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# Toxicopathological study on the effect of different toxic doses of *Euphorbia granulata F*. crude extract in albino mice.

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## Summary

The purpose of this study was to evaluate the effect of different toxic doses with regard to toxicopathological changes in mice injected with ethanolic crude extract of *Euphorbia granulata F*. The experiment was done on (24) mice of (6-8weeks age and 20-25g weight ) divided equally into (4) groups as follows:- First group injected distilled water subcutaneously for 45 days and considered as control ,second group injected subcutaneously (600 mg/kg B.W/day) ethanolic extract of *E. granulata* for 45 days, Third group injected subcutaneously (800 mg/kg B.W/day) ethanolic extract of *E. granulata* for 45 days. Fourth group injected subcutaneously (1000 mg/kg B.W/day) ethanolic extract of *E. granulata* for 45 days.The microscopic findings of internal target organs revealed that the severity of the lesion increased with the dose of the plant extract. Animals injected with (1000 mg/kg B.W) showed fibrosis in liver, kidney, myocardium with encephalomalacia and aggregation of astrocytes and microglial cells.Furthermore the study showed mononuclear infiltrations in some internal organs, with multiple granulomatous reaction and perivascular lymphocytic cuffings. Amyloid deposition in spleen with nodular hyperplasia of liver and hyperplasia of epithelial lining of endometrium.In conclusion, toxic doses of ethanolic crude extract of *Euphorbia granulata F* cause severe toxicopathological changes in important organs and tissues in addition the active compound of plant may act as immune stimulant, with formation of precancerous lesions in liver and uterus.

Key words : *Euphorbia granulata F*. Poisonous plants, Herbal medicine.

## دراسة التغيرات المرضية السمية حول تأثير جرعات سمية مختلفة من الخلاصة الكحولية الخام لنبات سرطان الثيل في الفئران البيضاء

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**بغداد-العراق**

### الخلاصة

هدفت الدراسه الى بيان تأثير الجرعات السميه للخلاصه الكحوليه لنبات سرطان الثيل في الفئران وذلك من خلال دراسة التغيرات المرضيه النسجيه في الاعضاء الداخليه المستهدفه اجريت التجربه على (24) فأر (عمر 6-8 اسابيع ووزن 20_25 غم) من كلا الجنسين حيث قسمت الى اربع مجاميع متساويه وكما يأتي : المجموعه الاولى حقنت يوميا تحت الجلد بالماء المقطر ولمدة 45 يوما واعتبرت مجموعة سيطره , المجموعه الثانيه حقنت يوميا تحت الجلد بالخلاصه الكحوليه لنبات سرطان الثيل وبجرعة 600 ملغم /كغم من وزن الجسم ولمدة 45 يوما , المجموعه الثالثه حقنت يوميا تحت الجلد بالخلاصه الكحوليه لنبات سرطان الثيل وبجرعة 800 ملغم /كغم من وزن الجسم ولمدة 45 يوما

,المجموعه الرابعه حقنت يوميا تحت الجلد بالخلاصه الكحوليه لنبات سرطان الثيل وبجرعة 1000 ملغم /كغم من وزن الجسم ولمدة 45 يوما .لقد اظهر الفحص المجهري للاعضاء الداخليه المستهدفه ان شدة الافه المرضيه ازدادت مع زيادة الجرعه السامه المعطاة حيث اظهر الكبد والكليه وعضل القلب حصول تليف في الحيوانات المحقونه بالجرعه 1000 ملغم /كغم من وزن الجسم بالاضافه الى تلين الدماغ وتجمع الخلايا النجميه والدباق وحصول تنكسات ونخر ونزف في الكبد والكليه كما بينت الدراسه ارتشاح خلايا وحيدة النواة وحصول أفات ورميه حبيبيه متعدده واستكفاف لمفاوي حول الوعاء الدموي اما المظهر المجهري للاعضاء الاخرى فقد اظهر ترسب لمادة النشواني في الطحال وفرط التنسج العقيدي للكبد مع فرط التنسج لظهارة بطانة الرحم. نستنتج مما سبق أن الجرع السميه لنبات سرطان الثيل تؤدي الى تغيرات مرضيه سميه شديده في اعضاء وانسجة الجسم الداخليه بالاضافه الى أن المركبات الفعاله للنبات تؤدي الى تحفيز مناعة الجسم مع حصول أفات مهيأه للتسرطن في الكبد والرحم .

