التأثير العلاجي لنباتات مختلفة غنية بالجلوتاثيون على السمية الكبدية و الكلوية التي يسببها الجنتاميسين في الفئران

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التأثير العلاجي لنباتات مختلفة غنية بالجلوتاثيون على السمية الكبدية و الكلوية التي يسببها الجنتاميسين في الفئران

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الملخص العربى

تعتمد الكلى والكبد بشكل كبير على كمية كافية من الجلوتاثيون (GSH) للحفاظ على الوظيفة الطبيعية لهم، و يرجع هذا إلى انه من مضادات الأكسده التي تلعب دورا كبيرا في وقايه الجسم من الامراض. يهدف البحث إلى دراسة تأثير تناول اغذيه غنيه بالجلوتاثيون على الفئران المحقونة بالجنتاميسين والتي تعاني من السميه الكلويه والكبديه. وقد أجريت هذه الدراسة على 30فأراً بالغًا من ذكور. تم تقسيمهم الي خمس مجموعات، سته فئران كمجموعة ضابطه سالبه وتم حقن 24 فاراً باستخدام الجنتاميسين (100 ملجم / كجم / يوم لمدة 7 أيام) للحث على حدوث سميه كلويه وكبديه . بعد ذلك تم تقسيم الفئران إلى اربع مجموعات مجموعة ضابطه موجبه و ثلاث مجموعات تم علاجها باغذيه غنيه بالجلوتاثيون (الاسبرجس والافوكادو) بجرعات مختلفه لمدة سته أسابيع. أظهرت النتائج أن هناك زيادة معنوية في الوزن النهائي ، الوزن المكتسب ، نسبه الوزن المكتسب ، نسبة كفاءة الغذاء ، مستويات الدم من الهيموجلوبين ، كرات الدم الحمراء ، كرات الدم البيضاء وإلانزيمات المضادة للأكسدة. كما كان هناك انخفاض معنوي كبير في مستويات الكوليسترول ، وبعض وظائف الكلى الكرياتينين ، واليوريا ، وحمض البوليك ، و وظائف الكبد AST و ALT في جميع مجموعات الفئران المعالجة مقارنة مع المجموعه الضابطه الموجبه.واظهرت المجموعة الخامسة التي تم علاجها بالجنتاميسين بجرعة عالية (1.9 ملجم كمتوسط المأخوذ للفأر الواحد/اليوم) تحسّن في مستويات الدم ، الإنزيمات المضادة للأكسدة ، مستويات الكوليسترول ، وظائف الكبد والكلى عن المجموعه الثالثه والرابعه. جميع مجموعات الفئران التي تم علاجها لم تكشف عن أي تغييرات نسيجية. **الخلاصه:** تناول النباتات الغنية بالجلوتاثيون يساعد في الحد من سميه الكبد والكلي ويحسن من وظائفهم بشكل كبير.

الكلمات المفتاحية: الجلوتاثيون – السميه الكبديه - السميه الكلويه -جنتاميسين - الفئران.

Therapeutic Effect of Different Plants Rich in Glutathione on Gentamicin Induced Hepato- and Nephrotoxicity in Rats

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Abstract:

The kidneys and liver are highly dependent on an adequate supply of glutathione (GSH) to maintain normal function; this is due to that it is an antioxidant that plays a big role in protecting the body from diseases. The search aimed to study the effect of different plants rich in glutathione on rats injected with gentamicin suffering from hepato- and nephrotoxicity. The study was carried out on 30 adult albino male rats Sprague-Dawley strain. Beside 6 rats served as a normal control group and 24 rats were injected with gentamicin (100 mg/kg/day for 7 days i.p) to induce hepatotoxicity and nephrotoxicity. Then, rats were the reclassified into control positive group and 3 treated rat groups that administered different doses of GSH are taken by eating rich foods from it (Asparagus and Avocado) for six weeks. The results revealed that, there was a significant increase for treatment groups in blood levels of HGB, RBCs, WBCs, HDL and Serum antioxidant enzymes, and there was a significant decrease in cholesterol levels, LDL, triglyceride, some renal functions Creatinine, Urea, Uric acid, liver functions parameters AST and ALT in all treated rat groups compared with the control positive group. Group five which administered gentamicin with a high dose of GSH (1.9 mg/day as average taken per rat) had a higher improvement in the final weight, weight gain, weight gain percent, feed efficiency ratio, blood levels, Serum antioxidant enzymes, Cholesterol levels, renal and liver function parameters than three and four groups. All treated rat groups revealed no histopathological changes. In conclusion, intakes plants rich in glutathione helps reduce hepato- and nephrotoxicity and significantly improve kidney and liver function.

Key words: Glutathione - Hepatotoxicity - Nephrotoxicity - Gentamicin - Rats.

