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Adverse Effect of Carboplatin on Biochemical Enzymes And Reproductive Hormones In Male Swiss Albino Mice.

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ABSTRACT

This work was designed to investigate the adverse effect of carboplatin, a platinum coordination compound broadly used as a chemotherapeutic agent in the treatment of cancer on male reproductive system by measuring some important enzymes and reproductive hormones using a mouse model. Also, an attenuative effect of melatonin was investigated in this study. Swiss albino mice were randomly selected into five groups (n=15). Group I received vehicle and served as control, groups II, III and IV received 1, 5 and 10mg/kg.wt of carboplatin respectively while group V was co-treated with 10mg/kg.wt of carboplatin and 5mg/kg.wt of Melatonin via intraperitoneal injection. Five animals from each group were sacrificed at the interval of 6, 12 and 24 hr after carboplatin exposure. Testes were stored in 20^{o}C for biochemical and hormonal analyses. Effect of Testosterone (T) and Luteinizing hormones (FSH) was measured by enzyme immunosorbent assay (ELISA). Effects on apoptosis and vital steroidogenic enzymes were also measured, Data were analyzed by Duncan ANOVA at $p<0.05$. It was observed that carboplatin leads to significant decrease in the levels of the anti-oxidative enzymes in a dose-dependent manner with simultaneous increase in malondialdehyde level. Decrease level of regulatory enzyme of steroidogenesis pathway was also measured. Carboplatin leads induction of apoptosis in the testicular tissue of the mice treated with high dose of carboplatin. This study gives insight on the adverse effect of carboplatin on male reproductive system and possible recovery effect of melatonin.

Keywords: Carboplatin; Melatonin; Apoptosis; Reproductive System; Enzymes

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INTRODUCTION

Carboplatin interferes with DNA repair so as to suppress and eventually kill cancer cells [1,2]. However, the cell growth of normal tissues may also be affected by carboplatin [3]. To be activated, carboplatin must cross the cell membrane and inside the cell, the molecule undergoes hydrolysis of 1,1-cyclobutanedicarboxylate, becoming positively charge which allow carboplatin to interact with nucleophilic molecules within the cell, including DNA, RNA and protein, generating the formation of adducts of platinum [4]. This process occurs through covalent binding of carboplatin to the N7 site of purine bases, forming DNA-protein or DNA-DNA interactions [5]. Due to the pharmacodynamics of carboplatin, it has fewer side effects than its precursor cisplatin, although less potency, which might be due to differences in rates of adduct formation with DNA. These toxicity differences are probably due to the low reactivity rate of carboplatin with nucleophiles, since 1,1-cyclobutanedicarboxylate is a poorer leaving group than chloride [6]. The linkage between DNA and carboplatin can produce lesions in DNA. Crosslinking between strands of DNA is the most cytotoxic effect, because it inhibits the process of DNA replication, causing changes that generate errors in replication, with the accumulation of cells in G2/M phase and the induction of apoptosis [7,8]. Damage to healthy tissues can arise as a side effect of chemotherapy treatment, since these cytotoxic drugs can target normal cells as well as malignant ones. It is becoming increasingly apparent that a vast majority of childhood cancer survivors are living with long-term chronic health conditions as a result of the treatment they received [9, 10, 11, 12]. The impact upon future fertility has been extensively studied, and there are now clear links between the use of alkylating chemotherapy agents in treatment regimens for childhood cancers and subsequent impairment of fertility [13, 14, 15]. It is clear that certain chemotherapy agents damage the reproductive systems of those children undergoing treatment. Therefore, this research is designed to evaluate the adverse effects of carboplatin on apoptosis and Steroidogenesis in testicular tissues of Swiss albino mice and evaluate the ameliorative potential of melatonin.

METHODS

Ethical Approval and experimental procedure

The use of animals in this study was conducted in accordance with the standards and permission established by The Ethics Committee of Animal, Ekiti State University, Ado-Ekiti, Nigeria. Male Swiss-albino mice were housed in room at 22 ± 2°C with 40% relative humidity and with a 12-hrs light ± dark cycle. They were fed with a standard rat chow and tap water *ad libitum*.

Dose grouping

Male mice were divided into five groups of nine animals each and treated intraperitoneally as following: Group I (Control), Group II (1mg/kg/bwt CPT), Group III (5mg/kg/b.wt), Group IV (10mg/kg /bwt CPT) and Group V (10mg/kg/b.wt + 5mg/kg /bwt MT). The animals were autopsied at the interval of 6 hr, 12 hr and 24 hr following the treatment.

Tissues samples for biochemical studies

At autopsy, one part of testis was kept in tube and immediately stored in -20^{0}C. Other parts of the tissues were fixed in 10% formal saline for microscopy analysis.

Measurement of Malondialdehyde (MDA)

Testes tissues were homogenized in 10ml TCA (trichloroacetic acid) which is at the rate of 10%, and then centrifuged at +4°C for 15minutes. 750µl of the supernatant obtained, was mixed with 0.67% TBA (thiobarbituric acid) in a ratio of 1:1. Afterwards, the solution was left in the water bath for 15minutes. Finally, the absorbance was measured spectrophotometrically at 535nm [15].

Superoxide dismutase (SOD) activity

Superoxide dismutase (SOD) activity was assayed by a spectrophotometric method. Assay mixture containing sodium pyrophosphate buffer (pH 8.3, 0.052M), phenazine methosulfate (186µM), nitroblue tetrazolium (300µM) and NADH (780µM) were diluted with appropriate enzyme in total volume of 3ml.

The mixture was incubated at 37°C for 90seconds and reaction was stopped by addition of glacial acetic acid. The reaction mixture was mixed vigorously by adding n-butanol and allowed to stand for 10 min before the collection of butanol layer. The intensity of chromogen in butanol was measured at 520nm [16].

