

Oxidative stress profile following the co-administration of cisplatin and resveratrol in female rats: a preliminary study



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ABSTRACT

Introduction: Cisplatin is one of the most widely used drugs for the treatment of various cancers but has oxidative tissue damage as one of its side effects. This study investigated the oxidative stress profile in some important body tissues following the co-administration of cisplatin (CIS) and resveratrol (RSV).

Methods: Thirty-five adult female rats with an average body weight of 162g were divided into 5 groups (n=7) and used for this experimental study. Group A served as the normal control group and received distilled water only. Group B received only a single dose intraperitoneal injection of 10mg/kg CIS. Groups C, D and E were orally given 5, 10 and 20mg/kg of RSV respectively for 7 days, starting 24h after a single CIS dose intraperitoneal injection of 10mg/kg. Selected body tissues were harvested for oxidative stress profiling at the end of the experiment.

Results: CIS significantly increased malondialdehyde levels and decreased glutathione, superoxide dismutase and catalase levels in all the tissues assessed (ovary, uterus, liver, kidney, pancreas, stomach and spleen) when compared to the normal control. The RSV treatment caused the reversal of these effects; malondialdehyde levels were significantly decreased, while glutathione, superoxide dismutase and catalase levels were significantly increased across all the examined tissues.

Conclusion: RSV at different doses could be effective in the management of CIS-induced oxidative stress and lipid peroxidation across some body tissues. However, this effect may be dependent on the dose of CIS and RSV.

Keywords:

Cisplatin
Oxidative stress
Resveratrol
Antioxidants

Introduction

Cisplatin (CIS) is a platinum-based chemotherapeutic drug used in the treatment of different forms of solid tumors such as ovarian, testicular, lungs, and brain cancers (Yao et al., 2007). However, despite the efficacy

of CIS as an anti-cancer agent, its use is primarily restricted due to numerous toxic side effects like nephrotoxicity, hepatotoxicity, ototoxicity and testicular toxicity (Watanabe et al., 2000; Nematbakhsh et al., 2012; Okafor et al., 2014; Mir et al., 2015). CIS is often

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Received 22 July 2020; Revised from 7 October 2020; Accepted 27 October 2020

Citation: Okafor IA. Oxidative stress profile following the co-administration of cisplatin and resveratrol in female rats: a preliminary study. *Physiology and Pharmacology* 2021; 25: 134-145. <http://dx.doi.org/10.32598/ppj.25.2.90>

thought to trigger cellular apoptosis by inhibiting DNA synthesis and causing crosslink with DNA (Okafor et al., 2014). The clinical use of CIS has been reported to exert severe side effects on different organs of the body. In humans, CIS has been reported to trigger cardiotoxicity through lipid peroxidation, which induces oxidative stress, causing physical injury and damages to myocytes (Hanchate et al., 2017). Although the exact individual mechanism through which CIS exerts its damaging effects on different organs of the body is not entirely the same, the induction of oxidative stress damage, the alteration of tissue antioxidant defense system and the generation of reactive oxygen species (ROS) have been strongly linked to the pathogenesis of CIS-induced toxicity (Coskun et al., 2013). ROS are composed of highly reactive molecules such as hydrogen peroxide (H_2O_2), hydroxyl radical and superoxide radical, which can chemically interact with cellular components such as proteins, lipids, sugar or nucleotide (Bhattacharjee, 2005; Marrocco et al., 2017). The interactions of these free radicals with cellular components destabilize the biomolecules of the cell, triggering a large chain of free radical reactions that ultimately destroys cellular structural integrity, causing tissue and organ damage (Amin et al., 2008; Nandi et al., 2019). In addition to ROS production and oxidative stress induction, other suggested underlying mechanism involved in CIS-induced toxicity includes alteration in Ca^{2+} homeostasis, apoptosis, and the activities of pro-inflammatory genes such as COX-2 (Mir et al., 2015).

