Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

Research article

Improving liver metabolite performance and immune system reaction after treatment with camel milk in food-based poisoned rats

Hanoaf Shugaa Ali Alsadoon
Intermediate school Youssef Al siddiq, Al-Kut City, Iraq.
Email: hanofalsadon@gmail.com
Phone: +964 780 472 0205
Received: April 16, 2024; **Accepted**: April 26, 2024; **Published**: May 7, 2024

Abstract

Mycotoxins represent a continuous major problem that face food industry around the world due to their health impacts on humans and animals. Research studies that target citrinin mycotoxin are at a limit range. The current study was carried out to explore the ameliorative effects of camel milk (CMk) on the rats exposed to citrinin (CTN) at the levels of liver function, cytokine response, hepatic tissue lipid profile, hepatic tissue antioxidant activity, and gene expression of some repair related genes in tissue. According to these, an experiment that lasted for 20 days included the recruitment of 24 male rats into four groups (control group: received no treatment, CMk group: received 1.25 ml/kg B Wt of CMk orally, CTN group: received CTN at 10 mg/kg diet, and CTN+CMk: received both CTN and CMk at the same doses). After the end of the experiment, blood samples were collected from all animals before scarification. Blood samples were utilized for AST, ALT, ALP, LDH, GGT, and Ck liver function enzymes and in IL-6 and IL-1b responses. Liver tissues were employed for the detection of GSH and SOD activities and in *APE1* and *OGG1* gene expression. The findings of the liver function demonstrated significant (p<0.05) improvements in the enzyme and cytokines levels in the CTN+CMk group comparing with both control and CTN groups. Moreover, antioxidant and gene activities recorded significant (p<0.05) alterations in the CTN+CMk group. The present study results display important data about the improving effects of camel milk on the citrinin-intoxicated rats.

Keywords: *Aspergillus*, citrinin, fungi, immune system, liver enzymes, mycotoxin

Introduction

Mycotoxins are substances produced by molds that infect crops and grains either before they are harvested or, after storage. The growth of fungi and the development of compounds in stored crops depend on some factors like moisture levels, temperatures and water activity. CTN is a mycotoxin primarily forms during storage and derives from polyketides (1-6). In the 1930s Hetherington and Raistrick successfully isolated CTN, from *Penicillium citrinum* cultivation. It has been noted that three types of fungi Penicillium, Aspergillus and Monascus, have the ability to produce CTN. Over the years CTN has also been found in food dyes that commonly made in Asia using *Monascus purpureus* rice known as "red mold rice". These colorings are typically used to preserve meat and add color to food products (7,8).

Mycotoxins have the potential ability to contaminate food products causing health risks. However, the use of food processing techniques, like hazard analysis of control points (HACCP) and good manufacturing practices (GMP) has greatly enhanced the safety and nutritional value of final food

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

items. Various methods can be utilized to reduce or eliminate these toxins from food ensuring consumer safety and health protection (9-11). Despite its noted antibacterial, anticancer and neuroprotective properties CTN is not commonly used in pharmaceuticals due to its effects on the kidneys and genes. Laboratory studies have shown that CTN can negatively impact reproduction causing birth defects and harm developing embryos in both controlled experiments and living animals. Nonetheless, the International Agency for Research on Cancer (IARC) classifies CTN as a Group III carcinogen based on evidence from animal trials and insufficient data from research, on its potential carcinogenic activity (12-14).

Studies have shown that CTN can have impacts, on both kidneys and on the liver of human. There is often a connection between CTN and ochratoxin with research indicating that when they are present together their combined effect increases potential toxicity leading to kidney disease in humans (15). Aside from the kidneys CTN also affects the liver, mitochondrial respiratory chain, and bone marrow. To assess the risk of CTN as a food contaminant expert estimated concentrations in grains and grain-based products that could lead to kidney damage. Furthermore, CTN is fast absorbed into the body particularly targeting the liver and kidneys. A recent study, on how CTN moves through the body in humans revealed that 40% of it was excreted in urine (16, 17).

