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Effect of some Foods on Glutathione Synthesis to Reduce Hepatic and Renal Toxicity in Rats

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Abstract

The aim of this study was to determine the physiological and histological changes in the liver and kidneys induced by glyphosate exposure in male albino rats and the ameliorative effects of broccoli and cauliflower, as well as their role in stimulating the liver to produce glutathione. The chemical composition of broccoli and cauliflower was determined, and the results showed the presence of natural antioxidants. Thirty- six animals were used for the study. NC- negative control group (received distilled water). PC (positive control group) -were used for the LD10, which was evaluated to be 375mg/kg glyphosate. GBH + GSH group -glyphosate 375 mg/kg and glutathione 50mg/kg b. wt. GBH+ Broccoli group-glyphosate 375 mg/kg and broccoli 5 g/kg b. wt. GBH+ Cauliflower group-glyphosate 3750 mg/kg and cauliflower 5 mg/kg b. wt. GBH+ Broccoli+ Cauliflower group-glyphosate 375 mg/kg, broccoli 2.5 mg/kg b. wt. and cauliflower 2.5 mg/kg b. wt. for eight weeks. The levels of antioxidant enzymes and glutathione in the liver, kidney, and serum were measured at the end of the experiment. According to our findings, glutathione peroxidase and glutathione – s transferase were enhanced in positive groups, whereas reduced glutathione was decreased. In the positive group, liver enzymes were significantly different from those in the negative control group and kidney enzymes in the positive group were considerably different from those in the negative groups.But when broccoli and cauliflower were given, this led to a significant improvement in both liver and kidney functions. Also, oral administration of glutathione has been demonstrated to reduce the toxic effect of glyphosate and improve liver and kidney function, although glutathione can be synthesized in the liver which resulted in a reduction in the negative effects of glyphosate. Histological changes in the liver and kidneys were determined which confirmed what the physiological results showed. In conclusion, Glyphosate exposure was observed to cause toxicity in rats' hepatic and renal functions, which was exacerbated by broccoli and cauliflower consumption,which in turn stimulates the production of glutathione.

Keywords: broccoli, cauliflower, glutathione, glyphosate, toxicity.

1. Introduction

Brassica vegetables such asbroccoli andcauliflower are popular and are among the most consumed vegetables in the world. Brassicas are known to possess antioxidant activity, mainly due to the presence of phenols, flavonoids, and glucosinolates and have been linked to a variety of health-promoting properties [1]. Broccoli is a good source of vitamins, minerals, and flavonoids in the diet, but its high levels of glucoraphanin have been related to health benefits like cancer prevention and increased endogenous antioxidant production [2]. Cauliflower belongs to the most valuable vegetables because it has high nutritive value, is tasty and is easy to prepare. Cauliflower is considered as one of the highest antioxidative activities among plants [2]. In the form of its inactive precursor, glucoraphanin, sulforaphane (SFN) is a chemical found in broccoli and cauliflower (GFN). SFN is formed when myrosinase enzymatically hydrolyzes glucoraphanin under certain chemical conditions [3].

Humans, animals, plants, and microorganisms all have glutathione (GSH). GSH exists in both reduced and oxidized forms within cells. The majority of GSH in healthy cells is in the reduced form. GSH can be produced by almost every cell in the human body using glutamate, cysteine, and glycine. GSH is a cofactor or substrate for GSH peroxidase and GSH S-

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transferase (GST), two key antioxidant and detoxifying enzymes [4].

Glutathione is formed of glutamic acid, cysteine, and glycine in cells. Methionine and serine produce cysteine and glycine, respectively, while -ketoglutarate, a glucose metabolite, and produces glutamic acid [5]. Park, et al. discovered an increase in the protein-bound form of glutathione in human blood after oral administration, but no change in glutathione in the deproteinized fraction [6].

Herbicides based on glyphosate are the most widely used pesticides in the world [7]. Glyphosate (GBH) residues have been found in foods and drinking water [8]. Several studies have found that glyphosate and its commercial formulations have non-target effects on mammalian metabolism and are toxic, especially to the structure and function of the liver and kidneys [9].
In terms of the risks posed to humans, the International Agency for Research on Cancer (IARC) of the World Health Organization (WHO) has placed the glyphosate herbicide into the 2A Group (probably carcinogenic to humans) and the Food and Agriculture Organization (FAO) reported that glyphosate and its major metabolite, aminomethylphosphonic acid (AMPA), are of potential toxicological concern, mainly as a result of the accumulation of residues in the food chain[10].

The aim of this study was to show that some foods, such as broccoli and cauliflower, can stimulate the liver to create glutathione, which is an antioxidant that protects the body when it is exposed to hazardous compounds like glyphosate.

2-Materials and Methods

2.1. Materials

Broccoli and cauliflower: Broccoli and cauliflower were purchased from the local produce market in Benha, Egypt. A food processor was used to grind the broccoli and cauliflower after they dried. The powder was then filtered through a mesh and stored at 20 °C for future analyses [11].

Central Pesticide Laboratory (CPL), Agricultural Research Center (ARC), Giza, Egypt provided glyphosate (WSC Monsanto Co.). Biodiagnostic Co., Giza, Egypt, provided the diagnostic kits used in this investigation.

2.2.Methods

Moisture, crude protein, crude fibers, fats and total carbohydrates of Broccoli and cauliflower were determined using A.O.A.C.'s procedures [12].

