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The Effect of Different Dose of Rifampicin on Induction Apoptosis in Renal Cells of Male White Rats

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Abstract--- *The present study was aimed to detect the effect of rifampicin on the apoptotic pathway in the rat renal cells, the experimental animals divided randomly into four groups, each group consist of 10 rats, the group 1 consider as control group and administrated with distilled water for period 28 days while the rest groups administrated with gastric dose (50,100,150 mg/kg) respectively for period 28 days. the rifampicin started promote apoptosis in renal cells was evaluated histologically by aiding scanning electron microscope (S.E.M) techniques tissue of animals treated with the low dose of rifampicin , there are small blebs on the surface of the outer cell with few pores in it and the surface in general appears smooth with the presence of red blood cells indicating bleeding, while the animals treated with the moderate dose shows the presence of more numerous blebs than the low group, and the outer surface appears rough and perforated, while for the group of animals treated with high dose, it showed the presence of many blebs with a clear perforation of the surface.*

Keywords--- *Apoptosis, Rifampicin, S.E.M,*

I. INTRODUCTION

Rifampicin effective antibiotics is often used for cure many disease and infections, is bacterial antibiotics drug used to treatment bacterial infections, at the beginning was used to cure tuberculosis (Eminzade et.al,2008). Rifampicin induce many morphological and metabolic deflections in hepatic cells, because of the ability of liver to detoxify the harm effect of these compounds (Santhosh et.al, 2007) .

There was important effect of rifampicin on lipid peroxidation when it's administrated to mice after one week of treatment (Upadhyay et.al,2007),other studies find out when the rats receiving (250 mg/kg/day) for period one month there was increasing in the level of triglycerides ,cholesterol and free fatty acids, while there was significant influence of rifampicin on the level of liver enzyme AST, ALP ,also when these antibiotic administrated to the rats would be elevate the level of liver enzymes (Rana et.al,2010).in the same direction ,other findings reported that ,the rats treatment with rifampicin (250 mg/kg) for the same period , there was noticeable increasing in the level of serum bilirubin and no changes in the level of creatinine and urea(Tasduq et al., 2007). Antibiotics such as gentamycin and amoxcilline excreted by the kidney, but small amount thought that toxic and harmful effect ,would be reabsorbed and accumulated in the proximal renal cells(conde et .al,2006). Apoptosis programmed cell death and consider as active form of cell death and have morphological characteristics include both of cytoplasmic and nucleic shrinking ,both of them ,apoptotic cells and apoptotic bodies engulfed by neighboring cells , so the apoptosis unclear in histological sections even if have important influence on the cell lysis(Saad et.al,2009)

Apoptosis has been confirmed to happen in the renal cells, thus the significant induced by apoptosis in renal

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cytotoxicity has become serious, we designed this study to identify the mechanism of renal cells death promoted by rifampicin with different level dose (50,100,150 mg/kg/day) respectively.

II. MATERIAL AND METHODS

The current study was performed on white male albino rats having body weight ranged from 200 to 250 g This study was conducted in the animal house of the College of Science / University of Qadisiyah, where in this study 40 adult male rats were used ina this study, randomly divided into four equal groups for the drug, control group and three groups treated with gastric dosage Low, moderate and high (50, 100, 150 mg / kg) respectively for a period of 28 days, the control group was administrated with 0.9% NaCl (sterile normal saline) at the dose 2ml/kg for 28 days while the second group was administrated with rifampicin at dose(50mg/kg/day) for 28 days, the third group was administrated with rifampicin at dose (100mg/kg/day) for 28 days, while the fourth group was administrated at dose (150mg/kg/day) for 28 days. Then kidney tissues collected from all animals were immediately fixed by immersion in 2.5% glutaraldehyde in 1% M phosphate buffer pH 4.7 for 24 hours at 4 C, tissue specimens were post fixed were post fixed in1% osmuim tetraoxide (OsO4) and then dehydrate in ascending grade of ethanol then embedded in Epon resin mixture, after ultrathin sections were preparing, these ultrathin sections were examined and photographed under a scanning electron microscope (S.E.M) (Saad et.al, 2009).

III. RESULTS AND DISCUSSION

Rifampicin induced apoptosis in rat renal cells was determined by using scanning electron microscope (S.E.M) .The first group that was consider as control group was administrated with 0.9% NaCl (sterile normal saline) at the dose 2ml/kg for 28 days, the data show that no significant changes in the renal cell.

