

## **1. INTRODUCTION**

Sleep plays an important role in human life by buffering out body metabolites and thus maintaining general and mental health (Greene *et al.*, 2004). Sleep is divided into two stages that alternate with each other: non-rapid eye movement and rapid eye movement (REM) (Guyton *et al.*, 2006). REM sleep is involved in memory consolidation. This was suggested by the fact that REM sleep duration is increased by learning (Smith *et al.*, 1997), whereas it was decreased in patients with Alzheimer disease (Montplaisir *et al.* 1998). Numerous animal and human studies have described memory deficits following sleep deprivation (Smith *et al.*, 1997). Recent studies have shown that sleep deprivation-induced memory deficits may possibly be caused by increasing oxidative stress marker, which might be caused, at least in part, by accumulation of reactive oxygen species during the wake cycle (Zagaar *et al.* 2012). Anti-oxidative stress mechanisms are important for cognitive functions. The term oxidative stress describes the situation of imbalance between reactive oxygen species and the antioxidant opposing forces (Gupta *et al.* 2003). Superoxide dismutase (SOD) catalyzes the dismutation of superoxide into hydrogen peroxide, which is then neutralized by catalase (Zelko *et al.* 2002; Chelikani and Loewen 2004). The glutathione system is composed of the enzymatic antioxidants including GPx and non-enzymatic free radicals scavenger glutathione (GSH) (Meister *et al.*, 1983). Oxidative stress in the brain is associated with neuronal damage and the subsequent impaired spatial learning and memory (Benzi *et al.*, 1990).

## **2. EXPERIMENTAL DESIGN**

Sixty-four male wistar rats of weight 143g-330g were used for the study. The rats were acclimatized for 7 days in the Animal house of Faculty of Pure and Applied Science, Kwara State University, Malete, under ambient temperature of 25°C after which they were subjected to sleep deprivation using disk-over water method for 14 days. The study was conducted in accordance

with the standards established by the guide for the care and use of Laboratory Animals. Rats had unrestricted access to Normal animal feed and distilled water and they were assigned into 8 experimental groups (groups 1-8) based on their weight, (n=8 per group)

Group 1 (Control) were kept in normal cage and fed with Normal animal feed.

Group 2 (Control) were kept in normal cage and fed with pelletized Sorghum

Group 3 (Control) were kept in normal cage and fed with milled Corn

Group 4 (Control) were kept in normal cage, fed with pelletized sorghum and administered with Caffeine per kg body weight

Group 5 (Sleep deprived) were kept in disk over water cage and fed with normal diet

Group 6 (Sleep deprived) were kept in disk over water cage and fed with pelletized sorghum

Group 7 (Sleep deprived) were kept in disk over water cage and fed with milled corn

Group 8 (Sleep deprived) were kept in disk over water cage, fed with white and yellow corn and administered with Caffeine per kg body weight.

After 14 days of sleep deprivation, the animals were transferred to normal cages for 7 days to recover their sleep debt.

### **3. DISK OVER WATER CAGES**

The disk over water cages were constructed locally. A pair of clear glass cages, a 46 cm diameter glass disk, with its centre in an alley between the cages, protrudes 15.5 cm under each cage to provide a partial floor. Beneath each side of the disk and extending beyond it to the walls of each

cage is a tray of 2-3 cm deep water. Drinking water was available ad libitum, food was also available from feeders specially designed to minimize waste

### **3 TEST FOR MALONDIALDEHYDE (MDA)**

#### **ASSAY PROCEDURE**

1. Preparation of Standards and Samples: Each of the following reagents was added into glass test tubes and mixed well.

- Standards: 200µl of standard and 200µl of Indicator Solution.

- Samples: 200µl of sample and 200µl of Indicator Solution.

2. After the standards, samples and blanks have been mixed; they were allowed to react for 45 minutes at room temperature.

3. Exact 300µl was transferred to a microplate and the absorbance of the resulting solution at 532 nm was measured. The pink color is stable for several hours at room temperature.

### **4. TEST FOR GLUTATHIONE (GSH)**

#### **ASSAY PROCEDURE**

1. Approximately 50µl of standard and samples was added in a clean glass tube.

2. Exact 400µl GSH dilution buffer was added to the glass tube

3. 10µl GSH Chromogen was added to the tube and mixed thoroughly

4. 400ul was transferred into a cuvette and read at 412nm within 5 minutes.

