



## The Effect of Dietary Supplement on Glutathione Level in Rats

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### Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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### ABSTRACT

**Background:** Oxidative stress occurs due to decreased glutathione inside the body. Some supplements may promote and stimulate glutathione production in the liver. This article aims to investigate the impact of different supplements on enhancing glutathione synthesis in rats' livers. For this purpose 42 rats (male albino) were separated into 7 groups, each including 6 animals with average weights ranging between 150 and 160 g. Group 1 (control) and different groups consumed a basal diet for 8 weeks, whereas group 2 received 500 mg/kg bw of L-cysteine daily. Group 3 received 250 mg/kg bw of methionine, while group 4 got 250 mg/kg of L cysteine plus 125 mg/kg of methionine daily. Spirulina (20 mg/kg bw), turmeric (500 mg/kg bw), and dried garlic (500 mg/kg bw), respectively, were given to groups 5, 6, and 7.

**Results:** Utilizing the various dietary supplements decreased levels of liver function enzymes, bilirubin, urea, creatinine, and malondialdehyde while enhancing levels antioxidant enzymes of liver, and increased glutathione of kidneys and liver. However, cysteine alone at 500 mg/kg bw decreased glutathione formation in the liver and kidneys. Compared to the amino acid supplements (group 2, 3, 4) used, spirulina, turmeric, and dried garlic had a significant impact on reducing liver function enzymes, bilirubin, uric acid, creatinine, urea, and malondialdehyde and increasing antioxidant enzymes, and glutathione while turmeric supplement showed the best influence. Using dietary supplements did not result in any pathological modifications in the liver tissues, but there were some unsatisfactory minor alterations. However, group 2 showed considerable pathological developments in the liver tissues.

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**Conclusion:** According to the findings, using the suggested dietary supplement except for cysteine alone can promote and encourage glutathione synthesis in different organs, especially the liver, hence alleviating the effects of oxidative stress associated with several illnesses.

**Keywords:** Dietary supplements; glutathione; L-cysteine; methionine; spirulina; curcumin; garlic.

## 1. INTRODUCTION

Oxidative stress happens when the output of ROS (reactive oxygen species) surpasses the ordinary level in the body. When the proportion of reduced glutathione to oxidized is disturbed, DNA, proteins, and lipids are damaged, oxidation processes increase, and malondialdehyde (MDA) content rises, destroying the antioxidant system and causing a defect in glutathione and cytokine generation, cell reproduction, DNA synthesis, and gene expression. It also leads to antioxidant enzyme depletion and a reduction in reductase glutathione molecules, leading to glutathione peroxidase (GPx) failure as its first defense system versus free radicals, which allow chronic illnesses developed such as cardiovascular disease, premature ageing, and cancer [1]. This is the main cause of cardiovascular disease, insulin impedance dyslipidemia, and systemic infections [2].

Glutathione is a kind of antioxidant produced from cysteine, glycine, and glutamine by cells. The reduced and oxidized states are two forms of glutathione in cells. The proportion between them in healthy cells is more than 100 in reduced but ranged from 1 to 10 at oxidation form. Glutathione is also known as a thiol store because it helps retain the sulfhydryl groups in proteins. Ascorbate and glutathione are the most essential internal antioxidant. Glutathione protects against nonspecific oxidative damage, acts as an electron donor for GPx, and detects hydrogen peroxide, which plays a major disease-inducing role in many health problems [3]. Glutathione is also involved in abiotic conjugation and secretion [4]. Its levels decrease by exposure for toxins environment, poor diet, oxidative stress and age increase. Glutathione blocks the effects of oxidative stress that may reduce disease. Glutathione helps people with deep-seated fatty liver illnesses to improve the levels of enzymes, bilirubin, and protein in their blood [5]. It also aids to the detoxification of organisms and the elimination various free radicals or ROS in cells [6]. It prevents proteins from forming bonds with many harmful chemicals. Furthermore, it influences numerous biological functions, including the immune

