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Effect of Acute Administration of Ethanol Extract of *Cannabis sativa* Leaf on Oxidative Stress Biomarkers in Male and Female Wistar Rats

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ABSTRACT: Oxidative stress is a phenomenon caused by an imbalance between production and accumulation of oxygen reactive species (ROS) in the cells and tissues and the ability of a biological system to detoxify these reactive products. This study investigated the acute effect of different doses of ethanolic extract annabis sativa (EECS) on oxidative stress biomarkers in male (M) and female (F) Wister rats. Twenty male rats (170 g ± 1.24) and twenty female rats (150 g ± 1.05) were separately assigned into four groups of five animals each for male and female, such that the rats in groups IM and IF, IIM and IIF, IIIM and IIIF and IVM and IVF received orally 1 mL of distilled water, 2 mg/kg body weight (BW) of EECS, 4 mg/kg BW of EECS and 6 mg/kg BW of EECS respectively for twenty one (21) days. Catalase, superoxide dismutase (SOD), Glutathione peroxidase (GPx), Glutathione reductase (GSH), malondialdehyde (MDA), total antioxidant capacity (TAC) and lactate dehydrogenase (LDH) were determined using standard methods. Administration of different doses (2 mg/kg BW, 4 mg/kg BW and 6 mg/kg BW) of EECS significantly ($p<0.05$) decreased catalase, SOD, GPx, GSH and TAC levels respectively when compared with the control. However, *Cannabis sativa* (CS) increased MDA and LDH levels significantly ($p<0.05$) when compared with the control. It was deduced that these alterations in oxidative stress biomarkers were dependent on the doses of CS consumed. Thus, the more the concentration of CS consumed, the more it may affect oxidative stress biomarkers negatively. In addition, all these effects of CS on oxidative stress biomarkers were more in male than in female.

Keywords: *Cannabis sativa*, Oxidative stress, Biomarkers, Reactive oxygen species

Introduction

Cannabis sativa (CS) is known for its medicinal uses since ancient times because of its rich supply of phytochemicals (Andre *et al*., 2016), hence the quest for harnessing its pharmacological potential by scientists. It is one of the most commonly used illicit drugs worldwide (Abdel-Salam, 2016). Disturbances in the normal redox state of cells can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including proteins, lipids, and DNA (Narayanan, Goodwin, & Lehnert, 1997). Oxidative stress from oxidative metabolism causes base damage, as well as strand breaks in DNA. Base damage is mostly indirect and caused by the reactive oxygen species generated, e.g., O_2^- (superoxide radical), OH (hydroxyl radical) and H_2O_2 (hydrogen peroxide) (Narayanan *et al.*, 1997). Oxidative stress is the result of an imbalance in the body between the oxidizing system, consisting mainly of free radicals, reactive oxygen

species (ROS) and reactive nitrogen species (RNS) (Zhang *et al*., 2019) and antioxidant systems that neutralize these free radicals capable of multiple deleterious effects (Alagbonsi & Olayaki, 2017). Oxidative stress is involved in aging (Martemucci *et al*., 2022) and is found in certain chronic pathologies such as diabetes mellitus, cancers, hypertension, coronary heart disease, etc. (Yaribeygi *et al.,* 2020) and certain infections, particularly by the RNA viruses (Zhang *et al*., 2019), a family to which belong corona viruses (Facultéde *et al*., 2020). However few research has been done on effect of different doses of CS in Wistar male and female rats. Thus, this research work examined the short term effects of different concentrations of CS in male and female Wistar rats.

