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Determination the Lethal Dose$_{50}$ (LD_{50}) and Study of Acute Toxicity and Histopathological Effects of Glycosides Extract of *Alhagi maurorum* (Aqual) in Mice

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Abstract

This study was curried out on the extraction glycosides constituents of shoot and seeds of *Alhagi maurorum* (Aqual), determination LD_{50} and study histopathological effects of intestine, lungs ,liver and kidney in mice. The results exhibited acute toxicity with LD_{50} of shoot and seeds extract were 8333.333 and 7414.666 mg / kg respectively. Upon intraperitoneal administration in mice. The histopathological examination indicated that tested extract induced several histopathological changes in the mice such as degeneration, desquamation of epithelial cells, atrophy, destruction of villi, hemorrhage and necrosis in intestine .Pulmonary emphysema. Necrosis of some hepatocytes, hydropic degeneration, edema and there are scattered bile canaliculi in liver. Necrosis of some of renal tubules with atrophy in some other , as well as necrosis of glomerulus with dilatation of bowman's capsule, sloughing of epithelium lining of collecting tubules , as well as presence of completely fibrosis glomerulus .

تحديد الجرعة المميتة للنصف (LD_{50}) ودراسة التأثيرات السمية الحادة والنسجية لمستخلص المركبات الكلايكوسيدية لنبات العاقول في الفئران

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الخلاصة

شملت هذه الدراسة على استخلاص المركبات الكلايكوسيدية من الجزء الخضري وبذور نبات العاقول ثم تحديد الجرعة المميتة الوسطية ودراسة السمية الحادة في الفئران و التأثيرات النسجية المرضية في كل من الأمعاء والرئتين والكبد والكلية وأظهرت النتائج بأن هناك سمية عالية عند الجرعة المميتة الوسطية التي كانت ٨٣٣٣,٣٣٣ و٧٤١٤,٦٦٦ملغم / كغم لمستخلص الجزء الخصري والبذور على التوالي عند إعطائها تحت البريتون في الفئران كما اظهر الفحص النسجي بأن المستخلص المستخدم قد احدث بعض التغيرات النسجية تمثلت بتنكس ،توسف للخلايا الظهارية،ضمور،تنخر ونزف في ظهارة الامعاء وتحطم الزغابات ,انتفاخ رئوي, تنخر و تميء وتناثر قنيات الصفراء في الكبد,.تنخر في النبيبات الكلوية وضمور في البعض الاخروتنخر الكبيبة و اتساع في محفظة بومان وانسلاخ في بطانة النبيبات الجامعة وتليف كامل للكبيبة.

Introduction

Alhagi maurorum medik (Synonyms *A. camelorum* Fisch) is a species belonging to Fabaceae known by the common name camel thorn. Arabic name: Aqual, or Shouk Aljemal. *Alhagi maurorum* grows in the area from west Asia (including Palestine, Syria, Egypt, Iraq, gulf countries, Cyprus, Turkey, and Iran) through Caucasus and southern Russia, Afghanistan, Pakistan and India to the Himalayas. It is used in folk medicine as a purgative, laxative, diaphoretic, expectorant and diuretic (1). It has anti-inflammatory, antinociceptive and antipyretic effects(2) *A. maurorum* used for the prevention and treatment of various disorders such as liver ailments (including jaundice) (3,4).

The flavonoid fraction of *A.maurorum* possess anti-inflammatory activity and antimicrobial activity and are not toxic (5).Ethanolic extract 10% of *A. maurorum* showed significant anti-inflammatory activity (6).Oral administration of methanol extract from *A. maurorum* in a 200 mg kg^{-1} dose exhibits a significant antidiarrhoeal effect (7).

flavonoid glycosides were isolated from the ethanol extract of *A. maurorum (*kaempferol, chrysoeriol, isorhamnetin, chrysoeriol-7-*O*.-xylosoid, kaempferol-3-galactorhamnoside, and isorhamnetin 3-*O*.-β-D-apio-furanosyl (1-2) β-D-galactopyranoside) showed a very promising antiulcerogenic activity .(8).

