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Research Paper

Melatonin Attenuates Thiol/Disulphide Alterations In Testicular Dysfunctionof Capecitabine-Induced Swiss Albino Mice

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ABSTRACT
Capecitabine (CPT) compound has shown various therapeutic potentialsincluding antitumor, antituberculosis and antimicrobialactivities. However, severalreports confirmed different adverse reactions due to the administration of the compound.Therefore, this work was carried out to investigate the ameliorative effect of melatonin against capecitabine-induced testicular dysfunctions using mouse model. Albino mice were randomly selected into 3 groups (n=5), Group I served as the control, Groups II received 5mg/kg.bwt of CPT and animals in Group III were given 5mg/kg.bwtof CPT co-treated with 10mg/kg.bwt of Melatonin via intraperitoneal injection.Animals from each group were sacrificed at interval of 7 days, 14 days and 21 days after capecitabine exposure. Biochemical and reproductive hormonal analyses were carried out. Native thiol, total thiol, and disulphide levels were measured. Role of apoptosis was evaluated by measuring CASP-3 and CASP-9 activities using ELISA method. Histoarchitectural changes of the testes were observed by light microscopy. It was observed that CPT led to significant decrease of all the anti-oxidative enzymes measured in a dose-dependent manner with simultaneous raised in malondialdehyde and reactive oxygen species. CBT also led to significant decrease in the testicular testosterone level and apoptotic markers evaluated. However, the animals co-treated with melatonin showed appreciable recovery in all the tests. This study therefore suggests that melatonin supplement may help in reducing the deleterious effects of capecitabine against male reproductive system.

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I. Introduction

Capecitabine a thymidine phosphorylase activated fluoropyrimidine carbamate, one of the approved orally-administered chemotherapeutic agent used in the treatment of numerous cancers (Rossi, 2013)is orally administered as a precursor of 5-fluorouracil (5-FU) which convert to 5-FU preferentially in human liver and cancer tissue (Miwa *et. al.,* 1998).Fluorouracil metabolized into two active metabolites, 5-fluoro-2'-deoxyuridine 5'-monophosphate (FdUMP) and 5-fluorouridine triphosphate (FUTP) which cause cell injury by two different mechanisms. Firstly, FdUMP and the folate cofactor, N5-10-methylenetetrahydrofolate bind to thymidylate synthase forming a covalently bound ternary complex. The binding therefore inhibits the formation of thymidylate, the necessary precursor of thymidine triphosphate which is essential for the synthesis of DNA, therefore a deficiency of this compound can inhibit cell division. Secondly, nuclear transcriptional enzymes can mistakenly incorporate FUTP in place of uridine triphosphate (UTP) during the synthesis of RNA. This metabolic error can interfere with RNA processing and protein synthesis through the production of fraudulent RNA. Several authors outlined the reproductive toxicities of the postanticancer-treatments of this compound. A single dose administration of 5-fluorouracil (5-FU) to Wistar rats for 1, 3,15, and 30 days treatments were found to decrease the testes weight, seminiferous tubular diameter, and germ cell height (D'Souza and Narayana,2001). Sperm abnormalities including amorphous, coiled, and double headed/double tailed spermatozoa.Decrease level of testosterone level was also detected in rats that were exposed to capecitabine.(Takizawa and Horii 2002). Decreased testosterone level was found to be associated with degeneration of Leydig cells postbleomycin, etoposide and cisplatin treatment (Al-Bader and Kilarkaje, 2015). This study was designed to investigate the exchange in thiol/disulphidehomeostasis as abiomarker for oxidative stress status in testicular tissue impairment and the toxicity induced by capecitabine drug. Also, to investigate

The implication of apoptosis in capecitabine testicular impairment and evulated the protective effect of the melatonin.

Keywords: Capecitabine; melatonin; Testicular dysfunction; Oxidative stress; Apoptosis

II. Methodology

2.1 Animals and Experimental procedure

Male Swiss-albino mice were housed in room at 22 ± 2$^{\circ C}$ with 40% relative humidity and with a 12-hr light ± dark cycle. They were fed with a standard rat chow and tap water ad libitum. The studies were conducted in accordance with the standards and permission established by The Ethics Committee of Animal, Ekiti State University Ado-Ekiti, Nigeria.

2.2 Dose grouping

Male mice were divided into three groups of animals (n=10) and intraperitoneal dosing of 5mg/kg/bwtCapecitabine and 10mg/kg/btw Melatonin+5mg/kg/bwtCapecitabine were administered daily to all the animals in group II and III respectively.Group I servedas the control.

2.3. Glutathione (GSH) Determination

Testis tissues were homogenized in 10 ml TCA (trichloroacetic acid) which is at the rate of 10%, and then centrifuged at 4°C for 15 minutes. Afterwards, 0,5 ml of supernatant was taken, and mixed with 2 ml of 0.3M Na_2HPO_4. The mixture was thoroughly vortexed. This mixture was vortexed by the addition of 0.2 ml DTBN (Dithiobisnitrobenzene: prepared by dissolving in 1% sodium citrate). Finally, the absorbance was measured at 412 nm.

