Histological Study of Survivin Expression in Experimentally Induced Renal Failure

Ayman M. Ghallab, Maha A. Abdallah, Gamal A.Attia and Ghada A. Elsammak

Histology and Cell Biology Department, Faculty of Medicine, Zagazig University, Egypt.
maha18770@gmail.com

Abstract: Introduction: Survivin is a member of inhibitor of apoptosis protein (IAP) family that has been implicated in both apoptosis inhibition and regulation of mitosis. Nowadays, involvement of survivin in renal repair mechanisms is considered as a matter of controversy. **Aim of the study:** This study was done to detect the expression of survivin in the normal renal cortices and in experimentally induced acute renal failure of adult male albino rats. **Materials & Methods:** Twenty healthy adult male albino rats were used in this study. They were equally divided into two groups; control (I) and experimentally induced acute renal failure; ERF (II). Rats in group (II) were injected by 20 mg cisplatin per kg body weight intraperitoneally dissolved in saline and then subdivided into two subgroups according to the time of sacrifice after ERF. Subgroup A (IIA) sacrificed after 24 hours while subgroup B (IIB) rats were sacrificed at the fourth day. Rats of control group (I) were injected with saline by the same dose and route of administration. They were also subdivided into two subgroups (IA&IB) according to the time of sacrifice. Renal cortices were dissected out and were processed for examination by light microscope. Immune reaction of survivin and P53 were carried out. Area percentage and optical density of both survivin and P53 were estimated and statistically analyzed. **Results:** Twenty four hours after induction of ERF, renal cortices contained apparently normal corpuscles and markedly dilated convoluted tubules with luminal casts in some of them. Most of the tubular cells had deeply stained nuclei and vacuolated cytoplasm. Others had deeply acidophilic cytoplasm. Thin collagen fibers still present around renal tubules, corpuscles and within the corpuscles between the glomerular capillary tuft. Few blood capillaries were also observed among the tubules. Concomitant with these changes, marked reduction of survivin expression with highly expressed P53 were observed. Four days after ERF, moderate improvement of renal tubular architecture with focally affected tubules. Most of the tubules were lined by cuboidal cells with pale stained cytoplasm and round pale nuclei. Some tubules still had vacuolated cytoplasm. Flattened cells with flattened nuclei were observed around renal tubules. Many blood capillaries were observed among the renal tubules. Moderate aggregations of collagen fibers were observed around the renal corpuscles and the affected tubules. Strong positive immune reaction for survivin was observed in the apparently improved renal tubules. While weak positive one was still noticed in renal corpuscles and the affected tubules. P53 immune reaction was negative in apparently normal tubules and corpuscles. Weak positive P53 immune reaction was noticed in the affected tubules. Estimated and analyzed data of area percentage and optical density of survivin and P53 confirmed the results. **Conclusion:** Variable structural changes were observed in renal cortices after experimentally induced acute renal failure. These changes were correlated with marked reduction in survivin expression and high expression of P53. After that, moderate improvement of renal structure was associated over expression of surviving and marked reduction of P53 and. These results encourage further evaluation of survivin for prevention and /or treatment of acute renal failure.

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1. Introduction

In adult human body, several thousands of cells are produced every second by mitosis and a similar number die by apoptosis for maintenance of homeostasis. The process of cell division and loss must be balanced not only in order to generate and maintain tissue architecture but also to allow adaptation to environmental changes. So, various anti-apoptotic molecules and mechanisms as well as proapoptotic factors are involved in cell homeostasis **[1,2]**.

Among the cell homeostasis, considerable interest has been focused on the Inhibitor of Apoptosis (IAP) family. IAP is a family of regulatory proteins that bind and inhibit caspases activity. It is also modulates cell division, cell cycle progression and signal transduction pathways. Nine IAP family members have been identified: X-linked IAP, cIAP1, cIAP2, neuronal apoptosis inhibitor protein, melanoma IAP, IAP-like protein 2, livin, apollon and survivin**[2,3]**.

Survivin is a unique protein that is highly expressed during embryonic development and with

less expression in most normal non-dividing adult tissues. It is notably present in all tumors making it a potential diagnostic marker and therapeutic target for cancer**[4,5]**. The main established function of survivin is the regulation of cell division potentially by regulating spindle microtubule assembly **[6]**.

For many years, there was a prevalent concept that survivin was not or hardly expressed in adult differentiated tissues. However, numerous studies have demonstrated survivin expression in various normal cells and tissues as ovaries, oviducts and kidney. Therefore, careful descriptions of survivin expression and its role in adult non-cancerous tissues are urgently required **[7]**.

P53 is a tumor suppressor protein synthesized in the cytoplasm of various cells. It is usually expressed at high level during embryogenesis with persistent expression in some tissues undergoing differentiation. P53 can block cell cycle progression and/or induce apoptosis by transactivation of specific target genes. It is triggered by a wide variety of insults as ultraviolet radiation, chemotherapeutic agents, free radicals and hypoxia **[8]**.

