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Grape Seed Extract Enhances Antioxidant Capacity and Attenuates the Ochratoxin A-Induced Genotoxicity and Oxidative Stress in Albino Rats

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Abstract

Ochratoxin A (OTA) is highly toxic to animals and humans within mycotoxins. Some studies have reported that Grape Seed Extract (GSE) has a protective role on these toxicities. The aim of the present study is to determine the antioxidant effect of GSE against genotoxicity, and oxidative stress induced by OTA in albino rats. Forty mature Wistar albino male rats with similar body weight were randomly divided into four groups (10 rats each): 1- untreated control group, 2- OTA treated group (1.7 mg/Kg b w, i. p), 3- OTA + 75 mg/Kg b w GSE group, 4- OTA + 150 mg/Kg bw GSE group. Rats were treated for 15 days and at the end of experiments, liver and kidney tissue homogenate, as well bone marrow cells were prepared to determine the antioxidant capacity and ameliorate role of GSE on genotoxicity, DNA damage and oxidative stress was induced by OTA in albino rats. The results show that GSE could significantly improve genotoxicity and DNA damage induced by OTA. SOD and GSH-Px as antioxidant enzymes were increased, as well, MDA and NO as oxidative stress parameters were significantly decreased, and effectively alleviating the oxidative stress caused by OTA and enhance the antioxidant capacity. These results suggest that GSE has a protective effect against OTA induced genotoxicity, DNA damage and oxidative stress in rats through its antioxidant effect. Thus, clinical application of GSE as therapy should be considered to prevent and treat oxidative damage in cases of ochratoxicosis.

Keywords: Ochratoxin A (OTA), genotoxicity, DNA damage, oxidative stress, grape seed extract (GSE), antioxidant, liver; kidney, rats

1. Introduction

Ochratoxin A (OTA) is highly toxic to animals and humans within mycotoxins (Wang et al., 2016), and it is the most potent carcinogenic (Duarte et al., 2011; Malir et al., 2016). The mechanism of toxicity may be attributed to oxidative stress, inhibition of protein synthesis, interference of cell signal transduction and apoptosis (Zhang et al., 2022). Oxidative stress is an increase in oxidation as a result of imbalance between oxidation and antioxidant systems (Yoshikawa and Naito, 2002). Under oxidative stress, overproduction of malondialdehyde (MDA), genotoxicity and DNA damage, and on the other side, antioxidant enzymes activity: superoxide dismutase (SOD), glutathione peroxidase (GPx), reduced glutathione (GSH), and Glutathione reductase (GR)can be estimated (Eken, 2017; Kıran et al., 2023)

OTA exposure increases the production of reactive oxygen species (ROS) and consequently induces genetic damage in rats and mice, indicating that the genotoxic effect may be secondary to oxidative stress due to the formation of DNA-adducts as a result of ROS generation. OTA also induces single and double strand breaks (SSBs and DSBs), gene mutations, chromosomal aberration and DNA damage in mammals (Schrenk et al., 2020). It was found to induce micronucleus (MN) formation and hypodiploid (a measure of aneugenicity) and DNA damage in Chinese hamster. The association between oxidative DNA damage and OTA exposure suggests its involvement in aneugenicity and clastogenicity production (Ali et al., 2011). The methods that measure chromosome aberrations and DNA fragmentation indicate that OTA gives positive results such as single-strand DNA breaks in rat and mice kidney and liver (Mally *et al*., 2005 ; Schrenk *et al.,* 2020) and induces micronuclei in cells of different origins(Degen *et al*., 1997; Ali *et al*., 2014; Campra *et al.,* 2020).

Oxidative stress is one of the main mechanisms of OTA toxicity showing genotoxicity, hepatotoxicity, teratogenicity and carcinogenicity (Heussner and Bingle 2015; Zhang *et al*., 2022). Excessive oxidants accumulation exceeds the scavenging ability of the body, leading to a significant increase of MDA (lipid peroxidation) levels in various tissues, resulting in DNA

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** **Abbreviations:** OTA: Ochratoxin A; GSE: Grape seed extract; DNA: Deoxyribonucleic acid; SOD: Superoxide dismutase; GPx: glutathione peroxidase; GSH: Reduced glutathione; GR: Glutathione reductase; MDA: Malondialdehyde; NO: Nitric oxide; ROS: Reactive oxygen species; MN: Micronucleus; DTNB: 5, 5′-dithio-bis (2-nitrobenzoic acid); NaCl: Sodium chloride; KCl: Potassium chloride; TCA: Trichloroacetic acid; Mn PCE: Micronucleated polychromatic erythrocytes.

