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Effect of Consumption Different Legumes in Diets Fortified With Black Cumin and Guava Seeds on Nephrotoxicity in Rats

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ABSTRACT

This study aimed to investigate the effect of diets contains one of casein, fenugreek, lupins or soybean) and fortified with 5% of both black cumin seeds and guava seeds. Thirty adult male rats of Sprague Dawely strain (205 ± 8g) were randomly classified into to normal control group and nephrotoxic groups that injected with cisplatin (3 mg/kg i.p) and then reclassified into 5 groups (5 rats each) as follows: Control positive, casein, fenugreek, lupins and soybean groups. In comparison with normal rats, it was found that cisplatin injection caused a significant decrease in nutritional indicators, hemoglobin, RBcs and platelets. Moreover, serum total protein, albumin, calcium and potassium and liver and kidney antioxidant enzymes were decreased. Also, injection rats by cisplatin resulted in a significant increase in liver and renal function indicators, sodium and WBcs. It is clear that the addition of black cumin seeds and guava seeds to the diet resulted in a remarkable improvement in the above mentioned indicators of nephrotoxic rats. Consumption of different legumes in diets gave more desirable results that lower nephrotoxicity from cisplatin. The soybean diet was the best among the tested diets in improvement of the above mentioned parameters compared to their corresponding in control positive group. This may be due to the combine effect of the nutritional and phytochemical components in soybeans, black cumin seeds and guava seeds which may provide a protection against cisplatin-induced structural and functional alterations in rats.

Key words: Fenugreek, Lupins, Soybeans, Black cumin seeds, Guava seeds, Nephrotoxicity, Rats.

Introduction

Cisplatin (*cis*-diamine-dichloro platinum (II), is one of the most remarkable successes in the war on cancer. Since the accidental discovery over four decades ago, cisplatin has been widely used for chemotherapy and used in the management of a variety of tumors. Its therapeutic activity is a dose dependent, but the achievement of its full therapeutic potential is limited mainly by its nephrotoxicity (Hanigan and Devarajan, 2003 and Yao *et al*., 2007).

Cisplatin nephrotoxicity is caused by inflammatory reactions, oxidative stress, necrosis and apoptosis in tubular cells. It may induce injury in renal vasculature and result in decreased blood flow and ischemic injury of the kidneys, contributing to a decline in glomerular filtration rate. These events, together, culminate in the loss of renal function triggering acute renal failure (Pabla and Dong, 2008 and Abolfazl *et al*., 2011).

Administration of cisplatin to rats induced a marked renal failure, characterized with a significant increase in serum creatinine, urea, alanine and aspartate aminotransferase and alkaline phosphatase (Domitrović *et al*., 2013, Lee *et al*., 2013 and Domitrović *et al*., 2014).Also, A significant decrease in body weight and antioxidant enzymes activities of liver and kidney were noticed in cisplatin - treated rats (Sahu *et al*., 2013 and Uppuluri *et al*., 2013).

Almost 20% of the patients treated with cisplatin develop acute renal failure, the most important cause of mortality related to this drug. Patients with renal failure undergo either painful dialysis or kidney transplantation from a willing living donor, which is both costly and harmful (Carvalho- Rodrigues *et al*., 2010). So, prevention of cisplatin nephrotoxicity would reduce morbidity and complications, decrease hospitalization costs, and may allow administration of higher dosage of this effective anti-tumor drug with added therapeutic potential. Food legumes are crops of the family *Leguminosae* also called *Fabaceae.* They are mainly grown for their edible seeds, and thus are also named grain legumes. Grain legumes contain moderately high levels of protein and amino acids and are promising alternatives (El-Safy *et al.,* 2012). For the above mentioned reasons, and for the active constituents in legumes,black cumin seeds and guava seeds, this work was designed to use them in diets to investigate their effects on the side effects of cisplatin induced nephrotoxicity in rats.

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Materials and Methods

Materials

Cisplatin:

Cisplatin was purchased from the pharmacy, El-Mansoura city, Egypt. Cisplatin is given to rats in a dose of 3mg/kg BW for inducing nephrotoxicity according to previous studies as reported by Karimi *et al.,* (2005).

Plant materials:

Dried fenugreek *(Trigonella foenumgraecum),* Lupins (*Lupinus spp.*), Soybeans **(***Glycine max (L.) Merr.***)** Legumes and black cumin seeds *(Nigella sativa)* were obtained from local market in El-Dakahlia governorate, Egypt. Fenugreek was washed, sprayed with distilled water for at least 72h or more (as needed) until completed germination. Lupins and soybeans were soaked in distilled water for 12h, cooked, washed and drained. All prepared legumes dried at hot oven at 50°C and crushed to powder. They were added to standard diet at 20% as instead of casein. Guava seeds (*Psidiumguajava*) were obtained as by-products from fresh guava which were purchased from the local market in El-Dakahlia governorate, Egypt. Guava seeds were washed, drained, dried at hot oven at 50°C, and crushed to powder, and then added to standard diet at 5% instead of 2.5% of corn starch and 2.5% of cellulose. Black cumin seeds were crushed to powder and added to standard diet at 5% instead of 2.5% of corn starch and 2.5% of corn oil.

