Impact of Altered Behavioral Condition on Markers of Oxidative Stress and Different Biochemical Parameters

D. S. Mohale, A. V. Chandewar

Abstract—Aim- Study was undertaken to investigate the effect of altered behavioral condition like depression on various oxidative stress markers and biochemical parameters in rats. Methods- Rats were subjected for short (21 days) and long term (84 days) social isolation; the rats displayed an increase in depression on force swim test relative to control. Various markers of oxidative stress like lipid per oxidation (LPO), reduced glutathione (GSH), Supers oxide dismutase (SOD), catalase (CAT) and biochemical parameters like SGOT, SGPT, and blood glucose were determined. Results- There was significant increase in the level of LPO and decrease in the levels of GSH, SOD and CAT after long term isolation. Biochemical parameters were significantly altered after social isolation. Conclusion- Increased oxidative stress in depression which may leads to alteration of biochemical parameters.

Keywords—Depression, Glucose, LPO, Oxidative stress.

I. INTRODUCTION

THE term "depression" is still somewhat ill-defined covering a range of phenomena from a normal emotion - a natural response to loss or disappointment - to an accompanying symptom common in a variety of physical conditions, up to a clinical psychiatric disorder. [1] It is one of the most common mental disorders among humans and it is associated with a significant negative impact on quality of life, morbidity/mortality, and cognitive function. The pathophysiology of depression is multifactorial and includes changes in brain monoaminergic transmission, abnormalities in neurotransmitter receptors function, reduced neurotrophic factors, dysregulation of HPA axis (cortisol), increased proinflammatory cytokines, increased NO (e.g., L-arginine-NO-cGMP pathway) etc. [2]-[4].

A variety of physiologic and pathophysiologic procedures are believed that reactive oxygen species play an important part in which the expansion of oxidative stress may have a significant function in disease mechanisms. Oxidative stress is defined as the deleterious impact in cell function as a consequence of the loss in homeostatic balance between reactive oxygen species (ROS) and antioxidants in the cellular milieu. ROS are formed continuously as a result of normal cellular respiration, enzymatic metabolism, and exogenous insults. Oxidative stress has been implicated in the onset and development of several pathological processes including cancer and age-related neurodegenerative diseases such as Parkinson's disease (PD) [5].

Reactive oxygen species (ROS) such as $O_2{}^-$, H_2O_2 and OH are highly toxic to cells. Cellular antioxidant enzymes and the free-radical scavengers normally protect a cell from toxic effects of the ROS. However, when generation of the ROS overtakes the antioxidant defense of the cells, oxidative damage of the cellular macromolecules (lipids, proteins, and nucleic acids) occurs, leading finally to various pathological conditions. ROS-mediated lipid per oxidation, oxidation of proteins, and DNA damage are well-known outcomes of oxygen-derived free radicals, leading to cellular pathology and ultimately to cell death.

II. MATERIAL AND METHOD

A. Chemicals

Vitamin E, Imipramine, Ethylene diamine tetra acetic acid (EDTA), Hydrogen peroxide H_2O_2, Metaphosphoric acid, Pyrogallol, Thiobarbituric acid (TBA), Tris buffer, Trychloroaceticacid (TCA), 5,5'-Dithiobis(2-nitrobenzoic acid) (DTNB), Phosphate buffer, Phosphate buffer saline, Drafkin's Reagent, SGOT dtermination kit, SGPT determination kit.

B. Experimental Animal

Healthy Male Sprague–Dawley rats (200-250gm) were used for the pharmacological screening. The animals were housed in polypropylene cages with wire mesh top and husk bedding and maintained under standard environmental conditions (25 ± 2^0C, relative humidity 60 ± 5%, light- dark cycle of 12 hours each) and fed with standard pellet diet (Trimurti feeds, Nagpur) and water *ad libitum*, were used for the entire animal study. The experiments were performed during day (08:00-16:00 hours). The rats were housed and treated according to the rules and regulations of CPCSEA and IAEC. The protocol for all the animal study was approved by the Institutional Animal Ethics Committee (IAEC). (Certification number– 650/02/c/CPCSEA/12 & date 30/09/2011.)

