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Influence of various stressors on the thyroid function and to set up its correlation with the markers of oxidative stress in blood.

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ABSTRACT

In this set of experiment, the rats were grouped and exposed to physiological, chemical and psychological stressor over a sufficient long period and at the end of the exposure, the levels of thyroid hormones such as T_3, T_4 and TSH as well as the levels of oxidative stress-markers such as lipid peroxidation (LPO), superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH) were studied only in blood as the oxidative stress in blood may represents the generalized stress in the body. It was postulated that thyroxin may play important role in induction of oxidative stress. Variations of the levels of thyroid hormones can be one of the main physiological modulators of in vivo cellular oxidative stress. The thyroid hormones may play a crucial role in inducing the generation of generalized oxidative stress.

Keywords : stressors, Oxidative stress, thyroid hormones, markers of oxidative stress.

INTRODUCTION

Variations of the levels of thyroid hormones can be one of the main physiological modulators of in vivo cellular oxidative stress due to their known effects on mitochondrial respiration[1]. In particular, it has been suggested that the increase in reactive oxygen species induced by thyroid hormones can lead to an oxidative stress condition in the liver and in the heart and some skeletal muscles with a consequent lipid peroxidativeresponse[2;3].Both oxygen consumption and free radical production take place in mitochondrial membranes. The sensitivity to oxidative damage of these membranes is strongly dependent on their unsaturated fatty acid content (PUFAs) which are among the more susceptible cellular macromolecules to oxidative stress. At the same time, the lipid environment can directly affect membrane function, including mitochondrial electron transport and possibly oxygen free radical production. Moreover, changes in lipid composition of

cellular membranes appear to be well-established features of altered thyroid states in rat tissues[4;5].

In aerobic cells, active oxygen species, e.g. super-oxide and hydrogen peroxide, are generated as by-products of oxidative metabolism in mitochondria. These species are toxic to biomembranes and eventually lead to peroxidation of lipids unless they are removed by free radical scavenging enzymes, such as superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase. Antioxidant enzymes act to scavenge free radicals by converting them to less harmful molecules[4;5].

High concentrations of thyroid hormones may change the metabolism of oxygen in the cells and stimulate the production of free radicals[6]. In the course of hyperthyroidism, oxidative stress and the peroxidation of lipids can be generated[7]. Acceleration of the mitochondrial respiration[8] and basal metabolic rate and the energy metabolism of tissues in several mammalian species represent some of the major functions of thyroid hormones[9]. Accumulating evidence has suggested that the hypermetabolic state in hyperthyroidism is associated with increase in free radical production and lipid peroxide levels[10], whereas the hypometabolic state induced by hypothyroidism is associated with a decrease in free radical production[11] and in lipid peroxidation products[12]. The changes in the levels of the scavengers α-tocopherol[13], glutathione[14;15] and coenzyme Q[13], and activities of antioxidant enzymes[16] in various tissues were found to be imbalanced and often opposite. It is worth mentioning that some of the antithyroid drugs have antioxidant effects[17;18]. It was shown that both methimazole and propylthiouracil abolished or reduced the oxygen radical production by thyroid cells and decreased cytokine production[19]. Therole of thyroid hormones in metabolic pathways is well known. However, their involvement in lipidperoxidation and antioxidant enzyme activities is not known.

Reports suggest that high concentration of thyroid hormones can affect the metabolism of oxygen in aerobic condition and stimulate free radical generation in mitochondria[20]. Oxidative stress produces immunosuppression[21]. Reactive oxygen types play an important role in physiological mechanism, however, extremely reactive oxygen types leads to oxidative stress[22]. Reports suggests that hypothyroidism reduced oxidative stress in kidney and testis tissue, but short term, high dose of thyroxine administration in addition to hypothyroidism increased oxidative stress in same tissue of rats[23]. Other studies showed that hypothyroidism reduced oxidative damage in cerebral., hepatic and cardiac tissues of rats and high dose of thyroxine increased oxidative damage in tissues[24]. It has been proposed that hypothyroidism provide *in vivo* protection against free radical induced damage and this cellular defence mechanism may be acting differently from antioxidant defencesystem[25].Basedow disease patients, exhibited increased T3 and T4, and their treatment with propylthiouracil resulted in decreased catabolism and lowered oxidant generation[26]. These evidences suggest that oxidative stress in any diseased condition or in infected condition or in stressed condition may be mediated through thyroid gland. Hence, it is contemplated that thyroid gland plays a central role in generating generalized oxidative stress in diseased condition There are merits and demerits of free radical generation. Thyroid hormones play a crucial role in the regulation of mitochondrial oxidative metabolism.

