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Levels of glutathione-related antioxidants in some tissues of stressed Wistar rats

Bartholomew Chukwuebuka Nwogueze[1], Anthony Emeka Ojieh[2], Chukwuemeka Peter Aloamaka[2], John Chukwuka Igweh[2], Innocent Onyesom[3]

[1]Department of Human Physiology, Evangel University Akaeze, Ebonyi State, [2]Department of Human Physiology, [3]Department of Medical Biochemistry, Delta State University, Abraka, Delta State, Nigeria.

***Corresponding author:**
Bartholomew Chukwuebuka Nwogueze,
Department of Human Physiology, Evangel University Akaeze, Ebonyi State Nigeria.

bukasono123@gmail.com

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ABSTRACT

Objectives: Oxidative stress (OS)-related pathologic conditions in the tissues of living organism have been linked to exposure to stressful events within the environment. This study examined the impact of different kinds of stress exposure on glutathione (GSH)-related antioxidants. The effect of stress was examined using comparative levels of GSH, glutathione-S-transferase (GST) and glutathione peroxidase (GPx) in female Wistar rats.

Materials and Methods: One hundred and sixty-eight adult female Wistar rats with body weights ranging between 150 and 200 g, were used for the study. The animals were distributed into 28 groups of six animals each. The experimental animals were routinely exposed to three different stressors; restraint chamber test, mirror chamber test and intruder chamber test for a duration of 1, 3 or 5 h per day for 1, 2 and 3 weeks, respectively. All animals were allowed free access to food (rat chaws), with water *ad libitum*. Animals were euthanise through cervical dislocation after the experimental period and the different target tissues were carefully harvested and homogenised for antioxidant estimation following standard procedure. Data obtained were statistically analysed and values expressed as mean ± standard error of mean and $P < 0.05$ level was considered as statistically significant.

Results: Findings from this study elucidated the fact that exposure to stress is capable of causing marked OS and reducing GSH-based antioxidant activities in Wistar rats. A decline in the GSH level and GPx activity as observed in the study is an indication of alterations of kidney and brain tissue cellular integrity by free radicals generated during exposure to the stressors, while the observed significant increase in GST activity level in the affected tissues indicates compromised rapid exhaustion of the cellular system.

Conclusion: Hence, we conclude that stress of different nature, intensity and duration can alter the levels of GSH-related antioxidants, especially in the kidney, ovary and brain tissues of stressed Wistar rats. The GSH levels in liver tissues were observed not to have changed significantly despite the oxidative damage caused by the stressors.

Keywords: Glutathione, Stress, Stressors, Oxidative stress, Wistar rats

INTRODUCTION

Oxygen is a key factor involved in sustenance of cellular energy production in every living system. This is achieved through the process of oxidative phosphorylation through electron transports in the mitochondria of cells. Oxidative stress (OS) results in an imbalance between the systemic production of reactive oxygen species (ROS), also known as free radicals and antioxidant defence system, thereby, causing the damage of biomolecules and this leads to modification of proteins, lipids and amino acids as well as breakage of DNA strands.[1] OS has been implicated in inflammatory and metabolic disease and cell membrane and organ damage.[2] Such cell

injuries cause cytokines release, specifically, the tumour necrosis factor-α which is responsible for generation of ROS in tissues, such that, excessive release of free radicals leads to electron leakage, increased superoxide and loss of cell membrane integrity while deregulating cellular function.[3]

Glutathione (GSH) is a tripeptide (γ-glutamate, glycine and cysteine) considered as a principal scavenger of excessive free radicals produced by OS.[4] Reduced GSH catalyses the production of glutathione peroxidase (GPx) and glutathione-S-transferase (GST). Thus, the biological activities of GST and GPx are modulated by GSH pathway. GSH present in mammalian cells plays crucial role as enzymatic factor and antioxidant redox buffer in numerous cellular redox process.[5] The regulatory synthesis of GSH enzyme involves *de novo* salvage signalling pathway and homeostasis of GSH antioxidant involves intra- and extra-cellular mechanisms such as; direct non-enzymatic GSH oxidation, ascorbate GSH cycle oxidation and reduction, thiol-redox enzymes glutaredoxin and thioredoxin signalling as well as other biosynthetic processes sustained by negative feedback using ATP molecules.[4]

As illustrated in [Figure 1], in OS, glutamate combines with cysteine by the action of dipeptide bond (glutamate cysteine ligase [GCL]) requiring ATP and GCL combines with glycine to produce reduced GSH. GSH undergoes a redox reaction using GPx to eliminate ROS such as hydrogen peroxide (H_2O_2) by the actions of superoxide dismutase. GSH is converted to GSSG, an oxidised form of GSH which is then recycled back to GSH with the help of nicotinamide adenine dinucleotide phosphate (NADPH) cofactor forming a redox cycle.[6] Under OS conditions, exposure to stressor(s) triggers depletion in tissue GST and subsequent reduction in the activities of GST and GPx.