The animals were grouped in to 4 groups each group possesses 6 animals as follows: -

Group I Normal Control Group: Animals were not treated with any drugs or injections

Group II Negative Control Group: Animals were isolation in dark room and no treatment was given.

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International Journal of Pharmacological and Pharmaceutical Sciences
Vol:7, No:12, 2013

Group III (Social Isolation + Imipramine): Animal were treated with imipramine (16mg/kg, i.p.).

Group IV (Social Isolation + Imipramine + Vit. E): Animals were treated with Imipramine (16mg/kg, i.p.) and Vit. E (100 mg/kg, oral).

Behavioral and Oxidative stress parameters (in blood & tissue) were studied in Socially Isolated rats and normal rats [social isolation for 21 days (short term) and 84 days (long term)] [6], [7]. After short and long term isolation, rats were assessed for behavior using various animal behavioral models like force swim test.

C. Determination of Behavior

1. Forced Swim Test

It was carried out in a cylinder (45x20cm) rat was placed in the cylinder containing 38 cm water (25 ± 20º), so that the rat could not touch the bottom of the cylinder with its hind limb or tail, or climb over the edge of the chamber. Two swim sessions were conducted, an initial 15min pretest followed by 5min test 24h later. Drugs were administered after the pretest. The period of immobility (remained floating in water without struggling and making only those movements necessary to keep its head above water) during 5min Test period was noted [8].

2. Tail Suspension Test (TST)

In this test the rat was hung upside-down using an adhesive tape to fix its tail to a vertical surface (the edge of a laboratory bench). A square platform made of plywood was positioned horizontally 20–30cm (depending on the animal's size) below the bench, just under the rat's forepaws, in such a way that the rat could lightly touch the platform and thus minimize the weight sustained by its tail. The illumination in the test room was of about 70 lx. During 6min, the total time of immobility was registered through direct observation. The animal was considered immobile when it was not making any movements of struggling, attempting to catch the adhesive tape, body torsions or jerks [9].

D. Determination of Oxidative Stress Parameters in Blood

Suspension of RBC (red blood cells, 5%) was prepared by adding phosphate buffer saline (8ml) to packed cells. 0.5ml of 5% RBC was mixed with 5ml of distilled water, shaken for 5 min. then kept at 4°C for 5min. Subsequently, 0.4ml of 3:5 chloroform ethanol mixtures was added, shaken vigorously to precipitate hemoglobin, and then 0.15ml of distilled water was added. The mixture was centrifuged to get a clear erythrocyte lysate.

1. Determination of LPO

LPO (lipid peroxidation) was determined on the basis of the molar extinction coefficient of MDA (1.56/105) and expressed in terms of nanomoles of MDA/gHb [10].

2. Determination of CAT

The activity of CAT enzyme was determined in the erythrocyte lysate as the decrease in absorbance was measured spectrophotometrically at 240nm for 1 minute [11].

3. Determination of SOD

The activity of SOD was determined in the erythrocyte lysate by monitoring spectrophotometrically the increase in the absorbance at 420nm for 3 minutes [12].

4. Determination of GSH

Blood GSH (reduced glutathione) was measured by addition of 0.2ml of whole blood to 1.8ml of distilled water followed by 3.0ml of precipitating mixture. It was centrifuged at 2000 rpm for 5 minutes and 1 ml of supernatant was added to 1.5ml of phosphate solution, followed by addition of 0.5 ml of DTNB {Dithionitrobenzoic acid; 5, 5'-Dithiobis (2-nitrobenzoic acid)} reagent. The absorbance was measured at 412nm [13].

E. Determination of Oxidative Stress Parameters in Tissue

After receiving the treatments for 84 days, the rats were sacrificed using deep ether anesthesia. The liver was removed and thoroughly washed with ice-cooled 0.1 M phosphate buffer saline (PBS) containing 0.1 mmol/L phenyl methanesulfonyl fluoride. This tissue was blotted dry and homogenized in 0.1 M PBS in an ice bath to prepare a 10% suspension. This suspension was then centrifuged at $16000 \times$ rpm for 1h in a cooling centrifuge at 0°C. The supernatant was employed to assess the parameters of oxidative stress after estimating the protein content [14].