Resveratrol (RSV) is a polyphenol phytoalexin found in edibles like grapevine, peanuts and red wine (Shrikanta et al., 2015). It is classified as a phytoestrogen due to its estrogenic properties and has been shown to possess numerous health benefits (Szkudelska and Szkudelski, 2010). Studies involving RSV have reported its anti-cancer, antidiabetic, anti-inflammatory and neuroprotective properties (Frémont, 2000; Athar et al., 2009; Fukui et al., 2010; Hamadi et al., 2012). RSV also possesses antioxidant and cardioprotective properties, as it has been reported to inhibit lipid peroxidation, elevate serum levels of low-density lipoprotein cholesterol and inhibit the generation of ROS in the liver and brain (Tadolini et al., 2000; Gedik et al., 2008; Fukui et al., 2010; Akbel et al., 2018). The inhibition and removal of oxidative stress are primary preventive and interventive action against oxidative-

induced tissue damage. Several defense systems have been put in place within cells to prevent and remove uncontrolled increase and production of ROS (Marrocco et al., 2017). Enzymatic antioxidants such as catalase (CAT) and superoxide dismutase (SOD), as well as non-enzymatic antioxidants like glutathione (GSH), vitamin A, C and E, are known endogenous antioxidants that inhibit the production of such reactive species, protect the cell against tissue damage and maintain balance (Marrocco et al., 2017). There is a need to demonstrate RSV activities against CIS-induced oxidative damage in several tissues to give a clearer view of its antioxidative potential. This study assessed the oxidative stress profile of some important body tissues, following the co-administration of CIS and RSV in adult rats.

Materials and methods

Study setting

This experimental study was carried out in the research laboratory of the Department of Anatomy, College of Medicine of the University of Lagos, Nigeria as a preliminary part of a thesis.

Ethical considerations

Ethical approval was obtained from the College of Medicine of the University of Lagos Health Research Ethics Committee (CMULHREC) with ID number CMULHREC/09/16/025. The experimental procedures of this study complied with the National Research Council (US) and National Health Research Ethics Committee of Nigeria (NHREC) guidelines for the care and use of laboratory animals (NRCC 2011, NHREC 2014).

Animal Care and Handling

Thirty-five adult female *Sprague–Dawley* rats were procured from the Animal House, College of Medicine of the University of Lagos. They were acclimatized for two weeks to exclude any intercurrent infection under standard housing of $24\pm 2^\circ C$ and 12h light/dark cycle. The rats were fed with standard rat chow and water *ad libitum* throughout the experimental period. The rats were monitored throughout the study period for any undue drug reaction.

Experimental drugs

RSV with the brand name ‘Restorlyf’, manufactured by Nature’s Way U.S.A., was procured from Alliance in

Motion Global Ltd., Ikeja, Lagos, Nigeria. The 325mg of RSV was diluted immediately before each use in 20ml of distilled water and doses of 5, 10 and 20mg/kg body weight were administered orally using the oral cannula. The remaining formulation was discarded after each use. The drug dosages and formulations were chosen based on previously published studies on RSV (Bolis et al., 1997; Loehrer et al., 1998). The CIS (Zuplatin, 50mg/ 50ml) injection manufactured by Taj Pharmaceuticals Ltd. India was procured from Bayston Pharmacy, Mushin, Lagos, Nigeria. The injection was given intraperitoneally according to body weight in a single dose of 10mg/kg. The drug dosages were chosen based on previously published studies on CIS (Coppin et al., 1996; Rose et al., 1999).

Experimental design

Thirty-five adult female rats with an average weight of 162g were divided into 5 groups (n=7) and used for this experimental study. Group A served as the normal control group and received distilled water only. Group B was given only a single dose intraperitoneal CIS injection at 10mg/kg, and allowed to stay for 7 days before sacrifice. Groups C, D and E were given 5, 10 and 20 mg/kg RSV respectively for 7 days, starting 24h after a CIS single dose intraperitoneal injection of 10 mg/kg. The least significant effect of RSV is being understudied, hence female rats were considered to be preferable for this research as studies have shown cisplatin-induced toxicity to be more pronounced in male rats. The female sex hormones estrogen and progesterone have been shown to inhibit the sodium-potassium ATPase enzyme which may exacerbate cisplatin-induced toxicities like hyponatremia and urinary sodium excretion; the male sex hormone, testosterone, stimulates the enzyme (Stakisaitis et al., 2010).

Animal sacrifice and sample collection

The animals fasted overnight on the last day of drug administration and were sacrificed the next morning by cervical dislocation for the collection of tissues. Tissues collected include ovary, uterus, stomach, liver, kidney, spleen and pancreas. The tissue samples were homogenized immediately for oxidative stress profiling or were stored at -40°C until further analysis in the case where the immediate analysis was not possible.

