Role of sprouted fenugreek seeds as a nutraceutical in the protection against rats' cisplatin-induced nephro- and hepatotoxicity

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العنوان: كلية التربية النوعية . جامعة المنيا . جمهورية مصر العربية

دور بذور الحلبة المنبتة كمغذيات في الحماية من السمية الكلوية والكبدية التي يسببها السيزبلاتين للفئران

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بذور الحلبة المنبتة غنية بمضادات الأكسدة النشطة بيولوجيا وتستخدم على نطاق واسع كمكون حيوي في تحضير الطعام اليومي والتركيبات العشبية .أجريت الدراسة الحالية بهدف فهم دور بذور الحلبة المنبتة في الحماية ضد السمية الكلوية والكبدية التي يسببها السيزبلاتين في الفئران . تمت التجارب البيولوجية على عدد 24 فأر تتراوح اوزنهم 150±10 وتم تقسيمهم إلى أربع مجموعات (ستة فئران لكل مجموعة). تم تغذية المجموعة (1) من مجموعة الكنترول السالبه على الغذاء الأساسي ، وبينما باقي المجموعات الثلاثة(2،3،4) تم حقنهم داخل الغشاء البريتوني بواسطة سيزبلاتين (5 مجم / كجم من وزن الجسم) من اليوم الأول والانتظار لمدة 24 ساعة لإحداث التسمم الكلوي والكبدي. تم تغذية المجموعة الضابطة الموجبة وهي المجموعة الثانية على الغذاء الأساسي بعد الحقن ،بينما تغذت المجموعة الثالثة والرابع على الغذاء الأساسي بالاضافة الي + 5٪ ، 10٪ بذور الحلبة المنبتة على التوالي واستمرت الدراسة لمدة 28 يومًا. تم إجراء التحاليل الكميائية للعناصر الاساسية لبذور الحلبة المنبتة والجافة حيث ارتفعت نسبة البروتين والماء والألياف للحلبة المنبته (فقد ارتفع البروتين من 27.1 الي 32.6 والرطوبة ارتفعت نسبتها من 8.4 الي 11.4 والالياف زادت من 10.6 الي 15.4) وتم اجراء التحاليل البيوكيميائية في الكبد والكلى والهستوباثولوجي للكبد والكلي. وقد اسفرت نتائج التحاليل ان هناك زيادة معنوية للمجموعة الضابطة الموجبة في كل من الكرياتينين ، واليوريا ، وحمض البوليك ، ومالونديالديهيد (MDA) في الكلى ومضادة للأكسدة الكلية (TAC) والجلوتاثيون (GSH) وانزيمات السوبرأكسيد ديسميوتاز (SOD) ، والكاتلاز (CAT) والألبومين ، البروتين الكلي ، ألانين أمينوترانسفيراز (ALT) ، وسبارتات امينو ترانسفيراز (AST) بالمقارنة بالمجموعة الضابطة السالبة وقد أظهرت المعاملة ب 5٪ و 10٪ من بذور الحلبة المنبتة وارتفاع حالة مضادات الأكسدة وجعلت جميع القيم قريبة من المستويات الطبيعية ، وتوصي الدراسة بضرورة تناول بذور الحلبة المنبتة حيث تلعب. المواد الكيميائية النباتية الطبيعية الموجودة في الحلبة دورًا مهمًا في تخفيف السمية التي يسببها السيزبلاتين في الكبد والكلى في الفئران

الكلمات الرئيسية: بذور الحلبة المنبتة. سيزبلاتين. السمية الكلوية الكبدية. الفئران

Role of sprouted fenugreek seeds as a nutraceutical in the protection against rats' cisplatin-induced nephro- and hepatotoxicity

Abstract:

The present study was designed to investigate the effects of sprouted fenugreek seeds on the nutritional status and some biochemical analyses of serum and liver and kidney in Nephrohepatotoxicity rats. Twenty-four healthy Sprague–Dawley albino rats weighing 150±10g were classified into four groups. One was fed on basal diet and kept as control (-ve) group. The other three group fed on basal diet and injected intraperitoneal with Cisplatin (5mg/kg body wt.) to induce Nephrohepatotoxicity The Nephrohepatotoxicity rats were classified into control (+ve) group and two treated rat groups which administered 5% and 10% sprouted fenugreek seeds The treatment period was designed for 28days. the chemical composition of sprouted fenugreek seeds showed higher value of moisture, protein, and fiber than Dried fenugreek seeds (8.4 , 27.16 and 10.82 for dried fenugreek & 11.3, 32.98 and 15.95 respectively for sprouted fenugreek). The results revealed that, the control (+ve) group showed a significant increase in alanine aminotransferase (ALT), albumin (Alb), total protein , spartate aminotransferase (AST). creatinine, urea, uric acid,and malondialdehyde (MDA) in the kidney but a significant decrease in total antioxidant capacity (TAC), glutathione (GSH), superoxide dismutase (SOD)and catalase (CAT) compared with control (- ve) group. The 5% and 10% sprouted fenugreek seeds rat group showed a significant decrease in in alanine aminotransferase (ALT), albumin (Alb), total protein , spartate aminotransferase (AST). creatinine, urea, uric acid,and malondialdehyde (MDA) in the kidney but a significant increase in total antioxidant capacity (TAC), glutathione (GSH), superoxide dismutase (SOD)and catalase (CAT) compared with control (+ ve) group. All treated rat groups revealed no histopathological changes. In conclusion, feeding rats with 5% and 10% sprouted fenugreek seeds may significantly reduce nephrohepatotoxicity.

