Hematological and Histomorphological Amelioration by Withania Somnifera of Cisplatin-induced Splenotoxicity in Rats

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Abstract

Objective: Withania Somnifera is a naturally occurring antioxidant. This study was designed to analyze the oxidative effects of the anti-cancer drug cisplatin on rat spleen and blood parameters and its amelioration by the root extract of Withania Somnifera.

Methods: This experimental study was conducted during November and December 2023 in the Anatomy departments of Islam Medical and Dental College and Jinnah Medical and Dental College, after the approval of the Institutional Animal Ethical Committee. 60 male Albino Wistar rats, 14-16 weeks old and weighing 200-250 gm were randomly divided into 4 groups. Initial body weights were taken for all groups. Group A received no treatment, group B was given an intraperitoneal injection of Cisplatin as a single daily dose for 7 days, group C received W. somnifera root extract orally for 15 days as a pre-treatment and then concurrently with intraperitoneal cisplatin for 7 days and group D was given W. somnifera root extract only for 22 days. At the end of the study period, the rats were sacrificed. Final body and splenic weights were taken, blood was collected and splenic tissue was processed for histological analysis. Statistical analysis of data was done using SPSS version 25.0.

Results: The rats of Cisplatin-only group B showed significant toxic changes in splenic histology: thickened splenic capsule, white pulp reduction with reduced PALS areas, and central artery vacuolization. These changes were improved in cisplatin and W. somnifera group C. Blood profiles showed decreased Hb, TLC, and platelets in cisplatin-only group B but it was improved in the cisplatin and W. somnifera group C.

Conclusion: Our study concluded that W. somnifera root extract protects against cisplatin-induced oxidative damage to splenic tissue and hematological alterations in rat model. This ponders on its potential as an adjuvant therapy with anticancer cisplatin treatment for minimizing its side effects.

Keywords: Cisplatin, Withania somnifera, antioxidant, splenotoxicity, hematology, histomorphology

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Introduction

Cisplatin, a platinum-coordinated compound, is a widely used chemotherapeutic agent for the treatment of nearly half of all solid tumors. Although Cisplatin is primarily known for its nephrotoxicity and neurotoxicity, however, adverse effects on hepatic and splenic tissues and cardiovascular and pulmonary systems have also been identified[1].

Cisplatin is thought to kill cancer cells by attaching to DNA and disrupting its repair process during the synthesis of DNA or after an insult. This leads to triggering apoptotic pathways ultimately causing the cells to die. Similar to other anti-cancer agents, Cisplatin also generates oxidative stress which is an important contributor to the pathophysiology of inflammation and drug toxicity [2]. The core cause of toxicity induced by Cisplatin lies in its poor targeting of cancer cells exclusively, leading to the bio-distribution of this chemotherapeutic agent

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to other organs in the body[3]. It has been documented that acute renal injury is initiated in 20-30% of patients receiving the recommended treatment dose of cisplatin[4].

In addition to nephrotoxicity, cisplatin has serious effects on the immune system of the body[5]. The spleen's response to the adverse effects of cisplatin has not been thoroughly understood so far. Blood parameters like red blood cell (RBC) count, Total leucocyte count (TLC) and hemoglobin (Hb) concentration values outside the standard ranges are indicative of stress, illness, or toxicity[6]. Therefore, our goal was to investigate the mechanism of pathophysiology of Cisplatin-induced Splenotoxicity.

Withania somnifera (W. somnifera), also known as Ashwagandha, winter cherry, and Indian ginseng, is a drought-resistant plant that thrives in various regions, including Africa, Sri Lanka, Pakistan, and India. W. somnifera is known not only for its role in enhancing the immune system but also for its various other health benefits. Studies suggest that it has antioxidative, anti-inflammatory, bactericidal, antiarthritic, hepatoprotective, cardioprotective, neuroprotective, nephroprotective, and antidiuretic properties[7]. Recent research has also proven the use of W. somnifera in combination with another chemotherapeutic agent to reduce hematotoxicity as well as to enhance tumoricidal efficacy[8].

