The Egyptian Journal of Hospital Medicine (Apr. 2015) Vol. 59, Page 233- 243

The Therapeutic Role ofProximol and Lasilactone in Rat Model of Renal Stress

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ABSTRACT

Background

High salt intake induces renal-stress. The present study was carried out to examine the therapeutic effects of proximol(Halfa bar extract), lasilactonedrug(Spironolactone+Furosemide) and their combination on renal-stressed rats.

Material and Methods: Thirty five male rats were used and divided into five groups. The first group served as negative control and received fresh tap water orally for four weeks. The animals in the other four groups drank hypertonic saline solution (2% NaCl) as a sole source of drinking water for four weeks to induce the animal model of renal stress. Then the renal-stressed rats were further divided into: positive control, renal-stressed rats treated daily with proximol (7.8 mg/kg b.wt), renal-stressed rats treated daily with lasilactone (3.9 mg/kg b.wt), and renal- stressed rats treated daily with a combination of proximol and lasilactone for four weeks. The levels of aldosterone, sodium, potassium, calcium, urea, uric acid and creatinine were measured in the sera of rats. Nitric oxide (NO), reduced glutathione (GSH) and lipid peroxidation (MDA) levels were also measured in the homogenate of renal tissue.

Results: In the renal-stressed group, there was a significant increase in levels of aldosterone, sodium, calcium, urea, uric acid, NO and MDA and a significant decrease in potassium and GSH as compared to control group. Although the treatment of renal stressed rats with proximol, lasilactone and their combination reduced the increased level of aldosterone induced in renal stressed rats, aldosterone level was still higher than the control value. In addition, the treatment with proximol, lasilactone and their combination restored the significant increase in sodium, NO and lipid peroxidation to non significant changes as compared to control group. Also the decreased levels of GSH induced in renal-stressed rats returned to non significant changes. However, potassium decreased significantly below the control and the model groups with the combined treatment. Furthermore, treatment with proximol, lasilactone and their combination reduced the elevated levels of uric acid and urea induced by hypertonic saline solution to control- like values in the case of uric acid and to a significant decrease in the case of urea.

Conclusion: In conclusion, proximol, lasilactone and their combination have an effective role in ameliorating the changes in the levels of aldosterone, serum electrolytes, oxidative stress and consequently the disturbance in kidney functions in renal-stressed rats induced by hypertonic saline solution.

Keywords: renal-stressed rats, proximol, lasilactone, aldosterone, electrolytes, oxidative stress.

INTRODUCTION

High-salt diet is one of the major risk factors in the development of kidney stones, kidney disease, and ultimately kidney failure[1]and hypertension.[2] Studies carried out *in vitro* have recently shown that salt loading induces an increasing mechanical stretch and a flow-induced superoxide production in the thick ascending limb of Henle's loop. In this regard, it has been hypothesized that the oxidative stress induced by salt overload could stimulate inflammatory and fibrogenic signaling pathways in normal rats.[3] Consequently, the deterioration of renal function and impairment of salt and water clearance leads to edema and volume overload.[4]

The effects of a high-salt diet are related to the function of the renin-angiotensin system, which is normally suppressed by a high-salt diet.[5]Aldosterone, the principal human

DOI: 10.12816/0012181

mineralocorticoid (MR), is produced in the zona glomerulosa of the adrenal gland. Traditionally, the principal target organ for aldosterone was the kidney.[6]The bestcharacterized physiologic effect of aldosterone is to increase the reabsorption of sodium in the kidney and at other secretory epithelial sites at the expense of potassium and hydrogen ions.[7]Consequently, male albino rats chronically loaded with sodium by receiving 1% NaCl solution as the sole source of drinking water for six weeks, showed raised plasma Na+ concentration, lowered plasma K+ concentration and lowered haematocrit value.[8]

It has been observed that interaction between salt and aldosterone plays an important role in the development of organ damage and cardiovascular disease.[9&10]The excess of aldosterone in combination with an elevated salt intake resulted in renal inflammation, fibrosis, podocyte injury, and mesangial cell proliferation.[11&12]

Accordingly, aldosterone blockers have been used to attenuate chronic renal injury.[13]Diuretics are the oldest, least expensive, and still among the best antihypertensive medications. Initiation of diuretic therapy and the subsequent contraction of blood volume explain the initial fall in blood pressure. With continued diuretic therapy, blood volume is restored, and vasodilator mechanisms sustain the antihypertensive action.[14]

The study of **Birbari*et al*.**[15]suggested that lasilactone which is a combination of spironolactone and furosemide improves the hypotensive potency and minimizes the metabolic and electrolyte alterations.