### **5. STATISTICAL ANALYSIS**

All data were expressed as the mean of five replications  $\pm$  standard error of the mean (S.E.M). Statistical evaluation of data were performed using SPSS version 16.0, using one way analysis of variance (ANOVA), followed by Duncan's posthoc test for multiple comparison. Values were considered statistically significant at  $p < 0.05$  (confidence level = 95%).

## 6. RESULTS

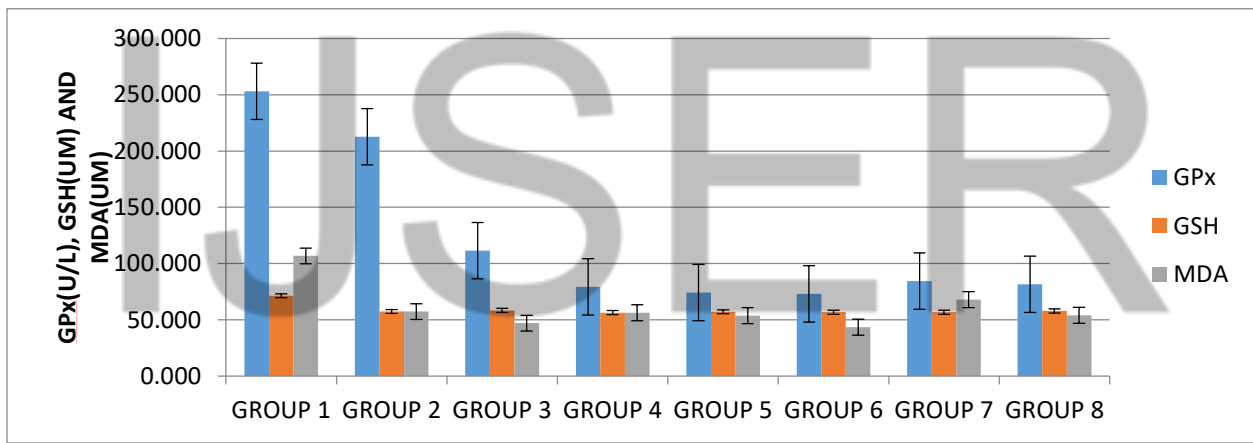


Figure 1: A Graphical representation showing the effect of disk over water cage induced sleep deprivation on GPx(u/L), GSH(uM) and MDA(uM) levels on experimental animals across all groups (Day 1). Data are expressed as mean  $\pm$  S.E.M (n=8).

A significance difference was observed in group 1, 2 and 3 but no significance difference was observed in group 4, 5, 6, 7 and 8 in GPx level however, no significance difference was observed in GSH and MDA level across all groups.

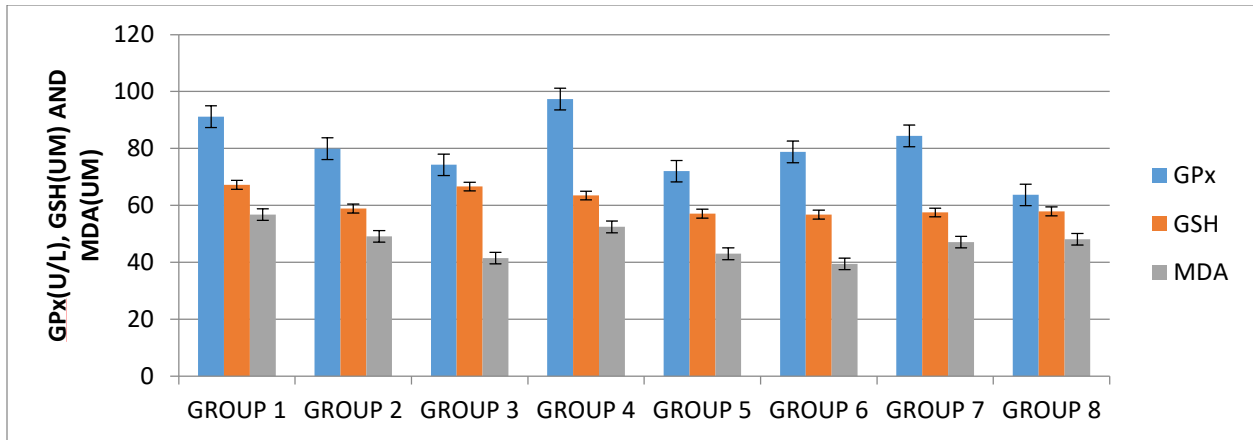


Figure 2: A Graphical representation showing the effect of disk over water cage induced sleep deprivation on GPx(u/L), GSH(uM) and MDA(uM) levels on experimental animals across all groups (Day 7). Data are expressed as mean  $\pm$ S.E.M (n=8).