1. Determination of LPO

The extent of lipid peroxidation in terms of thiobarbituric acid reactive substances (TBARS) formation was measured. As 99% TBARS are malondialdehyde (MDA), so TBARS concentrations of the samples were calculated on the basis of the molar extinction coefficient of MDA (1.56×105) and expressed in terms of nanomoles of MDA/mg tissue [10].

2. Determination of CAT

The enzyme catalase converts H_2O_2 into water. The activity of CAT tissue homogenate, the decrease in absorbance was measured at 240nm for 1 minute using spectrophotometer by calculating the rate of degradation of H_2O_2, the substrate of the enzyme. [11] One unit of CAT activity is defined as the amount of enzyme, which reduces 1 millimole of H_2O_2 per minute.

3. Determination of SOD

The activity of SOD was determined in the tissue homogenate. An increase in the absorbance was measured at 420 nm for 3 minutes using spectrophotometer [12].

4. Determination of GSH

GSH was measured by addition of 0.2ml of tissue homogenate to 1.8ml of distilled water followed by 3.0ml of precipitating mixture. It was centrifuged at 2000rpm for 5 minutes and 1ml of supernatant was added to 1.5ml of phosphate solution, followed by addition of 0.5ml of Dithionitrobenzoic acid; 5,5'-Dithiobis(2-nitrobenzoic acid) (DTNB) reagent. The absorbance was measured at 412nm. [13].

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F. Determination of Biochemical Parameters

1. Determination of Glucose

The serum glucose concentration was determined using the glucose oxidase method. The principle of the method is based on the ability of glucose oxidase to catalyse the oxidation of β-D-glucose to D-glucono-σ-lactone with the concurrent release of hydrogenperoxide (H_2O_2). In the presence of peroxidase (POD) this H_2O_2 enters into a second reaction involving *p*-hydroxybenzoic acid and 4- aminoantipyrine with the quantitative formation of a quinoneimine dye complex which is measured at 510nm [15].

2. Determination of SGOT and SGPT

The measurement of transaminase levels in serum by Ambica diagnostic kit studied. The kit utilize the colorimetric procedure of Reitman and Frankel1 in which the oxaloacetate and/or pyruvate formed in either the GOT or GPT reaction is combined with 2, 4-initrophenylhydrazine to yield a brown-coloured hydrazone which is measured at 505nm [16].

G. Determination of Adrenal Gland Weight

Animals were weighed just before termination, sacrificed by decapitation, adrenals were quickly removed, cleaned from surrounding and weighed [17].

III. STATISTICAL ANALYSIS

Values are expressed as mean ± SD (n=6). Statistical significance was determined by one way analysis of variance (ANOVA) followed by Dunnet's post-*hoc* test. P<0.01 and P<0.05 were considered statistically significant when compared with Negative control.

IV. RESULT

The level of depression is displayed in Table I. There was significant increase in the (p<0.01) immobility time when subjected to Forced swim test and Tail suspension test of group II when compare to group I which signifies that there was increase in the level of depression in socially isolated rat. Even the time of immobility significantly increased (p<0.01) after social isolation of 84 days compared to 21 days. There was significant decrease in immobility time in group III and group IV compared to group II. As shown in Table II there was increase in the levels of Oxidative stress markers in the socially isolated rats. There were significant increase (p<0.01)

in the level of LPO and Decrease in the level of GSH, CAT and SOD in socially isolated rats (isolated for 21and 84 days) compare to normal rats. There were significant alteration (p<0.01) in the levels of oxidative stress markers in socially isolated rat of 84 days compare to 21 days of social isolation. In group III and group IV rats there were decrease in the levels of LPO and increase in the level of GSH, CAT and SOD compare to Group II rat (isolated for 21 and 84 days).

The change in the biochemical parameters like SGOPT, SGPT, Blood glucose level in socially isolated rats were observed of 84 days (Table III). On 21 days of social isolation there was no significant changes in the level of SGOPT, SGPT and Blood Glucose compare to Group I but after 84 days of social isolation there was significant (p<0.01) increase in the level of SGOT, SGPT and blood glucose level of group II compare to Group I. After 84 days of social isolation Group III and Group IV shows significant (p<0.001) decrease in the levels of SGOT, SGPT and Blood glucose level.

