The effectiveness of Aqueous Extract of Grape Seeds *Vitis vinifera* as an antibiotic for some microorganisms and its Protective Role Histology for Liver, Kidney in Mice

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Abstract

1- Among the common disease treatments and long-term use of plant extract in the treatment of bacterial infections, This research used black grape seed extract to observe its effect on some Gram negative and positive bacteria like *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Proteus mirabillis* obtained from patients with urinary tract infection Using four specific extract concentrations (100 mm / L, 75 mm / L, 50 mm / L, 25 mm / L) to test the effect of black grape plant extract. The results showed that *E. coli* was the most resistant bacterial species to plant extract and so on with other gram negative bacterial species. In the minimum inhibition concentration test for the plant extract, showed that the highest concentration 100 mm / L had the good inhibitory activity for *Staph. aureus* and less than other strains specially for *Kleb. pneumoniae* and *Pro. mirabillis* while the 25mm / L concentration had the lowest acitivity against all.

2- This study used 10-day oral methionine and 30-day grape extract treatment for 16 white mice to study the histopathological effects in the liver, kidney and heart tissues, And the results showed a very good organ tissue improvement for all treated groups compared to the control group.

Keywords *: bacteria, extract grape seeds aqueous, histopathological effects .*

Introduction

Researchers have turned to plant extracts as natural products to tract bacterial infection and tissues damage because plants are rich with vital compounds such as volatiles oils, Tannins, Alkaloids, Phenols, Glycosides and Saponins [(1)].

Urinary tract infection is known as the presence of pathogenic microorganisms within the vessels of the urinary system so that they appear in the urine , *Escherichia coli* is responsible for causing about 80-90% of infections, and so on for all types of Enterobacteriaceae family, especially urethritis and bladder in Women and children, as for the positive group of gram stain, *Staphylococcus aureus* is the most common type of this type of infection [(2),(3),(4)].

Grape Seed Extract is one of the richest sources of powerful and beneficial component called flavonoids, the most important of which is Proanthocyanidin, which is 50 times stronger than the effect of vitamin such as C and E participate in protecting the body from the effect of oxidization by free radicals [(5),] and these Antimicrobial phytochemicals such as flavonoids have another functions such as Anti-inflammatory, anti-allergenic, anti-viral and anti-cancer action [(6),(7)]. Also the Phenol which found in bear berry with an antiseptic urinary effect [(8)], Saponins also have anti-inflammatory, anti-microbial, anti-protozoan and immunostimulative properties [(9)].

As well as its effectiveness against bacteria, viruses and inflammatory diseases through the inhibition of peroxide formed by phagocytic cells [(10)].

For the above reasons, this research has came to study the effect of aqueous extract of grape seed on some bacterial species isolated from urinary tract infections and to study its effect of treatment for methionine treated tissues.

Materials and methods

Bacteria diagnosis: Samples were obtained from Samarra General Hospital's urinary tract infections,

We have been identified through the routine procedures listed in Wise, A. (2017) [11].

Four bacterial species were obtained: *Staph. aureus*, *E. coli*, *Kleb. pneumoniae* and *Pro. mirabillis*, which was predominant on most specimens of urinary tract infection and was subsequently taken as a test bacterial species for subsequent testing, And all its characteristics that were diagnosed as a result were identical to those mentioned in above reference [11].

Sterilization of the extract and preparation of fears:

Prepare a storage solution by taking 1gm of dry plant extract powder and dissolved in 10 ml of sterile distilled water. The concentration of the storage solution is 100 mg / ml. 0.11mm filter papers were used to separate the large pieces of the extracted particles and the extract was sterilized by Millipore 0.22 mm which prevent the passage of contaminant bacteria through it to obtain a sterile storage solution of 100 mg / ml and use it as a source for preparation of fears [12].

Preparation of concentrations of grape seed extract:

The four concentrations (25,50,75,100 mm/L) have been prepared through addition amount of storage solution to equal volume of distilled water as following equation:

Volume of storage solution/volume of distilled water.