While all parts of the plant, including the seeds, fruits, leaves, and shoots have been traditionally used for remedial purposes, the roots and leaves are particularly recognized for their medicinal properties [9]. Although there are 35 other different chemical compounds found in the plant, Withaferin A and Withanolides, constituents of the roots, serve as the key components responsible for its biological activity[10]. Studies show that the antioxidant capabilities of W. somnifera enable it to neutralize free radicals and reactive oxygen species (ROS), thus preventing many free radical-related disorders[11]. No significant adverse effects or alterations in biochemical parameters were reported with the use of W. somnifera. Only mild and mostly temporary adverse events are documented as less frequent side effects[12]. Hence, the rationale of our study is to contribute to the treatment of cancer by prescribing a harmless and potently protective natural antioxidant as a concurrent therapy to combat the possible harmful effects of cisplatin.

In light of the above rationale, this study was designed to assess the oxidative damage of cisplatin on the splenic parenchyma and blood parameters and to observe the alleviating effects of a natural antioxidant, W. somnifera root extract on the above parameters.

Methodology

This experimental study was conducted between November and December 2023 in the Department of Anatomy, Islam Medical and Dental College, Sialkot and Jinnah Medical and Dental College, Karachi. The research was carried out after obtaining Institutional Animal Ethical Committee approval. The minimum number of animals in each group for research studies that require sacrifice should be six. Hence, a sample size of sixty was selected, with 15 animals in each group. These adult Albino Wistar rats (aged 14-16 weeks and weighing 200-250gm) were housed at the Animal House of Islam Medical and Dental College. Only male animals were selected for this study and female rats were excluded as female hormonal cycles may influence the results of the experiment. Animals were provided with prior 2-week adaptation period, following standard laboratory conditions, before the commencement of the experiment.

Cisplatin injections (Inj. Cisplasul 50mg/50ml) were obtained from a local pharmacy. The Ethanol extract of W. somnifera roots, used in this study was prepared by the Department of Pharmacognosy at the University of Karachi. Botanical identification was conducted, and a voucher number (WSR -01-18/18) was generated. The dried roots of W. somnifera purchased from a local market, were provided for this purpose.

Sixty Albino rats were divided into four equal groups by randomization. This was achieved by allotting numbers to the rats. The numbers were drawn by the lottery system separately for four differe-

ent groups and these numbered rats were put in different cages. Group A served as control group without any intervention. Group B was the treated-group and received Inj. Cisplatin 1mg/kg intraperitoneally for 7 days[13]. Group C was the protect- ed-group and was given W. somnifera root extract 500mg/kg orally through gastric gavage for 15 days as a pretreatment and then concurrently with Inj. Cisplatin 1mg/kg intraperitoneally for 7 days, so the treatment of group C lasted for a total of 22 days[14]. Group D was a positive-control group, which received only root extract of W. somnifera 500mg/kg orally for 22 days.

The initial and final body weights of the animals were recorded using a digital scale, at the beginning and at the end of the experiment. Group B was sacrificed on the 8th day, and groups A, C, and D were sacrificed on the 23rd day of study. The animals were dissected, and the spleen was removed, weighed, and harvested for tissue preparation and then stained with Hematoxylin and Eosin. Stained sections of spleen were observed under a light microscope at 100X and 400X final magnification to examine morphological alterations. Blood samples were collected through cardiac puncture and preserved in an EDTA tube for Complete Blood Count (CBC) estimation performed through an automated hematology analyzer.

Statistical data analysis was done on (SPSS) version 25.0. The mean of body weight and organ (spleen) weight was calculated and expressed as Mean and standard deviation (Mean± SD). Hematological parameters including Hb (g/dl), TLC ¶ (103/µL), Neutrophil count (%), Lymphocyte count (%), Monocyte count (%), Eosinophil count (%), and Platelet count (10^2) were also calculated as Mean± SD.

As there were more than 2 groups and data were normally distributed, One-way Analysis of Variance (ANOVA) followed by post hoc Tukey's test was performed to compare means of different parameters among various groups at a 95% confidence interval with p-value of less than 0.05 considered significant ($p<0.05$).

