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Research Article

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Comparison of Histopathological Effects of Milk Thistle, and Chicory Plants and Vitamin E in all Rats Treated with Gentamicin Using Optical Microscopy Method

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ABSTRACT

Introduction: one of the reasons of acute renal failure is toxicity with aminoglycosides, particularly gentamicin. It seems that antioxidants such as vitamin E, chicory and Milk thistle can have protective function on reduction of nephrotoxicity of gentamicin. The protective effect of this extract with vitamin E on nephrotoxicity of gentamicin on male Wistar rats was studied individually and together with optical microscopy method.
Method: to perform this experience, 60 male Wistar rats were randomly divided into 6 groups of 10 each, including: the first group received 40mg/200gbw dose of gentamicin 0.5 cc and 80mg/kg dose of Milk thistle 0.25cc, the second group daily received 40mg/200gbw dose of gentamicin 0.5 cc and 80mg/kg dose of chicory 0.25cc, the tired group daily received 40mg/200gbw dose of gentamicin0.5 cc and 100Iu/200gbw dose of vitamin E 1cc, the fourth group daily and only received 40mg/200gbw dose of gentamicin 0.5 cc, the fifth group daily and simultaneously received gentamicin, Milk thistle, chicory plants and vitamin E and the sixth group daily received 1cc of distilled water.
Results: the obtained results from this research showed that chicory had higher protective effect compared to vitamin E and Milk thistle. Less renal damage was observed compared to treated group with Milk thistle. Renal tubular necrosis decreased significantly in the group which received Milk thistle, chicory and vitamin E as treatment.
So chicory, vitamin E and Milk thistle can prevent renal damage caused by gentamicin and simultaneous use of these extracts has better influence in reducing of gentamicin-induced nephrotoxicity.

Keywords: *Milk thistle, Chicory, Vitamin E, Nephrotoxicity, Gentamicin, Optical Microscopy*

INTRODUCTION

Antibiotics are a most common part of medicines that are prescribed by physicians and/or consumed by patient themselves [1]. These medicines had saved millions of lives and prevent multiple disabilities caused by infections. If these drugs had not been discovered, many cases of complications of infectious diseases such as deafness, blindness, paralysis of organs, and failure of heart valves, etc could be observed [2-4]. However, the presence of antibiotics has been rarely associated with complications and can also affect various body organs including blood, skin, eye, mouth, etc [5-9].

Aminoglycoside antibiotics, especially gentamicin are widely used to treat severe infections induced by gram-negative bacteria [10]. The most important side effects of this class of medicines are nephrotoxicity which are responsible for acute renal failure in a significant number of people taking this drug. Given that nephrotoxicity is one of the major side effects of gentamicin in a significant number of people, taking this medication is faced with limitations [10-12]. Actual mechanism of nephrotoxicity induced by gentamicin has not yet fully been identified [10-13]. Some researchers believe that increased production of reactive oxygen species (ROS) which can be seen in cells following the use of gentamicin, are effective in induction of toxic effects of this medicine on structure and function of kidneys [11-15]. It has been also indicated that ROS have been suggested in some of different pathological conditions [13].

Studies have shown that gentamicin passes through placenta [16-18]. Nephrotoxicity and autotoxicity of gentamicin were examined on embryos in two separate studies and although these two complications were not proved, but nephrotoxicity could not rule out [19-20]. Studies have shown that consumption of compounds with antioxidant activity decrease gentamicin-induced nephrotoxicity [13, 15, 21].

Chicory is an herbaceous plant that its average height is one meter; it has strong and relatively long roots [22-24]. The roots of this plant contain 8 percent of a polysaccharide called inulin. Bitter compounds such as lactucin, lactucopicrin, chicoric acid, flavonoids, glucoside, sugars and tannins are the other compounds of the root. The root of the plant also contains a lot of steaming aromatic compounds that “acetophenone” is the most prominent of them [22-24].