Many human physiological processes produce reactive chemical molecules known as ROS which represents

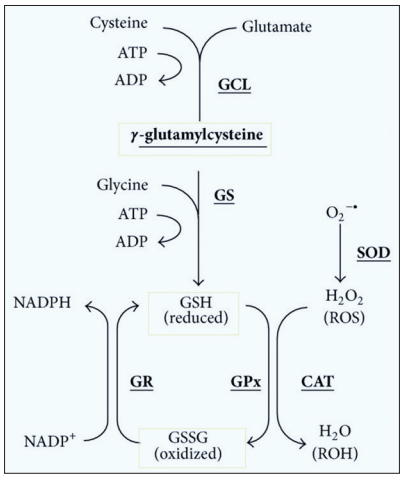

Figure 1: Glutathione redox cycle.
Source: Tamara *et al*.[6]

collections of broad ranges of radicals (oxidising agents) generated as by-products from oxygen metabolism due to impaired electron in the outer shell forming highly reactive molecule.[7] The relevant players of OS are; free radicals, ROS and antioxidant. Reactive molecules can arise from exogenous and endogenous sources, specifically, lifestyle changes such as; smoking, alcoholism, stressful events and aging.[8] Contributions can also come environmental factors such as; toxins, chemicals and changes in electromagnetic radiation.[9] Susceptibility to infections, autoimmune-related conditions and drug exposure have also being implicated.[10] Physiologically, the body has developed antioxidant defence system to protect itself from oxidative damage as a result of free radicals within the system initiated through one or more unpaired electrons by scavenging the formation of derived oxygen free radicals. According to Sies,[11] this occurs through three specific antioxidant mechanisms; prevention, interception and repair.

In the previous study, OS has been linked to damage aggravated by alteration in oxidative antioxidant enzyme activities which act as free radical scavengers across many tissues such as; brain, liver, kidney, lungs and small intestine.[12] Considerable number of experimental and clinical investigations has established the involvement of free radicals in pregnancy loss/abortion, pre-eclampsia and infertility.[13] In the event of stress, ROS are produced posing as threat to cellular biomolecules, causing damage to proteins, nucleic acids and subsequently lipid peroxidation. Although, study of GSH alone cannot confirm the above mechanism, hence the need for further studies as it relates to stress conditions. Due to the paucity of studies available on the role of OS GSH activity modifications following exposure to different nature of stress, this study was designed to investigate the impact of GSH -related antioxidants in the kidney, liver, ovary and brain tissues in female Wistar rats in variable stressor situations.

MATERIALS AND METHODS

Animal care and handling

One hundred and sixty-eight apparently healthy female Wistar rats were used for the study. The animals were procured from the Emma Maria Laboratory, Abraka, Delta State. The animals were transported in plastic cages to the Animal House of the Department of Human Physiology, Faculty of Basic Medical Sciences, Delta State University (DELSU), Abraka, Nigeria. All the rats used for this experiment were between the ages of 12 and 14 weeks with a body weight range of 150–200 g. The animals were properly handled and housed in wooden cages under normal room temperature and humidity in well-ventilated and hygienic conditions. The rats were allowed to acclimatise for a period

of 14 days before the study. The animals were properly fed with rat chaws and water *ad libitum* and were subjected to natural photoperiod of 12 h light and 12 h dark cycle.

Ethical clearance

Before the study, ethical approval was obtained from the Research and Bioethics Committee of Basic Medical Science, DELSU, Abraka, Delta State, Nigeria (Permit No: REC/FBMS/DELSU/18/16/53). The experiment was conducted in line with the laid down ethical standards of the Helsinki Declaration of 1964, and its later amended or comparable ethical procedure.

Induction of stress

Three different stressors were adopted in this study, namely restraint chamber test (RCT), mirror chamber test (MCT) and intruder paradigm test (IPT) as described by Nwogueze *et al.*[14] The restraint chamber stressor was used to induce physical stress. While, the mirror chamber stressor was used to induce anxiety stress in the Wistar rats. The intruder paradigm stressor was used to induce psychosocial stress on the animals by introducing an aggressive male cat to elicit defensive reaction in the female Wistar rats. The animals were exposed to these stressors for 1, 3 or 5 h per day for 1, 2 or 3 weeks, depending on the group to which the rats belonged.

Grouping of animals

The animals were randomly separated into 28 groups of six rats each as described in [Table 1].

Table 1: Animal grouping.

Animal group	Description of groups
Group 1	Control group
Groups 2–4	Animals exposed to stress of RCT of 1, 3 or 5 h per day for 1 week
Groups 5–7	Animals exposed to stress of RCT of 1, 3 or 5 h per day for 2 weeks
Groups 8–10	Animals exposed to stress of RCT of 1, 3 or 5 h per day for 3 weeks
Groups 11–13	Animals exposed to stress of MCT of 1, 3 or 5 h per day for 1 week
Groups 14–16	Animals exposed to stress of MCT of 1, 3 or 5 h per day for 2 weeks
Groups 17–19	Animals exposed to stress of MCT of 1, 3 or 5 h per day for 3 weeks
Groups 20–22	Animals exposed to stress of IPT of 1, 3 or 5 h per day for 1 week
Groups 23–25	Animals exposed to stress of IPT of 1, 3 or 5 h per day for 2 weeks
Groups 26–28	Animals exposed to stress of IPT of 1, 3 or 5 h per day for 3 weeks

