

Histological Study Of Liver And Intestine Of Rats Treated With Colchicine

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Abstract

The aim of the present work is to detect the effect of colchicine administration on the histology of the liver and intestine in albino rats.

Most drugs have side effects which represent a great problem for human. Some of these side effects may be serious. An example of these drugs is colchicine. Its only approved use is to treat gout, though it is also occasionally used in veterinary medicine to treat cancers in some animals. It is also used as an antimetabolic agent in cancer research involving cell cultures. Colchicine was given i.p with a dose of 3mg/kg body weight daily for a period of 5 days. Histological examinations were carried out at one, four and seven days post treatment.

Histological examination of liver one, four and seven days post treatment with colchicine showed sporadic necrosis, loss of hepatic architecture, pyknosis and vacuolations of some hepatocytes, corrugated hepatic portal vein surrounded by large fibrotic area, edema of portal tract with new bile ductules formation, dilatation and congestion of hepatic sinusoids, multihaemorrhagic areas, hypertrophied hepatocytes with deeply stained shrunken nuclei and mononuclear cells infiltration replacing focal areas of hepatic necrosis.

Histological examination of intestine showed no changes one day post treatment with colchicine. At four and seven days post treatment, the intestine revealed hyperplasia and hyperactivation of mucous secreting cells and intestinal glands and mononuclear cells infiltration and edema in lamina propria with multihemorrhagic areas.

In conclusion, the present study has shown that colchicine has a toxic effect and some histopathological changes have been detected, so care should be taken when colchicine is prescribed in gout treatment.

Introduction

The *autumn Crocus* has been known since the times of ancient Greece. In the fifth century, herbalists discovered it and it could be used to treat rheumatism, arthritis and gout. The useful active ingredient in the plant is called colchicine. It is one of the oldest drugs which were used in the treatment of gout and is still useful in the treatment of several other diseases (Bertram, 1992 and Eric *et al.*, 1992) and was used by physicians for the last 150 years.

Colchicine is a water-soluble alkaloid and it is found in the *autumn crocus*. It has the ability to block or suppress cell division by inhibiting mitosis. Specifically, it inhibits the development of spindles as the nuclei are dividing. Normally, the cell

would use its spindle fibers to line up its chromosomes, make a copy of them, and divide into new cells with each daughter cell having a single set of chromosomes. Colchicine blocks formation of spindle fibers and so the cell can't move its chromosomes around. The cell may end up copying some or all of the chromosomes anyway, but can't parcel them out into new cells, and so it never divides. It has been proposed as a novel drug for therapy of pulmonary fibrosis (Entzian *et al.*, 1997). Borisy and Taylor (1967) reported that colchicine is one of the micro tubule-disruptive agents and it binds with tubulin, which is the microtubule-protein. Colchicine was also reported by Ostermann *et al.* (1993) and Perico *et al.* (1996) to

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inhibit cell-mediated immune responses and promotes long-term survival of major histocompatibility complex-incompatible renal allografts in rat.

Because cancer cells divide much more rapidly than normal cells, cancers are more susceptible to being poisoned by mitotic inhibitors such as colchicine, paclitaxel, and the Vinca alkaloids.

Gout is a disease caused by faulty uric acid metabolism in which excess uric acid is turned into crystals of sodium urate, which are then deposited in the joints (most often in the big toe), causing inflammation and pain. Researchers aren't sure exactly how colchicine works against gout, but it seems to reduce the frequency of severe attacks and relieves residual pain. Colchicine is unique among therapeutic agents used in the treatment of acute arthritis. Its usefulness is generally felt to be relatively limited to this rheumatic disease and that it is not a potent general anti-inflammatory agent (Malawista, 1975).

Colchicine is rapidly absorbed from the gastrointestinal tract after ingestion. It undergoes significant first pass hepatic metabolism, which primarily involves deacetylation. It is thought that the extended time period during which the gastrointestinal mucosal cells are exposed to colchicine may explain the prominence of the gastrointestinal symptoms of toxicity. Subsequent to this, the metabolites undergo widespread enterohepatic recirculation before being excreted in bile and faeces (Borron *et al.*, 1996).

As far as side effects go, colchicine caused a temporary reduction in the number of leukocytes (white blood cells) in the blood stream; afterward, the leukocyte count can rebound to abnormally high levels. Colchicine also caused teratogenic birth defects in lab animals and injection of colchicine in pregnant mice resulted in cytolytic effects on fetal hepatocytes and macrophages (Sonada *et al.*, 1998), and so pregnant women with gout should not use colchicine-containing drugs.

