

Effect of the Natural Cat and Dog Liquid Additives on 3% Salt Fed Rats

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ABSTRACT

Salt poisoning could be important for the pets' life. High salt and low water consumptions can be effective in the formation of salt poisoning. Low levels of salt poisoning increase blood pressure which reduces the quality of life. Plant-origin cat and dog liquid additives are previously used and improve water consumption of rats. In this study, 36 wistar albino rats were randomly divided into 4 groups (regular diet with water (RDW), 3% salt containing diet with water (SW) or cat (SCat) or dog (SDog) liquid additives added to their water). Animals were fed ad libitum for two weeks. At day 0 and day 14, blood samples were collected from the rats. At Day 14, the rats were individually placed in metabolic cages and urine and fecal samples were also taken. The rats were subjected to necropsy and kidney tissue was removed. Sodium (Na) concentration of urine samples were 36% SCat and 45% SDog higher than those measured in the SW groups. Na concentration of the feces was higher in SW groups (27% or 47%) than those in the SCat and SDog groups, respectively. According to the kidney tissue microscopic findings, the health conditions of the SCat and SDog groups, supplemented with the additive were improved. Especially, SDog product reduced the negative effects of elevated salt intake on rats.

Keywords: Rat, salt, poisoning, liquid additives

1. INTRODUCTION

With the developing technology, human beings and pets are in contact with thousands of substances that can have toxic effects in their environment [1]. One of them is salt. Salt is frequently used in our daily life as a flavoring additive and nutritional requirement of most foods for all living things. The sodium chloride is of the most frequently used and readily available salts supplying electrolytic balance of the body. Excessive use of each substance causes toxic effects, like salt intake. Low levels of salt poisoning cause increased blood pressure, reducing quality of life.

There are various medicinal plants used in traditional medicine through preventing and treating diseases. The effects of liquid additives obtained from the mixture of a variety of medicinal plants [2] were investigated in rats fed with 3% salt containing feed.

2. MATERIALS AND METHODS

2.1 Liquid Additives Products

The heyLove Cat Detox and heyLove Dog Detox products were kindly provided by ATY Grup Yem San. ve Tic. A.Ş., Istanbul, Turkey [2]. The products are

known to be produced with liquid additives prepared from the extracts of 11 plants, which are arranged according to the needs of cats and dogs, respectively, to neutralize free radicals accumulated in the body and to strengthen immunity [3,4].

2.2 Animals and groups

Thirty-six male Wistar albino rats at fifty-four weeks age (428.36 ± 12.34 g) were housed (Ethics Committee Protocols No: 93773921-239) at the Laboratory of Animal Experiments, Faculty of Veterinary Medicine, Mehmet Akif Ersoy University, Burdur, Turkey. The rats were individually housed in the cages. Feed and water with liquid additives were given as ad-libitum. The feed of rats was prepared (soybean, oats, anchovy flour, and commercial layer chicken vit-min) at the Department of Animal Science Laboratory Isparta University of Applied Sciences, Faculty of Agriculture. Each cage was randomly divided into four groups: a) The main feed mixture and commercial water were given to the RDW group, b) the salt added feed and commercial water were given to the SW group, c) the salt added feed and 20% cat detox were given to the SCat group, and d) the salt added feed and 20% dog detox was

given. The detox products were diluted with commercial water.

2.3 Blood sampling

Blood was drawn for serum chemistry on day 1 and day 14 [5]. Urea, creatinine, sodium, chlorine, calcium, phosphorus and potassium analyzes were performed at the the Labotaroty of Veterinary Faculty of Mehmet Akif Ersoy University, Burdur, Turkey.

2.4 Urine and fecal sampling

In the experiment, at the end of the 14th day, the rats were placed in individual metabolic cages (three rats each from the RDW, SW, SCat and SDog groups) and urine and fecal samples were collected for 24-hour periods. The urine samples were stored at -20°C after the quantities were determined, and then sent by cold chain, to Düzen Norwest Laboratory, Çankaya, Ankara, Turkey for analysis. After the fecal samples were weighed and recorded in their wet state, they were first subjected to drying for analysis and their dry quantities were recorded. Dry matter and crude ash levels were determined after grinding feces. Later, sodium, calcium and potassium analysis of fecal samples was determined at Isparta University of Applied Sciences, Agricultural Faculty, Soil Science and Plant Nutrition Laboratory, Isparta, Turkey, [6] and phosphorus contents of fecal digests were spectrophotometrically determined by the blue dye method [7].