RCT: Restraint chamber test, MCT: Mirror chamber test, IPT: Intruder paradigm test, hr: Hour (s)

Preparation of tissues for biochemical examination

The experimental animals of the control and stressed groups were fasted overnight before euthanising them by cervical dislocation. 24 h after last exposure, the target organs (kidney, live, ovary and brain) were quickly isolated from the rats. Each organ was placed in sterile containers and 0.5 g of the tissue of each organ was homogenised in 4.5 ml of buffer solution (ice-cold phosphate buffer, pH 7.4) using a potter Elvehjem homogeniser. The resultant homogenates of the different tissues from the respective groups were centrifuged in sterile bottles at 15,000 rms for 10 min in an ultracentrifuge. The resulting supernatant was collected and used for the biochemical investigations. Storage of the samples was at 4°C to ensure enzyme preservation.

Determination of reduced GSH

Reduced GSH was estimated in the different tissue homogenates using the colorimetric method of Ellman.[15] One hundred microliters of tissue homogenate were added to 1 ml of 0.2 M Tris EDTA buffer and this mixture was incubated at 25°C for 15 min. It was centrifuged to remove precipitate. Then, 5,5 dithiobis-2-nitrobenzoic acid was added and the reaction mixture was incubated at 37°C for 15 min on the development of a relatively stable yellow colour. Thereafter, the absorbance measured at 412 nm was read against a blank containing 3.5 ml of buffer using spectrophotometer and the equivalent GSH content was estimated from the standard GSH curve provided in the kit.

Determination of GST

The GST activity of the homogenised tissues was done by the method of Habig *et al.*[16] The method involved the formation of a complex from the enzymatic conjugation of reduced GSH with the aromatic substrate 1-chloro-2,4, dinitrobenzene (CDNB). The reaction mixture is formed by 1 ml of 0.3 M phosphate buffer (pH 6.5), 100 µL, 30 mM CDNB, 100 µL 30 mM GSH and 100 µL enzyme source (20% of tissue homogenates). The absorbance of the reaction mixture was read spectrophotometrically at 340 nm. The calculation of GST activity was by molar extinction coefficient that is, 9.6×10^3 $M^{-1}cm^{-1}$.

Determination of GPx

The activity of GPx was determined by the methods of Wendel,[17] in the tissue homogenates: 0.25 mM phosphate buffer (pH 7.0) containing 2.5 mM EDTA and 2.5 mM sodium azide (NaN_3) was used. The reaction mixture contains 1.8 ml of assay buffer, 100 µL NADPH, 100 µ GR, 100 µL GSH and 250 µg of enzyme source (20% tissue homogenates). Addition of 100 µL cumene hydroperoxide

(CHP)/hydrogen peroxide (H_2O_2) and linear reduction in NADPH initiated GPx reaction. The absorbance was read 3 times on spectrophotometer at 340 nm against a blank without enzyme source. The calculation of GPx activity was by molar extinction coefficient that is, $6.32 \times 10^3 cm^{-1}$.

Statistical analysis

Data obtained from this study were analysed using mean ± standard error of mean (SEM). One-way analysis of variance was used in mean comparison. $P < 0.05$ served as an indicator of statistical significance. *Post hoc* test was performed using Fisher's LSD method to check for the homoscedasticity of variance.

RESULTS

The trend in the changes of non-enzymatic biomarker; reduced GSH level and the enzymatic biomarkers; GPx and GST activities are represented in [Figures 2-4] for experiments on kidney, liver, ovary and brain tissues in female Wistar rats exposed to RCT, MCT or IPT stressor for 1, 3 or 5 h per day for 1, 2 or 3 weeks, respectively.

The kidney GSH levels of rats exposed to RCT stressor at the rate of 1, 3 or 5 h per day for 1 week were significantly ($P < 0.05$) decreased as illustrated in [Figure 2a]. Data from this study showed that the levels of kidney GSH were also significantly ($P < 0.05$) decreased in rats exposed to MCT stressor at the rate of 1, 3 or 5 h per day for 1 week as well as where rats were exposed to similar stressor at the rate of 3 h or 5 h per day for 2 weeks when compared to control. Kidney GSH levels of rats exposed to MCT at the rate of 1 or 3 h per day for 3 weeks were significantly ($P < 0.05$) elevated when compared to the control levels. Whereas, kidney GSH levels of rats exposed to IPT stressor at the rate of 1, 3 or 5 h per day for 1 week were significantly ($P < 0.05$) reduced when compared to control.