Some considered effects for chicory is as following, stomach tonic, diuretic, blood purifier, bile and fever scraper, appetizer, and treatment of gout and rheumatism, disposal of urinary sediment and elimination of anemia. The usage of chicory in the above cases is investigating and new and decisive cure for them is anticipated in not too distant future [22-24].

Vitamin E is a different form of tocopheryl that is found in herbal and animal resources. Plants are rich in vitamin E and contain a large amount of vitamin E, especially oilseed crops like wheat germ oil, safflower oil, soybean oil, etc. In general, animal sources are poor in vitamin E, however the amount of vitamin E in egg yolks, milk fat and liver is remarkable [25, 26].

Vitamin E performance as an antioxidant is as neutralization of free radicals and prevention from peroxidation of membrane lipids. All forms of vitamin E have antioxidant activity. Vitamin E is a peroxyl devastating substance and especially prevents from oxidation of unsaturated fatty acids which present in membrane phospholipids and plasma lipoproteins. Hydroxyl group in chromanol ring react with peroxyl radical and hydrogen that exists in this group is given to free radicals. As a result, free radicals obtain relatively stable status [27, 28].

Milk thistle is one of the known plants and it has high medicinal use from many years ago. Its therapeutic effects are attributed to its powerful antioxidant properties, regenerative and anti-inflammatory effects. Milk thistle is an herbal plant from starflower family and is a wild plant. Milk thistle is effective in treatment of various illnesses including hepatic diseases. Treatment sections of this plant are seed and leaves and its therapeutic substance is silymarin which is beneficial in reducing of blood cholesterol. The leaves of the plant contain bitter and nutrient materials and are consumed to treat loss of appetite and digestive disorder. Treatment of liver, gallbladder, and spleen disorders and treatment of colic due to gallstones and jaundice is some of applications of its grain [22-24]. The other elements of flavonoligand in silymarin are included: isosilybin, dihydrosilybin, silydianin, silychristin [22-24], which all of them have antioxidant properties. Milk thistle is a powerful antioxidant which protects body against cell damages caused by free radicals [22-24].

According to previous study, prescription of the extract of Milk thistle, despite plenty of medicinal properties, with dose of 140 mg and three times in a day not only is useful for treatment of diabetic nephropathy but also can worsen kidney function in terms of random excretion of albumin in urine. Milk thistle has been used for centuries to treat a variety of diseases. This plant is very effective in reducing the signs of aging and plays an important role in detoxification of body organs, particularly the liver [22-24]. Given to the above and nephrotoxicity effects of gentamicin, the aim of this study is comparison of histopathological effects of Milk thistle and chicory plants, and vitamin E in nephrotoxicity induced by prescription of gentamicin using optical microscopy method.

