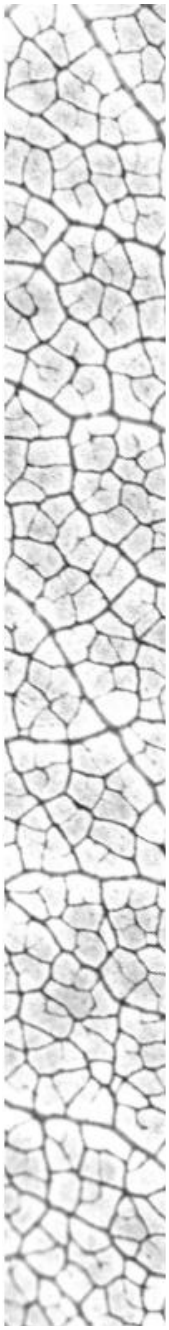

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The effects of oral dosage of glutathione and some biological agents on immunity and oxidative stress parameters in male rats induced with immunosuppression

Zahraa D. Abdel-Hamid [1], Karkaz M. Thalij [1*]

[1] Department of Food Science, Tikrit University, Tikrit, IRAQ
*Corresponding author: kthalij@gmail.com

Abstract

Objective: The research was aimed to determine the effects of glutathione extracts and some biological agents on immune and oxidative stress parameters in male rats induced Immunosuppression used Sandimmune. **Methods:** Glutathione was extract from spinach leaves and estimated the concentration used a high-performance liquid chromatography (HPLC) and determined the effect of orally dosage at one mg/ml alone or with both Zinc or Vit C or cell concentration of Lactobacillus plantarum (CCLP) on the immunity and biological parameters of male rats induced Immunosuppression used Sandimmune and breeding for 28 days. **Results:** The results indicated that aqueous spinach extract was contained glutathione at a concentration of 246 µg/g in the case of extraction using aqueous extract. The Immunosuppression induced was significantly ($p<0.05$) decreased of IgA, IgG and IgM values and became at 1022, 2031 and 121.5 mg/dl respectively compared with the control rats group which at 1564, 3206 and 174.7 mg/dl respectively while the orally dosage from biological parameters was caused in amelioration of immunity parameters to became similar of values with the control rats group. Also the oxidative stress parameters value as GSH, GPx, SOD and catalase enzyme were significantly decreased to 222, 0.17, 375 and 0.31µmol/L respectively and increased in MDA value to 4.6 µmol/L compared with the rats in control group, and the used of glutathione alone or with the biological parameters were done improvement to similar values in control group. **Conclusion:** It was concluded that oral dosage of glutathione alone or with biological agents was significantly effective in improving immunity and decreasing the oxidative stress values in the laboratory rats that induced immunosuppression.

Keywords: glutathione, biological agents, sandimmune, oxidative stress, male rats

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INTRODUCTION

Oxidative stress was defined as increasing the oxygen radicals' level in cells causing defect in cell membrane and damaging the cells in all body organs, which leading some diseases, such as Alzheimer's, diabetes or cancer (Martínez-Holguín, et al. 2020). The level of oxygen in the cell caused in improved the metabolism pathways, but its levels were increasing up normal limits may be caused the oxidative stress in body cells (Breitkreutz, et al. 2000. Kasperczyk, et al. 2013). the oxidative stress occurs in the cells of the organism due to the influence of multiple agents, most of them are pollutants from water or food pollutions. As well as herbicides and some heavy metals and drugs (Wang, et al. 2017) Glutathione is the most effective of the antioxidants, which was consists of three-molecule peptide which was γ-glutamyl-cysteinyl-glycine amino acids (Ballatori, et al. 2009). Which is distinguished in the presence of the peptide bond γ between the carboxyl group of the amino acid glutamate and the amine group of the cysteine, and this group is what distinguishes it from other proteins (Nocttor Get al. 2011). Its antioxidant effect occurs through its association with free electrons that cause oxidative stress, as well as its effectiveness in removing toxins produced in cells in high-end organisms, especially humans (Wang, & Ballatori, 1998; Obinna-Echem, & Chukunda, 2018). It also have relation and capable to protection against various infections (Testa, et al. 2001). Glutathione is obtained from two main sources, one of which is internal which is made in the cells, especially the liver cells, and approximately

