

## EFFECT OF DIAZEPAM ON BLOOD AND BRAIN OXIDATIVE STRESS AND PLASMA ACTH OF ANXIOTIC MALE ALBINO RATS

By

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### ABSTRACT

The present study was designed to investigate the effect of diazepam on the physiological changes of anxiogenic male rats. 120 male albino rats weighing  $120 \pm 10$ g were divided into 6 groups. Control -ve (Untreated), control +ve (Treated with saline), drug (0.9mg diazepam/kg body weight), anxiety (Sleep deprived), drug+ anxiety, anxiety+ drug groups (exposed to the same conditions of drug and anxiety groups). Blood and brain samples were taken from all groups after 48 and 72 hours of anxiety. Blood was collected into two tubes, the first containing EDTA and the second without anticoagulant. Erythrocyte lysate, plasma and serum were separated and frozen at  $-20^{\circ}\text{C}$  for biochemical and hormonal analysis. The results revealed that 48 and 72 hours of sleep deprivation caused depletion of SOD, CAT and GSH activities and increase of MDA levels in blood and brain of anxiogenic rats compared with control ones. The increment of plasma ACTH was also reported in anxiety group. Anxiogenic rats treated with diazepam revealed an improvement in SOD, CAT and GSH activities and depletion of MDA level in blood and brain compared with anxiety group. Plasma ACTH concentrations was decreased also in anxiogenic rats treated with diazepam. It might be referred that treatment with diazepam minimizing deleterious effects of sleep-deprivation induced oxidative damage suggesting its anti oxidative effect, and its inhibitory effect on the hypothalamic pituitary adrenal axis (HPA) activity.

**Key words:** diazepam, oxidative stress, hypothalamic pituitary adrenal axis.

### INTRODUCTION

**S**leep deprivation is a kind of stress which leads to decrease in body weight in spite of increase in food intake <sup>(1)</sup>, initial hyperthermia followed by hypothermia <sup>(2)</sup>, increase in anxiety levels <sup>(3)</sup> and decrease locomotor activity, behavioral alteration (irritability and poor performance) <sup>(4)</sup>. Sleep deprivation is known to impair cognitive functions <sup>(5, 6)</sup>, oxidative stress <sup>(7)</sup> and decreased anti-oxidative defense <sup>(8)</sup>. The brain is more sensitive to oxidative stress due to an abundant presence of polyunsaturated fatty acids, a deficient antioxidant defense, high rate of oxygen utilization due to higher metabolic rate and high content of transition metals like copper and iron in several regions which could lead to formation of hydroxyl radicals <sup>(9)</sup>. Sleep deprivation causes oxidative stress by generating free radicals and non-radical derivatives of oxygen and nitrogen <sup>(10)</sup> and the imbalance between generation of these products and the antioxidant defense system results in oxidative stress. The hyper-metabolism and immuno-pathology in sleep-deprived animals are reported to produce excessive metabolic and oxidative burdens <sup>(11)</sup>.

It has been reported that oxidative stress induced due to sleep deprivation stimulate the hypothalamic-pituitary-adrenal (HPA) axis resulting in increased production of corticosterone <sup>(1)</sup>. Also, **Machawal and kumar** <sup>(12)</sup> found that immobilization stress raised corticosterone levels and oxidative stress as evidenced by rise in lipid peroxidation and depletion of reduced glutathione and catalase activity. Loss of sleep induces elevations in circulating levels of cholesterol, stress related hormones <sup>(13)</sup> and catecholamines <sup>(14)</sup>, with an increase in blood pressure and heart rate <sup>(15)</sup>. Further more, habitual sleep loss and insomnia are markers of subclinical heart disease and are independent predictors of cardiovascular disease risk <sup>(16)</sup>.

Diazepam is a benzodiazepine used in human therapeutics due to its anxiolytic, sedative and muscle relaxant effects. Its pharmacological activity is consequent to the enhancement of GABAergic transmission of benzodiazepine-sensitive GABA<sub>A</sub> receptors <sup>(17)</sup>. Diazepam exposure is related to oxidative alterations mediated by free radicals in experimental animals, namely rodents, as shown by **Musavi and Kakkar** <sup>(18)</sup>. Those

authors found that diazepam can play a prooxidant/antioxidant role dependant upon the dosage, and toxic responses can thus be modulated according to the assessed organs or regions. Benzodiazepine (BZD) is known to have sedative, hypnotic effects on the brain function represented by the induction of sleepiness and decline in psychomotor performance. When maintenance therapy is required (e.g. epilepsy and anxiety), benzodiazepines have prolonged half life time ( $t_{1/2}$ ) are preferred, as effective drug concentrations can be maintained with out the need for frequent dosing<sup>(19)</sup>.

