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Effect of Nano-Selenium on Oxidative Stress Induced by Thioacetamide in albino rats

Fatma S. Salama, Safwat H. Ali, Sharaf Eldeen H.A.M., Mohamed S. Hikal and Mohamed M.A. Omar

Agricultural Biochemistry Dept., Fac. of Agric., Ain Shams Univ., P.O. Box 68 Hadayek Shoubra 11241, Cairo, Egypt.

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ABSTRACT

The conducted study was aimed to evaluate effect of selenium nanoparticles (SeNPs) on biochemical parameters, liver toxicity and renal failure induced by Thioacetamide (TAA) in male albino rats. In the present study the experimental rats were divided into 4 groups (6 rats/group) as follows: Group 1: Normal control (NC), only treated with 0.9% saline solution, group 2: treated with SeNPs (0.5mg/Kg body weight), group 3: treated with TAA [100 mg/Kg body weight], group 4: treated with the same dose of TAA + SeNPs as in group 2 & 3. The experiment was continued for three months, and blood samples were taken for analysis every month. The results indicate that TAA causes significant alterations in biochemical parameters. Besides, TAA-induces hepatic fibrosis and histological manner of liver and elevates serum levels of aminotransferases, increases oxidative stress as malondialdehyde (MDA) biomarker of lipid peroxidation. Also, TAA significantly decreases levels of antioxidants enzyme activities e.g. superoxide dismutase (SOD), glutathione reductase (GR), catalase (CAT) and reduced glutathione (GSH). While administration of SeNPs enhances liver enzymes and reduces the oxidative stress. In addition, data obtained reveal that SeNPs significantly reduce the harmful effect of TAA in rats. It could be concluded that SeNPs has powerful as antioxidant and hepato-protective effects. However nano selenium plays as antagonistic agent against toxicity induced by thioacetamide.

Keywords: Thioacetamide, Nano-selenium, Oxidative stress, antioxidant enzymes, Transaminases, Malondialdehyde (MDA), reduced glutathione (GSH).

1. Introduction

Selenium (Se) is a trace element, which is an essential nutrient for humans and animals to form important selenoproteins, including glutathione peroxide, thioredoxin reductase (Zhu *et al.,* 2014), also, has a very important role in cancer cell, it acts as chemotherapeutic and chemo preventive agent and has been demonstrated in many epidemiological, preclinical, and clinical studies (Nel *et al.,* 2006; Giannitrapani *et al.,* 2014). Selenium nanoparticles (SeNPs) are considered as a source of selenium that provides optimum in vivo bioavailability with a decrease of the risk of selenium toxicity. The biocompatibility and degradability of SeNPs *in vivo* are significantly preferable than noble metals as silver, gold and platinum. In addition, when compared to organic and inorganic forms of selenium, Se in many nanoforms has appeared lower toxicity and superior antioxidant and anti-tumor activity (Chaudhary *et al.*, 2014). Besides, the anti-hyperglycemic activity of SeNPs has also been confirmed due to its antioxidant properties that can scavenge the free radicals generates due to hyperglycemia (Quraishy *et al.*, 2015).

Thioacetamide (TAA) is a thiono-sulphur containing compound, that industrially has been used in the past (Kuˇcera *et al.,* 2011). Thioacetamide produces hepatotoxicity within a short period of time. Thioacetamide inhibits oxidative phosphorylation of the liver by causing uncontrolled entry of Ca^{++} ions into the hepatocytes cells and impairing its oxidative metabolism. It interferes the movement of the RNA leading to increase RNA content which may cause membrane injury. A metabolite of thioacetamide, thioacetamide s-dioxide is responsible for hepatotoxicity. Thioacetamide s-dioxide is

Corresponding Author: Fatma S. Salama, Agricultural Biochemistry Dept., Fac. of Agric., Ain Shams Univ., P.O. Box 68 Hadayek Shoubra 11241, Cairo, Egypt

highly reactive, reduces the number of viable hepatocytes as well as rate of oxygen consumption. It also decreases the volume of bile and its content i.e., bile salts, cholic acid and deoxycholic acid. TAA is hepatotoxic, dose dependent, acute liver injury characterized by centrilobular necrosis with subsequent regenerative response (Chen *et al.,* 2008; Wong *et al.,* 2012). It's found that TAA chronic exposure leads to hepatic cirrhosis and hepatocarcinoma (Natarajan *et al.,* 2006). TAA-induced hepatotoxic effects are manifested only after metabolic activation of TAA, by cytochrome P450, the TAA-intermediates will increase cellular oxidative stress (Pallottini *et al.,* 2006; Chilakapati *et al.,* 2007).
The aim of the present study was to evaluate the possible hepatoprotective effects of nano selenium on the oxidative chemical stress induced by thioacetamide on male albino rats. Also, study if nano selenium could play as antagonistic agent against thioacetamide toxicity or not.