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25% of it is found in the liver alone, it is also present in lower concentrations in the kidneys and in the secretions of the mucous membrane of the intestine and lungs, in addition to that it is found inside the cells or in extracellular fluids. The external source is obtained from foods rich in it, such as in spinach, tomatoes, carrots, broccoli, meat and spices such as curry and turmeric (Allen, & Bradley, 2011). Spinach is considered to be one of the most potent glutathione content, its quantity ranges from 166 mg/kg of fresh spinach, and in cooked its at 27.1 mg/kg (genannt Bonsmann, et al. 2008). Glutathione increases its potency as an antioxidant when present with immune-enhancing substances such as vitamin C and zinc, whose effect is to increase the effectiveness of antioxidant pathways as well as therapeutic enhancers, as in the bacterial species of L. plantarum, which has capable to production some agents effects as antimicrobial and antioxidants (Lin, et al. 2018). The goal of this study was to extract and estimate the amount of glutathione in the local spinach using a HPLC technique, and determine its effect after given orally on the immunity and biological parameters in laboratory male rats induced immunosuppressive using Sandimmune after breeding for 28 days.

MATERIAL AND METHODS

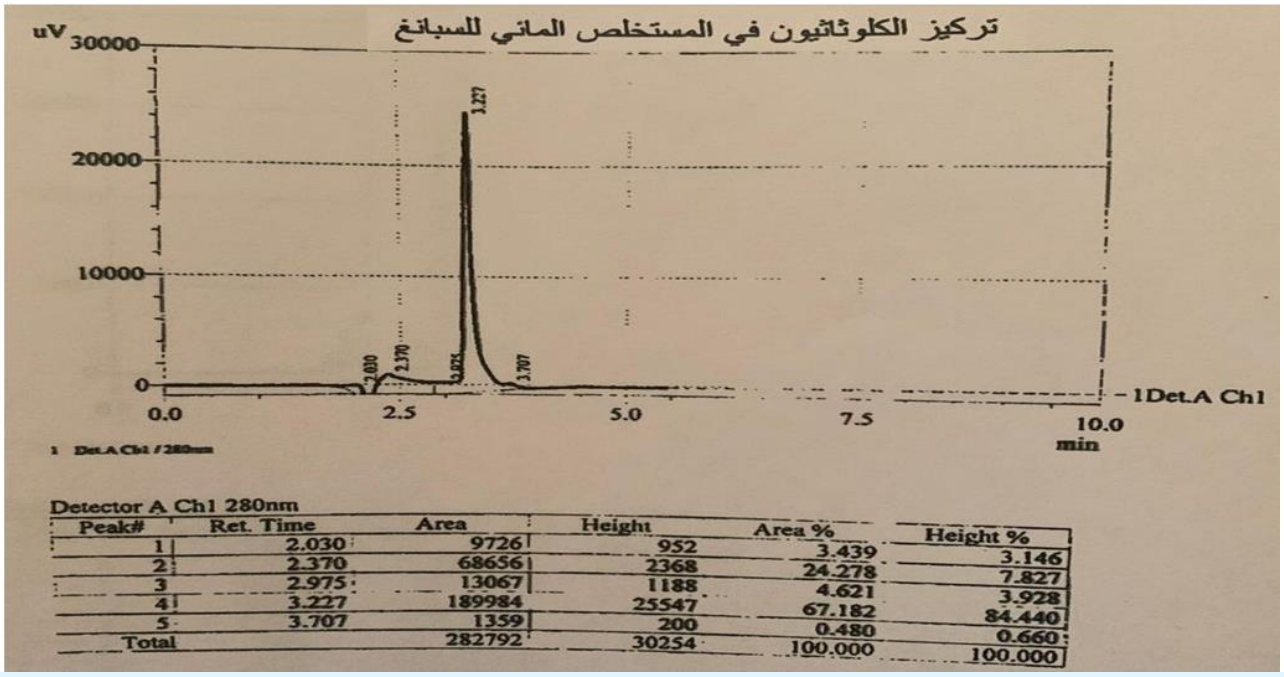
Determination of glutathione concentration in Spinach leafs: Spinach leaf samples were collected from the local markets of Baghdad city, and then grounded using the electric mill machine (Sony, Japan) to obtain fine powder to be ready for aqueous extraction as followed in .(Muryanto, et al. 2017, November). Glutathione in Spinach leaf extract was estimated using HPLC system (HPLC, LC-10-Shimadzu-Japan), it was used the pump injection type 4015, were injected 20 µl of the extract of samples at the rate time of 0.8 mL/min, used C8 separation column, with mobile phase consisting of phosphate buffer at 2.5 pH and acetonitrile as stationary phase. and UV/Visible detector at a wavelength of 280 nm (Tahir, Khan, Shah, & Aftab, 2016). unknowns extract samples were compared with the standard solution of glutathione which provided by Sigma Company (USA).

