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Potential Toxic Effects of Different High Doses of Glutathione Injection: An Experimental Study

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Abstract

Background: Glutathione injections for skin whitening have been widely used recently in capricious dosing regimens with no scientific evidence. The overall safety data on injectable glutathione are scarce. **Objective:** This study aims to investigate the potential toxic doses of injected glutathione, the possibility of inducing different organ dysfunction, and the recovery pattern if it occurs. **Methods:** Glutathione was injected intramuscularly into rats in two different high doses, 124mg/kg and 248mg/kg (G II& III), twice per week for 13 weeks with the control group (G I) in which rats did not receive the drug. Rats were sacrificed 1 hour, 1 week and 2 weeks after the last administered dose. The liver, kidneys, and heart underwent histopathological and biochemical analysis. **Results:** The results revealed that at a dose of 124 mg/kg, no toxic effect was shown on the liver, kidney, or heart. However, doubling the dose to 248mg/kg caused a toxic impact on the liver, which recovered 2 weeks after the last dose, and the kidney, with no recovery observed. No affection on the heart. **Conclusions:** Glutathione injection is proven to have a potentially toxic effect when given at a dose of 248 mg/ kg twice / week for 13 weeks in rats. So, the drug dose must be adjusted for its possible toxicity.

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Key words glutathione, injections, reductive stress, oxidative stress, whitening agents

Background

Glutathione is a thiol-tripeptide with a low molecular weight. It is produced in every cell in the body. Its principal role is to quench free radicals and maintain intracellular redox balance. It is important for detoxification and immune modulation as well. This molecule is crucial in modern medicine, as it is essential to the human body's chemical, electrical and mechanical activities (Sharma and Sharma, 2022).

Glutathione is widely used as a skin-whitening agent worldwide. This action of glutathione was accidentally discovered when skin whitening was observed as a side-effect of high doses of glutathione supplements used for chronic diseases (Sonthalia et al., 2016). The mechanism of action is that glutathione can, directly and indirectly, suppress the tyrosinase enzyme, which is the rate-limiting enzyme in the formation of melanin. It shifts the production of eumelanin (which is responsible for a dark color) to pheomelanin (which produces a yellow-red color), resulting in skin whitening (Sitohang and Ninditya, 2020).

Glutathione supplements have invaded the markets in topical, oral and injectable forms. The effect of topical formulations is restricted to the application site without any systemic skin whitening effect. The oral form has a limited bioavailability, which is a main disadvantage. Thus, manufacturers and consumers prefer using glutathione injectable forms to obtain faster and better skin-lightening effects. Although parenteral glutathione provides a high therapeutic dose that promotes its efficacy, it also has a narrow safety margin due to the possibility of overdose toxicity (Gandhi et al., 2021). The recommended dose of glutathione injection, according to the manufacturers, is 600- 1200 mg, to be injected once or twice per week, with no fixed treatment and maintenance durations (Mohan et al., 2020)

The desire to have a fairer skin tone and complexion in adults has become a major concern. This craze exploits the implications of skin-whitening agents, so glutathione's popularity as a "magical skin-whitening" molecule has quickly increased worldwide (Pollock et al., 2021). Owing to the exaggerated consumption of glutathione supplements by the public, certain national drug control agencies have restricted the sale and usage of these supplements (Sharma and Sharma, 2022).

The Food and Drug Administration (FDA) of the Philippines and the Philippine Dermatology Society have announced an advisory warning on the safety of injected glutathione used for skin whitening. The reported adverse effects included renal dysfunction with the possibility of developing renal failure, thyroid dysfunction, hepatic toxicity, and neurotoxicity, in addition to adverse cutaneous effects such as fatal Stevens-Johnson syndrome and toxic epidermal necrolysis (Lazo, 2011; Dadzie, 2016). Recently, another advisory warning has been published by the United States FDA on the potential health risks related to using unapproved injectable skin whitening agents,

including glutathione (FDA Consumer Health Information).

Thus, the current study aimed to investigate the potential toxic doses of injected glutathione and the possibility of inducing different organ dysfunction histopathologically and biochemically in experimental research and the pattern of recovery if it occurs.

Material and Methods

Animals:

This prospective experimental study included ninety male albino rats (180–220 g; 8–10 weeks old) obtained from the laboratory animals growing center at Minia University.

Rats were randomly housed in standard rodent cages (30 rats per cage) identified by the group number and dose of the administered drug to avoid mixing. They were preserved in a cleanly well-ventilated media at humidity (30%-70%), temperature (22C - 30C) and 12-hour light / dark cycles. They were fed with a standard pelleted diet and water ad libitum.