2.4. Measurement of 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 (186 µM), nitroblue tetrazolium (300 µM) and NADH (780 µM) were diluted with appropriate enzyme in total volume of 3 ml. The mixture was incubated at 37^{o}C for 90 sec 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 520 nm [16].

2.5. Measurement of Malodiadehyde (MDA)

Testes tissues were homogenized in 10 ml TCA (trichloroacetic acid) which is at the rate of 10%, and then centrifuged at 4°C for 15 minutes. 750 µl of the supernatant which was 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 15 minutes. Finally, the absorbance was measured spectrophotometrically at 535 nm.

2.6.Measurement of reactive oxygen species (ROS) level

The ROS assay was performed by the method of Hayashi., et al [14]. In brief, 50 µl of tissue homogenate and 1400 µl sodium acetate buffer were transferred to a cuvette. After then, 1000µl of reagent mixture (N,N-diethyl para phenylenediamine 6 mg/ml with 4.37 µM of ferrous sulfate dissolved in 0.1M sodium acetate buffer pH-4.8) was added at 37°C for 5 minutes. The absorbance was measured at 505 nm using spectrophotometer (Molecular Devices.) ROS levels from the tissue were calculated from a calibration of H2 O2 and expressed as U/mg of protein (1 unit = 1.0 mg H_2O_2 /L)

2.7. Measurement of Thiol Biomarkers

Testicular homogenate samples from capecitabine-treated animals along with the group that was co-treated with melatonin wereused to measure of levels of native thiol (SH), total thiol (total SH), and disulphide (SS) biomarkers. The homogenate was separated by centrifugation at 1500g for 10 minutes and stored at -20°C for analysis(Erel and Neselioglu [9])Testicular dynamic disulphide/thiol homeostasis as SH, total SH, and SS was assessed and then calculated the %SH/total SH, %SS/SH, and %SS/ native SH ratios.

2.8. Determination of Casp-3 and Casp-9 activities

DNA fragmentation was quantified in the cytosol by measuring the content of cytosolic mononucleosomes and oligonucleosomes (180-bp nucleotides or multiples) using a Cell Death ELISA (Roche Molecular Biochemicals, Germany) according to instructions from the manufacturer. Results were reported as arbitrary absorbance units normalized to mg of protein. Cytosolic cytochrome c was quantified using an ELISA kit from R&D Briefly, 1 ml of assay buffer (20 mM HEPES, 10% glycerol, 1 M DTT, and 14 lofn-acetyl-DEVD-AMC/ml of buffer), and 50 l of sample were added to a microcentrifuge tube and protected from the

light. Samples were incubated at 37°C for 60 min after which fluorescence was measured on a spectrofluorometer with an excitation wavelength of 380 nm and an emission wavelength of 440 nm.

2.9. Determination of Bcl-2 and Bax Levels

To quantify the amounts of mitochondrial Bcl-2 and Bax, ELISAs were performed. Plates were coated with 1g of mitochondrial protein in PBS and sealed overnight at 4°C. Bcl-2 and Bax peptide were included with a concentration range from 1g/ml to 4 ng/ml. The specificity of both antibodies has been confirmed by peptide inhibition experiments, and the sensitivity of this ELISA was 0.1 ng/ml with coefficient of variance 3.06 and 3.11% for Bax and Bcl-2, respectively. The plates were washed with buffer containing PBS with 0.02% sodium azide and 0.05% Tween-20. The wells were blocked with 300 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 5g/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 at 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.

III. RESULT

3.1.Effect on Reactive Oxygen Species (ROS) level

The 5mg/kg/bwt concentration of Capecitabine-exposure in testicular tissue of male swiss albino mice results in significant ($p<0.05$) increase of reactive oxygen species level across all the groups when compared to the control group. However, 10mg/kg/btw Melatonin (MT) co-treated with 5mg/kg/bwt CBT, significantly ($p<0.05$) decrease the generation of ROS (Fig 1)

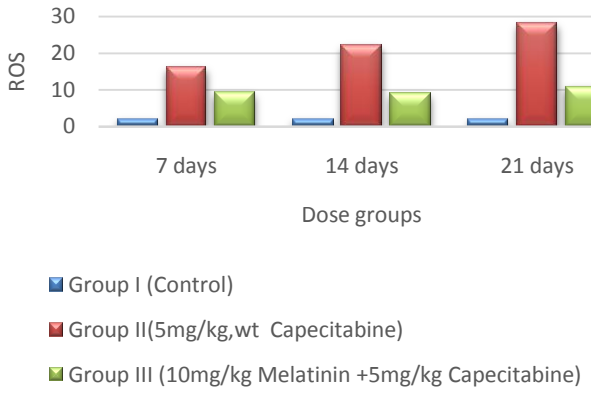


Fig.1: Bar chat representing the effect of CBT and co-treatment of CBT+MT on Reactive Oxygen Species. All values are expressed as LCM. (n = 5), ***p < 0.001, **p < 0.01 and*p < 0.05, 'a'- CBT vs. control and 'b' CBT+MT vs. CBT -5 mg/kg.