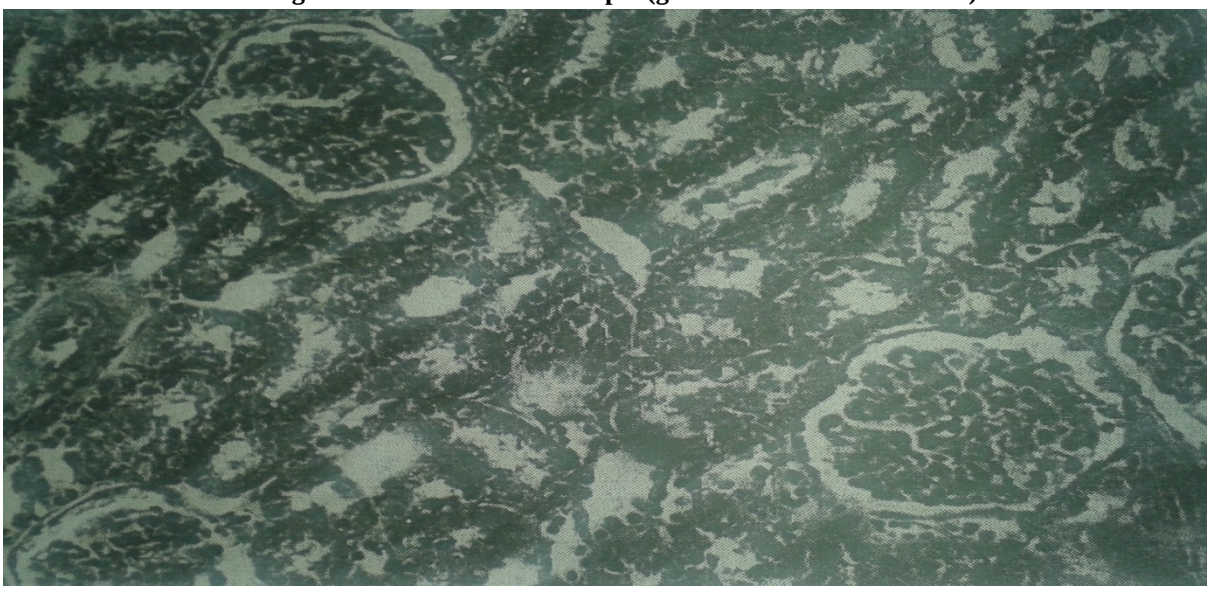
Method: to perform this research, a number of 60 Wistar rats with approximate weight of 200-250 g were prepared and after preparation of suitable location with appropriate temperature, the rats were kept in Animal House for 14 days. Necessary and sufficient food and water were prepared for the animals, during this period and temperature of the House was maintained at approximately 20-25 ˚C. The rats were divided into 6 groups of 10 each and after calculating of medicine dose and the required amount for injection of gentamicin, vitamin E, and the extract of Milk thistle and chicory, then at first:

Group (1) was simultaneously injected with gentamicin 0.5 cc with a dose of 40 mg /200 gBW and Milk thistle extract 0.25 cc that was received to approximate dose of 80mg/kg by distilled water.
Group (2) was daily injected with gentamicin 0.5 cc with a dose of 40 mg /200 gBW and chicory with approximate dose of 80 mg/kg.
Group (3) was daily injected with gentamicin 0.5 cc with a dose of 40 mg /200 gBW and vitamin E 1 cc with approximate dose of 100 Iu/200gBW.
Group (4) was daily and lonely injected with gentamicin 0.5 cc with a dose of 40 mg /200 gBW.
Group (5) was daily injected with gentamicin, vitamin E, and the extracts of Milk thistle and chicory.
Group (6) (standard group) was daily injected with distilled water 1 cc.
Vitamin E and gentamicin were injected intramuscularly (IM) and both Milk thistle and chicory extracts and distilled water were injected intraperitoneally (IP) during 2 weeks (14 days), continuously and at specified times.
After 14 days, 3 rats were randomly selected from each group and after collection of urine they were anesthetized and blood were taken from their hearts. Then, their kidneys were removed from body and renal capsules were removed, too. Then the kidneys were transversally and longitudinally cut. Some samples with size of 0.5 mm×0.5 mm and by thickness of 0.5 mm were prepared from border area between renal cortex and medulla as the samples contain both areas. The prepared samples were placed in containers with 10% of formalin solution. The samples were then removed from the solution and placed in formalin 2%. The samples were then sent to laboratory for preparation of tissue sections and staining for optical microscopy.

