



Inhibitory Effect of Dry Garlic Powder on the Nickel Chloride-induced Somatic and Germinal Cell Damages in Male Mice

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

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ABSTRACT

Background: Nickel is commonly used in industry, utensils and also present in cigarette. Over-exposure of nickel is known to cause several health complications including somatic and germinal cell injuries. Garlic (*Allium sativum*) being a spice has several pharmacological properties. However, its role on the nickel chloride induced somatic and reproductive cells damages are poorly studied in the literature.

Objective: To evaluate the effect of garlic powder on nickel chloride induced somatic and germinal cell damages in male mice.

Methods: Dried garlic clove powder is used in this study in three doses via., 50. 100 and 150 mg/kg, per oral (p.o) for 4-weeks against the nickel chloride [(10 mg/kg, intraperitoneal (i.p))] induced somatic and germinal damages. Somatic cells damages were studied using peripheral micronucleus test and germinal cell damages by sperm count and sperm shape abnormalities in male mice. Further, the *in-vitro* hydrogen peroxide scavenging activity of the garlic was evaluated. Ascorbic acid was tested as a standard drug. The data of the results were analyzed by One-way Anova and Tukey as post hoc test.

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Results: The result indicated that nickel chloride enhanced significantly ($p < 0.001$) both somatic and germinal cell damages compared to control animals. The administration of garlic powder at 150 mg/kg significantly ($p < 0.001$) minimized the frequency of micronuclei in the erythrocytes and reduced the spermatozoa anomalies compared to the nickel chloride group. The garlic powder also exhibited significant hydrogen peroxide scavenging activity.

Conclusion: The data indicated that garlic powder at 150 mg/kg reduced the cellular damaging effects of nickel chloride on somatic and germinal cells and the mechanism could be related to the free radical scavenging activity.

Keywords: Nickel; garlic; micronuclei; sperm abnormalities; free radical scavenging effect.

1. INTRODUCTION

Nickel abundance in the earth crust is ranked 24th and is extensively used in alloy industries, batteries, as pigments and catalysts. Exposure to nickel can occur through nasal, cutaneous, oral and a significant amount of the metal also enters the body through tobacco smoking, leaching and corrosion from various sources that we use in daily life [1].

Studies in the past confirmed that water-soluble nickel compounds carries substantial risk of nuclear damage. Besides, nickel is reported to affect the reproductive system in both male and females manifested as infertility, spontaneous abortions, birth defects and premature ageing. The damages on the nuclear part are reported to contribute in mutation [2]. The genetic alterations have the tendency to cause several pathological conditions such as heart ailments, neurological diseases and cancer, along with the risk of transmission to progeny through hereditary defects [3].

Among the battery of tests available, the micronucleus test is widely used as the tool to assess both DNA damaging ability of the chemical as well the protective potential [4]. The male reproductive damage can be analyzed quantitatively and qualitatively by sperm shape abnormality and sperm count tests, respectively [5].

Substances derived from nature have been traditionally used in the prevention and treatment of several diseases. Some of the common medicines reported to possess the protective effect against metal-induced toxicities are *Withania somnifera*, *Azadirachta indica*, *Asparagus racemosus* and *Glycyrrhiza glabra* [6]. In addition to these the common dietary products such as garlic, ginger, green teas have been reported to exhibit the inhibitory activity against the chemical-induced nuclear damages. Since it is difficult to avoid the exposure to

environmental pollutants and toxic metallic compounds, one of the easiest ways to minimize their effect is to enhance the intake of those dietary components that provide protection against the damages [7].

Garlic (*Allium sativum* L) is a spice of monocot, bulb-forming plant. Garlic contains 33 sulfur compounds, several vitamins such as A, B1 and C besides minerals like iron, calcium, selenium, copper and germanium [8]. The research on garlic have demonstrated that it can lower blood pressure, blood cholesterol, blood sugar, boost immune system besides exhibiting antimicrobial properties. Studies also indicated that garlic administration has the tendency to ameliorate the antioxidant defense mechanism of the host system [9]. However, the ability of garlic to protect somatic and reproductive cellular damages induced by metallic ions like nickel is poorly studied in the literature. Hence, in this study, the inhibitory effect of dry garlic powder suspension was tested against the nickel chloride induced nuclear damages and reproductive cell defects in male mice.

