Effect of Antox on Paraquat - Induced Histological and Biochemical Changes in Kidney of Albino Rats

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Abstract: Paraquat is a widely used and effective herbicide. Treating rats with paraquat at a dose level of 1/36 LD₅₀ 3 d/week for 3 weeks induced many histological changes in the kidney. The renal tubules lost their characteristic appearance and their lining epithelial cells appeared with cytoplasmic vacuolation. The glomeruli were degenerated and the renal blood vessels were congested. The intertubular spaces were infiltrated by inflammatory leucocytic cells. Paraquat also caused marked elevation in serum creatinine and blood urea nitrogen. These alterations were time-dependent. Treating rats with paraquat and the antioxidant, antox led to an improvement in both the histological and biochemical alterations induced by paraquat. This inhibitory effect of antox is attributed to its antioxidant and free radicals scavenging properties of its components (selenium, vitamin A acetate, ascorbic acid and vitamin E).

Keywords:

INTRODUCTION

Paraquat (1,1’-dimethyl-4,4’-bipyridylium dichloride) is a widely used and effective herbicide with a broad spectrum of activity. However, paraquat is quite toxic; the toxicity on animals and humans has been well documented⁵,⁷. A mouthful of the herbicidal compound usually results in death from caustic burns, renal tubular necrosis, and circulatory failure due to pulmonary fibrosis Campbell⁶. Experimental studies have shown that it is accumulated in the lung and kidney epithelial cells, leading eventually to pulmonary fibrosis and acute renal failure⁸,¹⁰. Many studies showed that paraquat poisoning was accompanying by histological changes in different organs of laboratory animals⁹,¹⁰.

Antioxidants nutrients, as ascorbic acid, tocopherol, B-carotene, etc., are considered to give protection against oxidative damage induced by different toxicants and reduce the activity of free radical-induced reactions¹⁶. Antox is an antioxidant drug composed of selenium, vitamin A acetate, ascorbic acid and vitamin E. Antox was used in therapy of different liver diseases¹⁴,¹¹,¹²,¹⁶. The aim of the present work is to explore the effect of antox on paraquat –induced kidney injury in albino rats.

MATERIALS AND METHODS

Adult male albino rats weighing 120 ± 5 g were used. Animals were kept in the laboratory under constant temperature (24±2 ℃) for at least one week before and throughout the experimental work. They were maintained on a standard diet and water was available ad libitum. Animals were divided into 4 groups. Group 1: animals of this group (20 rats) were given orally the herbicide paraquat dissolved in mammalian saline solution at a dose level of 1/36 LD₅₀ (3.46 mg/kg body weight) 3 times per week for 3 weeks. Group 2: animals in this group (20 rats) were given the same dose of paraquat given to animals of group 1 followed by antox dissolved in water at a dose level of 3.4 mg/kg body weight 3 times weekly for 3 weeks. Antox tablets composed of selenium, medicinal yeast, ascorbic acid, vitamin A acetate and vitamin E (Arab Company for Pharmaceuticals and Medicinal Plants). Rats in the third group (20 animals) were given antox only and those in the fourth group (10 animals) were given saline. The treated animals and their controls were killed by cervical dislocation, quickly dissected and their kidneys were fixed in Bouin’s fluid, dehydrated, embedded in wax and 5 micrometers thick sections were stained with haematoxylin and counterstained with eosin. for creatinine and urea nitrogen

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determination, sera were obtained by centrifugation of
the blood samples and stored at -20°C until assayed
for the biochemical parameters. Serum creatinine and
urea nitrogen were measured using a fully automated
Hitachi 911 analyzer (Tokyo, Japan). Commercial
Randox kits (Randox Laboratories Ltd, Ardmore,
Crumlin, United Kingdom) were used in these
analyses. The results were analysed statistically using
Student’s “t” test.

RESULTS AND DISCUSSIONS

Data in table (1) shows that treating animals with
paraquat induced an elevation in creatinine in the
serum compared with that of control. This increase
was significant (p < 0.05) after 2 and 3 weeks of
treatment. Animals treated with paraquat and antox
showed a significant decrease in creatinine values in
comparison with those treated with paraquat alone.
Results in table (2) shows that blood urea nitrogen
exhibited a significant increase after 1, 2 and 3 weeks
of treatment with paraquat. On the other hand, rats
treated with paraquat and antox revealed a significant
decrease in urea nitrogen after 2 and 3 weeks in
comparison with rats treated with antox.