Glutathione (GSH) Determination

Testis tissues were homogenized in 10ml TCA (trichloroacetic acid) which is at the rate of 10%, and then centrifuged at +4°C for 15minutes. Afterwards, 0.5ml of supernatant was taken, and mixed with 0.3 M_2 ml Na_2HPO_4. The mixture was thoroughly vortexed. This mixture was vortexed by the addition of 0.2ml DTBN (Dithiobisnitrobenzene: prepared by dissolving in 1% sodium citrate). Finally, its absorbance was measured at 412nm [17].

Testosterone (T) and Luteinizing hormone (LH) concentrations

The testicular testosterone and luteinizing hormone levels in three mice from each group were measured. Briefly, testicular proteins were extracted with phosphate buffer (50 mM, pH 7.4) and centrifuged at 10,000g for 20mins. The supernatant was used to estimate T and LH levels using ELISA, and were expressed in ng/ml.

Sperm Parameters

Caudal epididymidis was removed from each mouse and cleaned off from the epididymal fat pad, and minced in a pre-warmed Petri dish containing 500µl phosphate buffer saline solutions (PBS, pH 7.4) at 37°C. Sperm motility was estimated and expressed as percentage incidence. For sperm count, an aliquot of this suspension was charged into the Neubauer's counting chamber and the spermatozoa were counted under light microscope. Total sperm count was calculated as the average of the spermatozoa count (N) in each chamber X multiplication factor X dilution factor and was expressed in millions/ml. The sperm morphology was also evaluated (Wyrobek and Bruce, 1975). Briefly, a smear of sperm was made on a clean slide and stained with haematoxylin and eosin and were examined under a light microscope with an oil immersion lens. The morphology of spermatozoa was scored [18].

Caspases Estimation

Caspases activity was measured. Briefly, 1ml of assay buffer (20mM HEPES, 10% glycerol, 1M DTT, and 14 ml of n-acetyl-DEVD-AMC/ml of buffer), and 50ml of sample were added to a microcentrifuge tube and protected from the light. Samples were incubated at 37°C for 60 mins after which fluorescence was measured on a spectrofluorometer with an excitation wavelength of 380nm and an emission wavelength of 440nm [19].

Estimation of CYP450scc and StAR by ELISAs

CYP450scc and StAR mitochondrial level was measured by ELISAs. Briefly, 1 g of mitochondrial protein in PBS was sealed overnight at 4°C. CYP450scc and StAR peptide standards were included with a concentration range from 1 g/ml to 4 ng/ml. The plates were washed with buffer containing PBS with 0.02% sodium azide and 0.05% Tween-20. The wells were blocked with 300 l of 1% BSA in PBS with 0.02% sodium azide and incubated at room temperature for 60 min. After washing of samples four more times, primary antibody at a concentration of 5 g/ml diluted in 1% BSA in PBS/azide was added to each well, and the plate was incubated for 60 min at room temperature. Each well was washed four times before the addition of the secondary antibody. Secondary diluted 1:2000 in 1% BSA in PBS/azide was then added to each well, and the plate was incubated again for 60 min at room temperature. The washing procedure was then repeated. Substrate containing paranitrophenyl phosphate at a concentration of 1 mg/ml in substrate buffer [carbonate-bicarbonate (pH 9.6)] was added. The plate was then incubated at room temperature for 60 min, after which, absorbance at 405 nm was read [20].

Statistical analysis

All statistical comparisons between the groups were made using analysis of variance (ANOVA) by Prism statistics software. Results were presented as mean ± S.E.M (Standard Error Mean). Values of $p<0.05$, $p<0.01$, $p<0.001$ were considered as statistically significant.

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RESULTS

Reacting oxygen species (U/mg protein)

Carboplatin concentration at different dosage significantly increases the level of ROS in testicular tissue of mice when compared to the control group. However, co-treatment of melatonin with carboplatin (10mg/kg.bwt + 5mg/kg.bwt) attenuates the effect of carboplatin when compared to the control group.

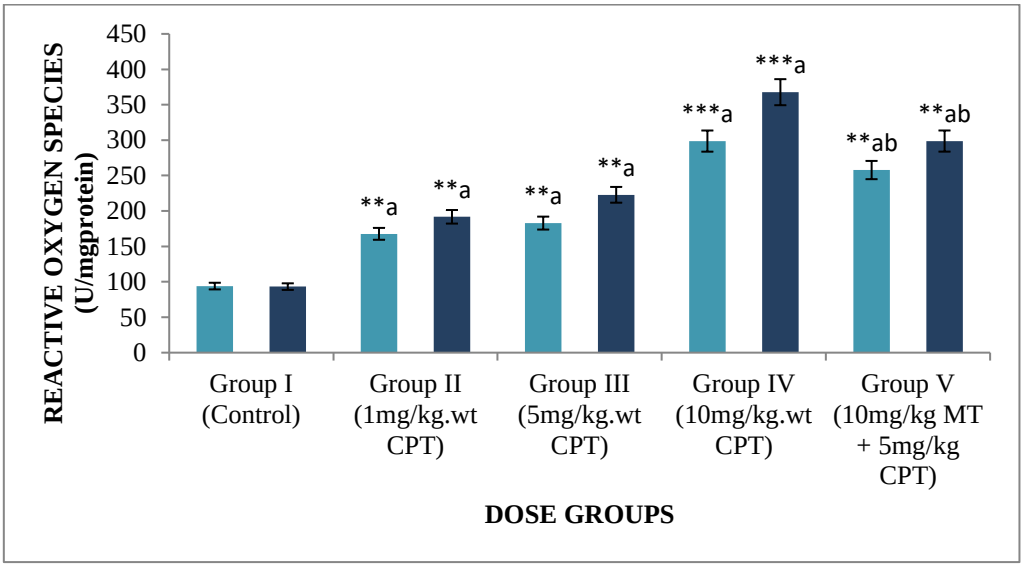

Fig 1: Bar chart showing effect of carboplatin and the ameliorative effect of melatonin when co-treated with carboplatin on ROS. All values are expressed as mean ± standard error mean (SEM), (n=5), * (p<0.05), ** (p<0.01), * (p<0.001). Note: 'a'- CPT –treated groups vs. control and 'b' CBT+MT vs. 5 mg/kg of CPT.**

Malondialdehyde (Umol/min/mg)

Malondialdehyde (MDA) level was significantly increased by carboplatin at different dosage (1mg/kg, 5mg/kg.bwt, and 10mg/kg.bwt) when compare to the control group, co-treatment of melatonin with carboplatin (10mg/kg.bwt + 5mg/kg.bwt) attenuates the effect of carboplatin when compare to the control group.