مفاتيح الكلمات : سرطان الثيل. النباتات السامه .طب الاعشاب

## Introduction

The substances responsible for poisoning or toxic reactions originate from many different pathways within plants. However, most poisonous principles are considered be secondary metabolites or by- products from the essential functions of the plant (1). Although there are many theories as to why plants produce these toxic compounds, of this theory that suggested by (2) ,who said the plants have evolved to produce these compounds in order to deter animals from grazing on them and to keep out the insects from eating them. Identifying plants that are poisonous is difficult since poisonous plants do not appear distinctly different from their nontoxic relatives or counterparts(3).One of these poisonous plant was *Euphorbia granulata* belong to the family *Euphorbiaceae* ephemeral herb, 6-13 cm tall, with a short life cycle (4). Poisonous principles include terpenes, triterpenoids, glycosides and phenolics are present in the milky juice (5, 6). Medicinal uses of *E.granulata* showed that the plant extract has inhibitory effects against fungal, viral and bacterial infections (7, 8, 9,10).Furthermore it has anti- carcinogenic effects on certain cell lines in vitro and on experimentally implanted mammary adenocarcinoma in mice (11).The objective of this study was to make knowledge about the effect of different toxic doses of crude extract of *E. granulata* on internal organs in mice.

## Materials and Methods

### 1-Plant collection

*Euphorbia granulata* plant has been obtained from field of College of Veterinary Medicine, University of Baghdad. Representative specimens (leaves and stem) were taken to the college of Science, Botany department, University of Baghdad and identified by prof. Dr. Ali- AL-Mosawy as *Euphorbia granulata* F.

**2-Plant extraction**

According to (12), 70% ethanolic extract of plant has been prepared as follows:

1-Fifty gram of fresh plant (leaves and stems) have been put in flask with 250 ml of 70 % ethanol and stirred on magnetic stirrer at room temperature for 72 hrs.

2-After 72 hours, the sediments have been filtered through gauze then by filter paper.

3- Steps (2) was repeated (3) times.

4-The solvent was evaporated by air convection oven at 38 °C.

5-The weight of crude extract powder resulted was measured and kept at 4 °C until used.

6-The plant extract was dissolved in distilled water and injected subcutaneously using insulin syringe according to experimental design.

**3-Experimental design:**

(24)Albino mice of age (6-8 weeks) and (20-25 g) of either sexes were divided equally into (4) groups: the first group was injected daily subcutaneously with distilled water for 45 days and considered as control, the second group was injected subcutaneously (600) mg/kg B.W/day of ethanolic extract of *E. granulata* for 45 days, The third group injected subcutaneously (800) mg/kg B.W /day of ethanolic extract of *E. granulata* for 45 days. The fourth group injected subcutaneously (1000) mg/kg B.W /day of ethanolic extract of *E. granulata* for 45 days.

**Histopathology**

At the end of experiment animals were sacrified and tissue specimens from brain, lung, heart, liver, kidney, spleen, pancreas, stomach, intestine, testis and uterus were taken for histopathological examination using 10% neutral buffered formalin as a fixative, then processed routinely in histokinette, cut at 5 µ thickness by microtome and stained with hematoxylin and eosin stain then examined under light microscope (13).

## Results and Discussion

**Histopathological findings:**

**First group (control):** No pathological changes

**Second Group**:

**Brain:** Degeneration and necrosis of neurons of cerebrum characterized by central chromatolysis with perivascular and perineuronal edema with congestion of blood vessels (figure 1).

**Lung:** Thickening of interalveolar septa due to congestion of blood capillaries and infiltration of mononuclear cells with areas of emphysema(figure 2).

**Heart:** Infiltration of mononuclear cells between the muscle fibers of myocardium.

**Liver:** Hepatocytes showed vacuolar degeneration with multiple areas of coagulative necrosis , blood oozing to the necrotic area with increase in apoptosis (figure 3) .In addition, there is tiny aggregations of mononuclear cells within parenchyma.

**Kidney:** Atrophy of glomerular tuft with dilation of urinary space with perivascular lymphocytic cuffing and cystic dilation of cortical renal tubules, with cloudy swelling and vacuolar degeneration of epithelial lining the proximal and distal convoluted tubules.

**Spleen:** Hyperplasia of lymphoid tissue of white pulp, with increase in numbers of megakaryocytes. In other sections, there is deposition of amyloid (figure 4).

**Pancreas: -** Atrophy of islets of langerhans with fatty degeneration.

**Stomach:** Marked hyperplasia and hyperkeratosis of epithelial lining of non – glandular region (figure 5).While the glandular region showed mononuclear cells infiltration in the mucosa and submucosa.

**Intestine:** Increase in numbers of goblet cells with extensive proliferation of lymphoid tissue of submucosa forming germinal centers.

**Testis:** Suppression of spermatogenesis, vacuolar degeneration and necrosis of epithelial lining of semineferous tubules ,with presence of spermatid multinucleated giant cells (figure 6). furthermore there is fibrous thickening of tunica albuginea with infiltration of neutrophils and mononuclear cells. .