Both the ethanolic and chloroform extracts produced center nerves system (CNS) stimulation in mice, slight tremors, straub tail, rapid respiration, twitches, excitability and slight itching were recorded (9). The flavonoid fraction extract caused an increased force of contraction of isolated rabbit heart and slight fall in blood pressure of anaesthetized rabbit. The extract showed no significant effect on the level of serum glucose, cholesterol and potassium in rats. However, chloroform extract caused a decrease in serum sodium content. The extracts are not toxic to Brine shrimps (5).The methanolic extract of *A.maurorum* in doses up to 2 g kg^{-1} b.wt. did not cause any deaths or major signs of acute toxicity (10).

Oral administration of methanol extract of *A. maurorum* at higher concentrations (>3.2 mg ml^{-1}) caused a rapid depressant effect. The depressant effect appeared to be due to calcium channel blocking effect, since $CaCl_2$ could not restore the contractile response of the tissue impregnated in calcium free-medium (7).

Materials and Methods

Albino mice (20-25g), of either sex roughly the same age (8-10 weeks). The experimental animals were healthy mice (active and without any physical defects),whose condition was monitored before and during the study, and who were handled with reasonable care during the experiments.They were kept in large airy cages in groups of 6 animals per cage with free access to food and water.

Extraction of Glycosides

A.maurorum were collected from house gardens of AL-Najef city in June – September (2009). The plants were identified and authenticated immediately after collection in botany laboratory, department of Biology College of science, Kufa University. Shoot and seeds of A.maurorum washed and then dried under shade (at room temperature). The dried plants were ground well into a fine powder in a mixer grinder and extracted with n-butanol according to Okonta and Aguwa (11).

Assessment of acute toxicity of glycosides extract (LD_{50})

The glycosides extract (GE) was administered to albino mice(six mice of both sexes per group) once intraperitoneally .In a preliminary test, animals in groups of three, received one of 10, 100, or 1000 mg/kg of the tested extracts dissolved in distilled water (D.W). Animals were observed for 24 h for signs of toxicity and number of deaths. Depending on the results of the preliminary test, doses of 1000, 2000, 3000, 4000, 5000, 6000, 7000, 8000, 9000 and 10000 mg/kg for shoot glycoside extract (ShGE) and 500, 1000, 2000, 3000, 4000, 5000, 6000, 7000, 8000 mg / kg for

seeds glycoside extract (SGE) the tested extracts were administered to fresh groups, each of 6 mice. (12).All doses were upon intraperitoneal injection in mice. Injection in a maximum volume of 12 ml/kg. Similarly, one group of six mice was given same size of D.W. intraperitoneally (control) and kept under the same conditions. The injected mice were placed separately for close observation and observed continuously for 6 hours then kept and observed occasionally for 4 hours. The behavioral changes, symptoms of toxicity and mortality were recorded. Signs were recorded during acute toxicity studies, respiration, convulsions, hypothermia, twitching. hyperthermia, aggression heart rate, excitation , iloerection,itching, salivation, waltzing movements, micturation, locomotor activity, defecation, pupil size, writhing, sedation, staggering and calmness, straub tail and mortality. (13).The LD50 was calculated by the method of Karber (14) as following:-

LD50 = Least lethal dose of all animals - ∑ Product / N

Product = Mean X Differences between doses

Mean = (number of mortality animals in dose + number of mortality animals in previously dose) ÷ 2,

N= Number of animals in a group.

Histopathology study

The organs (kidney, lung, liver and intestine) of treated animals were immediately (after death) removed and fixed in 10% formalin for histopathological assessment.The histopathological sections were made depending on (15).

Results and discussion

Acute intraperitoneal (I.P) toxicity study of shoot glycosides extract (ShGE) and seeds glycosides extract (SGE) in mice revealed that LD50 of ShGE and SGE were 8333.333 and 7414.666 mg / kg respectively. The animals receiving ShGE and SGE injection, increasing respiration rate was observed which persisted for few hours this in agreement with previously study (5). At the 6th hour they were drowsy, less responsive, calmness, decreasing respiration rate and Sedation before death or they recovered after 24 hours. However at 24th hour most of the survivors had recovered from these symptoms.

At dose 1000-5000 mg /kg increase in locomotor and sexual activities in mice treated with SGE.At dose 6000 mg/kg the mice treated with SGE was twitching. No mortality recorded by mice treated with ShGE and remained normal and don't show any sign yet dose 8000 mg/kg .However at 9000 mg/kg was drowsy.ShGE and SGE produced no diarrhea and urination, this and locomotor activity is in agreement with previously study (16).This high LD50 indicated to the safety of the use of the SGE and ShGE, therefore, can be categorized as highly safe since substances possessing LD50 higher than 50 mg/kg are non toxic (17).