Despite the fact that many research articles have been written aboutstress, stress-related diseases and oxidative stress etc., very little work has been done indicating the role of thyroid in induction of oxidative stress . The body generates free radicals by cellular mechanisms and or endocrine mechanism. Many scientific studies suggest that CRF supports neuronal system increases the neuronal effects, may generate more free radicals. Most scientists view stress as the situation when hypothalamo-pituitary-adrenocortical (HPA) axis, represented mainly by elevated ACTH levels, is activated[27].

Others suggest that activation of other systems with or without an elevation in ACTH may reflect stress-induced disturbed homeostasis. Apart from other factors the role of neuroendocrine response in coping with stress is well recognized. During stress response the physiological processes are playing vital role to redirect energy utilization among various organs. The thyroid gland is the body's primary regulator of metabolism. Thyroid stimulating hormone (TSH) affect metabolism and may be affected by the thyroxin secretions

Therefore, it is postulated that thyroxin may play important role in induction of oxidative stress[28]. Variations of the levels of thyroid hormones can be one of the main physiological modulators of *in vivo* cellular oxidative stress due to their known effects on mitochondrial respiration[29]. The thyroid hormones may play a crucial role in inducing the generation of generalized oxidative stress.

MATERIALS AND METHODS

Animals : Sprague-Dawley rats of both sex weighing between 180-230gms were used for experiment. They were maintained in a clean polypropylene cage with food and water *ad libitum*. All the rats were kept under the same experimental conditions of temperature (25 ± 2^0C), humidity (55±2) and light (dark/light -12/12hr cycle).Institutional Animal Ethical committee under guidelines of CPCSEA, New Delhi, INDIA, approved the protocol.

Chemicals: Diacetylmonoxime, thiosemicarbazide, metaphosphoric acid, picric acid, uric acid, trichloroacetic acid, thiobarbituric acid, pyrogallol, hydrogen peroxide, 5,5 dithio-bis-2 nitro benzoic acid (DTNB). Chemicals were purchased from LOBA Chemie, Burgoyne, Merck and Sigma Aldrich Co. U.S.A.

Assessment of thyroid function test

Blood was withdrawn from retro-orbital plexus of all the rats and was tested for thyroid function tests like levels of T_3, T_4& TSH. Tests were carried in association with Sainath blood bank and Thyrocare technologies Ltd. T_3, T_4& TSH were assessed by competitive ChemiluminescentImmuno Assay.

Assessment of oxidative stress

Lipid Peroxidation Assay(LPO)

Method: For determination of lipid peroxidation (LPO,) the blood was withdrawn from retro-orbital plexus and was taken in the centrifuge tube containing anticoagulant. From this 5% suspension of RBC in 0.1M phosphate buffered saline was prepared.

To the 2ml of this 5% suspension, 2ml of 28% trichloroacetic acid was added and centrifuged. After centrifugation the supernatant was separated. To the 4ml of supernatant 1ml of 1% thiobarbituric acid was added, heated in boiling water for 60 minute and cooled immediately. The absorbance was measured spectrophotometrically at 532 nm. The lipid peroxidation was calculated on the basis of the molar extinction coefficient of malondialdehyde (1.56×10^5) and expressed in terms of nanomoles of MDA/g Hb[30].

Reduced glutathione Assay(GSH)

Method: Glutathione activity was measured in whole blood. Whole blood (0.2ml) was added to 1.8 ml of distilled water followed by 3.0ml of precipitating mixture (1.67 gms of metaphosphoric acid, 0.2gms of EDTA, and 30 gmsNaCl to make 100ml of solution). It was centrifuged at 2000 rpm for 5 minutes. 1ml supernatant was added to 1.5ml of phosphate solution followed by addition of 0.5ml of DTNB reagent. The optical density was measured at 412nm using spectrophotometer [31].

Assay of Superoxide dismutase activity (SOD)

Method: The SOD was determined in the erythrocyte lysate prepared from the 5% RBC suspension. To 50µl of the lysate, 2ml of 75mM of trisHCl buffer (pH 8.2), 0.6 ml of 30mM of EDTA and 0.3ml of 2mM of pyrogallol were added. An increase in the absorbance was measured at 420nm for 3 minutes using spectrophotometer. One unit of enzyme activity is 50% inhibition of the rate of auto-oxidation of pyrogallol, as determined by change in absorbance/minute at 420nm [32].

