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The use of dietary antioxidants to improve on CCl4 hepatotoxicity rats .

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Abstract:

Liver disease still a medical problem that face several patients in awide as especially large base of humans. Liver injury due to chemicals (or) infectious agents may lead to progressive liver fibrosis and ultimately cirrhosis and liver failure.Therefore the aim of this study was to investigate the protective effect of four plants including green coffee, Red cabbage, Artichoke, Black mulberry and silymarin individually toward hepatotoxicity induced by CCl4. All plants were added at two concentration levels, 2.5 % and 5 %, respectively to the basel diet. Forty-eight adult male albino rats, weighing (150±5g), were divided into 12 groups each with 4 rats, each with similar total body weight. All the group of rats except for negative control were injected for two consequence weeks each with 0.2mg/kg of Carbon tetrachloride (CCl4) injectable solution to induce the liver impaired. Glutamic Oxaloacetic transaminas (GOT),glutamic pyruvic transaminas (GPT), creatinine, and histopathological changes all were measured. The results showed that all treatments significantly reduced Glutamic Oxaloacetic transaminas (GOT) as compared to positive control grop ($p \leq 0.05$). Treatment plants were order upon their effects on GOT values as following: blackmulbarry 5% > silymarien 5%> silymarien 2.5%> red cabbages 5%> artichoke 5%. On the other hand, their treatment plants were order upon their effects on GPT values as following: silymaren 5% > red cabbage 5%> green coffee5%>artichoke5%> silymaren 2.5%> Black mulberry 2.5%.data also showed that all treatment plants were order

upon their effects on GSH values as following: silymaren 5% > red cabbage 5%> artichoke 5%> silymaren 2.5%> Black mulberry 2.5%. Significant increase in creatinen on control positive grope as compeard with control negative. Histopathological investigation confirmed the biochemical changes in the liver and spleen functions. In concolusion, data suggested a potential role of these plant under study on liver diseas.

Keywords: Hepatotoxicity, artichoke,milkthistle,green coffee,red cabbage, silymarien, black mulberry, antioxidant enzymes activities ,GPT, GOT, MAD,G-SH.

Introduction:

Liver is one of the important organs of the body which plays a major role in the metabolism of protein, carbohydrates and lipids. It is also having a wide range of functions including detoxification, storage of glycogen, production of several coagulation factors and growth factors hormones. Liver diseases are mainly caused by toxic chemicals, excess consumption of alcohol, infections and autoimmune disorders. Hepatotoxicity in most cases is due to free radicals that are fundamental to many biochemical processes and represent an essential part of aerobic life and metabolism. (**Wolf 1999) and Abd (El-Ghany and Nanees 2010).**

Plant antioxidants constitute one of the most active food compounds (**Kris *et al*., 2002).** The main source of these substances is plant material. Garlic, broccoli, green tea, soybean, tomato, carrot, brussels sprouts, kale, cabbage, onions, cauliflower, red beets, cranberries, cocoa, black berry, blue berry, red grapes, prunes, and citrus fruits are mentioned as the richest source of antioxidants. The content of phenolic antioxidants calculated per one kilogram of plant dry matter amounts to form about 0.1 to 1.0 g in the majority of fruits and vegetables up to 226 g in green leaves **(King and Young, 1999).** Interception of free radical generation is to be described under two heads. In a biological system. Flavonoids, a large family of polyphenolics synthesized by plants,including: anthocyanidins, chalcones, flavanols, flavones, flavanones, Flavonols, Flavononols, isoflavones. They act as free radical acceptors and chain breakers, but the antioxidant activity of flavonoids depends on their chemical structure. The potential benefits to human health of flavonoids include antioxidant activities. **(Beecher, 2003)**.