**Third Group :**

**Brain:** Focal aggregation of microglial cells forming nodular like structures in brain parenchyma (figure 7).

**Lung:** Perivascular and peribronchiolar lymphocytic cuffing (figure 8). Other sections showed hyperplasia of epithelial lining of bronchi and hyperatrophy of muscular layer.

**Heart:** Focal aggregation of mononuclear cells (Figure 9) .Other sections showed mononuclear cells infiltrations in the epicardial adipose tissue.

**Liver:** Formation of numerous hyperplastic nodules (figure 10) with severe hemorrhage, necrosis and apoptosis, with formation of early granuloma (figure11).

**Kidney:** Subcapsular hemorrhage with perivascular and periglomerular lymphocytic cuffing and cloudy swelling of epithelial cells lining the renal tubules with focal interstitial mononuclear cells aggregations and slight fibrous thickening of Bowmans' capsule (figure12).

**Pancreas: -** Hemorrhage and congestion of blood vessels.

**Spleen, Stomach, Intestine, Testis** showed similar lesions as in the previous dose.

**Fourth group:**

**Brain:** Cerebrum showed the presence of multifocal variable sized empty

spaces of malacia. (Fig13) with clustering of astrocytes cells around the necrosed areas (Fig 14)

**Liver:** Thickening in the wall of bile ducts due to periportal fibrosis with infiltration of mononuclear cells (Figure15)

**Kidney:** Fibrous thickening of the capsule of the organ with cystic dilation of medullary renal tubules with formation of hyaline cast (figure 16).

**Pancreas:-**Focal aggregation of mononuclear cells between pancreatic acini (Figure 17).

**Heart:**Moderate fibrosis of myocardium with infiltration of mononuclear cells (figure18) **.**

**Uterus:** Cystic dilation of uterine glands with hyperplasia of epithelial lining of endometrium was seen (figure 19).

**Lung, Spleen, Stomach, Intestine,Testis** showed similar lesions as in the previous dose..

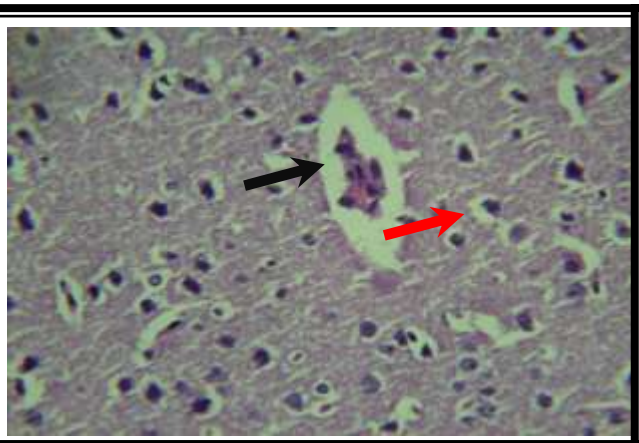


**Figure (1):** Brain of mouse injected subcutaneously (600 mg / kg B.w /day) ethanolic extract of **E.** granulata for 45 days showing )and perineuronal( ) edema ( perivascular with congestion of B.V (H&Ex 400).

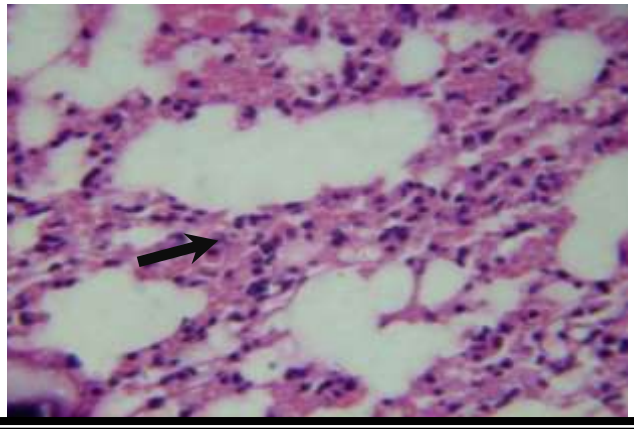


**Figure (2)** lung of mouse injected subcutaneously (600 mg / kg B.w /day) ethanolic extract of **E.** granulata for 45 days showing thickening of interalveolar septa due to infiltration of mononuclear cells ( ) (H&Ex 400).