Oxidative stress determination

The tissues were homogenized in a Teflon-glass homogenizer with a buffer containing 1.5% potassium chloride to get the 1:10 (w/v) whole homogenate. Malondialdehyde (MDA) was measured using the thiobarbituric acid test to determine the concentration of ovarian and uterine MDA levels. CAT and reduced GSH level activity were determined as described by Rukkumani and his colleagues (2004). CAT was assayed colorimetrically at 620nm and expressed as μmoles of H_2O_2 consumed per min/mg protein. Reduced GSH was evaluated with Ellman's reagent using 0.2ml of the tissue homogenate (homogenized in 0.4M, phosphate buffer pH 7.0) while SOD activity was measured by the method described by Sun and colleagues (1998) using the nitroblue tetrazolium reduction inhibition with xanthine-xanthine oxidase as a superoxide generator.

Statistical analysis

The results were analyzed using the IBM Statistical Package for the Social Sciences version 21 (IBM SPSS, Chicago, IL, USA). Data were reported as mean \pm SEM and differences between mean and the main effects of treatment group were determined using the one-way analysis of variance (ANOVA) and LSD posthoc tests. The mean difference is significant at the 0.05 level ($P<0.05$).

Results

Mortality

The mortality results are presented in Table 1. The control group had no deaths. The 57% of animals in groups 2, 4 and 5 died before the end of the experiment, while 43% of experimental animals in group 3 died before the end of the study. The total combine mortality observed in all groups was 43% while the mortality in all groups that received CIS was 54%.

The animal body weights

Table 2 shows that the animals across all groups had decreased body weight when the body weight before and after drug administration were compared ($P<0.05$) except for the control group whose body weight did not change significantly.

Oxidative stress profile following the co-administration of CIS and RSV

TABLE 1: Mortality rate following the co-administration of CIS and RSV in Sprague-Dawley rats.

Groups	Number of deaths recorded (X_1)	Mortality (%)	Death ratio (X_1/N)	Survival ratio (X_2/N)
A (Control)	0	0	0.00	1
B (CIS Only)	4	57	0.57	0.43
C (CIS+ Rsv Low)	3	43	0.43	0.57
D (CIS+ Rsv Med)	4	57	0.57	0.43
E (CIS+ Rsv High)	4	57	0.57	0.43

X_1 = number of animals that died in a group; X_2 = Number of animals that survived in a group. CIS: Cisplatin; RSV: Resveratrol; Low: low dose (5mg/kg); Med: medium dose (10 mg/kg); High: high dose (20 mg/kg). number of animals per group: 7.

TABLE 2: The mean body weight following the co-administration of CIS and RSV in Sprague-Dawley rats.

Groups		Mean±SEM (g)	t-value	P-value
A (Control)	Before administration	140.0 ±2.9	-0.36	0.754
	After administration	140.7 ±4.5		
B (CIS Only)	Before administration	200.7 ±3.0	4.44	0.047*
	After administration	176.7 ±3.0		
C (CIS+ RSV Low)	Before administration	170.7 ±3.5	22.52	0.002*
	After administration	144.7 ±3.0		
D (CIS+ RSV Med)	Before administration	150.7 ±3.5	28.75	0.001*
	After administration	112.3 ±2.3		
E (CIS+ RSV High)	Before administration	149.3 ±2.3	7.21	0.019*
	After administration	115.7 ±2.3		

* $P < 0.05$. CIS: Cisplatin; RSV: Resveratrol; Low: low dose (5mg/kg); Med: medium dose (10 mg/kg); High: high dose (20 mg/kg).

Ovary oxidant status

The mean GSH level was significantly ($P < 0.05$) decreased in all treated groups when compared to the control. The SOD level was significantly ($P < 0.05$) decreased only in group 2. Notably, no significant difference was observed in the mean SOD values of groups 3, 4 and 5 when compared to the control. The CAT level was significantly ($P < 0.05$) decreased in groups B and D when compared to the control group. There was a significant increase ($P < 0.05$) in MDA in all treated groups except in group E when compared to the control. However, when compared to group B, SOD and CAT levels in groups C, D and E showed a significant ($P < 0.05$) increase, while GSH levels in groups C and E showed a significant ($P < 0.05$) increase. MDA levels were significantly decreased in groups C, D and E when compared to group B. (Table 3).

Uterus oxidant status

When compared to the control group, CIS

administration caused a significant change ($P < 0.05$) in the SOD, CAT and MDA levels but caused no significant difference in the GSH levels. CIS-induced changes were not reversed significantly by RSV across all groups and oxidative parameters except for MDA levels where RSV administration caused a significant reduction ($P < 0.05$). Also, High dose RSV caused a significant increase in CAT levels in group E when compared to the CIS control group ($P < 0.05$, Table 4).

