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The protective effect of spironolactone and role of the Na+/K+-ATPase pump on intestinal ischemia/reperfusion injury

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ABSTRACT: The aim of this study was to evaluate the possible protective effect of spironolactone (SPL) and role of the Na-K ATPase pump on intestinal ischemia/reperfusion injury. In our study, the period of ischemia was established by clamping the mesenteric artery for 45 minunder anesthesia in Wistar albino rats and the animals left for reperfusion at the end of this period were decapitated after one hour. Spironolactone (20 mg kg-1) was administered orally for three days before ischemia, 30 minbefore ischemia. The control group rats were subjected to the Sham operation and administered saline solution. TNF-α and IL-1β levels were measured in the serum samples. Ileal Na+/K+-ATPase, myeloperoxidase (MPO) analysis were performed. Structural injury was assessed histopathologically. Ischemia/reperfusion increased serum TNF-α and IL-1β levels together with MPO activity, whereas these values were maintained at the control group levels through SPL activation. However, ischemia/reperfusion decreased Na+/K+-ATPase activity in ileal tissues; however, these parameters were found to be significantly increased with SPL activation. The protective effect of SPL against ischemia/reperfusion injury by different mechanisms, mainly the activity of the Na+/K+-ATPase pump, suggests that this nontoxic agent may constitute a new clinical therapeutic principle.

KEYWORDS: Intestinal ischemia/reperfusion; inflammation; Na+/K+-ATPase; spironolactone.

1. INTRODUCTION

Intestinal ischemia/reperfusion injury is a serious and frequently observed clinical condition, and is a frequent occurrence particularly during conditions such as superior mesenteric artery occlusion, hemorrhagic shock, septic shock, and small bowel transplantation [1]. The occurrence of this injury plays an important role in anaerobic metabolism, and in the inability to produce sufficient amounts of high-energy phosphates necessary for several cellular functions. Low values of the high-energy phosphates affect most cellular function such as hemostasis, signal interaction, cellular proliferation, and the process of apoptotic death cycle [2].

Depletion of adenosine triphosphate (ATP) disrupts Na+/K+-ATPase function. In addition, toxic metabolites which occur during ischemia lead to cellular edema. Under conditions where ischemia is prolonged, the ATP-synthase activity is irreversibly inhibited, resulting in increased sodium (Na^+) and water entry into the cell [3]. Ischemia also causes neutrophil adhesion to endothelial cells due to increased expression of adhesion molecules. In addition, vasoconstriction and subsequent microcirculatory insufficiency are observed despite the fact that blood circulation is reestablished. Reperfusion increases the levels of cytokines such as TNF-alfa and IL-1 beta, rendering the injury irreversible [4].

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Aldosterone, whose main role is to regulate the density of Na^+ ions in the body, demonstrates this effect, particularly influencing Na channels and the Na+/K+-ATPase pump through mineralocorticoid receptors, which are located in tissues such as the kidneys and intestines. Increased levels of cytokines, particularly with inflammation are associated with increased aldosterone levels, leading to increased severity of inflammation as a result of inhibition of the Na+/K+-ATPase pump [5]. Spironolactone, a mineralocorticoid receptor antagonist, has been shown to reduce morbidity and mortality in patients with heart failure [6]. It has been shown to demonstrate a protective effect by preventing tissue injury particularly in inflammatory models, either through its effect on cytokine levels or by providing Na+ balance [7-9].

The aim of this study was to investigate the role of the Na+/K+-ATPase pump, whose effect on mesenteric ischemia/reperfusion injury has so far not been clearly determined, and whether spironolactone has an effect on this mechanism.

2. RESULTS

2.1. Serum results

Serum levels of TNF-α and IL-1β were found to be significantly higher in the IR group than the control group. On the other hand, increase in the IR group was found to significantly decrease in the group administered spironolactone (SPL), approaching the control values (p<0.01-0.001, Table 1).

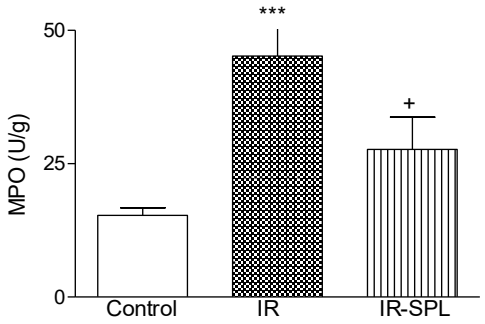
Table 1: Serum TNF-α and IL-1β values of all groups in mesenteric ischemia/reperfusion (I/R) models in rats.SPL; Spironolactone.