Colchicine poisoning resembles arsenic poisoning because of its poisonous effect on mitosis and occurs 2 to 5 hours after the toxic dose which has been

ingested, the symptoms include burning in the mouth and throat, diarrhea, stomach pain, vomiting, and kidney failure. A specific antidote doesn't exist, so treatment typically involves giving the victim activated charcoal or pumping the stomach to decrease absorption if it was orally taken (Wagenaar, 2004).

This study was designed to investigate the effect of colchicine on the structure of liver and intestine of rat trying to give warnings about using this drug in gout patients.

Material and Methods

Animals

Male Swiss albino rats (120-140) were used. The animals were housed in specially designed cages and were kept in controlled room in the animal house facility of National Centre for Radiation Research and Technology (NCRRT) at a maintained good temperature and humidity. They were given standard food and normal tap water daily.

Colchicine treatment

Colchicine (Sigma) was given as intra peritoneal injections with a dose of 3mg/kg/day for 5 days (double the therapeutic dose).

Experimental design

The animals were divided into four groups of 4 each according to the time of sampling: (Group 1): control group, the animals received *i.p.* injection of saline. (Group 2): The animals were sacrificed at one day post treatment with colchicine. (Group 3): The animals were sacrificed at four days post treatment with colchicine. (Group 4): The animals were sacrificed at seven days post treatment with colchicine.

Histological analysis

Rats of each group were sacrificed at 1, 4 and 7 days after treatment with colchicine. Animals were sacrificed by decapitation, the liver and the intestine, were excised and fixed in 10% formalin, dehydrated in ascending grades of ethyl alcohol, cleared in xylol, embedded in

molten paraplast at 56°C and cut at 5µ on rotator microtome. The paraffin sections were stained with haematoxylin and eosin (Drury and Wallington, 1980). Histopathological studies were undertaken through light microscopy and photomicrographs were made.

Results

Liver Examination

Liver sections of control, untreated rat revealed normal histological structure of hepatic lobules (Fig. 1).

Liver section of a rat treated with colchicine one day post treatment showed sporadic necrosis of hepatocytes (Fig. 2), focal area of hepatic necrosis occupied by leucocytic cells infiltration (Fig. 3) as well as edema in the portal tract with new bile ductules formation (Fig. 4).

Liver of a rat treated with colchicine after four days revealed dilatation and congestion of hepatic sinusoids with lots of hemorrhagic areas (Fig. 5), vacuolation in some hepatocytes, and loss of hepatic architecture with increased signs of necrosis, dilated sinusoidal spaces and multi hemorrhagic areas (Fig. 6). A large degenerated area which is occupied by hemorrhage was detected. Some

hepatocytes were free from nuclei and others contained pyknotic nuclei (Fig. 7).

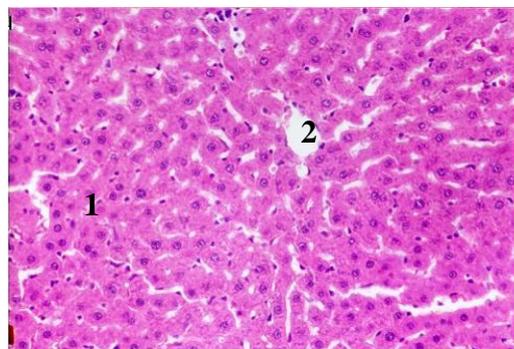
At seven days post treatment with colchicine the liver showed hypertrophied hepatocytes with deeply stained shrunken nuclei and small necrotic area (Fig. 8). Focal area of hepatic necrosis replaced by mononuclear leucocytic cells (Fig. 9), large haemorrhagic areas with increased signs of necrosis and disturbed architecture of hepatic tissue (Fig.10), edema in the portal tract associated with new bile ductules formation, corrugated portal vein surrounded by a fibrotic area and the adjacent hepatocytes were highly affected with foamy appearance (Fig. 11).

Intestine of control, untreated rat revealed no histopathological changes (Fig. 12). No changes were detected in examined sections of rat treated with colchicine at one day post treatment (Fig. 13).

At four days post treatment with colchicine the intestine revealed activation of mucous secreting epithelium and glands with hemorrhagic area (Fig. 14).

Hyperplasia and hyperactivation of mucous secreting cells, mononuclear cells infiltration and edema in lamina propria with large degenerative areas containing cellular debris and small hemorrhagic areas were observed in examined intestine treated with colchicine seven days post-treatment (Fig. 15).