Assay of Catalase activity (CAT)

Method: The activity of catalase enzyme was determined in erythrocyte lysate. The lysate (50µl) was taken and added to a test tube containing 2ml of phosphate buffer (pH 7.0) and then 1ml of 30mM of H_2O_2 was added to it. The decrease in absorbance was measured at 240 nm for 1 minute using spectrophotometer [33].

RESULTS

A)*Influence of physiological stress on the thyroid function levels andmarkers of oxidative stress in rats.* (Table-1).

Table 1: Influence of physiological stress on the thyroid function levels and on the markers of oxidative stress in blood

	Control (unstressed)	Experimental (stressed)
T_3	33.17 ± 0.87	47.64 ± 0.29**
T_4	1.45 ± 0.45	3.85 ± 0.37
TSH	2.11 ± 0.25	1.21 ± 0.32#
LPO	2.05±0.39	2.45 ± 0.21
SOD	39.30±0.78	37.20±0.89*
CAT	291.2±0.57	229.5±0.68**
GSH	10.80±0.67	10.59 ± 0.65

*Values are mean ±SEM(n=6). *P<0.001, #P<0.05 and **P<0.01, when compared to respective control (unstressed).*

i) T_3 (ng/dL) **:**In control (unstressed) group, levels of T_3 were 33.17±0.87, whereas in experimental (stressed) group, these were increased to 47.64±0.29.

ii) T_4 (µg/dL) **:**In control (unstressed) group, levels of T_4 were 1.45 ±0.4, whereas in experimental group (stressed), these were increased to 3.85 ±0.37 .

iii) TSH (µIU/mL) **:**In control (unstressed) group, levels of TSH were 2.11±0.25, whereas in experimental group (stressed), these were decreased to 1.21±0.32 .

iv) Lipid peroxidation (nmols MDA/mg protein) **:**In control (unstressed) group, LPO levels were 2.05±0.39, whereas in experimental (stressed) group, these were increased to 2.45±0.21 in blood.

v) Superoxide dismutase (U/mg protein) **:**In control (unstressed) group, the SOD activity was39.30±0.78, whereas in experimental (stressed) group, this value decreased to 37.20±0.89 in blood .

vi) Catalase (nM of H_2O_2 decomposed/min/mg protein) **:**In control (unstressed) group, the CAT activity was 291.2±0.57, whereas in experimental (stressed) group, this value decreased to 229.5±0.68 in blood .

vii) Reduced Glutathione **(**µM/ mg protein) **:**In control (unstressed) group, the GSH content was 10.80±0.67, whereas in experimental (stressed) group, this value decreased to 10.59 ± 0.65 in blood .

B)*Influence of chemical stress on the thyroid function levels andmarkers of oxidative stress in rats.* (Table-2).

Table 2: Influence of chemical stresson the thyroid function levels andmarkers of oxidative stress in rats

	Control (unstressed)	Experimental (stressed)
T_3	36.17 ±0.94	56.17 ±0.54
T_4	1.75 ± 0.24	3.85 ± 0.37
TSH	2.16 ± 0.18	0.98 ± 0.26
LPO	2.27±0.22	3.94±0.23
SOD	35.88 ± 0.73	30.90±0.68
CAT	280.1 ± 1.45	226.1±0.75
GSH	10.73± 0.13	9.59 ± 0.65

*Values are mean ±SEM(n=6). *P<0.001, #P<0.05 and **P<0.01, when compared to respective control (unstressed).*

i) T_3 (ng/dL) **:**In control (unstressed) group, levels of T_3 were 36.17 ±0.94, whereas in experimental group (stressed), these were increased to 56.17 ±0.54 .

ii) T_4 (µg/dL) **:**In control (unstressed) group, levels of T_4 were 1.75± 0.24, whereas in experimental group (stressed), these were increased to 3.85 ±0.37.

iii) TSH (µIU/mL) **:**In control (unstressed) group, levels of TSH were 2.16± 0.18, whereas in experimental group(stressed), these were decreased to 0.98± 0.26 .

iv) Lipid peroxidation (nmols MDA/mg protein) **:**In control (unstressed) group, the LPO levels were 2.27±0.22, whereas in experimental (stressed) group, these were increased to 3.94±0.23 in blood .

