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## THE PROTECTIVE ROLE OF AQUEOUS EXTRACT OF PLANT ROOTS *CICHORIUM INTYBUS* L. IN A NUMBER OF BIOCHEMICAL VARIABLES IN THE BLOOD SERUM AND TISSUES OF RABBITS INFECTED WITH EXPERIMENTAL KIDNEY STONES

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### ABSTRACT

This study aimed to know the protective role of *Cichorium intybus* root aqueous extract in inhibiting the formation of kidney stones induced by ethylene glycol in male *Oryctolagus cuniculus*, expressed by monitoring kidney function and studying the effect of oxidative stress induced by ethylene glycol, as well as by microscopic examination of kidney tissue. In this study, male domestic rabbits used *Oryctolagus cuniculus* with (20) animals whose ages ranged from (6-8) months and were examined by a veterinarian and their weights ranged between (1500 + 100) gm. The animals were divided into four experimental groups by 5 rabbits / Group, dosed for 60 days, as follows: The first group was given water and food throughout the trial period, The second group dosed (50 mg / kg of body weight) with *Cichorium intybus* root aqueous extract, while the third group was dosed with ethylene glycol at a concentration of (0.9%) mL / kg of body weight, and the fourth group was dosed with ethylene glycol in addition to *Cichorium intybus* root aqueous extract. The results showed that the formation of stones induced by ethylene glycol in male white rabbits led to negative effects in the biochemical variables, represented by a significant increase at a probability level ( $P \leq 0.05$ ) in total protein, urea, creatinine and K ion, and treatment with this substance did not lead to a change in albumin. And Mg ion and Ca ion, while the treatment with E.g led to a decrease in the occurrence of histological changes and calcium crystal deposition were evident in the kidneys. The results of the group treated with *Cichorium intybus* root aqueous extract at a concentration (50 mg / kg of body weight) showed a significant increase, Mg, and did not affect, albumin, and a decrease in creatine, total protein, and urea. Ca compared with the control group, while no tissue changes occurred to the kidneys. The treatment with *Cichorium intybus* root aqueous extract and the treatment with ethylene glycol at a concentration of (0.9%) led to a decrease in Ca, K, creatinine, total protein, urea, albumin and a significant increase in, Mg, to a group Control treated with ethylene glycol, and this treatment led to a significant improvement in kidney tissue.

**Keywords:** ethylene glycol (E.g.), *Cichorium intybus*, kidney stones.

### Introduction

The urinary system is one of the important excretory organs which has many basic regulatory roles through the regulation of water and salts excreting waste in the body of the organism, and it consists of the kidneys, ureters and the bladder, and the kidneys are the main active organ in the urinary system for its important physiological functions (Al-Abdullah, 2012). The kidney is one of the important organs because it has many important functions, including filtering blood from the products of unwanted metabolic processes, controlling blood pressure and sugar synthesis by the process of Gluconeogenesis, and the regulation of the endocrine glands through the secretion of the hormone Erythropoietin and Renin. And vitamin D3 production (Guyton & Hall, 2019). Kidney stones are a common and serious condition of the urinary system that affects the kidneys after kidney and prostate failure (Al-Hadidi, 2015). This disease constitutes a rate parallel to the appearance of diabetes and atherosclerosis in statistical terms, as urinary stones (4%) are among the rest of the most common diseases (Gilligan & Raphael, 2017). Stones are formed as a result of metabolic or genetic factors as well as environmental and nutritional factors. The disease

results by forming insoluble stones in the urinary tract and usually forming in one of the two kidneys. Now the mechanism of its formation is subject to many theories according to the material that it forms and thus differs in its sizes Sand, including the large ones that fill the lumen of the kidney pelvis, which leads to it remaining in the renal pelvis or passing to the ureter and then to the urinary bladder as this works to block the urethra, which leads to severe pain in the urethra (Sunitha *et al.*, 2018). There are many types of kidney stones, including calcium stones, uric acid stones, ammonium phosphate stones and magnesium, and for each of these types there are many factors and substances that cause them in addition to the methods of treatment (Badrinthan *et al.*, 2015). Since ancient times, man has been interested in getting to know many herbs and natural plants that grow wild, and he found many of them useful as food or medicine, in addition to his access to knowledge of many of their properties and to determine their benefits using them in folk medicine (Mustafa & Yasar, 2010).

Despite the widespread discovery and use of many medicinal drugs (industrial chemical) and the interest of researchers and doctors in therapeutic methods for this

disease, the materials extracted from plants are still of high value in the medical field in order to avoid the side effects that the manufactured drugs generate (Qi, 2011). The dandelion plant is a perennial herbal plant (chicory), which grows naturally in different regions in the country and belongs to the compound family, and it is one of the plants commonly used in the field of folk medicine to treat many diseases. As the plant juice is used as a whole, tonic and appetizer, especially for those who complain of anemia, and is useful for treating high blood pressure, strengthening the nerves and heart, treating atherosclerosis, regulating muscle work, increasing calcium, strengthening the nervous system, hypnotic and calming (Zhou *et al.*, 2010).

