Salvia Miltiorrhiza Polysaccharides Alleviates Florfenicol-Induced Kidney Injury Via Modulation of Nuclear Factor Kappa-B (NF-κB) Signaling Pathway In Broilers

Chunyu Lu

Hebei Agricultural University

Lu Zhang

Hebei Agricultural University

Shuying Li

Hebei Agricultural University

Yuqing Cui

Hebei Agricultural University

Wanyu Shi (✉ shiwanyu2010@126.com)

Hebei Agricultural University <https://orcid.org/0000-0002-3280-6362>

Yongzhan Bao

Hebei Agricultural University

Research Article

Keywords: *Salvia miltiorrhiza* polysaccharides, Kidney injury, NF-κB signaling pathway, Chicken, Florfenicol, Anti-inflammatory

Posted Date: August 4th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-670553/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Kidney, as an important excretory organ in the body, is easily affected by various pathogenic factors. This research aimed to explore the mechanism of mitigative role of *salvia miltiorrhiza* polysaccharides (SMPs) on Florfenicol-caused renal injury in chickens. Kidney histological study and uric acid (UA) creatinine (Cr) urea nitrogen (BUN) contents in serum was performed to assess the effects of FFC and SMPs on kidneys. The expression of key factors in NF- κ B pathway were detected by RT-PCR and Western blot. We also used ELISA to detect the expression of inflammatory cytokines TNF- α , IL-1 β and IL-6 in the kidney. The results showed that SMPs could reduce the levels of UA, Cr, BUN and renal pathology of FFC-induced kidney injury, and reduce the expressions of TNF- α , IL-1 β and IL-6 in the kidney of broilers ($P < 0.05$). 5.00 g/L SMPs inhibited the expression of NF- κ Bp65 and I κ K β in renal tissue, and the expression of I κ B α was increased ($P < 0.05$). Conclusion: SMPs treatment can protect kidney injury of broilers by inhibiting NF- κ B signaling pathway.

Introduction

Kidney plays a major role in the excretion of metabolic waste and the regulation of extracellular fluid volume electrolyte composition and acid-base balance. In addition, the kidneys also synthesize and release many hormones (Sharma et al. 2020). In the broiler production process, Mycotoxin (Saleemi et al. 2020), high protein diet (Hong et al. 2020) and heat stress (Tang et al. 2020) can cause kidney injury. These can cause damage and do not lead to immediate death of broilers, but can lead to impaired kidney function, slow growth, reduced economic benefits, and no guarantee of food safety. These phenomena are not to be sniffed at.

Salvia miltiorrhiza is a traditional Chinese medicine, which has the effect of activating meridians, promoting blood circulation and dissipating blood stasis (Wang et al. 2017). The research of using *salvia miltiorrhiza* to alleviate kidney injury has made great progress in modern times. *Salvia miltiorrhiza* can reduce BUN and Cr levels in mice with lead poisoning (Han et al. 2021). *Salvia miltiorrhiza* can significantly improve the infiltration of inflammatory cells in SAP and OJ rats, protect glomerular capillaries, and reduce the shedding and necrosis of renal tubular epithelial cells (Li et al. 2014). A large number of experiments have shown that the polysaccharides of *Salvia miltiorrhiza* exhibit strong anti-inflammatory effects. Preliminarily proved that SMPs protected the inflammatory response of RAW 264.7 cells induced by LPS by significantly inhibiting the transcription of TNF- α , IL-6 and COX-2 mRNA, and the expression of NF- κ B, p-p65 and p-I κ B α proteins (Han et al. 2018). Through the study of liver injury caused by LPS/D-GalN combination, it was found that SMPs could effectively inhibit the TLR4/MyD88 inflammatory signaling pathway and inhibit excessive inflammatory response in the liver (Wang et al. 2019). SMPs can treat colitis in mice by inhibiting TLR4, mTOR, p-mTOR, NF- κ Bp65 (Peng et al. 2020).

