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Biochemical Studies in The Effect of Nitrite Ion in Pre-chlorinated Drinking Water in Sohag Governorate on Male Albino Rats and Treatment with Vitamin C

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Abstract: The presence of nitrites in groundwater may be a sign of sewage pollution. Drinking water typically has nitrite concentrations below 0.1 mg L^{-1}. To examine the effect of nitrites on the liver of male albino rats that were affected by nitrite dosages, and then to evaluate the ameliorative effect of vitamin C on the liver of male albino rats, this study intends to measure the nitrite concentrations in groundwater in Sohag governorate, Egypt. 60 albino adult male rats were placed into 4 groups, each group contains 15 animals: T_1: standard nutrition (Control group), T_2: Drinking water containing (>0.2 mg L^{-1} nitrite) group. T_3: Sodium nitrite (300 mg/kg of body weight) group, T_4: Sodium nitrite followed by vitamin C (300 mg/kg of body weight) daily for 3 months. At the end of the experiment, all rats were sacrificed to collect blood samples and then evaluated aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), albumin, total antioxidant capacity (TAC), and catalase, which showed a significant difference between animal groups especially elevation of hepatic parameters and decrease of antioxidant parameters in T_3. The results of the biochemical analysis of the nitrite group and vitamin C group showed that there is a significant improvement in all parameters measured in the vitamin C group compared to the nitrites group.
Keywords: Drinking water, Nitrite ion, Rats, Vitamin C, Sohag governorate, Egypt.

1. Introduction

Nitrite is a key intermediate between nitrates and ammonia, so there are several potential pathways, both oxidative and reductive [1]. Ionized nitrite (NO_2^-) and molecular nitrous acid (HNO_2) are the two forms of nitrite, these two forms exist in equilibrium, with the generation of nitrite being favored by higher pH values [2]. In the body, it is frequently transformed into one of two types of compounds; one is nitric oxide, which is useful for the body, but Meth hemoglobin formation results from a reaction of nitric oxide with oxyhemoglobin. The remaining compounds are nitrosamines, which may be harmful to the body. Nitrite has been shown to cross the placenta and form fetal methemoglobinemia in rats. It may combine with nitrosatable compounds in the stomach as secondary and tertiary amines or amides in meals to generate N-nitroso compounds, It has been demonstrated that this type of endogenous nitrosation happens in both human and animal gastric juice, both *in vivo* and in vitro, typically at higher pH levels when both nitrite and nitrosatable substances are present at the same time [3]. Human DNA, genetic alterations, and cancer have been linked to nitrosamine exposure [4]. It is also generally recognized that nitrite has a deleterious impact on human health, causing diseases such as stomach cancer and methemoglobinemia [5]. Nitrites are absorbed mostly orally, dermally, and through the lungs. In humans, 90-95% of orally administered sodium nitrite is absorbed in the gastrointestinal system, approximately 25% of the ingested amount of nitrate is released with saliva, and 20-46% of this 25% is converted to nitrite by oral cavity microbes. The conversion of nitrates to nitrites within the body is a crucial feature from a metabolic standpoint. Nitrates and nitrites consumed are rapidly absorbed in the stomach and upper small intestine. Nitrite is rapidly transported throughout the body after absorption by fast binding to red blood cells in the blood plasma [6]. Nitrite concentrations in vivo range from 300–500 nM in the blood [7] and 1–10 μmol in tissues [8].

In blood, NO reacts with oxygenated hemoglobin at near diffusion-limited rates ($k = 1 \times 10^7\ M^{-1} s^{-1}$) to form nitrate and methemoglobin [9]. This kinetics suggests that nitrite should not form in the blood via NO auto-oxidation [8]. Nitrosamines can be generated when nitrites are broken down in the presence of amines (compounds having a basic nitrogen atom with a lone pair). Low pH conditions, such as those seen in the stomach, as well as inflammation and bacteria (Helicobacter pylori), may accelerate the process of transformation of nitrite to nitrosamine. However, antioxidants (substances that can block or limit free radical damage to cells) have been discovered to reduce this transformation [10]. Nitrite is designated as a critical parameter that ought to be evaluated in groundwater used as a drinking water source (2000/60/EC, WFD). The existence of nitrites in water is caused by both natural and artificial causes.

