EFFECTS OF GLUTATHIONE IN REGENERATION OF BLOOD CELLS AFTER ADMINISTRATION OF PHENOBERBITAL IN RATS

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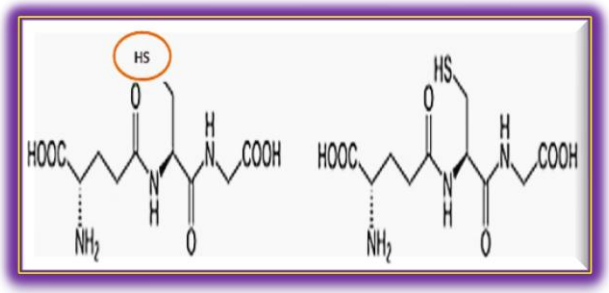
Abstract:

To examine effects of glutathione in regeneration of blood cells after administration of phenobarbital in rats.Investigational drug Glutathione was used as an anti-oxidant drug. Dosage of 30mg/kg body weight of Glutathione was given to GLU group of female Albino Wister rats. Three behavioral studies were examined after administration of glutathione i.e. Open field experiment; particular for kinesis, light & dark experiment; particularly used for depression and home cage experiment; particular for stimulation. These experiments were scored for 5 minutes in 2.5 months. For the determination of the level of regeneration of blood cells, blood sample was collectedby sacrificing animals for hematological estimations by Sysmax (XN-3000 and XN-1000), Cell DYN (Sapphire1-45136AZ, Sapphire2-45197AZ) by Flow Cytometry. Results were carefully compared with control and model groups. Control was untreated group. Model received phenobarbitalas an inducer of dosage 15mg/kg body weight for 15 days and 6mg/kg body weight afterward.Careful observation of results states, locomotion and stimulation, that was gradually reduced after the phenobarbital administration, has steadily but significantly increased when the female Albino Westar were treated with GLU and the depression was markedly disappeared after the treatment in those female rats.On hematology especially on CBC, Glutathione has shown better improvementswhen they were emphasized by comparing with control and model reports.Glutathione has constructive effects on locomotion and stimulation and decrease the level of depression in the body. These activities were depending upon the anti-oxidant concentrations. Glutathione showed better effects on Hematological parameters, especially on CBC.

KEY WORDS: Oxidative stress, Oxidation, Anti-oxidant, Glutathione, Blood Cells, Regeneration

INTRODUCTION

Oxidative stress can be tackled by scavenging the free radicals via the use of strong anti-oxidant. Three types of anti-oxidants are consisted in our body naturally i.e. Catalase, SOD and Glutathione. Structure of Glutathione has a specific group i.e. Sulfhydryl group which gives special properties and increase the importance of glutathione. Glutathione exists in two special forms i.e. GSH and GSSG. GSH is the most important form of glutathione and make it a defensive molecule as well as free radical scavenger(Khannaet al. 2014).



Human body uses Glutathione (GSH) which helps to defend the body against environmental and chemical threats. Between accessibility and requirements a gap of GSH can be established for the concern of lifestyle, diet, aging and diseases(Kurutas 2015)**.** This decreased GSH is associated with definite diseases, containing CVDs and diabetes mellitus and had associated in many others(Lutchmansingh et al. 2018). A direct causative link between low GSH, cellular encumbrances and decreased defenses is supported by excessive biochemical data, in model systems(Viñaet al.1996). GSH as a quantitative indicator of health is consumed by developing personalized health approaches, with the anticipation that diet choice, GSH supplementation, and lifestyle tactics can be exploited to deal GSH status, thus by defending against the development of diseases, GSH provide a health surplus(Jones 2011)**.**Glutathione is usually defined as, the most important intracellular non-enzymatic anti-oxidant. This is a tri-peptide comprised of cysteine, glutamic acid and glycine (Alanazi et al. 2015).The chemical formula of glutathione is **$C_{10}H_{17}N_3O_6S$** (Görbitz 1987).