3.2. Ameliorative effect on MDA

On the degree of malondialdehyde, the lipid peroxidase of testicular tissue was determined. Capecitabine increased significantly ($p < 0.05$) the level of lipid peroxidase of testicular tissue using malondialdehyde across the dose group compare to the control group. But when 10mg/kg/bwt MT + 5mg/kg CBT was co-treated, it decreases the effectiveness of capecitabine significantly across the dose group. The control was compared with the co-treated doses at 7 days of the dosage also shows significant increase ($p<0.05$).

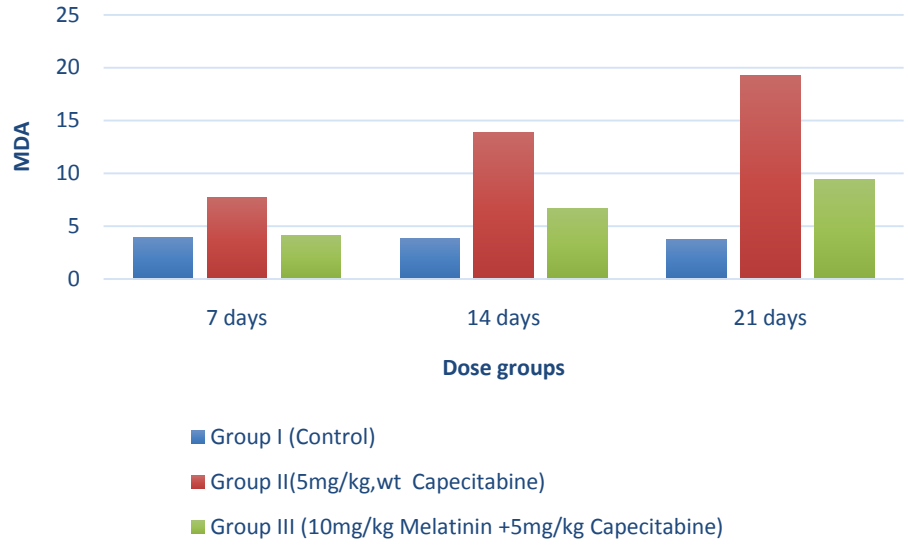


Fig. 2: Effect on MDA. All the values are expressed as mean ± SEM, (n = 5), ***P < 0.001, **P < 0.01 and*P < 0.05, 'a'- CBT vs. control and 'b' CBT+MT vs. CBT -5 mg/kg.

3.3 Effect on Anti-oxidants

Capecitabine administered rapidly reduces the level of superoxide Dismutase (SOD) within the hours of dose groups significantly ($p<0.05$) compared the control groups. When 10mg/kg/btw MT + 5mg/kg CBT, was combined, it significantly ($p<0.05$) raised the SOD level as shown in Fig 3.