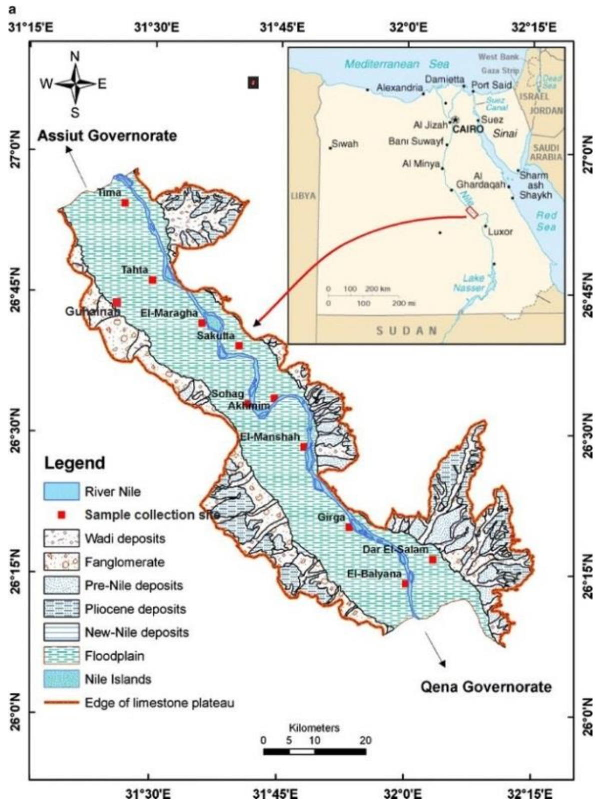

Figure 1: Location map for drinking water samples gathered from the areas of Sohag governorate [29].

From the standpoint of the anthropogenic process, nitrite existence in water can be caused by excessive nitrogen fertilizer consumption, poor agricultural management practices, improper wastewater treatment associated with biological steps based on nitrification-denitrification processes, or discharges from industrial and mining processes [11].

Small amounts of nitrite, typically between 1 and 2 mg kg-1 of fresh weight and infrequently exceeding 10 mg kg-1, are found in plants. Vegetables provide 80–85% of the average daily consumption of nitrite in different European countries, ranging from 31 to 185 mg. Nitrite consumption ranges from 0.7-8.7 mg per day on average in various European nations, with cured meats and vegetables serving as the primary sources. 0.05 mg l-1 (as nitrite-N, Japan), 0.5 mg l-1 (as nitrite, EU), 1 mg l-1 (as nitrite-N, US EPA), and 3 mg l-1 (acute) and 0.2 mg l-1 (chronic) (as nitrite, WHO) are the maximum allowable concentrations of nitrite in drinking water [12]. Adult cancer prevalence has been linked to being subjected to greater amounts of nitrites, and in children, brain tumors, leukemia, and nasopharyngeal tumors may also be linked to nitrite intake [13].

Vitamin C (Ascorbic acid) is known to be a potent antioxidant and may augment the function of endogenous free radical scavengers [14]. Since it is hydrophilic and a major free radical scavenger in extracellular fluids, vitamin C traps radicals and guards against oxidative damage to bio-membranes [15]. Additionally, it is an excellent supplier of electrons and can give electrons to free radicals like hydroxyl and superoxide radicals, which would extinguish their reactivity [16]. Multiple studies have shown that vitamin C's antioxidant effects reduce the likelihood of DNA damage and prevent both cancer and heart disease [17, 18]. The current study aims are to quantify the nitrites concentrations in groundwater in Sohag governorate, Egypt, and to evaluate the effect of nitrites on the liver of male albino rats, and then assess the ameliorative effect of vitamin C on the liver of male albino rats.

