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Changes in Antioxidant Enzymes Activities and Lipid Peroxidase Level in Tissues of Stress-induced Rats

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Estimating the changes in the levels of oxidative stress biomarkers are vital in identifying stress related disease in living organism. This study examined changes in the activity of antioxidant enzymes and lipid peroxidase level in female Wistar rats exposed to stressors of different nature were examined. 88 apparently healthy rats within the ages of 8-12weeks and weighing between 120-180g were utilized for the study. Rats were acclimatized for 2weeks and fed with rat chaw and water *ad libitum*. Animals were stressed at the rate of 1hr, 3hr or 5hr per day for 1, 2 or 3weeks respectively. After the experimental protocol of stress induction, the rats were killed via cervical dislocation and some vital organs were carefully harvested for tissue homogenates in assessing SOD, CAT and MDA antioxidants. Data collected were analyzed using Mean±SEM, ANOVA was used to compare means and LSD was used for post hoc. SOD activity of the different tissues examined were significantly ($p<0.05$) altered irrespective of the stressor applied especially in the restraint or intruder stressors. CAT activity were significantly ($p<0.05$) reduced in all stressors irrespective of the rate of exposure. The study also revealed that lipid peroxidase levels were significantly ($p<0.05$) increased in all tissues irrespective of the rate of exposure and type of stressor applied. The findings validate the ability of the stressors to increase production of free radicals, thus, changes in antioxidant enzymes activities and lipid peroxidase level implies compromised cellular activity in tissues of stress-induced rats.

Keywords: Oxidative stress, Biomarkers, Superoxide dismutase, Catalase, Malondialdehyde, Wistar rats.

Stress is a collective occurrence that attracts cascade of events and is implicated as a cofactor in the severity and development of numerous diseases, and threatens cellular homeostasis by neutralizing oxidants in various tissues of the body that are key in stress response[1]. Stress also plays vital role in aggravating several ailments, particularly hepatic inflammation.

Stress responses are meant to support maximal strength and vigilance in every healthy human. An organisms' response to stress starts with the central nervous system processing sensory information related to the external stressor through activation of physiological, biochemical and neurochemical mechanisms via hypothalamic-pituitary-adrenal (HPA) pathways[2]. If the situation is appraised as potentially harmful, then, a cascade of neural, hormonal and behavioral responses is initiated in order to deal with the situation[3].

Stress either acute or chronic in humans arises because they are exposed to antagonistic or disastrous life occasions within the social environment. Stressful conditions can likewise be portrayed by the inability to manage suitably the demands of the day-to-day activities humans are exposed to, and this can be in the form of stressors, especially when they exceed the coping state[4]. An individual's reaction to this stressor by activating interconnected neuroendocrine circuits in other to establish homeostatic equilibrium[5], hence the need of antioxidants in scavenging the oxidants effects produced during stress condition which endangers the survival of living cells[6].

Antioxidant system in humans acts by controlling the dangerous effects of excessive production of reactive oxygen system (ROS) during stressful situation for enhanced cellular function of different organelles necessary for achieving reproductive success. They are available in two forms, as enzymatic as well as non-enzymatic[7]. This antioxidant defense system operates by three different mechanisms: by using antioxidants for prevention, secondly, by interception and thirdly by repair. Mitochondria and metabolic processes are vital sites for the production of physiological reactive oxygen species (ROS) in steroidogenic tissues[8]. The mechanisms underlying stress-induced tissue damage are not yet fully understood. Although, evidence has implied that the excessive generation of free radicals play a critical role in these processes[9].

An excess in the ROS production according to AbdulSalam *et al.,*[10] can affect different tissues following excess lipid peroxidation, oxidation, and different lesions generated in DNA. Aprioku,[11] maintains that deviations within the human system from the normal physiological ranges of antioxidants may result in the progression of several diseases in respective tissues. Obviously, antioxidant systems functions by minimizing the over production of oxygen derived free radicals released in excess during stress to protect itself from oxidative damage. Hence, elevated levels of stress have been established to contribute towards infertility[12]. Stress response could be perceived as a compensatory mechanism to the disturbances in the living system caused by a stressor by increasing corticosterone levels[13].

Antioxidant system differs from tissue to tissue and cell-type to cell-type. Most important in-vivo enzymatic antioxidant defense system include; SOD, CAT, GPx, and GST. The non-enzymatic antioxidants are; ceruloplasmin, transferrin, glutathione (GSH), albumin, ferritin, myoglobin, and metallothionein. Perhaps, they act as antioxidant by inactivating pro-oxidants to scavenge the activities of the free radicals[14]. Antioxidant protection system can also include; dietary antioxidants, endogenous enzymatic and non-enzymatic constituents that regulates' the overproduction of these ROS[7]. Perhaps, one of the markers of lipid peroxidase (end products of unsaturated fats) widely used in determining oxidative stress measurement in experimental animals is Malondialdehyde (MDA)[15]. Most important in-vivo enzymatic antioxidants of focus in experimental research are; Catalase (CAT) and Superoxide Dismutase (SOD) activities of the different tissues considering that they are vital ROS neutralizing antioxidant and index of peroxidation.

The impact of stress on an organism depends to a large extent on the type of stressor applied, the intensity of the stressor, and whether controllable or uncontrollable[16], in addition, to some reasonable extent on the traits and conditions of the individuals been affected such as; coping ability and history of previous stressful events[17]. Nevertheless, the effect of stress can be manifested in four different folds namely; physiologic, behavioural, subjective experience and cognitive functions depending on the individuals predictability and controllability[18]. It is therefore pertinent for this study to investigate the changes in the activities of SOD and CAT antioxidant enzymes and MDA levels in tissues specifically; kidney, liver, ovary and brain of stress-induced Wistar rats as a factor perceived to be responsible for infertility in females with reference to a previous study reported by

Nwogueze *et al.,*[13] who applied similar nature of stressors.