Studies have shown that camel milk has been found to have properties that can help against diabetes and fight bacteria along, with the potential to combat hepatitis. It contains beneficial components, including the lactoperoxidase/thiocyanate/hydrogen peroxide system, lactoferrins, lysozyme, immunoglobulins and free fatty acids (18).

Mycotoxins represent a continuous major problem that faces food industry around the world due to its health impacts on humans and animals. Research studies that target citrinin mycotoxin are at a limit range. The current study was carried out to explore the ameliorative effects of CMk on the rats exposed to CTN at the levels of liver function, cytokine response, hepatic tissue lipid profile, hepatic tissue antioxidant activity, and gene expression of some repair related genes in the hepatic tissue.

Materials and methods

Ethical approval

The present study was conducted according to the standards for animal care and use and was approved by the Ethical Committee at University of Wasit (No. 37 in 10-01-2024), Al-Kut City, Iraq.

Experimental design

Animal experiment was for 20 days, 24 male rats were reared and divided into four groups (control group: received no treatment, CMk group: received 1.25 ml/kg B W of CMk orally, CTN group: received CTN at 10 mg/kg of diet, and CTN+CMk: received both CTN and CMk at the same doses). After the end of the experiment, blood samples were collected from all animals before scarification. Blood samples were utilized to measure AST, ALT, ALP, LDH, GGT, and Ck liver function enzymes and IL-6 and IL-1b responses. Liver tissues were employed for detection of GSH and SOD activities level of *APE1* and *OGG1* genes expression. We utilized polypropylene cages sized at $70 \times 50 \times 30$ cm. The temperature was carefully regulated within a range of 19–22 °C with a humidity set at 60% and a dark cycle of 12 hours. Throughout the experiment all rats had access, to water and standard rat diet without any restrictions. Before the actual

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

experiments, the rats underwent a two-week acclimatization period to familiarize themselves with the laboratory environment. The researcher grew *Penicillium citrinum*, a fungus identified in a study that hasn't been published yet on groundnut to produce citrinin. The researcher has measured toxin amount using thin layer chromatography (TLC) and spectrophotometry techniques.

Blood and tissue tests

On the day 20th, blood samples were taken from the rat retro venous plexus while they were anesthetized with isoflurane inhalation. Subsequently all the rats were humanely euthanized to obtain their liver tissues for examination. The collected blood was centrifuged at 3000×*g* for 15 minutes. The resulting serum was stored at -20°C, for analyzing enzyme levels, as well as pro inflammatory cytokines like IL-6 and IL-1β. Additionally, to study tissue biomarkers in details, liver tissues were carefully washed with saline solution to remove blood clots and red blood cells. Following this, the tissues underwent homogenization in a chilled buffer solution at a ratio of 5–10 mL per gram of tissue before being centrifuged at 5000 rpm for 30 minutes. The resulting liquid was transferred in to tubes. Stored at -80°C, for analysis using a spectrophotometer. Blood biochemistry methods were performed utilizing techniques from (19-24). The SOD and GSH were tested in the tissues utilizing methods by Beutler *et al* (25) and Nishikimi *et al* (26). For the lipid profile, liver tissues were exposed to a method by Folch *et al* (27) to extract the lipids, and the extracts were employed to measure cholesterol and triglycerides in accordance with a method by Rotimi *et al* (28).

Gene expression profile

Levels of base excision repair genes /apyrimidinic endodeoxyribonuclease 1 (*APE1*) and 8 oxoguanine DNA glycosylase (*OGG1*) were assessed through qRT-PCR. Liver samples were subjected for RNA extraction. The RNA extraction process followed the manufacturers guidelines utilizing an RNA Purification Kit from Thermo Scientific (USA). To create cDNA from RNA, cDNA synthesis kit from Thermo Scientific, USA was utilized. Specific primers were used in the qRT PCR assay to amplify the *APE1* and *OGG1* genes. The β-actin functioning as a housekeeping gene serving as a reference gene for normalization. The collected data were analyzed using the $2^{-\Delta\Delta Ct}$ method (29). These primers were *APE1* F: GCTCAGAGAACAAACTCCCG and *APE1* R: TTGTTTCCTTTGGGGTTACG, OGG1 F: CCTGGCTGGTCCAGAAGTAG and OGG1 R: TTTCCCAGTTCTTTGTTGGC, and β-actin F: CACCATGTACCCAGGCATTG and β-actin R: ACAGTCCGCCTAGAAGCATT (30, 31).