Materials and methods

Sample collection: Cannabis sativa (*CS*) leaves were donated by National Drug Law Enforcement Agency (NDLEA), Nigeria, for research purpose only.
Sample preparation and extraction: Extraction of *Cannabis sativa* (*CS*) leaves was done with Soxhlet apparatus by soaking 700 g of CS in 98% ethanol for 48 h. It was filtered and the filtrate was poured into a round bottom conical flask, which was fixed with a rotary evaporator. It was then evaporated and cooled. The dried yield of the extract was 45.2 g.
Experimental animals: Twenty male rats (170g ± 1.24) and twenty female rats (150g ± 1.05) that were used for this research were obtained from Temilade Animal Venture, Ogbomoso, Oyo State, were housed at room temperature with unrestricted access to diet and water and maintained on a daily light/dark cycle. Principles of laboratory animal care (NIH publication No. 85-23, revised 1985) were followed. The experimental protocol was approved by Ethical Committee of the Al-Hikmah University, Ilorin, Nigeria.
Experimental protocol: After 2 weeks of acclimatization, the animals [male (M) and female (F)] were separately assigned into four groups of five animals each for male and female, such that the rats in groups IM and IF, IIM and IIF, IIIM and IIIF and IVM and IVF received orally (in the morning) 1 mL of distilled water, 2 mg/kg body weight (BW) of EECS, 4 mg/kg BW of EECS and 6 mg/kg BW of EECS respectively, for twenty one (21) days. The animals were sacrificed after the 21st daily dose with access to food and water.
Preparation of serum: The male and female rats were sacrificed under ketamine anesthesia and blood was collected by cardiac puncture into sample bottles. The blood was left for 30 min to clot and thereafter centrifuged at 625×*g* for 10 min using a Uniscope Laboratory Centrifuge (Model SM800B, Surgifield Medicals, Essex, England). The serum was collected into plain bottles with the aid of a Pasteur pipette. Sera were stored in a freezer maintained at -5 ℃ and used within 12 hours of preparation.
Drug and assay kits: Lactate dehydrogenase (LDH) activity was assayed spectrophotometrically (Spectramax Plus; Molecular Devices, Sunnyvale, CA, USA) following the kit manufacturer's procedures (product code BXC0243; Fortress Diagnostics, UK). The determination of serum superoxide dismutase (SOD) concentration was done with SOD colorimetric assay kit (Fortress Diagnostics Ltd., Antrim, UK; Product code: BXC0531), following the manufacturer's protocols. The determination of serum glutathione peroxidase (GPx) activity was done with GPx colorimetric assay kit (BioVision Inc., Milpitas, CA, USA), following the manufacturers protocols. Based on the manufacturer's protocol, total anti-oxidant capacity (TAC) measurement in the serum was done with a spectrophotometric microplate reader (Spectramax Plus, Molecular Devices, Sunnyvale, CA, USA) using OxiSelect TAC assay kit that uses the single electron transfer mechanism (Cell Biolabs, Inc. San Diego, CA. cat no: STA-360). The continuous catalase activity was determined through spectrophotometric reading (modification of the method used by Claiborne, 1985). Reduced glutathione (GSH) was measured according to the method of Ellman (1959).The assay method of Hunter *et al*. (1963), modified by Gutteridge and Wilkins (1982) was adopted for Malondiadehyde (MDA).
Statistical analysis: Results were expressed as the mean ± standard error of mean. Data were analyzed using a One-way Analysis Of Variance, followed by the LSD post-hoc test to determine significant differences in all the parameters with Students Package for Social Science, version 22.0 (SPSS Inc., Chicago, USA) and Excel. Differences with values of $P<0.05$ were considered statistically significant.

Results

Effects of administration of different doses of EECS on SOD levels in male and female Wistar rats: There were significant decrease ($p<0.05$) in SOD levels in both male and female rats following the administration of different doses of EECS (2 mg/kg BW, 4 mg/kg BW and 6 mg/kg , respectively) when compared with the

control (Figure 1). However, administration of 6 mg/kg BW of EECS significantly (p<0.01) decreased SOD level when compared with that of 2 mg/kg BW and 4 mg/kg BW. In addition, this change in the level of SOD was more in male and female rats.

