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Evaluation of Antihypertensive and Antioxidant Activity of Methanol Seed Extract of *Telfairia occidentalis* in Wistar Albino Rats

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ABSTRACT

The study evaluated the ameliorative potentials of methanol extracts of *Telfairia occidentalis* seed in sodium chloride induced hypertensive rats. Twenty four albino rats weighing 190 g-280 g were used for the study. The rats were divided into six (6) groups of four (4) rats per group. Group I serve as the normal control. Group II received 10 ml/kg body weight of 16% sodium chloride only. Group III through V received daily dosage of 10 ml/kg body weight of 16% sodium chloride and 100 mg/kg, 200 mg/kg and 400 mg/kg of extract respectively while group VI received 3.6 mg/kg body weight captopril. All administration last for seven (7) days. The effect of the administration on phosphocreatine kinase, lactate dehydrogenase, serum sodium ion (Na+) concentration was evaluated. The in-vitro antioxidant potentials of *Telfairia occidentalis* seed was assayed using 2, 2-diphenyl-1-picrylhydrazine (DPPH) scavenging activity, Nitric oxide inhibition activity, Anti-lipid peroxidation. Results obtained showed a significant dose dependent increase in percentage 2, 2-diphenyl-1-picrylhydrazine (DPPH) scavenging activity, nitric oxide inhibition, anti-lipid peroxidation of the plant extract $P<0.05$. Administration of the extract at doses of 200 mg/kg and 400 mg/kg body weight shows a significant reduction in serum phophocreatine kinase level and serum lactate dehydrogenase concentration compared to the negative control group $P<0.05$. The results gotten in the experiment were highly comparable to that of standard drug captopril. The study shows that *Telfairia occidentalis* seed possesses antioxidant activity and can ameliorate the changes in hypertensive parameters induced by sodium chloride.

Keywords: *Telfairia occidentalis*, Sodium chloride, Hypertension, Methanol extract, Albino Rat, Antioxidant

INTRODUCTION

Hypertension is one of the principal health problems in the society and an important cause of cardiovascular deaths in various communities worldwide. It is a silent killer whose onset of complications is insidious. Such complications as cardiac remodeling, hypertrophy, renal impairment, nephropathies and ocular complications such as retinopathies and cardiovascular accident or stroke are associated with hypertension [1].

Traditionally, the use of plant parts as source of herbal preparations for treatment of various ailments are based on the experience passed from generations to generation, virtually by oral tradition and through practice and forms part of the indigenous knowledge of people of any locality [2,3]. Most of the herbal remedies are known by our traditional healers and elderly men and women of families in our rural areas.

Recently, it has been hypothesized that oxidative stress is a key player in the pathogenesis of hypertension endothelial cells play a major role in the arterial relaxation [4]. Nitric oxide is released by the endothelium and causes vascular relaxation [5]. Nitric oxide is rapidly degraded by oxygen-derived free radical superoxide anion. Superoxide anion serves as a vasoconstrictor and is a major determinant of Nitric oxide (NO) biosynthesis and bioavailability. Human hypertension is associated with a decrease in Nitric oxide

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bioavailability and an increase in oxidative stress [6].

Almost all organisms possess anti-oxidant defense and repair systems that have evolved to protect them against oxidative damage. These systems are often insufficient to prevent the damage entirely. However, antioxidant supplements or food containing antioxidant may be used to help human reduce oxidative damage [7].

Telfairia occidentalis (Fluted pumpkin), a member of cucurbitaceous family is one of the commonly consumed leafy and seed vegetables in Nigeria. It is indigenous to southern Nigeria and grown in the forest zone of the west and central Africa with Nigeria, Ghana and Sierra Leone being the major producers [8]. This study is aimed to evaluate the Anti-hypertensive and Anti-oxidant activity of methanolic seed extract of *Telfairia occidentalis*.

MATERIALS AND METHOD

Collection and identification of plant materials

Fresh seed of *Telfairia occidentalis* (fluted pumpkin) were brought from Ndoro Market, Oboro in Ikwuano LGA of Abia State in the month of July, 2017 and were authenticated by Dr. M.A Jimoh of the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Umudike, Nigeria.