It had been established that p53 transcriptionally regulates a number of genes that control apoptosis as Bcl-2. Survivin can block apoptosis induced by a variety of apoptotic triggers. The exact biochemical mechanism by which survivin suppresses apoptosis has been debated. However, it was found that disturbances of the correlation between P53 and survivin expression are common events in various medical problems suggesting a functional link between them **[9]**.

Kidney is an organ with a variety of highly specific tasks as blood pressure control, regulation of erythropoiesis in addition to maintenance of water and electrolyte homeostasis. Acute renal failure (ARF) is one of the most important socioeconomic problems that usually occur as a result of reversible decline in glomerular filtration rate. ARF occurs at least in 5% of hospitalized patients and in 30-50% of those admitted to the intensive care unit**[10-12]**.Until now, the majority of patients with ARF will die. Most of the survivors will become independent from renal replacement therapy within a year. Involvement of survivin in renal repair mechanisms is considered as a matter of controversy **[13,14]**. So, this study was done to detect the expression of survivin in the normal renal cortices and in experimentally induced acute renal failure of adult male albino rats.

2.Materials and Methods

Twenty healthy adult male albino rats (3-6 months) weighing 180-200 g were utilized in this study. They were housed in stainless-steel cages and were maintained in room temperature at 23ºC. They were allowed water ad libitum and were fed a standard diet. They were equally divided into two main groups; a control group (I) and an experimentally induced acute renal failure (ERF) one (II). ERF was done by a single intraperitoneal injection of 20 mg cisplatin per kg body weight dissolved in saline. Cisplatin was manufactured by Mylan Co. Rats in group (II) were subdivided into two subgroups according to the time of sacrifice after ERF. Subgroup A (IIA) rats were sacrificed after 24 hours from induction while subgroup B (IIB) rats were sacrificed at the fourth day. Rats of control group (I) were injected with saline by the same dose and route of administration. They were also subdivided into two subgroups (IA&IB) according to the time of sacrifice **[15]**.

Before the time of sacrifice of each subgroup of both groups, the blood samples from the rats' tails were collected for estimation of serum creatinine level. After that, rats were anesthetized with 50 mg sodium pentobarbital per kg body weight intraperitoneally. Renal cortices of all rats were dissected out carefully and their renal cortical regions were processed for light microscope examination. They were fixed in 10%formal saline and were processed to prepare 5µm thick paraffin sections for Haematoxylin &Eosin, Mallory Trichrome and immunohistochemical stains **[16]**.

For immunohistochemical staining of survivin (anti-apoptotic) and P53 (pro-apoptotic), paraffin sections were cleaned in xylene, hydrated and then placed in PBS (pH 7.6). They were treated with 0.01M citrate buffer (pH 6.0) for 10 minutes to unmask antigen. Then, they were incubated with 0.3% H2O2 for 30 minutes to abolish endogenous peroxidase activity before blocking with 5% horse serum for 1-2 h then washed with PBS. Slides were incubated with the primary antibody (Thermo scientific company) (1:50 monoclonal mouse anti surviving or anti P53) at 4°C for overnight, then washed and incubated with biotinylated secondary antibodies followed with avidin–biotin complex. Finally, sections were developed with 0.05% diaminobenzidine slides, were counter stained with Mayer's hematoxylin, dehydrated, cleared and mounted. Positive cells for survivin and P53 exhibited brown color in their cytoplasm with blue stained nuclei **[17,18]**.

Morphometric study:

Area percentage and optical density of survivin and P53were measured using the Leica Qwin 500 image analyzer computer system (Leica Imaging System Ltd, Cambridge, UK) at the Histology and Cell Biology Department, Faculty of Medicine, Cairo University. The procedure was done

in ten non overlapping fields for each group at ×40 magnification.

Statistical analysis

Data for both groups (I&II) were expressed as mean ± SD (X ± SD). The data obtained from the image analyzer were subjected to the SPSS program version 17 (Chicago, USA). Statistically significant differences were determined by one-way analysis of variance, followed by a Post-hoc test for multiple comparisons between different groups. The P values <0.05, <0.001, and >0.05 were considered significant, highly significant and non-significant respectively.

3.Results

I-Histological results:

Haematoxylin and Eosin stained sections of the renal cortex of the control adult male albino rats (IA&IB) showed that the renal cortex was formed of renal corpuscles and convoluted tubules with minimal interstitium in between. Each renal corpuscle was formed of a glomerulus; tuft of blood capillaries surrounded by Bowman's capsule with narrow urinary space. Two types of cortical renal tubules were present; proximal convoluted tubules (PCT) and distal convoluted tubules (DCT).Both tubules were lined by cuboidal epithelial cells with round pale nuclei and prominent nucleoli. PCT had relatively narrow lumina with few epithelial cell lining. DCT had wider lumina with more epithelial cell lining **(Fig. 1)**. Mallory trichrome stained sections revealed thin collagen fibers arranged around renal tubules, corpuscles and between loops of glomerular capillary tuft **(Fig. 2)**. Immunohistochemically, strong positive cytoplasmic immune reaction for surviving was observed in cells of both renal tubules and corpuscles **(Fig. 3)**.Weak positive cytoplasmic immune reaction for P53 was detected in some tubular cells and corpuscles **(Fig. 4)**.