One week before the experiment, animals were acclimatized to the laboratory conditions to preclude any possible stress.

Ethical approval:

This experimental study was conducted according to the laboratory animal care and usage recommendations and guidelines authorized by the ethical committee of the Faculty of Medicine, Minia University, approval No. 501/2022.

Chemicals used:

Glutathione I.M., a Spanish product, was obtained from Cosmo Medica Company, Nasr City, Cairo Governorate. The vial is about 10 ml. Each contains 2400 mg glutathione.

Study design:

Rats were distributed into three groups (30 rats each) as follows:

Group I: rats served as untreated control. They received 0.2 ml saline twice weekly for 13 weeks by intramuscular route.

Group II: Rats were injected intramuscularly with glutathione 124 mg/kg (equivalent to the highest dose recommended by the manufacturers) (Mohan et al., 2020; Al Ghamdi et al., 2020). It was given twice per week for 13 weeks.

Group III: Rats were injected intramuscularly with glutathione 248mg/kg (double the highest recommended dose). It was given twice per week for 13 weeks.

The dose was calculated according to the Human-Animal Dose Conversion table using a human and albino rat body weight of 60 kg and 200 g, respectively (Nair and Jacob, 2016).

Then, 10 rats were chosen randomly from each group and sacrificed 1 hour after the last administered dose of glutathione to study the possible toxic effects of glutathione on the liver, kidney and heart. The remaining 20 rats of each group were kept alive with no more injections and subjected to the same previous living conditions to study the extent of any detected toxicity of the glutathione and to see any possible delayed toxic effects or recovery. Then they were sacrificed as follows:

-1 week: (10 rats from each group) were sacrificed one week after the last administered dose.

-2 weeks: (10 rats from each group) were sacrificed two weeks after the last administered dose.

Rats were anesthetized by intraperitoneal Urethane; the rats were sacrificed by cervical dislocation. Blood samples were collected by cardiac puncture and prepared to get serum for biochemical analysis. The liver, kidneys and heart were dissected and designed for histopathological study and oxidative markers detection.

Serum biochemical analysis:

Liver enzymes:

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined in the serum using Assay Kits, BioMed, Egypt, according to the provider's instructions (Reitman & Frankel, 1957).

Renal function:

Creatinine and urea levels in serum were estimated using Assay Kits, Biodiagnostic, Egypt (Bartels et al., 1972).

Cardiac troponin-I:

According to the provider instructions, the Cardiac troponin-I level was determined in the serum using The Rat Cardiac troponin-I ELISA kits, Kamiya Biomedical, USA (Cat. No: KT-480) (Apple & Wu, 2001).

Oxidative/antioxidative biomarkers:

Reduced glutathione (GSH):

According to Ellman (1959), hepatic, renal and cardiac GSH levels were determined using Assay Kits, Biodiagnostic, Egypt (Cat. No. GR 25 11).

Malondialdehyde (MDA):

According to Ohkawa et al. (1979), hepatic, renal, and cardiac MDA levels were measured using Assay Kits Biodiagnostic, Egypt (Cat. No. MD 25 29).

Histopathology:

Hepatic, renal and cardiac tissues were isolated and fixed in a 10% neutral buffered formalin solution, then dehydrated in ascending alcohol grades and infiltrated with paraffin wax to make the block sufficiently rigid for a uniformly thin section about 5 μm thickness and ready for cutting, trimming and finally staining with hematoxylin and eosin (H&E) stain to be examined by light microscope. The tissues were analyzed using a light microscope with an attached camera (Olympus BX51, Tokyo, Japan) in the Pathology department, Faculty of Medicine, Minia University.

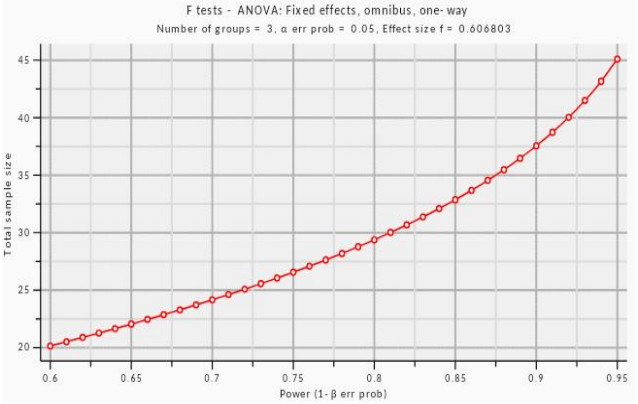
Statistical analysis:

The collected data were coded, tabulated and statistically analyzed using Statistical Package for Social Sciences (SPSS) program software, version 25. For parametric quantitative data, descriptive statistics were carried out by mean± Standard deviation (S.D.), minimum and maximum range. The data was distributed using the Shapiro-Wilk test, according to Mishra et al. (2019).

