ORIGINAL ARTICLE

Effect of Glutathione on Depression Paradigms in Male Wistar Rats and Swiss Albino Mice

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Abstract:

Background: Depression is a major public health concern. In the recent years, two intertwined events namely oxidative stress and inflammation have emerged as major players in the pathophysiology of depression with evidence that, there are reduced antioxidant defenses in depression. *Aim and Objectives:* This study was aimed at evaluating the antidepressant activity of glutathione which is an antioxidant, using behavioural models of depression namely Forced Swim Test (FST) in male Wistar rats and Tail Suspension Test (TST) in male Swiss mice. *Material and Methods:* All drugs were administered orally. Amitriptyline in 1% gum acacia suspension was used as the standard drug. For the FST, rats were divided into three groups of six animals each, amounting to a total of 18 animals. Control group received 0.5 ml of 1% gum acacia suspension. Test group received glutathione in 1% gum acacia suspension. For the TST, mice were divided into three groups of six animals each, amounting to a total of 18 animals. Control group received 0.5 ml of 1% gum acacia suspension. *Results:* There was a statistically significant difference in the duration of immobility with amitriptyline and glutathione treated groups when compared to that of control groups in both models of depression. *Conclusion:* Based on the findings of this study, it can be concluded that glutathione has significantly better antidepressant activity as compared to control in both FST as well as TST. But, significantly better antidepressant activity as compared to amitriptyline is found only in FST. Based on the results of the present study and the fact that oxidative stress has a major role to play in the pathophysiology of depression, glutathione appears to be a promising agent for the treatment of depression. Antidepressant activity of glutathione can be explained by its role in neutralizing reactive oxygen species, role as a neuromodulator and neurotransmitter as well as a regulator of transcription of inflammatory cytokines.

Keywords: Glutathione; Depression; Amitriptyline; Oxidative Stress

Introduction:

As per the World Health Organization (WHO), the proportion of the world population suffering from depression was estimated to be 4.4% in 2015 and 322 million people across the globe live with depression with nearly fifty percent of them residing in South-East Asia and the Western Pacific Region. Over a period of 10 years from 2005 to 2015 there has been an increase of 18.4% in the number of people living with depression [1]. Depression is not just consistent with persistent low mood and negative thoughts but also interferes with a person's routine activities like sleep, appetite and libido. It also affects inter personal relationships with family, friends and work place interactions [2-3]. In 2015, it was estimated that 7,88,000 people died after having committed suicide and many more tried committing suicide. Suicide contributed to the top 20 prominent causes of death in 2015 [1].

Presently available antidepressants are a diverse group of compounds acting by different mechanisms of action and although widely used in the treatment of depression 30% of patients are non-responders and 70% fail to achieve remission. Moreover, these compounds also produce a plethora of adverse effects and have a potential for abuse [4].

The well-known theories of depression are brain monoaminergic transmission disturbances, reduced Brain Derived Neurotropic Factor (BDNF) levels and dysregulation of Hypothalamo-pituitary Adrenal (HPA) axis [5].

In the recent years, two interlinked events namely oxidative stress and inflammation have emerged as major players in the pathophysiology of depression. The susceptibility of neurons to oxidative stress is because of their high oxygen consumption and metabolism. At the same time, neurons have comparatively low levels of antioxidant substances and enzymes in comparison to other tissues [6].

Evidence also points out that, there is a deficiency of antioxidant defense mechanisms in depression. This is as evidenced by a deficiency of vital antioxidants as well as enzymes serving as antioxidants which include scarcity of coenzyme Q10, vitamin E, glutathione, serum zinc and Glutathione Peroxidase (GPX) [7]. Also, there is evidence that, glutathione levels are deficient in prefrontal cortex of patients with major depressive disorder as pointed out during postmortem examination of the brain [8]. Hence, the present study aimed at investigating the effect of glutathione on depression using behavioural models of depression namely Forced Swim Test (FST) in male Wistar rats and Tail Suspension Test (TST) in male Swiss mice.