Introduction:

Glutathione is a tripeptide made up of three different amino acids: glutamate, cysteine and glycine with numerous important cell functions. Glutathione contains an unusual peptide link between the cysteine amine group and the glutamate side chain carboxyle group. Glutathione is a nucleophilic scavenger and a donor of electrons to the sulphydryl group **(Couto et al., 2013).**

It is the predominant intracellular nonprotein thiol and plays a significant role in maintaining the intracellular redox state. It could be considered an intracellular redox buffer.Glutathione protects the tissues by neutralizing free radicals, and by promoting antigen presentation and stimulating CD8 cells, it improves the immune system. Glutathione is involved in various stages of hepatic detoxification. Glutathione acts as a reducing agent, is combined with drugs to make them more water-soluble, is involved in the transport of amino acids across cell membranes (the g-glutamyl cycle), is part of peptidoleukotrienes, serves as a cofactor for certain enzymatic reactions and helps to rearrange protein disulfide bonds (**Panigrahi et al., 2018 and Duygu et al., 2022).** And it plays a role in catalysis, metabolism, signal transduction and gene expression **(Pastore et al., 2001).**

Most foods containing glutathione in large quantities are Asparagus, Avocado, Spinach, Okra Broccoli and Cantaloupe **(Pophaly et al., 2012).** Recommended Dietary Allowance (RDA) for glutathione has not been set. Dosage varies from 50:150 milligrams per day (2mg / kg). Persons with a proven glutathione deficiency may require glutathione administration **(Eran et al., 2003).**

Gentamicin is a bactericidal broad-spectrum antibiotic, commonly used in veterinary practice to treat acute serious infections.

Despite of therapeutic application, its usage is limited due to its severe acute nephrotoxicity **(Ali, 2003).**

Gentamicin belongs to a family of aminoglycoside antibiotics, is a very effective antibiotic better adapted for the treatment of serious infections and is commonly used for the prevention of Gram-negative bacterial infections **(Kaloyanides, 2000).**Unlike other amino glycosides, gentamicin causes nephrotoxicity in the renal cells by inhibiting protein synthesis. Specifically this mechanism causes cell necrosis in the proximal tubule, resulting in acute tubular necrosis that can lead to acute renal failure **(Sundin et al., 2001).**

A rise in bilirubin levels above 1.5 mg/dl or an increase in AST and ALT levels above three times the usual range was referred to as hepatotoxicity. A rise in serum creatinine of 0.5 mg/dl, or a 50% increase from baseline, was considered nephrotoxic. **(Gourang et al., 2011).**

The last ten years have seen a lot of work and interest put into reducing or protecting against aminoglycoside nephrotoxicity and hepatotoxicity. Antioxidants are one type of defense (**Fauconneau et al., 1995).** The normal diet includes a significant number of antioxidant activities, such as Glutathione, which are large-scale plant metabolites in plant foods that have an outstanding antioxidant free radical scavenging properties **(Bonorden and Pariza, 1994) and (Imen et al., 2022).**

The aim of the present study was to evaluate and compare the influence of plants rich in glutathione on the side effects of gentamicin damage that induced Hepatotoxicity and Nephrotoxicity in rats.

Materials and Methods:

Materials

Gentamicin: it was purchased from El-Gomhoria Co., Cairo, Egypt. Gentamicin is given to rats inducing renal damage according to previous studies as reported by **(Farombi. and Ekor, 2006).**

Natural Foods: Asparagus and avocado were obtained from the market of the Ministry of Agriculture in Cairo city, Egypt. They were mixed together in different proportions by (1g) = (0.5g Asparagus + 0.5g Avocado) gave 0.27mg glutathione, and added to the standard diet at 5%. And (3g) = (1.5g Asparagus+ 1.5g Avocado) gave 0.82 mg glutathione, were added to the standard diet at 15 %. While (7g) = (3.5g Asparagus + 3.5g) Avocado, gave 1.9 mg glutathione was added to the standard diet at 35%. The percentage of glutathione contained in avocado and asparagus was calculated according to (**USDA, 2020**), and these percentages were confirmed by their analysis at the Institute of Food Technology Cairo, Egypt.

Experimental Animals: Thirty adult male rats were Sprague Dewily strain weighing 145 ± 7g were purchased from the Agricultural Research Center, Giza, Egypt.

Standard Diet: Standard diet was prepared according to **(NRC., 1995).**

Methods

Experimental Rats Design: Rats were kept under observation for seven days for adaptation and fed on the standard diet. And 6 rats served as the normal control group and 24 rats were injected with gentamicin at dose a 100 mg/kg / day for 7 days intraperitoneal to induce hepatotoxicity and nephrotoxicity

(Abdullah et al., 2021), which classified into control positive group and 3 treated rat groups that treated with Asparagus and Avocado for six weeks by (0.27mg glutathione, 0.82 mg glutathione and1.9 mg glutathione) for group three, four and five respectively (as average taken per rat/day). Food and water were provided periodically. Food intake was recorded daily and the body weight of rats was measured once weekly. At the end of the experimental period (eight weeks), the rats were anaesthetized by diethyl ether and sacrificed. Blood samples of each rat were withdrawn in two test tubes. The first was a heparinized tube for estimation of some biochemical analysis and also to obtain blood pictures. The other tubes of blood were left for coagulation and then centrifuged at 3000 rpm for 15 minutes to obtain serum for further analysis. Kidneys for every rat were collected. One kidney is used to assess antioxidant enzymes and the other was submerged in 10 percent neutral formalin buffered as a fixative and then sent to the Veterinary Medicine Department of Pathology, University of Cairo for pathological analysis.

