Research Article

# Investigation and Comparison of the Protective Effect of Some Statins against Cyclophosphamide-Induced Urotoxicity (Hemorrhagic Cystitis) in Rats

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**ABSTRACT**

Cancer is a common cause of mortalities. The systemic chemotherapy is usually required for the effective management of cancer. Cylophosphamide (CP) is an alkylating agent, which is hydroxylized by hepatic P450 to the active 4-hydroxy metabolites; phosphoramide mustard and acrolein (AC). The common adverse reactions of CP are urological, mainly hemorrhagic cystitis (HC) which is caused by AC. MESNA is the most common used uroprotective agent, but its uroprotective effect cannot always be achieved. Statins, have pleiotropic effects (anti-inflammatory and immunomodulatory effects). The aim of this study was to investigate and compare the potential preventive effect of some statins; atorvastatin (AT) and fluvastatin (FL), against CP-induced HC in rats – In this study, adult male wistar rats, weighing (190-260 g), were divided into 4 groups of 12 animals in each group. Group (A) was received saline (10 ml/kg, i.p) as a normal control, group (B) was received CP (200 mg/kg, i.p) as a single dose, and group (C,D) were received AT and FL, respectively, in a dose of 10 mg/kg,PO, for each compound, and for 7 days before administration of CP in a dose of 200 mg/kg, i.p. Animals were Sacrificed 24 hours after administration of CP or saline. The weights of isolated bladders were measured, and histopathological changes were determined – The results revealed that statins have some but not full protective effect against CP toxicity. AT was better than FL as a protective agent.

**Keywords:** Cyclophosphamide, protective, urotoxicity, hemorrhagic cystitis, statin.

## INTRODUCTION

Cancer is a common cause of mortalities. it is usually caused by a mutation or by some other abnormal activation of cellular genes that control cell growth and cell mitosis [1]. The systemic chemotherapy is usually required for the effective management of cancer [2]. almost of chemotherapeutic agents commonly cause severe vomiting, stomatitis, bone marrow suppression, and alopecia[3]. Cylophosphamide (CP) is an alkylating agent, used singly or in a combination with other chemotherapeutic agents for treatment of various neoplastic (e.g. breast cancer) and non-neplastic diseases (e.g. nephrotic syndrome in children)[4]. It's a prodrug that is hydroxylized by hepatic P450 to the active 4-hydroxy metabolites[3,5]; phosphoramide mustard (which is responsible for antitumor activity) and acrolein (AC) (which is responsible for the urotoxic effect of CP)[2,3]. CP causes many adverse reactions such as: alopecia, bone marrow suppression, nausea, and vomiting[4,6]. it also leads to urological side effects which may range from microhematuria or transient voiding symptoms to life-threatening hemorrhagic cystitis (HC)[7]. HC is an acute and insidious hemorrhage in the urinary bladder[8]. It is the major potential toxicity and dose limiting side effect of CP which causes urgency, frequency, dysuria, mucosal ulceration, transmural edema, and epithelial necrosis associated with acute hemorrhage[9,10]. Acrolein (AC), the urotoxic metabolite of CP[4], is the most reactive unsaturated α,β aldehyde[11], and it is the causative agent of HC[12]. MESNA; also called sodium 2-(mercaptoethane sulfonate), is the most common used uroprotective agent[3], it also can delay degradation of the 4-hydroxy metabolites of CP[13,14,15]. But it's observed that a significant percent (about 33%) of patients present with one feature of HC at least, such as hematuria[16]. Statins; also called HMG-CoA reductase inhibitors, reduce plasma cholesterol levels by inhibition of hydroxy methyl glutaryl Co-A reductase[17,18,19]. they also have anti-inflammatory and immunomodulatory effects (pleiotropic effects)[16]. this group involves atorvastatin (AT) and fluvastatin (FL) which are used in this study.

