AMELIORATIVE EFFECT OF BERBERIS VULGARIS FRUIT EXTRACT AGAINST GENTAMICIN INDUCED NEPHROTOXICITY IN ALBINO RATS

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ABSTRACT

Objective: To observe the nephroprotective role of berberis vulgar is on renal parenchyma against microscopic and morphometric changes induced by Gentamicin in albino rats.

Study Design: Lab based experimental study.

Place and Duration of Study: It was conducted in Baqai Medical University in collaboration with department of Anatomy Liaquat National Hospital and Medical College, from Jan to Jul 2017.

Methodology: A total of 40 male adult albino rats were used in the study. Four groups were made. Each group contained 10 rats. Group A was a control group, group B received only berberis vulgaris fruit extract orally per day for 21 days, group C received Gentamicin 100 mg/kg/day intraperitonially daily for 21 days. Group D received Gentamicin 100 mg/kg/day intraperitonially along with berberis vulgaris fruit extract 100 mg/kg/day orally. Both kidneys were removed. H&E and PAS stains were used for observing histological alterations and protective role of berberis vulgaris fruit extract.

Results: Glomerulus and proximal convoluted tubules were observed histologically in all 4 groups. Microscopy of group B showed parameters nearly similar to control group. Microscopy of group C showed significant derangement in all parameters when compared with control group. Group C showed decrease glomerular, proximal convoluted tubular count was noted. Glomerular diameter increases and there was glomerular hypertrophy and tubular necrosis. Microscopy of group D showed significant improvement due to berberis vulgaris which restored normal renal architecture.

Conclusion: Berberis vulgaris has a nephroprotective effect and it can be used as a new medicine against nephrotoxic drugs like Gentamicin.

Keywords: Berberis vulgaris, Gentamicin, Kidney, Nephrotoxicity.

INTRODUCTION

Kidney play pivot role to remove waste products and overloaded water from the body in the form of urine. Gentamicin is the most common antibiotic against gramnegative bacterial infections. One of the main side effects of gentamicin is nephrotoxicity1. Impairment occurs in 30% of patients treated with gentamicin2.

Gentamicin an aminoglycoside has been reported to induce acute kidney failure due to acute tubular necrosis by increasing serum creatinine and blood urea concentration. This drug also has a potential to cause ototoxicity and nerve damage. Mechanism of Gentamicin induced nephrotoxicity involves different pathways including oxidative stress, inflammation, decreased renal blood flow and increased nitric oxide level (NO)3. However Gentamicin play significant role against life threatening infections especially when used in combination therapy4. It can lead to renal damage due to tubular necrosis and interstitial damage. Aminoglycosides collect in proximal tubular cells, from where they set apart in lysosomes and communicate with ribosomes, mitochondria and remaining cell organelles to generate cell injury5. It works by inhibiting protein synthesis. Berberis vulgaris (Barberry) belongs to the family Berberidaceae. A well-known plant in Iran and has been used widely as a medicinal plant in folk medicine. All parts of berberis vulgaris i.e. root bark, leaves, seeds, and fruits have been used as medicinal pur-pose in Iran and many other countries for long period. Most commonly studied naturally occurring protober-berine alkaloids is berberine6. Iran Berberis vulgaris berries commonly called Zereshk. According to World Health Organization (WHO) in about 75-80% of world’s population, herbal medicine is widely used as a source for primary health care especially in developing countries. All parts of plant have pharmaceutical properties including anti-inflammatory, antioxidant, antinociceptive, antiarrhythmic, anticholinergic, anti-pruritic, anti diarrheal, antiprotozoal properties7. Berberis vulgaris has nephroprotective effect by inhibiting oxidative stress. Berberis act as free radical scavenger and provide strengthful protection in acute kidney damage8. Cytoprotective compounds isolated from
Berberis vulgaris are phenolic compounds which include tyramine, cannabins G and lyoniresinol. Lyoniresinol has antioxidant properties and cannabins exhibit cytoprotective activity.9

**METHODOLOGY**

Forty healthy male albino rats Sprague Dawley species weighing (180-250) gms 10-12 weeks of age were kept. Rats aged 10-12 weeks and weight between 180-250 gms. All animals were weighed, tagged and kept in separate well ventilated cages of Animal house at the beginning of study. Before the start of experimental procedure all albino rats were kept under observation and acclimatization for 10 days to observe their weight gain or loss, their nutritional status on the basis of their oral intake and excretion, behavioural change and general health conditions. All standard of laboratory environment were maintained.