Haematoxylin and Eosin stained sections of the renal cortex in ERF subgroup A(IIA) revealed that renal cortex contained apparently normal renal corpuscles and markedly dilated convoluted tubules with luminal casts in some of them **(Fig. 5)**. Most of the renal tubular cells had deeply stained nuclei and vacuolated cytoplasm. Others had deeply acidophilic cytoplasm **(Fig.6)**. Mallory trichrome stained sections revealed that thin collagen fibers still present around renal tubules, corpuscles and within the corpuscles between the glomerular capillary tuft. Also, few blood capillaries were observed among the tubules **(Fig. 7)**. Survivin immunohictochemical stained sections showed weak positive immune reaction in cytoplasm of the tubular cells and renal corpuscles in comparison with that observed in control group **(Fig. 8)**.Most of renal tubular cells and corpuscles showed strong positive P53 immune reaction in their cytoplasm in comparison with the control group **(Fig. 9)**.

Haematoxylin and Eosin stained sections of the renal cortex in ERF subgroup B (IIB) showed moderate improvement of renal tubular architecture with focally affected tubules. Most of the tubules were lined by cuboidal epithelial cells with pale stained cytoplasm and round pale nuclei. Some tubules still had vacuolated cytoplasm**.** Flattened cells with flattened nuclei were observed around renal tubules. Many blood capillaries were observed among the renal tubules **(Fig. 10).** Mallory trichrome stained sections showed moderate aggregations of collagen fibers around the renal corpuscles and the affected tubules **(Fig. 11)**.Strong positive immune reaction for survivin was observed in the apparently improved renal tubules. While weak positive one was still noticed in renal corpuscles and the affected tubules **(Fig 12)**. P53 immune reaction was negative in apparently normal tubules and corpuscles. Weak positive P53 immune reaction was noticed in the affected tubules **(Fig. 13)**.

II- **Morphometrical and Statistical results**

a- Regarding Survivin:

The mean value of the area percentage of survivin in the renal cortex in random fields using ANOVA test showed that survivin area percentage was decreased in subgroup A and markedly increased in subgroup B with statistically significant difference.

Table (1): Comparison between mean values of survivin area percentage in different group using ANOVA.

Survivin area percentage	**Mean±SD**	**Range**	**F test**	***P* value**
Control	42.4±2.1	40.53-45.77	47.811	*<0.001
ERF; Subgroup (A)	11.8±3.8	7.30-17.44		
ERF; Subgroup (B)	64.1±14.1	45.44-80.51		

The mean value of survivin optical density in the renal cortex in different studied group using ANOVA test showed that survivin optical density was decreased in subgroup A after cisplatin injection and markedly increased in subgroup B with statistically significant difference.

Table (2): Comparison between mean values of survivin optical density in different group using ANOVA.

Survivin optical density	**Mean±SD**	**Range**	**F test**	***P* value**
Control	49.8±6.9	44.12-57.76	59.705	*<0.001
ERF; Subgroup (A)	22.7±4.8	15.12-26.97		
ERF; Subgroup (B)	70.1±1.8	67.74-72.46		

b- Regarding P53:

Statistical analysis of the morphometrical results of the area percentage of P53 in the renal cortex using ANOVA test showed that P53 area percentage was increased in subgroup A after cisplatin injection and markedly decreased in subgroup B with statistically significant difference.

Table (3): Comparison between mean values of survivin area percentage in different group using ANOVA.

P53 area percentage	**Mean±SD**	**Range**	**F test**	***P* value**
Control	20.8±4.3	13.59-24.58	112.657	*<0.001
ERF; Subgroup (A)	67.2±8.4	57.53-78.19		
ERF; Subgroup (B)	0.31±0.2	0.03-0.76		

Statistical analysis of the morphometrical results of P53 optical density in the renal cortex in different group using ANOVA test showed that P53 optical density was increased in subgroup A after cisplatin injection and markedly decreased in subgroup B with statistically significant difference.

Table (4): Comparison between the mean values of P53 optical density in different studied groups using ANOVA test.

P53optical density	**Mean±SD**	**Range**	**F test**	***P* value**
Control	21.3±4.3	18.05-28.55	153.228	*<0.001
ERF; Subgroup (A)	64.3±1.0	62.83-65.27		
ERF; Subgroup (B)	9.5±1.4	8.04-11.45		

III- **Serological results:**

Measurement of serum creatinine level in the control and experimental groups was so necessary for assessment of renal function

	Control	***ERF;*** Subgroup (A)	***ERF;*** Subgroup (B)
S. creatinine levelmg/dl	0.53±0.06	1.12 ±0.05	0.89±0.02