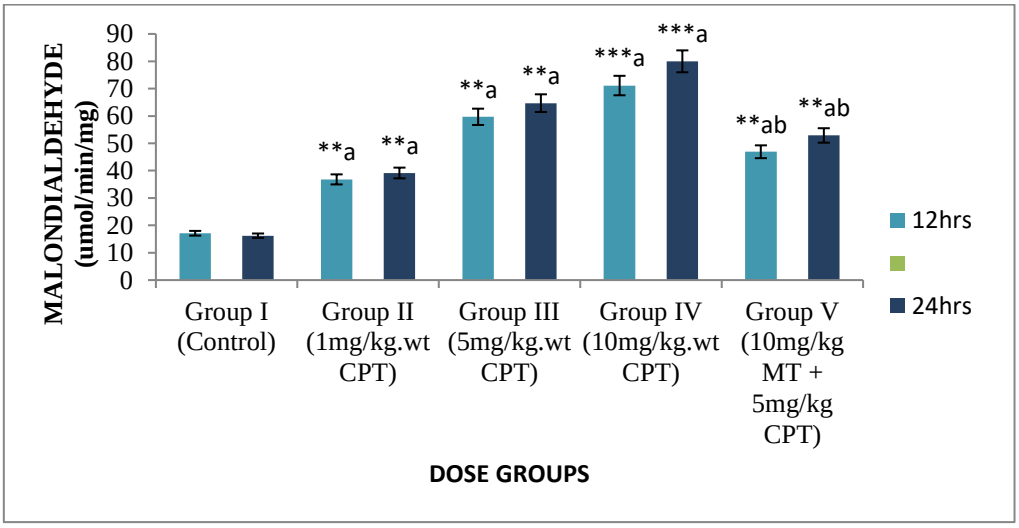

Fig 2: Bar chart showing effect of carboplatin and the ameliorative effect of melatonin when co-treated with carboplatin on MDA level. All values are expressed as mean ± standard error mean (SEM), (n=5), * (p<0.05), ** (p<0.01), * (p<0.001). Note: 'a'- CPT –treated groups vs. control and 'b' CBT+MT vs. 5 mg/kg of CPT.**

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Superoxide dismutase (Unit/ml/min)

Superoxide dismutase (SOD) level was significantly reduced following treatment with carboplatin at a dosage of 1mg/kg.bwt, 5mg/kg.bwt and 10mg/kg.bwt when compared to the control group. However, the effect was reversed in group V co-treated with melatonin when compared to control.

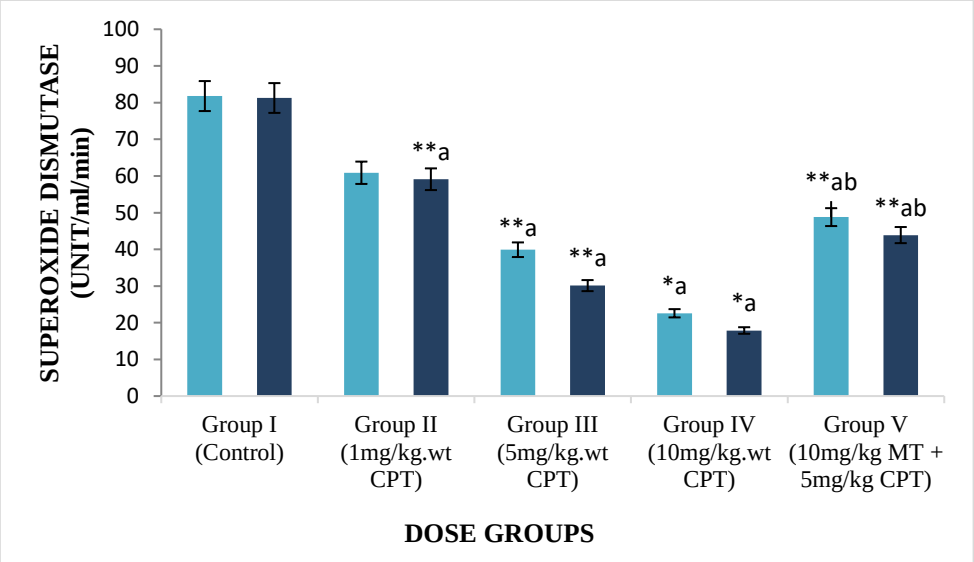

Fig 3: Bar chart showing effect of carboplatin and the ameliorative effect of melatonin when co-treated with carboplatin on SOD level. All values are expressed as mean ± standard error mean (SEM), (n=5), * (p<0.05), ** (p<0.01), * (p<0.001). Note: 'a'- CPT –treated groups vs. control and 'b' CBT+MT vs. 5 mg/kg of CPT .**

Catalase activity (Umol/min/mg protein)

On treatment of testicular tissue with carboplatin catalase activity (CAT) is reduced with increase in dosage when compare to the control. However, the effect was reversed in group V co-treated with melatonin when compared to control.