2. Materials and Methods

2.1. Chemicals

Thioacetamide (TAA): TAA known as thioacetamide acid, or acetothioamide with molecular formula (CH_3CSNH_2). TAA exists at room temperature as colorless to yellow crystals with a slight odor of mercaptans. It is highly soluble in distilled water and physiological saline. Thioacetamide was purchased from Sigma Aldrich Company.

2.2. Rats and experimental design

Twenty four (24) male albino rats of Wistar strain, weighing about 120 ±5 g were purchased from Animal Health Research Institute Dokki, Giza, Egypt. The rats were kept under normal laboratory condition and fed on standard diet for one week as an adaptation period before starting the experiment. The twenty four rats were divided into 4 groups (6 rats/group) and orally received different dose of **TAA** & **SeNPs** as follow: **Group 1 (NC):** Normal Control group, only received 0.9% saline, **group 2 (Nano selenium SeNPs):** [0.5mg/Kg body weight], **group 3 (Thioacetamide TAA):** [100mg/Kg body weight], **group 4 (TAA + SeNPs):** [100mg/Kg body weight], [0.5mg/Kg body weight].

Rats in groups 2 and 4 orally received 0.5ml containing [0.5mg/Kg body weight] Nano-selenium in drinking water by gavage syringe. However, rats in groups 3 and 4 (i.p.) treated with TAA [100mg/Kg body weight].

2.3. Induction of chemical oxidative stress

Chemical oxidative stress in albino rats was induced using thioacetamide TAA (i.p.) treatment.

2.4. Preparation of Selenium Nanoparticles

SeNPs were prepared according to Jinsong *et al.* (2012).

2.5. Collection of blood samples

Blood samples were taken every month for 3 months, after that allowed to be clotting and were centrifuge at 4000 rpm for 10 min to separate serum which collected in Eppendorf tubes and was stored at-18˚C in a deep freezer until biochemical assays.

2.6. Serum biochemical assays

The antioxidant enzymes analyzed were superoxide dismutase (SOD) according to Nishikimi (1972), Glutathione reductase (GR) according to Goldberg (1983), Reduced glutathione (GSH) according to Beutler (1963), all those determinations were analyzed using Biodiagnostic kits. catalase (CAT) according to Sinha (1972). Meanwhile, lipid peroxidation was assayed as Malondialdehyde (MDA) according to the method of Buege and Aust (1978). However, serum transaminases ALT and AST, were determined according to Tietz (1995), Alkaline phosphatase (ALP) and Lactate dehydrogenase (LDH) were determined according to (Rosalki *et al.*, 1993), Gamma-glutamyl transpeptidase (γ-GT) was determined according to Kaplan *et al.* (1984). All those enzymes were measured by SPINREACT kit, Spain, using spectrophotometer (UV-Vis) model (9100 B) Lab Tech).

2.7. Statistical analysis

Data were subjected to homogeneity test (Lev- ene 1960) and Anderson-Darling normality test (Scholz and Stephens 1987) prior to two-way analysis of variance (ANOVA), and the significance of the differences between means was tested using Duncan's honestly significant difference test ($p<0.05$). The software program used was Costat, version 6.303 (2004). Values are expressed as means ± standard error.

3. Results and Discussion

3.1. Characterization of Selenium Nanoparticles:

Results of morphological shape and size molecule of SeNPs were illustrated in SEM & TEM images (1 & 2). Image (1) shows that SeNPs was found in a spherical shape. On the other hand, the Transmitted Electron Microscope (TEM) Image (2) reveals that selenium Nanoparticles has ranged diameter between (6.53 – 10.6) nm.

