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Biochemical studies and expression of Atg7/LC3 autophagy genes in mephalan-induced testicular dysfunction

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Abstract

Autophagy is an intracellular lysosomal degradation pathway and plays a very important role in maintaining intracellular homeostasis. This present study demonstrates the biochemical status and expression of ATG7/LC3 autophagic genes in Melphalan-induced testicular dysfunction. Twenty make Swiss albino mice were maintained under standard conditions of humidity (50 ± 5%), temperature (25 ± 2° C) with free access to food and water. The mice were divided into five groups (n=5 each) and treated by intraperitoneal injection as follows; Group I: vehicle-treated control; Group II: 1 mg/kg/bwt MEP; Group III: 3mg/kg/bwt MEP; Group IV: 5mg/kg/bwt MEP. Histopathological and histochemical evaluation by light microscopy, biochemical assays and sperm parameters evaluation are the various investigation depicted in this study. Result shows a rise percentage of DNA damage and teratozoospermia Index (TZI); decrease of protein concentration and total antioxidant capacity (TAC). Testosterone concentration was depleted. The result also shows a significant decrease of Luteinizing Hormone (LH) level across the groups when compared to the control. This study confirms oxidative stress by increased ROS and MDA levels in the testes of the melphalan-treated mice and defective antioxidants response as evident from diminished activities of antioxidant enzymes. The autophagic gene markers showed an upstream Atg7 gene and downstream of LC3-II/LC3-I ratio. Autophagy is mediated by the expression of Atg7 and LC3 genes and these varying mRNA expression led to autophagic defect.

Keyword: Autophagy; Melphalan; Total Antioxidant Capacity; Hormones; Testes

1. Introduction

Autophagy is considered to be a process conserved during evolution that plays an important role in physiological and pathological conditions. Its main role is the degradation of harmful cytoplasmic components such as damaged organelles and poorly folded proteins that are no longer needed. Thus, autophagy contributes to reduce the risk of formation of toxic protein aggregates and promotes cell survival (Loos *et al*., 2013). This catabolic process can be activated under various stress conditions such as oxidative stress, thermal stress, endoplasmic reticulum stress, hypoxia, and unbalanced diet. In pathological situations including infection and cancer and neurodegenerative, cardiovascular, and autoimmune diseases, the roles of autophagy have been well demonstrated. At the cellular level, during autophagy, some of the cytoplasmic proteins and organelles are sequestered into double membrane vesicular formations called autophagosomes that fuse with the lysosomes to degrade their contents. The resulting simple molecules, including free fatty acids, amino acids, and nucleotides, are then recycled and reused as an energy source by the cell (Van Erp *et al*., 2017). According to recent reports, under physiological conditions, autophagy might contribute to spermatogenesis. In the mouse, the knockout of the autophagic gene Atg7 demonstrated its involvement in acrosome biogenesis by regulating the transport and/or fusion of proacrosomal vesicles derived from Golgi. In human, it has been shown that autophagy is crucial for maintaining seminiferous tubules in stressful situations, such as exposure to formaldehyde.

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Recently, research on male infertility has highlighted the pro-survival role of autophagy in the process of differentiating spermatogonia into spermatozoa (Song *et al*., 2012).

Melphalan is an antineoplastic agent that acts as a bifunctional alkylating agent used in the treatment of multiple myeloma, advanced ovarian adenocarcinoma, early and advanced breast cancer, childhood neuroblastoma, and polycythaemia vera. Melphalan is also used for regional arterial perfusion in localized malignant melanoma, and localized soft-tissue sarcoma of the extremities (Dent et al., 2000). It is usually combined with corticosteroids (Eastin et al., 2001). Melphalan has been tested in mice by oral, intraperitoneal, and dermal application, reports confirmed the generation of ROS leading to induction of apoptosis in epididymal sperm by increasing pro-apoptotic protein levels and decreasing anti-apoptotic protein expression (Ojo, 2020), Also, results from study in monkey was inconclusive (Schoeffner and Thorgeirsson, 2000). Due to all the observation so far on the investigation of melphalan, this study is therefore designed to explore the roles of expression of Atg7/LC3 autophagy genes in melphalan-treated testicular tissue and also to measure the activity levels of some oxidative stress biomarkers.

2. Material and methods

2.1. Animals grouping and Treatments

Male and female Swiss albino mice weighing about 25 g were obtained from the Laboratory Animal Division of the University. The animals were maintained under standard conditions of humidity (50 ± 5%), temperature (25 ± 2^0C) and dark and light cycles (12h each) with free access to food and water. Male mice were divided into four groups of five animals each and treated intraperitoneally as follows; Group I: Vehicle-treated control; Group II: Void, Group III: 1mg/kg/bwt MEP; Group IV: 3 mg/kg/bwt MEP; Group V: 5mg/kg/bwt MEP for 28 days.

2.2. General observations

Body and reproductive organ weights of the mice were observed daily for behavioral changes. Body weight was recorded daily prior to administration of MEP with the help of a mono pan balance. At autopsy, testicular tissues were removed, blotted free of blood and adhering tissues.

2.3. Testicular cells preparation

Testicular cells were prepared for apoptosis analysis. Briefly, testes were removed and decapsulated by making a small incision in the testis. The contents of the testes were collected through the incision into a 15 ml tube containing 5 ml ice-cold 1X PBS buffer (pH -7.4) and the contents were incubated for 40 min at 37° C with vigorous shaking. Then, the tubes were placed on ice and incubated to allow the seminiferous tubules to settle. The supernatants were discarded and the seminiferous tubules were washed twice in 10 ml of PBS twice.