response, while supporting immune system functions. It also contributes to the normal synthesis and repair of DNA through the production of protein and prostaglandins, and helps certain enzymes to function. It reduces cell damage in people with fatty liver disease. It also reduces liver damage by interacting with toxins and carcinogens and enhances the solubility of foreign compounds such as drugs and thus is easily excreted by the kidneys as does paracetamol in high doses. It also helps the liver and gallbladder with fat metabolism and the prevention of hepatitis, in addition to improving the body's sensitivity to insulin and contributing sperm cells formation. Contributes to the detoxification of harmful minerals via the mercapturic acid pathway [7]. It has a concentration range of 0.5-10 mM in mammalian cell types but only  $\mu$  molar concentrations in extracellular fluids [8]. Furthermore, it is an important reducing agent characterized by high antioxidant activity and controls apoptosis, the cell cycle, reproduction, thiol-disulfide exchange, and organism metabolism. It also functions as a cysteine storage area [9]. Glutathione is also accountable for eliminating toxins such as mercury from cells and the brain, and regulating cell proliferation and programmed cell death. Glutathione directly removes various oxidants: superoxide, hydroxyl radical, carbon radical, and nitric oxide. Glutathione detoxifies hydroperoxides, lipid peroxides, and peroxynitrites [10] and defend as antioxidants, maintains the thiol status of proteins, and modify immune system structure and DNA [11]. With no, glutathione is essential to the hepatic working of insulin-sensitive factors and plays a vital function in regulating the redox state of the cell with lipids, amino acids, and glucose. Glutathione is helped in amino acid transportation and estrogen synthesis, prostaglandins and leukotrienes [12]. Upkeep of neurotransmitters, safeguard of membranes, detoxification, arrangement of metabolism, and modification of sign transduction. The brain glutathione depletion is implicated in both Parkinson's disease and post-stroke neuronal damage [13]. Higher glutathione levels have been linked to better physical health and fewer illnesses [14]. Given the importance of glutathione to health, several researchers have

sought strategies to boost amounts within cells. However, the lower half-life of oral glutathione treatment has been found to be beneficial in many disorders, at least in the short term. As a result, providing some supplements that promote glutathione production may be more useful. The cysteine availability may encourage glutathione synthesis and increase their level [15].

On the other hand, hepatic glutathione contents are tightly tied to nutritional circumstances, particularly cysteine concentration in the diet. The presence of cysteine is indeed for glutathione production. Cysteine is generated naturally from the food, through protein catabolism in the liver, via sulphur conversion from methionine [12]. Sulphur-rich amino acids like cysteine and methionine are essential for protein and glutathione synthesis [16]. A vital amino acid called methionine can provide only by diet [17]; While cysteine is semi-essential and can be obtained by synthesis from methionine and serine via the sulfur conversion pathway [18]. Cysteine is easily converted to the disulfide form that acts primarily as a precursor to glutathione.

Spirulina has several antioxidants such as phycocyanin, fucoxanthin, polysaccharides, and polyphenols [19,20]. Spirulina recently demonstrated the ability to be used as a component in enhancing functional meals, which is a significant advancement in the food sector [21]. It is wealthy in protein (55-70%) and different types of amino acids [22], which increases the probability of promoting glutathione synthesis in the liver, especially since several studies have confirmed the possibility of its use as a supplement or functional food. In addition, [23] found that spirulina has a positive effect on inhibiting lipid peroxidation after exposure to toxic stresses.

Turmeric has no side effects [24], and due to the presence of many polyphenol compounds in turmeric, it enhances the body's defense system in terms of antioxidant enzyme activities or glutathione synthesis, which helps to eliminate ROS, thus reducing fat oxidation. It reduces oxidative stress through non-enzymatic and enzymatic mechanisms [25,26]. This affects quality of life and lowers mortality rates because glutathione improves liver function by reducing free radicals and oxidative stress [27]. It is used in pharmacological applications, as anti-inflammatory, immunomodulator, antimicrobial,

antiparasitic and anticancer activities [28] and ROS scavenging [29]. It encourages detoxifying enzymes such SOD, CAT, POD, APX, GR, GPx and GST due to its antioxidant capacity [30]. It also helps reduce oxidative stress thus healing from many acute and chronic diseases like arthritis diabetes, diabetic microangiopathy, diabetic nephropathy, psoriasis, gastrointestinal disease, acute heart disease, anxiety, hyperlipidemia, liver disease, inflammation and bacterial infection [31].