Fig. 1: Section in the kidney of a control rat
showing a glomerulus (G) and renal tubules
(R), x 400.

Figure (1) shows the histological structure of the
kidney of control rat. The kidney is divided into an
outer, granular appearing cortex and an inner, striated-
appearing medulla. It is composed of a huge number
of functional filtering units, nephron. Each nephron
consists of a dilated portion, the renal corpuscle; the
proximal convoluted tubule; the thin and thick limbs
of the loop of Henle; and the distal convoluted tubule.
The renal corpuscle consists of a tuft of capillaries,
the glomerulus, surrounded by a double walled
epithelial

Fig. 2: Section in the kidney of a rat treated with
paraquat for 2 weeks showing dilated and
congested renal vein (CR), x 400.

Fig. 3: Section in the kidney of a treated rat
showing renal tubules desquamated from the
underlying basement membrane (arrow) x
400.

capsule called Bowman’s capsule. Between the two
layers of the capsule is the urinary space. The
proximal convoluted tubule is lined by simple cuboidal
or columnar epithelium. Its cells have an acidophilic
cytoplasm and the apex possesses abundant microvilli
which form a brush border. The distal convoluted
tubule is lined by simple cuboidal epithelium. The
cells of the collecting tubules and ducts stain weakly
with the used stains.

Histological examination of kidneys of animals
after one week of treatment showed insignificant
changes. After 2 weeks, examination of the kidney
tissue showed that most of the renal blood vessels
were dilated, congested and engorged with blood
(Fig. 2).

The lining cells of the renal tubules exhibited
signs of cloudy swelling and most of them showed
Table 1: Effect of different treatments on serum creatinine.

<table>
<thead>
<tr>
<th>Period of treatments (weeks)</th>
<th>Control</th>
<th>Antox</th>
<th>Paraquat</th>
<th>Paraquat + Antox</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.45 ± 0.02</td>
<td>0.55 ± 0.01</td>
<td>0.67 ± 0.03</td>
<td>0.70 ± 0.2</td>
</tr>
<tr>
<td>2</td>
<td>0.43 ± 0.01</td>
<td>0.51 ± 0.02</td>
<td>1.80* ± 0.3</td>
<td>1.21 ± 0.1</td>
</tr>
<tr>
<td>3</td>
<td>0.51 ± 0.01</td>
<td>0.53 ± 0.2</td>
<td>2.3* ± 0.7</td>
<td>1.45 ± 0.4</td>
</tr>
</tbody>
</table>

- Values are expressed as mean ± SD (mg/dl).
- (*) Significant at P<0.05

Table 2: Effect of different treatments on blood urea nitrogen

<table>
<thead>
<tr>
<th>Period of treatments (weeks)</th>
<th>Control</th>
<th>Antox</th>
<th>Paraquat</th>
<th>Paraquat + Antox</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>31.6 ± 1.2</td>
<td>32.4 ± 2.2</td>
<td>54.6* ± 1.4</td>
<td>43.5 ± 1.4</td>
</tr>
<tr>
<td>2</td>
<td>33.2 ± 2.3</td>
<td>31.8 ± 3.1</td>
<td>68.7* ± 2.4</td>
<td>36.7 ± 3.4</td>
</tr>
<tr>
<td>3</td>
<td>33.6 ± 1.2</td>
<td>30.9 ± 2.2</td>
<td>77.3* ± 1.5</td>
<td>41.4 ± 5.2</td>
</tr>
</tbody>
</table>

- Values are expressed as mean ± SD (mg/dl).
- (*) Significant at P<0.05

Fig. 4: Section in the kidney of a treated rat showing leucocytic infiltration (F) and degenerated glomeruli (G), x 400