Analyses were performed for quantitative data between the different groups using the One-way ANOVA test followed by Post Hoc Tukey's analysis between the two groups. The level of significance was considered at a P value ≤ 0.05.

Sample Size Calculation:

Before the study, the number of rats chosen per group was determined after a power calculation according to data gained from a pilot study. In that study, the immediate liver GSH level in the control group was 1.77±0.31; in group II, it was 1.78±0.14; and in group III, it was 1.55±0.08. A sample size of 10 rats in each group was determined to provide 80% power for the One-way ANOVA test at 0.05 significance using G Power 3.1 9.2 software. Same number of rats per group was used at 1 week and 2 weeks.



F tests - ANOVA: Fixed effects, omnibus, one-way
Analysis: A priority: Compute the required sample size

Input: Effect size f	= 0.6068025
α err prob	= 0.05
Power (1-β err prob)	= 0.8
Number of groups	= 3
Output:	
Non-centrality parameter λ	=11.0462782
Critical F	= 3.3541308
Numerator df	= 2
Denominator df	= 27
Total sample size	= 30
Actual power	= 0.8098104

Results

Liver:

The effect of glutathione injections on liver enzymes and oxidative/antioxidative biomarkers in rats is illustrated in Table 1. On comparing both group II & group III with control (group I), there was a significant increase in ALT, AST & L-MDA measured 1 hour, 1 & 2 weeks after the last dose in group III when compared to group I & II with a significant decrease in L-GSH in group III

In group II, liver enzymes and oxidative/ antioxidative biomarkers showed insignificant differences at 1 hour, 1 week, and 2 weeks after the last dose of glutathione injection. While in group III, ALT & AST significantly decreased at 2 weeks compared with 1 hour & 1 week. Regarding oxidative/ antioxidative biomarkers, L-MDA decreased significantly and L-GSH increased significantly at 2 weeks compared with 1 hour & 1 week after the last dose.

Regarding the histopathological findings, sections of hepatic tissues obtained from rats in the control group as well as those taken from rats in group II either 1 hour, 1 and 2 weeks after the glutathione treatment course showed normal histological structure with normal portal tract and normal hepatocytes separated by sinusoids as shown in (Figure 1a).

In group III, hepatic sections obtained from rats 1 hour after the last dose of glutathione showed congestion and marked infiltration with inflammatory cells in the portal tract. Hepatic tissue sections obtained from rats sacrificed 1 week after the last dose revealed congestion, marked inflammation in the portal tract, and fatty change in hepatocytes. Hepatic sections got 2 weeks after the last administered dose showed congestion and mild inflammation in the portal tract with no fatty change in hepatocytes (figure 1 b, c, d consequently).

Kidneys

Table 2 showed the effect of glutathione injections on renal function as well as renal oxidative/antioxidative biomarkers in rats; comparison between group II & group III with control (group I) revealed significant elevation in creatinine, urea and K-MDA associated with a significant decline in K-GSH at all times of measurements in group III compared to their respective values in group I (the control) and group II.

In group II, creatinine, urea, K-MDA and K-GSH measured 1 hour after the last dose of glutathione didn't change significantly neither 1 week nor 2 weeks after the last administered glutathione injection.

The affected renal biomarkers (creatinine, urea, K-MDA & K-GSH) measured 1 hour after the last dose of glutathione in group III showed insignificant differences in the following two weeks.

Histopathological results showed normal histologic structure with normal glomeruli and tubules in renal sections obtained from rats in both the control group and group II (whether sacrificed 1 hour, 1 and 2 weeks after the glutathione treatment course). (Figure 2a).

In group III, sections of renal tissues obtained from rats sacrificed 1 hour, 1 and 2 weeks after the last dose of glutathione showed congestion, cloudy swelling and marked infiltration of renal tubules by inflammatory cells. (figure 2b).

Heart

The effects of glutathione injections on cardiac troponin-I and cardiac oxidative/antioxidative biomarkers in rats are shown in Table 3. Cardiac troponin-I, H-MDA and H-GSH levels measured 1 hour, 1 & 2 weeks after the last glutathione dose showed no significant difference among the three groups. They also showed no significant change at different measurement times within each group.

Regarding the histopathological examination of the heart, all cardiac tissue sections obtained from rats (the control group, group II and group III) at all times of sacrifice showed normal cardiac muscles with no histologic changes (figure 3).