This work aims to evaluate the effect of different types of supplementation on the normal stimulation of glutathione generation in the liver of rats.

## 2. MATERIALS AND METHODS

### 2.1 Materials

Imtenan Company, Mansoura branch, Egypt, supplied the spirulina. Turmeric powder was obtained from a local market. Garlic was got from the native market, dried, and crushed .L-cysteine and methionine were procured from Sigma-Aldrich (Steinheim, Germany). Nimesh (Mumbai, India) Corporation provided the casein. SISCO Research Laboratories provided the AIN-76 mineral mix and AIN-76 vitamin mix (Mumbai, India).

### 2.2 Animal Nutrition and Care

The rats utilized for the study were treated according to guidelines of the Food Technology Research Institute's experiment animal house, follows Agriculture Research Center animal patronage commission assizes, which are in line with international care and use recommendations. The rats (male albino) between 150-160 g were got of Food Technology Research Institute's. Animal House at the Agriculture Research Center in Giza, Egypt. The animals were placed in well- aerated cages with a sieve down and continue supply for a basal diet (control) for 10 days for *adaptation*. Then, the rats were separated into seven groups with six rats for each. The animals were allowed to fed different experimental diet for 8 weeks. Group one (G1) consumed a basal diet which comprises 15% sugar, 21.7% casein, 53.3% corn starch, 5% corn oil, 1% vitamin mixture, 4% mineral mix, and 0.2% choline chloride as recommended by [32]. Group 2 (G2) received a basal diet as well as 0.5 mL L-cysteine solution (500 mg/kg bw). Group 3 (G3) was given a basal

diet and 0.5 mL methionine solution (250 mg/kg bw). G4 received a basal diet and a 0.5 ml solution (L cysteine 250 mg/kg + methionine 125 mg/kg). G5 was given a basal diet and 0.5 ml spirulina solution (20 mg/kg bw). G6 was given a basal diet and 0.5 mL turmeric solution (500 mg/kg bw). Group 7 (G7) was given a basal diet and 0.5 mL garlic solution (500 mg/kg bw). Humidity and temperature were kept at 60% and 25±2°C, respectively. Water and feed were given, *ad libitum*. After 8 weeks of study, the rats were fasting overnight and then sacrificed.

### 2.3 Garlic Powder Preparation

The garlic powder used in this study was prepared from freshly harvested garlic of the Baladi cultivar. The garlic cloves were air-dried in the shade for one day, then the outer casing was removed and cut into slices, then dried at a temperature not exceeding 45°C until 5% humidity and then crushed to obtain a powder according to [33].

### 2.4 Liver Function Enzymes in Serum

After blood collection, it allowed clotting and serum was obtained by centrifugation at 3000 rpm for 15 minutes. Serum markers such AST, and ALT were determined using (Diamond kits Co, Hannover, Germany) calorimetrically.

### 2.5 Histological Examination

The experiment's rats were massacred, and the required organs were separated and kept in formalin buffer (10%) until histological analysis.

### 2.6 Biochemical Parameters in Liver Tissues Homogenates

Tissues from liver were homogenized in 50 mM buffer phosphate (KH<sub>2</sub>PO<sub>4</sub>/ K<sub>2</sub>HPO<sub>4</sub>) at pH 7.4 using Potter-Elvehjem homogenizer. The homogenates were centrifuged at 10000x g for 10 min (4° C) and the resulting supernatants were then used for the estimation of antioxidant enzymes activity and malondialdehyde.

#### 2.6.1 Lipid peroxidation

MDA was determined as described by [34].

#### 2.6.2 Glutathione

The tissues of the kidney and liver were homogenated at 0.1 M buffer of Tris-HCl at pH 7.4. The homogenates use in glutathione determination according to procedure [35].

#### 2.6.3 Antioxidant enzymes in liver

CAT activity was measured in accordance with [36]. GPx and SOD activity were determined using the method published by [37]. Lactate dehydrogenase (LDH) was determined according the methods as described in the kits instruction (Diamond Co, Hannover, Germany).

#### 2.7 Bilirubin

Total bilirubin was determined according the kits instruction method of (Diamond Co, Hannover, Germany).

### 2.8 Kidney Functions

#### 2.8.1 Serum urea

Serum urea was determined using the kinetic method [38].

