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Research Article

Effect of Fenugreek Seed Extract on Lithium Induced Hepatorenal Toxicity and Oxidative Stress in Pregnant Rats

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Abstract

The present study aimed to investigate the effect of fenugreek seed extract against lithium carbonate (Li_2CO_3) induced liver and kidney toxicity in pregnant albino rats. Five groups of pregnant rats each contains six animals was used. Group (I) was served as control. Group (II) rats of this group were orally given fenugreek seed extract at a dose level of 0.4mg/kg body weight daily for four days from the third to the sixth day of pregnancy. Group (III): rats of this group were i.p. injected with Li_2CO_3 at a dose level of 800 mg/kg body weight daily for four days from the third to the sixth day of pregnancy. Group (IV): rats of this group were injected with Li_2CO_3 followed by fenugreek seed extract daily for four days from the third to the sixth day of pregnancy. Group (V): animals of this group were injected with Li_2CO_3 followed by fenugreek daily from the third to the sixth day of pregnancy, after the sixth day, Li_2CO_3 injection was stopped and fenugreek was given until the fifteenth day of pregnancy. The results revealed that Li_2CO_3 caused different histopathological alterations in the liver and kidney in addition to increase in caspase-3 and ki-67. Biochemical results showed increase in MDA and decrease in the activities of the antioxidant enzymes SOD and CAT. On the other hand, treatment with $Li_2CO_3$and fenugreek improved the histological picture of the liver and kidney, and reduced expression of caspase-3 and ki-67. Moreover, MDA level was decreased and SOD and CAT was elevated. In conclusion, fenugreek has ameliorative effect against lithium toxicity in pregnant rat by its antioxidant activity.

Keywords: Lithium Carbonate; Hepatotoxicity; Nephrotoxicity; Caspase-3; Fenugreek Seed Extract; Antioxidant

Introduction

Lithium carbonate (Li2CO3) is commonly used as a psychiatric medication for the treatment of mania, both acutely and in long term [1]. As a mood stabilizer, lithium is probably more effective in preventing mania than depression, and reduces the risk of suicide in bipolar patients [2]. It was also used to treat urinary calculi and gout with little success [3]. Lithium becomes widely distributed in the central nervous system and interacts with a number of neurotransmitters, decreasing norepinephrine release and increasing serotonin synthesis [4].

The toxicity of lithium carbonate was evaluated by many investigations; Lithium inhibited glycogen synthase kinase-3, which is involved in a wide range of signal transduction pathways. This effect occurs at high concentrations of lithium and may be more relevant for its toxic effect [5]. Ferner and Smith [6] reported that when lithium carbonate given to women during the first trimester of pregnancy it cause congenital defects to the cardiovascular system such as Ebstein's anomaly (a rare cardiac defect). Lithium carbonate administration to the pregnant rats from 6th - 15th of gestation caused an increase in the number of resorption and reduced the number of implantation and the size, body weight and the number of live fetuses [7]. Many pathological alterations were observed in different mammalian organs after treatment with Li-2CO3, in kidney of mice [8], rat [9], in thyroid gland of rat [10], liver of rabbit [11] and rat [12].

Fenugreek (*Trigonella foenum graecum*) is one of the oldest medicinal plants and an annual Mediterranean and Asiatic herb. The seeds are used worldwide as medicinal herb to soothe the stomach and help maintain blood sugar levels [13]. Its seeds are used in many oriented countries as a spice in food preparations due to their strong flavour and aroma [14] and as herbal medicine for their carminative, tonic and aphrodisiac effects [15]. It was used in old Egypt as incense, and for mummifying corpses and also for easy confinement and increase in milk production. Even nowadays Egyptian woman use this plant for curing menstrual pain, as a tea for stomach problems, and also as a complement matter for wheat

and corn flours for baking breads and confectionaries [16]. In ancient Chinese drugs, seeds of fenugreek were used as strengthen drug [17].