2.2.1. HPLC Determination of total Flavonoids content and total Phenolic content:

Total phenolic content was estimated by the Folin–Ciocalteu colorimetric method based on the procedure previously described by Singleton and Rossi using gallic acid as a standard phenolic compound [13]. The vegetable extracts obtained for total phenol analysis were also used for total flavonoid analysis using a colorimetric method described by Zhishen et al [14].The high performance chromatography system (Agilant Germany) 1200 equipped with a variable wave length detector. Additionally, the HPLC was equipped with an auto sampler, a degasser for the quaternary pump, and a column compartment. The experiments were conducted on a C 18 reverse phase (BDS 5 µ m, Labio, Czech Republic) packed stainless steel column (4×250mm, id).

2.2.2. Biological experiment

Animals and treatments:In the current experiment, 36 male albino rats weighing 100- 120 g were used. The animals are housed in the animal house of the Food Technology Research Institute., Agric. Res. Center, Giza, Egypt,under normal healthy conditions. For 56 days, the animals had free access to tap water and were fed a baseline diet. The rats were randomized into six groups (n = 6) at random as follows:

Negative control group (NC) rats were given distilled water every day. Positive control group (PC)Glyphosate was given orally to rats at a dose of 375 mg/kg body weight [15]. Glyphosate and glutathione group (GBH +GSH), rats were administered glyphosate orally at 375 mg/kg body weight (10% LD50) +glutathione at a dose of 50 mg/kg body weight [16]. glyphosate andbroccoli group (GBH+Broccoli), Glyphosate was given to rats at a dose of 375g/kg body weight (10% LD50) in combination with broccoli at a dose of 5g/kg body weight. Glyphosate andcauliflower group (GBH+Cauliflower), Glyphosate at a dose of 375 mg/kg body weight (10 percent LD50) was administered to rats, along with cauliflower at a dose of 5 g/kg body weight. Glyphosate, broccoli andcauliflower group (GBH+Broccoli+Culiflower), Glyphosate at 375 mg/kg body weight (10 percent LD50) was given to rats, along with broccoli extracts at 2.5 g/kg body weight and cauliflower extract at 2.5 g/kg body weight.

The animals were housed in a controlled environment with a 12-hour light-dark cycle. The rat's body weight was measured at the start of the experiment and at 1-week intervals for 12 weeks

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2.2.3. Blood sampling

According to Van-Herck, blood samples were taken through retro-orbital puncture at the end of the experiment to get hemolysis-free clear serum [17]. The serum was extracted from the blood samples after centrifugation at 1500rpm for 10 minutes. It was then stored in clean eppendorf tubes until analysis.

2.2.4. Preparation of tissue homogenate

A portion of liver and kidney were taken fresh from each animal at the end of the 8 weeks and stored on ice. Using an electrical tissue homogenizer (Orto-Alresa, Spain), homogenates of the tissues were prepared in 1.0 ml of phosphate buffer per 100 mg of tissue. The samples were spun at maximum speed at 4°C for the biochemical examination, and the supernatant was used.

2.2.5. Determination of superoxide dismutase (SOD)

Superoxide dismutase activity was measured spectrophotometrically by blocking epinephrine autoxidation, as explained byMisra and Fridovish [18].

2.2.6. Determination of catalase

Góth method for measuring catalase (CAT) was used by using commercial kit from Biodiagnostic, Cairo, Egypt [19].

2.2.7. Determination of malondialdehyde

To analyze malondialdehyde in the liver, kidney and serum samples were measured according to Kartavenka, [20].

2.2.8. Glutathione peroxidase

According to Flohe and Gunzler, (GPx) was measured in serum, liver, and kidney [20].

2.2.9. Determination of glutathione (GSH) concentration

The total glutathione content was determined using the glutathione reduced colorimetric method developed byBeutler et al.by using commercial kit from Biodiagnostic (Cairo, Egypt) [22].

Total GST activity was measured spectrophotometrically using substrates of 1 mM 1-chloro-2, 4-dinitrobenzene (CDNB) and 1 mM glutathione [23].

2.2.10. Liver enzyme

The modified kinetic technique of Wilson and Islam was used to measure serum alanine aminotransferase (ALT) activity [24]. The modified kinetic technique of Schumann and Klauke was used to quantify serum aspartate aminotransferase (AST) activity [25]. The method of Tietz and Shuey was used to measure serum alkaline phosphatase (ALP) [26]. According toKeyser, the concentration of serum albumin was measured [27]. According to Westwood, the concentration of serum bilirubin was calculated [28]. The kits for ALT, AST, and ALP were products of Randox Laboratories Limited (Crumlin, United Kingdom).

2.2.11. Renal Functions

Commercial kits were used to determine these functionalities (Biomed. Company, Germany). Urea was measured using the method provided by Chaney and Marbach [29], uric acid was measured using the method published by Trinder[30], and creatinine was measured using the method described by Jaffé [31]. The serum acid phosphatase was measured using the method of Fabiny-Byrd andErtingshausen [32].

2.2.12. Histopathologicalanalysis

Small pieces of fresh hepatic and renal tissues were fixed in formalin 10% immediately after removing from the abdominal cavity. After 24 h, tissue specimens were washed, dehydrated with ethanol and embedded in paraffin wax. Paraffin blocks were cross-sectioned using a rotary microtome,Germany. Cross sections were rehydrated in distilled water, stained with hematoxylin and eosin (H&E×400), and then examined by light microscopy andphotographed.

2.2.13. Statistical analysis

Data generated were expressed as mean ± SD. Statistical significance of difference was determined using the program SPSS 25 by performing one-way ANOVA with post-hoc comparisons between the control group and each of the treated groups by Ducan's multiple comparison test. A p-value less than 0.05 was considered statistical significant.

