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Protective role of grape seed extract on genotoxicity, hepatic, and renal dysfunction induced by ochratoxin A in rats

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Abstract

Ochratoxin A (OTA) is a natural toxin and produced by various fungi of the genus Aspergillus and Penicillium and the second one in toxicity among mycotoxins. Recent studies have shown that grape seed extract (GSE) has a protective effect on mycotoxin-induced toxicity. The aim of this study was to investigate the protective effect GSE on OTA-induced genotoxicity, liver and kidney injury in rats. Forty mature Wistar albino male rats with similar body weight were divided into four groups (10 rats each): 1- untreated control group, 2- OTA treated group (1.7 mg/Kg bw, i. p.), 3- OTA + 75 mg/kg bw GSE treated group, 4- OTA + 150 mg/kg bw GSE treated group. Rats were treated for 15 days and at the end of experiments, blood, liver and kidney tissue homogenates, as well spermatocyte cells were harvested to determine the effect of OTA on geno-, hepato-, and renal toxicity in rats and the protective role of GSE. The results showed that GSE could significantly alleviate genotoxicity, DNA damage and improve the liver and kidney injury induced by OTA toxicity in male albino rats due to its antioxidant and anti-inflammatory activity.

Keywords: Ochratoxin A (OTA); Grape seed extract (GSE); Protective; genetic; Hepatic; Renal; Toxicity; Rats

1. Introduction

Ochratoxin A (OTA) is the most common and contaminated mycotoxin, second only to aflatoxin in toxicity (Wang *et al.,* 2016). Due to the stable chemical properties of OTA, it can withstand the physical and chemical conditions of modern food processing without degradation (Escriva *et al*., 2017), and the decomposition and elimination rate of OTA is very slow. It is also difficult to be excreted with metabolism, thus accumulating in animal tissues and organs and endangering health (Manderville and Wetmore, 2017; Park *et al*., 2019). Therefore, OTA will not cause acute diseases in terms of clinical manifestations. Instead, chronic poisoning will be caused as animals and humans consume a small amount of food contaminated by OTA for a long time, showing hepatotoxicity, genotoxicity, renal toxicity, teratogenicity and carcinogenicity (Heussner and Bingle, 2015).

OTA has nephrotoxic deleterious effect on human health due to exposure to OTA-contaminated food (Fuchs and Peraica, 2005). Previous studies have confirmed that exposure to OTA can lead to multiorgan damage (Gagliano *et al*., 2006; Patil *et al.,* 2006; Sheu *et al*., 2017) such as the kidney (Raghubeer *et al.,* 2019) , liver (Shin *et al.,* 2019), spleen (Gan *et al.*, 2017), intestine (El Cafsi *et al*., 2020), lung (Xu *et al,.* 2019) and brain (Park *et al.*, 2019), but the kidney is the primary target of OTA (Pfohl-Leszkowicz and Manderville, 2007). The mechanisms of OTA-induced nephrotoxicity include

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inhibition of protein synthesis, DNA damage, renal dysfunction, inflammatory response and renal tumor formation (Özcan *et al.,* 2015; Gong *et al.,* 2019).

OTA was found to induce DNA fragmentation and chromosome aberrations in rat and mice kidney and liver (Mally *et al.,* 2005; Creppy *et al.,* 1985). The ability of OTA to cause DNA damage in kidney cells was studied (Arbillaga *et al*., 2007) using the alkaline comet assay in the human renal epithelial cell line (HK-2) and found that OTA is not acting as a direct genotoxic and cytotoxic but due to oxidative stress which is implicated in the genotoxicity and cytotoxicity observed in renal cells, as well as in rat liver and kidney (Tozlovanu *et al.,* 2012). A highly significant elevation in total serum ALT and AST in OTA treated mice compared with control group leading to hepatic damage **(**Jeswal 1998; Alghamdy *et al.,* 2013), and a high significant level of uric acid causing renal damage was reported (Li, 2020).

Due to its widespread threat to human health, the detoxification of OTA has been of major interest. The addition of nutrients or additives with protective properties to contaminated foodstuffs have been developed to reduce and/or eliminate the toxic effects of contaminated products, and improve food safety (Gholampour Azizi *et al.*, 2012). Reducing genotoxicity, ROS production, activation of the Nrf2 pathway, a key factor in regulating inflammatory response (decrease the levels of expression of proinflammatory cytokines such as IL-1 β, TNF- α), and maintaining DNA stability are several ways used to prevent OTA toxicity (Liu *et al*., 2017; Khoi *et al.,* 2021). So, some studies demonstrated that antioxidants are able to counteract the deleterious effects of chronic consumption of OTA and confirmed the potential effectiveness of dietary strategies to counteract OTA toxicity (Sorrenti *et al.,* 2013).