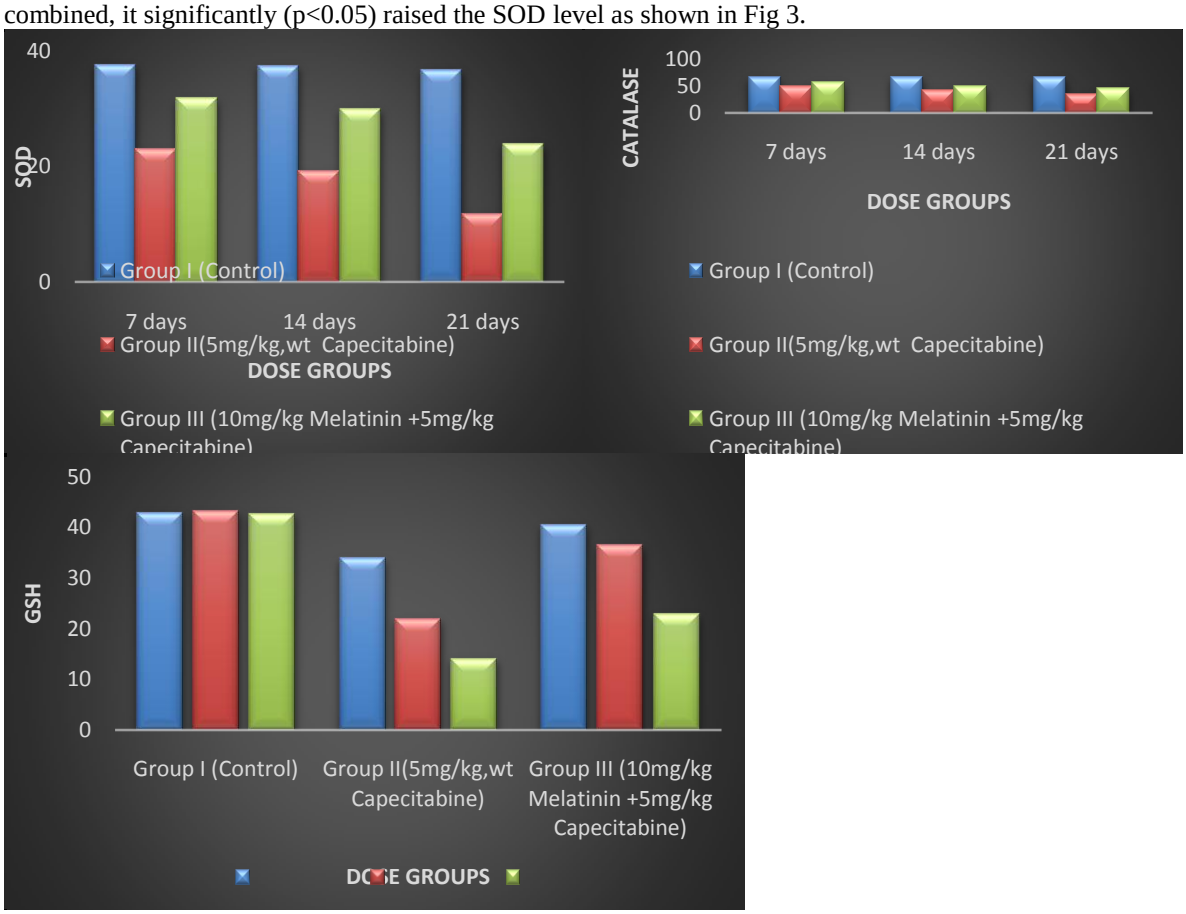


Fig. 3: Protective effect of MT (a)SOD, (b) CAT and (c) GSH depletion in CBT-treated testis. All the values are expressed as mean ± SEM, (n = 5), ***p < 0.001, **p < 0.01 and*p < 0.05, 'a'- CBT vs. control and 'b' CBT+MT vs. CBT -5 mg/kg.

3.6 Thiol Results

The levels of Native SH and Total SH in CBT group decrease drastically compared with the control ($p< 0.05$) as capecitabine was administered and this reduction gradually proceed to the 21 days when compared with the control group and or the co-treatedCBT ($p<0.05$).

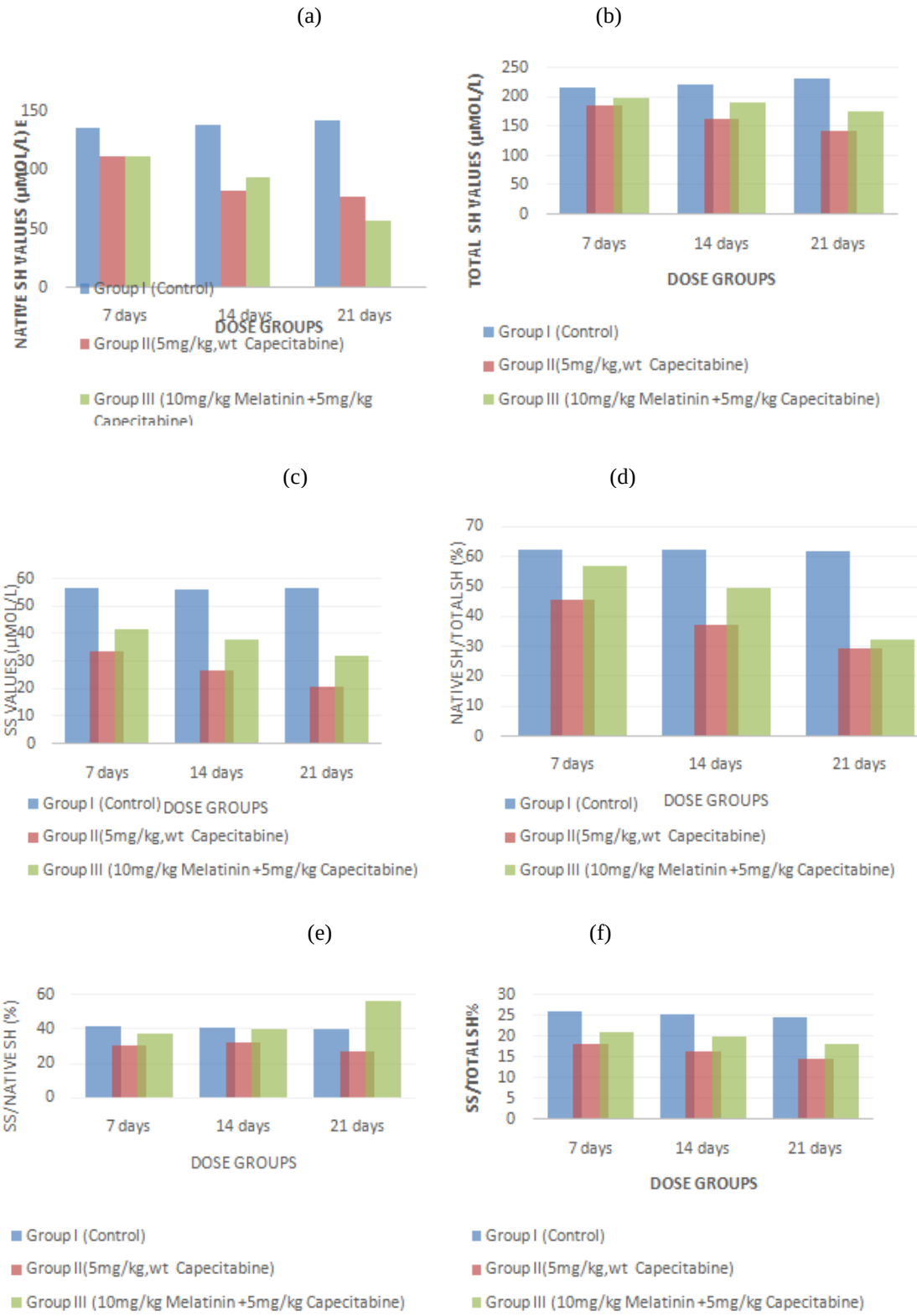


Fig. 4: There were significantly decrease ratios in CBT group with %SS/SH, SH/Total SH, SS/Native SH across the group. The co-treated ratios increase mildly compared to capecitabine. However, there was increase at thetwenty-one days of the co- treated ratio in %SS/Native value compared to all in dose group (figure 4 a-f).