Experimental Animals:

Thirty adult male rats of Sprague Dawely strain weighing 205 ± 8g were purchased from the Agriculture Research Center, Giza, Egypt.

Standard Diet:

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

Methods

Experimental Rats' Design:

Rats were kept under observation for five days for adaptation and fed on standard diet. Five rats served as normal control group and twenty five rats were injected with cisplatin (3 mg/kg i.p) at the beginning and at third week of the experiment to induce nephrotoxicity which classified into 5 groups (five rats /group). One group served as control positive group which fed on standard diet, whereas, the rest of nephrotoxic groups fed on experimental diets that contains 5% from both of black cumin seeds and guava seeds and supplemented with casein or one of legumes (fenugreek, lupins or soybean). Food and water were provided *ad-libtum*. Daily Food intake and weekly body weight were recorded. 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 heparinized tube for estimation of some biochemical analysis and also to obtain blood pictures. The other tubes of blood were left for coagulation then centrifuged at 3000 rpm for 15 minutes to obtain serum for further analysis. Kidneys and liver tissues from every rat were collected. Antioxidant enzymes determination was carried in one Kidneys and liver tissues. The other kidney was immersed in 10% neutral buffered formalin as fixative and then sent to Pathological Department of Veterinary Medicine, Cairo University for histopathological examination.

Laboratory Analysis:

Blood hemoglobin (HG), blood platelets count, red blood cells (RBCs) and white blood cells (WBCs) were estimated according to Drabkin, (1949), MC Inory, (1954) and Cynthia *et al.*,(1993), respectively. Serum alanine and aspartate amino transferase (ALT and AST) and alkaline phosphatase (ALP) enzymes activity were performed according to the methods of Bergmeyer and Horder, (1980) and Kind and King, (1954), respectively. Serum creatinine, urea, uric acid, total protein and albumin were enzymatically determined according to Bonsens and Taussky, (1984), Kanter, (1975), Fossati *et al.*, (1980), Henry, (2001) and Bartholomev and Delany, (1966), respectively. Superoxide dismutase (SOD), glutathione-peroxidase (GPx) and catalase (CAT)

enzymes activity were determined by enzymatic colorimetric procedures in liver and kidney according to Misra, (2012), Rotruck *et al*., (1973) and Lueck, (1965), respectively. Serum Ca, P, Na and K were estimated according to Pupsa *et al.,* (1994).

Histopathological Examination

The fixed samples of kidney in 10 % neutral buffered formalin were cleared in xylol and embedded in paraffin 4-5 µm thick section and stained with Hematoxylin and Eosin (H and E) for subsequent histopathological examination (Bancraft *et al.*, 1996).

Calculation of Some Parameters

Food and protein efficiency ratio (FER and PER) were determined according to the method described by Chapman *et al.,* (1950). Serum globulin value was determined according to Coles, (1974), while albumin/globulin (A/G) ratio was calculated according to the methods of Friedewald *et al.* (1972).

Statistical Analysis

All the obtained data were statistically analyzed by SPSS computer software. The calculated occurred by analysis of variance ANOVA and follow up test LSD by SPSS ver.11 according to Abo-Allam, (2003).

Results

Nutritional Indicators'

Data in table 1 showed that the administration of cisplatin resulted in a significant decrease in final weight, body weight gain , body weight gain percent, food efficiency ratio and protein efficiency ratio values for control positive rats group at (P<0.001) when compared to normal control rat group. Also, feeding nephrotoxic rats on diets supplemented with 5% from both of black cumin and guava seeds and different legumes decreased final weight and daily food intake at p<0., body weight gain, body weight gain percent, food efficiency ratio and protein efficiency ratio compared to normal control rat group however increased significantly the above mentioned nutritional indicators values (p<0.05) as compared with control positive rats group.

Table 1: Mean ± SD of nutritional indicators of normal control and nephrotoxic rat groups consumed diet fortified with black and guava seeds and different legumes

Variables Groups	Initial weight (g)	Final weight(g)	Weight gain (g)	Weight gain (%)	Daily food intake (g)	FER	Daily protein Intake (g)	PEF
Normal control	205.0±3.54ab	242.00±4.95a	37.00±4.00a	18.06±2.04a	17.68±1.71a	0.034±0.01a	3.54±0.34a	0.17± 0.004a
Control positive	208.0±5.61a	173.00±9.35 d***	-35.00±4.12e***	-16.87±2.37e***	12.50±0.80c***	-0.050±0.01e***	2.50±0.16c**	-0.23±0.001f***
Casein	203.20±7.01ab	197.20±8.01bc**	-6.00±1.00c***	-2.97±0.60c***	14.46±0.35b**	-0.010±0.00c***	2.88±0.06b*	-0.03±0.002c***
Fenugreek	200.20±6.01b	189.20±4.02 c**	-11.00±2.00d***	-5.47±0.84d***	13.44±0.35bc**	-0.014±0.01cd***	2.69±0.07bc*	-0.06±0.003e***
Lupins	202.40±5.50ab	195.40±3.51bc**	-7.00±2.00c***	-3.44±0.90c***	13.98±0.40bc**	-0.010±0.00c***	2.80±0.08b*	-0.04±0.001d***
Soybeans	204.20±7.01ab	200.20±8.01b**	-4.00±1.00b***	-1.97±0.56 b***	14.92±0.26b**	-0.004±0.00b***	2.98±0.05b*	-0.02±0.005b***

*Significant with control group * p< 0.05. ** p< 0.01. *** p< 0.001.*
Mean values in each column having different superscript (a, b, c, d,.....) are significantly different at p< 0.05.