The present study is conducted to investigate the effect of a single dose of diazepam on the oxidative stress in rats before and after anxiety.

## MATERIALS AND METHODS

### Experimental animals:

The present study was carried out at Zoology Department, Faculty of science, Benha University, Benha, Egypt. In this study, 120 white male albino rats (weighing  $120\pm 10$ g) were used. Rats were purchased from the laboratory of Animal colony, Ministry of Public Health, Helwan, Egypt. Animals were housed in wired cages at a temperature controlled environment ( $20\pm 2$ c°) and 12-hour light/dark cycle. Food and water were freely available through the period of the experiment. Rats were acclimatized to laboratory conditions before the test.

### Sleep deprivation protocol:

The sleep deprived animals were placed for 48 and 72 hours on the grid suspended over water as described by *Shinomiya et al.*,<sup>(20)</sup>. Animals were placed on a grid floor ( $29\text{cm}\times 15\text{cm}\times 7\text{cm}$ ) inside the plastic cage filled with water to 1cm below the grid surface. The stainless steel rods of the grid (3mm wide) were set 2cm apart.

### Drug:

Diazepam was purchased from Amoun Pharmaceutical company (A.R.E). Diazepam had many uses however, in this study it was used as anxiolytic agent.

Diazepam was administered as a single intramuscular dose ( $0.9\text{mg}/\text{Kg}$  body weight) according to *Morris*<sup>(21)</sup>

### Experimental groups:

Rats under the study were classified into the following groups (20 rats each):

**A-Control –ve group:** Untreated 20 rats.

**B-Control +ve group:** Twenty rats treated with 0.5ml saline and divided into two sub groups.

**C-Drug group:** Twenty rats treated with a single intramuscular dose of diazepam ( $0.9\text{mg}/\text{kg}$  b.w in 0.5 ml saline)

It was subdivided into two parts:

**Part 1:** Included 10 rats; from which, blood and tissue samples were collected after 48hours.

**Part 2:** Included 10 rats; from which, blood and tissue samples were collected after 72hours.

### D-Anxiety group:

It was also subdivided into two parts:

**Part 1:** Included 10 rats; were sleep-deprived for 48 hours then blood and tissue samples were collected.

**Part 2:** Included 10 rats; were sleep-deprived for 72 hours then blood and tissue samples were collected.

### E-Drug+Anxiety group (D+An):

It was subdivided into two parts:

**Part 1:** Included 10 rats. Firstly, rats were injected with a single dose of diazepam, waited for 2 hours then; they were sleep-deprived for 48hours, blood and tissue samples were collected

**Part 2:** Included 10 rats. Firstly, rats were injected with a single dose of diazepam, waited for 2 hours then; they were sleep-deprived for 72hours, blood and tissue samples were collected.

### F-Anxiety+Drug group (An+D):

It was subdivided into two parts:

**Part 1:** Included 10 rats. Firstly, rats were sleep-deprived for 48 hours then; they were injected with a single dose of diazepam, waited for 2 hours, and blood and tissue samples were collected.

**Part 2:** Included 10 rats. Firstly, rats were sleep-deprived for 72 hours then; they were injected with a single dose of diazepam, waited for 2 hours, and blood and tissue samples were collected.

### Blood and tissue sampling:

The rats were fasted over night prior to blood and brain collection. The rats were

anaesthetized by ether inhalation <sup>(22)</sup>. The blood was collected from the heart and the abdominal vein into two tubes: the first tube containing ethylene diamine tetra acetic acid (EDTA) (El-Gomhorya Co.Egypt) while the second one without anticoagulant. EDTA tubes were centrifuged immediately at 3000rpm for 15 minutes by Hittech® centrifuge and plasma free of hemolysis was separated and RBCs lysate was prepared and collected. Tubes without anticoagulant were also centrifuged after one hour and the serum free from hemolysis was separated. Erythrocyte lysate, plasma and serum were stored at -20°C for biochemical and hormonal assays. The rats were dissected and the brains were collected, perfused with PBS (phosphate buffered saline, PH=7.4 containing 0.16mg/ml heparin to remove any blood clots or RBCs) and homogenized in potassium phosphate for determination of superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), and malondialdehyde (MDA).