2. MATERIALS AND METHODS

2.1 Animals

Swiss albino mice (male) weighing 30-35 gm was selected for the present study. The animals were maintained in the standard laboratory condition with 12 hours dark/bright environment and room temperature (20-22°C). Animals were provided with pelleted food and water *ad-libitum*. Animal experimentation was done after obtaining the prior permission from the institutional animal ethics committee (Approval ID 2019-CP-2). Randomly selected animals were grouped as follows:

- **Group-1:** Control
- **Group-2:** Nickel chloride (NiCl_2 - 10 mg/kg, i.p) [10]

- **Group-3:** Treatment-1: NiCl₂ + Garlic powder suspension (50 mg/kg, p.o) [11]
- **Group-4:** Treatment-2: NiCl₂ + Garlic powder suspension (100 mg/kg, p.o)
- **Group-5:** Treatment-3: NiCl₂ + Garlic powder suspension (150 mg/kg, p.o)
- **Group-6:** Standard: NiCl₂ + Ascorbic acid (15 mg/kg, p.o) [12].

2.2 Induction of Nuclear Damages

The nuclear damage in the somatic and germinal cells was induced by the intra-peritoneal administration of nickel chloride (10 mg/kg) [10]. The peripheral blood micronucleus assay and sperm count and shape abnormalities were tested to study the somatic and germinal cells damages, respectively.

2.3 Garlic Powder

Fresh cloves of the garlic were dried in shade and grinded into fine powder. The powder was triturated in distilled water and dispersed suspension then administered to the animals as per the dose and body weight [11]. Three doses viz., 50, 100 and 150 mg/kg were administered by oral route daily for 4-weeks. On the last day, after 1 hour of dosing, a drop of blood was collected from tail vein under light diethyl ether anesthesia for peripheral blood micronucleus test and then the animals were sacrificed to excise the testis.

2.4 Peripheral Blood Micronucleus Test

A drop of blood collected on a clean glass slide was smeared immediately. Micronucleus is the fragment of nucleus that remain in the cytoplasm after DNA damage. The micronuclei test described by Hayashi, et al (1990) was used to study the frequency of micronuclei in two types of RBCs viz., polychromatic (P) and normochromatic (N) erythrocytes [13]. The dried, smeared slides were sequentially stained in Wrights' and Giemsa stains. The presence of micronuclei was identified by using light microscope under oil immersion objective [14]. A total of 1000 cells were counted and the ratio of polychromatic to normochromatic (P/N) was used to study the effect of treatment on erythropoiesis.

2.5 Total Sperm Count

The analysis of total sperm count was done as per the method described by D'Souza (2004) [15]. The suspension of the sperms isolated from

the caudal epididymis was filtered to remove the tissue debris. A 3-4 drops of 1% aqueous Eosin-Y stain was added and the total sperm count was done using the Neubauer's chamber. The stained solution was taken in the WBC pipette upto 0.5" mark and immediately diluted with phosphate buffer upto 11" mark. The solution was mixed for 1-2 minutes and then charged into the chamber. The sperm count will be done after allowing the sperm to settle in the chamber. The total number of sperms present in the four chambers were taken and represented as cubic millimeter after multiplying with dilution factor (50,000).

2.6 Sperm Abnormality Test

The qualitative defects in the sperms are determined by observing the alterations in the sperm shapes. The stained solution of the sperms is smeared on a clean glass slide. After air drying, the smears were observed under light microscope. Six types of sperm abnormalities such as hook less, tailless, banana shaped, double hooked and double tailed [14] as per the procedure described by Wyrobek (1984) [16].