Fig. 1: Liver of control, untreated rat showing normal architecture of liver tissue, normal hepatic strands (1) and central vein (2). (Hx & E stain X



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Fig.2: Liver of a rat one day post-treatment with colchicine showing sporadic necrosis of hepatocytes (↑). (Hx & E stain X 200).

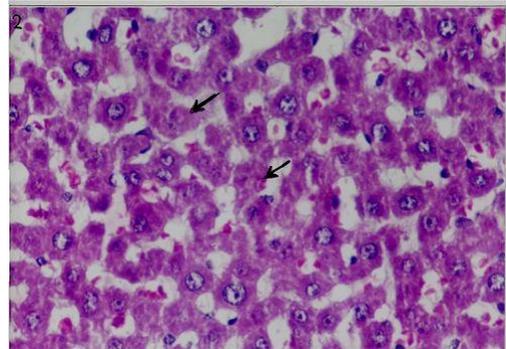


Fig. 3: Liver of a rat one day post-treatment with colchicine showing focal area of hepatic necrosis occupied by leucocytic cells infiltration (↑). (Hx & E stain X 200).

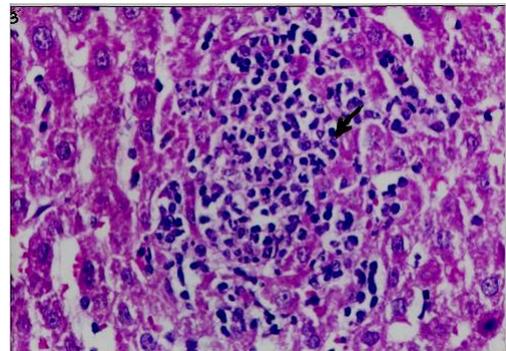


Fig. 4: Liver of a rat one day post-treatment with colchicine showing edema in the portal tract (↑) and new bile ductules formation (▲). (Hx & E stain X 200).

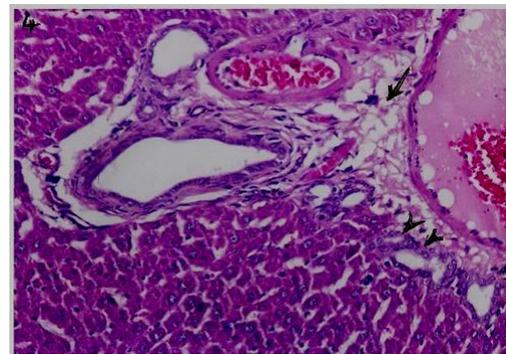


Fig. 6: Liver of rat treated with colchicine four days post-treatment showing vacuolation of some hepatocytes (↑), loss of hepatic architecture with increased signs of necrosis and dilated sinusoidal areas (▲) and multihemorrhagic areas (h). (Hx & E stain X 200)

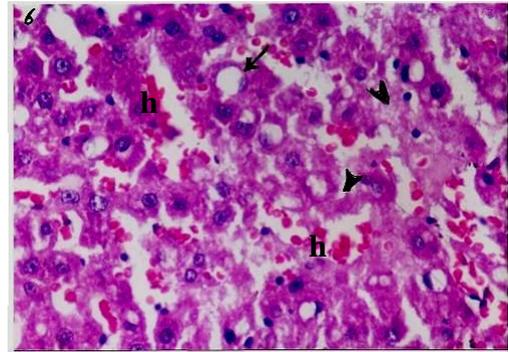


Fig. 7: Liver of rat treated with colchicine four days post treatment showing large degenerated area which is occupied by hemorrhage (↑). Some hepatocytes were free from nuclei and others contained pyknotic nuclei (▲). (Hx & E stain X 200)

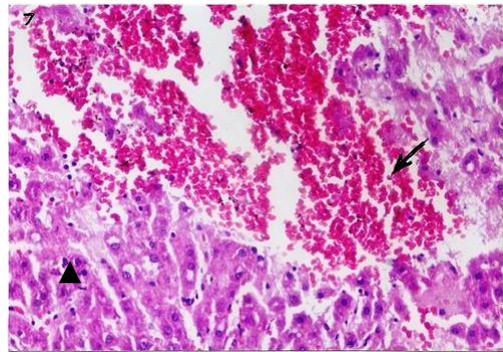
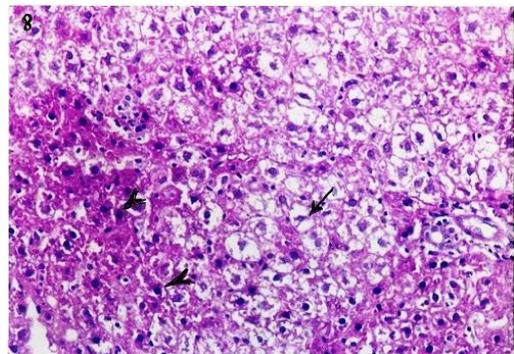