#### 2.8.2 Creatinine assay

The creatinine assayed by colorimetric method of Jaffe as described by [39].

#### 2.8.3 Uric acid

A uricase enzyme technique was utilized to determine uric acid [40].

### 2.9 Statistical Analyses

The Duncan test, included in the SPSS program (SPSS Inc., Chicago, IL), was employed for means comparison at 0.05 probability.

## 3. RESULTS AND DISCUSSION

### 3.1 The Effect of Some Supplements on Liver Function in Rats

ALT and AST are key liver enzymes that significantly increased under toxicity [41]. Increasing ALT and AST levels in hepatic cells, important indicator for hepatocellular inflammation, damage and impairment [42].

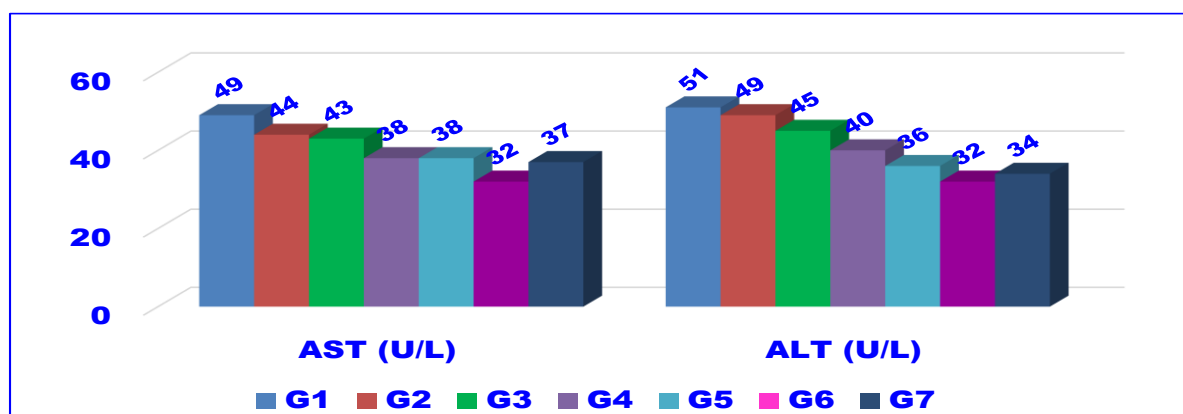


Fig.1. The effect of several types of dietary supplements on liver function in Rats

Fig. 1 depicts the present study's findings, which reveal how all supplemented diets had reduced amounts of liver enzymes like ALT and AST when compared to the control diet (G1). In addition, it is noticed that all natural supplemented such turmeric, dried garlic, spirulina (G5, G6, and G7) reduced the activity of ALT, and AST compared with the amino acids supplements used (G2, G3, and G4). Lower activities of AST and ALT were observed herein in G6 and G7. These data illustrated that bioactive compounds in turmeric and dried garlic did not cause liver damage and may be linked to the generation of cytokines that preserve liver cells. Liver cells turmeric application showed an important decrease in hepatic enzymes levels corresponding with other treatments. The trend of findings was identically for [43]. The existence of different phenolic components and flavonoid in turmeric may be responsible for the turmeric - induced benefits, which may reduce lipid peroxidation [44].

### 3.2 Effect of Some Supplements on Bilirubin in Rats

The current study showed that the dietary supplementation with cysteine 500 mg/kg (Group 2) enhanced bilirubin compared with control whereas; its values were significantly reduced in other groups especially G6 which was supplemented with turmeric (0.36 mg /dl). Groups that used natural substances such as spirulina, turmeric and garlic led to a decrease in the level of bilirubin compared with that supplemented by amino acids like cysteine or methionine or a mixture of them. The highest decrease was recorded by G6 that used turmeric at level 500 mg/ kg. Stercobilin and urobilin that are breakdown products of bilirubin can cause the brown feces color and straw yellow color of urine. Elevated levels of bilirubin may designate certain diseases such as jaundice [45] or indicators of damage induced by metal [25,29].