Results

Table 1 shows the mean ± SD initial, final body, and spleen weights of Group A, B, C & D. Comparison of means between the groups displayed a significant ($p<0.05$) decline in final body weight of Cisplatin-treated group B when compared with group C and D. However, there was a significant ($p<0.05$) increase in body weight of W. somnifera protected-group C animals in comparison to treated-group B (Table 2).

The complete blood picture showed decreased hemoglobin, Total leukocyte count (TLC), and platelets in treated group B but these increased to near normal, in protected group C (Table 1 & 2). The Differential Leucocyte Count (DLC) including Neutrophil, Lymphocyte, Monocyte, and Eosinophil count, results varied in different groups (Table 1).

Histomorphological examination of H & E-stained sections from group A control and group D positive-control spleens showed normal histological structure with a fibrous capsule (Fig 1a &1d). Parenchyma consisted of a white pulp and a red pulp. The white pulp is comprised of a central artery surrounded by a periarteriolar lymphatic sheath (PALS), comprised of lymphatic tissue, and also forming lymphoid follicles. These lymphoid nodules were distinctly separated from the red pulp with a well-visible marginal zone, which is less basophilic than the PALS. The red pulp showed blood sinusoids. (Fig 1a & 1d). However, sections of cisplatin-treated group B showed distinct alteration of splenic architecture including disorganization of a lymphoid follicle in the white pulp, with decreased PALS around the central artery, along with dilated and congested sinusoids in the red pulp (Fig 1b). Furthermore, the thickening of both the reticular fiber of the capsule and trabeculae was noted. Like other cytotoxic drugs, cisplatin also caused vacuolization of the central artery (Fig 2).

The splenic sections of protected group C, which received W. Somnifera as pretreatment with Cisplatin treatment, demonstrated preservation of splenic architecture by amelioration of the inflammatory response to its normal state. (Figure 1c)

There was significant improvement in the distortion of lymphoid follicles in the white pulp with increased PALS around the central artery, alleviation of sinusoidal congestion in the red pulp, and reduction in central artery vacuolization. Additionally, marginal zone enlargements were also observed in this protected group as well as positive control group D. (Fig 1c & 1d).

Table 1. Descriptive Statistics Of Mean Body And Spleen Weights And Hematological Parameters Of Groups A, B, C & D

Parameters	Group A (n=15) Mean ± SD	Group B (n=15) Mean ± SD	Group C (n=15) Mean ± SD	Group D (n=15) Mean ± SD
Initial body wt. (gm)	217.95 ± 21.090	218.95 ± 22.988	215.50 ± 24.04	207.10 ± 15.134
Final body wt. (gm)	234.80 ± 21.683	188.55 ± 25.276	204.50 ± 24.85	221.80 ± 13.942
Spleen wt. (gm)	0.859 ± 0.100	0.689 ± 0.104	0.736 ± 0.082	0.811 ± 0.046
Hb (g/dl)	13.63 ± 1.29	13.09 ± 1.13	13.26 ± 1.34	13.91 ± 0.91
TLC¶ (103/µL)	8.22 ± 2.53	6.11 ± 1.32	10.34 ± 3.88	9.45 ± 2.54
Neutrophil count (%)	37.80 ± 7.86	45.93 ± 11.09	31.00 ± 14.14	34.47 ± 13.16
Lymphocyte count (%)	52.33 ± 8.95	43.27 ± 7.55	62.73 ± 13.90	55.53 ± 11.08
Monocyte count (%)	6.73 ± 3.67	9.60 ± 7.07	4.00 ± 3.95	7.80 ± 3.59
Eosinophil count (%)	3.13 ± 1.36	1.07 ± 1.10	2.07 ± 1.98	2.13 ± 1.46
Platelet count (102)	1084.08 ± 206.22	677.87 ± 179.82	1003.0 ± 195.50	1051.27 ± 70.52

Table 2. Statistical Analysis of Mean Final Body Weight, Spleen Weight, Hb, TLC And Platelet Count Between Different Groups

