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Sodium Chloride Decrease Body Weight of Non-water Deprived *Rattus Norvegicus*

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Abstract: Effect of hypertonic sodium chloride was studied in non water deprived Wistar albino rats. Twenty (20) Wistar albino rats weighing between 128.4g and 470.2g were obtained from Veterinary Physiology, Pharmacology and Biochemistry Departmental animal house, University of Agriculture, Makurdi, Nigeria. The rats were housed in cages and administered 0.02, 0.04, 0.06, 0.10 and 0.20mg/g body weight of 10% sodium chloride on 1st, 2nd, 4th, 6th and 8th day respectively to induce hypertension. Grower's marsh® and fresh clean water were provided *ad libitum*. The weights of the rats were recorded daily, but the heart rates were recorded on the 1st, 5th, 8th and 19th day of experiment. Pretreatment and post-treatment blood samples were collected on the 1st and the 5th days respectively for determination of haematological and biochemical parameters. The results of daily average weight gain/loss of the experimental rats revealed significant difference ($p < 0.05$) of 16.0, 0.1*, 5.3, 3.9*, 4.7* and 8.9g* on the 2nd, 3rd, 4th, 6th, 8th and 19th day respectively. There was significnant increased heart rate ($p<0.05$) on the 5th, 8th and 19th day given 209 ± 14, 222 ± 20 and 271 ± 7 beats/minute respectively. The post treatment creatinine value (91.94 ± 13.96µmol/L) was lower than pretreatment value (106.88 ± 42.14 µmol/L). Hypertonic saline caused weight loss which was progressively regained, increased heart rate and decreased plasma creatinine. Adult rats lost body weight much more than young rats. Hence the strain of rats used for this study may be good for hypertensive experiment.

Key words: Hypertension, weight loss, hypertonic sodium chloride, wistar rats.

INTRODUCTION

Sodium chloride cause body weight loss in water deprived *Rattus norvegicus*. Extracellular fluid is characterized by high content of sodium ion (Na^+), calcium ion (Ca^{2+}) and chloride ion (Cl^-) being the major anion (Murray, R.K. and D.K. Granner, 2000). The difference between the extracellular and intracellular ions was traced to primordial sea in which the life originated (Felix, K., 2000). Blood pressure and volume as well as sodium-potassium (Na^+-K^+) pump used in the conduction of nerve impulse maintained the characteristics of sodium (Aka, L.O., 2004). Chloride, a component of sodium chloride assists in maintaining fluid balance inside and outside the cells. The main source of sodium ions is sodium chloride (Clement, I., 2006). Sodium deficiency can result through diarrhoea, excessive vomiting and over enthusiastic diuretic therapy (Fdudleyhart, E., 1987). Symptoms of deficiency in human include headache, muscle cramps, weakness, reduced ability to concentrate, memory and appetite losses (Clement, I., 2006). Sodium chloride deficiency in rats caused eye lesions, reproductive disturbances and death (Agricultural and Food Research Council, 1991), decreased appetite, weight loss, and lowered milk production in ruminants (McDonald, P., 1998). Fitzsimons and Kaufman (1977) and Nistico and Bolis (1983) reported that drinking in response to systemic administration of hypertonic sodium chloride produced cellular dehydration.

But a desert Kangaroo rat hardly drinks and manages the water formed in metabolic oxidation processes as the main water source (Schmidt-Neilsen, K., 2002). Sodium chloride toxicosis is known to result when excessive quantities of salts are ingested and water intake is limited (Kahn, C.M., 2005). Hypertension may also lead to congestive heart failure which in turn leads to oedema in response to high quantity of salt intake without water (Clement, I., 2006). Sodium chloride is one of the essential minerals that play great role in osmo-regulation and a higher concentration of it is being used to induce hypertension. Hence, there is need to study the effects of sodium chloride on non water deprived wistar albino rats.