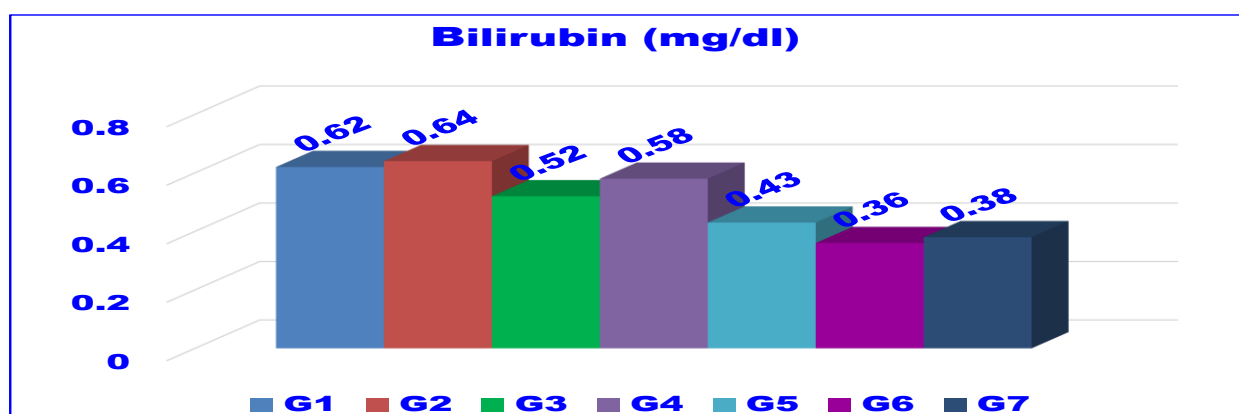


Fig. 2. The effect of several types of dietary supplements on Rats' Bilirubin

### 3.3 Effect of Some Supplements on Kidney Functions in Rats

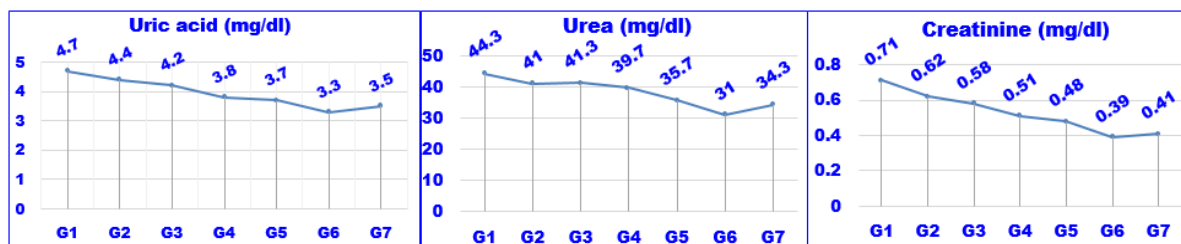


Fig. 3. The effect of several types of dietary supplements on kidney functions of Rats

Fig. 3 shows that despite the increase in the dose of amino acid supplementation given to rats in the first, second and third groups, kidney function was not affected. The content of uric acid, urea and creatinine did not increase, but on the contrary, their levels decreased compared to the control. The decrease continued with the use of nutritional supplements such as spirulina and turmeric, but with the use of dried garlic supplements, there was some increase when comparing group 7 with the previous group 6, but this increase was less than the control in general. According [46] abnormal kidney functions for increase uric acid, urea and creatinine induce kidney-related diseases. Here it is noted that all the treatments used have reduced or improved kidney function activity. In the present research, serum bilirubin and kidney functions were reduced in all groups compared with control, especially with used turmeric [41]. Also observed similar findings.

### 3.4 Effect of Some Supplements on Glutathione Level in Liver and Kidney in Rats

Fig. 4 summarizes the effect of different nutritional supplements on the content of glutathione in both the liver and kidneys. It is noted that hepatic tissues of the rat liver contain a high level of glutathione, which may reach 7-8 micromoles of glutathione per gram of tissue. This is congruent with what [47] indicated. Glutathione in cells limits the harmful effect of ROS on cell health by scavenging the oxidants and by opposing the proinflammatory influence of hydrogen peroxide. Because of its quantitative importance within cells, its importance has increased as an antioxidant in the elimination of ROS and as a substrate for the action of an enzyme for the reaction of GPx.