Biochemical Analysis

In comparing to normal control group, the control positive rat group showed a significant decrease (p< 0.001) in blood levels of hemoglobin (HG), red blood cells (RBCs) and platelets, but showed a significant increase (p< 0.001) in white blood cells (WBCs). The nephrotoxic groups which fed on casein and different legumes diets showed a non significant decrease (p> 0.05) in HG level and significant increase in WBCs (p< 0.01&0.05). Also, each of casein, lupins and soybean nephrotoxic groups showed a non significant decrease (p> 0.05) in RBCs count while both casein and soybean nephrotoxic groups showed a non significant decrease (p> 0.05) in platelets count. In comparing to control positive, all nephrotoxic groups which fed on casein and different legumes diets showed a significant increase in HG, RBCs and platelets and a significant decrease in WBCs. However, it is clear that these diets did not able to reach to the WBCs count of nephrotoxic rats to its corresponding value of normal rats as illustrated in table 2.

Data in table 3 showed that the control positive rat group showed a significant increase (p< 0.001) in the liver functions parameters, as AST, ALT and ALP compared with normal control group. Also, each of casein, lupins and soybean nephrotoxic groups showed a non significant increase (p> 0.05) in AST while both casein and soybean nephrotoxic groups showed a non significant increase (p> 0.05) in ALT compared with

normal control group. The nephrotoxic groups which fed on casein and different legumes diets showed a significant decrease in AST and ALT compared with control positive group. Data concerning ALP showed that all nephrotoxic groups which fed on casein and different legumes diets showed a significant decrease ($p< 0.05$) in ALP compared with positive control group. However, it is clear that these diets did not able to decrease ALP values of nephrotoxic rats to their corresponding values in normal rats.

Table 2: Mean ± SD of HG, RBcs, WBcs and platelet cells in control and nephrotoxic rat groups consumed diet fortified with black and guava seeds and different legumes

Variables Groups	HG (gm/ml)	RBCs (---×10^6) (gm/ml)	WBCs (---×10^3) (cells/ml)	Plateletes (---×10^3) (cells/ml)
Normal control	14.00±0.60 a	4.94±0.65 a	4.94±0.75 e	304.00±1.50 a
Control positive	9.46±0.65 c***	2.10±0.40 d***	11.06±0.75 a***	213.40±1.50 e***
Casein	13.18±0.90 a	3.70±0.60 ab	6.14±0.70 bc**	297.60±0.08 ab
Fenugreek	12.88±0.50 ab	3.18±0.60 bc*	7.64±0.75 b**	253.00±1.25 d**
Lupins	13.10±0.60 a	3.80±0.60 ab	6.36±0.55 bc**	273.60±1.50 bc**
Soybeans	13.38±0.45 a	4.54±0.62ab	5.68±0.40 cd*	300.00±1.67 a

Significant with control group * *p< 0.05.* ** *p< 0.01.* *** *p< 0.001.*
Mean values in each column having different superscript (a, b, c, d,.....) are significantly different at p< 0.05.

Table 3: Mean ± SD of AST, ALT and ALP of control and nephrotoxic rat groups consumed diet fortified with black and guava seeds and different legumes

Variables Groups	AST(µ/l)	ALT(µ/l)	ALP(µ/l)
Normal control	27.50± 5.50 d	31.00± 6.00 c	115.00± 11.50 c
Control positive	54.10± 9.05 a***	79.40± 12.50 a***	259.00± 12.00 a***
Casein	33.40± 7.51 d	35.00± 6.00 bc	137.20± 12.05 b*
Fenugreek	37.60± 8.06 b*	40.20± 8.01 b*	146.40± 12.11 b*
Lupins	35.60± 5.51 cd	39.40± 5.50 b*	136.80± 11.05 b*
Soybeans	34.80± 5.56 cd	35.20± 7.95 bc	133.00±1 2.50 b*

Significant with control group * *p< 0.05.* ** *p< 0.01.* *** *p< 0.001.*
Mean values in each column having different superscript (a, b, c, d,.....) are significantly different at p< 0.05.

Data in table 4 showed that there was a significant increase at ($P<0.001$) in serum creatinine, urea, uric acid and globulin (G) and also a significant decrease at ($P<0.01$, $p<0.001$) in total protein, albumin (A) and A/G ratio after injection rats by cisplatin. All nephrotoxic groups which fed on casein and different legumes diets showed a significant decrease ($p< 0.05$) in serum creatinine and urea compared with positive control group. However, it is clear that these diets did not able to decrease serum creatinine and urea values of nephrotoxic rats to their corresponding values in normal rats. With respect to serum uric acid and globulin data, it is found that each of casein, lupins and soybean nephrotoxic groups showed a non significant increase ($p> 0.05$) in serum uric acid and globulin compared with normal control group. Data concerning total protein and albumin showed that all nephrotoxic groups which fed on tested casein and different legumes diets showed a non significant decrease ($p>0.05$) in serum total protein and albumin compared with normal control group. In addition, it is clear that both lupins and soybean nephrotoxic groups showed a non significant decrease ($p> 0.05$) in A/G ratio compared with normal control group.