Assessment of antimicrobial potential:

Neomycin (10 Mg) has been used in all media plates as a normal control for plant extracts. according to Bridson (2006) [13], standard antibiotics and plant extracts were used in the "cylinder-plate" process to carry out the antibiotic sensitivity test. The test was performed by individually injecting extract solutions or standard antimicrobials into the media cylinders and measuring the inhibition zones by the zone reader system after incubation.

Test for histopathological effects

Methionine have a molecular weight of 472.09 and was dissolved in the solution to prepare a natural solution and was orally administered for a period of seven days at a concentration of 0.5 mg / kg twice a day [14].

Preparation of the water extract of grape seeds

The water extract of grape seeds was prepared by method mentioned by Bayadar. N.G.*et al.,* [15] by crushing a quantity of clean seeds using the Ultra-Tubax blender (Germany), Then, 50 g of dry powder was weighed and placed in a 1000 ml glass container Add the distilled water and complete the volume to 1 liter then add 3 ml of absolute ethyl alcohol to prevent fungal growth .

The samples were left for half an hour in the horizontal vibrator and at medium speed. The samples were stabilized for an hour, then filtered by three layers of gauze to separate the large plankton and then filtered to 3,000 centrifuges / min for 15 minutes to separate the small plankton. Of the extract at 40 ° C using rotary evaporator and put in incubator at 37° C and then save the extract in a sealed bottle at a temperature of 8° C in wet conditions for use in the study .

Figure 1 Black grape seeds

Laboratory Animals group : In this study, 16 mice from Swiss white mice were used, ranging from 25 to 28 g. Obtained from Pharmacology Department / General Pharmaceutical Company for General Industries. The animals were in good health and were placed in laboratory plastic cages dedicated to the breeding of mice, and sprinkled with sawdust, with cages cleaned and sterilized twice a week. Group design : The animals were randomly divided into four groups in plastic cages. Each cage contained four mice, and treated as follows:

1- Control group: consists of 4 mice treated with normal saline solution, 0.1 mg/ kg

2- Group I: The First Group , Was composed of four mice treated orally with methionine 0.5 mg / kg for 10 days.

3- Group II: The Second Group , Was composed of four mice treated orally with aqueous extract of grape

seeds 10 % mg / kg for 30 days.

4- Group III: The Third Group , Was composed of four mice treated orally with aqueous extract of grape seeds 30 % mg / kg for 30 days, as mentioned by AL.Jeboory *et al*., [16].

Preparation of tissue sections After dissecting the animals and placing organs in the Formalin 10% solution, the samples to be studied were converted to ethyl alcohol at 70% concentration. The following steps were taken as mentioned by Khaleel .Zinah I. *et al.,* [17].

Result and Discussion

Assessment of antimicrobial potential:

Grape seed extract showed highest inhibitory activity on *Staph. aureus*, But to a lesser extent *Kleb. pneumoniae* and *Pro. mirabillis* bacteria which were 6mm at 100mm / L, 5mm at 75mm / L and 4mm at 50mm / L, But in general, the four concentrations of the extract had significant effect compared to the antibiotic Neomycin with gram positive group, whose average inhibition diameter for the four types of bacteria ranged between 10-20mm, These results were in agreement with what was stated by Jayaprakasha and. Sakariah [18], They indicated that grape seeds water extract have a greater effect on gram positive bacteria than negative, whereas 25mm / L concentration had the lowest inhibitory activity, which was 3mm for *Staph. aureus*, *Pro. mirabillis*, while it showed no effect on *Kleb. pneumoniae* and *E.coli*,