Material and Methods:

Animals:

Adult male Wistar rats having a body weight of 150+/-25g and adult male Swiss mice of 25+/-5g were obtained from the Central Animal House of the Institution. They were adapted to a light - dark cycle consisting of 12 hours each of light and dark for 10 days prior to the start of the experiment. They were allowed to feed on standard chow pellets of Amrut Brand and water at liberty. The study was approved by the Institutional Animal Ethics Committee (IAEC) constituted as per the guidelines framed by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPSCEA), New Delhi. For the FST, rats were divided into three groups of six animals each, amounting to a total of 18 animals. Control group received 0.5ml of 1% gum acacia suspension. Standard group received amitriptyline in 1% gum acacia suspension. Test group received glutathione in 1% gum acacia suspension. All drugs were administered orally. For the TST, mice were divided into three groups of six animals each, amounting to a total of 18 animals. Control group received 0.5ml of 1% gum acacia suspension. Standard group received amitriptyline in 1% gum acacia suspension. Test group received glutathione in 1% gum acacia suspension. All drugs were administered orally.

Drugs and Doses:

Considering the therapeutic doses of glutathione and amitriptyline [9-10] equivalent doses for rats and mice were calculated with the help of converting table devised by Paget and Barnes [11]. Standard groups received amitriptyline (27 mg/kg body weight of rat and 39 mg/kg body weight of mice) equivalent to 300 mg of clinical

dose orally [10]. Test groups received glutathione (54 mg/kg body weight of rat and 78 mg/kg body weight of mice) equivalent to 600 mg of clinical dose orally [11]. Antidepressant activity was assessed with the help of following paradigms:

FST:

Principle: When a rat is involuntarily made to swim in an environment from which it cannot escape, it initially shows vigorous activity and ultimately stops all movements except for those movements necessary to maintain its head above the water. This form of immobility is typical and points out to a state of despair. The rat is forced to resign itself to the existing situation since it realizes that, there is no escape. This typical behavior is considered to be the equivalent of clinical depression. Therefore, drugs that decrease this immobility would have to possess antidepressant activity. Rats are preferred over mice in this test, because the rat version is said to be more selective for this experiment i.e. fewer false positives [12-14].

Procedure of FST:

A vertical cylinder made of plexiglass having a height of 21cm, diameter of 12cm and containing a 15cm water column maintained at 25^0C was used for the test. FST has two swim sessions namely a pre-test session and a test session. First session is known as the "Pre-test" session. This lasts for 15 minutes and is held prior to drug administration and no recording of animal behavior is done. This serves as a habituation session and guarantees a stable and high duration of immobility during the 6-min test session which is held 24 hours later [12, 14].

Adult male Wistar rats weighing 150± 25 g were subjected to swimming for 15 minutes during which no immobility scoring was done. The rats were removed, dried and the first dose of the drug was given orally and they were returned to their cages, where they were provided with food and water. The water in the cylinder was changed for every rat before subjecting it to swimming. These rats were subjected to the test session on the next day 24 hours after the pretest session [14]. On the day of the test session, the rats were given the second dose of the drug orally - 4 hours prior to test and third dose of drug orally - 1 hour prior to test. The reason for administration of three doses of drug prior to the test is to provide more stable pharmacological results than a single administration. Then they were subjected to FST and the duration of immobility (in seconds) was recorded for 6 minutes [12,14].

TST:

Principle: Basic principle of this test is, the behavioral despair paradigm which has already been described in FST. When mice are hung by their tails, they are subjected to inescapable stress. The mice finally attain immobility after an initial period of vigorous activity. Any drug that decreases the time spent in immobility is supposed to possess antidepressant activity [11, 13].

Procedure: It consist of 2 metallic rods placed 35 cm away from each other and connected with a horizontal rod required to hang a nylon thread from its centre. A mouse treated with either the drug or vehicle was suspended from the hook hanging from the center of the horizontal rod with the help of an adhesive tape fixed 1 cm proximal to the tip of the tail. The mouse was said to be immobile, when it ceased to move and hung totally motionless. Immobility time in seconds was recorded over a period of 6 minutes [12, 13].