Table 4: Mean ± SD of some renal functions parameters of control and nephrotoxic rat groups consumed diet fortified with black and guava seeds and different legumes

Variables Groups	Creatinine (mg/dl)	Urea (mg/dl)	Uric acid (mg/dl)	Total protein (g/dl)	Albumin (A) (g/dl)	Globulin (G) (g/dl)	A/G ratio
Normal control	0.28±0.03 d	31.00±0.30 d	3.74±0.35 c	8.86±0.65 a	5.96±0.25 a	2.90±0.40 c	2.06±0.05a***
Control positive	2.96±0.25a***	87.60±0.51a***	6.88±0.30a***	6.30±0.40c***	2.40±0.60c***	3.90±0.20a***	0.62±0.53 d
Casein	1.25±0.03bc**	48.60±0.88 b**	4.20±0.90 bc	8.32±0.80 a	5.00±0.40 ab	3.32±0.40 bc	1.51±0.03bc**
Fenugreek	1.39±0.05 b**	46.60±0.67 b**	5.16±0.25 b*	7.94±0.35 ab	4.52±0.65 a	3.42±0.30 b*	1.32±0.18 c*
Lupins	1.31±0.03 b**	43.20±0.59 bc	4.58±0.30 bc	8.62±0.45 a	5.40±0.80 a	3.22±0.35 bc	1.68±0.16 ab
Soybeans	1.21±0.07bc**	41.60±0.50 bc	3.94±0.15 c	8.78±0.40 a	5.66± 0.09 a	3.12±0.40 c	1.81±0.07 a

Significant with control group * *p< 0.05.* ** *p< 0.01.* *** *p< 0.001.*
Mean values in each column having different superscript (a, b, c, d,.....) are significantly different at p< 0.05.

Data in table 5 showed that there was a significant decrease at ($P<0.001$) in tested antioxidant enzymes activities, namely; SOD, GPx and CAT in liver and kidney tissues after injection rats by cisplatin. All nephrotoxic groups which fed on tested casein and different legumes diets showed a significant increase ($p< 0.05$) in the above mentioned antioxidant enzymes activities compared with positive control group and also showed a non significant decrease ($p> 0.05$) in SOD in liver and kidney tissues compared with normal control group. With respect to GPx data, it is clear that there was a non significant decrease ($p>0.05$) in liver GPx of casein nephrotoxic group but there was a non significant decrease ($p>0.05$) in kidney GPx of both lupins and soybean nephrotoxic groups when compared with normal control group. Consumption of fenugreek, lupins and

soybeans could increase liver GPx while consumption of casein and fenugreek could increase kidney GPx compared to control positive. Data concerning CAT showed that there was a non significant decrease ($p>0.05$) in liver CAT of both casein and soybean nephrotoxic groups and also there was a non significant decrease ($p>0.05$) in kidney CAT of both lupins and soybean nephrotoxic groups when compared with normal control group. Consumption of fenugreek and lupins could increase liver CAT while consumption of casein and fenugreek could increase kidney CAT compared to control positive.

Table 5: Mean ± SD of SOD, GPX and CAT in liver and kidney of control and nephrotoxic rat groups consumed diet fortified with black and guava seeds and different legumes

Variables / Groups	Liver antioxidant enzymes (μ/mg protein)			kidney antioxidant enzymes (μ/mg protein)		
	SOD	GPx	CAT	SOD	GPx	CAT
Normal control	3.44±0.65 a	48.50±0.90 a	12.54±0.35 a	1.80±0.30 a	40.92±0.60 a	2.40± 0.30 a
Control	1.14±0.17 c***	18.16±0.55 d***	5.40±0.90 c***	0.73±0.28 c***	17.90±0.70 c***	0.96±0.06 c***
Casein	2.46±0.07 ab	41.56±1.90 ab	10.80±0.60 ab	1.66±0.12 ab	32.54±0.90 b*	1.94±0.25 b*
Fenugreek	2.60±0.30 ab	31.18±1.75 bc*	8.52±0.80 b*	1.46±0.06 ab	32.48±0.70 b*	1.44±0.1 b*
Lupins	2.82±0.30 a	34.66±0.85 b*	9.64±0.45 b*	1.56±0.08 ab	36.32±0.75 ab	1.83±0.07 ab
Soybeans	2.94±0.35 a	36.98±0.80 b*	10.52±0.60 ab	1.68±0.10 ab	38.78±0.90 ab	1.98±0.20 ab

Significant with control group * $p< 0.05$. ** $p< 0.01$. *** $p< 0.001$.
Mean values in each column having different superscript (a, b, c, d..,) are significantly different at $p< 0.05$