RESULTS

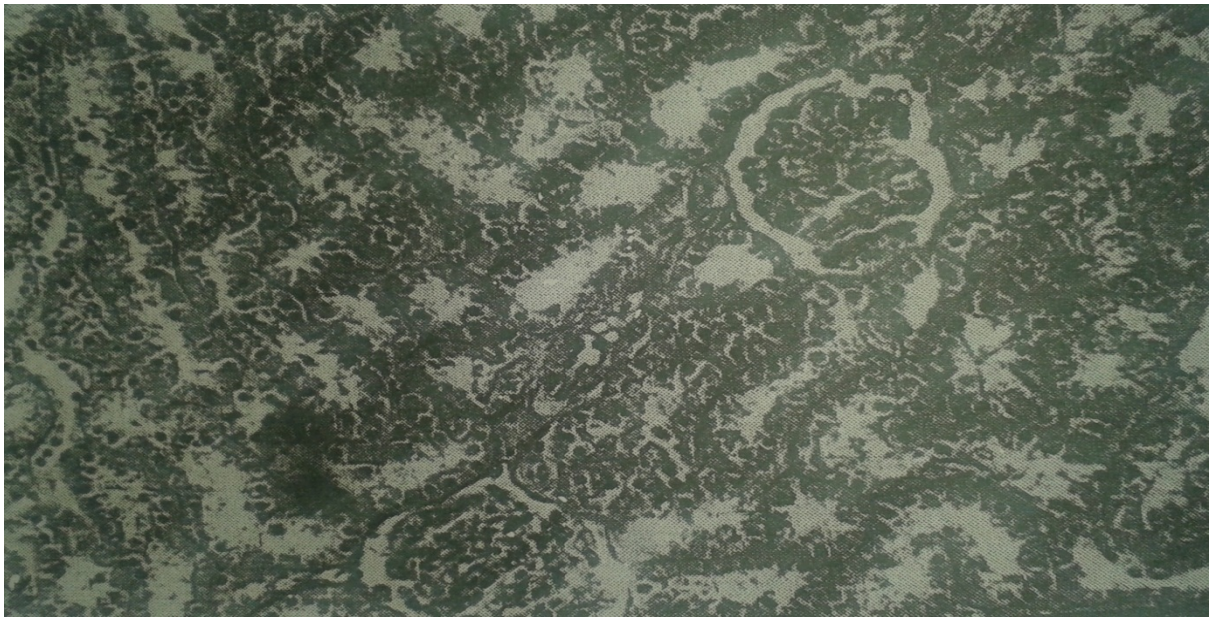
Group 1 (gentamicin and Milk thistle)
Two slides of 3 prepared slides in each specimen of optical microscopy showed 5 to 10 cells with coagulation necrosis in urinary tube wall. Ten to twenty cells with coagulation necrosis were observed in its slide.

Figure 1: renal tissue in Group 1 (gentamicin and Milk thistle)



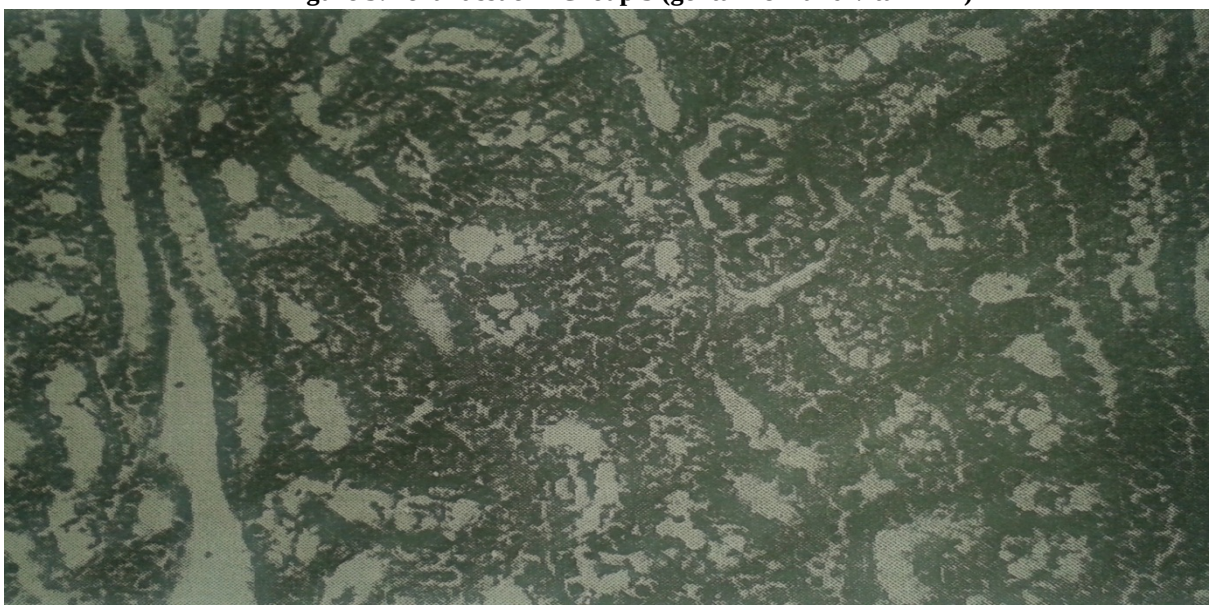
Group 2 (gentamicin and chicory)
Two slides of 3 prepared slides in each specimen of optical microscopy showed average 20 to 30 cells with coagulation necrosis in urinary tube wall. Ten to twenty cells with coagulation necrosis were observed in one slide.