Fenugreek seeds exhibit hypoglycemic, hypolipidaemic, antifertilitic, antiandrogenic, and antinociceptive and wound healing properties, good source of dietary fibres and anticarcinogenic effect [18,19]. The present work was conducted to study the effect of fenugreek seeds extract on toxicity induced by lithium in liver and kidney of pregnant rats.

Materials and Methods

A- Materials used:

1- Lithium Carbonate

Lithium carbonate, with trade name Prianil CR was obtained as tablets from El-Nile Company for pharmaceuticals and chemical industries, Cairo, Egypt. Each tablet contains 400 µg of lithium carbonate.

The tablets were dissolved in distilled water and were given interperitoneally to the experimental animals at a dose level of 800 mg/kg body weight, equivalent to therapeutic dose for human [20].

2- Fenugreek

Fenugreek (*Trigonella foenum graecum*) purchased from local market, cleaned from extraneous matter, air dried and ground into a fine powder. The fenugreek seed extract was freshly prepared by dissolving one gram of fenugreek seeds powder in 100 ml distilled water by a vortex cyclomixer for 10 minutes and then centrifuged. The supernatant was collected and given orally to the animals at a dose level 0.4 mg/kg body weight [21].

B-Experimental Animals

Healthy mature virgin females and fertile males of Wister albino rats (*Rattus norvegicus*), weighing 150 ± 10g and aged 16 weeks, were obtained from Hellwan Animal Breeding Farm, Ministry of Health, Cairo, Egypt. Animals were kept in the laboratory for at least one week before initiation of the experiment for acclimatization. They were housed in specially designed plastic rodent cages in animal house at Faculty of Science, Menoufia University. They were maintained at 25 ± 2ºC in 12h light: 12h dark cycle. Water was allowed ad *libitum*. Free access of standard diet composed of 50% ground barely, 10% ground yellow maize, 20% milk and 10% vegetables was supplied. All the experiments were done in compliance with the guide for the care and use of laboratory animals approved by Faculty of Science, University of Menoufia, Egypt (Approval No. MNSC179).

Mating was induced by housing 2 virgin females with one fertile male overnight. Females were checked daily in the morning for the presence of a copulatory plug and vaginal smears were carried out to give a precise determination of the onset of gestation. The day at which vaginal plug was detected was considered as zero day of pregnancy. While the 15th day considered as the end of the experimentation period.

Groups of Animals under Investigation

Pregnant females were equally divided into 5 groups, each contain 6 animals.

(I): was served as normal control.

(II): animals of this group were orally given fenugreek seed extract (0.4 mg/kg body weight) daily for four days from the third until the six days of pregnancy.

(III): animals of this group were intraperitoneally given lithium carbonate (800 mg/kg body weight) daily for four days from the third until the six days of pregnancy.

(IV): females were intraperitoneally given lithium carbonate (800 mg/kg body weight) then orally given fenugreek seed extract (0.4 mg/kg body weight) daily for four days from the third until the six days of pregnancy.

(V): females were given lithium carbonate (800 mg/kg body weight) then orally given fenugreek seed extract (0.4 mg/kg body weight) daily from the third to the sixth day of pregnancy. After the sixth day, lithium carbonate injection was stopped and fenugreek seed extract was given until the fifteenth day of pregnancy.

C-Histological Preparation

At the fifteenth day of pregnancy, the pregnant females of the control and treated groups were scarified and their livers and kidneys were fixed in alcoholic Bouin's fluid. After fixation, specimens were dehydrated in ascending series of alcohol, cleared into two changes of xylene and embedded in paraffin (m.p 58 - 60). Sections of 5 microns thickness were cut by using rotary microtome and mounted on clean slides with gelatine as adhesive media. For histological examination, sections stained with Ehrlich's haematoxylin and counter stained with Eosin.

