Research Article

SOHAG JOURNAL OF SCIENCES

https://doi.org/10.21608/sjsci.2023.226367.1106

Nephrotoxicity of Patulin and its Modulation by Aqueous Green Tea Extract in Male Albino Rats

Nagwa Elsawi[1], Hisham Ismail[2], Ahmed Sayed[3], Soad Shaker Ali[4] and Ahmed G.Khamis[1,*]

[1] *Chemistry Department, Faculty of Science, Sohag University, Sohag 82524, Egypt.*
[2] *Chemistry Department, Faculty of Science, Minia University, Minia 61519, Egypt.*
[3] *Chemistry Department, Faculty of Science, Assiut University, Assiut 98467, Egypt.*
[4] *Histology, Faculty of Medicine, Assiut University, Assiut 98467, Egypt.*
[*] *E-mail: ahmed.khamis@science.sohag.edu.eg*

Received: 1st August 2023, **Revised:** 28th September 2023, **Accepted:** 2nd October 2023.
Published online: 7th November 2023

Abstract: Patulin (PAT) is a major secondary metabolic mycotoxin produced by *Penicillium* that can contaminate food. Patulin forms an adduct when it interacts with amino acids that contain thiol groups; the creation of these adducts could represent the main toxic pathway of PAT. Catechins are a major component of green tea polyphenols. Polyphenols have many protective effects against chronic diseases. In this study, 60 rats were randomly divided into five groups, with 12 rats in each group. Group I rats were given 0.1% DMSO in saline 2 mL/kg BW for 7 days; Group II rats received 50 mg/kg BW of GTE for 7 days; Group III, IV, and V rats received 0.2 mg/kg BW of patulin for 14 days; then Group IV and V rats received 50 mg/kg and 100 mg/kg BW of GTE for 7 days; respectively. We evaluated the nephrotoxicity induced by patulin within the medulla and cortex and the enhancement role of GTE on both renal histological and physiological parameters. Urea, BUN, and creatinine levels showed a high significance increase (p<0.001) in the patulin group, while GTE-treated groups showed a high significance decrease (p<0.001). PAT-induced harmful effects on kidney tissue are portrayed in the structure of the cortex and medulla. The cortex showed improvement in renal corpuscles, while the medulla showed highly dilated lumina in most tubules with luminal deposits. Treated groups with GTE showed improvement in renal corpuscles, especially with high doses. So, we concluded that GTE had dose-dependent antioxidant and therapeutic effects on renal functions.
Keywords: Green tea, Kideny, Patulin, Antioxidants, Catechins, Histology.

1. Introduction

Mycotoxins are hazardous substances in human food; they are a major problem across the world, and they are considered a risk to both humans and animals [1]. Mycotoxins are classified as secondary metabolites, and their presence in nutrition, even in trace amounts, impacts their quality and safety, resulting in enormous losses and damages [2]. These mycotoxins are formed under particular circumstances: temperature and humidity in the air [3]. More than 300 mycotoxins have been identified, the majority of which are produced by the well-known genera *Aspergillus*, *Fusarium*, and *Penicillium* [4]. Even in minute concentrations, mycotoxins have been demonstrated to be dangerous to the human body[5]. The effects of these toxins on human health included changes in gene expression, renal disorders, human reproductive systems, gastric disruption, and the growth of tumor-causing cells within the human organs [6].

Patulin with the molecular formula ($C_7H_6O_4$) is created by numerous fungi such as *Penicillium*, *Byssochlamys*, and *Aspergillus* species and is mostly produced by *Penicillium patulum* and *Penicillium expansum*. Previously in the twentieth century, PAT was presented as an antibacterial agent and anticancer substance, and as an antibiotic for treating the common cold caused by *Penicillium patulum*. PAT's toxicity became known in the decade between 1950 and 1960, Aside from antibacterial, antiviral, and antiprotozoal capabilities; PAT was categorized in the 1960s as a mycotoxin generated by several *Aspergillus*, *Byssochlamys*, and *Penicillium* species [7]. After that PAT was recognized as a toxic secondary toxin that was derived from fungal origin. Fliege and Metzlar did an extensive study on PAT's chemical and biological properties, involving PAT's electrophilic characteristics [8]. It has been shown it is highly toxic by covalently binding to different amino acids containing thiol groups. Glutathione is thought to be a scavenger of PAT-induced toxicity. The main target for PAT-induced toxicity is the kidney [9] The results found by Melo suggested that the intraperitoneal injection of PAT reduced GSH levels and increased thiobarbituric acid reactive substances (TBARS) in the kidneys mice [10]. Moreover, PAT administration increased oxidative stress by generating ROS and high decreases in the activity levels of antioxidant enzymes, such as superoxide dismutase (SOD) and catalase (CAT) [11]. The intraperitoneal injection of PAT raised significantly creatinine, serum urea, BUN, and lactate dehydrogenase (LDH) levels, meanwhile, the sections of kidney tissue indicated inflammation of the tubules and deformation of the vacuoles[12, 13].

