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IMIPRAMINE AMELIORATE ALTERED BIOCHEMICAL PARAMETERS AND OXIDATIVE DAMAGE IN DEPRESSION

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Abstract

Background: The study was undertaken to investigate the effect of imipramine on various biochemical parameters and oxidative stress markers in short and long term depression in rats.

Method: Rats were subjected for short (21 days) and long term (84 days) social isolation for and checked for depression on force swim test and tail suspension method. Various markers of oxidative stress like lipid peroxidation (LPO), reduced glutathione (GSH), Supersoxide dismutase (SOD), catalase (CAT) and biochemical parameters like Serum glutamate oxaloacetate transaminase (SGOT), Serum glutamate pyruate transaminase (SGPT), and blood glucose were determined in depressed, control, imipramine and Vitamin E treated group.

Result: The rats displayed an increase in depression on force swim test and tail suspension method relative to control. There was a significant increase in the level of LPO and decrease in the levels of GSH, SOD and CAT after short and long term depression.

Conclusion: Increased oxidative stress in depression, which may lead to, alteration of biochemical parameters. Treatment with imipramine (tricyclic antidepressant) significantly decreases the level of LPO, SGOT, SGPT and increase in the levels of GSH, SOD and CAT in long term depression.

Keywords- Depression, Oxidative stress, lipid peroxidation, reduced glutathione, Serum glutamate oxaloacetate transaminase, Serum glutamate pyruate transaminase, Glucose

INTRODUCTION

The term "depression" covers 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.

Depressive disorders are amongst the leading causes of disability and mortality worldwide and are at increased risk for serious medical illness, including cardiovascular disease, diabetes, cancer and stroke. This risk is often independent of traditional risk factors suggesting that depression may

function as a causal factor in the pathogenesis of multiple diseases. To understand the pathophysiological effect of depression, we examined one of the biologic mechanisms common to multiple diseases: oxidative stress. [2]

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 aging-related neurodegenerative diseases such as Parkinson's disease (PD). [3]

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 the toxic effects of the ROS. However, when the 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 peroxidation, oxidation of proteins, and DNA damage are well-known outcomes of oxygen-derived free radicals, leading to cellular pathology and ultimately to cell death.

There is significant evidence that psychological stress is associated with increased oxidative stress in both animal and human models. Further, depression may contribute to the production of and exposure to reactive oxygen species. [4]

The generation of reactive oxygen species (ROS) plays an important role in producing liver damage, other hepatic alterations and progressed liver disease (initiating hepatic fibrogenesis). [5 & 6]. OS disrupts lipids, proteins and DNA, induces necrosis and apoptosis of hepatocytes and amplifies the inflammatory response. ROS also stimulate the production of profibrogenic mediators from Kupffer cells and circulating inflammatory cells and directly activate hepatic stellate cells, resulting in the initiation of fibrosis. [6]

Treatments of major mood disorders have improved in recent years with the advent of antidepressant drugs like tricyclic antidepressant (Imipramine). [7] Imipramine is well known for an antidepressant by inhibiting neurons from the reuptake of neurotransmitter such as norepinephrine and serotonin. [8] Imipramine also known as melipramine, is a tricyclic antidepressant (TCA) of the dibenzazepine group. Imipramine is mainly used in the treatment of major depression and enuresis (inability to control urination). It has also been evaluated for use in panic disorder. [9] Along with antidepressant activity imipramine is capable of modulating oxidative stress. [5]
Females may be more vulnerable to events affecting their close emotional ties and more likely to develop depression in response to them. [10]

In this study, we investigated the effect of imipramine treatment on oxidative stress and biochemical parameters present in depression induced by social isolation and it is characterized by behavioral changes in female rat. To fulfill our aim, we quantified: i) behavioral changes (forced swim test, tail suspension method) ii) oxidative stress parameters (Lipid peroxidation, LPO; reduced glutathione, GSH; superoxide dismutase, SOD & Catalase, CAT)
iii) Biochemical parameters (Glucose; Serum Glutamate oxaloacetate Transaminase, SGOT & Serum Glutamate Pyruate Transaminase, SGPT)

MATERIAL AND METHOD

Chemical

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, Serum Glutamate oxaloacetate Transaminase (SGOT) determination kit, Serum Glutamate Pyruate Transaminase (SGPT) determination kit.

Experimental animals

Healthy female Sprague–Dawley rats (200-250 g) 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 the day (08:00- 16:00 hours). The rats were housed and treated according to the rules and regulations of the committee for the purpose of control and supervision experiments on animal (CPCSEA) and Institutional Animal Ethics Committee (IAEC). The protocol for all the animal studies was approved by the IAEC. (Certification number – 650/02/c/CPCSEA/12 & date 30/09/2011.)