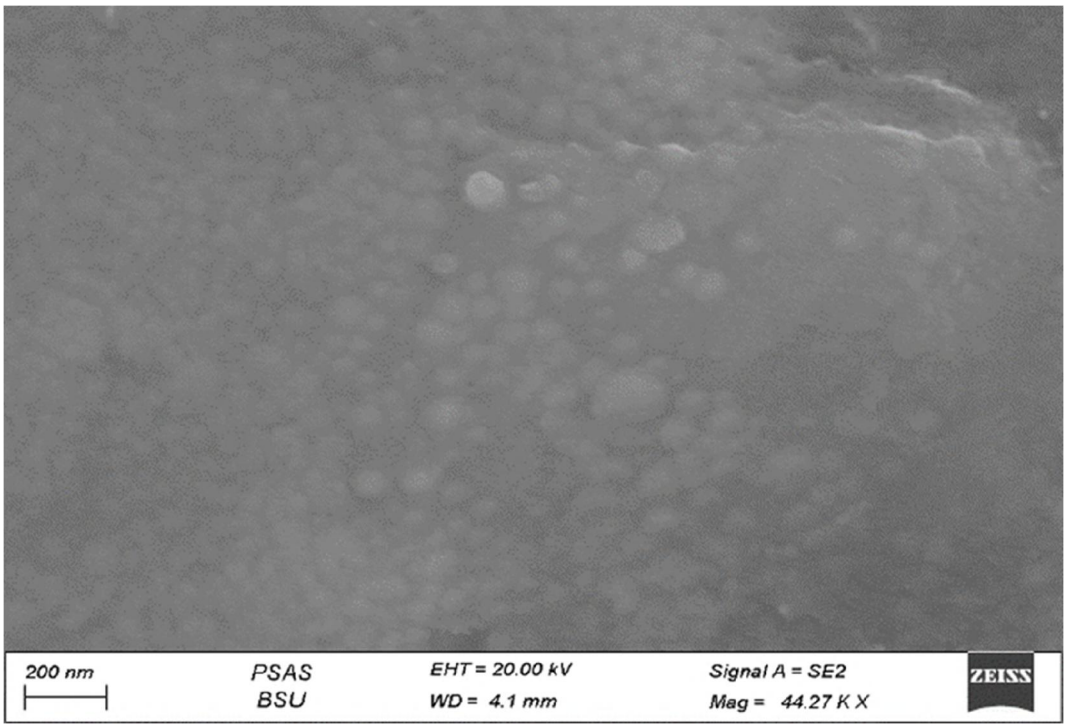

Image 1: SEM of selenium Nano-particles size show spherical particles.

