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# Chlorella Vulgaris Extract protects against Sodium Nitrite Toxicity in Male Rats

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**A B S T R A C T**

The current study was carried out to investigate the protective potential of Chlorella Vulgaris (CV) extract against sodium nitrite toxicity. Forty-five male Wister albino rats were assigned into 5 groups (n=9). Group (A) rats received normal saline orally for 3 months, Group (B): rats administered CV extract (70 mg/kg.BW) orally for 3 months, Group (C): rats received sodium nitrite (80 mg/kg.BW) orally for 3 months, Group (D): rats simultaneously received CV along with sodium nitrite treatment, orally, daily for 3 months, and Group (E): rats pre-treated with CV extract for 4 weeks followed by simultaneous treatment with sodium nitrite and CV extract for additional 8 weeks. Treatment of rats with sodium nitrite significantly increased serum urea and creatinine levels, associated with interstitial edema with mononuclear cells infiltrations, the renal tubular epithelium showed cloudy swelling and sever coagulative necrosis with desquamation of tubular epithelium and renal cast, the glomeruli showed congested glomerular capillaries. Besides, it decreased total leucocytic count and platelets count with normal hemoglobin, red blood cell count, hematocrit levels, mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration. Pre-treatment and co-treatment of sodium nitrite-treated rats with CV in groups (D & E) significantly prevented sodium nitrite-induced alterations of renal function and histological architecture. This study indicated that CV extract ameliorates the renal dysfunction induced by sodium nitrite toxicity.

**Keywords**: Chlorella vulgaris, Sodium nitrite, kidney, platelets, total leucocytic count.

## 1. Introduction

The wide use of these food additives in food technology increased the importance of studying their side effects on mammals (Ezz El-Arab et al., 2006). Sodium nitrite is a food additive used in processed meats and in fish products (Jørgen & Marianne, 2011). Chronic administration of sodium nitrite in food can induce inflammation and tissue destruction to many body organs (Hassan et al., 2009, 2010; Sherif & Al-Gayyar, 2013; Al-Gayyar et al., 2014, 2015). Sodium nitrite exerts its toxic effect by the generation of free radicals, which made oxidant / antioxidant imbalance (Naik, 2006). Nitrite in food might react with the amines of food in the stomach producing carcinogenic nitrosamines (Hassan and Ali, 2010) which cause excessive the generation of free radicals (Hassan and Yousef, 2010). Nitrite is a potent nitric oxide donor which possess detrimental biological effects (Jensen, 2007).

Chlorella vulgaris extract contains very powerful naturally occurring antioxidant and free radical scavenging activities (Jiang et al., 2010). It has high antioxidant and therapeutic uses such as lutein, α, β-carotene, ascorbic acid, and α-tocopherol, which are effective against free radicals and may be responsible for Chlorella functional activities (Zhao and Sweet., 2008: Plaza et al., 2009). it possesses hypoglycemic and hypolipidemic effects (Ebrahimi -Mameghani et al., 2014), antibacterial activities (Dineshkumar et al., 2017), protective activities against tumors (Ramos et al., 2010) and hepatotoxicity (Cai et al., 2015).

## 2. Materials and Methods

Sodium nitrite (CAS No: 7632-00-0), Methanol (99.5% PIOCHEM) were purchased from the El-Gmhoria Company (Cairo, Egypt). Chlorella vulgaris powder was obtained from Algal Biotechnology Unit, National Research Centre (Dokki, Cairo, Egypt). Diagnostic kits for assessment of serum urea (CAT. No:318002) level was purchased from Egyptian Company for Biotechnology (SPECTRUM DIAGNOSTICS). Diagnostic kits for assessment serum creatinine (CAT. No:1001110) level was purchased from (SPINREACT DIAGNOSTIC)

2.2. Experimental design and animal groups:

Forty-five healthy albino Wister male rats weighing about 100-120g were obtained from Vac Sera company (Helwan, Egypt). Rats were housed in polypropylene cages under standard hygienic conditions and supplied with free access to basal diet (AL wadi- Company, Egypt) and water. The rats were housed under natural ventilation, a 12 h light/dark cycle, and at a temperature of 20–22Ċ. Rats were acclimatized for 10 days before the beginning of the treatment.