With the deepening of clinical and experimental studies, more and more researchers have begun to realize that the prevention of multiple organ injury is one of the key measures to shorten the course of disease and reduce the mortality rate, among which the kidney is particularly important. Studies have shown that

continuous feeding 0.15 g/L FFC from 1 to 6 days of age can cause renal injury (Wang et al. 2021). Based on the important role of NF-κB in tissue injury, we speculated that the effective inflammatory response induced by FFC was due to the activation of NF-κB. In this study, the expression of NF-κB pathway and its downstream genes in renal tissues of broilers treated with SMPs were detected. Hence, in this study, we wanted to investigate mitigative effect of FFC on renal damage in a SMPs-treated chicken model in order to provide a theoretical basis for the study of renal toxicity of FFC and the mitigation of kidney inflammation caused by FFC with SMPs.

Materials And Methods

2.1 Drugs and chemicals

Flufenicol solution ($\geq 10\%$ purity) were purchased from Shandong Shenniu Animal Pharmaceutical Co., Ltd.; Hematoxylin and eosin dyeing solution was purchased from Beijing Boaotuda Technology Co., Ltd.; UA, BUN, CRE was purchased from Nanjing Jiancheng Institute of Biological Engineering; IL-6, IL-1 β , TNF- α ELISA Kit was purchased from Shanghai Enzyme-Linked Biotechnology Co., Ltd.; EASTEP® Super Total RNA Extraction Kit, Go Taq® PCR Master Mix; Go Script TM Reverse Transcription System was purchased from Promega; BCA protein detection kit was purchased from Vazyme Biotech Co., Ltd; anti- β -actin, anti-IkBa was purchased from Cell Signaling Technology; rabbit anti-p65, rabbit anti-IkK β was purchased from Bioss, anti-mouse or anti-rabbit was purchased from Beyotime.

2.2 Animal treatment and experimental design

180 one-day-old broilers were randomly divided into six groups ($n = 30$): the control group (A), the FFC group (B), the SMPs control group (C), the FFC + 1.25 g/L SMPs (D), the FFC + 2.50 g/L SMPs (E) and the FFC + 5.00 g/L SMPs (F)(Table 1).

Table 1
Experiment animal groups and treatment

Groups	0.15g /L FFC	SMPs Concentration
A	-	-
B	0.15g /L FFC	-
C	-	5.00 g/L SMPs
D	0.15g /L FFC	1.25 g/L SMPs
E	0.15g /L FFC	2.50 g/L SMPs
F	0.15g /L FFC	5.00 g/L SMPs

At weeks 6, respectively, ten birds from each group were randomly chosen to euthanize. Blood was collected from hearts to separate serum with a centrifuge at 4°C and 3500 r/min for 10 min. Kidneys were collected and were rinsed with physiological saline. Part of the kidney tissue was fixed with 4%

formaldehyde solution, and part was cryopreserved in -80°C refrigerator. The protocols of animals were permitted by the Council for Animal Care in Hebei province.

2.3 Renal function indexes in chicken serum

The separated serum was operated according to the biochemical kit of UA, BUN and Cr according to the instructions, and the content of each index in the serum was calculated.

2.4 Histological analysis

Renal tissues fixed in formaldehyde solution were dehydrated with alcohol at gradient concentration, transparent with xylene, paraffin impregnation, embedded, sectioning, and hematoxylin-eosin (H&E) staining. The morphological and structural changes of renal tissue were observed under light microscope.

2.5 Detection of inflammatory factors

10% renal tissue homogenate was prepared, and the contents of inflammatory factors in renal tissue were calculated according to IL-6, IL-1 β , TNF- α ELISA kit.

2.6 Relative mRNA expressions

Total RNA of the kidney was extracted using total RNA extraction kit. The total RNA extracted by 1 μ l was absorbed and detected on the RNA measuring instrument. The contamination and degradation of RNA quality were identified according to the value of OD260 nm/OD280 nm and OD260 nm/OD230 nm. According to the measured data, the cDNA was synthesized using the reverse transcription kit. The transcription levels of p65, I κ B α and I κ K β genes were detected by RT-QPCR using β -Action as the reference gene. The primers were designed and synthesized by Takara (Dalian, China). The setup procedure of Light Cycler® 96 automatic quantitative fluorescence PCR instrument was as follows: pre-denaturation at 95°C for 30s, and 5s at 95°C; 55 °C for 30 s; 4 cycles of 72°C 30s amplification. $2^{-\Delta\Delta C_t}$ was used to calculate the transcription level of each target gene (Table 2).