Bacterial cell concentrate preparation: Bacterial cells of L. plantarum cultivation anaerobically on the MRS agar was used to prepared the bacterial cell concentration, through compared the bacterial cells broth in peptone with a concentration of 0.1 concentrate of McFarland tubes to obtain bacterial accounts at 1 x 10^6 cells/ml of then centrifuging the cells at a 5,000-cycles for 15 minutes. the concentrate of 25 mg was used to orally dosage for each animal/day.

Glutathione and biological factor preparations: Glutathione which obtained from (Sunpure company, USA), was prepared by dissolved in distilled water to obtain 1 mg of glutathione/ ml which orally dosage for each animal daily. The aqueous extract of glutathione from Spinach leaf was used at the similar concentrate. Zinc and vitamin C also were prepared by dissolved in distilled water to obtain a concentration of 0.1 mg and orally dosage for each animal daily. The dosage concentrates were separated equally at each 12 hours. The same concentrations were used when mixed the glutathione with each of biological agents used in the experiment. Sandimmune was added to the distilled water to obtain the daily orally dosage concentrate at 0.3 µg/ gram of animals which consecutive for 7 days, and the dosage determination according to LD_{50} experiment on for laboratory rats.

Initialization of laboratory animals: In this study, 120 adult albino male rats, with a weight of 195-198 g, were obtained from the Biotechnology Research Center - Al-Nahrain University. The animals were randomly separated into twelve groups, with ten animals per group. The solutions were given through oral dosage as in the following: 1) Control animals group, 2) Group of animals given glutathione extract at a concentration of 1mg/ml, 3) The group of animals given standard glutathione at a concentration of 1 mg/ml, 4) The group of animals given zinc at a concentration of 0.1 mg/g body weight per animal/day, 5) The group of animals given Vit.C at a concentration of 0.1 mg/g body weight per animal/day, 6) Group of animals given L.plantarum extract at a concentration of 25 mg/animal/day, 7) The group of animals given the glutathione extract with Zinc at the above concentrations, 8) A group of animals given the glutathione extract with Vit.C in the mentioned concentrations, 9) A group of animals given the extract of glutathione with L.plantarum extract in the above concentrations. 10) A group of animals given glutathione with Zinc in the above concentrations, 11) A group of animals given glutathione with Vit.C at the above concentrations, 12) A group of animals given glutathione with L.plantarum extract at the above-mentioned concentrations. The biological experiment was designed to be in three stages, the first as control stage or pre-orally in which the animals were placed for 7 days under observation, blood was drawn on the eighth day and considered as control (without dosage or treatment), The second stage, consist to oral dosage of rats from 0.3 ml of Sandimmune, daily for a period of 7 days. While the third stage (Treatments) was consist to orally dosage from standard or extract of glutathione alone or with each biological agent which continue for 28 days. Finally, in each the end of breeding periods the blood was take in EDTA tubes to obtain the blood serum for tests the immunity and oxidation stress parameters.

Parameters assay: At the end of the experiment period, the animals were anesthetized used the chloroform, then blood samples were drawn directly from the heart using a Cardiac puncture. The blood was placed in tubes that do not contain anticoagulants to estimate immunoglobulins IgG, IgM, IgA, using ELISA



Detector A Ch1 280nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.030	9726	952	3.439	3.146
2	2.370	68656	2368	24.278	7.827
3	2.975	13067	1188	4.621	3.928
4	3.227	189984	25547	67.182	84.440
5	3.707	1359	200	0.480	0.660
Total		282792	30254	100.000	100.000