All experimental design and procedures were approved by the Research Ethics Committee of the Faculty of Veterinary Medicine, University of Sadat City, Egypt (VUSC-002-2-20). Rats were randomly allocated into 5 equal groups of 9 rats each.

Group (A), rats were received 0.2 ml of normal saline/ 100 g BW orally, daily for 3 months.

Group (B), rats were received CV extract dissolved in 0.9% sodium chloride solution at dose of 70 mg/kg.BW orally, daily for 3 months (Vijayavel et al., 2007).

Group (C), rats were treated with sodium nitrite in distilled water at dose rate of 80 mg/kg.BW (Elsherbiny et al., 2017) orally, daily for 3 months.

Group (D), rats were simultaneously treated with CV along with sodium nitrite treatment, as described in the CV and Sodium nitrite-treated groups.

Group (E), rats were pre-treated with CV extract for 4 weeks followed by simultaneous treatment with sodium nitrite and CV extract for additional 8 weeks.

2.3. Blood and tissue sampling:

Blood samples were collected after 12th week of experiment for serum collection. The animals were anaesthetized with Diethyl ether (DEE). Blood samples were collected from the inner cansus of the eye using capillary tubes. The collected blood samples divided into 2 parts: the first sample was poured gently and carefully on the wall of clean, dry and labeled glass centrifuge tubes without using anticoagulant and left for clotting at room temperature (RT), followed by centrifugation at 3000 rpm for 15 minutes. The clear supernatant serum was aspirated and finally stored at -200C till used for investigation of urea and creatinine, second part: blood was collected in other tube contains anticoagulant (EDTA) used for estimation of Hb, WBCs, RBCs, Hb indices & platelets (CBC). Rats were sacrificed and the kidney was immediately picked up, rinsed by 0.9% saline solution and preserved in 10% neutral buffered formalin and used for histopathological examination.

2.4. Estimation of serum urea and creatinine levels:

Colorimetric kinetic method according to manufacturer's instruction used for measuring serum creatinine concentration.

Serum urea concentration was measured according to manufacturer's instruction by modified urease –Berthelot method

2.5. Methods used for measuring hematological parameters (CBC): using cell counter analyzer (GENRUI KT 6400 analyzer, China)

2.6.Histopathological examination:

Kidneys were collected and fixed in 10% neutral buffered formalin for 3 days. fixed samples were routinely processed, embedded in paraffin wax, cut into 4 µm sections and stained with hematoxylin and eosin (H&E) (Bancroft et al., 1996).

2.7. Statistical analysis:

All data were presented as mean ± standard error (SE) and were tested for significance by using one-way analysis of variance (ANOVA test) and P value < 0.05 were considered significant by using the statistical analysis system program SPSS (SPSS version 13.0, IBM, Chicago, IL, USA)

3.1. Chlorella Vulgaris extract ameliorated sodium nitrite increased levels of renal function biomarkers:

Sodium nitrite increase serum urea and creatinine levels significantly in group (D) in comparison with group (A). Administration of C.V in group (B) has no effect in serum urea and creatinine levels compared to group (A). co-treatment and pre-treatment of sodium nitrite-treated rats with CV caused significant decreased in urea and creatinine levels in groups (D) & (E) compared to group (C).

3.2. Chlorella vulgaris improved platelets and total leukocytic counts decreased by sodium nitrite treatment

Chlorella vulgaris and sodium nitrite had no significant changes in hemoglobin (Hb) %, red blood cells (RBCs) count & hematocrit (HCT) % in different group of rats compared to control group. Sodium nitrite treatment increased red cell distribution width (RDW) % and lymphocytes % significantly in group (C) in comparison with group (A) but significantly decrease platelets (PLT) count, white blood cells (WBCs) count and neutrophil % in group (C) in comparison with group (A). Co-treatment and Pre-treatment of sodium nitrite-treated rats with CV caused significant decreased in RDW % and lymphocytes % compared to group (C) on other hand increased significantly PLT count, WBCs count and neutrophil % in comparison with group (C). Administration of CV in group (B) has no effect in RDW %, PLT count, WBCs count, neutrophil % and lymphocytes % in comparison with group (A).