2.7 Relative Weight of Testis

The right testis was isolated immediately from the sacrificed animal and weighed. The relative reproductive organ weight was calculated from the following formula [15];

$$\text{Relative testis weight} = \frac{\text{Weight of the testis}}{\text{Weight of the animal}} \times 100$$

2.8 In-vitro Antioxidant Assay

The in-vitro antioxidant assay was done by the procedure of Ruch, et al (1989). In this method a 40 mM solution of the hydrogen peroxide was prepared in phosphate buffer (50 mM, pH 7.4). Different concentration of the garlic powder solution (20 to 80 µg / mL) were added to the hydrogen peroxide solution, the absorbance was recorded at 230 nm after allowing the mixture to stand for 15 minutes [17]. The percentage scavenging activity was calculated from the formula;

$$\text{Percentage scavenging activity} = \frac{(\text{Abs of control} - \text{Abs of test})}{\text{Abs of control}} \times 100$$

2.9 Statistics

The data obtained from the study was statistically evaluated by One-way Anova followed by post

hoc analysis by Tukey. $p < 0.05$ was considered to indicate the significance of the result.

3. RESULTS

The data summarized in the Table 1 indicated that administration of nickel chloride (10 mg/kg) significantly ($p < 0.001$) increased the percentage micronuclei in both polychromatic erythrocytes (PCEs) and normochromatic erythrocytes (NCEs), and reduced the P/N ratio compared to control group. Among the three tested doses of garlic powder (50, 100 and 150 mg/kg), the highest dose (150 mg/kg) reduced significant ($p < 0.01$) the micronuclei frequency in erythrocytes compared to nickel-treated animals without affecting the P/N ratio. The percentage inhibition was found to be 36.5 % for PCEs and 32.3% for NCEs. Garlic powder at 100 mg/kg inhibited significantly ($p < 0.05$) the micronuclei only in NCEs (21.5%) compared to the nickel group. Further, administration of ascorbic acid significantly ($p < 0.01$) reduced the micronuclei in both PCEs

(40.6%), NCEs (40.7%) and also enhanced the P/N ratio compared to the nickel-treated group.

The observations indicated that nickel chloride administration to the male mice significantly ($p < 0.001$) reduced the total sperm count and increased the sperm shape abnormalities compared to the normal animals. The highest tested dose of garlic powder (150 mg/kg) significantly enhanced ($p < 0.01$) the diminished spermatozoa number and reduced ($p < 0.001$) the sperm abnormalities compared to the nickel-treated animals. The percentage variation was found to be 24.4% for sperm count and 26.9% for sperm shape abnormality. The medial tested dose of garlic (100 mg/kg) produced reduction ($p < 0.05$) in only the sperm shape abnormality (14.3%) compared to the challenge group. Ascorbic acid on the other hand exhibited significant ($p < 0.01$) variation in both sperm count (27.8%) and sperm morphology (30.9%) compared to the nickel group (Table 2).

Table 1. Effect of garlic powder on the percentage micronuclei in erythrocytes and P/N ratio in nickel chloride treated animals

Sl. no.	Treatment	% MN in PCE	% MN in NCE	P/N ratio
1	Control	0.62 ± 0.02	0.57 ± 0.04	0.26 ± 0.02
2	NiCl ₂ (10 mg/kg)	1.23 ± 0.03 ^a	1.30 ± 0.05 ^a	0.15 ± 0.01 ^a
3	NiCl ₂ + GP suspension (50 mg/kg)	1.32 ± 0.07	1.29 ± 0.06	0.16 ± 0.02
4	NiCl ₂ + GP suspension (100 mg/kg)	1.02 ± 0.08	1.02 ± 0.05*	0.17 ± 0.05
5	NiCl ₂ + GP suspension (150 mg/kg)	0.78 ± 0.09**	0.88 ± 0.02**	0.17 ± 0.03
6	NiCl ₂ + Ascorbic acid (15 mg/kg)	0.73 ± 0.07**	0.77 ± 0.06**	0.27 ± 0.01**