Laboratory analysis: The activity plasma was measured for blood hemoglobin (HGB), red blood cells (RBCs) and white blood cells (WBCs) were estimated according **to (Drabkin. 1949, Inory. 1954 and Cynthia et al., 1993)** respectively. Beside lipid panel, Cholesterol, high-density lipoprotein HDL, low-density lipoprotein LDL and Triglyceride it was measured according to **(Kanter. 1975 and Fossati et al. 1980)**. The activity of serum alanine and aspartate amino transferees (ALT and AST) were carried out using the Bergmeyer and Horder methods **(Bergmeyer and Horder, 1980)**, and **(Kind and King, 1954)**, respectively. Serum creatinine, urea and uric acid were enzymatically determined according to **(Bonsens and Taussky. 1984),** respectively. Total antioxidant capacity of serum was measured according to Habig **(Habig et al. 1974).**

Histopathological examination: Fixed kidney and liver samples in 10 % neutral buffered formalin were removed in xylol and embedded in a 4-5 µm section of paraffin and stained with Hemotoxic and Eosin (H and E) for subsequent histopathological examination. **(Bancraft et al., 1996).**

Total glutathione content: To assessment and determined glutathione in asparagus and avocado, the method was followed and described by **(Singleton and Rossi 1965);** it showed in table (1)

Table (1): The content of Asparagus and Avocado from glutathione

Nutrient	Quantity	Glutathione (mg)
Asparagus	**100 g**	28.3
Avocado		27.7

Statistical Analysis: SPSS computer software statistically analyzed all of the data obtained. The estimate occurred by ANOVA variance analysis and SPSS ver.11 follow-up test LSD **(Abo-Allam, 2003).**

Results and Discussion:

Nutritional Results: Data in **table (2)** showed that the control positive rat group which administered gentamicin showed a significant decrease($P< 0.01$) in the final weight, weight gain, weight gain percent, food intake and food efficiency ratio (FER) ($P<0.05$) compared with the normal control group, FER as reported by **Mohamed, (2013).** Moreover, the reduction of final body weight obtained in this study agreed with that obtained with a in recent study that confirmed that Gentamicin administered rats (toxic group) exhibited significant weight loss ($P< 0.01$) **(Mishra**

et al., 2014). Progressive weight loss in rats treated with gentamicin may be due to damage to renal tubules and subsequent loss of tubular cells to reabsorb water, resulting in dehydration and loss of body weight. Polyphenols present in grape seeds might have reduced food intake and prevented weight gain (**Fisher et al., 2002**). It should be noted that, all treated groups tended to have body weight, weight gain, weight gain percent, food intake and food efficiency ratio (FER) significantly higher than the control positive group ($P<0.05$). These results were in line with that of (**KrofičŽel et al., 2014 and Azmandian et al., 2017).** This difference of weight gain could be attributed to the catabolic state occurring as a result of acute renal failure due to gentamicin (**Sawardekar and Patel, 2015**).

Table 2: Nutritional indicators of normal control and infected rat groups treated with plants rich in glutathione

Groups		Variables					
		Initial weight (g)	Final weight (g)	Weight gain (g)	Weight gain (%)	Food intake (g)	Feed Efficiency Ratio (FER)
Control Negative		147.8 ±2.77 [c]	290.21 ±14.17 [a]	148.41 ±5.11 [a]	100.6 ±4.36 [a]	18.21 ±1.07[a]	0.051 ±0.01 [b]
Control Positive		146.1 ±2.34 [b]	212.81 ±20.93 [c]	66.71 ±4.34 [c]	45.66 ±3.27 [c]	19.02 ±0.93[a]	0.047 ±0.004 [b]
Treatment groups GSH	Low dose (0.27mg)	145.2 ±3.71 [b]	254.42 ±16.13 [b]	109.22 ±6.32 [b]	75.22 ±7. 49 [b]	17.91 ±1.9 [b]	0.077 ±0.001 [a]
	Medium dose (0.82mg)	148.4 ±4.33 [a]	262.11 ±19.81 [b]	113.71 ±7.41 [b]	76.61 ±3.33 [b]	18.71±1.61[a]	0.082 ±0.002 [a]

	High dose (1.9mg)	148.2 ±4.14 [a]	299.21 ±18.54 [b]	151.01 ±.54 [b]	102.5 ±4.48 [b]	19.01 ±0.96[a]	0.048 ±0.003 [c]

Each value is the mean ±SD.
The different letters means that there is a significant difference between groups at P <0.05 and vice versa.
GSH=Glutathione.