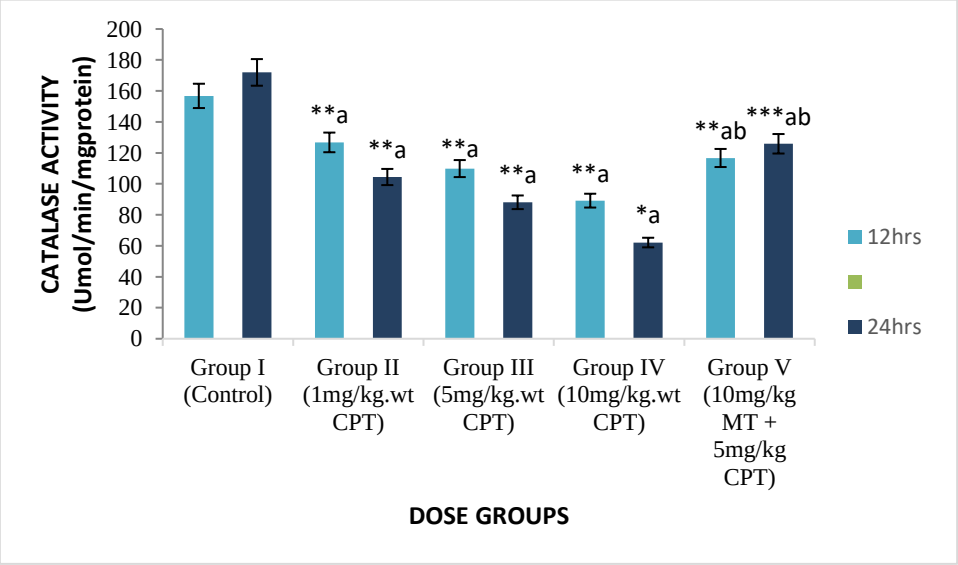

Fig 4: Bar chart showing effect of carboplatin and the ameliorative effect of melatonin when co-treated with carboplatin on CAT activity. All values are expressed as mean ± standard error mean (SEM), (n=5), * (p<0.05), ** (p<0.01), * (p<0.001). Note: 'a'- CPT –treated groups vs. control and 'b' CBT+MT vs. 5 mg/kg of CPT.**

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Glutathione (um/mg)

Carboplatin reduced significantly the activity of glutathione (GSH) with increase in dosage when compared to group I which is the control group. However, the effect was ameliorated in group V co-treated with melatonin when compared to control.

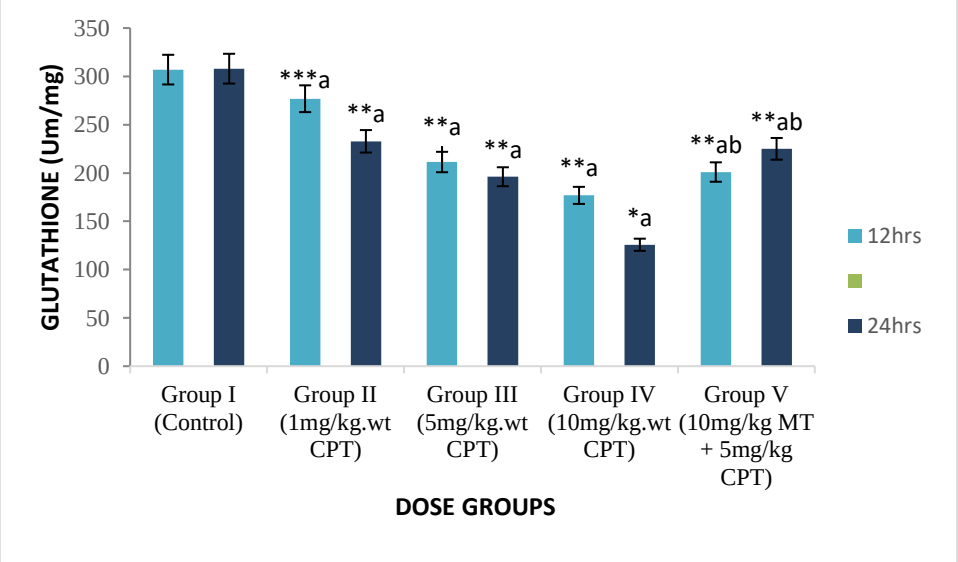

Fig 5: Bar chart showing effect of carboplatin and the ameliorative effect of melatonin when co-treated with carboplatin on GSH. All values are expressed as mean ± standard error mean (SEM), (n=5), * (p<0.05), ** (p<0.01), * (p<0.001). Note: 'a'- CPT –treated groups vs. control and 'b' CBT+MT vs. 5 mg/kg of CPT.**

Testosterone (ng/ml)

Carboplatin reduced significantly the activity of testosterone (T) with increase in dosage when compared to group I which is the control group. However, the effect was ameliorated in group V co-treated with melatonin when compared to control.

Animal Dose groups	6 hrs	12 hrs	24 hrs
Group I (Control)	7±0.01	7±.05	6±0.01
Group II (1mg/kg.wt CBT)	5±0.03^{**a}	4±.06^{*a}	3±0.05^{*a}
Group III (5mg/kg.wt CBT)	4.5±0.05^{**a}	3.5±.01^{*a}	2±0.03^{*a}
Group IV (10mg/kg.wt CBT)	3±0.07^{*a}	2±0.04^{*a}	1.3±0.08^{*a}
Group V (10mg/kg MT + 5mg/kg CBT)	6±0.09^{***ab}	5±.07^{**ab}	4±0.05^{**ab}

Table 1: Showing carboplatin effect on T level and the attenuative effect of melatonin when co-treated with carboplatin on T level. All values are expressed as mean ± standard error mean (SEM), (n=5), * (p<0.05), ** (p<0.01), * (p<0.001). Note: 'a'- CPT –treated groups vs. control and 'b' CBT+MT vs. 5 mg/kg of CPT .**

Luiteinizing hormone (ng/ml)

Luiteinizing (LH) hormone was significantly increased following treatment with carboplatin with increase in dosage when compared to group I. However, the effect was ameliorated in group V, co-treated with melatonin when compared to control.