3. Results and discussion

Our results showed the chemical composition of broccoli and cauliflower (table 1). There was a difference in the chemical composition of broccoli and cauliflower, crude fibers in broccoli was 2.36±0.33 and cauliflower was 2.29±0.93. Total carbohydrates in broccoli (20.82±1.97) significantly increased than total carbohydrates in cauliflower (13.15±1.16).HPLC analysis of broccoli and cauliflower indicate that plenolic content was 17.14±2.07 while Total Flavonoid Content was 5.02±1.03 in broccoli but in cauliflower, total Phenolic Content was 17.23±1.26 and Total Flavonoid Content was 0.95±0.03 and this in

agreement with Bhandariand Kwak,[33].Consumption of polyphenol-rich diet protects lymphocytic deoxyribonucleic acid (DNA) from oxidative damage and work as antioxidants. A similar protective effect was evident for the beverages rich in polyphenols [34]. Polyphenols not only protect the cell and cellular components from oxidative damage but also reduce the risk of oxidative stress linked to different degenerative diseases [35].

Table (1)
Chemical composition of broccoli and cauliflower

	Broccoli	Cauliflower
Moisture (g/100 g)	72.77±4.64^{e}	78.34±2.10^{e}
crude protein (g/100 g)	2.24±0.52^{b}	2.68±0.65^{b}
crude fibers (g/100 g)	2.36±0.33^{b}	.29±0.93^{b}2
Fat (g/100 g)	0.35±0.03^{a}	0.43±0.01^{a}
Total carbohydrate (g/100 g)	20.82±1.97^{c}	13.15±1.16^{c}
Total Phenolic Content (mg GAE/g DW)	17.14±2.07^{d}	17.23±1.26^{d}
Total Flavonoid Content (mg CE/g DW)	5.02±1.03^{c}	0.95±0.03^{a}

All values are expressed as mean ±SD. Values given represent means of three determinations.GAE, gallic acid equivalent; CE, catechin acid equivalent

Effect of the treatments on body and organs weight

After glyphosate administration, there was a significant difference in rat body weight between the control group and the 375 mg/kg GLP group (p 0.05). In the glutathione therapy group, body weight increase was likewise significantly lower than in the control group (p 0.05). Significant difference was also observed in the average daily gain and average daily feed intake in positive control group compared with the control group ($p < 0.05$). Glyphosate toxicity of rats did not result in mortality throughout the treatment period, but Weight loss was observed in those who were given the glyphosate [36].

The absolute and relative organ weights for the liver and kidney were significantly lower (p 0.05) in the 375 mg/kg GLP group, showing that GLP toxicity is predominantly directed at growth and development. Through this study, the significant effect of glyphosate on body weight or organs weight were shown, as body weight decreased, while liver and kidneys weight increased in rats treated with glyphosate, and body weight loss in mice may be due to a weakening of appetite in these rats, or may be attributed to its corrosive action on the digestive system[37, 38].

Table (2)
Effect of the treatments on body and organ weight

	NC	PC	GBH + GSH	GBH+ Broccoli	GBH+ Cauliflower	GBH+ Broccoli +Cauliflower
Initial body weight(g)	102.34 ±10.22^{a}	105.65 ±11.58^{b}	103.15 ±10.23^{a}	105.83 ±12.10^{b}	105.47 ±9.93^{b}	104.34 ±10.35^{b}
Final body weight(g)	280.80 ±18.45^{d}	212.54 ±15.21^{a}	255.22 ±17.80^{c}	242.03 ±16.65^{bc}	238.59 ±16.11^{b}	240.47 ±19.09^{bc}
Average daily gain (g)	3.18 ±0.86^{d}	1.90 ±0.12^{a}	2.71 ±0.57^{c}	2.43 ±0.60^{b}	2.37 ±0.62^{b}	2.43 ±0.44^{b}
daily feed intake (g)	19.86 ±2.36d	14.35 ±1.80a	17.09 ±1.95^{c}	16.36 ±1.99^{b}	16.22 ±2.13^{b}	17.91 ±2.08^{c}
Liver weight (g)	12.33 ±1.31^{a}	13.58 ±1.02^{b}	12.48 ±0.93^{ab}	12.70 ±0.86^{ab}	12.23 ±1.04^{a}	12.99 ±1.29^{ab}
Relative liver weight (%)	4.40 ±0.92^{a}	6.38 ±0.67^{c}	4.88 ±1.02^{a}	5.24 ±1.19^{ab}	5.13 ±0.79^{ab}	5.40 ±0.39^{b}
Kidney weight (g)	4.22 ±1.36^{ab}	5.78 ±1.94^{c}	4.69 ±0.93^{b}	4.15 ±1.02^{a}	5.05 ±1.14^{bc}	4.87 ±1.11^{b}
Relative Kidney weight (%)	1.50 ±0.09^{a}	2.71 ±0.51^{c}	1.83 ±0.15^{ab}	1.71 ±0.61a	2.12 ±0.08^{bc}	2.02 ±0.61^{b}

Each value is the mean of n=6 animals ± standard deviation.
When comparing treatments at P 0.05, lowercase letters indicate significant differences
NC-Negative control group.PC-Positive control group.GBH + GSH - 375mg/kg b. wt. Glyphosate +50 mg/kg b. wt.Glutathione. GBH+ Broccoli - 375mg/kg glyphosate +broccoli 5g/kg b. wt. GBH+ Cauliflower - 375mg/kg glyphosate +cauliflower5g/kg b. wt. GBH+ Broccoli +Cauliflower - 375mg/kg glyphosate +Broccoli 2.5g/kg b. wt. + Cauliflower 2.5g/kg b. wt.