Numerous studies have demonstrated that phytochemicals protect against the harmful effects of environmental contaminants such as mycotoxins [14]. Consumption of these phytochemicals has been linked to health advantages such as

reduced oxidative damage, according to epidemiological research [15]. After water, tea made from a plant called Camellia sinensis (Theaceae) is the world's second most popular beverage. [16] The three primary varieties of tea are green tea, black tea, and oolong tea, and they differ in terms of manufacturing techniques and chemical components [17]. The global consumption of green tea accounts for 20%, is distinguished by a high level of flavan-3-ols called catechins. An average cup of boiled green tea contains 600-900 mg water extractable solids and is created from 2 g of green tea in 0.2 L of boiling water. Green tea catechins represent approximately thirty percent to forty percent of the total weight among these solids. The most prevalent catechin is (−)-Epigallocatechin-3-gallate (EGCG), accounting for as much as half of the total catechin amount. Epidemiological research has revealed that green tea and green tea polyphenols, particularly EGCG, can help prevent serious diseases such as heart disease, diabetes, neurological disease, and cancer[18]. Several mechanisms were proposed in studies on animals to account for the cancer-preventive benefits of EGCG and green tea, inhibiting growth factor signaling is one of these techniques. critical cellular enzyme inhibition, gene transcription inhibition, and tumor suppressor gene activation [19, 20]. green tea polyphenols' antioxidant activity and their pro-oxidant effects were recently proposed as possible cancer prevention methods [21].

This study aimed to determine PAT nephrotoxicity and investigate the green tea extract (GTE) therapeutic advantages against PAT-induced damage. In the current study, GTE has a therapeutic effect on toxicity features that appeared in kidney function.

2. Materials and methods

2.1. Reagents, Instrumentation, and Methodss

Patulin purity >98.0% was purchased from Sigma-Aldrich, dried green tea leaves were bought from an Egyptian local market, and no commercial reagents were involved., Spectrum Diagnostic kits of Urea/(blood urea nitrogen)BUN, Uric acid, and Creatinine (Jaffe) were obtained from The Egyptian Company for Biotechnology Spectrum Diagnostics (Cairo, Egypt), all animals were obtained from Experimental Animals Unit, Faculty of Medicine, Sohag University, Egypt.

2.2. Ultrasonic extraction of green tea

Green tea components were extracted utilizing an electronic ultrasonic bath (WUC-D06H type; produced in Korea) Briefly, in a round bottom flask, 100 mL of double-distilled water at 80 °C was poured over 10 g of green tea leaves powder. The flask was then immersed in an ultrasonic bath which was kept at 80 °C for 1h. The extract was filtered and then left to cool at a surrounding temperature[24]. The filtrate was placed in a Lyophilizer (Freeze Dryer model: Vertis 6KBTES-55, NY, USA), The Lyophilizer was set to temperature: -55 °C Vacuum: 100 mTorr Duration: 48 hours until dryness and then solid extract was obtained.

2.3. Animal design

All animal studies were carried out by the Sohag University Animal Welfare Committee guidelines and authorized by the ethics committee approval number (CSRE-17-23). Suffering has been reduced to the greatest extent possible, 60 male rats weighing 180 ± 10 g were kept in regular circumstances, with an optimum temperature and dark/light cycle, free access to a basal feed diet and drinking water in a climate-controlled, following 7 days of acclimatization, 60 rats were randomly divided into five groups with 12 rats in each group, PAT and GTE were dissolved in saline, 0.1% dimethyl sulfoxide (DMSO) was included in the PAT only, the control group was given saline with 0.1% DMSO, all rats in each group were received a daily intraperitoneal injection, the groups were divided as follow:

i- Group I Control group rats were given 0.1%DMSO in saline 2 mL/kg BW for 7 days
ii- Group II rats received 50 mg/kg BW of GTE for 7 days
iii- Group III PAT group rats received 0.2 mg/kg BW of patulin for 14 days.
iv- Group IV firstly, rats received 0.2 mg/kg BW of patulin for 14 days and then 50 mg/kg BW of GTE for 7 days[22].
v- Group V rats received 0.2 mg/kg BW of patulin for 14 days and then 100 mg/kg BW of GTE for 7 days.