Table 2
Gene sequence and primers

Target Gene	Primer sequence(5'-3')
β-action	Forward 5'-ATTGTCCACCGCAAATGCTTC-3'
	Reversed 5'-AAATAAAGCCATGCCAATCTCGTC-3'
IkBα	Forward 5'-GTCCTGCAGGCCACCACTA-3'
	Reversed 5'-AGGTATTGACAACAGCCAGGTATC-3'
IkKβ	Forward 5'-CTGCCC GTGATGTTCTCTGA-3'
	Reversed 5'-CAGCAATGCTCCAGCTGATTC-3'
p65	Forward 5'-TGAACGGATCCGCACCAATA-3'
	Reversed 5'-CGTTCACCCACACCTGGAAG-3'

2.7 Relative protein expressions

Extraction of Total Protein from Kidney Tissue 100mg of kidney was homogenized in 1ml RIPA lysate at low temperature using a tissue homogenizer. The above renal tissue homogenate was transferred into a centrifuge tube and centrifuged at a low temperature of 4°C for 12000×g for 2min. The supernatant was collected and a small amount of the supernatant was taken for the determination of protein content. After SDS-PAGE electrophoresis, the protein samples were separated with 12% separation gel and 15% concentrate gel. The sample size was 10 μL. After the gel electrophoresis, the proteins were transferred to NC film, closed with 5% skimmed milk at 37°C for 2h, then briefly rinsed with PBST, and incubated with primary antibody at 4°C overnight. Wash the film with 1×PBST for 4 times, 15 min each, dilute secondary antibody, incubate at 37°C for 2h, wash the film with 1×PBST for 4 times, 15min each, and use ECL chemiluminescence solution for color development.

2.8 Statistical Analysis

Data were analyzed by one-way ANOVA analysis and χ^2 test (IBM SPSS Statistics 21.0, Armonk, NY, USA), and values are presented as means $\pm$ SD. Significances were established at the $P < 0.05$ level, and extremely significances established at the $P < 0.01$ level. P values are symbolized as **($P < 0.01$), *($P < 0.05$) representing significant difference compared with the control group. P values symbolized as ## ($P < 0.01$), #($P < 0.05$) representing significant difference compared with FFC treated groups.

Results

3.1 The effects of SMPs on Average Daily Gain

The *Average Daily Gain* for each group was shown in Table 3. There was no difference in broilers at 1 day of age ($P > 0.05$). At 42 days of age, the final body weight and average daily gain of the FFC group were

lower than those of the control group ($P < 0.01$). Compared with FFC group, average daily gain of broilers in 5.00 g/L SMPs group were increased ($P < 0.05$) (Table 3).

Table 3
the body index

Groups	The initial body weight /g	The final body weight /g	Average daily gain /g
A	40.40 ± 3.69	2599.60 ± 407.12	62.44 ± 9.94
B	39.96 ± 4.65	2129.75 ± 187.08**	50.95 ± 4.58**
C	38.05 ± 3.33	2528.23 ± 174.34	60.75 ± 4.23
D	40.46 ± 2.76	2385.83 ± 171.04	57.21 ± 4.13
E	38.61 ± 3.75	2265.93 ± 118.89	54.27 ± 2.87
F	39.93 ± 3.87	2365.47 ± 99.91	59.93 ± 5.99 [#]
Note: P values are symbolized as **($P < 0.01$), representing significant difference compared with the control group. P values symbolized as # ($P < 0.05$) representing significant difference compared with FFC treated groups.			

3.2 The effects of SMPs on the Biochemical Indexes

We examined renal function in broilers at 42 days of age. Compared with the control group, the Cr, BUN and UA was significantly increased ($P < 0.05$) or ($P < 0.01$). The contents of Cr, UA and BUN in serum were decreased ($P < 0.05$) or ($P < 0.01$) in the 5.00 g/L SMPs. The contents of Cr and BUN in 2.50g/L SMPs were decreased ($P < 0.05$), and the contents of Cr in 1.25g/L SMPs were decreased ($P < 0.05$) (Fig. 1).

