SOME ADVERSE EFFECTS OF FOSFOMYCIN IN RATS

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ABSTRACT
The aim of this study was to determine the adverse effects of fosfomycin on liver, kidney functions which was evaluated by measuring liver and kidney enzymes and through histopathological examination of liver and kidney tissues. Sixty six male Wister albino rats were randomly divided into main six groups including Group(1): control (saline only 0.2ml) Group(2): fosfomycin was given orally 300 mg/kg body weight for 5 days; Group(3), vancomycin was administrated intraperitoneally 200 mg/kg body weight daily for 7 days; Group(4), fosfomycin as in group 2 followed by intraperitoneal injection of vancomycin 200 mg/kg body weight for 7 days; Group(5), *Nigella sativa* oil was given orally 1 ml/kg for 10 days and Group (6), *Nigella sativa* oil as group 5 followed by fosfomycin orally 300 mg/kg body weight for 5 days. The finding revealed that there was significant increase in liver enzymes of rat given fosfomycin treatment group. There was significant decrease in liver enzymes of fosfomycin with *Nigella sativa* group and improvement of histopathological study compared to fosfomycin. There was no significant change in kidney enzymes of fosfomycin treated group. There was decrease in kidney enzymes of fosfomycin with vanomycin group compared to vancomycin group.

Histopathological study showed improvement kidney tissue compared to vancomycin group. It is concluded that supplementation of fosfomycin orally 300 mg/kg body weight for 5 days resulted an increase of liver enzymes and improvement with *Nigella sativa*. There was no change in kidney enzymes level and improvement of nephrotoxic effect of vancomycin.
1-INTRODUCTION
Fosfomycin is a phosphonic acid derivative, which has a broad spectrum antibiotic, structurally unrelated to other classes of antimicrobial agents and with its target site of action unaffected by other antibiotics. Fosfomycin inhibits cell wall synthesis as it interferes with peptidoglycan production at an earlier stage than beta-lactams or glycopeptide antibiotics.\cite{1} Fosfomycin has bactericidal activity against both Gram positive and Gram negative bacteria \textit{Staphylococcus aureus}, \textit{Staphylococcus epidermidis}, enterococci and \textit{Esherichia coli}.\cite{2,3} The use of fosfomycin in animals and humans has been proposed because of its low toxicity and potential efficacy.\cite{4} It is also widely used in animal production due to its rapid effect, good tolerance and lack of side effects.\cite{5,6} Fosfomycin has other properties such as, inhibition of bacterial adhesion to epithelial cells, penetration of wells in the biofilms of exopolysaccharides and promotion of phagocytosis. The aim of the present study was to investigate the adverse effect of fosfomycin on liver, kidney functions and histopathological changes. The protective effect of fosfomycin on nephrotoxicity induced by vancomycin and the protective effect of \textit{Nigella sativa} against hepatotoxicity resulted after fosfomycin were investigated in rats.

2-MATERIALS AND METHODS
Materials

\textbf{Fosfomycin} (phosfomycin) C3H7O4P, a phosphonic acid derivative (cis-1,2-epoxypropyl phosphonic acid) [(2R,3S)-3-methyloxiran-2-yl] phosphonic acid obtained from Adwia company.

\textbf{Vancomycin} (C66H75Cl2N9O24.HCl) is a complex tricyclic glycopeptide antibiotic produced by \textit{streptococcus orientalis} obtained from Sigma chemical company (St. Louis, Mo).

\textbf{Nigella sativa} (black cumin) belongs to the family \textit{Ranuncu-laceae} and is widely distributed and cultivated in Medi-terranean countries, middle Europe and western Asia. \textit{Nigella sativa} oil was obtained from local market of Benha Qaliubiya government.

\textbf{Animals}
66 male Wister albino rats weighting 200-250 g (age of rat 50–60 days) were used in the experimental investigation of these study. Rats were obtained from animal house of faculty of veterinary medicine, Benha university EGYPT. They were fed fresh-pelleted food and their
water as placed in glass bottles of 500 ml. Rats were kept at a constant environmental and nutritional condition throughout the period of experiment. The animals were left for 15 days for acclimatization before the beginning of the experiment.