2.4. Sample collection

Rats were sacrificed and dissected at the end of each given time, and blood samples from the five groups were taken directly from the heart into vacuum tubes before being centrifuged at 3000 rpm for 15 minutes to separate the resulting serum. The samples were then separated into various parts and kept at -20°C awaiting analysis.

2.5. Histology studies

After taking blood from anesthetized animals, they were euthanized. the abdomen was opened, and kidneys were extracted and washed th normal saline, The kidney w op ed thr gh a convex border and fixed in 10% formalin, routine processing for paraffin bloc ng and section was done in a professional patho gy lab, hematoxylin and eo n (H&E) stained sections were examined by light microscope and b cor x and edulla photographe with a digi camera to look for nges in rtical and m ullary structural compone s that may be induced by PAT mycotoxin compared to normal ntrol, observation for any improvement ere corded.

2.6. Statistical analysis

Prism 5 was used to examine the data. The mean ± SD (standard deviation) was used to express the results. For comparing means, the Tukey-test analysis was used, and the significance level was established at $p<0.05$, while there is a significant difference between control&patulin against different groups. $^{*,\#}$ = $p<0.05$ Significant. $^{***,\#\#\#}$ = $p<0.001$ Highly Significant. Non-Significant $p>0.05$.

155.46, 148.78, 144.08, 136.26, 134.32, 132.55, 117.92, 80.03, 73.22. Anal. Found for $C_{12}H_9N_7O_2$: C, 50.88; H, 3.20; N, 34.62. Calc: C, 50.86; H, 3.18; N, 34.60.

3. Results and Discussion

3. 1. Kidney Function Parameters

In summarizing the renal function results for each group **(Table 1)**, the patulin group presented a high significance increase ($p<0.001$) in each of urea, BUN, and creatinine concentrations (mg/dL) compared to the control group (Fig. 1-3), in another hand the uric acid concentration observed a high

significance decrease (p<0.001) (**Fig. 4**), while the results showed in treatment groups with GTE, initially group II exhibited non-significant change in urea, BUN, and uric acid levels, and high significance increase (p<0.01) in creatinine compared to the control group, group IV (low dose) observed a high significance decrease (p<0.01) in urea, BUN, and creatinine, also non-significant change observed in uric acid compared to patulin group. Finally, group V group (high dose) renal test results implied a high significance decrease (p<0.001) in urea, BUN, and creatinine, while uric acid levels showed a significant increase. We noted that there was no significant change in the results of different renal functions in group V compared to the control group and this proves that great efficacy for high doses.

Table 1: Effect of patulin on kidney function.

Tests \ Groups	Control	50mg/Kg BW GTE	PAT	PAT+50mg/ Kg BW GTE	PAT+100mg /Kg BW GTE
Urea	44.38 ± 0.215	45.63 ± 0.689$^{ns,###}$	62.87 ± 0.405***	49.13 ± 0.190$^{ns,###}$	42.46 ± 1.001$^{ns,###}$
BUN	21.51 ± 0.175	21.25 ± 0.341$^{ns,###}$	29.05 ± 0.120***	22.94 ± 0.090$^{ns,###}$	19.82 ± 0.468$^{ns,###}$
Creatinine	0.7771 ± 0.030	1.086 ± 0.042$^{***,###}$	2.484 ± 0.056***	1.053 ± 0.039$^{**,###}$	0.9425 ± 0.036$^{ns,###}$
Uric Acid	2.065 ± 0.065	1.950 ± 0.010$^{ns,###}$	1.395 ± 0.025***	1.720 ± 0.010^{**,ns}	2.049 ± 0.079 $^{ns,###}$

To represent the significant difference between the groups in contrast to the control group:
non-significant (p> 0.05); *p<0.05; **p<0.01; ***p<0.001.
To represent the significant difference between the groups in contrast patulin group:
non-significant (p> 0.05); $^{\#}p$<0.05; $^{\#\#}p$<0.01; $^{\#\#\#}p$<0.001.