In recent researches, it is discovered that extracellular GSH is responsible in certain conditions significantly; respiratory,patho-physiology and other functions of GSH other than antioxidant and even pro-oxidant roles are discovered(Lushchak 2012).GSH has attained a great value as a defensive molecule in the body of human being and it is one of the vital molecules which fascinate the scientist's attention toward it and over 100 years of research and about 8100 scientific papers had been published to describe immune-efficient property of GSH (Gaucher et al. 2018). Previously, it was mentioned by many scientists that lower GSH level influences the nervous system, kidney, lungs, lever, heart, pancreas, GIT, skin, hearing, vision and cause many other infectious diseases (Townsend et al. 2003). It is also shown earlier that a gap between the

GSH line of defense and the levels required for ideal fitness is due to the poor and improper intake of diet which leads toward age related diseases(Singh2002)**.**GSH is an endogenous antioxidant and most of foods consist of less or more amounts of GSH(Piste 2013). The natural sources of GSH are fresh fruits, vegetables and nuts. Asparagus, avocados, walnuts, tomatoes and oranges are various most common nutrients which help to boost up the levels of GSH in the body(Sonthalia et al. 2016). Protein is an additional rich cradle for GSH and has been used to increase GSH levels in cystic fibrosis (Grey et al. 2003).Present study has established to test the GSHas an anti-oxidant drug as to have beneficial role for the purpose of regeneration of blood cells in rats.

EXPERIMENTAL PROTOCOL

From animal house of Research Institute of Agha Khan University, Karachi Pakistan,locally bred female Albino Wister rats; weighedabout 150gm to 250gm on arrival, were purchased that were used throughout the experiment. Those rats were lodged alone in home cages with saw-dustbeddingin a quiet room. Before starting the experiment,rats were allowed a free access to cubes of standard rats' food and water for almost 3 to 4 days, so that the rats could train themselves to the new environment.Capsules of Glutathione (30mg) were purchased from a local Medical Store, Karachi, Pakistan. Phenobarbital (30mg) of Venus Pharma was purchased from medical store. Rats' cages were purchased from Saddar Market, Karachi.

Dosage ToThe Rats;A total of 18 rats were purchased. Rats were divided in 3 groups (6 rats/group), named as; Control, Model and GLU group.

Control Group; Control group was given water and food throughout the bench work. After a week various behavioral experiments had performed to observe the stimulation, locomotion and depression activities.

Model Group; Model group was given 15mg/kg body weight of Phenobarbital for 15 days. And 6mg/kg body weight afterward. After a week, various behavioral experiments had performed to observethestimulation, locomotion and depression activities.

Treated Group; Dosage of 30mg/kg body weight of GSH was given to the GLU group. GSH was dissolved in deionized water. Rats were given water extract ofGSH orally. After a week, various behavioral experiments were performed to observe the stimulation, locomotion and depression activities.

Behavioral Techniques

Open Field Apparatus;Theapparatus used in this experiment, consists of squares of (76x76cm) with walls of height 42cm. The floor has divided into 25 equal squares. The experiment hadperformed under day light in a room of quiet environment to avoid noise effects. This activitywasscored for 5 minutes.

Light and Dark Box Apparatus; Thisexperiment is commonly performed to measured expression. The apparatus used in light and dark experimentation is comprised of two square-shaped boxes of area (26cm each side) with an access of (12x12cm). Walls of a box are transparent and that of second box are colored black. The experiment was performed inday light in a room of quiet environment to avoid noise effects; observations were taken by noticing the time taken by the rats to remain in the light within 5 minutes.

Home Cage Activity; Specially designed home cagesof (26cm each side) and the floor Covered with Saw Dust is used normallyin this experiment. After a week of GSH administration,activity from control, model andtest group was noted down for 5 minutes.

Decapitation And Blood Sampling; Rats were sacrificed after 10 weeksby specially designed instrument for guillotine. Blood sample was taken within 60 seconds of decapitation.

Clinical Analysis; The blood samples were carried out to the **DOW LAB** Diagnostic Reference & Research Laboratory for the Analysis of Blood Cell markers.In hematology, CP was performed in which Hb, RBC, Platelet count and WBC is included. All the clinical analysis was done by KIT method. All hematological tests of CP were performed on "Sysmax (XN-3000 and XN-1000), Cell DYN (Sapphire1-45136AZ, Sapphire2-45197AZ)" by Flow Cytometry.

Statistical Analysis; All the investigational outcomes has stated as means ± SD. Statistical Investigation of variance has performed by ANOVA. The results with$P<0.1$, $P< 0.05$ and $P<0.01$ have found to be statistically significant. Clinically analyzed results and behavioral observations taken, have statistically evaluated using SPSS version 23.

RESULTS

Effects of PHB andGSH on Locomotion and stimulation are given in figure 1 and 3 and depression in figure 2. These results show that after 2.5 months of GSHtreatment, motor activity and stimulatory activityhad significantly and prominently increased in GLU group as compare to model group and near to control group showing that the locomotion and stimulatory activity which had decreased in model group had considerably increased in almost all cured rats and model group had spent more time in light portion but treated group's entries in light portion were markedly decreased as compare to model group and the results are near to control group's

readings showing that the depression which had caused in model group has significantly and prominently decreased in almost all treated rats and near to control group of rats and the effects on Hematological Parameters are given in figure 4 showing hematological analysis has prominently come out to be normal in GLU group as compare to Model group and near to Control group, shows that hematological parameters which became agitated in model group of rats had significantly gone near to or equal to control group's values in almost all treated rats.