There were no observable significant changes which were recorded in the levels of GSH in the liver of rats following exposure of the different nature stressors when compared to control. In [Figure 2c], the ovary GSH levels of rats following exposed to RCT stressor at the rate of 1 or 5 h per day for 1 week or 3 weeks, respectively, were significantly ($P < 0.05$) increased. Similarly, the ovary GSH levels of rats exposed to similar stressor at the rate of 5 h per day for 2 weeks were significantly ($P < 0.05$) increased when compared to the

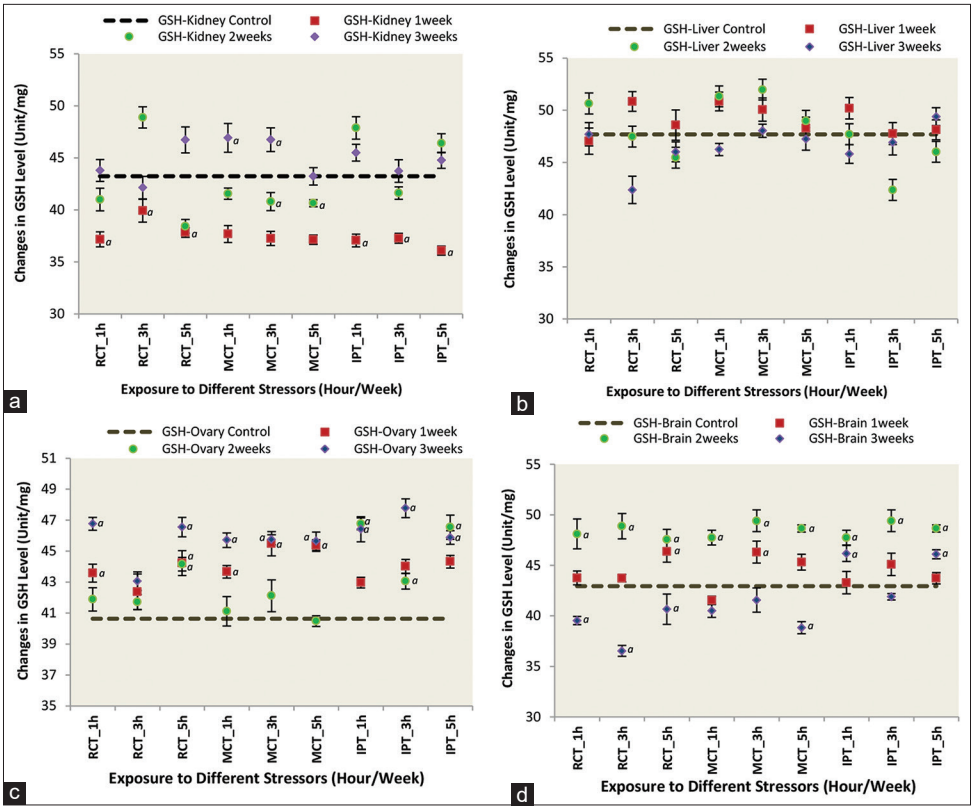

Figure 2: Stress-induced reduced glutathione modulation in the kidney (a), liver (b), ovary (c) and brain (d) tissues of female Wistar rats. Values are presented as mean ± SEM, where $n = 6$. Values with different superscript along the same row are significantly different at $P < 0.05$. RCT: Restraint chamber test, MCT: Mirror chamber test, IPT: Intruder paradigm test, hr: Hour. [a]Significant when compared to control.