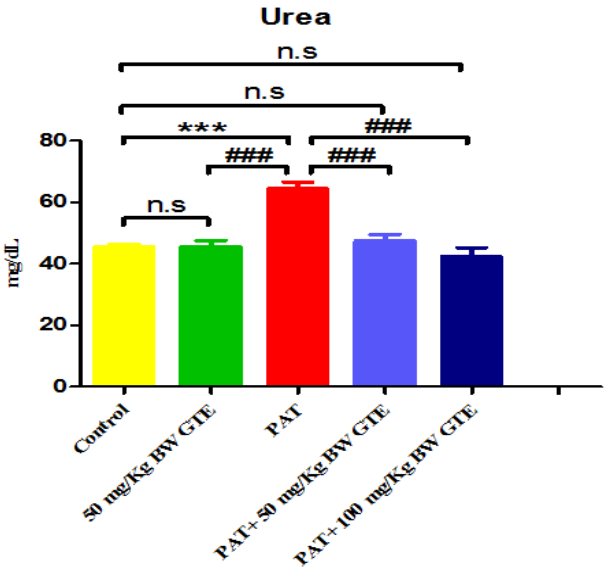

Fig. 1: GTE improves renal functions in PAT-intoxicated rats. GTE administration decreases serum urea levels.

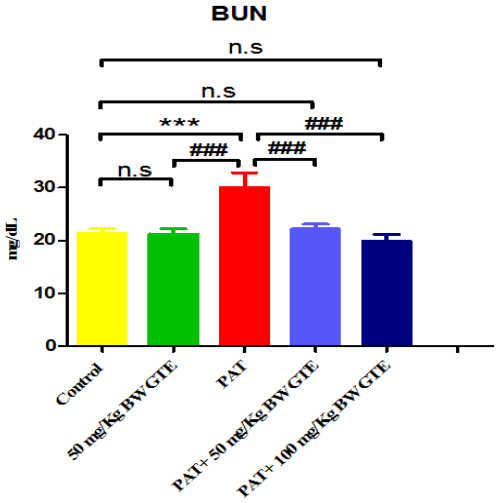

Fig. 2: GTE improves renal functions in PAT-intoxicated rats. GTE administration decreases serum BUN.

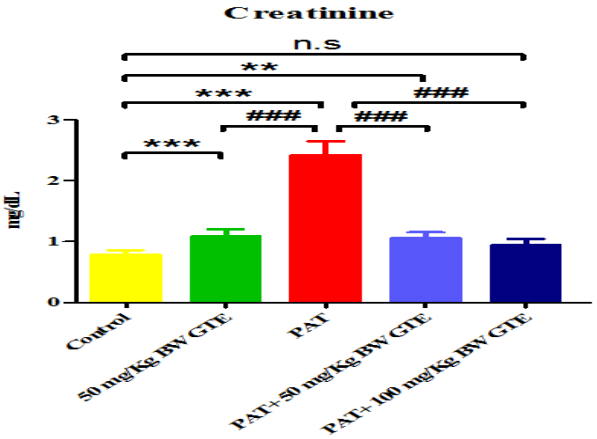

Fig. 3: GTE improves renal functions in PAT-intoxicated rats. GTE administration decreases serum creatinine levels.

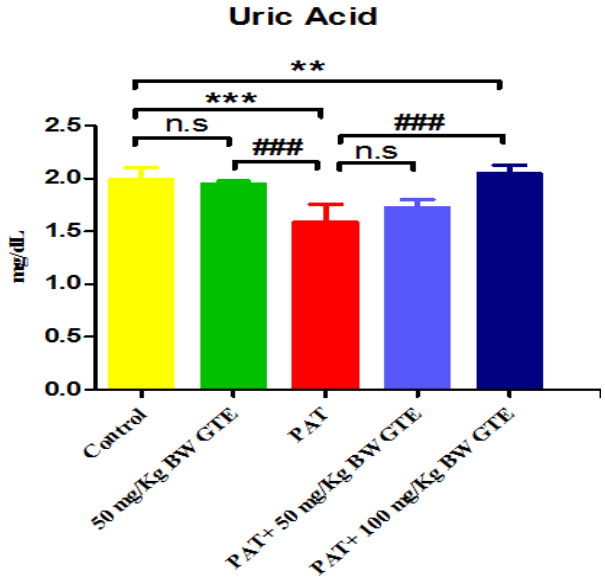

Fig. 4: GTE improves renal functions in PAT-intoxicated rats. GTE administration increases uric acid levels.