Keywords: Sprouted fenugreek seeds; cisplatin; nephrotoxicity; hepatotoxicity; rats.

Introduction:

Cisplatin or cis-diamminedichloroplatinum (II) is broadly used as a highly effectual cancer chemotherapeutic agent. Nephrotoxicity is a major complication and dose-limiting factor for CIS therapy. The Cis-induced nephrotoxicity is strongly related to an increment in the kidney lipid peroxidation in the kidney. It has been observed that CIS decreases antioxidant enzyme activities and induces the reduction in GSH **(Bentli *et al*., 2013, Farooq *et al*., 2019, Michel and Menze, 2019).** Nephrotoxicity is significant with the dose-limiting side effect of CIS, with an occurrence announced as 6-13% **(Guerra *et al*., 2019).** The increased free radical generation and the declined antioxidant defense system may increase the nephritic lipid peroxidation and MDA creation in kidney tissue **(Fanizzi *et al*., 2019).** Now, it is recognized that Cis significantly stores within the human liver causes hepatotoxicity indicated by deteriorated liver functions, higher contents of enzymatic biomarkers such as AST and ALT **(Palipoch and Punsawad, 2013),** in addition to pericentral disorganization, hepatic necrosis, and apoptotic changes **(Attyah and Ismail, 2017).** While the basic mechanisms of Cis-induced hepatoxicity are still unwell understood; oxidative stress is assumed to be a primarily mediate Cis promoted hepatotoxicity **(Cetin *et al*., 2011).**

The nutraceuticals term was derived from the words 'Nutrition' and Pharmaceuticals'. It is employed as a substance or part of nutrition that will have restorative or medical advantages involving the prevention and managing of diseases/disorders **(Pandey *et al*., 2010).** fenugreek (*Trigonella foenum graecum* L) is a leguminous plant conventionally used as a medicinal herb and spice. It has been well recognized to have many medicinal properties, such as anti-inflammatory, antidiabetic, anti-hyperlipidaemic, anticancer, antioxidant, as well neuroprotective activities. Also contains active components such as alkaloids, flavonoids (luteolin, kaempferol, quercetin, tricin, gallic acid… etc), steroids, and saponins **(Sushma and Devasena, 2010, Tavakoly *et al*. 2018, Nagamma *et al*., 2019).**

The effect of germination (sprouting) of fenugreek seeds makes its nutritional value higher and increases raise its nutritional and dietary contents compared to its dried seeds. The fenugreek sprouted seeds have high protein content (32%), lysins (5.7 g 16 g^{-1} of N), 20 % soluble and 28% insoluble dietary fiber, L-tryptophan, and rich in calcium, iron, and beta carotene. The galactomannans, steroidal sapogenins, and isoleucine are the three principal chemical constituents of fenugreek seeds with preferred potential medicinal usages **(Khan and Khosla, 2018).** These components have put fenugreek amongst the most commonly perceived "nutraceutical". The fenugreek sprouted seeds comprise many active compounds with medicinal, pharmaceutical, and therapeutic applications. The chemical and medicinal constituents of fenugreek seeds consist of carbohydrates, vitamins A, B1, C, and E, phosphates, alkaloids, flavonoids (apigenin, quercetin, orientin, luteolin, vitexin, and isovitexin), saponins, trigonelline, steroids, polyphenolic substances, free amino acids, such as 4-hydroxy isoleucine, histidine, and lysine, arginine), glycosides among other substances **(Khan and Khosla, 2018).**

Therefore, this study was performed to examine the nutraceutical role of sprouted fenugreek seeds in protection against Cis-induced nephro- and hepatotoxicity in rats.

Material and methods:

Materials:

1-Fenugreek seeds *(Trigonella foenum graecum., L)* were got from the local market, Cairo, Egypt.

2-Platinol,1mg ml^{-1}(cisplatin) was obtained from Cairo Company for Chemical Trading, Egypt.