Spironolactone is a synthetic steroid that competes for the cytoplasmic aldosterone receptor. It increases the secretion of water and sodium, while decreasing the excretion of potassium, by competing for the aldosterone sensitive Na+/K+ channel in the distal tubule of the nephron. Approximately 5% of the filtered Na+ load is ultimately excreted in the urine.[16] For decades, spironolactone has been considered as an antagonist at the aldosterone receptors of the epithelial cells of the kidney and was clinically used in the treatment of hyperaldosteronism and occasionally as a potassium-sparing diuretic.[17] In addition, spironolactone acts to decrease the amount of oxidative stress in patients being treated for chronic kidney disease with no change in serum creatinine.[18]

An alternative approach to renal protection is the use of loop diuretics such as furosemide. Loop diuretics are clearly ineffective in established acute renal failure [19&20] but, at least in the experimental situation, can exert a protective effect if given before a potential renal insult.[21-23]Furosemide inhibits sodium reabsorption in the thick ascending limb of the loop of Henle, thus inducing a reduction in tubular oxygen consumption and improving renal tolerance to hypoxia.[22]

It has been concluded that spironolactone and furosemide combination was an effective diuretic therapy compared to furosemide alone.[24]According to **Amonkan*et al*.**[25], furosemide increased urinary volume and increased the urinary excretion of electrolytes (Na^+, Cl^- and Ca^{2+}), urea and creatinine. However, during spironolactone treatment, **Chapman *et al*.**[26]recorded anincrease in the serum level of potassium, creatinine, glucose and high density lipoprotein cholesterol.Proximol, *Cymbopogonproximus*, Family Gramineae, locally known as halfa-bar, is an aromatic densely-tufted grass growing wildly and widely in Upper Egypt. The *Cymbopogonproximus*is highly reputed in folk medicine as an antispasmodic and urolithiasis (renal stone removal), and diuretic agent, and for gout.[27]The plant is used in the treatment of prostate inflammation, kidney disease, inhibition of kidney shrinkages, anthelminthic and for stomach pains.[28]

Due to the side effects and contraindications that have been observed with the use of these conventional diuretics, [29-31] the present study was conducted to evaluate the efficacy of proximol (halfa bar extract) as a diuretic and antioxidant agent in comparison to lasilactone using a rat model of renal stress.

Materials& Methods

The experimental animals

Thirty five male albino rats (*RattusRattus)*weighing 140 ±20 g were used in this study. Rats were obtained from

SchistosomaBiological Supply Program (SBSP), Theodor Bilharz Research Institute.

Experimental design

Animals were divided into five groups (7 rats per group). The first group (C) served as negative control and were fed on standard foodand allowed to drink fresh tap water throughout the study(for four weeks), while the animals in the other four groups drank hypertonic saline solution (2% NaCl) as a sole source of drinking water for four weeks to induce the rat model of renal stressaccording to**Florin *et al.*[32]**. Then the renal-stressed rats were further divided into: positive control (S) drinking hypertonic saline solution (renal-stressed rats), renal-stressed rats treated daily with proximol(H) (halfa bar extract) (7.8 mg/kg b.wt, orally), renal-stressed rats treated daily with lasilactone (L) (spironolactone/furosemide) (3.9 mg/kg b.wt, orally), and renal-stressed rats treated daily with an oral combination of proximol and lasilactone(HL) for four weeks.

At the end of the experimental period, the animals of both control (negative and positive groups) and treated groups were sacrificed by decapitation. Individual blood samples were collected from each rat for biochemical analyses and the kidney of each rat was dissected out. The kidney of each rat was homogenized in phosphate buffer solution (pH 7.4) and centrifuged at 5000 rpm. The supernatant was used for measuring nitric oxide, reduced glutathione and lipid peroxidation.

Biochemical assays:

Serum aldosterone concentration was measured by radioimmunoassay (RIA) technique, according to **Bravo *et al.*[33].**

sodium and potassium were estimated according to **Tietz[34]**, calcium was estimated according to **Faulker and Meites method [35]**, urea and uric acid were estimated according to **Young [36]**. creatinine was determined according to the method described by **Bartels and Bohmer method [37]**.

Determination of nitric Oxide, reduced glutathione and lipid peroxidation a in kidney tissue homogenate:

Nitric oxide was determined calorimetrically according to the method described by **Montgomery and Dymock[38]**, reduced glutathione was measured by the method of **Beutler*et al.*[39]** and lipid peroxidation was determined in kidney tissue homogenate using the method of **Ruiz-Larrea*et al.*[40]**.

