

Potential and challenge assessment of tannin extracts from black tea in male rabbits fed contaminated diet by Mycotoxins

**Aseel Adnan Abdulhussein¹; Mohammed Munis Dakheel^{1, 2},
Jessica Quijada³**

*corresponding author: m.m.dakheel@covm.uobaghdad.edu.iq

¹ Department of Veterinary Public Health; College of Veterinary Medicine, University of Baghdad, Iraq;

² Zoonoses Research Unit, College of Veterinary Medicine, University of Baghdad, Iraq;

³ School of Agriculture and Applied Sciences Research, Langston University, Langston, USA.

ABSTRACT

The focus of this research was to investigate how tannin extract from black tea influenced the hematological and histopathological evaluations of male rabbits fed a mycotoxin-contaminated feed, including 17 ppb aflatoxin, 5 ppb ochratoxin and 2 ppb fumonisin. A total of 28 local male rabbits were allocated into four groups, the first of which was a control group. The second was fed a Mycotoxin-contaminated meal. The third was given a Mycotoxin feeding with tannin extracts (125mg/mL per head) administered orally, while the fourth was fed a Mycotoxin contaminated diet with tannin extracts (250 mg/ mL per head) given orally. The findings revealed that tannin extracts seemed to have a significant positive impact on haematological results, particularly RBCs and WBCs, throughout the period; however, the 250 mg/mL dose showed no significant differences in Hb levels. Nonetheless, as compared to the Mycotoxin group, the histopathological sections of tannin extract demonstrated a reduction in the toxicity of the Mycotoxin diet on the liver and kidney tissues; conversely, the tissue sections of the control animals showed no abnormalities. In male rabbits fed contaminated Mycotoxin diets, tannin extract from black tea was found to have a favourable impact on hematological activities and vital organs such as the liver and kidney.

Keywords: Tannins, black tea extract, hematological tests, histopathological sections, Mycotoxins

INTRODUCTION

Tannins are referred to as water-soluble polyphenols that are present in many plant feeds. Tannins have different effects on health. Besides the antioxidant and antimicrobial activities, tannins are also used as antiseptics and astringents (Dakheel et al., 2020). Black tea contains a large molecular weight of tannins, which has broad-spectrum and specific medicinal effects as an antioxidant, anti-inflammatory and anti-cancer, and antimicrobial (Khasnabis et al., 2015).

Mycotoxin contamination of food and feed (such as fumonisin, aflatoxin, and ochratoxin A) decreases the nutritional value, consistency and protection of food and feed in the same way. These naturally occurring toxic compounds frequently contaminate agricultural commodities posing a hazardous risk to humans and animals (Haque et al., 2020). Unfortunately, there is little information on the impact of tannins on the clinical symptoms of these toxins in the blood parameters of rabbits. Rabbits can display perplexing clinical indications, and additional information may be obtained from laboratory studies. Furthermore, as information is gathered and researchers become more aware of diagnostic and prognostic hematologic signs, the lack of metabolic data for pet rabbits is changing (Cullere and Zotte, 2018).

Mycotoxicosis in rabbits is characterized by a 20-60% reduction in feeding, which can result in acute or chronic diseases, Mycotoxicosis, is based on the mycotoxin concerned, its concentration, exposed length, accumulative effects, and mycotoxin synergisms (Gimeno et al., 2011). Thus, this work aimed to determine the effects of tannin extract, from black tea, on hematological and histopathological tests of male rabbits fed on a Mycotoxin diet.

MATERIALS AND METHODS

Experimental design

Twenty-eight healthy local male rabbits (average weight 1322 ± 100 g and aged 5-6 months old) were randomly allocated into four groups; the first group was daily fed freely on water and basal diet as a control (C). The second group was fed on a diet that contained Mycotoxins, which was named (T1), The third group (T2) was fed on a Mycotoxins diet plus administered with tannin extracts orally (125 mg/ mL per head), as well as the fourth group (T3) was fed on mycotoxin diet plus giving oral administered of tannin extracts, (250 mg/ mL per head). This experiment was continued for 63 days. Ethical permission was obtained by the College of Veterinary Medicine/ University of Baghdad.