Effects of the treatments on activities of glutathione peroxidase (GPx), glutathione reductase (GR), and glutathione-S-transferase (GST)

Table (3) shows a significant increase in the concentration of the enzyme glutathione peroxidase (GPX) in the serum of the positive control group (125.42±11.73) compared to the negative control group (98.05 ±9.34), but gradual improvement in the concentration of the (GPX) was observed with the addition of broccoli, cauliflower, and glutathione, but it did not reach the level of the negative control group. The activity of glutathione peroxidase in the liver and kidney did not differ significantly between the negative control group and the groups treated with broccoli, cauliflower, and glutathione.

Although there were significant differences between the negative control group and positive control groups in (GSH) level in liver and kidney tissue, the decrease in the concentration of reduced glutathione (GSH) in serum was significant in the positive control group (20.47±3.10) compared to the negative control group (35.19±5.27). However, improvement in groups treated with broccoli, cauliflower, and glutathione compared to the positive control group, and although there were significant differences between the negative control group and positive control groups in (GSH)

El-Shenawy studied examined how glyphosate, alone or in combination with the herbicide Roundup, affected the levels of hepatic reduced glutathione (GSH) and lipid peroxidation in rats as indicators of antioxidant status and oxidative stress. Table 3 reveals that the amount of glutathione S-transferase enzyme (GST) in all treatment groups treated with broccoli, cauliflower, and glutathione was nearly similar, despite being much lower than the positive control group. The findings of this study indicated that glyphosate causes oxidative damage [39, 40].

Table (3)
Effects of the treatments on activities of glutathione peroxidase (GPx), glutathione reductase (GR), and glutathione-S-transferase (GST)

	NC	PC	GBH + GSH	GBH+ Broccoli	GBH+ Cauliflower	GBH+ Broccoli+ Cauliflower
SERUM						
GPxµmol/mL	117.43 ±12.23^{a}	380.35 ±18.63^{d}	144.82 ±11.34^{a}	208.22 ±15.11^{b}	227.19 ±15.61^{c}	204.52 ±14.97^{b}
GSH mg/dl	35.19 ±5.27^{d}	20.47 ±3.10^{a}	25.10 ±4.14^{b}	23.16 ±4.82^{ab}	28.58 ±5.70^{c}	30.82 ±3.73^{cd}
GST µmol/min/ml	1.41 ±0.92^{d}	0.95 ±0.17^{a}	1.12 ±0.62^{b}	1.19 ±0.55^{bc}	1.04 ±0.93^{ab}	1.23 ±0.72^{c}
LIVER						
GPxµmol/mL	275.78 ±15.92^{a}	370.19 ±22.61^{d}	339.21 ±20.12^{cd}	320.55 ±18.36^{c}	283.14 ±15.10^{ab}	289.47 ±12.33^{b}
GSH mg/dl	40.23 ±5.82^{d}	22.47 ±2.11^{a}	35.01 ±4.39^{c}	34.11 ±3.29^{c}	26.92 ±2.96^{b}	32.71 ±4.20b^{c}
GST µmol/min/ml	8.34 ±1.64^{a}	22.46 ±4.25^{c}	10.43 ±1.93^{ab}	17.26 ±2.74^{b}	18.35 ±2.77^{b}	9.13 ±1.92^{a}
KIDNEY						
GPxµmol/mL	98.05 ±9.34^{a}	125.42 ±11.73^{c}	102.93 ±9.22^{ab}	112.28 ±10.46^{b}	108.30 ±9.39^{b}	102.58 ±8.19^{ab}
GSH mg/dl	8.43 ±1.57^{d}	5.33 ±1.04^{a}	6.14 ±1.96^{b}	6.89 ±0.99^{b}	6.17 ±1.39^{b}	7.03 ±1.45^{c}
GST µmol/min/ml	0.95 ±0.06^{a}	3.35 ±1.55^{e}	1.43 ±0.95^{ab}	2.94 ±0.72^{d}	2.33 ±0.93^{c}	1.73 ±0.89^{b}

Each value is the mean of n=6 animals ± standard deviation.

When comparing treatments at P 0.05, lowercase letters indicate significant differences

NC-Negative control group.PC-Positive control group.GBH + GSH - 375mg/kg b. wt. Glyphosate +50 mg/kg b. wt.Glutathione. GBH+ Broccoli - 375mg/kg glyphosate +broccoli 5g/kg b. wt. GBH+ Cauliflower - 375mg/kg glyphosate +cauliflower5g/kg b. wt. GBH+ Broccoli +Cauliflower - 375mg/kg glyphosate +Broccoli 2.5g/kg b. wt. + Cauliflower 2.5g/kg b. wt.

Effect of the treatments on the antioxidant enzymes in serum, liver and kidney tissue

Table 4 shows that, the concentration of the enzyme super oxide dismutase in the positive control group decreased to (39.07±5.31) when compared to the negative control group of liver tissue. SOD was also decreased in the positive control group kidney tissues (78.12±7.02), which showed a significant difference when compared to the control group. When compared to the negative control group, the groups that dealt with broccoli, cauliflower, and glutathione showed progressive improvemen.