Statistical analysis

The obtained data were processed by using GraphPad Prim v9 (USA). The figures were generated using the same software. Mean±SEM was utilized for the data. Five percent was the limit for significant or non-significant probabilities.

Results

Liver function enzymes

The findings of the liver function demonstrated significant (p<0.05) improvements in the enzyme and cytokine levels in the CTN+CMk group when compared with both control and CTN groups (Figure 1).

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

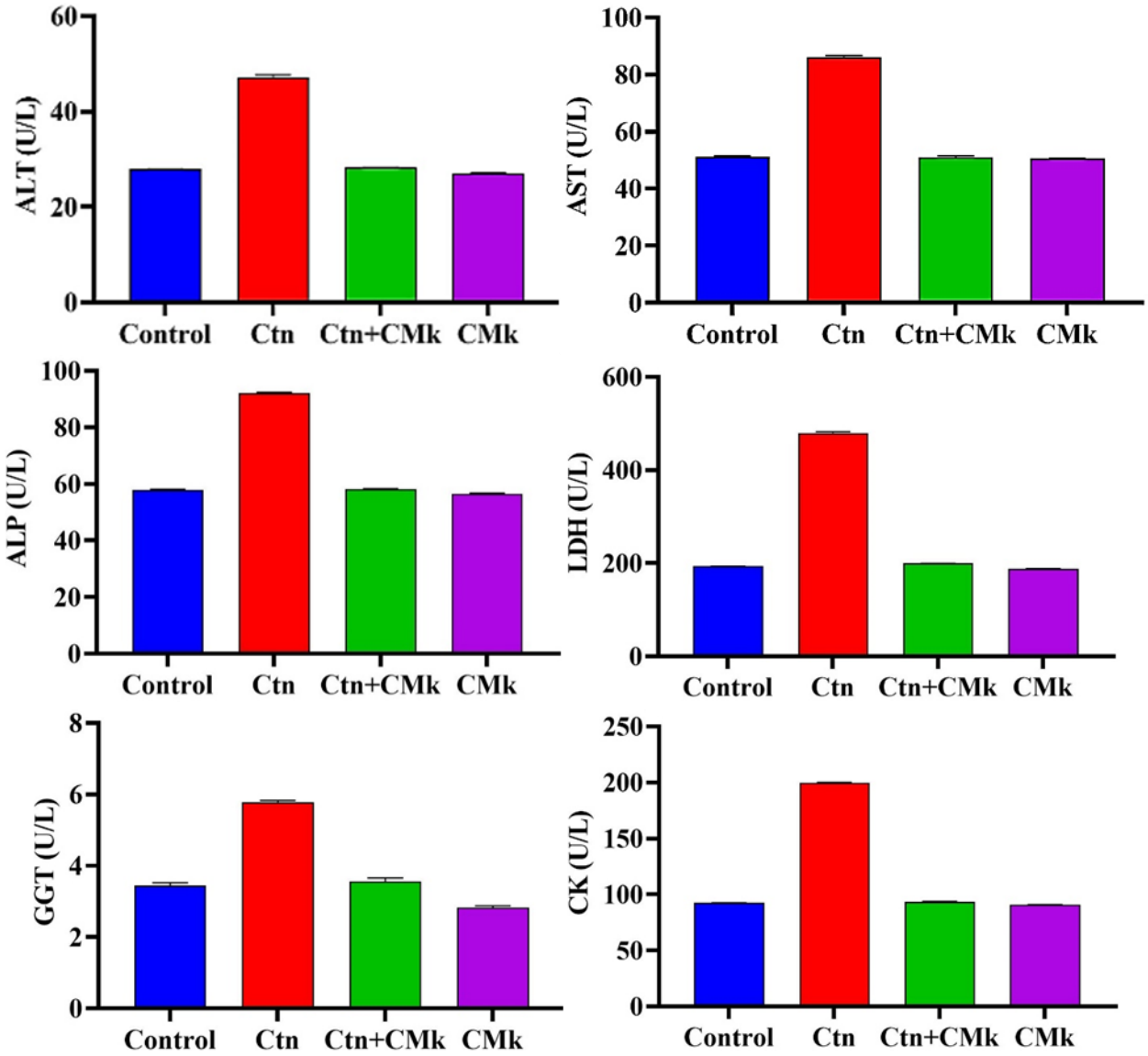


Figure 1: Liver function enzymes in rats exposed to citrinin and treated with camel milk.

Immune response

The immune response was recorded via the examination of IL-6 and IL-1b cytokines in the tested rats. The findings revealed significant increases in the levels of these indicators. However, these levels were restored significantly (p<0.05) when CMk was applied (Figure 2).