*Cichorium intybus* root extract is also used to treat chronic diseases because it contains many effective compounds, including (tannin, lactones) (Abdel-Rahim *et al.*, 2016). In addition to containing sugar-lowering compounds, it has been observed to reduce levels of malondialdehyde and increase the level of glutathione, as well as reduce the level of triglycerides and cholesterol (Alipour *et al.*, 2018). It is also used in the treatment of pathological conditions, as it is a diuretic, a cleanser for the kidneys and bladder, crushing stones and dissolving them, disposing of the body of sand, filtering blood and its juice is useful, removes fever and eliminates it, helps to reduce the temperature, and is used in the treatment of urinary tract infection and prostate (Das *et al.*, 2016).

## Materials and Methods

### Animals used in the study

*Oryctolagus cuniculus* was used in this study, with (20) animals whose ages ranged from (6-8) months, and they were examined by a veterinarian, and their weights ranged between (1500 + 100) gm, and they were obtained from the local market of the city of Mosul. In wooden crates with dimensions (40 x 30 x 50) cm prepared for this purpose. And taking into account the aspect of hygiene and sterilization of its place from time to time. The animals were subjected to adequate and uniform laboratory and environmental conditions from a light cycle to 12 hours of light and 12 hours of darkness, and the temperature was (25 + 2) degrees Celsius. A special diet was fed (35% wheat, 34% yellow corn, 20% soybeans, 10% protein, 1 g/kg powdered milk, plus 50 g/100 kg of anti-fungal substances. Water and food were given freely. And in sufficient quantities for the length of the experiment (NRC, 1994).

### Development of kidney stones

Kidney stones were developed using ethylene glycol at a concentration of 0.9% orally for 60 days.

### Preparing the aqueous extract of the plant

The *Cichorium intybus* plant was obtained from the local market of the city of Mosul. It was cleaned and cut the required parts for the purpose of the study from the roots of the dandelion plant and dried in the shade of the sun for a week, then it was ground to obtain a powder for the purpose of preparing the aqueous extract, then the roots are ground. The plant was carried out by an electric grinder. The method of Zheng-Mu and his group (1990), was adopted in the preparation of the hot aqueous extract, where 20 g of plant powder was added to 200 ml of boiling water placed in a 250 ml beaker, and mixed with a magnetic mixer with heating,

then the mixture was left for 30 minutes to cool. He was filtered by several layers of gauze, and then using filter paper, ignore the sediment and dry the filtrate in an electric oven at a temperature of 45 °C until the weight was fixed to obtain it in powder form.

### Experiment Design

This study was conducted for (20) white male rabbits and was divided randomly into (4) groups, each group included (5) animals, and the groups were as follows:

**The first group:** (the control group) This group was given regular drinking water and food daily for a period of (60) days

**The second group:** group of animals treated with aqueous extract of dandelion roots at a concentration (50 mg / kg of body weight) orally by tube feeding + regular food + water for a period of 60 days

**The third group:** (group induced agglutination with ethylene glycol E.g.) This group was treated with ethylene glycol (0.9%) with drinking water for 60 days (Ravindra *et al.*, 2006).

**Fourth group:** (group of animals treated with ethylene glycol E.g. + aqueous extract of the roots of the dandelion plant), this group was treated with ethylene glycol E.g at a concentration of (0.9%) with drinking water and dosed with aqueous extract of the roots of the dandelion plant at a concentration (50 mg / kg of body weight) about Oral route by tube feeding for a period of 60 days.

### Collection of Blood Samples:

After the end of the specified period of the experiment, the animals were anesthetized with chloroform, and blood samples were obtained by heart-stabbing. About (6-8) ml of blood were collected, they were placed in plane test tubes and left for about a quarter of an hour in a water bath at 37 C. Then, the blood was separated by a centrifuge at a speed of (3000 revolutions / minute) to obtain the blood serum preserved at (-20) m after it was divided into three new, clean plastic tubes until the required biochemical tests were carried out.

### Estimation of a number of biochemical variables

#### 1. Determination of Total Serum Protein Concentration

The total protein concentration in the blood serum was determined according to the biorite method using a ready-made analysis kit from the French Bilabio company, The reaction between the protein in the serum and the solution of the base copper potassium citrate (copper ions in the base ocean) is obtained to give a purple-colored complex whose intensity depends on the number of peptide bonds present in the protein whose absorption intensity is measured at a wavelength of 550 nm.