Fig. 5: Section in the kidney of a rat 3 weeks post-treatment with paraquat showing degenerated tubules x 400

separation of the tubular cells from the underlying basement membranes (Fig. 3). Large number of glomeruli were degenerated and inflammatory leucocytic cells were abundant in the intertubular spaces (Fig. 4). These histopathological changes were increased after three weeks of treatment. Most of the renal tubules were damaged and lost their characteristic appearance. Their lining epithelial cells became undistinguished and their contents were intermixed with each other (Fig. 5). The walls of Bowman’s capsule were eroded and the glomeruli were atrophied and in some sections appeared as empty spaces containing amorphous cellular derbis (Fig. 6). Kidney sections of animals treated with paraquat and antox for 2 weeks revealed certain degree of improvement in the tubular appearance, but few glomeruli were atrophied (Fig.7). An obvious degree of improvement were observed after 3 weeks and the kidney tissue appeared normal (Fig.8). Animals treated with antox for 3 weeks showed normal appearance of the kidney tissue.

Discussions: The present results showed that paraquat induced many histological alterations in the kidney of albino rats. These results are similar to those shown by many investigators. Abdel-Magid[1] reported that kidney of paraquat intoxicated rats revealed degeneration of renal tubules, shrunken glomeruli and congested dilated blood vessels. Damin et al.,[7] emphasized that as a result of paraquat poisoning,
renal damage occurs. Laurence and Bennett\cite{15} reported that toxicity of paraquat caused renal tubular necrosis followed by kidney failure. Inflammatory cells were observed in the interstitial tissue of kidney of paraquat-treated rats. Similar observation was described in human cases of intoxication with paraquat in the lung and considered as sign of toxicity and consequent activation of defensive mechanism\cite{3}.

Regarding the biochemical results, administration of paraquat caused marked elevation in creatinine and blood urea nitrogen. Similarly, Cobe\cite{10} reported that increased blood urea nitrogen and creatinine concentration are caused by paraquat poisoning. Hyo et al.\cite{14} reported a case of Korean woman who presented with generalized proximal tubular dysfunction including aminoaciduria, phosphaturia and glycosuria after paraquat intoxication. Paraquat is known to be filtered at the glomerulus and actively secreted by tubular cells\cite{2}. In addition, it has been reported that the kidney has the highest concentration of paraquat in rats\cite{21}. Therefore, the histological and biochemical results observed in the present work proved that paraquat affected both function and structure of rat kidney.

Animals treated with both paraquat and antox revealed an improvement in histopathological alterations induced by paraquat alone. Moreover, the elevation of blood urea nitrogen and creatinine was significantly reduced in rats treated with paraquat and antox. This proved the effectiveness of antox in prevention of paraquat-induced renal toxicity. The protective effect of antox was studied by many investigators. Antox succeeded in minimize cadmium-induced toxicity in albino rats and increase the activity of endogenous antioxidants including glutathione, superoxide dismutase and catalase\cite{11}. Watson et al.\cite{24} reported that bio-antox have protective effect against primary biliary cirrhosis. A significant reduction in oxidative stress parameters as well as in blood and hepatic lead level and in hepatic 8-oxodeoxyguanosine phosphate were recorded after giving antox to Schistosoma-infected and chronic lead
exposed hamsters.\textsuperscript{[9]} Antox was found to maintain blood glutathione, plasma vitamin C and serum selenium levels towards the normal range.\textsuperscript{[12]}

Some studies showed that antioxidants are useful in prevention of renal damage induced by different toxicants including paraquat. Nagano et al.\textsuperscript{[18]} reported that dimethylthiourea, defereroxamine and alphatocopherol have a protective action against paraquat-induced acute renal failure in mice. Shukla and Chandra\textsuperscript{[22]} reported that antioxidants (vitamin E and ascorbic acid) produced a significant reduction in lipid peroxidation induced by cadmium and prevent kidney damage in rats. Antox is a multivitamin compound (ascorbic acid, vitamin A acetate and vitamin E). The protective of these vitamins rests with strong antioxidants, free radical scavenging activity and inhibition of lipid peroxidation\textsuperscript{[23]}. Thus the preventive effect of antox against paraquat-induced renal damage recorded in the present work is attributed to its antioxidant properties.

REFERENCES