Kidney weight were found to be significantly increased in rats treated with only gentamicin; whereas treatment with different plants rich in GSH was found to protect the rats from such effects of gentamicin. As shown in table (3). In contrast, there no statistically significant differences were observed between treated or no treated groups with different doses of GSH derived from plans on liver weight. This result was in the line with the previous study and may be due to injected of gentamicin causing inflammation in kidney tissue which increases the weight and size of the kidney **(Jedage and Manjunath, 2016 and Dumludag et al., 2020).**

Table 3: Effect of different plants rich in glutathione and induced to gentamicin on liver and kidney weight

Groups		**Variables**	
		Kidney weight (g)	Liver weight (g)
Control Negative		0.92±0.11 [d]	7.15±0.59 [a]
Control Positive		1.25±0.04 [a]	9.22±0.94 [b]
Treatment groups GSH	Low dose (0.27mg)	1.02±0.16 [b]	6.73±0.90 [a]
	Medium dose (0.82mg)	0.96±0.19 [c]	6.94±0.78 [a]
	High dose (1.9 mg)	0.93±0. 07 [d]	7.41±0.35[a]

Each value is the mean ±SD.
The different letters means that there is a significant difference between groups at P <0.05 and vice versa.
GSH=Glutathione.

There was a significant difference in both kidney and liver weight for the positive control group, as compared to the control negative group. This result agreed with another study which confirmed that, intraperitoneal administration of gentamicin at a dose of 80 mg/kg caused a significant ($p < 0.01$) increase in liver and kidney weight **(Alinoorania et al., 2011).**

Biochemical Analysis: The data presented in **table (4)** illustrated that, the control negative group tended to have total hemoglobin, red blood cells, white blood cells and antioxidant capacity significantly higher than control positive groups that were injected with gentamicin. Even there were statistically significant differences in another study that studies the effects of glutathione anemia and erythropoietin requirements in hemodialysis patients. It observed that anemia significantly improved with treatment, due to a significant increase in RBC, WBCs and antioxidant survival **(Usberti et al. 2002)**. Adequate control of oxidative stress achieves better control of anemia. Glutathione seems to be the best antioxidant therapy so far for improving anemia and function kidney.

It was observed that, all groups suffering from renal damage and treated with different doses of GSH have approximately similar results. Even there were no statistically significant differences were observed between them. In most cases, all treated groups with different doses of GSH have total hemoglobin, red blood cells, white blood cells and antioxidant capacity lower than control positive, but higher than control negative groups.

The best results for total hemoglobin and red blood cells were noted for the group treated with a high dose of plants rich in GSH. There was no statistical difference was observed between this

group and control negative group. This result in line with **Shoshana et al (1985)**, who showed that the formation of glutathione hemoglobin (G-Hb) can be induced within erythrocytes, leading to a marked reduction in the propensity for sickling in the erythrocytes and improving RBCs and WBCs.

Table 4: Blood levels of HGB, RBCs and WBCs and Serum antioxidant enzymes in control infected rat groups treated with different plants rich in glutathione

Groups		Variables			
		HGB (g/dL)	RBCs /µL) ($\times 10^6$)	WBCs (µL)	Antioxidant (µ/ml)
Control Negative		12.08±0.74 a	4.78±0.17 a	7944.4±140.36 a	1.92±0.19 a
Control Positive		8.80±0.47 c	3.54±0.42 b	1390.1±180.87 d	0.83±0.03 d
Treatment groups GSH	Low dose (0.27mg)	10.64±0.68 b	3.97±0.16 b	4320.4±165.03 c	1.18±0.17 b
	Medium dose (0.82mg)	10.84±0.48 b	4.44±0.27 a	6013.4±150.61 b	1.42±0.19 b
	High dose (1.9 mg)	11.08±0.55 b	4.71±0.33 a	6996.5±81.7 b	1.98±0.32 a

Each value is the mean ±SD.
The different letters means that there is a significant difference between groups at P <0.05 and vice versa.
GSH=Glutathione.

The current data showed that Anemia has been observed following the administration of gentamicin in albino rats. Gentamicin induced a highly significant decrease in Hb concentration and RBCs. These results are in line with data obtained by **El-Maddawy (2014)**.One of the major causes of the Anemia induced by gentamicin treatment is Erythropoietin (Epo) deficiency following injury to the kidney **(Naeshiro *et al*., 1997)**.