#### Calculation

The concentration of total protein in serum is calculated according to the following equation:

$$\text{Total protein concentration} = \frac{\text{The intensity of absorbance of the form}}{\text{The intensity of absorbance of the standard}} \times \text{standard solution concentration (6g / L)}$$

### Determination the Concentration of Albumin in Blood Serum

The albumin concentration in the blood serum was determined using a ready-made analysis kit from the Spanish company (Biolabo), This method relies on the amount of albumin that binds with the reagent 5,5,3,3-tetrabromo-metacresol phthalene sulfate 3,3,5,5-Tetrabromo-M-Cresolsulphonphthalein which is called Bromo Cresol Green (BCG), to be a complex Albumin-BCG-Complex green, whose absorption intensity is measured at wavelength 630 (Webster, 1977).

#### Calculation

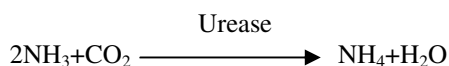
The albumin concentration in blood serum was calculated from the following relationship:

Albumin concentration =  $\frac{\text{The intensity of absorbance of the form Solution}}{\text{The intensity of absorbance of the standard}}$

$\times$  standard solution (5 g / liter)

### Determination the Concentration of urea in blood serum

The concentration of urea in serum was estimated by the Urease\_modified\_elot method using the analysis kit provided by the French company Bilabio (De *et al.*, 2000). This method involves the use of prepared prepared solutions that include the enzyme urease (urease) that serves to identify ammonia in the basic environment.



Ammonia reacts with Hypochlorite Salicylate to form 2,2\_ Dicarboxyindophenol, green in color, which was measured by the absorbance intensity by a Spectrophotometer at the wavelength of 580 nm.

#### Calculation

The Urea concentration in blood serum was calculated from the following relationship:

$$\text{Urea concentration} = \frac{\text{Abs sample}}{\text{Abs standard}} \times \text{standard concentration}$$

### Determination the Concentration of Creatinine in blood serum

The amount of creatinine in the blood serum according to the usual method mentioned in the analysis kit prepared by the French-made Biolabo company, where the creatine reacts by the method of Jaff reaction with picric acid in a kinetic manner at the wavelength of 490 nm without sedimentation and this reaction is faster and more accurate Initial rate (Tietz *et al.*, 1999).

#### Calculation

The factor was extracted and multiplied by the subtraction of the two readings for each Standard-Standard's second reading form:

$$\text{Factor} = \frac{\text{Standard Concentration}}{\text{Standard}}$$

### Determination of the concentration of magnesium ion in the blood serum

The concentration of magnesium ions was determined using the enzymatic method provided with the measuring kit

by the French Bilabio Company, as Galmagite, which is a colored metal detector, is a metallochromic indicator, which is complex colored with the magnesium present in the sample. And that the presence of the ethylene glycol tetraacetic acid (EGTA) complex is to reduce or reduce the interference with calcium ions, and that Potassium cyanide (KCN) works to prevent or reduce interference with heavy metals, while the compound polyvinylpyrrolidon (PVP) works to prevent interference with Blood proteins and fats (Tietz, 2006).

#### Calculation

The concentration of magnesium in the blood serum per unit (mg / dl) according to the following law:

$$\frac{\text{Absorbency test solution (Test)}}{\text{Absorption intensity of standard solution}} \times \text{Standard solution}$$

### Determination the Concentration of potassium in blood serum

The concentration of potassium in serum was determined using the colourimetric method using the measuring kit provided by the German company Human, Potassium ions react in a free base protein medium with Sodium tetrapheny boron to produce after the separation process a turbid suspended solution of Potassium tetrapheny boron and that the turbid filtrate determines the potassium concentration and is measured spectrally at a wavelength of 578 nm (Tietz, 2006).

#### Calculation

Calculation of potassium in blood serum is carried out according to the following equation:

$$\text{Sample concentration} = \frac{A \text{ sample}}{A \text{ std.}}$$

A = Absorbance

Std = Standard

### Determination the Concentration of Calcium in Blood Serum

Calcium ion concentration in blood serum was determined according to the method mentioned in the ready-made kit provided by the French company Biomerieux, where the calcium reacts with the red complex compound forming a complex in the base solution O-cresol phtaleine in which we measure the calcium concentration (Tietz, 2006).

#### Calculation

Calculate the calcium concentration in the blood serum, as in the following equation

$$\text{Sample concentration} = \frac{A \text{ sample}}{A \text{ std.}}$$

### The histological study

After dissecting the animals and extracting the kidney required in the current study, the organs were washed with the physiological solution, and then the methods used for making tissue sections were performed based on (Al-Khatib, 1997 & Al-Hajj, 2010). My agencies: Fixation, Washing, Dehydration, Clearing, Infiltration, Embedding, Trimming and sectioning, Staining, Mounting, then the tissue slides were examined using the Compound light microscope Altay

Reichert Neovar, And using different enlargement powers to find out the changes in the tissue structure of the kidneys for each experimental group treated compared to the control group. The sections were photographed by a compound imaging microscope (Taiwan Biol lab line Altay 1007) equipped with a digital camera connected to a P4 hp electronic computer, and then the images were printed using color printing.