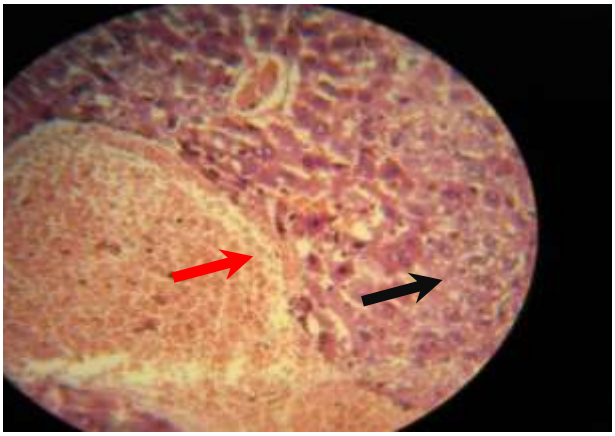


**Figure (3)** liver of mouse injected subcutaneously (600 mg / kg B.w /day) ethanolic extract of **E.** granulata for 45 days showing massive areas of necrosis with blood oozing to the necrotic area ( ) and apoptosis ( ) with congetion of blood vessel (H&Ex 400).

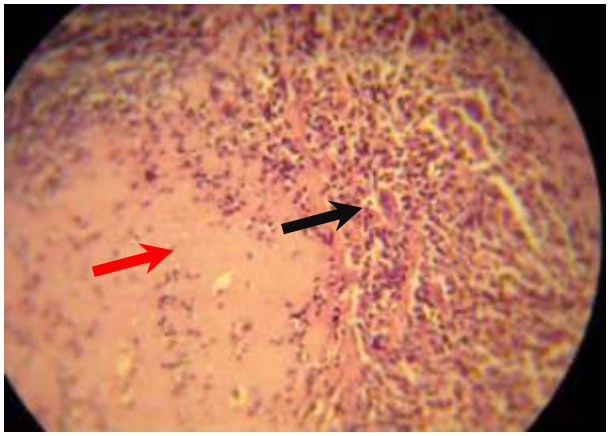


**Figure (4)** Spleen of mouse injected subcutaneously (600 mg / kg B.w /day) ethanolic extract of **E.** granulata for 45 days showing deposition of amyloid substance ( ) with increase in numbers of megakaryocytes ( ) (H&Ex 400).

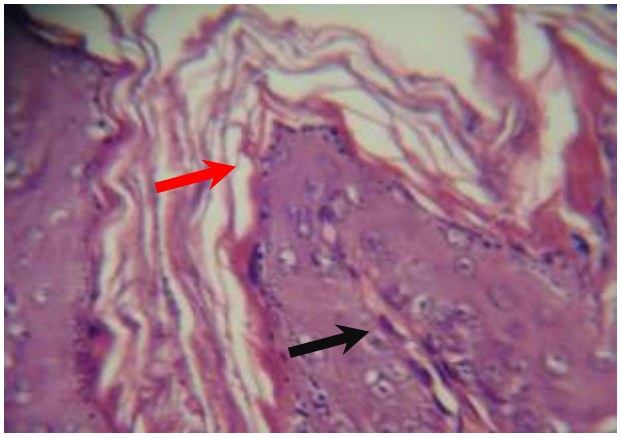


**Figure (5)** Stomach of mouse injected subcutaneously (600 mg / kg B.w /day) ethanolic extract of **E.** granulata for 45 days showing papillary proliferation of epithelial lining ( ) with marked hyperkeratosis (

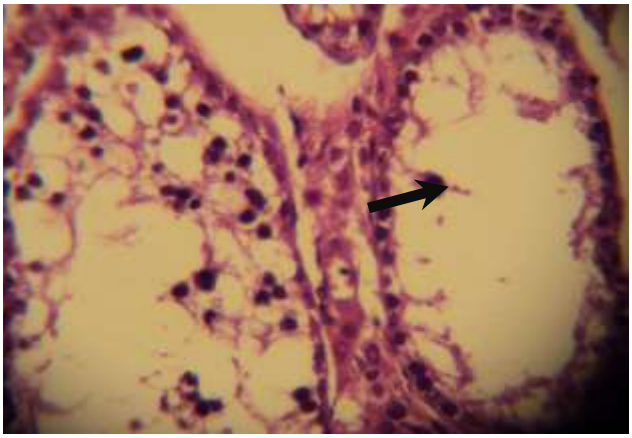


**Figure (6)** Testis of mouse injected subcutaneously (600 mg / kg B.w /day) ethanolic extract of **E.** granulata for 45 days showing inhibition of spermatogenesis and presence of multinucleated spermatid giant cells