The animals were grouped into 4 groups, each group possesses 6 animals as follows: -
Group I Normal Control group: Animals were treated with saline solution only

Group II Negative control group: Animals were subjected for social Isolation in a dark room and no treatment was given.

Group III (Social isolation + imipramine): Animals were subjected for social Isolation and treated with imipramine (16mg/kg, i.p.)

Group IV(Social isolation + imipramine + Vit. E): Animals were subjected for social Isolation and treated with imipramine (16mg/kg, i.p.) and Vit. E (100mg/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)]. [11 & 12] After short and long term isolation, rats were assessed for behavior using various animal behavioral models like force swim test.

Determination of Behavioral Parameters

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 15 min pretest followed by 5 min test 24 h 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 5 min Test period was noted. [13]

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 20–30 cm (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 about 70 lx. During 6 min, 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. [14]

Determination of oxidative stress parameters in blood

Sample preparation

Suspension of RBC (red blood cells, 5%) was prepared by adding phosphate buffer saline (8 ml) to packed cells. 0.5 ml of 5% RBC was mixed with 5 ml 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 0.15 ml of distilled water was added. The mixture was centrifuged to get a clear erythrocyte lysate.

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. [15]

Determination of CAT

The activity of the CAT enzyme was determined in the erythrocyte lysate as the decrease in absorbance was measured spectrophotometrically at 240 nm for 1 minute. [16]

Determination of SOD

The activity of SOD was determined in the erythrocyte lysate by monitoring spectrophotometrically the increase in the absorbance at 420 nm for 3 minutes. [17]

Determination of GSH

Blood GSH (reduced glutathione) was measured by the addition of 0.2 ml of whole blood to 1.8 ml of distilled water followed by 3.0 ml of precipitating mixture. It was centrifuged at 2000 RPM for 5 minutes and 1 ml of supernatant was added to 1.5 ml of phosphate solution, followed by the addition of 0.5 ml of DTNB {Dithionitrobenzoic acid; 5, 5'-Dithiobis (2-nitrobenzoic acid)} reagent. The absorbance was measured at 412 nm. [18]

Determination of oxidative stress parameters in tissue

Tissue preparation

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 × rpm for 1 h in a cooling centrifuge at 0°C. The supernatant was employed to assess the parameters of oxidative stress after estimating the protein content. [19]

Determination of LPO

According to the method of Esterbauer and Cheeseman (1990), 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. [15]

Determination of CAT

The enzyme catalase converts H_2O_2 into water. The activity of the CAT tissue homogenate, the decrease in absorbance was measured at 240 nm for 1 minute using spectrophotometer by calculating the rate of degradation of H_2O_2, the substrate of the enzyme. [16] One unit of CAT activity is defined as the amount of the enzyme, which reduces 1 millimole of H_2O_2 per minute.

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 a spectrophotometer. [17]

Determination of GSH

GSH was measured by the addition of 0.2 ml of tissue homogenate to 1.8 ml of distilled water, followed by 3.0 ml of precipitating mixture. It was centrifuged at 2000 RPM for 5 minutes and 1 ml of supernatant was added to 1.5 ml of phosphate solution, followed by the addition of 0.5 ml of Dithionitrobenzoic acid; 5,5'-Dithiobis (2-nitrobenzoic acid) (DTNB) reagent. The absorbance was measured at 412 nm. [18]

Determination of Biochemical Parameters

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 catalyze the oxidation of β-D-glucose to D-glucono-σ-lactone with the concurrent release of hydrogenperoxide (H2O2). In the presence of peroxidase (POD) this H2O2 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 510 nm. [20]

Determination of SGOT and SGPT

The measurement of transaminase levels in serum by Ambica diagnostic kit studied. The kit utilizes the calorimetric 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 505 nm. [21]

Determination of Adrenal gland weight

Animals were weighed just before termination, sacrificed by decapitation, Adrenals were quickly removed, Cleaned from surrounding and weighed. [22]

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.

Result

The level of depression is displayed in table no.1. There was a significant increase in the ($p<0.01$) immobility time when subjected to Forced swim test and Tail suspension test of group II, when compared to group I which signifies that there was an increase in the level of depression in socially isolated rats. Even the time of immobility significantly increased ($p<0.01$) after social isolation for 84 days compared to 21 days. There was a significant decrease in immobility time in group III and group IV compared to group II.

Table 1 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

As shown in Table No. 2 there was an increase in the levels of Oxidative stress markers in the socially isolated rats. There were significant increases ($p<0.01$) 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 rats of 84 days compared 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 comparison to Group II rat (isolated for 21 and 84 days).