Liver oxidant status

Table 5 shows the oxidative changes caused by CIS and RSV co-administration in the rat liver. GSH, SOD, CAT and MDA were significantly altered due to CIS administration when compared to the control group. RSV at different doses reversed the changes made in only GSH, SOD and CAT across all groups. CIS-induced MDA increase was reduced significantly only in groups C and D but not in E (higher dose) when compared to group B ($P > 0.05$, Table 5).

TABLE 3: Oxidative stress profile of the ovary following the co-administration of CIS and RSV in Sprague-Dawley rats.

Groups	GSH ($\mu\text{mol/ml/mg pro}$)	P-value	SOD ($\mu\text{mol/ml/min/mg pro}$)	P-value	CAT ($\mu\text{mol/ml/min/mg pro}$)	P-value	MDA ($\mu\text{mol/ml/mg pro}$)	P-value
(Control)	5.53±0.15	0.00 [#]	12.57±0.12	0.00 [#]	112.36±4.83	0.00 [#]	0.08±0.00	0.00 [#]
B (CIS Only)	2.60±0.11	0.00*	9.35±0.17	0.00*	69.31±4.78	0.00*	0.22±0.02	0.00*
C (CIS+ RSV Low)	3.48±0.09	0.00*	12.60±0.80	0.97*	111.20±0.96	0.87*	0.16±0.01	0.00*
		0.00 [#]		0.00 [#]		0.00 [#]		0.00 [#]
D (CIS+ RSV Med)	3.09±0.11	0.00*	11.63±0.79	0.23*	96.23±7.47	0.04*	0.12±0.01	0.01*
		0.07 [#]		0.01 [#]		0.00 [#]		0.00 [#]
E (CIS+ RSV High)	4.64±0.29	0.00*	12.75±0.22	0.81*	110.04±4.23	0.75*	0.08±0.00	0.57*
		0.00 [#]		0.00 [#]		0.00 [#]		0.00 [#]

* $P < 0.05$ compared to the control group. [#] $P < 0.05$ compared to the CIS only groups. CIS: Cisplatin; RSV: Resveratrol; Low: low dose (5mg/kg); Med: medium dose (10mg/kg); High: high dose (20 mg/kg); GSH: Reduced glutathione; SOD: Superoxide dismutase; CAT: Catalase; MDA: Malondialdehyde.

TABLE 4: Oxidative stress profile of the uterus following the co-administration of CIS and RSV in Sprague-Dawley rats.

Groups	GSH ($\mu\text{mol/ml/mg pro}$)	P-value	SOD ($\mu\text{mol/ml/min/mg pro}$)	P-value	CAT ($\mu\text{mol/ml/min/mg pro}$)	P-value	MDA ($\mu\text{mol/ml/mg pro}$)	P-value
A (Control)	2.47±0.0	0.27 [#]	11.39±0.47	0.01 [#]	113.32±2.87	0.01 [#]	0.09±0.00	0.01 [#]
B (CIS Only)	2.27±0.09	0.27*	9.54±0.63	0.01*	84.52±12.40	0.01*	0.17±0.00	0.00*
C (CIS+ RSV Low)	2.03±0.17	0.03*	7.72±0.26	0.00*	77.47±4.10	0.00*	0.11±0.01	0.20*
		0.18 [#]		0.01 [#]		0.44 [#]		0.00 [#]
D (CIS+ RSV Med)	2.17±0.07	0.10*	9.55±0.31	0.02*	99.16±0.61	0.13	0.10±0.01	0.33*
		0.55 [#]		0.99 [#]		0.12 [#]		0.00 [#]
E (CIS+ RSV High)	2.22±0.16	0.17*	9.64±0.48	0.02*	106.86±2.96	0.47*	0.11±0.01	0.13*
		0.77 [#]		0.88 [#]		0.03 [#]		0.00 [#]

* $P < 0.05$ compared to the control group. [#] $P < 0.05$ compared to the CIS only groups. CIS: Cisplatin; RSV: Resveratrol; Low: low dose (5mg/kg); Med: medium dose (10mg/kg); High: high dose (20 mg/kg); GSH: Reduced glutathione; SOD: Superoxide dismutase; CAT: Catalase; MDA: Malondialdehyde.

Stomach oxidant status

The CIS administration caused a significant change in GSH, SOD, CAT and MDA levels in CIS-treated groups which were reversed to normal by RSV administration across all groups when compared to both group B and the control group ($P < 0.05$). However, RSV at a lower dose (group C) did not reverse the effect of cisplatin on

the MDA level as group C showed significantly higher MDA levels compared to the control group. Also, RSV did not reverse the GSH levels in the stomach across all the RSV-treated groups ($P < 0.05$, Table 6).