MATERIALS AND METHODS

Animals and Study Design

Eighty eight (88) apparently healthy female Wistar rats (120-180g, 12-14weeks old) obtained from the Department of Anatomy, Delta State University, Abraka were used for the study. Rats were housed four per group in wooden cages and were maintained under constant environmental and nutritional conditions (60% relative humidity, 21–22°C temperature, 12hr light and 12hr dark cycle). All the animals were acclimatized for 2weeks prior to the experiment and were fed with constant ration of standard food and clean water *ad-libitum*. Rats were divided into 22 groups as described in Table 1 below. The study was conducted within a period of 28days.

Ethical Considerations

Ethical permission was obtained from the Research and Bioethics Committee of the Faculty of Basic Medical Sciences, College of Health Science, Delta State University, Abraka. All the procedures used for experimental and other scientific purposes as well as for consideration of the protection and welfare of animals were approved by the board (Permit: REC/FBMS/DELSU/58).

Induction of Stress

Stressors of three (3) different natures were used to induce stress in the female Wistar rats as described below:

Restraint Chamber Stressor

The female Wistar rats were transported to separate cylindrical plastic tubes (inner, 5.7cm, length 20.3cm) to induce physical stress to a greater extent adopting the methods of Ely *et al.*[19]. The restraining device was constructed to fit the rats so that lateral movement was eliminated allowing only backward and forward movements, thus, this helped to prevent the animals from injury. The strainer was made of adhesives wire mesh at both ends of the plastic tubes to allow for adequate air flow and to secure its restraint. Consequently, the animals were returned to their respective cages after induction of stress at the required rates per day. Meanwhile, the animals of the control group were not stressed.

Mirrored Chamber Stressor

This stressor was used to induce anxiety stress to a greater extent in the Wistar rats following the methods of Lamberty and Gower,[20]. The Mirrored chamber consisted of wooden chamber with mirrored measuring 42 x 42 x 42cm cube. The chamber consisted of boxes having three mirrored walls and the remaining wall was coloured opaque black. The rats were placed in the mirrored chamber at the specified rate of exposure per day. Rats in the control group were not stressed throughout the experimental period.

Intruder Paradigm Stressor

The test apparatus comprised of six (6) enclosed rectangular partitions measuring 25 x 13 x 32cm with a transparent wall in-between the cages separated by wire mesh. The chamber was used to induce psychosocial stress in the experimental animals adopting the methods of McGregor *et al.*[21]. An aggressive male cat was introduced to elicit defensive reaction in the female Wistar rats in hourly and weekly dependent format. The control groups were not stressed throughout the experimental period.

Tissue Samples Preparation

After the period of stress induction, the rats were humanely killed and the organs of kidney, liver, ovaries and brain were immediately carefully harvested and weighed to the nearest 0.001g. Tissues selected were considered as key organs required for measuring reproductive success and functional status of the rats in altered oxidative state. The tissues were washed in ice cold phosphate-buffer saline (PBS) solution of pH 7.4. The washed tissues were immediately frozen in liquid nitrogen and stored at "70 °C prior to biochemical analysis of the samples.

Biochemical Analysis

The frozen tissue samples were weighed crushed in a liquid nitrogen medium on the day of use. The respective powdered frozen tissue was placed in a test tube containing 500ìL of phosphate-buffered saline (pH 7.4) previously cooled to 4°C and then homogenized using a potter Elvehjem homogenizer. The resultant homogenates of the kidney, liver, ovary and brain tissues were centrifuged at 15,000rpm xg for 10minutes under 4°C to prevent the destruction of proteins and enzymes. After the centrifuging of the samples, the supernatants were carefully separated and

used to assay for SOD, CAT and MDA antioxidant enzymes activities and protein determination.

Determination of Superoxide Dismutase (SOD) Activity

Superoxide dismutase was analyzed adopting the methods of Winterbourn *et al.,*[22] using an ELISA kit (Shanghai Sunred Biological Technology Co., Ltd.). A sandwich enzyme immunoassay method was used with this kit. A specific antibody was used to coat the microplate kit that is specific to SOD. The absorbance of each sample was read on spectrophotometer at 560nm for 5minutes and the results were expressed in unit/mg protein.

Determination of Catalase (CAT) Activity

Catalase was analyzed adopting the methods of Aebi,[23] using an ELISA kit (Shanghai Sunred Biological Technology Co., Ltd.). A sandwich enzyme immunoassay method was used with this kit. A specific antibody was used to coat the microplate kit that is exclusive to CAT. The samples were read on spectrophotometer at 240nm

Table 1. Experimental Protocol

Categories	Duration (Hr)		
Control	*	*	*
Restraint Stressor[a]	1hr	3hr	5hr
Mirrored Chamber Stressor[b]	1hr	3hr	5hr
Intruder Paradigm Stressor[c]	1hr	3hr	5hr
Restraint Stressor[a]	1hr	3hr	5hr
Mirrored Chamber Stressor[b]	1hr	3hr	5hr
Intruder Paradigm Stressor[c]	1hr	3hr	5hr
Restraint Stressor[a]	1hr	3hr	5hr
Mirrored Chamber Stressor[b]	1hr	3hr	5hr
Intruder Paradigm Stressor[c]	1hr	3hr	5hr

[*]Non Pregnant Female Rats not Exposed to Stress
[a] Non Pregnant Female Rats Exposed to Stressor at the Rate of 1, 3 and 5hr for 1week
[b] Non Pregnant Female Rats Exposed to Stressor at the Rate of 1, 3 and 5hr for 1week
[c] *Non Pregnant Female Rats Exposed to Stressor at the Rate of 1, 3 and 5hr for 1week*