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MATERIALS AND METHODS

Twenty Wistar albino rats comprised offsprings and their parents weighed between 128.4 and 470.2g were used for the study. The rats obtained from a colony bred by the Department of Physiology, Pharmacology and Biochemistry, College of Veterinary Medicine, University of Agriculture, Makurdi, Nigeria were housed in cages, acclimatized for 2 weeks and fed growers marsh® produced by Grand Cereals and Oils Company Limited Jos, Nigeria. Clean fresh drinking water was provided *ad libitum*. Animal care was provided according to NIH (National Institute of Health (NIH), 1985) guidelines and recommendations of the University of Agriculture, Makurdi ethical committee on the use of laboratory animals. The animals were marked serially from 1-20 with identification numbers. The weights of the animals were taken before intraperitioneal administration of 0.02, 0.04, 0.06, 0.1 and 0. 2mg/g body weight of 10% sodium chloride on the 1st, 2nd, 4th, 6th and 8th day respectively to induce hypertension. The weights of the animals were recorded on days 1, 2, 3, 4, 6, 8 and 19. Heart rate was taken from all the experimental rats on day 1, 5, 8, and 19.

Pretreatment and post treatment blood samples were collected on day 1 prior to salt administration and on day 5 of salt treatment respectively. One milliliter (1ml) of blood sample was collected from each rat through intracardiac puncture under anaesthetic effect of ether into ethylene diammine tetraacetate (EDTA) containing tubes for determination of haematological and plasma biochemical parameters.

Full blood cells count was carried out using the method of Cheesbrough (2005), while total plasma protein was determined using Biuret method (Tietz, N.W., 1995). Albumin was determined using Bromocresol green method (Doumas, B.T., 1973). Conjugated bilirubin and total bilirubin were analyzed using the method of Jendrassik and Grof (1938). Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) were determined using the method of Reitman and Frankel (1957). Sodium ion (Na^+) and potassium ion (K^+) were determined using the method of Healy (1995). Both bicarbonate ion (HCO_3^-) and chloride ion ($C1^-$) were determined by titration method of Chaney and Marbach (1962). The values of haematological and biochemical parameters, weight gain/loss and heart rate were expressed as Mean ± SEM. Analysis of variance (one-way ANOVA) was used to analyze the data on weight gain/loss and heart rate (Bamgboye, E.A., 2002). Tests for significance of haematological and biochemical parameters between pretreatment and post-treatment samples were performed using students't'test unpaired at 5% level of significance (Petrie, A. and P. Watson, 2002).

Results:

The mean weight loss was noticed on day 2 post administration of 10% hypertonic saline in all the experimental animals ($p<0.05$). Rat serial No. 1 weighed 470.2g on day 1 but later weighed 270.5g on day 2. But rat serial No. 20 weighed 128.4g on day 1 and later weighed 123.4g on day 2 (Table 1). The daily mean weight (188.5 ± 78.0g) on day 1 decreased to 172.5 ± 38.7g on day 2. But on day 3 there was no significant increase or decrease in the weight of the rats. On day 4th, all the rats lost weight but relatively small. The group mean weight further decreased from 172.6 ± 38.7g on day 3rd to 167.3 ± 38.9g on day 4th and their mean weight increased to 184.8 ± 32.9g on the 19th day (Table 1).

However daily weight losses per treated rats were 16.0, 0.1*, 5.3, 3.9*, 4.7* and 8.9*g on day 2nd, 3rd, 4th, 6th, 8th and 19th respectively. But weight loss/saline dose ratios were 4.24, 0.02, 0.51, 0.23, 0.14 and 0.25g/mg on the 2nd, 3rd, 4th, 6th, 8th and 19th day respectively (Table 2).

The heart rates of the experimental rats showed significant difference ($P<0.05$) on the 5th, 8th and 19th days having the mean values of 209 ± 14, 222 ± 20 and 271 ± 7 beats/minute respectively in comparison with the value of heart rate on day 1 (196 ± 11 beats/minute). The differences in the mean values of the heart rate on day 5th, 8th, and 19th were 13, 13 and 49 beats per minute respectively (Table 3).

Haematological indices such as packed cell volume, neutrophils, lymphocytes, monoctyes, eosinophils and basophils did not increase ($P>0.05$) significantly (Table 4).

All the plasma biochemical parameters investigated did not increase significantly ($P>0.05$). However creatinine was significantly decreased ($p<0.05$) in post treatment samples as compared to the pretreatment samples (Table 5). All the plasma ions under investigation did not increase ($P>0.05$) significantly (Table 6).