Silymarin is extracted from the seeds of the milk thistle. Its major flavonolignans include silybin (syn. silibinin) A and B, isosilybin A and B, silychristin A and B, and silydianin, as well as other minor polyphenolic compounds. Silymarin as antioxidants, scavengers and regulators of the intracellular content of glutathione also as inhibitors of the transformation of stellate hepatocytes into myofibroblasts, the process responsible for the deposition of collagen fibres leading to cirrhosis according to **(Mills and Bone, 2005).**

Green Coffee Bean Extract is a natural compound which has demonstrated its ability to support weight loss, stimulate the liver to increase fat metabolism and reduce fat accumulation in the body, even when no changes are made to caloric intake and exercise level. The effects component of the green coffee bean extract due to the chlorogenic acid. which include a naturally-occurring blend of the 3, 4, and 5-caffeoylquinic acids. Chlorogenic acid is a polyphenolic compound that is known to be a natural antioxidant which neutralizes free radicals in the body. It can also play a significant role within the body's natural biological processes due to its anti-inflammatory and antioxidant activities. (**Oben *et al*.,2008).**

Red cabbage belongs to the Cruciferae family, is inexpensive and ,is easy to grow harvest and store. Red cabbage is a rich source of anthocyanins which have health promoting properties and potential applications as natural food color. This red cabbage color can be widely used in wines, beverages, fruit sauces, candies and cakes. While anthocyanins have been traditionally used as food colorants, **(Dr. Julie and Pliszka,2009)**, also possess health promoting benefits including anticarcinogenic activity. The effect of anthocyanins on different cancers have been studied, including colon, pancreatic esophageal, lung and skin, cancers. Red cabbage extract has been reported to possess anti-diabetic and anti-inflammatory effects **(Kataya, 2008).**

Globe artichoke *(Cynara scolymus, L.)* is an ancient herbaceous plant, originating from the Mediterranean area. It is rich in bioactive polyphenol compounds (mainly cynarin, luteolin and chlorogenic acid), dietary fibers, vitamins and minerals **(Lattanzio *et al*., 2009).** Its leaf is widely used for therapeutic purposes**.** Cynareae tribes present in Egypt are known for their efficacy in relieving some liver disorders **(El-Sohafy *et al*., 2013).** Artichoke leaf extracts have traditionally been used to treat

dyspeptic symptoms, increased bile flow and to exert hepatoprotective, lipid-lowering, antioxidant and antispasmodic effects **(Holtmann *et al.*, 2003)**. Artichoke has also several non-food uses: the leaves, rich in polyphenols, are employed by the pharmaceutical industry for the production of commercial extracts, while roots and flower heads contain inulin, an important oligosaccharide used as a prebiotic ingredient in functional foods **(Raccuia and Melilli, 2004).**

Morus nigra, L. (*Moraceae*) belongs to the genus *Morus* and is found in Africa, South America and in Asia. *M. nigra* has been used in Unani medicine as antitussive, diuretic, expectorant and hypotensive. It has wide range of medicinal uses and can be used either as single drug or compound drugs to treat different ailments. The phenolic compounds of *M. nigra* have anti-oxidant and anti-bacterial activities. The bark of M. nigra has been used as antihelmintic and its extracts have antibacterial and fungicidal activity. In present article, medicinal uses of M. nigra have been discussed. **Fekri N ,and Khayami, (2008).**

In the present work , the protective effect of some plant parts including Green coffee, Red cabbage, Artichoke, Black mulberry and silymarin as Dietary antioxidants to treat liver disorders induced by carbon tetrachloride on rats.

Materials and Methods

Plantes:

The test plants in this investigation were Artichoke (*cynara scolymus*), Black mulberry (*mours nigra*), The Green coffee ,and Red cabbage were purchased from the local market of Menofia , Egypt.

Chemeicals and drug:

Carbon tetrachloride (ccl4) was obtained from AL-Gomhoriya Company for Med-preparations Chemicals and Medical equipment's, Cairo, Egypt as10% liquid solution . silymarin capsules(Legalon 140mg) was obtained from Pharmaceutical Industries Cairo, Egypt.

Experiment of animals:

Forty-eight adult male albino rat's ,weighting (150±5 g). from Research Institute of Ophthalmology, Medical Analysis Department, Giza, Egypt ,were used in this study.

Methods:

Preparation of dried plant materials :

All plants were carefully washed with tap and distilled water then sliced into small pieced than put it an vacuum oven at 45 C^o to deride were then grounded and sifted using 60 mesh screens.