Nagano *et al*. (1990) reported that a gentamicin-treated rat is a useful and convenient anemic model and recombinant human

erythropoietin (r-HuEPO) is useful for the treatment of anemia in acute renal failure. Anemia may also result from hem dilution, extravascular hemolysis as well as toxic dyshematopoiesis **(Elyazji and Abdel-Aziz, 2013)**. Anemia may be the result of lipoperoxidation changes in the erythrocyte membrane **(Ambali *et al.*, 2010)**. Moreover, the reduction appear in red blood cells (RBCs) in the control positive group may be due to the destruction of RBCs being postulated to occur by either membrane oxidation or Hb denaturation **(Ambali *et al.*, 2010).** The decrease of blood antioxidants may increase the oxidative of the erythrocyte membrane has been shown to increase the fragility of RBCs. Accordingly, increasing of blood antioxidants derived plants in the treated group saved oxidative of the erythrocyte membrane and decreased the fragility of RBCs and WBCs because oxidants can alter the surface characteristics of RBC **(Shi and Pamer, 2011)**.

Rats treated with some plants at different doses in the present study showed a significant increase in Hb concentration and RBCs. It may be due to treatment with plants improved hematological parameters due to its antioxidant properties that enhanced hematopoiesis **(Malaguarnera *et al.* 2011) and (Duygu et al., 2022)**.

The effect of different concentrations from some plants on total Cholesterol, Triglyceride, LDL-c and HDL-c in gentamicin-injected rats are presented in **table (5)**. Data in table (5) showed that the control positive rat group which administered gentamicin showed a significant increase in total Cholesterol, LDL-c and Triglyceride ($P<0.05$ and $P<0.01$), as compared with the normal control group. This result is in close agreement with **(Maha and Haneen, 2017)** who reported that total cholesterol was significantly increased in nephrotoxic rats. The present research has shown that groups that treatment with glutathione induced

substantial increases in concentrations of serum HDLc and decrease in Cholesterol, Triglycerides and LDLc compared with control positive. This is in agreement with Rashid and Khan who stated that gentamicin (80 mg / kg) treatment in rats increased serum levels of total cholesterol, triglycerides and LDL compared to control animals **(Rashid and Khan, 2017). Ademiluyi, *et al.* 2017**, showed the plasma atherogenic lipids (triglycerides and total cholesterol) were reported to increase in rats treated with gentamicin. The high triglyceride levels may be due to a 7a-hydroxylase activity inhibition**(Hussein et al., 2014).**In addition , high LDL-C levels can be due to a decreased regulation in LDL receptors **(Mustad et al., 1997)**, and this rise in LDL-c levels can also be explained by involving two enzymes, cholesterol ester hydrolase and cholesterol ester synthetase **(Azab et al . , 2015) and (Ademiluyi, *et al.* 2017).**

The injection of rats with gentamicin significantly caused a increase in serum LDL-C levels in comparison with control negative rats and all treatment groups. This study is performed in accordance with the results of **Ahmad et al. (2016),** which found that gentamicin significantly decreased HDL-C levels in rats treated for 12 days with 100 mg / kg / day compared to the control group.Also, **Ademiluyi, *et al.*,(2017)** reported that the plasma HDL-c decreased in gentamicin-treated.

Lerman *et al* in 1994 concluded that in avocado the reduction of triglycerides and cholesterol in blood plasma was favorable. Another nutraceutical factor in avocado is a mixture of high quality lipids: fatty acids w3, w6, and w9. When **Carranza et al (1997)** performed clinical trials in patients with elevated cholesterol levels; they observed that a diet supplemented with avocado resulted in a substantial decrease in low density lipoproteins ("bad cholesterol") and total cholesterol.

Visavadiya and Narasimhacharya (2009), the effectiveness of Asparagus racemosus was observed in lowering cholesterol levels and as an antioxidant in hyperlipidemic rats, the study showed that the addition of 5 g Asparagus racemosus root powder and 10 g amount as feed supplement decreased the plasma lipid profile including serum triglycerides and VLDL. **Visavadiya and Narsimhacharya (2005)**, the study stated that phytosterol (0.79 mg) and saponin (8.83 mg) of the AR root content (in addition to polyphenols, 1.69mg ; flavonoids, 0.47 mg ,ascorbic acid, 0.76 mg) could be responsible for lower cholesterol levels in hyperlipidemic rats. Phytosterols interact with and displace cholesterol from the micelles of the intestinal bile acid and thereby reduce the production of cholesterol, Saponins precipitate cholesterol from micelles and interact with bile acids in the entero-hepatic circulation, Saponins thus also reduce cholesterol levels in plasma by inhibiting its absorption **(Oakenfull and Sidhu. 1990).**

The rat groups which administered Glutathione with a low and medium, high dose of GSH showed a significant increase at ($P<0.05$) in Cholesterol, HDL, LDL and Triglyceride ($P<0.05$) compared with the normal control group. While, the rat groups which administered Glutathione with low, medium and high doses showed a significant decrease ($P<0.05$ and $P<0.01$) in total cholesterol, LDL and Triglyceride compared with the control positive group. This result in line with that of **Chu et al., (1992) and Zachara et al., (2006)** whose confirm that glutathione deficiency is closely associated with the height of HDL, LDL, and Triglyceride in blood.