Figure 2: the effect of extraction on *Pro. mirabillis*

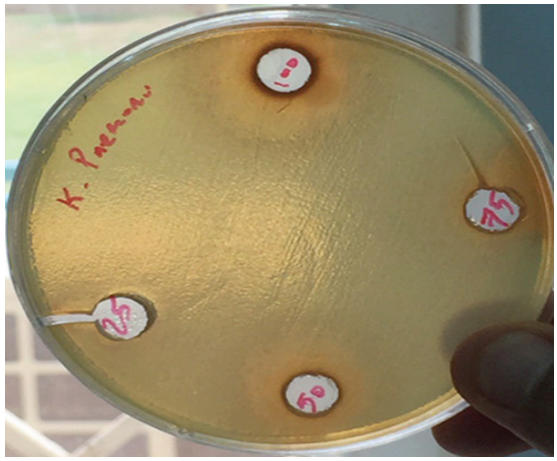

Figure 3: the effect of extraction on *Kleb. pneumoniae*

As for *E. coli* bacteria were resistant to all extract concentration, only the concentration of 100 mm /L caused inhibition of a diameter of 1 mm as shown in table 1.

Table 1 the inhibitory activity of four concentrations of black grape seed extract

Isolates	Plant extract concentrations				
	100mm/L	75mm/L	50mm/L	25mm/L	Neomycin
E. coli	1 mm	-	-	-	20
Staph. auerus	11 mm	7 mm	6 mm	3 mm	10
Kleb. pneumoniae	6 mm	5 mm	4 mm	-	15
Proteus mirobillis	6 mm	5 mm	4 mm	3 mm	15

The result that showed with table 1 referred that *E. coli* was the most resistant bacterium used in the study .This shows that it has developed its ability to resist antibiotics in contrast to the other species belong to both gram positive and negative, these results agree with Zdenka Cvetni and Sanda Vladimir [19], Whom made it clear the grape seed extract less affect on Enterobacteriaceae familly like Salmonella enteritidis, although Gram positive bacteria, the extract showed the efficacy of its orientation not similar to that of the Gram negative bacteria and this result is not necessarily consistent with the result of the study done by Fleming [20], In his study, *Staph. aureus* showed that the bacteria were more strongly affected than the negative bacteria of Gram.

histopathological effects

Liver

Histological sections of the first group of mice treated with methionine for 10 days showed tissue changes such as necrosis, degeneration, congesting, hemolysis, sinusoidal enlargement, and hepatocyte swelling. With Infiltration Lymphocyte lymphocytes and hemorrhagic hemorrhage, as well as pyknic nuclei .

The histological examination of the liver section in the animals treated with the grape extract for the second group for 30 days showed hemorrhage and lymphocytic infiltration with intracellular necrosis of the central vein (desquamation). A few cases of Karyolitic were also found with some nuclei that suffered small size and Pyknic Nuclei intensified and also found improvements in liver cells in terms of return to normal form compared to the group of methionine. While the results of the second and third group of the same period showed a good improvement of the hepatic cells and regularity of the natural form as it was approached the normal form, although there are some cases of hemorrhage bleeding and lymphocyte Infiltration lymphocyte. and the third group was better.

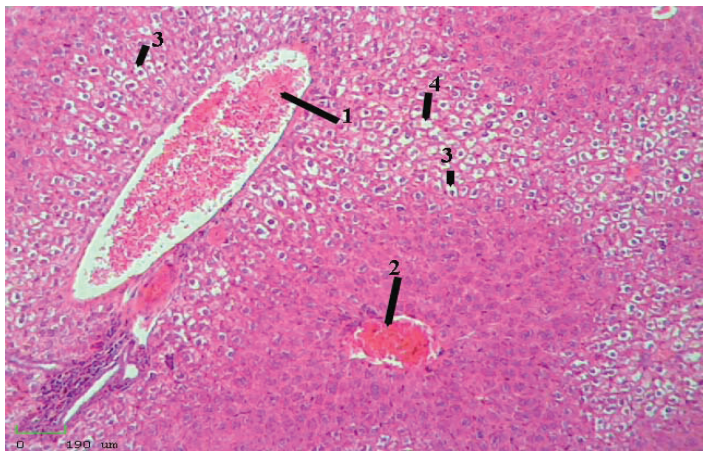

Figure 4: liver tissue , methionine , showing congestion in the tissue , H&E, 400X .