	Control	IR	IR-SPL
TNF-α	5.37 ± 0.91	40.58 ± 8.54***	15.30 ± 2.76++
IL-1β (pg/ml)	9.51 ± 1.69	52.07 ± 5.99 ***	23.28 ± 4.99++

Mean± standard error ***p<0.001 vs control group,++ p<0.001 vs I/R group

2.2. Myeloperoxidase (MPO) activity results

In the IR group, the MPO values were found to be significantly increased compared to the control group, whereas the MPO activity was significant decreased in the SPL group; and the results were found to be close to the control group MPO values (p<0.01-0.001, Figure 1).

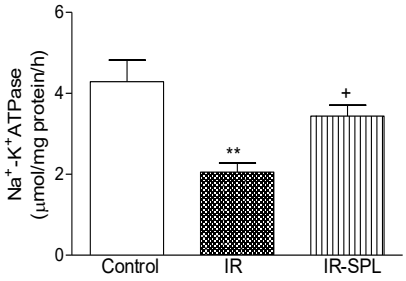


** p<0.01, ***p<0.001 Comparisons according to the control group, + p<0.05 Comparisons according to the IR group.

Figure 1. Tissue myeloperoxidase (MPO) values of all groups in mesenteric ischemia/reperfusion (I/R) models in rats.SPL; Spironolactone.

2.3. Na+/K+-ATPase activity results

The Na+/K+-ATPase activity was found to be lower in the IP group when compared to the control group. This decrease was found to be significantly prevented in the IR group treated with SPL (p<0.01-0.001, Figure 2).



** p<0.01, ***p<0.001 Comparisons according to the control group, + p<0.05 Comparisons according to the IR group.

Figure 2. Tissue Na^+/K^+-ATPase values of all groups in mesenteric ischemia/reperfusion (I/R) models.SPL; Spironolactone.

2.4. Histopathological results

The epithelium of the ileal tissue was found to be uniform in the control group, with smooth goblet cell structures observed (Figure 3a). In the IR group, the epithelium was markedly spilled and the gland structures were also damaged (Figure 3b).On the other hand, in the IR-SPL group, the epithelial structures were found to have improved and the regenerated bulbous structures of the goblet cells were markedly visible (Figure 3c).

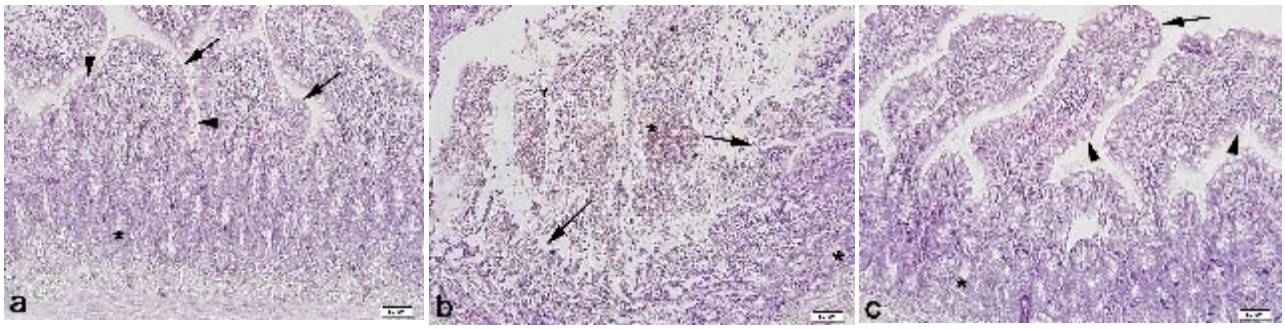


Figure 3. a: Control group smooth epithelial structure (arrows), goblet cells (arrowheads), and glandular structures (*); **b:**Ischemia/reperfusion (I/R) Group, markedly warn-out epithelium (arrows) as well as damaged glandular structures (*); **c:**Spironolactone-treated Ischemia Reperfusion (IR-SPL) Group; reformed smooth epithelium (arrow) and goblet cells (arrowheads) as well as glandular structures (*).