The changes in thiol level of the testiscular tissues of capecitabine treated mice (a) Native thiol (SH) levels. (b) Disuphide (SS) levels. (c) Total thiol (SH). (d) %Native SH/ Total SH ratios. (e) %SS/Native ratios (f) %SS/Total SH ratios.values are expressed as mean ± standard deviation. Values significantly different from saline control and duration. ***p < 0.001, **p < 0.01 and*p < 0.05, 'a'- CBT vs. control and 'b' CBT+MT vs. CBT -5 mg/kg.

3.7 Effect of capecitabine on Caspase 3 and 9 Activities

Caspase 3 activity decreases down the dose group significantly ($p<0.05$) lower than the control group when treated with CBT. When 10mg/kg/btw MT + 5mg/kg CBT was co-treated, there was a significant increase in caspase 3 activity close to value of the control within the 7 days of dosage group with mild decrease across the dose group compared to capecitabine alone. There was a rapid increase in caspase-9 intrisinc pathway activity significantly as capecitabine was administered ($p<0.05$) compared with the control. However, the co-treated melatonin attenuated the effect of capecitabine by decrease in caspase 9, although a zig zag decrease was seen at 21days in co-treated does group the dose (Fig. 5).

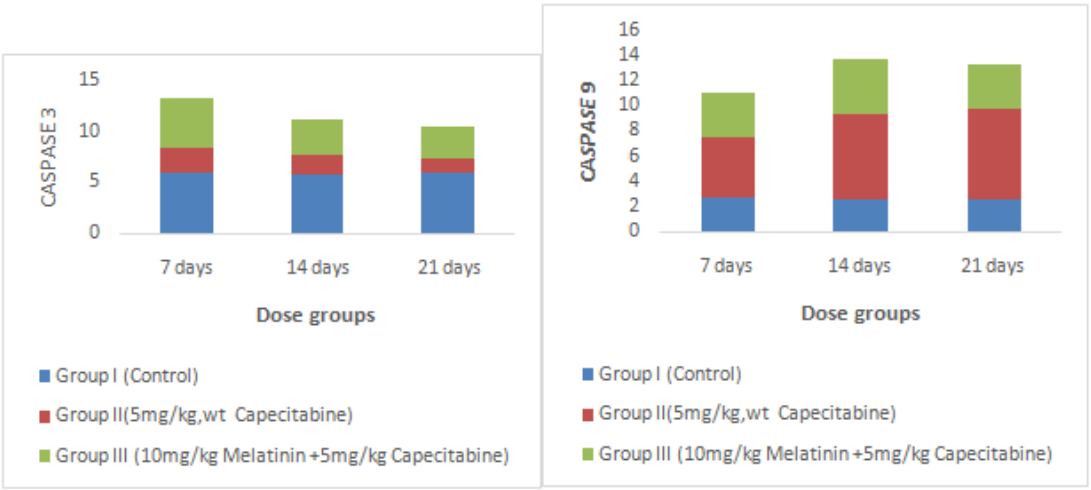
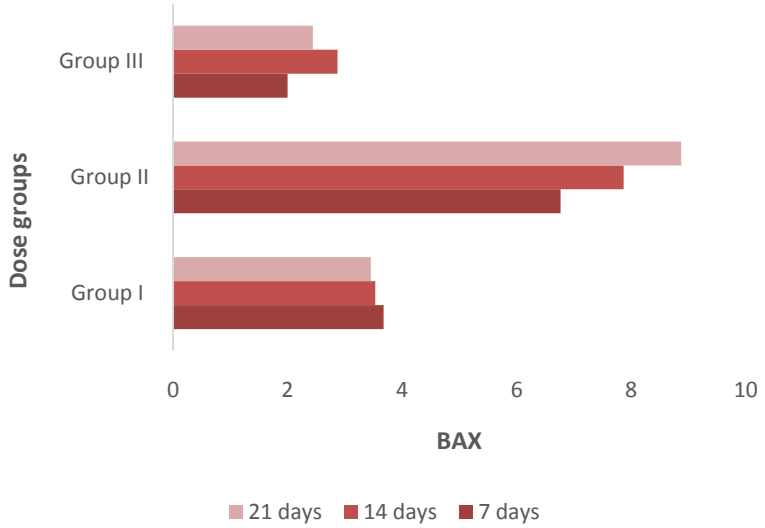


Fig.5: Caspase 3and 9 activitieson capecitabine induced testiscular toxicity and CBT co-treated with MT in swissabino mice. All the values are expressed as mean, ($p<0.05$). All the values are expressed as mean ± SEM, ($n = 5$), ***p < 0.001, **p < 0.01 and*p <0.05, 'a'- CBT vs. controland 'b' CBT+MT vs. CBT -5 mg/kg.