Table 2. Changes in SOD Activity in Tissues of Restraint Stress-induced Rats

Duration of Exposure to Stress	Rate of exposure to Stress	Changes in SOD activity (unit per milligram protein) Kidney	Liver	Ovary	Brain
Week 1	Control	29.60±1.51	57.65±13.94	4.25±0.49	8.88±0.92
	1 hr	26.11±4.23[ø]	94.75±15.84	6.32±0.85[*]	9.91±1.56
	3 hr	23.77±3.74[ø]	74.96±19.33	6.80±0.79[*]	9.53±0.47
	5 hr	42.66±6.12[*]	122.44±18.24[*]	7.18±0.61[*]	11.23±1.83
Week 2	1 hr	161.11±3.54[*#]	52.26±9.11	3.80±0.72	9.60±0.50
	3 hr	125.32±8.70[*]	27.98±2.71[*]	7.36±2.68	15.61±3.43
	5 hr	167.25±3.70[*#]	45.04±7.38	3.90±0.57	23.28±3.72
Week 3	1 hr	24.73±3.43	49.74±5.34	1.59±0.28[*]	10.29±1.17
	3 hr	22.00±2.70[*]	53.05±7.84	4.05±0.77[+]	10.31±1.07
	5 hr	22.00±2.00[*]	42.38±5.14	3.73±1.11	12.26±3.32

Values are Means ±Standard Error of Mean; Four animals in each group. Significant level was fixed at P<0.05 following exposure to Restraint Stressor.
*****Comparison of control and other groups.
[+]Comparison of 1hr stress group and other groups.
[#]Comparison of 3hr stress group and other groups.
[ø]Comparison of 5hr stress group and other groups

and results were expressed in unit/mg protein, were 1unit = 1mole of H_2O_2 in min/mg protein.

Determination of Malondialdehyde (MDA) Level

Malondialdehyde was analyzed adopting the methods of Satoh, [24] using an ELISA kit (Shanghai Sunred Biological Technology Co., Ltd). A sandwich enzyme immunoassay method was used with this kit. A specific antibody was used to coat the microplate kit that is exclusive to MDA. The lipid peroxidation level was read on spectrophotometer at 532nm and the results were expressed as nmol/mg protein.

Statistical Analysis

All data obtained were expressed as Mean ± SEM (Standard Error of the Mean). Differences

Table 3. Changes in SOD Activity in Tissues of Mirrored Stress-induced Rats

Duration of Exposure to Stress	Rate of exposure to Stress	Changes in SOD activity (unit per milligram protein) Kidney	Liver	Ovary	Brain
	Control	29.60±1.51	57.65±13.94	4.25±0.49	8.88±0.92
Week 1	1 hr	17.20±2.56$^{*\#}$	152.06±21.78*	9.05±2.43*	12.57±1.63
	3 hr	29.11±3.30	179.55±17.13*	6.98±0.57	9.29±2.00
	5 hr	14.68±1.68$^{*\#}$	115.48±16.95$^{*\#}$	5.70±0.90	14.89±1.62$^{*\#}$
Week 2	1 hr	178.27±2.39^{*ø}	24.80±4.41*	3.21±0.59	14.23±2.87
	3 hr	177.62±6.75^{*ø}	28.81±5.71*	3.63±0.74	9.91±1.36
	5 hr	152.40±8.07*	18.79±3.48*	3.66±0.84	22.00±5.87
Week 3	1 hr	35.23±4.33^{ø}	38.62±4.88	3.80±0.70	11.40±1.13
	3 hr	36.89±1.60^{ø}	55.16±5.78	4.77±1.03	14.85±4.88
	5 hr	24.56±2.76	49.71±4.04	4.70±1.47	10.26±1.26

Values are Means ±Standard Error of Mean; Four animals in each group. Significant level was fixed at P<0.05 following exposure to Mirrored Stressor.
*Comparison of control and other groups.
$^{+}$Comparison of 1hr stress group and other groups.
$^{\#}$Comparison of 3hr stress group and other groups.
^{ø}Comparison of 5hr stress group and other groups

Table 4. Changes in SOD Activity in Tissues of Intruder Stress-induced Rats

Duration of Exposure to Stress	Rate of exposure to Stress	Changes in SOD activity (unit per milligram protein) Kidney	Liver	Ovary	Brain
	Control	29.60±1.51	57.65±13.94	4.25±0.49	8.88±0.92
Week 1	1 hr	17.41±3.08*	40.14±2.73	5.39±0.52	11.54±1.44
	3 hr	18.83±1.87*	48.57±5.40	7.40±0.68	14.48±3.91
	5 hr	20.10±1.39*	48.91±7.94	11.40±4.17*	11.36±1.13
Week 2	1 hr	44.42±8.65$^{\#}$	17.89±1.34*	3.45±0.58	9.53±1.26
	3 hr	154.34±9.65*	18.62±3.89*	4.73±0.76	11.23±2.17
	5 hr	33.58±4.20$^{\#}$	28.26±6.31*	2.90±0.51$^{\#}$	13.23±0.88*
Week 3	1 hr	15.06±1.73*	83.70±8.76	3.21±0.75^{ø}	10.50±0.88
	3 hr	18.93±1.89	83.56±0.82	3.18±0.82^{ø}	7.74±1.15
	5 hr	26.67±8.06	62.31±1.28	6.56±1.28	9.64±0.86

Values are Means ±Standard Error of Mean; Four animals in each group. Significant level was fixed at P<0.05 following exposure to Mirrored Stressor.
*Comparison of control and other groups.
$^{+}$Comparison of 1hr stress group and other groups.
$^{\#}$Comparison of 3hr stress group and other groups.
^{ø}Comparison of 5hr stress group and other groups

between groups were analyzed using one-way ANOVA and the LSD test using SPSS (Version 20). A p-value d" 0.05 was considered statistically significant and this was differentiated with asterisk.