**Experimental design.**
The rats were randomly divided into main 6 groups.

Group (1): Eleven rats were administrated saline only (0.2ml). Group (2): Eleven rats were administrated fosfomycin orally 300 mg/kg b.wt for 5 days. Group (3): Eleven rats were administrated vancomycin intraperitoneally 200 mg/kg b.wt daily for 7 days for induction of renal toxicity. Group (4): Eleven rats were administrated fosfomycin as in group 2 followed by intraperitoneal injection of vancomycin 200 mg/kgb.wt. for 7 days. Group (5): Eleven rats were administrated *Nigella sativa* oil orally 1 ml/kg for 10 days. Group (6): Eleven rats were administrated *Nigella sativa* oil as group 5 followed by fosfomycin orally 300 mg/kg b.wt. for 5 days.

**Blood samples**
Blood samples were taken at First day, seventh day, fourteenth day post-treatment in all groups after the end of administration of fosfomycin, vancomycin, fosfomycin with vancomycin, *Nigella sativa* and *Nigella sativa* with fosfomycin. The blood sample was taken from each rats in the group for biochemical studies from median canthus of the eye. The blood sample was collected without anticoagulant for separation of clear serum for biochemical analysis. These serum samples were used for biochemical analysis to determine serum total bilirubin, serum transaminases activities (AST and ALT), alkaline phosphatase (ALP), total protein, albumin, blood creatinine, blood urea, creatinine kinase.

**Biochemical Analysis**
Stocks and working solutions were maintained at 0°C in a refrigerator. Serum was obtained by high speed centrifugation. Serum total and direct bilirubin was determined according to the method described by.[7] Serum AST and ALT were determined colorimetrically using spectrophotometer using specific kits (Centronic company) according to.[8] Serum alkaline phosphatase was determined according to[9] by kit from (Centronic company). Colorimetric determination of serum albumin was carried out according to.[10] Colorimetric determination of serum creatinine was carried out using spectrophotometer according to[11] using specific kits. Serum urea concentration was determined according to[12] by kits (Centronic company).
Serum creatinine phosphokinase activity was determined according to\textsuperscript{[13]} by kit (Centronic company).

**Histopathological study**
Tissue samples were taken from the liver and kidney of rats in different groups and fixed 10% formaldehyde saline for twenty four hours and then processed for histopathological examination.\textsuperscript{[14]}

**Statistical analysis**
The data were calculated as mean ± standard error. All statistical analysis was carried out according to\textsuperscript{[15]} using the following formulae.

\[
\bar{X} = \frac{\sum x}{n}
\]

\[
SD = \sqrt{\frac{\sum(x_i - \bar{X})^2}{n-1}}
\]

\[
S.E. = \frac{S.D.}{\sqrt{n}}
\]

Where
\[X= \text{mean}\]
\[\sum x = \text{sum of values}\]
\[n = \text{number of rat in each group}\]
\[S.D. = \text{standard deviation from the mean}\]
\[\sum{\bar{X}} = \text{sum square difference of mean from value}\]
\[S.E. = \text{Standard error of the mean}\]

The compared by student’s (t) probability test which was performed according to the following equation.

\[
t = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{(SE1)^2 + (SE2)^2}}
\]

Where
\[\bar{X}_1 = \text{mean of data of control rat}\]
\[\bar{X}_2 = \text{mean of data of the tested rat}\]
RESULTS

Effect on liver function

Administration of fosfomycin (300mg/kg b.wt) for 5 days induced significant increase in serum total bilirubin, AST, ALT, ALP and decrease in serum albumin and total protein (figure 1-6). Administration of fosfomycin together with vancomycin reduced significantly the significant increase of the previous parameters of liver (figure 1-6). The hepatotoxicity of fosfomycin can be resolved by administration of *Nigella sativa* oil.

Effect on kidney function

Oral ingestion of fosfomycin (300mg/kg b.wt) for 5 days resulted in not significant changes of serum creatinine, urea, creatinine kinase. But vancomycin induced nephrotoxicity which can be reversed by fosfomycin (Figures 7-9).

The histopathological findings confirmed the obtained biochemical results Figure (10-17).

Effect of fosfomycin (300 mg/kg body weight) orally for 5days, vancomycin taken (200 mg/kg body weight) intraperitoneally for 7days and *Nigella sativa* oil (1ml/kg body weight) orally for 10 days on serum compared to control level in rats (n=5).