Data in table 6 showed that there was a significant decrease in $Ca^{+2}$and K^+ concentrations ($P<0.001$) and also serum pH at $P<0.01$, whereas, there was significant increase in P and Na^+ concentrations ($P<0.01$& $P<0.001$, respectively) in serum rats after their injection by cisplatin. Serum Ca^{+2} of all nephrotoxic groups which fed on tested casein and different legumes diets had a significant increase value when compared with positive control group, and a non significant decrease ($p> 0.05$) except fenugreek group that showed significant decrease at $p<0.05$ compared with normal control group. With respect to serum P data, it is clear that serum P concentration of casein, lupins and soybean nephrotoxic groups had a significant decrease ($p <0.05$) when compared with positive control group, and a non significant increase ($p> 0.05$) when compared with normal control group. Data concerning serum Na^+ showed that there was a significant increase ($p<0.05$) in serum Na^+ of all nephrotoxic groups compared to normal control and significant decrease compared to control positive. Serum K^+ of nephrotoxic groups which fed on tested casein and different legumes diets had a significant increase when compared with control positive group while casein and soybeans had a non significant decrease ($p> 0.05$) when compared with normal control group. It was clear that serum pH of nephrotoxic groups which fed on tested casein and different legumes diets had a non significant decrease ($p> 0.05$) when compared with normal control group. Soybean nephrotoxic group had a significant increase when compared with positive control group

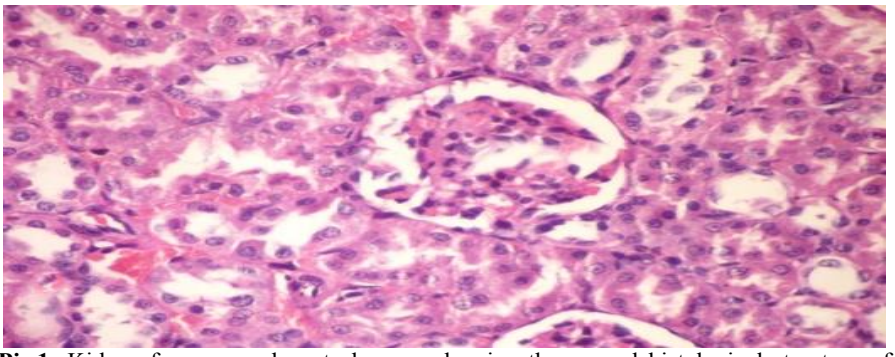
Table 6: Mean ± SD of serum Ca, P, Na, K and pH of control and nephrotoxic rat groups consumed diet fortified with black and guava seeds and different legumes

Variables / Groups	Ca (mg/dl)	P (mg/dl)	Na (m.equivalent/l)	K (m.equivalent/l)	pH
Normal control	9.86±0.25 a	4.00±0.20 b	171.76±1.36 e	5.06±0.25 a	7.38±0.20 a
Control positive	5.68±0.90 c***	6.63±0.49 a**	303.44±1.30 a***	2.86±0.14 d***	5.54±0.75 b*
Casein	8.54±1.10 ab	4.46±0.65 b	218.56±1.75 bc***	4.56±0.75 ab	6.64±0.75 ab
Fenugreek	7.66±0.55 b*	5.34±0.45ab	224.64±1.55 b***	3.76±0.35 c*	6.14±0.25 ab
Lupins	8.94±0.65 ab	4.88±0.30 b	213.18±1.95 b***	4.04±0.45 bc*	6.84±0.55 ab
Soybeans	9.32±0.40 a	4.40±0.70 b	202.52±2.20 d***	4.86±0.55 ab	7.28±0.29 a

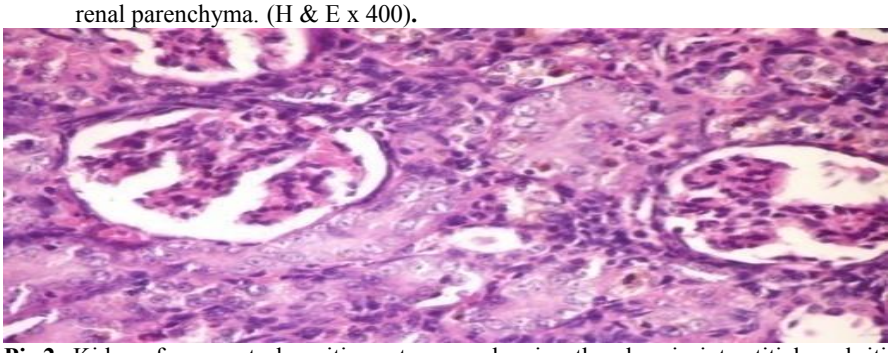
Significant with control group * $p< 0.05$. ** $p< 0.01$. *** $p< 0.001$.
Mean values in each column having different superscript (a, b, c, d….,) are significantly different at $p< 0.05$