Table 5: Effect of different plants rich in glutathione on lipid panel (Cholesterol, HDL, LDL and Triglyceride) of control and infected rat groups

Groups		Variables			
		Cholesterol mg/dl	HDL mg/dl	LDL mg/dl	Triglyceride mg/dl
Control Negative		78.4±2.37 ^{d}	42.6±2.07 ^{a}	24.2±3.11^{c}	76.21±3.96^{c}
Control Positive		105.8±2.59 ^{a}	28.6±5.02 ^{b}	45.4±1.81 ^{a}	100.11±7.28 ^{a}
Treatment groups GSH	Low dose (0.27mg)	93.2±1.8 ^{b}	42.6±2.07 ^{a}	36.8±2.94 ^{b}	86.42±3.78 ^{b}
	Medium dose (0.82mg)	90.2±2.65 ^{b}	43.1±1.58 ^{a}	36.1±1.87 ^{b}	77.43±3.21 ^{c}
	High dose (1.9 mg)	84.2±2.31 ^{c}	42.4±1.67 ^{b}	29.1±4.18 ^{c}	76.41±5.51 ^{c}

Each value is the mean ±SD.
The different letters means that there is a significant difference between groups at P <0.05 and vice versa.
GSH=Glutathione

Data in **table (6)** showed that the control positive rat group showed a significant increase in Creatinine, Urea, Uric acid, AST and ALT ratio (P<0.05) compared with the normal control group, this result is in agreement with **(Werner et al., 1994 and Zachara et al 2000)** whose showed that decreased plasma glutathione peroxidase activity causing an increase in uric acid, urea and creatinine, but **(Yasushi et al., 2017)** observed that decreased plasma glutathione peroxidase activity causing an increase AST and ALT in liver.

All treated groups GSH showed a significant increase (P< 0.05 and 0.001) in creatinine and ALT compared with the control negative group. While the rat group which administered

gentamicin with a low dose of GSH showed a significant increase (P< 0.05) in uric acid. Besides the rat group which administered gentamicin with a medium dose of GSH showed a significant increase (P< 0.05) in urea and uric acid compared with the normal control group. And observed a significant increase (P< 0.05 and 0.001) in AST and urea respectively for rat group which administered gentamicin with high dose GSH compared with control negative group. This result is in line with **(Lawrence, 2005)**.That showed that the kidneys are highly dependent on adequate glutathione supply (GSH) for normal function. GSH renal cell concentrations are maintained through both intracellular synthesis and transportation from outside the cell.

On the other hand its close agreement with (**Omid et al., 2014)** who observed that glutathione peroxidase (GSH-Pxs) enzyme activity in patients with chronic kidney disease (CKD) is usually lower than in healthy individuals, This research aimed at assessing the impact of Se supplementation on GSH-Px activity in patients with different stages of CKD and found that Se supplementation could increase GSH-Px activity in patients with different stages of CKD and improve kidney function. And agree with **(Sacco et al., 2016)** who affirm liver disease contributes significantly to a global mortality and morbidity burden. The pathogenesis of liver diseases caused by alcohol and non-alcohol is complex, and several factors have been identified to lead to the progressive loss of functions in the liver, including the over-generation of reactive oxygen species. Glutathione (GSH) is the most effective antioxidant synthesized in cells, with low molecular weight. GSH administration tends to be a promising method for repairing liver damage caused by oxidative stress in alcoholic and non-alcoholic liver diseases.

Table 6: Effect of different plants rich in glutathione on some renal functions parameters and AST and ALT of control and infected rat groups

Groups		Variables				
		Creatinine (mg/dl)	Urea (mg/dl)	Uric acid (mg/dl)	Ast (µ/ml)	Alt (µ/ml)
Control Negative		0.72±.08 ^{c}	34±1.58 ^{c}	1.26±0.11 ^{b}	9.41 ±1.34 ^{c}	9.41±2.07 ^{c}
Control Positive		1.74±0.10 ^{a}	82.4±6.18 ^{a}	2.82±0.51 ^{a}	29.22 ±3.27 ^{a}	22.52±2.96 ^{a}
Treatment groups GSH	Low dose (0.27mg)	1.11±0.08 ^{b}	42.8±2.58 ^{b}	2.06±0.21 ^{a}	17.62 ±1.94 ^{b}	15.41±2.71 ^{b}
	Medium dose (0.82mg)	0.81±0.07 ^{c}	38.6±1.14 ^{b}	1.52±0.17 ^{b}	13.12 ±1.58 ^{c}	13.31±1.81 ^{b}
	High dose (1.9 mg)	0.73±0.08 ^{c}	38±1.1 ^{b}	1.41±0.14 ^{b}	10.01 ±1.58 ^{c}	9.81±1.92 ^{c}

Each value is the mean ±SD.
The different letters means that there is a significant difference between groups at P <0.05 and vice versa.
GSH=Glutathione.