v) Superoxide dismutase((U/mg protein) **:**In control (unstressed) group, the SOD activity was 35.88 ± 0.73, whereas in experimental (stressed) group, this value decreased to 30.90±0.68 in blood .

vi) Catalase **(**(nM of H_2O_2 decomposed/min/mg protein) **:**In control (unstressed) group, the CAT activity was 280.1 ± 1.45, whereas in experimental (stressed) group, this value decreased to 226.1±0.75 in blood .

vii) Reduced Glutathione(µM/ mg protein) **:**In control (unstressed) group, the GSH content was 10.73± 0.13, whereas in experimental (stressed) group, this value decreased to 9.59 ± 0.65 in blood .

C)*Influence of psychological stress on the thyroid function levels andmarkers of oxidative stress in rats.* (Table-3)

Table 3: Influence of psychological stresson the thyroid function levels andmarkers of oxidative stress in rats

	Control (unstressed)	Experimental (stressed)
T_3	34.34 ±0.32	42.17 ±0.34
T_4	2.56 ± 0.14	3.18 ± 0.25
TSH	1.33 ± 0.25	1.14 ± 0.15
LPO	1.95±0.15	2.34 ± 0.32
SOD	36.03±0.93	31.97±0.85
CAT	290±2.68	268±1.79
GSH	11.59 ± 0.06	10.40±0.18

*Values are mean ±SEM(n=6). *P<0.001, #P<0.05 and **P<0.01, when compared to respective control (unstressed).*

i) T_3 (ng/dL) **:**In control (unstressed) group, the levels of T_3 were 34.34 ±0.32 whereas in experimental group (stressed), these were increased to 42.17 ±0.34 .

ii) T_4 (µg/dL) **:**In control (unstressed) group, the levels of T_4 were2.56 ± 0.14 whereas in experimental group(stressed), these were increased to 3.18 ± 0.25 .

iii) TSH (µIU/mL) **:**In control (unstressed) group, the levels of TSH were1.33 ± 0.25 whereas in experimental group(stressed), these were decreased to 1.14 ± 0.15. .

iv) Lipid peroxidation (nmols MDA/mg protein) **:**In control (unstressed) group, the LPO levels was 1.95±0.15, whereas in experimental (stressed) group, these were increased to 2.34 ± 0.32 in blood .

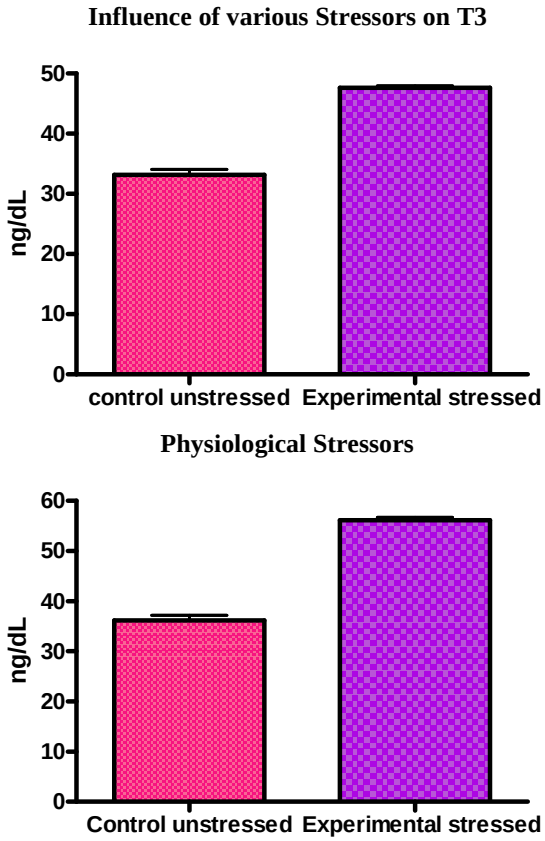
v)) Superoxide dismutase **(** (U/mg protein) **:**In control (unstressed) group,, the SOD activity was 36.03±0.93, whereas in experimental (stressed) group, this value decreased to 31.97±0.85 in blood .

vi) Catalase (nM of H_2O_2 decomposed/min/mg protein) **:**In control (unstressed) group, the CAT activity was 290±2.68, whereas in experimental (stressed) group, this value decreased to 268±1.79 in blood .

vii) Reduced GlutathioneµM/ mg protein**:**In control (unstressed) group, the GSH content was 11.59 ± 0.06, whereas in experimental (stressed) group, this value decreased to 10.40±0.18 in blood .