Tannin Extraction

An aqueous extract of tea tannins was given to the rabbits orally. The assessment of insoluble tannins was done using Butanol-HCl (Sigma-Aldrich) reaction to insoluble plant materials; the colorimetric reaction has used an oxidative de-polymerization of tannins to produce red anthocyanidins. These methods have been described by Mueller-Harvey (Mueller-Harvey, 2001).

Mycotoxin detection analysis

The mycotoxin detection analysis of a concentrated diet was achieved using HPLC/ MS at a group of the Veterinary Center/ Laboratory and Science Unit/ Baghdad-Iraq. The results shown that the diet was contaminated with different mycotoxins including aflatoxin (17 ppb), ochratoxin (5 ppb) and fumonisin (2 ppb); the rabbit diet was naturally contaminated with these Mycotoxins.

Hematological tests

The blood samples were taken three times during the experiment (at 0, 30 and 60 days), which were withdrawn in the morning before treatments were given. These samples were withdrawn from the heart after sterilization at the site of blood drawn by using disposable syringes sterilized. The samples were kept in (5mL) tubes that contain an anticoagulant EDTA (ethyl diamine-tetraacetic acid).

Histopathological protocol

After anaesthetizing rabbits using ether (diethyl ether / CAS 60-29-7), the animals were labelled. The liver and kidneys of the animals were removed and cleaned in distilled water before being put in 10% Formalin and dehydrated with an increasing concentration of alcohol (50, 70, 90, and 100 %). The "Clearing" procedure was then completed with Xylol, followed by the casting, and embedding processes. Hematoxylin and eosin were used to investigate pathological changes after the paste was placed on cleaned glass slides (Junqueira, et al., 1979).

Statistical Analysis

SAS (2012) program (Statistical Analysis System) was used to determine the effect of statistical values on the study parameters. A least significant difference (LSD) and one-way ANOVA were applied to compare significant means.

RESULTS

Blood hemoglobin (Hb)

The treated groups and control demonstrated no differences in Hb values between them at ($P \geq 0.05$) from 0 day throughout 30th and 60th days, as shown in Table 1.

Table 1. Effect of different concentrations of tannin extracts on the blood hemoglobin (Hb) (g/ dl)

Groups/ Times	Control	T1	T2	T3	LSD
0 day	10.00±0.74	10.05±0.90	10.10±0.38	10.03±0.40	0.15 NS
30 th day	13.82±0.28	12.90±1.04	11.90±1.50	13.44±0.16	1.95 NS
60 th day	13.76±0.18	13.24±0.25	13.56±0.15	13.98±0.22	0.80 NS

(NS) indicates no significant differences ($P \geq 0.05$) between groups at the same time; data are means ±SEM

Erythrocytes (RBCs)

The results of RBCs of different groups showed fluctuating significant differences ($P \leq 0.05$) in the average of red blood cells (Table 2). Those groups recorded no significant differences between them on the 1st day. Whereas the 30th day and 60th day recorded different values ($P \leq 0.05$) compared with the 1st day.

Table 2. The effect of different concentrations of tannin extracts on the Erythrocytes (RBCs = $\times 10^6$ / μ L)

Groups/ Times	Control	T1	T2	T3	LSD
0 day	5.50 ±0.34	5.45 ±0.33	5.52 ±0.38	5.49 ±0.42	0.1 NS
30 th day	6.10 ±0.14	5.60 ±0.47	5.58 ±0.35	6.11 ±0.36	0.55 NS
60 th day	5.90 ±0.31 ^a	5.51 ±0.12 ^b	5.53 ±0.13 ^b	6.12 ±0.40 ^a	0.35*

The small letters refer to significant differences between groups ($P \leq 0.05$). Also, (NS) indicates no significant differences; data are means ±SEM.