Pancreas oxidant status

CIS administration caused a significant decrease in

TABLE 5: Liver oxidative stress profile following the co-administration of CIS and RSV in Sprague-Dawley rats.

Groups	GSH ($\mu\text{mol/ml/mg pro}$)	P-val- ue	SOD ($\mu\text{mol/ml/min/mg pro}$)	P-value	CAT ($\mu\text{mol/ml/min/mg pro}$)	P-value	MDA ($\mu\text{mol/ml/mg pro}$)	P-value
A (Control)	1.87±0.03	0.00 [#]	8.00±0.06	0.00 [#]	85.47±2.93	0.00 [#]	0.04±0.01	0.00 [#]
B (CIS Only)	0.72±0.043	0.00*	5.59±0.06	0.00*	43.14±1.81	0.00*	0.23±0.01	0.00*
C (CIS+ RSV Low)	1.36±0.046	0.00*	6.58±0.24	0.00*	63.72±1.73	0.00*	0.04±0.01	0.96*
		0.00 [#]		0.00 [#]		0.00 [#]		0.00 [#]
D (CIS+ RSV Med)	1.60±0.042	0.00*	5.25±0.06	0.00*	57.58±2.00	0.00*	0.06±0.01	0.12
		0.00 [#]		0.09 [#]		0.00 [#]		0.00 [#]
E (CIS+ RSV High)	1.80±0.023	0.23*	7.58±0.11	0.04*	75.56±2.73	0.01*	0.12±0.015	0.00*
		0.00 [#]		0.00 [#]		0.00 [#]		0.00 [#]

*P<0.05 compared to the control group. [#]P<0.05 compared to the CIS only groups. CIS: Cisplatin; RSV: Resveratrol; Low: low dose (5mg/kg); Med: medium dose (10mg/kg); High: high dose (20 mg/kg); GSH: Reduced glutathione; SOD: Superoxide dismutase; CAT: Catalase; MDA: Malondialdehyde.

GSH, SOD and CAT across all groups when compared to the control group ($P<0.05$). RSV co-administration in groups C, D and E caused a sharp decrease and reversal of GSH, SOD and CAT levels when compared to the control and cisplatin groups ($P<0.05$). However, there is no change in MDA levels due to CIS administration across all the treated groups ($P<0.05$, Table 7).

Spleen oxidant status

TABLE 6: Stomach oxidative stress profile following the administration of CIS and RSV in Sprague-Dawley rats.

Groups	GSH ($\mu\text{mol/ml/mg pro}$)	P-value	SOD ($\mu\text{mol/ml/min/mg pro}$)	P-value	CAT ($\mu\text{mol/ml/min/mg pro}$)	P-val- ue	MDA ($\mu\text{mol/ml/mg pro}$)	P-value
A (Control)	2.64±0.05	0.000 [#]	13.54±0.40	0.000 [#]	107.87±5.37	0.000 [#]	0.03±0.0	0.001 [#]
B (CIS Only)	0.39±0.03	0.000*	5.72±0.31	0.000*	58.47±1.16	0.000*	0.166±0.04	0.001*
C (CIS+ RSV Low)	0.66±0.04	0.002*	5.82±0.08	0.000*	62.32±1.39	0.000*	0.14±0.02	0.005*
		0.000 [#]		0.829 [#]		0.350 [#]		0.465 [#]
D (CIS+ RSV Med)	2.26±0.04	0.000*	10.90±0.40	0.000*	100.94±1.26	0.108*	0.09±0.00	0.068*
		0.000 [#]		0.000 [#]		0.000 [#]		0.042 [#]
E (CIS+ RSV High)	2.45±0.05	0.000*	12.06±0.28	0.008*	104.21±2.18	0.373*	0.04±0.00	0.708*
		0.020 [#]		0.000 [#]		0.000 [#]		0.003 [#]

*P<0.05 compared to the control group. [#]P<0.05 compared to the CIS only groups. CIS: Cisplatin; RSV: Resveratrol; Low: low dose (5mg/kg); Med: medium dose (10mg/kg); High: high dose (20 mg/kg); GSH: Reduced glutathione; SOD: Superoxide dismutase; CAT: Catalase; MDA: Malondialdehyde.

Table 8 shows that GSH, CAT, SOD and MDA were significantly altered across CIS-treated groups when compared to the control group ($P<0.05$). The subsequent administration of RSV caused the reversal of these changes only in groups D (for MDA and SOD) and E (for GSH, CAT and MDA; $P<0.05$).