Statistical analysis

The obtained results were statistically analyzed by using SPSS program according to the method of **Glantz[41]**. Significant differences among groups were determined by one-way analysis of variance **(ANOVA).**This was followed by post hoc test using Duncan to compare significance between groups when p-value <0.05.

Results

In the sera of the renal-stressed rats model induced by drinking hypertonic saline solution for 4 weeks, the levels of aldosterone, sodium and calcium increased significantly recording 128.7%, 4.3% and 6.3%,respectively compared with the control values.This was accompanied by a significant decrease (-10.7%) in potassium level**(Table 1)**.

Although the daily treatment of renal-stressed rats with proximol (halfa bar extract), lasilactone or proximol+lasilactone decreased aldosterone levels significantly below the renal-stressed rats, its levels were still higher than the control value. The daily treatment of renal-stressed rats with proximal, lasilactone or their combination restored the elevated levels of sodium to control-like value.However, potassium ions showed a nonsignificant changes after proximal and lasilactone and decreased significantly after the combined treatment. In addition, calcium ions returned to non significant change after proximal treatment but was still elevated after lasilactone and proximol+lasilactone treatments**(Table 1).**

Table (2) shows the effects ofproximol (halfa bar extract), lasilactone (spironolactone+furosemide) and their combination on the levels of nitric oxide (NO), reduced glutathione(GSH) and lipid peroxidation (MDA) in the kidney tissues of renal-stressed rats.

A significant increase in the levels of NO (123.1%) and MDA (121.3%) and a significant decrease in the level of GSH (-38.3%) were recorded in the kidney tissues of renal-stressed

rats as compared to control values. The treatment of the renal-stressed rats with proximol, lasilactone or proximol + lasilactone restored the increased levels of NO and MDA induced in the kidney tissues of renal-stressed rats to nearly control values. GSH levels were restored to control levels only after lasilactone treatment.

As shown in **table (3)**, a significant increase in levels of uric acid and urea was observed in the sera of renal-stressed rats recording 23.3% and 14.8%, respectively above the control values. However, creatinine showed a nonsignificantchanges. The treatment of renal-stressed rats with proximol, lasilactone or their combination restored the significant increase in uric acid to control-like values and reduced the elevated urea levels below the control value.

Discussion

Effect of either poximol ,lasilactone or their combination on the serum levels of aldosterone, sodium , calcium and potassium ions of stressed rats.

In the present study drinking hypertonic saline solution resulted in a significant increase in the serum levels of aldosterone, sodium , calcium and a significant decrease in potassium ions.

The present findings are in agreement with the study of **Bayorh*et al*.** [42]who found that high dietary salts intake to Dahl salt sensitive rats resulted in an increase in angiotensin II that caused an increase in the aldosterone level. In addition, it has been observed that salt loading was associated with inadequate suppression of aldosterone production and increased aldosterone secretion in response to angiotensin II.[43]

Therefore, the significant increase in the level of serum aldoserone observed in the present study as a consequence of drinking hypertonic saline solution could be mediated by the production of angiotensin II that stimulates the secretion of aldosterone.[44]

The present results shows that there was a significant increase in serum aldosterone level in the renal-stressed rats model induced by hypertonic saline solution was reduced from 128.7% to 53.6 % by daily treatment with proximol and to 64.7 % by daily treatment with lasilactone. Moreover, the combined treatment of the renal-stressed rats with both lasilactone and proximol reduced the aldosterone level to 23.8%.

The present findings indicated that the combined treatment with both lasilactone and proximol was more potent in reducing aldosterone level than either proximol or lasilactone.

Spironolactone which is one of the lasilactoneingradients has an inhibitory effect on aldosterone biosynthesis.[17&45] In addtition, the study of **Leclerc *et al*.**[46] showed that spironolactone acts as an antagonist at the aldosterone receptors of the epithelial cells of the kidney.

Thus, it could be concluded that lasilactone reduced the aldosterone level by inhibiting its synthesis and inhibiting its effect by acting as an antagonist at the aldosterone receptors.

Also, it could be deduced that proximol reduced the level of aldosterone by inhibiting its synthesis and/or antagonizing its receptors. However, this suggestion needs more investigation.

The significant increase in the serum levels of sodium and calcium ions and the significant decrease in potassium ions that were recorded in the present study in response to the intake of hypertonic saline solution are in linewith the findings of **Gan and Tan** [8]in which male albino rats were chronically loaded with sodium as 1 % of sodium chloride solution as a sole source of drinking water.

