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The Effective Role of Nano Selenium and Probiotic in Reducing Ochratoxicosis in Rats

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Abstract: The aim of the current study was to evaluate the possible protective influences of Nano selenium (Nano Se), probiotics and vitamin C against ochratoxin-A (OTA) induced hepatorenal injury and oxidative stress in male rats. Forty-five albino rats were divided into 9 equal groups.

G1: Control, G2: Ochra 50 µg/kg b.wt, G3: Ochra + Nano Se 0.31 mg/kg b.wt, G4: Ochra+ vitamin C 1000 mg /L water, G5: Ochra+ Probiotic 400 g/ton of diet, G6: Ochra + Nano Se + vitamin C, G7: Ochra + Nano Se + Probiotic, G8: Ochra + Nano Se + vitamin C+ Probiotic, G9: Ochra + vitamin C + Probiotic. The obtained results showed significant increase in serum AST, GGT, ALT, and LDH activities, Creatinine, urea, TNF-*α*, IL-6, SOD, CAT, MDA, alpha 1, gamma 2, T. alpha and T4 concentrations in OTA intoxicated rats. Meanwhile, a significant decrease in GSH, TAC, total protein and its fractions including (albumin, Total beta, Total gamma, Total globulin, alpha 2, beta 1, gamma 1), albumin/globulin (A/G) ratio in addition to serum testosterone, estradiol, TSH and T3 concentrations were observed in OTA exposed rats. The results suggested that NanoSe, probiotics, and vitamin C are strong antioxidants agents that might ameliorate OTA-induced renal and liver damage in rats by combating free radical and attenuate oxidative stress. Also, Nanoselenium, probiotics, and vitamin C, were found to be effective in protecting rats against OTA-induced hepato-renal injury by anti-inflammatory mechanism with the best result of Nano selenium or probiotic alone and their combination.

Keywords: Se-NPs; Vitamin C; Probiotics; Ochratoxicosis; Oxidative stress

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1. Introduction

Climatic changes over the world increase the formation of mycotoxins with the increase in temperature and relative humidity in the air and surrounding environment. Mycotoxins are secondary metabolites generated naturally via several fungal species that grow in humid, hot climates **(Mgbeahuruike et al., 2018)**. They elicit toxic responses in humans and animals. Mycotoxins polluted over 25% of world's food crops **(Eskola et al., 2019)**.

Reverberi et al (2010) stated that Ochratoxins are groups of mycotoxins that are created via many *Penicillium or Aspergillus* species; these involve several members of the group *Aspergillus ochraceus* and *Penicillium verrucosum* I and II type. Ochratoxins consists of A, B and C compounds. Amongst these three compounds, Ochratoxin-A (OTA) is the highly hurtful one. Fungi is widespread in food or feedstuffs because it could pollute susceptive agricultural goods **(Wu et al., 2011)**. Cereals contaminated by OTA (polluted before, during or after harvest) as well as cereal products represent 60% of this exposure **(Tabarani et al., 2020)**. A variety of toxicological influences have been recorded in ochratoxicosis, inclusive kidney toxicity, immunotoxicity, teratogenicity, mutagenicity or neurotoxicity in humans and animals **(O'Brien and Dietrich, 2005)**. The main target organ for OTA is the kidney as a highly sever nephrotoxin. Exposure to OTA has been proven to be included in Human Balkan endemic nephropathy, as well as it is divided as a probable carcinogen [group 2B] in humans **(IARC, 2002)**. From experimental studies in animals' models, OTA-caused kidney toxicity as well as genotoxic influences are mostly mediated by free radicals' production, which can cause renal, hepatocellular cancers and immunotoxin in mammalian species involving rabbits, pigs, and rats **(EFSA 2020; Malir et al., 2016)**. One of the main cellular processes that lead to OTA-caused renal damage is reactive oxygen species (ROS), if even the molecular mechanisms and its influences are unknown yet **(García-Pérez et al., 2021)**. Ochratoxin is a mycotoxin responsible for various kinds of cancers in mice, rats as well as humans. Protections from cytotoxicity, lipid peroxidation, as well as DNA damage induced by OTA was reported, another confirming of OTA toxicity and oxidative damage link **(Sorrenti et al., 2013)**. As well as **Diamanti-Kandarakis et al (2009)** mentioned that mycotoxin such OTA is one of natural origin endocrine disrupting chemicals [EDCs]. EDCs can interfere with hormonal system of humans or animals via effecting on hormonal synthesis, metabolism, or transport; or through directly interacting with hormonal receptor (such as antagonist or) **(Wuttke et al., 2010)**.

Antioxidants can confront the harmful influences of chronic exposure to OTA and assured the effectiveness of dietary strategies to inhibit OTA toxicity. Three mitigation methods of physical, chemical, or biological basis are used for driving out mycotoxin pollution from food or animals feed **(Luz et al., 2017)**. Selenium (Se) is a trace element necessary for all mammalian species as well as have essential function in antioxidant protection **(Gan et al., 2014)**, reducing toxicity **(Ren et al., 2019)**, as well as a higher influence of organic Se than inorganic Se has been recoded. Se appears its biological role via enhance expression of selenoprotein. Administration of Se could

safeguard against aflatoxin B1 intoxication **(Sarker et al., 2021),** OTA **(Gan et al., 2015 and Gan et al., 2017)**, T-2 toxins **(Yu et al., 2017),** or deoxynivalenol [DON] **(Ren et al., 2018)** through enhancing expressions of glutathione peroxidase 1 (GPx1) and/or selenoprotein. Recently, research on SeNPs has acquired highly interested because of its influence action in different biological processes. In general, SeNPs have a high value of absorption in orderly administration relative to selenium. SeNPs has biomedical or pharmaceutical applications because of its antimicrobial, antioxidant, anti-diabetic, as well as anticancer influences **(Awanish and Kumar, 2021)**. Moreover, nano selenium has an effective role in enhancing anti-oxidative status and boosting immune response in broiler chickens when exposure to heat stress **(Eid et al., 2023).**

Biological control of food fermentation, bacteria, yeast, or non-toxic strains of fungi was used as post-harvest mycotoxins controls **(Agriopoulou et al., 2020)**. Probiotics, non-pathogenic microorganisms, could counter host small intestinal digestion or implement useful influences on host animal's health. *Lactobacillus rhamnosus* could decrease deoxynivalenol (DON) intoxication **(Plaza-Diaz et al., 2019)**. Also, Bacillus subtilis CW14 could prevent damage caused via OTA in intestinal **(Peng et al., 2019).** Probiotics including six strains of *S. cerevisiae* yeast and 12 strains from *Lactobacillus* sp. Bacteria could reduce aflatoxin B1concentrations, DON, fumonisins as well as zearalenone in feed to various degrees **(Chlebicz and Slizewska, 2020)**.