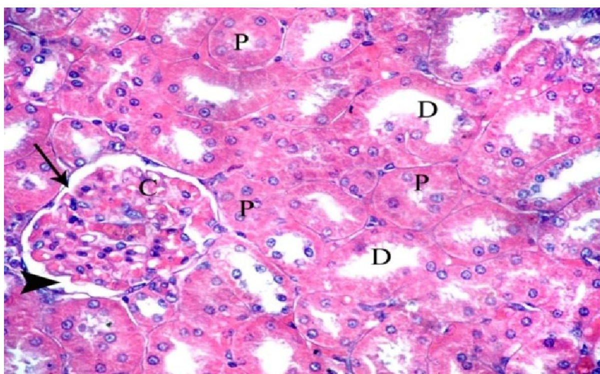

Figure (1): A photomicrograph of a section in the control renal cortex showing renal corpuscles and convoluted tubules with minimal interstitium in between. Renal corpuscle is formed of glomerulus; tuft of blood capillaries (C) surrounded by Bowman's capsule (arrow) with narrow urinary space (arrow head). Two types of cortical renal tubules are present; proximal (P) and distal (D) convoluted tubules. Both tubules are lined by cuboidal epithelial cells with round pale nuclei and prominent nucleoli. PCT have relatively narrow lumina with few epithelial cell lining. DCT have wider lumina with more epithelial cell lining. (H&E X400).

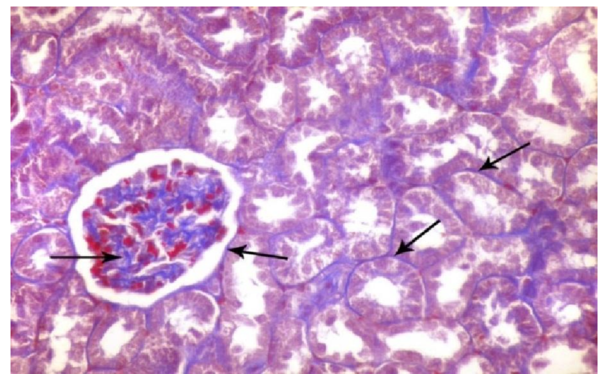

Figure (2): A photomicrograph of a section in the control renal cortex showing thin collagen fibers (arrows) arranged around renal tubules, corpuscles and between loops of glomerular capillary tuft. (Mallory trichrome stain X400).

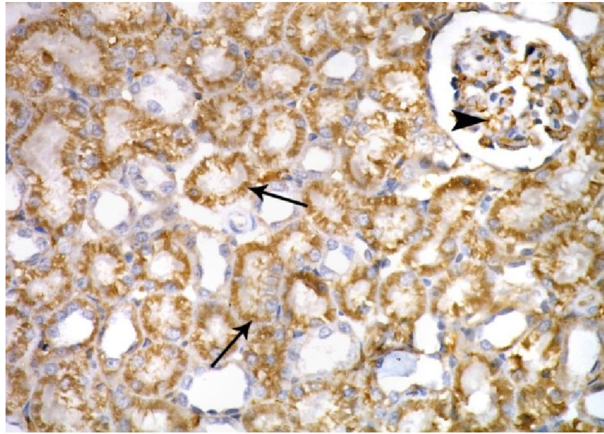

Figure (3): A photomicrograph of a section in the control renal cortex showing strong positive cytoplasmic immune reaction of survivin in cells of tubules (arrows) and corpuscles (arrow head). **(**Survivin immunostaining X 400).

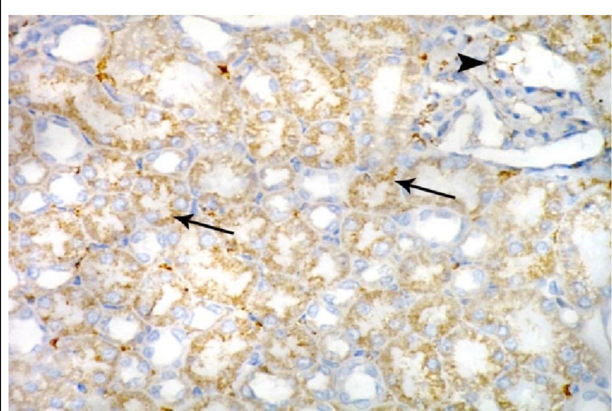

Figure (4): A photomicrograph of a section in the control renal cortex showing weak positive cytoplasmic immune reaction for P53 in some tubular cells (arrows) and corpuscle (arrow head). (P53immunostaining X 400).

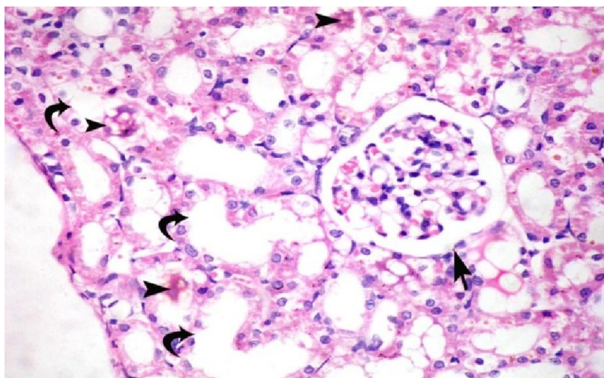

Figure (5):A photomicrograph of a section in the renal cortex of subgroup IIA adult rats showing apparently normal renal corpuscle (arrow) and markedly dilated convoluted tubules (curved arrows) with luminal casts (arrowheads) in some of them. (H&E X400).