Fig. 1. Glutathione concentration in Spanish leaf equeous extract estamited with HPLC

device according to Tietz et al, (2005), Tietz (2005). Biological parameters of oxidation indicators such as Catalase, Gpx, GPX, MDA, and SOD were estimated using the color method according to the manufacturer's instructions where the concentration of glutathione in the blood serum was measured according to the Ellman method as reported in (Nasif, 2002). The focus of Malondialdehyde (MDA) was done according to the procedure in (Omodeo-Salè, et al. 1998). The experiment was carried out under the Complete Randomized Design (CRD), and the ionization analysis was performed using the General Linear Model within the ready statistical program (SAS, 2012). The Duncan (1955) test was used to determine the significance of the differences if they existed between different averages at a probability level of 0.05.

RESULTS

Glutathione concentration in Spinach leaf aqueous extract samples: The results in **Fig. 1** was illustrated the concentration of glutathione in aqueous extract of the spinach leaf samples, which was found at 246 µg/g of spinach leaf. the results were indicate that spinach leaf has high concentrate of glutathione compare with the other plant sources, and this amounts were agreement with (Dewanjeeet al. 2015). whom founds that the effect of water in extraction was caused in increased the polarity of the extracting solution, that results in it to produce the precipitation of glutathione from the other compounds in the aqueous extract.

Effect of oral dosage of glutathione and some biological agents on the immunoglobulins in male rats induced immunosuppression.

Table 1 was illustrated the efficacy of oral dosage of standard or extract glutathione from spinach alone or with each zinc or vitamin C or CCLP in the mean values of immunoglobulins of types IgA, IgG and IgM in male rats induced with immunosuppression used Sandimmune. The results, found that the values of immunoglobulin IgA, IgG and IgM were significantly decreased ($p<0.05$) in the serum of rats group that induced immunosuppression and became at 1022, 2031 and 121.5 mg/dL, respectively, compared to their values in the control group that was at 1564, 3206, and 174.7 mg/dL, respectively. The treatment status using both standard glutathione or its extract from spinach alone or with each biological agents mentioned above has caused an increased in IgA values and became between 1072 and 1201 mg/dL, and for the values of IgG, increased significantly to become at ranges between 2574 to 2835 mg/dL, while the values of IgM had their values between 130.8 to 161.2 mg/dL. The use of both standard glutathione or spinach extract for each of them with each of the biological agents has caused a significant improvement in immunological parameters of IgA, IgG and IgM species and has reached similarities with the immunological values in serum of laboratory rats from control group. The results were consistent with previous studies in the occurrence of differences in levels of immunoglobulins from the natural limit (Douer, Chaimoff, Pick, & Pinkhas1981. Franco, Panayiotidis, & Cidlowski, 2007). When comparing the levels of immunoglobulins in patients with the control group, it showed that IgA and IgM of the patients group had values lower than control group. It has also been reported by (Oborilová, et al. 2004). that found happen

Table 1. Efficacy of oral dosage of the glutathione and some biological agents on immunoglobulin parameters in male rats induced immunosuppressive

Treatments	IgA	IgG	IgM
		(mg/dL)	
Control	1564a±13.6	3206a±13.86	174.7a±5.54
Sandimmune treatment	1022f±7.82	2031f±18.55	121.5f±4.49
After treatment			
Standard glutathione	1187d±11.34	2782d±17.70	145.7d±4.36
Glutathione extract	1201d±9.74	2835d±16.37	147.0d±4.33
Zinc	1072e±10.59	2650e±14.54	137.7e±5.82
Vit C.	1085e±14.69	2574e±20.66	130.8e±4.60
L. plantarum Cell Conc.	1176d±12.53	2682e±18.22	161.2c±5.21
Std. glutathione + Zinc	1480b±12.27	3125b±13.75	169.8a±6.93
Std. Glutathione + Vit C.	1403b±16.38	3172b±19.82	167.5b±4.52
Std. Glutathione + L. plantarum	1461b±15.60	3176b±15.44	170.0a±4.80
Glutathione Extract + Zinc	1478b±13.48	3150b±18.58	170.8a±3.73
Glutathione Ext. + Vit C.	1379c±15.47	3086c±22.16	168.1b±2.68
Glutathione Ext. + L. plantarum	1383c±14.55	3052c±17.48	171.0a±4.58

*Different letters on the averages in one column indicate significant differences at probability 0.05.
Means values were taken from five replicates. ± stands for standard error, Std Err.