A significance difference was observed in GPx, GSH and MDA levels across all experimental groups.

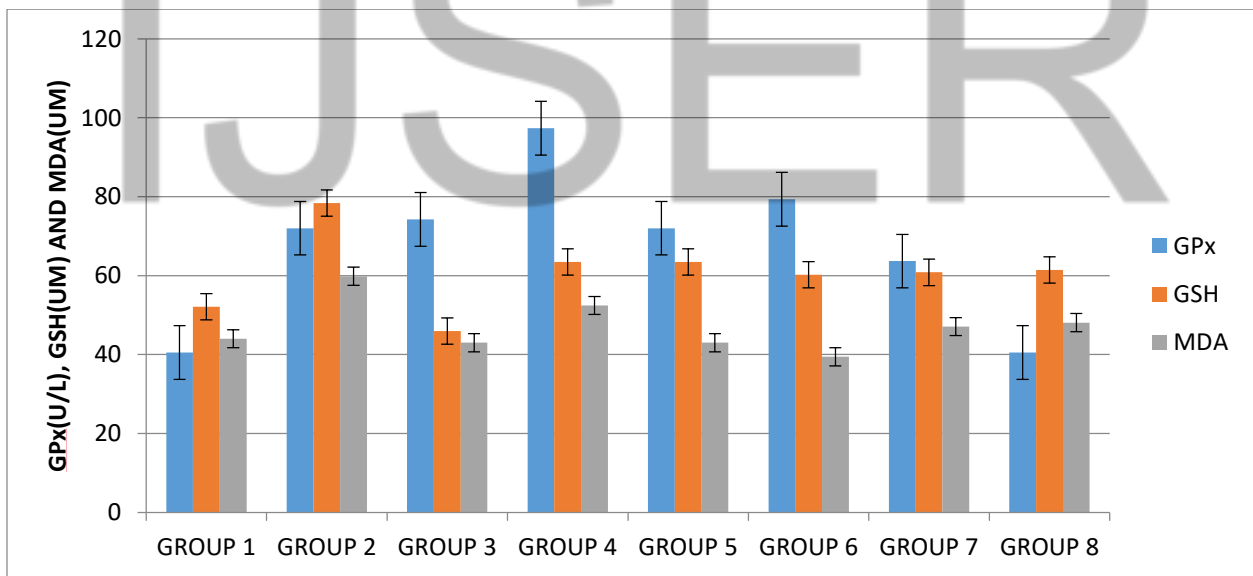


Figure 3: A Graphical representation showing the effect of disk over water cage induced sleep deprivation on GPx(u/L), GSH(uM) and MDA(uM) levels on experimental animals across all groups (Day 9). Data are expressed as mean  $\pm$ S.E.M (n=8).

A significance difference was observed in GPx, GSH and MDA levels across all experimental groups.