The enzyme catalase (CAT) concentration in the serum, liver, and kidney tissue (42.02±6.27, 16.23±3.44 and 75.22±8.25 respectively) significantly decreased in the positive control group compared to the negative control group (30.22±5.65, 12.47 ±2.58 and 120.73 ±12.36 respectively) but not

in all of the treatment groups treated with broccoli, cauliflower, and glutathione compared to the negative control group, as shown in table (4). Malondialdehyde (MDA) levels in serum, liver, and kidney tissue were significantly greater in the positive control group than in the negative control group. Also in table (4) showed the broccoli, cauliflower and glutathione consumption was associated with decreasing in malondialdehyde (MDA) in the treated groups compared with positive control group [36-41]. Treatment with broccoli extracts and SF increased activities of CAT and the MDA content in cells was gradually decreased with the increase of broccoli extracts, indicating that broccoli extracts could inhibit lipid peroxidation and improve antioxidant capacity [42]. Increased lipid peroxidation, which was triggered by excessive generation of ROS, was thought to be the cause of the elevated MDA levels. The lowered antioxidant enzyme activity in rats exposed to organophosphorus pesticides could be attributable to ROS's (reactive oxygen species) direct negative effects [43].

Table (4)
Effect of the treatments on the antioxidant enzymes in serum, liver and kidney tissue

	NC	PC	GBH + GSH	GBH+ Broccoli	GBH+ Cauliflower	GBH+ Broccoli+ Cauliflower
SERUM						
SOD(U/ml)	12.63 ±2.19 ^{c}	10.86 ±1.83^{a}	11.21 ±2.52^{b}	10.53 ±1.37^{a}	10.96 ±1.03^{a}	11.81 ±2.93^{b}
CAT(U/ml)	30.22 ±5.65^{a}	42.02 ±6.27^{d}	35.21 ±4.22^{c}	32.06 ±3.72^{b}	30.49 ±3.92^{a}	31.34 ±4.29^{a}
MDA(nmol/ml)	20.73 ±3.82^{a}	25.30 ±4.11^{c}	22.48 ±3.98^{b}	23.21 ±3.15^{bc}	21.38 ±2.99^{ab}	20.95 ±3.05^{a}
LIVER						
SOD(U/mg protein)	50.34 ±8.40^{d}	39.07 ±5.31^{a}	47.34 ±6.46^{c}	48.56 ±6.90^{c}	44.27 ±5.38^{b}	47.12 ±6.18^{c}
CAT(U/mg protein)	12.47 ±2.58a	16.23 ±3.44c	14.38 ±2.04bc	13.08 ±2.57b	13.59 ±3.04b	12.62 ±1.94a
MDA(nmol/mg protein)	2.01 ±1.05^{a}	3.93 ±1.09^{d}	2.52 ±0.93b^{c}	2.99 ±0.79^{c}	2.49 ±0.72^{b}	2.12 ±0.87^{ab}
KIDNEY						
SOD(U/mg protein)	95.47 ±10.72^{d}	78.12 ±7.02^{a}	82.19 ±7.95^{b}	84.62 ±8.38^{c}	84.17 ±6.30^{c}	90.93 ±8.31^{cd}
CAT(U/mg protein)	120.73 ±12.36^{c}	75.22 ±8.25^{a}	90.24 ±9.51^{b}	87.48 ±9.12^{b}	89.15 ±8.22^{b}	79.13 ±6.71^{ab}
MDA(nmol/g protein)	3.45 ±0.94^{ab}	5.82 ±1.03^{d}	4.03 ±1.07^{c}	3.20 ±0.98^{a}	3.88 ±0.72^{b}	3.51 ±0.69^{ab}

Each value is the mean of n=6 animals ± standard deviation.

When comparing treatments at P 0.05, lowercase letters indicate significant differences

NC-Negative control group.PC-Positive control group.GBH + GSH - 375mg/kg b. wt. Glyphosate +50 mg/kg b. wt.Glutathione. GBH+ Broccoli - 375mg/kg glyphosate +broccoli 5g/kg b. wt. GBH+ Cauliflower - 375mg/kg glyphosate +cauliflower5g/kg b. wt. GBH+ Broccoli +Cauliflower - 375mg/kg glyphosate +Broccoli 2.5g/kg b. wt. + Cauliflower 2.5g/kg b. wt.

Effect of the treatments on Liver function

Examination of the data from table (5) showed that AST activity in positive control group (76.01) was significantly higher compared with negative control group (46.79). However, as can be seen from table (5) there was an increasing trend in AST activity in GBH + GSH group, GBH+ Broccoli group, GBH+ Cauliflower and GBH+ Broccoli + Cauliflower group compared with control group.

The PC and GBH+ Broccoli groups had significantly increased ALT activity than the control group (p 0.05). There was a significant increase in ALT activity in the GBH+ Cauliflower group (62.51) compared to the control group (34.27), but no significant increase in ALT activity in the GBH + GSH group (42.19), GBH+ Broccoli+ Cauliflower group (39.10) groups (Figure 1). As compared with the control group, the PC (184.83) group had a significant (p 0.05) increase in ALP activity. In comparison with the control group (77.92), ALP activity increased in the GBH + GSH (115.35), GBH+ Broccoli (125.62), GBH+ Cauliflower (117.33), and GBH+ Broccoli+ Cauliflower (89.27) groups table (5).

. Following oral exposure of rats to the herbicide glyphosate, we observed significant increases in blood parameters associated with liver damage (AST, ALT, ALP), which agrees with previous findings [41, 44]. The hepatotoxicity of glyphosate is linked to cell membrane destruction, which causes intracellular

enzymes (ALT, AST) to seeps into the bloodstream [45]. Other investigations found that glyphosate increased the rate of production of apurinic/apyrimidinic DNA sites in the liver, causing oxidative DNA damage. This could be linked to a change in the expression of genes involved in the induction/repair of DNA damage. Genes whose expression is consistently affected by both glyphosate and Roundup MON 52276 could be used as a biomarker for early carcinogenesis events [39].