damage (Sharifi-Rad *et al*., 2020). If any amount of OTA enters the body, the antioxidant defense system is destroyed, leading to a significant increase in nitric oxide (NO) and malondialdehyde (MDA) levels (marker of lipid peroxidation) and a significant decrease in antioxidant enzymes: (SOD, GSH and GPx) levels. However, antioxidant supplementation may activate antioxidant enzymes (SOD and GSH-Px) in the liver and kidney, and decrease toxicity-induced oxidative damage in rats (Wang *et al.,* 2019)

Antioxidants are reported to prevent cells from genotoxicity and cytotoxicity induced by OTA (Ramyaa *et al*., 2014; Costa et al., 2016; Damiano *et al.,* 2020). Grape seed extract (GSE), a polyphenolic flavonoid, is an excellent antioxidant, and can protect the activities of various antioxidant enzymes and work as antioxidant enhancement. In the rat, GSE increased antioxidant enzymes (SOD, GSH and GPx) levels, decreased oxidative stress or lipid peroxidation parameters (NO and MDA) expression and enhanced antioxidant capacity (Chis *et al.,* 2009). As well, Taghizadeh et al. (2016) proved that the scavenging properties of GSE were clear in the lipid peroxidation inhibition by reducing the high levels of MDA (oxidative stress indicator) and increasing the antioxidant enzymes activity especially the GPx. Therefore, this study was designed to investigate the antioxidant effect of GSE on genotoxicity, DNA damage and oxidative stress induced by OTA in albino rats.

2. Materials and Methods

2.1. Animals

Adult albino male rats of Wistar strain weighing 180-220 g were obtained from animal house colony of the National Research Centre, Dokki, Giza, Egypt. They were kept for 1 week of adaptation under the hygienic conditions, controlled temperature and humidity and fed with well-balanced diet and water *ad libitum*. The experiments were carried out according to the National regulations on animal welfare and Institutional Animal Ethical Committee guidelines (IAEC) and conform the requirement of the ethics committee of the Institutional Animal Care and Use Committee (IACUC), approval no: 01122022587.

2.2. Experimental groups

Forty mature Wistar albino male rats with similar body weight were randomly divided into four groups (10 rats each): 1- untreated control group; 2- OTA (1.7 mg/Kg, i.p.) treated group; 3- OTA (1.7 mg/Kg bw, i.p.) + GSE (75 mg/kg bw, orally) treated group; and 4- OTA (1.7 mg/Kg bw, i.p.) + GSE (150 mg/kg bw, orally) treated group. Rats were treated for 15 days and at the end of experiments, blood, liver and kidney tissue, as well bone marrow cells were harvested to determine the antioxidant capacity and ameliorative role of GSE on genotoxicity and oxidative stress induced by OTA in the liver and kidney of albino rats.

2.3. Preparation of tissue homogenate

At the end of experiment, a piece of whole liver and kidney was taken freshly from each animal on ice and homogenized by blender in 20% cold saline for determination of oxidative stress parameters: malondialdehyde (MDA) level and nitric oxide (NO) activity, and antioxidant defense system: reduced glutathione (GSH), glutathione peroxidase (GPX), and the activity of superoxide dismutase (SOD) in liver and kidney homogenates.

2.4. Antioxidants and oxidative stress parameters assay

Reduced glutathione (GSH) was determined using the spectrophotometric/microplate reader assay method which involves oxidation of GSH by the sulfhydryl reagent 5, 5′-dithio-bis (2-nitrobenzoic acid) (DTNB) to form the yellow derivative 5′-thio-2-nitrobenzoic acid (TNB), measurable at 412 nm. The activity of GSH was expressed as µg/g tissue (Rahman *et al.,* 2006).

Spectrophotometric assay of superoxide dismutase (SOD) was applied. The assay was based on the SOD-mediated inhibition in the rate of nitroblue tetrazolium reduction to the blue formazan at alkaline pH. The optimized assay of SOD was performed in 50 mM glycine-Na OH buffer, pH 9.5, at 25 degrees C. The SOD concentration was determined from the V/v ratio of rates measured in the absence (V) or the presence (v) of SOD. One unit of SOD has been defined as the concentration that decrease the rate to 50% (V/v = 2) as described by (Nagi *et al.,* 1995). The activity of GPx in the serum and aliquots of the liver and kidney was assayed using the Glutathione Peroxidase Assay Kit by Cayman Chemical Company (Ann Arbor, MI, USA) according to the kit instruction.