Table (1): Effect of glutathione injection on liver biomarkers in the studied groups:

		Control (I)	Group II	Group III	P value
		N=10	N=10	N=10	
ALT	1 hour	81.6±16.1 a, d	88.7±12.09 a, d	120.5±16.85 b, d	***<0.001****
	1 week	82.3±14.46 a, d	86.2±14.65 a, d	118.4±18.64 b, d	***<0.001****
	2 weeks	80.9±11.07 a, d	84.8±12.52 a, d	99.4±11.07 b, c	***0.003****
	P value	*0.975*	*0.799*	***0.011****	
AST	1 hour	63.9±4.39 a, d	66±7.69 a, d	94.3±12.58 b, d	***<0.001****
	1 week	64.3±2.25 a, d	67.3±11.63 a, d	95.5±12.53 b, d	***<0.001****
	2 weeks	64.8±3.76 a, d	67.9±15.81 a, d	80.3±9.74 b, c	***0.009****
	P value	*0.854*	*0.938*	***0.012****	
L-MDA	1 hour	3.45±0.66 a, d	3.8±0.7 a, d	9.11±1.22 b, d	***<0.001****
	1 week	3.6±0.6 a, d	4.1±0.9 a, d	8.23±0.79 b, d	***<0.001****
	2 weeks	3.96±0.76 a, d	4.5±0.57 a, d	6.93±0.92 b, c	***<0.001****
	P value	*0.240*	*0.121*	***<0.001****	
L-GSH	1 hour	2.12±0.43 a, d	1.95±0.28 a, d	1.42±0.23 b, d	***<0.001****
	1 week	2.17±0.32 a, d	1.95±0.32 a, d	1.47±0.26 b, d	***<0.001****
	2 weeks	2.21±0.42 a, d	2.15±0.31 a, d	1.75±0.22 b, c	***0.008****
	P value	*0.878*	*0.257*	***0.009****	

ALT: Alanine aminotransferase, AST: aspartate aminotransferase, L-GSH: liver reduced glutathione, L-MDA: liver malondialdehyde.

- *One-way ANOVA test followed by post hoc Tukey's analysis.*
- *Superscripts with different small letters (a, b) refer to significant differences between groups.*
- *Superscripts with different small letters (c, d) refer to significant differences between each two times.*
- **: Significant level at P value < 0.05*

Table (2): Effect of glutathione injection on kidney biomarkers in the studied groups:

		Control (I)	Group II	Group III	P value
		N=10	N=10	N=10	
Urea	1 hour	35.5±7.48 a, d	35.2±6.61 a, d	65.4±7.61 b, d	***<0.001****
	1 week	36.2±7.89 a, d	40.3±7.85 a, d	63.7±10.78 b, d	***<0.001****
	2 weeks	35.3±4.34 a, d	40.5±8.86 a, d	62.9±11.03 b, d	***<0.001****
	P value	*0.952*	*0.247*	*0.849*	
Creatinine	1 hour	0.88±0.18 a, d	1.1±0.16 a, d	2.5±0.31 b, d	***<0.001****
	1 week	0.9±0.25 a, d	1.1±0.19 a, d	2.3±0.38 b, d	***<0.001****
	2 weeks	0.91±0.21 a, d	1.00±0.17 a, d	2.2±0.28 b, d	***<0.001****
	P value	*0.950*	*0.354*	*0.131*	
K-MDA	1 hour	2.1±0.61 a, d	2.4±0.4 a, d	6.4±0.67 b, d	***<0.001****
	1 week	2.13±0.41 a, d	2.55±0.35 a, d	6.2±0.67 b, d	***<0.001****
	2 weeks	2.15±0.85 a, d	2.81±0.52 a, d	6.12±0.74 b, d	***<0.001****
	P value	*0.985*	*0.116*	*0.654*	
K-GSH	1 hour	2.14±0.37 a, d	1.97±0.19 a, d	1.3±0.11 b, d	***<0.001****
	1 week	2.16±0.46 a, d	1.92±0.33 a, d	1.28±0.25 b, d	***<0.001****
	2 weeks	2.2±0.41 a, d	2.05±0.4 a, d	1.32±0.25 b, d	***<0.001****
	P value	*0.948*	*0.661*	*0.918*	

K-GSH: kidney reduced glutathione, K-MDA: kidney malondialdehyde.