Kidney oxidant status

Table 9 shows the effect of CIS and resveratrol on

TABLE 7: Pancreas oxidative stress profile following the administration of CIS and RSV in Sprague-Dawley rats.

Groups	GSH ($\mu\text{mol/ml/mg pro}$)	P-value ue	SOD ($\mu\text{mol/ml/min/mg pro}$)	P-value	CAT ($\mu\text{mol/ml/min/mg pro}$)	P-value	MDA ($\mu\text{mol/ml/mg pro}$)	P-value
A (Control)	4.76±0.04	0.000 [#]	22.84±1.28	0.000 [#]	195.3±2.85	0.000 [#]	0.10±0.01	0.639 [#]
B (CIS Only)	1.50±0.04	0.000 [*]	9.18±0.97	0.000 [*]	85.92±6.81	0.000 [*]	0.19±0.00	0.639 [*]
C (CIS+ RSV Low)	3.02±0.39	0.000 [*]	18.45±0.72	0.006 [*]	170.99±0.76	0.001 [*]	0.17±0.01	0.700 [*]
D (CIS+ RSV Med)	3.75±0.15	0.004 [*]	21.44±0.67	0.292 [*]	180.16±3.57	0.016 [*]	0.11±0.00	0.935 [*]
E (CIS+ RSV High)	4.24±0.09	0.089 [*]	23.35±0.62	0.694 [*]	190.72±0.88	0.402 [*]	0.39±0.29	0.148 [*]
		0.000 [#]		0.000 [#]		0.000 [#]		0.303 [#]

* $P < 0.05$ compared to the control group. [#] $P < 0.05$ compared to the CIS only groups. CIS: Cisplatin; RSV: Resveratrol; Low: low dose (5mg/kg); Med: medium dose (10mg/kg); High: high dose (20 mg/kg); GSH: Reduced glutathione; SOD: Superoxide dismutase; CAT: Catalase; MDA: Malondialdehyde.

the GSH, SOD, CAT and MDA levels in rats. CIS administration caused a significant decrease in GSH, SOD and CAT across treated groups; but a significant increase in MDA compared to the control ($P < 0.05$). The RSV co-administration did not make any significant difference in any of the observed oxidative parameters across all treated groups except in group E, where GSH, SOD and CAT levels were not different compared to the control group ($P < 0.05$). MDA levels of group E

TABLE 8: Spleen oxidative stress profile following the co-administration of CIS and RSV in Sprague-Dawley rats.

Groups	GSH ($\mu\text{mol/ml/mg pro}$)	P-value	SOD ($\mu\text{mol/ml/min/mg pro}$)	P-value	CAT ($\mu\text{mol/ml/min/mg pro}$)	P-value	MDA ($\mu\text{mol/ml/mg pro}$)	P-value
A (Control)	2.75±0.10	0.000 [#]	8.65±0.49	0.001 [#]	78.65±1.37	0.000 [#]	0.04±0.00	0.000 [#]
B (CIS Only)	1.23±0.12	0.000 [*]	6.54±0.24	0.001 [*]	52.23±0.94	0.000 [*]	0.15±0.01	0.000 [*]
C (CIS+ RSV Low)	1.11±0.20	0.000 [*]	6.86±0.03	0.002 [*]	57.95±1.83	0.000 [*]	0.15±0.00	0.000 [*]
D (CIS+ RSV Med)	1.56±0.04	0.000 [*]	7.88±0.30	0.102 [*]	63.46±1.70	0.000 [*]	0.06±0.01	0.070 [*]
E (CIS+ RSV High)	2.51±0.13	0.234 [*]	7.60±0.25	0.034 [*]	74.73±2.72	0.157 [*]	0.04±0.00	0.654 [*]
		0.000 [#]		0.033 [#]		0.000 [#]		0.000 [#]

* $P < 0.05$ compared to the control group. [#] $P < 0.05$ compared to the CIS only groups. CIS: Cisplatin; RSV: Resveratrol; Low: low dose (5mg/kg); Med: medium dose (10mg/kg); High: high dose (20 mg/kg); GSH: Reduced glutathione; SOD: Superoxide dismutase; CAT: Catalase; MDA: Malondialdehyde.

was significantly lower than the CIS group but was significantly higher compared to the control ($P < 0.05$).

Discussion

The RSV is a naturally occurring phytoalexin with numerous pharmacologic and therapeutic attributes including anti-inflammatory, anti-neoplastic and anti-diabetic properties (Singh et al., 2019). RSV has also been shown to inhibit lipid peroxidation and reduced

TABLE 9: Kidney oxidative stress profile following the co-administration of CIS and RSV in Sprague-Dawley rats.