3.3. Chlorella Vulgaris extract ameliorated sodium nitrite induced alteration in renal tissue architecture:

Histopathological examination of renal tissue samples collected from rat of both control group and algae administrated group showed normal renal tissue structure (Fig. 1: A& B). while renal tissue of sodium nitrite-treated group showed interstitial edema with mononuclear cells infiltrations, the renal tubular epithelium showed cloudy swelling and sever coagulative necrosis with desquamation of tubular epithelium and renal cast, the glomeruli showed congested glomerular capillaries (Fig. 1: C&D). Co-treatment of sodium nitrite-administrated rats with CV showed cloudy swelling and coagulative necrosis with desquamation of tubular epithelium and renal cast, the interstitial tissue showed mononuclear cells infiltrations (Fig. 1: E). In addition, the renal tissues of rats in pretreated group exhibited better recovery, showed swelling of tubular epithelium and mild congestion of glomerular capillaries (Fig. 1:F).

## 4. Discussion

Urea is the primary metabolite derived from dietary protein and tissue protein turnover, creatinine is the product of muscle creatine catabolism (Walker et al., 1990). Serum creatinine is an indicator of kidney function, removed from the blood by the kidneys, elevated its level indicate renal dysfunction (Lewis et al., 2014).Nitrite is water soluble so easily transported into renal cells during tubular reabsorption causing renal damage (Al-Gayyar et al., 2016), nitrite may react with amines of the foods in acidic media and produce nitrosamines which increase lipid peroxidation leading to oxidative stress and can be harmful to different organs including kidney (Choi et al., 2002). The current study indicated the administration of rats with sodium nitrite induced a significant increase of serum urea and creatinine levels inducing renal damage. this result is consistent with previously reported study (Abu Aita and Mohammed, 2014). In contrary, Co-treatment and pre-treatment of sodium nitrite-treated rats with CV decreased significantly serum urea and creatinine levels which was in line with finding (Blas-Valdivia et al., 2011) which attributed to CV contains antioxidants such as α-tocopherol, ascorbic acid, carotenoids, chlorophyll, and other vitamins which protect against oxidative stress and cellular damage.

Hematoxylin and eosin (H&E) staining of the kidney sections from sodium nitrite-treated group showed interstitial edema with mononuclear cells infiltrations, the renal tubular epithelium showed cloudy swelling and sever coagulative necrosis with desquamation of tubular epithelium and renal cast, the glomeruli showed congested glomerular capillaries. The outcome results were comparable to the previously reported histopathological examinations of kidney injuries induced by sodium nitrite (Abu Aita and Mohammed, 2014; Ansari et al., 2018; Hassan et al., 2018). On the other hand, renal tissues were found to be normalized with minimum cellular damage after Co-treatment and pre-treatment of sodium nitrite-treated rats with CV suggesting that the CV was able to improve renal toxicity and pathological lesions in renal tissues (Blas-Valdivia et al., 2011) attributed to their high content of α and β-carotene react with various ROS and also interfere with the processes of oxidation in the lipid and cellular compartment.

Our study showed no significant changes in Hb, PCV concentration, RBCs count in sodium nitrite-treated group compared to control group , in contrast, Abu Aita and Mohammed, (2014); Hassan et al., (2018) reported that decreases in RBCs count , Hb, PCV levels due to Sodium nitrite induces oxidative damage and free radical generation that stimulates oxidation of ferrous ions in oxyhemoglobin to form methemoglobin (Baky et al., 2010), may be need more time of nitrite exposure to be affected.