Animals

Male Sprague–Dawley rats, (obtained from Animal house of National Research Centre, Cairo, Egypt) were used with an average body weight of 150±10 g. They were individually kept in metabolic cages; where water and food were given *ad-libtium.*

Basal diet was prepared according to **Reeves *et al*., (1993).**

Methods:

Germination of Fenugreek Seeds:

After purchasing fenugreek seeds from the local market, Egypt, the seeds were cleaned out from all impurities and soaked in tap water (1:3 v/v seeds: water) at room temperature (25-28°C) for 12 hours. The soaked seeds were spread on wet cotton tissue in stainless steel containers using a woody cover not only to reduce the loss of moisture but also to keep the seeds away from light during the germination period. Two days later of germination, germinated seeds were gathered and dried in an electric air drought oven at 40 °C for 24 hours. The obtained dried seeds were ground by Braun grinder and packaged in polyethylene bags, then kept in a deep freezer at -16 °C until used for supplementation **(Magda, 2017).**

Chemical composition of fenugreek:

The chemical composition of fenugreek (moisture, ash, crude protein, and fat) was determined according to **(AOAC, 2010).** While total carbohydrates were estimated by difference as follows:

Carbohydrates% = 100% - the percentages of (moisture + protein + fat +ash).

Experimental design:

A number of 24 rats were housed in well-aerated cages under hygienic conditions and fed on a basal diet for one week for adaptation. Then, rats were distributed into four experimental groups, each group has six rats as follow:

1-Group (1) was a negative control group (-ve control) fed only on basal diet.

The other three group fed on basal diet and injected intraperitoneal with Cisplatin (5mg/kg body wt.) to induce Nephrohepatotoxicity according to **Yogesh *et al.*, (2010)** The Nephrohepatotoxicity rats were classified into the following:

1- **Group (2):** as a positive control group (+ve control) where the rats were fed a basal diet.

2-Group (3) and Group (4): Rats were fed basal supplemented with (5% and 10% of sprouted fenugreek seeds/100gm diet, respectively). The study was assigned for 28 days. The animal experiments were carried out according to the Ethics Committee, National Research Centre, Cairo, Egypt and followed the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

Body measurements, organs and blood sampling

Feed intake (FI), body weight gain (BWG), and feed efficiency ratio (FER) were measured (Chapman *et al,* 1959). At the end of the experiment, all rats were fasted overnight (until 12 hours), sacrificed, and the blood samples were collected and centrifuged for obtaining the serum which was kept frozen till analysis. Livers and kidneys were removed, washed in saline solution, dehydrated by filter paper and weighted, and quickly kept on ice bags until moving to froze at – 18°C, till using for assessment of lipid peroxidation and antioxidant activity in liver tissue.

Biochemical Analysis

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined **(Bergmeyer *et al*.,1986)** , albumin **(Drupt (1974)**, and total protein **(Sonnenwirth and Jaret, 1980).** As well, the liver content of glutathione (GSH) was determined **(Bulaj *et al.*,1998)** and the liver activity of SOD and CAT enzymes were chemically measured **(Kakkor *et al*., 1984, Sinha, 1972)**. Serum concentrations of creatinine (Cr) were measured using colorimetric kinetic as in **Waiker and Bonventre (2008),** serum uric acid (UA) was measured by the enzymatic colorimetric method **(Fossati *et al, 1980)*,** while serum urea was measured **(Kaplan *et al*., 1984).** The serum concentration of MDA was analyzed based on **Draper and Hadley, (1990),** whilst, the total antioxidant capacity of (TAC) was determined **(Erel, 2004).**

Preparation of Liver and kidney tissue homogenate:

Liver and kidney tissues were cut into small pieces and directly homogenized and kept in 5-10 ml ice-cold medium containing buffer. The homogenates tissues were centrifuged at 4000 rpm for 15 min at 4°C and the supernatant was carefully separated to determine the biochemical analysis **(Sushma and Devasena, 2010).**

Histopathological examination

From each kidney tissue, 5 μm thickness slices were cut, settled in 10% paraformaldehyde, and fixed in paraffin wax blocks. Tissue sections of 5 μm thick were stained with hematoxylin and eosin (H and E) and Masson’s trichrome, then investigated under a light microscope for evaluating the histopathological changes **(Suzuki and Suzuki, 1989).**

The rats' liver was submerged in neutral buffered formalin (10%) for 24 hr. The fixed tissues were routinely processed, fixed in paraffin, sectioned, deparaffinized, and rehydrated according to the basal techniques **(Bancroft and Gamble, 2002).**

Statistical analysis:

The data were statistically analyzed by one-way analysis of variance "ANOVA" and the results are presented as mean ± SD, accoriding to **(Armitage and Berry, 1987).**

Result and discussion:

Effect of Germination on Proximate Chemical Composition of Fenugreek Seeds

The results in Table (1) reveal the effect of 2 days of germination on the chemical composition of fenugreek seeds. These results exhibited increasing in crude protein, crude fiber, fat, and moisture were increased than crude fenugreek seeds and decreasing in the total carbohydrates and ash content by germination compared to crude fenugreek seeds.

