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# Impact of Toxicity with Commercial Sodium Chloride Salt on Kidney and DNA of Male Mice-Part 1

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**Abstract:** Sodium chloride is widely used in various industrial, medical and food applications that increasing human exposure to it by excessive amount. However, almost all studies focused on its nephrotoxicity and adaptation of kidney cells to high concentration salt and only few scientific attentions were concerned on its cytotoxicity. Hence, the oxidative DNA damage induction by excessive salt intake in mice was investigated. mice were orally administrated with three different concentrations commercial NaCl solution started with concentration at tap water. Second and third concentrations were (50 & 100mg/L) orally administrated for 10 successive days then sacrificed 24 hour after the last orally administrated. Comet assays were used to evaluate the possible toxicity of excessive salt on DNA levels. Also, some biochemical markers of kidney were assessed to shed more light on salt mechanism of action. DNA damage induction by excessive intake of high salt was evidenced by the statistical significant elevations in both two group. This was further evidenced by appearance damage of DNA on agarose gel. Moreover, excessive NaCl caused significant elevation in kidney function level in a dose dependent manner. It was also noticed that Important result of this salt on (spoons) than any other table salt. *Conclusion*: we can say that: DNA damage induction by excessive salt intake was evidenced on DNA damage in mice Also, its reactive oxygen species generation ability was evidenced by the significant elevation of granzyme B and kidney function levels Therefore, it's recommended not use this table salt due to its dangerous toxic effects.

**Key words:** Commercial salt · DNA damage · GranzymeB · Kidney function and mice

## INTRODUCTION

Sodium Chloride (NaCl) is widely used in various applications in industry, medicine and nutrition e.g. smoked bacon or fish [1, 2]. It is also used in many brands of shampoo, toothpaste. Moreover, its uses in medicine, including: dilutions of many anticancer drugs and wounds disinfection [3,4]. It is used as well in treatment of sodium and chloride ions deficiencies caused by excessive salt restriction and in the muscle cramps prevention and heat prostration [5]. Thus, human exposure to excessive NaCl continuously increased its risk. In the workplace, concentrated Na Cl solutions may be irritating to the eyes and respiratory tract. Possible target organs may include the cardiovascular, gastrointestinal and nervous system [6].

Despite, NaCl is an essential nutrient for the healthy body e.g. conduction of nerve, contraction of muscle, extra-cellular fluid osmotic balance and other nutrients absorption. The toxic effect of taking excessive amount of salt causes many insults as increased blood pressure, muscle weakness, nephrotoxicity, neurotoxicity and cardiovascular complications [7,8]. Moreove, NaCl has been proved to be a promoter gastrointestinal tumors in experimental animals and could be associated with increased incidence of stomach cancer in human populations with traditional diets of highly salted foods.

Genotoxicity of high NaCl has been well investigated using different experimental system in *in vitro*. It has been induce DNA damage in Chinese hamster ovary (CHO), L5178Y cells, murine kidney and others in culture [9-11],

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chromosomal aberrations in CHO cells [12,13] and cause mutations in a mouse lymphoma cell line [14,15].

The available experimental data on the *in vivo* toxicity of high NaCl are limited. In *in vivo* genotoxicity and clastogenicity were evidenced by the appearance of DNA damage using comet assay and micronuclei induction in rat bladder epithelial cells [16], mouse renal inner medullas [7,17] in *Caenorhabditiselegans*[18] and in marine invertebrates [19]. Moreover, following excessive NaCl, Mre11 gets out of the nucleus, thus double-strand DNA breaks cumulates in the G2and S phases of the cell cycle and DNA repair is inhibited [10]. The exact process through which high NaCl causes DNA damage is not obvious, a variety of biological responses to DNA damage caused by high NaCl have been shown: induction of checkpoints of cell cycle [20-22], upregulation of the tumor suppressor p53 [23], growth arrest of the GADD45 and DNA proteins induced by damage. [20,24].

Recently, There were studies about DNA damage induction by NaCl [25,26]. Tamura and his team [7] found that high NaCl intake may lead to generation of reactive oxygen species mainly $H_2O_2$ and $O_2$- in normal rat gastric epithelial cells using the electron paramagnetic resonance (EPR).