Weight of Adrenal gland and Adrenal gland Index were checked in 84 Days socially isolated rats. There was significant increase in the weight of Adrenal gland (50.33 ± 0.24) and Adrenal gland index (15.58 ± 0.13) in Group II as compare to Group I. Whereas in the Group III and IV there was significant decrease (p<0.001) in the weight of Adrenal gland and Adrenal Index compare to Group II (Table IV).

There were significant increase (p<0.01) in the level of LPO and Decrease in the level of GSH, CAT and SOD in liver of socially isolated rats for 84 days compare to normal rats. In group III and group IV rats there were decrease in the levels of LPO and increase in the level of GSH, CAT and SOD compare to Group II rat.

TABLE I
DETERMINATION OF DEPRESSION BEHAVIOR

Sr. No.	Groups	Forced swim test		Tail suspension test	
		21 days	84 days	21 days	84 days
1	**Group-I**	100.83 ± 4.26	106.33 ± 2.42	142.83 ± 5.07	144.33 ± 1.50
2	**Group-II**	145.16 ± 4.13@	181.50 ± 2.81@#	204.50 ± 6.89@	244.16 ± 4.16@#
3	**Group-III**	96.66 ± 2.33**	102.00 ± 4.09**	155.66 ± 5.50**	159.50 ± 3.27@
4	**Group-IV**	97.16 ± 1.60**	101.16 ± 3.06**	159.83 ± 6.73**	157.66 ± 9.20@

Data are expressed as the Mean ±SD (n=06), where
@ p< 0.01, compared to control group, ** P < 0.01, compared to negative control group
@# p<0.01, Compared to 21 days reading

TABLE II

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DETERMINATION OF OXIDATIVE STRESS PARAMETERS IN BLOOD

Sr. No.	Groups	21 days				84 days			
		LPO (nMMDA/g hb)	GSH (μM/mg protein)	CAT (U/mg protein)	SOD (Unit/mg protein)	LPO (nMMDA/g hb)	GSH (μM/mg protein)	CAT (U/mg protein)	SOD (Unit/mg protein)
1	**Group-I**	3.15 ± 0.43	8.58 ± 0.49	251.16 ± 0.75	49.23 ± 0.53	3.74 ± 0.08	8.82 ± 0.10	251.56 ± 0.87	50.05 ± 0.48
2	**Group-II**	$5.81 \pm 0.07@$	$5.66 \pm 0.40@$	$220.16 \pm 0.75@$	$30.36 \pm 1.02@$	$7.87 \pm 0.37^{@£}$	$4.67 \pm 0.16^{@£}$	$206.30 \pm 2.40^{@£}$	$21.75 \pm 0.66^{@£}$
3	**Group-III**	$5.46 \pm 0.08*$	$6.21 \pm 0.20*$	$221.33 \pm 0.51*$	$32.00 \pm 1.09*$	$7.28 \pm 0.23^{@£}$	5.2 ± 0.16	$217.61 \pm 1.74^{@£}$	$27.93 \pm 0.97^{@£}$
4	**Group-IV**	$4.03 \pm 0.08**$	$6.85 \pm 0.13**$	$238.66 \pm 0.51**$	$38.83 \pm 1.16**$	$5.81 \pm 0.38^{@£}$	5.85 ± 0.15	$227.66 \pm 2.12^{@£}$	$41.33 \pm 1.63^{@£}$

Data are expressed as the Mean ±SD (n=06), where
[@] $p< 0.01$, compared to control group, ** $P < 0.01$, compared to negative control group £ $p<0.01$, Compared to 21 days reading