Antioxidants are reported to protect cells from OTA-induced cytotoxicity and genotoxicity (Costa *et al.,* 2016). One of those is GSE; it is a natural extract from the seeds of *Vitis vinifera*. A multitude of flavonoids are contained in GSE and has a free radical scavenging capacity with potent antioxidant and anti-inflammatory effect (Nada *et al*., 2014). Several studies have also established that GSE had beneficial and protective effects against many toxic elements in different organs such as testis (Hajizadeh *et al.,* 2016), kidney (Çetin *et al.,* 2006), and liver (Baleni *et al.,* 2015). Therefore, this study is designed to investigate whether GSE could protect kidney and liver tissues from OTA-induced genetic, liver and kidney damage and to determine the protective effect of GSE as an antioxidant and anti-inflammatory against OTA toxicity in male albino Wistar rats.

2. Material and methods

2.1. Animals

Adult albino male rats of Wistar strain weighing 140-150 g were obtained from animal house colony of National Research Centre. They were kept under the hygienic conditions and fed with diet and water *ad libitum*. They were allowed to acclimate for three weeks before the experiment. 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 design

Forty mature Wistar albino male rats with similar body weight were divided into four groups (10 rats each): group 1 untreated control, group 2 received OTA (1.7 mg/Kg b w, i.p.), group 3received OTA (1.7 mg/Kg b w, i.p.) + GSE (75 mg/kg bw, orally), and group 4 received OTA (1.7 mg/Kg b w, i.p.) + GSE (150 mg/kg bw, orally). Rats were treated for 15 days and at the end of experiments, blood, liver and kidney tissue homogenate, as well spermatocyte cells were prepared to determine the effect of OTA on geno-, hepato-, and renal toxicity in rats and the protective role of GSE**.**

2.3. Liver and kidney function test:

Whole blood was centrifuged twice at 3000 rpm for 10 min in order to separate serum. Using a biochemical autoanalyzer (Type 7170, Hitachi), serum biochemical analysis was carried out. To evaluate the liver function, the levels of aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), alanine aminotransaminase (ALT) were measured; while to evaluate the kidney function, the level of uric acid was measured.

2.4. Estimation of serum inflammatory mediators

The serum inflammatory mediators, such as IL-1 β, TNF- α, and PGE_2 levels of the rats were estimated using the sandwich enzyme-linked immunosorbent assay (ELISA) kit according to supplier's instructions (supplied by WKEA Med Supplies Corp., Changchun, China).

2.5. Cytogenetic and DNA damage analysis

2.5.1. Chromosomal analysis in germ cells

Spermatocyte cells were prepared according to Brewen and Preston (1987). Chromosomes were spread on clean glass slides by the gradual fixation/air-drying method. The preparations were stained with 20% Giemsa (Merck, Darmstadt, Germany) in PBS (pH 6.8) for 10 min for conventional chromosome analysis. Aberrations are scored in metaphase chromosomes of dividing cells. Fifty metaphase spreads per animal were analyzed for studying the structural and numerical chromosomal aberrations.

2.5.2. Molecular analyses

Comet Assay

Isolated blood cells of all groups of male rats were subjected to the modified single-cell gel electrophoresis or comet assay (Fairbairn et al., 1995). To obtain the cells, the pellet of blood cells was washed with an excess of ice-cold Hank's balanced salt solution (HBSS) and minced quickly into approximately 1 mm3 pieces while immersed in HBSS, with a pair of stainless steel scissors. After several washings with cold phosphate-buffered saline (to remove red blood cells), the blood cells were dispersed into single cells using a pipette. In brief, the protocol for electrophoresis involved embedding of the isolated cells in agarose gel on microscopic slides and lysing them with detergent at high salt concentrations overnight (in the cold). The cells were treated with alkali for 20 min to denature the DNA and electrophoresis under alkaline conditions (30 min) at 300 mA, 25 V. The slides were stained with ethidium bromide and examined using a fluorescence microscope (Olympus BX60 F-3) with a green filter at × 40 magnifications. For each experimental condition, about 100 cells (about25 cells/animal) were examined to determine the percentage of cells with DNA damage that appear like comets. The non-overlapping cells were randomly selected and were visually assigned a score on an arbitrary scale of 0–3 (i.e., class 0 = no detectable DNA damage and no tail; class 1 = tail with a length less than the diameter of the nucleus; class 2 = tail with length between 1× and 2× the nuclear diameter; and class 3 = tail longer than 2× the diameter of the nucleus) based on perceived comet tail length migration and relative proportion of DNA in the nucleus (Collins et al., 1997). A total damage score for each slide was derived by multiplying the number of cells assigned to each class of damage by the numeric value of the class and summing up the values. Slides were analyzed by one observer to minimize the scoring variability.