#### Biochemical analysis:

##### 1) Superoxide dismutase activity:

Superoxide dismutase (SOD) activity was assayed spectrophotometrically in the erythrocyte lysate and the brain homogenate according to **Nishikimi et al.** <sup>(23)</sup>. This assay relies on the ability of the enzyme to inhibit the phenazine methosulphate-mediated reduction of nitroblue tetrazolium dye and the absorbance was measured at 560nm. The SOD activity was expressed as U/ml for the erythrocyte lysate and U/g tissue for the brain homogenate.

##### 2) Catalase activity:

Catalase (CAT) activity was determined spectrophotometrically in the plasma and brain homogenate according to **Aebi** <sup>(24)</sup> where catalase reacts with a known quantity of H<sub>2</sub>O<sub>2</sub>, and then the reaction is stopped after exactly one minute with catalase inhibitor. The absorbance was measured at 510 nm and CAT activity was expressed as U/L for the plasma and U/g tissue for the brain homogenate.

##### 3) Reduced glutathione concentration:

Reduced glutathione (GSH) was estimated spectrophotometrically in the plasma and the

brain homogenate according to the method described by **Beutler et al.** <sup>(25)</sup>. The method based on the reduction of 5,5-dithiobis-(2-nitrobenzoic acid)(DTNB) with glutathione to compound. The reduced chromogen directly proportional to GSH concentration and the absorbance was measured at 405nm. The GSH concentration was expressed as mg/dl for the plasma and mg/g tissue for the brain homogenate.

##### 4) Malondialdehyde content:

The malondialdehyde content, a measure of lipid peroxidation, was assayed spectrophotometrically in the serum and the brain homogenate according to **Satoh** <sup>(26)</sup>. Thiobarbituric acid (TBA) reacts with malondialdehyde (MDA) in acidic medium at temperature of 95°C for 30 minutes to form thiobarbituric acid reactive product and the absorbance of the resultant pink product can be measured at 534nm. Malondialdehyde (MDA) content was expressed as nmol/ml for the serum and nmol/g tissue for the brain homogenate.

##### 5) Plasma adrenocorticotrophic hormone (ACTH):

Plasma ACTH concentration was estimated by the electrochemiluminescence immunoassay "ECLIA" according to **Talbot et al.** <sup>(27)</sup> with the aid of Cobas e411 (Japan) using commercial kits purchased from Roche Diagnostics, Germany. ACTH concentration was expressed as Pg/ml.

##### Statistical analysis:

The mean ± standard error was calculated for each measured parameter in all rat groups at 48hr and 72hr periods. Analysis of variance (ANOVA-One way) was carried out according to Costat Version 7.0.1 (copyright SPSS INC 1997).

## RESULTS

### 1-Superoxide dismutase activities (SOD; U/ml):

Erythrocyte and brain superoxide dismutase activities were significantly high and low (P<0.05) in rats of drug group and other treated groups respectively compared with those of control groups post 48 and 72hr periods of diazepam treatments, except those of An+D group in erythrocyte post 48 hours

which showed non significant difference compared with control ones.

SOD activities were significantly high in rats of anxiety, D+An and An+D groups after 48hr compared with those after 72hr, while they were significantly high in rats of drug group after 72hr compared with those after 48hr (Table 1 and 2).

### **2-Catalase activities (CAT; U/L):**

Plasma and brain catalase activities were significantly high and low ( $P<0.05$ ) in rats of drug group and other treated groups respectively compared with those of control groups after 48 and 72hr of administration, except those of brain of An+D group after 48 hours which was non significant high compared with control ones.

Catalase activities were significantly high in rats of anxiety, D+An and An+D groups after 48hr compared with those after 72hr of treatment. Plasma catalase activities showed non significant differences between two periods in rats of drug group while brain catalases were significantly high after the 72hr compared with those after 48hr period (Table 1 and 2).