3.9 Effect of CBT on BAX and BCL-2

There was a drastically increase in the BAX when capecitabine induced on the swissalbine mice across the dose group significantly ($P<0.05$). When 10mg/kg/btw MT + 5mg/kg CBT was administered a reductant in the effectiveness of the induced capecitabine on BAX was seen (Fig6) compared to control level significantly with a zig zag pattern at 21 days of dosage was compared to the group. As shown in Fig. 6. Capecitabine induced reduced the level of BCL-2 form of cancer with a low decrease compared to control group. When 10mg/kg/btw MT + 5mg/kg CBT was co-treated there was an effective rapid increase significantly ($p<0.05$) between the 7 days and 14days of the dosage and decrease down the 21 days (Fig 6).



(b)

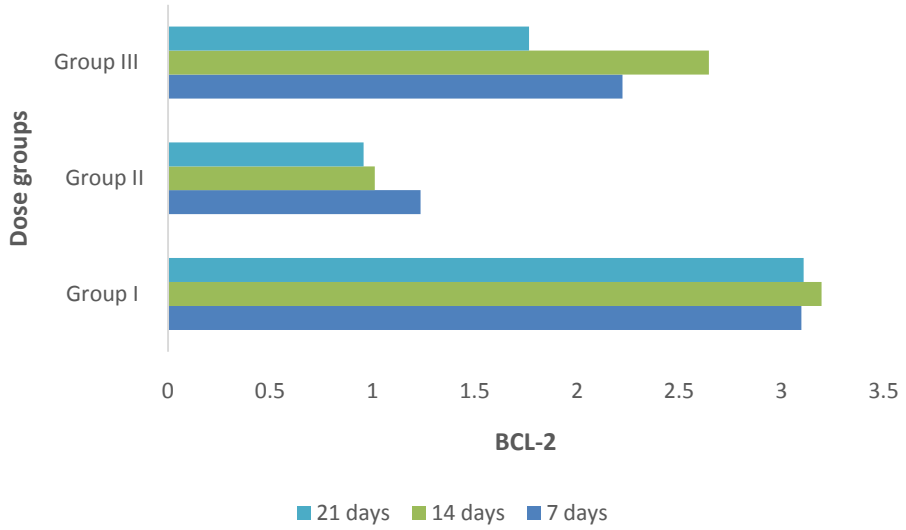


Fig 6. Effect of CBT on BCL-2 Associated X Protein (BAX) And CBT + MT All the values are expressed as mean, ($p<0.05$). All the values are expressed as mean ± SEM, (n = 5), ***$p < 0.001$, **$p < 0.01$ and*$p < 0.05$, 'a'- CBT vs. control and 'b' CBT+MT vs. CBT -5 mg/kg.

IV. DISCUSSION

In this study, the toxicity of cepcitabine in the testicular tissues was shown, capecitabine has a well-distributed systemic circulation and an easy passage from the serous membranes (Ilhan, *et.al.,* 1999)It is commonly known that most drugs, especially chemotherapeutic agents, mediate their toxic effect through the generation of reactive oxygen species (Wang and Yi 2006), which generates more reactive oxygen species causing testicular damage. In this study, 5mg/kg/bwt concentration of Capecitabine exposure significantly increase of reactive oxygen species level across the dose group compared to the control in Fig 1 Reactive oxygen species (ROS) elevation characterized the endothelial response ROS is also important chemical messengers, with an essential role in cell homeostasis, growth and proliferation. Many reports have revealed a role for ROS as mediators of the death program, while other reports have shown that oxidative stress inhibits apoptotic signaling (Choi, *et. al.,* 1996; Aronis *et. al.,* 2003).

Cytochrome C is another form of antioxidant that attacks the free radicals, also special role cytochrome C has recently been confirmed in reducing testicular H_2O_2. Cytochrome C which is a small protein, liken to coenzyme Q (ubiquinone) in terms of motility. The cytochrome C isoform is regarded as a capable apoptosis activator and plays a part in increasing the protective capacity of testicular tissue via eliminating damaged of germ cells (Liu, *et .al.,* 2006). These responses were prevented by a ROS scavenger. Oxidative stress causes

increase in DNA breakdown (WHO, 1999), evidence indicates that fragmentation of DNA is commonly seen in infertility of the spermatozoa due to the high concentration of ROS in sperm ROS (Bennetts and Aitken, 2005).The flunctuation balance of ROS production and elimination, which necessitate normal cellular homeostasis, disturbance. The cellular damages that occur in the form of breaking down of biomolecules such as: proteins, lipids, carbohydrates, and DNA which annex in cellular death. In a study on DNA samples in people with teratozoospermia, the DNA damage rate was higher in the samples of people with spermatozoospermia than in those of healthy people; moreover, this damage was demonstrated to be mainly due to the amount of ROS produced by these sperms which can be the cause of infertility in people with spermatozoospermia. Therefore, a main reason for infertility is believed to be excessive production of ROS or decreased antioxidant capacity in semen that causes oxidative stress conditions and ultimately decrease in sperm motility, increase in sperm death, and fragmentation of DNA (Said, *et. al.,* 2005).