It has been reported that the increased concentration of aldosterone leads to increased re-absorption of sodium ions and water from epithelial cells in the distal nephron of the kidney and increased excretion of potassium ions.[47] This in turn may explain the present significant increase in sodium and decrease in potassium in the serum of rat model of renal stress.

The significant increase in the serum calcium level in the present study could be due to the enhancement of calcium reuptake under the effect of aldosterone.

The study of **Leclerc *et al*.**[46]showed that aldosterone enhances sodium and calcium re-absorption and the effect of aldosterone on the calcium ions is mediated by L type(Long-Lasting) and T type (transient opening) of

calcium channels in the distal lumen membrane.

Supporting this explanation is the reduction of serum calcium levels by proximol where the decreased level of aldosterone induced by proximol treatment prevented the reuptake of calcium and sodium and normalized their levels in the serum.

In the light of the obtained results, it could be concluded that proximol exerts its diuretic effect by reducing serum aldosterone level. This in turn will prevent the retention of sodium and calcium ions and normalize the electrolyte levels in serum.

In the present study, although the treatment of renal-stressed rats with lasilactone alone and in combination with proximol reduced the serum aldosterone level, the level of calcium was still elevated. This effect may be attributed to the effect of spironolactone on serum calcium level as spironolactone decreasesurinary calcium excretion (has a calcium-sparing effect).[48] In addition, the treatment of renal-stressed rats with proximol+ lasilactone reduced the serum level of potassium significantly below the control and renal-stressed values.

Although spironolactone which is one of the lasilactone ingredients is a potassium-sparing diuretic and can increase the potassium level,[49] its combination with furosimide and proximol decreased the serum potassium level in the present study. This could be due to the diuretic effect of furosemide which is aggravated by proximol. Therefore, the dose of proximol and lasilactone needs to be adjusted or refined in the case of the combined treatment between lasilactone and proximol.

The present data revealed that proximal (halfa bar extract) could exert diuretic effects solely or in combination with the other diuretics.

Oxidative stress

Under normal circumstances, the reactive oxygen species (ROS) generated are detoxified by the antioxidants present in the body and there is an equilibrium between the ROS generated and the antioxidants present.[50] Detrimental effects caused by ROS occur as a consequence of an imbalance between the formation and inactivation of these species.[50] However, owing to ROS overproduction and/or inadequate antioxidant defense, this equilibrium is hampered favoring the ROS upsurge that culminates in oxidative stress.[50]

In the present study, rats drinking hypertonic saline solution showed a significant increase in the level of nitric oxide and lipid peroxidation in the kidney tissue. This was accompanied by a significant decrease in reduced glutathione level.These results indicated the evolution of a state of oxidative stress which agrees with the study of**Dobrain*et al.***[51]who observed that high salt intake induced oxidative stress in the vasculature and kidney tissues and resulted in kidney glomerulosis.

Recent studies showed that high salt intake correlated with the higher concentration of marinobufagenin, the hormone known to mediate natriuresis and oxidative stress.[52] In addition, salt induced oxidative stress plays a role in salt-induced kidney damage.[53] The injurious pathways of high salt intake in the kidney that include oxidative stress and renal expression of NADPH oxidase as well as superoxide dismutase have been demonstrated in rat experiments.[54]

Oxidative stress occurs when there is an imbalance between the generation of reactive oxygen species or reactive nitrogen species and the antioxidant defense system so that the latter become overwhelmed.[55] Lipid peroxidation arises from the attack of the cell membrane by the evolved free radicals.[56]

Although, nitric oxide serves beneficial roles as a messenger and host defense molecule, excessive nitric oxide production can be cytotoxic. The result of nitric oxide reaction with reactive oxygen leads to the production of the most potent injurious molecule peroxynitrite**.**[57] Excessive nitric oxide production contributes to the pathogenesis of a variety of renal and vascular diseases characterized by inflammation and injury including glomerulonephritis,[58] tubulointerstitial renal disease[59]and postschemic renal failure**.**[60&61]