D- Immunohistochemical Study

Immunohistochemical reaction was performed by using an avidin biotin complex immune peroxidase technique on paraffin sec-

tions according to Hus., *et al*. [22]. Formalin fixed paraffin-embedded liver sections were deparaffinized, endogenous peroxidase activity was blocked with H2O2 in methanol. Then, the sections were heated in 0.01 mol/L citrate buffer in a microwave pressure cooker for 20 minutes. The slides were allowed to cool to room temperature and nonspecific binding was blocked with normal horse serum for 20 minutes at room temperature. The MIB-1 monoclonal antibody was used for detection of nuclear Ki-67, a marker of proliferating cells (code no: M7187, dilution 1:40, DAKO) while monoclonal antibody ((Neo Markers, Cat. #Ms-113-P, Fremont, CA, USA) was used for detection of caspase 3. Counterstaining was performed using Mayer's haematoxylin (BioGenex, Cat. No.94585). For evaluation of each marker, the percentage area of positively stained cells in the total number of cells was calculated using image-j analyser.

E-Biochemical Study

For biochemical analysis, blood samples were collected in clean centrifuge tubes. The samples left to clot in room temperature and then serum separated by centrifugation at 3000 rpm for 20 minutes. The collected serum stored at -18 -20°C until analysis. The activity of superoxide dismutase (SOD) was determined according to Minami and Yoshikawa [23], while the activity of catalase (CAT) was measured according to Góth [24]. Lipid peroxidation marker, malondialdehyde (MDA), was determined according to Ester Bauer and Cheeseman [25].

F-Statistical Analysis

Data were expressed as mean ± standard deviation (SD). The significance of differences means evaluated by using independent sample t test. Statistical program of social sciences (SPSS) software for windows, version 20 was used.

Results and Discussion

a. Histological Results

Liver of control animals or animals administered with fenugreek seed extract showed normal organization of the hepatic tissue (Figure 1A, 1B). Examination of liver sections of rats treated with lithium carbonate showed apparent signs of degeneration. The hepatic cells lost their characteristic appearance and most of them showed cytoplasmic vacuolization and pyknosis of the nuclei with congested and enlarged central vein (Figure 1C). Leucocytic infiltration and bile duct proliferation were observed (Figure 1D). Scattered areas of degenerative hepatocytes were seen with showed fatty infiltration (Figure 1E). Examination of liver sections of rats treated with lithium carbonate and fenugreek (from 3rd - 6th day of gestation) showed that most of the previously observed degenerative changes induced by lithium carbonate were still seen.

When animals treated with lithium carbonate (from 3rd - 6th day of gestation) and fenugreek (from 3rd - 14th day of gestation), advanced degree of recovery was observed. The majority of hepatocytes appeared with normal cytoplasm and nuclei (Figure 1F).

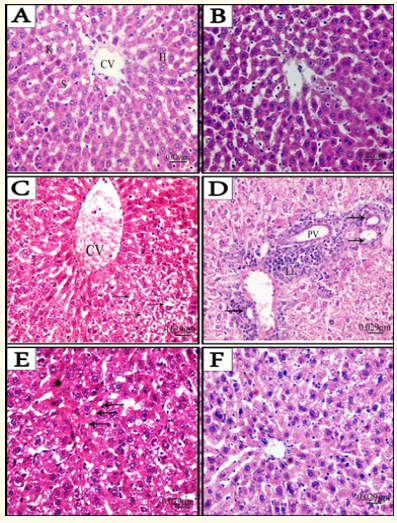

Figure 1(A): A photomicrograph obtained from liver of a control rat showing central vein (CV), hepatocyte (H), blood sinusoids(S) and Kuffer cells (K). (B). liver section of a rat treated with fenugreek seed extract showing normal organization of hepatic architecture. (C). liver section of a rat treated with lithium carbonate showing enlarged central vein with congestion (CV) and cytoplasmic vacuolation (arrows). (D). liver section of a rat treated with lithium carbonate showing congested portal vein(PV), bile duct proliferation(arrows), and leucocytic infiltration(LI). (E). liver section of a rat treated with lithium carbonate showing fatty infiltration (arrows), and degenerated area (*). (F). liver section of a rat treated with lithium followed by fenugreek for fourteen days showing recovery of hepatic cells, with increase of bi-nucleated cells, (H and E).