Table 2. Effectiveness of Oral Dosage of Glutathione and Some Biological Agents on Some Oxidation Evidence in male rats induced immunosuppression.

Treatments	MDA	GSH	GPx	SOD	Catalase
			(µmol/L)		
Control	3.1b±0.03	591d±14.47	0.43c±0.03	2397a±25.4	0.74a±0.04
Sandimmune treatment	4.6a±0.05	222a±12.52	0.17d±0.05	375e±4.38	0.31d±0.01
After treatment					
Standard glutathione	3.8a±0.04	572e±16.36	0.49b±0.02	1350d±15.8	0.53c±0.02
Glutathione extract	3.7a±0.03	567e±6.20	0.44c±0.03	1260d±18.6	0.55c±0.03
Zinc	4.1a±0.06	425h±11.50	0.62a±0.04	1150d±12.2	0.42c±0.01
Vitamin C.	3.9a±0.02	435h±9.73	0.59a±0.03	1100d±15.4	0.44c±0.03
L.plantarum Cell Conc.	3.7a±0.04	505g±10.64	0.48b±0.01	2050c±26.5	0.49c±0.03
Standard glutathione + Zinc	3.3±0.02	669a±7.83	0.43c±0.01	2250b±27.8	0.65b±0.05
Standard Glutathione + Vitamin C.	3.5b±0.03	657b±6.72	0.44c±0.03	2274b±22.9	0.65b±0.05
Standard Glutathione + L.plantarum	3.3b±0.01	610c±8.94	0.43c±0.04	2293b±14.7	0.66b±0.06
Glutathione Extract + Zinc	3.1b±0.07	681a±13.51	0.42c±0.01	2310b±24.4	0.64b±0.05
Glutathione Extract + Vitamin C.	3.3b±0.05	685a±8.32	0.45bc±0.04	2226b±15.3	0.65b±0.06
Glutathione Extract + L.plantarum	3.2b±0.02	602c±11.29	0.42c±0.01	2314b±26.5	0.70a±0.06

*Different letters on the averages in one column indicate significant differences at probability 0.05.
Means values were taken from five replicates. ± stands for standard error, Std Err.

lower values of IgM, IgA, and IgG often indicate the incidence of immunotoxicity of cells in the body. Reduced immunity values of IgM, IgA, IgG in the case of oral dosage of Sandimmune, which consists of Cyclosporine produced from the fungus Tolypocladium inflatum and its effectiveness in binding with external receptors of lymphocytes called cyclophilin in cells, Cytokine production inhibition, which causes a decrease in the formation of interleukin 2 (IL2) and 4 (IL4), as well as the tumor necrosis factor (TNF) alpha interferon and interferon gamma, all of which were decrease the effectiveness of lymphocytes (Advani, Horwitz, Zelenetz, & Horning, 2007).

The concentration used for each substance is as follows: Sandimmune (0.3µl/g of BW), standard or extracted glutathione at 1 (mg/animal/day), Zinc and vitamin C (0.1 mg/g animal weight/day), L.plantarum cell concentration (25 mg/animal/day).

Effectiveness of oral dosage of glutathione and some biological agents at the level of some oxidative indications in male rats induced immunosuppression.

The efficacy of oral dosage of glutathione extract from spinach alone or with both zinc or Vit C or CCLP in the values of some oxidation indices in male rats induced immunosuppression used Sandimmune was shown in **Table 2**. The results show that the values of some oxidative stress parameters in male rats were significantly affected ($p<0.05$). the Malondialdehyde (MDA), which is an indication of the oxidative stress of fats in body cells, was increased significantly in the group of immunocompromised animals, and became at 4.6 µmol/l compared to their value in the control group rats which was at 3.1 µmol/l. Treatment with both glutathione or the biological agents of zinc, vitamin C or CCLP were reduced the MDA rates to a significantly nearer level of their value in the control group animals. It was appear between 3.7 to 4.1 µmol/l. in the case of treatment with both types of standard glutathione or that extracted from spinach with each of the biological agents, the MDA value improved significantly due to its similarity with its value in the control group animals, as it was between 3.1 to 3.5 µmol/l.