3. DISCUSSION

Mesenteric I/R injury can occur in most clinical procedures including organ transplantation, bowel resection, trauma, and vascular disease [10,11]. Several factors are known to contribute to mesenteric I/R injury. These may include oxidative stress, upregulation of the proinflammatory cytokine signaling system, and dysfunction of the Na+/K+-ATPase pump [12,13].

The aldosterone antagonist of spironolactone is a diuretic and has been shown to lead to enzyme induction and to suppress many proinflammatory cytokines such as TNF-α and IL1β, and has been shown to have anti-inflammatory activity [7,14]. In our study, TNF-α and IL-1β levels were also found to be increased following mesenteric I/R as a consequence of inflammation. With administration of spironolactone, this proinflammatory cytokine has regressed to control levels.

In our study, myeloperoxidase (MPO) whose activity is assessed during evaluation of intestinal ischemic injury, was shown to be an enzyme released due to neutrophil activation and is present during the production of free oxygen radicals [15,16]. Various studies have demonstrated that increase in MPO activity as a sign of neutrophil infiltration during mesenteric I/R injury causes endothelia dysfunction and inflammation [17,18]. In our study, MPO values increased significantly with I/R, consistent with literature results. Taking into consideration the positive effects of spironolactone on blood parameters which was used in our study, this drug was found to markedly suppress myeloperoxidase enzyme in the tissue, suggesting that the enzyme plays an important role during IR. As a result, the presence of inhibitor properties on the MPO activity of agents used during IR therapy will facilitate the tissue to be protected against oxidation and

enhance the success of the treatment. Spironolactone has been reported to reduce increased myeloperoxidase activity during inflammatory events [7]. Results of all these studies suggest that our findings are compatible with literature results.

During the ischemic period, various functional changes occur at the cellular level. Particularly specific is the reduction in oxidative phosphorylation; this results in ATP depletion and disorder in calcium homeostasis. In addition, the absence of oxygen in cells during ischemia leads to mitochondrial degeneration, changes in Na+ homeostasis, and finally endothelial cell growth. Activation of these cells leads to inflammation, leading to an increase in the level of proinflammatory cytokines, chemokines and adhesion molecules resulting in the production of reactive oxygen derivatives and neutrophil-mediated injury [3,19].

Sodium-potassium adenosine triphosphatase (Na+/K+-ATPase) is an important membrane enzyme which plays a key role in cell structure and physiology by providing a sodium-potassium gradient in all cell membranes [20]. Tissue is the marker of viability during organ transplantation [20]. There is no study which adequately demonstrates the role of the Na+/K+-ATPase pump in the development of mesenteric I/R injury. In previous in vitro studies and transplantation models; Na+/K+-ATPase pump effects have been evaluated [13,21]. Results of our study paralleled results of this study, and the Na+/K+-ATPase activity in the IR group was found to be lower than that in the control group.This decrease was found to have been significantly prevented in the IR group treated with spironolactone.In the study conducted by Federova et al., it was demonstrated that decreased Na+/K+-ATPase activity with inflammation improved following administration of aldosterone antagonist [22].

4. CONCLUSION

In conclusion, our study results demonstrated that MPO activity and pro-inflammatory cytokine levels increased, whereas Na+/K+-ATPase activation reduced following the formation of IR in the ileum, leading to structural and functional damage in the ileal tissue. SPL provides protection by significantly reducing I/R injury in intestinal tissue. Our results are different from previous studies; Spironolactone has shown protective effect on mesenteric ischemia reperfusion injury with different mechanismis. Although spironolactone has been shown to have effect on MPO, sodium pump in previous studies; spironolactone has not shown any effect on these parameters in intestinal ischemia. In addition, we suggest that SPL may provide a new treatment modality in the prevention of organ damage and dysfunction which may arise as a result of I/R in the ileal tissue.

5. MATERIALS AND METHODS

This study was approved by Local Ethical Committee of Animal Experiments of Marmara University (Protocol No: 23.2017.mar).

A total of 24 Rattus species of Norvegicus Wistar albino rats were used in the study, weighing 200-250 g and which were 10 weeks old. The rats were randomly divided into three groups of eight each, and each cage was kept in wire cages under standard laboratory conditions (20-24 °C temperature, 50-60% humidity; 12 hours light and 12 hours darkness cycle) with four rats per cage; tap water and standard rat feed were released throughout the entire experimental period. Enteral and parenteral antibiotics were not used during the study.

