and colleagues, where mice showed cisplatin treatment results in chronic pain phenotypes in mice, with evidence of mechanical allodynia and heat hyperalgesia.[17] The main reason of this neuropathy may be due the suppression of ability of peripheral nervous system to regenerate and protects itself. Bulks of anti cancer chemotherapeutic agents provoke sensory neuropathy. Symptoms range from painful sensations to paresthesia. The onset is abrupt with start of therapy and persists throughout the remaining life even after discontinuation of drug, which has great impact on quality of life.[18]

In addition, mice with cisplatin treatment also showed mental disorientation, they were extremely confused and were not able to recognize object. These findings indicate effects of this platinium based drug on central nervous system as well. Although antioxidants were able to partially attenuate these effects complete protection was not observed. This might be the fact that other mechanisms are involved in cisplatin induced nervous system damage.

This study also reveals that the cisplatin treated mice have less ability to respond to noise as compared to control. Animals treated with antioxidants were able to respond but not comparable to control. These results are in line with other studies conducted on mice and rats, which show ototoxicity in platinium treated animals, Kelles and colleges showed ototoxicity when cisplatin was administered in mice.[19]

CONCLUSION

In this study the preventive role of antioxidants was assessed in the prevention of cisplatin induced toxicities in nervous system and behavior of adult mouse. The cisplatin induced abnormalities in gross features, peripheral and central nervous and behavior were partially protected by administration of two antioxidants vitamin E and green tea.

Due to limitation of time and funding the levels of ROS was not measured. The studies will be conducted in future to detect the ROS and also other mechanisms of action of cisplatin drug in order to reduce the morbidly associated with cisplatin therapy.

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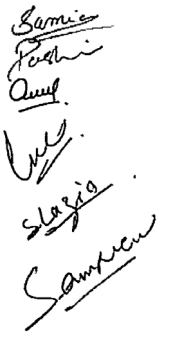
AUTHORSHIP AND CONTRIBUTION DECLARATION

Sr. #	Author(s) Full Name	Contribution to the paper	Author(s) Signature
1	Sameena Gul Memon	Principal investigator and data collection	
2	Pashmina Shaikh	Data interpretation	
3	M. Yaqoob Shahani	Corresponding Author & Writing corrections,	
4	Umbreen Bano	Study design and sample collection.	
5	Shazia Rani	Statistical analysis and data interpretation.	
6	Samreen Memon	Writing support and data interpretation.	