Histological examination of kidney of control rats and rats given fenugreek extract showed normal appearance of kidney cortex (Figure 2A). On the other hand, animals treated with lithium

carbonate showed histological alterations. The renal blood vessels were enlarged and congested (Figure 2B).

Scattered haemorrhage areas were obviously seen between the different components of the kidney tissue. Some of the renal corpuscles appeared damaged and irregular in shape while other contained shrunken glomeruli and widen capsular spaces. Cytoplasmic vacuolation was clearly observed in large number of tubular cells (Figure 2C). Such abnormalities were decreased in animals treated with lithium carbonate (from 3rd - 6th day of gestation) and fenugreek (from 3rd - 14th day of gestation) (Figure 2D).

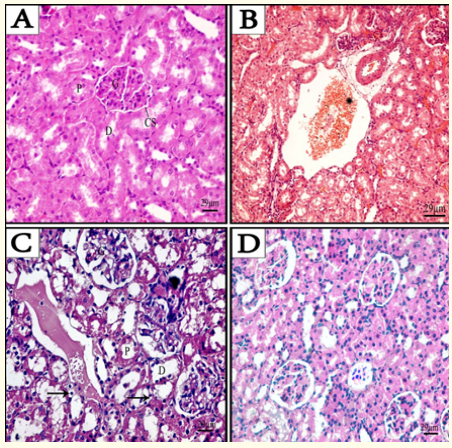

Figure 2(A): A photomicrograph obtained from the cortical region of a kidney of a control rat showing normal glomerulus (G), capsular space (CS), proximal convoluted tubules (P) and distal convoluted tubules (D). (B). Cortical region of a kidney of a rat treated with lithium carbonate showing enlarged and congested renal vein. (C). cortical region of a kidney of a rat treated with lithium carbonate showing shrinkage in glomerulus (G), dilated proximal and distal renal tubules (P &D), cytoplasmic vacuolation in the lining epithelium of tubules (arrows). (D). cortical region of a kidney of a rat treated with lithium and fenugreek showing an improvement in glomeruli and tubules, (H and E).

Lithium carbonate is one of the most common mood stabilizers which used for bipolar disorder (manic depression), mania, hypomania and sometimes recurrent severe depression [2]. In the present study, lithium carbonate treatment induced histopathological alterations in liver of pregnant rats. These results were in agreement with Toplan., *et al*. [12] who observed that lithium administration caused many histological alterations in liver tissue of rats including disorganization in hepatic cords, cytoplasmic vacuolization in hepatocytes, and increase in the amount of collagen fibres. Bhat., *et al*. [26] reported that the long-term administration of lithium carbonate caused many changes in the liver tissue such as necrosis, distortion of the hepatic cords, dilated sinusoids, bile duct proliferation and dilated and congested central veins. Lithium application constituted histopathological changes which caused severe liver damage, including sinusoidal dilation, congested central veins, vacuolization and inflammatory cell infiltration [27].

The cortical region of the kidney tissues showed many histopathological changes after treatment with lithium carbonate. Many authors observed similar results. Toplan., *et al.* [28] reported that lithium carbonate affected the kidney by causing degeneration in glomeruli and tubular structures, increase in mesangial matrix and damage of proximal and distal tubule. Kumarguru., *et al.* [29] showed a significant chronic tubulointerstitial nephropathy and a considerable glomerular pathology in the kidney and damage of the proximal tubules. Histopathological examination of kidneys tissue of rats treated with lithium carbonate showed necrotizing cell in damaged tubules with hemorrhagic glomerulitis [30]. Marti., *et al.* [31] reported that lithium-fed diet to rats exhibited grossly dilated in collecting ducts, a striking focal tubulointerstitial fibrosis, many glomeruli showed evidence of a focal glomerulosclerosis while others showed attachments to the capsular wall. Greatly dilation in the cortical collecting ducts and their epithelial lining was observed.

b. Immunohistochemical Result:

i. Caspase-3

Expression of caspase-3 appeared in the cytoplasm of the hepatocytes as brown colour. Control animals and animals given fenugreek showed slight expression of caspase-3 (Figure 3A). On the other hand, rats treated with lithium carbonate showed expression of caspase-3 in large number of hepatocytes (Figure 3B).