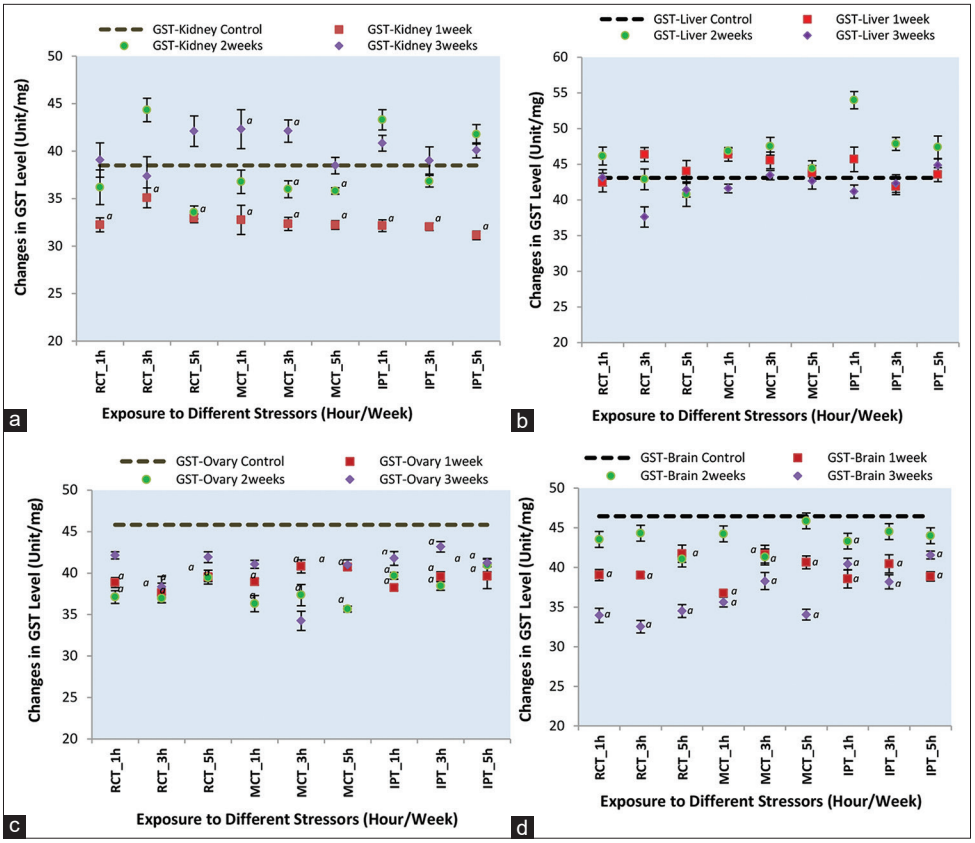

Figure 3: Stress-induced glutathione-S-transferase activity in the kidney (a), liver (b), ovary (c) and brain (d) tissues of female Wistar rats. Values are presented as mean ± SEM, where $n = 6$. Values with different superscript along the same row are significantly different at $P < 0.05$. RCT: Restraint chamber test, MCT: Mirror chamber test, IPT: Intruder paradigm test, hr: Hour. [a]Significant when compared to control.

control level. For rats exposed to MCT stressor, stressing the rats at the rate of 1, 3 or 5 h for 1 week or 3 weeks, respectively, caused significant ($P < 0.05$) increase in the ovary GSH levels when compared to the control level. Furthermore, the ovary GSH levels of rats exposed to IPT stressor at the rate of 1, 3 or 5 h per day for 2 or 3 weeks were significantly ($P < 0.05$) increased when compared to the control level.

Our data showed that the brain GSH levels of rats exposed to RCT stressor at the rate of 5 h per day for 1 week were significant ($P < 0.05$) increased as represented in [Figure 2d]. Similarly, the levels of brain GSH were equally significantly ($P < 0.05$) increased following exposure to similar stressor at the rate of 1, 3 or 5 h per day for 2 weeks when compared to the control levels. The brain GSH levels were significantly ($P < 0.05$) decreased in rats exposed to RCT stressor at the rate of 1, 3 or 5 h per day for 3 weeks when compared to control levels. Exposure to MCT stressor at the rate of 3 h per day for 1 week significantly ($P < 0.05$) caused an increase in the levels of brain GSH of rats. Similarly, the brain GSH levels of rats exposed to MCT stressor at the rate of 1, 3 or 5 h per day for 2 weeks were significantly ($P < 0.05$) increased when compared to the control level. However, the levels were significantly ($P < 0.05$) reduced following exposure to the same stressor at the rate of 5 h per day for 3 weeks. The brain GSH levels of rats following exposure to IPT stressor at the rate of 1, 3 or 5 h per day for 2 weeks were significantly ($P < 0.05$) increased while exposure of the rats to IPT stressor at the rate of 1 h or 5 h per day for 3 weeks significantly ($P < 0.05$) increased when compared to control level.

Results obtained revealed that the activity of kidney GST of rats exposed to RCT stressor at the rate of 1, 3 or 5 h per day for 1 week was significantly ($P < 0.05$) decreased when compared to control level. In the case of exposure to MCT stressor, stressing the rats at the rate of 1, 3 or 5 h per day for 1 week significantly ($P < 0.05$) reduced the activities of kidney GST when compared to the control level. Exposure of the rats to MCT stressor at the rate of 3 h or 5 h per day for 2 weeks significantly ($P < 0.05$) decreased the activities of kidney GST when compared to the control level, while exposure of the rats to MCT stressor at the rate of 1 h or 3 h per day for 3 weeks significantly ($P < 0.05$) increased the activities of kidney GST when compared to the control level. Exposure to IPT stressor at the rate of 1, 3 or 5 h for 1week significantly ($P < 0.05$) decreased the kidney GST activity of the rats when compared to control level. The results obtained in [Figure 3b] revealed that there were no significant changes observed in