As for glutathione, which is a non-enzymatic antioxidant, there was a significant decrease in its values in the group of immunocompromised animals, which was at 222 µmol/l compared to its value in the control group rat group which was at 591 µmol/l. Oral dosage of standard glutathione or glutathione extract to treat immunocompromised animals has caused the values of glutathione in their blood to be significantly

returned to levels close to their value in control group animals and were at 572 and 567 µmol/l respectively. As for the treatment with biological agents such as zinc, vitamin C and CCLP alone, it improved the values of glutathione in the serum of these animals to levels that did not reach with them in their control group. It was at 425, 435, and 505 µmol/l, respectively, which indicates that these biological agents, when used alone to reduce oxidative stress, are less effective than antagonists than glutathione. As for treatment by oral dosage of each of them with both standard glutathione or glutathione extract from spinach, the values of glutathione in the blood serum of rats increased from levels in the control group and was between 602 to 685 µmol/l. Glutathione peroxidase (GPx) is an important enzyme that acts as an anti-oxidant in body cells, its value was demonstrated in the serum of the control group of laboratory rats at 0.43 µmol/l. The case of experimental immunosuppression using Sandimmune caused its value to be reduced to 0.17 µmol/l, for use in eliminating oxidative stress. The use of standard glutathione or its extract from spinach or the biological agents mentioned above are all separate or glutathione with each of the agents Bio has significantly improved the values of GPs enzyme to similar levels or more significantly and was between 0.42 to 0.69 µmol/l, compared to its values in control group animals.

The induced immunosuppression case for male rats was caused the Superoxide dismutase enzyme activity values to drop to very significantly sluggish levels, and it was became at 375 µmol/l, compared to its value in the serum of control group animals that was at 2397 µmol/l. Oral dosage of treatment with both standard glutathione or spinach extract glutathione with each of the biological agents mentioned above has caused significant improvement in enzyme activity values that were between 2226 to 2314 µmol/l. Likewise in the activity of Catalase, the group of animals whose immunosuppression induced with Sandimmune was caused a significant decrease in the activity of catalase enzyme to the level of 0.31 µmol/l compared to its effectiveness in the control group animals that was at 0.74 µmol/l. The case of oral dosage to reach treatment from immunological decline and oxidative stress in both glutathione or biological agents caused significant improvement in enzyme efficacy and became between 0.42 to 0.55 µmol/l.

The improvement in enzyme activity in the case of oral dosage of both types of glutathione with each of the biological agents was highly significant and its effectiveness values became between 0.64 to 0.70 µmol/l.

The concentration used for each substance is as follows: Sandimmune (0.3µl/g of BW), standard or extracted clotathione at 1 (mg/animal/day), Zinc and vitamin C (0.1 mg/g animal weight/day), L.plantarum cell concentration (25 mg/animal/day).

The case of immunosuppression using Sandimmune, which is a Cyclosporine compound consisting of a cyclic polypeptide, causes reduced immunity and increases oxidative stress by producing free radicals and accumulating in the cells of the organism. Free radicals are unstable because they contain one or more Unpaired electron which makes them very interacting with other compounds in the cell. The effectiveness of these free radicals is through stripping the cell compounds of proteins, fats, and nucleic acids from their electrons to stabilize those roots, and for these compounds to become free radicals in themselves, causing a chain of reactions with a harmful effect that is in multiple cases including cell death or mutagenesis or Cancer cases in it. The body destroys free radicals and gets rid of them with certain mechanisms, but when this does not happen sufficiently, their accumulation in the body generates the phenomenon of oxidative stress. And oxidative stress plays a major role in the development of chronic diseases such as aging, cancer, cardiovascular disease, neurological diseases such as Alzheimer's, asthma and chronic obstructive pulmonary disease, rheumatoid arthritis, kidney disease, age-related macular degeneration. The body contains many mechanisms to counteract oxidative stress by producing antioxidants, which are enzymatic or non-enzymatic (Whaley-Connell, McCullough, & Sowers, (2019). The results were consistent with (Saleh, Edeas, & Van Goor, 2019. Islam, et al. 2017), whom found a significant increase in the value of MDA and a significant decrease in the activity values of SOD and CAT enzymes in rats treated with oxidative stress substances. The results were also consistent with what was mentioned by (Wood, S2020). In the resistance of antioxidant biomaterials to cases of oxidative stress of metabolic activities and free radicals oxidized in animals, and the main antagonistic enzyme in them was GSH as well as cyclathione and ascorbic acid (Sonani, Rastogi, & Madamwar, 2015. Dröge, & Breitkreutz, 2000). Oxidative stress is a state of stress resulting from a high oxidative activity of free radicals on cells in the body of the organism. Reduced or eliminated by antioxidants that contain multiple compounds of the enzyme types Superoxide dismutase (SOD), Catalase (CT) and Glutathione peroxidase (GPx), Non-enzymatic glutathione and vitamins, phenols, etc., which are effective in converting free radicals to H_2O and O_2 and safe on body cells (Sumalatha, 2013).