A decrease in expression of caspase-3 was observed in animals treated with lithium carbonate (from 3rd-6th day of gestation) and fenugreek (from 3rd-14th day of gestation) (Figure 3C). Data in Figure (4) showed the percentage area of caspase-3 expression in the cytoplasm of hepatocytes of the different experimental groups. There were no statistical differences between control group and fenugreek treated group. The obtained results from rats treated with Li_2CO_3 showed a significant elevation ($p < 0.05$)

in the percentage area of caspase-3 in the cytoplasm of hepatocytes. Treating rats with Li_2CO_3 + fenugreek showed a significant decrease ($p < 0.05$) in the percentage area of caspase-3 in the cytoplasm of liver sections.

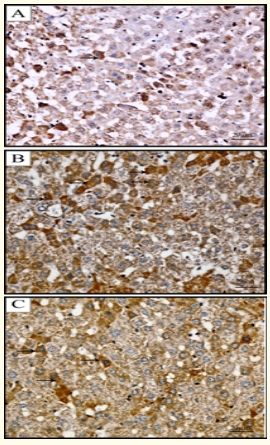

Figure 3(A): A photomicrograph obtained from liver of a control rat showing expression of casepase-3 in the cytoplasm of few number of hepatocytes (arrows). (B). liver of a rat treated with lithium carbonate showing expression of casepase-3 in the cytoplasm of large number of hepatocytes (arrows). (C). liver of a rat treated with lithium carbonate followed by fenugreek showing few expressions of casepase-3 in the cytoplasm (arrows). (casepase-3 immuno stain, counter stained with haematoxyline).

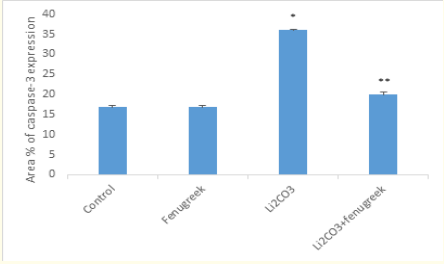

Figure 4: The percentage area (Mean ± SD) of caspase-3 expression in liver of rats in different experimental groups. (*) significant increase comparing with control group. (**) significant decrease comparing with Li_2CO_3 group.

Similarly, expression of caspase-3 was increased in tubular cells of kidney of rats treated with lithium carbonate compared with control rats or rats treated with fenugreek extract (Figure 5A - 5C). A significant increase ($p < 0.05$) was recorded in the percentage area of caspase-3 in kidney of lithium carbonate-treated animals, while animals treated with Li2CO3 + fenugreek showed a significant decrease in the expression of caspase-3 (Figure 6).

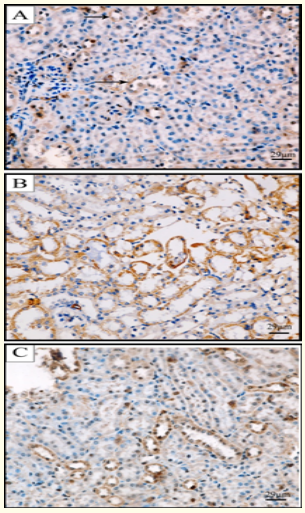

Figure 5(A): A Photomicrograph Obtained from Renal Cortex of a Control Rat Showing Expression of Casepase-3 in the Cytoplasm of Cells of Some Tubules (Arrows). (B). Renal Cortex of Lithium Carbonate-Treated Rat Showing Large Number of Renal Tubules with Positive Expression of Casepase-3 in the Cytoplasm of their Lining Cells. (C). Renal Cortex of a Rat Treated with Lithium Carbonate Followed by Fenugreek Showing Positive Expression of Casepase-3 in a Few Number of the Renal Tubules (Casepase-3 Immuno Stain, Counter Stained with Haematoxyline).