LD50 of *A.maurorum* is not in agreement with previous study which mentioned the aqueous and 10% ethanolic extracts of *A.maurorum* has no toxicity and no mortality when administered orally (up to 10g/kg).(18; 16) that may be belong to differences in types of extracts.The aqeous and methanolic extracts contain many types of constituents such as Alkaloids, flavonoids, glycosides, steroids, terpenoids, resins and tannins (10,19,20; 21) . SGE showed Copulatory activity in concentration 500-4500 mg/Kg.) This may be due to the constituents in *A.maurorum* may interact with steroids sex hormone metabolism. This activity is due to such bioactive secondary metabolites as isoflavonoid and sterols (22). It is also probable that the reproductive system may have target receptors for binding with the phytochemical components in the extract (23).

Histopathological effects

Histpoathological changes of mice treated with SGE revealed there was sever degeneration of epithelium which lining the intestine, desquamation of epithelial cells in the lumen (Figure 2) comparison with control (Figure 1), atrophy,destruction of villi and extensive necrosis(Figure3) destruction of villi and sever hemorrhage of intestine treated with ShGE (Figure 4). Lung treated with ShGE Show the alveoli united together to form large alveoli(pulmonary

emphysema)and lung treated with SGE show thickening of alveolar wall and emphysema.(Figure 6,7) comparison with control (Figure 5) .

Liver treated with ShGE and SGE show necrosis of some hepatocytes, hydropic degeneration, edema and there are scattered bile canaliculi (Figure 9, 10) comparison with control (Figure 8). Necrosis of some of renal tubules with atrophy in some other, as well as necrosis of glomerulus with dilatation of bowman's capsule, sloughing of epithelium lining of collecting tubules, degeneration hydropic (ballooning cell) , as well as presence of completely fibrosis glomerulus in the Kidney treated with SGE and ShGE (Figure 12,13) comparison with control (Figure 11). This histpoathological changes may be belonging to using the high dose of ShGE and SGE.The probable reason for the observed histological effects may be due to extract constituents in A. marorum which may interact with rat metabolism and this activity is due to such bioactive secondary metabolites as isoflavonoid, (22). This may explain a tendency towards atrophy and degeneration (23).These phytochemicals are known to perform several general and specific functions in plants, and may exhibit different biochemical and pharmacological actions in different species of animals when ingested. These actions range from cell toxicity to cell protective effects (24).

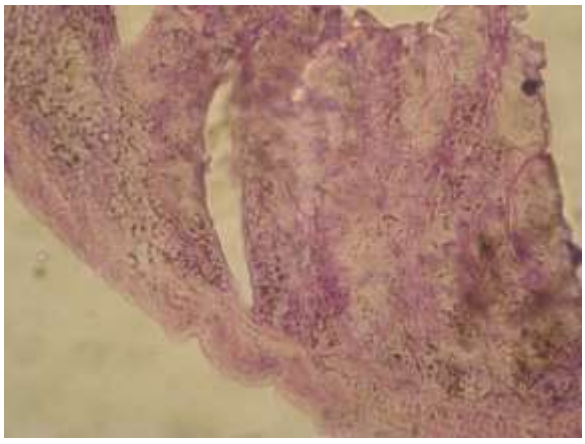

Figure (1): Histological appearance of normal intestine (control). 400X, H&E stain.

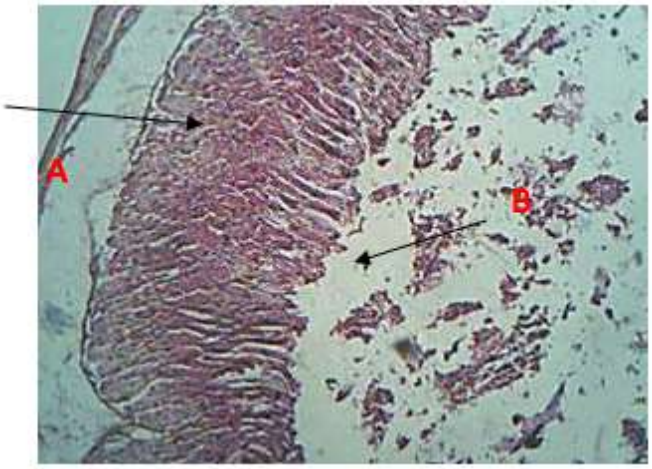

Figure (2):- Histpoathological changes of intestine treated with SGE. : There is sever degeneration of epithelium which lining the intestine (A). Also there is desquamation of epithelial cells in the lumen (B). 100X, H&E stain.