CONCLUSIONS

Kidney efficacy of immunoglobulin values IgA, IgG and IgM, were improved in the case of oral dosage of glutathione. also affected the moral shipment of and oxidation criteria values for MDA, GSH, GPx, SOD and Catalase in the male rats.

REFERENCES

Advani, R., Horwitz, S., Zelenetz, A., & Horning, S. J. (2007). Angioimmunoblastic T cell lymphoma: treatment experience with cyclosporine. *Leukemia & lymphoma*, *48*(3), 521-525.

Allen, J., & Bradley, R. D. (2011). Effects of oral glutathione supplementation on systemic oxidative stress biomarkers in human volunteers. *The Journal of Alternative and Complementary Medicine*, *17*(9), 827-833.

Ballatori, N., Krance, S. M., Notenboom, S., Shi, S., Tieu, K., & Hammond, C. L. (2009). Glutathione dysregulation and the etiology and progression of human diseases. *Biological chemistry*, *390*(3), 191-214.

Breitkreutz, R., Pittack, N., Nebe, C., Schuster, D., Brust, J., Beichert, M., ... & Dröge, W. (2000). Improvement of immune functions in HIV infection by sulfur supplementation: two randomized trials. *Journal of Molecular Medicine*, *78*(1), 55-62.

Dewanjee, S., Dua, T. K., Khanra, R., Das, S., Barma, S., Joardar, S., ... & Jaafar, H. Z. (2015). Water spinach, Ipomoea aquatic (Convolvulaceae), ameliorates lead toxicity by inhibiting oxidative stress and apoptosis. *PloS one*, *10*(10), e0139831.

Douer, D., Chaimoff, C., Pick, A. I., & Pinkhas, J. (1981). Serum immunoglobulin levels in splenectomized Hodgkin patients and in subjects following post-traumatic splenectomy. *Oncology*, *38*(3), 165-167.

Dröge, W., & Breitkreutz, R. (2000). Glutathione and immune function. *Proceedings of the Nutrition Society*, *59*(4), 595-600.

Duncan, B., (1955). A methodological Analysis of Segregation Indexes. American Sociological Review, 20(2): 210-217.

Franco, R., Panayiotidis, M. I., & Cidlowski, J. A. (2007). Glutathione depletion is necessary for apoptosis in lymphoid cells independent of reactive oxygen species formation. *Journal of Biological Chemistry*, *282*(42), 30452-30465.

genannt Bonsmann, S. S., Walczyk, T., Renggli, S., & Hurrell, R. F. (2008). Oxalic acid does not influence nonhaem iron absorption in humans: a comparison of kale and spinach meals. *European journal of clinical nutrition*, *62*(3), 336-341.

Islam, M. A., Al Mamun, M. A., Faruk, M., Islam, M. T. U., Rahman, M. M., Alam, M. N., ... & Alam, M. A. (2017). Astaxanthin ameliorates hepatic damage and oxidative stress in carbon tetrachloride-administered rats. *Pharmacognosy research*, *9*(Suppl 1), S84.

Kasperczyk, S., Dobrakowski, M., Kasperczyk, A., Ostałowska, A., & Birkner, E. (2013). The administration of N-acetylcysteine reduces oxidative stress and regulates glutathione metabolism in the blood cells of workers exposed to lead. *Clinical Toxicology*, *51*(6), 480-486.

Lin, X., Xia, Y., Wang, G., Yang, Y., Xiong, Z., Lv, F., ... & Ai, L. (2018). Lactic acid bacteria with antioxidant activities alleviating oxidized oil induced hepatic injury in mice. *Frontiers in microbiology*, *9*, 2684.