RESULTS

The effect of stress-induced changes on the kidney, liver, ovary and brain SOD levels in control and restraint stressed groups is depicted in Table 2. Mean SOD activity was decreased in the kidney of rats following exposure at the rate of 1hr and 3hr per day for 1week but stressing the rats at the rate of 5hr for 1week significantly ($p<0.05$) increased the activity of SOD in kidney when compared to activity in the control. However, rats exposed to restraint stressor for 2weeks were

Table 5. Changes in Catalase Activity in Tissues of Restraint Stress-induced Rats

Duration of Exposure to Stress	Rate of exposure to Stress	Changes in CAT Activity (unit per milligram protein) Kidney	Liver	Ovary	Brain
Week 1	Control	22.48±0.50	84.07±2.81	42.21±0.60	31.83±0.63
	1 hr	12.39±0.08*	39.19±4.17*	24.02±0.74*	24.70±2.51
	3 hr	13.94±1.55*	30.96±0.91*	24.63±2.44*	25.00±4.50
	5 hr	12.79±0.06*	44.51±4.40*#	24.54±0.29*	29.04±2.49
Week 2	1 hr	14.72±0.17*	44.36±1.02*	17.54±0.62*	17.18±0.42*
	3 hr	14.76±0.10*	43.69±1.34*	18.14±1.50*	17.19±0.32*
	5 hr	14.66±0.06*	45.72±1.30*	16.68±0.90*	18.90±1.72*
Week 3	1 hr	20.70±1.64	46.64±1.40*	28.33±1.90*	31.89±1.72º
	3 hr	22.10±2.25	44.08±0.99*	30.62±2.60*	31.98±1.23º
	5 hr	23.12±2.28	48.14±2.90*	24.26±0.71*#	26.10±2.85*

Values are Means ±Standard Error of Mean; Four animals in each group. Significant level was fixed at P<0.05 following exposure to Restraint Stressor.
*Comparison of control and other groups.
+Comparison of 1hr stress group and other groups.
#Comparison of 3hr stress group and other groups.
ºComparison of 5hr stress group and other groups

Table 6. Changes in Catalase Activity in Tissues of Mirrored Stress-induced Rats

Duration of Exposure to Stress	Rate of exposure to Stress	Changes in CAT Activity (unit per milligram protein) Kidney	Liver	Ovary	Brain
	Control	22.48±0.50	84.07±2.81	42.21±0.60	31.83±0.63
Week 1	1 hr	13.25±0.03*	35.73±3.79*	27.68±2.52*	29.78±2.02
	3 hr	13.13±0.08*	40.76±1.99*	23.53±0.38*	26.46±0.38
	5 hr	13.67±0.06*	34.92±3.95*	27.60±143*	24.67±1.23
Week 2	1 hr	14.87±0.08*	44.63±0.88*	26.21±1.91*	27.83±2.28
	3 hr	14.70±0.07*	46.33±2.07*	21.91±1.70*	21.78±2.89*
	5 hr	14.85±0.09*	45.09±1.39*	25.70±1.49*	23.93±2.11*
Week 3	1 hr	17.95±0.38*º	47.37±1.20*	26.10±1.53*º	31.96±1.59
	3 hr	18.54±0.65*º	47.41±1.95*	26.51±0.98*º	31.29±1.05
	5 hr	23.51±1.01	45.33±1.60*	31.15±1.65*	30.36±1.30

Values are Means ±Standard Error of Mean; Four animals in each group. Significant level was fixed at P<0.05 following exposure to Mirrored Stressor.
*Comparison of control and other groups.
+Comparison of 1hr stress group and other groups.
#Comparison of 3hr stress group and other groups.
ºComparison of 5hr stress group and other groups

significantly ($p<0.05$) elevated notwithstanding the rate of exposure in comparison to activity of SOD in the control. Rats exposed to the same stressor for 3weeks experienced significant ($p<0.05$) reduction in the SOD activity in kidney tissues of rats exposed to 3h or 5hr per day when compared to their activity in the control. The mean activity of SOD in liver tissues of rats exposed to restraint stressor at a rate of 1hr, 3hr and 5hr per day for 1week was markedly increased when compared to the control levels. The observed increase was only significant ($p<0.05$) in the rats exposed at the rate of 5hr per day, whereas, the SOD activity in liver tissue following exposure to similar stressor at the rate of 1, 3 or 5hr for 2 or 3weeks were lowered when compared to the control.

The SOD activity in the ovary of rats exposed to restraint stressor at a rate of 1, 3 or

Table 7. Changes in Catalase Activity in Tissues of Intruder Stress-induced Rats

Duration of Exposure to Stress	Rate of exposure to Stress	Changes in CAT Activity (unit per milligram protein) Kidney	Liver	Ovary	Brain
	Control	22.48±0.50	84.07±2.81	42.21±0.60	31.83±0.63
Week 1	1 hr	14.32±0.09*	42.78±1.07*	26.77±1.65*	21.74±2.71*
	3 hr	13.84±0.15*	39.70±2.30*	33.76±1.69$^{*+ø}$	19.03±1.84*
	5 hr	14.64±0.16*	46.98±1.07$^{*\#}$	22.76±0.70^{*+}	24.33±4.06
Week 2	1 hr	14.67±0.08*	48.29±2.89*	23.76±1.61*	28.44±0.66
	3 hr	14.67±0.05*	45.69±1.94*	20.13±0.83*	24.78±1.28*
	5 hr	14.49±0.07*	52.38±2.87*	24.09±2.13*	27.72±206*
Week 3	1 hr	20.93±0.57	53.16±0.82*	28.58±2.21*	23.15±1.28*
	3 hr	21.33±0.95	51.29±1.99*	29.79±1.26*	24.18±0.99*
	5 hr	18.01±2.21*	52.45±2.53*	31.47±2.38*	24.47±1.05*