Statistical Analysis:

The results are represented in the form of mean± Standard Error of Mean (SEM) of 6 mice / 6 rats in each group. The results were analyzed by one-way analysis of variance (ANOVA) followed by a post hoc test called Bonferroni's test. $P < 0.05$ was considered statistically significant [15].

All data were analyzed using statistical software called GraphPad Prism (GraphPad Software, Inc. La Jolla, California, USA).

Results:

In the present study, glutathione has been evaluated for its antidepressant activity using two models of depression in animals namely forced swim test performed in rats and tail suspension test performed in mice. Amitriptyline has been used as a standard antidepressant drug for the purpose of comparison.

FST:

The duration of immobility in seconds was noted over 6 minutes. The mean immobility time in the control group was 156 ± 1.5 seconds, while it was found to be 74 ± 1.6 seconds and 51 ± 6.6 seconds in the amitriptyline and glutathione groups respectively. There was a statistically significant difference ($P< 0.0001$) in the duration of immobility in amitriptyline and glutathione treated groups as compared to that of control group. There was also a significant difference ($P< 0.01$) in the duration of immobility in glutathione treated group as compared to amitriptyline group (Fig. 1) indicating superiority of glutathione over amitriptyline in FST.

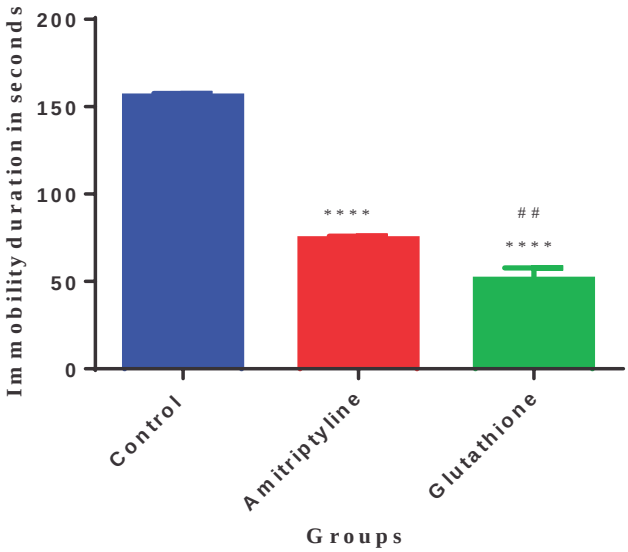

Fig. 1: Effect of Various Treatments on Duration of Immobility in FST

*Values are represented as Mean± Standard error of mean (SEM) (n=6). ANOVA followed by Post Hoc Analysis by Boniferroni's test. ****P < 0.0001- Glutathione and Amitriptyline as compared to control.##P < 0.01 - Glutathione as compared to Amitriptyline*

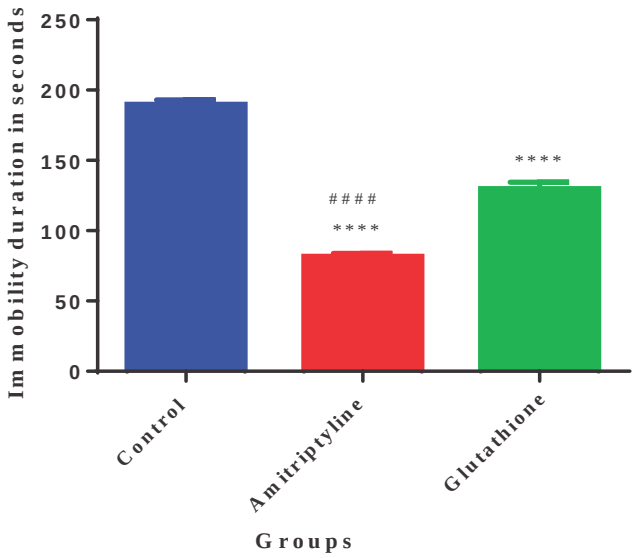

Fig. 2: Effect of Various Treatments on Duration of Immobility in TST

*Values are represented as Mean± Standard error of mean (SEM) (n=6). ANOVA followed by Post Hoc Analysis by Bonferroni's test. ****P < 0.0001- Glutathione and Amitriptyline as compared to control. #### P < 0.0001- Amitriptyline as compared to Glutathione.*