### Statistical Analysis

Statistical analysis of the data and results was performed using SPSS 11.5 for Windows software. Comparison of means using Anova-one way was performed by linear variance Analysis using (LSD) test at significance level ( $P < 0.05$ ) for comparison between different exposure periods.

### Results and Discussion

The results of our current study shown in Table (1) a significant increase in the total protein concentration in the group treated with ethylene glycol (0.9%) compared to the healthy control group. The results of our study agree with (Mahdi, 2014) indicated as it showed a significant increase in the concentration of total protein in the blood serum of rats subjected to a renal test with ethylene glycol. While our current results do not agree with what (Ibrahim, 2012) reported, as it showed a significant decrease in the total protein concentration in the blood serum of rats exposed to experimental immunization with E.g, (Al-Khafaji, 2004), in a study of biochemical variants of kidney stone disease, showed a significant decrease in the concentration of total protein in patients' blood serum, and they showed (Cavendish, 2008) the accumulation of oxalates in the kidneys leads to disturbance and dysfunction of the kidneys, which increases the rate of blood proteins, especially our results agree with these studies, The reason for the high total protein concentration in the serum of animals treated with E.g at a concentration of (0.9%) is that this substance is metabolized to give oxalates as a final product (Morgan & Pearle, 2016).

The results showed a significant decrease in the group treated with *Cichorium intybus* root extract in total protein concentration compared with the healthy control group. Our current study agrees with what they indicated (Hu *et al.*, 2015) the diuretic property of *Cichorium intybus* root extract works to remove kidney deposits and improve their functions. And liver function, which led to a decrease in the stimulation of the liver to form proteins due to crystal formation and the associated infections and infections (Buzanovskii, 2017) and the results showed a significant decrease in the groups treated with *Cichorium intybus* root extract in the total protein concentration compared to the control group treated with ethylene glycol. The groups treated with *Cichorium intybus* root extract showed that they did not show any significant differences in the total protein concentration compared to the control group treated with Eg, our results are inconsistent with what (Saggu *et al.*, 2014) indicated to the role of the *Cichorium intybus* plant in improving liver and kidney function because it contains medically active substances. These are the glucocinoids and flavonoids. (Hu *et al.*, 2015) indicated that the glucocinoids present in the roots of the *Cichorium intybus* plant contribute to strengthening the body's immunity and improving liver and kidney functions, thereby increasing the formation of

blood proteins, The reason for the absence of significant differences may be due to the fact that the therapeutic and diuretic properties of the *Cichorium intybus* plant could not eliminate the toxic effects of the oxalate precipitates from the degradation of E.g. Thus, the liver continued to form immune bodies against oxalates and infectious infections of the kidneys.

The results shown in Table (1) showed a significant decrease at the probability level ( $P \leq 0.05$ ) in the concentration of albumin in the blood serum in the treated group, the group treated with E.g with a concentration of (0.9%). Kidney stones as a result of deposition of crystals and harmful substances in the renal units impairs the filtering function of the nephrons and thus increases the rate of excretion of albumin in the urine and works to reduce its concentration in the blood (Agrahrker *et al.*, 2003).

While they indicated (Khan *et al.*, 2006) kidney stone disease is associated with a decrease in serum albumin due to impaired renal function resulting from damage to some kidney tissues due to crystal deposition or repeated surgery to remove the stones, As our results are compatible with these studies, and the reason for the significant differences may be explained by the fact that the crystals precipitated from oxalate and calcium phosphate, which caused major damage to the kidney tissues, affect the process of filtering albumin and lead to its loss with the urine, so there was no change in the albumin concentration when using ethylene glycol.

The results of our study also showed that there were no significant differences in the group treated with *Cichorium intybus* root extract compared with the healthy control group. *Cichorium intybus* root extract contributes to the breakdown of kidney stones and eliminates them, as it improves the kidney function and improves the filtering function of the renal units and thus reduces the albumin particles lost in the urine due to the damage resulting from the deposition of crystals in the kidneys (El-Sayed *et al.*, 2015).

The *Cichorium intybus* plant contains active substances such as glucocinoids, flavonoids and saponins, which are considered to have a therapeutic effect for many diseases such as kidney and liver diseases, and this increases the efficiency of the liver in the manufacture of blood and kidney proteins in regulating the concentration of these proteins, of which albumin is the most important (Al-Snaf, 2016).

Whereas they indicated (Ahmed *et al.*, 2017) the roots of the *Cichorium intybus* plant contain many substances, elements and vitamins that contribute to the treatment of liver diseases, remove the toxic effects of chemicals on it and improve its functions, as it works to increase the manufacture of proteins, including albumin, thus increasing the concentration of the latter in patients' blood serum. Our results are consistent with these studies, as no significant differences were found in the albumin concentration compared to the healthy control group. Our results also showed no significant differences in the group treated with *Cichorium intybus* root extract compared with the control group treated with E.g., *Cichorium intybus* root extract contributes to breaking up and eliminating kidney stones (El-Sayed *et al.*, 2015), as it improves the kidney function and improves the filtering function of the renal units and thus reduces the albumin particles lost in the urine due to the damage resulting from the deposition of crystals in the kidneys, Thus, these results do not agree with our study.