- *One-way ANOVA test followed by post hoc Tukey's analysis.*
- *Superscripts with different small letters (a, b) refer to significant differences between groups.*
- *Superscripts with different small letters (c, d) refer to significant differences between each two times.*
- **: Significant level at P value < 0.05*

Table (3): Effect of glutathione injection on heart biomarkers in the studied groups:

		Control (I)	Group II	Group III	P value
		N=10	N=10	N=10	
Troponin I	1 hour	0.23±0.1 [a, d]	0.22±0.11 [a, d]	0.22±0.09 [a, d]	0.975
	1 week	0.2±0.1 [a, d]	0.23±0.12 [a, d]	0.2±0.06 [a, d]	0.768
	2 weeks	0.21±0.08 [a, d]	0.21±0.11 [a, d]	0.23±0.1 [a, d]	0.920
	P value	0.796	0.923	0.813	
H-MDA	1 hour	6.8±1.11 [a, d]	6.9±1.55 [a, d]	7.2±1.24 [a, d]	0.780
	1 week	6.9±1.64 [a, d]	7.2±1.3 [a, d]	7.3±1.05 [a, d]	0.790
	2 weeks	7.1±1.04 [a, d]	7.1±1.28 [a, d]	7±1.15 [a, d]	0.976
	P value	0.870	0.885	0.839	
H-GSH	1 hour	2.1±0.46 [a, d]	2.22±0.33 [a, d]	2.23±0.44 [a, d]	0.738
	1 week	2.15±0.44 [a, d]	2.19±0.34 [a, d]	2.18±0.34 [a, d]	0.969
	2 weeks	2.21±0.35 [a, d]	2.14±0.29 [a, d]	2.2±0.61 [a, d]	0.929
	P value	0.842	0.854	0.972	

H-GSH: heart reduced glutathione, H-MDA: heart malondialdehyde.

- *One-way ANOVA test followed by post hoc Tukey's analysis.*
- *Superscripts with different small letters (a, b) refer to significant differences between groups.*
- *Superscripts with different small letters (c, d) refer to significant differences between each two times.*

****: Significant level at P value < 0.05***

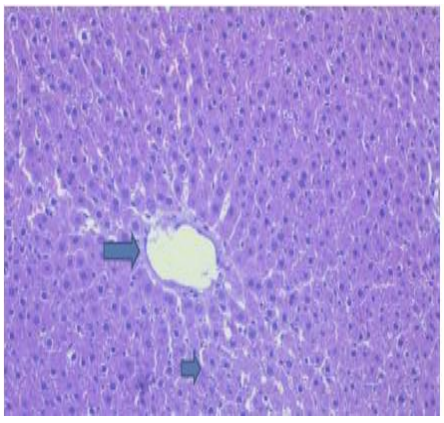


Figure (1a)

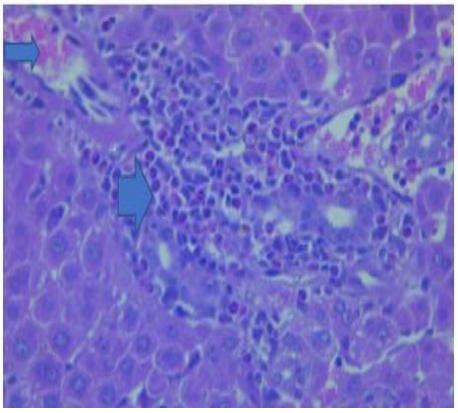


Figure (1b)

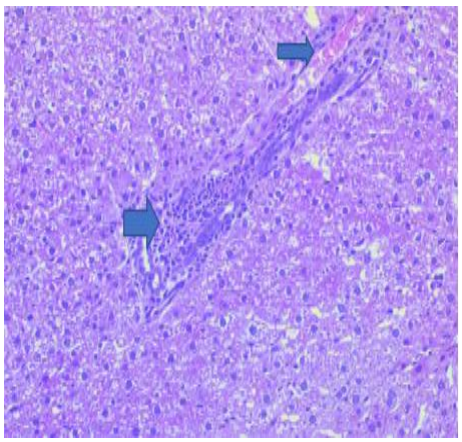


Figure (1c)

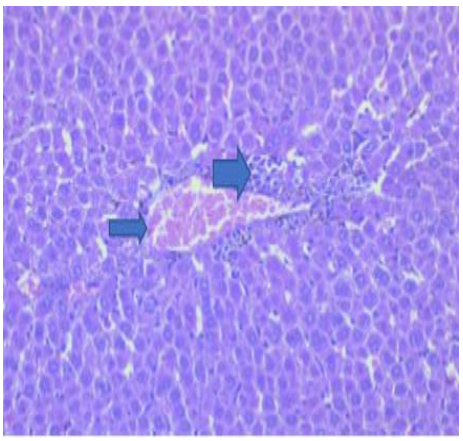


Figure (1d)