Fig. 8: Liver of a rat treated with colchicine seven days post treatment showing hypertrophied hepatocytes with deeply stained shrunken nuclei (↑) and small necrotic area with dark pyknotic nuclei (▲). (Hx & E stain X 200).



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Fig. 9: Liver of a rat treated with colchicine seven days post treatment showing focal area of hepatic necrosis replaced by mononuclear cells (↑). (Hx & E stain X 200).

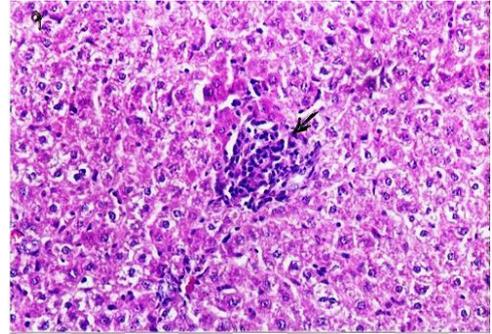


Fig. 10: Liver of a rat treated with colchicine seven days post treatment showing large haemorrhagic area (↑) & disturbed architecture of hepatic tissue with increased signs of necrosis (▲) (Hx & E stain X 200).

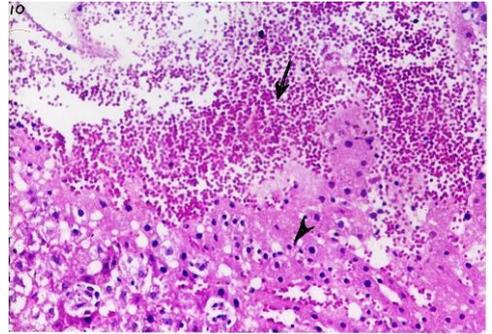


Fig. 11: Liver of a rat treated with colchicine seven days post treatment showing edema in the portal tract (↑) associated with new bile ductules formation (▲) and corrugated portal vein surrounded by fibrotic area (v). (Hx & E stain X 200).

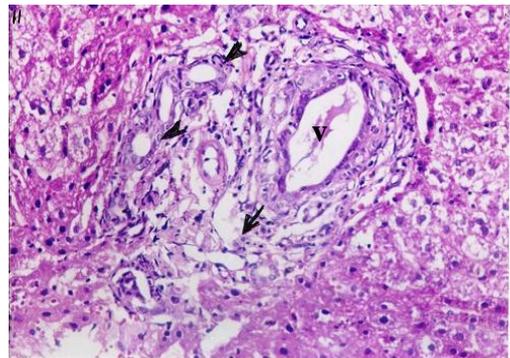


Fig. 12: Intestine of control, untreated rat showing normal histological pattern. (Hx & E stain X 100).

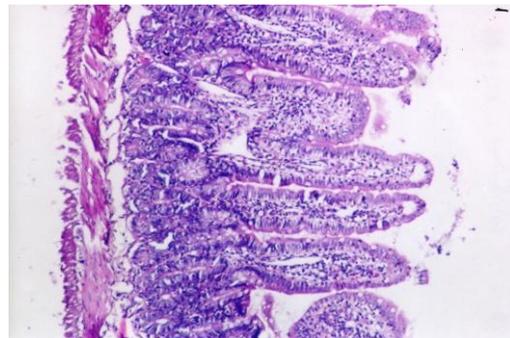
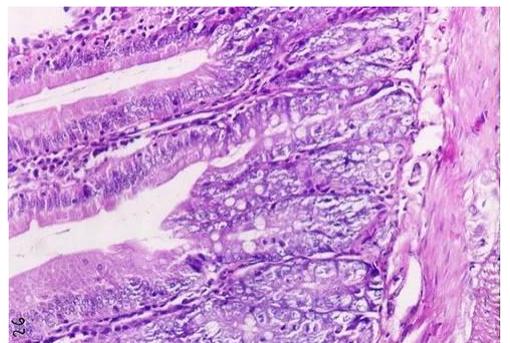


Fig. 13: Intestine of rat treated with colchicine one day post treatment, no histopathological changes were detected. (Hx & E stain X 200).