The results showed a significant increase in the concentration of urea and creatinine in the group treated with E.g at a concentration of (0.9%), compared with the healthy control group, The results of our study are consistent with what (Mahdi, 2014). As it was shown that treating rats with ethylene glycol at a concentration of (0.9%) led to a significant increase in the concentration of urea. Likewise, the results of our current study also agree with what (Ibrahim, 2012) brought, as it indicated that treating rats with Eg at a concentration of (1%) led to An increase in blood urea concentrations occurs while (Gunjan *et al.*, 2011). The disturbances in the work of the kidneys lead to an increase in the concentration of urea in the blood, as the substance ethylene glycol Eg is a substance with high toxicity to the kidneys as a result of its formation of oxalates, which are deposited in the form of calcium oxalate crystals that cause acute tubular necrosis and thus disrupt the kidney function, including the urea filtration process (Bomalaski, 2005).

While showed (Cavendish, 2008) kidney stones lead to defects and impairments in kidney function, and the symptoms of this imbalance are an increase in the concentration of urea and creatine, The results of our study are consistent with these studies. As the treatment of rats with ethylene glycol resulted in an increase in the concentration of urea, and the reason is due to the extent of damage to the kidneys as a result of calcium oxalate deposition and acute tubular necrosis, which in turn caused major disturbances in kidney function With *Cichorium intybus* root extract, it did not affect the concentration of urea and creatinine in the blood serum, Our results are consistent

with what previous research and studies indicate that an increase in the concentration of calcium and salts in the blood leads to the formation of stones and that the formation of stones increases the concentration of urea in the blood (Han *et al.*, 2015).

Our results agree with what (Saeed *et al.*, 2017) stated that *Cichorium intybus* root extract is a diuretic that removes the blood from harmful substances, and the results of our current study showed a significant decrease in the concentration of urea and creatine from the group treated with *Cichorium intybus* root extract compared to the control group treated with ethylene glycol. *Cichorium intybus* root extract is a diuretic that increases urinary excretion rate (Hitova & Melzig, 2014).

Consequently, the concentration of urea and creatinine will decrease as a result of its continuous release. The results of the group treated with *Cichorium intybus* root extract agree with this study, as it showed significant differences compared with the control group treated with E.g at a concentration of (0.9%). As they indicated (Wolf, 2004) a decrease in the concentration of urea and creatinine may be associated with most of the therapeutic effect of the extract and not affected by cases of kidney dysfunction resulting from the toxic effect of ethylene glycol, Whereas the therapeutic effect of *Cichorium intybus* root extract had a clear effect of lowering the concentration of urea, a body of excess salts, especially the salts associated with the formation of kidney stones, and it works to reduce urea and creatinine in the blood serum. As shown in Table (1).

**Table 1 :** Shows the total protein concentration, creatine, albumin and urea for male rabbits treated for 60 days.

	Variables Transactions	TOTAL PROTEIN (g\dl)	CREATININ (g\dl)	ALBUMIN (g\dl)	UREA (ml\dl)
1	Control	4.733 ±0.488	5.190± 0.193	6.36 ±0.454	24.757±2.18
2	Ethylene glycol	9.013 ±0.544	10.460 ±0.597	4.570 ±0.324	38.980 ±3.00
3	<i>Cichorium intybus</i>	4.050 ±1.362	3.150 ±0.497	6.320 ±0.475	22.530±1.074
4	E.g.+ ex. <i>Cichorium intybus</i>	6.543 ±0.291	9.310 ±0.265	3.193±0.176	0.524±27.580
	L.S.D	0.6969	0.7619	0.5104	2.808

- Values are expressed in mean ± standard deviation.
- The number of rats (5) in each group

The results shown in Table (2) showed a significant decrease in the concentration of Mg ion in the group treated with E.g with a concentration of (0.9%) compared with the healthy control group, The results of our current study are consistent with what (Ibrahim,2012) brought. Where it indicated a significant decrease in the concentration of Mg in rats treated with Eg with a concentration (1%), and our study agrees with they (Bano *et al.*, 2018) the ethylene glycol has a toxic effect on the digestive system, leading to a defect in the absorption process of magnesium and the process of its excretion outside the body Consequently, its concentration will decrease and this corresponds to the results of our study.

It also showed that there was a significant decrease in the concentration of Mg in the group treated with E.g. This decrease may be attributed to the deposition of intermediate products, namely calcium oxalate from the ethylene glycol metabolic pathways, which thus form oxalate stones, and oxalate deposition results in a decrease in the concentration of magnesium (Medline, 2012).