Figure (1): Histopathological effect of glutathione injection on liver in rats. (1a): A photomicrograph section from rats` liver in both the control group and group II at all examined intervals showing normal portal tract (large arrow) and normal hepatocytes (small arrow) separated by sinusoids with no histological changes (H&E X100). (1b): A photomicrograph section in rat liver obtained 1 hour after last glutathione injection in group III showing congestion (small arrow) and marked infiltration by inflammatory cells in portal tract (large arrow) (H&E X400). (1c) A photomicrograph section in rat liver obtained 1 week after last glutathione injection in group III showing congestion (small arrow), marked inflammation in portal tract (large arrow) and fatty change in hepatocytes (H&E X400). (1d) A photomicrograph section in rat liver obtained 2 weeks after last glutathione injection in group III showing congestion (small arrow), mild inflammation in portal tract but there was no fatty change in hepatocytes (large hand) (H&E X200).

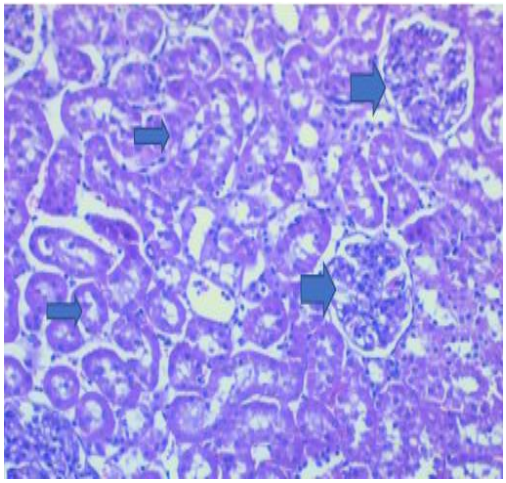


Figure (2a)

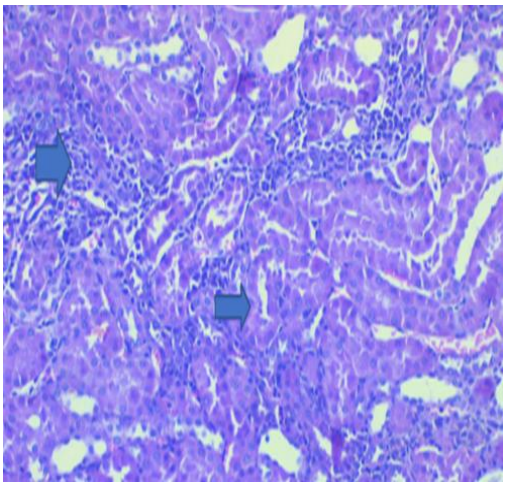


Figure (2b)

Figure (2): Histopathological effect of glutathione injection on kidneys in rats. (2a): A photomicrograph section in rats` kidneys in both control group and group II at all examined intervals showing normal glomeruli (large arrow) and tubules (small arrows) with no histological changes (H&E X200). (2b) A photomicrograph section in rat kidney showing congestion, cloudy swelling (small arrow) and marked infiltration of the kidney tubules by inflammatory cells (large arrow) at 1 hour, 1 week and 2 weeks after last administered dose in group III (H&E X200).

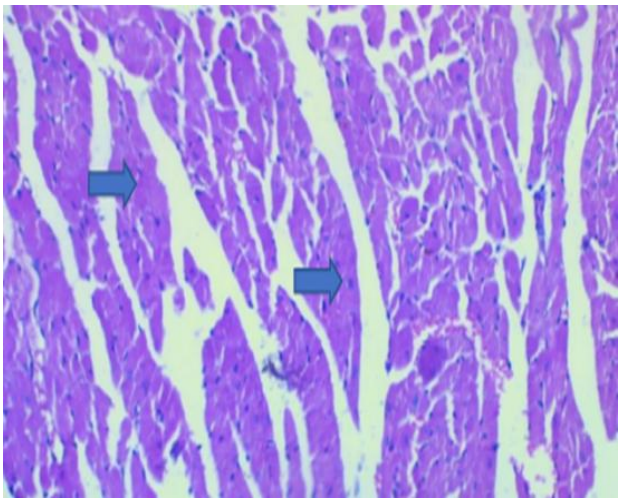


Figure (3): Showing the section in rat heart showing normal cardiac muscles (arrows) with no histological changes in groups 1, II, III (H&E X200).