DISCUSSION

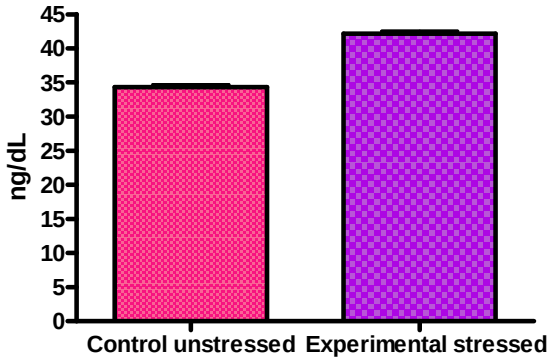
In all the stressed conditions, there was an increase in T_3 and T_4 and decrease in TSH. There was a concurrent increase in LPO and decrease in the SOD and CAT activity and reduction in the reduced glutathione content in blood. The data on oxidative stress and blood levels of thyroid hormones T3 and T4 condition exhibited a linear correlation.



The changes in thyroid hormone levels correlates with the parameters of oxidative stress. In unstressed condition, there was a decrease in T_3 and T_4 levels and increase in TSH in hypothyroid state and the levels of LPO decreased whereas the SOD and CAT activity and the

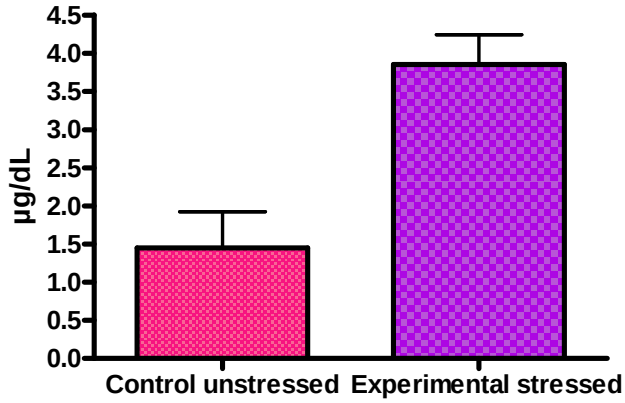
reduced glutathione content increased significantly as compared to normal. In all the stressed conditions, there were an increase in T_3 and T_4 levels and decreased in TSH in hyperthyroid state and levels of LPO increased whereas SOD and CAT activity and the reduced glutathione content decreased significantly as compared to normal.