Carbon tetrachloride (CCl4):

It was dispensed in white plastic bottles each containing one liter as a toxic chemical material for liver poisoning according to **(Passmore and Eastwood, 1989)**. In the same time, it is mixed with 10% olive oil which obtained from the Pharmacy for dilution during the induction.

Experimental design:

Induction of carbon tetrachloride and treatment Protocol:

Rats were divided into 12 groups each with 4 rats, Each with similar total body weight. All the group of rats except for negative control were injected for two consequence weeks each with 0.2mg/kg of CCl4 injectable solution to induce the liver impaired. Rats were kept on experimental diets for 4 weeks according to the following:

- **Control positive:** (hepatic group fed on basal diet "casein diet")and control negative, rats were fed on basal diet and tap water (untreated group).
- **Group 3 and 4:** Hepatic rats treated with orally dose of Silymarin at two concentration levels, (2.5%and 5%), respectively.
- **Group 5 and 6:** Hepatic rats treated with powder of Green Coffee at two concentration levels, (2.5 % and 5 %), respectively.
- **Group 7 and 8:** Hepatic rats treated with powder of Red cabbage at two concentration levels, (2.5 % and 5 %), respectively.
- **Group 9 and 10:** Hepatic rats treated with powder of Artichoke leaves at two concentration levels, (2.5 % and 5 %), respectively.
- **Group 11and 12:** Hepatic rats treated with powder of Black mulberry at two concentration levels,(2.5 % and 5 %), respectively.

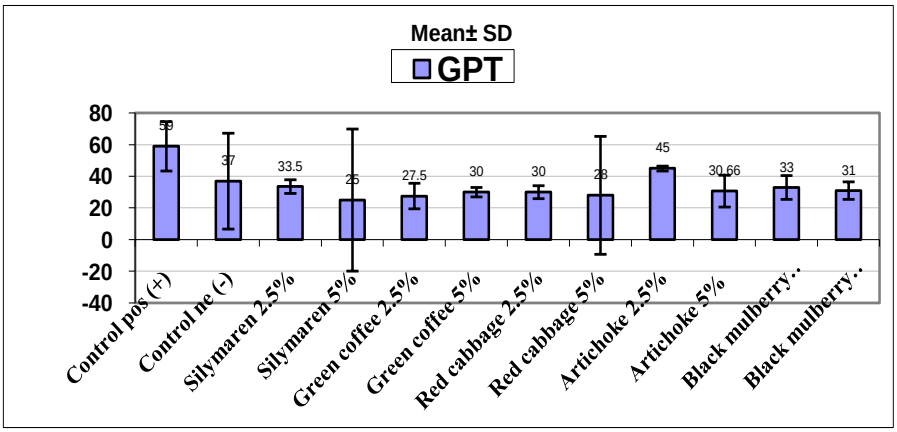
In the final step is fed normal rat's food plus a powder plants under study for a period of 30 days

Biochemical evaluation:

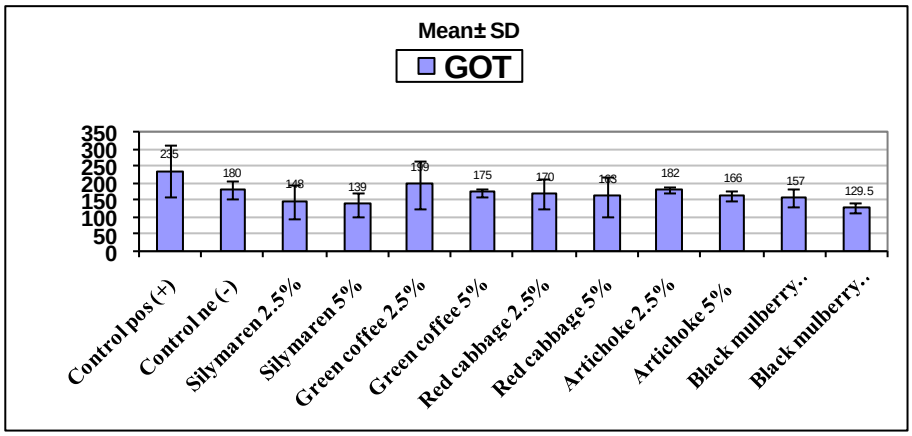
At the end of experiment peried, rats were fasted and only allowed to drink water for 16 hours and they were humanely sacrificed under diethyl ether anesthetized., blood was collected portal vein in two sterile tube one with EDTA to analyze blood picture within 24 hours and the second tube without EDTA to separate serum by centrifuging at 4000 rpm for 10 min. Serum samples was then stored at -20^{o}C untel analysis. Serum was used to determine enzymes GOT, GPT, ALK, BILI, ALB, LDL, VLDL, HDL, T.G, CHOL, MAD, G-SH.