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

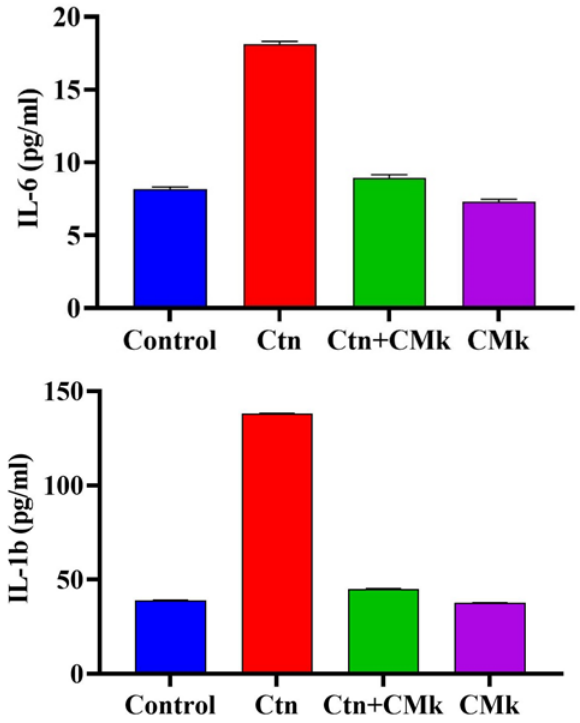


Figure 2: Immune response (IL-6 and IL-1b) in rats exposed to citrinin and treated with camel milk.

Antioxidants and lipid profile

Moreover, antioxidants, including GSH and SOD, were measured in the liver tissues, which showed alterations in the levels of these indicators. However, these levels were restored significantly ($p<0.05$) when CMk was applied (Figure 3).

Additionally, the lipid composition, in the liver samples, was assessed, focusing on cholesterol and triglyceride levels revealing an increase, in these markers. Nonetheless, these levels saw a restoration ($p<0.05$) following the application of camel milk (Figure 4).