2.4. Testicular Testosterone (T) and luteinizing hormone (LH) concentrations

The testicular testosterone and luteinizing hormone levels in three mice from each group were measured (Rocha *et al*, 2007). Briefly, testicular proteins were extracted with phosphate buffer (50 mM, pH 7.4) and centrifuged at 10,000rpm for 20mins. The supernatant was used to estimate T and LH levels using ELISA, and were expressed in mg/ml.

2.5. Biochemical Assays of testes tissue

Testicular tissue from each mouse were stored at -20°C for different biochemical assays of lipid peroxidation: glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT). Protein quantity was estimated and according to Lowry's method (Lowry and Rosebrough, 1951). 10% tissue homogenates (w/v) were prepared in chilled 100mM Tris-HCl buffer (pH 7.4) using Cole Parmer tissue homogenizer. The values were expressed per mg of protein.

2.5.1. Measurement of Reactive Oxygen Species (ROS) level

The ROS assay was performed by the method of Anderson (1983). In brief, 50 µl of testicular tissue homogenate and 1400 µl sodium acetate buffer were transferred to a cuvette. After then, 1000 ul of reagent mixture (N,N-diethyl paraphenylenediamine 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 H_2O_2 and expressed as U/mg of protein (1 unit = 1.0 mg H_2O_2/L).

2.5.2. Superoxide Dismutase (SOD) Activity

Superoxide dismutase (SOD) activity was estimated 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 totalvolume of 3 ml. The mixture was incubated at 37° 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.

2.5.3. Catalase (CAT) activity

Catalase activity was quantified by measuring the decomposition of hydrogen peroxide (H_2O_2). Assay mixture consisting of 0.01M phosphate buffer (pH 7), 0.2 M hydrogen peroxide and tissue homogenate was incubated at 37° C for 1 min. The reaction was stopped by addition of potassium dichromate (5% w/v) and acetic acid. The remaining hydrogen peroxide was determined by measuring chromium acetate after heating the assay mixtures in a boiling water bath for 15 min. The absorbance was read at 570 nm (Aebi, 1983).

2.5.4. Glutathione (GSH) content

Glutathione (GSH) content was estimated by centrifuging an aliquot of 10% homogenates of the tissues in 100 mM Tris-HCL buffer (pH 7.4) containing 0.16 M KCL at 1000 g for 5 min. The supernatant was used to measure the rate of reduction of 5' 5'- dithiobis-(2 nitrobenzoate) to 2-nitro-5 thiobenzoate. The absorbance was read at 412 nm. Glutathione content was expressed in μM/mg protein. For determination of GSSG content 0.1M NaOH was used instead of Tris-HCL buffer.

2.5.5. Glutathione peroxidase (GPX) level

Glutathione peroxide activity was determined according to the method of Wendel, 1980. The reaction mixture containing 48 mM sodium phosphate, 0.38 mM EDTA, 0.12 mM NADPH, 0.95 mM sodium azide, 3.2 units glutathione reductase, 1 mM glutathione, 0.02 mM DTT and 0.0007 % (v/v) H_2O_2 were used to monitor the enzyme activity. Enzyme activity was determined by measuring the change in absorbance at 340 nm for 3 min at 30 sec interval and expressed in units/mg protein

2.5.6. Malondialdehyde (MDA) measurement

Testicular 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. The results were presented as μmol/L.

2.6. Assessment of Sperm Parameters

Sperm concentration and motility will be assessed using standard methods. For sperm morphology, from each sample, two smears will be prepared, and 200 sperm cells will be counted under an optical microscope (Olympus EXSI, Tokyo, Japan). Abnormalities in the head, neck, and tail of sperm will be determined, and a percentage of abnormal sperm cells morphology will be reported for each sample (Ojo *et al*., 2013).

2.7. Sperm Viability

Eosin-Nigrosine staining was used to assess sperm viability Briefly, Eosin and Nigrosine (Merck, Germany) were prepared in distilled water. One volume of sperm suspension was mixed with two volumes of 1% eosin. After 30 sec, an equal volume of nigrosine was added to this mixture. Thin smears were then prepared and observed under a light microscope at 1000X magnification. Viable sperm remained colorless while nonviable sperm was stained red.

2.8. Sperm DNA Damage Evaluation

The assessment of DNA damage in epididymal sperm will be performed using the orange acridine dye (Afiyani *et al.,* 2014). The result of test will be expressed as percentage of DNA fragmentation.

2.9. RNA extraction and RT- PCR

Total RNA will be extracted from testis samples using the Trizol reagent (Invitrogen, Carlsbad, CA, USA) and reverse transcribed into cDNA will be done using standardization of cDNA amplification condition and optimization of annealing temperature for primer use.

Table 1 Primers to be used for quantitative RT-PCR for amplifications of genes involved in autophagy, their Tm and amplicon sizes

Genes	GenBank Accession	Forward Primer	Reverse Primer	Tm (oC)	Amplicon size
ATG7	NM_001083906	GGA GTC ACA GCT CTT CCT T	CAG CTA TTG GAA CAC TGTA	59.5	85
LC-3	NM_007809	AGTCAAAGACACCTAATGCCAAG	ACGTCTGGGGAGAAACGG	57.1	83

2.10. Statistical Analysis

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

3. Results

3.1. Effect on Testosterone Level

The study shows the activity of Testosterone in the testes of Melphalan-treated mice. The activity of Testosterone significantly (p<0.01) (p<0.05) decreased in the testes of mice treated with melphalan when compared with the control at different days interval (7 days, 14 days and 21 days).