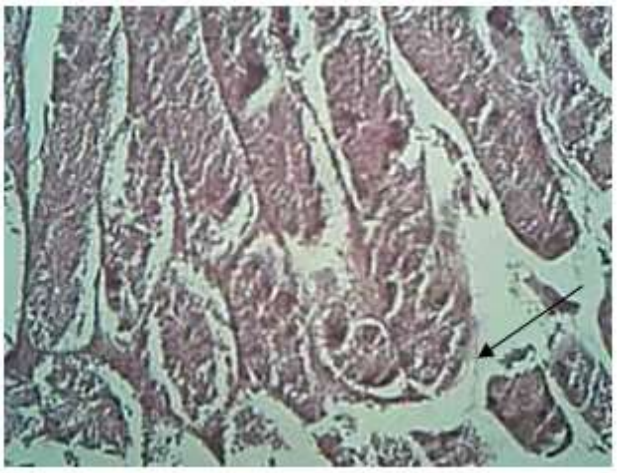

Figure (3):- Histpoathological changes of intestine treated with SGE: atrophy, destruction of villi (arrow) and extensive necrosis. 400X, H&E stain.

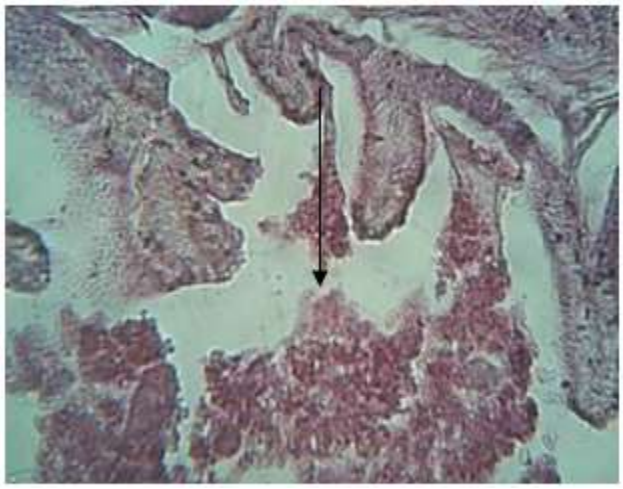

Figure (4):- Histpoathological changes of intestine treated with ShGE. Show destruction of villi and sever hemorrhage (arrow). 400X, H&E stain.

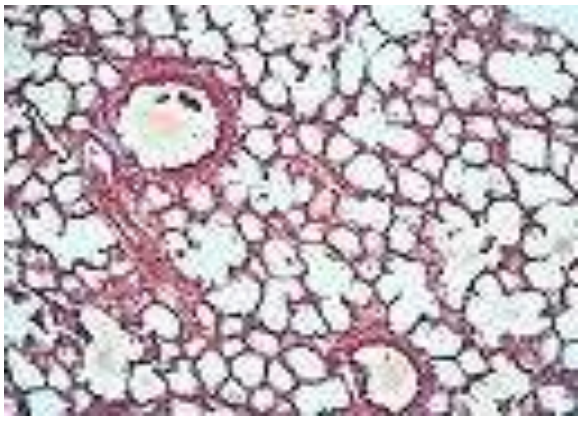

Figure (5): Histological appearance of normal lung (control) 100X, H&E stain.