3.3 Histological changes

Kidney histological study was performed to assess the effects of FFC and/or SMPs on kidneys at week 6. For control group (Fig. 2a) and SMPs control group (Fig. 2c), tubular epithelial cells (TEC) were clearly visible. For FFC group (Fig. 2b), we found the following histological changes: inflammatory infiltration (II), vacuole (Va), erythrocyte blood cell (RBC) and the degeneration of tubular epithelial cells (DTEC). For 1.25g/l and 2.50g/L SMPs (Fig. 2d and Fig. 2e) vacuole and the erythrocyte blood cells were also observed. However, 5.00 g/L SMPs was better (Fig. 2f), and the degree of cell inflammatory infiltration was less, which was close to the kidney tissue morphology of control group.

3.4 The effects of SMPs on the levels of IL-6, IL-1 β and TNF- α

Compared with the control group, the renal inflammatory factors in the FFC group were all up-regulated ($P < 0.01$) or ($P < 0.05$), and there was no difference in the SMPs control group. Compared with the model group, IL-6 could be inhibited by all doses ($P < 0.05$) (Fig. 3a). The levels of TNF- α were decreased by 1.25 g/L and 2.50 g/L SMPs ($P < 0.05$) (Fig. 3c). 5.00 g/L SMPs decreased the level of IL-1 β ($P < 0.01$) (Fig. 3b).

3.5 The effects of SMPs on the mRNA relative expression of NF- κ Bp65, I κ B α and I κ K β .

Compared with control group, the mRNA expression of NF- κ Bp65 and I κ K β in kidney tissue of the FFC group was increased (Fig. 4b and Fig. 4c), and the expression of I κ B α mRNA was decreased ($P < 0.05$) or ($P < 0.01$) (Fig. 4a), but no difference was found in the SMPs control group. Compared with FFC group, the mRNA expression levels of NF- κ Bp65 and I κ K β in SMPs groups were decreased (Fig. 4b and Fig. 4c), while the mRNA expression levels of I κ B α mRNA in 5.00 g/L SMPs group were increased ($P < 0.05$) (Fig. 4a).

3.6 The effects of SMPs on the protein expression of NF- κ Bp65, I κ B α and I κ K β

Compared with control group, FFC caused the degradation of I κ B α protein ($P < 0.05$). The 5.00 g/L SMPs significantly inhibited the degradation of I κ B α protein induced by FFC ($P < 0.05$). FFC significantly increased the expression of p65 and I κ k β protein ($P < 0.05$), while 5.00 g/L SMPs significantly decreased the expression of p65 and I κ k β protein ($P < 0.05$). In addition, the 2.50 g/L SMPs significantly decreased the expression of I κ K β protein ($P < 0.05$) (Fig. 5).

Discussion

Inflammatory reaction is one of the most common renal dysfunction in broilers during production. there is no reliable drug prevention and treatment and the prognosis is poor. NF- κ B signaling pathway is one of the most important mechanisms leading to renal injury. SMPs has anti-inflammatory effect, can reduce the inflammatory response of a variety of diseases, and has the effect of inhibiting the inflammatory signal pathway. Studies have shown that SMPs can inhibit the expression of NF- κ B induced by oxidative stress and reduce LPS-induced liver injury. Based on the above mechanisms, this study was conducted to investigate whether SMPs has protective effects on kidney injury in broilers, and whether its protective effects on kidney injury in broilers are mediated by inhibition of NF- κ B signaling pathway.

In this study, a broiler model with kidney injury was successfully established by drinking 0.15 g/L FFC mixed water for 5 days. Previous studies found that FFC exposure significantly inhibited the growth performance of broilers. (Han et al. 2021). Compared with model group, SMPs dosage groups increased the daily gain of broilers in different degrees. In addition, there was no difference between the control group and the SMPs control group, but the standard deviation was smaller, indicating that the growth difference of the chicken group was smaller, and it was more suitable for collective breeding. A large number of Traditional Chinese medicine polysaccharides were found to have the effect of promoting weight gain. Studies have shown that APS can increase body weight, feed conversion rate and digestive enzyme activities of broilers. (Wu. 2018.). SMWP-U&E can improve digestion and nutrient absorption of weaned piglets, and have beneficial effects on intestinal morphology and microbiota (Jiang et al. 2020). Therefore, we found that SMPs can promote the growth of broilers.