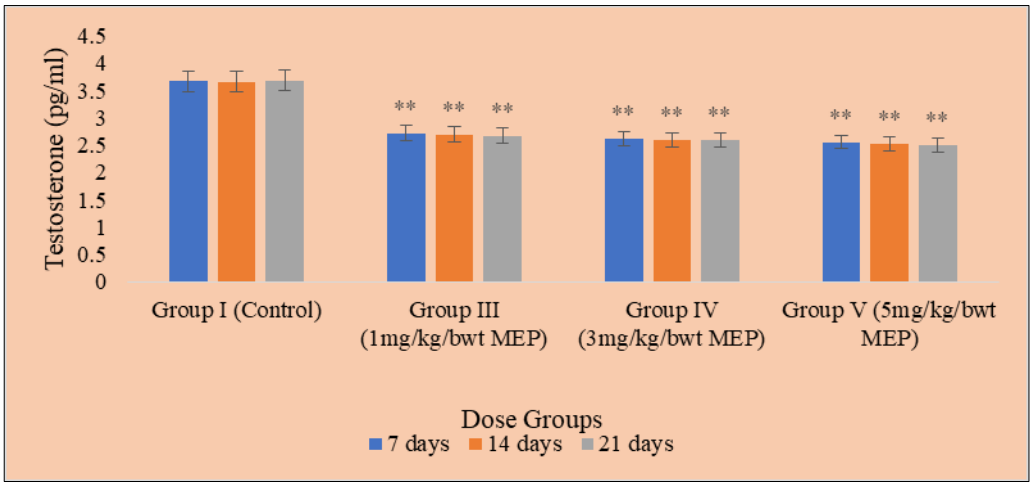

Figure 1 The effect of Melphalan induced decrease changes in the activity of Testosterone. The data showed significant changes from untreated Group I (control) to Group V (5mg/kg/bwt MEP) at varying interval (7 days, 14 days and 21 days). Note: ** Indicate significantly different as compared to controls at 'p<0.05'

3.2. Effect on Luteinizing Hormone Level

The level of Luteinizing Hormone significantly (p<0.05) increased in the testes of mice in the control group compared to the group of mice treated with melphalan at different days interval (7 day, 14 days and 21 days).

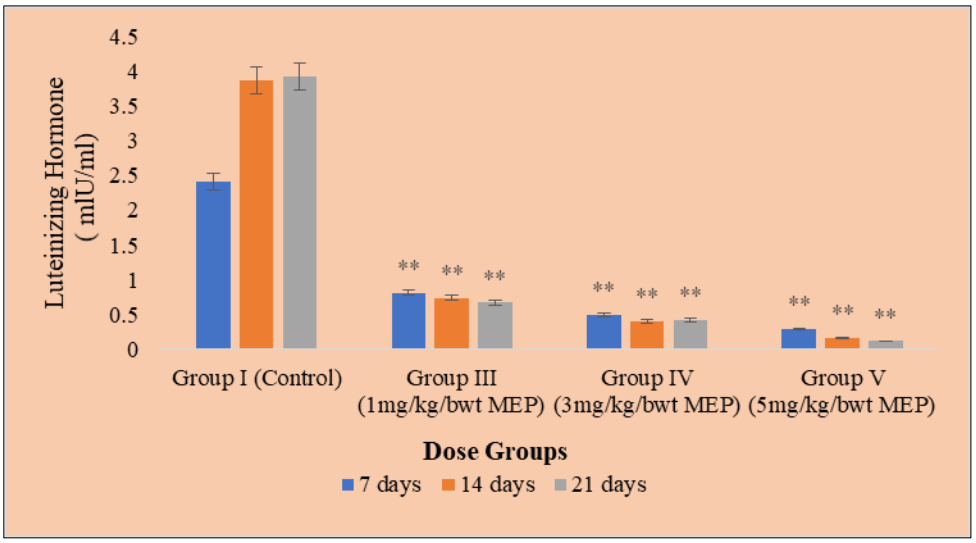

Figure 2 The effect of Melphalan induced decrease changes in the level of Luteinizing Hormone. The data showed significant changes from untreated Group I (control) to Group V (5mg/kg/bwt MEP) at varying interval (7 days, 14 days and 21 days). Note: ** Indicate significantly different as compared to controls at 'p<0.05'

3.3. Effect on Follicle Stimulating Hormone Level

The study shows the level of Follicle Stimulating Hormone in the testes of Melphalan-induced mice. The level of Follicle Stimulating Hormone significantly (p<0.01) (p<0.05) decreased in the testes of mice treated with melphalan when compared with the control at different days interval.