2.6. Statistical analysis

Data were expressed 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) according to Snedecor & Cochran (1980) followed by Student-Newman- Keuls multiple comparisons test. Differences between groups were considered significant at $P \leq 0.05$.

3. Results

This study was designed to investigate the protective effect of GSE on genetic, hepatic and renal toxicity induced by OTA in male rats. These parameters were estimated by measuring Liver and kidney function, estimation of TNF-α, IL-1β and PGE_2 and cytogenetic and DNA damage analysis using chromosomal analysis in germ cells and comet assay. The results in table (1) show that OTA treatment induce a significant increase in structural chromosomal aberrations frequency in spermatocyte cells represented as autosomal and x-y univalents, rings and chains compared to control, however, co-treatment with GSE decreased significantly these parameters (18.8^{d} vs. 11.2^{c} and 3.2^{b} , for OTA vs. OTA+ GSE1 and OTA+GSE 2, respectively). The same results were found concerning the numerical variations, where OTA treatment induce a significant increase in total numerical chromosomal variation (hypo polyploidy and hyper polyploidy) in germ cells compared to control, whereas, co-treatment with GSE decreased significantly these parameters (12.0^{d} vs7.0^{c} 3.6^{b}, for OTA vs. OTA+ GSE1 and OTA+GSE 2, respectively).

Table 1 Mean percentage of chromosomal aberrations in rat spermatocytes treated with OTA and GSE

Treatment	No. of examined cells	Structural aberrations								Total structural aberrations		Numerical variation				Total numerical variation	
		Autosomal univalent		x-y, univalent		Ring		Chain				Hypo polyploidy		Hyper polyploidy			
		No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%
Control	250	1	0.4	0	0.0	1	0.4	1	0.4	3	1.2[a]	0	0.0	1	0.4	1	0.4[a]
OTA	250	13	5.2	11	4.4	12	4.8	11	4.4	47	18.8[d]	16	6.4	15	6	31	12.0[d]
OTA+ GSE1	250	7	2.8	5	2	7	2.8	9	3.6	28	11.2[c]	11	4.4	8	3.2	19	7.0[c]
OTA+GSE 2	250	3	1.2	2	0.8	2	0.8	1	0.4	8	3.2[b]	4	1.6	5	2	9	3.6[b]

Significant at P ≤ 0.05. GSE1= 75 mg/kg bw GSE; GSE2 = 150 mg/kg bw GSE. The different small letters are significantly different at p ≤ 0.05.

Compared with the control group, significantly higher level ($p < 0.01$) of DNA damaged cells was detected in OTA group (4.6[a] and 23.8[d] for control and OTA group, respectively) (Table 2), whereas the treatment with GSE in combination to OTA decreased significantly ($p < 0.01$) DNA damage especially with OTA + GSE2 group (23. 8[d], 17.2[c] and 11.4[b] for OTA vs. OTA + GSE1 and OTA + GSE2 groups, respectively).

Table 2 Visual score of DNA damage in control and treated rats with OTA and GSE using comet assay

Treatment	Number of animals	No. of cells		Class of comets				DNA damaged cells (%)
		Analysed	Total comets	0	1	2	3	
Control	5	500	23	477	9	8	6	4.6[a]
OTA	5	500	119	381	44	39	36	23.8[d]
OTA+GSE1	5	500	86	414	33	29	24	17.2[c]
OTA+GSE2	5	500	57	443	24	21	12	11.4[b]

Number of cells examined per a group (500). Class 0 = no tail, class 1 = tail length < diameter of nucleus, class 2= tail length between 1X and 2X the diameter of nucleus, and class 3 = tail length > 2X the diameter of nuclear. The different small letters are significantly different at p ≤ 0.05.

3.1. Biochemical Analysis

In order to explore the changes in rat liver function in each group, we measured the activities of ALT, AST and GGT in rat plasma, which are the key indexes to reflect the changes in liver function. After OTA administration, we obtained the results shown in Table 3 and Figure 3. Compared with the control group, the activities of serum ALT, AST and GGT in the OTA group were significantly increased by 2 fold for ALT and AST and 3 fold for GGT ($p \leq 0.05$), indicating severe impairment of liver function. Compared with the OTA group, the activities of AST, ALT and GGT in serum were decreased significantly after GSE treatment especially with GSE2 (OTA + 150 mg/kg GSE group), which had the most significant effect, and the activities of ALT, AST and GGT were reduced by about 50 % ($p \leq 0.05$).