## MATERIALS AND METHODS

In this study, adult male wistar rats (obtained from the Researches and Scientific Studies Center, Damascus, Syria), weighing (190-260 g), were randomly divided into 4 groups of 12 animals in each group. Animals were housed in laboratory animals incubators in faculty of pharmacy, University of Damascus, Syria, in environmentally (25 °C) and air humidity controlled room (60%) and kept on standard laboratory diet (pellets which consist of: 16% protein (minimum), 56% carbohydrate, 0.5% calcium, and moisture 13% - maximum) and were maintained on a 12-hours light dark cycle for 2 weeks prior to the experiments. Group (A) was received saline (10 ml/kg, i.p) as a normal control[6], group (B) was received CP (obtained from Khandelwal Laboratories Pvt. Ltd, India, available as a vial for injection) (in a dose of 200 mg/kg, i.p) as a single dose[20,21], group (C) was received AT (obtained from Alpha Pharmaceutical Industries, Aleppo, Syria) and group (D) was received FL

(obtained from Diamond Pharma Pharmaceutical Industries, Damascus, Syria), in a dose of 10 mg/kg by oral gavage, for each compound, and for 7 days before administration of CP in a dose of 200 mg/kg,i.p. Animals were Sacrificed 24 hours after administration of CP or saline[6,13] by inhalation of diethyl ether. The bladders were collected to compare their weights, and to performing the histopathological study. The weights of isolated bladders were immediately measured after surgical dissection of rats, then they were fixed in 10% formalin for at least 24 hours to perform the histopathological study[6]. The histopathological study was performed in the General Authority of Almouwasat Hospital, Damascus, Syria. Samples were embedded in paraffin using an embedding centre (SLEE MPS/C MAINZ, Germany), then were cut into 5 - 7 µm sections, using a manual microtome (CUT 4050 microTec, Germany), sections were stained with hematoxylin-eosin, using a programmable slide stainer (Medite, Germany). The histopathological changes including inflammation, hemorrhage, tissue injury (hyperplasia of the basal cells layer), epithelial ulceration and necrosis, hydropic degeneration, and vascular congestion were studied. These changes were evaluated according to the criteria which are shown in table 1.

**Table 1:** The histopathological criteria

| Histological Parameter | Normal (0) | Mild (+1) | Moderate (+2) | Severe (+3) |
|---|---|---|---|---|
| **Inflammation** | no inflammation | less than 14 inflammatory cells / field (×40) | more than 14 inflammatory cells / field (×40) | more than 30 inflammatory cells / field (×40) |
| **Hemorrhage** | no hemorrhage | with telangiectasia | with mucosal hematomas | with intravesical clots |
| **Tissue injury (hyperplasia of the basal cells layer)** | 1 layer of basal cells | 2 layers of basal cells | more than 2 layers of basal cells | --- |
| **Epithelial ulceration** | 6 layers of transitional epithelium cells (or more) | 3 layers of transitional cells | 1-2 layers of transitional cells | transitional cells were reduced to zero |
| **Epithelial necrosis** | no necrosis | local necrosis in some epithelial cells in a limited area | spread necrosis in several layers of epithelium in a limited area | spread necrosis in several layers of epithelium and along the urinary tract including transitional epithelium and connective tissue |
| **Hydropic degeneration** | no hydropic degeneration | some small drops of water in some cells but these cells was survived (these cells had nuclei) | large drops in many cells leading to cell death (these cells didn't have nuclei) | many drops of water were accumulated to form one large drop of water |
| **Vascular congestion** | no vascular congestion | it has seen some red blood cells within the blood vessels | large amount of red blood cells within the blood vessels without extravasation of these cells to the neighboring connective tissue | large amount of red blood cells within the blood vessels with extravasation of these cells to the neighboring connective tissue |

**Statistical analysis**

The bladders weights were expressed as mean± SD, while the histopathological changes were expressed as median± SD. Kolmogorov-Smirnov test was used in tests of normality for both bladders weights and histopathological studies, one way ANOVA test followed by Fischer test were used for parametric data (bladders weights), Kruskal Wallis test followed by Mann-Whitney test were used for nonparametric data (histological changes). The SPSS software was used for analysis of data, the charts were drawn using MS Excel 2007.

## RESULTS AND DISCUSSION

Although it wasn't perfect, the pre-treatment with statins had a protective effect against CP toxicity. AT was better than FL as a protective agent. The bladders weights in group (B) were significantly upper than that in group (A). There was no significant difference in the bladders weights between groups (A,C,D), but it wasn't significantly lower in groups (C,D) than that in group (B). (mean± SD: 0.12±0.01, 0.19±0.05, 0.15±0.02, 0.16±0.03, respectively). (Figure 1).