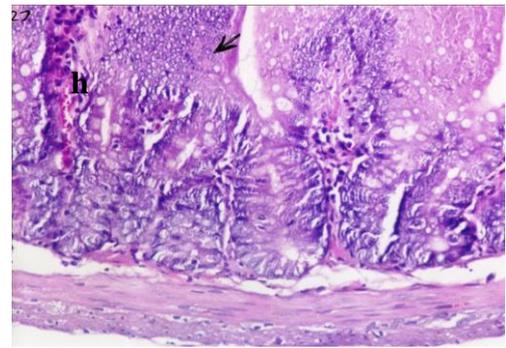


Fig. 14: Intestine of rat treated with colchicine four days post treatment showing activation of mucous secreting epithelium and glands (↑) with hemorrhagic area (h). (Hx & E stain X 200).

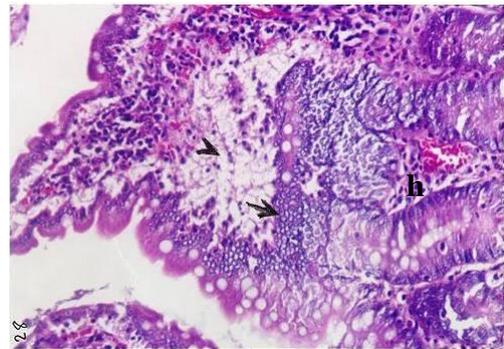


Fig. 15: Intestine of rat treated with colchicine seven days post treatment showing hyperplasia and activation of mucous cells (↑), mononuclear cells infiltration and edema in lamina propria with large degenerative areas containing cellular debris (▲) and multi small hemorrhagic areas (h). (Hx & E stain X 200).

Discussion

Colchicine overdose is uncommon but potentially life threatening. Colchicine is a safe drug when used according to established therapeutic guidelines but causes serious systemic effects if ingested in doses that exceed the recommendations.

In the present study, histological parameters were followed in the liver and intestine of albino rats treated with colchicine to detect its effects on different tissues.

The administration of colchicine resulted in histopathological changes in the liver of rat manifested by sporadic necrosis, pyknosis and vacuolations of some hepatocytes, leucocytic cells infiltration, and edema of portal tract with new bile ductules, corrugated portal vein surrounded by fibrotic area, hepatocytes with foamy appearance, dilatation and congestion of hepatic sinusoids, hepatic haemorrhage, loss of hepatic architecture and mononuclear cells infiltration replacing focal areas of hepatic necrosis. These findings run in full agreement with the results of other authors (El-Shafeey *et al.*, 2000 and Bumbasirevie *et al.*, 1996).

Das *et al.* (2000) studied the antioxidant and antifibrotic properties of colchicine. They summarized that colchicine had

only weak antioxidant properties, but afforded some protection against oxidative stress; more importantly, long treatment with this drug may be of value in producing regression of established cirrhosis. Baumgartner *et al.* (2001) reported that colchicine inhibits taurodeoxycholate transport in pericentral but not in periportal hepatocytes.

Also colchicine administration revealed hyperplasia and hyperactivation of mucous secreting epithelium and glands and mononuclear cells infiltration and edema in lamina propria with large degenerative areas containing cellular debris and small multihemorrhagic areas in examined intestine of rat. This may be explained by Hampton (1966) who stated that the intestinal pathology after large doses of colchicine is probably related to cessation of cellular proliferation and has been compared to that observed in acute radiation injury of the bowel. Also Hawkins *et al.* (1965) and Finger and Headington (1963) reported that it has been generally assumed that gastroenterologic symptomatology and pathology encountered with large doses of colchicine are probably the result of inhibition of growth of cellular elements of crypts. Karmeli *et al.* (1997) stated that

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colchicine induced a significant decrease in jejunal nitric oxide synthase activity (NOS) and suggested that the effect of colchicine on intestinal transport is, at least partly, mediated through nitric oxide (NO) inhibition. Colchicine is well known to be associated with malabsorption and it has been shown that this drug, when given in large doses, can cause an almost complete loss of intestinal villi, with concomitant steatorrhea and xylose malabsorption, as well as a depression in activity of disaccharides in the intestine of man (Race *et al.*, 1966).