It was discovered that cysteine works on limiting the rate of generation of glutathione. The same findings were given by [48,49]. Glutathione level

was increased using the other treatments, mainly when rats were fed turmeric supplement which gave the highest glutathione content in both the liver and kidneys. In addition, turmeric supplements had critical Immunological functions including prohibiting the disorders connected with the reduction of glutathione levels [50]. Despite feeding a meal containing cysteine and methionine supplements, either individually or in combination, as in groups (2, 3 and 4), the group fed a meal containing spirulina, which the protein may reach about 65 to 70% records higher glutathione. This may explain the positive effect of higher protein intake on mortality and risk of impairment in older adults, especially among vegetarians who eat a low-protein diet. The results are similar to [51]. Phycocyanin compound from Spirulina can improve the capability of naturalistic antioxidant action [23]. Furthermore [52], showed that the algae carotenoid had an important positive impact on the activity of antioxidants. In addition, phycocyanin showed protective effects [53], due to the enhanced activity of enzymes GPx and SOD in cells [54].

### 3.5 The Effect of Several Types of Dietary Supplements on Antioxidant Enzymes Activity of Liver Rats

In general, the body's metabolism produces free radicals in equilibrium with the body's antioxidant system. [55]. However, sometimes this balance could be unexpectedly exposed to growth in free radicals of oxygen or decline in the antioxidant system, leading to a chain of an oxidative reaction, lipid peroxidation and caused oxidative damage to cells [56]. The antioxidant enzymes indicated the functional state of the body's antioxidant system that reflect the ability of the body to metabolize oxygen free radicals and defend rates tissues from oxidative damage [57]. In this trial, the supplements used enhanced the activities of the enzymes GPx and CAT

compared to the control diet as appeared from Fig. 5. GPx, the first defense line versus free radicals, which had ability to eliminate the external or internal ROS and the elimination of xenobiotic compounds in cells. GPx holds the redox system (reduced glutathione /oxidized glutathione) status in the glutathione protocol that helps protect biological components from oxidative damage. Its deficiency causes oxidative stress that not only enhances the oxidation of proteins and ribonucleic acid (DNA), but also leads to insulin resistance, dyslipidemia, inflammation, and metabolic changes, exposing a high risk of cardiovascular disorders due to cardiovascular and degenerative diseases, and aging-related [58].

However, all the supplements used have enhanced SOD activity except for the use of 500 cysteine per kg as in the second group, which led to a reduction activity of enzyme. While the activity of LDH enzyme in the liver was increased only when using each of 500 mg turmeric / kg of body weight, as well as when using dried garlic at this concentration. By comparing all the supplements used, it is clear that the highest

activity of antioxidant enzymes in the liver was obtained by using turmeric as in-group 6, then using dried garlic as in-group 7, followed by group 6, which used spirulina as dietary supplement. In recent research findings shows that turmeric lessens the content of MDA and rises the SOD and GPx level [59]. The addition spirulina significantly improved the action of antioxidant enzymes through inhibiting the formation of ROS [60]. In vitro [61] indicated that spirulina improved the activities of CAT and SOD under states of moderate stress. Also [23] discovered that spirulina boosts the activities of CAT, SOD, and GPx after the exposition of poisoning stresses.

### 3.6 The Effect of Several Types of Dietary Supplements on Level of Malondialdehyde

Increased MDA content in rat kidney and liver tissue reflects oxidative damage caused by elevated ROS, H<sub>2</sub>O<sub>2</sub>, and fatty acids oxidation like polyunsaturated, thus exposing tissues and cells to oxidative stress [62,63]. ROS can injure cellular proteins, the arrangement of DNA that