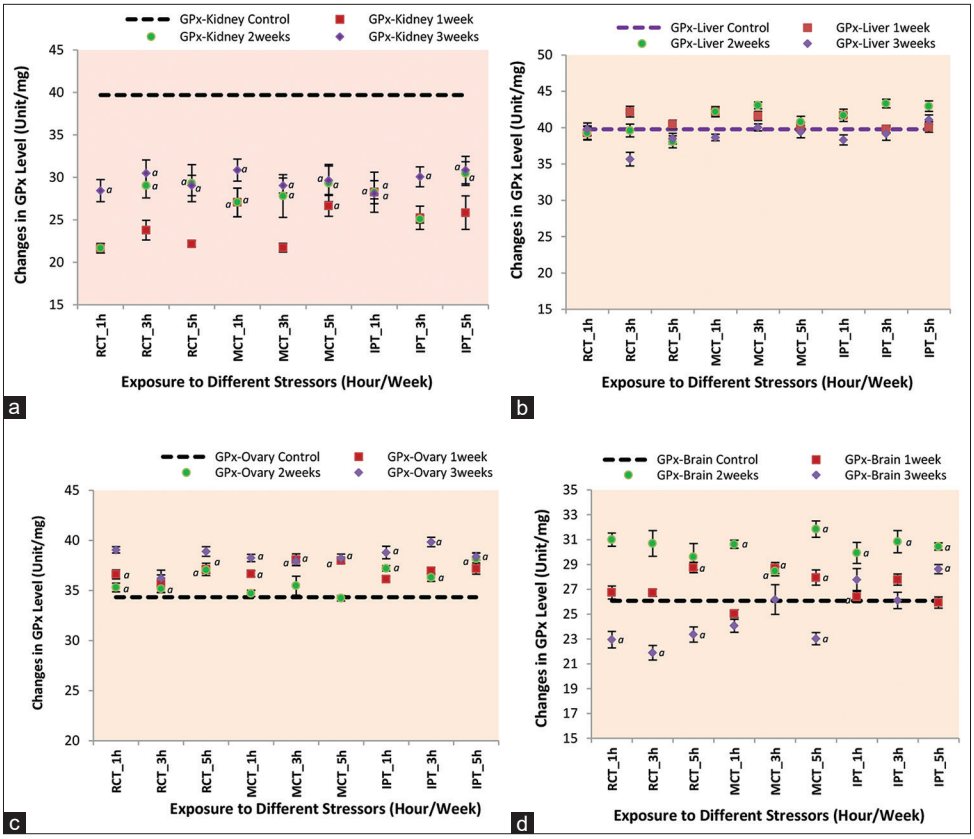

Figure 4: Stress-induced glutathione peroxidase activity in the kidney (a), liver (b), ovary (c) and brain (d) tissues of female Wistar rats. Values are presented as mean ± SEM, where $n = 6$. Values with different superscript along the same row are significantly different at $P < 0.05$. RCT: Restraint chamber test, MCT: Mirror chamber test, IPT: Intruder paradigm test, hr: Hour. [a]Significant when compared to control.

the activities of liver GST of rats following exposure of the different nature stressors when compared to control.

Stressing the rats with RCT stressor at the rate of 1, 3 or 5 h per day for 1 or 2 weeks, respectively, significantly ($P < 0.05$) decreased the activities of ovary GST when compared to the control level. Similarly, stressing the rats with RCT stressor at the rate of 3 h per day for 3 weeks significantly ($P < 0.05$) reduced the activities of ovary GST when compared to the control level. Exposure of the rats to MCT stressor at the rate of 1, 3 or 5 h per day for 1, 2 or 3 weeks significantly ($P < 0.05$) decreased the activities of ovary GST when compared to control level. Similarly, exposure of rats to IPT stressor at the rate of 1, 3 or 5 h per day for 1, 2 or 3 weeks significantly ($P < 0.05$) decreased the activities of ovary GST when compared to the control level.

Our study showed that the activities of brain GST of rats exposed to RCT stressor at the rate of 1, 3 or 5 h per day for 1 or 3 weeks were significantly ($P < 0.05$) reduced when compared to the control level. Similarly, exposure of rats to MCT stressor at the rate of 3 h per day for 2 weeks significantly ($P < 0.05$) reduced the activities of brain GST when compared to the control level. Stressing the rats with MCT stressor at the rate of 1, 3 or 5 h per day for 1 or 3 weeks significantly ($P < 0.05$) reduced the activities of brain GST when compared to the control level. With respect to the case of IPT stressor, exposure of the rats at the rate of 1, 3 or 5 h per day for 1 or 3 weeks significantly ($P < 0.05$) reduced the activities of brain GST when compared to that of the control level, but stressing the rats with IPT stressor at the rate of 1 h per day for 2 weeks caused significant ($P < 0.05$) decrease in the brain GST activities of the rats.

Results obtained from [Figure 4a] revealed that the activity of kidney GPx of rats exposed to RCT, MCT and IPT stressors at the rate of 1, 3 or 5 h per day for 1, 2 or 3 weeks, respectively, were significantly ($P < 0.05$) decreased in progressive manner when compared to the control level. [Figure 4b] clearly revealed that there were no significant changes observed in the activities of liver GPx of rats following exposure of the RCT, MCT and IPT stressors when compared to control, irrespective of the rate per day and duration per week of exposure. The changes in the activities of ovary GPx of rats exposed to RCT stress at the rate of 1 h or 5 h per day for 1 or 2 weeks, respectively, revealed significant ($P < 0.05$) increase when compared to control levels, as demonstrated in [Figure 4c]. Similarly, stressing the rats with RCT stressor

at the rate of 3 h per day for 2 weeks significantly ($P < 0.05$) increased the activity of ovary GPx when compared to the control level. In respect of the MCT stressor, exposure of the rats at the rate of 1, 3 or 5 h per day for 1 or 3 weeks significantly ($P < 0.05$) increased the activity of ovary GPx when compared to the control level. Exposure of rats to IPT stressor at the rate of 1, 3 or 5 h per day for 2 or 3 weeks, respectively, significantly ($P < 0.05$) increased the activity of GPx in the ovary when compared to the control levels.