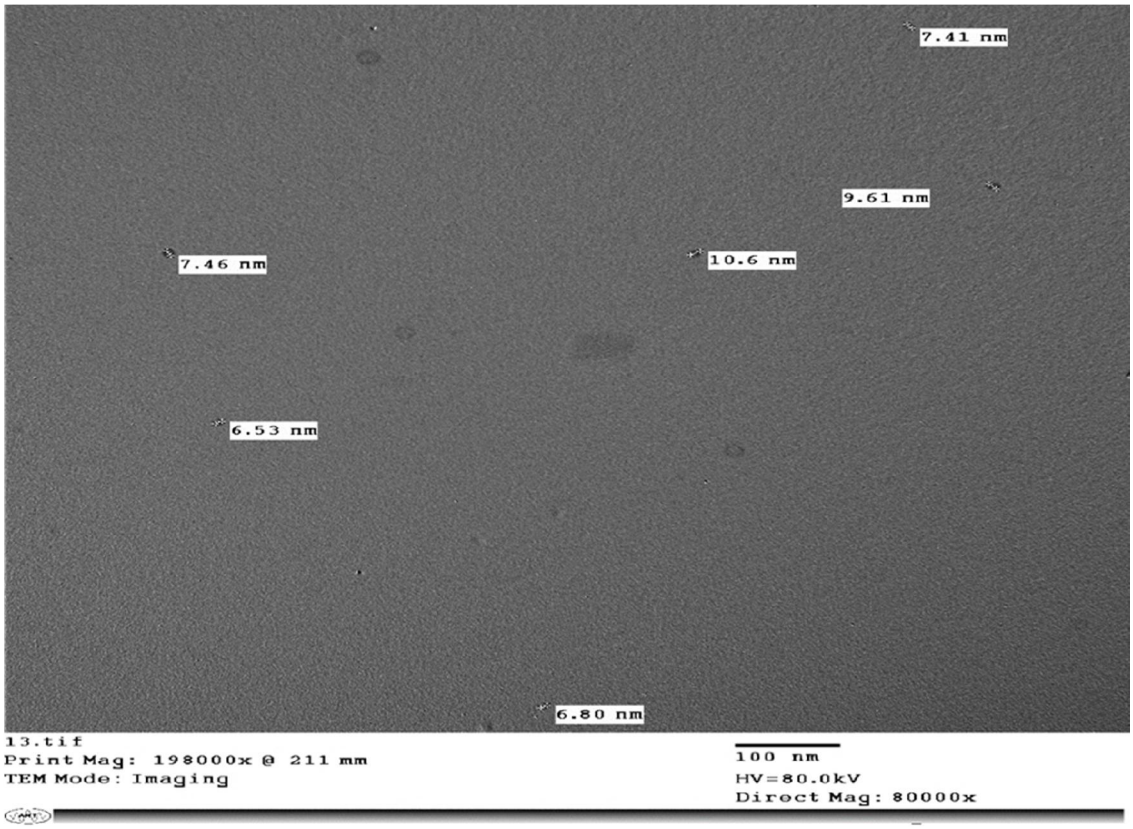

Image 2: TEM of selenium Nano-particles size, demonstrate ranged diameter in nanometer between (6.53 – 10.6) nm.

3.2. Liver enzymes (AST, ALT, ALP, LDH & γ-GT) activity

Data in Table (1) represent effect of SeNPs on liver enzymes activity (AST, ALT, ALP, LDH & γ-GT) in rats treated with TAA.

Results show that liver enzymes levels were increased due to TAA treatment as shown in Table (1). However, SeNPs groups show enzymes activity level paralled to control group in all measured enzymes levels except for LDH level which reveal lower level of activity than control groups. On the other hand rats treated with both SeNPs & TAA show that enzymes activity levels were lower than TAA group and higher than the normal control level. In other word SeNPs inhibit oxidative stress or work as antagonistic agent against chemical stress induced by TAA.

In addition, data in Table (1) show that AST level was affected by both SeNPs and TAA treatments through phase duration. Results reveal that AST level (Table.1) show approximately the same level for SeNPs & control group, while TAA group represent higher level of AST starting from first, second month and still increase markedly till the third month. In contrary, SeNPs could return this elevation of AST level in rats' serum to be close to control group especially at the 3rd months.

These results indicate that SeNPs could inhibit ROS which produces from TAA treatment and so lower this elevation of AST level, in other word SeNPs play an important inhibitor in this trend and could protect liver from injury induced by TAA stress.

Results obtained from analysis of hepatic enzymes indicate a significant rise in AST, ALT and ALP in TAA-treated group compared with control group. This increase in liver enzymes could be explained by the destructive changes that occurs by TAA-treatment. These destructive changes may be attributed to the inflammatory reactions, as proved by inflammatory cell infiltration in liver sections, and oxidative stress with accumulation of free radicals, as shown by the significant increase in the MDA level associated with significant decrease in the GSH, CAT, SOD and GR levels in the TAA group compared to control group. These results are in accordance with studies show that TAA increases the

activities of different marker enzymes of hepatocellular injury, AST, ALT, ALP, LDH and ɣ-Gt (Anbarasu *et al.,* 2012). Also, there is an increase in oxidative stress after TAA administration indicated by increasing lipid peroxidase levels (Anbarasu *et al.,* 2012; Sehrawat and Sultana, 2007).

The higher level of liver enzymes reflects the oxidative damage induced by TAA treatment. These results are in coincided with Wang *et al.* (2009) who reported that the increasing activity of enzymatic markers of liver function enzymes indicate to cellular leakage and structural damage of liver cell impairment. Also, Jalili *et al.* (2015) as reported that the resulted damage of cell membranes would release the enzymes into the circulation.