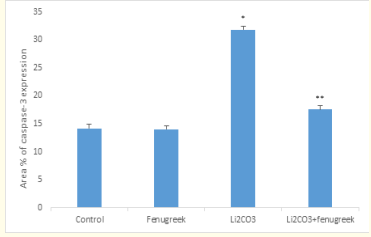

Figure 6: The Percentage Area (Mean ± SD) of Caspase-3 Expression in Renal Cortex of Rat of the Different Experimental Groups. (*) Significant Increase Comparing with Control Group. (**) Significant Decrease Comparing with Li_2CO_3 Group.

ii. Ki-67:

Ki-67 was expressed in few nuclei of hepatocytes in liver sections of rats of the control groups (Figure 7A). Animals treated with fenugreek extract showed expression of Ki-67 in few nuclei of the hepatocytes which was nearly similar to control group. After treatment with lithium, expression of Ki-67 was observed in a large number of the nuclei of the hepatocytes (Figure 7B). Liver sections obtained from rats treated with lithium followed by fenugreek showed expression of Ki-67 in the nuclei of only scattered hepatocytes (Figure 7C). By using image j analysis, the percentage area of Ki-67 positive nuclei of hepatocytes of rats treated with lithium was significantly elevated ($p < 0.05$). On the other hand, rats treated with lithium and fenugreek extract recorded a significant decrease ($p < 0.05$) in the percentage area of Ki-67 positive nuclei of the hepatocytes (Figure 8).

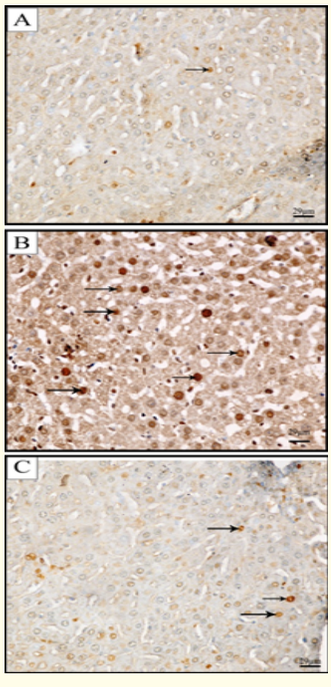

Figure 7(A): A Photomicrograph obtained from a Liver of a Control Rat Showing Few Expressions of Ki-67 in Some Nuclei of the Hepatocytes (Arrow). (B). Liver of a Rat Treated with Lithium Carbonate Showing Positive Expression of Ki-67 as Brownish Color in many Nuclei of the Hepatocytes. (C). Liver of a Rat Treated with Lithium Carbonate Followed by Fenugreek Showing Expression of Ki-67 in Few Nuclei of Hepatocytes (Ki-67 Immuno Stain, Counter Stained with Haematoxyline).

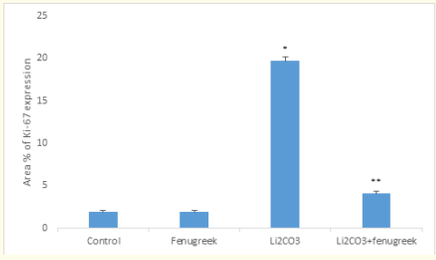

Figure 8: The Percentage Area (Mean ± SD) of Ki-67 Expression in Rat Liver of the Different Experimental Groups. (*) Significant Increase Comparing with Control Group. (**) Significant Decrease Comparing with Li_2CO_3 Group.

Concerning Ki67 in the kidney, it was expressed in the nuclei of few tubular epithelial cells in control and animals treated with fenugreek extract (Figure 9A). On the other hand, pregnant rats treated with lithium revealed expression of Ki67 in large number of cells (Figure 9B). Animals given lithium and fenugreek extract showed a decrease in the expression of ki67 (Figure 9C). Data in Figure 10 showed that the percentage area of ki67 positive nuclei in tubular cells was significantly increase ($p < 0.05$) while animals treated with lithium and fenugreek extract recorded a significant decrease in expression of ki67.