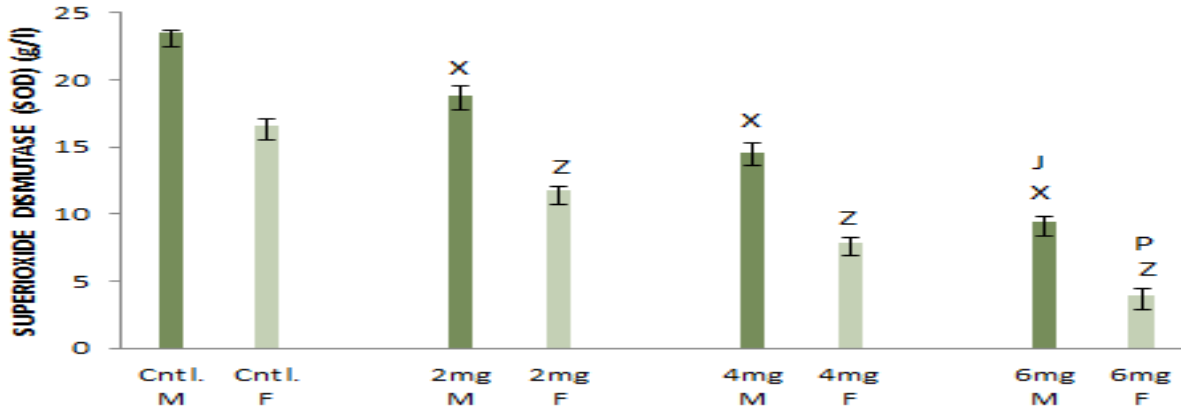

Figure 1: Effect of administration of different doses of EECS on SOD levels in male and female Wistar rats. Values are expressed as mean ± S.E.M.; $^{X}p<0.05$ vs control male; $^{Z}p<0.05$ vs control female; $^{J}p<0.01$ vs 2 mg and 4 mg/kg BW of male treated groups; $^{P}p<0.01$ vs 2 mg and 4 mg/kg BW of female treated groups
NB: Cntl = Control; M = Male; F = Female

Effect of administration of different doses of EECS on LDH levels in male and female Wistar rats: There were significant increase (p<0.05) in SOD levels in both male and female rats following the administration of different doses of EECS (2 mg/kg BW, 4 mg/kg BW and 6 mg/kg BW, respectively) when compared with the control (Figure 2). However, administration of 6 mg/kg BW of EECS significantly (p<0.01) increased LDH level when compared with that of 2 mg/kg BW and 4 mg/kg BW. In addition, this change in the level of LDH was more in male and female rats.

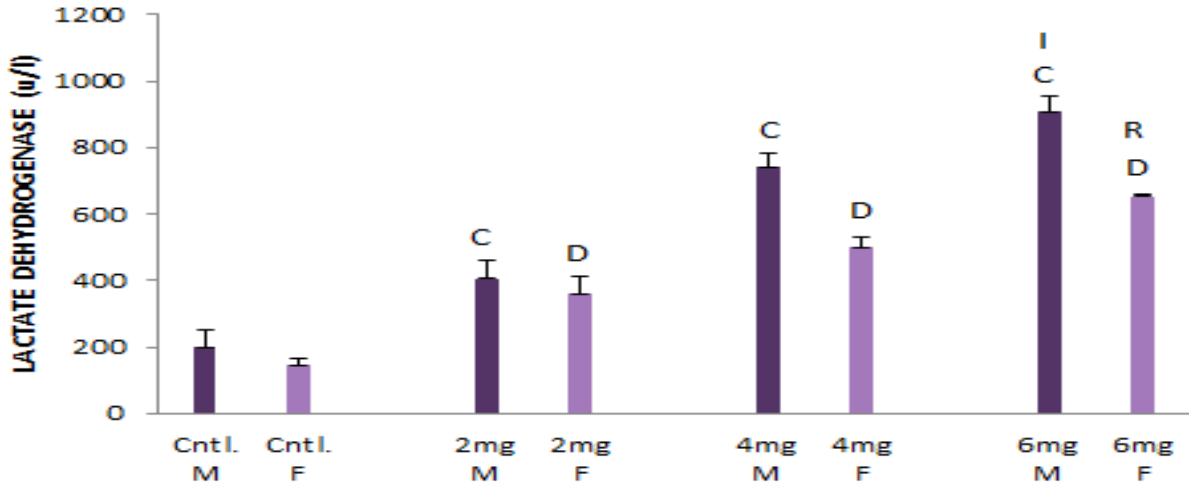

Figure 2: Effect of administration of different doses of EECS on LDH levels in male and female Wistar rats. Values are expressed as mean ± S.E.M. $^{D}p<0.05$ vs control male; $^{C}p<0.05$ vs control female; $^{I}p<0.01$ vs 2 mg and 4 mg/kg BW of male treated groups; $^{R}p<0.01$ vs 2 mg and 4 mg/kg BW of female treated groups

Effects of administration of different doses of EECS on catalase levels in male and female Wistar rats: There were significant decrease (p<0.05) in catalase levels in both male and female rats following the administration of different doses of EECS (2 mg/kg BW, 4 mg/kg BW and 6 mg/kg BW, respectively) when compared with the control (Figure 3). However, administration of 6 mg/kg BW of EECS significantly (p<0.01) decreased catalase level when compared with that of 2 mg/kg BW and 4 mg/kg BW. In addition, this change in the level of catalase was more in male and female rats.