MDA levels in the supernatant of the homogenates were determined using a spectrophotometric assay kit according to the manufacturer's instruction. The absorbance of the resultant pink product was measured at 534 nm according to (Ohkawa *et al.,* 1979). Tissue MDA levels were calculated as nmol MDA/g tissue. NO levels were determined using a specialized kit purchased from Assay Designs, Ann Arbor, MI, USA, according to manufacturer's instructions (Jablonska *et al.,* 2007). Results were expressed as µmol/g tissue.

2.5. Cytogenetic and DNA damage analysis

2.5.1. Chromosomal analysis in somatic cells

Rats were subjected to cytogenetic analysis from bone marrow cells (Preston et al., 1983). Briefly, rats were injected with Colchicine (0.05 mg/kg bw) for two and a half hours before sacrifice. Animals were sacrificed and femoral bone marrow cells were flushed with isotonic solution (0.9% NaCl). Hypotonic solution (0.56% KCl) was added to the cell pellet and incubated at 37°C for 30 minutes and the solution was fixed, slides were air dried and stained with 10% Giemsa stain for 20 minutes. 50 metaphases were studied per animal scoring different types of structural and numerical aberrations in bone marrow cells.

2.5.2. Micronucleus assay

Bone marrow slides were prepared according to the method described by Krishna and Hyashi (2000). The bone marrow were washed with 1ml of fetal calf serum and then smeared on clean slides. The slides were left to air dry and then fixed in methanol for 5 minutes, followed by staining in 5% Giemsa stain for 5 minutes then washed in distilled water and mounted. For each animal, 1000 polychromatic

erythrocytes (PCEs) were examined for the presence of micronuclei.

2.5.3. DNA fragmentation

Liver samples were collected immediately after sacrificing the animals. The tissues were lysed in 0.5 ml lysis buffer containing 10mM tris-HCL (PH.8), 1mM EDTA, 0.2 % triton X-100, centrifuged at 10000 rpm (Eppendorf) for 20 minutes at 4°C. The pellets were re-suspended in 0.5 ml of lysis buffer to the pellets (P) and supernatants (S). 1.5 ml of 10% Trichloroacetic acid (TCA) was added and incubated at 4°C for 10 minutes. The samples were centrifuged for 20 minutes at 10000 rpm (Eppendorf) at 4°C and the pellets were suspended in 750µl of 5 % TCA, followed by incubation at 100°C for 20 minutes. Subsequently, to each sample, 2 ml of diphenylamine (DPA) solution [200 mg DPA in 10 ml glacial acetic acid, 150µl of sulfuric acid and 60µlacetaldehyde] were added and incubated at room temperature for 24 hours (Gibb *et al.,* 1997). The proportion of fragmented DNA was calculated from absorbance reading at 600 nm using the formula:

% DNA fragmentation = [OD(S)/ OD(S) + OD (P)] X 100

Where: OD(S) optical density of supernatant; OD (P) optical density of pellet.

2.6. Statistical analysis

Results were represented as mean ± SE, and the data analysis was performed using SPSS 20.0 software. Statistical analysis was carried out using one way analysis of variance (ANOVA) followed by Student-Newman-Keuls multiple comparisons test. Differences between groups were considered significant at $P \leq 0.05$.

3. Results

In this study, the ameliorative effect of GSE on geno-, hepato-, and renal toxicity induced by OTA in albino rats was estimated by measuring oxidative stress parameters (MDA and NO levels) and antioxidant activity (GSH, GPX and SOD activities) as recorded in Materials and methods section and the results are presented in Table (4). In addition, genotoxicity (chromosomal aberration analysis, DNA damage and incidence of micronucleus) in albino Wistar male rats was determined. The results presented in Table (1) and Figure (1), revealed that OTA treatment induced a significant increase in chromosomal aberration especially in gap, fragment, deletions and breaks as well total structural aberration compared to control. In addition, OTA was found to induce aneugenic aberration where it caused numerical variation (5.80±0.37, 7.40±0.60 and 13.2±0.86[d] vs. 0.40±0.24, 0.00±0.00 and 0.4±0.2[a], for hypo polyploidy, hyper polyploidy and total numerical aberration in OTA and control groups, respectively). However, GSE administration in combination with OTA, reduced significantly all types of these aberrations especially with high dose of GSE (150 mg/kg bw).

Table 1. Percentage of chromosomal aberrations in rat bone marrow cells after treatment with OTA and GSE.