Preparation of plant extract

Fresh seed of *Telfairia occidentalis* (Fluted pumpkin) were air dried and then pulverized with mechanical blender to obtain a coarse powder. 20 g of the pulverized sample was used to investigate and establish the antioxidant property. 100 g of the pulverized plant was macerated in 400 ml of methanol for 72 h at room temperature, and then filtered afterwards into a beaker using funnel and Whatman filter paper No 1 (125 mm), the filtrate was concentrated by evaporation in a water bath at a temperature of 40°C to obtain the crude extract.

Experimental animals

Twenty four (24) Wistar albino rats weighing between 190 g-280 g were purchased from the animal center of the faculty of Pharmaceutical Science, University of Nigeria Nsukka. The animals were housed in the animal center of the Department of Biochemistry, Michael Okpara University of Agriculture, Umudike and were acclimatized for two weeks prior to start of experiment, with access to standard rodent feed and water ad libitum.

Experimental design

All animals were handled according to method described by guide for care and use of laboratory animals. The animals were housed and randomly divided into six groups of animals (4 rats per group). Group I received distilled water serving as normal control; group II received 10 ml/kg body weight of 16% sodium chloride without administration of extract. Group III, IV and V received 10 ml/kg body weight of 16% sodium chloride and plant extract at 100 mg/kg, 200 mg/kg and 400 mg/kg by oral administration respectively. Group VI received 10 ml/kg body weight of 16% sodium chloride and standard drug- captopril (3.6 mg/kg). The blood of the rats were collected after one week of administration and Creatine phosphokinase (CK), Lactate Dehydrogenase (LDH), Serum Sodium Ion level were analyzed.

EVALUATION OF ANTIOXIDANT ACTIVITY

Nitric oxide inhibition activity

The Nitric Oxide Scavenging activity was conducted based on the Greiss Assay Method (1858), which involves generating oxide from sodium Nitroprusside by the Greiss reaction. 2 ml of 10 mM sodium Nitroprusside and 5.0 ml of phosphate buffer were mixed with 0.5 ml of different concentration (12.5-200 µg/ml) of plant extract and incubated at 25°C for 150 min. The samples were run as above but the blank was replaced with the same amount of water. After the incubation period, 2 ml of the incubated samples was added to 2 ml of Greiss reagent (1% sulphanilamide, 0.1% alpha-napthyl-ethydiamine dihydrochloride and 3% phosphoric acid) and then incubated for a period of 30 min. The absorbance of the pink chromophore formed by the diazotization of nitrite with alpha-napthyl-ethydiamine dihydrochloride was measured at 540 nm. Ascorbic acid was used as positive control, the experiment was performed in triplicate and the capacity to scavenge the nitric oxide was calculated:

$$\% \text{ inhibition} = \frac{\text{Absorbance of control - Absorbance of sample} \times 100}{\text{Absorbance of control}}$$

2,2-Diphenyl-1-picrylhydrazine (DPPH) scavenging activity

2,2-Diphenyl-1-picrylhydrazine (DPPH) scavenging activity was quantified in the presence of stable DPPH radical on the basis of 2,2-Diphenyl-1-picrylhydrazine (DPPH) assay system [9] as cited by Omodamiro et al. [10].

Preparation of DPPH solution

- 1 mmol/L of DPPH=0.394 g of DPPH
- 0.5 mmol - 0.197 g (197 mg)
- 150 ml=X

$$X = \frac{150 \times 197}{1000} = 29.55 \text{ mg}$$

About 2 ml of *Telfairia occidentalis* seed extract dissolved in methanol was mixed at different concentrations (12.5-200 µg/ml) with 1 ml of DPPH solution in test tube and incubated for 30 min in the dark at room temperature. 1 ml of ethanol+2 ml of test extract were used as negative control. The degree of discoloration indicates the scavenging efficacy of the extracts and absorbance was measured at 517

nm. The experiment was performed in triplicate and percentage of scavenging activity was calculated using the following equation:

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample} \times 100}{\text{Absorbance of Control}}$$

Anti-lipid peroxidation activity

The determination of anti-lipid peroxidation activity was according to the method of Schippers [8]. The methanol extract of *Telfairia occidentalis* were used at different concentrations (500, 400, 300, 200, 100 mg/ml) individually. 3 ml of liver homogenate was added to 100 ml of 15 mM ferric chloride and was shaken for 30 min from collected mixture, 100 ug/ml was added with 1 ml of different concentrations of plant extract individually in different test tubes. Ascorbic acid was used as the standard (100 µg/ml). All the test tubes were incubated for 4 h at 37°C.