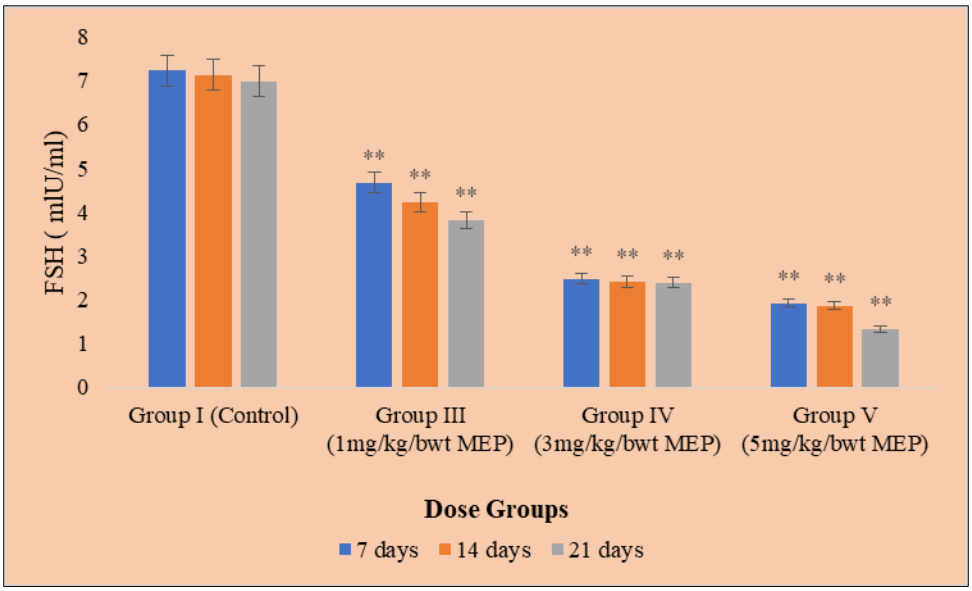

Figure 3 The effect of Melphalan induced decrease changes in the level of Follicle Stimulating Hormone. The data showed significant changes from untreated Group I (control) to Group V (5mg/kg/bwt MEP) at varying interval (7 days, 14 days and 21 days). Note: ** Indicate significantly different as compared to controls at 'p<0.05'

3.4. Effect on Malondialdehyde Level

The study shows the concentration of Malondialdehyde in the testes of Melphalan-treated mice. The concentration of Malondialdehyde significantly (p<0.01) (p<0.05) increased in the testes of groups of mice treated with melphalan (Group III, Group IV and Group V) when compared with the concentration of Malondialdehyde in the testes of mice in the control group, at different days interval (7 days, 14 days and 21 days).

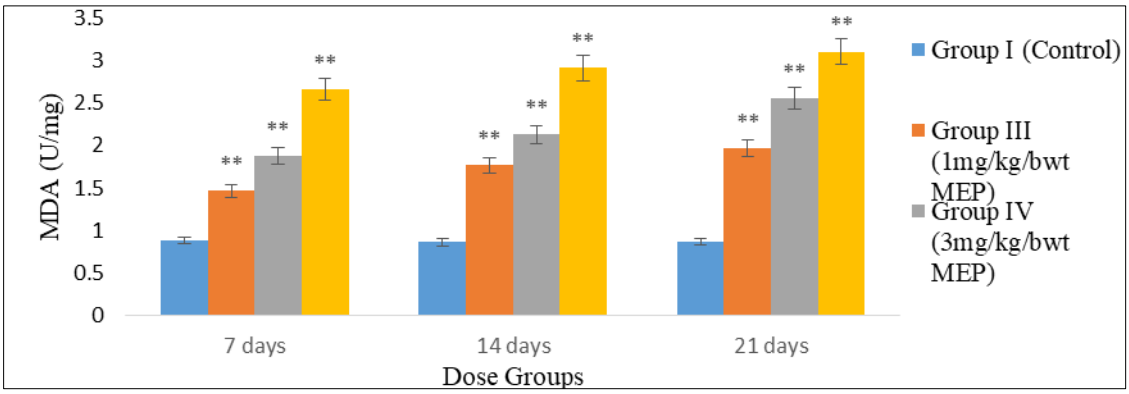

Figure 4 The effect of Melphalan induced increased changes in the concentration of Malondialdehyde. The data showed significant changes from untreated Group I (control) to Group V (5mg/kg/bwt MEP) at varying interval (7 days, 14 days and 21 days). Note: ** Indicate significantly different as compared to controls at 'p<0.05'

3.5. Effect on Reactive Oxygen Species (ROS) Level

The study shows the concentration of Reactive Oxygen Species in the testes of Melphalan-treated mice. The concentration of ROS significantly (p<0.01) (p<0.05) increased in the testes of groups of mice treated with melphalan (Group III, Group IV and Group V) when compared with the concentration of ROS in the testes of mice in the control group, at different days interval (7 days, 14 days and 21 days).