TST:

The duration of immobility in seconds was noted over 6 minutes. The mean duration of immobility in the control group was found to be 190 ± 3.2 seconds while it was 82 ± 2 seconds and 130 ± 4.6 seconds in the amitriptyline and glutathione groups respectively. There was statistically significant difference (P< 0.0001) in the duration of immobility with amitriptyline and glutathione treated groups, when compared to that of control groups (Fig. 2). There was also a significant difference (P< 0.0001) in the duration of immobility in amitriptyline treated group as compared to glutathione group (Fig. 2) indicating superiority of amitriptyline over glutathione in TST.

Discussion:

In the present study, glutathione was evaluated for its antidepressant activity using two models of depression in animals, namely FST performed in rats and tail suspension test performed in mice. Glutathione was found to have significantly better antidepressant activity as compared to control in both FST as well as TST. But, significantly better antidepressant activity as compared to amitriptyline was found only in FST.

FST and TST are widely used in research in depression since they are economical, sensitive and easy to perform. Among the two tests TST is supposed to be more sensitive to lower drug doses and provides a clearer dose response relationship [16, 17]. As mentioned earlier, the role of oxidative stress and inflammation in the pathophysiology of depression, have gained significance in the recent years. The susceptibility of the brain cells to harmful effects of oxidative stress can be explained by the fact that, brain cells have a high metabolic rate (more than 20% of the total oxygen consumption is accounted for by the brain), abundant amount of highly peroxidizable substrates are

generated in the brain and the antioxidant levels in the brain are modest [18].

Studies have been done which have proven not only that oxidative stress plays a role in depression but also that glutathione has been found to be deficient in animals with depression like behavior as elaborated below.

A study which used Ultrasound (US) model as a source of stress in rats, found that US caused depression-like behavioral disorder in rats. In the study, the beginning of behavioral symptoms (increased immobility in FST and decreased sucrose intake) correlated with a rise of oxidative stress markers in the brain. Levels of protein carbonyl and total glutathione in the hippocampus and prefrontal cortex were altered which support the existence of a link between development of the depression like state and oxidative stress in the brain [19].

A study was performed to evaluate biochemical and molecular changes associated with Reactive Oxygen Species (ROS) generation in the brains of rats submitted to chronic variable stress. After sacrificing the animals various biochemical parameters were measured in the prefrontal cortex. Of relevance was the fact that, the prefrontal cortex of rats submitted to chronic stress did show a statistically significant decrease in the GSH/ GSSG ratio (ratio of reduced to oxidized glutathione). The GSH/GSSG ratio is considered to be a sensitive indicator of the cellular redox state [20]. Studies similar to the present study have also used forced swim test but the differences are that they have not used tail suspension test, they have used N-Acetyl Cysteine (NAC - a prodrug of glutathione) and have measured levels of some oxidative stress parameters. A study was performed to evaluate the possible role of oxidative stress in depression induced by morphine administration. It was found that, in albino rats administered NAC along with morphine all parameters demonstrated clear trends to nearly full normalization in comparison to the controls administered morphine alone. Parameters studied included immobility duration in the forced swim test and levels of oxidative stress markers in the prefrontal cortex [21].

A study was performed with the objectives of assessing the preventive effect of metoprolol, NAC and escitalopram on Chronic Unpredictable Mild Stress (CUMS)-induced depression and effect of endoplasmic reticulum stress on antidepressant role of these drugs. Of relevance is the fact that, NAC mitigated depression like behaviors (as assessed by open field activity and sucrose preference) and also decreased hippocampal cell apoptosis. NAC also decreased markers of endoplasmic reticulum stress such as Glucose-Regulated Protein 78 (GRP78), C/EBP-Homologous Protein (CHOP), and caspase-12 [22].