Each rat was kept in a jar containing ether for induction anesthesia, for a period of 40-60 sec. Following induction anesthesia, ketamine was administered intraperitoneally (ip) at a dose of 100 mg/kg and chlorpromazine intraperitoneally (ip) at a dose of 1 mg/kg, for maintenance anesthesia.

5.1. Surgical technique and treatment application

The rates were anesthetized and placed on their backs with the anterior abdominal wall shaved. The shaved areas were sterilized using povidone iodine solution. Subsequently, the abdomen was assessed by a midline incision, the superior mesenteric artery was explored and ischemia period was established through clamping for 45 min. At the end of this period, the rats which were left for reperfusion were later decapitated after 60 min [23]. Blood and small intestinal tissue samples were collected at the end of the experimental period. Spironolactone (20 mg/kg, p.o. [Gavage]) or SF was administered were applied via oral route for 3 consecutive days and 30 minutes before ischemia [24].Control group rats were subjected to the Sham operation and administered saline solution.

5.2. Groups

Control Group: The superior mesenteric artery was isolated and exposed, but not ligated. Blood and tissue samples were obtained after 60 min.

Ischemia Reperfusion (IR) Group: The rats were administered saline solution 3 consecutive days and 30 minutes prior to the day of mesenteric ischemic procedure. Blood and tissue samples were obtained after the standard operation.

Spironolactone administered Ischemia Reperfusion (IR-SPL) Group: The rats were given 20 mg/kg SPL orally 3 consecutive days and 30 minutes before the time of the mesenteric ischemia procedure. Blood and tissue samples were obtained after the standard operation.

5.3. Serum Examinations

Serum TNF-α and İL-1β levels were measured by the ELISA method using rat kits.

5.4. Tissue Examinations

5.4.1. Myeloperoxidase (MPO) activity analysis

Tissue MPO assessment in these tissues was performed using the Hillegas method [25]. Immediately following decapitation, the extracted small intestinal tissue was washed with saline solution to remove blood and environmental waste, and then dried with filter paper. The small intestinal tissue was homogenized with 50 mM K2HPO4 (pH: 6)to prepare 10% homogenate and centrifuged at 41400 g for 10 min at 4 °C. The supernatant was discarded, and the samples which were re-homogenized by the addition of 0.5% Hexadecyl Trimethyl-ammonium bromide (HETAB) to the solution, were frozen, thawed and sonicated three times. The samples were then centrifuged at 41400 g for 10 minat a temperature of 4 °C.The color reaction was stopped by the addition of 2% sodium azide to the supernatant for three minand at 37 °C water bath, by adding 50 mM K2HPO4 (pH: 6), 20 mM H2O2 and o-Dianizidin-2 HCl. The samples were then centrifuged at 41400 g for 10 minat a temperature of 4 °C, and the absorbent color of the supernatant was read at 460 nm on a spectrometer.

5.4.2. Tissue Na+/K+-ATPase activity analysis

The tissue Na+/K+-ATPase activity was determined by the Soltoff and Mandel [26] method. Immediately after decapitation, the extracted small intestinal tissue was washed with saline solution to remove blood and environmental waste, and then dried with filter paper. The small intestinal tissue was homogenized with 0.32 M sucrose solution. A 10% homogenate was prepared and centrifuged at 3000 rpm for 5 min. The supernatant was incubated with ATPand trichloroacetic acid for five minat 37 °C and then centrifuged at 3000 rpm for 5 min. The absorbent color which was obtained after the addition of ammonium molybdate, Fiske-subbarow solution, and distilled water to the supernatant was read at 690 nm on a spectrometer.