Discussion

Nowadays, glutathione injections are widely available for many purposes. However, the safety profile of parenteral glutathione has yet to be fully investigated. Data from clinical trials in the literature examining the effects of glutathione is crumbled with no obvious guidelines for the clinician, particularly in managing skin pigmentation problems. Moreover, the Philippine Food and Drug Administration has cautioned that off-label glutathione-containing skin-whitening agents could cause serious health problems (Mahmood, 2022).

Thus, the objective of the current study was to investigate the potentially toxic doses of injected glutathione and the possibility of inducing different organ dysfunction histopathologically and biochemically in experimental research and the pattern of recovery if it occurs.

This study revealed that I.M. glutathione given at a dose of 124 mg/Kg twice per week for 13 weeks is nontoxic to the liver, kidney or heart among the animals. In addition, no delayed toxic effects could be detected over the two weeks following cessation of treatment; these results coincide with AlGhamdi et al. (2020), who evaluated intraperitoneal glutathione injection into guinea pig at 20 mg/kg three times per week for three weeks and revealed that glutathione given at this dose was nontoxic to liver or kidney with no histopathological or laboratory changes.

On the contrary, Zubair et al. (2016) investigated glutathione for skin lightening in 25 Pakistani patients who received intravenous glutathione at a dose of 1,200 mg twice per week (equivalent to the amount given in group II in the current study) for six consecutive weeks versus 25 controls who received IV

normal saline. They noticed that all subjects in the treatment group had experienced adverse effects, and about a third had abnormal liver function. The small sample size, high dropout rate (9 out of 25), and lack of toxic effects follow-up in the study of Zubair et al. (2016) may explain this contrary.

Regarding changes in oxidative redox markers at the previous dose, it was noticed that such a dose didn't cause significant changes in the GSH or MDA contents of the examined tissues. These findings are consistent with Allen and Bradley (2011) who demonstrated that four weeks of oral glutathione supplementation didn't improve erythrocyte GSH concentration or reduce oxidative stress biomarkers in healthy adults. Also, Masubuchi et al. (2011) found that intraperitoneal glutathione injection could ameliorate acetaminophen-induced hepatotoxicity in animal models without restoration of hepatic GSH but with other mechanisms of protection rather than GSH increase. These results are attributed to glutathione concentration in extracellular space being extremely lower than that found intracellularly which is great to be overcome and permits extracellular glutahione to enter the cells after its administration either orally or parentally (Braidy et al., 2015).

In the current study, glutathione injected at a dose of 248 mg/kg twice per week for 13 weeks provoked a deleterious effect on the liver and kidneys, sparing the heart. That was manifested by elevated (ALT, AST, urea and creatinine) levels and histopathological changes in both organs. Two weeks after cessation of treatment, hepatic biomarkers and histopathological findings showed some improvement but not complete recovery. However, renal function tests and histopathological findings noticed 1 hour after the last dose of glutathione remained the same through the following two weeks.

These results differed from AlGhamdi et al. (2020) when they increased the dose of intraperitoneal glutathione to 40 mg/kg three times per week for three weeks, they found that even with increasing the dose, it was nontoxic to the liver and kidneys with no histopathological or laboratory changes. This controversy in results may be due to differences in the route of administration, number of overall given doses, duration of treatment and type of involved animals.

Regarding the oxidative redox markers changes in this study, doubling the dose revealed altered redox cellular equilibrium in hepatic and renal tissues; significantly high MDA associated with significantly low GSH; high MDA levels may be explained by the fact that doubling the dose may enhance free radical production and induce oxidative stress in liver and kidneys. In the same direction, the GSH decrease may be because intracellular reduced GSH was consumed trying to maintain the cellular redox balance by neutralizing the generated free radicals and reactive oxygen compounds that caused lipid peroxidation and resulted in high MDA levels (Tsikas, 2017). Unfortunately, no study investigated levels of MDA or GSH in blood or tissues with high glutathione doses to be compared with our results.

These findings can be explained by the fact that oxidative condition in different body tissues is affected by the balance between antioxidant (intracellular and extracellular) and free radicals. Thus, high doses of antioxidants (such as glutathione) can interfere with the physiological concentrations of free radicals, especially reactive oxygen species (ROS), which are important for normal cell signaling, redox regulation and immune response stimulation resulting in cellular dysfunction (Rahal et al., 2014). Moreover, that relative lack of ROS compared with reducing equivalents for a long time, known as reductive stress, can break the mitochondrial homeostasis and promote excessive ROS generation to a level that overwhelms ROS scavenging capability with subsequent hydrogen peroxide spillover from mitochondria, resulting in oxidative stress which in turn causes membrane lipid peroxidation and protein damage (Pérez-Torres et al., 2017). Also, renal impairment may be induced by high doses of parenteral glutathione overburdening the renal circulation (Sonthalia et al., 2016).