Granzymes(Gzms) are a group of fivecellular enzymes that activate programmed cell death. GzmA and GzmB are the most abundant inside the cell. GzmB acts mainly by activating the caspase pathway.GzmA on the other hand acts by activating the caspase-independent programmed cell death (27).

The aim of this study was to investigate excessive salt intake on DNA in mice. Comet assay were used as cytotoxic indicators. Indeed, evaluation of kidney function markers: creatinein and urea level, granzyme B also done to shed more light on NaCl toxicity.

## MATERIALS AND METHODS

**Animals:** Male Swiss Webster mice aged 10-12 weeks (25-30 gm) were obtained from the animal house of National Research center (NRC). Animals were supplied with standard diet pellets and water that were given *ad. Libitum*. They were kept in plastic cages for 7 days to be accommodated with our laboratory conditions before being treated.

**Chemicals**

**Analysis of Sodium Chloride:** A stock solution of commercial sodium chloride powder was prepared to give 1ml=1mg this was carried out by weight 1,648gm and dissolving it by distilled water and diluted to one liter according to standard methods for the examination of water and waste water 19th edition 1995. Other wise a measurement of chloride for drinking water was occurred by taking 50ml sample from tap water. It was found to be 31mg/Lchloride then a similar concentration of 30mg/L was prepared from previous commercial sodium chloride powder for injection of tested laboratory animals, then concentration of 50 and 100mg/L respectively was prepared for this purpose.

Sodium chloride (NaCl) was purchased in the form of an odorless white soft crystal with a distinctive taste (Almassa company, Egypt)

**Treatment Schedule:** Animals were divided randomly into three groups of five animals each. Negative control group (group 1) was orally administrated. with dist. $H_2O$ and the remaining two groups were orally administrated with the fixed NaCl dose level for 10 days (50 and 100mg/L) concentrations one times and scarified after 24 hour of the last orally administrated.

**Laboratory Methods:** Five milliliters of blood was withdrawn from the rats into two sterile vaccutainers; one containing EDTA and the other without additives to separate serum.

Serum samples were assayed, within 2 hours, for urea and creatinine using the automated clinical chemistry analyzer Olympus AU 400 analyzer,GranzymeB assay by RayBiotech. Mouse GranzymeB ELIZA Kit Protocol Lot no.0000515069. The other tube containing EDTA used immediately to measured DNA damage by the Comet Assay.

**Measurement of Comet Assay:** The alkaline (pH >13) comet assay was performed according to the method described by [28], with minor modifications

**Cell Preparation:** Peripheral blood leukocytes were isolated by centrifugation (30 min at 1300g) in Ficoll-Paque density gradient (Pharmacia LKB Biotechnology, Piscataway, NJ, USA). After centrifugation, leukocytes were aspirated and washed twice by phosphate-buffered saline at pH 7.4 (PBS).

**Preparation of Cell Microgels on Slides:** The comet assay was performed according to Singh and colleagues [28] with modifications according to Blasiak and colleagues [29]. Cell microgels were prepared as layers.

The first layer of gel was made by applying 100 µl of normal melting point agarose (0.7%) onto a precleaned microscope charged slides and coversliped gently. The coverslip was removed after the agarose solidified at 4°C. Low melting-point agarose (0.5%) was prepared in 100 mmol/L PBS and kept at 37°C. Approximately, 1500 of peripheral blood leukocytes were mixed with the low melting-point agarose and 100 µl of the mixture was applied to the first gel layer. The slides were then covered with a coverslip and placed at 4°C for solidification. After the second layer solidified, the coverslips were removed from the cell microgels. A final layer of low-melting agarose was added followed by coverslips, left to solidify for 10 minutes then the coverslips were removed.