CAT and SOD are intracellular enzymes which form the primary defense system against oxidative stress (Erel, *et. al.,* 1997) SOD catalyses the dismutation reaction that converts the superoxide ion into hydrogen peroxide, which is rapidly removed by the catalase enzyme that turns it into water and oxygen (Casado. *et. al.,*2001). The result of this study confer a significant decrease in the activities of CAT and SOD in testicular tissue of the animal dose group that only capecitabine was administered seen in fig.3, the co-treated melatonin with CBT increased the level of these scavengers relatively to the control across the the group. The fact that catalase removes H_2O_2 generated in testicular tissue by SOD indicate that accumulation of H_2O_2 might be responsible for the inactivation of SOD, which is confirmed by its concomitant low levels observed in this study. In addition, the significant decrease in the levels of CAT and SOD may likely affect the vulnerability of oxidative stress induced by H_2O_2, HO^- radicals and the superoxide ion (Rattan, 2006). Anyway, in this study, the co-treatment of MT+CBT revealed a significant protection by raising the levels of both SOD and CAT to near the expected control level of animal dose groups although, there was differentiated decrease across the group at 21 days (Fig 3 a,b,c)).

.Lipid peroxidation involve the molecular mechanism of the lipid cellular oxidation, which is a accepted as an indicator of oxidative stress resulting from intense ROS and depleted antioxidant levels (Packer and Cadenas 2002). On the degree of malondialdehyde, the lipid peroxidase of testicular tissue was determined. Capecitabine increased significantly, the level of lipid peroxidase of testicular tissue using MDA biomarker. However, the level of MDA was reduced when co-treated with melatonin. MDA levels resultant of lipid peroxidation, used as a relevant procedure in introducing oxidative damage. Hence the treatment with CBT significantly increase MDA level, enhances reduce in SOD and CAT activity in testicular tissue (Fig 2).The presence of polyunsaturated fatty acid (PUFA) tails on phospholipid components in biological membranes has been indicated to cause the fluidity property of those membranes (Ayala, 2014). Excess free radicals attack the PUFA and introduce lipid peroxidation leading to MDA generation. Glutathione (GSH) is an antioxidant in plants, animals, fungi, and some bacteria and archaea. Glutathione is capable of preventing damage to important cellular components caused by reactive oxygen species such as free radicals, peroxides, lipid peroxides, and heavy metals, this is done by reacting with ROS or inactivation of ROS catalyzed by GSH peroxidase to produce the following during the reaction, GSH disulphide, oxidized GSH (Pompella*et al.,* 2003). GSH has a functional group of thiol which can be modified and involve reversible oxidation of thiol to disulphide (SS). The relevance of the GSH molecule affix with its capacity to regenerate active forms of both vitamins C and E, and with the detoxifification of endogenous and exogenous toxic substances in the body (Masella, *et. al.,* 2005). In the present study, a signifificant reduction in the levels of GSH and vitamin C, and in the activities of GSH when group treated with capecitabine decreased the level of GSH comparatively lower than the control down the dose groups was observed relative to the control group. Which confirms that capecitabine is toxic (Hidir, *et. al.,*2010). However, When MT + CBT 10mg/kg/btw MT + 5mg/kg CBT, was administered GSH activities increased relatively to the control although decrease down the dose group (Fig.3c).melatoninfeasilbly improved the balance of redox reactin in the testicular tissue of the swissabino mice. Whereby they are efficient in obtaining GSH content

Caspases are cysteinyl aspartate proteinases identified as crucial mediators of the complex biochemical events associated with apoptosis (Thornberry,*et.al.,*1997) exist at the core of the apoptotic pathways, and caspase-3 is the most notable among the ‘‘executioner’’caspases (Kwak, 2013). In this research, the caspase-3 activity result shown that capecitabine decreases the activity of caspase 3 down the dose group lower than the control group. This results show that the decreased caspase-3 activity was aided by antioxidant and radical remover. It has been presented that ATP is required for apoptotic cell death, and the level of intracellular ATP determines whether cell death signaling favors apoptosis or necrosis (Leist et al., 1997). Therefore, it can be concluded that increased intracellular ROS through a mitochondrial pathway triggers either an apoptotic or necrotic mode of cell death in capecitabine -treated testiscular tissues of an albino mice. Capecitabine rapidly increase the activity of caspases-9 intrisinc pathway above the control the co-treated CBT reduced the effect of as capecitabine relatively toward control level especially at 7 days and 21 days. (Fig. 5)

Caspase-9 is a very vital component of the mitochondrial death pathway. In this pathway, cell death signals lead to cytochrome c release from the mitochondria, which binds and facilitates the formation of the septamericapoptosome that recruits and activates caspase-9 (Acehan, *et. al*., 2002). The apoptosome-bound caspase-9 cleaves and activates caspase-3. It has been displayed that these caspases have an outcom on the mitochondria and on upstream events of intrinsic apoptosis, even though they are thought to act downstream of cytochrome c release. Caspase-9 has been revealed to lose the mitochondria and increase ROS production, while cells defective in caspase-3 or caspase-7 show a hindrance in the mitochondrial events of intrinsic apoptosis (Cepero, *et. al.*,2005) Caspase-3 have been shown to have differential activity toward multiple substrates,, which mention the ideal of an unecessary role for these related caspases (Walsh, *et.al*.,2008). Taken together, these data indicate that caspase-9, caspase-3 and caspase-7 have vital roles during apoptosis and that there may be a feedback loop on the mitochondria as well as additional upstream functions.