Treatment	Structural aberrations							Numerical variations		
	Chromatid gap	Break	Fragments	Deletions	Endomitosis	End to end association	Total structural aberration	Hypo polyploidy	Hyper polyploidy	Total Numerical variations
Control	0.60±0.24	0.00±0.00	0.00±0.00	0.00±0.00	0.40±0.24	0.20±0.20	1.20±0.58[a]	0.40±0.24	0.00±0.00	0.4±0.2[a]
OTA	5.40±0.92	3.20 ±0.37	4.80 ±1.06	3.60 ±0.67	3.00 ±0.31	4.60 ±0.50	25.40 ±2.27[d]	5.80 ±0.37	7.40 ± 0.60	13.2 ±0.86[d]
GSE1 + OTA	3.40 ±0.50	3.20 ±0.20	3.40 ±0.40	4.80 ±0.20	2.20 ±0.37	2.80 ±0.20	19.80 ±0.48[c]	2.20 ±0.58	3.20 ± 0.58	9.8 ±0.9[c]
GSE2 + OTA	1.80 ±0.37	2.20 ±0.20	1.60 ±0.24	1.80 ±0.37	1.00 ±0.00	2.00 ±0.31	10.20 ±0.66[b]	1.40 ±0.50	2.20 ± 0.66	3.6 ±1.2[b]

Means with different small letters (a, b, c, d) are differ significantly(P≤0.05).

GSE1 + OTA= 75 mg/kg bw GSE + OTA; GSE2+ OTA = 150 mg/kg bw GSE + OTA.

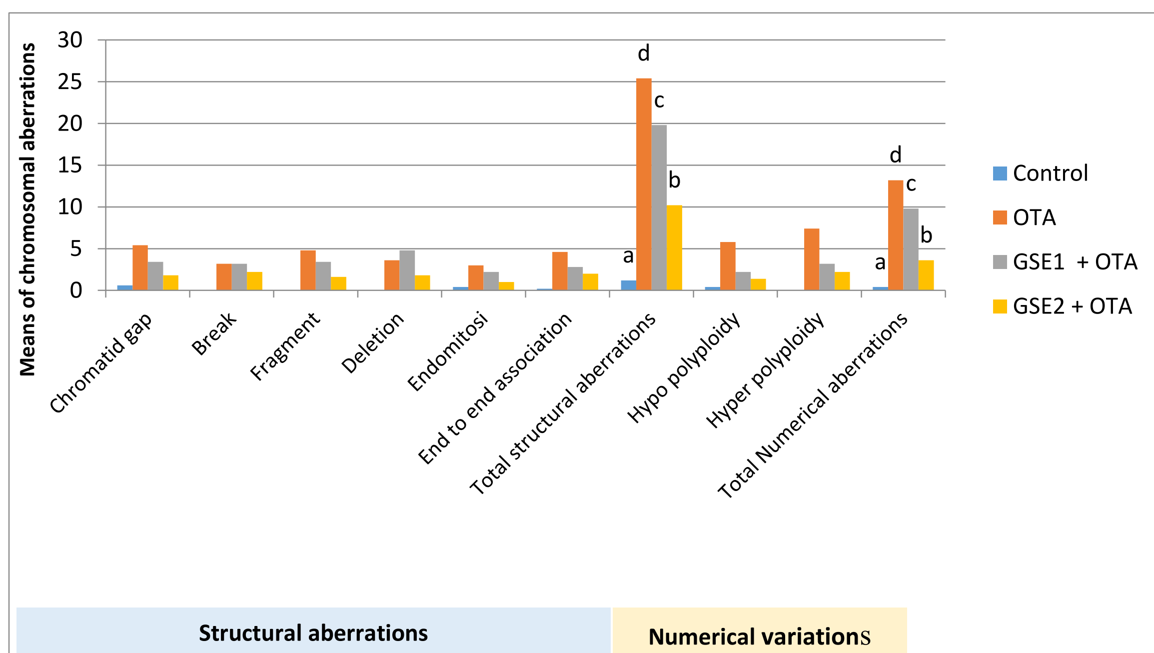

Figure 1. Comparison of chromosomal aberrations in rats after treatment with OTA and GSE.

Micronuclei (MN) frequency results (Table 2 and Figure 2) revealed that MN increased significantly in OTA group compared to control (18.20±2.48[d] and 4.80±1.78[a] for OTA and control groups, respectively). However, the treatment with GSE in combination with OTA, lowered significantly Mn frequency, especially in the treated group with high dose of GSE (150 mg/kg bw) compared to control, where it was found to be (12.40±1.51[c] and 8.0±1.41[b] and 4.80±1.78[a] for GSE1+ OTA, GSE2+ OTA and control groups; respectively).