2. Materials and methods:

2.1. Drinking water samples

Eleven groundwater samples from pre-chlorinated wells in different regions of Sohag governorate were gathered (Fig. 1) and analyzed nitrite ion concentration by Azo dye formation method according to (Table 1; Fig. 2) [19], and other parameters including ammonia by phenate method (Table 2; Fig. 3) [20], and nitrates with Ultraviolet Spectrophotometric Screening method (Table 3; Fig. 4) [21]. All samples were measured in the inorganic chemistry laboratory of the Central Drinking Water Laboratory, which has a quality control certificate (ISO 17025).

2.2. Chemicals and apparatus

$NaNO_2$, vitamin C (L. Ascorbic acid) was procured from Sigma-Aldrich, St. Louis, Missouri, USA. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), and Alkaline phosphatase were measured by Spectrophotometer 5010 (Germany) using a kit from Human Diagnostics Worldwide (Germany) kinetically according to [22]. Antioxidant tests (TAC. & Catalase) were carried out by Spectrophotometer 5010 (Germany) using a kit from BIODIGNOSTIC, 29 Tahreer St., Giza, Egypt.

2.3. Animals and experimental design

This study expert 60 healthy rats with suitable weight from Sohag University having ethical agreement number CSRE-18-23. Rats were divided into four groups, fifteen rats for each group.

T1: Fifteen rats received the standard diet without any treatment (daily for 3 months), and served as a control group throughout the study.

T2: Fifteen rats received the standard diet with drinking water containing nitrite ion (0.27 mg L^{-1}) from the Dar El-Salam area (Sohag governorate) (daily for 3 months).

T3: Fifteen rats were given orally $NaNO_2$ dissolved in distilled water (300 mg kg^{-1} of body weight) daily for 3 months [23].

T4: Fifteen rats were given orally $NaNO_2$ only (300 mg kg^{-1} of body weight) daily for 1 month, and in the second and the third months vitamin C (Ascorbic acid dissolved in distilled water) (300 mg kg^{-1} of body weight) was given daily simultaneously with $NaNO_2$.

2.4. Collection of samples:

At 24 hours after the last treatments, rats were slaughtered. Then blood samples were collected during slaughter.

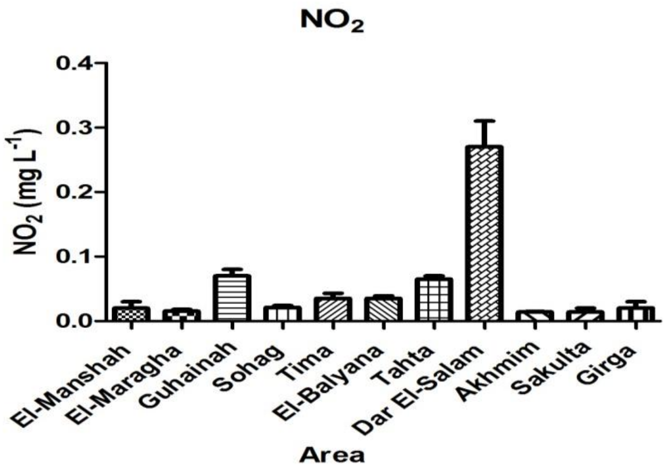

Figure 2: Concentrations of nitrites in drinking water in the areas of Sohag governorate.