Table 1. Blood serum parameters of the rats.

Blood chemistry	RDW	SW	Scat	SDog	Average
Urea (mg/dL)	30.17 ± 3.87	33.20 ± 9.87	31.00 ± 4.47	30.90 ± 3.03	31.46 ± 6.04
Creatinine (mg/dL)	0.61 ± 0.02	0.64 ± 0.02	0.63 ± 0.04	0.62 ± 0.03	0.62 ± 0.03
Total Protein (g/dL)	7.20 ± 0.15	7.07 ± 0.28	7.23 ± 0.68	7.01 ± 0.30	7.12 ± 0.41
Albumin (g/dL)	2.84 ± 0.12	2.97 ± 0.27	3.36 ± 0.47	3.39 ± 0.16	3.17 ± 0.37
AST (U/L)	107.28 ± 18.85	93.07 ± 35.08	105.56 ± 11.93	105.48 ± 14.15	105.16 ± 22.59

^{A, B} The different letters on the same line indicate the difference between treatment groups (p<0.05).

The differences in the creatinine, total protein, albumin, AST, ALT, GGT values among treatment groups were not statistically significant, whereas the urea and ALP levels were statistically different between the treatment groups. According to the studies conducted, the serum urea level varies depending on the nutritional value of the feed. Despite the fact that it was aimed to meet the nutrition needs of the rats, the serum urea level was determined to be higher than those reported in the literature [2,10]. Serum ALP level can be high for many reasons and needs to be evaluated by considering in other parameters.

2.5 Necropsy and pathological examination

The anesthesia-euthanasia was performed by cervical dislocation method. Euthanized animals were immediately subjected to necropsy to check the anatomical location of the abdominal, thoracic and pelvic cavities and internal organs. The kidney tissues examination was performed by strictly following the Mohan's [8] criteria for studying of renal toxic injury.

2.6 Statistical Analysis

Data were analyzed by using a commercially available statistical software package [9]. The means of the groups were compared through Duncan's multiple range tests after all data were subjected to Analysis of Variance.

3. RESULTS AND DISCUSSION

3.1 Blood Parameters

The urea, creatinine, total protein, albumin, AST, ALT, ALP, GGT, sodium, chloride, calcium, phosphorus and potassium levels of blood serum are shown in Table 1.

3.2 Urine and Feces Parameters

Some physico-biochemical properties of the urine samples are given in Table 2. In the physical examination of the urine, it was observed that the color of the urine changed from light yellow to dark brown according to the amount of water they consumed. In addition, as water consumption increased, the urine specific gravity of rats increased. The urine volume, pH and specific gravity of rats were not significantly different between the treatment groups. The rat's urine volume, pH and specific gravity values were significantly influenced from the treatments which were in agreement with the other studies [11-17].