Kidney Histopathological Results

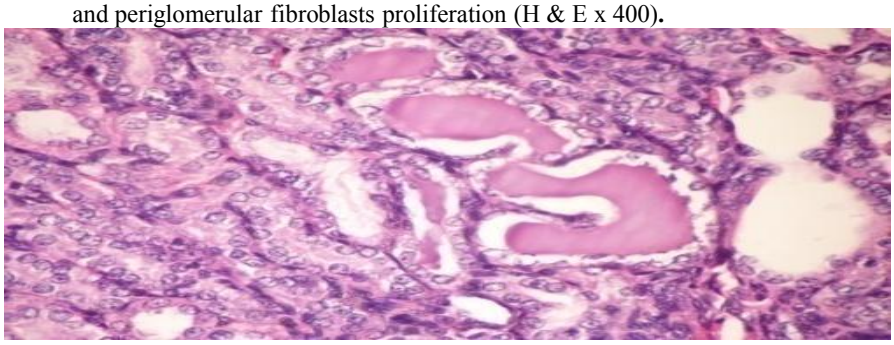
Microscopically, kidney of control negative rat revealed the normal histopathological structure of renal parenchyma (pic. 1). Meanwhile, kidney of control positive rat showed chronic interstitial nephritis and periglomerular fibroblasts proliferation (pic. 2), also cystic dilatation of renal tubules with eosinophilic protein casts (pic. 3). Kidney of rat from casein nephrotoxic group revealed interstitial nephritis, cystic dilatation of renal tubules and periglomerular fibroblasts proliferation (pic. 4). Whereas, kidney of rat from fenugreek nephrotoxic group revealed atrophy of glomerular tufts, distention of Bowmen's space and interstitial nephritis (pic.5).Meanwhile, kidney of rat from lupins nephrotoxic group revealed interstitial nephritis and periglomerular fibroblasts proliferation (pic. 6). Kidney of rat from soybean nephrotoxic group revealed interstitial nephritis and presence of protein cast in the lumen of renal tubules (pic. 7).



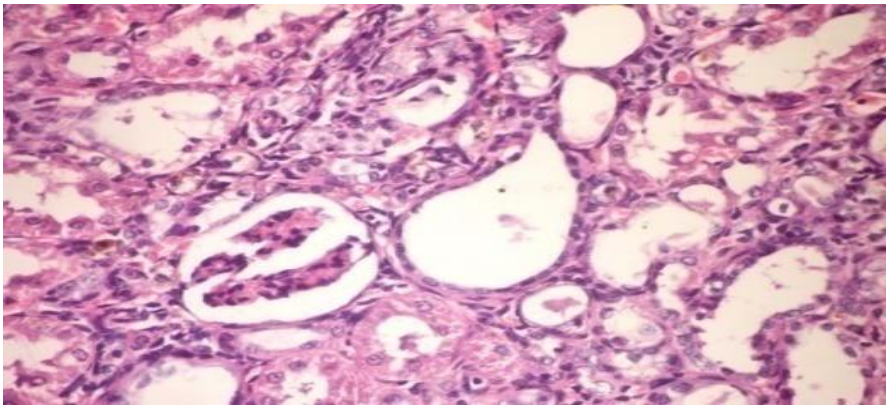
Pic.1: Kidney from normal control group showing the normal histological structure of renal parenchyma. (H & E x 400)**.**



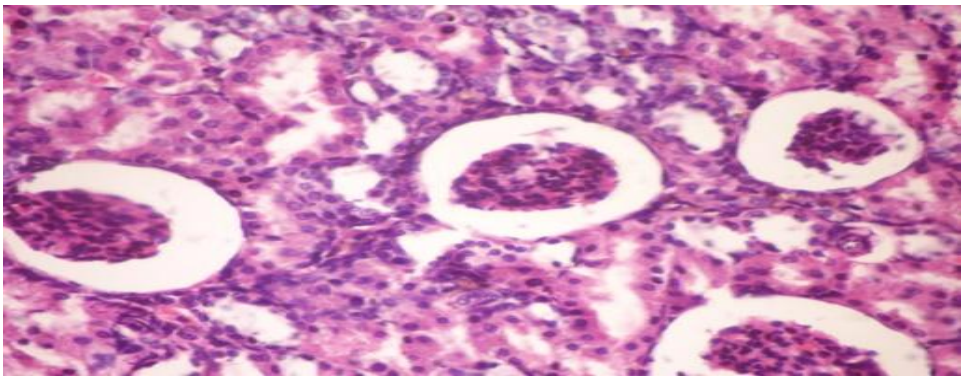
Pic.2: Kidney from control positive rat group showing the chronic interstitial nephritis and periglomerular fibroblasts proliferation (H & E x 400)**.**



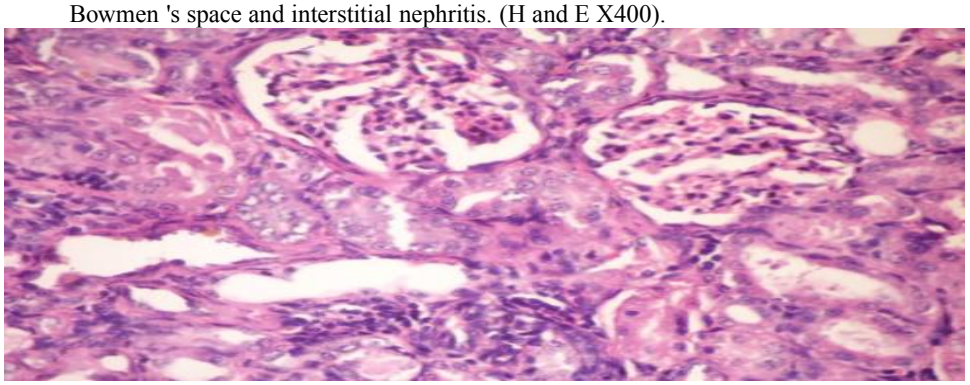
Pic.3: Kidney from control positive rat group showing the cystic dilatation of renal tubules with eosinophilic protein casts. (H & E x 400).