Our study, supplementation of sodium nitrite in rat showed significant decreased in platelets count in agreement with Azab et al., (2015) reported that sodium nitrite treated Guinea pigs had significantly lower WBCs counts and platelets count compared to control group, may explained this by liver diseases associated with thrombocytopenia (Afdhal et al., 2008; Hancox and Smith, 2013; Mitchell et al., 2016).

Sodium nitrite treatment decreased WBCs count in sodium nitrite-treated rat compared to control group in agreement with Abu Aita and Mohammed, (2014); Tan et al. (1992) reported that treatment with sodium nitrite followed by decreased WBCs count explained by failure of the hematopoietic tissues to produce new WBCs, in contrast, Hassan et al., (2018) reported that significant increase total leukocytic count. In addition, sodium nitrite-treated rat showed significant increase in lymphocytes % (lymphocytosis) associated with neutropenia in comparable to control group in agreement with Hassan et al., (2018), reported that significant increase in total leukocytes, lymphocytes (lymphocytosis) with neutropenia in rats treated with sodium nitrite, in contrast, Abu Aita and Mohammed, (2014) reported that leukopenia was associated with lymphopenia which reflects the immunosuppressive effect of sodium nitrite (Gluhcheva et al., 2012). Pre-treatment and co-treatment of rats with CV (group D and E) improved hematological parameters as increased WBCs and platelets count in agreement with (Emami and Olfati, 2017) reported that CV can improve deleterious effect of diabetes on hematological parameters by their antioxidant properties.

## 5. Conclusion

The current study clearly indicated the protective activities of Chlorella Vulgaris against sodium nitrite-induced renal damage. Chlorella Vulgaris mostly accomplish such protective role through restoring the renal function profile, and normal renal architectures.

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Table (1). Chlorella Vulgaris extracts improved sodium nitrite increasing renal function tests.

| Test Group | G A | G B | G C | G D | G E |
|---|---|---|---|---|---|
| **Urea (mg/dl)** | 51.68±1.79[b] | 48.49±2.28[b] | 60.65±1.44[a] | 52.88±1.65[b] | 52.53±1.89[b] |
| **Creatinine( mg/dl)** | 0.62±0.01[b] | 0.61 ±0.01[b] | 0.68±0.01[a] | 0.61±0.01[b] | 0.61±0.01[b] |

Values are represented as the mean ± SE.

Table (2): Chlorella Vulgaris extracts improved sodium nitrite decreasing platelets and total leucocytic counts

| Test Group | G A | G B | G C | G D | G E |
|---|---|---|---|---|---|
| **Hb(mg/dl)** | 14.37±0.34 | 14 ± 0.06 | 14.73±0.42 | 15.13±0.13 | 14.7±0.49 |
| **RBCs ($10^6$) /L** | 6.9±0.12 | 6.92±0.1 | 6.89±0.16 | 7.24±0.13 | 7.22±0.14 |
| **HCT (%)** | 42.4±0.6 | 41.4±0.4 | 44 ± 1.5 | 44.5 ± 0.8 | 43.5±1.2 |
| **RDW %** | 12.9±0.3[c] | 12.6±0.2[c] | 16.5±0.5[a] | 15.3±0.4[b] | 14.9±0.3[b] |
| **Platelet ($10^3$) /L** | 448.2±15.1[a] | 447±18.6[a] | 376.8±9.5[b] | 465±18.4[a] | 437.5±14.9[a] |
| **WBCs ($10^3$) /L** | 15.93±0.72[a] | 14.44±1.02[ab] | 10.32±0.34[c] | 12.87±0.73[b] | 14.42±0.88[ab] |
| **Neutrophils %** | 24.8±2.7[a] | 27.3±2.3[a] | 16.7±0.5[b] | 22.7±1.3[a] | 22.3±1.5[a] |
| **Lymphocyte%** | 68.2±3.1[b] | 65.6±2.7[b] | 77.7±0.8[a] | 70.8±1.3[b] | 71.2±1.8[b] |