Figure 2: renal tissue in Group 2 (gentamicin and chicory)



Group 3 (gentamicin and vitamin E)
Two slides of 3 prepared slides in each specimen of optical microscopy showed average 10 to 20 cells with coagulation necrosis and one slide showed 20 to 30 cells with coagulation necrosis.

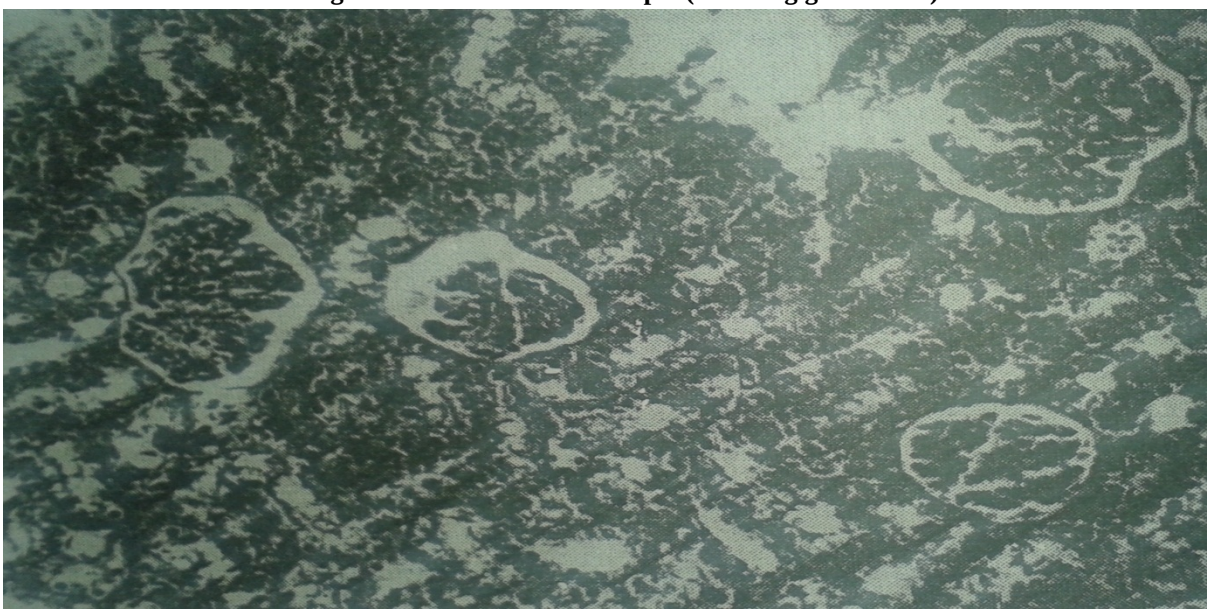
Figure 3: renal tissue in Group 3 (gentamicin and vitamin E)



Group 3 (gentamicin)

Two slides of 3 prepared slides in each specimen of optical microscopy showed more than 30 cells with coagulation necrosis and one slide showed 20 to 30 cells with coagulation necrosis.