Values are Means ±Standard Error of Mean; Four animals in each group. Significant level was fixed at $P<0.05$ following exposure to Intruder Stressor.
*****Comparison of control and other groups.
$^{+}$Comparison of 1hr stress group and other groups.
$^{\#}$Comparison of 3hr stress group and other groups.
^{ø}Comparison of 5hr stress group and other groups

Table 8. Changes in Malondialdehyde Levels in Tissues of Restraint Stress-induced Rats

Duration of Exposure to Stress	Rate of exposure to Stress	Changes in MDA activity (nanomole per milligram protein) Kidney	Liver	Ovary	Brain
Week 1	Control	0.22±0.03	0.13±0.04	0.05±0.01	0.14±0.03
	1 hr	1.79±0.44*	1.07±0.60$^{*\#}$	3.66±0.06*	0.41±0.14
	3 hr	2.42±0.58*	1.60±0.16^{*+}	3.48±0.20*	0.79±0.35
	5 hr	2.52±0.08*	1.28±0.08$^{*\#}$	3.62±0.04*	1.53±0.37^{*+}
Week 2	1 hr	0.95±0.27	0.41±0.09	0.28±0.03	2.61±0.28*
	3 hr	1.51±0.42*	0.57±0.29	0.56±0.20*	1.24±0.41^{+}
	5 hr	2.03±0.32^{*+}	0.63±0.14*	0.41±0.06*	1.77±0.72*
Week 3	1 hr	0.40±0.10	2.49±0.32*	0.27±0.05*	0.58±0.15^{*ø}
	3 hr	0.37±0.08	0.87±0.13$^{+ø}$	0.17±0.03^{ø}	0.66±0.15^{*ø}
	5 hr	0.30±0.06	3.97±0.72^{*+}	0.38±0.06*	0.15±0.06

Values are Means ±Standard Error of Mean; Four animals in each group. Significant level was fixed at $P<0.05$ following exposure to Restraint Stressor.
*****Comparison of control and other groups.
$^{+}$Comparison of 1hr stress group and other groups.
$^{\#}$Comparison of 3hr stress group and other groups.
^{ø}Comparison of 5hr stress group and other groups

5hr per day for 1week were significantly ($p<0.05$) increased when compared to the activity in the control but were lowered reduced following exposure of the rats at a rate of 1, 3 or 5hr per day for 2 or 3weeks when compared to the activity in the control level. The decrease was significant ($p<0.05$) following exposure of the rats at the rate of 1hr for 3weeks. Results of the activity of SOD in the brain of rats exposed at a rate of 1, 3 or 5hr per day to restraint stressor were not significantly affected for 1, 2 or 3 weeks respectively when compared with control level, irrespective of the rate of exposure.

Table 9. Changes in Malondialdehyde Levels in Tissues of Mirrored Stress-induced Rats

Duration of Exposure to Stress	Rate of exposure to Stress	Changes in MDA activity (nanomole per milligram protein) Kidney	Liver	Ovary	Brain
	Control	0.22±0.03	0.13±0.04	0.05±0.01	0.14±0.03
Week 1	1 hr	1.67±0.19*	1.51±0.14*	0.53±0.16$^{\#}$	1.22±0.17*
	3 hr	1.71±0.25*	1.26±0.11*	3.79±0.23*	1.44±0.24*
	5 hr	2.18±0.11^{*+}	1.56±0.04$^{*\#}$	0.92±0.26$^{*\#}$	1.31±0.34*
Week 2	1 hr	1.40±0.61	0.37±0.40	0.20±0.01*	2.57±0.81*
	3 hr	1.53±0.42*	1.25±0.50^{*+}	0.28±0.06*	2.10±0.80*
	5 hr	1.47±0.38*	0.71±0.19	0.33±0.05^{*+}	2.12±0.59*
Week 3	1 hr	0.24±0.03	2.67±0.92*	0.12±0.01	0.25±0.04
	3 hr	0.27±0.04	2.44±0.26*	0.35±0.20*	0.34±0.06*
	5 hr	0.20±0.05	2.62±0.66*	0.17±0.03	0.36±0.07*

Values are Means ±Standard Error of Mean; Four animals in each group. Significant level was fixed at $P<0.05$ following exposure to Mirrored Stressor.
*****Comparison of control and other groups.
$^{+}$Comparison of 1hr stress group and other groups.
$^{\#}$Comparison of 3hr stress group and other groups.
^{o}Comparison of 5hr stress group and other groups

Table 10. Changes in Malondialdehyde Levels in Tissues of Intruder Stress-induced Rats

Duration of Exposure to Stress	Rate of exposure to Stress	Changes in MDA activity (nanomole per milligram protein) Kidney	Liver	Ovary	Brain
	Control	0.22±0.03	0.13±0.04	0.05±0.01	0.14±0.03
Week 1	1 hr	1.79±0.11*	1.15±0.10*	2.00±0.62*	2.05±0.41*
	3 hr	1.73±0.19*	1.41±0.14*	0.84±0.21^{+}	1.33±0.23*
	5 hr	2.04±0.31*	1.01±0.07$^{*\#}$	0.93±0.24^{+}	1.04±0.31^{*+}
Week 2	1 hr	1.09±0.35	0.46±0.10^{*o}	0.29±0.07*	0.81±022
	3 hr	1.87±0.81*	0.53±0.13^{*o}	0.20±0.01*	1.82±0.68*
	5 hr	0.74±0.24	0.18±0.04$^{\#}$	0.21±0.05*	1.41±0.36*
Week 3	1 hr	0.92±0.35*	2.62±0.65*	0.46±0.09*	0.59±0.19
	3 hr	0.42±0.07	2.58±0.64*	0.36±0.06^{o}	0.80±0.35
	5 hr	0.57±0.12	2.20±0.37*	0.71±0.20*	0.72±0.30