Table 2. Effect of OTA and GSE treatment on the incidence of micronucleated polychromatic erythrocytes (MnPCE) and the relation of PCE to NCE in rats.

Treatment	PCE Screened	Mn PCE / 1000 PCE		NCE scored	PCE / NCE	
		Number	Mean ±SD		Ratio	Mean ±SD
Control	1000	3		372	2.68	
	1000	5		376	2.65	
	1000	7		505	1.98	
	1000	6		543	1.84	
	1000	3	4.80±1.78[a]	381	2.62	2.35±0.4[a]
OTA	1000	14		267	3.74	
	1000	19		134	7.46	
	1000	20		149	6.71	
	1000	18		195	5.12	
	1000	20	18.20±2.48[d]	223	4.48	5.50±1.54[d]
GSE1 + OTA	1000	13		074	13.51	
	1000	12		263	3.80	
	1000	13		124	8.06	
	1000	14		126	7.93	
	1000	10	12.40±1.51[c]	222	4.50	7.56±3.85[c]
GSE2 + OTA	1000	6		747	1.82	
	1000	8		742	1.34	
	1000	10		7.73	1.26	
	1000	8		119	8.40	
	1000	8	8.0±1.41[b]	651	1.53	2.87±3.09[b]

Means with different small letters (a, b, c, d) are differ significantly(P≤0.05)

Mn PCE = micronucleated polychromatic erythrocytes; PCE=polychromatic erythrocytes; NCE= normochromatic erythrocytes. GSE1 + OTA= 75 mg/kg bw GSE + OTA; GSE2+ OTA = 150 mg/kg bw GSE + OTA.

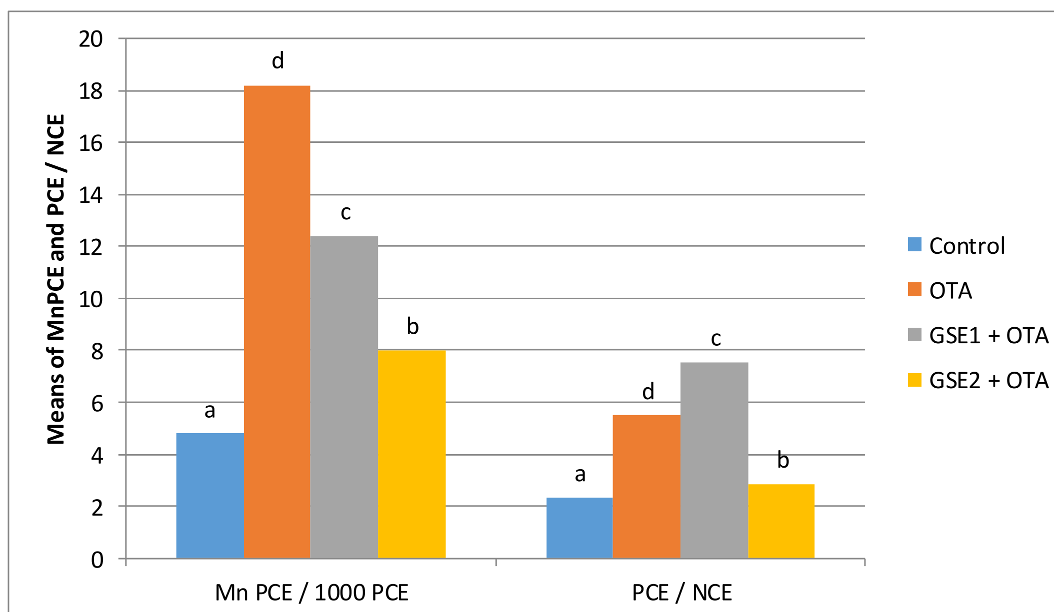

Figure 2. Incidence of micronucleated polychromatic erythrocytes (MnPCE) and the relation of PCE to NCE in rats treated with OTA and GSE.

DNA Fragmentation results (Table 3) revealed that DNA fragmentation frequency was increased significantly in OTA group compared to control (29.69 ± 0.48^{d} and 7.25 ± 0.60^{a} for OTA and control groups, respectively). However, the treatment with GSE in combination with OTA, lowered significantly DNA fragmentation frequency especially in the treated group with high dose of GSE (150 mg/kg bw) compared to OTA treated group (29.69 ± 0.48^{d}, 19.90 ± 0. 68^{c} and 10.86 ± 0.42^{b} for OTA, GSE1 and GSE2, respectively).