Researchers have recorded that antioxidant gained from daily diets such as polyphenols, carotenoids, vitamin C or E can scavenge the ROS. Also, these cofactors may be essential for enhancing and utilizing 1024.023AWenzymatic antioxidants in cells (**Eboh 2014)**. Vitamin C is the main efficacious vitamin for preventing OTA genotoxicity. Ascorbic acid is necessary for free radical scavenger, through inhibiting electrophilic metabolites release. Furthermore, it is needed for vitamin E renovation in lipid membranes as well as performances synergistically with other biological antioxidants, like glutathione. Moreover, various cytochrome P_{450} isoenzymes activities were reduced via vitamin c **(Grosse et al., 1997)**.

This study was designed to investigate the possible beneficial effect of selenium nanoparticles (Se-NPs), vitamin C and probiotics against deleterious effect of ochratoxicosis through investigation of hepato-renal functions, oxidative stress and antioxidant biomarkers, pro-inflammatory status in addition to hormonal profile and immune function in male rats.

2. Material and Methods

2.1. Ethical consent

The experimental protocol was accepted by AHRI in conformity with the ARC and IACUC committee (ARC, AHRI, IACUC, 62/23).

2.2. Selenium nanoparticles

2.2.1. Chemical identification and production of SeNPs

SeNPs were obtained via chemical reduction method. Briefly, double-distilled water (nine ml), 25 mM sodium selenite (three ml), 100 Mm reduced glutathione (three ml), 0.15g bovine serum albumin as stabilizer were added in sterile flask. Then the flask continuously stirred for one hour by using a magnetic stirrer and (1M) Sodium hydroxide poured until pH became nine. The solution color altered to a red color indicating Se-NPs production. The prepared Se-NPs were identified and their particles size, shape, as well as morphology were calculated via UV-visible spectra, XRD patterns **(Verma and Maheshwari, 2018; Atul et al., 2010)**.

2.3. Probiotic

Micro-Procell vials, each containing 1 g of powder consisting of *Lactobacillus plantarum* ($1x10^8$ CFU) as well as *Lactobacillus acidophilus* ($1 x 10^8$ CFU) strains were purchased from Cheil Bio Co.; Ltd; South Korea.

Saccharomyces cerevisiae ($1 x 10^7$ CFU) with 0.5 g skimmed milk carrier was supplied by Lallemand, SAS, France, under the name Levucell SB 10ME Titan (LSB).

2.4. Vitamin C

Vitamin C as L- ascorbic acid was provided by Memphis Company.

2.5. Ochratoxin A

Ochratoxin A was provided by biochemical, feed deficiency and toxicology; Animal Health Research Institute.

2.6. Experimental Design

Forty five male albino rats of 5-6 weeks old weighing 180 to 200 g were obtained from the animal house in the Ophthalmology Research Center, Giza, Egypt. In stainless steel cages rats were housed and kept on 12 hours, light-dark cycle, (20 ± 3°C) and 52 – 68% relative humidity. Water and food were provided ad libitum. All rats were acclimatized for 10 days prior to the beginning of study.

Rats were randomly divided into nine equal groups, placed in individual cages as follows in **Table 1**.

Table 1. Experimental design

Animal groups	Nutrition	Treatment
G1: Control normal	Normal nutrition and water ad-libitum for 3 weeks	without treatment
G2: Ochra	Ochratoxin A solubilize in corn oil and administered orally at a dose (50 µg/kg b.wt.) four times per week for 3 weeks **(Vettorazzi et al., 2011)**	No treatment
G3: Ochra + Nano Se	Ochratoxin A solubilize in corn oil and administered orally at a dose (50 µg/kg b.wt.) four times per week for 3 weeks	Rats received Se-NPs orally at a dose (0.31 mg/kg b.wt/0.2 ml of buffer/ day) for 3 weeks **(Hassan et al., 2022a)**.
G4: Ochra+ vitamin C	Ochratoxin A solubilize in corn oil and administered orally at a dose (50 µg/kg b.wt.) four times per week for 3 weeks	Rats Orally administered with vitamin C (1000 mg/l/daily in drinking water) for 3 weeks **(Mansour et al., 2015)**.
G5: Ochra+ Probiotic	Ochratoxin A solubilize in corn oil and administered orally at a dose (50 µg/kg b.wt.) four times per week for 3 weeks	Rats treated with 0.4 g of probiotic powder/one kg diet for 3 weeks **(Beshara et al., 2018)**.
G6: Ochra + Nano Se + vitamin C	Ochratoxin A solubilize in corn oil and administered orally at a dose (50 µg/kg b.wt.) four times per week for 3 weeks	Rats orally administered with Se-NPs and vitamin C for 3 weeks.
G7: Ochra + Nano Se + Probiotic	Ochratoxin A solubilize in corn oil and administered orally at a dose (50 µg/kg b.wt.) four times per week for 3 weeks	Rats orally administered with Se-NPs and probiotic in diet for 3 weeks.
G8: Ochra + Nano Se + vitamin C + Probiotic	Ochratoxin A solubilize in corn oil and administered orally at a dose (50 µg/kg b.wt.) four times per week for 3 weeks	Rats orally administered with Se-NPs, vitamin C and probiotic in diet for 3 weeks.
G9: Ochra + vitamin C + Probiotic	Ochratoxin A solubilize in corn oil and administered orally at a dose (50 µg/kg b.wt.) four times per week for 3 weeks	Rats orally administered with vitamin C and probiotic in diet for 3 weeks.