Lysis of cells, DNA unwinding, gel electrophoresis, DNA staining The slides were covered with 100 ml of ice-cold freshly prepared lysis solution buffer pH 10 (2.5 mol/L NaCl, 100 mmol/L EDTA, 1% Sodium hydroxide, 10 mmol/L Tris, 1% Triton X-100, 10% DMSO) for at least 1 h. After draining, microgels slides were treated with DNA unwinding solution (300 mmol/L NaOH, 1 mmol/L EDTA, pH 13) for 30 min at 4°C and placed directly into a horizontal gel electrophoresis chamber filled with

**DNA-Unwinding Solution:** Gels were run with constant current (300 mA at 4°C) for 30 min. After electrophoresis, the microgels were neutralized with 0.4 M Trisma base at pH 7.5 for 10 min. The slides were stained with 20 µl ethidium bromide (10 µg/ml).

**Visualization and Analysis of Comet Slides:** The slides were examined at 40× magnification using an inverted fluorescence microscope (IX70; Olympus, Tokyo, Japan) equipped with an excitation filter of 549 nm and a barrier filter of 590 nm, attached to a video camera (Olympus). Damaged cells were visualized by the "comet appearance", with a brightly fluorescent head and a tail to one side formed by the DNA containing strand breaks that were drawn away during electrophoresis. Samples were analyzed by counting the damaged cell out of 100 cells per slide to calculate the percent of damage.

## RESULTS

Data are presented as mean ±SD. The compiled data were computerized and analyzed by SPSS PC+, version 14. The following tests of significance were used: t test between means to analyze mean difference. A value of p =0.05 was considered significant, p<0.001 was considered highly significant and p>0.05 was considered insignificant.

In table 1As shown excessive salt intake of both group (50& 100mg/L) salt concentrations resulted in statistical significant increases in the kidney function, granzyme B and DNA damage.

The percent of DNA damage correlated strong positively with createinin p(0.002) and with granzyme B where P(0.049) at high concentration salt (100mg/L).

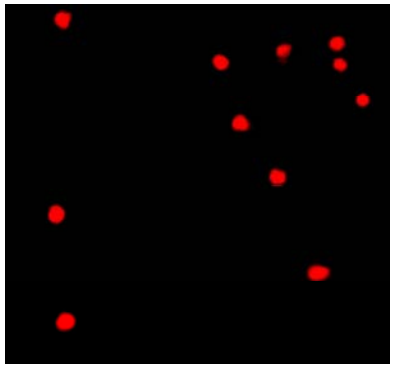

Fig. 1: Normal DNA

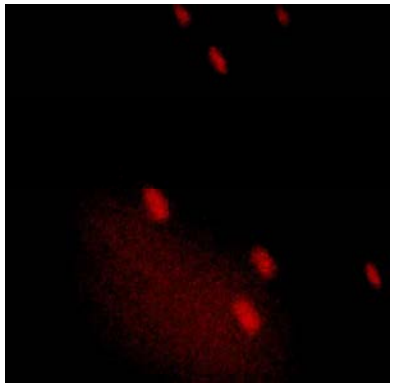

Fig. 2: DNA damage at 100 mg/L

Table 1: Shows results of the laboratory tests applied to experimental animals

| parameter | Granzyme B | DNA damage % | Urea | createinin |
|---|---|---|---|---|
| control | 28.3000±2.54034 | 5.6667±1.03280 | 37.0000±2.36643 | 0.2467±.2467 |
| Salt 50mg/L | 36.1500±3.86825 | 26.6667±2.58199 | 50.6000±11.69235 | 0.2440 ±0.01713 |
| Salt 100mg/L | 51.3000±5.88897* | 50.0000±4.47214** | 77.6000±15.25487 | 0.276+-0.0157 |

At the 0.05 level (2-tailed).*. Correlation is significant

At the 0.01 level (2-tailed)**. Correlation is significant

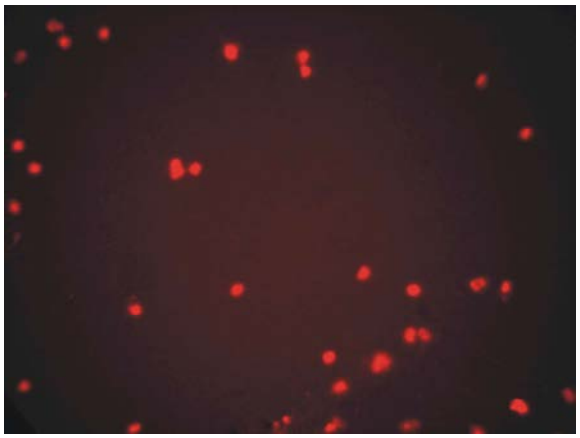

Fig. 3: DNA damage at 50mg/L


Fig. 4: Impact on the (spoons)