3.2. Histological results

A histological review of paraffin slices of rat kidneys labeled by hematoxylin and eosin (H&E) from the control, patulin, and GTE-treated groups, and photographed at power; Lowx400 at cortex and medulla regions (**Fig. 5**) showed that control cortex has well organized renal corpuscle (white arrow) and its glomerular capillaries (star), the renal tubules also showed normal epithelium and lumen free of any deposits(thin black arrows). The medulla showed intact normal proximal tubules and distal tubules with narrow and wide luminas lined with cuboidal cells with vesicular active nuclei (thin black arrows). PAT treatment hurts rat kidney tissue portrayed in the structure of cortex: A&B showing slightly (A) enlarged glomerular capillaries(black arrow & Black star) or degendered atrophied capillaries (B) with nearby dilated congested vessels (BV) The tubules(black dotted arrows) are dilated indicating epithelial lining atrophy. Their lumina contain acidophilic portentous deposition(casts). Medulla: A&B also showed highly dilated lumina of most tubules with luminal deposits (dotted arrows). In (B) we see perivascular(BV) inflammatory cells (lymphocytes) aggregates (a red star could be seen) this raised interalveolar fibrosis as compared to the usual features found in a control group. Finally, (**Fig. 6**) indicated that group II exhibited no change in the renal cortex, renal corpuscles, tubules, or medulla. In group IV, low dosage, the cortex showed improvement in renal corpuscles (white arrow) save for a few tubules that showed minor dilatation and deposits, the medulla revealed few dilated tubules with a few fine deposits. In group V, a high dosage of GTE significantly improves the alterations caused by PAT administration. the cortex had typical renal corpuscles (white arrows) and tubules that were more comparable to controls, whereas the medulla had normal proximal and distal tubules.

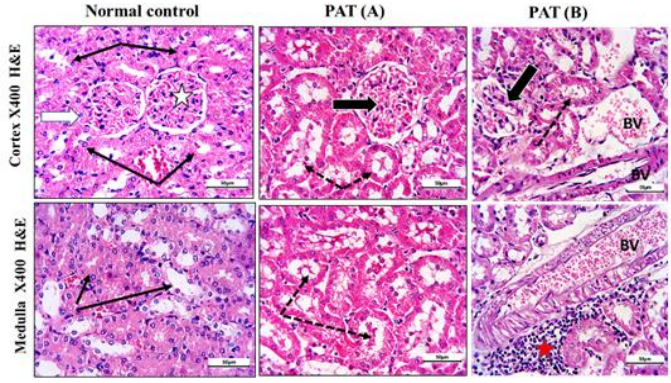

Fig. 5: Sections from albino rats kidney stained by H&E and photographed at x400 bar = 50µm: control showing normal cortex with well organized renal corpuscle and its glomerular capillaries, the renal tubules also showing normal epithelium and lumen free of any deposits, medulla showing intact normal proximal tubules, distal tubules (wide lumina) lined with cuboidal cells having vesicular active nuclei. PAT cortex A&B shows slightly (A) enlarged glomerular capillaries(black arrow & Black star) or degendered atrophied capillaries (B) with nearby dilated congested vessels (BV). The tubules (black dotted arrows) are dilated indicating epithelial lining atrophy. Their lumina contain acidophilic portentous deposition(casts). The medulla showing A&B also showed highly dilated lumina of most tubules with luminal deposits (dotted arrows). In (B) we can see perivascular (BV) inflammatory cells (lymphocytes) aggregates (red star).