2.7. Sampling

Blood samples were collected by ocular vein puncture in dry, clean tubes and allowed to clot for 30 minutes and serum was separated by centrifugation at 3000 rpm for five minutes. Serum was taken by automatic pipettes and received in dry sterile tubes, then kept in deep freeze at -20 °C until use for subsequent biochemical analysis.

2.8. Biochemical Analysis

Serum, ALT, AST, LDH, GGT, urea and creatinine were determined according to the method described by to the method described by **Reitman and Frankel (1957)**, **Szase et al (1976)**, **Wybenga et al (1971)**, and **Henry, (1974)**, respectively. Also, serum total antioxidant capacity (TAC), Malonaldehyde (MDA), catalase (CAT), superoxide dismutase (SOD) and reduced glutathione were determined according to the method described by **Koracevic et al (2001), Okhawa et al (1979)**, **Aebi (1974)**, **Nishikimi et al (1972)**, and **Beutler et al (1963)**, respectively.

Moreover, serum Tumor necrotic factor-α (TNF-α) and Interleukin 6 (IL-6) were determined by commercially available ELISA kits based on manufacturers' instructions (R and D Systems; Minnesota; Minneapolis; USA). Meanwhile, total protein and protein electrophoretic pattern were predestined based on **Sonnenwirth et al (1980)** and **Davis (1964)**, respectively and calculated according to Syn Gene S. No. 17292*14518 sme*mpcs.

Quantitative determination of serum estradiol was carried out using ELISA kit (DBC, Canada, catalog No. CAN-E-430). Additionally, serum testosterone, thyroxine (T4), triiodothyronine (T3) as well as thyroid stimulating hormone (TSH) levels were performed using ELISA kit (Perkinelmer, USA, catalog No. 10007, 10302, 10301 and 10304, respectively).

2.9. Statistical Analysis

Three samples from data were analyzed statistically via One-Way ANOVA test after that, Duncan's multiple range tests. The results have been given as mean ± standard error (SE) using SPSS 14 (2006). The results of $p < 0.05$ meant significant values.

3. Results

The obtained results demonstrated in **Table 2** revealed that serum AST, GGT, ALT, and LDH activities, urea and creatinine concentrations were significantly elevated in OTA intoxicated rats when compared with the normal control group. Meanwhile, significant decreases of all previous parameters were reported in all treated groups when compared with ochratoxicated rats.

The obtained data established in **Table 3** revealed that a significant increase in serum TNF-α, IL-6, MDA, CAT, and SOD with marked decrease GSH and TAC were observed in OTA exposed rats in comparison with control group. However, significant decreases of serum TNF-α, IL-6, MDA, CAT, and SOD with marked increase in serum GSH and TAC were noticed in all treated groups when compared with ochratoxicated rats.

The obtained results existing in **Table 4a** revealed that, a significant decrease in serum T. protein; albumin, T. beta, T. gamma, T. globulin and albumin/globulin (A/G) ratio with significant increase in T. alpha were detected in ochratoxicated rats comparing with normal group. Conversely, serum T. protein, albumin, T. beta, T. gamma, T. globulin and A/G ratio with reduction in serum T. alpha level were observed in all treated groups as compared with ochratoxin- A exposed rats.

The current results presented in **Table 4b** exhibited a significant increase in serum alpha 1 and gamma 2 with palpable decrease in alpha 2, beta 1 and gamma 1 levels were observed in OTA intoxicated rats when compared with normal group. While there were reductions of serum alpha 1 and gamma 2 with increase in beta 1, gamma 1 and alpha 2 concentrations in all treated groups comparing with ochratoxicated rats.

The recorded data in **Table 5** illustrated that OTA causes a significant decrease in serum testosterone, estradiol, T3 and TSH concentrations with significant increase in serum T4 level comparing with normal control group. Meanwhile, there were significant increases in testosterone, T3 and TSH associated with non-significant increase estradiol value with significant decrease in serum T4 levels in all treated rats' groups comparing with ochratoxicated rats.

Table 2. Protective effect of different treatment on serum liver marker enzymes (AST, ALT, LDH, and GGT) activities and kidney function tests (urea and creatinine) concentrations in ochratoxicated rats

	AST (U/L)	ALT (U/L)	LDH (U/L)	GGT (U/L)	Urea (mg/dl)	Creatinine (mg/dl)
G1	32.3 ± 5.17^{f}	27.3 ± 3.18^{e}	76.80 ± 2.58^{c}	6.88 ± 0.08^{d}	27.13 ± 0.59^{d}	0.73 ± 0.015^{c}
G2	92 ± 4.16^{a}	59.3 ± 3.82^{a}	111.56 ± 6.11^{a}	15.10 ± 0.67^{a}	48.34 ± 0.82^{a}	1.09 ± 0.07^{a}
G3	61 ± 2.51^{bc}	39.00 ± 3^{bcde}	96.29 ± 1.61^{b}	9.05 ± 0.79^{c}	39.26 ± 0.75^{bc}	0.89 ± 0.03^{b}
G4	63.33 ± 1.8^{bc}	43.3 ± 3.84^{bc}	96.06 ± 1.78^{b}	11.30 ± 0.50^{b}	40.16 ± 0.24^{b}	0.89 ± 0.04^{b}
G5	67.67 ± 4.63^{b}	51 ± 3.21^{ab}	94 ± 1.60^{b}	11.37 ± 0.47^{b}	41.59 ± 0.65^{b}	0.91 ± 0.03^{b}
G6	53.67 ± 4.91^{cd}	40.67 ± 2.19^{bcd}	84.44 ± 3.71^{c}	9.05 ± 0.79^{c}	39.86 ± 1.52^{bc}	0.90 ± 0.007^{b}
G7	44.67 ± 3.48^{de}	30.33 ± 2.85^{de}	83.26 ± 2.06^{c}	8.43 ± 0.48^{cd}	40.88 ± 1.93^{b}	0.89 ± 0.04^{b}
G8	40.33 ± 2.84^{ef}	51.67 ± 8.25^{ab}	83.92 ± 2.91^{c}	9.42 ± 0.54^{c}	39.37 ± 0.94^{bc}	0.82 ± 0.03^{bc}
G9	39.33 ± 3.33^{ef}	34.89 ± 2.02^{cde}	83.26 ± 2.66^{c}	9.05 ± 0.79^{c}	36.47 ± 1.48^{c}	0.82 ± 0.05^{bc}

Gps; G1: Control, G2: Ochra G3: Ochra + Nano Se, G4: Ochra+ vitamin C, G5: Ochra+ Probiotic, G6: Ochra + Nano Se + vitamin C, G7: Ochra + Nano Se + Probiotic**,** G8: Ochra + Nano Se + vitamin C+ Probiotic, G9: Ochra + vitamin C + Probiotic. Data are presented as (Mean ± SE). n= 3 rats; Mean values with different superscript letters in the same column are significantly different at ($p < 0.05$).