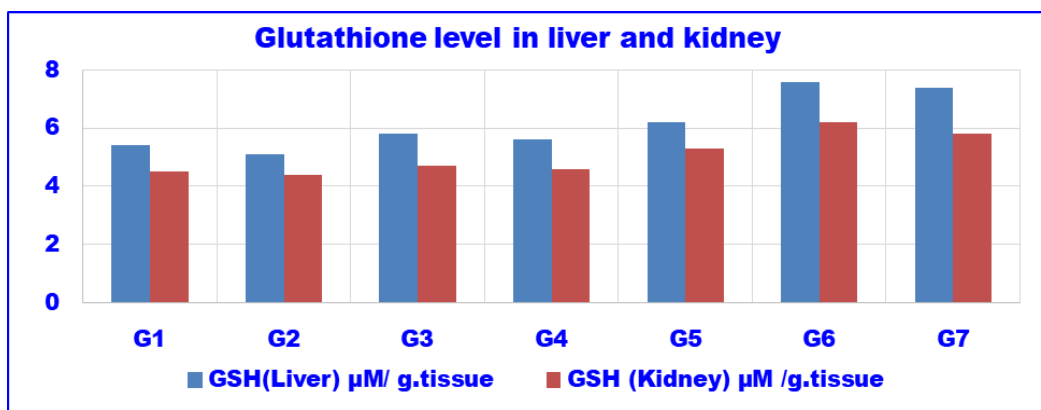


Fig. 4. The effect of several types of dietary supplements on glutathione level in liver and kidney in Rats

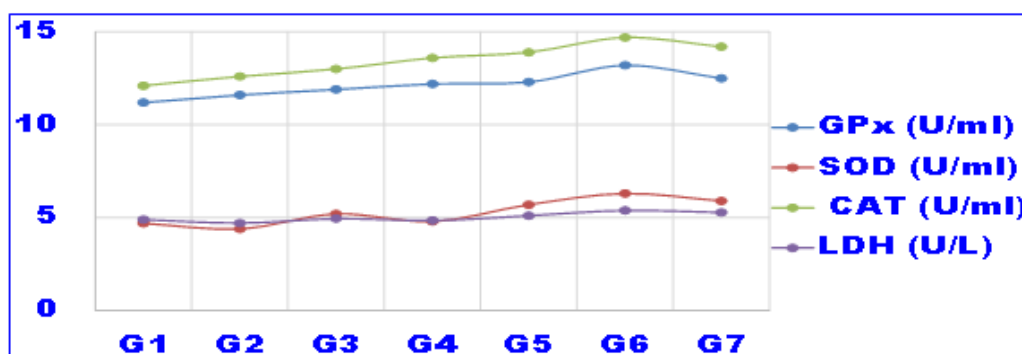
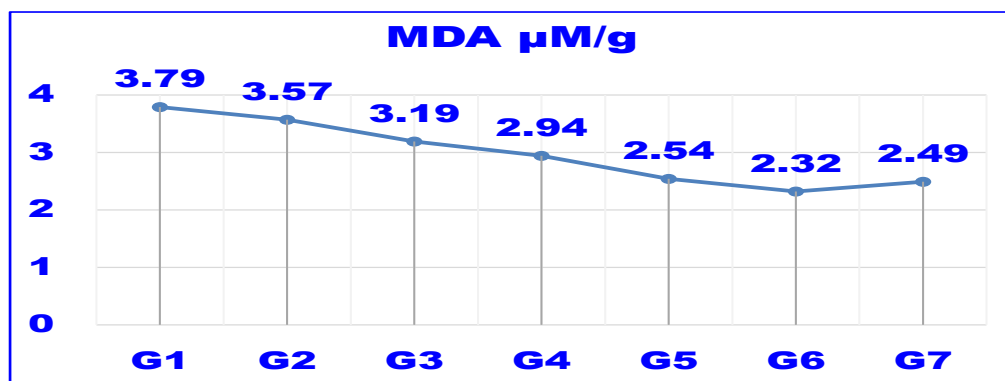


Fig. 5. The effect of several types of dietary supplements on antioxidant enzymes activity of liver Rats