Table 3. Effect of OTA and GSE treatment on the incidence of DNA fragmentation in rats.

GSE1 + OTA= 75 mg/kg bw GSE + OTA; GSE2+ OTA = 150 mg/kg bw GSE + OTA

Treatment	% of DNA Fragmentation
	Mean ± SE
Control	7.25 ± 0.60^{a}
OTA	29.69 ± 0.48^{d}
GSE1 + OTA	19.90 ± 0.68^{c}
GSE2 + OTA	10.86 ± 0.42^{b}

Means with different small letters (a, b, c, d) are differ significantly(P≤0.05).

Table (4) and Figure (3), showing that administration of OTA reveals a significant decrease (P ≤ 0.05) in GSH enzyme activity (µg/ g tissue) compared to control group in both liver and kidney tissues (3.24± 0.051, 1.18 ±0.03 vs. 12.78±0.076; 4.85±0.02, for OTA and control group in both liver and kidney, respectively). However, Oral administration of GSE in combination with OTA, increased significantly (P ≤ 0.05) GSH level, especially in the treated group with high dose of GSE (150 mg/kg bw) in both liver and kidney tissue compared to OTA group (8.8±0.077, 2.51±0.02, 10.7±0.09, 4.00±0.02, and 3.24± 0.051, 1.18 ±0.03, for GSE1+OTA, GSE2+OTA and OTA groups in liver and kidney tissues, respectively).

Similarly, GPX concentration (mg/mg tissue) decreased significantly in OTA group compared to control group in both liver and kidney tissues (183.20±2.19, 150.50±0.91, and 269.40± 1.60, 232.12±1.55, for OTA and control groups in liver and kidney, respectively). However, Oral administration of GPX in combination with OTA, increased significantly (P ≤ 0.05) GPX level, especially in the treated group with high dose of GSE (150 mg/kg b w) in both liver and kidney tissues compared to OTA group (183.20±2.19, 150.50±0.91, 275.50±1.92, 221.30±1.52, 289.40±1.67, 249.80±1.17, for OTA, GSE1 and GSE2 groups in liver and kidney tissues, respectively).

There was also a significant inhibition in SOD activity in OTA group in both liver and kidney tissues (24.70± 1.10, 20.62±1.90 and 64.30± 1.94, 61.20± 1.55, respectively) compared to the control group. But, co-treatment with both doses of GSE (75 and 150 mg/kg bw.) leading to a significant increase in SOD level in both kidney and liver tissue compared to OTA group (41.80±1.22, 53.80±1.03, 58.30±1.62, 64.70±1.88, and 24.70±1.10, 20.62±1.90, for GSE1, GSE2 and OTA groups in liver and kidney tissues, respectively).

OTA significantly increased (P ≤ 0.05) MDA in both liver and kidney tissues (809.00±2.18, 973. 20±2.74, vs 250.30±2.13, 250.30±2.75, respectively) compared to control group. On the other oral administration with the 2 doses of GSE (75 and 150 mg/kg bw) groups in combination to OTA, a significant decrease in MDA in both kidney and liver tissues (481.70±1.95, 463.80± 2.42, and 332. 60± 1.78, 302.70±2.71 vs. 809.00±2.18. 973. 20±2.74, respectively) compared to OTA group was accomplished.

The results of NO show that OTA significantly increased (P ≤ 0.05) NO in both liver and kidney tissue (973.20± 2.74 and 114.70±2.15 vs. 250.30± 2.75 and 59.30±1.21, respectively) compared to control group. However, on the other groups treated with the 2 doses of GSE (75 and 150 mg/kg bw) in combination to OTA, a significant decrease in NO in both kidney and liver tissues

(463.80±2.42, 66.40±1.26 and 302.70±2.71, 50.30±1.19 vs. 973.20±2.74 and 114.70±2.15, respectively) compared to OTA group was revealed.

Table 4. Effect of Grape seed extract (75 and 150 mg/Kg bw GSE, POs) in concomitant with Ochratoxin A (OTA) (1.7 mg/Kg, i.p) on oxidative stress and antioxidant parameters in liver and kidney homogenates.