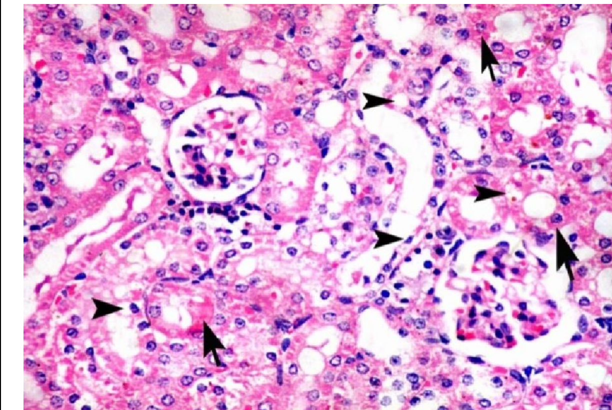

Figure (6): A photomicrograph of a section in the renal cortex of subgroup IIA adult rats showing that most of renal tubular cells (arrow heads) have deeply stained nuclei and vacuolated cytoplasm. Others have deeply acidophilic cytoplasm (arrows). (H&E X400).

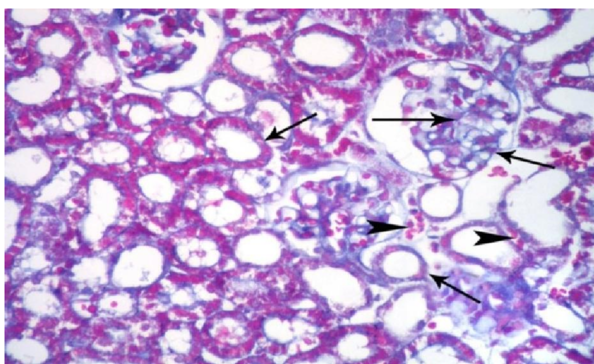

Figure (7): A photomicrograph of a section in the renal cortex of subgroup IIA adult rats showing thin collagen fibers (arrows) around the renal tubules, corpuscles and within the corpuscles between the glomerular capillary tuft. Few blood capillaries (arrow heads) are observed among the tubules (Mallory trichrome stain X400).

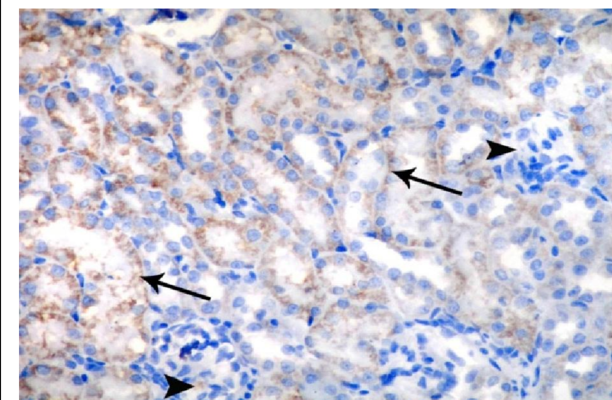

Figure (8):A photomicrograph of a section in the renal cortex of subgroup IIA adult rats showing weak positive immune reaction of survivin in cytoplasm of the tubular cells (arrows) and renal corpuscles (arrow heads)in comparison with that observed in fig 3. **(**Survivin immunostaining X 400).

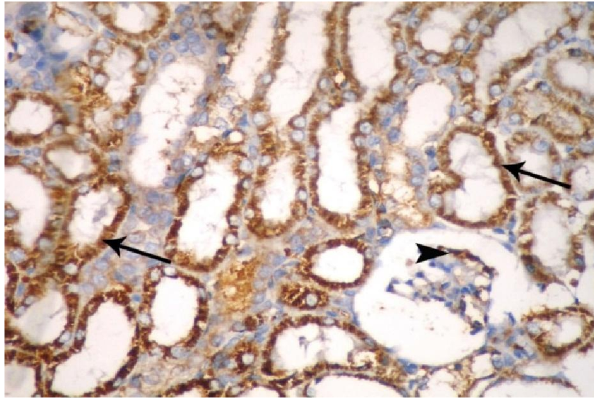

Figure (9): A photomicrograph of a section in the renal cortex of subgroup IIA adult rats showing strong positive immune reaction of P53in the cytoplasm of most tubular cells (arrows) and corpuscles (arrow head) in comparison with fig 4. (P53immunostaining X 400).

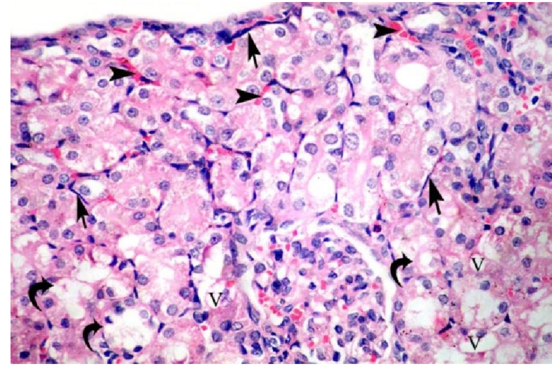

Figure (10):A photomicrograph of a section in the renal cortex of subgroup IIB adult male albino rats showing moderate improvement of renal tubular architecture with focally affected tubules (curved arrows). Most of the tubules are lined by cuboidal cells with pale stained cytoplasm and round pale nuclei. Some tubules still have cytoplasmic vacuoles (V)**.**Flattened cells (arrows) with flattened nuclei are observed around renal tubules. Many blood capillaries (arrowheads)are observed among the renal tubules. (H&E X 400).