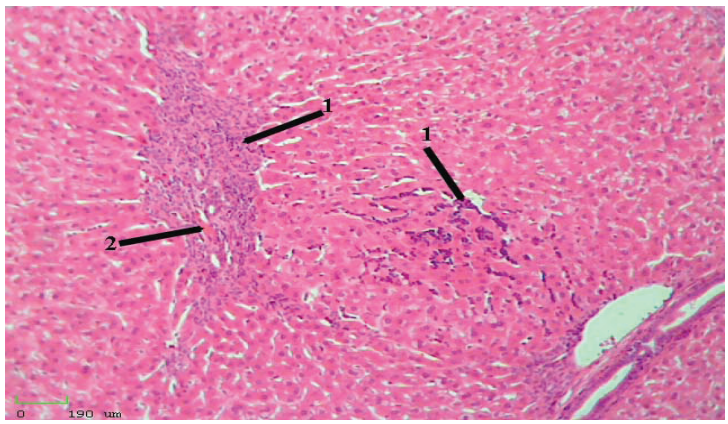

Figure 5: heart tissue , grape extract, group II , (1) lymphocytic infiltration (2)congestion, (3) vacuolated ,H&E, 400X

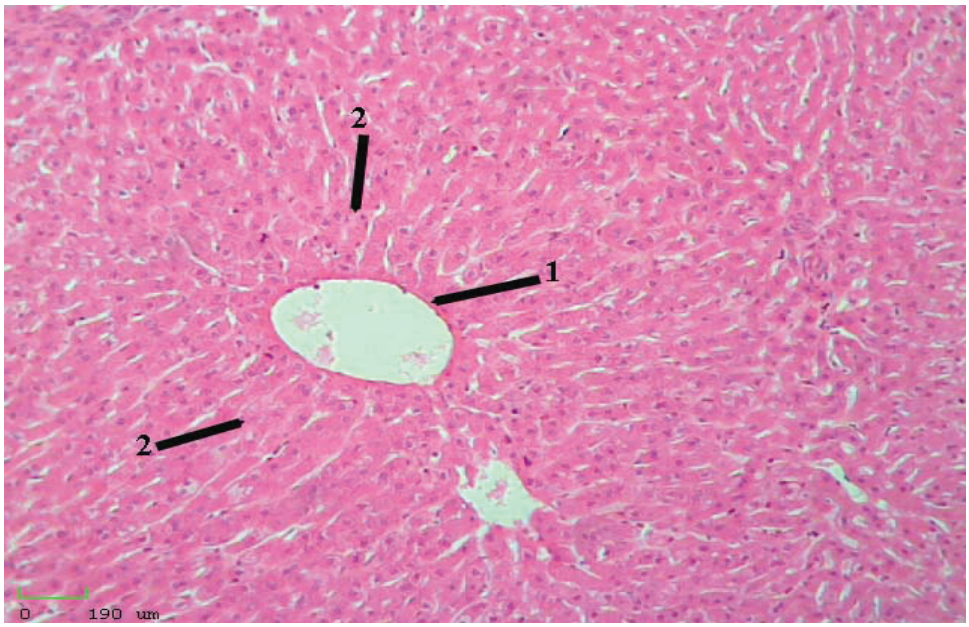

Figure 6: liver tissue, grape extract, group III, showing (1) Central vein (2) karyolysis, H&E, 400 X .

The liver tissue showed a significant effect of methionine at the concentration level, with bleeding, degeneration, lymphocytic infiltration, and endogenous degeneration and decomposition, These results were consistent with the findings of Al-Saidya. [21] ; Al-Shammry. and Al-Okaily [22]. Hepatotoxic substances are hydrogen peroxide and methionine. The toxicity of substances lies in their ability to form free radicals such as O-2 and OH. These roots are characterized by their ability to interact with lipid and proteins involved in the synthesis of membranes Kink and inside cellular and Intracellular membrane and thus discourage many vital special events such as the transmission of membrane necessary for the sustainability of cell materials also interfere with the action of enzymes and thus inhibit cellular respiration which leads to a reduction of cell events [23].