### **3-Reduced glutathione (GSH; mg/dl):**

Plasma and brain levels of reduced glutathione were significantly high in rats of drug group and significantly low ( $P<0.05$ ) in rats of other treated groups after 48 and 72hr of treatment compared with those of control groups.

Plasma and brain GSH levels were significantly high in rats of anxiety, D+An and An+D after 48hr compared with those after 72hr of treatment, while they were significantly high in rats of drug group after 72hr compared with those after 48hr (Table 1 and 2).

### **4-Malondialdehyde concentrations (MDA; nmol/ml):**

Serum MDA concentrations were significantly low ( $P<0.05$ ) in rats of drug and An+D groups, high in rats of anxiety and non significantly in D+An group compared with those of control groups after 48hr of treatment. Serum MDA concentrations were significantly low in rats of drug group and high in rats of other treated groups compared with those of control ones after 72hr of treatment. Brain MDA concentrations were significantly low and high ( $P<0.05$ ) in rats of drug group and rats of anxiety group respectively after 48 and 72hr of treatment. They were non significantly high in rats of D+An and An+D groups at 48hr and 72hr respectively compared with control groups. Serum and brain MDA concentrations were significantly low in rats of anxiety, D+An and An+D groups after the 48hr period compared with those after 72hr period, while they were significantly low in rats of drug group after the 72hr period compared with those after 48hr period (Table 1 and 2).

### **5-Plasma adrenocorticotrophic hormone concentrations (ACTH; Pg/ml):**

Plasma ACTH concentrations were significantly low ( $P<0.05$ ) in rats of drug group and high in rats of other treated groups compared with those of control groups after 48 and 72hr periods. Plasma ACTH concentrations were significantly low in rats of anxiety, D+An and An+D groups after the 48hr period compared with those after 72hr period, while they were significantly low in rats of drug group during the 72hr period compared with those after 48hr period (Table 3).

**Table (1):** Levels of erythrocyte SOD, plasma CAT, GSH and serum MDA of control rat groups (control-ve & control+ve), rats group treated with diazepam (Drug), rats group exposed to anxiety (Anxiety), rats group treated with diazepam then anxiety (D+An) and rats group exposed to anxiety then treated with diazepam (An+D) post 48hr and 72hr of diazepam treatment.

Rat groups	Control-ve	Control+ve	Drug	Anxiety	D+An	An+D	P
SOD (U/ml)	48hr	251.54 ± 0.382 <sup>b</sup>	250.44 ± 0.600 <sup>b</sup>	255.00 ± 0.467 <sup>aB</sup>	244.63 ± 0.263 <sup>Ad</sup>	249.12 ± 0.382 <sup>Ac</sup>	251.34 ± 0.424 <sup>Ab</sup> ***
	72hr	251.10 ± 0.523 <sup>b</sup>	250.50 ± 0.605 <sup>b</sup>	260.93 ± 0.872 <sup>Aa</sup>	233.20 ± 0.744 <sup>Be</sup>	238.80 ± 0.455 <sup>Bd</sup>	241.60 ± 0.281 <sup>Bc</sup> ***
	P	NS	NS	***	***	***	***
CAT (U/L)	48hr	610.50 ± 0.760 <sup>bc</sup>	610.90 ± 0.425 <sup>b</sup>	617.80 ± 0.614 <sup>a</sup>	597.90 ± 1.277 <sup>Ae</sup>	606.90 ± 0.301 <sup>Ad</sup>	608.50 ± 0.201 <sup>AcD</sup> ***
	72hr	610.80 ± 0.579 <sup>b</sup>	611.80 ± 0.634 <sup>b</sup>	619.90 ± 0.664 <sup>a</sup>	573.60 ± 1.075 <sup>Be</sup>	586.80 ± 0.489 <sup>Bd</sup>	591.10 ± 0.254 <sup>Bc</sup> ***
	P	NS	NS	NS	***	***	***
GSH (mg/dl)	48hr	2.71 ± 0.027 <sup>b</sup>	2.76 ± 0.023 <sup>ab</sup>	2.82 ± 0.029 <sup>Ba</sup>	2.46 ± 0.029 <sup>Ad</sup>	2.55 ± 0.009 <sup>Ac</sup>	2.56 ± 0.009 <sup>Ac</sup> ***
	72hr	2.70 ± 0.010 <sup>b</sup>	2.73 ± 0.025 <sup>b</sup>	2.98 ± 0.038 <sup>Aa</sup>	2.30 ± 0.008 <sup>Bd</sup>	2.36 ± 0.007 <sup>Bd</sup>	2.44 ± 0.005 <sup>Bc</sup> ***
	P	NS	NS	*	***	***	***
MDA (nmol/ml)	48hr	25.92 ± 0.245 <sup>b</sup>	25.79 ± 0.153 <sup>b</sup>	23.87 ± 0.226 <sup>Ad</sup>	28.06 ± 0.310 <sup>aB</sup>	26.06 ± 0.230 <sup>Bb</sup>	24.80 ± 0.097 <sup>Bc</sup> ***
	72hr	25.51 ± 0.218 <sup>c</sup>	25.39 ± 0.222 <sup>c</sup>	22.70 ± 0.218 <sup>Bd</sup>	32.01 ± 0.528 <sup>Aa</sup>	27.45 ± 0.302 <sup>Ab</sup>	26.53 ± 0.310 <sup>Ab</sup> ***
	P	NS	NS	**	***	**	***