To explore the changes in rat kidney function in each group, we measured the level of uric acid, which reflect the changes in kidney function. Table 3 and Fig. 3, show that, the activities of serum uric acid in the OTA group were significantly increased by 4 fold compared with the control group ($p \leq 0.05$), (5.23± 0.01 and 1.33± 0.02, for OTA vs. control, respectively), indicating severe impairment of kidney function. However, addition of GSE in combination to OTA, decreased significantly ($p \leq 0.05$) this level compared to OTA group and restore it to normal control especially with GSE2 (OTA + 150 mg/kg GSE group) (5.23± 0,01; 2.31 ±0.01 and 1.88± 0.02, for OTA, OTA+ GSE1 and OTA+ GSE2, respectively).

3.2. GSE Improves Rat Hepatic Inflammation Induced by OTA

After OTA treatment, TNF- α, IL-1 β and PGE2 levels (Table 3) were significantly increased ($p \leq 0.05$) compared with those in the control group (110.02 ±1.44, 21.70 ±0.88 and 89.50 ±1.48; and 52.10 ±1.32, 2.50±0.05 and 65.20 ± 1.13 for TNF- α, IL-1β and PGE2, in OTA and control, respectively). While GSE addition in combination to OTA, decreased significantly ($p \leq 0.05$) these levels compared to OTA group and restore most of them to normal control especially in the OTA+GSE2 group (110.02 ±1.44, 21.70 ±0.88 and 89.50 ±1.48; and 58.20 ±1.24, 2.67 ±0.06 and 70.40 ±1.51; and 50.40 ±1.85, 2.10 ±0.03; and 63.20 ±1.63, for TNF- α, IL-1β and PGE2, in OTA, OTA+GSE1 and OTA+GSE2, respectively).

Table 3 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 liver and kidney functions, TNF- α, IL-1 β, and PGE 2 levels in rat

Parameters	Control	OTA	OTA+GSE1	OTA+GSE2
ALT (IU/L)	27.40 ± 0.68^{a}	53.60 ± 0.98^{d}	$37.60^{bc} \pm 1.13$	29.50 ± 1.03^{a}
AST (IU/L)	45.20 ± 1.22^{a}	98.40 ± 1.73^{d}	63.90 ± 1.39^{c}	51.63 ± 1.69^{b}
GGT (IU/L)	1.24 ± 0.07^{a}	4.20 ± 0.03^{d}	3.21 ± 0.02^{c}	2.19 ± 0.04^{b}
Uric acid (mg/dl)	1.33 ± 0.02^{a}	5.23 ± 0.01^{d}	2.31 ± 0.01^{c}	1.88 ± 0.02^{b}
TNF-α (ng/L)	52.10 ± 1.32^{a}	110.02 ± 1.44^{d}	58.20 ± 1.24^{bc}	50.40 ± 1.85^{a}
IL-1 β (Pg/ml)	2.50 ± 0.05^{a}	21.70 ± 0.88^{b}	2.67 ± 0.06^{a}	2.10 ± 0.03^{a}
PGE2(ng/L)	65.20 ± 1.13^{a}	89.50 ± 1.48^{d}	70.40 ± 1.51^{bc}	63.20 ± 1.63^{a}

Results are expressed as mean ± SEM (n=8); The different small letters are significantly different at $p \leq 0.05$ using analysis of variance; ALT= alanine transaminase; AST= aspartate aminotransferase, GGT= gamma-glutamyl transferase.; IL-1 β = interleukin 1 beta; TNF- α = tumor necrosis factor alpha; PGE2 =prostaglandin E2.

4. Discussion

OTA mainly damages the kidney and liver of animals, and the kidney is the first target organ. Results from previous studies suggest that accumulation of OTA at lower doses can cause kidney damage. Additionally, the protective effect of GSE in the diet on animals was confirmed (Çetin *et al.,* 2006; Baleni *et al.*, 2015 and Hajizadeh *et al.,* 2016). However, to our knowledge the protective effect of GSE on OTA-induced liver and kidney injury has not been reported. So, the purpose of this study was to examine the protective role of 2 doses of GSE on OTA-induced DNA damage, genetoxicity, liver and kidney function in albino male rats.

