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Impact of Resveratrol in Attenuating Cisplatin Induced Testicular Toxicity in Male adult Rats

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ABSTRACT

Background: Resveratrol is a poly-hydroxy phenol plant toxin that reduces oxidative stress and prevents tissue damage by increasing endogenous antioxidant levels. The study aimed to investigate the protective effects of Resveratrol by histochemical, ultrastructural and biochemical methods in testicular toxicity induced by the Cisplatin in Wistar Albino rats.

Methodology: The quasi-experimental study was carried out at the department of Pharmacology, Anatomy and Postgraduate Laboratory of ISRA University Hyderabad from October 2020 to March 2021. Twenty-four male, healthy Wistar Albino rats of age from 8-10 weeks and having body weight 250-300 grams, were included in the study. Rats were divided into three groups; each group have same number (n=08) of rats. Group-A (Control), Group-B (Experimental group or Cisplatin group), Group-C (Experimental group or Cisplatin + Resveratrol combination group). Pre- and post-experimental body weight of all animals was measured, blood samples were collected for the biochemical analysis for the oxidative markers, semen parameters, and histo-morphology. Data was analyzed using SPSS version 24.0.

Results: Statistically significant decline in the bodyweight and testicular weight in group B and C respectively ($p<0.05$). While Group-B had lower sperm count, motility, and viability when compared to Group-C ($p<0.05$). Group-B also had significantly lower levels of oxidative markers than Group-C ($p<0.05$). Group- B's testicular histology significantly differed from Group-C's ($p<0.05$). Seminiferous tubules in Group-B were irregular, regressive, and atrophic.

Conclusion: Resveratrol is a powerful antioxidant shows potential in reducing cisplatin-induced oxidative stress and, eventually, testicular toxicity in mice models.

Keywords: Cisplatin, Oxidative Stress, Resveratrol, toxicity

Authors' Contribution:
[1]Conception; Literature research; manuscript design and drafting; [2,] Critical analysis and manuscript review; [3] Data analysis; [4]Manuscript Editing.[5,6]

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Introduction

Cisplatin is one of the most commonly used agents for chemotherapy owing to its action on a wide array of cancers.[(1)] The use of cisplatin, however, is limited due to an extensive list of side effects including the likes of nephrotoxicity, neurotoxicity, testicular toxicity, etc.[(2, 3)] The deleterious effects of cisplatin have been highlighted in numerous studies.[(4-6)]

Reactive oxygen species (ROS) induced oxidative stress has been deemed to be the underlying culprit in the production of testicular toxicity by cisplatin. [(5, 6)]

These ROS, produced in the testicular tissue, disrupts the normal function of Leydig cells as well as causes disruption in steroidogenesis, leading eventually to infertility.[7] Based on this, various anti-oxidants including extract of cinnamon, ginger, and Ginkgo Biolba have been designated to provide protection against testicular toxicity prompted by cisplatin administration.[4-6]

Resveratrol, (trans-3,4',5-trihydroxystilbene) (RSV), is found in over 70 species of edible plants and different foods like cranberries, grapes (skin and seeds), peanuts, dark chocolates, pistachios, etc.[7] It has a potential to fight against pathogens, anticancer properties and possess a potent antioxidant potential.

The positive effects of RSV usage for the treatment of numerous disease states has been documented in different studies.[8] The antioxidant effects of RSV are exerted by its ability to reduce lipid peroxidation and its ability to increase the levels of different endogenous antioxidants. [9] The objective of the study was to investigate the protective effects of Resveratrol in testicular toxicity induced by Cisplatin in Wistar Albino rats.

Methodology

Quasi-Experimental study was performed at the Department of Physiology and Postgraduate Laboratory of ISRA University Hyderabad from October 2020 to March 2021. Twenty-four male, healthy Wistar Albino rats of age from 8-10 weeks and having body weight 250-300 grams, were included in the study. The study animals were procured from the Sindh Agriculture University, Tando Jam, Sindh. Selection of rats was done by non-random purposive sampling technique while the standard method of power analysis for animal studies was used for the sample size calculation.[10]

The ethical approval for the study was sought from the Ethical Review Board of Isra University, Hyderabad, which is completely furnished to give permission for animal studies. The procured Wistar rats were placed in plastic cages at well-equipped and hygienic setting of postgraduate laboratory in Isra University, Hyderabad. The animals were kept in a for ten days acclimatization period at the optimal temperature of 24-26°C in a day-night (12/12) hours cycle. Each cage was having nozzles of stainless steel bedded with sawdust along with feed containers to avoid any harm to the study animals. While rats were provided with chow diet and clean water ad libitum during this period.