Table 2. The volume, physical and biochemical characteristics of urine

Urine characteristics	RDW	SW	SCat	SDog	Average
Output volume (mL/day)	6.55 ± 3.92	14.39 ± 5.21	14.86 ± 10.43	14.86 ± 10.43	13.31 ± 8.72
Density (g/L)	1014.17 ± 5.85	1019.44 ± 5.83	1014.50 ± 3.69	1017.00 ± 7.53	1016.43 ± 6.01
pH	7.17 ± 0.41	6.72 ± 0.97	7.20 ± 0.42	6.95 ± 0.93	7.00 ± 0.75
Protein (g/L)	0.92 ± 0.49	1.00 ± 0.50	0.75 ± 0.46	0.63 ± 0.53	0.80 ± 0.49
Glucose (mmol/L)	3.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00
Ketone (mmol/L)	0.67 ± 0.41	1.16 ± 0.51	0.79 ± 0.49	1.00 ± 0.54	0.90 ± 0.50
Bilirubin (mmol/L)	17.00 ± 0.00	17.00 ± 0.00	17.00 ± 0.00	17.00 ± 0.00	17.00 ± 0.00
Urobilinogen (µmol/L)	17.00 ± 0.00	17.00 ± 0.00	17.00 ± 0.00	17.00 ± 0.00	17.00 ± 0.00
Blood (Ery/µL)	216.67 ± 81.65	238.89 ± 33.33	200.00 ± 52.70	190.00 ± 96.61	210.00 ± 69.45
Leukocyte Esterase (Leu/µL)	25.00 ± 0.00	25.00 ± 0.00	25.00 ± 0.00	25.00 ± 0.00	25.00 ± 0.00
Erythrocyte	12.80 ± 4.97	13.75 ± 7.32	14.50 ± 6.54	9.80 ± 5.69	12.64 ± 6.31
Leukocyte	1.83 ± 0.98	2.63 ± 1.69	2.00 ± 1.49	2.40 ± 1.35	2.24 ± 1.39
Squamous epithelium	1.60 ± 0.89	4.00 ± 3.04	4.60 ± 3.13	3.11 ± 3.06	3.58 ± 2.93
Atp crystal	13.33 ± 2.89	11.00 ± 6.90	9.33 ± 4.08	13.67 ± 7.09	11.28 ± 5.38

The results of urine biochemical analysis of the rats are shown in Table 2. According to the results, all groups, including the RDW group, contain undesirable substances in the urine. However, the urine parameters of rats were similar in all groups. Protein, glucose, ketone, bilirubin, urobilinogen, blood, leukocyte esterase, erythrocyte, leukocyte, squamous epithelium and Atp crystals should not be present in the urine. However, kidney disorders were observed in rats

according to urine biochemical results depending on the protein level of the feed given to rats [18].

Mineral concentrations of urine fed with salt-rich diet are given in Table 3. It was found that the difference between the groups in urinary sodium, chlorine and potassium levels were statistically significant. Urine mineral levels were similar to the literature values [13, 16, 19].

Table 3. Urine mineral analysis of rats

Chemical parameter	RDW	SW	SCat	SDog	Average
Sodium (mg/dL)	171.33±64.83B	70.78±42.26A	102.90±40.54A	96.50±48.39A	104.54±56.59
Chloride (mg/dL)	24.50±9.04A	119.37±63.51B	88.60±28.48B	125.56±68.17B	99.00±59.42
Calcium (mg/dL)	8.02±4.47	11.50±8.13	10.24±5.50	11.37±10.50	10.51±7.57
Phosphorus (mg/dL)	78.97±26.74	110.60±48.76	93.59±52.67	102.09±55.21	97.89±47.34
Potassium (mg/dL)	59.03±27.47AB	36.57±13.45A	52.66±20.55AB	66.96±24.23B	53.70±23.57

^{A, B} Letters on the same line give the difference between groups ($P < 0.05$).

Physical examination of the feces revealed that there were consistent softness, color, odor and shape in the normal ranges. The fecal amount was highly dependent on the individuals. This in fact is a function of food consumption behavior of the individuals. The average

daily wet feces weight of the rats for the experimental period was 4.25 ± 2.16 g. The analysis results for the fecal dry matter and fecal raw ash percentages of the rats and the amounts of minerals fecal excreta are shown in Table 4. The fecal ash contents increased with excessive

salt supplementation. Sodium and chloride concentrations in excretion of the feces were accordingly increased with salt supplement.