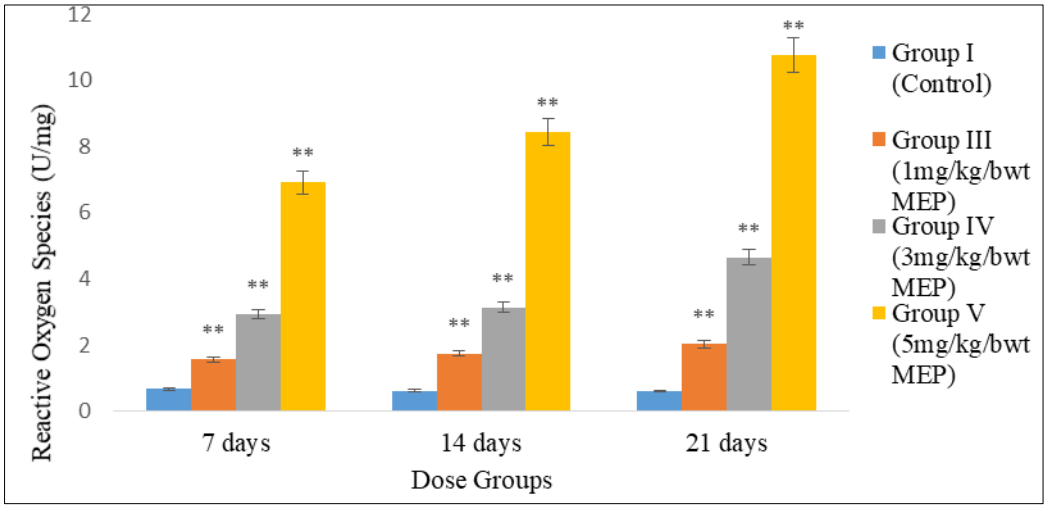

Figure 5 The effect of Melphalan induced increased changes in the concentration of Reactive Oxygen Species. The data showed significant changes from untreated Group I (control) to Group V (5mg/kg/bwt MEP) at varying interval (7 days, 14 days and 21 days). Note: ** Indicate significantly different as compared to controls at 'p<0.05'

3.6. Effect on Total Antioxidant Capacity Level

The level of Total Antioxidant Capacity significantly (p<0.01) (p<0.05) decreased in the testes of groups of mice treated with melphalan (Group III, Group IV and Group V) when compared to across the groups.

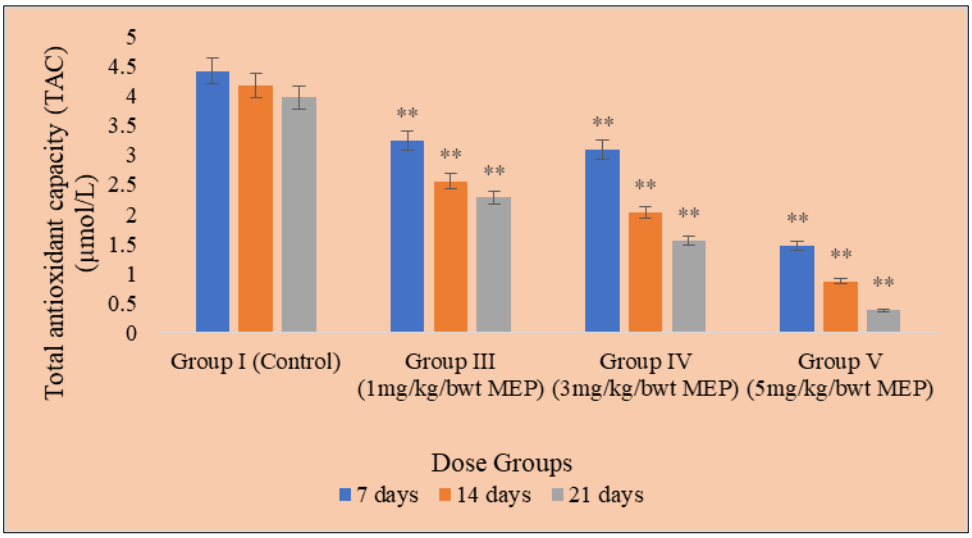

Figure 6 The effect of Melphalan induced decrease changes in the level of Total Antioxidant Capacity. The data showed significant changes from untreated Group I (control) to Group V (5mg/kg/bwt MEP) at varying interval (7 days, 14 days and 21 days). Note: ** Indicate significantly different as compared to controls at 'p<0.05'

3.7. Effect on Glutathione Peroxidase activity

The group induced with 3mg/kg/bwt and 5mg/kg/bwt of Melphalan respectively had significant decrease (p<0.05) (p<0.01) in the activity of Glutathione Peroxidase compared to the control group at day seven. Meanwhile, the activity of Glutathione peroxidase significantly (p<0.05) decreased in the testes of mice treated with Melphalan when compared with the control after seven days and fourteen days respectively.

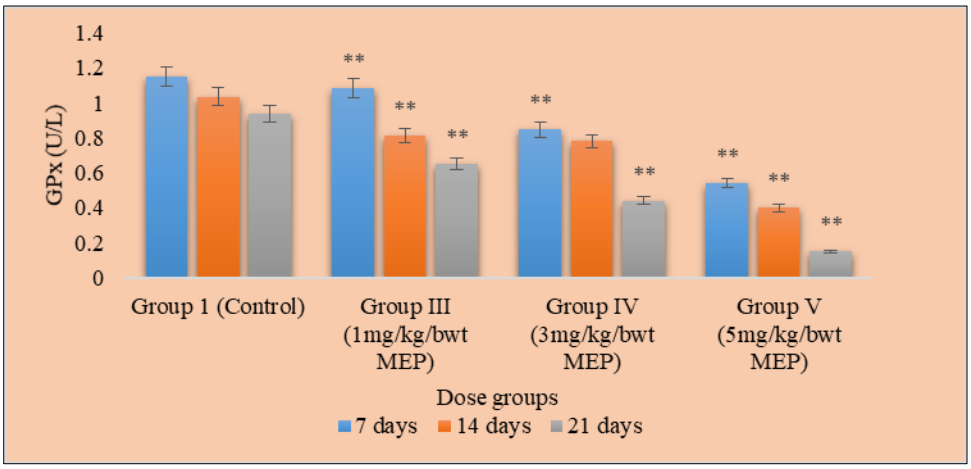

Figure 7 The effect of Melphalan induced decrease changes in the activity of Glutathione Peroxidase. The data showed significant changes from untreated Group I (control) to Group V (5mg/kg/bwt MEP) at varying interval (7 days, 14 days and 21 days). Note: ** Indicate significantly different as compared to controls at 'p<0.05'

3.8. Effect on the Concentration of Glutathione

The study shows the concentration of Glutathione in the testes of Melphalan-treated mice. The concentration of Glutathione significantly (p<0.01) (p<0.05) decreased in the testes of mice treated with melphalan when compared with the control at different days interval (7 days, 14 days and 21 days).