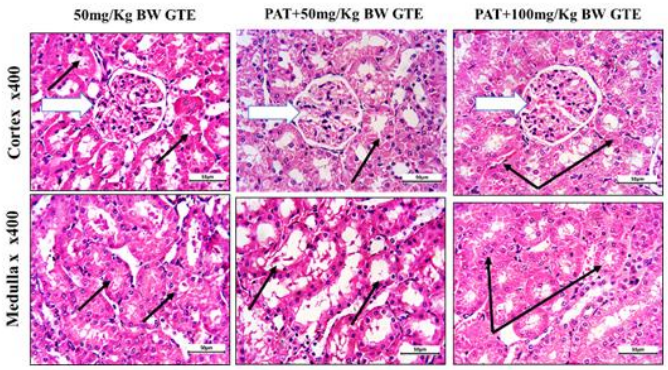

Fig. 6 Administrations of GTE only did not alter the normal structure of the renal cortex (white arrow) and medulla thin (black arrows), and its administration after PAT in both low 50mg/Kg BW and high 100 mg/kg BW doses preserved the normal structure of the kidney with superior effect n hia gh dose.

4. Discussion

This study investigated the effect of patulin on urea, BUN, creatinine, and uric acid, and the use of green tea extract as treatment of this effect was studied in male albino rats.

The result showed that the patulin group high significance increase (p<0.001) in urea levels (mg/dL), and also a high significance increase (p<0.001) in BUN levels (mg/dL) compared to the control group, BUN and urea are both protein metabolism markers indicating liver and kidney function. BUN is a test that evaluates the quantity of urea nitrogen in the blood. Urea is a molecule that includes urea nitrogen and is generated by the liver because of protein breakdown. BUN is a biomarker for renal and liver lesions since it is filtered out of the blood by the kidneys and eliminated in the urine [23, 24], The increased serum urea level may be due to renal impairment, GTE-treated groups IV and V showed a high significant decrease (p<0.001) compared to the patulin group, so GTE repaired the effect of PAT on urea and BUN dose-dependently, it is reasonable to conclude that GTE has a therapeutic role on kidney implication. Administration of GTE to rats reverted kidney injury as evidenced by the decreased level of urea and BUN.

The mean creatinine level in rats treated with patulin increased significantly (p<0.001) compared to the control group. This elevated Creatinine level might be attributed to serious renal insufficiency [25] and in most cases mycotoxin nephrotoxicity [26] Furthermore, the observed rise in creatinine may be attributed to numerous mechanisms influencing urea metabolism, such as amino acid equilibrium in the body, inhibition of gluconeogenesis, tissue injury, and liver or kidney diseases. All these factors are proved to be affected by patulin as reported by [27, 28], while the GTE-treated groups demonstrated a dose-dependent reduction in creatinine levels, proving that green tea extract had a therapeutic impact on the toxic effects of patulin. Finally, uric acid levels decreased significantly (p<0.001) in the patulin group, possibly because uric acid protects nerve cells by scavenging free radicals and serving as an extracellular antioxidant[29], a drop in serum uric acid levels has been

linked to enhanced oxidative sensitivity and a higher risk of ROS caused by patulin.

Histological examination of kidney tissue proved the damage of glomerular capillaries together with severe degeneration of renal tubules resulting in deposition of pretentious casts. This may explain the impairment of renal functions observed herein the antioxidant effect of water extract from green tea showed a marked renoprotective effect on structural parenchyma which was confirmed by improvement and return of renal functions to nearly normal levels, especially with a high dose.

5. Conclusion

Finally, this work revealed many biochemical tests of the kidney after rats were injected with patulin mycotoxin. The findings contribute to our understanding of PAT, which is very important for *Penicillium* and *Aspergillus* mycotoxins, which are present in natural agricultural goods, it turns out that GTE can be used to treat rats. GTE mitigated the reverse impacts of PAT toxin, GTEs may be employed as an antioxidant and antidote for patulin in rats, according to our findings.

CRediT authorship contribution statement:

Nagwa Elsawi: Supervision, conceptualization, visualization, and validation Hisham Ismail: supervision and visualization Ahmed Sayed: Supervision, methodology Soad Ali: methodology, writing—review and editing. Ahmed G. Khamis; software, formal analysis, investigation, resources, data curation, writing—original draft preparation. All authors have read and agreed to the published version of the manuscript.

Data availability statement

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

Declaration of competing interest

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

References

[1] K.H. Moon, I.K. Ko, J.J. Yoo, and A. Atala, *Methods*, 99 (2016) 112-119.

[2] M. Bashiry, F. Javanmardi, E. Sadeghi, S. Shokri, H. Hossieni, C.A. Oliveira, and A.M. Khaneghah, *Trends in Food Science & Technology*, 114 (2021) 100-115.