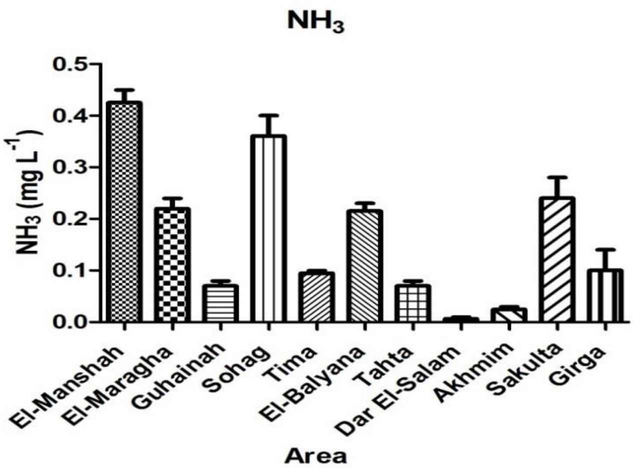

Figure 3: Concentrations of ammonia in drinking water in the areas of Sohag governorate.

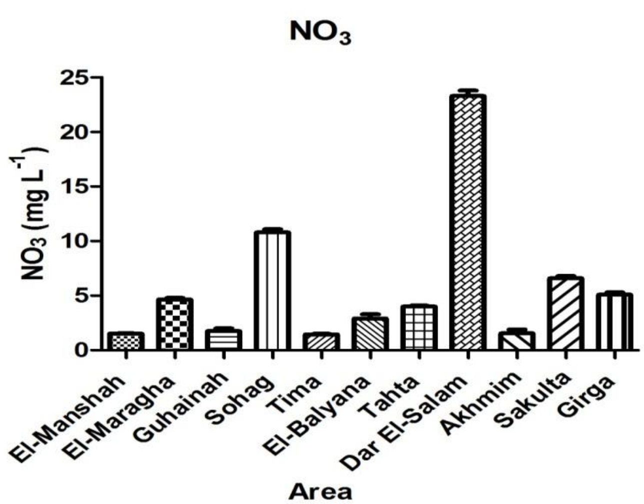

Figure 4: Concentrations of nitrates in drinking water in the areas of Sohag governorate.

2.4.1. Blood samples

A: Whole blood samples without anti-coagulant (EDTA): This sample was used for separating the serum. Following that, portions of the serum samples were produced and kept in Eppendorf tubes at -20 °C until additional biochemical analysis. Biochemical measurements in the serum: The parameters that were measured include liver function tests: alanine transaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatase, and albumin.

B: Biochemical measurements in the plasma samples added an anticoagulant to blood samples. The parameters that were measured include antioxidants, Total Antioxidant Capacity, and catalase.

2.5. Evaluation of biochemical variables

Using the appropriate kits, biochemical analysis was measured in the serum. The guidelines and instructions provided by the suppliers were followed for all tests. AST and ALT were kinetically determined *via* [**24**], alkaline phosphatase was measured kinetically according to [**25**], and Albumin was measured colorimetrically according to [**26**]. The TAC and Catalase were assayed and performed according to the protocol in [**27**, **28**].

2.6. Statistical analysis

Each result was presented as the mean value and standard deviation (M±SD). One-way analysis of variance (ANOVA) was used to assess mean differences, and then the Newman-Keuls test was performed. p values < 0.05 were regarded as statistically significant when using the GraphPad Prism® Software version 5.00 (San Diego, CA).

3. Results

This work took about four months, during which eleven samples of non-chlorinated groundwater wells were gathered from all areas of Sohag governorate (Fig. **1**), and then experiments were conducted on them at the Central Drinking Water Laboratory and the aforementioned indicators were measured. Here, the study site was determined for the samples of this spot from the total areas of the Sohag governorate. According to Minister of Health and Population Decision No. 458 of 2007 and Minister of Water Resources Decision No. 48 In 1982, the normal limits of nitrogen parameters that were measured for drinking water samples in this study are as follows: (Nitrites are up to 0.2 mg L^{-1}, ammonia is up to 0.5 mg L^{-1}, and nitrates are up to 45.0 mg L^{-1}).