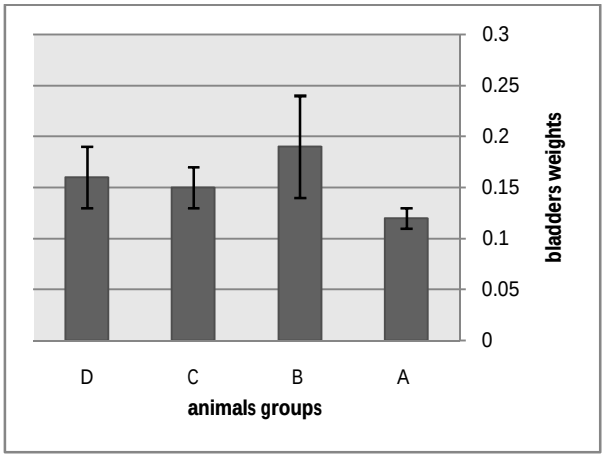


**Figure 1:** Comparison of the bladders weights between the groups of animals.

Hemorrhagic cystitis observed 24 hours after CP administration in the group (B) was significantly different (upper) from the control. The final scores of histopathological changes in the bladders of groups (C) and (D) were significantly lower than that in group (B), and significantly upper than that in group (A), there was no significant difference between groups (C) and (D). (median±SD: 1.5±1.37, 10.5±1.13, 5±1.16, 5±1.15, respectively). (Figure 2).

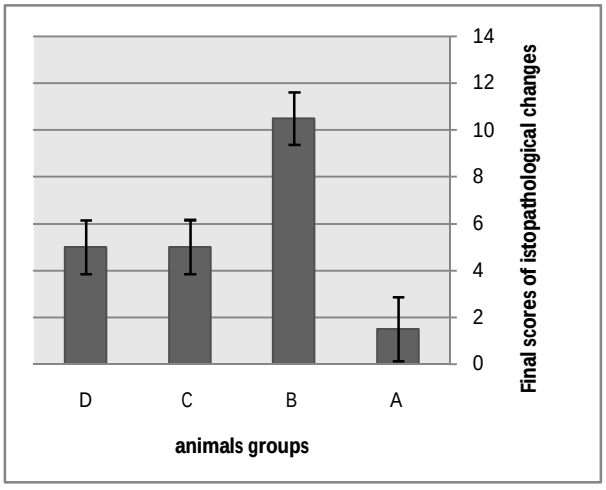


**Figure 2:** Comparison of the histopathological changes between the groups of animals.

Figure 3 shows the microscopic changes in the bladder.

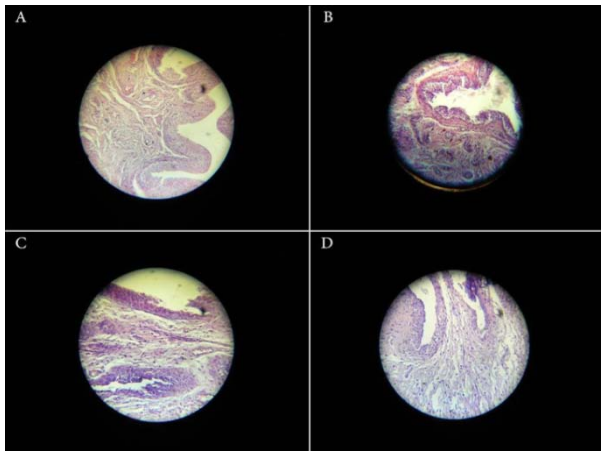


**Figure 3:** The microscopic changes in the bladder (×10); normal bladder (A); bladder with cyclophosphamide-induced hemorrhagic cystitis, epithelial ulceration and necrosis, vascular congestion and inflammatory cells infiltration (B); bladder of a rat which has been pre-treated with atorvastatin (C); bladder of a rat which has been pre-treated with fluvastatin (D).

## CONCLUSION

Current study suggests that statins (atorvastatin and fluvastatin) have a protective effect against cyclopospamide-induced hemorrhagic cystitis, although it's not a full effect. AT was slightly better than FL regarding to its effect on bladder's weight, but their effects on the histopathological changes were almost equal. This study suggests that both AT and FL are effective against CP-induced HC.

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