Another main factor to be restated here is that several researchers have established the antioxidant properties of glutathione. Yet, in specific circumstances, almost any antioxidant may function as a pro-oxidant. Ascorbic acid, for instance, can act as an antioxidant or pro-oxidant, depending upon the administered dose. Also, it was found that α-lipoic acid, in diabetic rats, has a protective impact on the kidney, while in non-diabetic animals, it has a pro-oxidant effect. Glutathione also has the potential to act as a pro-oxidant in specific situations (Rahal et al., 2014).

The pro-oxidant effect of glutathione has been demonstrated by Sagristá et al. (2002), who reported that oxidative metabolism of reduced glutathione could generate glutathione radical (G.S.), which can induce a pro-oxidant activity. These thiyl radicals are involved in metal ion-mediated reactions that produce ROS, responsible for lipid peroxidation and protein destruction**.** Also, Dewi et al. (2020) found that glutathione intramuscular injection (1.5 mg /day for two weeks) could significantly elevate MDA levels in retinopathy of premature albino rat models.

Reduced glutathione has a very intricate manner of input in different biological processes. Therefore, any experimental or therapeutic intervention should be carried out with caution owing to biological systems' complex, interconnected and strictly regulated networking. Changing one variable may bring unpredictable responses in several cases (Lushchak, 2012).

To our knowledge, this research is the first to study the possible toxic doses of injected glutathione in an experimental study.

Conclusion

The current study concluded that I.M. glutathione injection at a dose of 124 mg/kg/ twice a week for 13 weeks was nontoxic in an experimental animal model while doubling this dose for the same duration was found to be toxic to animals and could induce hepatic and renal inflammation and dysfunction. Therefore, I.M. glutathione injections should be used cautiously under the supervision of medical experts and

dermatologists with adjustment of the administered dose and treatment duration. Also, follow-up of hepatic and renal functions is essential during therapy.

Limitations and Recommendations

Limitations of this study include short post-treatment follow-up duration and lack of follow-up during the treatment period. Thus, further experimental and clinical studies with various designs, large sample sizes and longer-term post-treatment assessment are crucial for better evaluation of the safety of parenteral glutathione as a whitening agent.

Decelerations:

Availability of data and materials:

This published article will include all data generated or analyzed during this study.

Competing interests

The authors declare that they have no competing interests.

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الآثار السامة المحتملة لجرعات عالية مختلفة من حقن الجلوتاثيون: دراسة تجريبية

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الملخص العربي

المقدمة: تستخدم حقن الجلوتاثيون لتبييض البشرة على نطاق واسع مؤخرًا بجرعات مختلفة بدون دليل علمي وأيضا بيانات السلامة الخاصة بالجلوتاثيون القابل للحقن نادرة. لذلك ، لذلك هذه الدراسة تهدف الي التحقيق في الجرعات السامة المحتملة من الجلوتاثيون المحقون، وإمكانية إحداث خلل وظيفي في الأعضاء، ونمط الشفاء في حالة حدوثه. **طريقة البحث:** تم توزيع الجرذان عشوائيا الي ثلاثة مجموعات حيث تكونت كل مجموعة من ثلاثين جرذ. المجموعة الاولي تم اعطاءهم محلول ملح والمجموعة الثانية تم حقنهم الجلوتاثيون في العضل بجرعة 124 مجم / كجم و بجرعة 248 مجم / كجم في المجموعة الثالثة وذلك مرتين أسبوعياً لمدة 13 أسبوعًا. تم الحصول علي الكبد والكلى والقلب لتحليل الأنسجة واجراء بعض التحاليل البيوكيميائية؛ بعد ساعة وأسبوع وأسبوعين بعد آخر جرعة تم حقنها. **النتائج:** أظهرت النتائج عدم وجود تأثير سام على الكبد أو الكلى أو القلب عند حقن الجلوتاثيون بجرعة 124 مجم / كجم. ولكن مضاعفة الجرعة إلى 248 ملجم / كجم سببت تأثيرًا سامًا على الكبد الذي تعافى بعد أسبوعين من آخر جرعة، والكلى دون ملاحظة الشفاء. لم يظهر تأثير سام على القلب. **الخلاصة:** ثبت أن حقن الجلوتاثيون له تأثير سام محتمل عند إعطائه بجرعة 248 مجم / كجم مرتين أسبوعيا لمدة 13 أسبوعًا في الجرذان. لذلك، يجب تعديل جرعة الدواء لسميتها المحتملة.

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