Table (1): Chemical composition of dried and sprouted fenugreek seeds (gm 100gm^{-1})

Compositions	Dried Fenugreek seeds	Sprouted fenugreek
Moisture	8.40±.40	11.4±.51
Protein	27.1±.20	32.6±.57
Carbohydrate	46.1±1.0	32.5±.49
Fat	3.17±.20	3.6±.11
Crude Fiber	10.6±.53	15.4±.47
Ash	4.1±.10	3.7±.24

Nutritional parameters of the biological experiment:

Food intake, body weight gain, and feed efficiency ratio were determined and the results are tabulated in Table (3). It could be observed that there are no significant difference between (-ve) control and cis groups in food intake (FI). Final body weight showed significant decrease (P<0.05) in groups (2, 3, 4) when compared to normal rats (group 1) (17.11±1.21b, 17.08±.43b and 17.33±.72b respectively).The results in the table 3 showed significant decrease in feed efficiency ratio in (+ve) control compared to (-ve) control.

Table (2): Effect of sprouted fenugreek seeds on food intake, body weight and gain and feed efficiency ratio in rats in the experimental groups

Parameters / Groups	FI	BWG	FER
Group (1):(-ve) Control	14.48 ± .80[ab]	19.46 ±1.75[a]	1.35 ±.17[a]
group (2): (+ve) Control	15.86 ±.54[a]	17.11 ±1.21[b]	1.10 ±.10[b]
group(3)sprouted fenugreek5%	15.96 ±.48[a]	17.08 ±.43[b]	1.15 ±.08[b]
group(4)sprouted fenugreek 10%	14.86 ±.54[ab]	17.33 ±.72[b]	1.30 ±.08[a]

Each value is the mean ±SD Mean values in each column having different superscript (a, b, c & d) are significantly different at P <0.05

Effect of sprouted fenugreek seeds on liver enzymes, albumin, and total protein in Cis-induced nephrotoxicity and hepatotoxicity rats

Table 2 illustrates serum levels of liver enzymes, total protein (TP), and Albumin (Alb) in the four studied groups (i.e. -ve control group, + ve control group, sprouted fenugreek 5% and sprouted fenugreek 10%. The results recorded a significant decline ($p<0.05$) in the serum level of TP and Alb and an increase in the serum level of AST and ALT in Cis-treated rats than their corresponding in the normal rats. Sprouted fenugreek groups were significantly recorded higher serum TP and Alb levels and lower serum AST, ALT levels than the +ve control group. Group (4) has a significant increase in serum TP, Alb level, and serum liver enzymes than group (3) indicating that using fenugreek 10% is better in this regard than 5%.

Table (2): Effect of sprouted fenugreek seeds on serumAspartate transaminase (AST), Alanine transaminase (ALT), albumin and total protein levels in the experimental groups

Parameters / Groups	AST (IU l^{-1})	ALT (IU l^{-1})	Albumin (gm dl^{-1})	Total protein (gm dl^{-1})
Group (1): (-ve) control	102.7±5.72[d]	25.13±4.6	4.31±.35[a]	6.48±.50[a]
group (2): (+ve) control	292.3±7.51[a]	52.80±7.9	3.19±0.2	5.08±0.1
group (3) sprouted	210.20±8.5	43.23±6.8	3.69±0.2	5.89±0.3
group (4) sprouted	121.48±5.1	25.98±6.3	4.11±0.2	6.45±0.4

Each value is the mean ±SD Mean values in each column having different superscript (a, b, c & d) are significantly different at $P < 0.05$

Effect of sprouted fenugreek seeds on the GSH level and activities of antioxidant enzyme (SOD and CAT) levels in Cis-induced nephrotoxicity and hepatotoxicity rats

The results of (GSH, SOD, and CAT) levels in the liver tissue of the studied groups are presented in Table (3). It has appeared that group (2) had a significant decrease in levels of (GSH, SOD, and CAT) than the normal group. In contrast, sprouted fenugreek groups (groups 3 and 4) significantly increased the GSH level and activity of SOD and CAT enzymes than the Cis treated group. Its recorded results Group (4) tended to be close to the levels of normal rats. This indicates that the 10 % concentration of sprouted fenugreek was the best result in all analyzes, and it tends to be such as the same uninfected control group.

Table (3): Effect of sprouted fenugreek seeds on glutathione, superoxide dismutase and catalase in the experimental groups

Parameters / Groups	GSH	SOD (mmol	CAT(mmol
Group (1): (-ve) Control	1.98±0.09[a]	42.32±4.02[a]	1.01±0.08[a]
group (2): (+ve) Control	0.703±0.16[d]	18.72±2.53[d]	.430±0.11[c]
group (3) sprouted	1.31±0.08[c]	29.32±4.96[c]	.606±0.09[c]
group (4) sprouted	1.59±0.12[b]	37.16±3.57[b]	.893±0.07[b]

Each value is the mean ±SD Mean values in each column having different superscript (a, b, c & d) are significantly different at P <0.05

Effect of sprouted fenugreek seeds on serum (uric acid, creatinine, and urea) levels in Cis-induced nephrotoxicity and hepatotoxicity rats

As displayed in Table (4), this study showed that injected rats with Cis (+ve rats) have a significantly increased serum concentration of urea, Cr, and uric acid. Sprouted fenugreek groups have significantly decreased the serum levels of urea, Cr, and uric acid compared with their corresponding in the positive rats. The serum level of urea, Cr, and uric acid was significantly declined ($p<0.05$) in group (4) than group (3).