Glutathione is present in the cells in millimolar concentrations. It is a tripeptide and a small-molecule antioxidant and is considered one of the most vital small-molecule antioxidants in the somatic cells. It is also the most prevalent antioxidant in the brain. Glutathione is a thiol-containing molecule consisting of three amino acids namely glutamate, glycine and cysteine. It reacts with ROS and nucleophilic compounds. Reduced glutathione gets oxidized after reacting with free radicals. This reaction is catalyzed glutathione peroxidase or occurs independently. Recycling of oxidized glutathione to two molecules of its reduced form is catalyzed by Glutathione Reductase (GR) [23-24]. The role of glutathione as an antidepressant with respect to its

antioxidant property can be explained by the fact that, it serves several vital functions in the body such as direct chemical neutralization of various free radicals and serves as a cofactor for several antioxidant enzymes[25].

Glutathione also plays a role a as a neuromodulator/ neurotransmitter where in it moderates the activity of the N-Methyl-D-Aspartate (NMDA) receptors. Glutathione modulates the redox site of NMDA receptors by virtue of its free cysteinyl thiol group. Besides this, glutathione also aids as an endogenous nitric oxide pool from which S-Nitrosoglutathione (GSNO) is generated which has a shielding effect in the brain under oxidative stress conditions. Hence, it is proposed that glutathionemay have a role to play in synaptic transmission [26-27].

A vital role is played by the redox state of the cells in the regulation of the transcription of cytokines namely interleukin-1 beta (IL-1β), IL-6, and Tumor Necrosis Factor (TNF-α). The redox state also modulates the signaling pathways activated by these cytokines. Diminution of glutathione enhances both transcription as well as deleterious effects of cytokines. On the contrary, raised levels of glutathione and cysteine inhibit transcription of pro-inflammatory cytokines. The ratio of reduced to oxidized glutathione regulates the transcription of IL-6, IL-8, IL-4, and TNFα [28].

Conclusion:

Based on the findings of this study, it can be concluded that glutathione has significantly better antidepressant activity as compared to control in both FST as well as TST. But, significantly better antidepressant activity as compared to amitriptyline is found only in FST. Based on the results of the present study and the fact that oxidative stress has a major role to play in the pathophysiology of depression, glutathione appears to be a promising agent for the treatment of depression. Antidepressant activity of glutathione can be explained by its role in neutralizing reactive oxygen species, role as a neuromodulator and neurotransmitter as well as a regulator of transcription of inflammatory cytokines.

References

1. Publications [Internet]. Who.int. 2020 [cited 10 November 2020]. Available from: https://www.who.int/publications/i/item/depression-global-health-estimates
2. Chaterjee S, Dabholkar H. Let's talk about depression but more needs to be done –lessons from the Jan Man Swastha Program. *J Krishna Inst Med Sci Univ* 2017;6(2):1-2.
3. Mehta CP, Desale AV, Kakrani VA, Bhawalkar JS. Economic dependency and depression in elderly. *J Krishna Inst Med Sci Univ* 2016; 5(1):100-109.
4. Scapagnini G, Davinelli S, Drago F, De Lorenzo A, Oriani G. Antioxidants as antidepressants: fact or fiction? *CNS Drugs* 2012; 26(6):477-490.
5. Behr GA, Moreira JC, Frey BN. Preclinical and clinical evidence of antioxidant effects of antidepressant agents: implications for the pathophysiology of major depressive disorder. Oxid Med Cell Longev 2012; 2012:609421.
6. Freed RD, Hollenhorst CN, Weiduschat N, Mao X, Kang G, Shungu DC, Gabbay V. A pilot study of cortical glutathione in youth with depression. *Psychiatry Res Neuroimaging* 2017; 270:54-60.
7. Maes M, Fisar Z, Medina M, Scapagnini G, Nowak G, Berk M. New drug targets in depression: inflammatory, cell-mediated immune, oxidative and nitrosative stress, mitochondrial, antioxidant, and neuroprogressive pathways. And new drug candidates--Nrf2 activators and GSK-3 inhibitors. *Inflammoparmacology* 2012;20(3):127-50
8. Gawryluk JW, Wang JF, Andreazza AC, Shao L, Young LT. Decreased levels of glutathione, the major brain antioxidant, in post-mortem prefrontal cortex from patients with psychiatric disorders. *Int J Neuropsychopharmacol* 2011; 14(1):123-30.

