Egypt. J. of Appl. Sci., 35 (11) 2020 *117-126*

STUDYING THE EFFECT OF TWO TYPES OF SALT IN PROGRESSION OF CHRONIC RENAL FAILURE IN RATS

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Key Words: chronic kidney disease, rock salt, refined salt, rats.

ABSTRACT

Salt ingestion has been linked to kidney disease via hemodynamic (increased introglomerular pressure) and non-hemodynamic mechanisms (increased oxidative stress), independent of blood pressure. The aim of the present study was to compare the effect of rock salt (Halite) to refined salt in different concentrations on chronic renal failure rats. Thirty adult male Sprague-Dawley rats which weighing (170±5g) were divided randomly into two main groups as follow: the first group (-ve control= 5 rats) was fed on basal diet. The second group (25 rats) were fed on 14 % casein diet containing 0.7% adenine for 4 weeks to induce chronic kidney disease (CKD), then divided into 5 subgroups from group 2 to group 6. Subgroup 2 (+ve control) fed on basal diet. Subgroup 3 and 4 fed on basal diet supplemented with 4% and 8% of refined salt, respectively. Subgroup 5 and 6 fed on basal diet supplemented with 4% and 8% of rock salt, respectively. At the end of the experimental period (4 weeks), rats were scarified and serum was collected to determine kidney functions. The results showed that serum concentrations of creatinine, urea, uric acid, sodium and potassium were significantly elevated ($P<0.05$) by adenine administration (positive control) compared to negative control, in contrast, serum total protein, albumin and globulin were significantly reduced ($P<0.05$). Also, it was indicated that rock salt administration recorded the best changes for these parameters in CKD rats compared to refined salt. It be concluded that restriction of sodium intake is an important preventive and therapeutic measure in patients with chronic renal diseases or at risk of renal damage such as hypertensive. So, it be recommended to use rock salt instead of refined salt and further studies are required to elucidate beneficial effect and the mechanism of how rock salt attenuates blood pressure.

INTRODUCTION

Chronic kidney disease (CKD) is an important, widespread clinical problem which has multiple etiologies. Control of blood pressure, cholesterol, and glucose are important strategies to slow progression of CKD towards end-stage renal disease (ESRD) **(Walker *et al*., 1989)**.

The common sources of salt for consumers can be classified into refined (table) salt, sea salt, rock salt, and processed salt **(Lee *et al*.,**

2007). The relationship between salt and blood pressure has been discussed in medical literature for decades and it has been shown that increased salt intake contributes to the prevalence of hypertension and proteinuria **(Swift *et al*., 2005)**.

Less intensely studied are the direct nephrotoxic effects of sodium chloride. Salt ingestion has been linked to kidney disease via hemodynamic (e.g. increased introglomerular pressure) and non-hemodynamic mechanisms (e.g. increased oxidative stress), independent of blood pressure **(Weir and Fink, 2005)**.

Although there is growing awareness by the general public of the health benefits of rock salt, there is little information on whether consumption of rock salt can have a direct effect on blood pressure and CKD **(Chanumuang, 2010)**.

The aim of the present study was to compare the effect of rock salt (Halite) to refined salt in different concentrations on chronic renal failure rats.

MATERIALS AND METHODS

MATERIALS:

Rock salt and refined salt in the form of powder, were purchased from El-Gomhoriya Pharm., Cairo, Egypt. Adult male Sprague-Dawley rats (n= 30) which weighing (170±5g) were purchased from Farm of experimental animals in Helwan, Egypt. Adenine, casein, all vitamins, minerals, cellulose, choline and starch were obtained from El-Gomhoria Company, Cairo, Egypt.

METHODS:

1- Induction of CKD in rats:

Rats were inducted with chronic renal failure by using the method described by **(Yokozawa *et al*., 1986)** by feeding rats on 14 % casein diet containing 0.7% adenine for 4 weeks.

2- Preparation of salts:

Salts were grinded, then the powder was mixed with basal diet in different levels.

3- Diet composition and experimental animal design:

The basal diet was formulated according to AIN-93M diet **(Reeves *et al*., 1993)**. Animals (30 rats) were housed in well conditions in biological studies lab of Faculty of Home Economics. They were left for seven days as adaptation period and they were allowed to feed standard laboratory food and water. After the period of adaptation, animals were divided into two main groups, as follows: - the first group (5 rats) was fed on basal diet and served as a negative control group (-ve), the second group (25 rats) was fed on 14 % casein diet containing 0.7% adenine for 4 weeks. After chronic renal failure rats were divided as follow:-

Subgroup (1): Five Rats with chronic kidney disease (CKD rats) were fed on basal diet as positive control group (+Ve).

Subgroup (4): Five CKD Rats were fed on basal diet supplemented with 4 % Refined salt (Replacement with salt in basal diet).