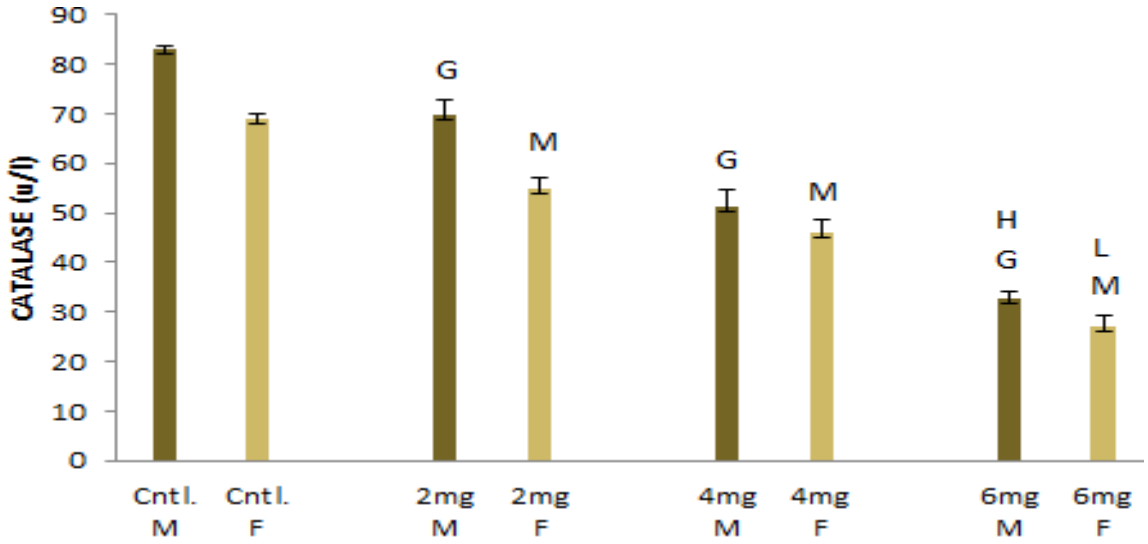

Figure 3: Effect of administration of different doses of EECS on catalase levels in male and female Wistar rats. Values are expressed as mean ± S.E.M. $^{G}p<0.05$ vs control male; $^{M}p<0.05$ vs control female; $^{H}p<0.01$ vs 2 mg and 4 mg/kg BW of male treated groups; $^{L}p<0.01$ vs 2 mg and 4 mg/kg BW of female treated groups

Effect of administration of different doses of EECS on GSH levels in male and female Wistar rats: There were significant decrease ($p<0.05$) in GSH levels in both male and female rats following the administration of different doses of EECS (2 mg/kg BW, 4 mg/kg BW and 6 mg/kg BW, respectively) when compared with the control (Figure 4). However, administration of 6 mg/kg BW of EECS significantly ($p<0.01$) decreased GSH level when compared with that of 2 mg/kg BW and 4 mg/kg BW. In addition, this change in the level of GSH was more in male and female rats.

