

## The Effects of the Concomitant Use of Caffeine and Streptomycin on Histopathological Changes in the Kidney and Liver of Rats

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### ABSTRACT

**BACKGROUND AND OBJECTIVE:** Considering the possibility of increased renal toxicity induced by streptomycin in tea and coffee, which contain methylxanthines such as caffeine, we aimed to investigate the histopathological changes in the kidney and liver of rats. We also evaluated some serum parameters after the concurrent use of streptomycin and caffeine.

**METHODS:** This experimental study was performed on 25 female Wistar rats, with the average weight of 200 g; the rats were divided in five groups. Group I was regarded as the control group, group II received streptomycin (25 mg/kg), group III was administered streptomycin+caffeine (5 mg/kg), group IV received caffeine (5 mg/kg) and group V received streptomycin + vitamin C (100 mg/kg) for a period of 21 days. Afterwards, the rats were anesthetized with ether and blood samples were obtained from the heart. Serum creatinine, blood urea nitrogen, sodium and potassium levels were measured in rats. After preparing and sectioning renal and hepatic tissues for pathological examinations, they were stained by hematoxylin and eosin (H&E); the samples were examined, using a light microscope.

**FINDINGS:** Serum creatinine and potassium levels were not significantly different between the groups, while urea nitrogen level in group V ( $15 \pm 0.7$  mg/dL) was significantly lower than the values reported in groups I and IV ( $p < 0.05$ ). Sodium level increased in group II ( $139.8 \pm 0.2$  mEq/L) and group IV ( $140 \pm 0.447$  mEq/L), while a significant reduction was reported in group V ( $137.25 \pm 0.75$  mEq/L) ( $p < 0.05$ ). According to the findings, hepatic and renal damages were severe after the concomitant use of streptomycin and caffeine. However, vitamin C reduced the induced damages.

**CONCLUSION:** As the results indicated, caffeine could aggravate renal injuries, induced by streptomycin, while vitamin C had an alleviating effect.

**KEY WORDS:** Streptomycin, Caffeine, Kidney, Liver, Rats.

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## Introduction

Caffeine is a member of methylxanthine family. Xanthine compounds act as phosphodiesterase inhibitors and increase cyclic adenosine monophosphate (cAMP), resulting in the expansion of smooth muscles. Xanthine compounds by increasing cAMP in the heart lead to positive chronotropic and inotropic effects and cause tachycardia. In the lungs, these compounds prevent the entrance of calcium to the cells and lead to the expansion of smooth muscles (bronchodilation).

Methylxanthine, especially caffeine, in small and medium amounts, lead to the mild cortical arousal of brain tissues by increasing consciousness and reducing fatigue. Higher levels of methylxanthine are required for inducing bronchodilation, which typically leads to restlessness and tremor in some patients. Methylxanthines should not be prescribed in patients with acute asthma, since they do not induce major bronchodilatory effects, compared to standard treatments. Also, the consumption of these compounds may be associated with substantial morbidity (1, 2). Caffeine as a mild stimulant is the most commonly used psychoactive drug in the world. Caffeine is present in non-alcoholic drinks, coffee, tea, cocoa, chocolate and many prescribed or non-prescribed medicines. This stimulant slightly increases norepinephrine and dopamine secretion and augments neural activity in many regions of the brain (3, 4). Many effects of caffeine occur via adenosine receptor antagonism. Adenosine is a neuromodulator, which can affect multiple functions of the central nervous system. Activation of specific adenosine receptor subgroups leads to moderate relieving effects and neutralizes the effects of caffeine (5, 6). The cytotoxicity of caffeine may be due to its ability to trigger apoptosis. Moreover, reactive oxygen species, produced by caffeine, cause cellular damage in apoptosis and necrosis (6).

Streptomycin is an aminoglycoside antibiotic that can destroy Gram-positive and Gram-negative bacteria by inhibiting their protein synthesis. Streptomycin is one of the important antibiotics in the treatment of brucellosis and tuberculosis. The most important side-effect of this medicine is renal toxicity, especially in patients with renal failure. Moreover, it can cause ototoxicity even in the fetus (1, 7, 8).