BEHAVIORAL TECHNIQUE

Figure 1:- Effects Of Phenobarbital and Glutathione On Locomotion

Figure 1 shows that values are means (n= 6/group).
Control group is compared with model group and GLU group; values of model Group are significantlydecreased as compare to control group and P< 0.0001 Showing decreasedlevel of significance (i.e. trouble in locomotion) in a group of Rats thatconsumedPHB and values of GLU group are significantly increased as Compare to Modelgroup and near to Control group. The P-value is gradually Increasedas P< 0.01 in1st month, P< 0.05 in 2nd month and P< 0.1 during 3rd month Oftreatment showing gradual increment in significance level of locomotion in Agroup of rats that consumed GSH.

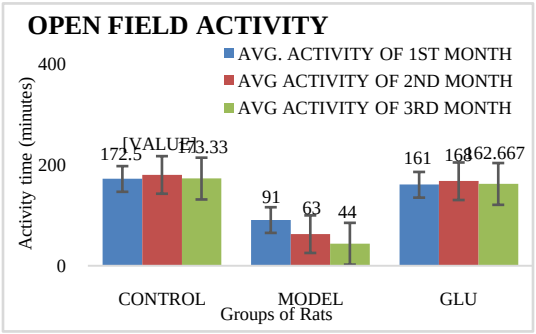


Figure 2:- Effects Of Phenobarbital and Glutathione On Depression

Figure 2 shows that values are means (n= 6/group).
Control group is compared with model group and GLU group,values Ofmodel group are significantly increased as compare tocontrol Group and P< 0.001 showing increased level of anxiety ina group of Rats that consumed PHB and values of GLU group aresignificantly Decreased as compare to model group and near to that ofcontrol Group and the P-value is gradually decreased as P< 0.1 in1st month, P< 0.1 in 2nd month and P< 0.05 during 3rd month of treatmentshowing Gradual decrement in significance level of anxiety in a group of rats that Consumed GSH.

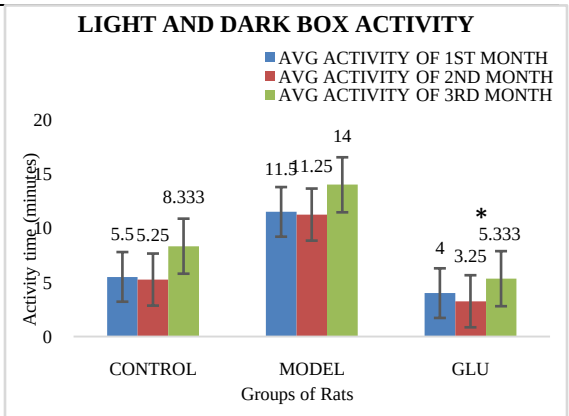
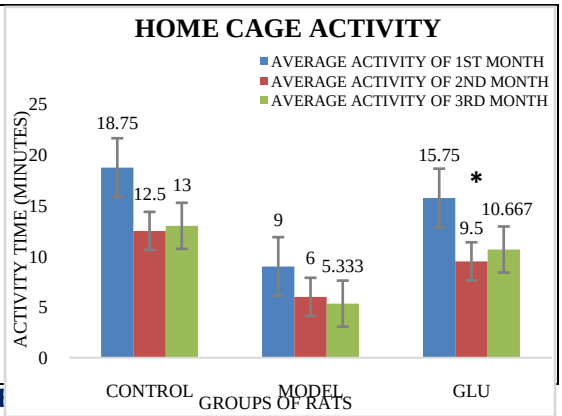


Figure 3:- Effects Of Phenobarbital and Glutathione On Stimulation

Figure 3 shows that values are means (n= 6/group).
Control group is compared with model group and GLU group,
Values of model group are significantly decreased as compare to control group and P< 0.001 showing decreased level of significance of stimulatory activity in a group of rats that consumed PHB and the values of GLU group are significantly increased as compare to model group and near to that of control group and the P-value is gradually increasedas P< 0.1 in 1st month, P< 0.05 in 2nd month and P< 0.05 during 3rd monthof treatment showing gradual increment in significance level



of stimulatoryactivity in a group of rats that consumed GSH.