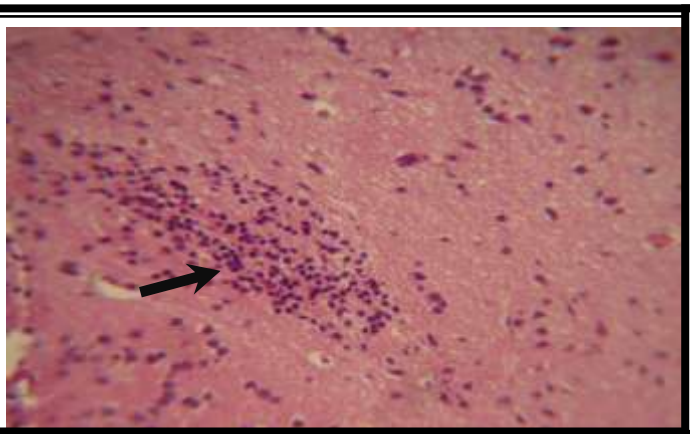


**Figure (7)** Brain of mouse injected subcutaneously (800 mg / kg B.w /day) ethanolic extract of **E.** granulata for 45 days showing focal gliosis ( ) due to proliferation of microglial

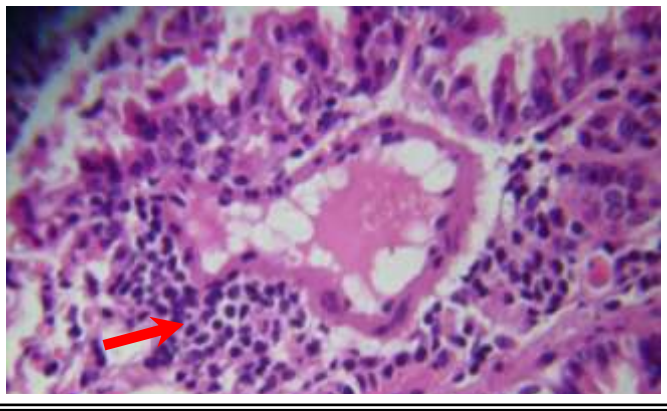
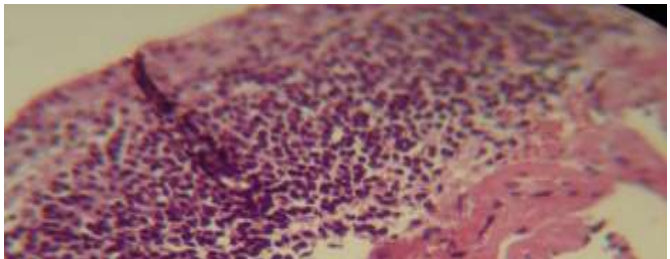


**Figure (8)** Lung of mouse injected subcutaneously (800 mg / kg B.w /day) ethanolic extract of **E.** granulata for 45 days showing peribronchiolar and perivascular lymphocytic cuffing ( ) (H&Ex



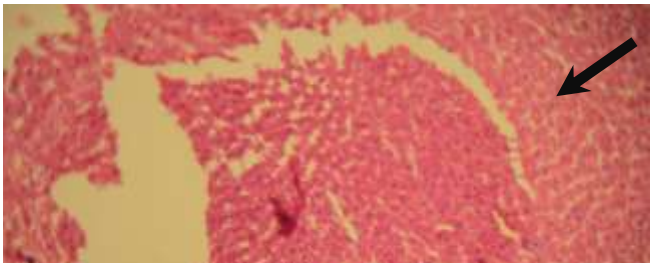


**Figure (9)** Myocardium of mouse injected subcutaneously (800 mg / kg B.w /day) ethanolic extract of **E.** granulata for 45 days showing focal aggregation of mononuclear cells

**Figure (10):** liver of mouse injected subcutaneously (800 mg / kg B.w /day) ethanolic extract of **E.** granulata for 45 days showing hyperplastic nodule lacking the central vein

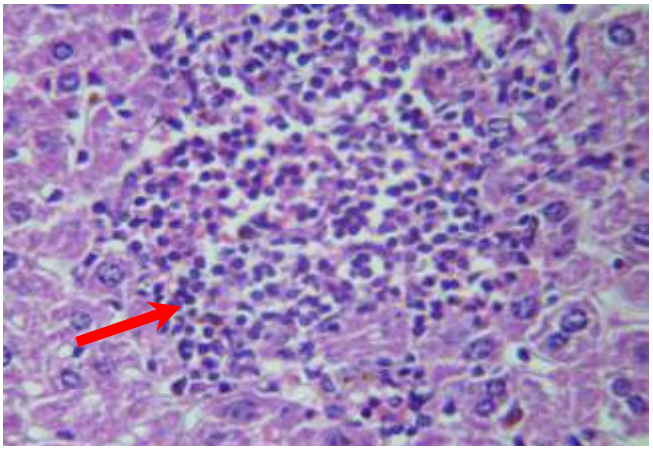


**Figure (11)** liver of mouse injected subcutaneously (800 mg / kg B.w /day) ethanolic extract of **E.** granulata for 45 days showing formation of early granuloma ( ) (H&Ex400).