Values are represented as Mean ± S.E.M

Statistics: One way ANOVA followed by post test Tukey

^a $p < 0.001$ compared with control

* $p < 0.01$, ** $p < 0.001$ compared with Nickel chloride

Table 2. Effect of garlic powder suspension on the total sperm count and percentage sperm shape abnormality in nickel chloride treated animals

Sl. no	Treatment	Total sperm count (10 ⁶ per cu mm)	Percentage sperm shape abnormality
1	Control	25.1 ± 0.91	11.5 ± 0.88
2	NiCl ₂ (10 mg/kg)	17.6 ± 1.02 ^a	22.3 ± 0.72 ^a
3	NiCl ₂ + GP suspension (50 mg/kg)	18.3 ± 0.77	23.7 ± 0.43
4	NiCl ₂ + GP suspension (100 mg/kg)	19.2 ± 0.71	19.1 ± 0.55*
5	NiCl ₂ + GP suspension (150 mg/kg)	21.9 ± 0.65**	16.3 ± 0.70***
6	NiCl ₂ + Ascorbic acid (15 mg/kg)	22.5 ± 0.48**	15.4 ± 0.88***

Values are represented as Mean ± SEM

Statistics: One way ANOVA followed by post test Tukey

^a $p < 0.001$ compared with control

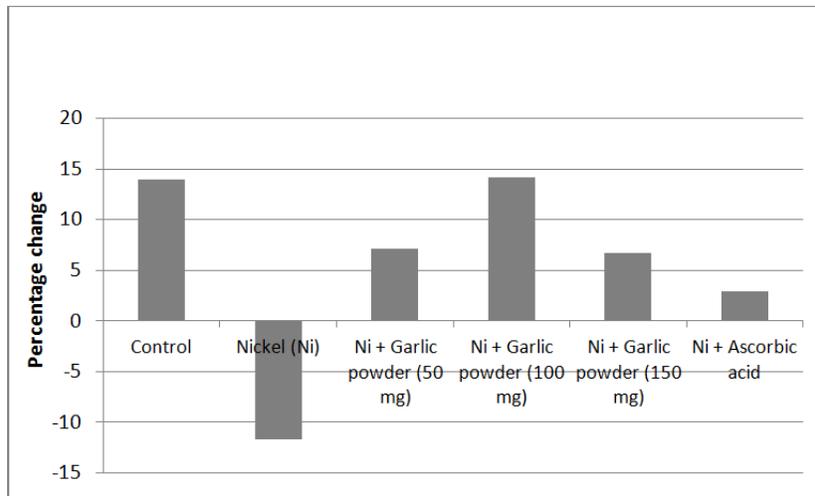
* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared with Nickel chloride

The values from the graph indicated that various treatment schedules produced positive change on the body weight except the nickel chloride administration. Nickel chloride at 10 mg/kg produced negative change on the body weight (-11.71%) whereas other treatments such as garlic powder and ascorbic acid produced positive change. The most prominent positive change was observed when garlic powder was tested at 100 mg/kg (+14.16%) followed by the control group (+13.97%).

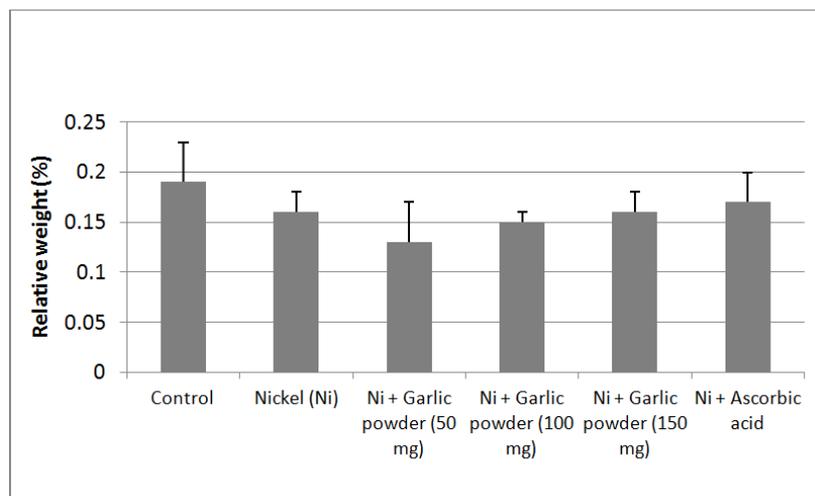
Our observations further indicated that none of the treatments including the nickel chloride, garlic