Leukocytes (WBCs)

On the 30th and 60th day of the experiment, the high concentration tannin group significantly showed a lower number of Leukocytes (WBCs) ($P \leq 0.05$) compared to the other groups; moreover, the mycotoxin group has also been found the highest value, on the 30th and 60th day of the experiment compared with the other groups in the experiment.

Table 3. Effect of different concentrations of tannin extracts on the Leukocytes (WBCs = $\times 10^3 / \mu\text{L}$)

Groups Times	Control	T1	T2	T3	LSD
0 day	5.50 \pm 0.6	5.00 \pm 0.8	5.30 \pm 0.9	5.60 \pm 0.7	0.70 NS
30 th day	6.14 \pm 1.02 ^a	6.85 \pm 1.2 ^a	6.27 \pm 1.4 ^a	5.29 \pm 8.40 ^b	0.82*
60 th day	6.57 \pm 1.11 ^a	6.80 \pm 1.3 ^a	6.10 \pm 9.22 ^a	5.35 \pm 0.2 ^b	0.71*

The small letters refer to significant differences between groups ($P \leq 0.05$). Also, (NS) indicates no significant differences; data are Means \pm SEM.

Histopathological study

Liver sections

Figure (1/A) showed that the control rabbits revealed normal parenchyma, no pathological lesion on each lobule of the liver, and a normal central vein with a normal hepatic cord under light microscopy.

The most lesion recognized in liver infected with mycotoxin shown in figure (1/B) as proliferation and infiltration of mononuclear cells in the portal area and liver parenchyma cells, and congestion of hemolytic blood in sinusoid lead to the presence of hemosiderin-like pigments, as well as, for necrotizing of a high number of hepatocyte also showing degeneration changes and deposition of fibrin-like materials with the proliferation of fibroblast, as well as showing fatty change like in hepatocyte and also there is hemorrhage.

In figure (1/C), the severity of degenerative changes appears less than in the infected wall, but the degenerative changes appear in hepatocytes (granular degeneration) and infiltration of inflammatory cells (mononuclear cell). However, in figure (1/D), the hepatocyte appeared normally, but there is infiltration of inflammatory cells (mononuclear cells) in the portal area and parenchyma of the liver, as well as, showing focal granulomatous lesion in the liver parenchyma those adjacent to normal liver lobules (central vein with hepatic cords).

Tannin has anti-inflammatory activities mediated by the alteration of tissue cells. In figure (1/D), tannin improved the liver function related to infiltration of inflammatory cells (mononuclear cells) in the portal area and parenchyma, plus detecting the focal granulomatous in the liver parenchyma, which is adherent to normal liver lobules (central vein with hepatic cords).