Discussion:

The significant difference ($P<0.05$) between the pretreatment and post treatment weight of the experimental rats may be attributable to the hypertonicity of 10% sodium chloride administered intraperitioneally. The weight loss may be due to suppression of feed intake and growth. Radostits *et al.* (1995) reported that too much salt intake can suppress feed intake and growth. This suppression might probably be via brain catecholamines. Decaro and Massi (1983) reported that the suppression of food intake in animals might be

Table 1: Effects of 10% sodium chloride on weight gain of Wistar albino rat (n=20)

S/No.	*Pretreatmentweight (g)*	*Post treatment weight (g)*					
	Day 1	Day 2	Day 3	Day 4	Day 6	Day 8	Day 19
1	470.2	270.5	270.5	262.7	271.2	277.5	269.9
2	299.4	240.0	247.0	244.6	255.5	251.3	255.7
3	250.3	241.3	241.3	240.0	248	240.9	226.4
4	216.4	185.4	187.4	179.8	193	202.4	210.1
5	187.2	181.6	181.6	173.7	170.8	180.8	201.4
6	181.6	178.5	178.5	172.8	170.3	180.5	191.1
7	179.7	172.4	172.4	172.5	168.1	174.9	175.7
8	178.6	171.8	171.8	167.4	161.2	170.9	175.6
9	170.3	171.2	171.2	163.0	160.9	168.0	174.4
10	167.3	167.2	167.2	158.1	159.8	165.1	174.0
11	160.3	164.6	164.6	157.6	159.6	161.7	173.6
12	158.5	160.0	160.0	156.0	159.3	160.4	172.1
13	154.7	155.5	155.5	148.3	156.7	156.6	170.3
14	152.1	148.8	148.8	146.6	152.3	155.0	168.5
15	148.7	148.2	148.2	142.9	151.0	154.3	168.3
16	148.0	154.5	154.5	140.5	148.9	151.8	166.9
17	144.9	144.7	144.7	137.1	144.8	149.9	164.3
18	143.0	140.2	140.2	132.3	143.0	147.3	160.6
19	131.1	132.5	132.5	128.3	131.3	141.4	157.2
20	128.4	123.4	123.4	122.1	125.3	126.5	140.6
MEAN	188.5	172.5	172.6	167.3	171.2	175.8	184.8
S. D.	78.0	38.7	38.7	38.9	40.3	38.8	32.9

Table 2: The weight loss/saline dose ratio per treated rats (n=20)

Parameters	*Day*					
	2nd	3rd	4th	6th	8th	19th
Daily weight loss (g)	16.0	0.1*	5.3	3.9*	4.7*	8.9*
Saline dose administered (mg)	3.77	6.5	10.36	16.73	34.24	35.16
Weight loss/ saline dose ratio (g/mg)	4.24	0.02	0.51	0.23	0.14	0.25

*weight regained

Table 3: Effect of 10% sodium chloride on heart rate of wistar albino rats (n=20).

S/No	*Day 1*	*Day 5*	*Day 8*	*Day 19*
1	192	222	228	268
2	192	216	192	264
3	204	180	216	280
4	192	204	240	274
5	192	204	228	272
6	192	204	192	288
7	192	216	204	268
8	204	258	228	264
9	192	204	216	268
10	180	204	216	264
11	204	204	216	268
12	204	204	216	276
13	204	204	204	264
14	216	204	228	278
15	192	198	264	260
16	216	216	228	280
17	204	204	216	264
18	192	216	204	274
19	180	210	264	278
20	180	210	240	276
MEAN	196.0	209.0	222.0	271.0
S. D.	11.0	14.0	20.0	7

via inhibition of catecholamine release. Khan (2005) also reported that hypertonic sodium chloride may be toxic and the toxicity may be characterized by depressed appetite, weight loss and dehydration. Therefore, the feeding centre in the lateral nuclei (*arcuate nuclei*) of the hypothalamus might be suppressed by 10% hypertonic saline. Although sodium chloride is one of the factors that precipitate hypertension, its application to decrease body weight of an obese person by inhibiting feeding centre should be an explorable area of research in human medicine. But it should also be considered when inducing hypertension in laboratory rodents. The hypertonic