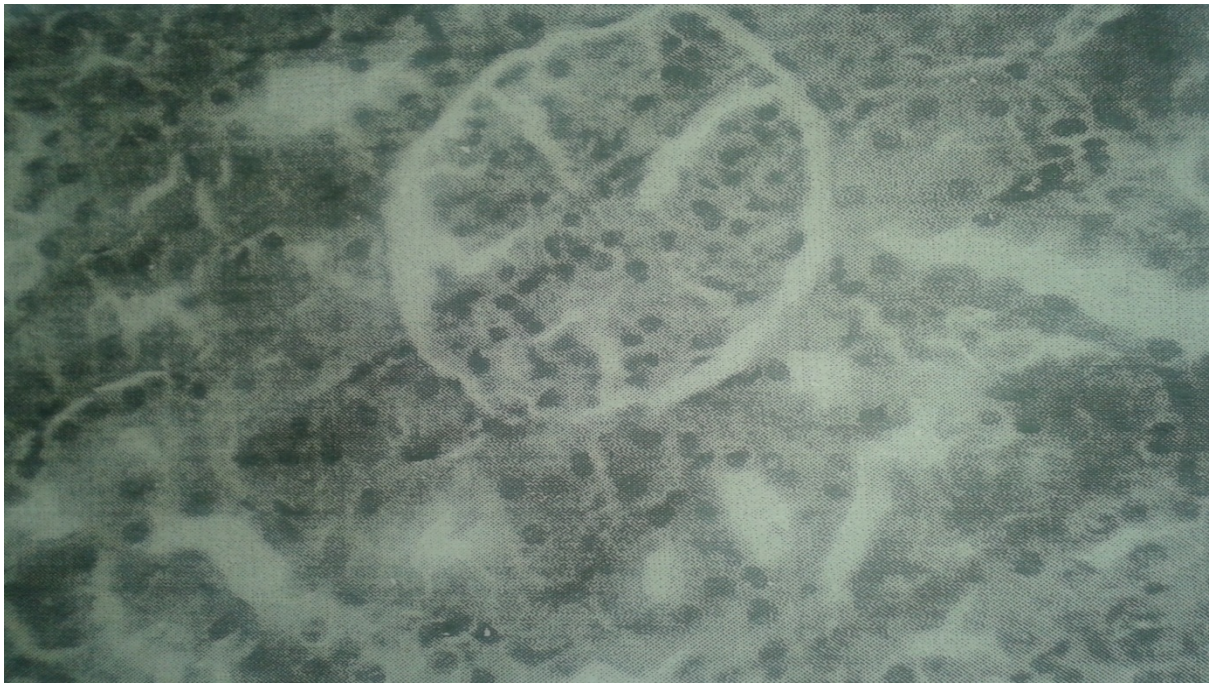
Figure 4: renal tissue in Group 4 (receiving gentamicin)



Group 5 (gentamicin, Milk thistle, chicory plants and vitamin E)

One slide of 3 prepared slides showed 5 to 10 cells with coagulation necrosis and two slides showed average 0 to 20 cells with coagulation necrosis in each specimen.