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Animal Dose groups	6hrs	12hrs	24hrs
Group I (control)	6.728±0.11	7.099±0.01	6.761±0.22
Group II (1mg/kg.wt CBT)	7.767±0.09^{**a}	9.721±0.02^{**a}	10.762±0.09^{**a}
Group III (5mg/kg.wt CBT)	10.800±0.12^{**a}	12.871±0.08^{**a}	13.897±0.08^{***a}
Group IV (10mg/kg.wt CBT)	12.672±0.07^{**a}	13.768±0.07^{***a}	14.787±0.22^{***a}
Group V (10mg/kg MT + 5mg/kg CBT)	9.235±0.22^{**ab}	8.112±0.02^{**ab}	10.161±0.11^{**ab}

Table 2: Showing carboplatin effect on L.H level and the attenuative effect of melatonin when co-treated with carboplatin on L.H level. All values are expressed as mean ± standard error mean (SEM), (n=5), * (p<0.05), ** (p<0.01), * (p<0.001). Note: 'a'- CPT –treated groups vs. control and 'b' CBT+MT vs. 5 mg/kg of CPT .**

Sperm head count (%)

There is significantly increase in the level of Sperm head count when exposed to carboplatin with increase in dosage as compared to group I. However, melatonin reverses the effect of carboplatin when compared to the control group.

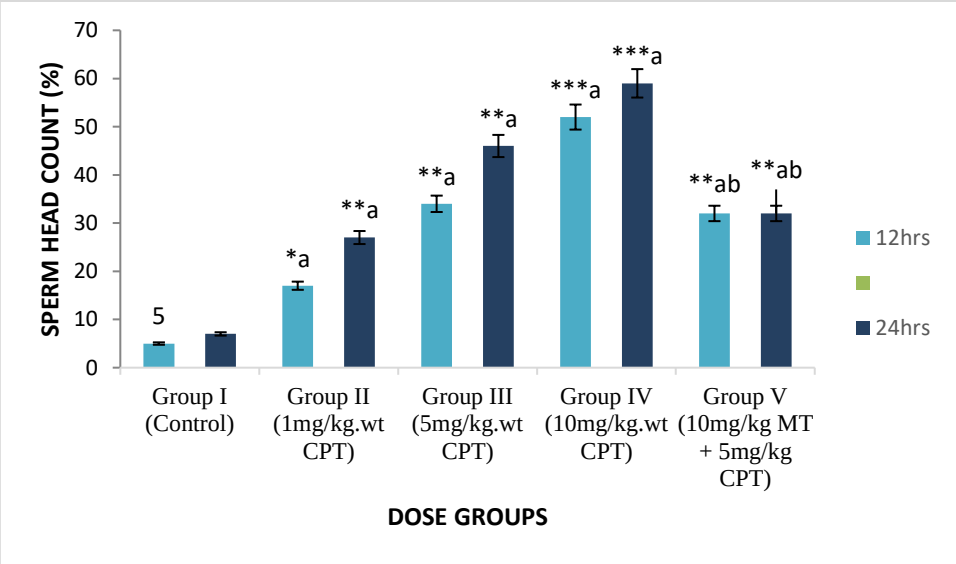

Fig 6: Bar chart showing effect of carboplatin and the ameliorative effect of melatonin when co-treated with carboplatin on sperm head count level. All values are expressed as mean ± standard error mean (SEM), (n=5), * (p<0.05), ** (p<0.01), * (p<0.001). Note: 'a'- CPT –treated groups vs. control and 'b' CBT+MT vs. 5 mg/kg of CPT.**

Sperm motility (%)

Carboplatin reduced significantly sperm motility with increase in dosage when compared to the control group. However, melatonin co-treatment with carboplatin reversed the effect of carboplatin when compared to the control group.

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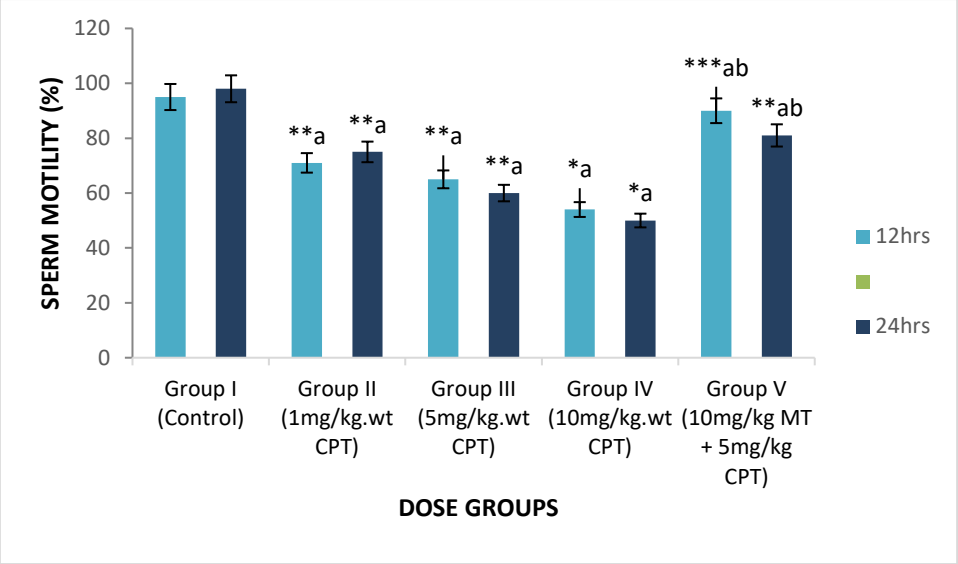

Fig 7: Bar chart showing effect of carboplatin and the ameliorative effect of melatonin when co-treated with carboplatin on sperm motility activity. All values are expressed as mean ± standard error mean (SEM), (n=5), * (p<0.05), ** (p<0.01), * (p<0.001).**

Sperm morphology (%)

Sperm morphology was significantly increased on treatment with carboplatin with respect to increase in dosage when compared to group I. However, treatment with melatonin reverses the effect of carboplatin when compared to the control group.