The interaction of free radicals with lipids results in lipid peroxides, which stimulate a series of membrane interactions with a decrease in mitochondrial membrane viability and destruction of the particle membranes of the Lysosomes and thus the cell's arrival in the necrosis [24].

This study also showed the reform in the liver tissue of the methionine-affected cells and the return of these changes to the natural state using the water extract of the grape plant was due to the action of the extracts in inhibiting the action of methionine by possessing many activities, especially anti-oxidation and then speeding up the repair process And stimulate the tissue cells to secrete chemical attractants to attract inflammatory cells, which was clearly visible to the region for the purpose of feeding the damaged tissue, as well as stimulate the fabric to divide to compensate the affected by methionine [21].

kidneys

The examination of tissue microscopiclly showed that a section of the cells lining the urinary bulb is similar to normal, but in some places there is swelling in the proximal epithelial cells of proximal and distal proxies, as well as swelling of the nuclei There is general necrosis and vacuolated necrosis with Degeneration within a section of the glomeruli within its constituent cells with dense and small nuclei observed on their way to death .

Some cases of hemorrhage, lymphocytes and lymphocytes were observed, as well as minor bleeding within the glomerulus, lymphocytic infiltration into the glomerulus, and cirrhosis in the proximal and proximal tubules.

Methionine causes a significant increase in serum creatinine, urea nitrogen in the blood and uric acid concentration in the blood, and high blood urea nitrogen is a marker of kidney disorders [25].

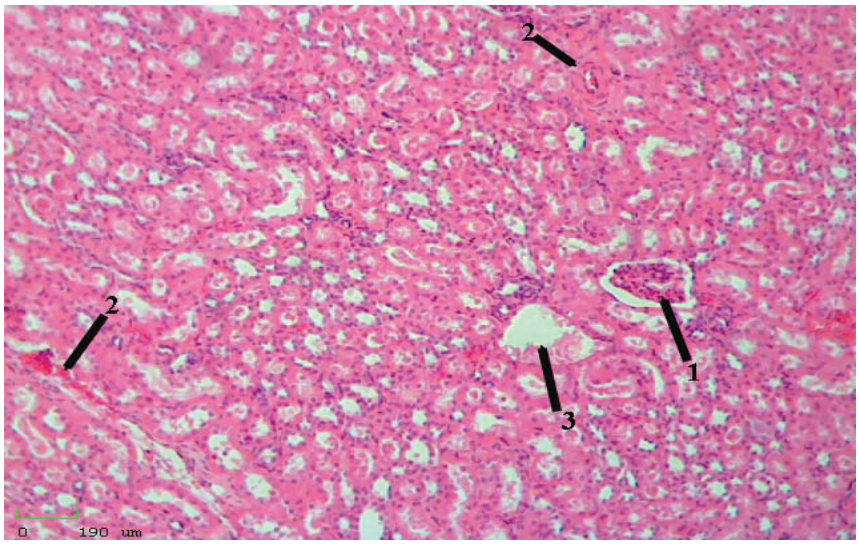

Figure 8: section of the tissue of the kidney with a dose of methionine, showing (1) congestion (2) Vacuolated, 400X H&E .