The results show that OTA treatment induce a significant increase in both structural and numerical chromosomal aberrations frequency in germ cells, and DNA damage compared to control. That coincide with those of Creppy *et al.,* 1985; Mally *et al.,* 2005; Tozlovanu *et al.,* 2012; Heussner and Bingle, 2015, who attributed this genotoxic effect to oxidative stress which is implicated in the genotoxicity and DNA damage observed in the treated rats. The consistent association of oxidative DNA damage, measured with the Comet assay, with OTA exposure suggests its involvement in producing OTA-induced clastogenicity and aneugenicity (Ali et al., 2011). However, co-treatment with GSE decreased significantly these parameters, due to its ability to reducing, genotoxicity via its antigenotoxic substances, ROS production, and maintaining DNA stability, the several ways to counteract OTA toxicity as an antioxidant (Sorrenti *et al.,* 2013; Costa *et al.,* 2016; Khoi *et al.,* 2021).

The liver, as a detoxifying organ, is one of the target organs of OTA toxicity. Changes in serum ALT, AST and ALP levels are usually used as important indicators to judge whether liver function is normal (Hu *et al.,* 2014). Previous studies have shown that OTA exposure can increase serum ALT, AST and ALP levels in rats, broilers and other animals (Zhang *et al.,* 2022). This is in consistent with our results. In this study, the enzyme activities of AST, ALT and ALP, which are important indexes of liver function, were significantly increased in the serum of the OTA treatment group, suggesting that liver cells were damaged. The reason may be that OTA plays a toxic role in liver cell necrosis. Studies have shown that 1% liver cell necrosis can double the activity of ALT enzyme in the blood and significantly increase the levels of AST and ALP (Sherman, 1991). When cells are damaged, excessive release of diseased liver cells will increase the levels of ALT, AST and ALP in the blood (Li *et al.,* 2021; Poupon, 2015).

Biomarkers in the blood are often used to detect kidney and liver damage. Uric acid (UA) level in serum are indicators of kidney health, and elevated levels of UA indicate impaired renal function (Zhang *et al.,* 2016). Our results showed that

after OTA exposure, serum UA level increased significantly. These results coincide with Li *et al.* (2020). However, after treatment with both GSE and OTA, the levels of the above-mentioned biomarkers were restored towards control levels. This indicate that GSE could effectively alleviate the renal damage caused by OTA.

Inflammation is an important indicator of liver injury caused by toxic physical or chemical stimuli. Inflammation plays an important role in the repair of liver injury (Hou *et al.,* 2018). TNF-α is the first inflammatory factor produced in the inflammatory response, which can induce the production of other cytokines, affect NF-κB, a key factor in regulating inflammatory response, and promote the production of free radicals, magnifying the inflammatory response and aggravating liver injury (Schwabe and Brenner, 2006). During liver injury, inflammatory cells are activated, and inflammatory cytokines (TNF-A, IL-1β and IL-6) are released, which are responsible for the accumulation of neutrophils in the liver, ultimately leading to increased cytokine expression (Olteanu *et al.,* 2012). Studies have shown that mycotoxins, including OTA, can promote the production of pro-inflammatory factor TNF-α in rats (Xu *et al.,* 2019), and mild OTA poisoning can lead to a persistent systemic inflammatory response (Bernardini *et al.,* 2014). Since GSE was found to significantly reduce OTA-induced oxidative stress, we examined the levels of three pro-inflammatory cytokines, TNF-α, IL-1β and PGE2 to further explore the protective mechanism of GSE. The results showed that different doses of GSE decreased the levels of TNF-α, IL-1β and PGE2, suggesting that GSE alleviates OTA-induced liver injury by inhibiting the inflammatory response. This may be due to its anti- inflammatory effect through inhibition of inflammatory cytokines (Liu *et al.,* 2017; Eid *et al.,* 2021). In this study, we found that adding GSE reduced, OTA-induced liver and kidney damage, genotoxicity by reducing oxidative stress and inflammatory responses.

5. Conclusion

GSE has an obvious protective effect on OTA induced genotoxicity, liver and renal inflammation and cause their injury in rats. In this study the protective effects of GSE against OTA as natural toxin which somehow is associated with genotoxicity, liver and renal injury in rats. The beneficial effects of GSE on the toxicity induced by natural toxins including OTA have been confirmed. The proposed mechanism of actions include decrease in genotoxicity, reduction in inflammatory factors (TNF-α, IL1B and PGE2) and improvement of biochemical parameters for liver and kidney function (ALT, AST, GGT, uric acid, etc.). This study provides evidence for GSE to significantly attenuate genotoxicity, DNA damage and improve the liver and kidney injury induced by OTA toxicity in male albino rats due to its antioxidant and anti-inflammatory activities and confirmed the potential effectiveness of dietary strategies to counteract its toxicity.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest.

Statement of ethical approval

The study was approved and authorized by the ethics committee of the Institutional Animal Care and Use Committee (IACUC), approval no: 01122022587.

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