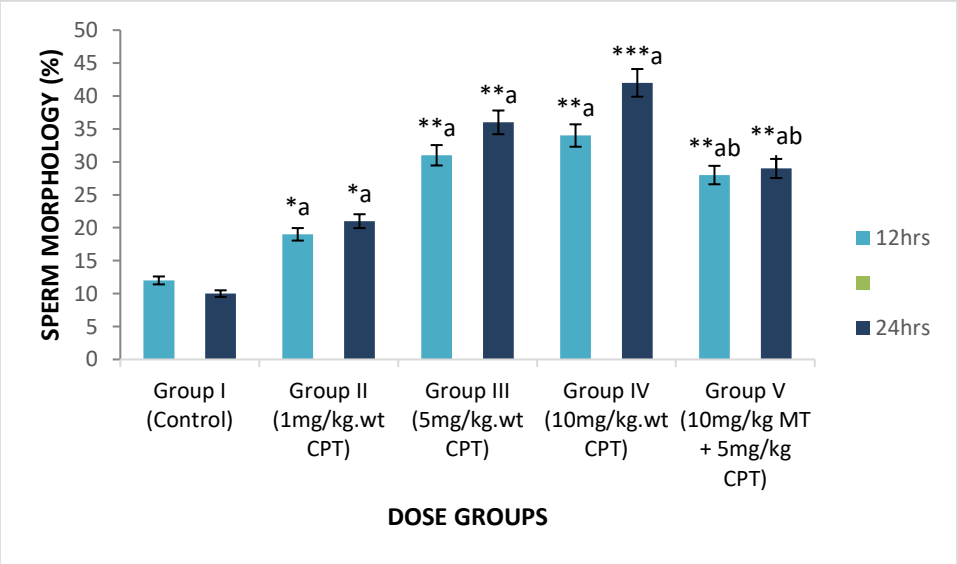

Fig 8: Bar chart showing effect of carboplatin and the ameliorative effect of melatonin when co-treated with carboplatin on sperm morphology. All values are expressed as mean ± standard error mean (SEM), (n=5), * (p<0.05), ** (p<0.01), * (p<0.001). Note: 'a'- CPT –treated groups vs. control and 'b' CBT+MT vs. 5 mg/kg of CPT .**

CYP450scc, StAR and Caspases level

Carboplatin reduce the levels of caspases 3 and 9 significantly across the groups when compared to the control group. When co-treated with melatonin, carboplatin effect was attenuated compared to the control group and carboplatin alone. StAR and CYP450scc level activities were significantly decrease too across the carboplatin-treated groups (Fig 9 10, 11,12)

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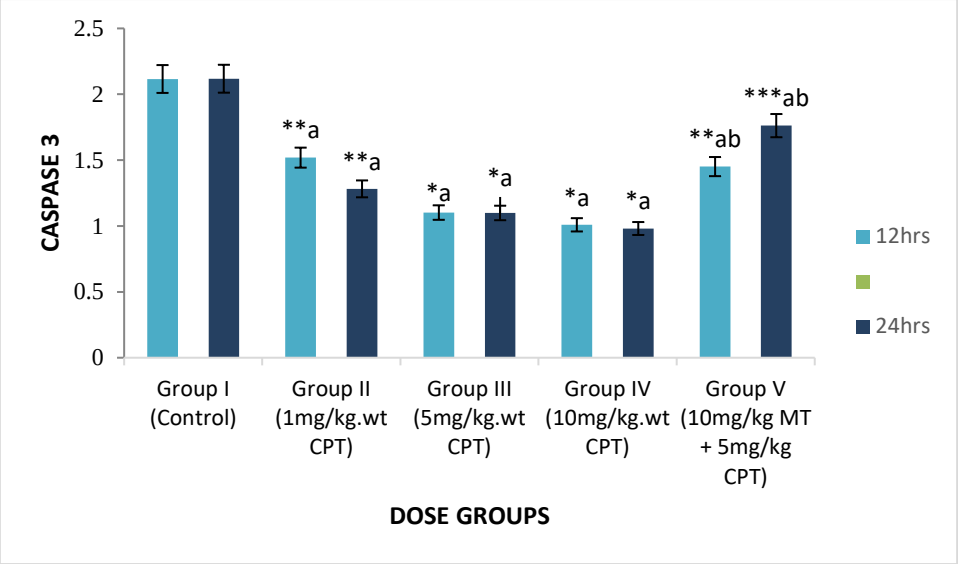

Fig 9: Bar chart showing effect of carboplatin and the ameliorative effect of melatonin when co-treated with carboplatin on caspase 3 level. All values are expressed as mean ± standard error mean (SEM), (n=5), * (p<0.05), ** (p<0.01), * (p<0.001). Note: 'a'- CPT –treated groups vs. control and 'b' CBT+MT vs. 5 mg/kg of CPT .**

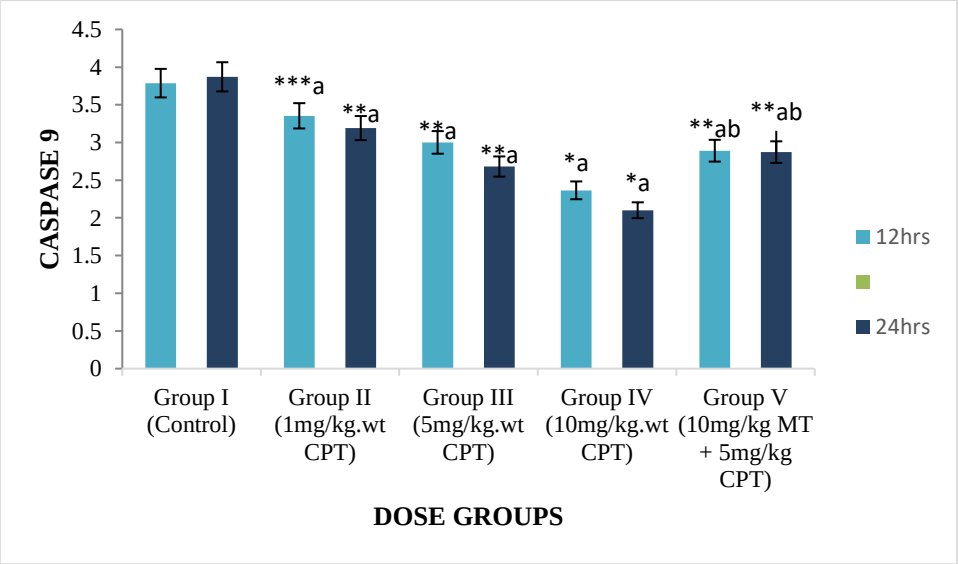

Fig 10: Bar chart showing effect of carboplatin and the ameliorative effect of melatonin when co-treated with carboplatin on caspase 9 level. All values are expressed as mean ± standard error mean (SEM), (n=5), * (p<0.05), ** (p<0.01), * (p<0.001). Note: 'a'- CPT –treated groups vs. control and 'b' CBT+MT vs. 5 mg/kg of CPT.**