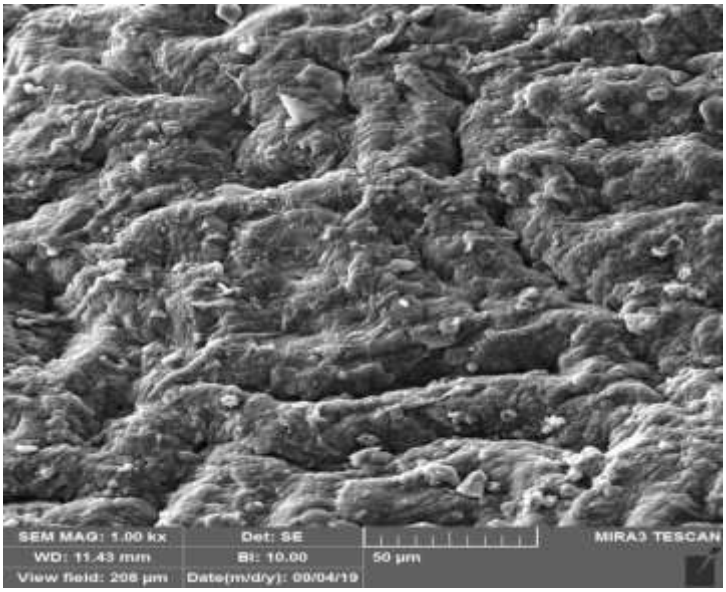

Image 1: The Rats Treated with 0.9% NaCl) at the Dose 2ml/kg: The Cell Membrane Appears Naturally and Regularly of the Renal Cells

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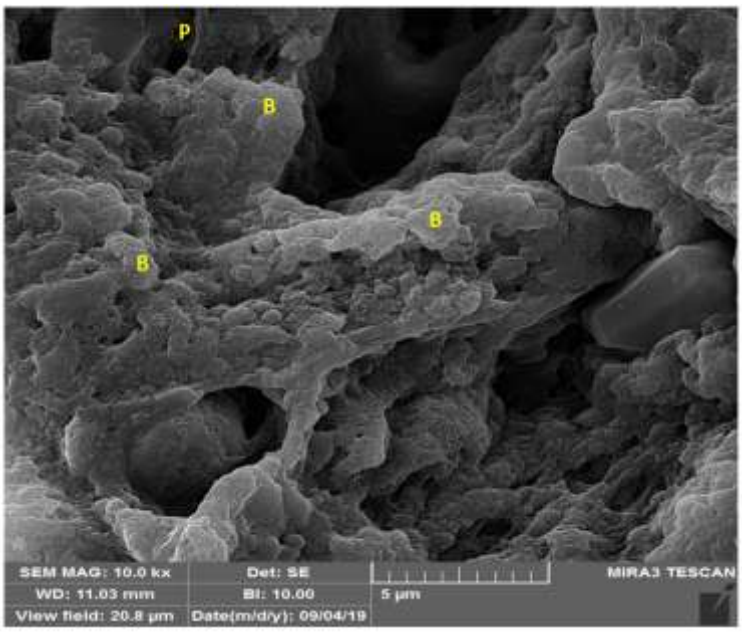

Image 2: The Rats Treated with 50mg/kg, the Image of the SEM shows the Presence of Few Pores (P) with Distinct Bubbles (B) Visible from the Surface of Renal Cells

The second group of rats that were treated with rifampicin at dose (50mg/kg/day) for 28 days show that the plasma membrane of renal cells irregular and few blebs projected and located on the renal cell's surfaces with few pores and few red blood cells. The third group of rats which were treated with (100mg/kg/day) for 28 days, their results indicated that many blebs more than in second group and many pores more than that were in second groupe with a lot of red blood cells (RBCs).

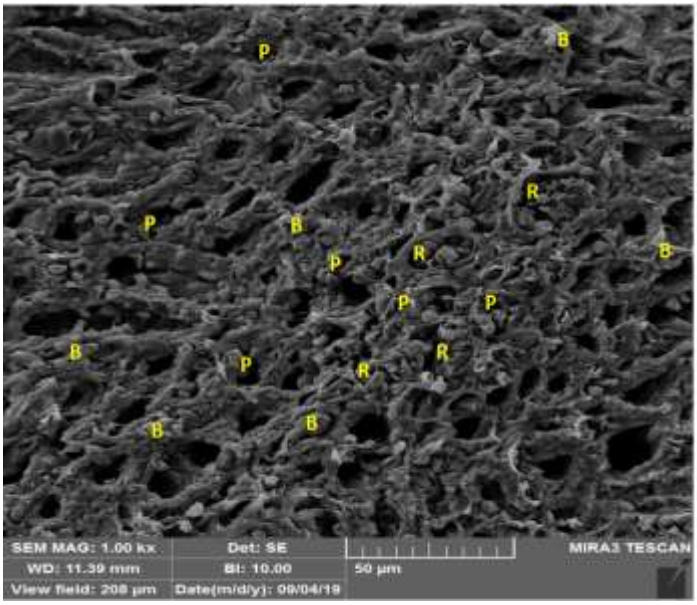

Image 3: The Rats treated with 100mg/kg, the Image of the SEM Shows the Presence of More Pores (P) with More Bubbles (B) Visible from the Surface In Addition to Aggregation of Red Blood Cells (R).of Renal Cells