Chemical Stressors

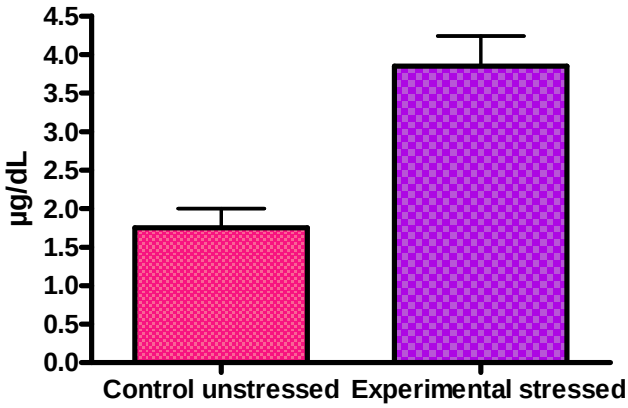


Psychological Stressors

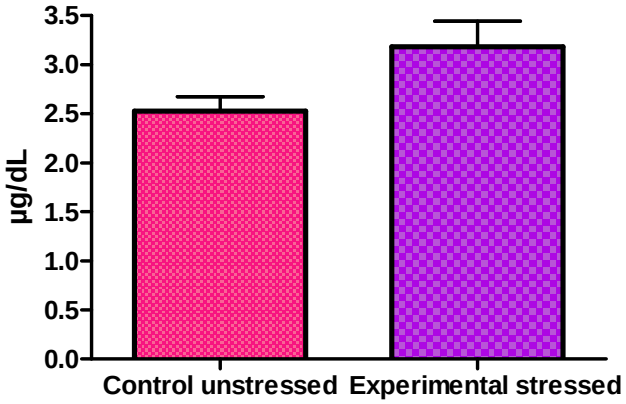
Influence of various Stressors on T4



Physiological Stressors

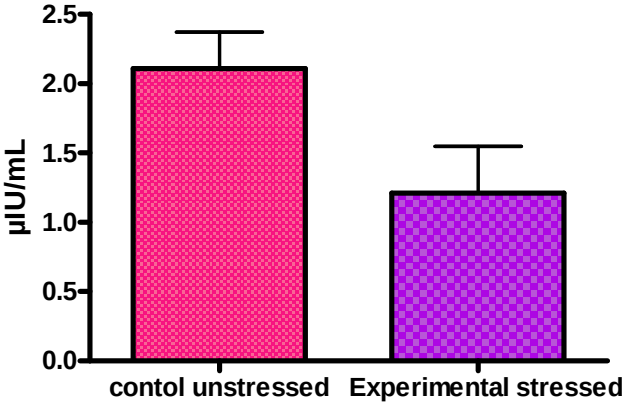


Chemical Stressors

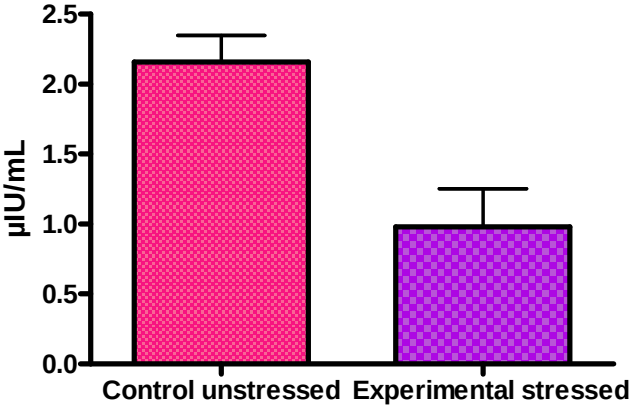


Psychological Stressors

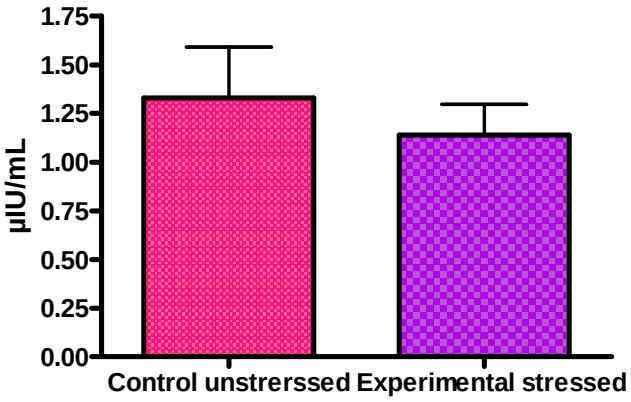
Influence of various Stressors on TSH



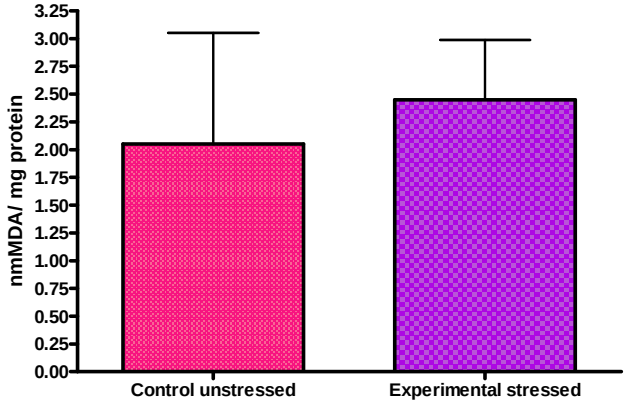
Physiological Stressors



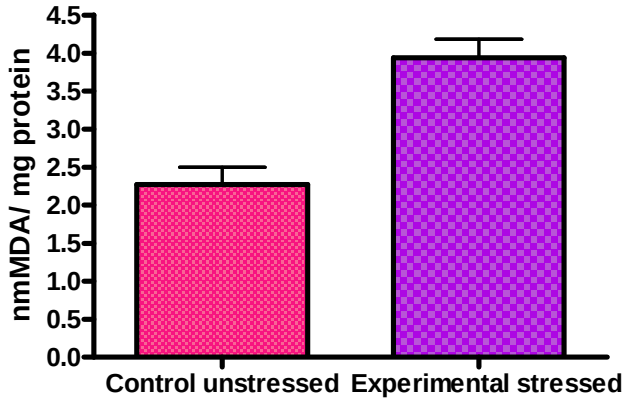
Chemical Stressors