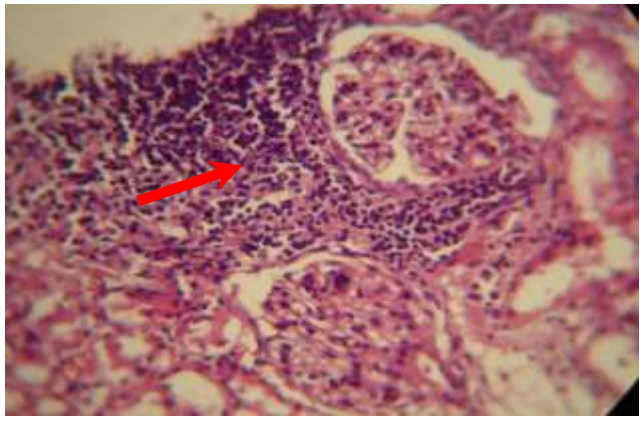
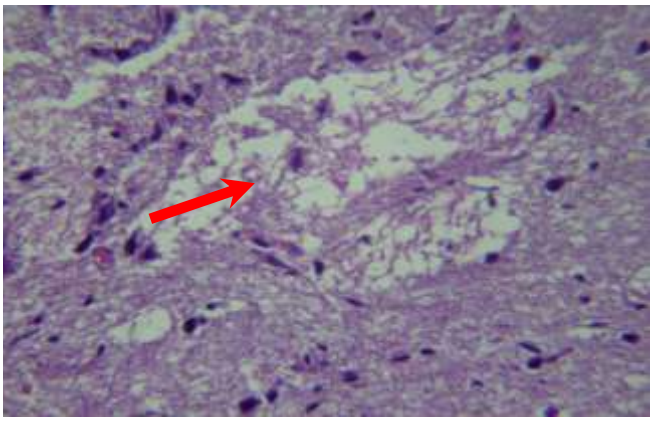


**Figure (12)** Kidney of mouse injected subcutaneously (800 mg / kg B.w /day) ethanolic extract of **E.** granulata for 45 days showing focal interstitial infiltration of mononuclear cells ( ) with thickening of Bowman's



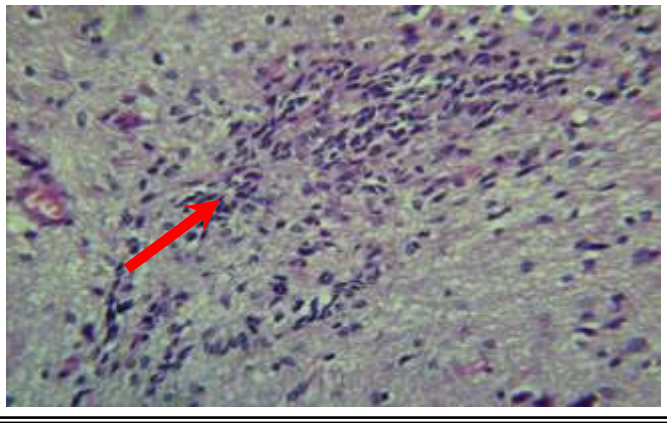


**Figure (13)** Brain of mouse injected subcutaneously (1000 mg / kg B.w /day) ethanolic extract of **E.** granulata for 45 days showing focal encephalomalacia ( ) (H&Ex400).

**Figure (14)** Brain of mouse injected subcutaneously (1000 mg / kg B.w /day) ethanolic extract of **E.** granulata for 45 days showing focal aggregation of astrocytes ( ) (H&Ex400).

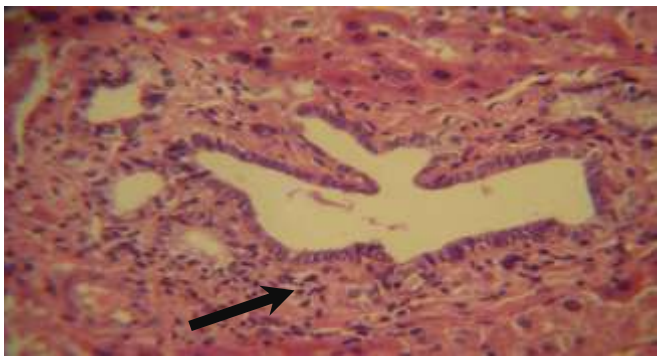


**Figure (15)** liver of mouse injected subcutaneously (1000 mg / kg B.w /day) ethanolic extract of **E.** granulata for 45 days showing thickening of the wall of bile duct due to