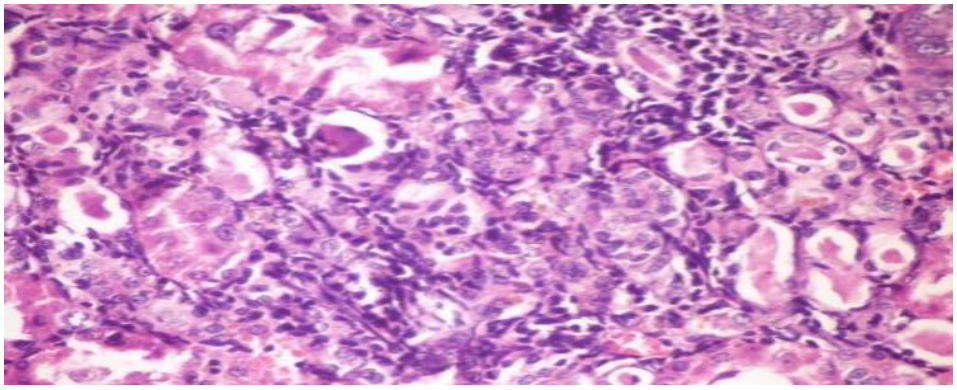
Pic.4: Kidney from Casein rat group showing interstitial nephritis, cystic dilatation of renal tubules and periglomerular fibroblasts proliferation. (H and E X400)**.**



Pic.5: Kidney from fenugreek rat group showing atrophy of glomerular tufts, distention of Bowmen 's space and interstitial nephritis. (H and E X400).



Pic.6: Kidney from lupin rat group showing interstitial nephritis and periglomerular fibroblasts proliferation. (H and E X400).



Pic.7: Kidney from soybean rat group showing interstitial nephritis and presence of protein cast in the lumen of renal tubules. (H and E X400).

Discussion

Nephropathy is a condition that affects the function of the kidneys, and that may progress over time to renal failure. Patients with kidney failure undergo either painful dialysis or kidney transplantation from a willing living donor, which is both costly and harmful (Oboh *et al.*, 2013).

In comparison with normal rats, it was found that cisplatin injection caused in a significant decrease ($P<0.001$) in weight gain, food and protein efficiency ratio, hemoglobin, RBcs, platelets, serum of both total protein, albumin, calcium and potassium and liver and kidney antioxidant enzymes. Also, injection rats by cisplatin resulted in a significant increase in serum alanine and aspartate amino transferase and alkaline phosphatase, creatinine, urea, uric acid, globulin, sodium and WBcs. The decrease in weight gain, food and

protein efficiency ratio may be due to loss of appetite of nephrotoxic rats which noticed in a decrease daily food and protein intake after cisplatin injection. The decrease in liver and kidney antioxidant enzymes may be due to cisplatin causes oxidative stress which is mainly due to the reactive oxygen molecules generated by cisplatin ((Pabla and Dong, 2008, Abolfazl *et al*., 2011 and Oboh *et al.*, 2013). The increase in serum alanine and aspartate amino transferases and alkaline phosphatase after cisplatin administration may be attributed to the hepatocytes destruction after injection rats by cisplatin (Ognjanovic *et al.*, 2012). The increase in serum creatinine and urea after cisplatin administration may be attributed to a decline in glomerular filtration rate after injection rats by cisplatin (Naqshbandi *et al.*, 2012 and Sindhu, 2013).

In fact, black cumin seeds (*Nigella sativa* L.) has occupied special place for its wide range of medicinal value. This might be due to the complex chemical composition of the seeds. It may utilize for the production of formulations containing phytochemicals with significant antioxidant properties and health benefits. Yet, these phytochemicals may bring nutraceutical and functional benefits to food systems. These points out the fact that its seed contains both active proteins and lipid soluble elements; thus, proving the multiple mechanisms of action behind this phytotherapeutic agent (Ramadan, 2007). Black cumin seeds contain both fixed and essential oils, proteins, alkaloids and saponins. Much of the biological activity of the seeds has been shown to be due to TQ, the major component of the essential oil, but which is also present in the fixed oil (Ali and Blunden, 2003 and Alam, 2011). The qualitative analyses of phytochemicals present in the methanolic extract of *N. sativa* seed showed the presence of sterols, alkaloids, saponins, phenols, flavonoids, terpenoids and cardiac glycosides (Islam *et al*., 2012).

Serious protein deficiencies and the high costs of animal protein sources have stimulated research on developing new sources of protein from unexploited sources or wastes and by-products (El-Safy *et al*., 2012).