After incubation, 1.1 ml of 30% trichloroacetic acid (TCA) and 1.1 ml of 0.65% thiobarbituric acid (TBA) were added to all tubes containing the mixture. After 30 min of incubation in a shaking water bath and subsequent cooling in ice cold water for 10 min, the tubes were centrifuged at 800 g for 15 min; the absorbance was measured at 530 nm. The percentage inhibition of lipid peroxidation was calculated by using the equation below:

$$\% \text{ inhibition} = \frac{\text{Absorbance of control - Absorbance of sample} \times 100}{\text{Absorbance of control}}$$

ESTIMATION OF ANTI-HYPERTENSIVE PARAMETERS

Determination of serum lactate dehydrogenase activity (U/L)

The serum lactate dehydrogenase activity was determined after 7 days of induction and treatment according to the method described by Weishaar. 2 ml of the sample was pipetted into a test tube and mixed with 1.0 ml of reagent (Randox kit). After mixing, the initial absorbance was read after 30 s and again after 1, 2, 3 min simultaneously at 340 nm using Agilent 8453 spectrophotometer. LDH activity was calculated using the following formula:

$$U/L = 9683 \times DA\ 340\ nm/min$$

Where, DA: change in absorbance

Determination of serum sodium ion concentration (mEq/L)

Sodium content was determined using spectrophotometric method and commercial kit following procedures prescribed by the producer, TECO Diagnostics, USA. Test tubes were set up and were labeled blank, standard and sample and 1 ml of the filtrate reagent was pipette into each. To the standard 50 µl of the standard solution was added while the same volume of distilled water was added to the blank. 50 µl of sample (serum) was also added to corresponding test tubes. All tubes were shaken vigorously for 3 min to ensure adequate mixing and thereafter were centrifuged at high speed for 10 min to obtain supernatant of each. Another set of test tubes were set up and labeled as previously done. 1 ml of the acid reagent was pipette into each, followed by the addition of 50 µl of each supernatant to respective test tubes and properly mixed. The color reagent (50 µl) was then added to each test tube and mixed. The spectrophotometer was zeroed before reading the absorbance of each sample at 550 nm.

Concentration of sodium for each sample in mEq/L was calculated using the relationship:

$$\frac{Absorbance\ of\ sample}{Absorbance\ of\ standard} \times Concentration\ of\ standard$$

Determination of serum creatine phosphokinase activity

The activity of creatine phosphokinase was determined according to the method described by Szasz (Randox C.K. 110 kit was utilized for the quantitative *in vitro* determination of the enzyme in serum). 20 ml of the sample was pipetted into a test tube and mixed with 1ml of the reagent (Randox C.K 110 kit). The mixture was left to incubate at 25°C for 3 min and the initial absorbance was read. The absorbance was taken again after 1, 2 and 3 min simultaneously at 340 nm using an Agilent 8453 spectrophotometer. The creatine phosphokinase activity was calculated using the formula:

$$U/L = 4127 \times DA340\ nm/min$$

Where DA: Change in absorbance

RESULTS AND DISCUSSION

Effect of methanol extract of *T. occidentalis* seed on serum sodium ion concentration of wistar albino rats

Figure 1 shows the effect of methanol seed extract of *Telfairia occidentalis* on serum sodium ion concentration of albino rats. A significant increase in rats treated with 0.2 ml distilled water (169.85 ± 5.42) was observed compared to the other groups ($P<0.05$). There was a significant reduction in 100 mg/kg extract treated group (112.05 ± 1.68) compared to the other groups ($P<0.05$). There was no significant difference for rats treated with 200 mg/kg (134.78 ± 2.17), 400 mg/kg extract (129.67 ± 0.76) compared to the standard drug captopril (131.52 ± 5.43) ($P<0.05$).