Group A rats required no intervention and served as control group. Group B received Berberis vulgaris fruit extract 100 mg/kg/body wt orally per day for 21 days. Group C received Gentamicin 100 mg/kg/day Intraperitonially, for 21 days. Group D received Gentamicin 100 mg/kg/day Intraperitonially in a single dose per day along with berberis vulgaris 100 mg kg/day through gastric gavages for 21 days. Rats were anesthetized by ether in a glass container and sacrificed. They were fixed on a dissecting board and abdomen was opened by mid line longitudinal incision that extended from manubrium sternum up to the lower abdomen. Both kidneys were removed and cut into two equal longitudinal halves. General architecture of kidney tissue was observed by staining with haemotoxylin and eosin (H&E) and periodic acid Schiff (PAS) was used to observe brush borders of proximal convoluted tubules, basement membrane intactness, architecture of glomerulus and bowman’s capsule under light microscope. Slides of all groups A to D architecture were observed. Random selection of slides was done. In each slides five random fields were selected and in each field almost nearly complete glomerulus and tubule were observed. All slides were observed by light microscope under magnification of 10 and 40.

SPSS version 21 was used for analyzing numerical data. The difference was scrutinized statistically significant when p-value was ≤0.05. The differences among treated groups were analyzed by one-way ANOVA.

**RESULTS**

Renal cortex showed normal architecture of renal corpuscles formed by glomerular tuft of capillaries and bowman’s capsules in group A. Basement membrane of glomerulus was intact. The renal columns and medullary rays were alternately arranged. (fig-1). Narrow lumen of proximal convoluted tubule were found. They are present in large number and lined with simple cuboidal cells. Cytoplasm is eosinophilic containing basophilic nuclei close to basement membrane and is spherical in shape. Wide lumen of DCT observed and their numbers also reduced lined with low cuboidal cells, nuclei at the center. Proximal convoluted tubular lumen showed well-demarcated brush border. No cellular debris in the lumen of PCT was observed. Cellular integrity was maintained (fig-1a).

Group B showed no structural change in glomerulus, proximal tubule and loop of henle. The purpose of this group was to find out if berberis vulgaris itself caused any alteration in morphological and histological structure of renal parenchyma (fig-1b).

The H & E stained renal sections of group C showed altered architecture of renal parenchyma along with degenerative changes in the renal cortex and medulla. Hypertrophy of glomeruli was observed apparently with congestion of glomerular capillaries, edema and hemorrhage. Renal interstitium showed inflammatory cells with moderate edema and congested and dilated blood vessels (table) (fig-1c).
Histopathology of group D showed significant improvement. The H & E stained sections showed near normal renal architecture with slight congestion and hemorrhage around glomeruli. Minimal changes was observed in the glomeruli and most of the glomeruli showed normal architecture with slight glomerular congestion, peritubular congestion along with blood vessels congestion. It was observed that Berberis vulgaris treated group has restored the normal glomerular structure (fig-1d).

Mean of glomerular count, proximal convoluted tubular count and glomerular diameter of group C animal were 3.76 ± 0.97, 6.15 ± 0.139 and 72.6 ± 0.486 respectively (fig 2 & 3). Mean of glomerular count, proximal convoluted tubular count and glomerular diameter of group D animal were 5.13 ± 0.071, 11.79 ± 0.088 and 65.37 ± 0.7589 respectively (fig-2 & 3).