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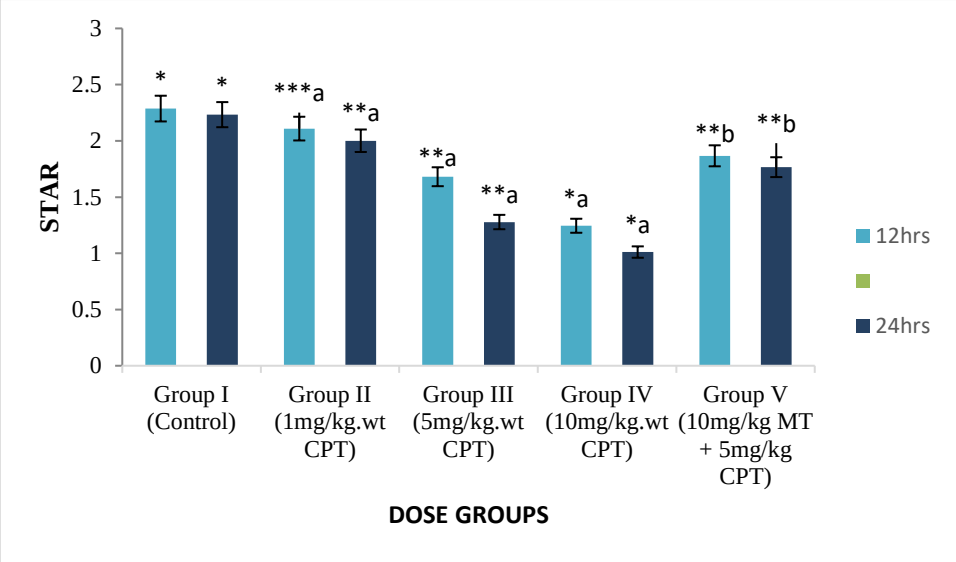

Fig 11: Bar chart showing effect of carboplatin and the ameliorative effect of melatonin when co-treated with carboplatin on StAR level. All values are expressed as mean ± standard error mean (SEM), (n=5), * (p<0.05), ** (p<0.01), * (p<0.001). Note: 'a'- CPT –treated groups vs. control and 'b' CBT+MT vs. 5 mg/kg of CPT .**

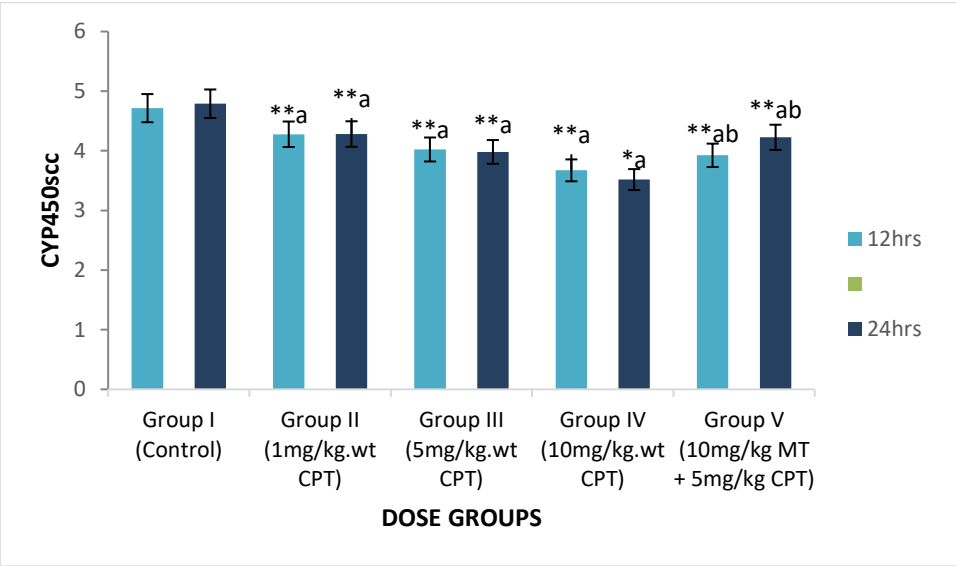

Fig 12: Bar chart showing effect of carboplatin and the ameliorative effect of melatonin when co-treated with carboplatin on CYP450 level. All values are expressed as mean ± standard error mean (SEM), (n=5), * (p<0.05), ** (p<0.01), * (p<0.001). Note: 'a'- CPT –treated groups vs. control and 'b' CBT+MT vs. 5 mg/kg of CPT.**

DISCUSSION

In this present study, ameliorative effects of melatonin were investigated against the adverse effects of carboplatin (CBT) on reproductive tissues of male mice. From the result above the level of reacting oxygen species (ROS) increases with increase in dose of carboplatin (CPT), however melatonin ameliorates the effect. The reacting oxygen species are the product of cellular metabolism in normal condition and readily they are important for cellular signaling pathways. But in some extreme condition, the accumulation or increase in the level of ROS up to a limit can cause pathological condition responsible for various diseases [21]. Diseases cause include, cancer, cardiac disease, alzheimer, parkinson's disease etc. [22]. Free radicals play significant role in controlling the blood pressure and also fight for the cures of infection. Formation of reactive oxygen species is a physiological process, which increases the production of free radicals and also its lead to the imbalance between the production of radicals and antioxidants which could lead to oxidative stress with the changes of various biological functions and structural changes in the cells [23].