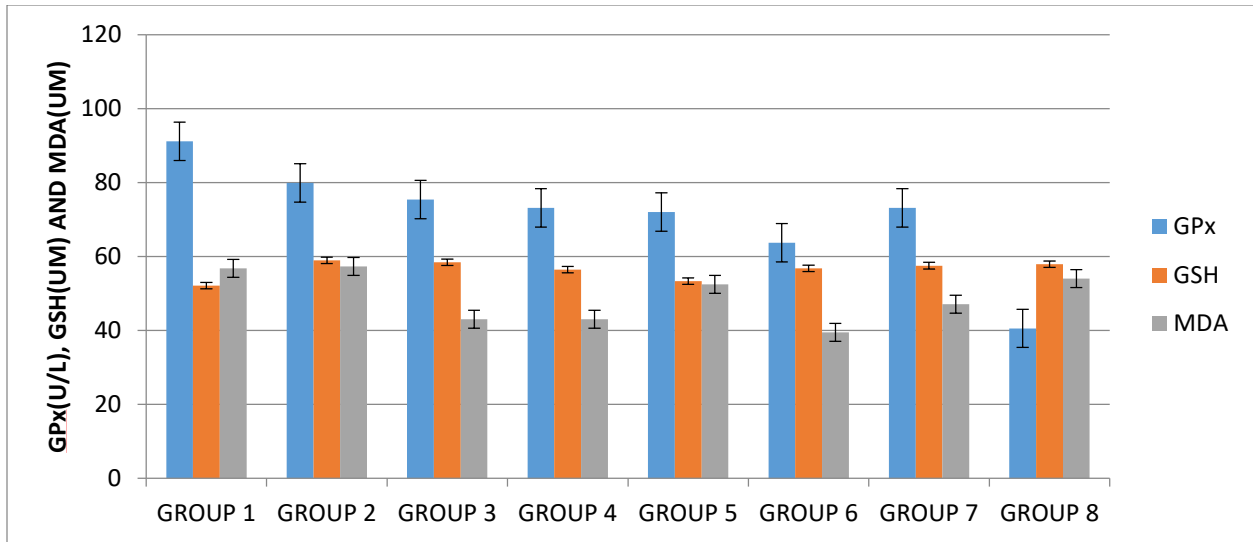


Figure 4: A Graphical representation showing the effect of disk over water cage induced sleep deprivation on GPx(u/L), GSH(uM) and MDA(uM) levels on experimental animals across all groups (Day 17). Data are expressed as mean  $\pm$ S.E.M (n=8).

A significance difference was observed in GPx and MDA levels across all experimental groups, however no significance difference was observed in GSH levels.

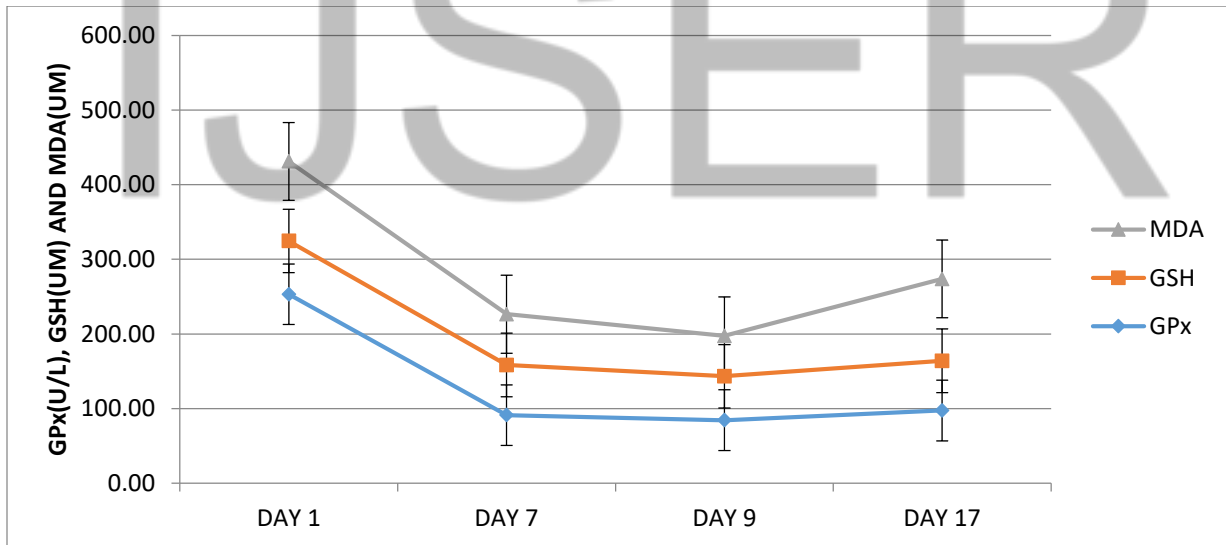


Figure 5: A Graphical representation showing the effect of disk over water cage induced sleep deprivation on GPx(u/L), GSH(uM) and MDA(uM) levels on experimental animals across all groups (Group 1). Data are expressed as mean  $\pm$ S.E.M (n=3).

An increase in GPx, GSH and MDA level was observed on day 1, whereas on day 7, 9 and 17, a decrease was observed in GPx and GSH.