In the current research, the protective impact of administering vitamin C to the rats given nitrite treatment was evaluated using certain biochemical indicators, including ALT, AST, ALP, and albumin. As shown in (Table **4**), (Fig. **5**), (Fig. **6**), and (Fig. **7**), when comparing to the other groups, the ALT, AST, and ALP of the group treated with nitrites considerably rise ($p<0.05$). A significant decrease in ALT, AST, and ALP values of the group treated with nitrites followed by vitamin C compared with the nitrites group ($p<0.05$) was noticed. Then we measured serum albumin levels for all groups of rats (Table **5**; Fig. **8**). We noticed a significant decrease of albumin ($p<0.05$) in the nitrite group compared to the control group, and the no significance between drinking water which contain low nitrite concentration group and the protective group of vitamin C indicates the ameliorative effect of vitamin C on the toxicity of nitrites. To confirm the effect of hepatotoxicity of nitrite, it was necessary to measure the extent of oxidative stress caused by nitrite on the groups of rats under study, and thus know the possibility of the therapeutic effect of vitamin C on this toxicity and its ability to reduce the oxidative stress caused by the effect of nitrite, especially since this is one of the very distinctive properties of vitamin C.

(Table 6) indicates that the Total Antioxidant Capacity and Catalase of the nitrites-treated group showed a significant decrease (p=0.0005, p<0.0001) compared to the other groups, vitamin C reduces this effect and that indicated a significant increase in TAC, and Catalase values in the group treated with nitrites followed by vitamin C compared with nitrites groups (p<0.05).

4. Discussion

By obtaining the results of the analyses, it was noticed that Dar El-Salam drinking water sample has the highest nitrite concentration in the Sohag governorate which is shown in (Table 1; Fig. 2), Then we noticed that Dar El-Salam drinking water sample has the lowest concentration value of ammonia in Sohag governorate, which shown in the (Table 2; Fig. 3).

Finally, we found that Dar El-Salam drinking water sample has the highest concentration value of nitrates in the Sohag governorate, which is shown in (Table 3; Fig. 4), This means that the water sample of the Dar El-Salam region in Sohag governorate has a high concentration of nitrogen. It is also clear that there are oxidation and reduction processes taking place in the water in this region, as evidenced by high concentrations of nitrite and nitrate, which are the oxidized form, and a significantly lower percentage of ammonia, even than in the rest of the samples from other regions, which confirms Oxidation A greater proportion of ammonia may be present and oxidized, This makes this sample fertile ground to be the subject of our study. An increase in these marker enzymes in sodium nitrite-treated rats compared with the control group indicates hepatic damage in experimental animals [30].

Table 1: Results of nitrate concentration in drinking water samples in Sohag governorate (n=5 samples are used, and results are presented as Mean±SD for each).

Sample Name	NO_2^- (mg l^{-1})
1-El-Manshah	0.02±0.014
2-El-Maragha	0.0155±0.004
3-Guhainah	0.07±0.014
4-Sohag	0.021±0.004
5-Tima	0.035±0.011
6-El-Balyana	0.035±0.006
7-Tahta	0.065±0.006
8-Dar El-Salam	0.27±0.06
9-Akhmim	0.014±0.0014
10-Sakulta	0.014±0.009
11-Girga	0.021±0.013

Table 2: Results of ammonia concentration in drinking water samples in Sohag governorate (n=5 samples are used, and results are presented as Mean±SD for each).

Sample Name	NH_3 (mg l^{-1})
1-El-Manshah	0.43±0.04
2-El-Maragha	0.22±0.03
3-Guhainah	0.07±0.014
4-Sohag	0.36±0.06
5-Tima	0.095±0.007
6-El-Balyana	0.22±0.02
7-Tahta	0.07±0.014
8-Dar El-Salam	0.007±0.005
9-Akhmim	0.03±0.007
10-Sakulta	0.24±0.06
11-Girga	0.1±0.06

Table 3: Results of nitrates concentration in drinking water samples in Sohag governorate (n=5 samples are used, and results are presented as Mean±SD for each).