The present achievements were used to assess the medicinal potential of various plants rich in GSH against Gentamicin induced oxidative damage in rat kidney. From the data illustrated in table 6, all kidney enzymes have been increased significantly for the control positive group which was injected with Gentamicin as compared to the control negative group. This may be because some experimental results show that nephrotoxic drugs may modify kidney marker rates. Commonly used to track the production and degree of tubular renal damage due to oxidative stress **(Manikandan *et al.*, 2014) and (Duygu et al., 2022)**

All treated groups with plants rich in GSH tended to have renal enzymes much lower than the control positive group and approximately similar to the control negative group (healthy group).Glutathione is an essential intracellular agent that protects the cell against the damage that free radicals can do. Furthermore,

Kidney has an important role in GSH metabolism **(Tiodorović, Cvetković, 2004).**

Liver Histopathological Results: The liver of rats from the control negative group revealed the normal histological structure of the hepatic lobule (Figs. 1). On the other hand, the liver of rats from the control positive group showed thickening of Glissonian's capsule (Fig. 2), cytoplasmic vacuolation of hepatocytes (Figs. 2 & 3), fibroplasia in the portal triad and hyperplasia of the biliary epithelium (Fig. 4). However, some sections from group 3 that intake low dose of GSH showed sinusoidal leukocytosis (Fig. 5), whereas, group 4that intake medium dose of GSH showed other sections revealed no histopathological changes (Fig. 6) except cytoplasmic vacuolation of centrilobular hepatocytes (Fig. 7) in some examined sections. Moreover, the livers of rats from group 5that intake high dose of GSH revealed no histopathological changes (Fig. 8).

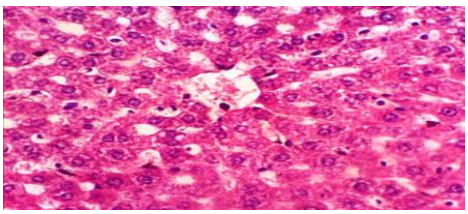

Fig. (1): Liver of rat from control negative group showing the normal histological structure of hepatic lobule(H & E X 400).

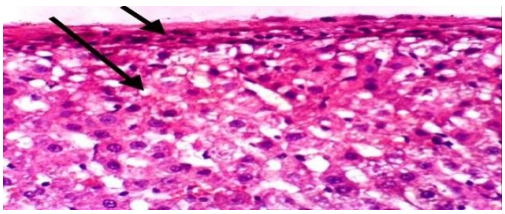

Fig. (2): Liver of rat from control positive group showing thickening o f Glissonian's capsule and cytoplasmic vacuolation of hepatocytes(H & E X 400).

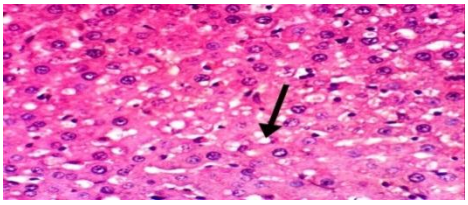

Fig. (3): Liver of rat from control positive group showing cytoplasmic vacuolation of hepatocytes(H & E X 400).

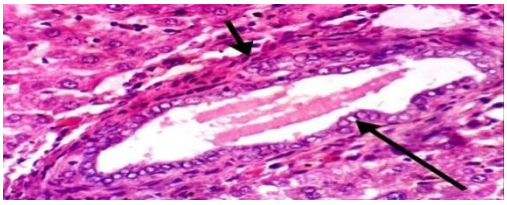

Fig. (4): Liver of rat from control positive group showing fibroplasia in the portal triad and hyperplasia of biliary epithelium(H & E X 400).

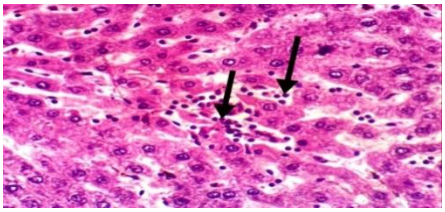

Fig. (5): Liver of rat from group 3 that intake low dose of GSH showing sinusoidal leukocytosis(H & E X 400).

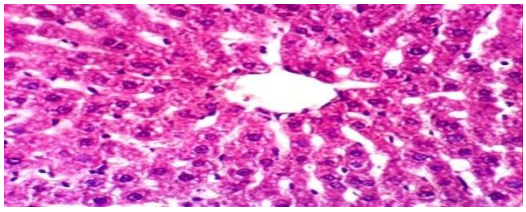

Fig. (6): Liver of rat from group 4 that intake medium dose of GSH showing no histopathological changes (H & E X 400).

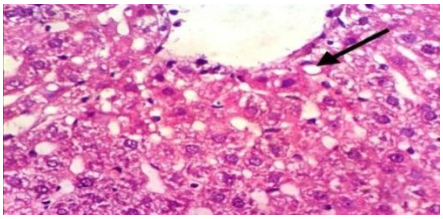

Fig. (7): Liver of rat from group 4 that intake medium dose of GSH showing cytoplasmic vacuolation of centrilobular hepatocytes(H & E X 400).