Values are means ±standard error of Mean; Four animals in each group. Significant level was fixed at $P<0.05$ following exposure to Mirrored Stressor.
*****Comparison of control and other groups.
$^{+}$Comparison of 1hr stress group and other groups.
$^{\#}$Comparison of 3hr stress group and other groups.
^{o}Comparison of 5hr stress group and other groups

In Table 3, the activity of SOD in the kidney tissue of rats exposed to mirrored chamber stress were significantly ($p<0.05$) lowered following exposure of the rats at a rate of 1 or 5hr per day for 1week when compared to the activity in the control; whereas, rats exposed to similar stressor revealed that the activity of SOD were significantly ($p<0.05$) increased irrespective of the rate of exposure for 2weeks. Rats exposed to mirrored stressor at a rate of 1 or 3hr per day for 3weeks revealed significant ($p<0.05$) increase in the activity of SOD in the kidney when compared to the activity in the rats exposed at the rate of 5hr. The activity of SOD in the liver tissue of rats exposed to mirrored stressor at a rate of 1, 3 or 5hr for 1week were significantly ($p<0.05$) increased when compared with the SOD activity of the control. In similar trend, the activity of SOD in the liver of rats exposed to similar stressor irrespective of the rate of exposure for 2weeks were significant ($p<0.05$) decrease when compared with the activity in the control. The SOD activity in the ovary tissue of rats exposed to mirrored stressor at a rate of 1hr per day for 1week was significantly ($p<0.05$) higher when compared to the activity in control. Similarly, the SOD activity in the brain tissue of rats exposed to mirrored stressor were not affected but exposure of rats at the rate of 5hr per day for 2weeks was significant ($p<0.05$) when compared to the SOD activity in the control.

The mean activity of SOD in the kidney of rats exposed to intruder stressor at a rate of 1hr, 3hr and 5hr per day for 1week were significantly ($p<0.05$) deceased as shown in Table 4. The SOD activity in the kidney tissue of rats exposed to intruder stressor at the rate of 3hr per day for 2weeks was significantly ($p<0.05$) higher when compared to the activity in the control. The SOD activity in the liver tissue of rats exposed to intruder stressor at a rate of 1hr, 3hr and 5hr per day for 2weeks were significantly ($p<0.05$) decreased when compared to the activity in the control. Changes in the activity of SOD in the ovary of rats exposed to intruder stressor for 1week were higher when compared to control, irrespective of the rate of exposure. However, the observed increase was significant ($p<0.05$) following exposure of the rats at the rate of 5hr per day when compared to the activity in the control. Meanwhile, there was decrease in the activity of SOD following exposure of the rats to intruder stressor for 2 or 3weeks notwithstanding the rate of exposure.

Table 5 above demonstrated that catalytic activity in the kidney, liver, ovary and brain tissues assessed were lowered in rats following exposure to restraint stressor irrespective of the rate of exposure for 1, 2 or 3weeks. The activity of CAT in the kidney of rats exposed to restraint stressor revealed that there was significant ($p<0.05$) decline following exposure at a rate of 1, 3 or 5hr per day for 1 or 2weeks respectively in comparison in the control. Similarly, the mean CAT activity in the ovary tissue of rats exposed to restraint stressor at the rate of 1, 3 or 5hr per day for 1, 2 or 3weeks respectively were significantly ($p<0.05$) reduced when compared to the activity in the control (see Table 5). In the case of CAT activity in the brain tissue, rats exposed to similar stressor at the rate of 1, 3 or 5hr per day for 1, 2 or 3weeks respectively were all clearly affected. The decrease was significant ($p<0.05$) in comparison to the control.

The effect of CAT activity in the kidney tissue of rats exposed to mirrored stressor at different rate of exposure is depicted in Table 6. The CAT activity of the kidney of rats exposed to intruder stressor at a rate of 1, 3 or 5hr per day for 1, 2 or 3weeks respectively were all significantly ($p<0.05$) lowered in comparison to the control, but the decrease was significant in the rats exposed at a rate of 5hr per day for 3weeks. Changes in the CAT activity in liver of rats following exposure to restraint stressor at the rate of 1, 3 or 5hr per day for 1, 2 or 3weeks respectively revealed that the activity were significantly ($p<0.05$) decreased when compared to that of the control. The CAT activities in ovary of rats exposed to mirrored stressor at the rate of 1, 3 or 5hr per day for 1, 2 or 3weeks respectively were significantly ($p<0.05$) lowered when compared to the activities in the control. Furthermore, changes in the activity of CAT in the brain of rats exposed to mirrored stressor at the rate of 3 or 5hr per day for 2weeks were significantly ($p<0.05$) reduced when compared to control.

Table 7 above revealed alteration in the CAT activity in the kidney of rats following exposure to intruder stressor for 1 or 2weeks were significantly ($p<0.05$) lowered irrespective of the rate of exposure However, there was significant ($p<0.05$) reduction observed in the activity of CAT in the kidney of rats exposed to similar stressor

at the rate of 5hr for 3weeks when compared to control. The mean changes in the activity of CAT in the liver of rats, ovary and brain tissues of rats exposed to intruder stressor at the rate of 1, 3 or 5hr per day for 1, 2 and 3weeks respectively were significantly ($p<0.05$) lowered when compared to CAT activity in the control.

The MDA level in the different tissues of rats assessed following exposure to restraint stressor at the rate of 1, 3 or 5hr per day for 1, 2 or 3weeks were elevated as shown in Table 8. There was significant ($p<0.05$) elevation in the mean level of MDA in the kidney of rats following exposure at a rate of 3 or 5hr per day for 2weeks in comparison to the control level. Changes in the level MDA of liver of rats exposed to restraint stressor at a rate of 1, 3 or 5hr per day for 1week were significantly ($p<0.05$) higher when compared to the control level. Also, MDA levels in liver of rats exposed at the rate of 5hr per day for 2weeks as well as rats exposed at the rate of 1hr per day for 3weeks were significantly ($p<0.05$) increased when compared to the control level.