After the period of acclimatization, rats were divided into three groups, each group have same number (n=08) of rats. Group 1 was the control group, in which rats were given a normal chow diet and clean water ad libitum only, Group 2 was the experimental group, in which rats were given (single dose of cisplatin (7mg/kg) through intra-peritoneal route with normal diet). [11], and Group 3 (single dose of cisplatin (7mg/kg) through intra-peritoneal route followed by (10 mg/kg) of oral Resveratrol for 21 days. [8, 11] Bodyweight of all rats was measured and recorded before initiation of the experiment. While on conclusion of the period of experiment, the body weight of all animals was measured again using electronic precision measuring balance.

Later, all rats were sacrificed by cervical dislocation after given anesthesia. Blood samples were collected by cardiac puncture for the biochemical analysis. Testes of all experimental animals were cleanly removed and weighed after proper dissection through midline incision.

For estimating the structural framework and motility, the epididymal content was suspended by extracting the cauda epididymis of one testis, which was afterward macerated with scissors in a Petri dish containing normal saline (3ml). Likewise, the sperm-count was achieved by removing the cauda epididymis of the other testis, which was then macerated by scissors in a Petri dish containing 0.9ml of 10% formalin.

The testicular tissue was fixed in the Bouin's solution and then sliced into longitudinal sections. The tissues were then circulated through water after being properly arranged in labelled cassettes. The sliced tissues were placed in lithium carbonate solution in 70% alcohol for removing any excess fixative. After passing these samples through a series of alcohol concentrations ranging from 70% to 100%, tissue blocks were created and embedded in paraffin wax. Tissues were sliced in cut sections of 5µm thick-were obtained through rotary microtome and then stained with Hematoxylin and Eosin (H&E) for examination. Serum SOD, GPX and CAT levels were analyzed at the research and diagnostic Laboratory of Isra University, Hyderabad.

The collected data was entered and analyzed in SPSS ver. 24.0. The descriptive findings were expressed as Mean±SD. One-way ANOVA with Post hoc Tukey's analysis was applied for the statistical analysis. Significance level of P-value ≤ .05 was considered as significant.

Results

Mean body weight of all three groups (A, B and C) prior to the experiment group was 238.4±6.10 gm., 240.6±7.81 gm., and 230.5±6.8 gm. respectively. Significant difference in mean before and after-experimental body weight of rats of all groups. While a statistically significant ($p<0.05$) decline in body weight of rats in both experimental groups (group B and C) (Table 1). A statistically significant difference ($p<0.05$) in mean testicular weight was also observed in all three groups. (Table 1). Moreover, the sperm count, its motility, and viability was reduced significantly in Cisplatin treated group B (Table 1).

Table 1. Post-Hoc analysis of weight (Testicular and Body), Sperm count, Motility and Viability between all groups

	Study Groups		
	A	B	C
Testis weight (g)	1.53 ± 0.36	1.03 ± 0.20*	1.40 ± 0.65ǂ
Body weight (g)	247.1 ± 6.41	196.7 ± 9.29*ǂ	210.0 ± 6.48ǂ
Sperm count ($x10^6$/ml)	72.84 ± 4.65	30.46 ± 3.18*ǂ	62.44 ± 5.74ǂ
Motility (%)	71.84 ± 5.25	27.79 ± 3.66*ǂ	66.74 ± 4.56ǂ
Viability (%)	80.33 ± 2.86	51.86 ± 3.36*ǂ	68.11 ± 2.26ǂ

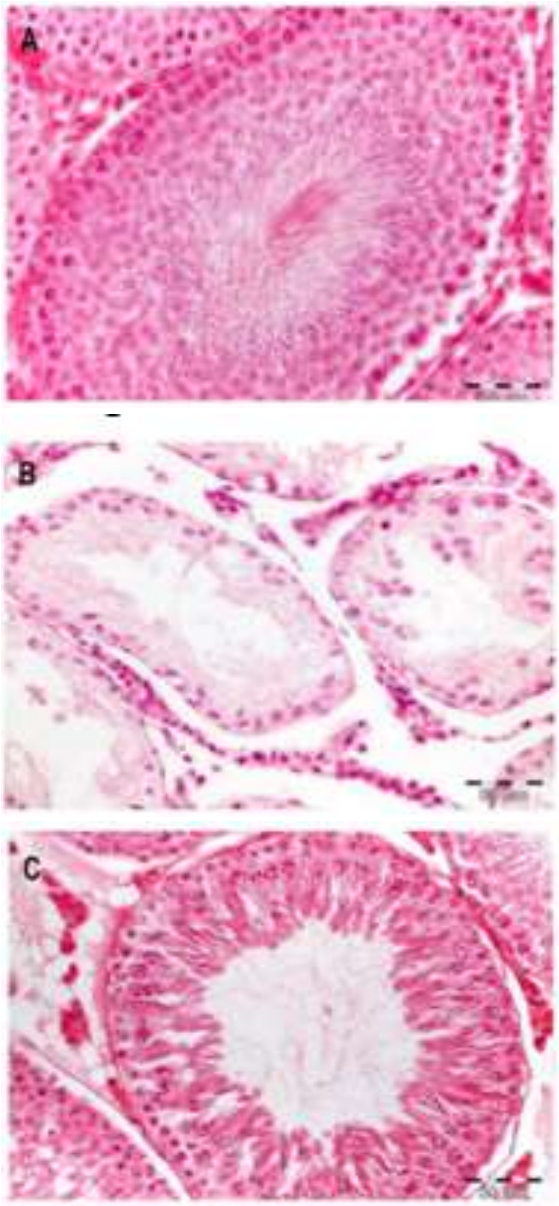
*: Statistically significant difference ($p<0.05$) between group B and C as compared with the group A