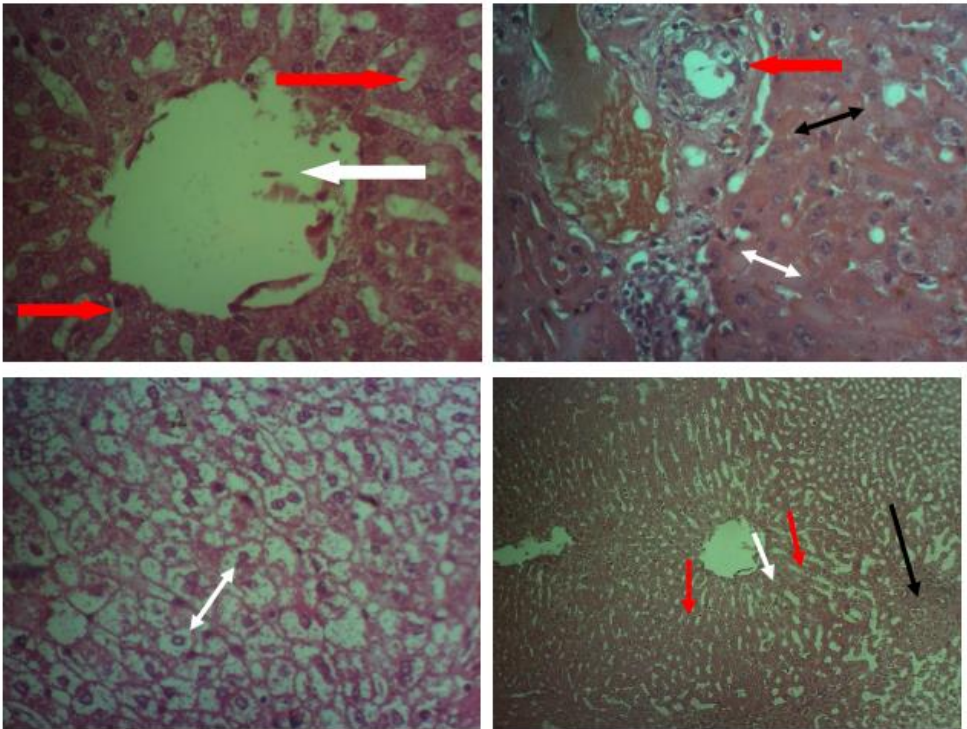


Figure 1. Histopathological section of liver tissues in the male rabbits treated; (A) a control group that shows (normal structure) no abnormal lesion noted in the central vein (White arrow) and hepatic cord (Red arrow); (B) a T1 group shows infiltration of inflammatory cells (mononuclear cells) in the portal area (Red arrow) and the necrosis of hepatocytes (Black arrow), as well as the congestion of sinusoids (White arrow); (C) a T2 group shows degenerative cells (white arrow); (D) a T3 group shows infiltration of inflammatory cells (mononuclear cells) forming focal granulomatous lesion in liver parenchyma (Black arrow) adjacent to normal liver lobules as central vein (white arrow) with hepatic cords (Red arrow).

Kidney sections

The tissues of the kidney similarly exhibited no abnormal alterations for the kidney. The rabbits in the control group had normal tissue and structure and no lesions; they were fed clean food and water regularly, and their kidney tissues were unaffected (Figure 2/A).

Infiltration of inflammatory cells (mononuclear cells) in the kidneys of diseased rabbits fed a mycotoxin diet, as well as fibrosis and thickening of blood vessel walls infiltrated by foamy cells, and blood vessel congestion with hemorrhage. The glomeruli tuft was fragmented, and the epithelial lining of

urinary tubules was degenerative and necrotizing in other sections (Figure 2/B).