Table 1: Effect of SeNPs on liver enzymes activity of male albino rats induced oxidative stress by TAA**.**

Parameter Groups	Time in month	AST (U/L)	ALT (U/L)	ALP (U/L)	LDH (U/L)	γ-GT (U/L)
Normal control (NC)	1	95.0 ± 1.14^{gh}	65.2 ± 1.59^{fg}	83.0 ± 8.60^{hi}	294.8 ± 1.28^{h}	53.9±1.14^{fg}
	2	105.2 ± 2.78^{f}	59.2 ± 1.06^{h}	81.3 ± 10.25^{i}	302.2 ± 1.98^{g}	55.7±1.39^{f}
	3	98.6 ± 0.50^{g}	68.6 ± 0.92^{f}	84.9 ± 7.79^{gh}	295.0 ± 1.41^{h}	54.6±1.36^{fg}
Mean ± SE		99.6^{c}	61.3^{c}	83.0^{c}	297.3^{c}	54.7^{c}
Selenium nanoparticles (SeNPs)	1	98.1 ± 0.44^{g}	64.0 ± 1.41^{g}	71.8 ± 12.83^{fg}	183.8 ± 1.65^{i}	50.6±2.33^{gh}
	2	96.6 ± 0.81^{gh}	47.8 ± 1.06^{h}	75.1 ± 8.29^{j}	178.8 ± 1.35^{j}	49.3±1.24^{h}
	3	89.8 ± 2.08^{h}	50.6 ± 1.07^{h}	64.5 ± 5.17^{j}	166.6 ± 2.22^{k}	44.5±1.64^{i}
Mean ± SE		94.8^{d}	54.1^{d}	70.4^{d}	176.4^{d}	48.1^{d}
Thioacetamide (TAA)	1	258.4 ± 4.65^{c}	149.0 ± 1.22^{c}	187.6 ± 7.82^{c}	574.6 ± 1.02^{c}	98.5±1.73^{c}
	2	305.2 ± 1.15^{b}	252.8 ± 1.71^{b}	218.1 ± 6.69^{b}	779.4 ± 1.98^{b}	127.3±1.37^{b}
	3	568.6 ± 1.32^{a}	418.6 ± 2.03^{a}	258.9 ± 6.98^{a}	858.2 ± 1.49^{a}	255.9±1.64^{a}
Mean ± SE		377.4^{a}	273.4^{a}	221.5^{a}	737.4^{a}	160.5^{a}
TAA + SeNPs	1	192.4 ± 2.27^{d}	98.6 ± 1.50^{d}	166.8 ± 7.34^{d}	428.6 ± 2.13^{d}	66.4±1.36^{e}
	2	196.2 ± 1.56^{d}	94.8 ± 0.86^{de}	146.9 ± 8.07^{e}	406.0 ± 1.84^{e}	71.5±1.58^{d}
	3	184.8 ± 1.24^{e}	91.6 ± 1.63^{e}	128.9 ± 7.69^{ef}	375.8 ± 1.77^{f}	65.8±1.28^{e}
Mean ± SE		191.1^{b}	95^{b}	147.5^{b}	403.4^{b}	67.9^{b}

* All data represented are means ± SE

** Different letters represent significant difference at p <0.05.

Data in Table (2) show lipid peroxidation, reduced glutathione (GSH) and enzymes activity (CAT, SOD & GR) in serum of rats treated by SeNPs and TAA. Results in Table (2) indicate that MDA was increased from (27.58, 50.43 & 67.80) compared to control groups (8.19, 9.69 & 9.63) nmol/ml plasma through three months. The higher value was recorded at the 3rd month which reveal oxidative stress induced by thioacetamide treatment. However, SeNPs result in lowering MDA level through all three month and show significant reduction in MDA value (26.41, 23.82 & 16.83) nmol/ml but the reduction was more than normal control. This finding reveals that SeNPs has powerful against elevation of lipid peroxidation. This result may be attributed to antioxidant effect of SeNPs itself or the antioxidant molecules that SeNPs involved in such as seleno amino acids. Also, may be due to elevation of antioxidant enzymes activity that remove ROS from metabolic pathway and inhibit lipid peroxidation, not only that but also elevate and increase of reduced glutathione from (24.46, 16.20 &11.86) to (28.65, 21.54 & 20.52) mg/dl as shown in (Table 2).