Comparison groups	Mean final body weight(gm)	Spleen weight (gm)	Hb (g/dl)	TLC count (10^3/µL)	Platelet count (10^2)
A & B	46.250.000*	0.1700.000*	0.540.596	2.110.157	406.930.000*
A & C	30.30.061	0.1230.001*	0.370.830	-2.120.155	81.800.564
A & D	130.080	0.0480.002*	-0.280.915	-1.230.608	33.530.950
B & C	-15.950.000*	-0.0470.000*	-0.170.978	-4.230.000*	-325.130.000*
B & D	-33.250.000*	-0.1220.023*	-0.820.239	-3.340.007	-373.400.000*
C & D	-17.30.067	-0.7500.001*	-0.650.444	0.890.805	-48.280.868

*Statistically significant

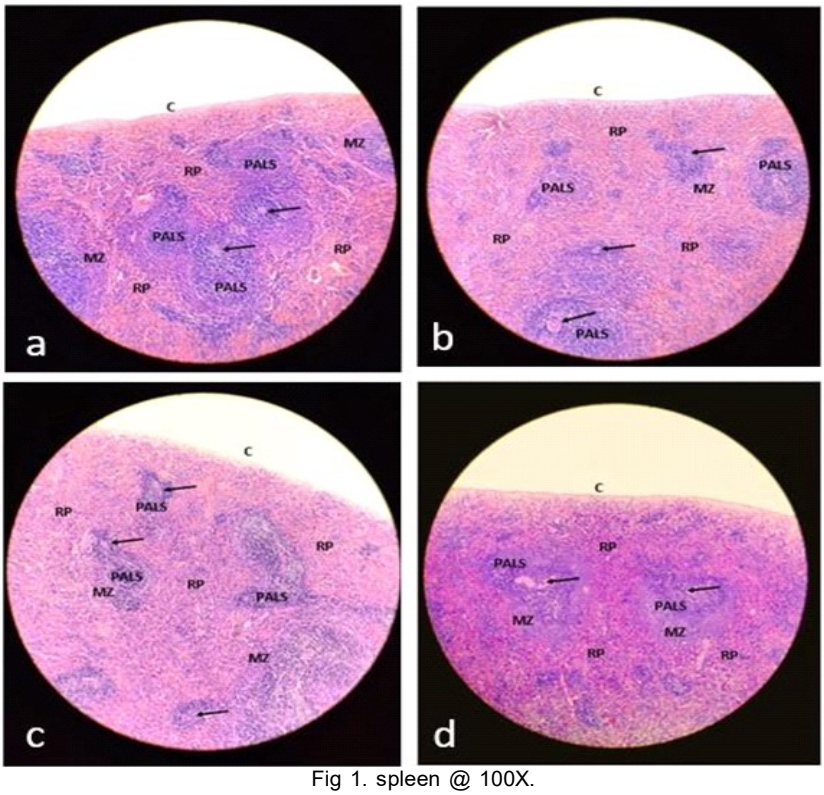

Fig 1. spleen @ 100X.

a: Control Group A showing normal architecture, lined by a fibrous capsule (C). Parenchyma consists of red pulp (RP) and white pulp in the form of PALS around the central artery (arrow), surrounded by a lightly stained Marginal zone (MZ).

b: Treated Group B with thickened fibrous capsule (C). Parenchyma consists of mildly congested red pulp (RP) and reduced white pulp in the form of decreased PALS around the central artery (arrow). The marginal zone (MZ) is very difficult to visualize.

c: Protected Group C showing less thickened capsule (C). Parenchyma showing normal red pulp (RP) and increasing white pulp in the form of PALS around the central artery (arrow). Marginal zone (MZ) is again appreciated.

d: Positive control Group D showing normal architecture with capsule (C), red pulp (RP), and white pulp in the form of PALS around the central artery (arrow). The marginal zone (MZ) is more than group A.