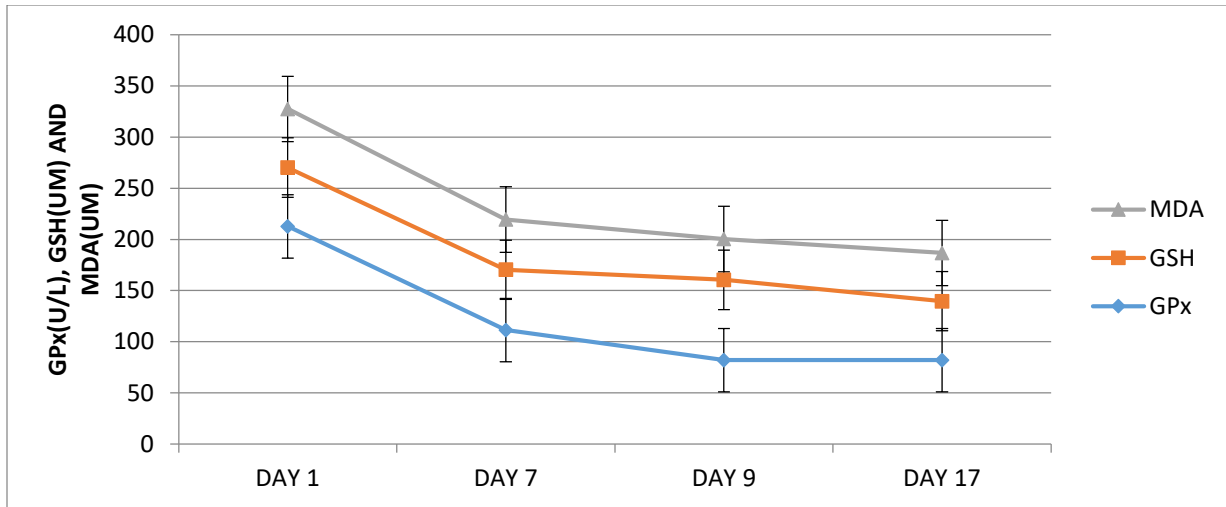


Figure 6: A Graphical representation showing the effect of disk over water cage induced sleep deprivation on GPx(u/L), GSH(uM) and MDA(uM) levels on experimental animals across all groups (Group 2). Data are expressed as mean  $\pm$ S.E.M (n=3).

On day 1, an increase was observed in GPx, GSH and MDA levels while a decrease was observed on day 7, 9 and 17 respectively.

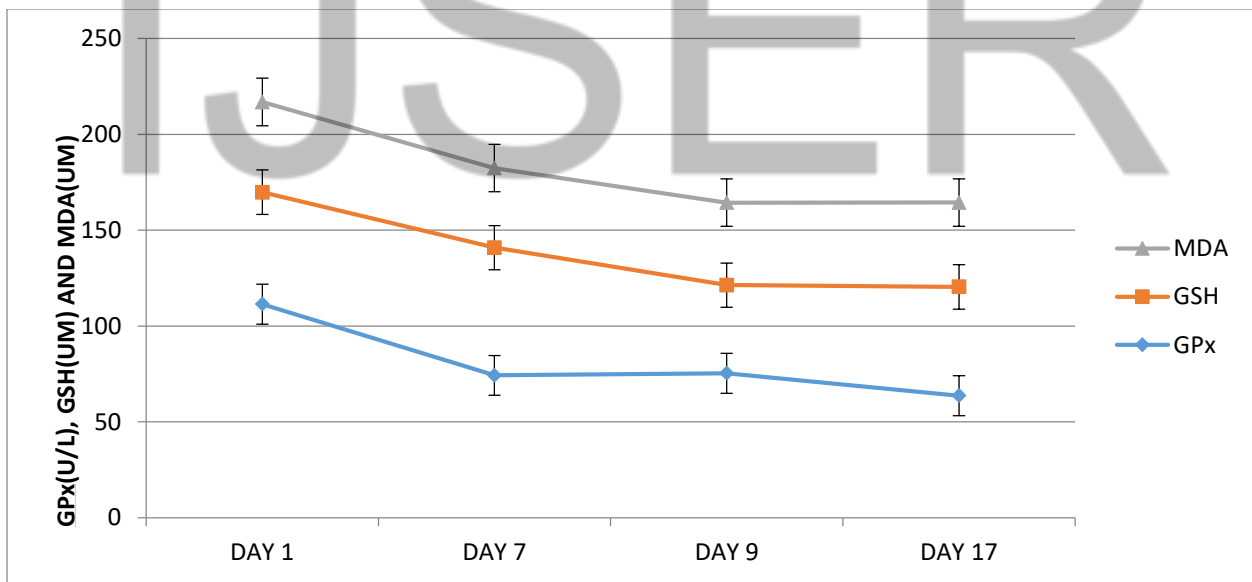


Figure 7: A Graphical representation showing the effect of disk over water cage induced sleep deprivation on GPx(u/L), GSH(uM) and MDA(uM) levels on experimental animals across all groups (Group 3). Data are expressed as mean  $\pm$ S.E.M (n=3).

On day 1, an increase was observed in GPx, GSH and MDA levels while a decrease was observed on day 7, 9 and 17 respectively.