Sample Name	NO_3^{-1} (mg l^{-1})
1-El-Manshah	1.53±0.04
2-El-Maragha	4.65±0.21
3-Guhainah	1.75±0.35
4-Sohag	10.8±0.42
5-Tima	1.45±0.07
6-El-Balyana	2.9±0.57
7-Tahta	4.0±0.14
8-Dar El-Salam	23.3±0.71
9-Akhmim	1.55±0.5
10-Sakulta	6.6±0.28
11-Girga	5.1±0.28

Table 4: Effect of vitamin C on nitrites treated rats (n=15 samples are used, and results are presented as Mean±SD for each) mean results of liver enzymes (ALT, AST, ALP) for rats groups.

Parameter	ALT (U L^{-1})	AST (U L^{-1})	ALP (U L^{-1})
Control	31.0±1.0	42.4±1.9	149.8±3.8
Dar El-Salam D. water-treated rats	71.2±2.86	97.9±1.9	220.8±4.8
Nitrites treated rats	172.4±4.5	243.2±6.8	314.2±9.7
Nitrites and Vit C treated rats	63.9±2.2	127.0±3.5	242.8±8.96

Table 5: Effect of vitamin C on nitrites treated rats (n=15 samples are used, and results are presented as Mean±SD for each) mean results of albumin for rats groups.

Parameter	Control	Dar El-Salam D. water-treated rats	Nitrites treated rats	Nitrites and Vit. C treated rats
Albumin	4.4±0.01	3.70±0.02	3.2±0.02	3.6±0.06

Table 6: Effect of vitamin C on nitrites treated rats (n=15 samples are used, and results are presented as Mean±SD for each) mean results of antioxidants (TAC, and Catalase) of rats groups.

Parameter	TAC. (mmole L^{-1})	Catalase (mg L^{-1})
Control	1.57±0.03	12.96±0.23
Dar El-Salam D. water-treated rats	0.82±0.04	10.08±0.18
Nitrites treated rats	0.33±0.03	6.86±0.08
Nitrites and Vit C treated rats	1.0±0.12	10.47±0.04

Vitamin C reduces this effect and that indicated a significant decrease in ALT, AST, and ALP values compared with the nitrites group ($p<0.05$), which may be due to the antioxidant effect of vitamin C which is reported to protect the liver from damage [30, 31].

(Table 5) indicates the measuring of albumin in which we noticed a high significant decrease ($p<0.05$) in the nitrite group due to the damage to the liver compared to the control group, which confirms the hepatotoxic effects of nitrites, and the no significance between drinking water which contain low nitrite concentration group and the protective group of vitamin C indicates the ameliorative effect of vitamin C on the toxicity of nitrites.

In (Table 6) the Total Antioxidant Capacity, and Catalase of the nitrites-treated group showed a significant decrease (p=0.0005, $p<0.0001$) compared to the other groups, vitamin C reduces this effect which indicated a significant increase in TAC, and Catalase values compared with nitrites groups ($p<0.05$). The results of the present study corroborate the earlier reports which suggest that chronic exposure of hepatocytes to reactive nitrogen species leads to functional alterations in the hepatocytes [23, 32, 33].

5. Conclusion

Drinking water before chlorination in Sohag governorate contains low concentrations of nitrite, which conforms with the health specifications of drinking water. The highest concentration of nitrite in drinking water is found in the Dar El-Salam area, where the concentration of nitrite in the water before chlorination is slightly higher than the permissible limit for drinking water. Through this study Groups of experimental rats were treated with drinking water from the Dar El-Salam area and compared with each of the groups treated with high concentrations of nitrite and others treated with high concentrations of nitrite and after one month treated with vitamin C with continuous treatment with high concentrations of nitrite. By obtaining the results of experiments and indicators, we conclude that drinking water before chlorination in Dar El-Salam has a very slight, almost negligible effect on health. It is not comparable to the effect of nitrites, which cause hepatotoxicity, including elevated liver enzymes and proteins, and also cause high oxidative stress, we conclude from the results that there is a mitigating effect of vitamin C on hepatotoxicity and oxidative stress resulting from the effect of nitrites.