Table (4): Effect of sprouted fenugreek seeds on serum (uric acid, creatinine, and urea) levels in the experimental groups

Parameters / Groups	Serum Creatinine (mg dl^{-1})	Serum uric acid (mg dl^{-1})	Serum Urea (mg dl^{-1})
Group (1): (-ve) Control	0.56±.08[c]	2.16±.17[c]	19.5±3.5[c]
group (2): (+ve) Control	1.20±.23[a]	4.25±.34[a]	69.2±8.6[a]
group(3)sprouted	1.04±.16[a]	3.88±.31[a]	48.8±7.9[b]
group(4)sprouted	0.80±.08[b]	2.88±.39[b]	21.5±5.0[c]

Each value is the mean ±SD. Mean values in each column having different superscript (a, b, c & d) are significantly different at P <0.05

Effect of sprouted fenugreek seeds on serum MDA and TAC levels in Cis-induced nephrotoxicity and hepatotoxicity rats

The data in Table (5) represents results of serum lipid peroxidation (MAD) and total antioxidant capacity (TAC) for experimental groups which showed that the Cis-treated group (group 2) had a significant increase in serum level of MDA and decrease in TAC compared with the normal group. Instead, sprouted fenugreek groups have significantly decreased the serum MDA levels and increased the serum TAC levels than in the Cis-treated group. Supplementation 10% of the sprouted fenugreek group makes the results tend towards normal rats compared with that treated with Cis.

Table (5): Effect of sprouted fenugreek seeds on malondialdehyde and total antioxidant capacity levels in the experimental groups

Parameters / Groups	MDA (nmol m^{l-1})	TAC (mmol l^{-1})
Group (1): (-ve) Control	3.70 ± 0.56^{d}	1.42 ± 0.13^{a}
group (2): (+ve) Control	15.6 ± 1.7^{a}	$0.38\pm.10^{d}$
group (3) sprouted fenugreek 5%	10.1 ± 1.2^{b}	0.75 ± 0.06^{c}
group (4) sprouted fenugreek 10%	7.72 ± 1.1^{c}	1.05 ± 0.06^{b}

Each value is the mean ±SD. Mean values in each column having different superscript (a, b, c & d) are significantly different at P <0.05

The overall results of our study suggest that treatment with (5 mg kg^{-1} body weight) of Cis for one week induces significant hepatic and renal toxicity in the treated rats. This is evident from the increase of MDA, UA, Cr, serum urea, AST, and ALT levels, in addition to the decrease of total protein, albumin, TAC, and GSH levels; and the activities of antioxidant enzymes (SOD and CAT).

These results in agreement with **Hegazy and Emam, (2015)** who found that the ethanolic extract of fenugreek species significantly attenuated the Cis-induced biochemical and histopathological alterations, inflammation, and apoptosis in rat livers and kidney These results suggested that fenugreek co-administration has a great antioxidant effect and may act as a novel and promising preventive approach against Cis-induced nephron- and hepatotoxicities. Also, **Palipoch and Punsawad (2013)** reported that Cis significantly accumulates within the human liver after injection with Cis, and a high dose caused hepatotoxicity distinguished by impaired liver function, higher concentrations of enzymatic biomarkers such as ALT and AST. **Zhai *et al.* (2019)** suggested that nitric oxide (NO) (as free radicals resulted from CIS injection) could play an important role in CIS-induced nephrotoxicity. Many anti-tumors' drugs accelerate NO production. It has been stated that CIS treatment led to a significant increase in the activity of calcium-independent NO synthase in the liver and kidney leading to enhancing NO formation. NO is well-known to react with the superoxide radical, creating a more effective oxidizing agent; peroxynitrite that can react clearly with 1) sulfhydryl residues in the cell membrane resulting in lipid peroxidation or with 2) DNA causing cytotoxicity**.**

The recorded reduction in SOD and CAT activities suggests their depletion in neutralizing the CM-induced reactive oxygen species (ROS). Several studies have demonstrated reducing the oxidative stress markers and increasing the antioxidant status following the treatment by fenugreek seeds **(Gupta *et al*., 2014, Joshi *et al*., 2015, Tavakoly *et al*,. 2018).** SOD and CAT are the first defense line against the toxic intermediates of oxygen metabolism. Furthermore, the reduction in SOD activity shows its consumption in scavenging superoxide radicals. CAT neutralizes the H_2O_2 produced by SOD catalyzed dismutation of superoxide anion. An elevated level of superoxide radicals hinders the activity of CAT **(Sushma and Devasena, 2010, Michel and Menze, 2019).** Furthermore, the Cis-induced nephrotoxicity is strongly related to an increase in the kidney lipid peroxidation. It has been realized that CIS declines antioxidant enzyme activities and induced the GSH depletion **(Farooq *et al*., 2019, Michel and Menze, 2019)**. GST, an important phase II enzyme, catalyzes the hydroquinones conjugation and epoxide polycyclic aromatic hydrocarbons with GSH for their detoxication. The GSH depletion is an indicator of tissue deterioration and damage; also it can have a considerable effect on the status of the cell antioxidants **(Sushma and Devasena, 2010).** Thus, the increased generation of free radicals and the decrease in the antioxidant defense system may lead to an increase in the production of renal lipid peroxidation and MDA in renal tissues **(Guerra and Bucci, 2019).**