Results

Biochemical changes:



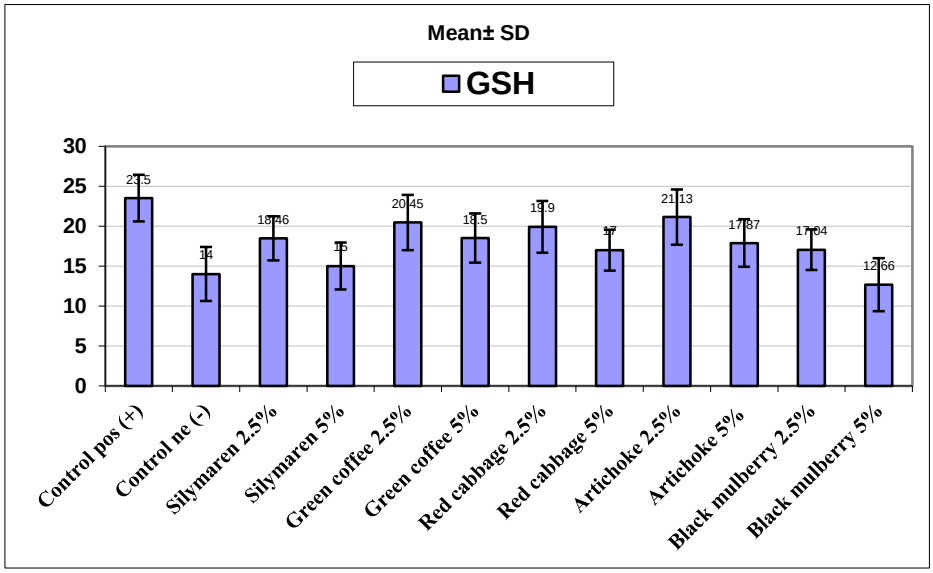
Figuer (1): The effect of treatment plants on serum (GPT) of rats.



Figuer (2): The effect of treatment plants on serum (GOT).

The effect of treatment plants on serum Glutamine Oxaloacetic Transaminase (GOT) values are illustrated in figure (2). As shown there, was a significant between control negative and positive. On the other hand, there was significant ($p \leq 0.05$) elevation achieved by all plant samples except blackmulbarry 5% compared with control positive. Plant samples were order upon their effects on GOT values as following: blackmulbarry 5% > silymarien 5%> silymarien 2.5%> red cabbages 5%> artichoke 5%.

El-Safi, *et al*., (2012): confirmed these results ,It was found that black berry juice as well as silymarin possess antioxidant properties could prevent liver dysfunction induced by CCl₄, and black berry juice and silymarin might be potential candidates for prevention of hepatic disorders.



Figuer (3): The effect of treatment plants on serum Glutathion.

The effect of treatment plants on serum Glutathion (GSH) values are illustrated in figure (3). As shown there, was a significant different between control negative and positive. On the other hand ,there was significant ($p \leq 0.05$) elevation achieved by all plant samples except black mulberry 2.5% and red cabbage 5% compared with control positive. Plant samples were order upon their effects on GSH values as following: silymaren 5% > red cabbage 5%> artichoke 5%> silymaren 2.5%> Black mulberry 2.5%.