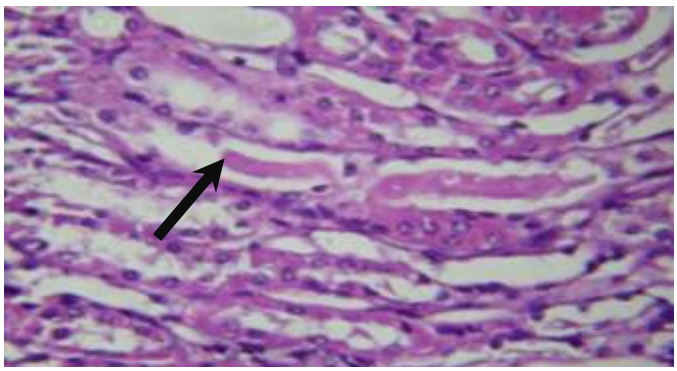


**Figure (16)** Kidney of mouse injected subcutaneously (1000 mg / kg B.w /day) ethanolic extract of **E.** granulata for 45 days showing cystic dilation of medullary renal tubules with formation of

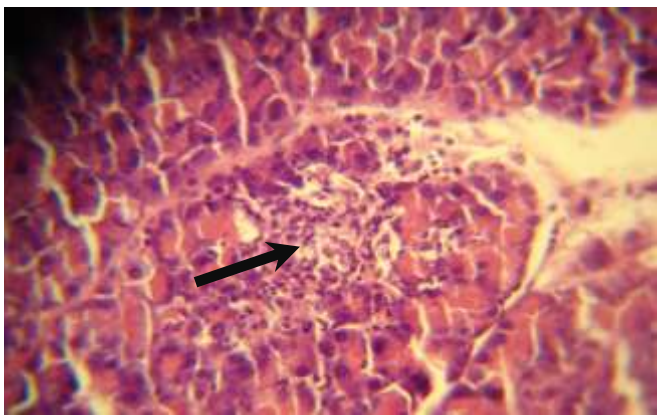


**Figure (17)** pancreas of mouse injected subcutaneously (1000 mg / kg B.w /day) ethanolic extract of **E.** granulata for 45 days showing focal aggregation of mononuclear cells between

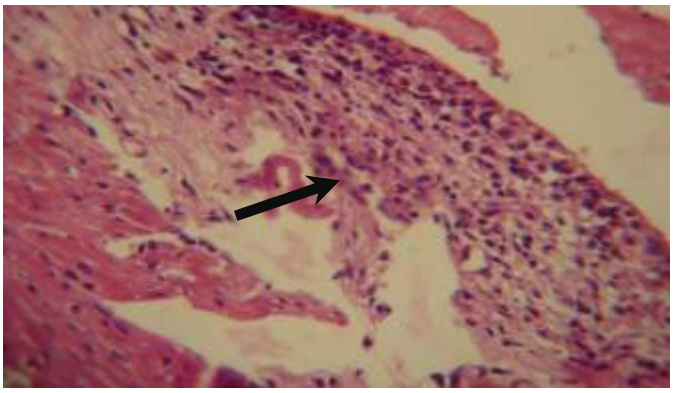


**Figure (18)** Heart of mouse injected subcutaneously (1000 mg / kg B.w /day) ethanolic extract of **E.** granulata for 45 days showing moderate fibrosis of myocardium ( )

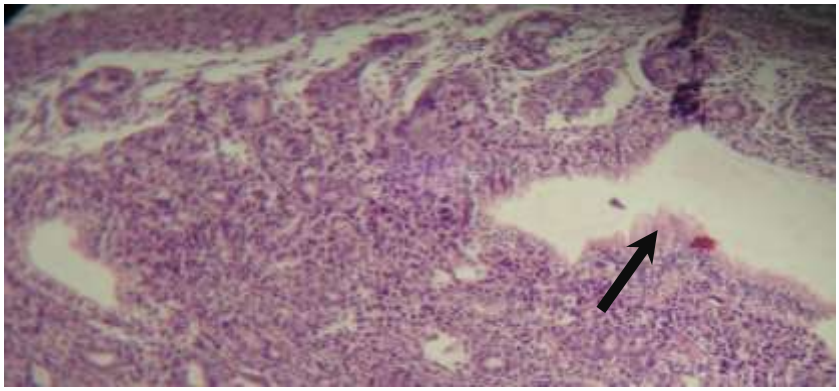


**Figure (19)** uterus of mouse injected subcutaneously (1000 mg / kg B.w /day) ethanolic extract of **E.** granulata for 45 days showing epithelial hyperplasia of endometrial lining ( )