Our study currently focused on the use of waste products generated by the food industry indicated they are an alternative source of proteins as Guava seeds (*Psidiumguajava* L.). Chemical composition of guava seed flours (% on dry basis) were crude protein 7.90, crude Lipids 16.20, crude fiber 64.67, total ash 0.96 and carbohydrate 10.27 and also antioxidant phenolics (Castro-Vargas *et al*., 2012 and El-Safy *et al*., 2012). In this study the effect of black cumin seeds and guava seeds appears when comparing platelets, RBCs, HGB, total protein, albumin, liver SOD, GPx, CAT, kidney SOD and serum Ca^{+2} , $K^{+}$and pH values in control positive rats (that fed on standard diet without black cumin seeds and guava seeds) with their corresponding values in casein nephrotoxic rats (that fed on casein diet supplemented with black cumin seeds and guava seeds), it is appeared that the addition of black cumin seeds and guava seeds to the diet resulted in a non significant decrease in these indicators of nephrotoxic rats.

It is clear that the addition of black cumin seeds and guava seeds was able to raise these indicators in nephrotoxic rats to approach to their corresponding values in normal rats. Also, When comparing serum ALT, AST, uric acid and serum P values in control positive rats with their corresponding values in casein nephrotoxic rats, it is appeared that the addition of black cumin seeds and guava seeds to the diet resulted in a non significant increase in these indicators of nephrotoxic rats. This may be due to their nutritional and phytochemical components especially polyphenols which act as a potent scavenger of free radicals to prevent the toxic effects of cisplatin. In regarding to serum creatinine and urea results, it is clear that these results agreed with that obtained by Salama *et al.*, (2011) and disagreed with that obtained by Sara *et al.*, (2011) and Khajavi *et al.*, (2011). The HG results were agreed with that obtained by Zaoui *et al*., (2002) and Ali and Blunden, (2003) who reported that treatment of rats with the black cumin seeds extract for 12weeks has been induced an increase the hemoglobin level. However, these results disagreed with platelets result that obtained by Zaoui *et al*., (2002) who found that oral administration of 2mg/kg of black cumin seeds extract for 12weeks significantly decreased the platelets count.

The consumption of different legumes in diets had ability to decrease nephrotoxicity induced by cisplatin. This result agreed with that obtained by El Nasri and El Tinay, (2007), Chatterjee *et al.,* (2010) and Ismael *et al.,* (2013) who recorded that legume represent, together with cereals, the main plant source of proteins in human diet. They are also generally rich in dietary fibre, certain minerals and vitamins and carbohydrates. Minor compounds of legumes are lipids, polyphenols and bioactive compounds with low level of fat. The fenugreek seeds are be mixed with cereals as a supplement for some limiting amino acids and hence for improving their protein quality through amino acid balance. Fenugreek is rich in flavonoids such as apigenin, luteolin, orientin, quercetin, vitexin and isovitexin. These natural antioxidants help to strengthen the immune system, improve cellular health and reduce signs of ageing. The spice seeds contain 0.1–0.9% diosgenin and are extracted on a commercial basis. Also contain the saponin fenugrin B. Several coumarin compounds have been identified in fenugreek seeds as well as a number of alkaloids as trigonelline, gentianine and carpaine.

Several reports have recently identified various pharmacologically active phytochemicals in lupins. For example, apigenin derivatives were detected with antioxidant properties in methanol extracts of *L. albus* seeds via Liquid Chromatography– Mass Spectrometry. Anti-tumour phytosterols were identified in *L. albus* including stigmasterol and campesterol, as well as the anti-inflammatory phytosterol β-sitosterol, via thin layer

chromatography and GC analysis. In addition, the pharmacologically active phytosterol lupeol was a minor constituent of the lipid fraction of *L. albus* seeds (Pilkington, 2013).

Soybean could improve the nutritional results due to contain complex carbohydrates, vegetable protein, dietary fiber, oligosaccharides and minerals mainly iron. It had a high protein content in comparison with most other legumes (Torres *et al*., 2005 and Bisla *et al*., 2012). The best results of liver and renal function, and elevation of antioxidant enzymes were revealed to the fact that soybean has various biologically active phytochemicals as isoflavones, glycitein, genistein and daidzein, coumestrol, phytate, saponins, lecithin, phytosterols, vitamin E, isoflavones and folate that provide several health benefits, including protection against oxidative stress (Prakash *et al*., 2007 and Ekor *et al*.,2010). The obtained results were agreed with histopathological results.

Conclusion

The central finding of the present investigation demonstrates that consumption of legumes could increase nutritional indicators and lower the functional alteration but consumption of soybean diet supplemented with black cumin seeds and guava seeds was the best diet among the tested diets in improvement of the nutritional indicators, blood picture, liver and renal function parameters, liver and renal antioxidant enzymes, serum minerals and pH values in nephrotoxic rats. This may be due to the combine effect of the nutritional and phytochemical components of both of soybean, black cumin seeds and guava seeds which may provide a partial protection against cisplatin-induced structural and functional alterations in rats.

Therefore, it is recommended to add legumes especially soy bean, black seeds and guava seeds in the diet of patient who treated by cisplatin.

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