Significantly difference from (*p<0.05,**p<0.01 and***p<0.001) when compared to control values,(#p<0.05,#p<0.01) when compared to group(2) fosfomycin and( ³p<0.05,²p<0.01 and ³³p<0.001) when compared to group (3) vancomycin
1-TOTAL BILIRUBIN (mg/dl)

Figure (1)

2-AST (U/L)

Figure (2)

3-ALT (U/L)

Figure (3)
4-ALP (U/L)

![Graph showing ALP levels over time for different groups.](image)

**Figure (4)**

5-Total protein (g/dl)

![Graph showing total protein levels over time for different groups.](image)

**Figure (5)**

6-Albumin (g/dl)

![Graph showing albumin levels over time for different groups.](image)

**Figure (6)**
7-Creatinine (U/L)

Figure (7)

8-Urea (mg/dl)

Figure (8)

9-Creatinine kinase (U/L)

Figure (9)
Figure 10: Liver of fosfomycin treated rat showing dilatration of portal vein (p.v) with diffuse degeneration in the hepatocytes (H) all over the parenchyma. (H&E, x40).

Figure 11: Liver of fosfomycin treated rat at seventh day showing sever congestion in central vein (c.v) with inflammatory cells infiltration in portal area (p). (H&E, x40).
Figure 12: Liver of fosfomycin treated rat at fourteenth day showing periduct fibrosis (f) with inflammatory cells infiltration and desquamation of lining epithelium in the lumen of bile duct in portal area (p). (H&E,x40).

Figure 13: kidney of fosfomycin treated rat showing normal histopathological structure (H&E,x40)
Figure (14): kidney of vancomycin treated rat showing highlights minor glomerular disturbance, cytoplasmic granulations in tubular cells and interstitial inflammatory infiltrate. Mild interstitial and glomerular capillary congestions (H&E,x40).

Figure 15: Kidney of rat administrated fosfomycin followed by vancomycin at first day showing cystic tubular dilatation in the degeneration tubules at corticomedullary. (H&E,x40).
Figure 16: Liver of rat administrated *Nigella sativa* followed by fosfomycin showing inflammatory cells infiltration in the portal area (p).(H&Ex40).

Figure 17: Liver of rat administrated treated fosfomycin followed by vancomycin at seventh day showing periductal fibrosis (f) with inflammatory cells infiltration in the portal area surrounding the bile duct with foal haemorrhage in hepatic parenchyma (H).(H&E,x40).
4. DISCUSSION

Fosfomycin is one of antibiotic drug, which the only representative of its drug class (phosphonic group). The present study was designed to investigate the benefit effect with other antibiotic as vancomycin (aminoglycoside group) to decrease the adverse effect on kidney. On the other hand, fosfomycin have adverse effect on the liver so in this study using the *Nigella sativa* which have hepatoprotective effect.

1 Effect on liver function

Effect of fosfomycin, vancomycin, fosfomycin with vancomycin, *Nigella sativa* oil and *Nigella sativa* oil with fosfomycin on liver function tests: total bilirubin, aspartateaminase, alanineaminase, alkaline phosphate total protein and albumin were investigated.

Fosfomycin showed significant increase in serum of total bilirubin, aspartateaminase, alanineaminase, alkaline phosphatase and decrease total protein and albumin after oral administration of fosfomycin (300 mg/kg body weight for 5 days) in rats. The obtained results came in agreement with that obtained by, [16],[17],[18],[19] and [20]. The obtained results were inconsistent with [21], who found that fosfomycin sodium had no interference on glutamate oxaloacetate transaminase and glutamate pyruvate transaminase activity measurement.

Vancomycin induced significant increase in serum total bilirubin, aspartateaminase, alanineaminase, alkaline phosphatase and significant decrease of total protein and albumin after administration of 200 mg/kg body weight daily for 7 days intraperitoneally in rats. This results came consistent with that obtained by, [22],[23],[24],[25] and [26], who reported elevation of liver enzyme levels in a case after oral vancomycin administration.

The effect of fosfomycin 300 mg/kg body weight for 5 days given orally followed by vancomycin (200 mg/kg body weight daily for 7 days) intraperitoneally showed significant increase in total bilirubin and decrease in aspartateaminase, alanineaminase, alkaline phosphatase, total protein and albumin compared to fosfomycin and vancomycin. There are little studies concerning the effect of fosfomycin with vancomycin on liver. This attained results were similar to that reported by [21], who revealed that there was no enhanced hepatic toxicity of carbon tetrachloride by fosfomycin sodium. Also [27] explored the optimal combination of agents (fosfomycin-selenium- zinc- sodium thiosulfate) used along with cisplatin for protection of hepatotoxicity in mice. The result of present study were
inconsistent with\cite{28} proved that fosfomycin not prevent the effect on glutamate oxaloacetate transaminase and glutamate pyruvate transaminase activity of cisplatin in rats.