Subgroup (5): Five CKD Rats were fed on basal diet supplemented with 8 % Refined salt (Replacement with salt in basal diet).

Subgroup (2): Five CKD Rats were fed on basal diet supplemented with 4 % Rock salt (Replacement with salt in basal diet).

Subgroup (3): Five CKD Rats were fed on basal diet supplemented with 8 % Rock salt (Replacement with salt in basal diet).

At the end of the experimental period (4 weeks), rats were fasted overnight before scarifying and blood samples were collected from each rat and were centrifuged at 3000 rpm for 15 min to obtain the serum for biochemical analysis.

4- Biological evaluation:

Feed intake (FI), body weight gain percentage (BWG %) and feed efficiency ratio (FER) were determined according to **Chapman *et al*., (1959)** using the following equation:

BWG% = Final body weight – Initial body weight / Initial body weight × 100

FER = Weight gain / Feed intake.

5- Chemical Analysis of Rock Salt and Refined Salt:

Chemical analysis of rock salt and refined salt were done in Agriculture Research Center and determined according to method describe by **Ranganna, (1977)**.

6- Biochemical analysis of serum:

Serum uric acid was determined in the serum according to method describe by **Milena, (2003)**. Serum urea nitrogen, creatinine and total Protein were determined in the serum according to method describe by **Burtis and Ashwood, (1999)**. Serum potassium and Serum sodium were determined according to method describe by **Hoeflmayr, (1979)**. Albumin and globulin were determined in the serum according to method describe by **Young, (2001)** and **Goldenberg and Drewes, (1971),** respectively. Serum sodium and potassium were determined according to method describe by **Guder, *et al*., (1982) and Hoeflmayr, (1979)**.

7- Statistical Analysis:

Results were expressed as the mean standard error ± SE. Data were statistically analyzed for variance "ANOVA" test at $P \leq (0.05)$

using SPSS statistical software, version 20 was used for these calculations **(Armitage and Berry, 1987)**.

RESULTS AND DISCUSSION

Data in **Table (1)** showed the chemical analysis of of rock salt and refined salt, it was observed that rock salt contained 87.98% of NaCl while refined salt contained 99.9% of NaCl.

Lee *et al*., (2017) reported that rock salt that contains 85.7% NaCl whereas refined salt contained 99.9% NaCl. In addition to sodium, rock salt also contains calcium (1.5 mg/g), potassium (2.9 mg/g), magnesium (3.9 mg/g), and traces amounts of iron, manganese and zinc.

Table 1: Chemical Analysis of Rock Salt and Refined Salt

Sample	NaCl%
Rock salt	87.98
Refined salt	99.9

Results recorded in **Table (2)** showed the effect of rock salt and refined salt on body weight gain (BWG), feed intake (FI) and feed efficiency ratio (FER) in CKD rats. It was observed that FI, BWG and FER significantly decreased in positive control compared to negative control. Group that fed on refined salt recorded the lower reduction in FI, BWG and FER than group fed on rock salt. Group that fed on 4% rock salt recoded the lowest reduction in FI, BWG and FER compared to positive control.

Table (2): Effect of Rock Salt and Refined Salt on Feed Intake (FI), Body Weight Gain% (BWG %) and Feed Efficiency Ratio (FER) of Chronic Renal Failure Rats

Parameters / Groups	FI (g/d)	BWG%	FER
Negative Control	**16.5**	**21.91±1.44^{a}**	**0.070±0.004^{a}**
Positive Control	**15.2**	**9.11±0.30^{b}**	**0.030±0.001^{b}**
8% Refined Salt	**11.55**	**3.15±0.17^{d}**	**0.018±0.002^{d}**
4% Refined Salt	**14**	**4.50±0.44^{cd}**	**0.020±0.001^{cd}**
8% Rock Salt	**13.5**	**4.18±0.34^{cd}**	**0.018±0.002^{d}**
4% Rock Salt	**14.15**	**6.05±0.43^{c}**	**0.026±0.003^{bc}**

***Mean values are expressed as means ± SE.**

***Mean values at the same column with the same superscript letters are not statistically significant at P<0.05.**

Results of BWG were in the same line with **Lee *et al*., (2017),** who found that the final body weight in the group that fed on the high level of refined salt was significantly lower than all other groups. In the current study, feed intake of refined salt group was the lowest compared to the rock salt group. This was due to larger amount of food consumption by the rock salt groups despite the refined salt diet

containing a higher salt content than the rock salt diet. The fact that rock salt diets were consumed at a higher rate than the refined salt diets may be an indication that the chow with rock salt was more palatable to the rats **(Lee *et al*., 2017)**.