Silymarin has been reported to inhibit the hepatotoxin binding to receptor sites on the hepatocyte membrane; reduce the glutathione (GSH) oxidation to enhance its level in the liver and intestine; and stimulate the ribosomal RNA polymerase and subsequent protein synthesis, leading to enhanced hepatocyte regeneration. Overall, silymarin possesses antioxidant, immunomodulatory, anticancer, antiinflammatory, antihepatoxic and some other pharmacological activities. Its effectiveness against multiple disorders makes it a very promising drug of natural origin.according to Pandey **(King and Young, 1999).**

Histopathological results

Liver:

The histopathological effect of CCl4 on liver tissue photos (1-4) showed normal histological structure of hepatic lobule on control positive. Where there were significant changes on the liver of control negative, was an cumpered fibrosis in portal tract, hyperplasia of epithelial lining bile duct and portal infiltration with leucocytes. On the other hand , silymaren ,blackmulbbary, red cabbage and artichoke extracts showed sleighed congestion of central vein and small local hepatic necrosis associated with inflammatory cells infiltration, sleighed cougestion of central vein and sleighed showing congestion of central vein and hepatic sinusoid on mellon killer cells activation where all other plants including, silymaren, blackmulbbary, red cabbage, artichoke and green coffee.

Kidney:

The histopathological effect of CCl4 on kidney tissue photos(5-10) showed normal histological structure of renal parenchyma on control positive. Where, there were significant changes on the kidney of control negative, cumpared pervious cular oedema associated with inflammatory cells infiltration. On the other hand, Silymarien, red cabbage, artichoke, showed sleighed showed dilatation and cougestion of renal blood vessel, sleighed eosinophilic protein cast in the leemen of renal tubules and sleighed protein Cast in the lumen of renal tubules and slight distension of Baumann's space where all other plants including, silymarien, redcabbage, artichoke, black mulbarry showed complete healing.

Spleen:

The histopathological effect of CCl4 on spleen tissue photos(9-12) showed normal while pulp and normal lymphoid follicle on control positive. Where, there were significant changes on the spleen of control negative, cumpared hemorrhage and haemosiderosis, as well as atrophied lymphoid follicle. On the other hand, green coffee showed sleighed dilatation hemorrhage associated with deposition of golden brown heemosidrin pigments, sleighed hemorrhage and heemosiderosis and sleighed hemorrhage and extremedullary haemopiosis where all other plants including silymaren, artichoke and redcabbage,greencoffe blackmulbarry

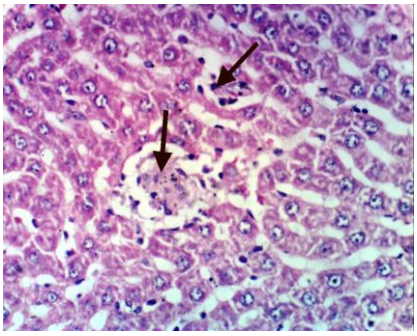


Fig. (1): Liver of rat from group 1 showing multiple focal hepatic necrosis (H & E X 400)

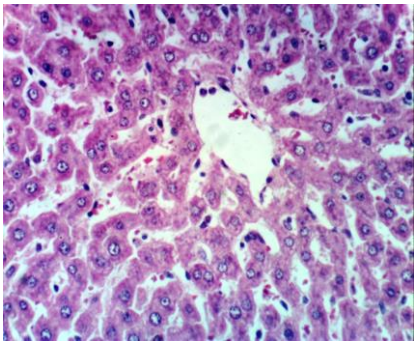


Fig. (2): Liver of rat from group 2 showing the normal histological structure of hepatic lobule (H & E X 400)

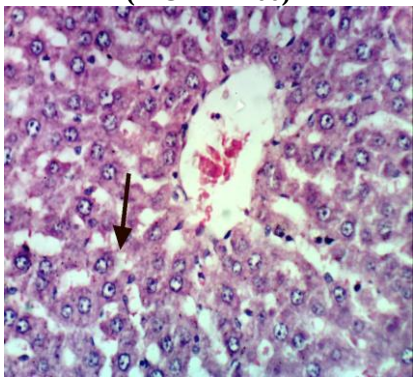


Fig. (3): Liver of rat from group 8 showing slight vacuolization of hepatocytes (H & E X 400)