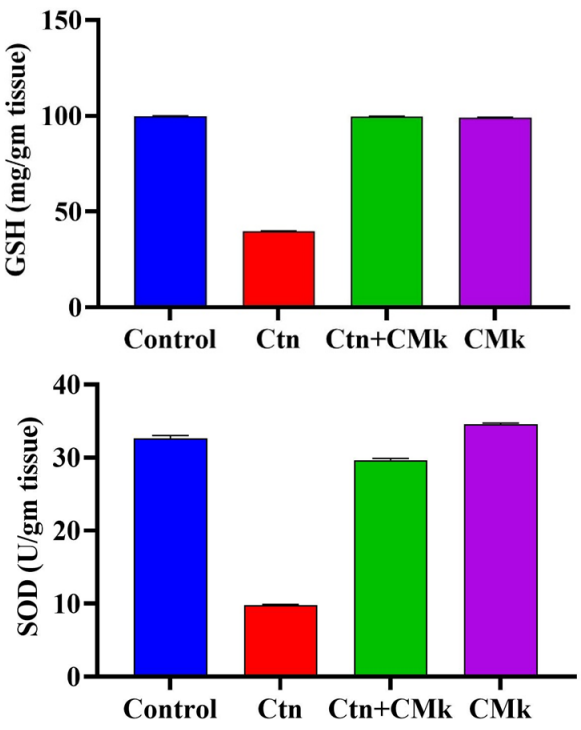
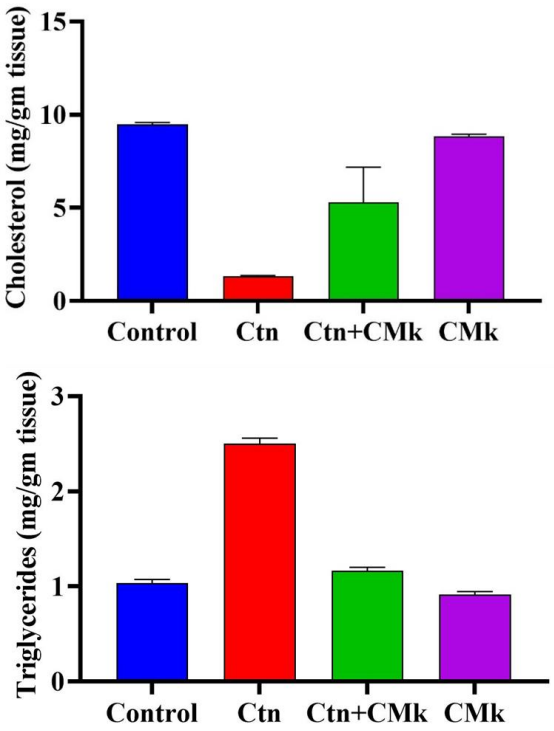


Figure 3: Antioxidant (GSH and SOD) activity in liver tissues in rats exposed to citrinin and treated with camel milk.



Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

Figure 4: Lipid composition of rat liver tissues, after exposure to citrinin and subsequent treatment, with camel milk.

Genes expression levels

Additionally, the levels of APE1 and OGG1 gene expression were assessed in liver tissues revealing an increase, in APE1 and a decrease, in OGG1 expression. However, these levels were restored significantly ($p<0.05$) when camel milk was applied (Figure 5).

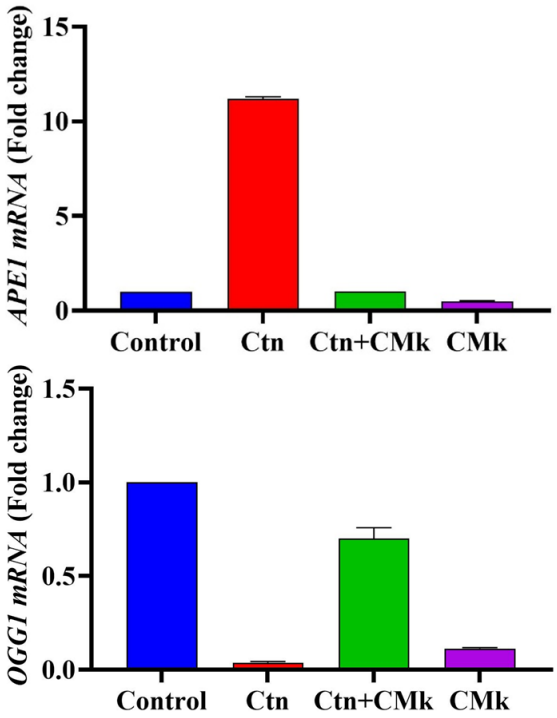


Figure 5: Repair gene expression of APE1 and OGG1 levels in liver tissues in rats exposed to citrinin and treated with camel milk.

Discussion

Citrinin is a toxin produced by types of fungi has been extensively researched for its effects on liver enzymes and interleukin levels in rats. This study, carried out over a 20-day period revealed an increase in liver enzyme levels like ALT and AST in rats exposed to citrinin. These findings align with studies by Abudayyak et al (32) who also noted elevated ALT and AST levels in rats treated with citrinin for 8 weeks. Additionally, Wu et al (33) observed liver cell damage and necrosis in rats exposed to citrinin further supporting the idea that this toxin is harmful to the liver.