Table 4. Fecal dry matter, raw ash and minerals of rats

Chemical parameters	RDW	SW	SCat	SDog	Average
Dry matter (%)	57.69 ± 26.68	57.89 ± 17.32	61.90 ± 14.87	61.59 ± 16.99	60.01 ± 17.74
Ash (%)	4.57 ± 1.08 ^A	6.40 ± 1.39 ^B	6.42 ± 1.71 ^B	7.15 ± 1.62 ^B	6.28 ± 1.69
Sodium (mg/dL)	316.38 ± 151.28 ^A	638.70 ± 357.95 ^B	467.18 ± 249.44 ^{A^B}	339.12 ± 104.07 ^A	452.07 ± 265.55
Chloride (mg/dL)	1.93 ± 1.02 ^A	4.24 ± 2.76 ^A	6.07 ± 4.05 ^{A^B}	10.06 ± 6.42 ^B	5.87 ± 4.91
Calcium (mg/dL)	299.76 ± 167.43	298.74 ± 168.08	419.07 ± 125.12	293.30 ± 148.62	333.17 ± 152.89
Phosphorus (mg/dL)	0.94 ± 0.17	0.87 ± 0.07	1.36 ± 0.90	0.83 ± 0.11	1.02 ± 0.54
Potassium (mg/dL)	1038.04 ± 411.51	1083.32 ± 303.94	829.72 ± 220.33	1064.22 ± 428.80	1000.91 ± 349.29

^{A, B} Letters on the same line give the difference between groups (P <0.05).

The SW group had the highest value of the treatment groups. The usage of SCat and SDog additives elevated chloride removal from the rats.

3.3 Pathological findings

After autopsy, kidney tissues of rats were taken and tissue surfaces and sections were examined microscopically. Among the experimental groups, it was determined that a significant difference was only found in proteinaceous mass of the kidney tissues of rats. Other criteria showed insignificant differences (Table 5).

Table 5. Pathological findings results of kidney tissues of rats

	RDW	SW	SCat	SDog
N	6	6	6	5
Proteinous mass	5.75 ^B	15.17 ^A	14.08 ^A	13.20 ^A
Bow. Gap Expansion	8.83	15.50	14.17	7.50
Tubular degeneration	7.25	15.83	13.92	10.80
Tubulus necrosis	1015	15.67	10.75	10.60
Fibrotice change	11.17	9.33	15.58	11.90

^{A, B} Letters on the same line give the difference between groups (P <0.05).

The kidneys of the SW group had moderately pale and swollen areas. Other groups were quite normal without any abnormalities. Microscopic examination of rat kidney tissue showed a reduction in degenerative changes in the proximal convolute and distal tubular

epithelium, in the tubular lumen in proteinaceous masses, and in the medullary and arterial perivascular edema compared to the SW group. Degenerative changes in the SDog group were significantly reduced compared to the SCat group (Figure 1).

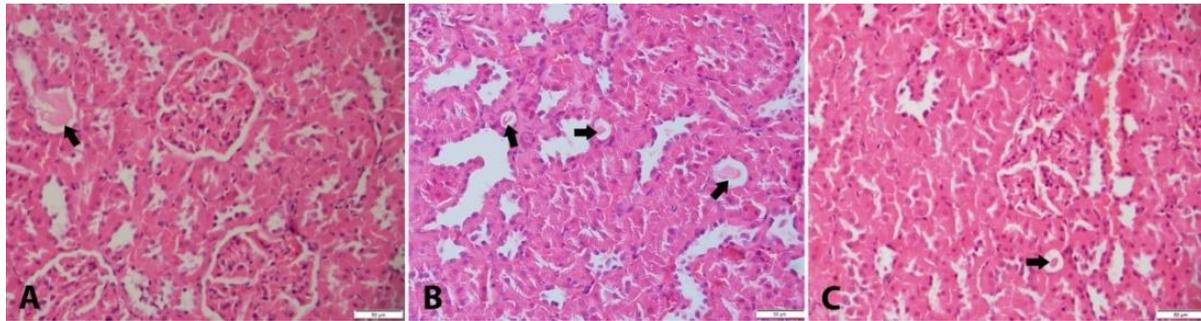


Figure 1. Images of kidney tissues of rats A: SW, B: SCat and C: SDog groups (Arrows indicate proteinaceous masses), H&E, barr 50 μ .

4. CONCLUSION

The consumption of 3% salt-enriched diet did not affect the serum, urine and fecal biochemical levels of rats. There was a change in the amount of excreted urine and feces of rats depending on the salt intake level. However, urinary sodium excretion of rats was higher for SCat and SDog groups than the SW group. The fecal sodium excretion of rats was found to be higher for SCat and SDog groups than the SW group. Furthermore, according to the kidney tissue microscopic findings, it was determined that the conditions of the groups consuming SCat and SDog products improved. Especially SDog product reduced deleterious effects of saline on rats.

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