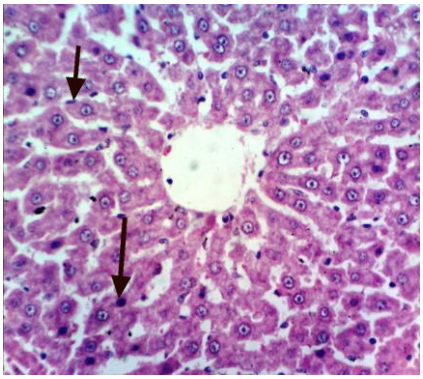


Fig. (4): Liver of rat from group 12 showing Kupffer cells activation and necrosis of sporadic hepatocytes (H & E X 400)

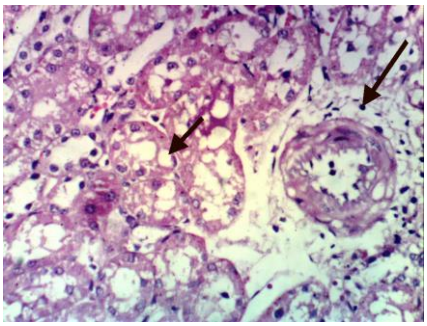


Fig. (5): Kidney of rat from group 1 showing vacuolation of epithelial lining renal tubules, perivascular oedema with few inflammatory cells infiltration (H & E X 400).

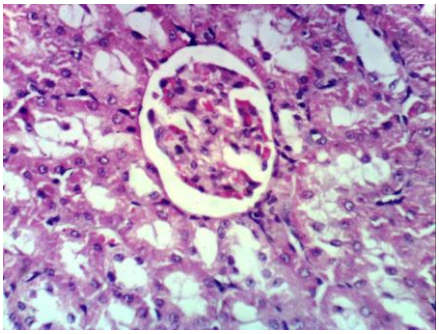


Fig. (6): Kidney of rat from group 2 showing the normal histological structure of renal parenchyma (H & E X 400).

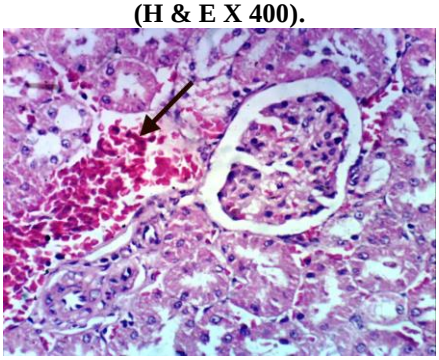


Fig. (7): Kidney of rat from group 4 showing congestion of renal blood vessel (H & E X 400).

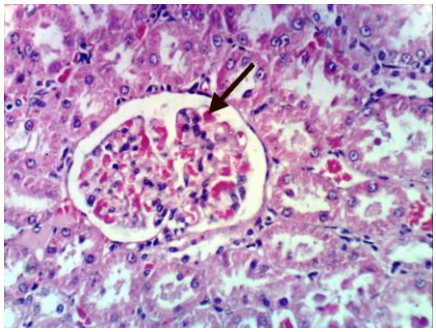


Fig. (8): Kidney of rat from group 10 showing slight congestion of glomerular tuft (H & E X 400)

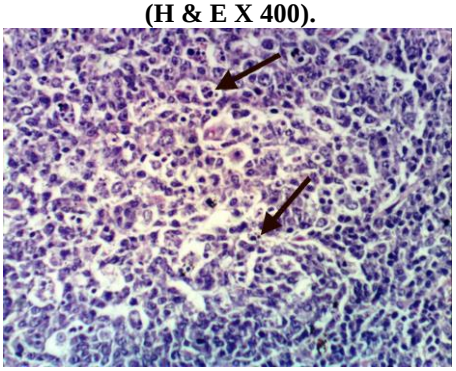


Fig. (9): Spleen of rat from group 1 showing lymphocytic necrosis and depletion (H & E X 400)

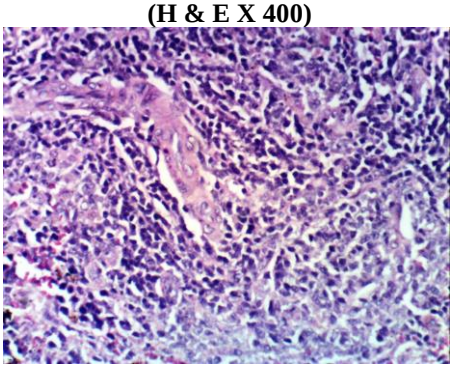


Fig. (10): Spleen of rat from group 2 showing no histopathological changes (H & E X 400).