The kidney has the function of circulation and excretion, and is the target organ of poisons (Rubatto Birri et al. 2010). Exposure to FFC causes microscopic changes in renal tissue, including congestion, swelling, and tubular shape. This is consistent with previous research. (Wang et al. 2021) Cr, UA and BUN are commonly used to assess the progression of kidney disease. Anhydride is a chemical waste produced by muscles breaking down a compound called creatinine. As the blood passes through the kidneys, Cr is filtered and excreted in the urine (Baum et al. 1975). Elevated serum Cr level suggests impaired glomerular filtration function in broilers. Wang et al. found that FFC can cause the increase of serum BUN in chickens and then cause kidney injury. Our results are consistent with previous research results (Wang et al. 2021). Renal uric acid excretion is determined by the balance between uric acid reabsorption and resecretion (Bobulescu et al. 2012). High protein diet can increase serum UA levels and cause kidney damage in chickens (Guo et al. 2005). BUN is the end product of protein metabolism in the body. If renal parenchyma is damaged and glomerular filtration rate drops to the critical point, the level of BUN in the blood will increase. In this experiment, the serum Cr, UA and BUN of broilers was significantly increased. These results suggest that FFC exposure has a negative effect on kidney growth and causes kidney damage in chickens. The results showed that different doses of SMPs could improve the kidney injury induced by FFC, and reduce the levels of Cr and BUN in broilers with kidney injury. With the increase of SMPs, the levels of Cr and BUN decreased more obviously. At the same time, SMPs can improve the pathological changes of renal tubules in broilers with kidney injury. Under light microscope, the kidney injury of broilers pretreated with different doses of SMPs was significantly improved, and with the increase of SMPs, the kidney of control group was more likely. The results showed that SMPs could reduce the kidney injury induced by FFC. In addition, SMPs has no negative effects on the kidneys.

Nuclear factor- κ B (NF- κ B) is a transcription factor that regulates immune and inflammatory responses under various conditions and plays a key role in inflammation and immune responses (Morgan and Liu 2011; Oeckinghaus et al. 2011) The activation of NF- κ B is mainly divided into normative pathway and non-normative pathway. Typical pathways are induced by most physiological NF- κ B stimuli, such as cytokine receptors, antigen receptors, and pattern recognition receptors. This pathway depends on the catalytic activities of I κ K β and mainly leads to the phosphorylation of I κ B α and the nuclear translocation of most heterodimer containing p65. In this study, we found that FFC-induced renal injury promoted NF- κ B phosphorylation and expression level of I κ B α protein degradation. I κ B α is the main protein that activates NF- κ B. NF- κ B is isolated from the cytoplasm by direct interaction with inhibitor proteins of the I κ B family, such as I κ B α . I κ B α phosphorylation and degradation are critical for NF- κ B activation, leading to rapid translocation of NF- κ B from cytoplasm to nucleus (Viatour et al. 2005). These results suggest that the activation of NF- κ B may be the cause of renal inflammation in broilers. However, the expression of NF- κ Bp65 and I κ K β in the renal tissue of FFC-induced acute kidney injury model could be inhibited by different doses of SMPs, and the inhibition effect was more obvious with the increase of dose. Studies have shown that SMPs can inhibit I κ B α degradation, down-regulate the nuclear translocation of NF- κ B, and play an anti-inflammatory role in liver injury in RAW264.7 Cells (Han et al. 2018). This study suggests that the protective effect of SMPs on FFC-induced nephrotoxicity in broilers may be related to the reduction of NF- κ B activation.

The expression level of NF- κ B can directly affect the transcription of proinflammatory factors. Over-activation of proinflammatory factors can induce the strong expression of TNF- α , IL-6 and ICAM-1, thereby promoting multi-organ cytotoxicity (Wen et al. 2020). Complex inflammation is regulated by a network of cytokines and is evidenced by the production of many pro-inflammatory markers, IL-6, IL-1 β , and TNF- α . Some studies have found that renal injury in broilers is associated with excessive release of pro-inflammatory factors (TNF- α , IL-1 β , IL-6) (Wang et al. 2017). In this study, it was found that the levels of TNF- α , IL-1 β , IL-6 and other inflammatory factors in the kidney of broilers in the model group increased significantly. SMPs can reduce the overexpression of TNF- α , IL-1 β and IL-6. This is consistent with whose research results (Han et al. 2018). The results showed that different doses of SMPs could significantly increase the expression of I κ B α protein, reduce the expression of I κ K β protein and p65 protein into the nucleus of kidney tissue of broilers, and reduce the production of TNF- α , IL-1 β , IL-6 and other pro-inflammatory factors, so as to alleviate the renal injury induced by FFC. This was consistent with a decrease in TNF- α , IL1 β , and IL-6 levels in the kidney. Through the above studies, it was preliminarily concluded that the mechanism of SMPs inhibiting the production of pro-inflammatory factors TNF- α , IL-1 β , IL-6 and alleviating renal injury might be related to the inhibition of NF- κ B signaling pathway.