**DISCUSSION**

Gentamicin is the commonly used antibiotic especially in developing countriey because of its low cost and easy availability, however it can induce nephrotoxicity. We observed the effects of gentamicin and berberis vulgaris on glomerulus and proximal convoluted tubules. It was noted in this study that there was decrease in the number of glomerular count, PCT count and Glomerular diameter in groups treated

<table>
<thead>
<tr>
<th>Experimental Groups</th>
<th>Control Group</th>
<th>Berberis Only Group</th>
<th>Berberis Treated Group</th>
<th>Gentamicin Only Group</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glomerular Count</td>
<td>5.73 ± 162</td>
<td>5.82 ± 0.98</td>
<td>3.76 ± 0.97</td>
<td>3.76 ± 0.97</td>
<td>0.0001</td>
</tr>
<tr>
<td>Pct Count</td>
<td>13.08 ± 0.364</td>
<td>12.35 ± 0.185</td>
<td>6.15 ± 0.139</td>
<td>6.15 ± 0.139</td>
<td>0.0001</td>
</tr>
<tr>
<td>Glomerular Diameter</td>
<td>61.32 ± 2.18</td>
<td>66.4 ± 1.769</td>
<td>72.6 ± 0.486</td>
<td>72.6 ± 0.486</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

**Figure-1(c):** Group C showing altered renal parenchyma. Hypertrophy of glomerulus. H & E stain 100x

**Figure-1(d):** Group D showing intact basement membrane. PAS stained 100x.

**Figure-2a:** Comparison of mean values of glomerular count in different groups of experiment.

**Figure-2b:** Comparison of mean values of glomerular diameter in different groups of experiment.
with gentamicin which was due to decrease in body weight and kidney weight. Body weight is the most precise indicator showing adverse effects of different xenobiotics. Difference of weight gain is due to catabolic activity which occurs as a result of renal dysfunction due to gentamicin, leading to acidosis and is accompanied by anorexia, food intake diminishes results in body weight loss due to gentamicin. Generation of ROS may lead to cell damage as well as cell death by scattered mechanism e.g. formation of ATP, cytochrome c release, lipid peroxidation, DNA damage by cell cycle arrest, all these results in cell swelling and necrosis\textsuperscript{12,13}.

Nephrotoxicity induced by gentamicin also involves lipid peroxidation. Gentamicin induced nephrotoxicity is structurally evident by glomerular hypertrophy or atrophy, tubular necrosis, haemorrhage and edema\textsuperscript{21,22}. One of the main mechanism of nephrotoxicity is oxidative stress generated by gentamicin drug which impaired renal function. Berberis vulgaris prevents oxidative damage and induces antioxidant enzymatic activities by decreasing free radicals generation. Berberis vulgaris extract react with metabolites of lipid peroxidation and free radicals as well, thereby increase thiol contents of tissue and produces antioxidant activities. Antioxidants can reverse most of the alterations in histological sections of renal tissue generated by gentamicin. Group B berberis treated group showed similar findings when compared with control group. Group gentamicin treated with berberis showed mild proximal tubular necrosis which showed that berberi vulgaris neutralized nephrotoxicity induced by gentamicin. Similar findings were also reported by chinnapa reddy \textit{et al}\textsuperscript{23}.

Gentamicin treated group produced alteration in glomerular structure in the form of mesangial hypercellularity and proliferation of endothelial cells of glomerulus. There was decrease in glomerular count due to inflammatory process as well in this study. This finding was according to the previously done studies by Alarifi \textit{et al}\textsuperscript{24}.

Microscopic examination of gentamicin treated group C of renal tissues showed changed parenchyma of kidney with proximal tubular dilatation, loss of brush border and necrosis of proximal tubular cells. Dilated proximal tubules contained degenerative and desquamative type cells. It was noted that presence of cytoplasmic vacuoles are due to toxic effect of gentamicin that is dilatation and destruction of the organelles. Most badly affected area by gentamicin is proximal convoluted tubules. It was found that berberis vulgaris possess nephroprotective activity (Soliman \textit{et al}, 2007\textsuperscript{25}).

CONCLUSION

It is concluded that Gentamicin produces oxidative nephrotoxic effects on renal parenchyma which can be limited and prevented by protective antioxidant effect of Berberis vulgaris that restored histomorphological changes in renal tissue induced by gentamicin toxicity.

CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any author.
REFERENCES