HEMATOLOGICAL PARAMETERS

Figure 4:- Effects Of Phenobarbital and Glutathione On CBC

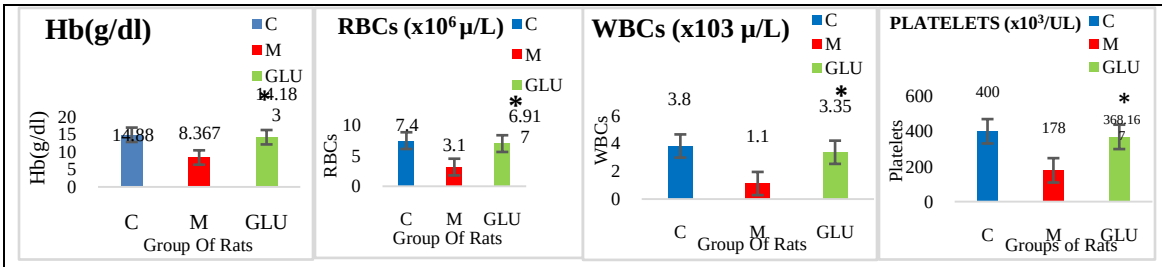


Figure 4 shows that values are mean (n= 6/group). Control group is compared with model group and GLU group, values of Model group are significantly disturbed as compare to Control group and P-value is showing agitated level of significance of hematological parameters in a group of rats that consumed PHB and the values of GLU group are significantly come out to be normal as compare to Model group and near to that of Control group and P-value is showing stabilized level of significance of hematological parameters in a group of rats that consumed GSH.

DISCUSSION

The findings from this nutraceutical research are based on regeneration of blood cells through GSH.Behavioral activities were done by performing different types of activities. Locomotion and stimulation, that had gradually decreased after PHB administration in 1st month, 2nd month and 3rd month i.e. Locomotion = (91 ± 3.464, 63 ± 19.201 and 44 ± 18.735) respectively and Stimulation = (9 ± 1.826, 6 ± 1.826 and 5.333 ± 1.528) respectively, has gradually but significantly increased in all 2.5 months when the female Albino Westar were treated with GLU i.e. Locomotion = (161 ± 0.817, 168 ± 1.826 and 162.667 ± 4.042) respectively and Stimulation = (15.75 ± 1.708, 9.5 ± 1.291 and 10.667 ± 0.577) respectively but the depression that was elevated after the administration of PHBin 1st month, 2nd month and 3rd month i.e. (11.5 ± 1.291, 11.25 ± 0.957 and 14 ± 1) respectively has markedly disappearedafter the treatment with GLU i.e. (4 ± 0.817, 3.25 ± 1.258 and 5.333 ± 1.528) respectively in those female rats. These activities weredepending on the anti-oxidant activity of GSH as ithas positive effects on locomotion, stimulation, depression, all sulk behaviors and all other body's functions related to the relaxation by increasing the level of locomotion and stimulation and decreasing the level of depression and anxiety as it reduces oxidative stress and neutralize the free radicals that actually cause oxidative stress. This drug enhances the level of estrogen which in turn decreases the levels of depression(Archer 1999).Before acting as anti-oxidant, glutathione is existed in reduced form

called GSH. When it performs its action on free radical it is converted in oxidized form GSSG. The status of oxidative stress of cell can be estimated by measuring GSH/GSSG ratio of a cell or organ (Owen and Butterfield 2010). The enzymes which are involved in this processare glutathione S-transferases(GSTs). It transfers a pair of electron from GSH to free radical. Free radical accepts electron pair as it is electrophilic molecule and becomes neutral. This process is actually the phenomena of anti-oxidation (Hayes et al. 2005). One more classical function of GSH as antioxidant is that it alters protein functions by a process called glutathione ylation(Klatt and Lamas 2000).GSH also conjugates with phase II enzymes targets and alter their structure. This helps in the removal of those targets from the body. This action is like detoxification process(Pastore et al. 2003).Through these mode of actions,GSH exerts most significant effects on different organs(Van 2000). Such as liver, kidney and Blood like Hemoglobin, RBCs, WBCs and Platelets i.e. (Hb. = 14.183 ± 0.355, RBCs = 6.917 ± 0.194, WBCs = 3.35 ± 0.187 and Platelet count = 368.167 ± 4.708) respectively.

CONCLUSION

In conclusion, prolonged use of Glutathione potentiates anti-oxidant system of the body and increases the regeneration of various organs of the body specially blood cells.Hence, in hematological disorders GSHcan play a role as a potent treatment.GSH is significantly effective for several health conditions. Hence in future, it is highly suggested by current studies that Glutathione can be used by medicinal practitioners.

Conflict of interest

The authors declare no conflict of interest.

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