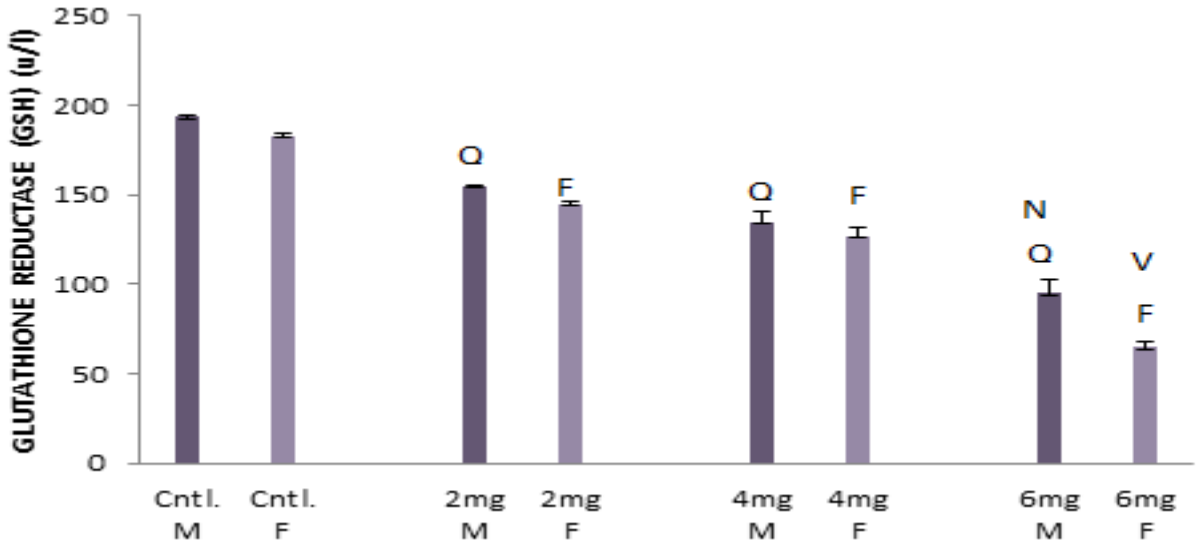

Figure 4: Effect of administration of different doses of EECS (2 mg/kg BW, 4 mg/kg BW and 6 mg/kg BW, respectively) on GSH levels in male and female Wistar rats. Values are expressed as mean ± S.E.M. $^{Q}p<0.05$ vs control male; $^{F}p<0.05$ vs control female; $^{N}p<0.01$ vs 2 mg and 4 mg/kg BW of male treated groups; $^{V}p<0.01$ vs 2 mg and 4 mg/kg BW of female treated groups.

Effect of administration of different doses of EECS on GPx levels in male and female Wistar rats: There were significant decrease ($p<0.05$) in GPx levels in both male and female rats following the administration of different doses of EECS (2 mg/kg BW, 4 mg/kg BW and 6 mg/kg BW respectively) when compared with the control (Figure 5). However, administration of 6 mg/kg BW of EECS significantly ($p<0.01$) decreased GPx level when compared with that of 2 mg/kg BW and 4 mg/kg BW. In addition, this change in the level of GPx was more in male and female rats.

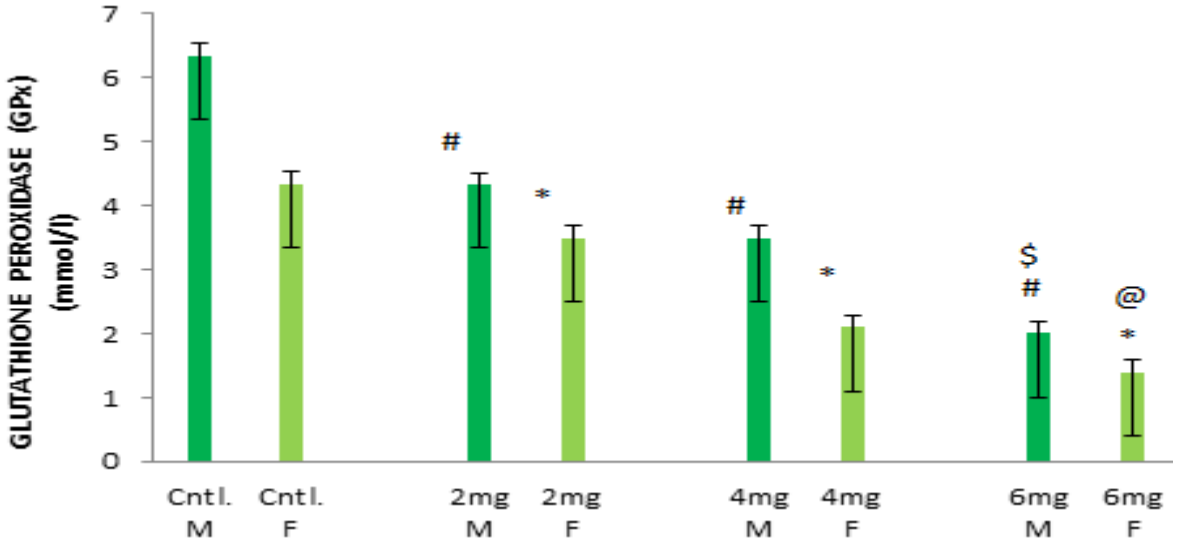

Figure 5: Effect of administration of different doses of EECS on GPx levels in male and female Wistar rats. Values are expressed as mean ± S.E.M. $^{\#}p<0.05$ vs control male; $^{*}p<0.05$ vs control female; $^{\$}p<0.01$ vs 2 mg and 4 mg/kg BW of male treated groups; $^{@}p<0.01$ vs 2 mg and 4 mg/kg BW of female treated groups.