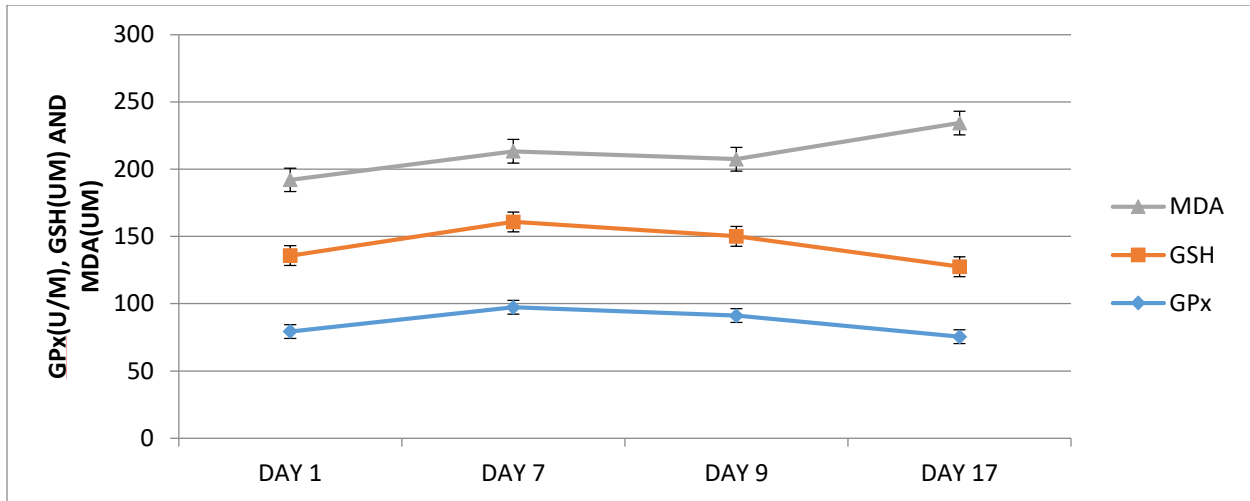


Figure 8: A Graphical representation showing the effect of disk over water cage induced sleep deprivation on GPx(u/L), GSH(uM) and MDA(uM) levels on experimental animals across all groups (Group 4). Data are expressed as mean  $\pm$ S.E.M (n=3).

On day 1, a decrease was observed in GPx, GSH and MDA levels while an increase was observed on day 7, 9 and 17 respectively.

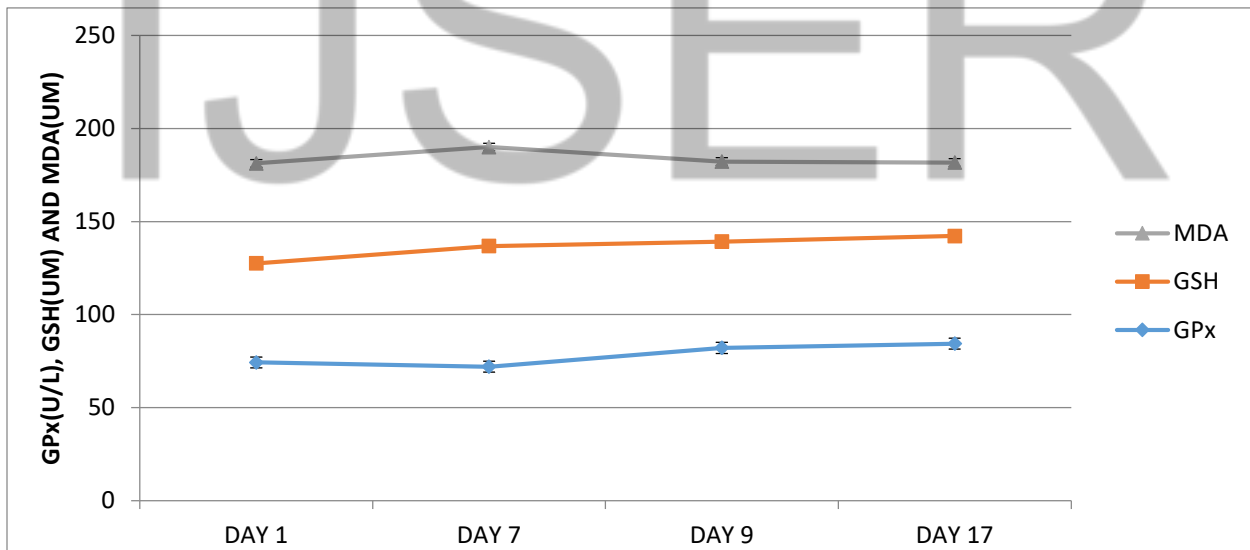


Figure 9: A Graphical representation showing the effect of disk over water cage induced sleep deprivation on GPx(u/L), GSH(uM) and MDA(uM) levels on experimental animals across all groups (Group 5). Data are expressed as mean  $\pm$ S.E.M (n=3).