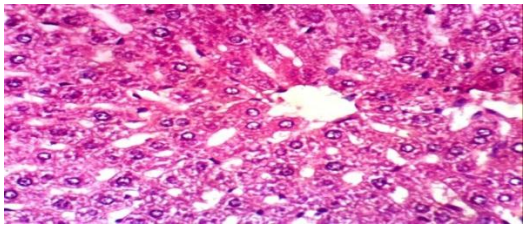

Fig. (8): Liver of rat from group 5 that intake high dose of GSH showing no histopathological changes(H & E X 400).

Kidney Histopathological Results: Microscopically, the kidneys of rats from the control negative group revealed the normal histological structure of renal parenchyma (Figs. 1). On the other hand, the kidneys of rats from the control positive group revealed cytoplasmic vacuolation of epithelial lining renal tubules, proteinaceous materials in the lumen of renal tubules (Fig. 2), congestion of glomerular tuft and focal inflammatory cells infiltration (Fig. 3). However, some sections from group 3 that intake low dose of GSH showed congestion of glomerular tuft and renal blood vessel (Fig. 4), whereas, group 4 that intake medium dose of GSH showed no histopathological changes (Fig. 5). Moreover, kidneys of rats from group 5 that intake high dose of GSH showed no histopathological changes (Fig. 6).

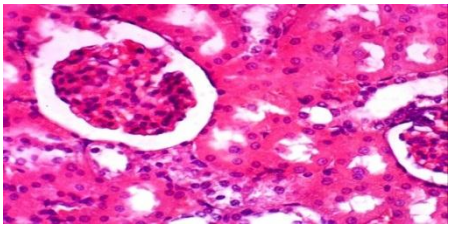

Fig. (1): Kidney of rat from control negative group showing the normal histological structure of renal parenchyma(H & E X 400).

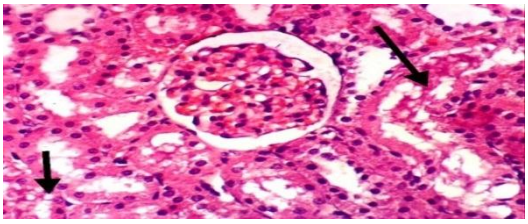

Fig. (2): Kidney of rat from control positive group showing cytoplasmicvacuolation of epithelial lining renal tubules and proteinaceous materials in the lumen of renal tubules(H & E X 400).

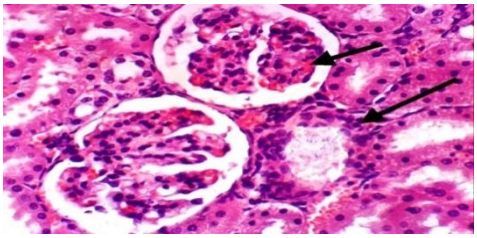

Fig. (3): Kidney of rat from control positive group showing congestion of glomerular tuft and focal inflammatory cells infiltration (H & E X 400).

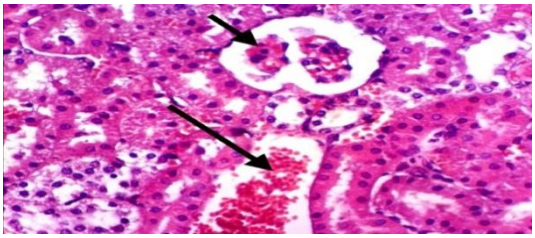

Fig. (4): Kidney of rat from group 3 that intake low dose showing congestion of glomerular tuft and renal blood vessel(H & E X 400).

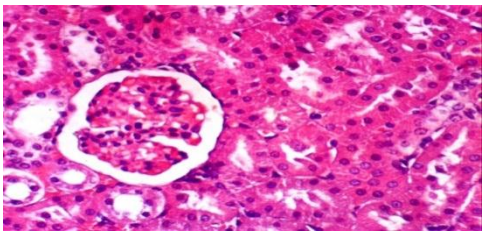

Fig. (5): Kidney of rat from group 4 that intake medium showing no histopathological changes (H & E X 400).

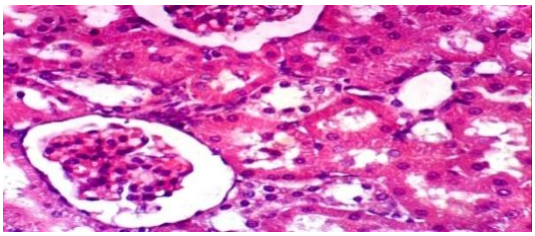

Fig. (6): Kidney of rat from group 5 that intake high dose showing no histopathological changes(H & E X 400).

Conclusion:

Glutathione (GSH) is an antioxidant and play an important role of maintain normal liver and kidneys function, this is due to many biological roles including protection against reactive oxygen and nitrogen species. Feeding rats with plants rich in glutathione about

1.9 mg (3.5g Asparagus + 3.5g Avocado), could significantly reduce acute Hepato- and Nephrotoxicity and improvement biochemical and physiological markers.

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