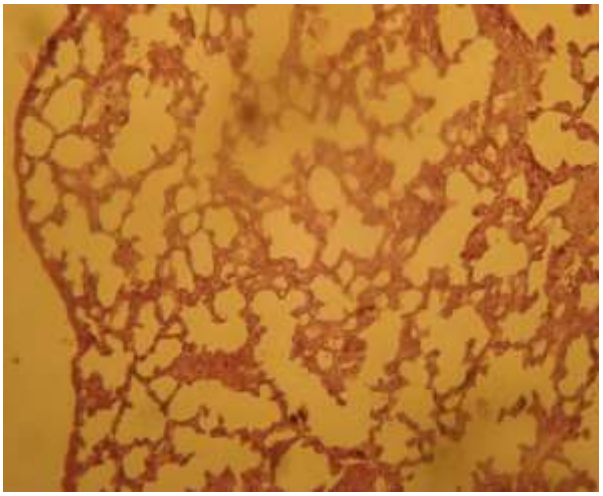

Figure (6):- Histpoathological changes of lung treated with ShGE. Show the alveoli united together to form large alveoli (pulmonary emphysema) 100X, H&E stain.

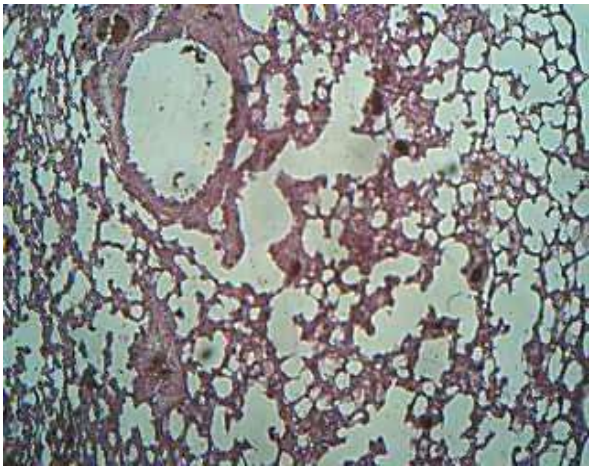

Figure (7):- Histpoathological changes of lung treated with SGE.Shaw thickening of alveolar wall, emphysema. 100X, H&E stain.

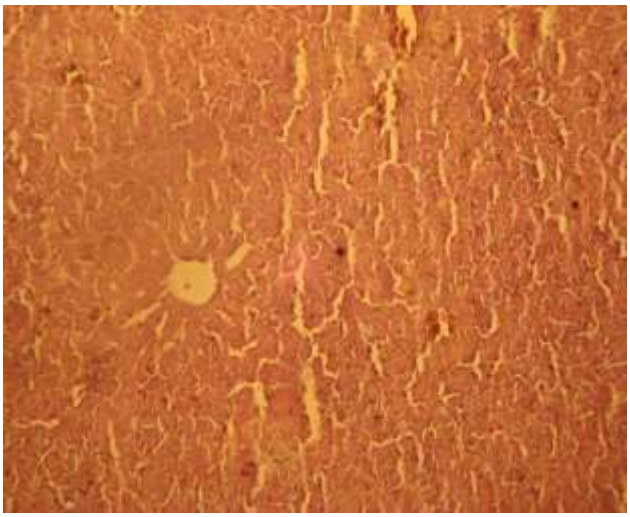

Figure (8): Histological appearance of normal liver (control) 100X, H&E stain.

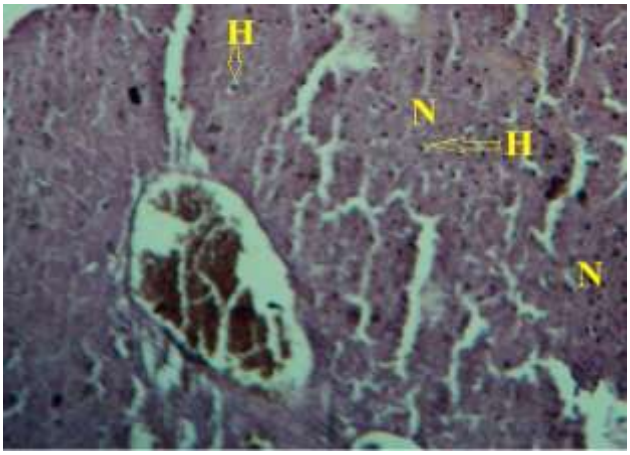

Figure (9):- Histpoathological changes of liver treated with SGE show necrosis of some hepatocytes (N), hydropic degeneration (H), edema (E) and there are scattered bile canaliculi.100X, H&E stain.