Table 4: Effect of 10% sodium chloride on some haematological indices of Wistar albino rats (n = 20)

Indices	*Pretreatment values*	*Post treatment values*
Packed cell volume (%)	34.5 ± 7.51	33.61 ± 8.21
Neutrophils (%)	55.25 ± 17.21	51.39 ± 8.71
Lymphocytes (%)	41.25 ± 17.65	44.89 ± 8.48
Monocytes (%)	2.0 ± 0.00	2.11 ± 0.58
Eosinophils (%)	1.5 ± 0.58	1.44 ± 0.61
Basophils (%)	0.00 ± 0.00	0.00 ± 0.00

Table 5: Effect of 10% sodium chloride on plasma biochemistry of Wistar albino rats (n = 20)

Indices	*Pretreatment values*	*Post treatment values*
Total protein (gm/L)	72.2 ± 7.19	70.07 ± 3.1
Albumin (gm/L)	39.78 ± 7.69	37.36 ± 2.17
Total bilirubin (µmol/L)	11.85 ± 4.73	14.59 ± 1.39
Conjugated bilirubin (µmol/L)	2.58 ± 0.46	3.37 ± 0.71
Alkaline phophatase (IU/L)	109.25 ± 69.93	134.89 ± 15.48
Asparatate amino transferase (IU/L)	17.25 ± 6.65	21.11 ± 8.76
Alanine amino transferase (IU/L)	8.0 ± 3.27	9.5 ± 3.33
Urea (mmol/L)	3.28 ± 0.77	3.33 ± 0.65
Creatinine (µmol/L)	106.88 ± 42.14	91.94 ± 13.96*

* = significantly decreased ($p < 0.05$) in comparison with the pretreatment value.

Table 6: Effects of 10% sodium chloride on plasma ions of Wistar albino rats (n = 20)

Indices	*Pretreatment values*	*Post treatment values*
Sodium ion (mmol/L)	135.25 ± 2.22	135.25 ± 2.22
Potassium ion (mmol/L)	3.55 ± 0.19	3.52 ± 0.27
Chloride ion (mmol/L)	99.25 ± 0.96	99.5 ± 2.36
Bicarbonate ion (mmol/L)	24.5 ± 1.91	24.12 ± 1.84

saline affected obese adult rats much more than young normal rats as revealed in this study. Experimental No.1 rat weighed 470.2g before the administration of the saline. But 2 days after, the weight reduced to 269.9g, losing 199.7g body weight contrarily to the experimental 20th rat that weighed 128.4g pre-administration of hypertonic sodium chloride, but weighed 123.4g post administration of the saline, losing 5.0g body weight (Table 1).

The highest mean weight loss (16.0g) caused by 3.77mg/g body weight of 10% sodium chloride between day 1 and day 2 (Table 2) is suggestive of acute effect of hypertonic sodium chloride on Wistar rats. But on the 3rd day the average weight of 0.1g was regained. This agrees with the finding of Fitzsimons and Kaufman (1977) indicating that systemic administration of hypertonic sodium chloride produced dehydration, which is a stimulus to drinking enough water that dilute the injected salt load to isotonicity. But the rats can restore body fluid to isotonicity in part by excreting hypertonic urine (Fitzsimons, J.T., 1961). The hypothesis is that hypertonic saline stimulated drinking by rising cerebrospinal mechanism (Anderson, B., 1967). The rat also drinks only about 75% of the water needed to dilute administered hypertonic saline to isotonicity (Hawkins, R.C. and J.D. Corbit, 1973). Osmo-receptors located in the preoptic area are involved in the response to hypertonic stimulus (Peck, J.W. and D. Novin, 1971). On the 4th day, the regaining weight increased to 5.3g when 10.36mg/g of 10% sodium chloride was administered. The weight loss response was highest on day 2 and reduced to 5.3g on day 4th. This may be suggestive of adaptive ability of the rats to hypertonic saline either by drinking excess water which might have caused the rats to regain part of their lost weights or by excreting hypertonic saline which led to decreased salt load that in turn might have improved the appetite. This was highly noticed when hypertonic saline was not administered on the 5th, 7th and between 9th and 19th day of experimentation. The rats regained average weight of 3.9, 4.7 and 8.9g on the 6th, 8th and 19th day respectively. But there was still net loss of 7.1g of body weight when compared initial and final average weights of the animals on the 1st and 19th day respectively (Table 1). So when inducing hypertension systemically using sodium chloride, weight by saline dose should be considered rather than dosing rats with same dose of saline or including unmeasured quantity of excess salt in their feeds.