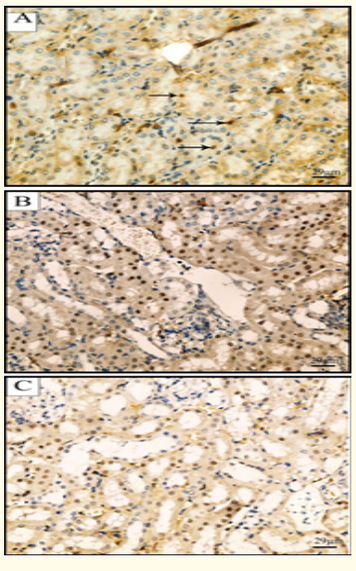

Figure 9(A): A Photomicrograph obtained from Section of Renal Cortex of a Control Rat Showing Few Expressions of Ki-67 in the Nuclei of the Tubular Cells (Arrows). (B). Renal Cortex of a Rat Treated with Lithium Carbonate Showing Positive Expression of Ki-67 in Large Number of Nuclei. (C). Renal Cortex of a Rat Treated with Lithium Carbonate and Fenugreek Showing Positive Expression of Ki-67 in Few Nuclei (Ki-67 Immuno Stain, Counter Stained with Haematoxyline).

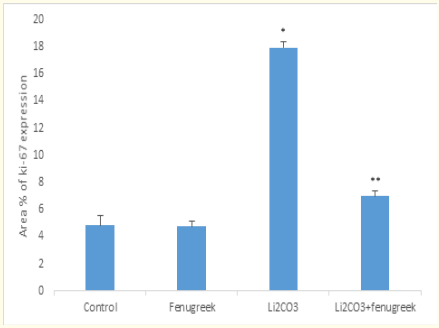

Figure (10): The percentage area (Mean ± SD) of Ki-67 expression in renal cortex of rat of the different experimental groups. (*) significant increase comparing with control group. (**) significant decrease comparing with Li_2CO_3 group.

Immunohistochemeical observation indicated that lithium carbonate increased the positive staining of both ki-67and caspase-3 in both liver and kidney tissues. Antigen KI-67 is a nuclear protein that is associated with cellular proliferation. Furthermore, it is associated with ribosomal RNA transcription [32]. Inactivation of antigen Ki-67 leads to inhibition of ribosomal RNA synthesis [33]. Caspase-3 is a marker of the early phase of apoptosis [34], and is essential for certain processes associated with the formation of apoptotic bodies [35]. Doi., *et al*. [36] reported that there was an increase in the number of Ki-67-positive cells after administration of lithium for 14 days. Santarelli., *et al*. [37] reported that chronic but not subacute treatment with antidepressants increase the cellular proliferation in the hippocampus. The increase in the caspase-3 positive cells was agreement with the study of Cabrera., *et al*. [38] who observed the increase in the caspase-3 in the cerebellum after treatment of lithium carbonate.

c. Biochemical Results

Data in Table 1 showed that treating pregnant rats with lithium significantly increased the level of MDA over the normal value. The activity of the antioxidant enzymes, SOD and CAT, was decreased significantly in sera of animals treated with lithium. Animals treated with lithium and fenugreek extract revealed a decrease in level of MDA and an increase in activity of SOD and CAT.

Animal group	MDA (n mol/ml)	CAT (µ mol / sec /ml)	SOD (n mol / ml)
Control	26.67 ± 1.63	26.67 ± 1.63	89.52 ± 0.93
Fenugreek	27.83 ± 1.60	27.83 ± 1.60	90.50 ± 0.61
Lithium	16.83 ± 1.94 *	16.83 ± 1.94 *	68.76 ± 2.59 *
Lithium + Fenugreek ((from 3rd - 6th day of gestation)	33.17 ± 2.48 **	33.17 ± 2.48 **	94.39 ± 6.80 **
Lithium + Fenugreek (from 3rd - 14th day of gestation)	42.67 ± 2.80 **	42.67 ± 2.80 **	109.56 ± 2.32 **

Table 1: Effect of different Treatments on Serum MDA Level, CAT and SOD Activity.

* Significant, at Level p ≤ 0.001, Comparing to Control Group.