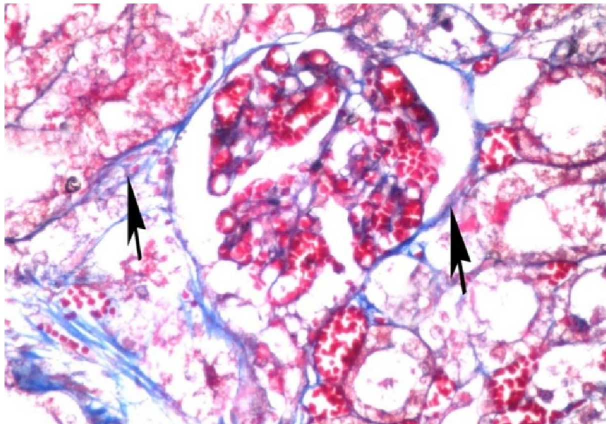

Figure (11): A photomicrograph of a section in the renal cortex of subgroup IIB adult male albino rats showing moderate aggregations of collagen fibers (arrows) around the renal corpuscle and affected tubules. (Mallory trichrome stain X400).

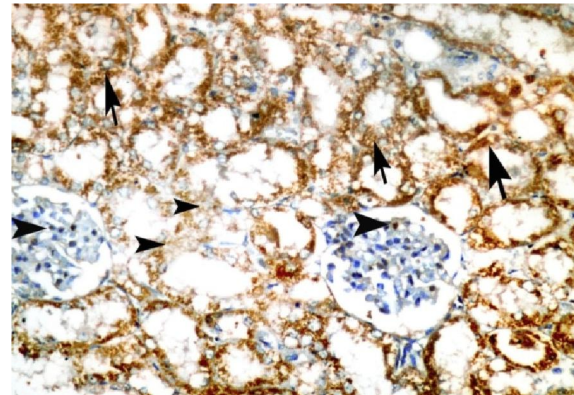

Figure (12): A photomicrograph of a section in the renal cortex of subgroup IIB adult male albino rats showing strong positive survivin immune reaction (arrows) in the apparently improved renal tubules. Weak positive immune reaction (arrow heads)is still noticed in renal corpuscles and the affected tubules. **(**Survivin immunostaining X 400).

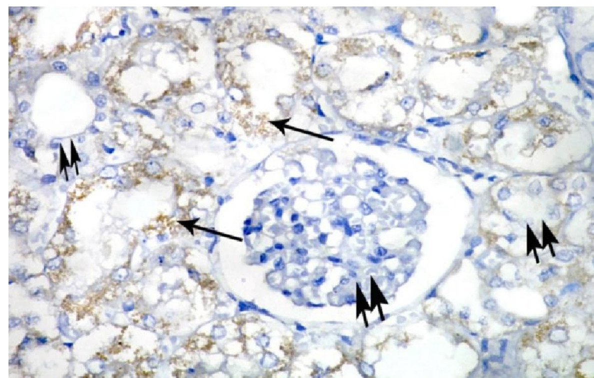

Figure (13): A photomicrograph of a section in the renal cortex of subgroup IIB adult male albino rats showing that P53 immune reaction is negative in apparently normal tubules and corpuscles (double arrows).Weak positive P53 immune reaction (arrows) is noticed in the affected tubules. (P53 immunostaining X 400).

4.Discussion

For many years, there was a prevalent concept that survivin was not or hardly expressed in adult differentiated tissues. However, some studies have demonstrated survivin in some tissues and cells. Therefore, careful description of survivin expression and function in adult tissues are urgently required. Survivin expression was prominently observed in renal tubular epithelial cells and podocytes. The high level of survivin expression in these cells may be of importance for renal physiology. Furthermore, the involvement of survivin in renal repair mechanisms could be postulated **[7,19]**.

In contrast to the heart or brain, the kidney can completely recover from an ischemic or toxic insult that resulting in cell death. During recovery, surviving tubular epithelial cells can redifferentiate and divide at low rate, eventually replacing the irreversibly injured tubular epithelial cells and restoring tubular integrity. This turnover rate must be strictly controlled. Imbalance between cell division and cell loss may lead to nephron loss or marked increase in nephron and kidney size **[20]**.

This study was performed on rats because rat's survivin is homologous to human survivin and the sequences homology of human and rat's survivin protein molecules exceed 70% **[21]**. The renal cortices were chosen due to easily detection of survivin in renal cells where it may play a central role in renal physiology and in response to injury **[7]**.

In this study, induction of ARF was done by cisplatin. It was found **[22]** that cisplatin induces ARF mimic *in vivo* condition as can as possible. The majority of cisplatin administered systemically at a high dose is largely cleared via the kidney within few hours. Toxicological studies suggest that critical effects of cisplatin on the kidney occur within the first four hours after administration. Also, it was reported **[23,24]** that the kidney accumulates cisplatin to a greater degree than other organs as it is the major route for its excretion. Cisplatin concentration in proximal tubular epithelial cells is about five times higher than the serum concentration. The disproportionate accumulation of cisplatin in kidney tissue contributes to cisplatin-induced nephrotoxicity that can be diagnosed by tubular cell death and deterioration renal functions.