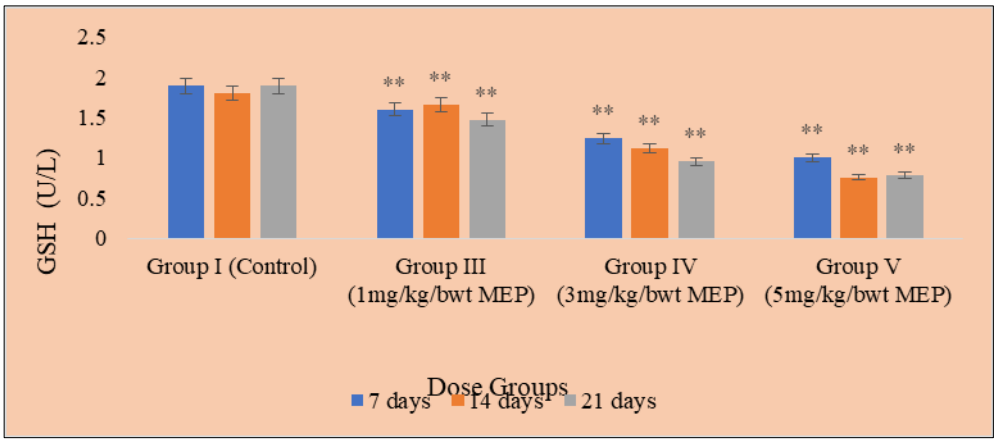

Figure 8 The effect of Melphalan induced decrease changes in the concentration of Glutathione. The data showed significant changes from untreated Group I (control) to Group V (5mg/kg/bwt MEP) at varying interval (7 days, 14 days and 21 days). Note: ** Indicate significantly different as compared to controls at 'p<0.05'

3.9. Effect on the Concentration of Teratozoospermia Index (TZI)

The study shows the concentration of Teratozoospermia Index in the testes of Melphalan-induced mice. The concentration of TZI significantly (p<0.01) (p<0.05) increased in the testes of groups of mice treated with melphalan (Group III, Group IV and Group V) when compared with the concentration of TZI in the testes of mice in the control group, at different days interval (7 days, 14 days and 21 days).

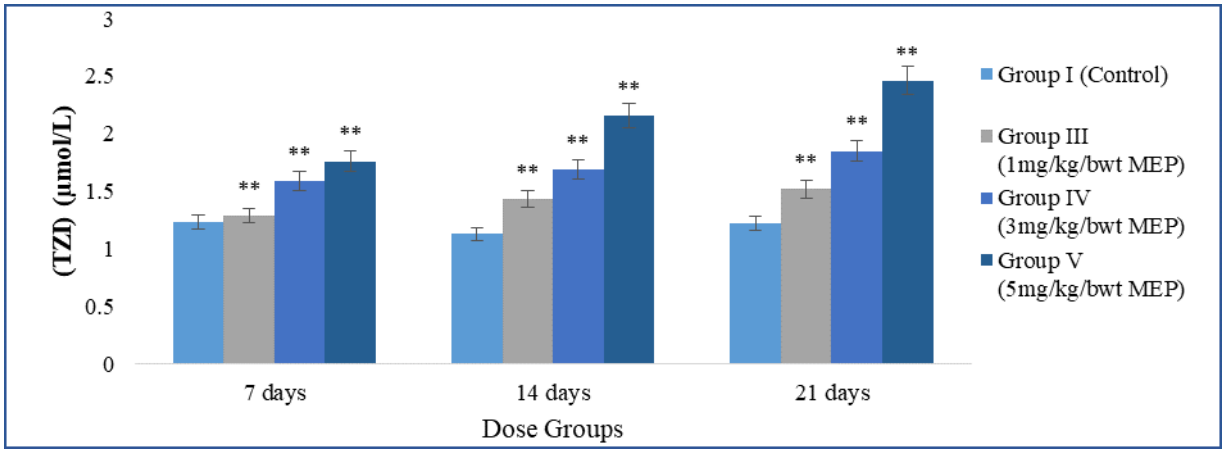

Figure 9 The effect of Melphalan induced increase changes in the concentration of Teratozoospermia Index. The data showed significant changes from untreated Group I (control) to Group V (5mg/kg/bwt MEP) at varying interval days. Note: ** Indicate significantly different as compared to controls at 'p<0.05'

3.10. Effect on Percentage DNA Damage Level

The percentage of Deoxyribonucleic Acid damage significantly (p<0.01) (p<0.05) increased in the testes of groups of mice treated with melphalan (Group III, Group IV and Group V) when compared with the percentage of Deoxyribonucleic Acid in the testes of mice in the control group, at different days interval (7 days, 14 days and 21 days).