As showed in **Table (3),** serum creatinine, urea and uric acid were elevated significantly (P< 0.05) elevated by adenine administration in positive control group compared to the negative control group. It was observed that the group that fed on high level of refined salt (8%) recorded the most increase in serum creatinine, urea and uric acid levels compared to all groups. While the rock salt group (4%) recorded the lower increase in these parameters than other groups that treated with the different levels of salts compared to positive control group.

Concerning results of kidney functions in were confirmed by **Saad *et al*., (2018),** who showed that adenine administration significantly increased serum urea, creatinine and uric acid. Salt intake is associated with the development of impaired kidney function in the general population, independent of its effects on blood pressure **(Sugiura *et al*., 2018)**. Rock salt contains trace amounts of natural minerals such as MgSO4, CaSO4, CaCl2 and KCl with slightly lower sodium content compared to refined salt. Although there is growing awareness by the general public of the health benefits of rock salt, there is little information on whether consumption of rock salt can have a direct effect on blood pressure regulation **(Chanmuang, 2010)**.

Table (3): Effect of Rock Salt and Refined Salt on Serum Creatinine, Urea and Uric Acid of Chronic Renal Failure Rats

Parameters / Groups	Creatinine	Urea	Uric Acid
	mg/dL		
Negative Control	0.62±0.05^{f}	23.15±0.43^{e}	2.75±0.75^{e}
Positive Control	0.91±0.09^{e}	30.05±0.29^{d}	3.11±0.04^{d}
8% Refined Salt	1.14±0.04^{a}	35.63±0.31^{a}	5.79±0.10^{a}
4% Refined Salt	0.96±0.06^{c}	33.22±0.30^{b}	4.62±0.80^{b}
8% Rock Salt	0.98±0.05^{b}	35.16±.48^{a}	4.59±0.84^{b}
4% Rock Salt	0.93±0.06^{d}	31.15±0.20^{c}	3.42±0.13^{c}

***Mean values are expressed as means ± SE.**

***Mean values at the same column with the same superscript letters are not statistically significant at P<0.05.**

Results in **Table (4)** showed a significant (P< 0.05) increased in serum sodium and potassium in positive control by administration of adenine compared to negative control group. Rats that fed on the high level of refined salt (8%) recorded the highest elevation in serum Na and K compared to other treated groups with salts, while rats that fed on 4% rock salt recorded the lowest elevation in serum Na and K.

Results of potassium were in agreement with **Kashioulis *et al*., (2018),** who reported that plasma concentration of potassium was clearly elevated in rats that treated with adenine. It is known that dietary potassium can influence blood pressure, and that it can play a role in controlling blood pressure in hypertensive patients **(Rodrigues *et al*., 2014)**. **Suzuki *et al*., (2007)** which reported that salt is essential and important for maintaining life. Sodium is an essential nutrient and one of the important cations in the extracellular fluid. Several studies have suggested that excess salt intake deteriorates a renal function and increase in blood pressure. In these regards, modulation of salt is of utmost importance in the fields of hypertension and nephrology **(McMahon *et al*., 2013)**. **Lee *et al*., (2017)** reported that how the rock salt diet actually exerts its anti-hypertensive effects is not clear. One obvious point to consider is the content of minerals other than sodium in the rock salt.

Table (4): Effect of Rock Salt and Refined Salt on Serum Sodium (Na) and Potassium (K) of Chronic Renal Failure Rats

Parameters / Groups	Na	K
	mg/dL	
Negative Control	142.80±0.86^{e}	4.07±0.04^{e}
Positive Control	149.00±1.14^{d}	4.60±0.11^{d}
8% Refined Salt	167.00±1.00^{a}	7.09±0.09^{a}
4% Refined Salt	153.80±0.77^{c}	5.01±0.04^{c}
8% Rock Salt	157.99±0.32^{b}	6.05±0.0^{b}
4% Rock Salt	151.20±0.41^{cd}	4.61±0.17^{d}

***Mean values are expressed as means ± SE.**

***Mean values at the same column with the same superscript letters are not statistically significant at P<0.05.**

Data in **Table 5** revealed that serum total protein, albumin and globulin levels were decreased by adenine administration in positive control group compared with the negative control group. Results also illustrated that rats treated with 8% refined salt recorded the highest reduction in serum total protein, albumin and globulin compared with other treated groups with salts, while rats that fed on 4% rock salt recorded the lowest reduction in these parameters.

Results of serum total protein and albumin were in agreement with **Saad *et al*., (2018),** who reported that the administration with adenine significantly decreased serum albumin and total protein. Salt restriction significantly reduced urinary protein excretion in all studies that reported proteinuria **(McMahon *et al*., 2015)**. In fact, a recently published double-blind controlled randomized trial in patients with CKD (stages 3 and 4) showed that dietary sodium restriction significantly

decreased blood pressure, and consistent reductions in proteinuria and albuminuria were shown **(McMahon *et al*., 2013 and Hosohata, 2017)**.