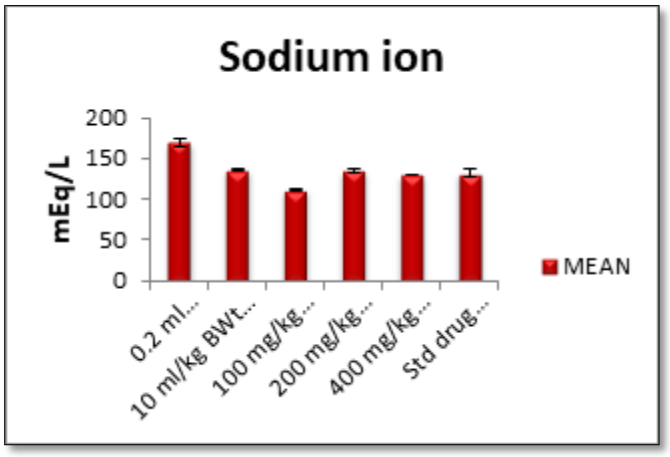

Figure 1. Total serum sodium ion concentration of experimental groups.

Effect of methanol extract of *T. occidentalis* seed on serum creatinine phosphokinase level of wistar albino rats

Figure 2 shows the effect of methanol extract of *Telfairia occidentalis* seed on serum creatine phosphokinase of wistar albino rats. There was a significant decrease in serum phosphokinase level of 0.2 ml treated rats (0.31 ± 0.02) compared to all other groups P<0.05. A significant increase was observed in 10 mg/kg NaCl treated albino rats P<0.05. Albino rats treated with 400 mg/kg (0.60 ± 0.01) showed a significant reduction in serum creatine phosphokinase level compared to 10 mg/kg NaCl (0.85 ± 0.12) treated group P<0.05, there was no significant difference between the 100 mg/kg extract (0.81 ± 0.03), 200 mg/kg (0.70 ± 0.02), 400 mg/kg (0.60 ± 0.01) extracts treated groups compared to the standard drug captopril (0.67 ± 0.02) P<0.05.

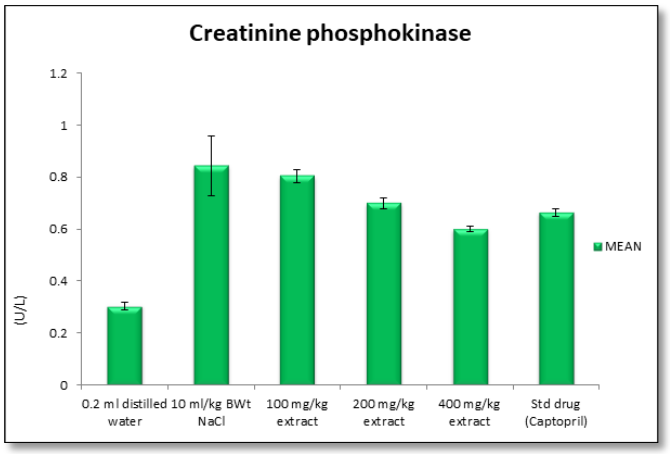

Figure 2. Total serum creatine phosphokinase of experimental groups.

Effect of methanol extracts of *Telfairia occidentalis* seed on serum lactate dehydrogenase level of treated albino rats

Figure 3 shows the effect of methanol extract of *Telfairia occidentalis* seed on serum lactate dehydrogenase level of wistar albino rats. There was a significant reduction of serum lactate dehydrogenase level of 0.2 ml (118.65 ± 1.69) treated albino rats compared to all other groups P<0.05. A significant increase was observed in 10 mg/kg NaCl (144.81 ± 5.57) (P<0.05). Albino rats treated with 200 mg/kg (130.88 ± 1.39), 400 mg/kg (130.82 ± 4.67) shows a significant reduction compared to 10 mg/kg NaCl (144.81 ± 5.57) treated albino rats (P<0.05).there was no significant difference in serum lactate dehydrogenase level of 100 mg/kg (144.81 ± 5.57), 200 mg/kg (130.88 ± 1.39), 400 mg/kg (130.82 ± 4.67) extract treated groups compared to the standard drug captopril (128.69 ± 1.57) (P<0.05).