It should be noted that oxidative stress is often counteracted by reduced glutathione resulting in its depletion.[62] This indicates that renal injury induced by drinking hypertonic saline solution is the result of oxidative stress inducedby excessive generation of ROS, which

have been reported to attack various biological molecules including lipids and cellular membrane causing lipid peroxidation.Accordingly, it could be suggested that the present increased level of lipid peroxidation in the kidney tissue as a result of drinking hypertonic saline solution may be mediated by the attack of the cells membrane by the peroxynitrite radicals. Furthermore, the decrease in reduced glutathione level may be due to its exhaustion in scavenging the free radicals. Glutathione in its reduced form is the most abundant intracellular antioxidant involved in scavenging free radicals or serving as a substrate for glutathione peroxidase enzyme that catalyses the detoxification of hydrogen peroxide (H_2O_2).[63]

When the renal-stressed rats drinking hypertonic saline solution were treated daily either with proximol, lasilactone or their combination, the levels of lipid peroxidation, nitric oxide and reduced glutathione returned to nonsignificant changes as compared to control values. **El-Nezhawya*et al.*[64]**reported that proximol has antioxidant activity. This antioxidant activity of proximol was attributed to the plant contents of flavenoids, rutenand quericetine that are well known antioxidants.[65]The antioxidant activity of lasilactone is due to the reported antioxidant activity of spironolactone,[66] in addition to the robust antioxidant status of furosemide and its free radical scavenging effects.[67]

A growing evidence indicates that reduced glutathione plays a vital role in cellular function. It detoxifies toxic metabolites of drugs, regulates gene expression, apoptosis, and transmembrane transport of organic solutes.[68] Reduced glutathione, which constitutes one of the physiologicallyimportant mechanisms to curtail progression of tissue damage, is generally affected under the conditions of oxidative stress.[62] Therefore, depletion of reduced glutathione levels in the kidney after drinking hypertonic saline solution as observed in the present study makes the kidney tissue susceptible to damage, indicating the occurrence of free radical reactions and oxidative stress in kidney tissue.

Oxidative stress through a series of events, dysregulates cellular physiology and its sustained presence may lead to pathogenesis of several chronic ailments.[69] In addition, lipid peroxidation is linked with excessive generation of ROS, which may be attributed to exogenous or endogenous sources and is the most destructive process in the living cells that has been implicated in causing a wide range of biological effects such as increased membrane rigidity, osmotic fragility, decreased cellular deformation, reduced erythrocyte survival, and membrane fluidity.[69&70]

Thus, it could be suggested that the state of oxidative stress observed in the kidney tissue in the present study was mitigated by the antioxidant activity of proximol and lasilactone and their combination.

Kidney functions

Serum creatinine, urea and uric acid are considered as markers for altered renal functions and were measured in the present work to evaluate the changes in kidney functions under the effect of drinking salt water (2%) for four successive weeks.Creatinine the end product of creatine metabolism; diffuses passively into the blood stream, where it is removed by the glomerular filtration action of the kidney. It passes through the tubular system, where only a very small additional amount of creatinine is added by the tubular secretion**.**[71]

The present results shows that there is an impairment in the kidney functions in rats that drank hypertonic saline solution. This was indicated from the significant increase in serum urea and uric acid.

The present results are consistent with the study of **Durack*et al.*[1]**who reported that the excess salt intake resulted in the development of kidney disease and ultimately kidney failure. There is a good evidence indicating that uraemia in general is associated with enhanced oxidative stress.[72]

Accordingly, the impairment in kidney function reported in the present study in response to renal stressinduced by hypertonic saline solution could be mediated by oxidative stress.

When the renal-stressed rats were treated with proximol, lasilactone or their combination, the

elevated levels of serum uric acid were restored to non significant changes as compared to control values. In addition, the three treatments reduced the urea levels below the control value.These results confirmed the efficacy of proximal and lasilactone against the deterioration in renal functions. These effects can be attributed to the recorded potent antioxidant effect of proximol andlasilactone and to the effective renal antispasmodic and diuretic actions of the Egyptian folk medicine known as "halfabar".[27&73] Also proximol has anti-inflammatory effects.[74&75]The antispasmodic properties of proximol are unique as it produces relaxation of the smooth muscle fibers without abolishing the propulsive movement of the tissue.[76]

In conclusion:

The present study revealed that proximol has a diuretic effect and can regulate the serum levels of some electrolytes. These effectsare mediated by the effect of proximal on aldosterone level. In addition, the antioxidant effects of proximal and lasilactonemitigated the state of oxidative stress induced in the kidney by hypertonic saline solution. Consequently, proximal and lasilactone restored the impairment in kidney functions to the normal state.

Therefore, proximal may be a safe and effective alternative diuretic agent that can be used alone or in combination with other diuretic agents.