All data are expressed as mean ± SE

<sup>a,b,c,d</sup> values in the same row with different letters are significantly different (P<0.05).

<sup>A,B</sup> values in the same column with different letters are significantly different (P<0.05).

**Table (2):** Levels of SOD, CAT, GSH and MDA in brain of control rat groups (control-ve & control+ve), rats group treated with diazepam (Drug), rats group exposed to anxiety (Anxiety), rats group treated with diazepam then anxiety (D+An) and rats group exposed to anxiety then treated with diazepam (An+D) post 48hr and 72hr of diazepam treatment.

Rat groups		Control-ve	Control+ve	Drug	Anxiety	D+An	An+D	P
Parameter								
SOD (U/gm. Tissue)	48hr	671.20 ± 2.377 <sup>b</sup>	667.60 ± 1.382 <sup>b</sup>	802.80 ± 2.505 <sup>aB</sup>	598.70 ± 3.001 <sup>Ae</sup>	645.20 ± 0.939 <sup>Ad</sup>	652.50 ± 0.628 <sup>Ac</sup>	***
	72hr	671.50 ± 0.833 <sup>b</sup>	666.90 ± 0.844 <sup>b</sup>	851.40 ± 3.151 <sup>Aa</sup>	533.00 ± 2.377 <sup>Bd</sup>	560.20 ± 0.458 <sup>Bc</sup>	564.90 ± 0.348 <sup>Bc</sup>	***
	P	NS	NS	***	***	***	***	
CAT (U/gm. Tissue)	48hr	84.83 ± 0.873 <sup>b</sup>	84.07 ± 0.655 <sup>b</sup>	101.20 ± 1.412 <sup>aB</sup>	71.09 ± 0.589 <sup>Ad</sup>	79.02 ± 0.373 <sup>Ac</sup>	85.87 ± 0.403 <sup>Ab</sup>	***
	72hr	83.94 ± 0.717 <sup>b</sup>	85.01 ± 0.712 <sup>b</sup>	111.70 ± 1.408 <sup>Aa</sup>	61.89 ± 0.618 <sup>Be</sup>	73.60 ± 0.364 <sup>Bd</sup>	80.13 ± 0.414 <sup>Bc</sup>	***
	P	NS	NS	***	***	***	***	
GSH (mg/ gm. Tissue)	48hr	5.13 ± 0.141 <sup>b</sup>	4.92 ± 0.107 <sup>bc</sup>	7.09 ± 0.026 <sup>aB</sup>	4.14 ± 0.047 <sup>Ae</sup>	4.69 ± 0.012 <sup>Ad</sup>	4.81 ± 0.010 <sup>AcD</sup>	***
	72hr	4.96 ± 0.068 <sup>b</sup>	5.06 ± 0.051 <sup>b</sup>	7.88 ± 0.048 <sup>Aa</sup>	3.58 ± 0.013 <sup>Be</sup>	4.22 ± 0.008 <sup>Bd</sup>	4.38 ± 0.009 <sup>Bc</sup>	***
	P	NS	NS	***	***	***	***	
MDA (nmol/ gm. Tissue)	48hr	32.89 ± 0.124 <sup>b</sup>	32.23 ± 0.142 <sup>b</sup>	23.14 ± 0.318 <sup>Ac</sup>	34.27 ± 0.418 <sup>aB</sup>	32.61 ± 0.096 <sup>Bb</sup>	32.53 ± 0.152 <sup>Bb</sup>	***
	72hr	32.53 ± 0.291 <sup>c</sup>	31.76 ± 0.227 <sup>c</sup>	18.52 ± 0.276 <sup>Bd</sup>	37.31 ± 0.276 <sup>Aa</sup>	35.04 ± 0.295 <sup>Ab</sup>	34.53 ± 0.201 <sup>Ab</sup>	***
	P	NS	NS	***	***	***	***	