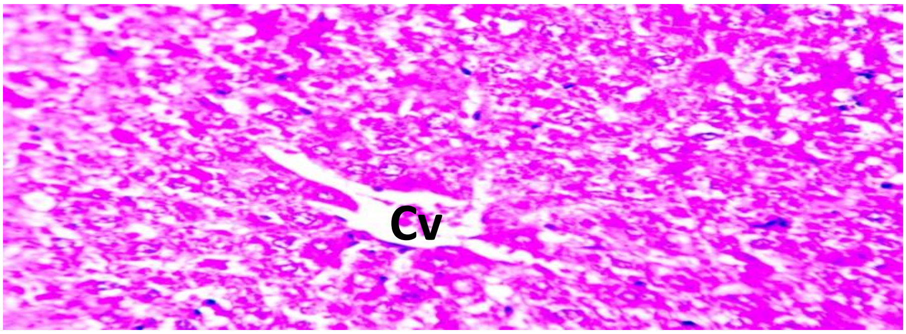
Cisplatin intoxication caused structural and functional impairments in the liver and kidney that was showed by massive histopathological changes and increased function tests of kidney and liver. However, it was associated with oxidative stress and lipid peroxidation as apparent by the increased level of ROS and MDA with a decreased level of total antioxidant activity. **Nagamma *et al*. (2019)** reported that when decreasing the free radical-scavenging activity of the antioxidants, increased oxidative stress is noticed. **El-Sayyad *et al*. (2009)** and **Kart *et al*. (2010)** showed that Cis promoted extreme anomalies in liver tissue histology and the ultrastructure. The Cis activated inflammatory responses and apoptosis in rats' liver and kidney as conducted by heightened expression of pro-inflammatory cytokine, tumor necrosis factor- α (TNF-α), and apoptotic marker p38 mitogen-activated protein kinase (p38 MAPK) due to the overproduction of ROS **(Hegazy and Emam, 2015).**

Fenugreek is a rich source of numerous flavonoids that impart significant antioxidant potential (Sushma and Devasena, 2010). **Gupta and Nair (1999)** have shown that fenugreek seeds are rich in flavonoids (>100 mg g^{-1}). **Randhir *et al.* (2004)** have reported increased phenolic antioxidants in the germinated sprouts of fenugreek. Our results showed that co-dealing of Cis with sprouted seeds of fenugreek induced the reduction in serum activity of AST, ALT, enzymes, MDA levels, creatinine, urea, and uric acid, while it increased the TP, albumin, TAC, SOD, and GSH compared to Cis-treated rats alone. Germinated fenugreek can prevent Cis-induced oxidative stress in the liver and kidney. The activities of SOD and CAT were restored to a level close to normal. This could be due to the potent radical-scavenging impact of fenugreek flavonoids, which may scavenge the free radicals induced in the Cis-treated group. This results in agreement with **Dixit *et al*,(2005)** who revealed that germinated seeds of fenugreek were noticed to be rich in polyphenols indicating the significant antioxidant activity of germinated fenugreek seeds which induced significant improvements of liver and kidney functions. **Nagamma *et al*, (2019)** confirmed that the -fed rats on fenugreek seed extract recorded a reduction in AST and ALT levels which suggesting decreased hepatic disorders. As well, **Sushma and Devasena (2010),** found that the treatment of germinated fenugreek seeds extract to rats with cypermethrin, induced hepatic and renal toxicity, can restore the antioxidant status and the activities of the enzymes to the levels close to the normal levels. Consequently, germination can lead to the development of such functional foods that have positive effects on humans, and that helps to maintain health **(Sangronis and Machado, 2007**).