** Significant, at Level p ≤ 0.001, Comparing to Lithium Group

The results revealed an increase in MDA and decrease in SOD and CAT in sera of animals treated with lithium. Similar results were obtained by Joshi., *et al*. [39], Toplan., *et al*. [12] and Mwaheb., *et al.* [30]. Free radical scavenging enzymes such as catalase, superoxide dismutase is the first line cellular defence enzymes against oxidative injury, decomposing O_2 and H_2O_2 before their interaction to form the more reactive hydroxyl radical (OH•). The equilibrium between these enzymes is an important process for the effective removal of oxygen stress in intracellular organelles. In our study, a decrease in the activity of SOD can result in the decreased removal of superoxide ion, which can be harmful to the hepatic and renal cells.

Fenugreek (*Trigonella foenum graecum*), used as traditional medicine and natural additive food, has been shown to exert significant antiatherogenic, antidiabetic, antianorexic, antioxidant, anticarcinogenic, antihyperlipidemic, galactagogue and anti-inflammatory effects in several human and animal models (40).

Our results showed that treating animals with lithium carbonate and fenugreek alleviated the histopathological changes induced by lithium carbonate in liver and kidney. These results agree with Belaïd-Nouira., *et al.* [41] who reported that fenugreek reduced the aluminium chloride toxicity in liver tissues. Also, Shekha., *et al.* [42] showed that treating rats with fenugreek in addition to ethylene glycol showed a disappearance of the renal histopathological changes.

Apoptosis is a controlled physiological cell death process that occurs as a result of normal cellular differentiation and development. Defects in apoptosis is contribute for many diseases, including liver injury [43]. This process is mediated by a complex mechanism involving intracellular proteases, the caspases, activators and inhibitors of these cell death proteases [44]. A decrease in expression of caspase-3 was recorded in liver and kidney of animals treated with lithium and fenugreek. This result indicated the antiapoptotic effect of fenugreek and is similar to results of Ibrahim and Osman [45] who studied the effect of fenugreek on hepatotoxicity of acetaminophen. Expression of Ki-67 was reduced in rats treated with lithium and fenugreek. This result proved the antiproliferative effect of fenugreek [46].

Treating pregnant rats with lithium and fenugreek extract reduced the lipid peroxidation marked, MDA and increased the activity of SOD and CAT. This data come in accordance with [47] who reported that extract of fenugreek prevented the increase in the amount of hydrogen peroxide and MDA and the decrease in SOD, CAT and GST activities in rats intoxicated with cypermethrin. Kumar and Bhandari [48] reported that aqueous extract of fenugreek reduce the MDA level and elevate the SOD and CAT activity after the toxicity with monosodium glutamate. Lamfon [49] showed that oral administration of fenugreek seeds extract improved the histological changes induced by carbendazim and suppress the oxidative stress as indicated by decrease of lipid peroxidation and increase activity of SOD and CAT.

Fenugreek seeds are rich source of many active phytochemicals such as saponins, coumarin, fenugreekine, nicotinic acid, sapogenins, phytic acid, scopoletin, and trigonelline, which are thought to account for many of its presumed therapeutic effects [50]. Fenugreek seed polyphenols proved to be an effective antioxidant and an anti-inflammatory agent [51]. Thus, it is concluded that fenugreek seeds extract exhibited antioxidant property which ameliorate the hepatorenal toxicity induced by lithium carbonate in pregnant rats.

Conclusion

Treating pregnant rats with lithium carbonate induced histopathological alterations in the liver and kidney. In addition, expression of the proliferative marker ki67 and the antiapoptotic protein bcl2 were increased in both organs. Biochemical results indicated an increase in MDA and decrease in SOD and CAT in sera of animals treated with lithium. On the other hand, animals with lithium carbonate and fenugreek alleviated the histopathological, immunohistochemical and biochemical changes induced by lithium carbonate in liver and kidney. Fenugreek seed polyphenols proved to be an effective antioxidant. Thus, it is concluded that fenugreek seeds extract exhibited antioxidant property which ameliorate the hepatorenal toxicity induced by lithium carbonate in pregnant rats.

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