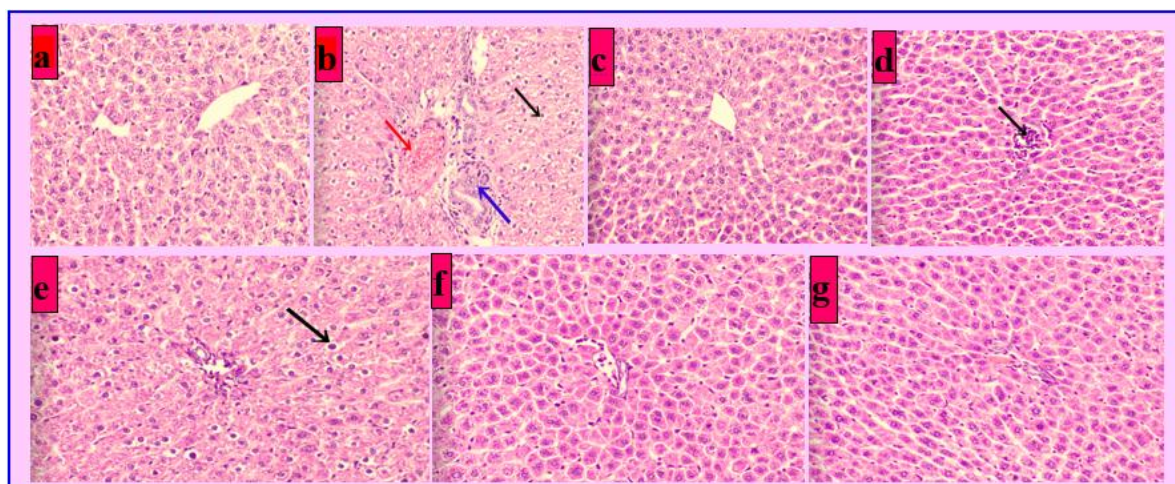


**Fig. 6.** The effect of several types of dietary supplements on level of malondialdehyde of liver Rats

finally hampers cellular protection systems like the antioxidant protection system versus toxicity [64]. Fig. 6 show that all the treatments used led to a decrease in the level of MDA at final experiment compared to the control treatment. It is also clear from the results that the use of turmeric at a concentration of 500 mg per kg as in G6 gave the lowest level of MDA and this is consistent with what was approved by [25,65]. This can be related to turmeric's capacity to increase hepatorenal polyphenolic compound concentration, ROS scavenging and metal binding [29]. Moreover, various studies have

revealed that several factors may help to protect and defend the body cells by removing radicle species to reduce oxidative stress [66]. Among these factors, turmeric encourages or stimulates increasing antioxidant enzymes action as shown in Fig. 5 such as CAT, SOD, and GPx as well as an increase in glutathione, which changing the cellular redox status which reduce the toxicity status formed by oxidative stress [67]. Our findings are similar to the research that shown turmeric's positive impact in promoting antioxidative defense versus diverse abiotic stressors in rats [25].

### 3.7 Histopathological Analysis of Rats' Liver



**Fig. 7.** a. Group (1) control displayed normal histoarchitecture of hepatic tissue. b. Group (2) indicated vacuolar degradation of hepatocytes, the blood vessels congestion of hepatoportal, hyperplasia of biliary epithelium and focal inflammatory cells infiltration. c. Group (3) exhibited no histopathological transformation. d. Group (4) no histopathological alterations except few inflammatory cells infiltrating portal triad in some sections. e. Group (5) showed no alterations with the exception of modest hydropic degeneration of few hepatocytes in certain sections. f. Group (6) no histopathological alterations except slight Kupffer cells activation. g. Group (7) no histopathological alterations except slight Kupffer cells activation



#### 4. CONCLUSIONS

Oxidative stress occurs as a result of an imbalance between reduced and oxidized glutathione inside the body, which generates many damages and health problems due to a decrease in the proportion of glutathione, which is the most powerful anti-oxidant in cells against many free radicals that cause damage to large molecules such as DNA, fats, proteins and excessive MDA. This study focuses on the search for dietary supplements that might promote and encourage the stimulate of glutathione in various organs, especially the liver, which is the body's first line defense for getting rid of free radicals that cause various harms. The ability of several dietary supplements was tested to enhance glutathione formation in different rat organs, particularly the liver, including cysteine and methionine, either alone or in certain proportions, spirulina, turmeric, and dried garlic in various concentrations. All supplements used succeeded in enhancing glutathione production except for cysteine alone at 500 mg/kg reducing glutathione formation in both the liver and kidneys. Spirulina, turmeric and dried garlic had a greater effect in increasing the formation of glutathione than the amino acids used, with the maximum effect recorded by turmeric. Tissue analysis data for all supplements showed no pathological transformations in liver tissue except for minor changes but cysteine at 500 mg/kg showed several significant pathological transformations in liver tissue. Based on the previous findings, it is recommended to use all the previous dietary supplements recommended in the research, while avoiding the use of cysteine, to increase the formation of glutathione in different organs of the body, especially the liver.

#### ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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