Table 3. Protective effect of different treatment on serum pro-inflammatory cytokines and oxidative stress/antioxidant markers in ochratoxicated rats

	IL-6 (Pg/ml)	TNF-α (Pg/ml)	MDA (nmol/ml)	SOD (U/ml)	CAT (U/l)	GSH nmol/ml	TAC (Mm/l)
G1	164.83 ± 1.74^{f}	71.50 ± 4.86^{d}	1.15 ± 0.05^{c}	22.69 ± 1.54^{d}	11.31 ± 0.3^{d}	13.88 ± 0.09^{a}	6.20 ± 0.21^{a}
G2	255.33 ± 7.05^{a}	155.33 ± 11.39^{a}	1.75 ± 0.04^{a}	60.09 ± 4.35^{a}	17.73 ± 1.20^{a}	13.13 ± 0.14^{bc}	1.86 ± 0.07^{g}
G3	217.33 ± 10.31^{b}	77.33 ± 5.29^{cd}	1.28 ± 0.04^{bc}	50.37 ± 3.62^{b}	14.40 ± 0.71^{b}	13.60 ± 0.04 ^{abc}	3.07 ± 0.08^{de}
G4	203.33 ± 8.97^{bc}	86.67 ± 3.72^{bcd}	1.45 ± 0.06^{b}	48.23 ± 1.62^{b}	13.44 ± 0.75^{bcd}	12.99 ± 0.27^{c}	3.16 ± 0.14^{de}
G5	203.33 ± 7.87^{bc}	93.33 ± 8.40^{bcd}	1.34 ± 0.06^{bc}	46.22 ± 2.57^{b}	13.25 ± 0.20^{bcd}	13.50 ± 0.06^{abc}	2.44 ± 0.07^{f}
G6	197.83 ± 5.34^{bcd}	104.50 ± 6.38^{b}	1.33 ± 0.08^{bc}	36.89 ± 1.17^{c}	13.83 ± 0.89^{bc}	13.28 ± 0.07^{abc}	3.48 ± 0.10^{d}
G7	174.13 ± 3.28^{ef}	98.79 ± 12.56^{bc}	1.28 ± 0.07^{bc}	37.82 ± 2.49^{c}	12.08 ± 0.63^{cd}	13.73 ± 0.18^{ab}	2.69 ± 0.18^{ef}
G8	190.67 ± 4.97^{cde}	84.00 ± 1.89^{bcd}	1.35 ± 0.20^{bc}	38.05 ± 0.48^{c}	13.79 ± 0.30^{bc}	13.44 ± 0.23^{abc}	5.06 ± 0.33^{b}
G9	177.67 ± 8.45^{def}	77.67 ± 4.96^{cd}	1.14 ± 0.10^{c}	34.10 ± 1.78^{c}	12.80 ± 0.32^{bcd}	13.83 ± 0.37^{a}	4.10 ± 0.07^{c}

Gps; G1: Control, G2: Ochra G3: Ochra + Nano Se, G4: Ochra+ vitamin C, G5: Ochra+ Probiotic, G6: Ochra + Nano Se + vitamin C, G7: Ochra + Nano Se + Probiotic**,** G8: Ochra + Nano Se + vitamin C+ Probiotic, G9: Ochra + vitamin C + Probiotic. Data are presented as (Mean ± SE). *n*= 3 rats; Mean values with different superscript letters in the same column are significantly different at ($p < 0.05$).

Table 4a. Protective effect of different treatment on serum Total protein and its fractions concentrations in ochratoxicated rats

	Total protein and main fractions (g/dl)						
	T. protein	albumin	T. alpha	T. beta	T. gamma	T. globulin	A/G ratio
G1	8.06 ± 0.23^{a}	2.74 ± 0.13^{a}	0.97 ± 0.04^{b}	2.00 ± 0.04^{a}	2.35 ± 0.11^{a}	5.32 ± 0.15^{a}	0.51 ± 0.02^{a}
G2	5.25 ± 0.25^{e}	1.46 ± 0.15^{d}	1.16 ± 0.06 ^{a}	0.99 ± 0.04^{f}	1.64 ± 0.05^{de}	3.79 ± 0.11^{c}	0.38 ± 0.04^{b}
G3	6.42 ± 0.12^{bcd}	2.14 ± 0.08 ^{bc}	0.75 ± 0.05^{c}	1.37 ± 0.07^{cde}	2.17 ± 0.08^{ab}	4.28 ± 0.04^{bc}	0.50 ± 0.02^{ab}
G4	5.78 ± 0.05^{de}	2.07 ± 0.08^{bc}	0.86 ± 0.07 ^{bc}	1.29 ± 0.06^{def}	1.56 ± 0.04^{e}	3.71 ± 0.03^{c}	0.56 ± 0.03^{a}
G5	6.13 ± 0.23^{cd}	1.85 ± 0.12^{c}	0.94 ± 0.06^{b}	1.41 ± 0.14^{cde}	1.92 ± 0.16^{bcde}	4.28 ± 0.34^{bc}	0.45 ± 0.08^{ab}
G6	5.88 ± 0.15^{cde}	2.11 ± 0.09^{bc}	0.93 ± 0.06^{bc}	1.15 ± 0.09^{ef}	1.69 ± 0.07^{cde}	3.78 ± 0.11^{c}	0.56 ± 0.03^{a}
G7	6.68 ± 0.48^{bc}	2.12 ± 0.03^{bc}	0.88 ± 0.01^{bc}	1.50 ± 0.19^{cd}	2.18 ± 0.25^{ab}	4.56 ± 0.45^{b}	0.47 ± 0.05^{ab}
G8	6.59 ± 0.16^{bc}	1.85 ± 0.12^{c}	0.91 ± 0.07^{bc}	1.84 ± 0.06^{ab}	1.99 ± 0.12^{abcd}	4.74 ± 0.13^{ab}	0.39 ± 0.03^{b}
G9	7.03 ± 0.28^{b}	2.28 ± 0.08^{b}	1.02 ± 0.06^{ab}	1.67 ± 0.09^{bc}	2.06 ± 0.13^{abc}	4.75 ± 0.25^{ab}	0.48 ± 0.03^{ab}