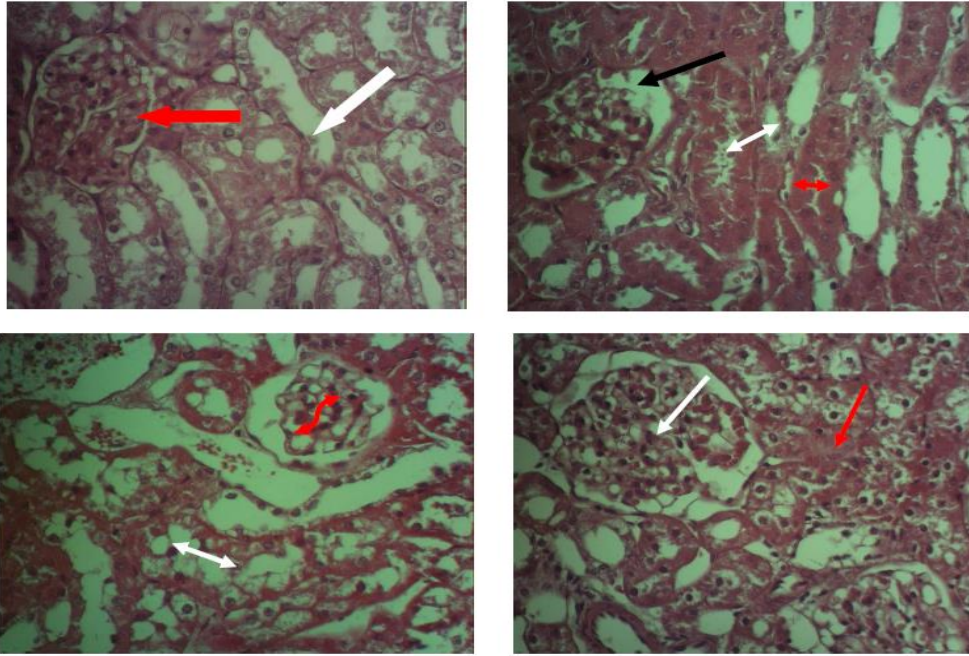


Figure 2. Histopathological section of kidney tissues in the male rabbits treated; (A) a control group shows (normal structure) no lesion or abnormal structure in the glomeruli (Red arrow) and the tubules (White arrow); (B) a T1 group shows the vacuolation of glomeruli tuft (Black arrow) and degenerative (Red arrow) plus necrotizing of epithelial lining urinary tubules (White arrow); (C) a T2 group shows the mesangial cells of glomerular tuft and widening of bowman space (Red arrow), as well as the degenerative changes in epithelium lining of urinary tubules infiltration of an inflammatory cells-mononuclear cell (white arrow); (D) a T3 group shows the mild vascular degeneration (Red arrow), as well as the vacuolation of mesangial cells of glomerular tuft (white arrow).

Figure (2/C) demonstrated the severity looks less than vacuolation of the mesangial cells of glomerular tuft and widening of bowman space in addition to degenerative changes in the epithelium lining urinary tubules infiltration of inflammatory cells (mononuclear cell).

Mild degenerative changes can be seen in this group that epithelial cells lining urinary tubules showing mild vascular degeneration, as well as mild vacuolation of mesangial cells of the glomerular tuft (Figure (2/D).

DISCUSSION

According to Stock *et al.* (2000), tannin, like flavonoids, may produce a rise in mean corpuscular hemoglobin concentration, especially in a group that was orally administrated high concentration of tannin extraction, which is responsible for the increase of RBCs in all treated rabbits after 30 and 60 days compared to a group of low tannin concentration (125 mg/mL). In T3 group, the high tannin concentration (250mg/mL) reduced the mycotoxins negative effect as resulted from the absence of weakness and anemia, effects already described by otehr researchers (Magouz *et al.* 2020). High concentration of tannin considerably improved the hematological traits as compared with both mycotoxin and low concentration tannin groups. Flavonoids attach to the membrane of RBCs, reducing lipid peroxidation and, as a result, trigger 'hypotonic lysis.' Tryptophan residue probably is where these antioxidant effects take place. Antioxidant phytochemicals, such as tannins, on the other hand, have been found to protect against oxidative damage. As a result, antioxidant tannins may be effective in preventing the degradation of red cell membranes as well as protecting against mycotoxin (Dakheel *et al.*, 2021).

The oral administration of high tannins concentration (as 250 mg/mL) for the group of experimental subjects receiving mycotoxin treatment may be responsible for the lower WBC count in the blood. According to Abdel-Wareth and Seddik (2014) results, the white blood cells (WBCs) are a crucial part of the host defence system because they defend the body from bacteria, fungi, parasite diseases, and viruses. In the current study, the difference in the leukocyte profile was found between the two treatment groups of those rabbits infected by *mycotoxin plus* (125 mg/mL) or (250 mg/mL); these findings are similar with Hidanah *et al.* (2018).

Furthermore, in T2 and T3 groups with lower mycotoxin levels, the total number of leukocytes decreased. This suggests that the irritating reaction caused by mycotoxin infection has ceased as a result of tannin extracts' capacity to suppress the mycotoxin, as tannin extract has also been documented to have antifungal action (Goloshvili *et al.*, 2021).