No significance difference was observed across the days.



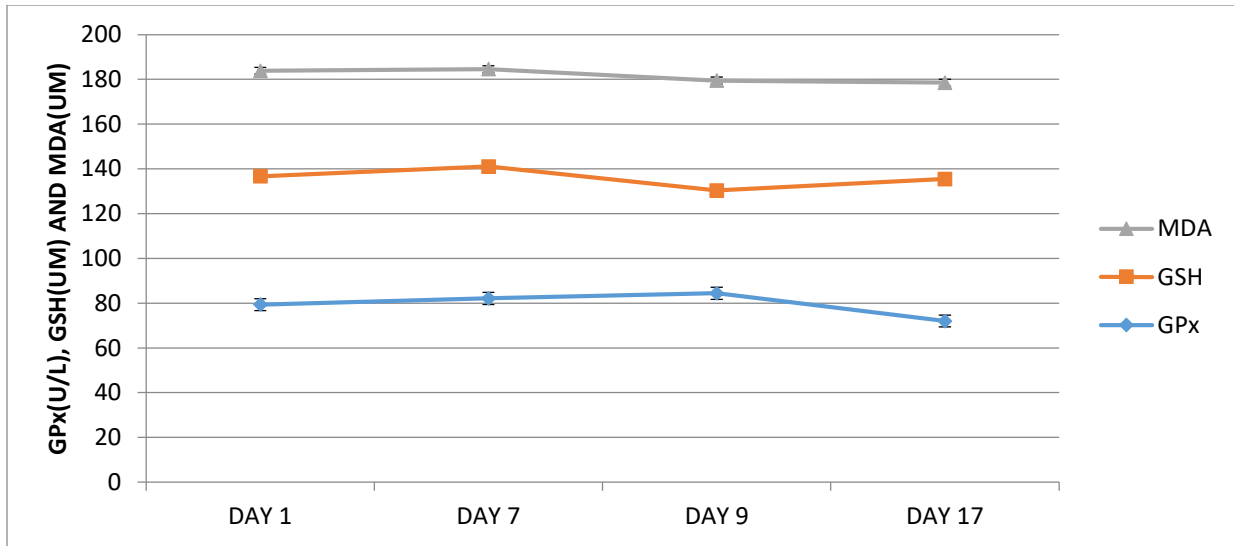


Figure 10: A Graphical representation showing the effect of disk over water cage induced sleep deprivation on GPx(u/L), GSH(uM) and MDA(uM) levels on experimental animals across all groups (Group 6). Data are expressed as mean  $\pm$ S.E.M (n=3).

No significance difference was observed in MDA level across the days, while a sharp decrease was observed in GSH level on day 9 and a decrease in GPx level on day 17.

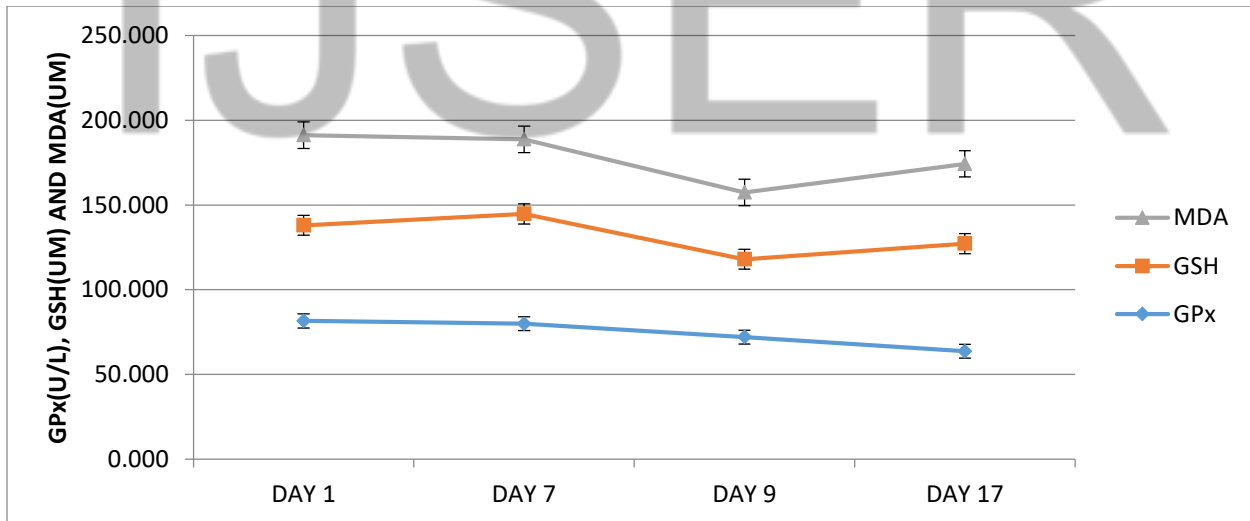


Figure 11: A Graphical representation showing the effect of disk over water cage induced sleep deprivation on GPx(u/L), GSH(uM) and MDA(uM) levels on experimental animals across all groups (Group 7). Data are expressed as mean  $\pm$ S.E.M (n=3).