Our study also showed that rats exposed to citrinin had levels of IL-6 and IL-1β in their blood serum indicating an immune response. These results are consistent with findings from Zargar and Wani (34) who found increased IL 6 and IL 1β expression, in rats treated with citrinin. In a vein Chan (35) found that rats exposed to citrinin exhibited levels of proinflammatory cytokines in their bloodstream hinting at a potential link, between citrinin triggered inflammation and liver damage.

In the present study, camel milk was found to lessen the effects of CTN, on blood chemistry. Research by Wang et al (36) supports these findings showing that camel milk shielded mouse livers from harm by reducing ALT and AST levels. In mice treated with cisplatin, camel milk demonstrated its ability to prevent genetic material damage and protect cells from toxicity. These results are attributed to the inflammatory and antioxidant properties of camel milk. It is believed that camel milk has anti-allergic effects, particularly, against allergies caused by various foods notably ruminant milk and dairy products.

APE1 and OGG1 are genes for fixing DNA bases damaged using a process known as base excision repair. In the group that received CTN treatment, APE1 showed an increase in its activity compared to the control group. Conversely, OGG1 was less active in the CTN treated group than in the control group. These results are consistent with a study by Liu et al (37) which demonstrated a reduction in OGG1 expression caused by aflatoxin. The combination of camel milk and citrinin led

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

to the correction of these levels. However, Korashy et al (38) reported that camel milk can activate apoptotic pathways. Furthermore, camel milk has been shown to reduce the activity of cancer promoting gene cytochrome P4501A1 (Cyp1a1).

Conclusion

Camel milk was able to reduce the effects of citrinin in rats due to the correction of liver enzyme function and antioxidant system.

Acknowledgement

The research study was self-funded only.

Conflict of interest

No conflict of interest is found for the present study.

References

1. Dalefield R. Veterinary Toxicology for Australia and New Zealand. Elsevier; Amsterdam, The Netherlands. Mycotoxins and mushrooms. 2017; pp. 373–419. https://doi.org/10.1016/b978-0-12-420227-6.00020-7
2. Kováč Š, Nemec P, Betina V, Balan J. Chemical structure of citrinin. Nature. 1961;190:1104–1105. https://doi.org/10.1038/1901104a0 .
3. Tangni EK, Pussemier L. Ochratoxin A and citrinin loads in stored wheat grains: Impact of grain dust and possible prediction using ergosterol measurement. Food Addit Contam. 2006;23:181–189. https://doi.org/10.1080/02652030500391911 .
4. Ostry V, Malir F, Ruprich J. Producers and important dietary sources of ochratoxin A and citrinin. Toxins. 2013;5:1574–1586. https://doi.org/10.3390/toxins5091574 .