Considering the importance of caffeine consumption and the possibility of streptomycin prescription for infectious diseases (e.g., brucellosis and tuberculosis), we evaluated renal and hepatic

histopathological changes, as well as some serum parameters in rats. We also aimed to determine how the concurrent use of caffeine and streptomycin may affect hepatic and renal tissues. Moreover, the effectiveness of vitamin C as an antioxidant in reducing the possible damages was evaluated.

## Methods

This experimental study was performed on 25 Wistar rats with an average weight of 200 g. The rats were divided into five groups (five rats each). Group I was regarded as the control group, group II received streptomycin (25 mg/kg), group III was administered streptomycin + caffeine (5 mg/kg), group IV received caffeine (5 mg/kg) and group V received streptomycin + vitamin C (100 mg/kg). The medicines were given intraperitoneally on a daily basis for a period of 21 days.

Afterwards, the rats were anesthetized with ether and blood samples were obtained from the heart for separating the serum. By maintaining anesthesia in rats, they were spared and the abdominal regions were cut open. Renal and hepatic tissues were sampled for pathological examination via routine sampling. Via common methods, cross-sectioning of the liver and kidney was conducted. The slides were stained by hematoxylin and eosin (H&E) and were evaluated using a light microscope to determine pathological changes including necrosis and hemorrhage.

Serum creatinine, blood urea nitrogen (BUN), sodium and potassium levels were measured, using Pars Azmoon kit and an autoanalyzer. Serum parameters were compared between the groups, using SPSS version 16. ANOVA and post-hoc Least Significant Difference (LSD) were applied for performing comparisons. The mean values ( $\pm$  standard error) were calculated, and P-value less than 0.05 was considered statistically significant.

## Results

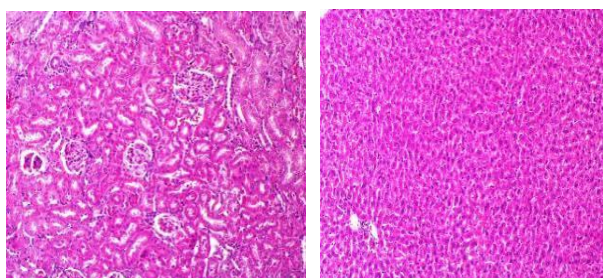
Serum creatinine and potassium levels were not significantly different between the groups. However, BUN level in group V (receiving streptomycin + vitamin C) was significantly lower than values reported in group I (control group) and group IV (receiving caffeine alone) ( $p < 0.05$ ). In fact, BUN level was  $18.25 \pm 0.854$  mg/dL in group I and  $17.6 \pm 0.927$  mg/dL in group IV; also, BUN level decreased to  $15 \pm 0.707$  mg/dL in group V (table 1).

**Table 1. The mean±SE of biochemical serum parameters in rats (letters indicate significant differences between the groups) ( $p<0.05$ ) (n=6)**