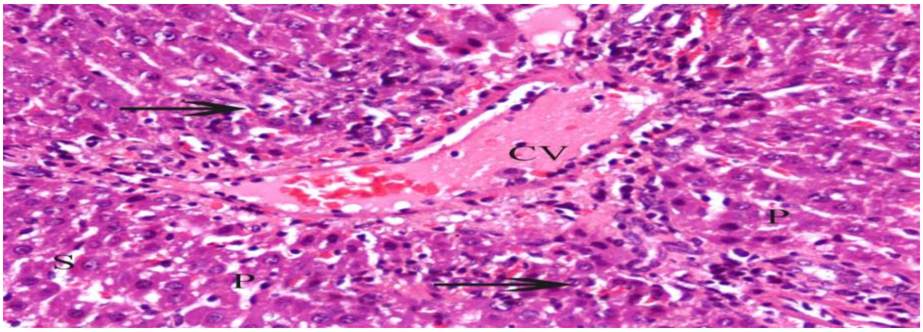
Histopathology

<u>Histopathological examination of the liver:</u>

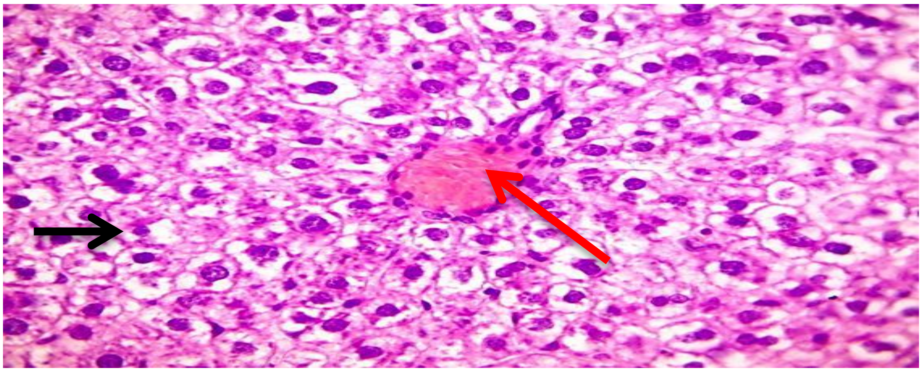
The results of the histopathological examination are presented in picture (1 – 4). The rat's liver in picture(1) showed group 1 (i.e. negative control group) with the normal histological structure of hepatic lobule, while picture (2) showed microscopic pictures of H and E stained hepatic sections that revealed that the rat received Cis (5 mg kg^{-1} body weight) has damage in hepatocytes (degeneration), cytoplasmic vacuolation, and centrilobular necrosis. On the other hand, picture (3) revealed the sections of rats received Cis +5% sprouted fenugreek that showing diffuse hydropic degeneration in hepatocytes (black arrows) and congested blood vessels (red arrows). Meanwhile picture (4) showed hepatic sections of rats received Cis +10% sprouted fenugreek that improved histology with regularly arranged hepatic cords around the central vein.



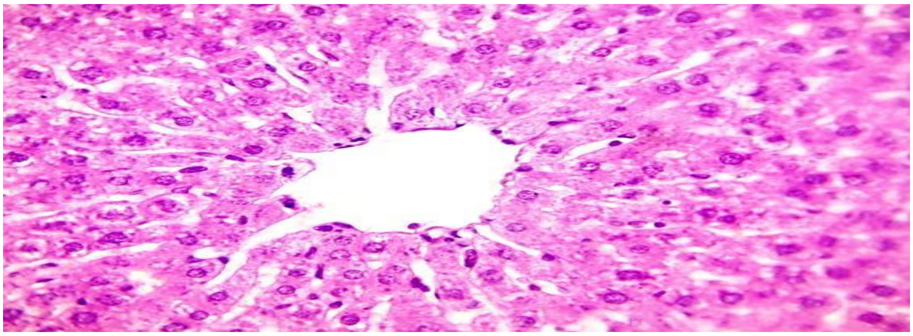
picture (1): Rat's liver in the negative control group showing normal radially arranged hepatocytes around central vein (CV)



picture (2): Hepatic sections of rats received cisplatin (+ control group)



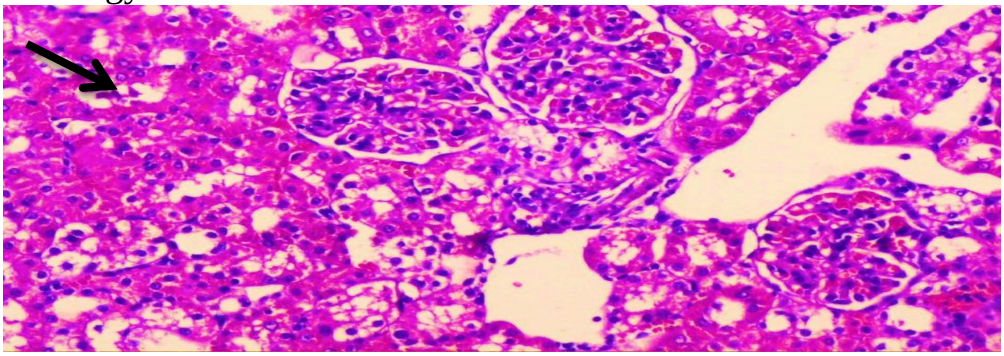
picture (3): Hepatic sections of rats received cisplatin +5% fenugreek