The mean changes in the MDA levels in the ovary tissues of rats exposed to restraint stressor at a rate of 1, 3 or 5hr per day for 1week as well as ovary of rats exposed at the rate of 2 or 5hr per day for 2weeks were significantly ($p<0.05$) increased when compared to the control levels. Similarly, the MDA levels in ovary of rats exposed to restraint stressor at the rate of 1 or 5hr for 3weeks were significantly ($p<0.05$) higher than the control level. The mean MDA level in the brain of rats exposed at a rate of 1, 3 or 5hr per day for 1week to restraint stressor were significantly ($p<0.05$) increased when compared to control level; while exposure of the rats to restraint stressor at the rate of 1 or 5hr for 2weeks significantly ($p<0.05$) increased the MDA levels in the brain. This was equally the case with rats exposed to similar stressor at the rate of 1 or 3hr per day for 3weeks in comparison to the control level. The peak in the MDA levels in rats were found in all the tissues assessed.

Changes in the mean MDA levels of different tissues assessed following exposure of the rats to mirrored stressor at the varying rate of exposure and durations is shown in Table 9. Stressing rats with mirrored chamber stressor at a rate of 1, 3 or 5hr per day for 1week significantly ($p<0.05$) elevated the MDA levels in the kidney. Whereas, rats exposed to mirrored stressor at the rate of 3hr or 5hr for 2weeks were significantly ($p<0.05$) increased. The MDA level in liver of rats exposed to mirrored stressor at a rate of 1, 3 or 5hr per day to for 1week were significantly ($p<0.05$) increased when compared to the control level. Similarly, the MDA levels in ovary of rats exposed to mirrored stressor at a rate of 3 or 5hr per day for 1week was significantly ($p<0.05$) increased when compared to the control levels. Meanwhile, the MDA level in the ovary of rats exposed for 2weeks irrespective of the rate of exposure were all significantly ($p<0.05$) elevated. However, in the case of the 3weeks duration, only rats exposed at the rate of 3hr per day was significantly ($p<0.05$) elevated. The MDA level in the brain of rats exposed to mirrored stressor at a rate of 1, 3 or 5hr per day for 1 or 2weeks were significantly ($p<0.05$) increased when compared to control level; although, the mean levels of MDA in the brain of rats exposed to mirrored stressor at the rate of 3 or 5hr for 3weeks were significantly ($p<0.05$) elevated.

Changes in the mean MDA levels in the different tissues of rats assessed following exposure to intruder stressor at the different rates and durations were all elevated as shown in Table 10. The MDA levels in the kidney of rats exposed to intruder stressor at the rate of 1, 3 or 5hr per day for 1week significantly ($p<0.05$) higher than the control. There was also significant ($p<0.05$) increase in the levels of MDA in the kidney of rats exposed at the rate of 3hr per day for 2weeks as well as 1hr for 3weeks respectively in comparison with control. The study revealed that the levels of MDA in the liver of rats exposed to intruder stressor at a rate of 1, 3 or 5hr per day for 1, 2 or 3weeks were all significantly ($p<0.05$) increased when compared to the control level. Similar observation was recorded for the MDA levels of ovary tissues.

Result obtained further revealed an increase in the levels of MDA in the ovary of rats exposed to intruder stressor at a rate of 1, 3 or 5hr per day for 1, 2 or 3weeks respectively. Results indicated that the levels of MDA in the ovary of rats exposed to similar stressor at the rate of 1hr for 1week was significantly ($p<0.05$) higher when compared to the control levels. The MDA levels in the ovary of rats following exposure to the different rates for 2weeks as well as rats exposed at the rate

of 1 or 5hr per day were all significantly ($p<0.05$) increased when compared to control level. The MDA levels in the brain of rats exposed at the rate of 1, 3 or 5hr per day for 1week to intruder stressor was significantly ($p<0.05$) higher than the control level. Meanwhile, rats exposed to intruder stressor at the rate of 3 or 5hr per day for 2weeks was significantly ($p<0.05$) elevated in same manner. As seen from Table 10 above, stress caused an increase in lipid peroxidation in the tissues assessed following exposure to intruder stressor; although, the alterations was milder in the kidney and brain tissues when compared to the liver and ovary tissues.

DISCUSSION

The results of the present study demonstrated changes in SOD, CAT and lipid peroxidase levels in the kidney, liver, ovary and brain tissues following exposure of female rats to restraint, mirrored or intruder stressor at the rate of 1, 3 or 5hr for a period of 1, 2 or 3weeks respectively as represented in Table 2-10. SOD is considered a key enzyme of first line of antioxidant defense which plays vital role functions in protecting cell integrity and tissue damage against superoxide free radicals following stress exposure. An increase in the kidney SOD activity as was observed especially when stressing the rats for 1week irrespective of the stressor is an indication that the organ was compromised the antioxidant which may be as a result of the activation of the enzyme protein synthesis following accumulation of superoxide anion radicals due oxidative stress as suggested by Zelko *et al.,*[25].

The noted decrease was significant ($p<0.05$) the rats were exposed at the rate of 3hr for 3weeks. This finding agrees with the reports from the studies of Mahvash *et al.,*[4] who reported that the mean activity of SOD liver following exposure to acute and chronic physical and psychological stress in rats for 1hr daily were altered due to the significant increase in their activity. With respect to specific to specific organ effects, exposure to restraint stressor at the rate of 5hr per day for 1week compromised the kidney, liver, ovary and brain tissues while exposure for a longer duration for up to 2weeks interfered with the activities of SOD in the kidney. Similar reports have been documented by studies of Sahin and Gumuslu,[26]; Samson *et al.,*[27], Ahmad *et al*.,[28].