All data are expressed as mean ± SE

<sup>a,b,c,d</sup> values in the same row with different letters are significantly different (P<0.05).

<sup>A,B</sup> values in the same column with different letters are significantly different (P<0.05).

**Table (3):** Plasma ACTH concentrations of control rat groups (control-ve & control+ve), rats group treated with diazepam (Drug), rats group exposed to anxiety (Anxiety), rats group treated with diazepam then anxiety (D+An) and rats group exposed to anxiety then treated with diazepam (An+D) during 48hr and 72hr periods.

Rat groups	Control -ve	Control +ve	Drug	Anxiety	D+An	An+D	P	
Parameter	48hr	9.42 ± 0.065 <sup>d</sup>	9.51 ± 0.081 <sup>d</sup>	5.34 ± 0.126 <sup>Ae</sup>	13.69 ± 0.094 <sup>aB</sup>	11.23 ± 0.036 <sup>Bb</sup>	10.92 ± 0.019 <sup>Bc</sup>	***
	72hr	9.34 ± 0.034 <sup>e</sup>	9.53 ± 0.064 <sup>d</sup>	3.78 ± 0.055 <sup>Bf</sup>	15.63 ± 0.097 <sup>Aa</sup>	12.69 ± 0.063 <sup>Ab</sup>	11.92 ± 0.022 <sup>Ac</sup>	***
P	NS	NS	***	***	***	***	***	

All data are expressed as mean ± SE

<sup>a,b,c,d</sup> values in the same row with different letters are significantly different (P<0.05).

<sup>A,B</sup> values in the same column with different letters are significantly different (P<0.05).

## DISCUSSION

Sleep has important homeostatic functions and sleep deprivation is a stressor that has consequences for the brain as well as many body systems. Sleep deprivation, by itself or as a consequence of the technique to induce it, is a stressor and influences a number of physiological mechanisms, such as food intake, thermo regulation and immune function.

In the present study, SOD activities showed decreases in rats of anxiety group compared with those of control ones after 48 and 72 hours periods. This may be due to the oxidative stress caused by sleep deprivation and the pre and post treatment with diazepam improves SOD activities. This hypothesis agreed with Méndez-Cuesta *et al.* <sup>(28)</sup> who studied the effect of a single dose of diazepam on different markers of oxidative damage in the striatum of rats and found that 24 hours of acute stress decreased superoxide dismutase activity (54% below the control). In addition Ramanathan *et al.* <sup>(8)</sup> reported a significant decrease in superoxide dismutase activity in the hippo campus and brain stem in sleep-deprived rats.

The results of this work showed that there were decreases of CAT and GSH activities in rats of anxiety group after 48 and 72 hours

period. This may be due to the oxidative stress. Similar results were reported by Goyal and Anil <sup>(29)</sup> who revealed that acute immobilization stress in mice caused depleted glutathione and catalase activities in stressed brains. In support to this view Kalonia and Kumar <sup>(30)</sup> found that 48 hours sleep deprivation caused significant oxidative stress.

The present data indicated an increase in levels of malondialdehyde in rats of anxiety groups after 48 and 72 hours period. This may be due to the elevated rate of lipid peroxidation in sleep deprived rats due to increased oxidative stress which might be decreased by the treatment with diazepam. Similar results were found by Kumar and Kalonia <sup>(31)</sup> who reported that 48 hours of total sleep deprivation caused elevation in lipid peroxidation.

Singh and Kumar <sup>(32)</sup> explored that sleep deprivation caused oxidative damage as indicated by increase in lipid peroxidation. The treatment with alprazolam reversed raised lipid peroxidation.