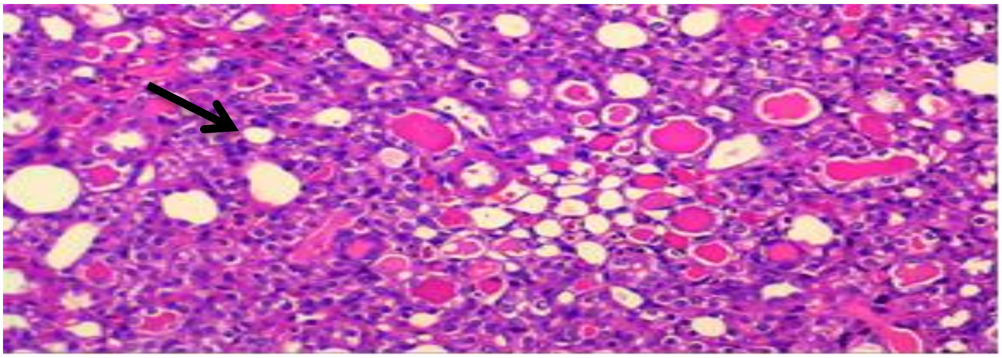
picture (4): Hepatic sections of rats received cisplatin+10% fenugreek

Histopathological examination of the kidney:

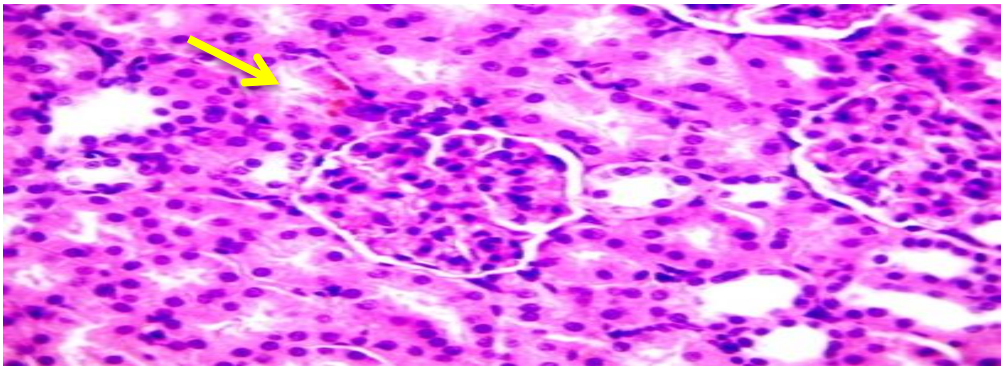
Rats' kidneys of rats from the negative control group showed the average-sized glomeruli as in picture (5), while rats' kidneys from the +ve control group showed the hyaline casts in the tubules surrounded by the chronic inflammatory cell in picture (6). On the other hand, picture (7) presented microscopic pictures of H and E stained renal sections of rats received Cis +5% fenugreek that showed tubular dilation and hyaline cast formation (black arrows) with the presence of lipofuscin pigment in the tubular epithelium (yellow arrows). Meanwhile, picture (8) showed microscopic pictures of H and E stained renal sections of rats received Cis +10% fenugreek showing greatly improved histology. X: 100 bar and X: 400 bar.



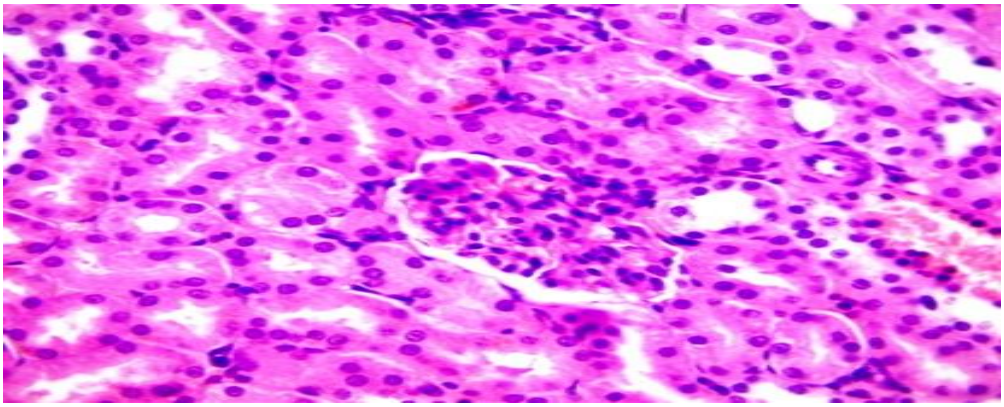
picture (5): Kidney of rat from the negative control group showing normal cortex including glomeruli (G) and tubules (T).



picture (6): Kidney of rats from the positive control group showed the hyaline casts in the tubules surrounded by chronic inflammatory cell



picture (7): Renal sections of rats received cisplatin +5% fenugreek



picture (8): Renal sections of rats received cisplatin +5% fenugreek

Conclusion

In conclusion, although CIS is used as a treatment of a wide range of cancers, it causes severe effects of nephro-and hepatotoxicity as indicated by the biochemical and histological analysis. The results of the present study showed that the treating of rats by 5 and 10% of sprouted fenugreek restored the biochemical and histological changes of Cis-induced liver and kidney toxicity in rats. This because germinated seeds exhibited stronger antioxidant activity that may protect against liver and kidney diseases.

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