In conclusion, it would appear that restriction of sodium intake is an important preventive and therapeutic measure in patients with chronic renal diseases or at risk of renal damage such as hypertensive. It is likely that the major beneficial effect of rock salt is associated with the mineral content of the rock salt that is known to be anti-hypertensive such as potassium, calcium and magnesium. It is also possible that there are as yet undetermined component(s) of the rock salt that might confer resistance to hypertension. So, further studies are required to elucidate the mechanism of how rock salt attenuates blood pressure. Based on our findings it would also be important to determine if rock salt consumption would have similar effects on blood pressure in humans.

Table (5): Effect of Rock Salt and Refined Salt on Serum Total Protein, Albumin and Globulin of Chronic Renal Failure Rats

Parameters / Groups	Total Protein	Albumin	Globulin
	g/dL		
Negative Control	8.84±0.10^{a}	3.65±0.13^{a}	2.45±0.05^{a}
Positive Control	7.58±0.11^{b}	2.99±0.07^{b}	2.00±0.04^{b}
8% Refined Salt	5.95±0.13^{d}	0.98±0.01^{e}	1.04±0.03^{c}
4% Refined Salt	6.71±0.07^{c}	2.04±0.2^{d}	1.89±0.05^{b}
8% Rock Salt	6.11±0.04^{d}	1.97±0.04^{d}	1.17±0.04^{c}
4% Rock Salt	7.03±0.19^{c}	2.28±0.12^{c}	1.99±0.17^{b}

***Mean values are expressed as means ± SE.**

***Mean values at the same column with the same superscript letters are not statistically significant at P<0.05.**

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دراسة تأثير نوعين من الملح في تطور مرض الفشل الكلوي المزمن في الفئران

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يرتبط تناول الملح بأمراض الكلى عن طريق الدورة الدموية (زيادة الضغط داخل الكبيبات) والآليات غير الديناميكية الدموية (زيادة الإجهاد التأكسدي) ، بغض النظر عن ضغط الدم. الهدف من هذه الدراسة هو مقارنة تأثير الملح الصخري بالملح المكرر بتركيزات مختلفة على الفئران المصابة بالفشل الكلوي المزمن. تم تقسيم ثلاثون من ذكور الفئران البيضاء التي تزن (170 + 5 جم) عشوائياً إلى مجموعتين رئيسيتين على النحو التالي: المجموعة الأولى (مجموعة ضابطة سالبة = 5 فئران) تم تغذيتها على النظام الغذائي الأساسي. تم تغذية المجموعة الثانية (25 فأرا) على 14٪ من الكازين على نظام غذائي يحتوي على 0.7٪ أدينين لمدة 4 أسابيع للحث على مرض الكلى المزمن ، ثم تم تقسيمها إلى 5 مجموعات فرعية من

المجموعة 2 إلى المجموعة 6. المجموعة الفرعية 2 (مجموعة ضابطة موجبة) تم تغذيتها على النظام الغذائي الأساسي. تم تغذية المجموعتين الفرعيتين 3 و 4 على الغذاء الأساسي المضاف إليه 4٪ و 8٪ ملح مكررعلى التوالي. المجموعة الفرعية 5 و 6 تغذى على الغذائي الأساسي مكمل بـ 4٪ و 8٪ ملح صخري على التوالي في نهاية الفترة التجريبية (4 أسابيع) تم تشريح الفئران وجمع السيرم لإجراء تحاليل وظائف الكلى. أظهرت النتائج أن تركيزات الكرياتينين واليوريا وحمض البوليك والصوديوم والبوتاسيوم في سيرم الدم ارتفعت معنويا عن طريق التغذية على الأدينين (المجموعة الضابطة الموجبة) مقارنة بالمجموعة الضابطة السالبة، على النقضي من ذلك انخفض البروتين الكلي في الدم والألبومين والجلوبيولين بشكل ملحوظ. كما تم الإشارة إلى أن إعطاء الملح الصخري سجل أفضل التغيرات لهذه التقديرات في الفئران المصابة بالفشل الكلوي المزمن مقارنة بالملح المكرر. نستنتج أن الحد من تناول الصوديوم هو إجراء وقائي وعلاجي مهم في المرضى الذين يعانون من أمراض الكلى المزمنة أو المعرضين لخطر الإصابة بأضرار كلوية مثل ارتفاع ضغط الدم. لذلك ، يوصى باستخدام الملح الصخري بدلاً من الملح المكرر وهناك حاجة إلى مزيد من الدراسات لتوضيح التأثير المفيد وآلية كيفية تخفيف الملح الصخري لضغط الدم.