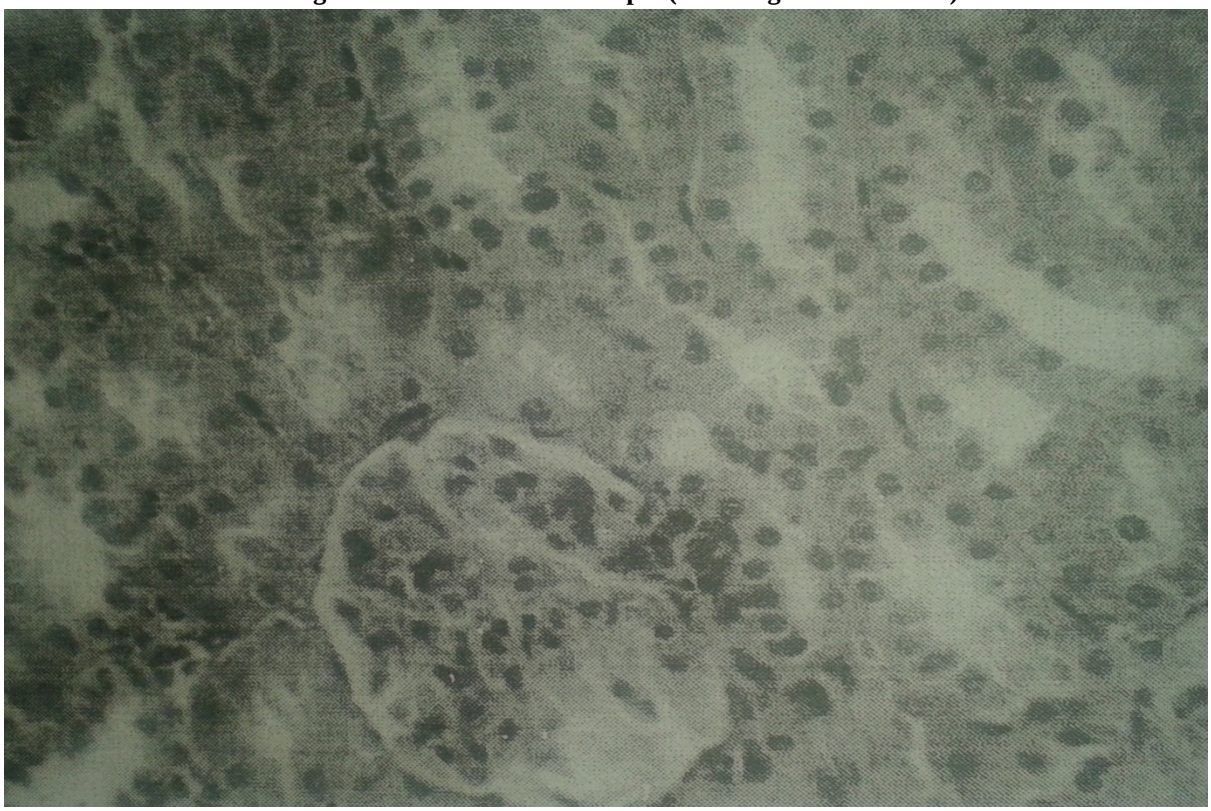
Figure 5: renal tissue in Group 5 (receiving gentamicin, Milk thistle, chicory and vitamin E)



Group 6 (receiving distilled water)

Two slides of 3 prepared slides showed average 5 to 10 cells with coagulation necrosis and one slide did not show any necrosis.

Figure 6: renal tissue in Group 6 (receiving distilled water)



Kidney is position of elimination and maintenance of active metabolites and most of metabolisms are excreted through kidneys [29]. Since kidney disease is one of major problem in today's society, so careful attention to its structure and function, can plays an important role in health of individuals [30]. Gentamicin taking is limited due to side effects that are produced by it. Nephrotoxicity and autotoxicity are the most side effects of aminoglycoside antibiotics and even kidney failure is observed in cases of prolonged consumption [28, 31, 32].

In addition these aminoglycosides including gentamicin due to their oxidative reactions and damage to renal tubule cells can cause renal toxicity. Due to remarkable approach of antioxidant drugs to suppress these reactions, it seems that a lot of these damages can be prevented.

Gentamicin attaches to anionic phospholipids of tubular cell membranes and enters to them through pinocytosis, so that an electrostatic reaction creates between this antibiotic and membrane phospholipids and then enters to cell. After entering to cell, these antibiotics accumulate in lysosomes and inhibit phospholipase A and C and sphingolipase enzymes. Phospholipids accumulate in cells by blocking of these enzymes and form myeloid bodies. The most important of these phospholipids are phosphatidylserine, phosphatidylcholine, phosphatidylinositol [28, 31, 32].

On the other hand following the enzymatic changes and/or perhaps due to the effects of these antibiotics inhabitation of sodium potassium adenosine triphosphatase (Na^+/K^+-ATPase) pump occurs. With disorder in activity of this pump and because of its key task, disorder occurs in electrolyte and hemodynamic balance. And cause sodium retention and reduction of potassium and other ions within these cells. In addition, permeability of inner mitochondrial membrane is confused by reducing the activity of sodium-potassium adenosine triphosphatase (Na^+/K^+-ATPase) pump. And then a disruption causes in cellular oxidation reaction. Membrane is responsible for exit and arrival of materials into cells and serves as the most important factor in the stability of cells. So, disorder in membrane causes disorder in intracellular reactions and cell death occurs followed by this process.