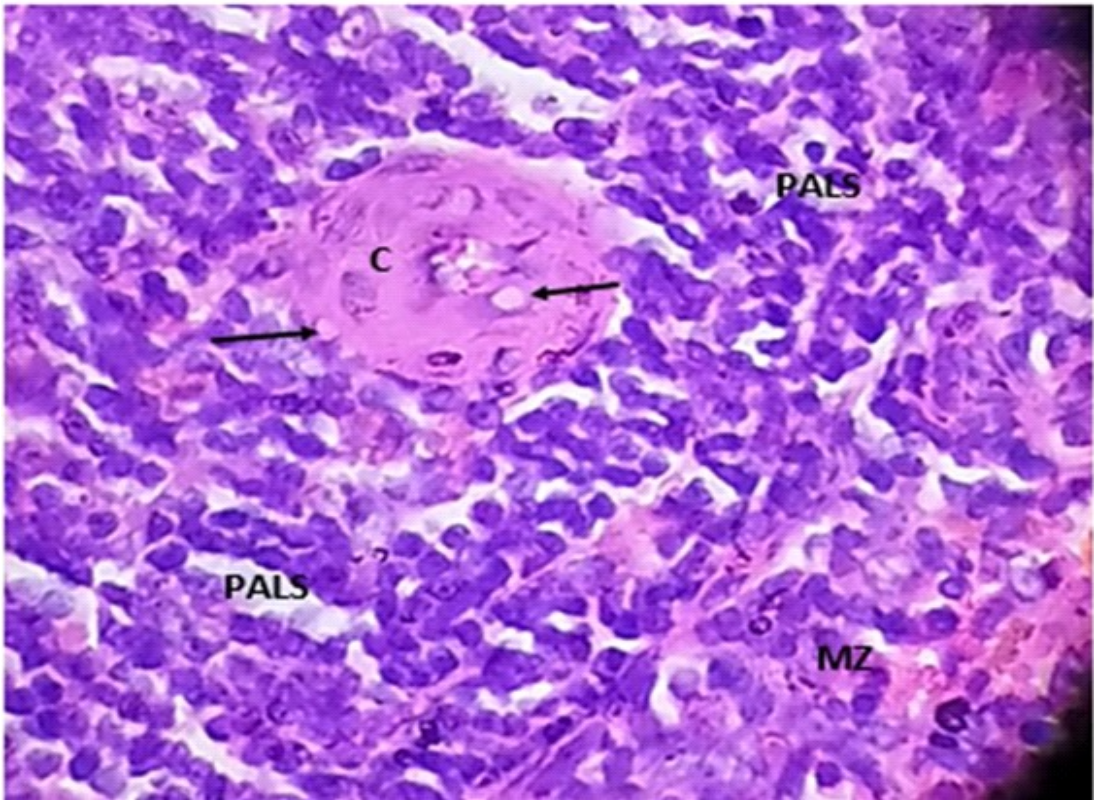

Fig 2. Spleen @ 400X: Treated Group B Central artery (C) surrounded by PALS showing vacuoles (arrow). A lightly stained Marginal zone (MZ) is also seen.

Discussion

This experimental study was designed to analyze the hematological and morphological alterations of cisplatin-induced splenotoxicity and to assess the ameliorative effect of W. somnifera root extract using complete blood picture and histopathological slides respectively.

Cisplatin toxicity is seen as a result of increased production of the reactive oxygen species (ROS) leading to oxidative stress, ultimately. Cisplatin interacts with glutathione, a cellular antioxidant, depleting its level and causing further increase of endogenous ROS and oxidative stress in the cell [15]. The root extract of W. somnifera was employed in this study. The constituent compounds, Withaferin A and Withanolides exhibit antioxidant and immunomodulatory properties, which play a preventive role against oxidative stress induced by free radicals[16].

The results of this study demonstrated a decrease in the final body weight within group B animals after receiving Cisplatin which aligns with the findings of Dalia et al, stating that the resulting weight loss could be from reduced food and water intake owing to cisplatin-induced loss of appetite and gastrointestinal toxicity[17]. There was a significant improvement in the body weight of Group C animals when W. somnifera root extract was given with cisplatin, which is in agreement with the observation of Salem et al[18]. They believed that W. somnifera possesses anabolic effects, stimulating the production of endogenous enzymes, enhancing nutrient digestion, and promoting liver biosynthesis, ultimately leading to an increase in body weight. In our study, mean splenic weight was also reduced in treated-group B but showed improved values in W. somnifera protected-group C and positive-control group D (Table 2). Comparable changes were seen by Okafor et al, who stated decrease in organ weight is a sign of organ toxicity[19]. The values of splenic weight in protected group C of our study improved, which is in agreement with the result of Azimi et al, who stated that an increase in splenic weight could be linked to enhanced proliferative capabilities of spleen cells [20]. He proclaimed that antioxidant phytochemicals in W. Somnifera provide protection against free radicals thus preventing harmful effects on spleen cell proliferation and differentiation.