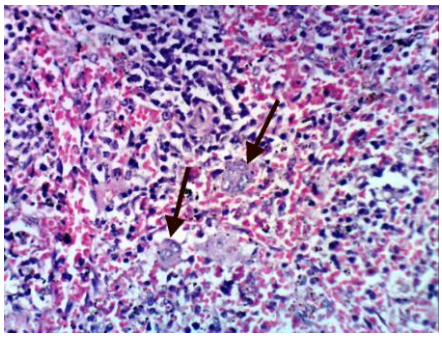


Fig. (11): Spleen of rat from group 5 showing extramedullary megakaryocytosis (H & E X 400).

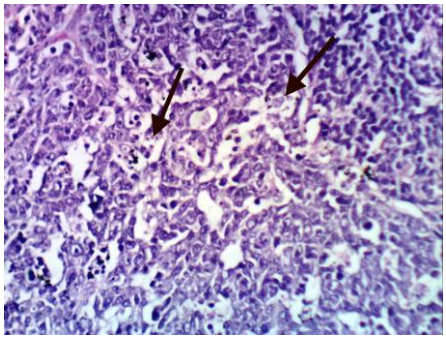


Fig. (12): Spleen of rat from group 11 showing lymphocytic necrosis and depletion (H & E X 400).

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استخدام مضادات الأكسدة الغذائية في تحسين كفاءة الجهاز المناعي للفئران المعاملة برابع كلوريد الكربون

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الملخص:

لا تزال أمراض الكبد مشكلة طبية معقدة تواجه العديد من المرضى في مراحل عمرهم المختلفة وفي مناطق واسعة من العالم . اصابة الكبد الناجمة عن المواد الكيميائية (أو) العوامل المعدية قد يؤدي إلى تليف الكبد بوتليف الكبد في نهاية المطاف وفشل الكبد. لذلك كان الهدف من هذه الدراسةالتعرف على التأثير الوقائي للنباتات محل الدراسة بما في ذلك القهوة الخضراء، والكرنب الأحمر والخرشوف والتوت الأسود وسيليمارين كلا منهم على حدى للفئران المعاملة برابع كلوريد الكربونلاحداث التسمم بالكبد . حيث استخدم كلاً من القهوة الخضراء، الخرشوف، الكرنب الاحمر،والتوت الاسود صبالاضافة الى السليمارين . باستخدام ٤٨ من ذكور فئران الألبينو، تزن ما بين ١٥٠±٥جم تم تقسيمها إلى ١٢مجموعة بكل منها ٤ فئران. واحده من هذه المجموعات تم الإحتفاظ بها كمجموعه ضابطه (–)، فى حين أن ٩مجموعات الآخرى تم حقنها برابع كلوريد الكربون، وتم إضافة النباتات محل الدراسه بتركيزين٢.٥% و ٥% الى الوجبة الأساسية وتم إعطاء كل منها على حدى للمجموعات المختلفة لـ ٥ من المجموعات بعد انتهاء فترة التجربه تم التشريح وإجراء التحاليل المعملية التاليه: Glutamic Oxaloacetic transaminas (GOT) glutamic pyruvic transaminas (GPT), creatinine, وكذلك التحليل الهستولوجى للأعضاء. ومن النتائج أتضح أن المجموعات المعالجه احدثت ارتفاعا معنويا واضحا وانخفاضا كبيرا فى GSH, GPT, GOT مقارنة بالمجموعة الضابطة الموجبة وقد اتفقت النتائج الهستولوجيه مع التغيرات فى نتائج التحاليل البيوكيميائيه فى كلاً من وظائف الكبد والكلى والطحال.

الكلمات الكاشفة: أمراض الكبد، السليمارين، الخرشوف، القهوة الخضراء، الكرنب الأحمر، التوت الأسود، إنزيمات الأكسدة، إنزيمات الكبد.