Histopathological sections

The liver has fundamental roles in the metabolism of drugs and a plant product and thus is at a high risk of damage (Juodeikiene *et al.*, 2018). These pathologic changes observed in the histology of the liver could be due to the contamination of the diet with mycotoxin and may also induced severe damage to the liver structures (Ejiofor *et al.*, 2021). In the low tannin group (T2) the histopathological changes in liver and kidney were more evident than in the organs of the animals receiving the highest concentration of tannins (T3 group).

The severity of degenerative changes in liver tissue seems to become less severe due to the tannin effect. Such histopathological changes were also reported by another author following various herbal extract administrations in experimental animals (Ruan *et al.*, 2019).

The protective effect is attributed to its antioxidant and free radicals scavenging properties; tannin has the ability to scavenge oxygen free radicals and to inhibit the lipid peroxidation. The various degrees of histopathological changes in the organs of rabbits administrated orally tannins showed their suppressive effects against the mycotoxins. This is consistent with similar reports on the protective effect of antioxidants (as curcumin contains flavonoids) on the cell walls of young ducks fed a contaminated diet by Ochratoxin-induced impairment of intestinal barrier function and histopathological changes (Saeed *et al.*, 2012).

The greater part of absorbed mycotoxins is removed with urine, but residues are accumulated in the liver, kidneys and muscles and pose a threat to animal and human health (Kovalsky *et al.*, 2016). The exposure to mycotoxins results into infiltration of inflammatory mononuclear cells in the kidney of intoxicated animals; these changes are associated with fibrosis and thickening of the blood vessels walls by foamy cells infiltration, congestion of blood vessels, fragmentation of glomeruli tuft and degenerative and necrosis of epithelial cells (Dakheel *et al.*, 2021).

Histopathological changes were not observed in the kidneys of rabbits administrated tannin orally (125 mg/mL) and were of lesser intensity compared with the mycotoxin group; due to the low concentration of toxins reached in kidneys and the findings of histological changes were not as pronounced as it was in case of mycotoxin group (Hänske *et al.*, 2021); however, in the liver and kidney, chemicals might cause severe histopathological alteration. According to Iglesias-De La Cruz (2001) findings, the chemical caused significant toxic pathological changes and biochemical disorders in rabbits.

Conversely, in a group of low tannin concentration, aflatoxins might cause the severity to look like less than vacuolation of the mesangial cells of glomerular tuft and widening of bowman space in addition to degenerative changes in the epithelium lining urinary tubules infiltration of inflammatory cells (mononuclear cell). The extent of mycotoxin-induced lesions in the liver and kidneys is dose-dependent, according to Ruan *et al.* (2019). Due to the properties of tannin, which had a beneficial protective effect on histopathological changes in the kidney of rabbits administered orally tannin 125 mg/ml, this research noted minor degenerative changes in the kidneys for the lowest concentration of tannin compared to the high concentration of tannin.

Administration of the high concentration of tannin induced mild degenerative changes: mild vascular degeneration of the epithelial cells lining

urinary tubules as well as mild vacuolation of mesangial cells of the glomerular tuft.

However, the nephron-protective property of the extract was therefore confirmed by significant improvement of the kidney architecture by reversing the nephrotoxic effects of mycotoxin such as glomerular congestion, interstitium with inflammatory cells, tubular necrosis, and others (Omar, 2018). Although the possible mechanism of its protection against mycotoxin-induced nephrotoxicity was not studied in the current study, the protective effect of the extract may be mediated through antioxidant and/or free radical scavenging activities. This is in accordance with Satrasala *et al.* (2021) who concluded that the high concentration of tannin is likely to be the cause of the observed bioactivity. Additionally, tannins have already been shown to have significant stabilizing effects on the lysosomes of experimental animals *in vitro* and *in vivo* (Dakheel *et al.*, 2021).

CONCLUSION

According to the current findings, tannin extracts modify the hematological parameters, in particular at the highest concentration used in the study (250mg/mL b.w); significant differences were obtained for RBCs and WBCs counts, but no significant variation was observed in hemoglobin levels. In addition, both tannin extract concentrations inhibited mycotoxin symptoms in rabbit liver and kidney tissues. When tannin extracts were administered orally to rabbits fed a mycotoxin-contaminated feed, they improved substantially their wellbeing.