Gps; G1: Control, G2: Ochra G3: Ochra + Nano Se, G4: Ochra+ vitamin C, G5: Ochra+ Probiotic, G6: Ochra + Nano Se + vitamin C, G7: Ochra + Nano Se + Probiotic**,** G8: Ochra + Nano Se + vitamin C+ Probiotic, G9: Ochra + vitamin C + Probiotic. Data are presented as (Mean ± SE). *n*= 3 rats; Mean values with different superscript letters in the same column are significantly different at ($p < 0.05$).

Table 4b. Protective effect of different treatment on serum protein sub-fraction concentrations in ochratoxicated rats

	Protein Sub-fraction (g/dl)					
	Alpha 1 (α1)	Alpha 2 (α2)	Beta 1 (β1)	Beta 2 (β2)	Gamma 1 (γ1)	Gamma 2 (γ2)
G1	0.25 ± 0.03^{d}	0.72 ± 0.03^{a}	1.29 ± 0.05^{a}	0.71 ± 0.02^{ab}	1.77 ± 0.13^{a}	0.58 ± 0.03^{b}
G2	0.65 ± 0.05^{a}	0.51 ± 0.01^{b}	0.60 ± 0.04^{c}	0.39 ± 0.02^{d}	0.85 ± 0.05^{e}	0.79± 0.03^{a}
G3	0.28 ± 0.05^{cd}	0.47 ± 0.04^{b}	0.83 ± 0.06^{bc}	0.54 ± 0.04^{cd}	1.56 ± 0.02^{ab}	0.61 ± 0.06^{b}
G4	0.40 ± 0.02^{bcd}	0.46 ± 0.06^{b}	0.79 ± 0.05^{bc}	0.50 ± 0.04^{cd}	1.01 ± 0.04^{de}	0.55 ± 0.01^{b}
G5	0.41 ± 0.04^{bc}	0.53 ± 0.05^{b}	0.87 ± 0.13^{bc}	0.54 ± 0.02^{cd}	1.31 ± 0.16^{bcd}	0.61 ± 0.03^{b}
G6	0.44 ± 0.03^{b}	0.49 ± 0.08^{b}	0.66 ± 0.06^{c}	0.49 ± 0.04^{cd}	1.11 ± 0.04 ^{cde}	0.58 ± 0.03^{b}
G7	0.34 ± 0.04^{bcd}	0.54 ± 0.06^{b}	0.95 ± 0.17^{b}	0.55 ± 0.05^{c}	1.51 ± 0.27^{ab}	0.67 ± 0.02^{ab}
G8	0.31 ± 0.06^{bcd}	0.60 ± 0.02^{ab}	1.04 ± 0.04^{ab}	0.80 ± 0.09^{a}	1.41 ± 0.06^{abc}	0.58 ± 0.11^{b}
G9	0.46 ± 0.07^{b}	0.56 ± 0.06^{ab}	1.07 ± 0.06^{ab}	0.60 ± 0.05^{bc}	1.54 ± 0.13^{ab}	0.52 ± 0.02^{b}

Gps; G1: Control, G2: Ochra G3: Ochra + Nano Se, G4: Ochra+ vitamin C, G5: Ochra+ Probiotic, G6: Ochra + Nano Se + vitamin C, G7: Ochra + Nano Se + Probiotic**,** G8: Ochra + Nano Se + vitamin C+ Probiotic, G9: Ochra + vitamin C + Probiotic. Data are presented as (Mean ± SE). *n*= 3 rats; Mean values with different superscript letters in the same column are significantly different at ($p < 0.05$).

Table 5. Protective effect of different treatment on serum testosterone, estradiol, T3, T4, and TSH concentrations in ochratoxicated rats

	Testosterone (ng/ml)	Estradiol (pg/ml)	T3 (ng/ml)	T4 (µg/dl)	TSH (MIU/m)
GI	4.60 ± 0.08^{a}	52.20 ± 1.47 ^{a}	0.705 ± 0.028^{a}	5.15 ± 0.086^{d}	0.622 ± 0.001^{a}
G2	2.05 ± 0.04^{d}	40.10 ± 1.62^{d}	0.355 ± 0.023^{c}	7.30 ± 0.230^{a}	0.436 ± 0.002^{f}
G3	3.31 ± 0.10^{b}	45.50 ± 1.51^{bcd}	0.601 ± 0.057^{ab}	6.20 ± 0.230^{bc}	0.545 ±0.001^{d}
G4	2.65 ± 0.08^{c}	43.30 ± 1.57^{cd}	0.538 ± 0.022^{b}	6.45 ±0.086^{bc}	0.510 ±0.013^{e}
G5	2.75 ± 0.08^{c}	44.09 ± 1.54^{cd}	0.655 ± 0.033^{ab}	6.70 ± 0.230^{b}	0.572 ±0.001^{c}
G6	3.40 ± 0.11^{b}	50.00 ± 1.96^{ab}	0.606 ± 0.018^{ab}	6.50 ± 0.057^{bc}	0.573 ±0.015^{c}
G7	3.18 ± 0.008^{b}	47.10 ± 1.61^{abc}	0.660 ± 0.054^{ab}	6.20 ± 0.173^{bc}	0.594 ± 0.002^{bc}
G8	3.43 ± 0.05^{b}	48.83 ± 1.70^{abc}	0.688 ± 0.047^{a}	6.00 ± 0.173^{c}	0.604 ± 0.002^{ab}
G9	2.88 ± 0.10 ^{c}	44.50 ± 1.91^{cd}	0.672 ± 0.041^{a}	6.25 ± 0.144^{bc}	0.584 ± 0.002^{bc}

Gps; G1: Control, G2: Ochra G3: Ochra + Nano Se, G4: Ochra+ vitamin C, G5: Ochra+ Probiotic, G6: Ochra + Nano Se + vitamin C, G7: Ochra + Nano Se + Probiotic**,** G8: Ochra + Nano Se + vitamin C+ Probiotic, G9: Ochra + vitamin C + Probiotic. Data are presented as (Mean ± SE). n= 3 rats; Mean values with different superscript letters in the same column are significantly different at ($p < 0.05$).