Conclusion

Our study reinforces the viewpoint that SMPs can improve the Cr and BUN levels of broilers induced by FFC, reduce renal tubular injury, and improve the renal injury induced by FFC. On the other hand, SMPs can alleviate renal injury by down-regulating FFC-induced I κ K β expression and then increasing I κ B α expression, thereby reducing p65 entry into the nucleus and reducing the production of pro-inflammatory factors TNF- α , IL-1 β , and IL-6. This study provides a theoretical basis for the treatment of kidney injury in broilers with SMPS.

Declarations

Ethical Approval

The use of animals in this study was approved by Hebei Animal Protection Association. (Permission number: AUH-2020141)

Consent to Participate

Informed consent was obtained from all individual participants included in the study.

Consent to Publish

Consent was obtained from participants about publishing their data.

Authors Contributions

Wanyu Shi and Yongzhan Bao conceived and designed the study, and they rigorously revised the manuscript. Chunyu Lu performed most of the experiments and analyzed the results and drafted the manuscript. Lu Zhang and Shuying Li helped collect samples and participated in the H&E and qPCR experiment. Yuqing Cui participated in the detection and result analysis of biochemical indicators. All authors read and approved the final manuscript.

Funding information

The authors are thankful to the Natural Science Foundation of Hebei Province, China (C2021204026) for the support in this work.

Competing Interests

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.

Availability of data and materials

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Authors' conflicts

The authors have no conflicts of interest.

Acknowledgements

The authors are thankful to Natural Science Foundation of Hebei Province, China (C2021204026).

References

1. Baum N, Dichoso CC, Dichoso CC, Carlton CE (1975) Blood urea nitrogen and serum creatinine. Physiology and interpretations.5(5):583-588. [https://doi.org/10.1016/0090-4295\(75\)90105-3](https://doi.org/10.1016/0090-4295(75)90105-3)
2. Bobulescu IA, Moe OW (2012) Renal transport of uric acid: evolving concepts and uncertainties. Adv Chronic Kidney Dis.19(6):358–371. <https://doi.org/10.1053/j.ackd.2012.07.009>
3. Guo X, Huang K, Tang J (2005) Clinicopathology of gout in growing layers induced by high calcium and high protein diets. Br PoultSci.46(5):641-646. <https://doi.org/10.1080/00071660500302661>
4. Han C, Wang X, Zhang D, Wei Y, Cui Y, Shi W, Bao Y (2021) Synergistic use of florfenicol and Salvia miltiorrhiza polysaccharide can enhance immune responses in broilers. Ecotoxicol Environ Saf 210:111825. <https://doi.org/10.1016/j.ecoenv.2020.111825>
5. Han C, Jiang YH, Li W, Liu Y (2020) Astragalus membranaceus and Salvia miltiorrhiza ameliorates cyclosporin A-induced chronic nephrotoxicity through the "gut-kidney axis". J Ethnopharmacol 269:113768. <https://doi.org/10.1016/j.jep.2020.113768>