Rats treated with diazepam revealed improvement in the anti oxidative capacity as shown by increase in SOD, CAT, GSH activities and depletion in MDA levels. These observations are supported by Mendez-

**Cuesta et al.** <sup>(28)</sup> who found that pretreatment of stressed rats with diazepam decreased the striatal lipid peroxidation levels (68% below the stress group) and improved mitochondrial function (18% above stress group), but only mild preservation of superoxide dismutase activity was detected (17% above the stress group).

The results of the present work showed increases in ACTH concentrations in rats of anxiety group after 48 and 72 hours period. This may be due to increased oxidative stress in sleep deprived rats. **Meerlo et al.**, <sup>(33)</sup> reported that 24 hours of sleep-deprivation caused significant increases in plasma ACTH and corticosterone in adult male rats. Immobilization stress has also been reported to induce 2-3 fold higher rise of plasma cortisol level. Increase cortisol level has been linked with anxiety like behavior <sup>(34)</sup>.

Diazepam treatment decreases the levels of ACTH. These results agreed with **Pomara et al.**, <sup>(35)</sup> who reported that acute diazepam administration has been shown to decrease plasma cortisol levels consistent with decreased activity of the hypothalamic-pituitary-adrenal (HPA) axis, especially in individuals experiencing stress.

In conclusion, the present study revealed that diazepam might be used to improve the oxidative stress damage resulted from sleep deprivation. Single dose of diazepam improve the oxidative stress effects after 48 and 72 hours of treatment.

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تأثير الديازيبام على إجهاد الاكسدة فى الدم والمخ وهرمون (اى سي تى انتش) فى ذكور الجرذان البيضاء المعرضة للقلق  
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قسم علم الحيوان-كلية العلوم-جامعة بنها-مصر.

تهدف هذه الدراسة الى التحرى عن تأثير الديازيبام على التغيرات الفسيولوجية فى ذكور الجرذان المعرضة للقلق، ولهذا الهدف تم تقسيم مائة وعشرون جرذ ابيض وزن كل واحد منها  $120 \pm 10$  جم الى 6 مجموعات وهى: المجموعة الضابطة السالبة (غير معالجة)، والمجموعة الضابطة الموجبة (معالجة بالمحلول الملحي)، ومجموعة الدواء (9, 0 مجم/كجم من وزن الجسم)، ومجموعة القلق (حرمت من النوم)، ومجموعة الدواء+القلق، ومجموعة القلق+الدواء (تعرضت لنفس ظروف مجموعة الدواء والقلق)، وقد أخذت عينات الدم وانسجة المخ من كل المجموعات بعد 4 و 8 و 24 ساعة حيث جُمع الدم فى نوعين من الانابيب احدهما تحتوى على مادة مانعة للتجلط والاخرى لا تحتوى عليها ثم فصل المصل والبلازما ومستخلص تكسير كرات الدم الحمراء وتم تفريزه عند درجة حرارة  $-20^{\circ}$  لاستخدامها فى القياسات الكيمائية الحيوية، وقد اظهرت النتائج ان الحرمان من النوم لمدة 4 و 8 و 24 ساعة ادى الى انخفاض معنوى فى مضادات الاكسدة (السوبر اوكسيد ديسميوتيز، كاتاليز، والجلوتاثيون المختزل)، وزيادة معنوية فى تركيز المالونديالدهيد فى دم وانسجة مخ الجرذان المعرضة للقلق مقارنة بالمجموعات الضابطة، وكذلك زيادة فى تركيز هرمون (اى سي تى انتش) فى بلازما نفس الجرذان. بعد العلاج بالديازيبام اوضحت النتائج تحسن فى انشطة السوبر اوكسيد ديسميوتيز، كاتاليز، والجلوتاثيون المختزل وانخفاض فى مستوى المالونديالدهيد، وكذلك قلة فى تركيز هرمون (اى سي تى انتش) فى جرذان المجموعات المعالجة مقارنة بجرذان مجموعة القلق، وبناء على هذا يمكن الاشارة الى ان العلاج بالديازيبام من المحتمل ان يقلل الآثار الضارة الناتجة عن اجهاد الاكسدة بسبب الحرمان من النوم لتأثيره المثبط على المحور الكظرى النخامى.