Glyphosate is an organophosphate herbicide that has been demonstrated to cause oxidative stress and/or inhibit antioxidant defense mechanisms in some studies. Increased hepatic TBARS (thiobarbituric acid reactive substance) was discovered when pregnant rats and their offspring were administered a glyphosate formulation in their drinking water during the gestational period [46]. Glyphosate was also administered intraperitoneally to rats at doses of 270 and 135 mg/kg every two days for one or two weeks, which increased serum hepatic enzyme activity, increased lipid peroxidation, and depleted GSH in the liver [39]. Statistically, the levels of AST and ALT enzymes showed an increase in the serum of treated groups compared to those of controls in the present study after days of exposure to glyphosate [47].

Table (5)
Effect of the treatments on liver function (AST, ALT, ALP, Albumin, and bilirubin

	NC	PC	GBH + GSH	GBH+ Broccoli	GBH+ Cauliflower	GBH+ Broccoli+ Cauliflower
AST(IU/ml)	46.79 ±6.84a	76.01 ±12.96d	55.74 ±9.07b	65.03 ±10.92c	52.34 ±9.34b	51.81 ±9.57b
ALT(IU/ml)	34.27 ±6.23a	70.26 ±11.03d	42.19 ±8.36ab	49.96 ±7.05b	62.51 ±9.65c	39.10 ±6.24a
ALP(IU/ml)	77.92 ±10.58a	184.83 ±18.93d	115.35 ±16.92b	125.62 ±15.37c	117.33 ±15.34b	89.27 ±8.66a
Albumin (mg/dl)	6.34 ±2.86a	20.23 ±5.38c	12.67 ±3.18b	13.18 ±3.82b	13.75 ±4.62b	8.33 ±2.02a
Bilirubin(mg/L)	0.23 ±0.07a	0.45 ±0.04d	0.28 ±0.06a	0.30 ±0.09b	0.35 ±0.02c	0.29 ±0.07ab

Each value is the mean of n=6 animals ± standard deviation.
When comparing treatments at P 0.05, lowercase letters indicate significant differences
NC-Negative control group.PC-Positive control group.GBH + GSH - 375mg/kg b. wt. Glyphosate +50 mg/kg b. wt.Glutathione. GBH+ Broccoli - 375mg/kg glyphosate +broccoli 5g/kg b. wt. GBH+ Cauliflower – 375mg/kg glyphosate +cauliflower5g/kg b. wt. GBH+ Broccoli +Cauliflower - 375mg/kg glyphosate +Broccoli 2.5g/kg b. wt. + Cauliflower 2.5g/kg b. wt.

Effect of the treatments on kidney function

Glyphosate at dose (375mg/kg) induced renal damage as reflected by significantly ($p < 0.05$) elevated serum urea, uric acidand creatinine (54.58 ±8.35, 1.24 ±0.64 and 2.04 ±1.05respectively) when compared to control group (26.72 ±5.86, 0.55 ±0.08and 0.65 ±0.14respectively) for 56 days. On the other hand, insignificant differences with recorded in glutathione group when compared to control groups. Rats treated with glyphosate + broccoli, glyphosate +cauliflower and glyphosate + broccoli + cauliflower observed a significant decrease ($p < 0.05$) when compared with positive control group. Data in table (6) also, recorded that a significant increase ($p < 0.05$) in serum acid phosphatase level in rats treated with glyphosate when compared with control groups for 56 days. On contrast insignificant differences was recorded in glutathione group when compared to control groups. Rats treated with glyphosate + broccoli, glyphosate +cauliflower and glyphosate + broccoli + cauliflower observed a significant decrease ($p < 0.05$) when compared with positive groups for 56 days.

Renal damage resulting from chronic glyphosate toxicity caused reduction of glomerular filtration as shown by elevated serum creatinine, urea and uric acid concentrations. After glyphosate administration, serum creatinine and urea were increased as previously reported following 12 weeks of exposure in rats [48].

Serum creatinine level was reported as the better biomarker of nephrotoxicity following glyphosate exposure [49].

It was also observed that in the glyphosate rats, uric acid concentration was significantly increased compared to control values. This increase was higher when the glyphosate applied alone but when applied with glutathione, broccoli or cauliflower, uric acid back to the normal value. It is known that uric acid is a valuable index of renal function in rats [50]. Therefore, the increase in uric acid level observed in the glyphosate animals study might indicate the possibility of a more severe effect on renal function.

Hepatorenal protection by broccoli and

cauliflower was attributed to its antioxidant properties which might have promoted the integrity of cell membranes and reduced the impact of oxidative stress and this is considered to be the principal factor promoting toxic injury following glyphosate exposure.

In recent study, among a group of farmers who used the pesticide glyphosate, a high index of kidney disease was found, through a high percentage of creatinine and urea [50].While another study examined the effect of glyphosate on kidney function in children at different ages and concluded that there was no evidence of a link between kidney disease and a high level of glyphosate in the urine of children [52]. To combat oxidative stress and prevent pathophysiologic alterations in the redox state, organisms have developed a number of antioxidant defenses. Antioxidants inhibit the formation of free radicals or mitigate their damaging effects by acting as an alternative substrate for oxidation, thereby preventing an oxidation reaction which would lead to the formation of free radicals, directly scavenging free radicals, or by up regulating antioxidant defenses or decreasing free radical generation, indirectly preventing the formation of oxidant chemicals. These antioxidants can be enzymatic or non-enzymatic, and they can be endogenous or exogenous from food sources. Superoxide dismutase (SOD) and catalase are examples of enzyme antioxidants that are both primary and constitutively active [53].