The results showed no significant differences in the concentration of Mg ion in the group treated with *Cichorium intybus* root extract compared with the healthy control group. The results of our current study do not agree with research indicating that the roots of the *Cichorium intybus* plant are rich in magnesium and provide the body with this element, which leads to its high concentration in the body (Li *et al.*, 2010).

The reason may be attributed to the lack of influence of animals with the extract from the roots of the *Cichorium intybus* plant to the fact that this plant has a diuretic effect that led to the release of quantities of Mg and thus did not show an increase in its concentration in this group, as they indicated (Malik *et al.*, 2017) the extract of the roots of the plant is a diuretic that works to clear, The body is made up of salts and some elements, and Mg is among them.

The results also showed a significant increase in the concentration of Mg in the group treated with *Cichorium*

*intybus* root extract compared with the control group treated with E.g and the control group treated with ammonium oxalate, The results of our current study are in agreement with studies that indicate that oxalate stones are the result of a decrease in the concentration of Mg, which is an inhibitor of the nucleation and deposition of oxalates and the formation of oxalate stones (Preminger & Curhan, 2009).

This resulting increase in the concentration of Mg may be due to an improvement in the condition of tissues that were exposed to damage by E.g and ammonium oxalate through treatment with these extracts, which led to an increase in the amount of extracellular Mg. Also, the reason for the increase in the concentration of Mg is due to the fact that the extract is rich in magnesium and thus increases its concentration in the blood serum (Arena *et al.*, 2008; Chehl *et al.*, 2009).

The results of our study shown in Table (2) showed a significant increase in the concentration of K in the group treated with ethylene glycol at a concentration of (0.9%) compared with the healthy control group. Our current study agrees with what (Gisela *et al.*, 2003) pointed out to him that the substance ethylene glycol destroys tissues and cells, and this in turn will lead to the exit of cell components and ions present from inside the cells, including the K ion, thus increasing its concentration in blood serum, Our results are also consistent with what (Buchholz *et al.* 2002) indicated that kidney disease leads to a decrease in the concentration of potassium in the body, Ethylene glycol has a toxic effect on the renal tubules, leading to malformations and acute necrosis in them, thus disturbing kidney function, including the process of reabsorption of the K ion and thus its concentration increases (Qahtan & Hamad, 2008).

The reason for the existence of significant differences in the group treated with ethylene glycol is attributed to the fact that the damage resulting from this substance on the cells may have reached an extent in which the cells lose their internal ions, including the potassium ion, and the reason may be that the kidneys were unable to get rid of the harm of E.g by increasing the rate of excretion with Urine, therefore, there was an increase in the destructive effect of this substance on the potassium concentration in the serum of the study animals.

The results of our study also showed a significant decrease in K concentration in the group treated with *Cichorium intybus* root extract with the healthy control group, the results of our current study agree with studies that indicate that *Cichorium intybus* has a diuretic property and works to reduce potassium in the body (Carazzone *et al.*, 2013). Our study also agrees with studies that indicate that the extract of *Cichorium intybus* root stimulates the kidneys to increase the rate of urine excretion in the body to get rid of

the crystals that contribute to the formation of kidney stones, and this will affect the K concentration, which leads to a decrease in its concentration (Khodadadi *et al.*, 2016).

The results showed a significant decrease in k concentration in the group treated with *Cichorium intybus* root extract compared with the control group treated with E.g. at a concentration of (0.9%) The results of our current study are consistent with the studies Which confirms the decrease of most of the body ions due to the diuretic property of the *Cichorium intybus* root extract, as they indicated (Carazzone *et al.* 2013) the diuretic property of the active substances in the *Cichorium intybus* plant causes the body to lose ions, including potassium ion, calcium, phosphorus and hydrogen, they showed (Malik *et al.* 2017) plant extracts with diuretic activity cause the body to lose many ions, which leads to a decrease in their concentration in the body.

The results showed a significant decrease in Ca concentration in the animals treated with ethylene glycol compared to the healthy control group. The results of our study agree with what was indicated by (Khatib *et al.*, 2010) that oxalates are the final results of the metabolism of E.g. This material has a strong ability to combine with calcium and form insoluble calcium oxalate crystals Which is deposited in the form of stones in the kidneys in the absence of inhibitors crystal formation. As this combination leads to a decrease in the concentration of calcium in the blood, (Ibrahim, 2012) showed a decrease in the concentration of Ca in the blood serum of rats treated with E.g at a concentration (1%) compared with the control group, The reason for the decrease was attributed to the deposition of calcium in the form of insoluble calcium oxalate and calcium phosphate crystals. This results of our current study agree with this study The results of our study do not agree with what (Mahdi, 2014) explained, as it showed that there was no significant difference in the concentration of calcium ions in groups treated with ethylene glycol.