TABLE III
DETERMINATION OF BIOCHEMICAL PARAMETERS

Sr. No.	Group	21 days			84 Days		
		SGOT (IU/L)	SGPT (IU/L)	Glucose (mg/dl) in blood	SGOT (IU/L)	SGPT (IU/L)	Glucose (mg/dl) in blood
1	Group I	16.83 ± 0.75	27.33 ± 0.81	75.98 ± 0.36	16.16 ± 0.75	27.33 ± 0.51	77.46 ± 0.92
2	Group II	17.33 ± 0.51^{NS}	26.83 ± 0.75^{NS}	75.2 ± 0.50^{NS}	$18.83 \pm 0.40^{@}$	$32.00 \pm 1.78^{@}$	$135.83 \pm 6.37^{@}$
3	Group III	17.16 ± 0.40^{ns}	26.83 ± 0.75^{ns}	74.50 ± 1.37^{ns}	$17.00 \pm 0.63**$	$30.50 \pm 0.54**$	$98.15 \pm 4.31**$
4	Group IV	16.50 ± 0.54^{ns}	26.33 ± 0.51^{ns}	75.00 ± 2.09^{ns}	$16.66 \pm 0.81**$	$29.66 \pm 0.81**$	$82.35 \pm 2.81**$

Data are expressed as the Mean ±SD (n=06), where
@ $p< 0.01$, compared to control group, ** $P < 0.01$, compared to negative control group
NS $p>0.01$, compared to control group, ns $p>0.01$, compared to negative control group

TABLE IV
DETERMINATION OF ADRENAL GLAND WEIGHT

Sr. No.	Groups	weight of adrenal gland in mg	weight of adrenal gland - mg/100 g body weight
1	**Group-I**	47.26 ± 0.55	13.65 ± 0.17
2	**Group-II**	$50.33 \pm 0.24@$	$15.58 \pm 0.13@$
3	**Group-III**	$49.68 \pm 0.29**$	$14.98 \pm 0.17**$
4	**Group-IV**	$47.86 \pm 0.28**$	$14.46 \pm 0.16**$

Data are expressed as the Mean ±SD (n=06), where
@ $p< 0.01$, compared to control group, ** $P < 0.01$, compared to negative control group

TABLE V
DETERMINATION OF OXIDATIVE STRESS PARAMETERS IN TISSUE

Sr. No.	Groups	LPO (nMMDA/g hb)	GSH (μM/mg protein)	CAT (U/mg protein	SOD (Unit/mg protein)
1	**Group -I**	23.10 ± 0.79	24.15 ± 0.46	330.91 ± 1.32	97.95 ± 0.41
2	**Group -II**	$31.56 \pm 0.49^{@}$	$15.03 \pm 0.63^{@}$	$245.28 \pm 4.59^{@}$	$61.25 \pm 0.75^{@}$
3	**Group -III**	$28.00 \pm 0.54**$	$16.23 \pm 0.13**$	$260.08 \pm 4.27**$	$78.25 \pm 0.88**$
4	**Group -IV**	$26.20 \pm 0.45**$	$19.61 \pm 0.61**$	$292.86 \pm 2.20**$	$85.76 \pm 1.96**$

Data are expressed as the Mean ±SD (n=06), where
@ $p< 0.01$, compared to control group, ** $P < 0.01$, compared to negative control group

V. DISCUSSION

The present findings show that short and long term social isolation is associated with significant enhancement of Depression as measured in the forced swim test and tail suspension test. This behavior state may contribute for generation of oxidative stress, which was confirmed by determining its markers i.e. lipid peroxidation, CAT, SOD and GSH. The present study indicates that long term social isolation for 84 days (i.e. 12 weeks) significantly altered oxidative stress markers as compared to short term social isolation in liver. Increased level of SGOT and SGPT indicates liver damage in rats with long term depression. The raised levels of these enzymes in depression might be as the result of cortisol induced gluconeogenesis in the liver. During stressful condition there might be altered membrane permeability which contributes to release of these transaminases.

The observed increase in the blood glucose level may be due to the release of glucocorticoids during persistent depression in two ways either by promoting gluconeogenesis in liver from amino acids or by inhibiting glucose utilization by peripheral cells. Increased release of glucocorticoids may be a consequence of increased weight of adrenal gland.

VI. CONCLUSION

Long term social isolation induced increase in glucose, SGOT, SGPT are mediated through the increased generation of free radicals. Thus, the present study also confirms the alteration of oxidant and antioxidant balance during altered behavior. The assessment of the altered behavior and different antioxidants during social isolation will be an interesting step forward to identify the mechanism involved.

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