In this study, serum creatinine level was progressively increased in ERF subgroup A. It was reported **[25]** that serum creatinine level is the most widely used and commonly accepted measure of renal function in clinical medicine. It was stated **[26]** that the normal kidneys are able to filter large amount of creatinine which is formed as a result of the non enzymatic dehydration of muscle creatine. But as a result of acute nephrotoxicity, kidneys became unable to excrete creatinine due to marked reduction in glomerular filtration rate.

Additionally, in this study after four days (IIB), serum creatinine begins to decrease. Previous studies **[27,28]** about acute renal injury were found that serum creatinine returned to its normal level after two weeks. They attributed this to return of the glomerular filtration rate to baseline values. However, the complete restoration of renal morphology may take up to four weeks. Some scientists **[20]** correlate between the proliferation of the renal cells as a trial of repair and the improvement renal function.

In this study, kidney of ERF subgroup A revealed that renal cortex contained apparently normal renal corpuscles and markedly dilated convoluted tubules with luminal casts in some of them. Most of the renal tubular cells had deeply stained nuclei and vacuolated cytoplasm. Others had deeply acidophilic cytoplasm. Many studies were done in attempt to explain the pathogenesis of acute renal failure. Some scientists suggested **[29]** that tubular cell apoptosis occurred as a result of aggregation of lethal cytokines such as TNF-α and Fas ligand. These cytokines may leak from the affected glomeruli and subsequently reach the tubular epithelium. Others **[30]** reported that cisplatin induced nephrotoxicity occurred through marked reduction of renal blood flow that subsequently led to not only marked hypoxia but also mitochondrial injury. PCT is the most liable part of the kidney to hypoxia and usually precedes the alteration of renal hemodynamics. However, some scientists **[23]** attributed cisplatin nephrotoxicity to the conjugation of cisplatin to intracellular glutathione and subsequently formation of reactive thiol; a potent nephrotoxin that may induce oxidative stress, apoptosis and inflammation. Other researchers suggested **[31-33]** that cisplatin nephrotoxicity occurred as a result of oxidative stress and subsequently increased generation of reactive oxygen species(ROS) as superoxide anion, hydrogen peroxide and hydroxyl radicals. Increased production of ROS not only decreases the activity of the antioxidant enzymes (catalase, SOD and glutathione peroxidase) but also depletes glutathione and subsequently enhances lipid peroxidation in renal tissue.

In this work, after 24 hours of induction of ERF (IIA), survivin immunohictochemical stained sections showed weak positive immune reaction in cytoplasm of the tubular cells and renal corpuscles in comparison with that observed in control group. Survivin area percentage and optical density was statistically decreased. In many types of cells, it was claimed **[34]** that leakage of cytochrome c from

mitochondria destroys cells not only by activation of caspases but also through cessation of mitochondrial electron chain transport with subsequent ATP depletion. Survivin is able to suppress caspases and subsequently prevent cytochrome c-induced apoptosis. However, it is not necessarily stop cell death induced by caspase-independent mechanisms. Some investigators **[35-37]** stated that cytoplasmic expression of survivin in normal fetal and adult cells reflect the activity of the cells. Survivin exists in two strikingly different subcellular pools comprising a predominant cytoplasmic and a smaller nuclear pool that localizes to kinetochores. The approximate ratio of cytoplasmic survivin to nuclear one was 6:1. Nuclear survivin controls cell division whereas cytoplasmic survivin is cytoprotective.

However, most of renal tubular cells and corpuscles of the same group showed strong positive P53 immune reaction in their cytoplasm in comparison with the control group. Statistically, P53 area percentage and optical density was increased. It was found **[38-40]** that there was an inverse relationship between survivin and P53. ARF may be initiated by depletion of intracellular Guanosine triphosphate (GTP) which is usually associated with rapid increase in the concentration of P53 that subsequently promote cell death. It was noticed **[15]** that renal expression of P53 was suppressed after survivin gene therapy. So, administration of survivin usually prevents renal tubular epithelial cell death in some forms of toxin-induced ARF. Some investigators **[24,33]**attributed the renal injury and dysfunction after cisplatin injection to excessive aggregation of P53. High level of P53 increases the permeability of outer mitochondrial membrane to various apoptotic factors as cytochrome c.