The effect of *Nigella sativa* oil (1 ml/kg for 10 days) orally in rats showed no significant difference in serum of total bilirubin, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, total protein, and albumin compared to control. The obtained results came in agreement with that obtained by\cite{29,30,31} This results were inconsistent with\cite{32}, who found that *Nigella sativa* increased the albumin level and aminotransferase. No changes were noticed in platelet count and aspartate aminotransferase activity in rats.

The effect of *Nigella sativa* oil (1 ml/kg for 10 days) orally followed by fosfomycin (300 mg/kg body weight for 5 days) orally in rat showed a significant decrease in serum of total bilirubin, alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase but there was increase of total protein and albumin compared to fosfomycin. The obtained results were consistent with\cite{33,34,35} investigated the hepatoprotective effect of.

*Nigella sativa* in isoniazid-induced hepatotoxicity in rabbits\cite{36} revealed that *Nigella sativa* oil able to give protection against paracetamol induced liver damage in rats\cite{37} studied the hepatoprotective effect of *Nigella Sativa* L. alcoholic extract on D-Galactosamine /Lipo polysaccharide induced hepatotoxicity in rats\cite{38} reported the hepatoprotective effect of.

*Nigella sativa* on rats\cite{39,40} reported that *Nigella sativa* extract possesses hepatoprotective action against paracetamol induced acute hepatotoxicity.

### 2. Effect on kidney function

Orally administrated fosfomycin in a dose of 300 mg/kg body weight for 5 days on serum creatinine, urea and creatinine kinase in rats showed no significant difference in kidney function tests .This results were similar to that obtained by\cite{41and42}

The effect of intraperitoneal injection of vancomycin at 200 mg/kg body weight daily for 7 days in rats showed a significant increase in serum of creatinine, urea and creatinine kinase. The obtained results were came in agreement with that obtained by\cite{43,44,45} who indicated that vancomycin induced nephrotoxicity in rabbits which represented by increases of serum creatinine concentration and blood urea nitrogen and morphological changes of the kidney after single intravenous injection of vancomycin of 300 mg/kg\cite{46,47,48,49,26} The obtained
results were inconsistent with\textsuperscript{[50]} who reported that vancomycin did not alter renal function in rats.

The effect of oral fosfomycin (300 mg/kg body weight for 5 days) followed by vancomycin (200 mg/kg body weight daily for 7 days) intraperitoneally showed a significant decrease in kidney testes (serum creatinine, urea and creatinine kinase compared to vancomycin). This results were consistent with that obtained by\textsuperscript{[51, 52, 53]} who showed that fosfomycin at dosages of 50 and 250 mg/kg protected against nephrotoxicity caused by vancomycin (50 mg/kg) in rats.\textsuperscript{[54, 55, 56]} The obtained results were inconsistent with\textsuperscript{[57]}, who stated that fosfomycin induces no change in the lysosomal structural latency and enzymatic study showing no change in the activities of hydrolases (alanine-aminopeptidase, alpha-galactosidase, N-acetyl-beta-D-glucosaminidase and sphingomyelinase), after treatment with 100 and 500 mg/kg fosfomycin.

The effect of oral administration of 1 ml/kg for 10 days \textit{Nigella sativa} oil in rats showed no a significant difference in serum of kidney (creatinine, urea, creatinine kinase) compared to control group. The obtained results came in agreement with that obtained by.\textsuperscript{[58, 59, 60, 61]}

The effect of oral \textit{Nigella sativa} oil (1 ml/kg for 10 days) followed by fosfomycin (300 mg/kg body weight for 5 days) orally in rat showed synergistic effect on creatinine and urea. There was a significant increase of creatinine, while no difference of urea and creatinine kinase. There are no available literatures concerning the effect of \textit{Nigella sativa} with fosfomycin on kidney function.

3. Histopathological changes

Liver examination of oral fosfomycin (300 mg/kg body weight for 5 days) on histopathological findings of the liver showed mild to moderated changes. The results came in agreement with those obtained by\textsuperscript{[19]}, who illustrated that acute hepatitis induced by fosfomycin after biopsy.

Kidney examination was done after oral fosfomycin (300 mg/kg body weight for 5 days) on histopathological changes of kidney. There was no change in kidney. The obtained results were consistent with\textsuperscript{[42] and 57]}.  