As presented above malondialdehyde increases with increase in concentration of carboplatin. Co-treatment with melatonin shows an amelioration of the effect of carboplatin. Free radicals generate the lipid peroxidation process in an organism. Malondialdehyde (MDA) is one of the final products of polyunsaturated fatty acids peroxidation in the cells. An increase in free radicals causes overproduction of MDA. Malondialdehyde level is commonly known as a marker of oxidative stress and the antioxidant status in cancerous patients [24].

Antioxidants such as SOD, CAT and GSH decreases with dosage increase as presented in the result above. Melatonin administration together with carboplatin was show to attenuate the effect of the drug carboplatin. Antioxidants are the compounds that can stabilize ROS. These molecules are the scavengers of free radicals and get easily oxidized. Antioxidants donate their electron to stabilize free radical and make it a stable compound so as to minimize the harmful effect of free radicals [25]. Superoxide dismutase is the cytosolic copper dependent enzyme whereas the mitochondrial superoxide dismutase is the manganese dependent enzymes, stabilize superoxide molecule [26]. Glutathione peroxidase is a selenium dependent enzyme stabilizes the peroxide molecule [27]. Selenium is a cofactor. Glutathione peroxidase is considered the major detoxification enzyme for H_2O_2. This enzyme is found in both the mitochondria and cytosol. The reduced plasma selenium and depressed glutathione peroxidase activities have a correlation between each other which can lead to oxidative stress. Catalase enzyme from peroxisome converts the acidic hydrogen peroxide to water and molecular oxygen [28]. Reduced levels of these antioxidants might lead to increase in the generation of ROS and reactive nitrogen species (RNS), which cause oxidative damage by increasing lipid peroxidation which result in increased level of malondialdehyde in testes leading to a disruption in spermatogenesis and steroidogenesis [16].

From the result of this present study, it was found that there is decrease in the level of testosterone and luteinizing hormone with treated with carboplatin. The two key enzymes involved in the biosynthetic pathway of testosterone are 3-Beta-HSD and 17-Beta-HSD. The activity levels of 3-Beta-HSD and 17-Beta-HSD have been used to study the testicular steroidogenesis of rats in different experimental conditions [29,30]. These two enzymes are having regulatory functions in the maintenance of steroidogenesis and also involves in the synthesis of testosterone. In carboplatin treated mice, a significant decrease in the testosterone (implying decrease in 3-Beta-HSD and 17-Beta-HSD) was observed which clearly indicates the impairment of steroidogenesis. The decreased steroidogenic enzyme activity levels indicate decreased androgen production in experimental mice which in turn lead to decreased reproductive activities in male mice. It seems carboplatin acts on Leydig cells and inhibits the testosterone production which was evident by decrease in the activity levels of 3-Beta-HSD and 17-Beta-HSD enzymes in the testes of experimental mice [29,30]. The testosterone level was significantly increased in carboplatin + melatonin treated mice when compared with carboplatin treated mice. This increase testosterone level in testis indicates the restoration of steroidogenesis and leads to normal fertility in carboplatin + melatonin treated mice.

Sperm head count and sperm morphology was found to be increased in mice treated with carboplatin at different concentration while sperm motility reduces with increase in concentration of carboplatin. Significant decrease in sperm motility indicated adverse effects of carboplatin on spermatozoa function probably through the structure and function of testis. Although, the damaged germinal epithelium in the testis appears to be the main reason for impaired sperm quality however increased lipid peroxidation in the testis might have contributed to abnormality of spermatozoa which may result in infertility [31]. It was evident however that melatonin improved sperm morphology, viability and sperm count. Melatonin ameliorates the effect of carboplatin on sperm parameters.

Decrease in the activities of Caspase-3 and caspase-9 from the results of this present study indicates decrease in apoptosis, however the effect of carboplatin was ameliorated with co-treatment of melatonin. Caspase-9 is an essential component for activation of apoptosis executioner. An activated caspase-9 is able to activate caspases-3 and 7 which are effective players regarding to appearance of apoptosis demonstrations. Caspase-9 and caspase-3 plays a significant role in association with apoptosis demonstrations. It shows that, the inhibition of caspase-9 activity leads to apoptosis prevention.

In this present study, steroidogenic acute regulatory (StAR) protein was found to be decrease with increase in concentration of carboplatin. Melatonin was found to reverse the effect of this anticancer drug. Steroid hormone biosynthesis is acutely regulated by pituitary trophic hormones and other steroidogenic stimuli. This regulation requires the synthesis of a protein whose function is to translocate cholesterol from the outer to the inner mitochondrial membrane in steroidogenic cells, the rate-limiting step in steroid

hormone formation. The steroidogenic acute regulatory (StAR) protein is an indispensable component in this process and is the best candidate to fill the role of the putative regulator. StAR is involved in the transportation of cholesterol. The positive and negative expression of StAR is sensitive to agents that increase and inhibit steroid biosynthesis respectively [32].

In the present study CYP450 level decreases, this indicate an adverse effect of carboplatin on its activity. However, increase in CYP450 enzyme in the Testis of mice indicates the restoration of normal metabolism in carboplatin + melatonin treated mice. CYP450 enzymes are known to be involved in the metabolism of a variety of anticancer drugs such as carboplatin. CYP activities are known to be modified by several factors including genetic polymorphisms, changes in physiological conditions such as are age, disease status or intake of certain drugs or foods or environmental factors such as smoking. These factors may cause inter-individual differences in the pharmacokinetic profiles of anticancer drugs, leading to the variations of efficacy or toxicity of the drugs. Low level of CYP450 enzyme leads to retardation of the steroid metabolism. In this study, melatonin is seen to exhibit ameliorative potential on carboplatin adverse effect and as a potent substance for the prevention of the adverse effect of this compound on the reproductive organ of male mice.

Conflict of Interest Statement

The authors stated that there are no conflicts of interest regarding the publication of this article.

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