In the current work, ERF subgroup B showed moderate improvement of renal tubular architecture with focally affected tubules. Most of the tubules were lined by cuboidal epithelial cells with pale stained cytoplasm and round pale nuclei. Some tubules still had vacuolated cytoplasm. In acute renal injury **[41]**, it was noticed that viable and nonviable tubular epithelial cells are desquamated leaving regions where the basement membrane remains as the only barrier between the filtrate and the peritubular interstitium. Recovery from acute injury led to a sequence of events includes epithelial cells spreading and possibly migration to cover the exposed areas of the basement membrane. Then, the cells redifferentiate and proliferate to restore cell number followed by differentiation and restoration of the functional integrity of the kidney. Furthermore, during the repair process of the kidney, it was reported **[42,43]**that certain matrix molecules may play an important role in the epithelial cell migration. Deposition of fibronectin was observed within three hours after acute renal injury. This may stimulate redifferentiation and subsequently proliferation. Osteopontin (OPN) is another molecule which was detected **[44]** in the loop of Henle of normal kidneys. In a trial of repair, it continues to be localized in all tubule segments and glomeruli as long as the seventh day after injury. OPN has some renoprotective actions in renal injury. It increases tolerance to acute ischemia through inhibition of inducible nitric oxide synthase responsible for nitric oxide synthesis, reduction of cell peroxide, decreasing cell apoptosis and participating in the regeneration of cells. At later times after ischemia, it was noticed **[45]** that laminin expression is markedly elevated. It has been proposed that laminin deposition may regulate redifferentiation and repolarization of the epithelial cells.

In this study, renal cortices of ERF subgroup B revealed flattened cells with flattened nuclei around renal tubules. Physiologically, It was reported **[29,46,47]** that renal cortex contains relatively few interstitial fibroblasts which usually present in a perivascular or peritubular location. In ARF, these fibroblasts have the ability to secrete excess amount of TNF-α which may be involved in the pathogenesis of tubular cell apoptosis. Injured tubular cells as well as interstitial infiltrating cells secrete many cytokines such as transforming growth factor-β, connective tissue growth factor, fibroblast growth factor and platelet-derived growth factor. These factors are able to transform interstitial fibroblasts to myofibroblasts. In addition, it was stated **[48]** that renal OPN plays a significant role in the recruitment and activation of interstitial fibroblasts to myofibroblasts. It was claimed **[27]** that peritubular myofibroblasts play an important role in cellular recovery in ARF model. Myofibroblasts were firmly attached to denuded tubular basement membranes. In addition to the possible supporting function of the basement membrane, myofibroblasts provide the extracellular matrix components by cytokines as paracrine mitogens which might promote cellular recovery from ARF.

In this work, thin collagen fibers were still present around the renal tubules, corpuscles and within the corpuscles between glomerular capillary tuft in ERF subgroup A. Among the tubules, few blood capillaries were observed. In ERF subgroup B, moderate aggregations of collagen fibers were noticed around the renal corpuscles and the affected tubules. Many blood capillaries were observed among the renal tubules. It has been shown **[49]** that acute renal injury usually affects the integrity of peritubular capillaries. Other scientists **[23,28]**added that marked reduction in renal microvessels that

occur in early stage of ARF usually exacerbate renal hypoxia. Hypoxia triggers overproduction of a transforming growth factor-beta (TGF-β) and other pro-fibrotic molecules that contribute the development of tubule-interstitial fibrosis. Post-ischemic renal fibrosis is usually reversible and replaced by regenerated renal tubules.

At the fourth day of ERF subgroup B, strong positive immune reaction of survivin was observed in the apparently improved renal tubules. While weak positive immune reaction was still noticed in renal corpuscles and the affected tubules. Area percentage as well as optical density of survivin in the renal cortex was statistically increased. Previously **[50]**, stated that mitochondria play a pivotal role in orchestrating apoptosis and provide a site of assembly for various pro and anti-apoptotic regulators. Furthermore **[51]**, cytoplasmic survivin is usually present in the inter-mitochondrial membrane space. Hazardous stimulus to the cell not only disturbs the mitochondrial membrane but also stimulate the cells to increase IAP expression. Leakage of survivin through the affected mitochondrial membrane towards the cytoplasm is considered as a cytoprotective trial from the cell. In addition **[5]**, there is a reserve pool of survivin within the mitochondria. Mitochondrial survivin is dynamic component meaning that this pool is rapidly expanded by cell stress stimuli like hypoxia and is promptly discharged in the cytosol in response to full blown apoptotic stimulation. So **[15],** overexpression of survivin can protect cells from Fas- and injury-induced apoptosis by interfering with effector caspases and also by stabilizing mitochondrial function.

In ERF subgroup B, P53 immune reaction was negative in apparently normal tubules and corpuscles. Weak positive P53 immune reaction was noticed in the affected tubules. Statistical analysis of area percentage and optical density was markedly decreased. It has been previously determined **[52]** that P53 can suppress survivin expression and so reduction of survivin expression plays an important role in P53-mediated apoptosis. In contrary **[9,53,54],**over-expression of exogenous surviving protein rescues cells from P53-induced apoptosis. Survivin regulates P53 expression not only by modification of P53 degradation but also through suppression of gene expression.

Conclusion:

From the results of this work, we concluded that the structural changes that occurred in renal cortices after experimentally induced acute renal failure was correlated with marked reduction in survivin expression and high expression ofP53. After 4 days of ERF, survivin expression was elevated that was associated with marked reduction of P53 and moderate improvement of renal structure.

Recommendations: These results encourage further evaluation of survivin for prevention and /or treatment of acute renal failure.

Corresponding author:
Maha A. Abdallah Histology and Cell Biology Department, Faculty of Medicine, Zagazig University, Egypt.
Email: maha18770@gmail.com

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