Previous studies were made on colchicine toxicity and poisoning due to over doses or accidental ingestion. Yamada *et al.* (1998) made a histopathological study of experimental acute poisoning of cattle by autumn crocus (*Colchicum autumnale L.*). They reported that all the calves developed severe diarrhea and died or euthanized within 63 hr. and at necropsy the gastrointestinal mucosa was edematous and hemorrhagic and histologically, necrosis and degeneration with karyopyknosis and karyorrhexis were shown in the basal cell layer of the tongue, esophagus, forestomach, renal pelvis, urinary bladder, neck cell layer of the abomasal gastric glands and intestinal crypts. They also reported that the lesions of the present acute crocus poisoning of cattle closely resembled those reported in humans with colchicine intoxication.

Colchicine is rapidly absorbed from the gastrointestinal tract after ingestion. It undergoes significant first pass hepatic metabolism, which primarily involves deacetylation. Subsequent to this, the metabolites undergo widespread enterohepatic recirculation before being excreted in bile and faeces. It is thought that the extended time period during which the gastrointestinal mucosal cells are exposed to colchicine may explain the prominence of the gastrointestinal symptoms of toxicity (Maxwell *et al.*, 2001).

Currently there is no specific treatment commercially available for the treatment of colchicine toxicity. However, the successful outcome after the use of colchicine specific Fab fragments has been reported (Scherrmann *et al.* 1992 and Milne and Meek., 1998). Colchicine specific Fab

fragments consist of the light chain and variable region of the heavy chain and are derived from goats (Kaplan *et al.*, 1986). Their mechanism of action is similar to that of digoxin specific Fab fragments, namely binding to the target drug allows redistribution into the intravascular compartment and thus the removal of substantial amounts from peripheral sites (Schaumann *et al.* 1986). There is a high affinity between the Fab fragment and colchicine and this acts to prevent the drug returning to these peripheral binding sites (Baud *et al.* 1995).

Klitschar *et al.* (1999) examined a case in which two persons confused this highly poisonous plant with wild garlic (*Allium ursinum*), a popular spice in the central European cuisine. While one person merely complained about a 3-day episode of nausea, vomiting and watery diarrhea, the second person died of multi-organ system derangements 48 h after the ingestion of the colchicum leaves. At autopsy hemorrhagic lung oedema, hypocellular bonemarrow, centrilobular fatty necrosis of the liver and necrosis of the proximal convoluted tubules of the kidneys were observed.

In conclusion, it is important therefore that the potential dangers of this drug are recognized by clinicians on its prescription, and that patients are given an understandable explanation of its effects including the point at which to cease ingestion. A careful watch must be made of the number of tablets prescribed to avoid unintentional overdose of this potentially lethal drug.

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دراسة هستولوجية فى كبد و أمعاء الجرذان المعاملة بالكولشسين

عزة عبد المجيد عبد الوهاب

قسم البحوث الصحية الإشعاعية

المركز القومى لبحوث و تكنولوجيا الإشعاع- القاهرة- مصر

معظم العقاقير لها آثار جانبية بذلك تمثل مشكلة للإنسان. الكولشسين واحد من هذه العقاقير والتي تستخدم فى علاج مرض النقرس. ولقد أعطى الكولشسين للجرذان عن طريق الحقن بجرعة مقدارها 3مجم/كجم يومياً لمدة 5 أيام. وأجريت الدراسات الهستولوجية بعد يوم و أربعة أيام و سبعة أيام من تعاطى الكولشسين. و قد أظهرت النتائج أن الكولشسين قد تسبب فى حدوث بعض التغيرات النسيجية فى الكبد وكذلك فى الأمعاء متمثلة فى ضمور و اضمحلال السيتوبلازم مع مظاهر تحلل الأنوية ووجود فراغات فى بعض الخلايا الكبدية و نزيف و ظهور بعض الخلايا الإلتهابية البيضاء و إتساع فى الجيوب الدموية و ظهور الخلايا أحادية النواة مع إزدياد عدد كريات الدم البيضاء فى النسيج الكبدى. أما نسيج الأمعاء فقد ظهرت فيه أيضاً بعض التغيرات النسيجية نتيجة لتعاطى الكولشسين متمثلة فى زيادة فى الخلايا ونشاط فى الغدد المخاطية وزيادة عدد كريات الدم البيضاء الخلايا أحادية النواة وإرتشاح فى نسيج الامعاء. ويدل هذا البحث أن عقار الكولشسين له بعض الآثار الجانبية الضارة ولهذا يجب الحذر عند إستعماله.