\textsuperscript{[50]} Mohamed \textit{et al.}, World Journal of Pharmacy and Pharmaceutical Sciences
The intraperitoneal administration of vancomycin at 200 mg/kg body weight for 7 days on histopathological findings of liver showed mild to moderate changes. The obtained results came in agreement with that obtained by[25], who determined the effect of different vancomycin dilutions on liver by biochemical parameters and histopathological analysis.

Liver and cannulated femoral vein fragments were collected. Intense inflammatory infiltrate with a large amount of inflammatory cells, polymorphonuclear leukocytes or mononuclear cells and a mild edema. Plasmocytes and mastocytes were mostly evidenced.

The effect of intraperitoneal administration of vancomycin at 200 mg/kg body weight for 7 days on histological findings of kidney showed mild to moderate degree of renal degeneration and necrosis. This results were similar to that obtained by[25, 62, 63, 64]

The effect of oral fosfomycin (300 mg/kg body weight for 5 days) followed by intraperitoneal vancomycin (200 mg/kg body weight daily for 7 days) induced a mild to moderate changes. There aren’t studies concerning the effect of fosfomycin and vancomycin on histopathological structure of liver.

The effect of oral fosfomycin (300 mg/kg body weight for 5 days) orally followed by intraperitoneal vancomycin (200 mg/kg body weight daily for 7 days) on kidney changes were investigated. There was improved in the histopathological findings compared to vancomycin. The obtained results came in agreement with that obtained by[65] who proved the protective effect of fosfomycin against cisplatin-induced toxicities in rats, nephrotoxicity and ototoxicity. It was recorded that significantly reduced functionally or histopathologically by the combined administration of cisplatin and fosfomycin. Another similar results were obtained by.[66]

Fosfomycin decreased glycopeptide antibiotic-induced nephrotoxicity as shown fewer histopathological signs of nephrotoxicity in the groups treated with the combination of glycopeptide and fosfomycin.[55] investigated the effect of coadministration of fosfomycin on nedaplatin-induced nephrotoxicity in rats. Fosfomycin decreased nedaplatin-induced nephrotoxicity and shown by fewer histopathological signs of nephrotoxicity in the groups treated with the combination of nedaplatin and fosfomycin.
The effect of oral *Nigella sativa* oil (1 ml/kg for 10 days) on histopathological finding of the liver. It showed normal histopathological structure of liver and the effect of oral *Nigella sativa* oil (1 ml/kg for 10 days) followed by oral fosfomycin (300 mg/kg body weight for 5 days) in rat showed hepatoprotection on histopathological section of liver compared to fosfomycin. The obtained results were consistent with that obtained by[30], who reported that *Nigella sativa* alone induce no harmful effects on the liver by histopathological examination of the liver tissue in male Albino mice and the protective role of *Nigella sativa* in dimethylaminoazobenzene induced liver carcinogenesis. Another similar results were obtained by[35], who reported that the histopathological findings were normal in three rabbits treated with *Nigella sativa* before isoniazid, very mild in two, and with moderate changes in one rabbit. *Nigella sativa* has hepatoprotective effects against isoniazid induced hepatotoxicity in rabbits.

The oral administration of *Nigella sativa* oil 1 ml/kg for 10 days showed normal histopathological changes in kidney tissue. The obtained results came in agreement with that obtained by[60], who determined the toxic effect of *Nigella sativa* powder on the kidney function through histopathological examination of kidney tissue in rats. Histopathological examination of kidney tissue showed normal kidney architecture with no tissue degeneration, inflammation, necrosis, and tubular dilation with normal kidney tissue in histology examination.

The effect of oral *Nigella sativa* oil (1 ml/kg for 10 days) followed by oral fosfomycin (300 mg/kg body weight for 5 days) on histopathological examination of kidney in rats showed normal histopathological tissue. There aren't studies concerning the effect of *Nigella sativa* with fosfomycin on kidney function. There is no available literatures concerning the combination of *Nigella sativa* and fosfomycin on kidney.

5. CONCLUSION

In the present work, it was concluded that the oral administration of fosfomycin 300 mg/kg body weight for five days in rats had protective effect on nephrotoxicity of vancomycin. It showed a significant decrease in kidney testes (serum creatinine, urea and creatinine kinase compared to vancomycin. On other hand hepatotoxicity of fosfomycin can be resolved by oral administration of 1 ml/kg for 10 days *Nigella sativa* oil.
6. REFERENCE