No significance difference was observed in GPx level across the days, but there was a sharp decrease in GSH and MDA level on day 9.

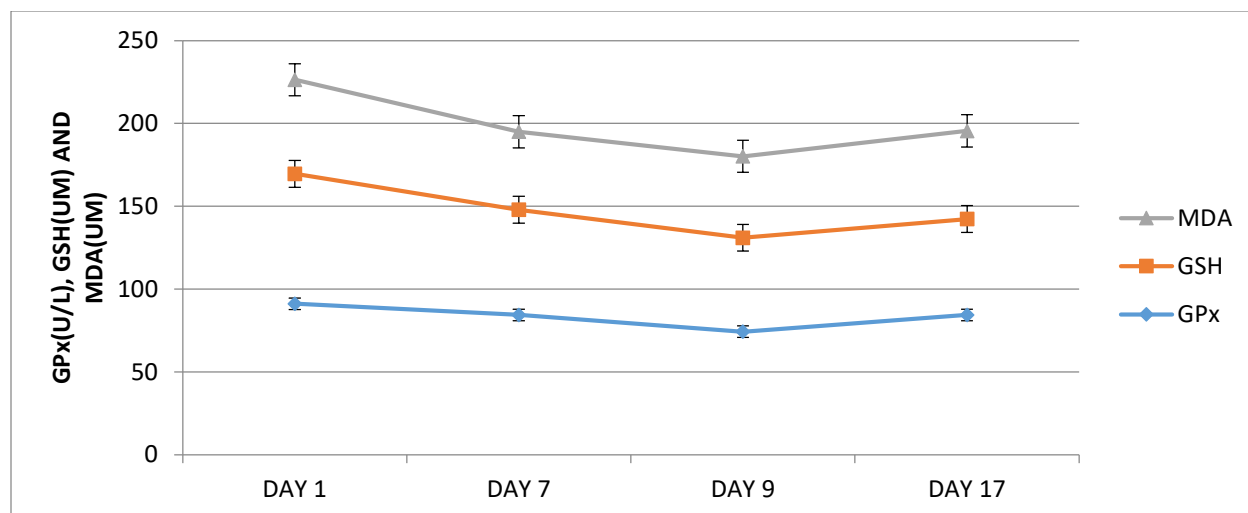


Figure 12: A Graphical representation showing the effect of disk over water cage induced sleep deprivation on GPx(u/L), GSH(uM) and MDA(uM) levels on experimental animals across all groups (Group 8). Data are expressed as mean  $\pm$ S.E.M (n=3).

On day 1, there was an increase in GPx, GSH and MDA level, while a decrease was observed on day 7 and 9.

## 7. DISCUSSION AND CONCLUSION

The results of the present study revealed the ability of sleep deprivation to ameliorate the imbalance between the reactive oxygen species and production of antioxidant enzymes in the sleep deprived male wistar rats. In this present study, Malondialdehyde (MDA) levels are significantly raised in the sleep deprived group, this alludes to the fact that sleep deprivation is a biological stressor (Olayaki *et al.*, 2015), while antioxidants, GSH and GPx was reduced in the sleep deprived groups.

In the sleep recovery group, the significant reduction in MDA level and increase in level of GPx and GSH can be attributed to sleep deprivation induced chronic stress and as such, sleep recovery restores or accentuates antioxidant level and leads to a decrease in free radical production. These findings are in consonant with the study of El-Aziz and Mostafa, 2012 and Olayaki *et al.*, 2015

The results of the present study revealed the ability of Sleep deprivation to ameliorate the imbalance between the reactive oxygen species and production of antioxidant enzymes in the sleep deprived male wistar rats. In the sleep recovery group the significant reduction in MDA level and increase in level of GPx and GSH can be attributed to sleep deprivation induced chronic stress.

In conclusion, sleep recovery restores or accentuates antioxidant level and leads to a decrease in free radical production.

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