The results showed a significant decrease in the group treated with *Cichorium intybus* root extract compared with the control group treated with Eg with a concentration (0.9%). Our results are consistent with what they indicated (Satish & Mahesh, 2009) the extracts used in the treatment of kidney stones, which depend on the diuretic property, reduce Serum calcium concentration explained by ( Rasmussen *et al.*, 2014).

*Cichorium intybus* extract contains active substances that prevent calcium from precipitating in the form of crystals of calcium oxalate and calcium phosphate and contributes to excretion of the excess in the urine, thus reducing the concentration of Ca in the blood, As shown in Table (2).

**Table 2 :** Shows the concentration of Ca, Mg, and K elements for male rabbits, treated for 60days.

	Variables	Ca (mg/dl)	Mg (mg/dl)	K (mg/dl)
	Transactions			
1	Control	8.037+0.0737	1.6200+0.0954	5.300+0.223
2	Ethylene glycol	7.033+0.763	1.2200+0.1217	6.510+0.430
3	<i>Cichorium intybus</i> ex.	6.470+0.949	1.6033+0.0643	4.590+0.080
4	E.g+ ex. <i>Cichorium intybus</i>	6.640+0.200	1.3567+0.0808	5.207+0.109
	<b>L.S.D</b>	<b>0.5691</b>	<b>0.09886</b>	<b>0.2675</b>

•Values are expressed in mean  $\pm$  standard deviation.

•The number of rats (5) in each group.



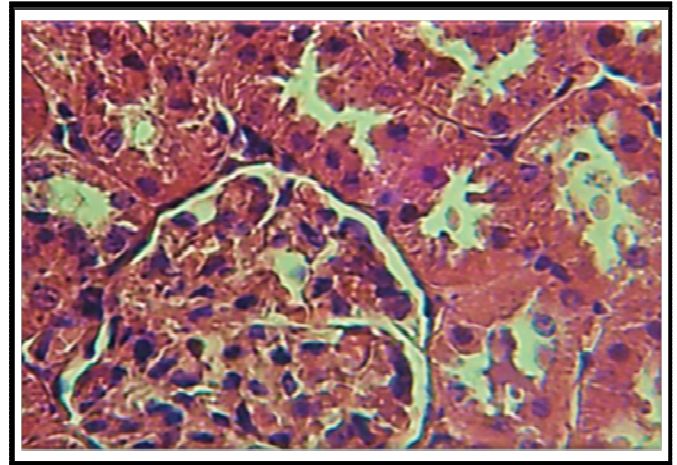
### Histological study

Figure (2) shows us the microscopic examination of the renal tissue in the rabbit treated with ethylene glycol showing the renal cyst and atrophy of the renal glomeruli, with necrosis of the epithelial cells lining the renal tubules, glomerular sclerosis S and infiltration of some inflammatory cells, as it shows the onset of calcification in the pelvic region, As the deposition of calcium salts appears, hemorrhage of some urinary tubules as a result of damage to blood vessels in some areas and infiltration of lymphocytes, and on the other hand, Calcium oxalate crystals that are present in the urinary tubules in a dense form are observed in most of the tissue sections prepared in the pictures.

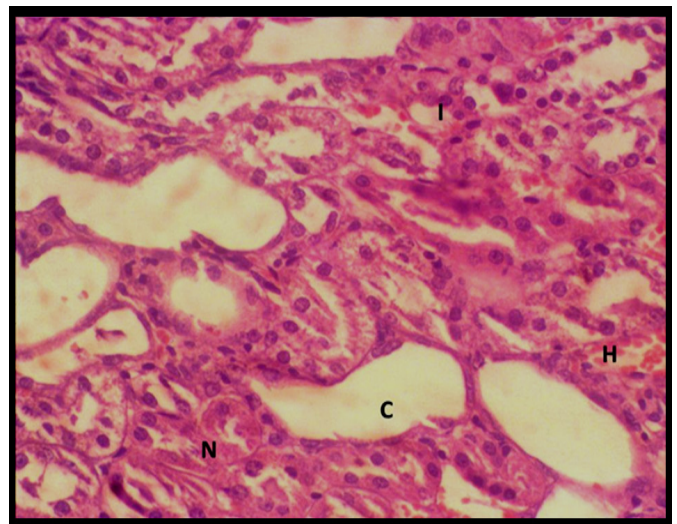
The results of our present study agree with what was reported by (Ibrahim, 2012), where it showed the formation of calcium oxalate crystals in rats treated with ethylene glycol at a concentration of (1%) for 30 days, These results are also consistent with what (Ravindra and others 2006) have indicated. Where it became clear that the stones resulting from the deposition of calcium oxalate crystals in the urinary tubules lead to the occurrence of necrosis and necrosis in the urinary tubules and causes the appearance of histopathological lesions, Ethylene glycol affects the kidneys through high levels of hyperoxaluria, which are one of the degradation products of E.g. , Where it binds with calcium to form calcium oxalate crystals that precipitate and form urinary stones, and E.g. leads to acute tubular necrosis of acute necrosis and necrosis of kidney tissues (Finkelstein & Temin, 2008).