Accordingly, Table (2) a significant reduction of the oxidative stress disclose in these biomarker parameters was resulted in due to SeNPs treatments. It may be possible that the mechanism of hepato-protection by Nano selenium is due to its antioxidant effect (Mayba *et al*., 2011). These findings are consistent with Kantah *et al*. (2011) reported that TAA caused a significant decrease in the levels of liver SOD, GSH, GR and CAT. Results, show that TAA induces elevation of MDA levels, and reduction of endogenous antioxidant enzymes (SOD & CAT). These results are in accordance with Fadhel (2002) who suggest that TAA induces liver fibrosis by forming free radicals, which then react with cellular lipids to promote lipid peroxidation. Meanwhile, treatment with Nano selenium significantly reversed

these changes, and it may be possible that the mechanism of hepato-protection by Nano selenium is due to its antioxidant effect.

Thus, SeNPs show higher potential to oxidative chemical stress induced by TAA. Therefore, the current recommends using SeNPs as a good potential agent against chemical oxidative stress induced by TAA.

Table 2: Effect of SeNPs on malonic dialdehyde (MDA), reduced glutathione (GSH) and antioxidant enzymes [catalase (CAT), superoxide dismutase (SOD) and glutathione reductase (GR)] in blood and plasma of rats treated with TAA.

Parameter / Groups	Time in month	MDA (nmol/ml plasma)	GSH (mg/dl blood)	CAT (U/ml blood)	SOD (U/ml)	GR (U/L)
Normal control (NC)	1	8.19 ±1.56^{e}	41.77 ±1.46^{b}	353.57±1.59^{ab}	336.82±1.28^{c}	168.63±1.50^{de}
	2	9.69 ±1.50^{e}	39.26 ±1.15^{b}	344.42±1.43^{c}	341.44±1.43^{b}	171.15±1.49^{d}
	3	9.63 ±0.92^{e}	37.50 ±1.04^{b}	358.32±1.84^{a}	339.58±0.92^{bc}	179.19±1.28^{c}
Mean ± SE		9.13^{c}	39.46^{a}	352.06^{a}	339.26^{b}	194.76^{a}
Selenium nanoparticles (SeNPs)	1	9.11 ±1.64^{e}	32.23 ±1.15^{c}	338.13±1.38^{d}	338.69±0.92^{bc}	181.14±1.60^{c}
	2	8.74 ±1.46^{e}	39.09 ±1.0^{b}	351.67±1.91^{b}	346.34±1.49^{a}	196.89±1.48^{b}
	3	8.75 ±1.13^{e}	48.22 ±1.42^{a}	353.77±1.56^{ab}	341.74±1.41^{b}	206.35±1.64^{a}
Mean ± SE		8.8^{c}	39.8^{a}	347.8^{b}	342.2^{a}	173.0^{b}
Thioacetamide (TAA)	1	27.58 ±1.50^{c}	24.46 ±0.92^{d}	250.55±1.51^{h}	291.29±1.42^{d}	138.18±1.15^{g}
	2	50.43 ±1.36^{b}	16.20 ±1.15^{e}	213.82±1.65^{i}	232.44±1.50^{h}	124.76±1.44^{h}
	3	67.80 ±1.71^{a}	11.86 ±1.65^{f}	163.20±1.88^{j}	127.85±1.28^{i}	87.66±1.80^{i}
Mean ± SE		48.60^{a}	17.46^{c}	209.16^{d}	217.13^{d}	116.83^{d}
TAA + SeNPs	1	26.41 ±1.63^{c}	28.65 ±1.43^{c}	286.30±1.85^{g}	254.55±1.5^{g}	142.33±1.39^{g}
	2	23.82 ±1.42^{c}	21.54± 1.93^{d}	312.44±1.86^{f}	267.81±1.59^{f}	158.45±1.86^{f}
	3	16.83 ±1.77^{d}	20.52± 1.84^{d}	324.68±1.43^{e}	283.94±1.74^{e}	165.51±1.78^{e}
Mean ± SE		22.33^{b}	23.53^{b}	307.76^{c}	268.73^{c}	155.4^{c}

*All data represented are means ± SE
** Different letters represent significant difference at p <0.05.

Conclusion

Nano-selenium has important role as antioxidant agent against chemical oxidative stress induced by TAA. However, the Nano-selenium protect the human body from the oxidative stress through scavenging the reactive oxygen species (ROS) resulted from chemical oxidative stress of TAA.

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