6. Han C, Yang J, Song P, Wang X, Shi W (2018) Effects of *Salvia miltiorrhiza* Polysaccharides on Lipopolysaccharide-Induced Inflammatory Factor Release in RAW264.7. Cells J Interferon Cytokine Res 38(1):29-37. <https://doi.org/10.1089/jir.2017.0087>
7. Hong F, Zheng A, Xu P, Wang J, Xue T, Dai S, Pan S, Guo Y, Xie X, Li L, Qiao X, Liu G, Zhai Y (2020) High-Protein Diet Induces Hyperuricemia in a New Animal Model for Studying Human Gout. Int J Mol Sci 21(6):2147. <https://doi.org/10.3390/ijms21062147>
8. Jiang YY , Li YB, Yu J, Chen H, Zhou J, Wang L, Zhang L, Zhao MJ, Zhou YH, Yu L (2020) Preliminary structure and bioactivities of polysaccharide SMWP-U&E isolated from *Salvia miltiorrhiza* Bunge Residue. Int J Biol Macromol.157:434-443.<https://doi.org/10.1016/j.ijbiomac.2020.04.092>
9. Li L, Zhang Y, Ma J, Dong W, Song Q, Zhang J, Chu L (2014) *Salvia miltiorrhiza* injection ameliorates renal damage induced by lead exposure in mice. Scientific World Journal 2014:572697-572697. <https://doi.org/10.1155/2014/572697>
10. Morgan MJ, Liu ZG (2011) Crosstalk of reactive oxygen species and NF- κ B signaling. Cell Res. 21(1):103-115. 10.1038/cr.2010.178
11. Oeckinghaus A, Hayden MS, Ghosh S (2011) Crosstalk in NF- κ B signaling pathways . Nat Immunol. 12(8):695-708. <https://doi.org/10.1038/ni.2065>
12. Peng KY, Gu JF, Su SL, Zhu Y, Guo JM, Qian DW, Duan JA (2020) *Salvia miltiorrhiza* stems and leaves total phenolic acids combination with tanshinone protect against DSS-induced ulcerative colitis through inhibiting TLR4/PI3K/AKT/mTOR signaling pathway in mice. J Ethnopharmacol 264:113052. <https://doi.org/10.1016/j.jep.2020.113052>
13. Rubatto Birri PN, Pérez RD, Cremonezzi D, Pérez CA, Rubio M, Rubio M, Bongiovanni GA (2010) Association between As and Cu renal cortex accumulation and physiological and histological alterations after chronic arsenic intake. Environ Res.110(5):417-423.<https://doi.org/10.1016/j.envres.2009.09.002>
14. Saleemi MK, Ashraf K, Gul ST, Naseem MN, Sajid MS, Mohsin M, He C, Zubair M, Khan A (2020) Toxicopathological effects of feeding aflatoxins B1 in broilers and its ameliosration with indigenous mycotoxin binder.Ecotoxicol Environ Saf.187.<https://doi.org/10.1016/j.ecoenv.2019.109712>
15. Sharma S, Baboota S, Amin S, Mir SR (2020) Ameliorative effect of a standardized polyherbal combination in methotrexate-induced nephrotoxicity in the rat. Pharm. Biol. 58, 184-199. <https://doi.org/10.1080/13880209.2020.1717549>
16. Tang SA-O, Zhou S, Yin B, Xu J, Di L, Zhang J, Bao E (2020) Heat stress-induced renal damage in poultry and the protective effects of HSP60 and HSP47.23(5):1033-1040.<https://doi.org/10.1007/s12192-018-0912-3>
17. Viatour P, Merville MP, Bours V, Chariot A (2005) Phosphorylation of NF-kappaB and IkappaB proteins: implications in cancer and inflammation. Trends Biochem Sci. 30(1):43-52.<https://doi.org/10.1016/j.tibs.2004.11.009>
18. Wang L, Ma R, Liu C, Liu H, Zhu R, Guo S, Tang M, Li Y, Niu J, Min F (2017) *Salvia miltiorrhiza*: A Potential Red Light to the Development of Cardiovascular Diseases. Curr Pharm Des. 23(7):1077-