**Comet Assay:** Administration of NaCl different concentrations at the dose level of 0,50 and 100% induced DNA damage as shown by the statistical significant increases in DNA migration from nucleus (Fig. 2) when compared with negative control group (group 1). a strong positive correlation between DNA and different NaCl concentrations.

Also there is impact on the (spoons) as shown in figure 4.

## DISCUSSIONS

The present study was designed to investigate DNA damage, granzyme B and kidney function induction by NaCl in mice. For less knowledge about toxicity of Na Cl (in level of DNA),

Also important of NaCl increasingly used in various applications, e.g. fermentation, disinfection, nutrition and dilutions of several drugs thereby, Therefore work done.

The recent discovery that DNA double-strand breaks causes by high NaCl, damage DNA known added it to the list of toxic stresses [7]. In our study comet assay evidenced the DNA damage induction by high NaCl intake as indicated by the statistical elevations in DNA migration assessed by percentage (Fig.2, 3) In this study, the appearance of DNAdamage is less than 50 mg/L compared with DNA marker further evidenced the dramatic DNA damage by the high NaCl intake(100mg/L) showed by high migration rate of breakage DNA fragments on agarose gel. In a harmony with the previous study of Dmitrieva *et al*. [7] showed that high NaCl damaged DNA as manifested by DNA fragmentation and double-breaks induction in mouse inner medullar cells.

Despite that the exact mechanism for NaCl–caused DNA damage is not clear, either physical disruption in the structure of chromatin or the free radicals generation (secondary to cellular disruption) have been postulated as being responsible for inducing damage to the DNA, phosphodiester skeleton, [9].

This study was focused on estimating granzyme B induced oxidative stress as one of toxic mechanisms of NaCl DNA damage induction.

Despite, Na ion as positivelycharged and Cl ion as negatively charged often function together within body is the predominant electrolytes in the fluid surrounding cells. Salt-induced high osmotic pressure environment inhibited a mitochondrial electron transfer system to involve superoxide anion production using therapy increasing superoxide anion radicals [31].

In mammalian cells Granzymes (Gzms) and caspases can induce apoptosis by triggeringprocesses targeting critical cellularprograms needed for survival—causing DNA damage, inducing reactive oxygen species (ROS), damaging the mitochondrial outer membrane, RNA splicing interference programs and translation of cellular protein. There is no one substrate/pathway that universally essential for cell-death. Mitochondrial damage is needed for example, in some pathways to kill some cells, but not others. DNA damage is not essential, because enucleated cells also undergo programmed cell death. Apoptosis also disrupts cellular repair (e.g., by cleaving and inactivating key DNA repair enzymes). This multistep attack means that virus-infected or cancer cells that resist damage to one pathway or another are still killed. The Gzms and caspases can cleave a few hundred substrates in mammalian cells.(27)

Oxidative stress has been addressed as one of the main causes of DNA damage as reactive oxygen species. (ROS) overproduction can cause severe damage to cellular macromolecules, especially the DNA [32]. ROS generation by salt intake was evidenced in this study by

significant elevations in granzyme B (GB) induce reactive oxygen species (ROS) formation [33], loss of transmembrane potential(I)( Gzms enter mitochondria where theyact by cleaving proteins in electron transport chain (ETC) complex I to produce superoxide anion, that plays a critical role in triggering apoptosis[34].

## CONCLUSION

DNA damage induction by excessive salt intake was evidenced by the observed significant increases in DNA may be attributed to ROS generation. Thus, it is recommended to prevent usage cheap table commercial Sodium Chlorid Salt. To avoid its risks including genotoxicity.

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