The activity of brain GPx of rats exposed to RCT stressor at the rate of 5 h per day for 1 week was significantly ($P < 0.05$) increased when compared to the control level as illustrated in [Figure 4d]. However, exposure of the rats to RCT stressor at the rate of 1, 3 or 5 h per day for 3 weeks significantly ($P < 0.05$) decreased the activity of brain GPx of rats when compared to the control level. The activity of brain GPx of rats exposed to MCT stressor at the rate of 3 h or 5 h per day for 1 week was significantly ($P < 0.05$) increased, whereas, the activity of brain GPx of rats exposed to similar nature of stressor at the rate of 1, 3 or 5 h per day for 2 weeks was significantly ($P < 0.05$) increased. Meanwhile, the activity of brain GPx of rats exposed to MCT stressor at the rate of 5 h per day for 3 weeks was significantly ($P < 0.05$) reduced when compared to the control level. The activity of brain GPx of rats exposed to IPT stressor at the rate of 1, 3 or 5 h per day for 2 weeks was significantly ($P < 0.05$) increased, similarly, the activity of brain GPx of rats exposed to IPT stressor at the rate of 5 h per day for 3 weeks was significantly ($P < 0.05$) increased when compared to the control level.

DISCUSSION

Antioxidants act by scavenging free radicals in the human system.[18] GSH is a key non-enzymatic antioxidant known for ROS detoxification as purported by the previous studies.[19,20] GSH is synthesised in cytoplasm of the liver and thereafter distributed to other organs.[21] Data from our study have showed that in the light of exposure of the animals to RCT, MCT and IPT stressors, there was markedly compromised defence mechanism of GSH-related antioxidants, thus compromising GSH homeostasis.[22,23] Significant increase in GSH level suggests stimulation of antioxidant system in defence against the effect of stress, whereas, depletion of GSH level is an indication of tissue OS.[24] This agrees with the findings of Pajovic *et al*.[25] and Ahmad *et al*.[26] who reported that restraint-induced stress depletes GSH resulting in oxidative damage and cell death in rats.

In the present study, the kidney GSH levels of rats were depleted following exposure to restraint or intruder stressor for 1 week, irrespective of the duration of exposure. However, the effects were more significantly pronounced in the MCT stressed rats when exposed for 3 h and 5 h per day for 2 weeks. Such observed reduction is consistent with the findings of Ghorbel *et al*.[27] who reported that reduced level of kidney GSH following generation of free radicals, redox imbalance due to exposure to stress may cause nephrotoxicity and loss of the integrity of kidney membrane due to oxidation of lipids and proteins. This finding also was in agreement with the reports provided by Samson *et al*.,[24] Chakraborti *et al*.,[20] and Sarumathi and Saravanan[28] who in their studies purported that GSH levels were decreased in various tissues following exposure to different stressors, thus altering the protective ability of vital organs from oxidation stress. GSH is substrates for maintenance of GST and GPx enzyme activities.

Data from this study revealed that the brain GSH levels were significantly reduced in rats exposed to restraint stressor, whereas, the observed significant increase in the brain GSH levels in rats exposed to MCT or IPT stressor suggests that the organs were spared. This observation was similar with the findings of Mehri *et al*.[29] that pointed out that GSH levels are significantly decreased following acrylamide stress-induced toxicity in brain tissues. This is an indication that stress of different magnitude may result in depletion of reduced GSH, resulting in impairment of cellular defence against ROS, leading to tissue injury.[30] Furthermore, Trevisan *et al*.[26] believed that such decrease of reduced GSH in vital tissues could result in lower rate of peroxidase activity, thus, interfering with immune cells and reduced survival capacity of animals in extreme stress exposure. The ovary and brain GSH levels were significantly elevated following exposure to all the stressors. Similar reports were provided by Ghizoni *et al*.[31] who in their studies observed an increase in the levels of GSH following exposure to restraint stress, although, this was in the cerebellum of rats. However, the levels of brain GSH were significantly reduced in the animals where restraint stressor was applied at the different rate of exposure daily, for 3 weeks.

Under physiologic situation, GST conjugates with GSH in protecting different tissues from OS effect.[31] GST is groups of large and complex family of proteins that forms the second phase of detoxifying enzyme that conjugates reduced GSH provided by the bile through sulfhydryl group catalysing deactivation of harmful compounds to electrophilic centres on different ranges of substrate toward ensuring their excretion from the cell.[32] In this study, activities of kidney GST were significantly affected and this was more deleterious following exposure to MCT at the rate of 1 h or 3 h per day for 3 weeks. This finding is consistent with the findings of Nadeem *et al*.[33] and Yuksel *et al*.[34] who reported that the activities of GST of kidney tissues were significantly increased following exposure to cold and social stressors. This observations agrees with the findings of Yousef and Demerdash[35] who reported an increase GST activity level of kidney and testis following stress induction.