1097. <https://doi.org/10.2174/1381612822666161010105242>

19. Wang X, Han C, Cui Y, Geng Y, Wei Y, Shi W, Bao Y (2021) Florfenicol induces renal toxicity in chicks by promoting oxidative stress and apoptosis. *Environ Sci Pollut Res Int.* 207:111282. <https://doi.org/10.1007/s11356-020-10550-4>
20. Wang X, Han C, Cui Y, Li S, Jin G, Shi W, Bao Y (2021) Florfenicol causes excessive lipid peroxidation and apoptosis induced renal injury in broilers. *Ecotoxicol Environ Saf.* 28(1):936-946. <https://doi.org/10.1016/j.ecoenv.2020.111282>
21. Wang X, Han C, Qin J, Wei Y, Qian X, Bao Y, Shi W (2019) Pretreatment with *Salvia miltiorrhiza* Polysaccharides Protects from Lipopolysaccharides/d-Galactosamine-Induced Liver Injury in Mice Through Inhibiting TLR4/MyD88 Signaling Pathway. *J Interferon Cytokine Res.* 39(8):495-505. <https://doi.org/10.1089/jir.2018.0137>
22. Wang Y, Zhao H, Shao Y, Liu J, Li J, Xing M (2017) Copper or/and arsenic induce oxidative stress-cascaded, nuclear factor kappa B-dependent inflammation and immune imbalance, triggering heat shock response in the kidney of chicken. *Oncotarget.*8(58):98103-98116. <https://doi.org/10.18632/oncotarget.21463>
23. Wen Y, Rudemiller NP, Zhang J, Robinette T, Lu X, Ren J, Privratsky JR, Nedospasov SA, Crowley SD (2020) TNF- α in T lymphocytes attenuates renal injury and fibrosis during nephrotoxic nephritis. *Am J Physiol Renal Physiol.*318(1):F107-F116. <https://doi.org/10.1152/ajprenal.00347.2019>
24. Wu S (2018) Effect of dietary *Astragalus membranaceus* polysaccharide on the growth performance and immunity of juvenile broilers. *Poult Sci.*97(10):3489-3493. <https://doi.org/10.3382/ps/pey220>

Figures

Figure 1

We examined renal function in broilers at 42 days of age. Compared with the control group, the Cr, BUN and UA was significantly increased ($P<0.05$) or ($P<0.01$). The contents of Cr, UA and BUN in serum were decreased ($P<0.05$) or ($P<0.01$) in the 5.00 g/L SMPs. The contents of Cr and BUN in 2.50g/L SMPs were decreased ($P<0.05$), and the contents of Cr in 1.25g/L SMPs were decreased ($P<0.05$).

Figure 2

Kidney histological study was performed to assess the effects of FFC and/or SMPs on kidneys at week 6. For control group (Fig.a) and SMPs control group (Fig.c), tubular epithelial cells (TEC) were clearly visible. For FFC group (Fig.b), we found the following histological changes: inflammatory infiltration (II), vacuole (Va), erythrocyte blood cell (RBC) and the degeneration of tubular epithelial cells (DTEC). For 1.25g/l and 2.50g/L SMPs (Fig.d and Fig.e) vacuole and the erythrocyte blood cells were also observed. However, 5.00 g/L SMPs was better(Fig.f), and the degree of cell inflammatory infiltration was less, which was close to the kidney tissue morphology of control group.

Figure 3

Compared with the model group, IL-6 could be inhibited by all doses ($P<0.05$) (Fig.a). The levels of TNF- α were decreased by 1.25 g/L and 2.50 g/L SMPs ($P<0.05$) (Fig.c). 5.00 g/L SMPs decreased the level of IL-1 β ($P<0.01$) (Fig.b).

Figure 4

Compared with control group, the mRNA expression of NF- κ Bp65 and $\text{IkB}\beta$ in kidney tissue of the FFC group was increased (Fig.b and Fig.c), and the expression of $\text{IkB}\alpha$ mRNA was decreased ($P < 0.05$) or ($P < 0.01$) (Fig.a), but no difference was found in the SMPs control group. Compared with FFC group, the mRNA expression levels of NF- κ Bp65 and $\text{IkB}\beta$ in SMPs groups were decreased (Fig.b and Fig.c), while the mRNA expression levels of $\text{IkB}\alpha$ mRNA in 5.00 g/L SMPs group were increased ($P < 0.05$) (Fig.a).

Figure 5

Compared with control group, FFC caused the degradation of $\text{IkB}\alpha$ protein ($P < 0.05$). The 5.00 g/L SMPs significantly inhibited the degradation of $\text{IkB}\alpha$ protein induced by FFC ($P < 0.05$). FFC significantly increased the expression of p65 and $\text{IkK}\beta$ protein ($P < 0.05$), while 5.00 g/L SMPs significantly decreased the expression of p65 and $\text{IkK}\beta$ protein ($P < 0.05$). In addition, the 2.50 g/L SMPs significantly decreased the expression of $\text{IkK}\beta$ protein ($P < 0.05$).