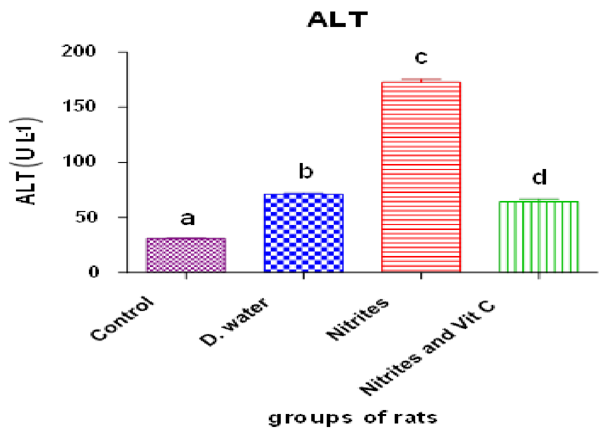

Figure 5: Serum ALT (U/L) levels of the four groups of rats shows a-b, a-c, a-d, b-c, c-d significant : $p<0.05$, and only b-d shows no significant : $p>0.05$, One-way ANOVA was used to determine significance, and then the Newman-Keuls test was utilized.

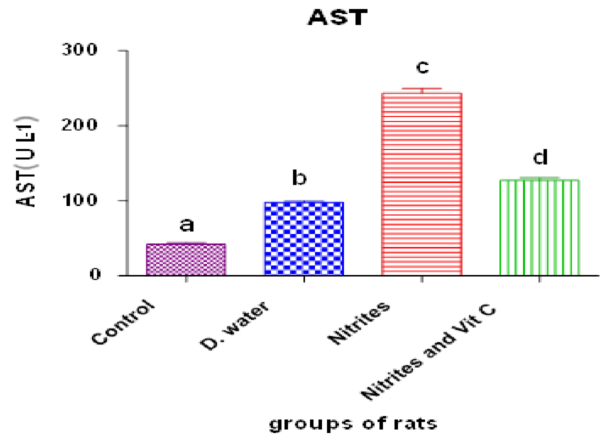

Figure 6: Serum AST (U/L) levels of the four groups of rats show significance: $p<0.05$, for all groups with others. One-way ANOVA was used to determine significance, and then the Newman-Keuls test was utilized.

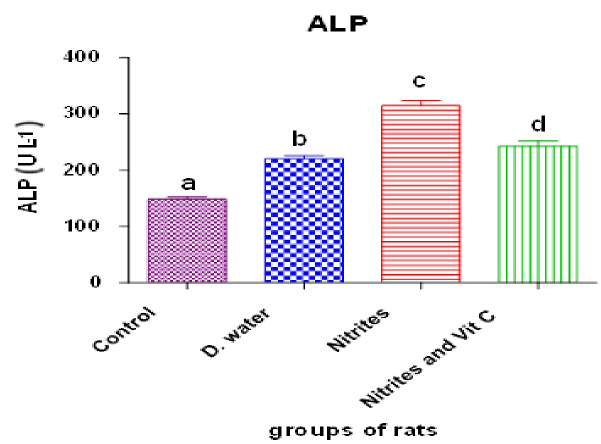

Figure 7: Serum ALP (U/L) levels of the four groups of rats shows a-b, a-c, a-d, b-c, c-d significant : $p<0.05$, and only b-d shows no significant : $p>0.05$, One-way ANOVA was used to determine significance, and then the Newman-Keuls test was utilized.