The pat ic doses of alcoholic extracts exhibited extensive lesions in their internal organs and especially of high dose, the results was in agreement with (14) who explained that the toxic doses of ethanolic extract of *E. granulata* ranged between (500-5000 mg/kg b.w) and classify it as moderately toxic plant. Organs of toxic groups showed focal infiltrations of mononuclear cells especially in kidney, lung, heart, glandular stomach, intestine, pancreas and formation of early granuloma in liver. This observation may be attributed to the active chemical compound of the plant which may act as immune stimulant and that agreed with (11) they showed that the plant extract induced lymphocytic hyperplasia as an indication of cellular immune response against cancerous cells in mice experimentally implanted mammary adenocarcinoma. The hyperplasia of lymphoid tissue of white pulp and proliferation of megakaryocytes were due to the immune stimulant and increase splenocytes proliferation caused by the active compound of the plant (15). Furthermore (16) showed that spleen follicular hyperplasia was due to catechines of plant extract. The proliferation of megakaryocytes result as a response to multicytokines such as interlukin- 3, interlukin- 6 and interlukin-11 to enhance immune response and stimulate the maturation of megakaryocytes progenitor cells (17). Spleen showed deposition of amyloid substance. Amyloid was reported in different chronic diseases or administration of plant extracts this may be due to that the extract causes over stimulation of immune cells lead to impair their function or influence the ability of monocytes to secrete enzymes that destruct the plasma AA(Amyloid-associated). This results was agreed with (11) who showed the deposit of this substance even when using the therapeutic dose of *E. granulata* (30mg/kg/B.w) in the treatment of experimentally mammary adenocarcinoma in mice.

The edema of the brain could be related to the damage of the permeability of blood brain barrier by the plant toxic compounds including terpens, triterpenoid, glycosides and phenolics. Focal encephalomalacia, which noted in higher dose, was due to necrosis of neurons and glial cells and the pressure resulting from the edematous fluid.Astrocytosis is a common neurocellular manifestation of

brain pathology in variety of diseases, it comprised of astrocytic hyperplasia. The precise cause(s) of astrocytosis remain unknown but it occurs typically in chronic degenerative conditions (18). Necrosis of hepatocytes was probably occurred due to the accumulation of toxins in mitochonderia and lysosomes causing progressive hepatocyte organelle damage and cellular degeneration and necrosis or it may result from hypoxia in the perivenular regions, with increase in hepatic oxygen demand without an appropriate increase in hepatic blood flow. Apoptosis of hepatocytes is an active and highly regulated form of cell death responsible for the cellular default demise of the hepatocyte, which may occur due to toxic effect of the extract. This process is thus incharge of tissue hemoeostasis and maintenance of vital function of the liver (19). Other important change occurred in liver was the formation of hyperplastic nodules. The hyperplastic nodules were associated with some of the predisposing factors of primary hepatocellular carcinoma (20). The degenerative changes of epithelial lining of renal tubules and the cystic dilation of cortical and medulary tubules with formation of hyaline cast is probably due to renal insufficient which occurred due to renal insufficiency resulting from the toxic effect of the extract on kidney. Fibrosis was found in myocardium, kidney, liver, and testes .Recent studies have identified macrophages as critical regulators of fibrosis. Like myofibroblasts these cells are derived from either resident tissue populations,or from bone marrow immigrants. Studies now suggest the pathogenesis of fibrosis is tightly regulated by distinct macrophage populations that exert unique functional activities throughout the initiation ,maintenance, and resolution phases of fibrosis (21) The non-glandular stomach hyperkeratosis and hyperplasia may be due toxic effect on the mucosal lining and that inagreement with (22) who stated that the available human and animal data suggest that gastro-intetinal tract is a sensitive target of toxicity. The uterus under the influence of high dose demonstrated hyperplasia of uterine endometrium. These changes could be considered as a preneoplastic lesion as some authors consider that the proliferative lesions of the uterus are preneoplastic (23). Testicular lesions may occurred due to the toxic effect of plant extract and that agreed with study which conducted to evaluate the probable effects of phenols, alkaloids and terpenoids extracts of *E. granulata* on the reproductive performance of albino male mice. The study showed that the extracts were reduced the fertility of male mice except in some low doses The fertility had correlated inversely with the extract doses and with the period of treatment (24).. The presence of spermatid giant cells was due to degenerative changes of spermatogonia. Ultrastructurally, spermatid giant cells were round cells with multiple nuclei that appeared to arise by widening of narrow intercellular that normally connect spermatogenic epithelial cells. Pale-staining spermatogonia consisted of cytoplasmic and nuclear swelling with disruption of plasma and nuclear membranes (25).

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