ACKNOWLEDGEMENTS

Appreciation goes to researchers and staff members of the Department of Veterinary Public Health at the Veterinary Medicine College; University of Baghdad, Iraq.

REFERENCES

Abdel-Wareth AA, Seddik Hammad HA. Effects of *Khaya senegalensis* leaves on performance, carcass traits, hemtological and biochemical parameters in rabbits. *Excli Journal*. 2014;13:502.

Cullere, Marco, and Antonella Dalle Zotte. Rabbit meat production and consumption: State of knowledge and future perspectives. *Meat Sci*. 143 (2018): 137-146. DOI <https://doi.org/10.1016/j.meatsci.2018.04.029>

Dakheel MM, Alkandari FA, Mueller-Harvey I, Woodward MJ, Rymer C. Antimicrobial *in vitro* activities of condensed tannin extracts on avian

pathogenic *Escherichia coli*. Lett. Appl. Microbiol. 2020; 70(3):165-72. DOI <https://doi.org/10.1111/lam.13253>

Dakheel MM, Atshan OF, Abdulwahed AA. Nutritional Comparison of Different Levels of Pomegranate Peel Extracts on Production Values and Performance in Female Rabbits. Ann. Romanian Soc. Cell Biol. 2021 May 24;25(6):4094-110. DOI <https://www.annalsofscb.ro/index.php/journal/article/view/6197>

Dakheel MM, Kaur A, Al-Mnaser AA, Mueller-Harvey I, Woodward MJ, Rymer C. Assessment of the anti-pathogenic effects of condensed tannin extracts using scanning electron microscopy. Arch. Microbiol.. 2021 May;203(4):1555-63. DOI: 10.1007/s00203-020-02147-9

Ejiofor T, Mgbeahuruike AC, Ojiako C, Ushie AM, Nwoko EI, Onoja IR, Dada T, Mwanza M, Karlsson M. *Saccharomyces cerevisiae*, bentonite, and kaolin as adsorbents for reducing the adverse impacts of mycotoxin contaminated feed on broiler histopathology and hemato-biochemical changes. Vet. world. 2021 Jan;14(1):23. DOI: 10.14202/vetworld.2021.23-32

Gimeno A, Martins ML. Micotoxinas y micotoxicosis en animales y humanos. Special Nutrients, Florida. 2011:50-3. DOI <https://www.specialnutrients.com/pdf/book/3%20edicion%20MICOTOXINAS%20LR%20Secure.pdf>

Goloshvili T, Akhalkatsi M, Badridze G, Kikvidze Z. Tocopherol contents and antioxidant activity in grape pomace after fermentation and alcohol distillation. Cellular and Molecular Biology. 2021 Jan 31;67(1):112-5. DOI: 10.14715/cmb/2021.67.1.17

Hänske GG, König P, Schuhmann B, Bertram CA, Müller K. Death in four RHDV2-vaccinated pet rabbits (*Oryctolagus cuniculus*) due to rabbit haemorrhagic disease virus 2 (RHDV2). Journal of Small Animal Practice. 2021 Apr 26. DOI: <https://doi.org/10.1111/jsap.13333>

Haque MJ, Bellah MM, Hassan MR, Rahman S. Synthesis of ZnO nanoparticles by two different methods & comparison of their structural, antibacterial, photocatalytic and optical properties. Nano Express. 2020;1(1):010007. DOI <https://iopscience.iop.org/article/10.1088/2632-959X/ab7a43/meta>

Hidanah S, Sabdoningrum EK, Wahjuni RS, Chusniati S. Effects of meniran (*Phyllanthus niruri* L.) administration on leukocyte profile of broiler chickens infected with *Mycoplasma gallisepticum*. Vet. world. 2018 Jun;11(6):834. DOI: 10.14202/vetworld.2018.834-839