powder and ascorbic acid in the tested doses and durations significantly altered the relative weight of testis when different groups were compared (Graph 2).

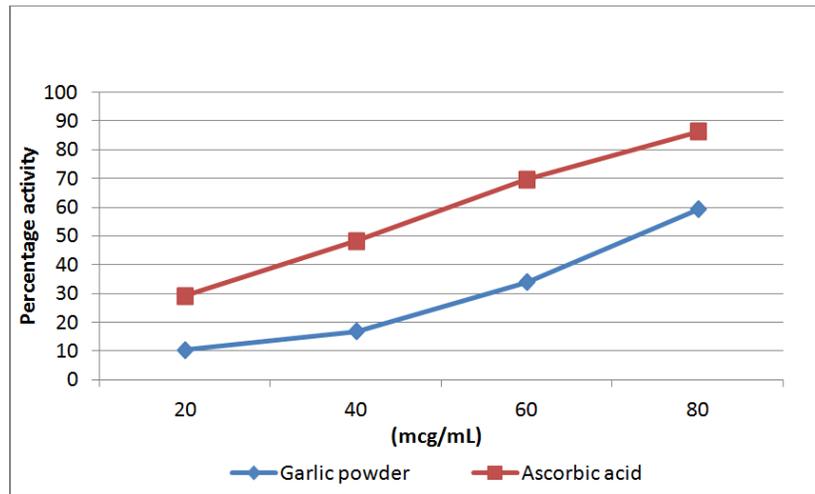
Four doses (20, 40, 60 and 80 mcg/mL) of garlic powder and ascorbic acid were tested for the hydrogen peroxide scavenging activity. The observations indicated that a dose-dependent scavenging activity was observed and the maximum effect was found at 80 mcg for both garlic (59.3%) and ascorbic acid (86.3%) when compared with the control blank (Graph 3).



Graph 1. Effect of garlic on the percentage change in body in nickel chloride treated animals



Graph 2. Effect of garlic on the relative weight of testis in nickel treated animals
Values are represented as Mean ± SEM



Graph 3. Percentage hydrogen peroxide scavenging activity

4. DISCUSSION

Micronucleus test is an established assay to determine the clastogenic ability of a compound. Micronuclei are the fragments of nucleus left in the cytoplasm of erythrocytes after the nuclear damage when the nucleus is expelled out during erythropoiesis. The increased percentages in two types of erythrocytes viz., polychromatic (PCEs) and normochromatic (NCEs) indicated the capability of the compound to produce the DNA damage. The ratio of these two types of erythrocytes (P/N) will suggest whether the test-chemical has affected the rate of erythropoiesis or not [4]. The decrease in P/N ratio demonstrates the cytotoxic potential. The chemical-induced reproductive damages can be studied qualitatively and quantitatively by estimating the sperm shape abnormality and total sperm count, respectively [5,15]. Together, these tests act as indices for the determining the somatic cell and reproductive cell anomalies [14].

The observation from the study indicated that administration of nickel chloride (10 mg/kg) significantly ($p < 0.001$) increased the percentage micronucleated polychromatic and normochromatic erythrocytes, and reduced the P/N ratio compared to control (Table 1). The administration also significantly ($p < 0.001$) reduced the total sperm count and enhanced the sperm shape abnormalities compared to normal animals (Table 2).