The alterations observed in the kidney and liver SOD activities is an indication that the cells of the organs involved are compromised by the oxidative stress especially at shorter durations of exposure possible due to decreased antioxidant defense and as suggested by Koracevic *et al.,*[29] and this potentials may be in various tissues and stress model[30]. The SOD activity in the various tissues assessed were milder in the mirrored stressor compared to the stressor according to Halliwel and Gutteridge,[15] is considered as vital enzyme for dismutation of superoxide anions produced during exposure to oxidative stress in living cells. Hence, an increase in the SOD levels in the kidney and liver tissues at for week 1 or 2 experiments points to compromised integrity and impairment of the organ at this level while a reduction in the ovary and brain irrespective of the rate or durations of exposure signifies protective effect of the organs by the SOD antioxidant marker.

The activity of SOD in the kidney of rats exposed at a rate of 1, 3 or 5hr for 3weeks was significantly ($p<0.05$) lower when compared to the activity in the control. Our result confirms with the reports of Reddy *et al.,*[31] who submitted that physical stress such as restraint stress procedure facilitates an increase in the production of superoxide which is an important factor portraying oxidative damage which is not healthy for the organ structural integrity. Nevertheless, the activity of SOD in the brain tissue was significantly ($p<0.05$) higher following exposure of the rats to intruder stressor at a rate of 5hr per day for 2weeks in comparison with the control activity; whereas, the other remarkable changes in the SOD activity in brain tissues were not significant. Such reduction in the SOD activity in the liver, ovary and brain to this stressor is indicative of protective mechanism. Consistent with our finding was the reports of Ayesha and Naheed,[32] who revealed that restraint stress caused a simultaneously decline level in the activities of SOD and CAT in brain tissues of rats.

The alterations in the activity of CAT in liver of rats exposed to restraint stressor at a rate of 1, 3 or 5hr for 3weeks were significantly ($p<0.05$) reduced when compared to the activity in the control. This findings is consistent with the study conducted by Sarumathi and Saravanan,[33]

that activity of CAT were decreased following on exposure to immobilization stress for 21days and attributed it to cellular alterations in response to oxidative stress damage in the different organs examined. The observed reduction in the CAT activities in all the tissues investigated reveals accumulation of H_20_2 as catalase is common enzyme found in virtually all living organisms that are exposed to oxygen whereby it catalyzes the decomposition of hydrogen peroxide to water and oxygen. Perhaps, decreased activities of CAT was also observed following exposure of the rats to 1-6hr stress as reported by Zaidi *et al.,*[34] and Devaki *et al.,*[35]. The observed alterations in the levels of catalase was similar with the studies of Iman and Yasser,[36] who observed that catalase in the kidney and liver tissues were significant decreased in rats following oxidative stress induced by Bisphenol. Bindhumol *et al.,*[37] suggests that the decrease in liver catalase activity reflects inability of the liver mitochondria and microsomes to eliminate hydrogen peroxide produced after oxidative stress.

The changes in the activity of CAT in the different tissues assessed following exposure to intruder stressor at the rate of 1, 3 or 5hr per day for 1, 2 and 3weeks were lowered when compared to CAT activity in the control. Comparatively, stressing rats with mirrored stressor compromised the activity of CAT the kidney, liver, ovary and brain. The variability in the stress response to catalytic activities in the different organs may be likened to the suggestions of Papandreou *et al.*,[38] who reported that stress antioxidant response would appear to vary depending on the type, duration and intensity of the stressor or even the history of the animal to stress condition. Obviously, catalytic activities following the exposure of rats to the intruder stressor also compromised the various tissues assessed irrespective of rates and duration. Perhaps, such reduction is an indication of reduced capacity of these organs to scavenge hydrogen peroxide in response to oxidative stress induced by the stressor applied as argued by Kaushik and Kaur,[39].

The level of MDA in the kidney tissue of rats exposed to restraint at the rate of 1, 3 or 5hr per day for 1week was significantly ($p<0.05$) elevated when compared to the control level. Our result is consistent with the findings from the reports of Sahin *et al.,*[40] who reported increased MDA level in kidney and following exposure of restraint stress for 21days. Also, the studies of Mahvash *et al.,*[4] on the impact of stress on elevated levels of MDA indicative of late adaptation of the animals to stress. Such increase establishes the over production of free radicals resulting from lipid peroxidation of membrane structure, capable of causing cellular injury while interfering with the antioxidant defense system due to the impact of the stress as reported in the studies of Chakraborti *et al.,*[41]; Zaidi *et al.,*[34], Devaki, *et al*.,[35].

The observed increase in the MDA levels of the tissues investigated proves that there was depletion in the endogenous antioxidant enzyme activities and confirms the role of ROS in stress induced in tissue damage[42]. However, the inconsistency in results of these organs may be due to the suggestion of Sahin *et al.,*[40] who identified both type and duration of the stressor applied as a factor. The pathway of stress induced MDA tissue alterations as argued by DelRio *et al.*[43] involves the interactions between cell proteins and lipids resulting in release and generation of free radicals and cellular damage which at extreme levels interference with structural and functional integrity of the cell and its respective membrane organelles. The levels of brain MDA tissue were elevated because it is one of the byproduct required in lipid peroxidation has been reported by Cotran *et al.,*[44] to be implicated in neurological and cardiovascular diseases.

CONCLUSIONS

From this study, the nature and intensity of stressors seems to be paramount factor in estimating changes in the levels oxidative stress biomarkers activities for SOD, CAT and MDA levels in tissues of stress-induced Wistar rats. The mechanism behind the observed oxidative cellular damage and perceived stress-induced alterations as established in this study was basically via alteration in cellular interactions with proteins and DNA in the different pathophysiological processes of stress-induced changes in the kidney, liver, ovary and brain organs examined. Hence, stress based on this study is not a specific stimulus but a disruption of/or threat to homeostasis capable of interfering with reproductive success via alterations in the antioxidant neuroprotective modulation of steroid

estrogen activities and lipid peroxidase in female fertility.

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