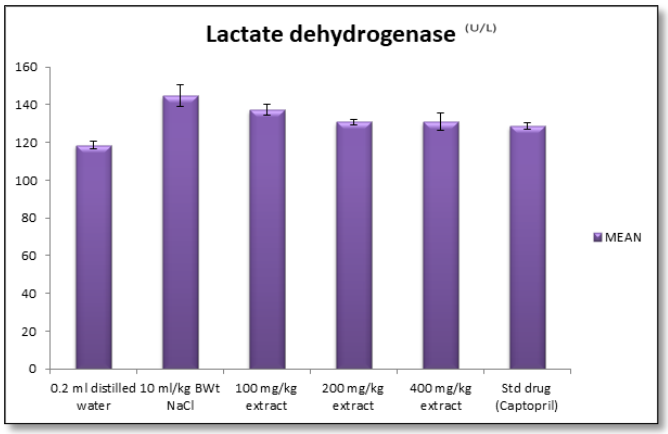

Figure 3. Total serum lactate dehydrogenase concentration of experimental groups.

ANTIOXIDANT ACTIVITY OF *Telfairia occidentalis* SEED

DPPH scavenging activity

Figure 4 shows a dose dependent increase in DPPH scavenging activity of *Telfairia occidentalis* seed P<0.05. A significant increase was observed in the positive control 96.32 ± 0.32 compared to different doses of extract of *Telfairia occidentalis* seed P<0.05.

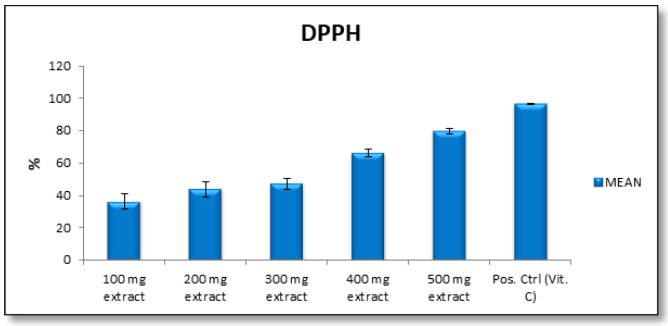

Figure 4. Dose dependent increase in DPPH scavenging activity of *Telfairia occidentalis*.

Nitric oxide inhibition activity

Figure 5 shows a dose dependent significant increase in nitric oxide inhibition activity of *Telfairia occidentalis* seed P<0.05. A significant increase was observed in the positive control 97.64 ± 0.29 compared to different doses of extract of *Telfairia occidentalis* seed P<0.05.

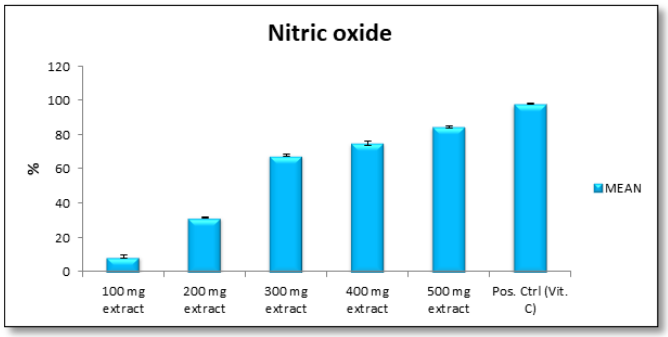

Figure 5. Dose dependent increase in nitric oxide inhibition activity of *Telfairia occidentalis*.

Anti-lipid peroxidation

Figure 6 shows a significant dose dependent increase in percentage anti-lipid peroxidation of *Telfairia occidentalis* seed extracts (P<0.05). A significant increase was observed in the positive control 97.19 ± 0.36 compared to *Telfairia occidentalis* seed extract of 100 mg/kg (18.05 ± 2.96), 200 mg/kg (56.37 ± 2.25), 300 mg/kg (65.92 ± 1.07), 400 mg/kg (86.80 ± 1.04), *Telfairia occidentalis* seed of 500 mg/kg

(99.30 ± 0.28), shows an insignificant increase in percentage anti-lipid peroxidation compared to the positive control 97.19 ± 0.36 (P<0.05).