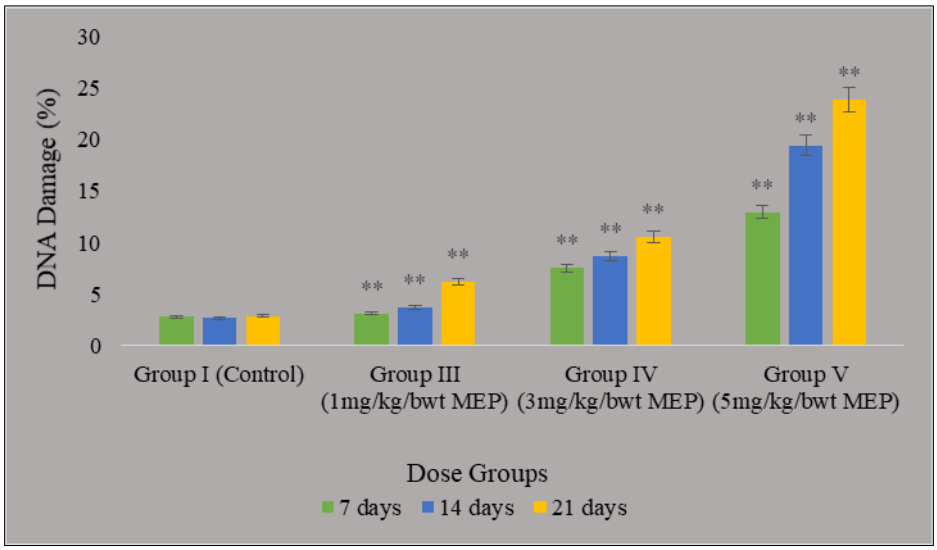

Figure 10 The effect of Melphalan induced increased changes in the level of Percentage DNA damage. The data showed significant changes from untreated Group I (control) to Group V (5mg/kg/bwt MEP) at varying interval (7 day, 14 days and 14 days). Note: ** Indicate significantly different as compared to controls at 'p<0.05'

3.11. Effect on the Expression of ATG7/LC3 Genes

The study shows the mRNA expression of ATG7/LC3 genes in the testes of Melphalan-treated mice. The group of mice treated with 5mg/kg/bwt MEP (Group IV) had a significantly (p<0.05) upregulation in the mRNA expression of ATG7/LC3 genes compared to the control group of mice. Also, the control group of mice (Group 1) had a significant (p<0.01) (p<0.05) downregulation in the mRNA expression of ATG7 gene when compared with the group of mice treated with 1mg/kg/bwt MEP and 3mg/kg/bwt MEP (Group II and III) respectively. Meanwhile, the control group of mice (Group 1) had a significant (p<0.01) downregulation in the mRNA expression of LC3 gene when compared with the group of mice treated with 3mg/kg/bwt MEP (Group III).

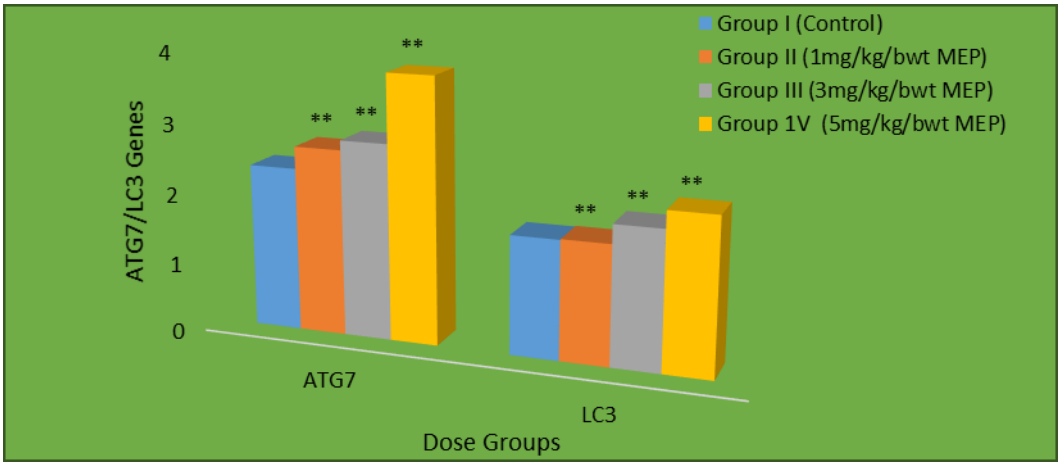

Figure 11 The effect of Melphalan induced changes in the expression of ATG7/LC3 (Autophagy Related 7 and Microtubule-associated Protein Light Chain 3) genes. The data showed significant changes from untreated Group I (control) to Group IV (5mg/kg/bwt MEP) at varying interval days. Note: ** Indicate significantly different as compared to controls at 'p<0.05'

4. Discussion

Autophagy is an intracellular lysosomal degradation pathway and plays a very important role in maintaining intracellular homeostasis. The main role of autophagy is to eliminate intracellular energy resources in nutrient shortage conditions and remove cytotoxic proteins and organelles under stressful situations (Wang *et al*., 2014). Previous studies have shown that autophagy plays an important role in acrosome biogenesis and spermatid differentiation during

spermatogenesis (Shang *et al*., 2016). Moderate autophagy maintains homeostasis of organisms and was reported to play a protective role against testicular damage caused by ROS, hyperglycemia and hypoxia. However, a series of research studies confirmed that abnormal autophagy is pivotal for male infertility. Leydig cells, as an important part of the testicular stroma, are the main source of androgens. Zhao *et al*. have shown that autophagy induced by suppressing the Akt-mTOR pathway can inhibit Leydig cells, thereby reducing serum testosterone levels (Zhao *et al*., 2018).