Groups	GSH ($\mu\text{mol/ml/mg pro}$)	P-value	SOD ($\mu\text{mol/ml/min/mg pro}$)	P-value	CAT ($\mu\text{mol/ml/min/mg pro}$)	P-value	MDA ($\mu\text{mol/ml/mg pro}$)	P-value
A (Control)	3.12±0.07	0.000 [#]	12.67±0.49	0.019 [#]	90.25±1.15	0.000 [#]	0.04±0.01	0.000 [#]
B (CIS Only)	1.34±0.17	0.000 [*]	9.15±0.32	0.019 [*]	44.51±7.30	0.000 [*]	0.16±0.01	0.000 [*]
C (CIS+ RSV Low)	1.55±0.05	0.000 [*]	7.89±0.43	0.004 [*]	76.01±2.42	0.038 [*]	0.14±0.01	0.000 [*]
		0.251 [#]		0.340 [#]		0.000 [#]		0.307 [#]
D (CIS+ RSV Med)	1.23±0.18	0.000 [*]	7.35±1.77	0.002 [*]	75.40±3.02	0.032 [*]	0.13±0.00	0.001 [*]
		0.538 [#]		0.182 [#]		0.000 [#]		0.155 [#]
E (CIS+ RSV High)	3.01±0.04	0.052 [*]	11.11±0.52	0.244 [*]	89.46±4.39	0.897 [*]	0.09±0.02	0.026 [*]
		0.000 [#]		0.150 [#]		0.000 [#]		0.004 [#]

^{*} P<0.05 compared to the control group. [#] P<0.05 compared to the CIS only groups. CIS: Cisplatin; RSV: Resveratrol; Low: low dose (5mg/kg); Med: medium dose (10mg/kg); High: high dose (20 mg/kg); GSH: Reduced glutathione; SOD: Superoxide dismutase; CAT: Catalase; MDA: Malondialdehyde.

ROS production in different organs, thus protecting cells from oxidative damage (Akbel et al., 2018). Oxidative damage occurs when the production of free radicals or ROS surpasses or overwhelms the body’s ability to counteract the actions of these reactive radicals. Free radicals produced in oxidative stress are known to interact with and damage important cellular components such as lipids, sugars, polynucleotides as well as proteins and may trigger cellular and tissue damage (Nandi

et al., 2019). Decades of epidemiological evidence have shown the involvement of oxidative stress in the progression and pathogenesis of several chronic diseases (Negre-Salvayre et al., 2010; Granger et al., 2015). The widespread involvement of oxidative stress damage in different diseases has made oxidative stress markers an important indicator of general disease state, disease progression and toxicity. In the present study, the levels of some sensitive markers of oxidative stress

in different body tissues were critically assessed and the changes that occurred following the co-administration of CIS and RSV were noted.

The administration of RSV twenty-four hours after CIS injection may have played a role in the effect seen on the mortality rate. The similarity of the death rate in groups B, D and E (Table 1) shows that RSV offers no protection against death as a result of cisplatin-induced toxicity. A higher CIS dose was used in the current study compared to other earlier studies (Okafor et al., 2014) which may be responsible for the number of deaths experienced. However, this present study lacks the evidence to claim that a different outcome could result if the dosing time differed.

The administration of 10ml/kg CIS showed a significant effect on the body weight of experimental animals when the body weight before and after drug administration was compared ($P=0.047$, Table 2). There was a reduction in body weights which were not salvaged by the co-administrative effect of RSV. No single dose of RSV attenuated the effect of cisplatin on the bodyweight of animals. This finding agrees with an earlier study by Okafor and colleagues (2014) which showed the weight reduction ability of CIS.

MDA is an endogenous end product of ROS-mediated lipid peroxidation and is considered a good marker of oxidative stress. CIS significantly increased MDA levels in different tissues assessed in this study. The mean values of MDA in ovary, uterus, liver, stomach, spleen and kidney were significantly increased in group B given only CIS injection when compared to the control. The increased MDA level observed in this study may be indicative of an increased polyunsaturated fatty acid oxidation, which is an important hallmark of oxidative stress (Marrocco et al., 2017). The elevated MDA levels observed in this study corroborated the findings of Ince et al. (2014), who reported a significant increase in MDA levels following intraperitoneal injection of 7kg/mg CIS. However, CIS did not cause the same effect on the pancreas as there is no difference with the MDA levels in group B compared to the control. The changes seen in the MDA levels were all reversed by RSV across the tissues in a dose-dependent manner with few exceptions (Tables 3-9). While only 10 and 20mg/kg RSV doses caused the tissues' toxicity recovery in the stomach and spleen (Tables 6 and 8), only 20mg/kg RSV was able to cause a significant difference in MDA

levels in the kidney when compared to group B (Table 9). Our finding is comparable to the studies of Gedik et al. (2008), which reported a significant decrease in MDA levels following the administration of RSV.