Effects of administration of different doses of EECS on MDA levels in male and female Wistar rats: There are significant increase ($p<0.05$) in MDA levels in both male and female rats following the administration of different doses of EECS (2 mg/kg BW, 4 mg/kg BW and 6 mg/kg BW, respectively) when compared with the control (Figure 6). However, administration of 6 mg/kg BW of EECS significantly ($p<0.01$) increased MDA level when compared with that of 2 mg/kg BW and 4 mg/kg BW. In addition, this change in the level of MDA was more in male and female rats.

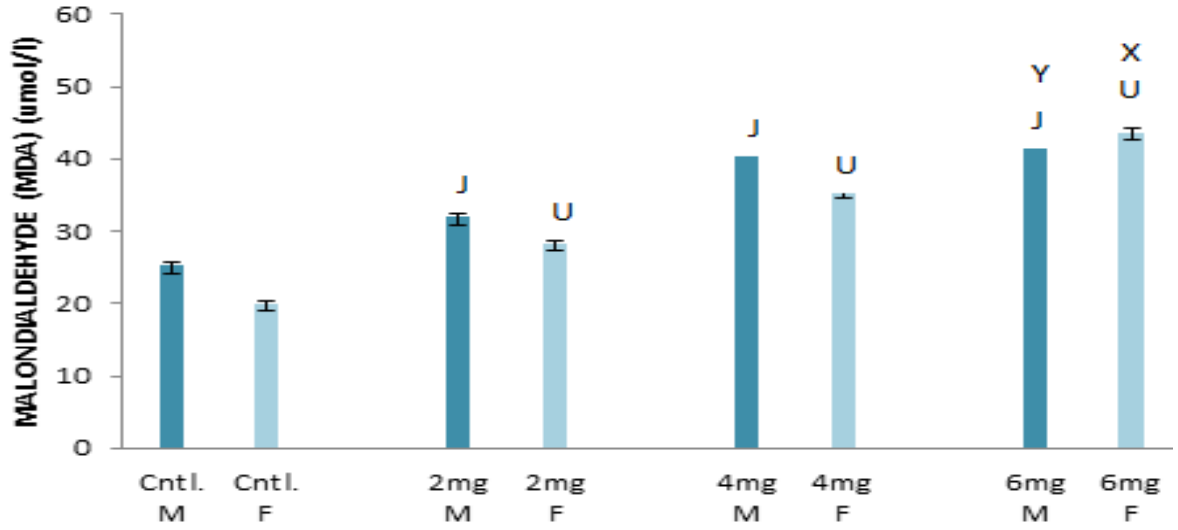

Figure 6: Effect of administration of different doses of EECS on MDA levels in male and female Wistar rats. Values are expressed as mean ± S.E.M. $^{J}p<0.05$ vs control male; $^{U}p<0.05$ vs control female; $^{Y}p<0.01$ vs 2 mg and 4 mg/kg BW of male treated groups; $^{X}p<0.01$ vs 2 mg and 4 mg/kg BW of female treated groups.

Effect of administration of different doses of EECS on the TAC levels in male and female Wistar rats: There are significant decreased ($p<0.05$) in TAC levels in both male and female rats following the administration of different doses of EECS (2 mg/kg BW, 4 mg/kg BW and 6 mg/kg BW, respectively) when compared with the control (Figure 7). However, administration of 6 mg/kg BW of EECS significantly ($p<0.01$) decreased TAC level when compared with that of 2 mg/kg BW and 4 mg/kg BW. In addition, this change in the level of TAC was more in male and female rats.