Psychological Stressors
Influence of various Stressors on LPO



Physiological Stressors



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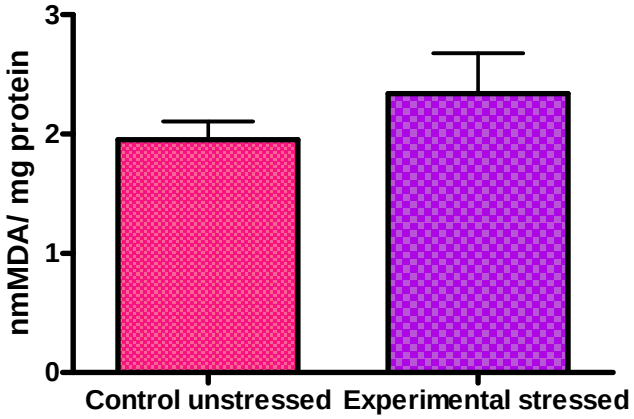
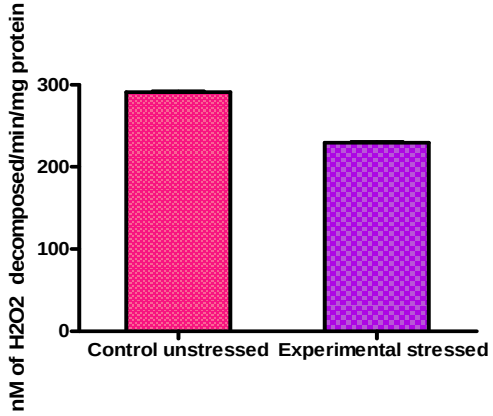
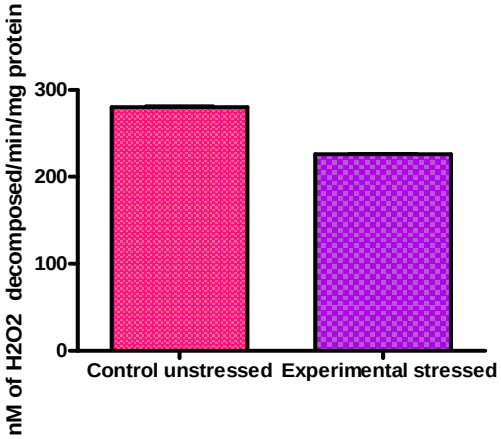


Psychological Stressors
Influence of various Stressors on CAT



Physiological Stressors



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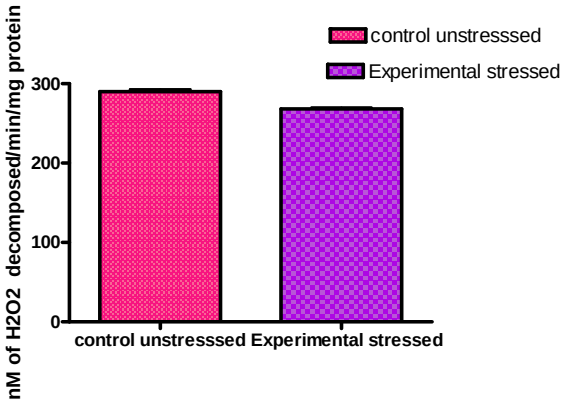
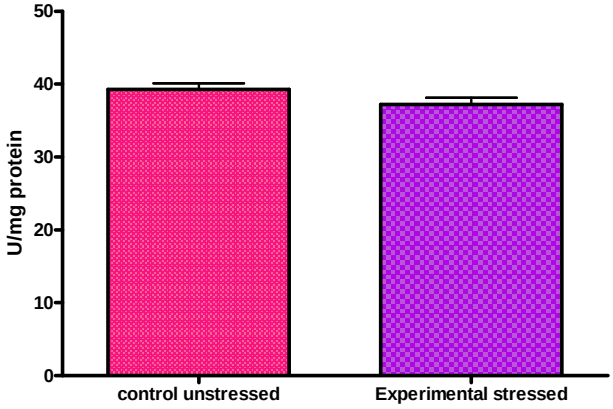
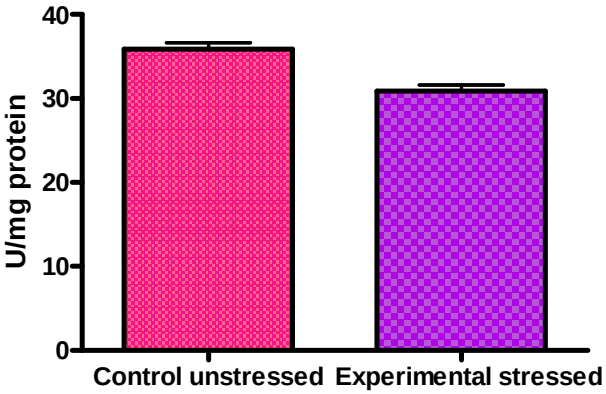