5.4.3. Histopathological evaluation method

After collecting tissues into 10% formalin the tissues were washed in tap water for at least three hours and dehydrated with increasing alcohol concentrations (15 minwith 70% alcohol, 15 minwith 90% alcohol, 30 minwith 96% alcohol, twice for 30 minwith 100% alcohol, twice for 30 minwith 100% toluene), then placed at 60 °C for one night; the tissues were then buried in paraffin blocks the next day. After blocking, sections of 5-6 mm in thickness were taken from the tissues and placed on the slide and left in toluene for two hours for salvage followed by water reduction with decreasing concentrations of alcohol (two minwith 100% alcohol, two minwith 90%, treated with 70% alcohol for two min.) and left in distilled water. After treating for 15 minwith hematoxylin, it was left in the tap water for 10 minfor the blooming procedure. After addition of distilled water for five minwith eosin, dehydration was again performed with increasing concentrations of alcohol (two minwith 70% alcohol, two minwith 90%, two minwith 96%, and 10 minwith 100% alcohol). It was then washed twice with Toluene (1st bath five min, 2nd bath 10 min); the tissue was covered with Entellan; the tissue was examined under light microscopy.

5.5. Statistical analysis

Statistical analysis was performed using SPSS version 18.0 for Windows (SPSS, Inc., Chicago, IL, USA). Descriptive data were expressed in means ±SEM. One way analysis of variance (ANOVA) was used to compare TNF-α and IL-1β levels in the serum, and MPO with the Na+/K+-ATPase activity measured from ileal tissue. The Tukey's test was used for binary comparisons. A p value of <0.05 was considered statistically significant.

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REFERENCES

[1] American Gastroenterological Association American gastroenterological association medical position statement: Guidelines on intestinal ischemia. Gastroenterology. 2000;118(5):951–953.

[2] da Silva de Souza AC, Borges SC, Beraldi EJ, de Sá-Nakanishi AB, Comar JF, Bracht A, Natali MR, Buttow NC. Resveratrol reduces morphologic changes in the myenteric plexus and oxidative stress in the ileum in rats with ischemia/reperfusion injury. Dig Dis Sci. 2015;60(11):3252-3263.

[3] Gerkau NJ, Rakers C, Petzold GC, Rose CR. Differential effects of energy deprivation on intracellular sodium homeostasis in neurons and astrocytes. J Neurosci Res. 2017;95(11):2275-2285.

[4] Ji YY, Wang ZD, Wang SF, Wang BT, Yang ZA, Zhou XR, Lei NN, Yue WN. Ischemic preconditioning ameliorates intestinal injury induced by ischemia-reperfusion in rats.World J Gastroenterol. 2015;21(26):8081-8088.

[5] Kakoki M, Pochynyuk OM, Hathaway CM, Tomita H, Hagaman JR, Kim HS, Zaika OL, Mamenko M, Kayashima Y, Matsuki K, Hiller S, Li F, Xu L, Grant R, Bertorello AM, Smithies O. Primary aldosteronism and impaired natriuresis in mice underexpressing TGFβ1. Proc Natl Acad Sci USA. 2013;110(14):5600-5605.

[6] Kolkhof P, Jaisser F, Kim SY, Filippatos G, Nowack C, Pitt B. Steroidal and novel non-steroidal mineralocorticoid receptor antagonists in heart failure and cardiorenal diseases: Comparison at bench and bedside. Handb Exp Pharmacol. 2017;243:271-305.

[7] Sehirli AO, Cetinel S, Ozkan N, Selman S, Tetik S, Yuksel M, Dulger FG. St. John's wort may ameliorate 2,4,6-trinitrobenzenesulfonic acid colitis off rats through the induction of pregnane X receptors and/or P-glycoproteins. J Physiol Pharmacol. 2015;66(2):203-214.

[8] Malaise O, Relic B, Charlier E, Zeddou M, Neuville S, Deroyer C, Gillet P, Louis E, Malaise MG, de Seny D. Glucocorticoid-induced leucine zipper (GILZ) is involved in glucocorticoid-induced and mineralocorticoid-induced leptin production by osteoarthritis synovial fibroblasts. Arthritis Res Ther. 2016;18(1):219.

[9] Ojeda-Cervantes M, Barrera-Chimal J, Alberú J, Pérez-Villalva R, Morales-Buenrostro LE, Bobadilla NA. Mineralocorticoid receptor blockade reduced oxidative stress in renal transplant recipients: A double-blind, randomized pilot study. Am J Nephrol. 2013;37(5):481-490.

[10] Ypsilantis P, Lambropoulou M, Tentes I, Kortsaris A, Papadopoulos N, Simopoulos C. Mesna protects intestinal mucosa from ischemia/reperfusion injury. J Surg Res. 2006;134(2):278-284.