ǂ: Statistically significant difference ($p<0.05$) between group B and C

A statistically significant difference ($p<0.05$) in levels of SOD, GPX, and CAT was observed in all study groups. While the post hoc Tukey test disclosed a significant decrease in GPX, SOD, and CAT levels in Group B when compared to controls. In comparison to Group B, Group C had significantly higher levels of antioxidants ($p<0.05$). Apart from that, Group III and Group I didn't show any statistical difference in antioxidant levels. (Table 2)

Table 2. Post-Hoc Analysis of serological markers between all study groups

	Study Groups		
	A	B	C
Glutathione peroxidase (umol/mg protein)	9.90±1.51	4.29±0.78*ǂ	8.13±0.51ǂ
Superoxide dismutase (u/mg protein)	13.20±0.50	5.90±0.15*ǂ	11.38±0.79ǂ
Catalase (u/mg protein)	19.42±1.04	12.70±0.67*ǂ	17.68±1.58ǂ



*: Statistically significant difference (p<0.05) between group B and C as compared with the group A

*: Statistically significant difference (p<0.05) between group B and C

Figure 1. Testicular cross-sections photographed under a microscope (H&E 400)

The testicular architecture of the experimental animals in group A was normal, with regular seminiferous tubules. Cisplatin-treated rats (Group B) had their normal testicular architecture disrupted but uneven small seminiferous tubules without any spermatogenesis.

Atrophic, Degenerative as well as regressive tubules were also detected. The testicular architecture of RSV-treated rats (Group C) was nearly normal, with most of the seminiferous tubules showing regular morphology identical to Group A. Furthermore, the seminiferous tubules atrophied and degenerated were greatly reduced.

Discussion

The optimal functioning of the reproductive system is critically threatened by the presence of ROS prompted oxidative stress. Spermatogenesis, as well as other important processes within the testicular tissue, are hampered by injuries caused by ROS.[12] ROS also causes occlusion of arteries, which compromises the blood supply making the tissue prone to oxidative stress due to increased oxygen demands and consumption. [12]

The current study demonstrated the actions of resveratrol in protecting the testicular tissue against cisplatin induced toxicity. It was found that cisplatin, in addition to causing histological discrepancies, led to oxidative stress which was ameliorated by RSV administration.

Although being beneficial against a wide variety of cancers, Cisplatin administration is accompanied with a long list of complications, all of which are attributed to the inherent capability of Cisplatin to generate ROS. [8, 13]

In the current study, the body weight as well as the semen parameters of the experimental animals were reduced post cisplatin administration. Additionally, the testicular histological architecture was also compromised. These findings are in accordance with those reported by Hamza et al. who reported that cisplatin exerts a toxic effect on not only on testicular histology but on testicular function as well. [14]

In the current study, the levels of endogenous antioxidants were also markedly decreased. These findings are also in agreement to the findings of Al-Bader et al. and Madhu et al. which also

demonstrated a decline in the total antioxidant status (TAS) of the cisplatin treated rats. [15, 16]

Being a polyphenolic phytoalexin, the beneficial role of Resveratrol in preventing the manifestation of toxicity in testicular tissue caused by the administration of various toxins has been reported by several studies. [17] [18] [19] [20] Observations of this current study are consistent with these findings as RSV was able to prevent testicular toxicity post cisplatin administration which was evident from the comparatively higher semen parameters as well as serum antioxidant levels in the RSV receiving group. The studies by Nagehan et al. and Reddy et al. reported findings consistent with the current study. According to their studies, RSV administration was able to amend the toxic effects produced by cisplatin. This was evident by improved semen parameters, and an elevated level of plasma antioxidants. Additionally, RSV therapy also barred the cisplatin prompted discrepancies in the histological architecture. These findings are in support of the current study.[7, 21]

Owing to limited availability of time and monetary resources, other parameters such as inflammatory as well as hormonal markers could not be explored. Therefore, further studies are recommended to investigate the effects of RSV, both individually as well as in combination with other antioxidants.

Conclusion

Resveratrol is a potent protective agent with promising results in attenuating cisplatin-induced oxidative stress and eventually the testicular toxicity in mice models.

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