[3] M.O. Akinyemi, D. Braun, P. Windisch, B. Warth, and C.N. Ezekiel, *Food Control*, 131 (2022) 108258.

[4] A. Alshannaq, J. Yu, *Toxicity, and Analysis of Major Mycotoxins in Food*, (2017).

[5] J. Pleadin, J. Frece, and K. Markov, *Advances in food and nutrition research*, 89 (2019) 297-345.

[6] Y. Luo, X. Liu, and J. Li, *Food Control*, 89 (2018) 123-132.

[7] R. Steiman, F. Seigle-Murandi, L. Sage and S. Krivobok, *Mycopathologia*, 105 (1989) 129-133.

[8] R. Fliege, and M. Metzler, *Chemical research in toxicology*, 13(2000) 373-381.

[9] Z. Ráduly, R. G. Price, M. E. Dockrell, L. Csernoch, and I. Pócsi, *Toxins*, 13 (2021) 848.

[10] F.T. de Melo, I. M. de Oliveira, S. Greggio, J.C. Dacosta, T.N. Guecheva, J. Saffi, J. A. P. Henriques, and R. M. Rosa, *Food and Chemical Toxicology*, 50 (2012) 3548-3555.

[11] M. Boussabbeh, I.B. Salem, F. Belguesmi, H. Bacha, and S. Abid Essefi, *Neurotoxicology*, 53 (2016) 343-349.

[12] X. Lu, E. Zhang, S. Yin, L. Fan, and H. Hu, *Journal of agricultural and food chemistry*, 65 (2017) 5299-5305.

[13] N. El-Sawi, M. Al-Seni, N. El Haliem, M. El-Wassimy, and H. Salah, *Int. J. Fd. Nutr. Sci*, 2 (2015) 122-128.

[14] A. Çelik, D. Eke, S. Y. Ekinci, and S. Yıldırım, *Food and Chemical toxicology*, 53 (2013) 249-255.

[15] C. Borek, *Integrative cancer therapies*, 3 (2004) 333-341.

[16] C. S. Yang, X. Wang, G. Lu, and S. C. Picinich, *Nature Reviews Cancer*, 9 (2009) 429-439.

[17] B. DA, *Crit Rev Food Sci Nutr*, 37 (1997) 693-704.

[18] J.D. Lambert, and R.J. Elias, *Archives of Biochemistry and biophysics*, 501 (2010) 65-72.

[19] C.S. Yang, S. Sang, J. D. Lambert, Z. Hou, J. Ju, and G. Lu, *Molecular nutrition & food research*, 50 (2006) 170-175.

[20] D. Chen, K.G. Daniel, D. J. Kuhn, A. Kazi, M. Bhuiyan, L. Li, Zhigang Wang, Sheng Biao Wan, Wai Har Lam, Tak Hang Chan, and Q. Ping Dou, *Front Biosci*, 9 (2004).

[21] M.S. Butt, and M.T. Sultan, *Critical reviews in food science and nutrition*, 49 (2009) 463-473.

[22] E. Song, X. Xia, C. Su, W. Dong, Y. Xian, W. Wang, and Y. Song, *Food and Chemical Toxicology*, 71 (2014) 122-127.

[23] C.P. Carvounis, S. Nisar and S. Guro-Razuman, *Kidney International*, 62 (2002) 2223-2229.

[24] R. D. Evans, U. Hemmila, H. Mzinganjira, M. Mtekateka, E. Banda, N. Sibale, Zuze Kawale, Chimota Phiri, Gavin Dreyer, Viviane Calice-Silva, Jochen G Raimann, Nathan Levin, Roberto Pecoits-Filho, Ravi Mehta, and Etienne Macedo, *BMJ Global Health*, 5 (2020) e002312.

[25] E. Harfenist, *Harper's Biochemistry*, (1990) 609-626.

[26] F. Bokhari, and S.S. Ali, *Advances in Biological Research*, 2 (2008) 17-25.

[27] H.M. Gashlan, *Journal of Applied Animal Research*, 34 (2008) 93-96.

[28] O. Puel, P. Galtier, and I.P. Oswald, *Toxins*, 2 (2010) 613-631.

[29] B.N. Ames, R. Cathcart, E. Schwiers and P. Hochstein, *Proceedings of the National Academy of Sciences*, 78 (1981) 6858-6862.