Figure (3) shows the microscopic examination of the renal tissue in the rabbit treated with aqueous extract of the roots of the *Cichorium intybus* plant, the natural features of the renal tissue through the glomerulus and the glomerulus tubular The results of our current study are consistent with a number of studies that indicated the role of *Cichorium intybus* root extract in stone fragmentation (Malik *et al.*, 2017). they indicated (Hitova *et al.*, 2014) the active substances present in the roots of the plant have a diuretic property that helps in ridding the kidneys of the salts that have the ability to form stones The most important active substances in the dandelion plant have a curative property for many diseases, foremost among which is kidney disease (Mehrandish *et al.*, 2017). also they indicated (Tranchimand *et al.*,2010), the antioxidant present in the extract improves the state of kidney tissue and contributes to the removal of infections resulting from the formation of kidney stones and the associated bacterial infections. While figure (4) shows the microscopic examination of the prepared cross-sections of the kidneys of this group, a significant improvement in the shape of the glomerulus, as it was normal, and a significant improvement in the shape of the urinary tubules, as it was normal in most of the sections, and it was also observed that the calcium oxalate crystals completely disappeared in areas with a small number Including in other areas, This improvement indicates the significant therapeutic role of the *Cichorium intybus* root, Our current study agrees with a number of studies that have indicated the role of *Cichorium intybus* root extract in stone fragmentation, improvement of the histological status of the kidneys and its return to normal through the hydrologic property of the extract to get rid of crystals formed by ethylene glycol (Khodadadi *et al.*, 2016). Showed (Jasim, 2018) that rutin, glycosides is an antioxidant that improves the condition of kidney tissue and contributes

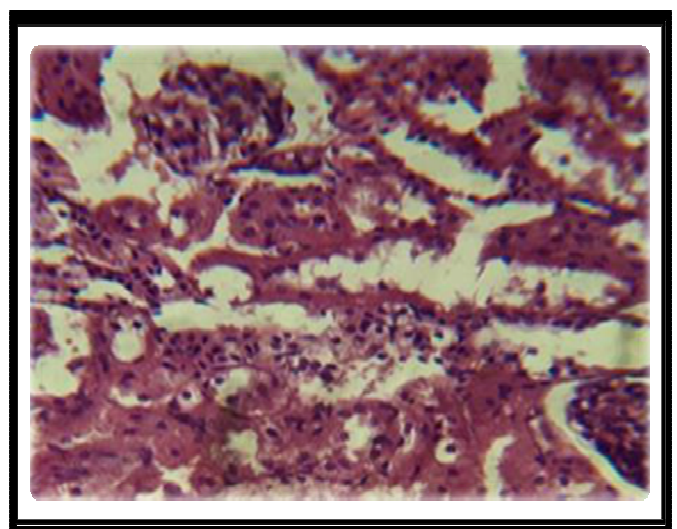
to removing infections resulting from kidney stones and the associated bacterial infections.



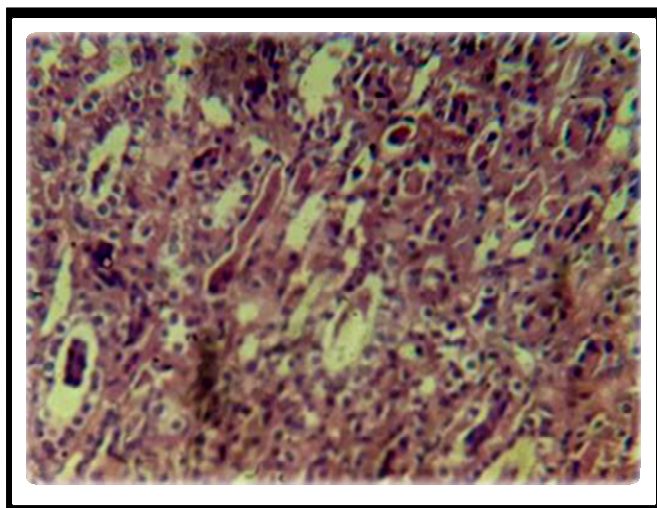
**Fig. 1 :** Cross-section of a rabbit kidney of a control group showing the normal shape of the glomerulus (G) and urinary tubules (CT) (H&E) 400X



**Fig. 2 :** Shows a histological section of a rabbit kidney showing polycystic renal cyst C with infiltration of inflammatory mononuclear cells I, necrosis of epithelial cells lining the renal tubules of N and hemorrhage H. (H&E) 400X



**Fig. 3 :** Shows a histological section of a rabbit kidney showing normal tissue parameters through glomeruli G and GT renal tubules( H&E) 400x



**Fig. 4 :** Shows a histological section of a rabbit kidney showing the deposition of acidic substances, which may be oxalate salts, CO, and regions devoid of deposits of A Dye (H&E) 400 X.

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