Table 2 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 + 0.07@	5.66 + 0.40@	220.16 + 0.75@	30.36 + 1.02@	7.87 + 0.37@£	4.67 + 0.16@£	206.30 + 2.40@£	21.75 + 0.66@£
3	**Group-III**	5.46 + 0.08*	6.21 + 0.20*	221.33 + 0.51*	32.00 + 1.09*	7.28 + 0.23**	5.2 + 0.16**	217.61 + 1.74**	27.93 + 0.97**
4	**Group-IV**	4.03 + 0.08**	6.85 + 0.13**	238.66 + 0.51**	38.83 + 1.16**	5.81 + 0.38**	5.85 + 0.15**	227.66 + 2.12**	41.33 + 1.63**

Data are expressed as the Mean ±SD (n=06), where
@ $p< 0.01$, compared to control group, * $P < 0.05$, compared to negative control group ** $P < 0.01$, compared to negative control group, £ $p<0.01$, Compared to 21 days reading

The change in the biochemical parameters like SGOT, SGPT, Blood glucose level in socially isolated rats were observed of 84 days (Table No. 3). On 21 days of social isolation, there were no significant changes in the level of SGOT, 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.

Table 3 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 + 0.40@	32.00 + 1.78@	135.83 + 6.37@
3	Group III	17.16 + 0.40^{ns}	26.83+ 0.75^{ns}	74.50 + 1.37 ^{ns}	17.00 + 0.63**	30.50 + 0.54**	98.15 + 4. 31**
4	Group IV	16.50 + 0.54^{ns}	26.33 + 0.51^{ns}	75.00 + 2.09 ^{ns}	16.66 + 0.81**	29.66 + 0.81**	82.35 + 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

The weight of Adrenal gland and the Adrenal gland Index were checked in 84 Days socially isolated rats. There was a significant increase in the weight of Adrenal gland (50.33 + 0.24) and Adrenal gland index (15.58 + 0.13) in Group II as compared to Group I. Whereas in the Group III and IV there was no significant increase ($p<0.001$) in the weight of Adrenal gland and Adrenal Index compare to Group II (Table No. 4).

Table 4 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 + 0.24@	15.58 + 0.13@
3	**Group-III**	49.68 + 0.29**	14.98 + 0.17**
4	**Group-IV**	47.86 + 0.28**	14.46 + 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

There were significant increases ($p<0.01$) 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 decreases in the levels of LPO and increase in the level of GSH, CAT and SOD comparison to Group II rat (Table No. 5).

Table No.5 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 + 0.49@	15.03 + 0.63@	245.28 + 4.59@	61.25 + 0.75@
3	**Group-III**	28.00 + 0.54**	16.23 + 0.13**	260.08 + 4.27**	78.25 + 0.88**
4	**Group-IV**	26.20 + 0.45**	19.61 + 0.61**	292.86 + 2.20**	85.76 + 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

Discussion

In this study, we examined the influence of imipramine on the duration of immobility in forced swim test and tail suspension method after short term and long term isolation. Imipramine reduces immobility time in forced swim test and tail suspension test, as well as cellular and oxidative damage. Our data suggest the antioxidant effect of imipramine along with antidepressive effect.

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. It has been reported that stress in rats increased frontal cortical 5-HT2A receptor levels. Clinically, the up-regulation of 5-HT2A receptors was reported in the platelets of depressed patients and in the frontal cortex of suicide victim. Furthermore, it was reported that number of 5-HT2A receptors increases as a result of some types of chronic stress. Therefore, stress produced by social isolation may shed light on the mechanism governing 5-HT2A receptor up-regulation, which may be associated with the pathophysiology of depression. [23]

Depression may contribute to the production of and exposure to reactive oxygen species, and thus leads pathogenesis of multiple diseases. Depression is associated with the activation of innate immune response and subsequent production of phagocytes. Activated Phagocytes are significant sources of reactive oxygen species and increased oxidative damage may represent a common mechanism for the pathogenesis of multiple diseases. [2]

We consider the possibility that the observed increased lipid peroxidation and decreased CAT, SOD and GSH were a part of a broader oxidative process occurring among the depressed rats. We studied persistent oxidative stress may damage liver tissue confirmed by measuring SGOT and SGPT (biomarkers of liver function test) at 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 the liver from amino acids or by inhibiting glucose utilization by peripheral cells. Increased release of glucocorticoids may be a consequence of the increased weight of the adrenal gland. The findings in the present investigation derives its importance from the evidence that oxidative stress has an important role in the development and progression of chronic liver disease and altered glucose level. By modulating oxidative stress imipramine might thus affect the progression and development of several hepatic pathologies.

MDA was decreased and GSH, SOD and CAT were increased in blood and liver by imipramine. This might suggest that imipramine can be administered for its antioxidant effect and treatment of liver disease in patients with depression.

CONCLUSION

Social isolation leads to depression, long term depression induced increase in glucose, SGOT, SGPT are mediated through the increased generation of free radicals. This alteration of oxidant and antioxidant balance is due to altered behavior. Pretreatment with imipramine an antidepressant drug, controls such behavior and thereby prevents generation of free radical.

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