Martínez-Holguín, E., Lledó-García, E., Rebollo-Román, Á., González-García, J., Jara-Rascón, J., & Hernández-Fernández, C. (2020). Antioxidants to Improve Sperm Quality. *Male and Sperm Factors that Maximize IVF Success*, 106.

Muryanto, M., Alvin, Nurdin, M., Hanifah, U., & Sudiyani, Y. (2017, November). Extraction of glutathione from EFB fermentation waste using methanol with sonication process. In *AIP Conference Proceedings* (Vol. 1904, No. 1, p. 020011). AIP Publishing LLC.

Nasif, Z. N. (2002). Molecular Characterization & Determinating The Level Of Oxidative Stress & Some Antioxidants In Iraqi Women With Breast Tumors.

Nocttor G.; Qucval G.; Muhamdi A.; Chaouch S. and Foyer C.H. (2011). Glotathionic. In: Arabidopsis book. American Society of Plant Biologists. Rockvillc, MD, pp142.

Obinna-Echem, P. C., & Chukunda, F. A. (2018). Nutrient Composition of Mushroom: Pleurotus Ostreatus (Jacaum, ex. Fr. Kummer) grown on Different Agricultural Wastes. *Agriculture and Food Sciences Research*, *5*(1), 1-5.

Oborilová, A., Mayer, J., Korístek, Z., Hofmanová, Z., Vásová, I., Navrátil, M., ... & Matuska, M. (2004). Evaluation of the clinical effectivity and toxicity of the FDN regimen (fludarabin, mitoxantron, dexamethason) in patients with follicular lymphoma. *Casopis lekaru ceskych*, *143*(10), 685-690.

Omodeo-Salè, F., Basilico, N., Folini, M., Olliaro, P., & Taramelli, D. (1998). Macrophage populations of different origins have distinct susceptibilities to lipid peroxidation induced by β-haematin (malaria pigment). *FEBS letters*, *433*(3), 215-218.

Saleh, J., Edeas, M., & Van Goor, H. (2019). Antioxidant Supplements and Oxidative Stress: The debate extends to the Middle East. *Sultan Qaboos University Medical Journal*, *19*(3), e177.

SAS, J. (2012). Statistical Analysis System, v. 10.0. 2. *Cary, North Carolina. USA*.

Sonani, R. R., Rastogi, R. P., & Madamwar, D. (2015). Antioxidant potential of phycobiliproteins: Role in anti-aging research. *Biochem Anal Biochem*, *4*(172), 2161-1009.

Sumalatha, D. (2013). Antioxidant and antitumor activity of Phyllanthus emblica in colon cancer cell lines. *Int. J. Curr. Microbiol. Appl. Sci*, *2*, 189-195.

Tahir, I., Khan, M. R., Shah, N. A., & Aftab, M. (2016). Evaluation of phytochemicals, antioxidant activity and amelioration of pulmonary fibrosis with Phyllanthus emblica leaves. *BMC complementary and alternative medicine*, *16*(1), 406.

Testa, B., Testa, D., Mesolella, M., D'Errico, G., Tricarico, D., & Motta, G. (2001). Management of chronic otitis media with effusion: the role of glutathione. *The Laryngoscope*, *111*(8), 1486-1489.

Tietz, Y., (2005). Clinical Biochemistry, 6thed., McGraw –Hill, New York.825.

Wang, W., & Ballatori, N. (1998). Endogenous glutathione conjugates: occurrence and biological functions. *Pharmacological reviews*, *50*(3), 335-356.

Wang, Y.; Wu, Y.; Wang, Y.; Xu, H.; Mei, X.; Yu, D.; Wang, Y. and Li, W. (2017). Antioxidant Properties of Probiotic Bacteria. Nutrients, Vol., 9, 521-536.

Whaley-Connell, A., McCullough, P. A., & Sowers, J. R. (2019). The role of oxidative stress in the metabolic syndrome. *Reviews in cardiovascular medicine*, *12*(1), 21-29.

Wood, S.K. (2020). The role of inflammation and oxidative stress in depression and cardiovascular disease. In Cardiovascular Implications of Stress and Depression (pp. 175-209). Academic Press.