Parameters	GSH –Liver µg/ g tissue	GSH Kidney µg/ g tissue	GPX Liver mg/ mg tissue	GPX Kidney mg/ mg tissue	SOD Liver nmol/g tissue	SOD Kidney nmol/g tissue	MDA Liver nmol/g tissue	MDA Kidney nmol/g tissue	NO Liver µmol/g tissue	NO Kidney µmol/g tissue
Control	12,78±0,076^{a}	4,85±0,02^{a}	269,40±1,60^{a}	232,12±1,55^{a}	64,30±1,94^{a}	61,20±1,55^{a}	250,30±2,13^{a}	250,30±2,75^{a}	250,30±2,75^{a}	59,30±1,21^{a}
OTA	3,24±0,051^{b}	1,18±0,03^{b}	183,20±2,19^{b}	150,50±0,91^{b}	24,70±1,10^{b}	20,62±1,90^{b}	809,00±2,18^{b}	973,20±2,74^{b}	973,20±2,74^{b}	114,70±2,15^{b}
GSE1+OTA	8,8±0,077^{c}	2,51±0,02^{c}	275,50±1,92^{a}	221,30±1,52^{c}	41,80±1,22^{c}	53,80±1,03^{c}	481,70±1,95^{c}	463,80±2,42^{c}	463,80±2,42^{c}	66,40±1,26^{c}
GSE2+OTA	10,7±0,09^{d}	4,00±0,02^{d}	289,40±1,67^{d}	249,80±1,17^{d}	58,30±1,62^{d}	64,70±1,88^{a}	332,60±1,78^{d}	302,70±2,71^{d}	302,70±2,71^{d}	50,30±1,19^{d}

Results are expressed as mean ± SEM (n=8). Different capital letters are differ significantly at $p < 0.05$.GSE1 + OTA= 75 mg/kg bw GSE + OTA; GSE2+ OTA = 150 mg/kg bw GSE + OTA.

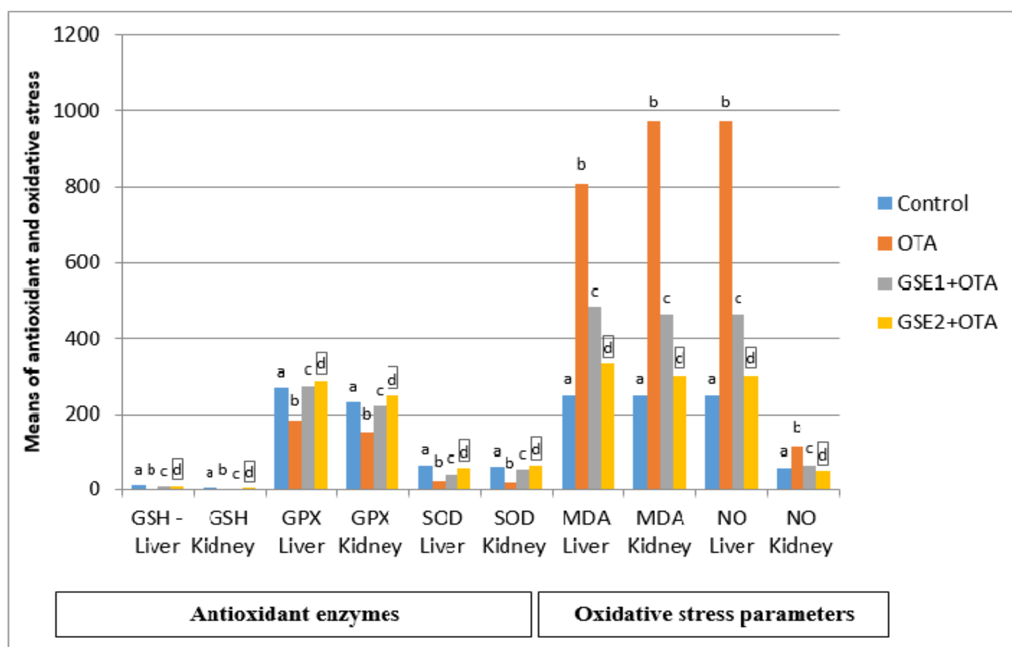

Figure 3. Effect of Grape seed extract (75 and 150 mg/Kg bw GSE) in concomitant with Ochratoxin A (OTA) on oxidative stress and antioxidant parameters in liver and kidney

4. Discussion

Induction of oxidative stress by OTA has a critical role in several kidney and liver toxicity, and many complications of these toxicities such as DNA and genetic damage are mediated by this oxidative stress (Ozbek, 2012; Gong *et al.,* 2019). However, the protective role of GSE on OTA-induced liver and kidney injury has not been reported. Therefore, this study was designed to investigate the antioxidant capacity and ameliorate role of GSE as antioxidant on ochratoxin A-induced genotoxicity and oxidative stress in adult albino male rats.