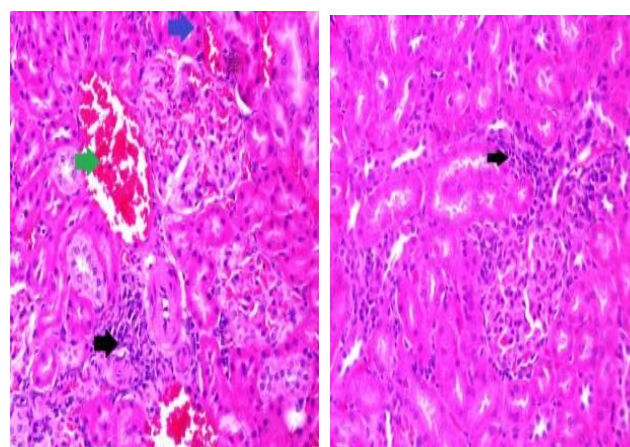
| Groups                 | Ceratinine (mg/dL) | BUN (mg/dL)              | Sodium (mEq/L)           | Potassium (mEq/L) |
|------------------------|--------------------|--------------------------|--------------------------|-------------------|
| Control                | 0.55±0.05          | 18.25±0.854 <sup>a</sup> | 138.5±0.289 <sup>a</sup> | 4.1±0.1826        |
| Streptomycin           | 0.56±0.04          | 17.2±0.735 <sup>ac</sup> | 139.8±0.2 <sup>b</sup>   | 4.26±0.051        |
| P-value                | 0.819              | 0.408                    | 0.607                    | 0.858             |
| streptomycin+ caffeine | 0.5±0              | 16.2±0.97 <sup>ac</sup>  | 138.6±0.5 <sup>b</sup>   | 3.94±0.1778       |
| P-value                | 0.156              | 0.404                    | 0.072                    | 0.705             |
| Caffeine               | 0.56±0.0345        | 17.6±0.927 <sup>ab</sup> | 140±0.447 <sup>cd</sup>  | 5.58±1.2138       |
| P-value                | 0.156              | 0.247                    | 0.039                    | 0.064             |
| Streptomycin+vitamin C | 0.5±0              | 15±0.707 <sup>c</sup>    | 137.25±0.75 <sup>b</sup> | 4.125± 0.2175     |
| P-value                | 1                  | 0.05                     | 0.001                    | 0.116             |

Serum sodium level significantly increased in group II (receiving streptomycin alone) and group IV ( $p<0.05$ ); the corresponding values were  $139.8\pm0.2$  and  $140\pm0.447$  mEq/L, respectively. However, serum sodium level in group V (receiving streptomycin and vitamin C) reduced to  $137.25\pm0.75$  mEq/L ( $p<0.05$ ) (table 1).

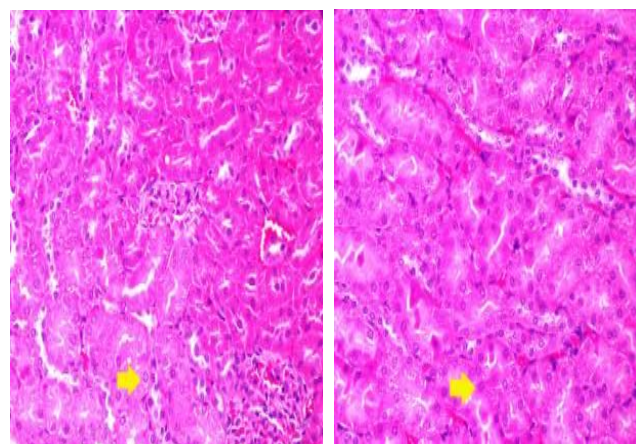
In the assessment of renal tissues, streptomycin was noted to cause cell swelling, interstitial nephritis, cell inflammation, necrosis and hyaline cast formation in the urinary tract. Use of streptomycin along with caffeine aggravated the above-mentioned damages and was associated with tubular necrosis and severe congestion. However, caffeine alone had a slight effect on renal tissues and the damages were not as severe. Moreover, vitamin C reduced the damages and renal tissues were almost intact (fig. 1A-1C). Streptomycin did not have any significant effects on histological changes in the liver. Only an insignificant coagulation necrosis and infiltration of mononuclear cells were reported. However, caffeine alone led to the significant formation of lipid vacuoles, cell swelling and reduced sinusoids. The same findings were reported by the concurrent use of caffeine and streptomycin. Also, vitamin C could reduce the induced damages (fig 1A, 1D and 1E).