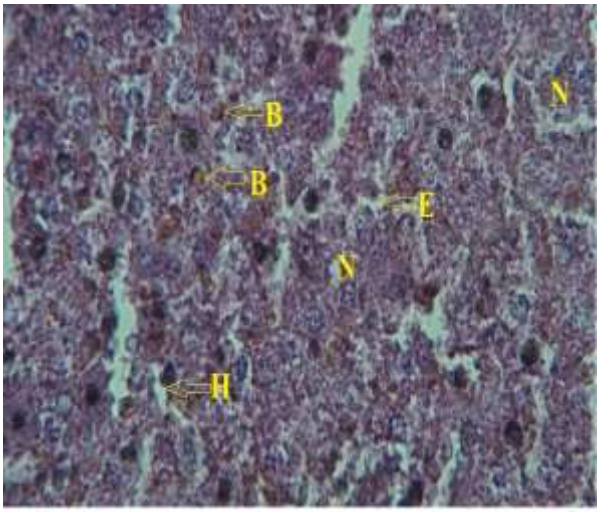

Figure (10): Histpoathological changes of liver treated with ShGE show necrosis of some hepatocytes (N), hydropic degeneration (H), edema (E) and there are scattered bile canaliculi (B).400X, H&E stain.

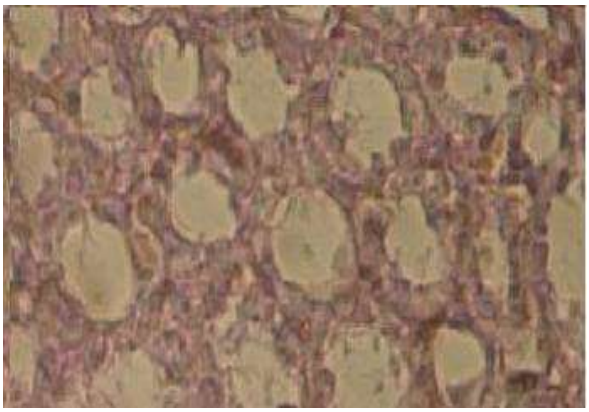

Figure (11): Histological appearance of normal kidney (control). 400X, H&E stain.

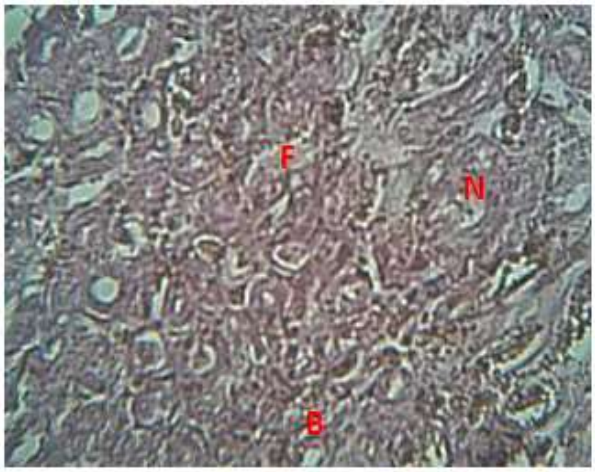

Figure (12):- Histpoathological changes of kidney treated with SGE show: Fibrosis (F), necrosis (N) and ballooning cells (B). 400X, H&E stain.

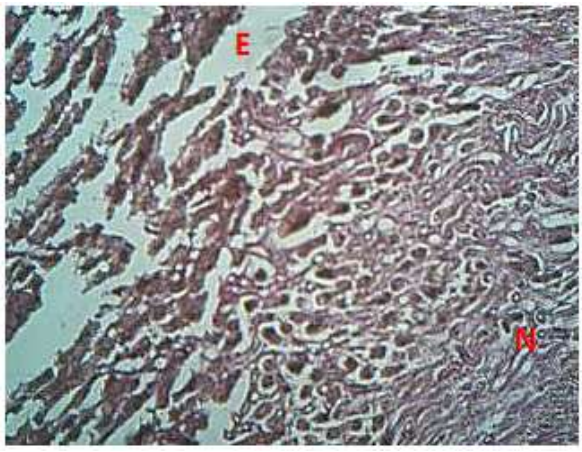

Figure (13):- Histpoathological changes of kidney treated with ShGE show necrosis (N) , edema (E). 400X, H&E

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