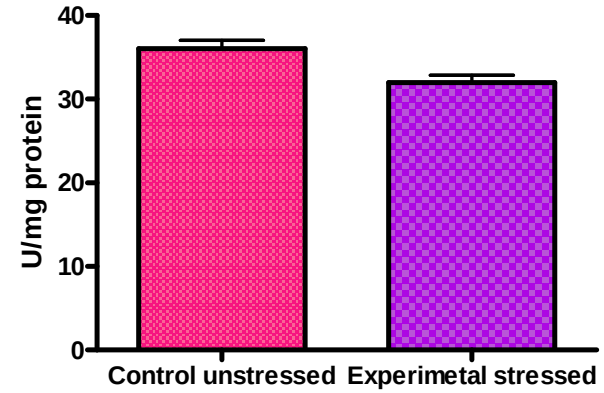
Psychological Stressors
Influence of various Stressors on SOD



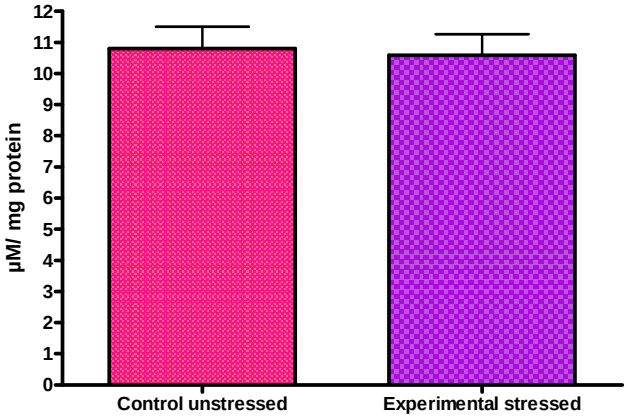
Physiological Stressors



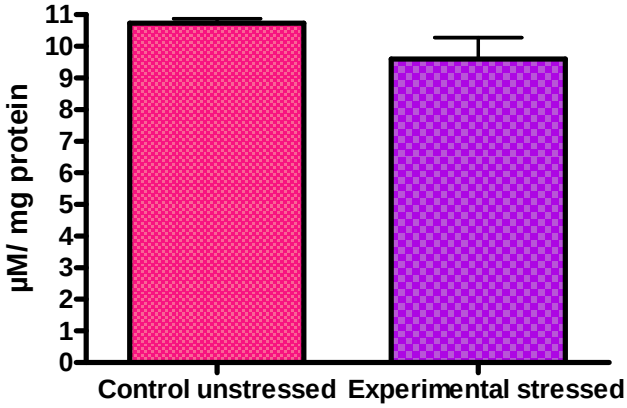
Chemical Stressors



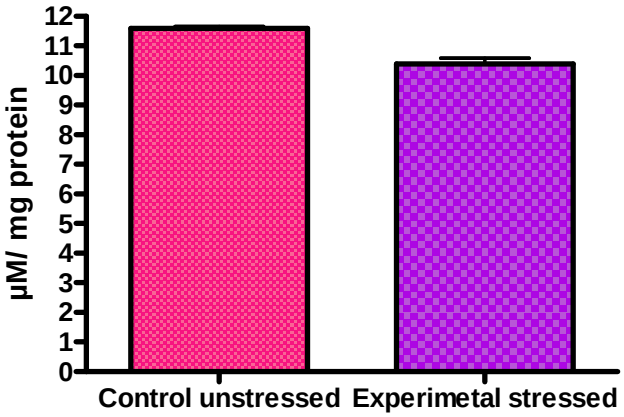
Psychological Stressors
Influence of various Stressors on GSH



Physiological Stressors



Chemical Stressors



Psychological Stressors

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