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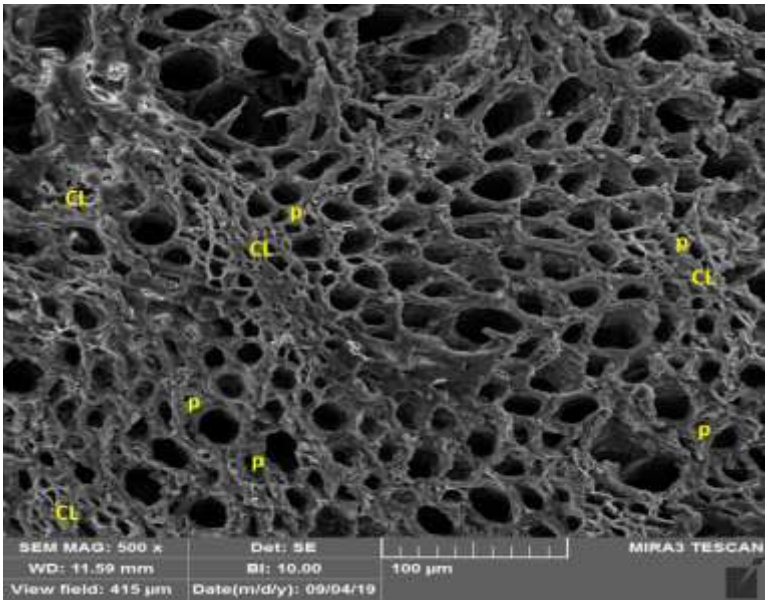

Image 4: The Rats Treated with 150mg/kg, the Image of the SEM Shows a Lot of Pores (P) with Bubbles (B) Visible from the Surface In Addition to Cytoplasmic Lysis (CL) of the Renal Cells

While the fourth group that were treated with 150mg/kg/day for 28 days , the data of these group reported that several significant changes included lysis of cytoplasm and a lot of blebs on the renal cell's surfaces and irregular plasma membrane and alt of red blood cells RBCs.

The gained results and data of this paper showed that rifampicin promoted apoptosis in rat's renal cells as indicated by morphological changes of renal cells compared with control group as well as the rifampicin induce cytotoxicity of renal cells at different doses ranged from 50mg/kg/day to 150 mg/kg/day.

Rifampicin induce apoptosis and cytotoxicity by promoted reactive oxygen system (ROS) is believed that have significant role in cellular changes and cytotoxicity, the effect occur by several ways such as DNA fragmentation, protein denaturation and oxidation of plasma membrane lipids(Kalayerasn et.al,2009).apoptosis and necrosis caused by reactive oxygen species and cytotoxicity depending on dose of drug, so the high doses of drugs caused plasma membrane irregular and cellular organelles dysfunction such as inability of mitochondria to provided sufficient of ATP level which is necessary for cellular activates (Denne et. al,2006 , Giorgi et.al,2012).

Depending on the present data of this study and compare with previous studies(Conde et.al,2006) which concluded that ; there weren't balance between oxidant and antioxidant leading to cellular membrane damage ,because ROS promoted apoptosis or necrosis by cells shrinkage , DNA fragmentation and alternative of cell membrane permeability.

When high concentration of drugs accumulated in the lysosomes of renal cells lead to induce apoptosis in renal cells and significant morphological changes involved with apoptosis have been occur especially alteration of plasma membrane architecture and many blebs that are showed , changing in the permeability of plasma membrane because of a lot of pores(Servias et.al,2005).These results are in agreement with those indicated that remarkable changes in

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architecture of renal cell membrane by activation of free radicals that cause induced phospholipid peroxidation and ruptured cell membrane in rats treating with rifampicin(Ravi et.al,2010).

In the present study the images of scanning electron microscope (S.E.M) reported that many blebs in the plasma membrane of renal cells, these blebs consider as remarkable sign to form apoptotic bodies. Because of degradation of cytoskeleton, caspase 3 necessary blebs formation where activation of caspase 3 lead to degradation of ROCK that important in proteins of cytoskeleton synthesis (Hartmann et.al, 2015). The proteins of ROCK caused moving of DNA fragments on the blebs and apoptotic to facilitate engulfed them by phagocytes (Iorgaa, & Daraa, 2019, Shabana et.al, 2012). While the pores formation on the surface of plasma membrane attributed to the effect of Caspase 3 on DFNA5 which moved to the plasma membrane and lead to dismantle and degradation of plasma membrane and pores formation(Giorgi et.al,2012 , Gueydan et.al,2018).

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