A previous study showed that the apoptosis of testicular germ cells increases in diabetic mice, leading to the disruption of spermatogenesis (Sainio-Pöllänen, *et. al.*,1997) Apoptosis occurs in the testes of diabetic animals, but the mechanism of apoptosis has not yet been clarified (Koh, 2007). Apoptotic cell death is mediated by the activation of apoptotic signaling pathways, including the Bcl-2 family proteins. Bcl-2 and Bcl-xL suppress apoptotic cell death through anti-apoptotic function, whereas Bax and Bad promote apoptotic death through pro-apoptotic function (Koh, 2007). In our study There was a drastically increase in the BAX when capecitabine induced on the swissalbine mice across the dose group, but value of CBT decreased below the control group, but only closer at 21 days of when co-treated with melatonin, the drug MT acting as s suppressant. Melatonin was used in the our study as antioxidant therapy. The administration of melatonin given orally is effective in exerting antioxidant effects in experimental breast cancer (Anisimov, *et.al.,*2003). The mechanisms whereby melatonin recovers antioxidant status may involve specifific membrane and nuclear receptor stimulation (Rodriguez, *et. al.,* 2004) Both bcl-2 and bax serve as apoptosis regulator, our study reveal that Capecitabine induced reduce the level of BCL-2 form of cancer below the control group, while the CBT + MT increase the level bcl-2 between7 dayss and 14 days closer to the control level. Bcl-2 is form of cancer result from damage b-cell or the overproduction of unwanted b-cell from the immune system.The excess secretion of ROS can result to process of apoptosis by increasing caspase-3 activity and inhibiting Bcl-2 expression, which indicate the relation pathway between apoptosis and oxidative stress (Tang, *et. al.,* 2007)

The thiol groups of sulfur containing amino acids (cysteine and methionine, etc.) in proteins are oxidized by oxygen radicals and converted to reversible disulphide bridges. The conformational and functional changes in physiological proteins occur due to the loss of -SH groups. -SH groups attempting to impede the harmful effects of free radicals might decrease the serum and tissue concentrations of these radicals (Topuz *et.al.,*2017). Thiols are very important molecules in the antioxidation process that contain –SH groups. –SH groups can reduce electrons so organism can be protected from oxidative damage caused by ROS. Thiols are converted to disulphides by this reaction. In the past, only a single side of this double-sided balance had been measured. Previous studies have reveal that thiol/disulphide homeostasis is disturbed in the lung disease such sleep apnea syndrome. (Punnonen, *et.al.,*1994). From Solak *et al.,* revealed that thiol/disuphide homeostasis shifts through disulphide oxidative stress of smokers. The decrease in thiol level shows the inadequate antioxidant in pregnant women with the obstructive sleep apnea syndrome. The alteration of thiol/disulphide homeostasis was studied with the thiol biomarker which include total SH, native SH, and SS in testicular tissue of albino mice in the CBT and CBT + MT dose groups within 21 days. the of native SH, total SH, and SS decreased across the dose group lower than the control from 7 days to 21days , but result of co-treated CBT was not as obvious as when CBT was treated alone compare to the control (Fig 4). Percentage relation between those biomarker of which are; %native/total, %SS/native, %SS/total thiol, was apparently similar to the initial case Fig4a-c, however a diverse change occur in the result of %SS/native with significant high ratio at 21 days although lower than the control, while there was an irreversible increase in the CBT + MT ratio whereby at the 21 days was significantly the same when compared to control ratio level (Fig 4). The stability between ROS procreation and degradation mechanism by the antioxidant can lead to high generation of ROS and the synthesis and weaken the antioxidant defense system during apoptosis. In apoptosis, generation of oxidative stress leads to disturbance of protein thiols. (Kannan2000). One the main reason for our research is to show that the alteration of thiol/disulphide homeostasis and to know if there is a played role in toxicity in the testicular tissue development via our result from this research, toxicity from caused by capecitabine which contain cytotoxic effect by incorporation of the RNA & DNA by the activity of metal and oxygen molecule, OH^- radicals, the free radical cause the breaking in the DNA component of protein which certainly leads to apoptosis (Rahman, *et. al.,* 1999). The SH thiol group help to protect against inflammation that has to do with oxidative stress. Hence the level of SH in the blood and tissue can be depleted by oxidation ,whereby there is a possibility for newly formed SS group to be converted to SH groups, thereby sustaining SH/SS homeostasis (Erel and Neselioglu 2014).This study reveal that the thiol/disulphide homeostasis can be altered as a result of the toxicity induced from capecitabine in testicular tissue, however, a therapeutic combination with melatonin improves the antioxidant status as a protective effect against apoptosis, oxidative stress and other testicular damages.

Conflict of Interest Statement

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

Acknowledgment

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