SOD and CAT compromise the body's antioxidant defense system as they form a formidable defense against ROS-mediated injury. SOD catalyzes the breakdown of superoxide anion radicals into H_2O_2 and molecular oxygen (Younus, 2018); while CAT catalyzes the breakdown of hydrogen H_2O_2 into H_2O and molecular oxygen (Nandi et al., 2019). The RSV treatment attenuated the effect of CIS on CAT and SOD in a dose-dependent manner in the ovary, liver, stomach and pancreas of all the treated groups (Tables 5-7). The CAT levels were seen to decrease compared to the control after treatment with the 10mg/kg RSV in the ovary while only the high dose (20mg/kg) was able to attenuate the CIS effect in the uterus, spleen and kidney (Tables 4, 8 and 9). It is also worthy to note that in the kidney, the CAT levels of all the RSV-treated groups were seen to be significantly higher when compared to group B (CIS only group, Table 9). Meanwhile, only the 20mg/kg and 10mg/kg RSV treatment caused restoration of SOD levels to comparable normal values in the kidney and spleen respectively (Tables 8 and 9); while RSV showed no antioxidative potential against CIS effects in uterus across all the treated groups (Table 4).

The observation of the endogenous, non-enzymatic antioxidant – GSH showed that outside the uterus, all other evaluated tissues showed a significantly lowered GSH levels due to CIS treatment when compared to the control group (Tables 3-9). In addition to the role of GSH in the detoxification of ROS, GSH also regenerates important antioxidants like vitamin C and E and has been reported to be involved in the redox reaction associated with DNA repair. Studies on the pro-oxidant and toxic properties of CIS abound (Mir et al., 2015; Wang et al., 2017) and the result of this study supports these data. However, all the doses of RSV were effective in the treatment of CIS-induced GSH decrease across several tissues including the ovary, liver and pancreas (Tables 3, 5 and 7). While RSV treatment at all doses was not able to cause the reversal of GSH to normal levels in the uterus and stomach, only the high dose RSV (20mg/kg) effectively reversed the GSH to normal levels in spleen and kidney (Tables 8 and 9).

Following the indicative findings, it can be posited

that RSV is a potent antioxidant. The dose-dependent antioxidant effect of RSV could be mainly as a result of its ability to improve the antioxidant capacity of tissues, rather than a direct free-scavenging activity. Fukui and colleagues (2010) made similar observations in their study which reported that RSV up-regulates cell mitochondrial antioxidants through the activation of the P13K/Akt signaling pathway. Similarly, Liu and colleagues (2015) suggested that the mediation of several intracellular signaling pathways including NOS, HO-1, SIRT1, among others, plays an important role in the organ-protective effect of RSV. The variation in the activities of RSV seen across different tissues could be as a result of the drug metabolic rate of RSV in the tissues or due to the level of oxidative activity produced by CIS administration. More so, the interplay of proximity of the tissues to one another and the substance distribution channels through the blood could determine the level of effects seen in the RSV antioxidative pattern. It is worthy to note that RSV showed a more effective antioxidative potential in all the observed tissues at a higher dose of 20mg/kg in most of the oxidative stress markers.

Conclusion

Overall, CIS induced oxidative stress and compromised the antioxidant capacity of body tissues, while RSV attenuated the induced toxic effect. In the search for effective drug combination therapies to cushion the effects of CIS chemotherapy, this study has provides compelling preliminary evidence that RSV supplementation in the right dosage is protective against CIS-induced oxidative stress damage in different body tissues and possesses the potential of effective combination therapy with CIS. However, more studies need to be carried out to ascertain the dose-related pharmacodynamics of RSV in CIS-induced oxidative stress.

Acknowledgment

I want to thank Mr. Jude Nnaka who helped with the manuscript editing and Mr. Johnson Nweke who performed the data analysis.

Conflict of interest

There is no conflict of interest to declare.

Availability of data

The dataset for this study has been deposited in

Figshare repository – (<https://doi.org/10.6084/m9.figshare.12413438.v1>)

Funding source

No funding was received for this study.

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