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Table (1): The effects of proximol, lasilactone and proximol+lasilactone on the serum levels of aldosterone, sodium, potassium and calcium in renal-stressed male rat models induced by hypertonic saline solution compared to control group (each included 7 rats).

	Control	**Renal-sressed rats**	**Renal-stressed rats treated with Proximal**	**Renal-stressed rats Treated with Lasilactone**	**Renal-stressed rats treated with proximol+lasilactone**
Aldosterone (ng/l)	$301.6^{a} \pm 4.409$	$690.0^{b} \pm 5.773$	$463.3^{c} \pm 13.333$	$496.7^{d} \pm 3.333$	$373.3^{e} \pm 12.018$
Sodium (mmol/l)	$136.3^{a} \pm 1.021$	$142.2^{b} \pm 1.137$	$134.8^{a} \pm 1.301$	$134.0^{a} \pm 0.930$	$136.7^{a} \pm 0.557$
Potassium (mmol/l)	$5.58^{a} \pm 0.237$	$4.98^{b} \pm 0.070$	$5.33^{ab} \pm 0.210$	$5.43^{ab} \pm 0.142$	$4.40^{c} \pm 0.089$
Calcium (mg/100ml)	$9.50^{a} \pm 0.224$	$10.1^{b} \pm 0.037$	$9.75^{ab} \pm 0.324$	$10.22^{b} \pm 0.054$	$10.17^{b} \pm 0.076$

- Values represent the mean ± S.E.
- p value < 0.05 was considered significant.
- Statistically significant means (p value < 0.05) are given different letters ; a, b, c, d, and e. The groups that showed a non significant change between each other take the same letter, but the group that showed a significant change compared to the others groups take a different letter.

Table (2): The effects of proximol, lasilactone and proximol+lasilactone on the serum levels of nitric oxide (NO), reduced glutathione (GSH) and lipid peroxidation (MDA) in renal-stressed male rat models induced by hypertonic saline solution compared to control group (each included 7 rats).

	Control	**Renal-stressed rats**	**Renal-stressed rats treated with proximol**	**Renal-stressed rats treated with Lasilactone**	**Renal-stressed rats treated with proximol+lasilactone**
NO (µmol/g)	$0.13^{a} \pm 0.01$	$0.29^{b} \pm 0.05$	$0.14^{a} \pm 0.02$	$0.16^{a} \pm 0.02$	$0.13^{a} \pm 0.02$
GSH (mmol/g)	$3.13^{a} \pm 0.29$	$1.93^{b} \pm 0.17$	$2.63^{ab} \pm 0.27$	$3.23^{a} \pm 0.34$	$2.53^{ab} \pm 0.24$
MDA (nmol/g)	$5.40^{a} \pm 1.038$	$11.96^{b} \pm 0.13$	$4.98^{a} \pm 0.24$	$6.75^{a} \pm 1.35$	$6.22^{a} \pm 1.43$

- Values represent the mean ± S.E.
- p value < 0.05 was considered significant.
- Statistically significant means (p value < 0.05) are given different letters ; a, b, c, d, and e. The groups that showed a non significant change between each other take the same letter, but the group that showed a significant change compared to the others groups take a different letter.

Table (3): The effects of proximol, lasilactone and proximol+lasilactone on the serum levels of uric acid, urea and creatinine in renal-stressed male rat models induced by hypertonic saline solution ompared to control group (each included 7 rats).

	Control	**Renal- stressed rats**	**Renal-stressed rats treated with proximol**	**Renal-stressed rats treated with lasilactone**	**Renal-stressed rats treated with proximal+lasilactone**
Uric acid (mg/dl)	$2.45^{a} \pm 0.176$	$3.02^{b} \pm 0.124$	$2.52^{a} \pm 0.116$	$2.58^{a} \pm 0.124$	$2.62^{a} \pm 0.111$
Urea (mg/dl)	$23.0^{a} \pm 0.912$	$26.4^{b} \pm 0.60$	$15.5^{c} \pm 0.428$	$17.83^{d} \pm 0.477$	$20.0^{e} \pm 0.949$
Creatinine (mg/dl)	$0.54^{a} \pm .019$	$0.56^{a} \pm 0.013$	$0.56^{a} \pm 0.037$	$0.51^{a} \pm 0.017$	$0.55^{a} \pm 0.019$

- Values represent the mean ± S.E.
- p value < 0.05 was considered significant.
- Statistically significant means (p value < 0.05) are given different letters ; a, b, c, d, and e. The groups that showed a non significant change between each other take the same letter, but the group that showed a significant change compared to the others groups take a different letter.