4. Discussion

Various research has been procedure applied antioxidants trying to suppress the harmful influences of oxygen radicals released in OTA-toxicity. The previous experiments suggested the ability of antioxidants to block the harmful influences of chronic OTA explore and assured highly potential effectiveness of nutritional strategies in suppression OTA intoxication **(Sorrenti et al., 2013)**. Up till now, less studied applied on acute toxicity model during OTA-intoxication. Depending on this, integrated analyses of oxidative stress (OS) biomarkers, Protein electrophoretic pattern or serum hormonal profiles were done in current study to illustrate OTA toxicity.

The obtained data clarified that a significant increase in serum AST, GGT, ALT, LDH, urea as well as creatinine values in rats administrated OTA (50 µg/kg of b.wt.) compared to normal group. These elevated values were highly critical serum biomarkers in determining hepatocyte's function and damage in OTA supplementation **(Prati et al., 2016)**. **Dreisbach and Lertora (2008)** reported that the very intense hepatocytes damage was the higher liberate of liver enzymes. Hepatotoxicity in most cases is due to free radical. Free radicals liberate via the metabolism of toxicants initiate the toxicity cascade **(Kumar et al., 2010)**. In the current study, increase hepatic and kidney biomarker could be a secondary condition following OTA caused lipid peroxidation of hepatocyte and kidney cells. The damaging effects of OTA may be resulted from its liberation of reactive oxygen species (ROS) that causes OS of various organs. ROS generation and lipid peroxidation of cell membranes causes absence of membrane fluidity, an increase in membrane permeability or changes in membrane potential which causing enzymes infiltration from the cells **(Nehru and Anand, 2005)**. Increases in cell damage as well as cell membrane permeability associated with extravagant release of hepatocyte diseases and biliary obstruction will increase ALT, AST values as well as ALP in serum **(Poupon, 2015 and Liu et al., 2021)**. Conditions that elevate serum GGT caused increased free radical liberation and reduction of glutathione **(Whitfield, 2001)**.

Betina (1989) reported that ochratoxin A acts particularly in the proximal renal tubules, blocking phosphoenol-pyruvate carboxylase enzyme, lipid prooxidant, or it changes the structural and functional kidney capability to metabolize calcium. It caused a high increase of serum TNF-*α*, IL-6, MDA, SOD and CAT values in rats. OS is closely regarding to inflammation. During oxidative stress, the cells liberate a huge amount of ROS, which activate nuclear factor kappa light chain enhancer of activated B cells (NF-*κ*B) inflammatory signaling pathways as well as elevate pro-inflammatory factors expression **(Glauert, 2012)**. Oxidative stress influences the body's immune system as well as inflammatory response **(Lauridsen, 2019).** Inflammation is a serious indicator of liver damage caused via physical and chemical toxic compound stimuli. Inflammation has an essential role in the repair of liver damage **(Hou et al., 2018).** TNF-*α* is the first inflammatory item liberate in the inflammatory response, which can cause the liberation of other cytokines, influence on NF-*κ*B, a key operator in regulating inflammatory response, enhance the release of free radicals and aggravating liver damage **(Schwabe and Brenner, 2006)**. Throughout liver damage, TNF-*α* and IL-6 activated and liberate lead to the cumulating of neutrophils in hepatocytes, ultimately causing up-regulation cytokine expression **(Olteanu et al., 2012)**. Experimental studies have stated that mycotoxins, involving OTA, can increase the release of TNF-*α* in rats **(Xu et al., 2019)**.

In the current study, an increase was observed in MDA (as oxidative stress marker) level was observed in ochratoxicosis group when compared with normal group this result convention with **Domijan et al (2005)**. These results indicate cellular injury produced via OTA. The liberation of ROS may be mediated via Fe^{3+}–OTA complex formation **(Gautier et al., 2001)**.

Superoxide dismutase has an antioxidant effect in the organism; it is vital due to SOD reacting with crucial cellular targets, as NO radical. In present study, SOD is a significantly increase in ochratoxicated group. Similarly, **Palabiyik et al (2013)** and **Aydin et al (2013)** suggested that, throughout OTA (0.5 mg/kg/b.wt) administered to rats for two weeks, the DNA damage in lymphocytes, renal and liver; the reduction of GSH values associated with the elevated of SOD activity were detected.

Also, serum CAT activity significantly increases in OTA treated rats' group as compared to normal control. These results are nearly similar to **Soyöz et al (2004)** and **Özçelik et al (2004)** who mentioned that CAT was elevated in serum of OTA-treated rats (0.289 mg kg^{-1}× four weeks). CAT is the most abundant in liver cells and it works in conjunction with SOD **(Baudrimont et al., 1997)**.

The obtained results illustrated that OTA decreased the concentration of serum TAC and GSH. There is a balance between ROS production and ROS degradation with total antioxidants in the cells. Any increase in ROS or antioxidants can cause abnormal oxidative stress state **(Akinrinmade and Akinrinde, 2013)**. Free radicals' accumulation in kidney or other cells, causing tissue injury because of oxidative binding of key intracellular molecules containing thiol groups as GSH and lipid peroxidation of cell membranes which might be of

greater importance in the cytotoxicity responsible for cell apoptosis **(López et al., 2007)**. Impairment in the synthesis of enzymatic or non-enzymatic antioxidants may be the high important factor in cellular total antioxidant reducing levels. So, the decrease in the serum GSH level makes the rats cells more vulnerable to oxidative stress damage. OS leads to irreversible cellular injury due to intracellular defense mechanisms are exhausted and therefore cannot protect cells contra ROS **(Rani et al., 2010)**.