[11] Jensen AR, Drucker NA, Khaneki S, Ferkowicz MJ, Markel TA. hydrogen sulfide ımproves intestinal recovery following ıschemia by endothelial nitric oxide dependent mechanisms. Am J Physiol Gastrointest Liver Physiol. 2017;312(5): G450-G456.

[12] Liu XH, Yang YW, Dai HT, Cai SW, Chen RH, Ye ZQ. Protective role of adiponectin in a rat model of intestinal ischemia reperfusion injury. World J Gastroenterol. 2015;21(47):13250-13258.

[13] Fu TL, Zhang WT, Chen QP, Gao Y, Hu YH, Zhang DL. Effects of L-arginine on serum nitric oxide, nitric oxide synthase and mucosal Na+-K+-ATPase and nitric oxide synthase activity in segmental small-bowel autotransplantation model. World J Gastroenterol. 2005;11(23):3605-3609.

[14] Mikkelsen M, Sønder SU, Nersting J, Bendtzen K. Spironolactone induces apoptosis in human mononuclear cells. Association between apoptosis and cytokine suppression. Apoptosis. 2006;11(4):573-579.

[15] Hansberry DR, Shah K, Agarwal P, Agarwal N. Fecal myeloperoxidase as a biomarker for ınflammatory bowel disease. Cureus. 2017;9(1):e1004.

[16] Pullar JM, Carr AC, Bozonet SM, Rosengrave P, Kettle AJ, Vissers MC. Elevated seminal plasma myeloperoxidase is associated with a decreased sperm concentration in young men. Andrology. 2017;5(3):431-438.

[17] Lu YZ, Huang CY, Huang YC, Lee TC, Kuo WT, Pai YC, Yu LC. Tumor necrosis factor α-dependent neutrophil priming prevents intestinal ischemia/reperfusion-induced bacterial translocation. Dig Dis Sci. 2017;62(6):1498-1510.

[18] Mészáros AT, Büki T, Fazekas B, Tuboly E, Horváth K, Poles MZ, Szűcs S, Varga G, Kaszaki J, Boros M. Inhalation of methane preserves the epithelial barrier during ischemia and reperfusion in the rat small intestine. Surgery. 2017;161(6):1696-1709.

[19] Sehirli AO, Koyun D, Tetik Ş, Özsavcı D, Yiğiner Ö, Çetinel Ş, Tok OE, Kaya Z, Akkiprik M, Kılıç E, Şener G. Melatonin protects against ischemic heart failure in rats. J Pineal Res. 2013;55(2):138-148.

[20] Liu J, Yan Y, Nie Y, Shapiro JI. Na/K-ATPase signaling and salt sensitivity: The role of oxidative stress. Antioxidants (Basel). 2017;6(1):pii: E18.

[21] Orsenigo MN, Porta C, Sironi C, Laforenza U, Meyer G, Tosco M. Effects of creatine in a rat intestinal model of ischemia/reperfusion injury. Eur J Nutr. 2012;51(3):375-384.

[22] Fedorova OV, Emelianov IV, Bagrov KA, Grigorova YN, Wei W, Juhasz O, Frolova EV, Marshall CA, Lakatta EG, Konradi AO, Bagrov AY. Marinobufagenin-induced vascular fibrosis is a likely target for mineralocorticoid antagonists. J Hypertens. 2015;33(8):1602-1610.

[23] Soydan G, Cekiç EG, Tuncer M. Nebivolol has protective effect against endothelial and ileal dysfunction due to I/R. J Surg Res. 2011;166(1):156-161.

[24] Mejía-Vilet JM, Ramírez V, Cruz C, Uribe N, Gamba G, Bobadilla NA. Renal ischemia-reperfusion injury is prevented by the mineralocorticoid receptor blocker spironolactone. Am J Physiol Renal Physiol. 2007;293(1):F78-86.

[25] Hillegass LM, Griswold DE, Brickson B, Albrightson-Winslow C. Assessment of myeloperoxidase activity in whole rat kidney. J Pharmacol Methods 1990; 24(4) :285-295.

[26] [Soltoff SP, Mandel LJ. Active ion transport in the renal proximal tubule. III. The ATP dependence of the Na pump. J Gen Physiol. 1984; 84 (4):643-662.