In addition, gentamicin reduces alkaline phosphatase, conversion and disulfide proteins and catalase in renal cortical tissue. This seems to be due to intervention of gentamicin in carboxylation reaction in epithelial cells of renal tubules. Lysosomal and brush border enzymes are released followed by damage to proximal convoluted tubules and these enzymes are increased in urine. Glomerular filtration reduces due to damages to the tubules (tubules obstruction by necrotic cells). Much research has been performed to reduce renal damages caused by gentamicin.

Milk thistle and chicory plants and vitamin E were used in this study to reduce these effects. It was found that chicory had more protective effect compared to Milk thistle and vitamin E, and less renal damage was observed in treated

group with chicory compared with treated group with Milk thistle. Renal tubular necrosis had been significantly reduced in the treated group with Milk thistle, chicory and vitamin E.

Vitamin E is a natural antioxidant and it has been proved to have protective role against nephrotoxicity of gentamicin. Also it has been shown in the past that vitamin E fights against reducing enzymatic activity of manganese superoxide dismutase and glutathione peroxidase and catalase caused by gentamicin in renal tissue [31].

The effects of vitamin E and Se (Selenium) were investigated in another study and it was observed that gentamicin has been the first nephrotoxic medicine during 12 years and plasma membranes of renal tubules are destroyed sooner than affected by lysosomal membrane. Acid phosphatase, alkaline phosphatase and muramidase enzymes are released from renal specific cells that damaged and gentamicin helps to destruction of membrane surface in kidney. It has been shown that selenium prevents from a number of degenerative changes in plasma membrane and the other cellular membrane and vitamin E plays role as a factor in biological membranes [33].

It was observed in the past that selenium has lower ability and vitamin E has more ability to create hepatic lithrosomes against damage. And also it can protect kidney and liver membranes against damage [34].

Since gentamicin accumulates in lysosomes may cause phospholipidosis and accumulation of phospholipids may be affected by a number of oxidation processes of lipid membranes that lead to membrane peroxidation. Lipids' peroxidation produces free radicals, which are highly toxic for interior parts of cells. Vitamin E with its antioxidant activity can prevent from formation of peroxides and/or may damage free radicals, if prescribed before or simultaneous with gentamicin.

Milk thistle plant is a powerful antioxidant that protects body against damaged cells which produce by free radicals. This plant is used for treatment of numerous diseases due to its prominent antioxidant activity. Milk thistle plant increases protein biosynthesis and cellular repair through DNA synthesis. The major usage of this plant is in liver toxicity. Silymarin, which its extract is silybin, is a component of flavonoids. 80% of flavonoids have free protection property and can protect cells from damages caused by free radicals. It seems that the protective effect of this plant in kidney is due to neutralization of free radicals caused by lipid peroxidation [22-24].

Chicory leaves have a lot of vitamin C which has powerful antioxidant effect. Vitamin C is essential for formation and maintenance of intracellular matrix and collagen within body. Also, vitamin C as a co-factor involves in most of body reactions and affect on materials and enzymes that are involved in repairing. So it is possible that vitamin C with mentioned roles in repair can reduce kidney damages caused by gentamicin [22-24].

Role of vitamin C in reducing of gentamicin-induced nephrotoxicity has been proved in the past and it was said that by increase of this vitamin its protective effect appears more, so that a higher dose of 150mg/kg of vitamin C with gentamicin 80mg/kg decreased renal tubular necrosis [22-24].

CONCLUSION

The results of the present study showed that the simultaneous use of chicory extract, Milk thistle and vitamin E due to their antioxidant properties had better effects in improving renal tissues in rats treated with gentamicin. So the simultaneous use of chicory extract, Milk thistle and vitamin E is recommended.

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