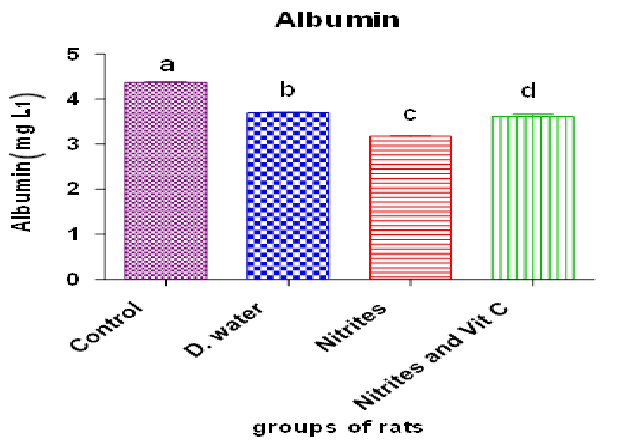

Figure 8: Serum albumin (mg/L) levels of the four groups of rats show a-b, a-c, a-d, b-c, c-d significant: *p*<0.05, and only b-d show no significant: *p*>0.05, One-way ANOVA was used to determine significance, and then the Newman-Keuls test was utilized.

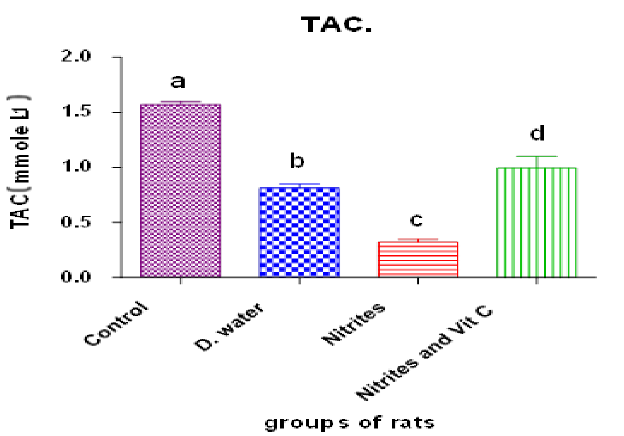

Figure 9: Total antioxidant capacity (mmole /L) levels of the four groups of rats shows a-b, a-c, a-d, b-c, c-d significant : *p*<0.05, and only b-d shows no significant : *p*>0.05, One-way ANOVA was used to determine significance, and then the Newman-Keuls test was utilized.

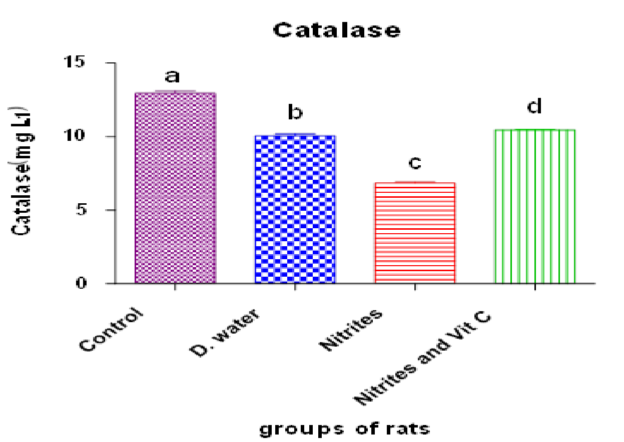

Figure 10: Catalase (mg /L) levels of the four groups of rats show a-b, a-c, a-d, b-c, c-d significant: *p*<0.05, and only b-d show no significant: *p*>0.05, One-way ANOVA was used to determine significance, and then the Newman-Keuls test was utilized.

CRediT authorship contribution statement

Nagwa Elsawi: Supervision, conceptualization, visualization, and validation; Metwally Kotb: supervision and visualization Mustafa Redwan: Supervision, methodology; M. Eid; software, formal analysis, investigation, resources, data curation, writing—original draft preparation. All authors have read and agreed to the published version of the manuscript.

Data availability statement

The data used to support the findings of this study are available from the corresponding author upon request

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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