Rats treated with OTA exhibited significant decreases in serum total protein, albumin, total beta, total gamma, and globulin values with corresponding depletion of A/G ratio. Similarly, **Hassan et al (2018)** recorded that OTA administration (50 µg kg^{-1} bodyweight) for 21days reduced serum total protein, albumin as well as globulin values with decreasing in A:G ratio. These reductions in serum protein concentrations might be because of OTA-caused liver injury **(Qi et al., 2014)**. Total proteins decreased may be due to OTA is a protein synthesis suppression that has an influence on activity of mitochondrial oxidative **(Vettorazzi et al., 2013),** or might be because of the imbalance between protein synthesis and its degradation rate in hepatocyte **(Arvind, 2014)**. Also, reduction of protein may be related to lowering globulin concentration with supports impaired immunoglobulin output. Hepatic insufficiency is often associated with decreased β-globulins values **(Werner and Reavill, 1999)**. Moreover, the reduction of albumin level may be because of reduced protein in liver or protein production loss in alimentary tract **(Karakilcik et al., 2004)**.

Moreover, OTA induced glomerular damage characterized by reduction in albumin levels. Hypoalbuminaemia may be appearing in kidney diseases and nephrotic syndrome, in which there is highly infiltration of this protein in urine because of glomerular injury **(Grauer, 2005; Kodner, 2009)**. The reduction in t.globulin or A/G ratio may be because of blocked protein synthesis or disturbance on immuno-globulin production. Thus, lead to an elevated of free amino acids or protein turnover reduction **(Selvakumar et al., 2013)**. Reduced values of t.globulin pointing to that the immune competence of the animals will be easily compromised. Additionally, the increase in α1-globulin (alpha-1antitirypsin) and gamma-2 globulins in current study (ight be related to tissue disruption and inflammatory reaction **(Pesce and Kaplan, 1987; Kaneko et al., 1997)**. The protein subfractions suggested a high reduction. This may indicate immunotoxicity and immunosuppressive action of OTA. Immunosuppression of OTA is presented by reduction size of immune organs, such as spleen, thymus, or lymph nodes, decrease of antibody responses, changes in total number and role of immune cells, or alteration of cytokine liberation. Ochratoxin A has immunotoxic activity due to degenerative alterations and cell apoptosis influenced immune cells, because of protein synthesis inhibition **(Al-Anati and Petzinger, 2006)**.

The current study showed that the mean values of serum testosterone, estradiol and T3 were significantly decreased in OTA rats. This data is in convention with **Kumar et al (2011)**, who mention that OTA inhibits testosterone secretions in rats. Mycotoxins produced via various fungi in many food products, grains, cheese, or meat caused a drop in testosterone value by (66.6%) in rats treated for two months causing rats infertility at different degrees with reduced the chance of normal reproductive activity **(Selmanoglu and Kockaya, 2004)**. **Hasanzadeh et al (2011) and Farag et al (2018)** mentioned reduction in concentration of estradiol, testosterone in group intoxicated by Aflatoxins (AFs) in male rat. **Bbosa et al (2013)** recorded that one of the main common mechanisms for AFB1 effect is the binding of DNA forming complexes and prevent nucleic acid production. This mode of action may demonstrate the direct influence of aflatoxin B1 on Leydig or Sertoli cells in the testes associated with the depletion of the gonadal hormones, testosterone, and estradiol. In the present study the similar result of aflatoxin B1 were shown with ochratoxin A, this may indicate the same mechanism that occurs with ochratoxin A.

In the present study serum T4 level showed significant increase in OTA treated rats while TSH level was significantly decreased as compared to normal control group. The reduction in serum TSH in OTA might be due to that OTA caused disturbance in excretory portion of thyroid gland function represented by T3, T4 and Pituitary hormonal pathway through TSH. **Hassan et al (2010)** reported that OTA has a significant effect of some endocrine function of reproductive organs and thyroid gland as reduction in T3 associated with increase in T4 level in male rats. The reason for elevating of T4 or reduced of T3, which were noted might have been because of a slowdown in the conversion of T4 to T3 in peripheral tissues. This may be because of the chemicals caused endocrine-disrupting (EDCs, as OTA) that suppressed the conversion of T4 into T3 by inducing the alterations, mostly in 5-deiodinase, malic enzyme, and activities of 6-phosphogluconate dehydrogenase **(Diamanti-Kandarakis et al., 2009)**.

Thus, OTA-induced enhanced production of ROS which induces lipid peroxidation may lead to conformational changes in thyroid receptors. Antioxidant supplementation may prevent disease progression. Administration of vitamin c, probiotics or/and Se-NPs along with OTA indicated a marked recovery in liver and renal biomarker comparing with OTA groups. According to ameliorative influence of vitamin C or/and Se-NPs against OTA toxicity, previous studies **He et al (2013)** reported a highly hepatoprotective activity of vitamin c or/and Se by decreasing the values of sera biological enzymes in AFB1 intoxicated rats. Similarly, **Hosseini et al (2023)** recorded that, decreased values of AST, ALP, ALT, creatinine, and BUN associated with an increased albumin and total protein concentrations in Propylthiouracil Se-NPs treated groups versus the Propylthiouracil induced hypothyroidism in rats. The protective hepatorenal influence of Se-NPs was associated with the increased serum values of GSH and TAC in all Se-NPs treated groups in comparing with OTA group. Se-NPs have been noted to have antioxidant or anti-inflammatory influences **(Abdou and Sayed, 2019; Hojjati Fard et al., 2022).** Additionally, Se-NPs has been noted to protect liver against cadmium induced hepatotoxicity via balancing the liver function markers and reduction antioxidant enzymes activities, involving GPx and CAT in animal models **(Vicas et al., 2021**).

The supplementation of Se-NPs, vitamin c and probiotics improve the damage induced by OTA as noted via increased GSH, TAC and reduced MDA values comparing with OTA group. Antioxidant enzymes are crucial in the protection from xenobiotic-induced oxidative damage. Probiotic strains that can highly inhibit levels of reactive radicals *in vivo* various restrict and treat several disorders communicated with oxidative stress. Probiotics enhance antioxidant defenses through releasing GSH and vitamins production that are absorbed and scattered through the body **(Spyropoulos et al., 2011; Kushkevych and Jampílek, 2021; Hassan et al., 2022b)**.