Findings from our study revealed that activities of ovary and brain GST of rats exposed to RCT, MCT or IPT stressor were markedly reduced irrespective of the duration of exposure. Our result was inconsistent with the findings of Zaidi *et al*.[36] and Devaki *et al*.[37] that reported a significant decrease in GST in rats exposed to restraint stressor at an interval of 1–6 h. The noted decrease GST activity as reported in this study could be linked to the adaptive mechanism or defensive response to xenobiotic detoxification, induction of the apoptosis signalling pathway, removal of OS product, transport of proteins as well as modulation and proliferation of cells as reported by Dasari *et al*.,[38] Laborde.[39] A study conducted by Saka and Aouacheri,[40] also revealed that decrease in GST levels in stressed rats suggests an adaptive mechanism by the cell detoxifying generated ROS to minimise tissue damage. Such detoxifying role of GST following the effect of stress on the tissues of rats is vital to the cell to regenerate stress-free environment from the actions of reactive electrophiles that reduce cell metabolising ability.[39]

GPx in the biological system present in cystol and mitochondria act as detoxifying agents by participating in the GSH redox cycle.[41] The activity of GPx is derived from the oxidation of reduced β-nicotinamide adenine dinucleotide phosphate (NADPH) in conjugated GSH system. Perhaps, isoenzymes of GPx catalyse the reduction of H_2O_2 and lipid peroxides using GSH.[42] GPx activity of all the kidney tissues was significantly altered following exposure to the different nature of stress model adopted in this study irrespective of the rate per day or duration in week(s) of exposure. The observed decrease of GPx in the kidney tissue is associated with higher level of activity of ROS and cellular damage as suggested by Chatziargyriou and Dailianis,[43] considering the fact that GPx and GST are key enzymes required in maintaining GSH homeostasis. The brain GPx content of rats exposed to RCT stressor for up to 3 weeks at the different rate per day was affected. The brain tissues require sufficient oxygen that is, approximately 20% of the total oxygen consumed by the body. In OS, the alterations to the brain GSH and GPx antioxidants activities potentiate dysregulation in the oxygen content of the brain resulting to positive risk factors for neuronal cell death, neurologic and neurodegenerative diseases as well as several pathologic conditions.[44]

The liver organ was spared from oxidative damage virtually in all the rats exposed to the RCT, MCT or IPT stressor irrespective of the duration of exposure per day and the duration in weeks. Thus, the activities of GSH, GST and GPx were unaltered following OS in comparison to normal rats thus providing evidence that counter regulatory mechanisms were initiated in maintaining the levels of GSH antioxidants as primary adaptation to prevent hepatic tissue damage. Such observation from our study is similar to the previous studies such as Depke *et al*.[45] and Mahvash *et al*.[46] who purported that the mechanism for such increase in liver GSH levels is indicative of possible adaptive hepatoprotective response of the animal to stress.

The study revealed that there was difference between stress exposure rate and duration of stress on the levels of GSH, GST and GPx. The mechanism underlying the variability to the impact of exposure to stressors on the GSH redox pathway as observed in our study is not well understood. The stressor with more pronounced effect was the RCT stressor for the kidney GSH level. In the case of GST levels, the MCT stressor had more impact especially for the kidney when exposure was up to the 3rd week. The activities of GPx in the kidney of rats were affected when all the stressors were applied, while, the activities of brain GPx were mostly affected when RCT stressor was applied up to the 3rd week. The precise mechanism for the variability of the impact of the stressors applied on the different tissues as found in the present study remains unclear, however, other studies such as Halliwell and Chirico,[47] Schmitt *et al*.[48] and El-Beltagi and Mohamed,[49] linked it to electron and proton donation process reducing reactive oxygen, suppressing cellular inflammation and mimicking nitric oxide synthesis.

The ability of the body antioxidant to neutralise the effects of OS differs in tissues depends largely on the stress model applied.[50,51] As Nadeem *et al*.[33] and Kamper *et al*.[51] maintained, different nature of stress facilitates the formation of ROS and enhances OS conditions. In all, the exposure to RCT, MCT or IPT stressor-induced ROS production in the different tissues with more deleterious effect observed in the kidney followed by the brain tissues. These changes varied considerably depending on time and duration of exposure to the stressor. Assessing the activities of GSH enzymes in this study has provided a baseline index for understanding the cellular changes and prevailing complications associated with OS indices in the different tissues studied.

CONCLUSION

In the present study, we assessed the markers of OS in the kidney, liver, ovary and brain of female Wistar rats exposed to RCT, MCT and IPT stress models at duration intervals of 1 h, 3 h and 5 h for 21 days. Findings from this study showed that the changes in GSH antioxidants levels in tissues varied considerably depending on the nature of stressor applied and the duration in hour(s) per day and week(s). Specifically, the kidney GSH and GPx were significantly altered, while kidney GST was only affected when stress was extended to the 3rd week. However, in the ovary and brain, there was observed significant increase in GSH and GPx levels following exposure to MCT and IPT stressor but exposure to RCT stressor by the 3rd week affected the brain GPx levels. In respect to GST levels, there was significantly decreased in the tissues irrespective of stressor applied, suggestive of a tissue-specific protective response to stress. However, in the liver, there were hardly any significant changes observed in the levels of GSH, GST and GPx. The present study did not

establish the reason for this observation, hence, there is room for further studies in this area.

Recommendations

It is recommended that the total antioxidant capacity should be done to show the cumulative impact between stress exposure rate and duration of stress on levels of GSH, GST and GPx of the overall kidney, ovary, liver and brain cellular defence system in redox state for the different stress model studied for a clearer understanding of the synergistic mechanism involved.

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Declaration of patient consent

Patient's consent not required as there are no patients in this study.

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Conflicts of interest

There are no conflicts of interest.

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