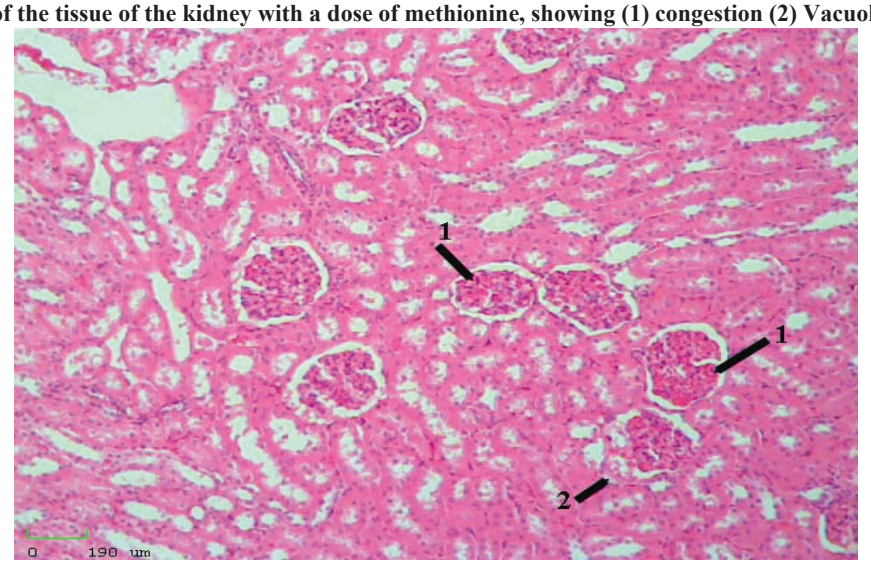

Figure 9: kidney tissue , grape extract, group I, showing (1) glomerulitis (2) lymphocytic infiltration, H&E, 400 X .

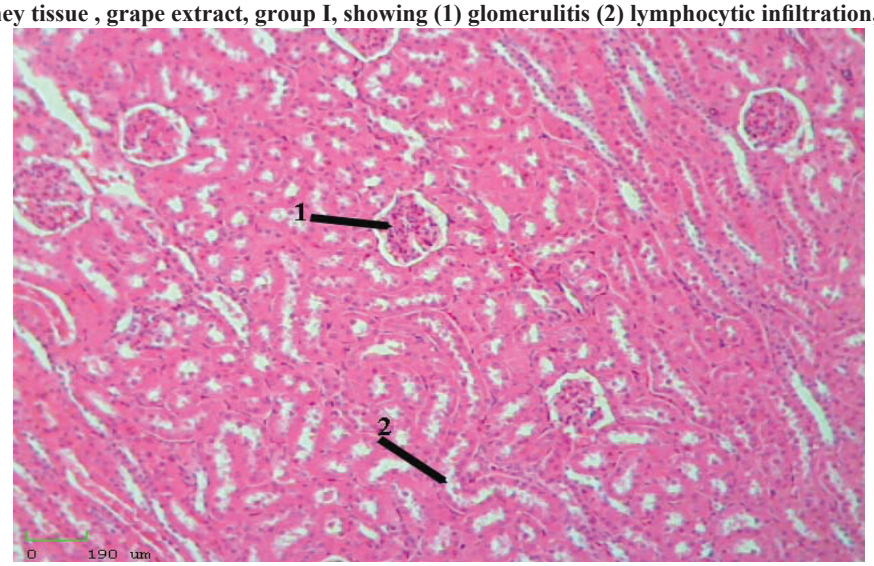

Figure 10: kidney tissue , grape extract, group III, showing (1) glomerular (2) lymphocytic infiltration, H&E, 400 X .

The effect of methionine on kidneys is consistent with Dever and Elfarra [26], they pointed out that high levels of uric acid have been associated with gout, high blood pressure, kidney damage, hyper homocysteine in the blood, Blood to overproduction and release of oxygen species (ROS) from the glomerulus, and then kidney damage, double glomerular filtration rate,

The excess adenosine will interact with methionine which forms S-adenocele methionine and then degrade to form uric acid as a final product leading to hyperuricemia [27].

The results of this study revealed that excess methionine caused renal injury manifested in a significant deterioration in epithelial lining cells of renal tubules. These results are consistent with Rowan [28]. Where glomerular lesion was observed and the progression of glomerular sclerosis was increased after methionine dosage.

As shown in this study, a severe renal dysfunction and this renal impairment may be due to homocysteine associated with increased adenosine level associated with increased level of sclerotic changes in glomeruli [28]

Ethical Clearance: It was obtained from the Scientific Research Committee at College of Dentistry/ University of Babylon, Iraq.

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Conflict of Interest: None to declare.

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