Nickel induced reproductive toxicity and ability to induce chromosome defects have been demonstrated in the earlier studies. The various

mechanisms suggested include bonding with sulfhydryl groups of proteins, inhibition of ATPase activity and depletion of glutathione levels [18]. The oxidative stress due to the generation of free-radicals is believed to be an important mechanism for nickel induced toxicity. The free radicals are generated from the reaction of nickel with thiol complexes of molecular oxygen and / or lipid hydroperoxides produces cellular damages including the nucleus of the host cells [3]. In addition, nickel ions interfere with nucleotide and base-excision repairs. These modifications are reported to contribute in stimulated cell proliferations, either by activation of proto-oncogenes or interfering with tumor-suppressor genes leading to carcinogenesis [2,18].

The administration of dried garlic powder suspension to the nickel chloride treated animals showed dose-dependent inhibition in the mutagenic and reproductive damages in male mice. The highest tested dose (150 mg/kg) significantly ($p < 0.01$) reduced the population of micronucleated erythrocytes, sperm shape abnormalities and increased the sperm count compared to the nickel-treated animals (Tables 1 and 2). The observations suggested that garlic powder inhibited the nickel-induced damages on the DNA and male reproductive cells. Several studies in the past have reported that substances of natural origin such as *Acacia salicina*, *Terminalia arjuna*, *Acanthopanax divaricatus var*, *Phellinus rimosus* and *Mangifera indica L* have suppressed the cellular damages induced by environmental chemicals [19]. These studies indicated that the plant extracts exhibit multiple

pathways such as scavenging the mutagens, enhancement of antioxidant status, modulation of DNA-repair enzymes, blocking the mutagens from binding to cellular components and antioxidant activity plays a vital role in protecting the toxic manifestations of environmental pollutants [6,7,19].

Antioxidant property of garlic is mentioned in the literature. Garlic reported to provide protection against free radical damages. The study indicated that the chemical components of garlic such as allicin suppressed the formation of superoxide by xanthin/xanthin oxidase system, and alliin, allyl cysteine and allyl disulfide scavenged hydroxyl radicals [8,20]. Further, our study also indicated that the solution of garlic powder exhibited significant hydroxyl radical scavenging activity in *in-vitro* assay (Graph 3). From these observations, it can be suggested that the free radical scavenging activity of garlic powder might be responsible for protecting against the nickel chloride-induced nuclear and reproductive damages, probably through the mechanisms discussed earlier [6,7,19]. However, garlic powder did not alter significantly the diminished P/N ratio induced by nickel (Table 1), indicating that the treatment might not attenuated the cytotoxic actions of nickel chloride [4]. On the other hand, ascorbic acid tested as a standard antioxidant agent significantly inhibited nickel chloride-induced chromosome and reproductive damages besides improving the P/N ratio.

Another observation of the study is that neither the administration of nickel chloride nor the treatment of garlic powder altered the relative weight of the testis, although nickel chloride produced negative change in the body weight (Graphs 1 and 2). These observations suggest that nickel chloride through its toxic expressions might have affected the body weight negatively [2]; however various tested-treatments and durations of nickel, garlic and ascorbic acid have no significant effect on the weight of the male sex organ.

5. CONCLUSION

The observations from the study indicated that nickel chloride at 10 mg/kg enhanced the micronuclei frequency in peripheral erythrocytes and contributed in the qualitative and quantitative defects in spermatozoa in mice. Garlic powder suspension at 150 mg/kg inhibited the nickel chloride mediated nuclear and reproductive damage most probably through the antioxidant

potential. Garlic being a spice, its regular intake might benefit in reducing the health complications associated with environmental chemicals such as nickel ions.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Animal experimentation was done after obtaining the prior permission from the institutional animal ethics committee (Approval ID 2019-CP-2).

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COMPETING INTERESTS

Author has declared that no competing interests exist.

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