**Figure 1A. The microscopic view of the kidney (left) and liver (right) in control rats (H&E×10)**

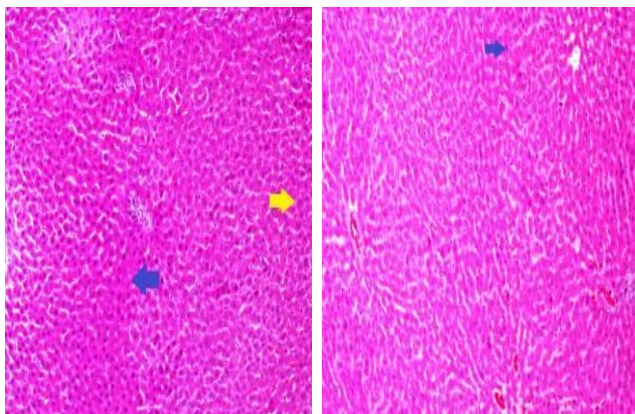


**Figure 1B. The microscopic view of the kidney in group II (right): the black arrow indicates interstitial nephritis; the microscopic view of kidney in group III (left): the blue, black and green arrows suggest necrosis, interstitial nephritis and congestion, respectively (H&E × 20)**

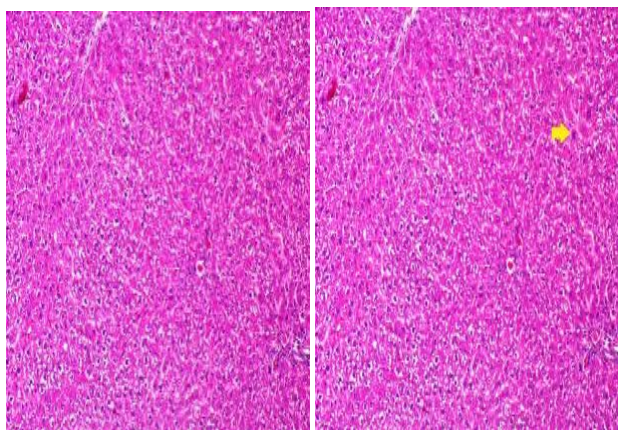


**Figure 1C. The microscopic view of the kidney in group IV (right) and group V (left): the yellow arrows indicate cell swelling (H&E × 20)**





**Figure 1D. The microscopic view of the liver in group II (right) and group III (left): the blue arrows indicate necrosis in group II and regional necrosis in group III; the yellow arrow shows cell swelling in group III (H&E×10)**



**Figure 1E. The microscopic view of the liver in group IV (right): the yellow arrow indicates cell swelling; the microscopic view of the liver in group V (left): a normal tissue is observed (H&E×10)**

## Discussion

In this study, regular use of streptomycin for 21 days resulted in considerable histopathological changes in the kidney; this effect was exacerbated by the simultaneous intake of caffeine. However, caffeine alone did not cause significant changes in renal tissues. On the other hand, the concomitant use of vitamin C and streptomycin could reduce renal injuries.

Streptomycin can probably cause renal injuries through the induction of oxidative stress by damaging renal tubule cell membranes and organelles such as

mitochondria and lysosome. These changes are caused by aminoglycoside antibiotics (9,10).

In some studies, the effects of gentamicin in oxidative stress changes and the protective effects of some antioxidants such as vitamins C and E and silymarin have been assessed. Unfortunately, there are no similar studies on this subject. However, in a previous study, streptomycin showed low potential for liver damage, although the induced renal toxicity could lead to hepatic toxicity. Also, patients with hepatic failure showed renal toxicity induced by streptomycin earlier than other individuals (11).

In the present study, caffeine alone had no effects on renal tissues, although it increased the effect of streptomycin. The exacerbation of renal toxicity has been reported in some medicines. For instance, Champion et al. demonstrated that caffeine increases renal toxicity induced by mefenamic acid in rats via an unknown mechanism (12).

On the other hand, in some studies, caffeine was shown to have protective effects. In a study in 2010, the administration of caffeine alone or along with fluride in one month reduced oxidative stress induced by fluride in different tissues (such as renal tissues) and acted as an antioxidant. It should be mentioned that caffeine in the aforementioned study was administered in water for mice (13).