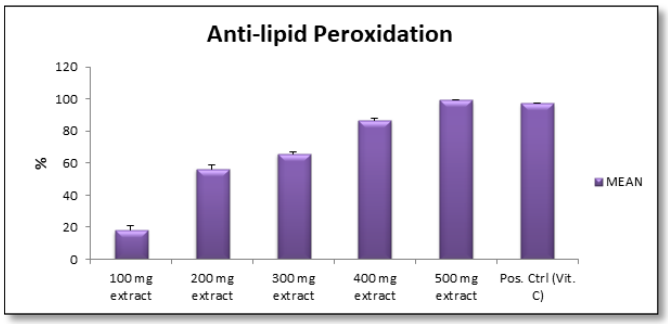

Figure 6. Dose dependent increase in percentage anti-lipid peroxidation of *Telfairia occidentalis*.

DISCUSSION

The high rate of sodium chloride (NaCl) consumption today has been implicated in the high incidence of hypertension [11]. The study aimed at evaluating the ameliorative potentials of methanol extracts of *T. occidentalis* seed in sodium chloride (NaCl) induced hypertensive rats. The effect of high sodium chloride on phosphocreatine kinase, lactate dehydrogenase, sodium ion (Na^+) concentration was evaluated. The study also determines the *in vitro* antioxidant activities of *T. occidentalis* seed.

The result of the DPPH scavenging activity of methanol extracts of *T. occidentalis* seed suggested a significant dose dependent increase. Maximum activity was observed in 500 mg/kg dose. This observed DPPH scavenging activity may be due to the neutralization of free radicals either by the transfer of hydrogen atom or the transfer of an electron. Thus this could be attributed to the presence of active phytoconstituents in *T. occidentalis* seed.

Studies have shown that nitric oxide plays a vital role in various inflammatory processes such as carcinomas, juvenile diabetes, arthritis [12]. Nitric oxide is a potent pleitropic inhibitor of physiological processes such as smooth muscle relaxation, neural signaling and a regulator of cell mediated toxicity. The result of the nitric oxide inhibition assay shows a significant dose dependent percentage inhibition of nitric oxide by *T. occidentalis* seed extract.

Reactive oxygen species induce membrane damage by peroxidizing lipid moieties particularly the polyunsaturated fatty acids in a chain reaction known as lipid peroxidation. The inhibition of lipid peroxidation is considered the most important index of antioxidant activity. The result of the anti-lipid peroxidation assay of methanol extracts of *T. occidentalis* seed showed a significant dose dependent percentage increase. This result indicates that *T. occidentalis* seed can possibly prevent cellular abnormalities caused by free radicals by breaking down the chain reaction responsible for lipid peroxidation.

Lactate dehydrogenase is found in the cells of almost all body tissue, it catalyses the inter-conversion of pyruvate to lactate with concomitant inter-conversion of NADH and NAD^+ [13]. Tissue injury could result in the release of lactate dehydrogenase into the blood stream. Analysis of the isoenzymes of lactate dehydrogenase in the blood facilitates the diagnosis of some diseases. Groff and Gropper [13] and Hazra et al. [14] reported that mRNA expression of lactate dehydrogenase on the glycolytic metabolic pathway of the heart was markedly higher in spontaneously hypertensive rats compared to the control. Lactate dehydrogenase release into blood circulation has been linked to cardiac tissue damage. Phosphocreatine kinase is associated with hypertension [15]. High blood flow leads to injury to the muscle due to stretch activity thus creatine kinase leaks into the blood circulation [16]. The result from the analysis of serum phosphocreatine shows a significant increase in 10 mg/kg sodium chloride treated rats ($p<0.05$). Rats treated with 400 mg/kg methanol extract of *Telfairia occidentalis* seed showed a significant reduction ($p<0.05$) in serum phophocreatine kinase [17].

The significant reduction of serum phosphocreatine kinase and lactate dehydrogenase by the treatment with methanol extracts of *Telfairia occidentalis* seed can protect against cardiac tissue damage.

Sodium ion is one of the four major electrolytes abundant in extracellular ion. It plays an important role in body water balance and muscle contraction. The result from the analysis of serum sodium ion concentration of treated albino rats showed a significant reduction in serum sodium ion level of treated albino rats [18].

CONCLUSION

Methanol seed extract of *Telfairia occidentalis* possess antihypertensive properties, due to its antioxidant composition and equally can ameliorate the changes in hypertensive parameters induced by sodium chloride (NaCl).

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