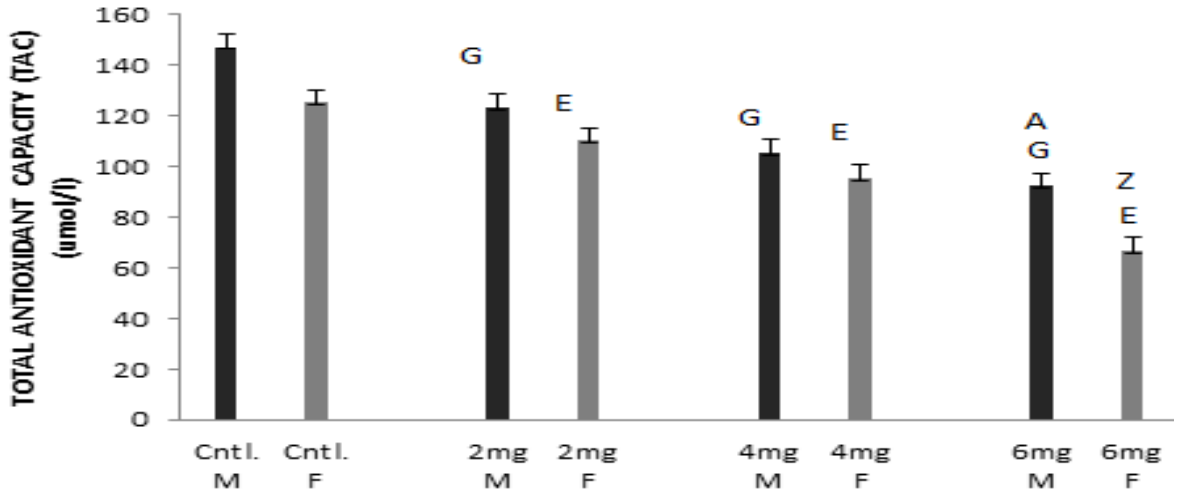

Figure 7: Effect of administration of different doses of EECS on TAC levels in male and female Wistar rats. Values are expressed as mean ± S.E.M. $^{G}p<0.05$ vs control male; $^{E}p<0.05$ vs control female; $^{A}p<0.01$ vs 2 mg and 4 mg/kg BW of male treated groups; $^{Z}p<0.01$ vs 2 mg and 4 mg/kg BW of female treated groups.

Discussion

Oxidative stress reflects an imbalance between the systemic manifestation of reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage. Disturbances in the normal redox state of cells can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including proteins, lipids, and DNA. Oxidative stress from oxidative metabolism causes base damage, as well as strand breaks in DNA. Base damage is mostly indirect and caused by the reactive oxygen species generated, e.g., O_2^- (superoxide radical), OH (hydroxyl radical) and H_2O_2 (hydrogen peroxide) (Narayanan *et al*., 1997). Studies have shown that oxidative stress is thought to be involved in the development of attention deficit hyperactivity disorder, cancer, Parkinson's disease, Lafora disease, Alzheimer's disease, atherosclerosis, heart failure, myocardial infarction, fragile X syndrome, sickle-cell disease, lichen planus, vitiligo, autism, infection, chronic fatigue syndrome and depression (Jimenez-Fernandez *et al*., 2015; Romá-Mateo *et al*., 2015; Verlaet *et al*., 2019). This oxidative stress can be prevented by the body system through the production of antioxidant enzymes. The increased lactate dehydrogenase (LDH) levels in both male and female rats were reflection of attack by free radicals which could be due to the generation of nicotinamide adenine dinucleotide phosphate (NADPH), a fuel for ROS generation. The work also confirmed previous study (Tan *et al.*, 2015) which reported that LDH plays a significant role in the generation of NADPH. It was also evident that CS increased MDA in both male and female rats which could be due to lipid peroxidation of polyunsaturated fatty acids which are degraded by ROS (Collodel *et al*., 2015). The result also showed decrease in catalase, superioxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GSH) and total antioxidant capacity (TAC) in both male and female rats. These were consistent with the increase in lactate dehydrogenase activity in these animals, which led to accumulation of ROS. These are in line with the findings of (Alagbonsi & Olayaki, 2017) which showed decreased in antioxidant enzymes following administration of CS in rats. All these effects of CS in both male and female rats were dose dependent. It is evident from this study that high dose of CS caused more oxidative stress than low dose. However, effects of CS on oxidative stress were more in male than in female rats which could be due to physiological factors such as antioxidant properties of oestrogen and gender differences in NADPH-oxidase activity (Vina *et al*., 2011).

Conclusion

The study concluded that CS showed alterations in oxidative stress biomarkers which were probably associated with the release of free radicals from the electron transport chain in the cell mitochondrion. However, these

effects were dependent on the dose of *Cannabis sativa* administered. In addition, CS caused more oxidative stress in male rats than in female rats. This study suggested that, the consumption of CS should be avoided because of generation of free electrons and oxidative stress that are associated with it.

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