9. Comprehensive peer-reviewed medical condition, surgery, and clinical procedure articles with symptoms, diagnosis, staging, treatment, drugs and medications, prognosis, follow-up, and pictures. [online] Available at: <https://reference.medhttps://reference.medscape.com/drug/gamma-l-glutamyl-l-cysteinylglycine-gsh-glutathione-344599scape.com/> [Accessed 10 November 2020].
10. Katzung BG, Masters SB, Trevor AJ. Basic and clinical pharmacology. 11th ed. Navi Mumbai: Tata McGraw Hill Education Private Limited; 2009.
11. Ghosh MN. Fundamentals of Experimental Pharmacology. 5th ed. Kolkata: S.K.Ghosh and others; 2011
12. Vogel HG. Drug discovery and evaluation: pharmacological assays. 3rd Ed. New York:Springer; 2008.
13. Gupta SK. Drug screening methods (Preclinical evaluation of new drugs). 2nd Ed. New Delhi:Jaypee Brothers Medical Publishers Limited; 2009.
14. Castagné V, Moser P, Roux S, Porsolt RD. Rodent models of depression: forced swim and tail suspension behavioral despair tests in rats and mice. Curr Protoc Neurosci. 2011 Apr;Chapter 8:Unit 8.10A.
15. Indrayan A, Satyanarayan L. Biostatistics for medical, nursing and pharmacy students. New Delhi: Prentice Hall of India Private Limited; 2006
16. Yan HC, Cao X, Das M, Zhu XH, Gao TM. Behavioral animal models of depression. *Neurosci Bull* 2010;26(4):327-37.
17. Bhattacharya SK, Satyan KS, Ramanathan M. Experimental methods for evaluation of psychotropic agents in rodents: II-Antidepressants. *Indian J Exp Biol* 1999;37(2):117-23
18. Bakunina N, Pariante CM, Zunszain PA. Immune mechanisms linked to depression via oxidative stress and neuroprogression. *Immunology* 2015; 144(3):365-373.
19. Gorlova A, Pavlov D, Zubkov E, Zorkina Y, Inozemtsev A, Morozova A, Chekhonin V. Alteration of oxidativestress markers and behavior of rats in a novel model of depression. *Acta Neurobiol Exp (Wars)* 2019; 79(3):232-237.
20. Herbet M, Korga A, Gawronska-Grzywacz M, Izdebska M, Piatkowska-Chmiel I, Poleszak E, *et al*. Chronic variable stress is responsible for lipid and dna oxidative disorders and activation of oxidative stress response genes in the brain of rats. *Oxid Med Cell Longev* 2017; 2017:7313090.
21. Famitafreshi H, Karimian M. Oxidative stress in the prefrontal cortex as a factor responsible for morphine administration-related depression in rats. *Neurophysiology* 2019;51(4):253-58.
22. Yang L, Zheng L, Wan Y, Chen Z, Li P, Wang Y. Metoprolol, N-Acetylcysteine, and Escitalopram prevents chronic unpredictable mild stress-induced depression by inhibition of endoplasmic reticulum stress. *Front Psychiatry* 2018; 9:696.
23. Gandhi S, Abramov AY. Mechanism of oxidative stress in neurodegeneration. Oxid Med Cell Longev 2012; 2012:428010.
24. Pocernich CB, Butterfield DA. Elevation of glutathione as a therapeutic strategy in Alzheimer disease. *Biochim Biophys Acta* 2012; 1822(5):625-30.
25. Pizzorno J. Glutathione! The Path Ahead. Integr Med (Encinitas) 2014; 13(1):8-12.
26. Aoyama K, Watabe M, Nakaki T. Regulation of neuronal glutathione synthesis. *J Pharmacol Sci* 2008; 108(3):227-38.
27. Janáky R, Ogita K, Pasqualotto BA, Bains JS, Oja SS, Yoneda Y, Shaw CA. Glutathione and signal transduction in the mammalian CNS. *J Neurochem* 1999; 73(3):889-902.
28. Morris G, Anderson G, Dean O, Berk M, Galecki P, Martin-Subero M, Maes M. The glutathione system: a new drug target in neuroimmune disorders. *Mol Neurobiol* 2014; 50(3):1059-84.

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