5. He Y, Cox RJ. The molecular steps of citrinin biosynthesis in fungi. Chem Sci. 2016;7:2119–2127. https://doi.org/10.1039/C5SC04027B .
6. Pitt JI. Biology and ecology of toxigenic Penicillium species. Adv Exp Med Biol. 2002;504:29–41. https://doi.org/10.1007/978-1-4615-0629-4_4
7. Pattanagul P, Pinthong R, Phianmongkhol A, Tharatha S. Mevinolin, citrinin and pigments of adlay angkak fermented by Monascus sp. Int J Food Microbiol. 2008;126:20–23. https://doi.org/10.1016/j.ijfoodmicro.2008.04.019 .
8. Marič A, Skočaj M, Likar M, Sepčić K, Cigić IK, Grundner M, Gregori A. Comparison of lovastatin, citrinin and pigment production of different Monascus purpureus strains grown on rice and millet. J Food Sci Technol. 2019;56:3364–3373. https://doi.org/10.1007/s13197-019-03820-8 .
9. Gil-Serna J, Vázquez C, González-Jaén MT, Patiño B. Mycotoxins toxicology. In: Batt C.A., Tortorello M.A., editors. Encyclopedia of Food Microbiology. 2nd ed. Academic Press; Cambridge, MA, USA: 2014. pp. 887–892. https://doi.org/10.1016/b978-0-12-384730-0.00234-2
10. Kumar P, Mahato DK, Kamle M, Mohanta TK, Kang SG. Aflatoxins: A global concern for food safety, human health and their management. Front Microbiol. 2017;7:2170. https://doi.org/10.3389/fmicb.2016.02170 .
11. Kamle M, Mahato DK, Gupta A, Pandhi S, Sharma N, Sharma B, Mishra S, Arora S, Selvakumar R, Saurabh V, Dhakane-Lad J, Kumar M, Barua S, Kumar A, Gamlath S, Kumar P. Citrinin Mycotoxin Contamination in Food and Feed: Impact on Agriculture, Human Health, and Detection and Management Strategies. Toxins (Basel). 2022;14(2):85. https://doi.org/10.3390/toxins14020085 .
12. Chang CH, Yu F., Wang LT, Lin YS, Liu BH. Activation of ERK and JNK signaling pathways by mycotoxin citrinin in human cells. Toxicol Appl Pharmacol. 2009;237:281–287. https://doi.org/10.1016/j.taap.2009.03.021 .
13. Nakajima Y, Iguchi H, Kamisuki S, Sugawara F, Furuichi T, Shinoda Y. Low doses of the mycotoxin citrinin protect cortical neurons against glutamate-induced excitotoxicity. J Toxicol Sci. 2016;41:311–319. https://doi.org/10.2131/jts.41.311 .
14. De Oliveira Filho JWG, Islam MT, Ali ES, Uddin SJ, de Oliveira Santos JV, de Alencar MVOB, Júnior ALG, Paz MFCJ, de Brito MdRM, de Sousa JMdC. A comprehensive review on biological properties of citrinin. Food Chem Toxicol. 2017;110:130–141. https://doi.org/10.1016/j.fct.2017.10.002 .
15. Vrabcheva T, Usleber E, Dietrich R, Märtlbauer E. Co-occurrence of ochratoxin A and citrinin in cereals from Bulgarian villages with a history of Balkan endemic nephropathy. J Agric Food Chem. 2000;48:2483–2488. https://doi.org/10.1021/jf990891y .
16. Bamias G, Boletis J. Balkan nephropathy: Evolution of our knowledge. Am J Kidney Dis. 2008;52:606–616. https://doi.org/10.1053/j.ajkd.2008.05.024 .