Oxidative stress and generation of reactive oxygen species (ROS), consequently induce genetic damage in rats, due to the formation of DNA-adducts (damage) as a result of ROS generation (Kamp *et al.,* 2005). OTA was reported to induce MN, hypopolyploidy, single and double strand breaks (SSBs and DSBs), gene mutations, deletions, chromosomal aberration and DNA damage leading to genotoxicity (Mally *et al*., 2005; Ali *et al*., 2011; Hibe *et al*., 2013; Schrenk *et al*., 2020). However, treatment with GSE antioxidant increased cell viability and decreased ROS level after OTA exposure (Schaaf *et al*., 2002), and this coincides with our results as presented in tables (1-3).

Our findings prove that GSE has improved all types of the chromosomal aberrations (structural and numerical) compared to OTA treated group, suggesting its ameliorative the genotoxic effect of OTA. Similar findings were obtained by Ramyaa *et al*. (2014); Costa *et al*. (2016) and Damiano *et al.* (2020), who proved that this extract possessed antimutagenic properties. In agreement with our results, Sorrenti *et al.* (2013) reported that GSE significantly ameliorated the DNA damage induced by OTA, as revealed by lowering of the occurrence of chromosomal aberrations, micronucleus and hepatic DNA fragmentation.

Glutathione in the reduced form (GSH), with GPx, and GR together bring about an important antioxidant defense system (Lu, 2013). In the present study, GSE was found to increase the GSH values significantly in liver and kidney tissues in both GSE+OTA treated groups especially with the high dose of GSE (150 mg/kg bw) compared to OTA treated group ($p<0.05$). In consistent to our results, GSE

supplementation may activate antioxidant enzyme GSH in the liver and kidney, indicating that GSE attenuated toxicity-induced oxidative damage in liver and kidney of the rats (Nagi *et al.,* 1995; Kıran *et al*., 2023).

In the same line as our results, Chis *et al.* (2009) found that GSE as an excellent antioxidant, can enhance the activities of various antioxidant enzymes, play an antioxidant role and increased antioxidant enzymes (GSH, SOD and GPx) levels due to polyphenolic flavonoid content. GPx is an important enzyme in the antioxidant system that works with GSH and SOD to prevent the formation of lipid peroxidation (Meki and Hussein, 2001). In our study, GPx and SOD levels were significantly high in liver and kidney tissues of both GSE+OTA treated groups compared to OTA treated group, and that clearly indicates the enhancement role of antioxidant enzyme activity for GSE in rats, matching those of (Taghizadeh *et al*., 2016; Zhang *et al.,* 2022).

Lipid peroxidation is an oxidative stress indicator resulting from MDA. The MDA level in both kidney and liver of OTA treated group was highly significant compared to control. However, GSE treatment reduced significantly MDA level especially with the 2nd dose of GSE. That is in the same line with Meki and Hussein, (2001), who found that OTA induced oxidative stress in rat liver and kidney by increasing lipid peroxidation (MDA level), and suppressing GPx, GSH and SOD. At the same time, treatment with GSE as antioxidant, can ameliorate the oxidative stress status of OTA toxicity by prevention of free radicals formation or at least by reducing their levels, and that coincides with the findings of Kuyumcu and Aycan, (2018). This indicates that the protective role of GSE on rat kidney injury might be related to prevention of lipid peroxidation.

It is important to say that OTA also increases the reactive nitrogen species (RNS). OTA increases nitric oxide (NO) production, inducible nitric oxide synthase (iNOS) expression, and protein nitration eventually leads to nitrosative stress (overproduction of nitric oxide) in kidney and liver cells (Cavin *et al.,* 2009; Sorrenti *et al*., 2013; Crupi *et al.,* 2020). So, oxidative and nitrosative stress are related to OTA-induced DNA damage and provide additional evidence for their role in this mechanism of toxicity (Tao *et al*., 2018). This is in the same line with our findings. However, GSE supplementation improved the NO levels and restored it nearly to the normal control.

5. Conclusion

In conclusion, we confirm that OTA could increase oxidation products (MDA and NO), genotoxiciy, reduce liver and kidney antioxidant capacity, and induce their injury. GSE displayed an obvious protective effect on OTA induced-genotoxicity, oxidative stress through antioxidant capacity. The beneficial effects of GSE on the toxicity induced by OTA have been proved for the first time in our study. The supposed mechanism of actions includes decrease in genotoxicity, oxidative stress, lipid peroxidation, and enhancement of antioxidant parameters such as GSH, GP-x and SOD. Thus, clinical application of GSE as therapy should be considered to prevent and treat oxidative damage in cases of ochratoxicosis.

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