Table (6)

Treatment effects on kidney function (urea, uric acid, creatinine, and acid phosphatise

	NC	PC	GBH + GSH	GBH+ Broccoli	GBH+ Cauliflower	GBH+ Broccoli+ Cauliflower
Urea mg/dl)(	26.72 ±5.86a	54.58 ±8.35d	39.32 ±8.10b	42.37 ±9.57c	35.12 ± 6.83b	29.92 ±4.92a
Uric acid(mg/dl)	0.55 ±0.08a	1.24 ±0.64d	0.96 ±0.72b	1.02 ±0.26c	0.98 ±0.37c	0.89 ±0.98b
Creatinine (mg/dl)	0.65 ±0.14a	2.04 ±1.05d	0.77 ±0.83a	0.93 ±0.76c	0.97 ±0.99c	0.85 ±0.49b
Acid phosphatase(u/l)	3.43 ±1.23a	12.82 ±2.54d	7.20 ±2. 95c	5.31 ±1.83b	5.11 ±1.68b	4.92 ±1.03a

Each value is the mean of n=6 animals ± standard deviation.

When comparing treatments at P 0.05, lowercase letters indicate significant differences

NC-Negative control group.PC-Positive control group.GBH + GSH - 375mg/kg b. wt. Glyphosate +50 mg/kg b. wt.Glutathione. GBH+ Broccoli - 375mg/kg glyphosate +broccoli 5g/kg b. wt. GBH+ Cauliflower - 375mg/kg glyphosate +cauliflower5g/kg b. wt. GBH+ Broccoli +Cauliflower - 375mg/kg glyphosate +Broccoli 2.5g/kg b. wt. + Cauliflower 2.5g/kg b. wt.

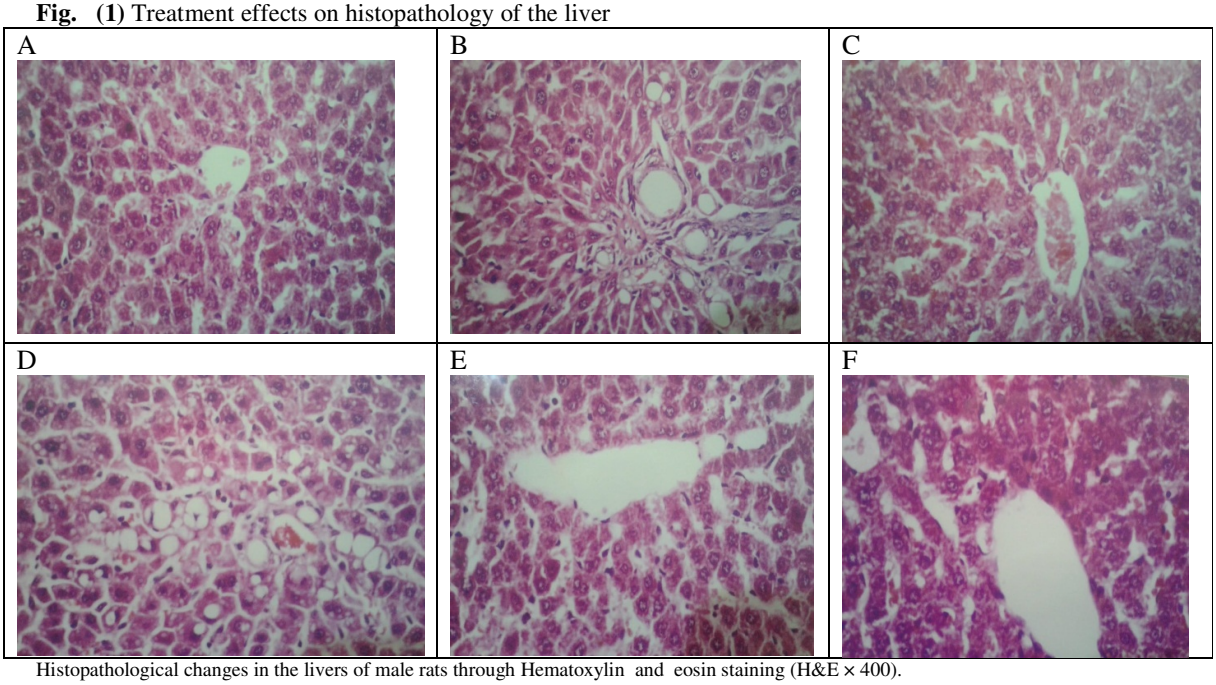
Fig (1, 2) Treatment effects on histopathology of the liver and kidneys

All treated and control animals were subjected to a histopathological evaluation of the liver and kidneys (Figure 1and 2). No histopathological changes were detected in the liver of control animals (fig.1A). In the present study, many cellular changes on thehepatic tissue of all treated groups compared to control. But in processing groups,the intake of the glutathione, broccoli and/or cauliflower reduced the negative effects of the pesticide as shown in fig. (1). The hepatotoxicity of glyphosate is associated with cell membrane damage, resulting in leakage of intracellular enzymes (ALT, AST) into the circulation [44]. Hepatic inflammation characterized by increasing numbers of mononuclear cells and proliferation of collagen fibers in the liver may be responsible for the increased serum ALP activity, in line with this, hepatic enzyme secretion is elevated following cholestasis in the presence of glyphosate-induced steatohepatitis. Damage to the liver was expected to affect the functional capacity of the liver to synthesize proteins, thereby influencing serum albumin level [44].