The significant difference ($p<0.05$) in daily heart rates of the experimental rats is suggestive of the hypertensive effect of sodium chloride. Our finding agrees with the report of Swales (1994) and Ganong (2001) indicating that there are a number of strains of rats that develop hypertension either spontaneously (SHR rats) or when fed a high-sodium diet (Dahl salt-sensitive rats). The mean heart rate on day 1 (196 ± 11) increased to 209 ± 14 on day 5th then to 222 ± 20 on day 8th and when the saline administration was stopped on day 8th the heart rate rose to 271 ± 7 on day 19th. This progressive increased heart rate is suggestive of residual effect of sodium chloride on contractility of the heart. The increased weight loss/saline dose ratio on the 2nd day of the salt treatment corresponds with increased weight loss on the same day. However the ratio decreased significantly on day 3 and the decrease continued until the end of the experimentation on day 19 suggesting progressive regain

of the lost weight perhaps by drinking excess water or eating adequate feed. As the salt intake increased, the weight loss decreased signifying that rats respond highly to the first loading dose much more than the subsequent higher doses. The situation is same in human whose response to salt intake increases as the dose increases. The response normally culminates in hypertension. This increased heart rate may be due to hypertonicity of extracellular volume caused by sodium chloride which needed to be balanced by sodium excretion (Fitzsimons, J.T., 1989). The imbalance might have lasted up to 19th day post-administration of initial dose of hypertonic saline. This agrees with the finding of Guyton and Hall (2007) indicating that an increased salt intake is far more likely to elevate arterial pressure than is an increased water intake. The reason is that pure water is rapidly excreted by kidney than salt. Because as salt accumulates in the body it increases extracellular fluid volume (Guyton, A.C., 1972; Guyton, A.C., 1991). Therefore, the strain of rats used in our study may be ideal for hypertensive experiment. However, the range of heart rate (250-400 beat/min) reported by Khan (2005) for rats disagrees with our finding of 196 ± 11 on the 1st day of hypertonic saline administration. This may be due to difference in climatic condition, genetic constitution of the rats, nutrition and health status. Generally the increased heart rate is multificatorial.

Lack of significant increase (P>0.05) in haematological and biochemical parameters between pretreatment and post treatment values (Tables 4, 5and 6) may suggest relative safeness of 10% sodium chloride in the Wistar rats. The reason might be due to the fact that the rats were not deprived of water, despite the hypertonic saline caused significant weight loss in the rats. But the observation of relative decreased creatinine in post-treatment samples (91.94 ± 13.96µmol/L*) in comparison with pretreatment samples (106.88 ± 42.14 µmol/L) may be due to metabolic effect of hypertonic saline on body protein of the rats. This agrees with the report of McDonald *et al.* (1998) indicating that the turn over rate of body protein varies from one tissue to another and the tissues are replaced at intervals of hours, days or months. The energy of urine is present in nitrogen-containing substances such as urea and creatinine. Creatine of muscles is converted to creatinine which is excreted in urine (McDonald *et al.* 1998) invariably decreasing plasma creatinine. Ganong (2001) reported that muscle breakdown can lead to creatinuria which may in turn lead to hypocreatinaemia. But the 24-hour urinary excretion of creatinine is proportionate to muscle mass. Creatinine which diffuses through out the body fluid is formed from glycine, arginine and methionine by methylation of guanidoacetate by S-adenysylmethionine (Rodwell, V.W., 2003).

Conclusion:

Hypertonic sodium chloride (10%) caused weight loss in non-water deprived Wistar albino rats. The weight loss though much more pronounced in adult rats was progressively being regained. Observed also are increased heart rate and, decreased plasma creatinine that might have resulted from muscular protein degradation. The strain of the rats used in our study may be suitable for hypertensive experiments. However, other parameters investigated did not increase significantly.

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