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

17. López Sáncheza P, de Nijsa M, Spanjerb M, Pietric A, Bertuzzic T, Starski A, Postupolski J, Castellari M, Hortós M. Generation of occurrence data on citrinin in food. EFSA Support Publ. 2017;14:1177E. https://doi.org/10.2903/sp.efsa.2017.EN-1177 .
18. Swelum AA, El-Saadony MT, Abdo M, Ombarak RA, Hussein EOS, Suliman G, Alhimaidi AR, Ammari AA, Ba-Awadh H, Taha AE, El-Tarabily KA, Abd El-Hack ME. Nutritional, antimicrobial and medicinal properties of Camel's milk: A review. Saudi J Biol Sci. 2021;28(5):3126-3136. https://doi.org/10.1016/j.sjbs.2021.02.057 .
19. Vázquez-Medina JP, *et al*. Prolonged fasting increases glutathione biosynthesis in postweaned northern elephant seals. J Exp Biol. 2011;214(Pt 8):1294-1299. https://doi.org/10.1242/jeb.054320
20. Tietz N.W., *et al*. A reference method for measurement of alkaline phosphatase activity in human serum. Clin Chem. 1983;29(5):751-761. https://doi.org/10.1093/clinchem/29.5.751
21. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am J Clin Pathol. 1957;28(1):56-63. https://doi.org/10.1093/ajcp/28.1.56
22. Szasz G, Gruber W, Bernt E. Creatine kinase in serum: 1. Determination of optimum reaction conditions. Clin Chem. 1976;22(5):650-656. https://doi.org/10.1093/clinchem/22.5.650
23. Würzburg U, *et al*. Determination of creatine kinase-MB in serum using inhibiting antibodies (author's transl) Klin. Wochenschr. 1976;54(8):357-360. https://doi.org/10.1007/BF01469790
24. Babson AL, Babson SR. Kinetic colorimetric measurement of serum lactate dehydrogenase activity. Clin Chem. 1973;19(7):766-769. https://doi.org/10.1093/clinchem/19.7.766
25. Beutler E, Duron O, Kelly BM. Improved method for the determination of blood glutathione. J Lab Clin Med. 1963;61:882-888. https://www.scienceopen.com/document?vid=0ae50d69-a9d9-486b-adf1-f9d2394d1630
26. Nishikimi M, Appaji N, Yagi K. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem. Biophys. Res Commun. 1972;46(2):849-854. https://doi.org/10.1016/S0006-291X(72)80218-3
27. Folch J, Lees M, Sloane Stanley GH. A simple method for the isolation and purification of total lipides from animal tissues. J Biol Chem. 1957:226. https://doi.org/10.1016/S0021-9258(18)64849-5
28. Rotimi OA, Olayiwola IO, Ademuyiwa O, Balogun EA. Effects of fibre-enriched diets on tissue lipid profiles of msg obese rats. Food Chem Toxicol. 2012;50:4062-4067. https://doi.org/10.1016/j.fct.2012.08.001
29. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods. 2001;25(4):402-408. https://doi.org/10.1006/meth.2001.1262
30. Caderni G, Giovannelli L, Lodovici M. Aging related changes in circulating reactive oxygen species (ROS) and protein carbonyls are indicative of liver oxidative injury. Toxicol Rep. 2018;5:141-145. https://doi.org/10.1016/j.toxrep.2017.12.017
31. Xie Z, Wu B, Shen G, Li X, Wu Q. Curcumin alleviates liver oxidative stress in type 1 diabetic rats. Mol Med Rep. 2018;17(1):103-108. https://doi.org/10.3892/mmr.2017.7911
32. Abudayyak M, Karaman EF, Ozden S. Mechanisms underlying citrinin-induced toxicity via oxidative stress and apoptosis-mediated by mitochondrial-dependent pathway in SH-SY5Y cells. Drug Chem Toxicol. 2023;46(5):944-954. https://doi.org/10.1080/01480545.2022.2113095 .
33. Wu D, Yang C, Yang M, Wu Y, Mao Y, Zhou X, Wang J, Yuan Z, Wu J. Citrinin-Induced Hepatotoxicity in Mice Is Regulated by the Ca2+/Endoplasmic Reticulum Stress Signaling Pathway. Toxins (Basel). 2022;14(4):259. https://doi.org/10.3390/toxins14040259 .
34. Zargar S, Wani TA. Food Toxicity of Mycotoxin Citrinin and Molecular Mechanisms of Its Potential Toxicity Effects through the Implicated Targets Predicted by Computer-Aided Multidimensional Data Analysis. Life (Basel). 2023;13(4):880. https://doi.org/10.3390/life13040880 .
35. Chan WH. Citrinin induces apoptosis via a mitochondria-dependent pathway and inhibition of survival signals in embryonic stem cells, and causes developmental injury in blastocysts. Biochem J. 2007;404(2):317-26. https://doi.org/10.1042/BJ20061875 .
36. Wang Z, Qiao X, Hao S, Ji R. Demonstration of hepatoprotective action of camel milk through improving antioxidant activity and regulating gene expression in mice. J Camel Pract Res. 2017;24(2):169–174. http://dx.doi.org/10.5958/2277-8934.2017.00026.1
37. Liu W, Wang L, Zheng C, Liu L, Wang J, Li D, Tan Y, Zhao X, He L, Shu W. Microcystin-LR increases genotoxicity induced by aflatoxin B1 through oxidative stress and DNA base excision repair genes in human hepatic cell lines. Environ Pollut. 2018;233:455–463. https://doi.org/10.1016/j.envpol.2017.10.067
38. Korashy HM, El Gendy MA, Alhaider AA, El-Kadi AO. Camel milk modulates the expression of

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

aryl hydrocarbon receptor-regulated genes, Cyp1a1, Nqo1, and Gsta1, in murine hepatoma Hepa 1c1c7 cells. J Biomed Biotechnol. 2012 https://doi.org/10.1155/2012/782642 .