As shown in fig. (2) no visible changes in glomerular structure were observed in control group however, a slight dilation of renal tubules was observed throughout the kidney regions of glyphosate versus control animals (Fig. 2). In addition glyphosate fed rats exhibited a significant tubular hypertrophy and cell proliferation, which is evident mainly in the inner medulla, as indicated by the increased number of nuclei in the tubular epithelial cells, as compared with control animals (Fig. 2).Renal damage resulting from glyphosate toxicity caused reduction of glomerular filtration as shown by elevated serum creatinine concentrations, without any effect on serum urea concentrations. Elevations of both serum creatinine and urea concentrations in subchronic glyphosate toxicity was previously reported following 12 weeks of exposure in rats [48]. Serum creatinine level was reported as the better biomarker of nephrotoxicity following glyphosate exposure and its association with renal damage.Glyphosate does not only affect humans, but also affects many other organisms, as a previous study conducted on fish kidney, it was revealed that glyphosate changes the

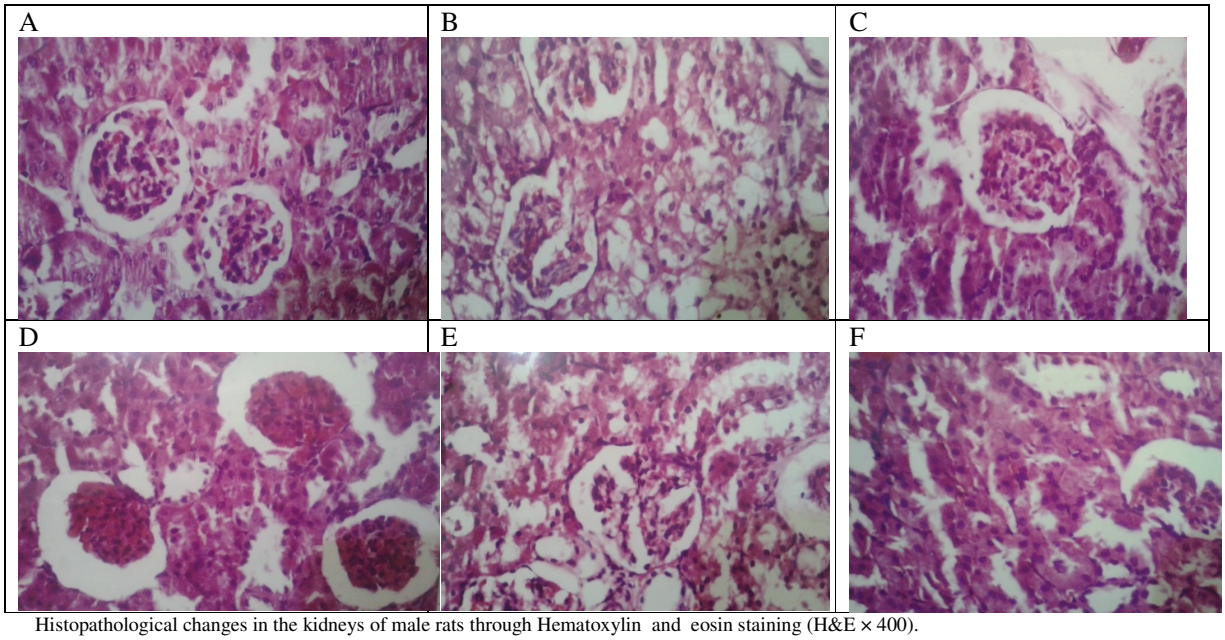
Bowman's space and leads to the accumulation of transparent droplets and pyknotic bodies in the epithelial cells of the proximal and distal convoluted tubules[54] . In another study, it was revealed that at higher doses, glyphosate leads to cellular accumulation, lympho-cytic infiltration necrosis, bleeding and deformation of the kidney cells in different parts of the renal tissue [55].

Fig. (1) Treatment effects on histopathology of the liver



Histopathological changes in the livers of male rats through Hematoxylin and eosin staining (H&E × 400).
A-Negative control group.B-Positive control group. C - 375mg/kg b. wt. Glyphosate +50 mg/kg b. wt. glutathione D - 375mg/kg glyphosate +broccoli 5g/kg b. wt. E - 375mg/kg glyphosate +cauliflower5g/kg b. wt. F - 375mg/kg glyphosate +Broccoli 2.5g/kg b. wt. + Cauliflower 2.5g/kg b. wt.

Fig. (2) Treatment effects on histopathology of the kidney



Histopathological changes in the kidneys of male rats through Hematoxylin and eosin staining (H&E × 400).
A- Negative control group. B-Positive control group. C - 375mg/kg b. wt. Glyphosate +50 mg/kg b. wt. glutathione D - 375mg/kg glyphosate +broccoli 5g/kg b. wt. E - 375mg/kg glyphosate +cauliflower 5g/kg b. wt. F - 375mg/kg glyphosate +Broccoli 2.5g/kg b. wt. + Cauliflower 2.5g/kg b. wt.

4. Conclusion

Antioxidants help fight free radicals and protect the body from its harmful effects. Glutathione is a verypowerful antioxidant, although glutathione is naturally produced in the liver, the body's daily exposure to toxins through eating foods contaminated with pesticides (glyphosate) affects the liver functions and thus affects the production of glutathione. Therefore, eating cauliflower and broccoli improves liver functions and increases its ability to produce glutathione almost completely similar to taking glutathione orallyand these effects might have been derived from the antioxidant properties of broccoli and cauliflower.

5. Conflicts of interest

There is no conflict of interest between authors.

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