These blood analytic tests serve as crucial indicators when assessing chemical exposure; even subtle alterations in these parameters can indicate toxicity[6]. The overall reduction of hematological parameters in cisplatin-treated group B when compared to the control group is in accordance with the results of Noviyani et al. They believed that a decline in blood parameters might be due to cisplatin-induced bone marrow suppression by directly inhibiting hematopoiesis[21]. Similar changes in blood parameters were reported by Abd Al-Rauof due to bone marrow cellular damage by cisplatin via increased reactive oxygen species production and release of inflammatory mediators[22]. Differential Leucocyte Count (DLC) results were varied in different groups of our research. However, W. Somnifera pre-treated, and cisplatin group C depicted significant improvement in all blood parameters of our experiment. These findings were in agreement with the previous study reported by Namdev et al, who mentioned that Ashwagandha, (Withania somnifera), improved blood parameters due to its antioxidant and immunostimulant effects[16].

Histopathological analysis showed the toxic effects of cisplatin on splenic tissue in this study. Similar histopathological changes were observed by Alsemeh et al. He reported that Cisplatin-induced oxidative stress works by the accumulation of free oxygen radicals, triggering mitochondrial dysfunction, protein degradation, and DNA damage, which ultimately led to cellular apoptosis and organ injury. He added that cisplatin led to impairment in the antioxidant defense enzymes catalase (CAT) and glutathione (GSH)[23]. There is a dose-dependent increase in ROS and nitric oxide (NO) leading to oxidative stress in the spleen with exposure to cisplatin[3]. Additionally, the deposition of hemosiderin, a hallmark of spleen damage was noticeable. Banerjee et al. reported that damaged erythrocytes due to cisplatin-toxicity accumulated in the red pulp of the spleen and resulted in iron overload leading to the deposition of hemosiderin in the spleen[3]. The thickening of the stroma was similar to the observations of our previous study where cyclosporin-induced toxic effects on the spleen included splenic capsule thickening secondary to the deposition of collagen fibers within the stroma[24]. There is also a reduction in white pulp and disruption of the well-defined marginal zone between white pulp and red pulp which is indicative of the toxic effect of cisplatin. These findings can be attributed to not only oxidative stress but the inflammation, which also plays a pivotal role in the pathophysiological effects of cisplatin on a rat's spleen. There has been a documented dose and time-dependent relation between the increase of proinflammatory cytokines such as TNF and IL-â after cisplatin administration in splenic toxicity[3]. Vacuolization of the central artery was also observed, which was the same caused by the toxic effects of other drugs like cyclosporin. This could be due to the osmotic influx within the intimal cells[24].

Similar restoration of splenic architecture was reported by Namdev et al and Azab et al which revealed that W. Somnifera might be considered as a potential source of antioxidants thus having splenoprotective and immunomodulatory effects[16,25]. Azab et al said that the antioxidant properties of W. somnifera could be attributed to its ability to neutralize free radicals and ROS, as well as to obstruct pathways that led to increased ROS production[25]. Namdev et al in their animal model demonstrated that the W. somnifera is capable of a significant reduction in oxidative markers like NO, Hydrogen peroxide, Lipid peroxidase, and Catalase while it increases antioxidative markers like Glutathione S-transferase, Glutathione reductase and Superoxide dismutase, which provide cellular defense against ROS[16].

The limitation of our study is that due to the toxic effect of cisplatin, the drug could not be administered for a longer duration in the animals to observe further protective effect of W. Somnifera. This is a preclinical study and a clinical trial should be conducted to assess the protective effects of W. somnifera on hematological parameters.

Conclusion

Our study concluded that cisplatin caused oxidative damage to splenic tissue and haematological alterations, which can be ameliorated by the root extract of a naturally-occurring agent W. somnifera in rat model. This study highlights the significance of W. Somnifera's anti-oxidative potential against the anti-neoplastic drug, cisplatin.

Conflict Of Interest: None

Disclaimer: None

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