Iglesias-De La Cruz MC, Ruiz-Torres P, Alcamí J, Díez-Marqués L, Ortega-Velázquez R, Chen S, Rodríguez-Puyol M, Ziyadeh FN, Rodríguez-Puyol D. Hydrogen peroxide increases extracellular matrix mRNA through TGF- β in human mesangial cells. Kidney Int. 2001 Jan 1;59(1):87-95. DOI <https://doi.org/10.1046/j.1523-1755.2001.00469.x>

Junqueira LC, Bignolas G, Brentani RR. Picrosirius staining plus polarization microscopy, a specific method for collagen detection in tissue sections. *Histochem J.* 1979 Jul;11(4):447-55. DOI <https://link.springer.com/article/10.1007/BF01002772>

Juodeikiene G, Bartkiene E, Cernauskas D, Cizeikiene D, Zadeike D, Lele V, Bartkevics V. Antifungal activity of lactic acid bacteria and their application for *Fusarium* mycotoxin reduction in malting wheat grains. *Lwt.* 2018 Mar 1;89:307-14. DOI <https://doi.org/10.1016/j.lwt.2017.10.061>

Khasnabis J, Rai C, Roy A. Determination of tannin content by titrimetric method from different types of tea. *J. Chem. Pharm.* 2015; 7(6):238-41. DOI <http://jocpr.com/vol7-iss6-2015/JCPR-...>

Kovalsky P, Kos G, Nährer K, Schwab C, Jenkins T, Schatzmayr G, Sulyok M, Krska R. Co-occurrence of regulated, masked and emerging mycotoxins and secondary metabolites in finished feed and maize—An extensive survey. *Toxins.* 2016 Dec;8(12):363. DOI <https://doi.org/10.3390/toxins8120363>

Magouz FI, Dawood MA, Salem MF, Mohamed AA. The effects of fish feed supplemented with *Azolla* meal on the growth performance, digestive enzyme activity, and health condition of genetically-improved farmed tilapia (*Oreochromis niloticus*). *Ann. Anim. Sci.* 2020 Jul 1;20(3):1029-45. DOI <https://doi.org/10.2478/aoas-2020-0016>

Mueller-Harvey I. Analysis of hydrolysable tannins. *Anim. Feed Sci. Technol.* 2001 May 16;91(1-2):3-20. DOI [https://doi.org/10.1016/S0377-8401\(01\)00227-9](https://doi.org/10.1016/S0377-8401(01)00227-9)

Omar AM. The potential protective influence of flaxseed oil against renal toxicity induced by thioacetamide in rats. *Saudi J. Biol. Sci.* 2018 Dec 1;25(8):1696-702. DOI: <https://doi.org/10.1016/j.sjbs.2016.09.021>

Ruan D, Wang WC, Lin CX, Fouad AM, Chen W, Xia WG, Wang S, Luo X, Zhang WH, Yan SJ, Zheng CT. Effects of curcumin on performance, antioxidation, intestinal barrier and mitochondrial function in ducks fed corn contaminated with ochratoxin A. *Animal.* 2019 Jan;13(1):42-52. DOI: <https://doi.org/10.1017/S1751731118000678>

Saeed N, Khan MR, Shabbir M. Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts *Torilis leptophylla* L. *BMC Complement Altern. Med.* 2012 Dec;12(1):1-2. DOI:10.1186/1472-6882-12-221

Satrasala N, Peram DR, Bannoth CS. Nephroprotectivity of *Coccinia indica* leaves against gentamycin-induced nephrotoxicity using in vitro and in vivo models. *Journal of Applied Pharmaceutical Science.* 2021 Jun;11(06):132-9. DOI: 10.7324/JAPS.2021.110616

Stock W, Hoffman R. White blood cells 1: non-malignant disorders. *The Lancet.* 2000 Apr 15;355(9212):1351-7. DOI [https://doi.org/10.1016/S0140-6736\(00\)02125-5](https://doi.org/10.1016/S0140-6736(00)02125-5).