Sertoli cells are essential for spermatogenesis and male fertility, and they coordinate the spermatogenesis process by providing nutrition and an environment conducive to the survival and development of germ cells. It has been reported by Duan *et al*. that in Sertoli cells, the mTOR signaling pathway mediated by ROS may be the main pathway to augment autophagy, which causes the suppression of Sertoli cells proliferation, thus impairing spermatogenesis and fertility (Duan *et al*., 2016). Melphalan is an antineoplastic agent that acts as a bifunctional alkylating agent, which is used in the treatment of multiple myeloma, advanced ovarian adenocarcinoma, early and advanced breast cancer, childhood neuroblastoma, and polycythaemia vera. Chemotherapeutic drugs such as melphalan, can alter sexual hormone levels and co-administration of melatonin effects these alterations. The increment of testosterone level is consistent with protection effect of melatonin on Leydig cells; because of existence of melatonin binding sites in the reproductive system (Kryl'skii *et al*., 2019). To establish the biochemical studies and expression of autophagy genes in Melphalan-induced testicular function, the key markers were observed; intraperitoneal administration of melphalan in increase of percentage DNA damage and Teratozoospermia Index (TZI); decrease of protein concentration and Total Antioxidant Capacity (TAC) (Menkveld, 1990).

Follicle stimulating hormone (FSH) was reduced on administration of melphalan at the dosage of 5mg/kg/bwt and also testosterone level was reduced as shown in figure 5, since testosterone is necessary for the initiation and maintenance of spermatogenesis (Egeland *et al.*, 1994). Decreased testosterone by melphalan can lead to reproductive consequences such as the impairment of spermatogenesis. This can be supported from the fact that the mice that received melphalan had decreased epididymis sperm count, motility and morphology compare to control. Also, there was significant decrease in the level of Luteinizing Hormone (LH) on administration of 5mg/kg/bwt of melphalan for 7 days, 14 days, 21days respectively, when compared to the control. Low level of LH indicates a reduction of testosterone in the testes resulting in absence of normal feedback leading to a pituitary depletion of LH. This study demonstrates that Glutathione (GSH) level in testis declined significantly in Melphalan treated mice. Depletion of GSH might be inducing oxidative stress by increasing the free radical generation in testis resulting in dis-regulation of spermatogenesis and fertility (Huang *et al.,* 2007).

The toxicological effect of melphalan in Melphalan-induced male swiss mice was assessed by investigating GPx, ROS and MDA. This current study showed signs of oxidative stress as shown by increased MDA activity in the testes of the MET-treated mice, the drug-treated mice also showed a defective antioxidant response as evident from diminished activity of antioxidant enzymes such as GPx. Similar reports have been likewise made available (Alexander *et al.,* 2016). Oxidative stress is a common pathology that has been implicated in male infertility. Induction of oxidative stress can be as result of increasing the free radical generation in testis and epididymis resulting in degeneration of spermatogenesis and ultimately infertility (Ojo *et al.,* 2013).

Increase in percentage DNA damage and decrease in sperm count, observed in the caudal epididymis of melphalan treated mice indicated the cytotoxicity of melphalan in the testis. The early germ cells in the spermatogenic cycle were affected by melphalan and became abnormal thereby increasing the number of abnormal spermatozoa. Significant decrease in sperm motility indicated adverse effect of Melphalan on spermatozoa function probably through the structure and function of both epididymis and testis. Although the damaged germinal epithelium in the testis appears to be the main reason for impaired sperm quality, oxidative stress in the epididymis might be another reason. Increased ROS in the testis might have contributed to abnormality of spermatozoa resulting in infertility (Ojo *et. al.,* 2013).

Using two markers of the cell autophagic response pathway, the upstream Atg7 protein and the downstream LC3-II/LC3-I protein ratio, this current study shown that in the autophagic testis and also in mature spermatozoa, both markers were significantly more present when compared to the control groups. This is in agreement with previous report that showed induction of autophagy in mouse male germ cells after exposure to heat stress and that down-expression of Atg7 lowers this heat-stress-associated with autophagic response. Zhu *et al*. (2017) also recently reported that the HIF-1 *a*/BNIP3/Beclin1 autophagy signaling pathway was upregulated in the varicocele rat testis. These authors hypothesized that upon varicocele, early hypoxia damages seminiferous cells, organelles, and proteins trig-gering the autophagic response as a pro-survival process. It is suspected that although autophagy is triggered as a pro-survival process, it does not succeed in protecting the testis, mainly because varicocele is a permanent situation of stress that finally leads to apoptosis (Lum *et al*., 2005; Kim *et al*., 2007).

5. Conclusion

The present study evaluates the biochemical studies and expression of Atg7/LC3 autophagy genes in Melphalan-induced testicular dysfunction. Oxidative stress was induced by melphalan treatment as confirmed by the simultaneous significant decrease in TAC, GSH and GPx levels and increase in MDA, ROS levels. Autophagy is mediated by the expression of ATG7 and LC3 genes and theses varying mRNA expression led to autophagic defects. The observed reduced Spermatogenesis might be due to administration of Melphalan.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

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

Statement of ethical approval

The protocol was approved by the Institutional Animal Ethics Committee of Ekiti State University, Ado Ekiti. This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the Institute.

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