In the status of an antioxidant, vitamin C could improve increased lipid peroxidation level caused by different toxins, including OTA **(Hoehler and Marquardt, 1996)**. It played an action in reducing the levels and accumulation of oxygen reactive species **(Layachi and Kechrid, 2012)**. Vitamin C has important properties that are probably involved in its immune-modulating influences. It is a highly effective antioxidant, due to its ability to readily donate electrons, thus protecting important biomolecules (proteins, lipids, carbohydrates, and nucleic acids) from damage by oxidants generated during normal cell metabolism and through exposure to toxins **(Carr and Frei, 1999)**. Also, vitamin C participates in redox recycling of other important antioxidants; for example, vitamin C is known to regenerate vitamin E from its oxidized form. However, vitamin C plus Nano se or probiotics or all together proved more effective as compared to individual of each. It is well established that vitamin C can protect indispensable molecules in the body **(Carr and Maggini, 2017)**.

The results showed that Se-NPs and probiotics significantly decrease TNF-α and IL-6 values this result is agree with **(Hassan et al., 2020; Sayed-ElAhl et al., 2022).** The Se-NPs have anti-inflammatory possibility modulating pro-/anti-inflammation cytokine production profiles, and that the mechanism is partially because of suppression of activations of NF-κB and p38 mitogen-activated protein kinases (p38 MAPKs) **(Wang et al., 2015)**. Se-NPs administration elevated the

delayed reaction of hypersensitivity and immune response **(Yazdi et al., 2015**).

Probiotics associated with the improvement of metabolic diseases **(Rad et al., 2016)**. Additionally, it played a beneficial role in improving the growth, immune system, and oxidative status **(Dawood et al., 2016)**. Consistently, our findings suggested that probiotics inhibited the release of the cytokines TNF-*α* and IL-6, decreased the level of MDA, but increased the concentrations of the antioxidants GSH. **Lutgendorff et al (2009)** recorded that administration of probiotics to rats could enhance transcription of genes concerned in glutathione production in the intestinal mucosa.

Vitamin C improvement pro-inflammatory cytokines levels these results nearly like **Amin et al (2023)** who recorded that, administration of vitamin C to rats reduce the increased of serum TNF-*α* and IL-6 compared with tartrazine or Allura red groups. Also, **El-Senousey et al (2018)** mentioned that vitamin C markedly decreases cytokines in chickens under oxidative stress condition when supplemented with their feed. Vitamin C can inhibit the release of TNF-*α* by enhancing nuclear factor-erythroid 2-related factor 2 (Nrf2) level **(Otomaru et al., 2023)**.

The current results explained that vitamin c, probiotic or/and Se-NPs treatment along with OTA improve OTA -induced alterations in protein concentrations in the serum of rats. These improvements may be because of enhanced DNA synthesis and suppression in harmful adduct formation **(Cheng et al., 2003)**. Similarly, **Eid et al (2023)** reported that Nano-selenium administration to heat-stressed chickens cause elevation in the plasma total protein, albumin, and globulin values. This change is because of the ability of Se-NPs to increase protein DNA formation and reduced their injury in lymphocyte cells as well as in bone marrow cells, which appears the potential factor of Nano-Se in protecting DNA disruption **(Bhattacharjee, 2016)**. Additionally, the ability of Se-NPs to ameliorate the concentrations of albumin in the liver toxicity may be attributed to S-selenite actions on antioxidant enzymes that are responsible for eliminating ROS. This is agreed with **(Boostani et al., 2015)**, who recorded that selenium is actively including in the antioxidant defense mechanisms since it is an important component of the enzyme selenium-dependent glutathione peroxidase, which decreases peroxide as well as safeguards cells from damage. This improvement might be attributed to increased protein synthesis, increasing incorporation of certain amino acids, increase of hepatic uptake of glucogenic amino acids, stimulation of amino acid incorporation into protein and decreased proteolysis by activating the enzyme that catalyzing amino acids transamination **(Daniel, 2015)**. Moreover, the increase of total protein in probiotic treated groups may be because of improved amino acids. The administration of probiotics to chickens for 6 weeks improved amino acids levels compared with deoxynivalenol intoxicated group. The amino acids are necessary for protein synthesis **(Sayed-ElAhl et al., 2022)**. Probiotics capacity, as a natural antioxidant, to reduced OTA effect by the immune strengthening action and prevent oxidative damage of lipid and protein, was investigated **(Hassan et al., 2016)**.

The current study showed significant increase in circulating testosterone when Se-NPs, Vitamin C, Probiotic was given to the OTA treated rats. **Gouda et al (2021)** establish that serum testosterone concentrations were significantly increased in rabbits treated with Nano selenium. Se-NPs, Vitamin C that has antioxidants can counteract ROS so that testicular cells can be repaired. Moreover, **Kanmani et al (2013)** has stated that probiotics are able to scavenge hydroxyl radicals and superoxide anions and release antioxidants. Furthermore, oxidative stress caused by OTA, the excessive production of ROS and free radicals in related glands, caused endocrine disruptors. Whereas Nano selenium, Vitamin C and probiotic have powerful activities to reduce ROS, so it could alleviate the deleterious effect of OTA and as follow in restore hormones.

5. Conclusion

It could be concluded that the hepato-renal toxicity induced via ochratoxin A seemed to be modulated effectively through the simultaneous antioxidants Nano selenium, probiotic, and vitamin c supplementation either alone or in combination. Also, Nano selenium, probiotic and vitamin c supplementation are powerful antioxidant agents, stops ROS production, attenuates OTA-induced oxidative stress and hepato renal damage and enhances antioxidant defense system. Also, treatment with such natural agents has a strong anti-inflammatory effect through inhibiting TNF-*α* and IL-6 which then contributes to the protection of such antioxidant agents against OAT-induced inflammation. So, these results confirm the strong antioxidant, anti-inflammatory and cytoprotective effects of Nano selenium, probiotic, and vitamin c against OTA toxicity with highly protective effect of Nano selenium or probiotic. Consequently, we suggested that Nano selenium, probiotic and vitamin c supplementation are very essential which may alleviate dangerous effects during ochratoxins exposure.

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