The discrepancy in the findings may be related to the difference in the type of toxicity (streptomycin) or the route of administration (injection) in our study. In the current study, streptomycin did not affect liver tissues, whereas caffeine led to the excessive formation of fat vacuoles, cell swelling and reduced sinusoids. Consistent with our study, Ohta et al. showed that caffeine can exacerbate hepatic damages in a weakened immune system (14).

The concomitant use of streptomycin and caffeine had no effects on hepatic injuries, induced by caffeine; however, vitamin C reduced the histopathological effects.

According to the present findings, it can be stated that hepatic and renal lesions were more severe by the concomitant use of streptomycin and caffeine, compared to their separate administration. Moreover,

vitamin C could reduce these injuries. Therefore, it can be stated that caffeine can exacerbate renal damages, induced by streptomycin.

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## References

1. Goodman LS, Limbird LE, Milinoff PB, Ruddon RW, Goodman Gilman A, Editors. Goodman and Gilman's: The Pharmacological Basis of Therapeutics. 9<sup>th</sup> ed. Mc Graw Hill (Tx);1996. p.396,592.
2. Waldman SA, Terzic A. Pharmacology and therapeutic: principles to practice. 1<sup>st</sup> ed. USA:Sanders/Elsevier; 2009. p.428-9.
3. Hoesch RE, Weinreich D, Kao JPY. A novel  $Ca^{2+}$  influx pathway in mammalian primary sensory neurons is activated by caffeine. *J Neurophysiol.* 2001;86(1):190-6.
4. Marret S, Gressens P, Van-Maele-Fabry G, Picard J, Evrard P. Caffeine induced disturbance of early neurogenesis in whole mouse embryo cultures. *Brain Res.*1997;773(1-2):213-6.
5. He Z, Ma WY, Hashimoto T, Bode AM, Yang CS, Dong Z. Induction of apoptosis by caffeine is mediated by the p53, Bax, and caspase 3 pathways. *Cancer Res.* 2003;63(15):4396-401.
6. Lu PZ, Lai CY, Chan WH. Caffeine induces cell death via activation of apoptotic signal and inactivation of survival signal in human osteoblasts. *Int J Mol Sci.* 2008;9(5):698-718.
7. Mingeot-Leclercq MP, Tulkens PM. Aminoglycosides: nephrotoxicity. *Antimicrob Agents Chemother.* 1999; 43:1003–12.
8. Briggs GG, Freeman RK, Yaffe SJ. Drug in pregnancy and lactation: A reference guide to fetal and neonatal risk. 8<sup>th</sup>ed. USA:Lippincott Williams and Wilkins; 2008.p. 228-33.
9. Kumar M, Kakkar V, Mishra AK, Chuttani K, Kaur IP. Intranasal delivery of streptomycin sulfate (STRS) loaded solid lipid nanoparticles to brain and blood. *Int J Pharm.* 2014. 461(1-2):223-33.
10. Williams PD, Bennett DB, Gleason CR, Hottendorf GH. Correlation between renal membrane binding and nephrotoxicity of aminoglycosides. *Antimicrob Agents Chemother.* 1987;31(4):570-4.
11. Andrade RJ, Tulkens PM. Hepatic safety of antibiotics used in primary care. *J Antimicrob Chemother.* 2011;66(7): 1431-46.
12. Champion de Crespigny P, Hewitson T, Birchall I, Kincaid-Smith P. Caffeine potentiates the nephrotoxicity of mefenamic acid on the rat renal papilla. *Am J Nephrol.* 1990;10(4):311-5.
13. Inkielewicz-Stepniak I, Czarnowski W. Oxidative stress parameters in rats exposed to fluoride and caffeine. *Food Chem Toxicol.* 2010;48(6):1607-11
14. Ohta A, Lukashev D, Jackson EK, Fredholm BB, Sitkovsky M. 1, 3, 7-trimethylxanthine (caffeine) may exacerbate acute inflammatory liver injury by weakening the physiological immunosuppressive mechanism. *J Immunol.* 2007; 179(11):7431-8.